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Taxonomics of piroplasmae and some peculiarities of their development in the vertebrate and invertebrate hosts[1]

Классификация пироплазмид и некоторые особенности их развития в позвоночном и беспозвоночном хозяевах

In the latest years, the piroplasmae have called a great attention. This is not only because of paramount economical importance of the group, but also due to the fact that the taxonomy of the group is still uncertain. Discoveries of the past decade or two on morphology and life cycles of the piroplasmidae made possible to establish their taxonomic position within the system of *Protozoa*. For a long time the piroplasmidae were reasonably admitted as a subordinate unit within the order *Haemosporidia*, class *Sporozoa*. A great contribution in this respect was made by Dennis 1932.

From studies on the life cycle of *Babesia canis*, Regendaz und Reichenow 1933, 1936 conclude that piroplasmidae are not related to *Haemosporidia* but are very close to *Sarcodina*. However, even after the above studies the piroplasmae still remained in the class *Sporozoa*.

The earlier studies (Nuttall and Graham-Smith 1906, Du Toit 1918) suggested that piroplasmae may belong to *Mastigophora*. However, thorough investigations of various piroplasmae with light and electron microscope failed to discover any kinetic elements at any developmental stage of the parasite. This seems a sufficient reason against including the piroplasmidae into the class *Mastigophora*.

*

Our studies (Cheissin i Muratov 1959, Poljansky i Cheissin 1959) on the development of *Piroplasma bigeminum* and *Babesiella divergens* (*B. bovis*) in the ticks and in the vertebrate host furnished a clear evidence of continuous asexual reproduction in both cases. In piroplasmae from the ticks neither sexual reproduction, nor developmental stages resembling gametes or zygotes have been found. As was recently demonstrated (Abramov et Markov 1964) the piroplasmae (*Babesiella ovis* and *Piroplasma caballi*) are capable to a continuous asexual reproduction within 14 and 30 successive

[1] Paper read at the 1st International Congress of Parasitology in Roma, 25 September 1964.

generations of the tick *Rhipicephalus bursa* and *Hyalomma plumbeum* respectively. Such a prolonged repetition of the same developmental stages of the parasite could hardly happen if sexual reproduction realy existed. The above findings forced us to conclude that the piroplasmae could not remain within *Sporozoa* in which the sexual reproduction is known as one of the most typical characters. Phagotrophy, characteristic of the malarian parasite, was seen also on electron micrographs of some piroplasmidae. However, this is nothing else than a convergence due to similar conditions of parasitic life in the blood cells of the vertebrate host, and cannot reflect any relationships between both parasites.

Almost all the developmental phases of piroplasmae are capable to amoeboid movement. Multiple division of piroplasmidae results in development of a multinuclear plasmodium which can fall into numerous agamonts, like in case of schizogony, or uni-and multinuclear agamonts can bud successively from the plasmodium surface.

Thus, the above findings enable us to include piroplasmidae into the class *Sarcodina* as a separate order *Piroplasmida.*

Ancestral forms of these blood parasites remain still unknown, but the similarity between piroplasmidae and amoebae, as concerns their movement and mode of reproduction, allows to unite them in one large taxonomical group. Within *Sarcodina* the piroplasmidae are the only group parasitizing in the blood cells of vertebrates. That is why they deserve rather to be called blood amoebae (P i e k a r s k i 1954).

The question arises whether is it possible to unite piroplasmidae and theileriae? To answer this question it is necessary to assume whether there is sexual reproduction in theileriae. It is usually thought that gametocytes or even gametes of theileriae circulate in the periphery blood of vertebrates. S e r g e n t and oth. 1945 reported the copulation of gametes of *Theileria dispar* in the intestine of *Hyalomma mauritanicum.* However, the authors themselves express some doubts as to the accuracy of their observation.

R e i c h e n o w 1940 denies the existence of sexual reproduction in *Theileria parva* during its development in *Rhipicephalus appendiculatus.* Indeed, it is rather difficult to be sure in case like this. However, it is necessary for establishing the right systematical position of theileriae (I mean *Gonderia* and *Theileria* together). Some data testify to the possibility to unite theileriae and piroplasmidae into one order *Piroplasmida.* It is not only their parasitic mode of life in the blood cells of the vertebrate host, but also a certain similarity of trophozoites (oval, round and amoeboid) and the absence of pigment formation from the host cell hemoglobin, that allows to unite the two groups in one. Their vectors are ixodid ticks.

However, theileriae and piroplasmae differ from each other in some respect, which made it possible to separate them into two different families — *Theileriidae* and *Piroplasmidae.*

The characteristic feature of *Theileriidae* which differentiates them from *Piroplasmidae* is that they multiply in lymphocytes and histiocytes where they undergo multiple division with Koch's bodies formation. In result, a number of uninuclear specimens appear which penetrate the erythrocytes and may or may not reproduce; in the latter case they divide into 2 or 4 daughter cells. Trophozoites of theileriae are rodshaped, oval, round or bacilliform.

At the same time, piroplasmae are known to occur only in the red blood

cells or sometimes in the blood plasma, but not in the lymphocytes of vertebrate host. The trophozoites are piriform, round, oval, amoeboid or rod-shaped. Reproduction is by binary fission or budding, or by multiple division with formation of 4—16 cells.

Passing over the family *Theileriidae* I shall now dwell on the taxonomy of *Piroplasmidae*.

I must stress that up till now no unanimous opinion exists as to the question what and how many genera constitute this family, and what are those characteristic features which might allow to distinguish one genus from another. Moreover, one and the same parasite is sometimes included by different authors in different genera.

There are two main view-points concerning this question. One prefers a single genus *Babesia*, parasitizing in mammals and birds, with a number of species. The other breaks this genus up into several genera, such as: *Piroplasma, Babesia, Babesiella, Francaiella, Nuttallia, Aegyptianella*. I say nothing of the genera *Smithia, Nicollia, Luhsia, Pattonella, Rangellia, Achromaticus*, which reality seems dubious; many of them have been returned to corresponding synonyms. The discrepances of both above view-points can be explained by the different value required of characteristic features upon which a generic difference could be founded.

To my mind, trophozoites possess a number of comparatively steady features which can be taken as generic criteria. They are the relative size of the piriform paired forms, the position of the latter within erythrocytes, the angle between the paired cells, and the mode of reproduction. Moreover, some biological peculiarities of the parasite, its development in the tick in particular, can be taken into account.

Thus, there is sufficient reason for establishment of six reliable genera within the *Piroplasmidae*. Four of them are parasitic in mammals and cause some disease of live-stock. The genus *Aegyptianella* is found in birds, and the genus *Babesiosoma* is known for poikilothermal animals.

Fig. 1. *Piroplasma* (A), *Babesia* (B), *Babesiella* (C), and *Nuttallia* (D) in the blood cell of the vertebrate host

The piriform paired forms of *Piroplasma* (Fig. 1 A) are large, they exceed the radius of the erythrocyte, the angle between two paired parasites is always acute. The genus is divided into several species: *P. bigeminum, P. caballi, P. canis, P. ovis* and others.

The piriform paired forms of *Babesia* (Fig. 1 B) do not exceed the radius of the red cell and frequently are equal to them. They lie, as a rule, in the center of the erythrocyte. The angle between two paired cells is predominantly obtuse. The parasite may be scarce in the perifery blood. *B. bovis, B. berbera, B. argentina*, and some others constitute the genus. *Boophilus* ticks are known

as vector of the *Babesia* of cattle. Many Soviet authors call *Francaiella* some species belonging to the genus *Babesia*. This name was given by Yakimoff 1926 for a group of parasites smaller than piroplasmae. Latter, on, this group became a genus. However, the separation of *Francaiella* genus seems dubious. The characteristic features of *Francaiella* coincide with those of *Babesia*. The latter name was given much earlier than *Francaiella*. Therefore it seems reasonable to consider the generic name *Francaiella* as a synonym of *Babesia*. According to the principle of priority, *Francaiella colchica* Yakimoff et Belawin, 1926 is to be called *Babesia bovis*.

The very identification of *Babesia* species is a special question which needs a thorough examination.

In the genus *Babesiella* (Fig. 1 C) the piriform paired forms are, as a rule, smaller than the radius of the red cell and usually lie along the circumference of the red cell (the so-called "accolé" position). The angle between the paired parasites is obtuse. The parasites are, as a rule, abundant in peripheral blood. *Ixodes* ticks serve as vectors. *B. divergens* and, very likely, *B. ovis* belong to the genus. By the Soviet authors *B. divergens* was called *B. bovis*.

A characteristic feature of the genus *Nuttallia* (Fig. 1 D) is multiple division of trophozoites with formation of 4 daughter cells, joined together in the form of a cross. In contrast to the rest of piroplasmidae, the transmission of *Nuttallia* is realized only stage-to-stage but not through the egg of the tick. As a result, nuttalliae cannot be preserved in ticks as it is the case in the rest of piroplasmidae, which are known to pass from one tick generation to another and thus the parasite can remain in the ticks for many years. For example, *B. ovis* remain in *Rhipicephalus bursa* more that 14 years. Thus, the ticks represent natural reservoir for piroplasmidae, except for nuttalliae. Nuttalliae, on the contrary are capable to be preserved only in the blood of the vertebrate host. *Nuttallia equi* and some species from small mammals belong to the genus *Nuttallia*. It is likely that *Babesia rodhaini* can be also included into the genus *Nuttallia*.

Some non-pigmented blood parasite of birds and those of poikilothermal vertebrates are similar to the species of *Nuttallia*. They are united in the genus *Aegyptianella*; multiple division with the formation of 4—20 agamonts being characteristic of this group. It might be related to the genus *Babesiosoma*, similar to *Nuttallia* by formation of 4 cells arranged as a cross resulting from multiple division. The question of a precise systematical position of the non-pigmented parasites of reptilia, amphibia and fishes needs futher investigation.

Echinozoon from *Heterohyrax syriacus* might be considered as a separate genus (Garnham 1951).

The division of *Piroplasmidae* into 6—7 genera given above seems to reflecte the character of divergence within this family.

A study on life cycles of various piroplasmidae will contribute much into the knowledge of interrelationships within the group. It has been stated that all piroplasmae develop only in the blood cell of the vertebrate host but not in any other tissue cells (Hoyte 1961, Krylov 1964). The parasite lie mainly inside the erythrocytes, however, they can live beyond the erythrocytes in the blood plasma in the internal organs, and here multiple division occurs. The offspring of agamonts inoculated through the tick into the vertebrate host is found in the blood during the incubation period. After the parasites

have been inoculated, the blood becomes infective for the recipients without any latent period as it was reported by many authors for the malarian parasite. The reproduction of the piroplasmae proceeds asynchronically both during the incubation period and some time later, and no periodicity in multiplication was observed.

Our earlier findings on the reproduction of piroplasmidae in the ticks concerned *Piroplasma bigeminum* from *Boophilus calcaratus* and *Babesiella divergens* (*B. bovis*) from *Ixodes ricinus*.

Now we have some new data (study of K r y l o v) on the development of *Nuttallia tadzhikistanica* in *Hyalomma anatolicum* which in many instances resembles those of *Piroplasma* and *Babesiella*.

Reproduction of *Nuttallia* is only asexual. Tick larvae take up the parasite during feeding on the vertebrate host. In the tick's intestine nuttalliae are released from erythrocytes and begin to multiply both by binary fission and by multiple division (Fig. 2). The latter form of reproduction is more usual.

Fig. 2. Developmental stages of *Nuttallia tadzhikistanica* in larva of *Hyalomma anatolicum*

Ripe multinuclear stages reach 10—12 μ in size. They break down into 16—30 uninuclear agamonts 1.5—2.0 μ long. This process is repeating over and over during 24 hours. Unicellular agamonts become cigarlike or piriform, enlarge and measure 3—5 by 1—2 μ. They enter haemocoel and become amoeba-like. The great majority of agamonts probably die in the tick intestine because at larva moult very few parasites, if any, can be found in the body cavity. By the end of larva metamorphosis nuttalliae stop their multiplication and remain dormant up to the nymph stage. In hugry nymphs, uninuclear amoeba-like agamonts, 3—8 by 2—7 μ in size, are found in haemocoel and in salivary gland. When the nymph starts to suck blood, the development of agamonts begins in its salivary glands (Fig. 3). In 42 hours multinuclear plasmodia with some tens of nuclei are found. In 72 hours large plasmodia with some hundred nuclei are met, and the formation of infective agamonts begins. In 86—96 hours a number of piriform agamonts, 1.5—3.0 by 0.8—1.1 μ of size appear in salivary glands. The parasites are injected into the blood of the vertebrate host (*Meriones erythrourus*) on the 4th day of sucking. *Meriones erythrourus* can be easily infected also by the emulsion taken from salivary glands of the nymphs on the 4th day of sucking. A club-form stage, usually present in *Piroplasma* and *Babesiella*, is absent in *Nuttallia*. The

existence of the club-form stage in former genera is thought to be related to transovarian transmission of the parasite. In *Nuttallia* neither the transovarian transmission nor the club-form stage of development occur.

Further investigation of life cycles of different piroplasmae is desirable to provide a more precise knowledge of these organisms.

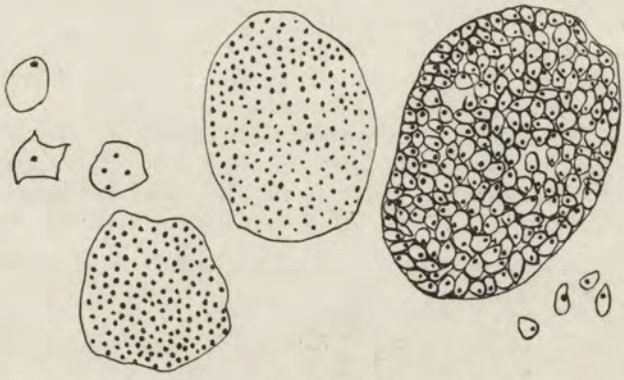

Fig. 3. Developmental stages of *Nuttallia tadzhikistanica* in nymph of *Hyalomma anatolicum*

Summary

The possibility is discussed of including piroplasmidae into the class *Sarcodina*, as a separate order *Piroplasmida*. Theileriae show some features similar to piroplasmidae which allows to include them tentatively also into the order *Piroplasmida*. However, their mode of reproduction differs them from piroplasmae which involves their separation in the second family *Theileriidae*, besides the family *Piroplasmidae*. Representatives of piroplasmidae parasitizing in mammals are the genera: *Piroplasma, Babesia, Babesiella* and *Nuttallia*, in birds occurs the genus *Aegyptianella*, and in the poikilothermal animals — *Babesiosoma*.

The development of piroplasmae, nuttalliae and babesiellae in vertebrate host all along the invasion period is limited to the blood; the parasite does not occur in any other tissue. Data on the development of *Nuttallia tadzikistanica* in ticks are summarized. Nuttalliae reproduce by simple and multiple division — sexual reproduction was not observed.

РЕЗЮМЕ

Рассматривается вопрос о возможности включения пироплазмид в класс *Sarcodina* в виде самостоятельного отряда *Piroplasmida*. Тейлерии обладают некоторыми сходными признаками с пироплазмами но они отличаются от пироплазм характером размножения, в связи с чем их следует обособить в само-

стоятельное семейство — *Theileriidae*, наряду с семейством *Piroplasmidae*. Представители пироплазмид из млекопитающих относятся к родам: *Piroplasma*, *Babesia*, *Babesiella* и *Nuttallia*. У птиц паразитируют представители рода *Aegyptianella* и у пойкилотермных животных — *Babesiosoma*.

Развитие пироплазм, нутталлий и бабезиелл в позвоночном хозяине на протяжении всей инвазии протекает только в крови, но не в каких либо других тканях. Проводятся данные по развитию *Nuttallia tadzikistanica* в клещах. Нутталии размножаются простым и множественным делением. Полового процесса не обнаружено.

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On the systematical position of *Toxoplasma* in *Protozoa*[1]

О положении токсоплазм в системе простейших

In order to conclude whether the *Toxoplasma* belongs to one or another large taxon, the leading features characterizing this taxon should be revealed in toxoplasmae. Our present knowledge, however, is seemingly far from full information on the developmental cycle of *Toxoplasma*. Nevertheless, it may not dissuade us from some suggestions concerning the taxonomic position of this group in *Protozoa*.

According to our views (Cheissin and Poljansky 1963), the phylum *Protozoa* is divided into two subphyla: *Plasmodroma* and *Ciliophora*. The former is characterized by monomorphy of nuclei followed, as a rule, at all the developmental stages, and if sexual reproduction is stated — by copulation of gametes. In the second subphylum the nuclear dimorphism (macronucleus and micronucleus) occurs at all the developmental stages; the sexual reproduction mode is the conjugation.

Toxoplasmae, which have the monomorphous nucleus (at least at the stages studied), belong without any doubt to the subphylum *Plasmodroma*.

With what class can they be related? According to our system, the subphylum *Plasmodroma* embraces following classes: *Sarcodina, Cnidosporidia, Mastigophora, Sporozoa* and *Toxoplasmatea*.

Amoeboid movement is characteristic of *Sarcodina* (though some stages may posses flagella). The presence of flagella or at least some elements of kinetic apparatus (when flagella are absent) is the main feature of *Mastigophora*. *Cnidosporidia* have spores with polar capsulae. *Sporozoa* are characterized by the obligate presence of sexual reproduction in the developmental cycle. Following copulation of gametes, the zygote formed gets incysted and becomes zygocyst or oocyst, in which the sporozoites are formed in result of sporogony. Very often asexual reproduction (schizogony) is inserted into the life cycle. The strict alternation of sexual reproduction and sporogony, or of sexual reproduction, sporogony and schizogony, occurs in *Sporozoa*.

The present data indicate no sexual reproduction in toxoplasmae. Multiplication of trophozoites in the vertebrate host is accomplished by binary

[1] Communication presented at the 1st International Congress of Parasitology in Roma on 23 September 1964.

fission within the "pseudocysts". Neither alternation, nor periodicity in formation of cysts or "pseudocysts" was found. For the time being, there is no reason to suggest that cysts might be oocysts with numerous sporozoites inside. It is likely that "pseudocysts" are stages of asexual reproduction. But in this case some stages of sexual reproduction preceding the cyst formation are expectable. However, all attempts to demonstrate sexual reproduction were unsuccessful. The question arises whether the sexual reproduction of toxoplasmae might occur in the vector? The transmission of toxoplasmosis is known also without the vector. Some epidemiological data testify to the fact that the presence of vector is not necessary in toxoplasmosis. If there is something like "vector", it should play rather a mechanical than a developmental role.

Thus, up to now no data exist which could prove that *Toxoplasma* belongs to *Sporozoa*.

I believe it not correct to include toxoplasmae into the class *Sporozoa* basing only on the finding that trophozoites of *Toxoplasma*, like sporozoites and merozoites of *Coccidia*, have the conoid, similar system of superficial fibrils, paired organelle, micropyle and some other analogous fine structures. These are adaptive structures providing the penetration into the host cells and in no way can be considered as characters of large taxon. At least, they serve as a species criterion. The presence of such ultrastructure in different unicellular organisms testifies to the similarity in the mode of adapting to similar conditions, but does not reflect a close phylogenetic relation between them.

To my mind, including *Toxoplasma* into the class *Sporozoa* basing on the similarities of adaptive characters seen at one of their developmental stages is as erroneously as combining the whale with the fish in one class.

Of course, toxoplasmae are not flagellates. Neither centriole, nor kinetosome, nor other kinetic elements have shown in the trophozoites. It seems hardly to believe that the intracellular mode of life resulted in the complete loss of kinetic elements. E.g. leishmaniae have not lost their kinetic apparatus. So, because the toxoplasmae have no kinetic elements at any developmental stage there is no reason to join them with flagellates. A diversity of hosts and longitudinal division cannot be considered characteristic of the flagellates; e.g. trypanosomae have a wide circle of hosts while *Hypermastigina* are strictly specific to their hosts. Irrespectively of the mode of division, the lack of kinetic elements in toxoplasmae does not allow to include them into the class *Mastigophora*.

The mode of movement of the toxoplasms differs also from that of the representatives of *Sarcodina* and, therefore, they can hardly be included into this latter class.

Our present knowledge of toxoplasmae suggests that they should not be included in the classes *Sporozoa, Mastigophora* nor *Sarcodina*. That is why I believe Biocca 1956 to be right when he separates the toxoplasmae into a class *Toxoplasmatea*.

Further investigations are necessary to ellicite the right position of toxoplasmae in the taxonomical system of *Protozoa* and to reveal the relation of this group with other protozoans, such as *Sarcocystis* and *Besnoitia*.

Summary

The taxonomic position of toxoplasmae is briefly discuted. The view is put forward that the toxoplasmae are not flagellates, because of lack of the kinetide elements, and — moreover — they cannot be included into the class *Sporozoa*. The sexual reproduction, characteristic of the sporozoa, is not found in toxoplasmae. The conoid of toxoplasmae trophozoites is considered to be an adaptive feature, analogous to that met in the merozoites and sporozoites of some coccidia. It is not a feature reflecting the phylogenetic relationships among protozoa. *Toxoplasma* may be combined with *Besnoitia* and, probably, with *Sarcocystis* in a separate class *Toxoplasmatea* Biocca 1956.

РЕЗЮМЕ

Кратко рассматривается вопрос о систематическом положении токсоплазм. Высказывается мнение, что токсоплазмы не являются жгутиконосцами, так как не имеют кинетиды и не могут быть включены в состав класса *Sporozoa*. У токсоплазм нет полевого процесса характерного для споровиков. Коноид у трофозоитов токсоплазм рассматривается как адаптивный признак, аналогичный таковому мерозоитов и спорозоитов некоторых кокцидий. Этот признак не отражает филогенетических отношений между различными простейшими. *Toxoplasma* вместе с *Besnoitia* и, вероятно, вместе с *Sarcocystis* могут быть выделены в особый класс *Toxoplasmatea* Biocca 1956.

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Studies on the representatives of the family *Paraisotrichidae* Da Cunha (*Ciliata, Trichostomata*). IV. General discussion

Badania nad przedstawicielami rodziny *Paraisotrichidae* Da Cunha (*Ciliata, Trichostomata*). IV. Dyskusja ogólna

In the preceding parts of this research (Wolska 1964b, c, 1965) the infraciliature and division morphogenesis of species belonging to the genera *Paraisotricha* Fiorentini and *Rhizotricha* Wolska constituting the family *Paraisotrichidae* Da Cunha, has been described.

The genus *Paraisotricha* Fiorentini was included to the family *Isotrichidae* Bütschli 1887 by Hickson 1903. Da Cunha 1917 excluded this genus from the family Isotrichidae and established the new family *Paraisotrichidae* for the genera *Paraisotricha* Fiorentini and *Blepharocorys* Bundle, giving the following diagnosis of the family: "Ciliados holotrichos do corpo inteiramente ciliade ou nao. Bocca collocada lateralmente e provida d'uma pharynge ciliada. Adiante da bocca existe sempre um tufo do cilios finos e longos, dirigidos para trás. Parasitos no intestino dos mammiferos. Genero typo: *Paraisotricha*". Hsiung 1929 excluded the genus *Blepharocorys* Bundle from the family *Paraisotrichidae* Da Cunha as devoid of the concretion vacuole (characteristic of the genus *Paraisotricha*), and having a reduced ciliature. The diagnosis of the family *Paraisotrichidae* assumed the following form: "Trichostomata. Body uniformly covered with cilia in slightly spiral longitudinal rows; a tuft of longer cilia at anterior end; cytostome just ventral to anterior concretion vacuole. Single contractile vacuole at posterior end. Type-genus: *Paraisotricha* Fiorentini 1890". (Hsiung 1930).

Da Cunha e Muniz 1925 described the new genus *Hydrochaeralla* with the species *H. intestinalis*, found in *Hydrochaerus capibara* and included this genus into the family *Paraisotrichidae*. Strelkov 1939 excluded this genus from the family *Paraisotrichidae* and stated: "No concretion vacuole, consequently those are not *Paraisotrichidae* in any case".

Kopperi 1935 included the genus *Protocaviella* Kopperi with the only species *P. acuminata* (synonym *Paraisotricha acuminata* Da Cunha) into the family *Paraisotrichidae*. Strelkov 1939 decided that this species should be included to another genus and another family (see the bibliographical revue in Wolska 1964b). In this way the genus *Protocaviella* Kopperi was put outside the family *Paraisotrichidae*.

Corliss 1961 in his monographical study of ciliates maintains the genus *Protocaviella* Kopperi in the family *Paraisotrichidae*. According the description of Kopperi 1935, in *Protocaviella* mouth is shifted to the middle of the flattened ventral body side, no long cilia over the mouth, no concretion vacuole, macronucleus is spherical, a zone of longer cilia is on the right margin of the ventral body side. The generalized and schematic illustration given by Kopperi does not allow to decide to which family this genus is mostly related, but presents a sufficient prove to exclude it from the family *Paraisotrichidae* Da Cunha.

Wolska 1964b separated the genus *Rhizotricha* Wolska with the single species *R. beckeri* (Hsiung) from the genus *Paraisotricha* Fiorentini comprising the species *P. colpoidea* Fiorentini, *P. minuta* Hsiung and *P. beckeri* Hsiung.

The family *Paraisotrichidae* Da Cunha is placed within the order *Trichostomata*.

The limits of the order *Trichostomata* Bütschli 1889, embracing initially all the ciliates except the *Gymnostomata*, became reduced in approach of subsequent authors to one of the suborders of *Holotricha* Stein 1859.

Kahl 1935 considers *Trichostomata* as one of 6 suborders and defines them as possessing an oral groove equiped with more or less dense rows of free cilia; these free, not agglutinated cilia, are the character distinctly differing *Trichostomata* from *Hymenostomata* whose oral groove is equiped with cilia fused in membranelles.

The order *Trichostomata* comprising several families forms an extensive, not uniform, unsufficiently investigated group — probably polyphyletic. (Kahl 1935, Corliss 1956 and 1961, Stout 1960).

Since the silver impregnation method had been introduced into protozoology (Klein 1926, Gelei 1932, Chatton et Lwoff 1930, 1936) and applied for the study of morphogenesis, a considerable progress was made in our general knowledge of ciliates, and among others — of *Trichostomata*. Nevertheless, the investigation of *Trichostomata* is not satisfactory even in the present time, which is connected with a difficulty in the study of their representatives. The criterion of free or coherenty cilia is not easy to be applied, as stated by Fauré-Fremiet 1950 b and Tuffrau 1952, especially when the peristome is deep and its structure is complicated by twisting. In those cases even silver impregnation fails to give distinct results. The criterion of free or coherent cilia, related to *Trichostomata*, should be avoided — according the opinion of Fauré-Fremiet — but the new criteria fail to eliminate all the difficulties.

The presence of vestibulum has been recognized by Fauré-Fremiet 1950 a, 1950 b, 1962 b and Corliss 1956, 1959, 1961 as an essential feature of *Trichostomata* (presently considered as an order — Corliss 1961). Fauré-Fremiet 1962 b, 1963 stressed the prostomatic position of mouth. Consequently, *Trichostomata* have a vestibular ciliature, not a true oral one (Corliss 1961). Vestibulum is treated as a concavity with the cytostome on its bottom and with the anterior segments of the somatic kineties or kineties of somatic origin, penetrating into it, never forming any complex organelles. Difficulties involved by this concept of vestibulum are manifested in the description of the oral ciliature of *Paraisotrichidae*.

as in *Hymenostomata*: "Vestibular but no buccal ciliature in oral area"

Fauré-Fremiet 1963 in his comparative review of the order *Trichostomata* pointed out the insufficient study of many of its species and families; out of 16 families included into *Trichostomata* (Corliss 1961) — according to the present state of knowledge — 7 free-living families of ciliates and 4 families of commensal-ciliates of the alimentary canal of echinoderms, frogs and mammals may be recognized as *Trichostomata*. These latter are: *Thyrophylaxidae* Berger 1961, *Balantidiidae* Reichenow 1929, *Isotrichidae* Bütschli 1887 and *Paraisotrichidae* Da Cunha 1917.

Somatic ciliature

The characteristic feature of the somatic ciliature in *Paraisotrichidae* is its differentiation. On the anterior body pole, between some kineties of the general ciliature — or between all of them — short interstitial kineties occur, single in the genus *Paraisotricha*, double or in a higher number in the genus *Rhizotricha*.

Besides the interstitial kineties, a group of delicate, closely located kineties in the nearest vicinity of the peristome are present. They bear in the genus *Paraisotricha* the differentiated frontal cilia involved in propelling food, covering the crescent-shaped area. In the genus *Rhizotricha* there are short delicate kineties, running perpendicularly to the right anterior peristome margin. They bear the praeoral cilia covering a very narrow elongated area.

In both genera, between the 1st and the 2nd kineties, 3 insertions exist with especially big kinetosomes — the triade.

The meridional kineties are of uneven length. Some of them terminate at a considerable distance from the posterior pole. This is most distinctly pronounced in *P. minuta*.

The interstitial kineties, as well as the frontal and praeoral ones and the triade, have no corresponding structures in the somatic ciliature of other representatives of the order *Trichostomata*. Besides, it is difficult to mark a boundary between the somatic and the oral ciliature in *Paraisotrichidae*. The frontal cilia in the genus *Paraisotricha*, and the praeoral in *Rhizotricha*, are functionally connected with the mouth although they lie out of peristome. The triade of kineties, being situated also out of peristome, diverges from the somatic ciliature as to its origin (which is to be discussed below). The true somatic insertions between the meridionnal kineties are rather comparable to some structures in *Gymnostomata*, i.e. the double or triple marginal kinety in *Prorodontidae* (Fauré-Fremiet 1961), or the multiplied segments of somatic kineties in *Nassulopsis* and in *Nassula* producing the "frange" (Fauré-Fremiet 1959).

An analogue of the characteristic left-side fibers, running from the somatic kineties in *Rhizotricha beckeri*, can hardly be found in lower *Holotricha* studied in the light microscope. They could be perhaps compared to the cross fibers in *Chlamydodon pedarius* Kaneda (*Gymnostomata-Cyrtophorina*) running to the right from the kinetosomes and being impregnable. Kaneda 1960 regards the cross fibers of *Chlamydodon pedarius* as performing the same function as "ciliary rootlets" belonging to the 3rd cathegory of fibers according to Jacobson 1931, i.e. supporting the motory organelles. "Ciliary rootlets" seem to be identical with the "racines ciliaires". Chatton et Lwoff 1935 consider the "racines ciliaires" to be absent in *Holotricha* with a scanty cilia-

ture, occurring yet in the forms with a strong ciliature; they are well developed in *Heterotricha*. Perhaps a function of supporting may be ascribed to the fibers of *Rhizotricha beckeri*. Its cilia, as arranged in less numerous rows than it occurs in *P. colpoidea* and *P. minuta*, possibly must work more intensely to evoke the equally quick and effective movement, and need a stronger anchorage.

Owing to the character of the general ciliature, the family *Paraisotrichidae* presents a peculiar group in the assembly of ciliates-parasites of the horse intestine. In general, in the ciliates parasites of the herbivorous mammals, a reduction of the full primary ciliature is observed (Strelkov 1939) which is often accompanied by the development of some specialized ciliary groups only (e.g. in *Cycloposthiidae* — Raabe 1947, Dogiel 1951, Dogiel, Poljansky i Cheissin 1962, Michajłow 1960). Besides, reduction of the general ciliature is not sufficiently justified in parasites moving and feeding actively. Considering the different adaptations to the life in the intestine lumen, Strelkov 1939 postulates that the numerous week cilia might be rather an obstacle for the motion in the dense and viscous medium containing solid particles. Condensed and strong organelles would be more appropriate as follows from Strelkov's considerations.

The ciliature in *Paraisotrichidae* as stressed by Strelkov 1939 diverges from the type of ciliature commonly occurring in the intestine ciliates, limited only to some body regions. Nevertheless, some features of the somatic ciliature of *Paraisotrichidae* may possibly indicate a tendency to reduction. Before all, in *Rhizotricha beckeri* a diminished number of kineties occurs, and in both genera some kineties of the general ciliature shorten and fail to reach the posterior pole. In *Paraisotricha*, as well as in *Rhizotricha*, a more loose disposition of the kinetosomes of the bipolar kineties posteriorly to the interstitial kineties is observable. Sometimes at this level a complete disruption of the kineties occurs. This may be a symptom of a primary reduction of somatic kineties with a simultaneous specalization of the detached anterior segments towards their transition to the oral ciliature, or to the stronger ciliature grouped on the anterior pole.

It is difficult to decide whether the reduction of the ciliature in *Paraisotrichidae* would be a progressive feature characterizing its evolution.

Among the material of the Łódź district, *P. colpoidea* with its very abundant ciliature, seems to be the species the best adapted to the medium conditions. It is the most frequent of the 3 species of the family. The most rarely occurring form is *R. beckeri* with its scanty ciliature. Similar proportion is observable in the material of Hsiung 1930 (USA) and Strelkov 1939 (USSR), although the differences in frequency were not so striking. The frequency may also be influenced by the character of fodder of the host (Raabe 1964) which is different in various regions. The moree effective adaptation of genus *Paraisotricha* than *Rhizotricha* may also result from different position of the mouth. In *Rhizotricha* the peristome is widely open closely beneath the anterior pole (Pl. I 3), in *Paraisotricha* (Pl. I 1—2) it is displaced towards the ventral side and therefore is protected by a "label" (term of Raabe 1947) of the anterior body part and of the frontal cilia. This position of the peristome promotes the effective intake of small food particles (Raabe 1947, Dobrzańska-Kaczanowska 1963) when moving forward with the anterior pole.

Ciliature of the buccal apparatus and stomatogenesis

The very high development of the buccal apparatus in *Paraisotrichidae* deserves being discussed.

In the vast deep cavity, supported by fibrils, bordered by the paroral kinety on its right side — lie the peristomal kineties, well developed, differentiated from the somatic ciliature. They partly reach the surface of the body, beyond the cavity. The cavity is formed by the complex morphogenetic movements occurring in the course of division.

The buccal depression of this kind as in *Paraisotrichidae* cannot be recognized as the vestibulum characteristic of *Trichostomata*. This is a real "buccal cavity" occurring in phylogenesis first time in *Hymenostomata* (Corliss 1961), i.e. in the so called higher *Holotricha*. This statement is supported by the results of the study of the buccal infraciliature and of stomatogenesis (Wolska 1964 c, 1965), when considered in relation to the commonly accepted principles of ciliates taxonomics.

The vestibulum of *Trichostomata* is characterized by Corliss and by Fauré-Fremiet as follows:

"The vestibular ciliature is somatic in origin, seldom modified from the type occurring elsewhere on the body. There is no buccal cavity, and therefore, no compound buccal ciliature in members of this order, according to my present concept of its limits" (Corliss 1961).

"...celle-ci permet de caractériser l'ordre des *Trichostomata* par un type structural fondamentalement prostomien, et par la présence d'une invagination vestibulaire prebuccale dans laquelle pénètre les extrémités antérieures de toutes ou quelques-unes de cinéties méridiennes..... la structure caractéristique des *Trichostomatida*, c'est-à-dire la présence, au niveau de l'aire orale, d'une ciliature vestibulaire somatique et non d'une ciliature proprement buccale..." (Fauré-Fremiet 1963).

The essential feature of the vestibulum is, consequently, the not complex ciliature of a somatic origin. Those criteria fail to determine the vestibulum precisely. In the family *Colpodidae*, regarded as typical *Trichostomata* (Fauré-Fremiet 1950 b), the vestibular ciliature is as far modified and individualized that it deviates from the model of vestibulum. Fauré-Fremiet stated — on the ground of observations of Taylor a. Garnjobst 1939 and Furgason 1940, that besides those two types of buccal ciliature: the "peniculina" and "tetrahymenina", another type of structure: purely "trichostomata", probably exists in the genus *Colpoda* and possibly in the genus *Tillina* and *Breslaua*. Later on, basing on the reports of Taylor and Garnjobst 1939, Fauré-Fremiet 1950 b stated that in *Colpoda duodenaria* occurs a certain degree of individualization of the buccal ciliature primordium. In conclusion Fauré-Fremiet says that different *Holotricha Trichotomatida* are characterized by the mouth opening directly on the bottom of vestibulum; the ciliature of the vestibulum derives from all, or from the part of the meridional and bipolar somatic kineties; this is the difference between these groups and *Hymenostomata* with the proper buccal ciliature differentiating in a complex process (stomatogenesis); but the first step towards such a differentiation may be recorded in *Trichostomatida* of the family *Colpodidae*. Tuffrau 1952 stated that in *Colpoda cucullus* the left ciliary area detaches from the kineties of which it originates, mi-

grates and concentrates; it seems to be an independent system despite the fibrillar union with the kineties.

From the morphological point of view *Paraisotrichidae* lie on one side of the not sharp boundary separating the forms with the typical vestibulum from those with the real buccal ciliature. The buccal kineties of *Paraisotrichidae* are not a system of free kineties independent from one another, but form a coherent, individualized and integrated group. This character of the buccal kineties is clearly reflected by the infraciliature (Wolska 1964c). This is also confirmed by the character of beating of the buccal cilia which is independent from the somatic ciliature work, as observed in vivo.

Observation of the living material provided no information concerning the ciliature of the paroral kinety which is so distinctly marked in the infraciliature. The right margin of the peristome in the genus *Paraisotricha* seems to bear a low immobile "batten", possibly formed by the short cilia of the paroral kinety. In the infraciliature, the paroral kinety has a character of a typical UM, i.e. of complex ciliary organelle, such one as those which permanently occur on the right peristome margin (buccal cavity) of *Hymenostomata*. The individuality of kinetosomes is here completely effaced; sometimes only in the genus *Paraisotricha* single kinetosomes are observed between the single segments of UM. Often one kinetosome is considerably distant from the last UM segment. So in *Paraisotrichidae* the buccal ciliature is a compound one. A compact, individualized group present the praeoral cilia in *Rhizotricha* and the frontal ones in *Paraisotricha*. In the latter genus, their function in food ingestion is doubtless; their synchronized movement proves their integration. In *Rhizotricha* they were not observed in vivo, yet the integration of this system is manifested in the infraciliature.

The course of stomatogenesis indicates that the complex of the buccal kineties is formed by rows of kinetosomes, being the continuation of somatic kineties and of interstitial kineties arising probably by delineation of the somatic ones. This process may be compared to the local multiplication of the somatic kineties in *Colpodidae* and in other *Trichostomata*. Certainly this buccal ciliature is of somatic origin. But the complete individualization of this complex makes the buccal kineties in *Paraisotrichidae* "a really buccal" system, in no less degree than they are in *Hymenostomata Tetrahymenina* in which the primordium of the specialized buccal ciliature originates from one or several stomatogenic — yet somatic — kineties. So this criterion of the vestibulum — the somatic origin of the ciliature — is difficult to be applied.

It may be proposed that the actual buccal ciliature is only such one which was differentiated in the process of phylogenesis and arises autonomically (sensu Corliss) in ontogenesis; any others may always be considered as somatic. Is there any substantial difference in formation of the buccal ciliature in *Hymenostomata* and such *Trichostomata* in which no direct continuity of somatic and buccal kineties exists? In both groups the buccal ciliature originates from the somatic kineties; the difference consists in the subsequent formation of the ciliature. The case of *Colpodidae* indicates that the delimitation of the vestibular and buccal ciliatures is faint.

The paroral kinety in *Paraisotrichidae* arises in a different way than the complex of kineties just discussed. As it was mentioned previously

(Wolska 1965), in the early stages of stomatogenesis, or in the perceptible onset of division, on the proter territory kinetosomes appear which migrate into the interkinetal spaces where the primordia of the paroral kinety and of the triade arise. The behaviour of the migrating kinetosomes seemed to indicate their share in formation of the preoral kinety and of the triade, but it might also be postulated that they have another destination.

The recent authors observations throw a new light on the significance of the migrating kinetosomes. Only in a few cases migrating kinetosomes prove to be organized in short kineties (Pl. I 4). In the ciliate in Pl. I 4 no symptoms of division in the equatorial zone are manifested. Between the triade and the kinetosomes migrating, organized in three short kineties, lies one kinetosome detached from the triade. It could be rather concluded that as well the paroral kinety as the triade arise directly of the praeoral kinety and of the triade of the parental individual by detaching kinetosomes and their shifting towards the opisthe territory. Consequently, it would be an autonomic organelle.

The organization of the migrating kinetosomes into kineties occurs in various time in different individuals: sometimes early (Pl. I 4), another time only after shifting on the territory of the division zone.

It seems doubtless that the paroral kinety and the triade of the opisthe arise of kinetosomes migrating from the territory of the proter. Nevertheless, it seems not quite certain whether those kinetosomes migrate from the paroral kinety and the triade. Possibly they might arise from the kinety 1, usually interrupted or loosened on the level of the triade. The segment of the kinety 1, partly or entirely detached, seems to be included in this way to the buccal apparatus. The most anterior part of this segment is composed of kinetosomes closely approached to one another. In *P. minuta* this feature is expressed very distinctly so that initially the anterior segment of the kinety 1 was mistaken by the author for a part of the paroral kinety — the UM (Wolska 1964 c, 1965). If it is so, then the migrating kinetosomes — if even detached of the kinety 1 — originate in this case as well from the buccal apparatus. It seems, however, more probable that they are derived from the preaoral kinety and the triade. The single kinetosomes occurring often, as mentioned above, behind the triade (Pl. I 4) and the paroral kinety, seem to prove their successive shifting from the paroral kinety and the triade towards the equatorial zone and arranging themselves there in form of the primordium of corresponding structures of the opisthe.

The above facts allow to ascertain that one of the elements of the buccal apparatus in *Paraisotrichidae* — the paroral kinety — arises autonomically, from the corresponding buccal element of the parental individual, being in this way of not somatic origin.

The significance of the triade is not clear. Cilia — if they exist at all on its kinetosomes — would be very delicate since they were never visible in vivo. Kinetosomes of the triade might be the elements supporting the right margin of the peristome. However, their early formation in division (the first element appearing in the division zone) indicates the important role of this organelle (Raabe 1947), more significant than a mechanical one. The triade lies in the immediate proximity of the "Konkrementenvacuole", even partly encroaching upon it. This fact evokes the analogy with short rows of thick kinetosomes placed near the "Konkrementenvacuole"

in some species of the family *Bütschliidae* from the horse intestine (Wolska 1964 a). The kinetosomes deprived of cilia, in the above mentioned *Bütschliidae* seem to be important for functioning of "Konkrementenvacuole". Possibly their significance in *Paraisotrichidae* is similar.

System of oral fibers

The buccal cavity in *Paraisotrichidae* is rich in fibers. Their system can scarcely be compared to any of the known. Their topography has been described earlier (Wolska 1964 c) Presumably the majority of fibers perform a supporting role. Dogiel 1929 stated that the pharynx in the genus *Paraisotricha* contains myonemes. Really the peristome of *Paraisotrichidae* shows some movements. Yet it is difficult to ascertain which fibers — if fibers at all — are responsible for those movements. The evaluation of the function of the fibers on the base of morphological study is not easy, even by means of electron microscopy (Randal 1959, Pitelka 1959, Noirot-Timothée 1960), consequently little may be concluded about such a complex system on the base of the methods of the present study. The contractility of the longitudinal fiber seems very probable on account of its characteristic feature which might be related to the presumable phases of contraction and relaxation. It has sometimes a zig-zag folding, likely being spiralized (phase of contraction), or strainghtened (phase of relaxation), similarly as it occurs in the stalk of *Vorticellidae*, or in the haptonemata in *Chrysochromulina* (Grimstone 1961). The movements of the peristome along the body axis, as well as a slight twist and tucking up of its posterior part, might be conditioned by the contraction of this fiber. In the movements of pulling up forwards the peristome, the oblique fibers might participate if they are contractile. Whether they are so indeed and in what degree — it is impossible to decide.

On the oblique fibers, undulation or folding (seen in photographs in Wolska 1964 c) are often observed which might suggest their contractility. Nevertheless such considerable changes in fibers are not in harmony with only a slight shifting of the peristome. So they may possibly be passive foldings caused by a certain deformation of the fixed ciliate. The above mentioned movements of the peristome — manifested in advancing and reeding its ventral margin — may be evoked only by a longitudinal fiber if its attachment point was above the peristome margin. Yet the attachment point of the longitudinal fiber is not known being lost in the network of other fibers.

A slight undulation is also visible on the semicircular fibers but it is impossible to ascertain whether they possess a capability of contraction. In living ciliates no sign of contraction was ever observed in those fibers. The most probable — in the cavity of such a large lumen — would be a supporting function of the semicircular fibers.

The connecting fibers — very delicate and scarcely perceptible — bind together the longitudinal and semicircular fibers, and the buccal kineties into one entity.

Such an abundant and complex system of fibers suggests a similitude with the "basket" ("nasse") of *Gymnostomata Cyrtophorina* although the position of its fibers is different. The "basket" of *Cyrtophorina* forms the fibrillar system of the cytopharynx, whereas the fibrillar structures of *Paraisotrichidae* lie in the subpellicle of the peristome (buccal cavity).

If we consider the complex fibrillar system of *Paraisotrichidae* as an analogue of the basket of *Cyrtophorina* — then *Paraisotrichidae* would find themselves in the same situation as *Pseudomicrothorax* Mermod (Corliss 1958), *Paranassula* Kahl (Fauré-Fremiet 1962 a), *Leptopharynx* Mermod (Prell 1961) — all forms joining the characters of *Gymnostomata Cyrtophorina* (a typical "nasse") with those of *Hymenostomata* (the membranelles system). This coincidence of features characteristic of different orders involves difference in opinions on the taxonomic position of the above mentioned genera and their role in the evolution of *Holotricha*. Corliss 1958 considers *Pseudomicrothorax* as a primitive form of *Hymenostomata*, being the exit point for *Tetrahymenina* and *Peniculina*. He postulates to include *Pseudomicrothorax* into a group (or perhaps a new suborder in *Hymenostomata*) linking *Gymnostomata Cyrtophorina*, and *Hymenostomata*. According to Fauré-Fremiet 1963 the adequate place for *Pseudomicrothorax* is among *Gymnostomata Cyrtophorina*. Prell 1962 joins *Pseudomicrothorax* and *Leptopharynx* into one family, embracing also the genera *Microthorax* and *Drepanomonas*, being within the *Gymnostomata Cyrtophorina* a branch "apparémment sans évolution ultérieure".

In *Paraisotrichidae*, the characters of *Hymenostomata* dominate distinctly over the characters of *Gymnostomata Cyrtophorina*. The buccal ciliature is complex and well developed, "nasse" is not of a typical feature. For that reason, placing *Paraisotrichidae* in the order of *Gymnostomata* is inadmissible. Nevertheless, it seems possible to ascertain the origin of *Paraisotrichidae* from *Gymnostomata Cyrtophorina* of the group *Nassulidae* with the axial symmetry and the full ciliature.

Transformations leading from the forms of the *Nassulidae*-type of structure to *Paraisotrichidae*, may be imagined as follows: the buccal ciliature arises as result of the development of the "frange suborale" of *Nassulidae*. In the initial stage of stomatogenesis, the primordium of the left peristomal ciliature in *Paraisotrichidae* has the form of multiplied anterior segments of the somatic kineties, resembling therefore to the short rows of condensed kinetosomes forming the "frange" (Fauré-Fremiet 1959 b). The hypertrophy of those multiplied segments and their sinking down into the cytopharynx might involve formation of such a system as it is in *Paraisotrichidae*. Simultaneously the cytostome sunks down to the bottom of the cavity. As result of penetration of the kineties into the primitive cytopharynx, this structure became transformed into the buccal cavity and the change in the pattern of the fiber system occurred. The paroral kinety origin may be traced from the first somatic kinety, to the right of the mouth.

Fauré-Fremiet 1962 points out the tendency of such transformations in some species of the genus *Nassula:* "Revenant aux espèces du genre *Nassula*, on constate que chez quelques-unes d'entre elles, la première cinétie qui contourne sur la droite l'ouverture buccale montre, au niveau de cette ouverture, une légère differentiation, en ce sens que les cils, beaucoup plus serrés qu'au long des autres cinéties, constituant peut-être l'équivalent d'une membrane paroralle. Chez certaines petites espèces, telles que *N. picta* le nombre des plaques ciliées de la frange sub-orale peut se reduire à trois ou quatre, disposées à gauche comme le seraient les membranelles adorales d'un Hymenostome tetrahymenien; mais leur nature reste ici, somatique".

The possibility of formation — in the course of evolution — of the paroral kinety in *Paraisotrichidae* from the somatic kinety, or kineties lying on the right side of the buccal cavity, is strengthened by the fact that in *Paraisotricha* a further tendency of differentiation of the first kinety, on the right of the mouth, and of including it to the buccal apparatus (see Wolska 1965 on stomatogenesis in *P. minuta*), is observed.

It is possible therefore that the "frange sub-oral" of *Nassulidae* underwent evolution giving the left-side peristome ciliature of *Paraisotrichidae*, whereas the kinety — or rather three kineties on the right side of the mouth — transformed themselves into three segments of UM (paroral kinety) of *Paraisotrichidae*.

Despite the above suggestion of the link between *Gymnostomata Cyrtophorina* and *Paraisotricha* — in the author's opinion — the latter group is the nearest *Hymenostomata* owing to the character of their buccal ciliature.

The fibers in *Paraisotrichidae* — considering their location in the buccal cavity and their course which is parallel to its surface — may be compared (except the longitudinal one) to the fibers of some *Hymenostomata Peniculina*, namely to the fibers of *Parameciidae* and of *Urocentrum* (Roque 1961). This similitude is stressed by the connection of the fibers with the kinetosomes in *Paraisotrichidae* and in the above mentioned *Peniculina*.

Roque 1961 considers that the fibers running obliquely over the right wall of the peristome in *Parameciidae* and directed towards the cytopharynx, are produced by the kinetosomes of the paroral kinety, lie subpellicularly and are not grouping into bundles. The same characters distinguish the fibers of *Urocentrum turbo*.

The fibers of *Paraisotrichidae*, as well the semicircular as oblique and connecting ones, are located subpellicularly and initiate — of it may be correctly observed in the light microscope — from the kinetosomes of the peristomal kineties. In the genus *Paraisotricha* they are single, in *Rhizotricha* — grouped by several in the anterior left part of the peristome — their number being lower than that of the kinetosomes from which they probably originate. The relationships of fibers of some *Peniculina* (*Frontoniidae*, and possibly, *Clathrostomata*) and *Gymnostomata Cyrtophorina* have been established by Roque: "Nous avons saisi les affinités des *Frontoniidae* et des Gymnostomes cyrtophores grâce à l'analogie des formations fibrillaires orales..." (Roque 1961). This substantiates the actually accepted view on the phylogenetic link between *Gymnostomata* and *Hymenostomata* (Corliss 1956, 1958). Roque 1961 discussing this subject stated: "Il est regrettable que nos connaissances ne nous permettent pas de considerer, sans aucun doute, le genre *Clathrostoma* comme le forme de passage entre les Gymnostomes cyrtophores et les *Frontoniidae*. Nous pouvons toutefois établir la série: Gymnostome rhabdophores → Gymnostomes cyrtophores → Hymenostomes peniculiens".

Since the fibers of *Paraisotrichidae* show a similitude to that of *Peniculina* and their buccal ciliature has a character of a real buccal ciliature — a link may be postulated for *Paraisotrichidae* (similarly as for *Peniculina*): *Gymnostomata Rhabdophorina* → *Gymnostomata Cyrtophorina* → *Paraisotrichidae*.

On the other hand, there exist a similitude of stomatogenesis in *Paraisotrichidae* to that in *Colpodidae*. Except the autonomous elements, the formation and individualization of the peristomal and frontal kineties in *Paraisotrichidae* may be compared to the formation of two groups of buccal cilia in

Colpodidae. The development and the degree of independence in corresponding ciliary groups is higher in *Paraisotrichidae* than in *Colpodidae*.

If the similarity of formation of the left-side and right-side buccal ciliature was accepted as the criterion of affinity of both families, it should be concluded that *Paraisotrichidae* are a higher branch deviating from *Colpodidae*, and developing the paroral kinety. Such postulation seems not sufficiently documented, the more so as *Paraisotrichidae* fail to show the torsion of the anterior part of the body which is so characteristic for *Colpodidae*. Besides, *Colpodidae* are prostomatic forms. Their cytostome arises between the anterior ends of the somatic kineties and shifts to the ventral side of the body together with the kineties — after the separation of proter and opisthe. In *Paraisotrichidae*, the buccal concavity arises on the ventral body side at once, in the early stage of stomatogenesis, and is almost fully developed prior the separation of proter and opisthe; consequently, this group is rather not prostomatic.

Concluding remarks

The above considerations indicate that the buccal ciliature of *Paraisotrichidae* shows the character of a real buccal ciliature. The system of peristomal kineties of the peristome left side as well as the right side system forming the UM — have both a complex character. So in *Paraisotrichidae* the buccal cavity exists, not the vestibulum.

It should be reminded that in many cases (in higher *Holotricha*) occurrence of vestibulum with its somatic ciliature is associated with the presence of a typical differentiated and autonomic buccal ciliature (e.g. in *Parameciidae*). So it is also in some *Thigmotricha*, which are a group derived from *Pleuronematina*, as e.g. in the representatives of the genus *Conchophthirus*. In this genus, besides the more or less deep funnel with the somatic ciliature in its depression — two adoral kineties occur, characteristic of *Thigmotricha* (Raabe 1936). A similar pattern was recently stated in *Thigmophrya* by Fenchel 1964.

Considering the origin of the left side kineties in *Paraisotrichidae* from the somatic ciliature, it might be accepted that here a combination of the buccal cavity and the vestibulum occurs, similarly as in *Conchophthirus*. Nevertheless, the "vestibular" kineties in *Paraisotrichidae* became decidely detached from the somatic ones, underwent a strong hypertrophy and are connected with one another by means of cross fibers, i.e. they are fully individualized and integrated. Considering their organization, they are not vestibular kineties.

As consequence of this statement, follows the postulation of excluding the family *Paraisotrichidae* from the order *Trichostomata* and of placing it in the ciliates system at least at the level of *Hymenostomata*.

The *Hymenostomata* are characterized by the buccal cavity with a ciliary apparatus, which is tetrahymenial in substance (UM on the right side, the tripartite AZM on the left — Corliss 1961). The poorly developed AZM consisting of three groups of coherent cilia, are characteristic of the suborder *Tetrahymenina*. The presence of peniculi — well developed compact ciliary structures, homologized with AZM of *Tetrahymenina*, and placed deeply in the buccal cavity, are characteristic of the suborder *Peniculina* (Corliss 1961). The number of ciliary rows forming the peniculi may be different in

various species (Yusa 1957). Hypertrophy of the UM is characteristic of the suborder *Pleuronematina* (Corliss 1961).

The analogy of the buccal ciliature in *Paraisotrichidae* and in *Hymenostomata* is distinct. The left-side buccal system of *Paraisotrichidae* may be considered as a polymerized and hypertrophic peniculus of *Hymenostomata-Peniculina*, the right-side one is an analogue of UM in *Peniculina* and *Tetrahymenina*.

The stomatogenesis in the order of *Hymenostomata* follows a various course. The buccal ciliature may arise from the kinetosomes originating from a single stomatogenic kinety (e.g. in *Glaucoma*, according Chatton, Lwoff, Lwoff et Monod 1931, and in *Tetrahymena*, according Furgason 1940), or from a greater number of stomatogenic kineties (e.g. in *Deltophylum* and *Ophryoglena*, after Mugard 1947 a, 1949), or at last this process may be more or less autonomic — when the buccal ciliature is formed from the parental buccal elements (*Philasteridae* and *Lembidae*, after Mugard 1947 b; *Parameciidae*, *Frontoniidae* and others, according to Roque 1961).

The stomatogenesis in *Paraisotrichidae* is of a mixed character. The left system arises in connection with the numerous stomatogenic kineties — essentially in the same way as in some *Tetrahymenina* — and becomes organized as a well developed, compact ciliary structure, similarly as in *Peniculina*. The right side system is formed autonomically.

Consequently, the stomatogenesis of *Paraisotrichidae* places this group at the same level as *Hymenostomata*.

Differentiation of the known types of stomatogenesis in *Ciliata* is, however, based on the quite general characters and cannot serve as evidence of homology of the buccal ciliature in groups belonging to the same type.

At present, it is difficult to look for proximate phylogenetic links of *Paraisotrichidae* and to define their place in the ciliate system. There are too many groups not sufficiently investigated among *Holotricha*, as well in the order *Trichostomata* (Fauré-Fremiet 1963) as in *Gymnostomata*. Among the latter, the *Cyrtophorina* are considered as the exit group for *Hymenostomata* (Corliss 1958, 1961; Fauré-Fremiet 1961, 1963; Roque 1961). For example, the *Pycnotrichidae* which are not sufficiently investigated, are included by Kudo 1947 and Jírovec 1953 to *Gymnostomata Hypostomata* Scheviakoff (= *Cyrtophorina* Fauré-Fremiet) whereas Corliss 1961 includes them to *Rhabdophorina* Fauré-Fremiet (= *Prostomata* + *Pleurostomata* Scheviakoff). Some *Pycnotrichidae* are parasites of the alimentary tract of herbivorous mammals: *Hyracoidea* and *Rodentia*, and live in a medium similar to that of *Paraisotrichidae* (e.g. *Nicolella ctenodactyli* Chatton et Perard, 1921 and *Collinella gundi* Chatton et Perard, 1921). Others — like *Buxtonella sulcata* Jameson, 1926 — live in the intestine of cattle. Similarly to the preceding, *Natheliidae* Singh 1953 from the intestine of the fish *Mystus cavasius*, placed by Corliss in *Pycnotrichidae*, are considered by Singh as *Hymenostomata*. The *Blepharocoridae* Hsiung 1929 occurring in the coecum of horse together with *Paraisotrichidae*, are not sufficiently investigated. Till present time they were included to the order *Trichostomata*, but Fauré-Fremiet 1963 pointed out that their belonging to that family is not sufficiently justified.

The study of morphogenesis, embracing a large number of forms, would

perhaps contribute to elucidation of the complex problem of phylogenesis of the parasitic *Protozoa,* so much modified by the adaptation processes.

Possibly, the *Paraisotrichidae* are a separate highly specialized evolutionary branch of *Ciliata,* originating from the more primitive forms and are adapted to the specific life conditions — concurrently to other groups but in another direction.

The evolution of *Paraisotrichidae* proceeded towards the high development of the buccal apparatus, with a simultaneous preservation and differentiation of the general ciliature.

The development of the buccal apparatus — a rather general tendency of parasitic ciliates (R a a b e 1947), so strongly marked e.g. in *Entodiniomorpha* — assumed in *Paraisotrichidae* a form characteristic only of this group.

In the horse coecum, food particles of different kind are abundant. *Cycloposthidae* (*Entodiniomorpha*) choose out of this alimentary mass before all big vegetable particles, and according to that function, their buccal ciliature assumed the form of strong brushes and the whole "alimentary system" has been developed. *Paraisotrichidae* feed on tiny food particles driven to the extensive funnel-shaped cavity. Their buccal cavity, permanently open, accepts a continuous stream of tiny suspended particles which are propelled to the cytostome by the compact rows of strong cilia coating its ventral wall. The aspect of the cavity and disposition of the ciliary rows is more or less similar in all the species. In *Paraisotrichidae,* besides the strongly developed ciliature, a rich network of fibers arose. They probably are of kinetosomal origin. Supporting the cavity, they secure it from collapsing. The course of fibers is essentially the same in all three species and very characteristic of the family *Paraisotrichidae.*

Another feature characterizing the evolution of *Paraisotrichidae* is the retention of the general ciliature, occurring merely in the free-living forms. The spacious organ as the intestine of horse is, produces conditions similar to those in the external water pools. The ciliates choose the tiny particles of food. For collecting it in a sufficient quantity they should move rapidly and cross long distances. They therefore retained the full ciliature, securing their rapid and efficient movement. In the genus *Paraisotricha,* numerous slightly spiralized ciliary rows developed. In the genus *Rhizotricha,* cilia developed in rows less numerous but stronger and more spiralized. So in both species, the efficiency of movement became secured by the development of the general ciliature, although it was effected in another way.

Around the buccal cavity, the somatic ciliature is highly differentiated and adapted to serve this organelle. Between the principal rows of the general ciliature, on the anterior pole, short interstitial rows arose which strengthened the ciliature of the anterior body part. The densification of the ciliature on the anterior body pole is more or less similar in all the species. In *Rhizotricha beckeri* with its rare ciliary rows, two rows developed between all the somatic kineties. In *Paraisotricha minuta* with its moderately dense principal kineties, single insertions arose at the periphery. In *P. colpoidea* with the most dense ciliature, the interstitial rows were formed only on a part of the periphery. A part of the somatic ciliature differentiated giving origin to a group of long densely accumulated cilia on the anterior margin of peristome. In the genus *Rhizotricha* this group of cilia occupies a more lateral position than in the genus *Paraisotricha,* which is associated with the less distinct dislocation of

mouth towards the ventral side. The most characteristic differentiated element of ciliature in *Paraisotrichidae* is the triade, a structure of undefined significance, lying on the right peristome margin, equally developed in the three species.

Paraisotrichidae — as follows from what was stated above — present a very distinct group, very uniform as to the course of their development.

Nevertheless, in the general character of their mouth and of stomatogenesis, *Paraisotrichidae* resemble the *Hymenostomata*, or — it would be more correct to say: they acquire the organization equal to *Hymenostomata*, if not exceeding this level.

Perhaps it would be right to form a new suborder within the *Hymenostomata* for this small but so distinct group, but as yet the question should be left open. Only a supposition may be put forward about the existence of an evolutionary branch originating from *Cyrtophorina* and running independently of other groups originating of *Gymnostomata*. This branch attains the organization level equal to *Hymenostomata*, and produces *Paraisotrichidae*.

At any rate, the above observations indicate the high organization level of the family *Paraisotrichidae* Da Cunha as well as the necessity of raising its rank in the system of *Ciliata*.

Summary

The somatic ciliature, the structure of the buccal apparatus and the course of stomatogenesis in the family *Paraisotrichidae*, are discussed. The analysis of the buccal apparatus and of stomatogenesis led to conclusion that the family *Paraisotrichidae* should be excluded from the order *Trichostomata* and that its rank in the ciliates system should be raised. It is ascertained that the organization level of *Paraisotrichidae* equals that in *Hymenostomata*. No definite suggestions as to the position of *Paraisotrichidae* in the system *of Ciliata* are put forward.

STRESZCZENIE

Autorka omawia orzęsienie somatyczne, budowę aparatu gębowego i przebieg stomatogenezy rodziny *Paraisotrichidae* Da Cunha. Na podstawie analizy aparatu gębowego i stomatogenezy autorka dochodzi do wniosku, że rodzina *Paraisotrichidae* powinna być wyłączona z rzędu *Trichostomata* i że jej ranga w systemie *Ciliata* powinna być podwyższona. Autorka stwierdza, że poziom organizacji *Paraisotrichidae* dorównuje poziomowi organizacji *Hymenostomata*. Autorka nie wysuwa konkretnych wniosków co do miejsca *Paraisotrichidae* w systemie *Ciliata*.

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Note added in proof

After having submitted this study to publication, the author became acquainted with the publication of Grain 1963: Sur l'ultrastructure du vestibule de *Paraisotricha colpoidea* Fiorentini, Cilié Trichostome de l'intestine du cheval. C. R. Acad. Sci. 257, 2534—2537; and Grain 1964: La stomatogenèse chez les Ciliés Trichostomes *Isotricha intestinalis* et *Paraisotricha colpoidea*. Arch. Zool. Exp. 104, 85—93.

The study of Grain 1963 introduces new data inaccessible with the light microscope. Nevertheless, there is no contradiction between the reconstruction of the median part of peristome in *P. colpoidea* as reported by Grain and the scheme presented in this study. The results of Grain indicate the specific structure of the buccal apparatus in *Paraisotrichidae* and the compactness of the buccal ciliature.

Formation of the vestibular kineties in *P. colpoidea*, as described by Grain 1964, is in general supported by my observations.

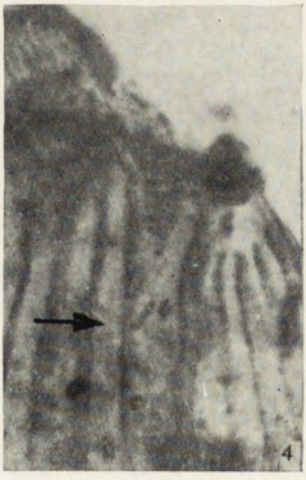
EXPLANATION OF THE PLATE I

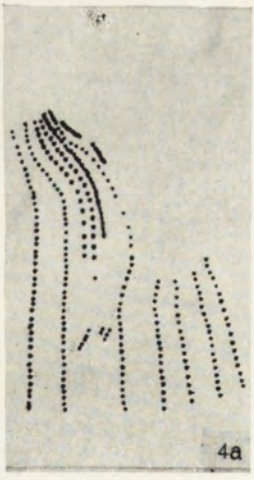
1: *Paraisotricha colpoidea* Fiorentini — 1000 ×

2: *Paraisotricha minuta* Hsiung — 1000 ×

3: *Rhizotricha beckeri* (Hsiung) — 1000 ×

4: *Paraisotricha colpoidea* Fiorentini, primordium of a new triade (4a: The same in sciematic drawing) — 2000 ×





M. Wolska auctor phot.

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Morphogenesis of ciliature in the physiological and traumatic regeneration of *Urostyla cristata* Jerka-Dziadosz 1964

Morfogeneza orzęsienia przy regeneracji fizjologicznej i traumatycznej u *Urostyla cristata* Jerka-Dziadosz 1964

In the former paper (Jerka-Dziadosz 1964 a) the process of formation of the new ciliature in course of division in *Urostyla cristata* was described. The division initiates by atrophy of UM and formation of the AZM primordium for the opisthe — and terminates in separation of the daughter individuals which is followed by the conclusive resorption of the old ciliature residue.

Three organization areas participate in the morphogenesis: the fronto-transversal cirri arise in the middle of the body near the AZM, the ventral cirri of the right body side arise on the area lying on the right side of AZM, whereas the cirri of the left side arise on the left lateral margin of the ciliate.

In the present study the process of formation of the new ciliature was followed in the physiological as well as in the traumatic regeneration.

Material and methods

The ciliate *Urostyla cristata* Jerka-Dziadosz 1964 (*Hypotricha, Urostylidae*) was used for study. Ciliates were grown in the Pringsheim's medium to which several rice grains were added. As supplementary nutrient, suspension of dried hen yolk or salad infusion with *Aerobacter aerogenes* was administered.

Single individuals were observed in a suspended drop placed on a slide with concavity, its margins being smeared with white vaseline. The usual light microscope or the phase contrasting optics were used for observation.

No immobilization, neither mechanical nor chemical, was applied.

The preparations of the ciliary system were executed — like in the former studies — using osmium tetroxide and sublimate as fixation fluid and Heidenhain's iron hematoxylin for staining, following the method of Párducz 1952.

Operations were perfomed with a suitably sharpened dentistic needle.

Formation of the new ciliature

Physiological regeneration

The physiological regeneration in *Hypotricha* consists in the exchange of the whole somatic and oral ciliature. The term "reorganization" is often used for determining this process.

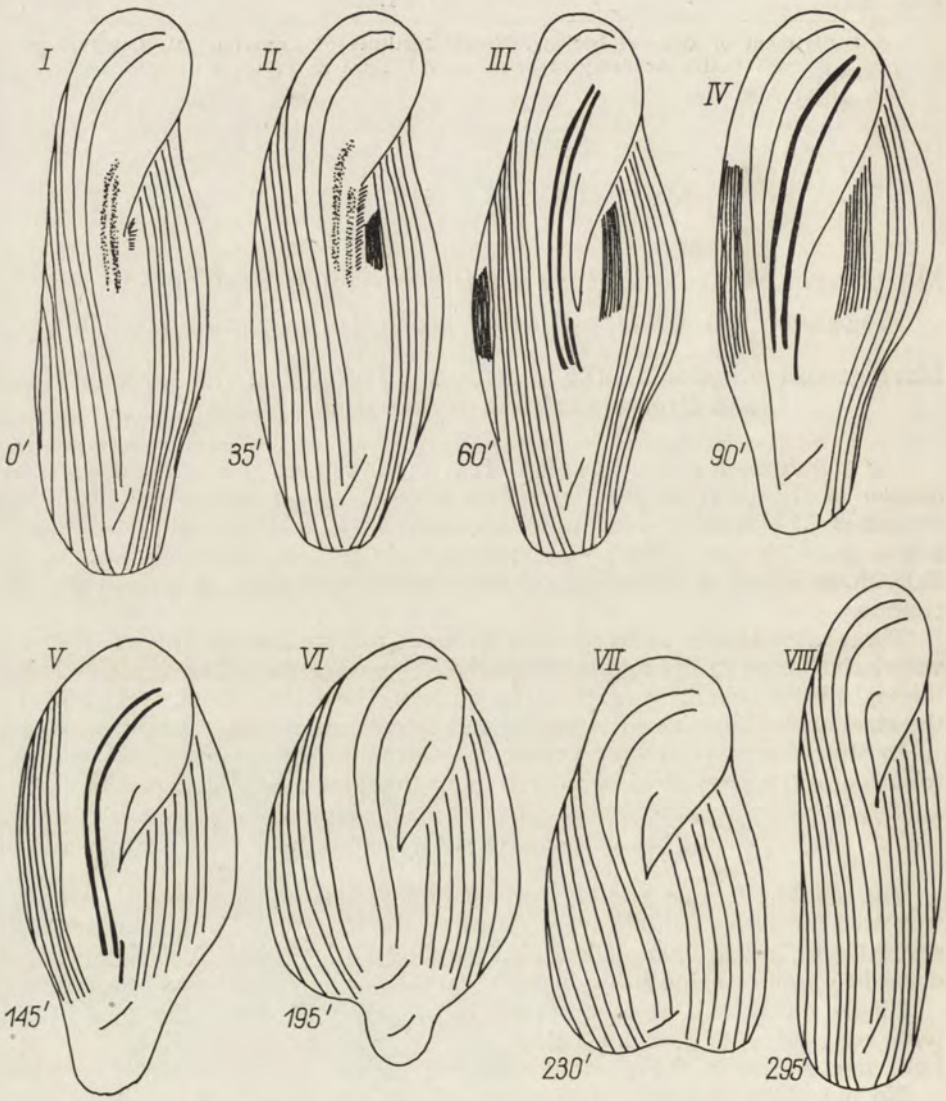


Fig. 1. Schematic representation of the physiological regeneration. Numerals indicate the stage of regeneration. Time in min. from the beginning of the process is also given

The exchange of the oral and somatic ciliature has been described in many ciliates of different systematic groups. In *Hypotricha* this process was studied — among others — by Dembowska 1939 in *Stylonychia mytilus* Ehrbg., by Hashimoto 1963 in *Oxytricha fallax* Stein, and also by Jerka-Dziadosz 1963 in *Urostyla grandis* Ehrbg. As stimuli of reorganization, starvation or general unfavourable conditions of culture are pointed out by the authors.

Reorganization in *Urostyla cristata* occurs mostly in conditions of irregular

feeding of the culture. If starvation is applied for evoking encystation, some ciliates fail to encyste but reorganize instead, which was proved by Pigoń and Edström 1959.

The scheme of the reorganization course is presented in Fig. 1.

The first perceptible change indicating the beginning of the reorganization, as observed in the living material, is a characteristic incision on the right anterior margin of the ciliate at the level of half of AZM (Fig. 1, stage I). In the fixed preparations of this stage, a group of small irregularly distributed cilia near the posterior margin of the peristome are seen. This are primordia, of the membranelles of the reorganized AZM. The exchange of membranelles follows from behind towards the anterior region. Left from the organizing peristome, the primordia of the fronto-ventralo-transversal cirri (FVT) arise as well as the primordia of UM.

Approximately 30 min. after the beginning of reorganization, on the right side of the peristome, an area appears on which 7 rows of tiny cirri are distinguishable. These are the primordia of cirri of the right ventral body side. Their formation is accompanied by a characteristic change of the body shape (Fig. 1, stage II). Initially the right margin bulges out slightly in the median part. In subsequent stages the bulging increases and covers the whole side of the ciliate. In the ciliary area 7 rows of cirri are distinguishable which elongate anteriorly and posteriorly and diverge laterally superseding the old ciliature.

In a similar way arise the ventral cirri of the left body side. The organization area of those cirri appears on the left body margin of the ciliate, behind the left old rows of ventral cirri (Fig. 1, stage III). The area is composed of 7 rows of tiny cirri which spread anteriorly and posteriorly, shift sideways and occupy their final position. Simultaneously with the appearance of these primordia on the left margin of the ciliate, this margin begins to bulge out as well. At the same time the ciliate shortens and becomes rounded on its anterior and. The posterior caudal part of the ciliate becomes elongated. In the course of the subsequent reorganization, it becomes gradually smaller and in the conclusive stage is resorbed (Fig. 1, stage IV—VIII).

The development of the ciliary areas of the reorganizing ciliate proceeds in the same way as in the prother in the course of division. The formation

Table 1

Duration time of corresponding stages of division, physiological regeneration and post-traumatic regeneration (in min.)

Stage	I-II	II-III	III-IV	IV-V	V-VI	VI-VII
Division	35	60	90	140	180	210
Physiological regeneration	35	60	90	140	180	280
Traumatic regeneration Promer	35	60	90	140	180	210
Traumatic regeneration Opimer	35	60	80	140	180	210

sequence of the groups of cirri as well as the duration of formation stages, of ciliary areas are also similar in both processes. Therefore, the similarity persists till the complete formation of the new ciliature. Difference appears, however, in the time necessary for resorption of the remainder of the old ciliature. The phase VI of the physiological regeneration may be looked upon as corresponding to the VI division stage but the time of duration of these processes differs in both cases considerably. In division 30 min. pass from the moment of the complete formation of the new cirri in proter and in opisthe till a full growing up of both individuals — whereas in physiological regeneration the regulation the regulation of the body shape and resorption lasts about 100 min (Table 1). Possibly this difference may be accounted for by the fact that proter and opisthe resorb only half of the mother ciliature while the regenerating individuals has to resorb the whole old set.

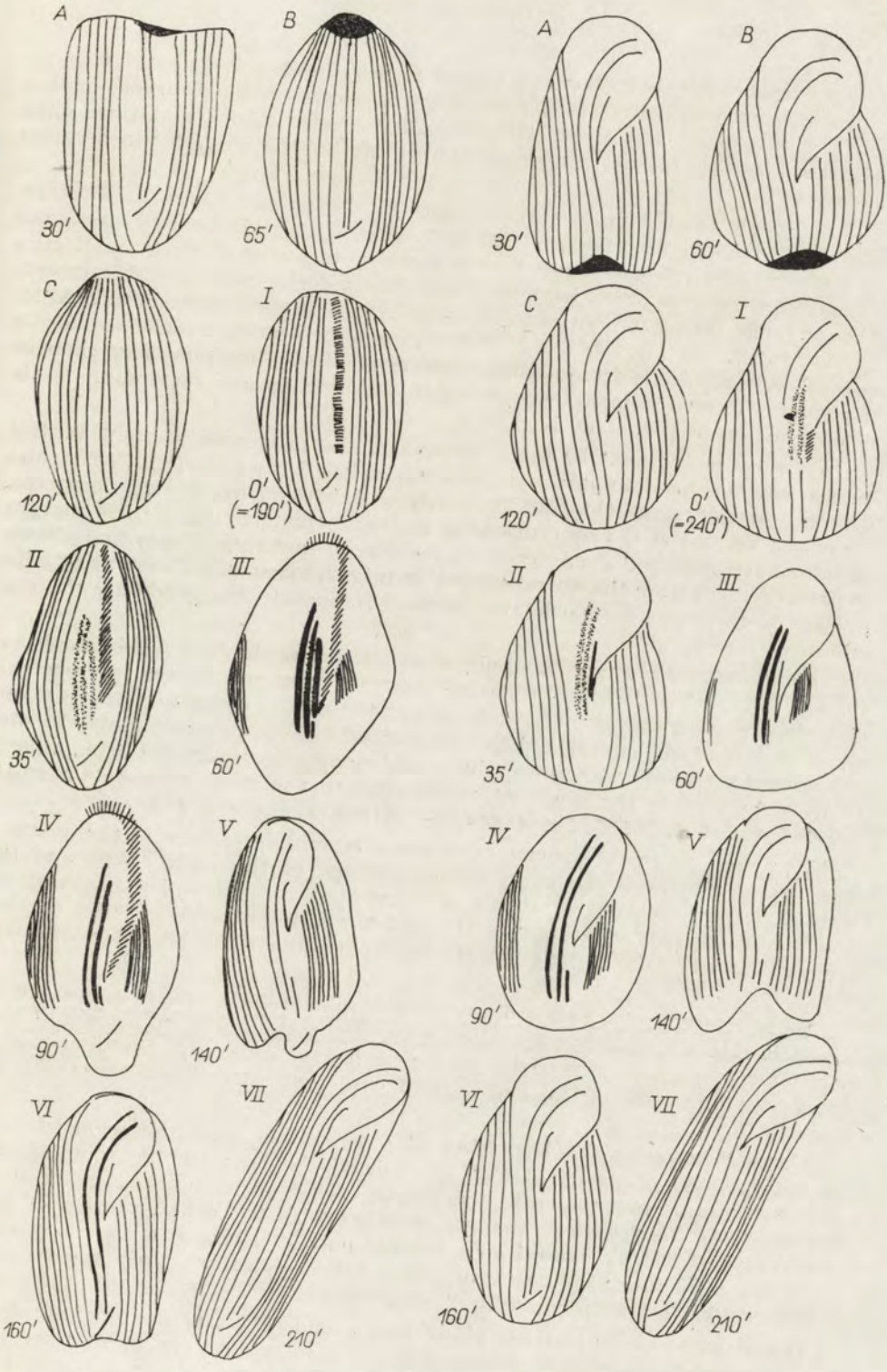
Traumatic regeneration

Ciliates were cut transversally through the body centre, vertically to the ventral surface and to the long axis. For determination of the fragments obtained the terms "promer" (for the anterior fragment) and "opimer" (for the posterior fragment) — as introduced by Golińska and Doroszewski 1963 — were applied. After the section the fragments were placed in a suspended drop and observed under microscope. In promer, the oral apparatus, frontal cirri and a part of the ventral and lateral ones were not injured. The opimer has the remaining ventral and lateral cirri as well as the transversal ones. Regeneration of ciliature is preceded by the process of wound healing (Fig. 2 ABC).

A few minutes after operation the margins of the wound begin to darken. Even in low magnification (Pl. I 2—3) a spot darker than the remaining body surface is observable. It increases in size initially in the first hour after operation, and subsequently begins to diminish till it vanishes entirely (about 2 hrs. after operation).

Observations under high magnification proved that the darker zone is formed by protrichocysts accumulated on the lip of the wound. Those bodies have the shape of granules or spherules 1—3 μ diameter, with a rapid Brownian movement. Formation of the protrichocyst accumulation on the lip of the wound occurs as follows: from the whole body, but mainly from its regions situated near the wound, greenish-brown granules shift towards the wounded place. When observing their accumulation and its nearest surrounding, it may be stated that the protrichocysts migrate in all directions: from the wound and towards the wound. The increase of protrichocysts accumulation is accompanied by rounding of the wound lips. After about 10 min. protrichocysts accumulated near the wound disperse gradually and, finally, the agglomeration vanishes entirely and in its place only an insignificant number of protrichocysts remains, like in the normal individual. It seems that about that time an alteration of the optic properties of protrichocysts occurs. Those bodies become more light, less visible and in some way "diffused".

Fig. 2. Schematic representation of the traumatic regeneration. A—C indicates the three stages of the preparatory period, I—VII — seven stages of regeneration itself. Time in min. is calculated from the moment of wounding and from the onset of regeneration. Promer and opimer are presented in parallel



Two hours after operation the wound is fully healed.

In the next two hours no changes in the fragments were observed, neither in the body shape nor in the ciliature. As it seems, about that time some internal processes occur, preparing the ciliate to the proper regeneration of the ciliature.

First begins the ciliature regeneration in the opimer — about $3^1/_2$ hrs. after operation. In the middle of the body (Fig. 2, stage I), between the rows of the ventral cirri, somewhat to the right of the ventral cirri, small cilia dispersed at random arise, beginning from the anterior part of the fragment. They arrange themselves so as to form the membranelles of the future AZM. In the next 30 min., left to the AZM being organized, arise the UM primordia as well as that of the fronto-dorso-transversal cirri. Simultaneously changes the shape of the fragment, narrowing in its anterior and distending in its median region (Fig. 2, stage II).

In the course of next 30 min. the area of the primordia of right ventral cirri arises. On the right side of AZM differentiate 7 rows of cirri. These rows diverge laterally and elongate forwards and backwards. Soon afterwards, 7 rows of ventral cirri primordia arise in similar manner, on the left margin of the ciliate body and partly even on its side, so that their disposition allows to observe them from the dorsal body side (Fig. 2, stage III). The rows of cilia diverge to the right, forwards and backwards, superseding gradually the old ciliature.

Simultaneously changes the body shape of the ciliate. The AZM rounds in its anterior part and shifts left as to meet the first row of the left ventral cirri. In the posterior part of the fragment the caudal elongation arises upon which a part of old lateral and the transversal cirri are pushed down. In the subsequent stages of regeneration the caudal elongation diminishes being at last entirely resorbed by the fragment, which since that moment becomes a normal vegetative individual (Fig. 2, stages IV—VII).

The primordia in the promer arise about 30 min. later than in the opimer. The first symptom is the characteristic change of the AZM shape and its transition from the lateral margin to the ventral body side. The preparation of this stage show the atrophy of UM and the differentiation of AZM on the segment between the peristomal concavity and the right lateral margin of the body.

About that time the primordium of reorganizing AZM arises. It is a group of small cilia forming membranelles. They lie on the posterior margin of the peristome, slightly to the right of the peristome concavity. Simultaneously appear the primordia of UM and of the fronto-ventro-transversal cirri (Fig. 2, stage I).

About 1 hr. after the beginning of regeneration arise the primordia of the lateral cirri of the right and left side of the body, in the same places and in the same manner as in the course of the physiological regeneration. The shape of the fragment changes together with the differentiation of the new ciliature. The old lateral cirri become pushed backwards producing two small processes which are subsequently resorbed. Sometimes only one process is formed like in the opimer (Fig. 2, stage V—VII).

The sequence of formation of different kinds of cirri and the duration of development stages of morphogenetic areas in promer and opimer are the

same as in proter and opisthe in division. It concerns also the time of resorption of the old ciliature. Consequently, with this regard, the traumatic regeneration resembles still more the division than the physiological regeneration.

It was stated in the reorganizing and regenerating ciliates that the fronto-ventro-transversal cirri arise by fusion of single cilia. The fusion of cilia occurs at a very early stage of differentiation of the area, soon after appearance of primordia.

Discussion

As follows from the above observations, in *U. cristata* — like in many other ciliates — there exists only one mechanism of morphogenesis for division and for regeneration.

The similitude of formation of the new ciliature in division to that in regeneration has been stated in many protozoa. Among others, in *Stylonychia mytilus*, as ascertained by Dembowska 1925 and 1938, new cirri arise in the same manner in division, in the physiological regeneration and in the traumatic one. In *Urostyla grandis* the development of the new ciliature in those three morphogenetic processes occurs in a similar manner as well (Jerka-Dziadosz 1963 and 1964 b).

The comparison of the morphogenetic processes occurring in *U. cristata* in division and in physiological regeneration leads to following conclusions:

1. Different kinds of cirri arise in their specific places (the fronto-transversal cirri in the middle of the body near the peristome, ventral cirri on the body sides).

2. Cirri arise by agglomeration and fusion of cilia.

3. In the course of division two sets of cirri arise: one of the proter and another of the opisthe, being separated from each other by a division furrow, whereas in the physiological regeneration only one set of cirri is formed, superseding the old ciliature.

4. A difference arises in the rate of resorption of the old ciliature: in the physiological regeneration it lasts about 70 min. longer than in division and in regeneration of fragments. As mentioned above, this difference may be connected with the fact that the reorganizing ciliate resorbs all former cilia itself, whereas the proter and opisthe, as well as the promer and opimer, resorb each only one half of the former ciliature.

In *Hypotrichida* — as shown by Roth 1956 and 1957 — cirri are composed of fused cilia. In *U. cristata* cilia fuse to form cirri in a very early stage of development of the primordial area (stage II and III). This process is similar to that seen in *U. grandis*. When comparing the moment of cirri differentiation in these ciliates, it will appear that in *U. grandis* cirri arise in the conclusive stage of development of the morphogenetic areas (Jerka-Dziadosz 1963), whereas in *U. cristata* they are formed at its initial stage. In *Stylonychia mytilus* — as reported by Dembowska 1925 and Yusa 1963 — cirri appear on the surface of the pellicle in a definite form.

The course of the traumatic regeneration in *U. cristata* may be divided into two periods. In the first of them occurs rounding of the wound margins and its healing, in the second one — the real regeneration of ciliature takes place.

Formation of the new ciliature in the traumatic regeneration proceeds in the same manner as in the physiological regeneration and in division (Jerka-Dziadosz 1964 a). Admitting that the section is an artificially evoked division furrow, the promer assumes the role of prother and opimer that of opisthe. Of course, in the traumatic regeneration the sequence of phenomena is artificially reversed: in division the primordia of ciliature appear first and the division furrow separates the individuals afterwards, whereas in regeneration the isolation of fragments is evoked first and the morphogenesis of ciliature follows it subsequently. The real regeneration of ciliature is preceded by cicatrization of the wound and regulation of shape. In *U. cristata* the first period of regeneration, in which healing of the wound and regulation of the body shape occurs, lasts in promer 4—4.5 hrs., and in opimer 2—3.5 hrs Jerka-Dziadosz 1963. Consequently, the preparatory period to the real regeneration is in both ciliates similar.

In *U. cristata* the promer begins to regenerate later than the opimer. Similar phenomena had been described in many other protozoa. This retardation may be accounted for by the inhibiting action of the old oral organelles (Weisz 1951). The morphogenetic area appears in promer only after a certain time necessary to suppress this action. In contrast to this the opimer, lacking the oral organelles, regenerates sooner. The influence of the oral organelles upon the rate of regeneration was also investigated by Hashimoto 1962 and 1963. He stated that in *Oxytricha fallax* the injury of AZM not only accelerates the regeneration of the fragment but also promotes the encystation in individuals in which AZM was partly removed.

A peculiar phenomenon observed in the course of wound healing in a transected *U. cristata* is the behaviour of protrichocysts. Those bodies, as reported in the description of the species (Jerka-Dziadosz 1964 a), scattered irregularly in the ectoplasm show no regular disposition, in normal individual.

Structures resembling the protrichocysts in *U. cristata* were described in various protozoa but no data concerning their function has been reported. The term "protrichocysts" was introduced by Breslau 1921 for the tectin bodies of *Colpidium campylum*. They are distributed in ectoplasm between the rows of cilia. The term "protrichocysts" suggests that they are the initial stage of formation of trichocysts. Nevertheless, Schneider 1930 and Krüger 1936 consider that protrichocysts have nothing in common with trichocysts, but are participating in formation of the mucoidal membrane on the body surface of protozoon. Pitelka 1963 reported in many protozoa mucine bodies, usually disposed in rows under the body surface. Under some conditions they may expel their content forming the viscous refractile fibrils. Their constituent material is most frequently a gelatinous or mucoidal substance, normally participating in formation of cysts and other — presumably protective — envelops. Cheissin and Mosevich 1962 studying the potrichocysts of *Colpidium colpoda* in electron microscope stated that those secretion ampullae contain a substance produced in ectoplasm and transported from it by means of special canalicles. The secretion of those ampullae takes part in formation of the mucoidal membrane and of the capsule of the cyst.

The behaviour of protrichocysts of *U. cristata* in regeneration — namely their agglomeration on the margin of the wound — seems to indicate that these bodies take part in restoring the membrane in the place of lesion. This problem is being actually worked out by the outhor.

Summary

The traumatic as well as the physiological regeneration in *Urostyla cristata* Jerka-Dziadosz was compared with the division of this ciliate. The morphogenesis of ciliature in physiological regeneration resembles the division phenomena in proter but the resorption of the old ciliature lasts longer. In the course of traumatic regeneration, the sequence of formation of different kinds of cirri on promer and on opimer as well as the duration of development stages are similar to that in proter and opisthe. Nevertheless, the whole process of regeneration is more prolonged than the division because it must be previously prepared by the wound healing and regulation of body shape. An agglomeration of bodies of unknown nature near the lip of the wound was observed, and the problem of their function was discussed, comparing them to protrichocysts and to the mucin secreting bodies.

The fronto-transversal cirri arise by fussion of single cilia, immediately after appearance of primordia, i.e. on an earlier stage than in *U. grandis*.

STRESZCZENIE

Porównano regenerację traumatyczną oraz fizjologiczną u *Urostyla cristata* Jerka-Dziadosz z podziałem tego orzęska. Morfogeneza orzęsienia przy regeneracji fizjologicznej przypomina zmiany podziałowe na proterze, jednak resorpcja starego urzęsienia trwa dłużej. W przebiegu regeneracji traumatycznej kolejność powstawania poszczególnych rodzajów cirri na promerze i opimerze oraz czas trwania stadiów rozwojowych są podobne jak u protera i opistora. Cały proces regeneracji trwa jednak dłużej niż podziału, ponieważ musi go przygotować okres gojenia rany i regulacji kształtu. Zauważono skupianie się przy brzegu rany ciałek o niewyjaśnionej naturze i rozważono zagadnienie ich funkcji, porównując je z protrichocystami i ciałkami wydzielającymi mucynę.

Cirri frontalno-transversalne powstają przez zlanie się pojedynczych rzęsek bezpośrednio po ukazaniu się zawiązków, to jest we wcześniejszym stadium niż u *U. grandis*.

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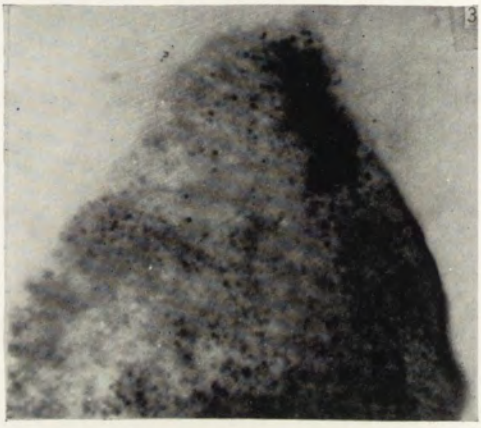
EXPLANATION OF THE PLATE I

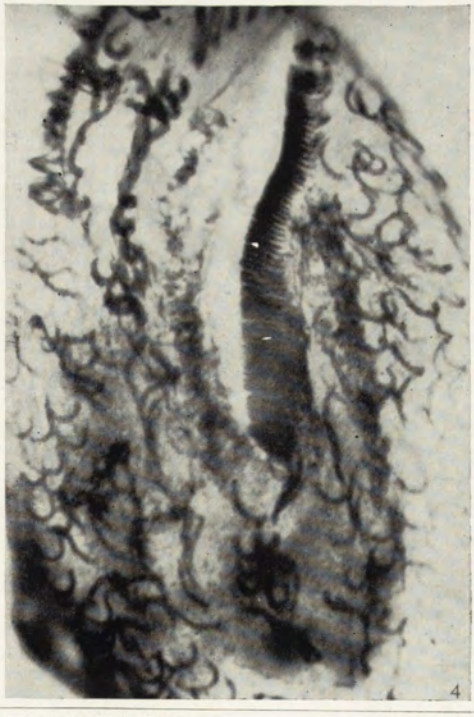
Physiological regeneration

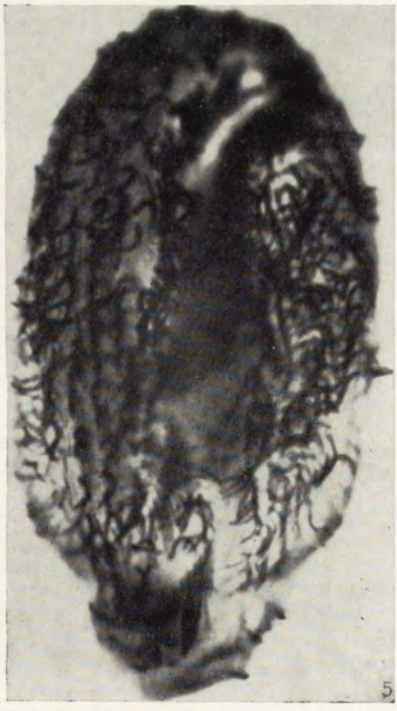
1: Stage IV (Párducz haematoxylin staining)

Post-traumatic regeneration

2: Agglomeration of protrichocysts near the wound (living material — phase contrasting optics)
3: Agglomeration of protrichocysts near the wound (fixed specimen)
4: Stage II of the promer (Párducz haematoxylin staining)
5: Stage V of the opimer (Párducz haematoxylin staining)







M. Jerka-Dziadosz auctor phot.

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Macronucleus in *Dileptus cygnus* and its changes in division

Makronukleus *Dileptus cygnus* i jego przemiany w czasie podziału

Dileptus cygnus presents an especially convenient object for the study of regeneration owing to its comparatively big body dimensions, high regenerative capability and its helpful „reference points" as the proboscis, the cytopharyngel complex or the moniliform macronucleus. The problem of formation of proboscis and of the cytopharyngeal complex during division and regeneration has been studied by Golińska and Doroszewski 1964. The aim of the present study is to analyse the behaviour of the nuclear apparatus in division as an introduction to the study on regeneration in *Dileptus cygnus*.

The nuclear apparatus of *Dileptus* has been investigated very scarcely. Claparède et Lachman 1859, Wrześniowski 1870, Kahl 1931, Šramek-Hušek 1949, Canella 1951, Jones and Beers 1953, Dragesco 1963 reported only that the macronucleus is moniliform consisting of 10 to 20 nods, micronuclei are numerous — about 20 situated near the Ma chain and are observable with difficulty. Within the genus *Dileptus,* the nuclear apparatus was studied in details only in *D. anser.* In that species Mi are numerous, small and compact, Ma is dispersed consisting of numerous (100—300) spherical bodies, of dimensions amounting 2—3 μ. Hayes 1938 found a fine granular Feulgen-positive substance inside these bodies. Jones 1951 described 1—4 nucleoli inside them, Dragesco 1963 considers that there exists only the central Feulgen-negative area without well delimited nucleoli. During division each part of Ma divides separately by elongation and disruption of connecting strands, elongation occurring in various directions (Studitsky 1930, Jones 1951). Hayes 1938 reported also some cases when single nods of Ma divided in interphase.

The macronucleus of moniliform type is common in different groups of ciliates, sometimes better investigated than *Dileptus*: therefore, more possibilities exist to compare the nuclear apparatus of *Dileptus* with that of more distant forms than within the same genus. Among *Holotricha* the moniliform nuclear apparatus was studied in *Paradileptus estensis* (Canella 1951), *Loxophyllus meleagris* (Ruthmann 1963) and in *Homalozoon vermiculare* (Weinreb 1955). The best analyzed ciliates with moniliform Ma belong to *Heterotricha.* Those are *Stentor, Spirostomum* and *Blepharisma.* The similitudes and differences in the structure of nuclear apparatus and in its behaviour during division in those species will be discussed below.

Material and methods

Dileptus cygnus was found in a ditch in Sadyba (Warsaw) and has been cultivated in the Department of Biology since 1961. Cultures were kept in the Pringsheim's medium and fed every day. As food, *Colpidium* and *Tetrahymena* were supplied, cultivated in the Pringsheim's medium with addition of dried yolk. For providing a great number of dividing individuals, a part of cultures was supplied with food less than 20 hrs. prior to sampling the material.

The observations were carried out as well on living ciliates as on fixed and stained material. The observations of living ciliates were performed in a suspended drop on a slide with water rim, or on a flat drop covered with a layer of paraffine oil with no cover slip. The light microscope with usual optics was used as well as phase contrasting or dark field set. The dividing individuals were sampled from the culture at a possibly early stage, observed under microscope and photographed at fixed time intervals. The pictures obtained were compared with the mass preparations. This routine enabled to determine the sequence of changes in macronucleus as seen in preparations. The measurements of the nods size were executed after squashing the ciliate with the cover slip; then the nucleus is seen well in the dispersed mass of cytoplasm (Pl. I 2).

The nuclear apparatus was stained after the Feulgen method, or with methyl green and pyronine following Brachet. For coördinating the nuclear division stages with the appearence of superficial structures, a combined staining with Feulgen and Párducz methods was applied. All staining methods were preceded by sublimate fixation.

Results

Macronucleus in interphase

As mentioned above, the macronucleus in *Dileptus cygnus* is moniliform. Its position in the interphase is labile. Usually Ma occupies a position parallel to the long body axis (Fig. 1 A), but it may be pushed backwards and forwards to the body ends by the big food vacuoles. The most anterior nod may be located nearly on the cytostome line, at the base of proboscis. The number of the Ma nods in the ciliates studied fluctuated from 7 to 15, the mean number being 9. The size of segments amounted 27×13 to 13×11 μ. Connectives of single nods are various not only in different individuals but also on one chain (Fig. 1 B, Pl. I 3, 4). All transition forms occur from the nods "grown together" by several connectives, to the linkage by a single connective. This lability of connections, not sygnalized as yet in moniliform Ma, suggests a possibility of changes in the number of nods occurring in interphase, as well towards increase as towards diminishing of number.

In preparations stained with the method of Brachet, spherical nucleoli are seen. They are evenly distributed in the nods of Ma (Pl. I 5—6). They are small and numerous or big and less numerous, several to over ten nucleoli in one nod. They were never observed situated near the lateral margins of the segments; if below the surface, they are situated near the connectives. An exception present the terminal segments having their nucleoli accumulateed

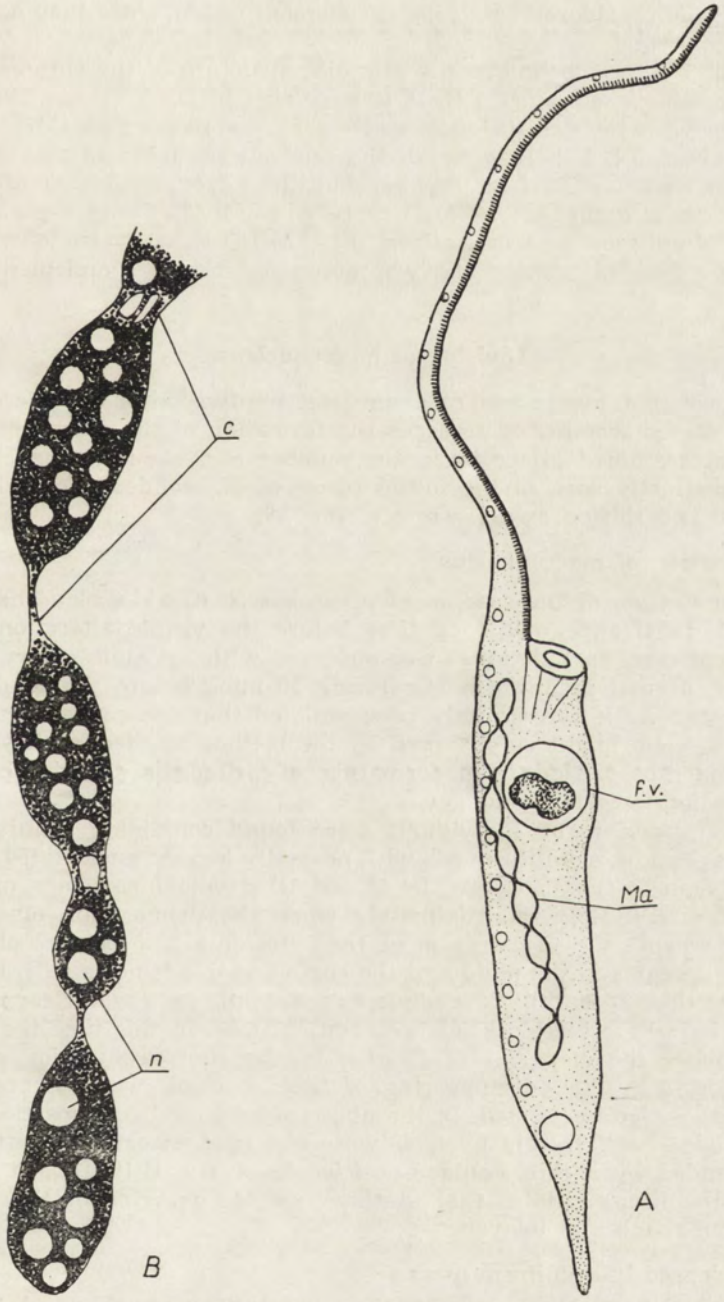

Fig. 1. Macronucleus in *D. cygnus*. A. Position of Ma in living ciliate. B. Structure of Ma, fixed and stained (Ma — macronucleus, f. v. — food vacuole, n — nucleoli, c — connectives)

near their free ends. Sometimes, but very rarely, large areas were found in single nods, staining neither with methyl green nor with pyronine. They can scarcely be considered as a constant element of Ma, since they occur very rarely.

After the Feulgen technique the granular structure of the chromatin component is distinctly seen (Pl. I 4), It is very rich in DNA because the grains stain deeply with the Schiff's fuchsin. Nucleoli are marked also distinctly, as stain-free areas (Pl. I 3). In general, the nucleoli resemble to that described by Ruthmann 1963 in *Loxophyllum,* but differ from that in *Stentor* where only one of them occurs in one Ma nod (Weisz 1949). Feulgen-positive granules are often seen on connectives (Pl. I 4). This was also observed by Schwartz 1935 in *Stentor* and was accounted for as complementation of a new nod.

Division of macronucleus

Division of the nuclear apparatus initiates by division of the micronucleus. It was not stated whether it precedes the formation of the ciliary primordia. In the ciliates studied Mi occur in the number of 2—3, near the Ma chain. They are distinctly seen in the initial phase of Ma condensation when they are divided and shifted away from Ma (Pl. II 9).

Condensation of macronucleus

The primordium of the equipment of proboscis (Golińska and Doroszewski 1964) appears a long time before the visible alterations of Ma occur. In one case, an individual was observed with a visible ciliary primordium and a normal moniliform Ma during 50 min., before the condensation of nods begun. As it can scarcely be postulated that the earliest changes in the ciliary system might be observed by the method applied, so it should be assumed that the period from formation of primordia till the beginning of condensation is certainly longer.

In preparations, many individuals were found containing simultaneously the primordia and a moniliform Ma with normally located nucleoli (Pl. II 7—8). The nods begin to fuse between the II and III development stage of the division furrow (Fig. 2 B), approximately when the primordium of proboscis equipment reaches the primordium of the cytostome. The process of nuclear condensation begins in the middle of the chain but this is not a rule. From the first signs of this process till the complete condensation of the nuclear mass into a more or less spherical block, elapse about 30 min. In this time the division furrow reaches the stage III (Fig. 2 D). In the course of fusion of single median nods, a gradual disappearing of their nucleoli was observed while the terminal nucleoli persisted. In the phase of advanced condensation, in the terminal nods especially big nucleoli were observed, situated near the margin, surrounded by a thin Feulgen-positive layer (Pl. II 10—12). It was observed in the living ciliates that at this time the margins of the terminal nods become rough and uneven.

Ma condensed in a uniform mass

In the condensed Ma mass sometimes nucleoli were observed but they were tiny and not numerous. Disappearence of these structures during condensation seems to be a rule. In the period when Ma is condensed, shifting of

the nuclear mass with regard to the division furrow was observed in living ciliates. The nucleus may entirely shift to proter or opisthe. Those movements, indicating a low density of cytoplasm, seem to be fortuitous and probably occur always in the active cell but at this phase are easily observable owing to the presence of the division furrow.

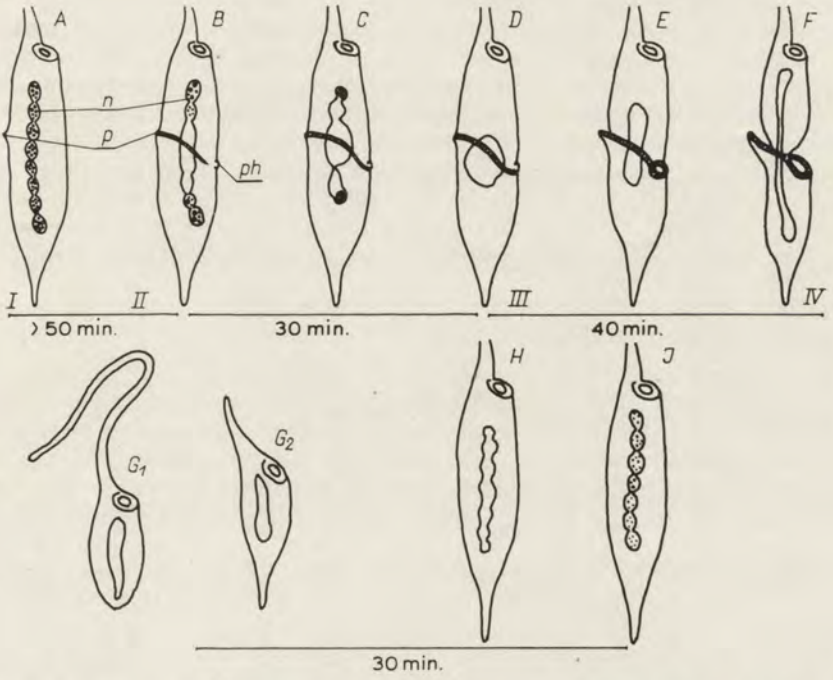

Fig. 2. Division cycle of *D. cygnus*. A—F. Successive stages of Ma transformation in division. G—I. Growing up of daughter individuals and segmentation of nuclei (G_1 — proter, G_2 — opisthe). I—IV. Successive stages of the surface changes after Golińska and Doroszewski 1964 (n — nucleoli, p — rudiment of the proboscis equipment, ph — rudiment of the cytopharyngeal complex)

As follows from the observation of the living material, the spherical form of the nucleus fails to persist for a long time. Ma begins to elongate soon after having become spherical. No changes in the shape of the DNA containing granules, nor in their distribution in longitudinal rows, were observed, either during the condensation of Ma or during its elongation (Pl. III 15—17) as observed by Canella 1951 in *Paradileptus* and by Ruthmann 1963 in *Loxophyllum*. The "RNS-haltige Chromosomen" — as reported by Ruthmann — were not stated either. Some similar pictures were observed when nucleoli remained in the condensed Ma because they assume an elongated form when the nucleus is becoming rod-shaped.

Division of macronucleus

Simultaneously with elongation of the nuclear mass, on the dorsal side differentiates the proboscis of the opisthe (Fig. 2 E, F), and the furrow is slowly narowing. Ma divides always at the stage of a uniform rod not differentiated

into nods. It occurs so because beneath the division furrow a constriction is formed very quickly. Immediately before separation both parts of Ma are connected by a thin thread (Pl. III 18) which breaks and its remnants are engulfed into the daughter nuclei. The time of elongation, from the maximal condensation to division, amounts about 40 min. Observations of living individuals suggest that Ma is simply broken by the narrowing division furrow. Cases of an uneven division of Ma were observed when the moment of interruption occurred simultaneously with shifting of the Ma rod forwards or backwards. In one especially striking case, growing up of the daughter individuals of such a ciliate was followed, and an uneven number of Ma nods was stated (proter 3, opisthe 8). This number was related to the quantity of nuclear material received by the daughter individual. The fact that from the nuclear division till separation of the individuals elaps about 5 min. speaks in favour of breaking up of Ma by the division furrow, the more so as the daughter individuals remain connected by a thin cytoplasmic thread in the major part of this period.

Segmentation of Ma

The maturing of daughter individuals presents a special problem. It was partly discussed in the previuos article (Golińska and Doroszewski 1964), concerning the body shape, length of proboscis and formation of the tail. Formation of the nuclear nods occurs at the same time (Fig. 2 G, H, I, J); all those processes are concluded about 30 min. after division. The segmentation procedes on the whole length of the nuclear rod simultaneously. In living individuals a gradual formation of swellings at regular intervals is seen. Subsequently, deep constrictions are formed between them. Agglomeration of a granular Feulgen-positive substance is seen in those swellings (Pl. II 21), which are initially connected by a broad Feulgen-negative strand (Pl. III 22). In more advanced stages it reminds the multiconnective junctions occurring sometimes in the interphase nucleus. The number of Ma nods is sometimes slightly lower soon after their formation than the mean for interphase, and usually amounts 6—7.

Discussion

The macronucleus in *Dileptus cygnus* shows in interphase an amazing variability of structure. The differences concern as well the number of nods, their size and structure of their connectives, as the size and number of nucleoli contained in them. This lability of the interphase Ma suggests a possibility of continuous transformations concerning, among others, the changes in the nods number in interphase. Such possibilities were documented by Schwartz 1935 and Weisz 1949 in *Stentor* but only in the sense of addition of new nods. They noticed also that the daughter individuals have a lower number of nods immediately after division than in the full interphase. This problem is to be worked out more extensively in the study being carried out presently by the author on regeneration of the nuclear apparatus in *Dileptus cygnus*.

The cytological picture of Ma found in different division stages seems to indicate that the reorganization of nucleus — and consequently duplication of its material — fail to occur in division. This is proved by the constant

grouping of the Feulgen-positive substance in granules with no signs of dispersion. It should be assumed that the duplication of DNA occurs in interphase as in many other ciliates (Gall 1959, Raikov, Cheissin and Buze 1961). Unfortunately, the interphase of Ma in *Dileptus cygnus* has not been exactly investigated as yet.

In *Loxophyllum*, big division chromosomes containing RNA, disposed longitudinally in the rod-shaped Ma, were observed by Ruthmann 1963. Canella 1951 found the longitudinal disposition of the nuclear substance at this period in *Paradileptus*. No similar pictures were found in *D. cygnus* with the methods described above. The granular structure of the chromatin Ma component persisted all along the division.

The problem of passage of the nucleoli content to the cytoplasm is still not clear. It follows from the pictures observed that nucleoli approach the nuclear surface only near the connectives or near the free margin of the terminal nods. Big nucleoli occurring in the terminal nods during the condensation of nucleus resemble the nucleoli observed by Kaneda 1961 in interphase and prophase Ma in *Chlamydodon pedarius*. Kaneda observed emission of the nucleolar content into the cytoplasm in living ciliates. The similitude between the nucleoli in *Chlamydodon* and *Dileptus* is raised by the fact that in *Chlamydodon* only two big nucleoli — the anterior and the posterior — emit their content, whereas the lateral ones remain unchanged. Only the study of the nuclear membrane in *D. cygnus*, and of the distribution of its pores, would solve this problem decisively.

Condensation of the moniliform Ma is not a common phenomenon. Accumulation of the nuclear substance into a uniform mass occurs in *Stentor, Spirostomum, Blepharisma* and *Paradileptus*. Its occurrence in *D. cygnus* allows to include this species into this group. In *Loxophyllum*, according to Ruthmann 1963, besides the complete condensation — at once a possibility exists of formation of a longitudinal rod which is subsequently distributed in this form between the daughter individuals. Weinreb 1955 stated in *Homalozoon (Litonotus) vermiculare* a complete lack of condensation of nuclear nods. Ma is simply broken in the middle during division. The same author revealed an interesting phenomenon of synchronic elongation and constriction of all the Ma nods without breaking the chain. Unfortunately it is not clear how this process may be connected with the normal division — whether it occurs before or after the separation of the daughter individuals.

Division of Ma between the daughter individuals may occur in different stages of the division transformations. So in *Blepharisma* and in *Stentor* Ma is broken in the median connective already after formation of the nods and the daughter individuals receive the moniliform nuclei. In *Loxophyllum, Dileptus* and *Paradileptus* distribution of nucleus occurs before the formation of nods and each daughter individual receives a part of Ma in the form of a rod which is subsequently reconstructed into a moniliform Ma.

The separation of Ma into two parts occurs probably by constriction of the division furrow. This is proved by the lack of any defined place on the rod-shaped Ma which might be liable to interruption. The disconnection of Ma may result in formation of two uneven parts because it breaks always beneath the division furrow, irrespectively of the nuclear shifting forwards or backwards in the dividing cell. The experiments of De Terra 1959 indicate that in *Stentor* Ma is broken also by the mechanical action of the division

furrow. This author obtained individuals with undivided Ma, after destroying the division furrow entirely, whereas a partial disruption caused an uneven distribution of Ma, proportional to the size of the individuals formed in the dublet.

Division changes in the nuclear apparatus, occurring after the fission of the daughter individuals, are known not only in the forms with the moniliform Ma as *Loxophyllum, Paradileptus* and *Dileptus* but were also reported in the ciliates with spherical nuclei as *Colpidium colpoda, C. campylum* or *Glaucoma scintillans* (Kidder and Diller 1934). In this case budding and resorption of a part of Ma occur, nevertheless, this may be looked upon as related to division because an analogical process takes place in *Urocentrum turbo* (ibid.) just before the fission of individuals. Consequently — it seems — that the moment of fission of the daughter individuals cannot be considered as the conclusion of the division process.

Summary

The division cycle of Ma in *Dileptus cygnus* has been investigated. It was stated that the first signs of changes in Ma appear after the formation of cytopharyngeal primordia as well as that of the proboscis equipment. In the course of division Ma undergoes condensation. A more or less spherical block arises which subsequently elongates as to form a rod structure and passes to the daughter individuals. Segmentation of Ma occurs after the fission of the ciliate.

Nucleoli disappear gradually after condensation of Ma. In the advanced stages of condensation, in the terminal nods exceptionally big nucleoli arise. Their content passes probably to the cytoplasm.

STRESZCZENIE

Zbadano cykl podziałowy Ma u *Dileptus cygnus*. Stwierdzono, że pierwsze oznaki zmian Ma występują po utworzeniu zawiązków — cytofaryngalnego i uzbrojenia proboscis. W trakcie podziału paciorkowaty Ma ulega skupianiu. Tworzy się mniej więcej okrągła bryła, która następnie wydłuża się i w formie wałeczka przechodzi do osobników potomnych. Segmentacja Ma odbywa się po rozdzieleniu wymoczków.

Nukleole zanikają stopniowo podczas kondensacji Ma. W zaawansowanych stadiach kondensacji w segmentach skrajnych tworzą się wyjątkowo duże nukleole, których zawartość prawdopodobnie przechodzi do cytoplazmy.

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EXPLANATION OF PLATES I—IV

Interphase macronucleus

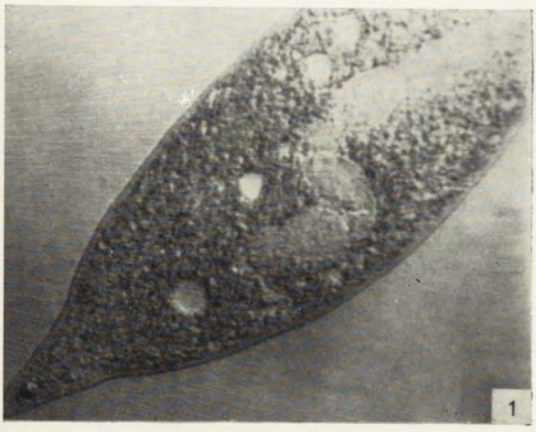
1: Ma in living individual. Outline of nods
2: Single Ma-nods in squashed individual
3—4: Structure of Ma nods. Granular structure, various connections between the nods and Feulgen-positive grains in connectives (Feulgen)
5—6: Dark stained nucleoli, 5 — big and not numerous, 6 — small and numerous (Methyl green—pyronine)

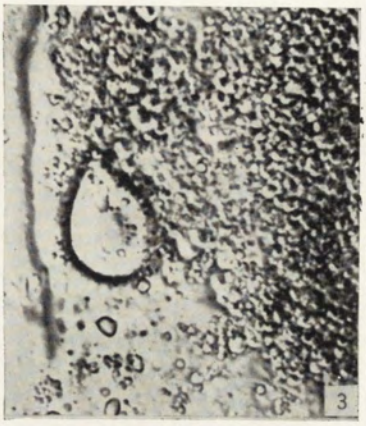
Condensation of Ma

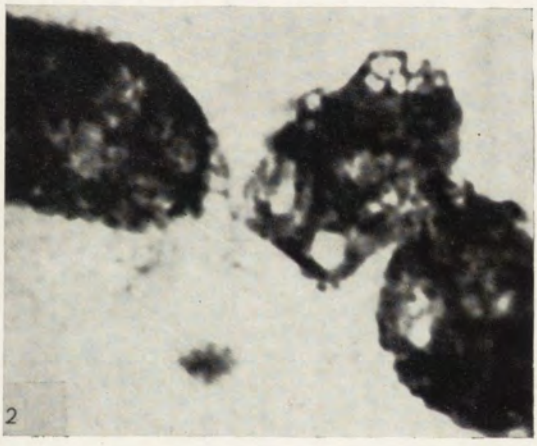
7: No changes in Ma, rudiment of the proboscis equipment (Feulgen—Párducz)
8: Still no changes in Ma. II stage of the rudiment development. In the terminal nod — pattern of nucleoli, i.e. unstained spherical areas (Feulgen—Páarducz).
9: Beginning of Ma condensation, fusion of nods in the middle part of chain. 4 Mi (Feulgen)
10—12: Advanced condensation. In terminal nods big nucleoli (Feulgen)
13: Condensed Ma in living individual. Both rudiments are seen
14: Beginning of elongation of the condensed Ma (Feulgen—Párducz)

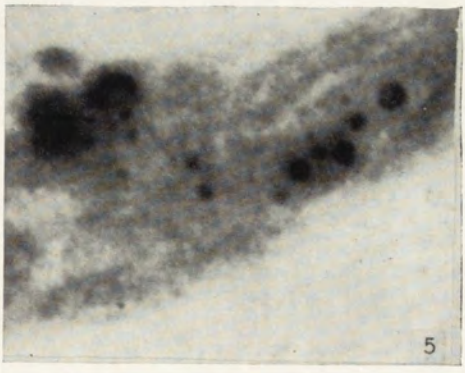
Division and segmentation of Ma

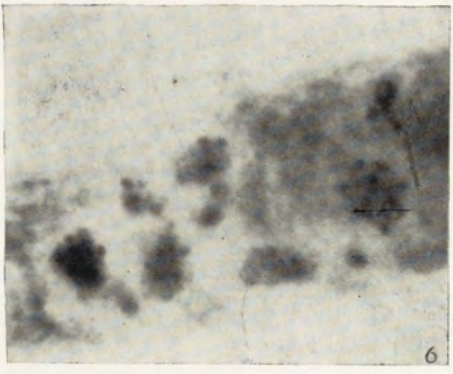
15—17: Elongation of condensed Ma. Peristing granular structure is visible (Feulgen)
18: Beginning of Ma division. Constriction of the nuclear rod and outline of the division furrow are seen (Feulgen—Párducz)
19: Moment of Ma division, both parts are still connected by a thin thread. Outlines of the daughter individuals (Feulgen—Párducz)
20: Opisthe soon after division. Non-segmented Ma and 2 Mi (Feulgen)
21: Segmentation of Ma. Swellings formed on the cylinder
22: Subsequent stage of segmentation. Accumulation of the Feulgen-positive substance in the future segments (Feulgen)











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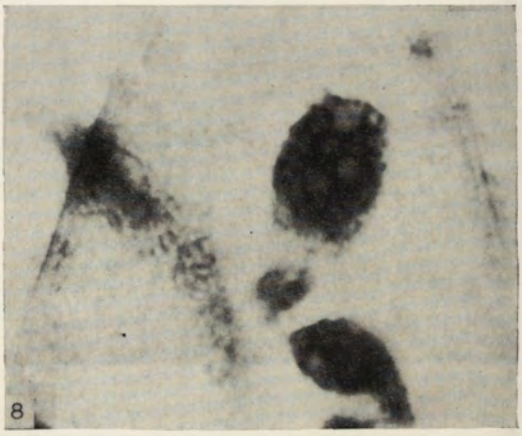

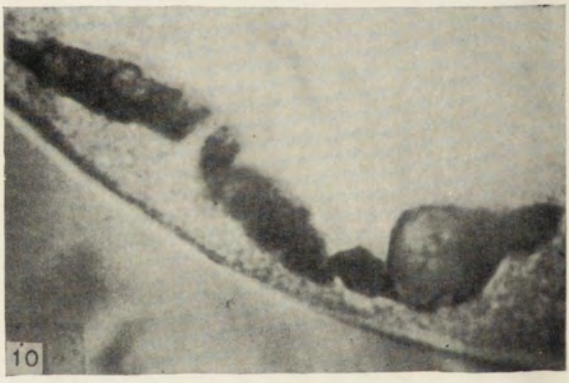

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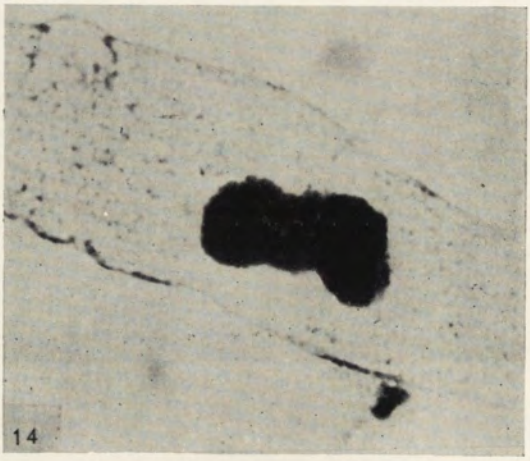
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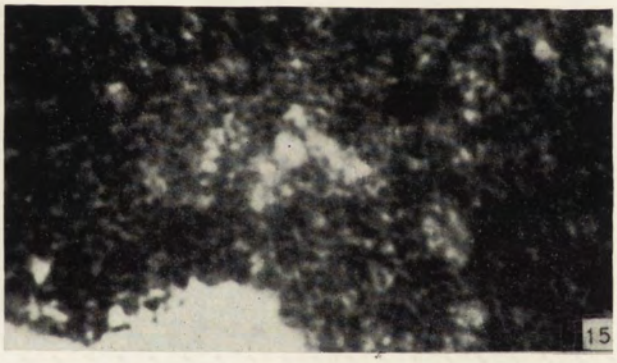
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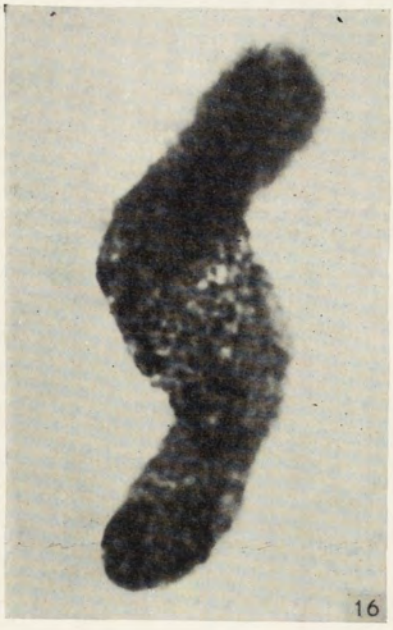
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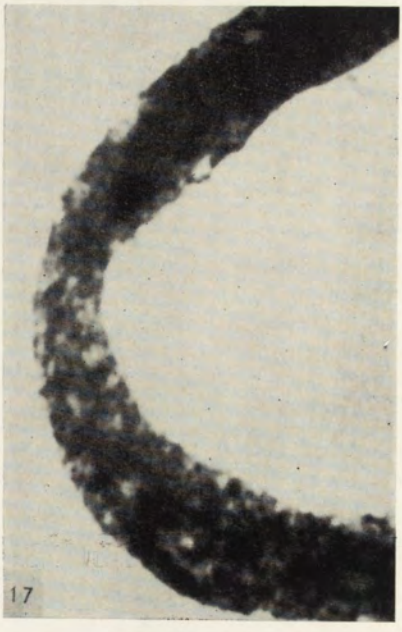
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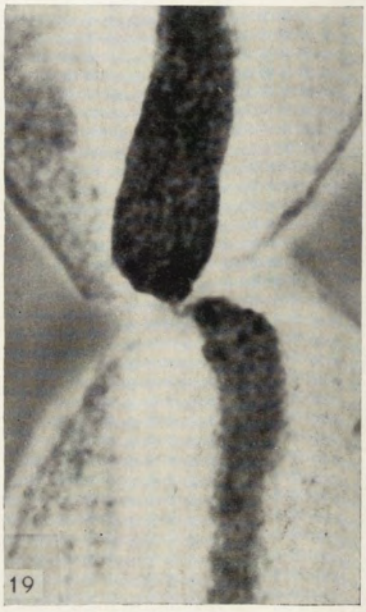

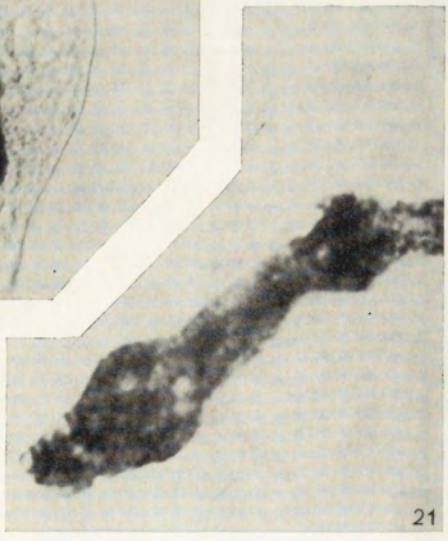

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Dependence of temperature adaptations of some unicellular organisms on feeding conditions

Влияние пищевого фактора на температурные адаптации у одноклеточных организмов

The temperature of the environment is known to be a principal factor which influences very essential changes in the thermoresistance of unicellular organisms (Poljansky 1957, 1959, 1963a, 1963b; Michaltshenko 1959; Sukhanova 1959, 1961, 1962, 1963a, 1963b; Irlina 1960; Luknitskaya 1963)[1]. The experimental data obtained by these authors showed the direct dependence of protozoa thermoresistance upon the environmental temperature. Free-living ciliates and flagellates adapted to relatively low temperatures (1—5°C) manifest low thermoresistance. The same protozoa adapted to relatively high temperatures (10—28°C) show a high thermoresistance which is closely related to the temperature of culturing. The unicellular organisms are able to change their thermoresistance rather quickly and simultaneously with the alterations of the thermic environment. This may be considered one of the most interesting and important physiological pecularities of *Protozoa*.

Besides the temperature itself, some other environmental factors influence the changes in the thermoresistance of *Protozoa*. Some interesting experimental data demonstrate the dependence of protozoa thermoresistance on salt concentration in the culture medium (Irlina 1962, 1963 a, 1963 b, 1963 c; Grigorjan 1964 a, 1964 b, 1964 c). It is also worth to note the effect of various pH value and of the density of population on the stability of cultures to the action of lethal temperatures (Mühlpfordt 1960).

The dependence of protozoa thermoresistance on food conditions has been little studied. However, both factors are frequently correlated. Many free-living and endoparasitic species of protozoa are known to undergo starvation during the Winter period, when the temperature of water is low and the amount of food is also lower than in Summer. Hence, the attempt of the present work is to study the changes in thermoresistance of protozoan cells grown simultaneously — under different food conditions and at various temperatures.

[1] The thermoresistance is considered here as ability of organism to withstand a high temperature.

Material and methods

Two species of free-living ciliates — *Paramecium caudatum* and *Paramecium putrinum* were used. The experiments were carried out on the clonal lines. Additionally, one natural population of *Paramecium caudatum* was used. The sterile Losina-Losinsky's salt solution was used as medium for all paramecium cultures. The mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* served as food.

Before starting the experiments the test-tubes with paramecia were divided into two parts: 1. ciliates fed quite regularly (every 24—48 hours), and 2. the ciliates grown in the salt solution without any food during the whole period of the experiment. At intervals of few days the ciliates of both groups were transferred to fresh sterile salt solutions. The thermoresistance was checked in both series simultaneously.

The well-fed ciliates and the ciliates kept in salt solution without food were incubated in different temperature conditions: 4°C (low temperature), 18—22° (room temperature), and 29°C (high temperature).

The thermoresistance was characterized by survival of ciliates at slightly lethal temperatures. Over 100 specimens were used for each test, and the average time of their survival was calculated in each case statistically.

The thermoresistance of ciliates was tested in a special glass-chamber (Alexandrov 1948; Poljansky 1957) in which the desired lethal temperature was kept at the same level during the whole experiment. *Paramecium caudatum* is a relatively thermoresistant species and, as a consequence, its thermoresistance was determined with the temperatures of 38 and 40°C. *Paramecium putrinum* proved to be less thermoresistant, and 36°C was used as the lethal temperature for checking its resistance.

The author express her gratitude to A. V. Jankowski for supplying the clones of *Paramecium putrinum,* and to T. V. Posnanskaya for her kind help in maintaining the cultures of *Paramecium.*

Results

Paramecium caudatum

Room temperature

Two clones of *P. caudatum* (A and B) cultivated at room temperature were used in the first series of experiments. Each experiment lasted 20—30 days. The thermoresistance was recorded every 2—3 days all along the experiment.

A number of measurements showed that the thermoresistance of the well-fed ciliates remains relatively constant in 24 and 48 hours from the moment of supplying the animals with food. Only, during the first hours (4—8) after adding the food the thermoresistance of these ciliates was not stabilized and showed a decrease. This decrease might be connected with food ingestion and digestion, i.e. its level could depend on the amount of food ingested and on the time of performing the measurements. For that reason the thermoresistance of the well-fed ciliates was always measured 24—48 hours after each feeding.

It was demonstrated that the survival time of the well-fed ciliates at 38°C was from 19.14 ± 0.39 to 21.52 ± 0.38 min. in the clone A, and from

17.5 ± 0.59 to 22.66 ± 0.29 min. in the clone B. This level of thermoresistance of paramecia kept in the room temperature and supplied with food proved to be relatively constant within the whole time of each experiment.

On the contrast, the thermoresistance of the ciliates resting in salt solution without food began to decrease on the 3rd day of experiment, and was decreasing furthermore for the next 20—30 days, i.e. until the experiment was over. This decrease of thermoresistance is shown in Fig. 1, in which the thermoresistance of the ciliates investigated under starvation is calculated in per cent of the value obtained with the well-fed specimens. Two curves illustrate the thermoresistance reducing in both clones used (A and B).

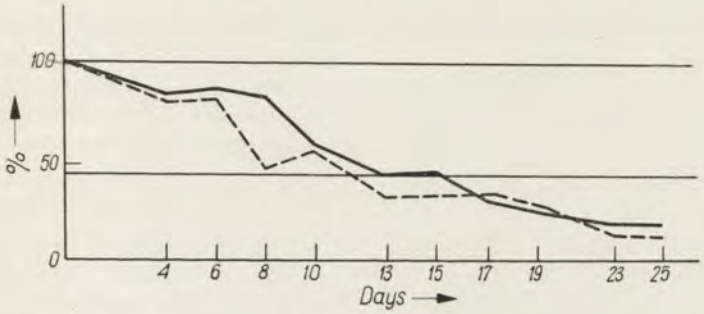

Fig. 1. Thermoresistance of *Paramecium caudatum* grown at room temperature. The curves illustrate the changes in thermoresistance during starvation, in clones "A" (the continous line) and "B" (the dotted line), in per cents of the values obtained in the same temperature for the well-fed cultures (the upper horizontal line — 100%). The lower horizontal line represents the thermoresistance of the well-fed ciliates kept ad 4°C

Moreover, also the thermoresistance of well-fed paramecia adapted to the low (4°C) temperature was calculated in per cent of the same control (the lower horizontal line). This lower line is given to show in what extent the decrease of the thermoresistance depends on thermic and on food conditions.

The ciliates under poor food conditions, when even kept at room temperature all along the experiment, nevertheless show much lower thermoresistance than well-fed paramecia grown at low temperature.

The results of these experiments indicate that the thermoresistance of the ciliates kept without food does not depend on the environmental temperature. The reduced thermoresistance depends on the deficiency of food.

The following experiment was carried out in order to confirm this supposition. A culture of paramecia belonging to clone A was kept without food for 10 days. During this period of time the thermoresistance of ciliates was decreasing, reaching a level much lower when compared with fed animals (Fig. 1). 10 days later the ciliates were fed. Several hours after feeding their survival time at the lethal temperature (38°C) began to increase. 30—40 hours after feeding the average survival time achieved the normal level.

Low temperature

The second series of experiments was carried out at low temperature (4°C). The same clones of *P. caudatum*, A and B, were used for these tests.

The first group of paramecia adapted to low temperature were fed quite regularly (simultaneously with the animals kept at room temperature). The surival time recorded several hours after feeding showed no such a temporary decrease in the thermoresistance as was characteristic of the well-fed ciliates grown at the room temperature.

It is known that, the process of adaptation to low temperature is connected with a very essential decrease in thermoresistance of ciliates (P o l j a n s k y 1957). The survival time of the well-fed ciliates belonging to the clone A was from 9.32 ± 0.04 to 11.46 ± 1.09 min. and from 9.82 ± 0.15 to 12.41 ± 0.70 min. in ciliates of the clone B, at 38°C. This level of survival time was kept all along the experiments.

However, paramecia grown under poor food conditions show a much stronger decrease of thermoresistance in several days, and after 10—14 days it remains only about half of that stated in the well-fed ciliates. The decrease in thermoresistance continues, and 18—20 days later, paramecia dye almost immediately (within 2—3 minutes), when exposed to 38°C. Such an extremely low thermoresistance was characteristic all along the further course of the experiment [2]. Fig. 2 illustrates this considerable decrease in thermoresistance

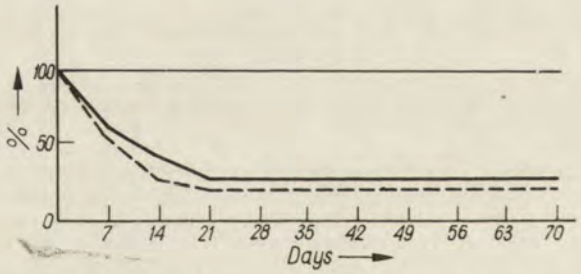

Fig. 2. Thermoresistance of *Paramecium caudatum* grown at low temperature (4°C). The curves illustrate the changes in thermoresistance during starvation, in clones "A" and "B", compared with the resistance of well-fed animals, as in Fig. 1

of paramecia grown under poor food conditions at low temperature (as expressed in per cent of the values obtained with well-fed ciliates in the same thermic conditions).

High temperature

In the third series of the experiments thermoresistance in ciliates adapted to high temperature (29°C) was studied. The high temperature in culture is a factor promoting a high thermoresistance (P o l j a n s k y 1957). As a consequence, a higher temperature (40°C) was necessary for testing the resistance.

The average survival time of the well-fed ciliates adapted to 29°C was from 18.46 ± 0.53 to 19.23 ± 0.79 min. in the ciliates of the clone A and from 18.99 ± 0.69 to 22.11 ± 0.55 min. in ciliates belonging to the clone B.

[2] Each experiment lasted for about 70 days and the thermoresistance was tested each 5—7 days, because the low temperature allowed a very long term keeping of cultures without food.

The thermoresistance of these ciliates was checked 24 hours after feeding, and simultaneously the termoresistance of ciliates grown without food was tested. Paramecia in starving cultures could survive at 29°C, 9—10 days as opposed to the feeding ones which usually could live at this high temperature for a long period of time. Also this series of experiments showed a very considerable decrease in thermoresistance of the ciliates grown under poor food conditions. It is demonstrated in Fig. 3 (in per cents of the heat resistance of the well-fed ciliates). Also the thermoresistance of fed ciliates kept at the

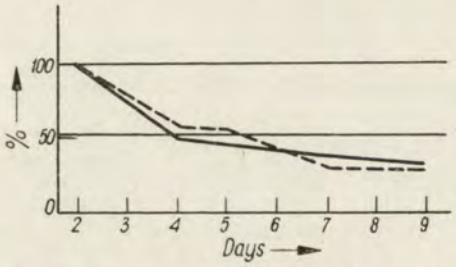

Fig. 3. Thermoresistance of *Paramecium caudatum* grown at high temperature (29°C). The curves demonstrate the changes in thermoresistance during starvation, in clones "A" and "B" in percentage of the resistance of well-fed paramecia kept at the same temperature (the upper horizontal line). The lower horizontal line presents the level of thermoresistance of the well-fed paramecia kept at room temperature

room temperature was calculated as percentage of this control (the lower horizontal line). Two curves illustrate the decrease of thermoresistance of both clones (A and B) cultivated at poor food conditions. The decrease is well pronounced and the comparison with both fed cultures suggests that it does not depend on the temperature of cultivation.

Population experiments

The clone material which was chosen for all these experiments provided the genetic homogenity and a relatively small variability of the thermoresistance. However, for comparison the next series of experiments was conducted on natural population of paramecia from which clones A and B had been previously isolated. The experiments were carried out at room and at low temperatures.

The measurements proved a wider variability in the thermoresistance of individuals belonging to the natural population. The well-fed paramecium population adapted to room temperature may survive from 31.89 $\pm$ 1.05 up to 34.21 $\pm$ 1.42 min. at 38°C, i.e. the average time of survival of the entire population is higher than that of the clones A and B.

Paramecia in population cultures kept without food, in several days become less thermoresistant and at the end of the experiment (25—30 days later) they usually perish within 3—4 min. at 38°C.

The ciliates in population cultures under normal food conditions but adapted to low temperature (4°C) were characterized by a low thermoresistance with the average survival time ranging from 8.96 ± 0.18 to 12.14 ± 0.96 min at 38°. The ciliates cultivated at low temperature without food showed a still more considerable fall of thermoresistance.

Thus it appeares that the changes of thermoresistance of paramecia in the well-fed population cultures and in the populations kept without food manifested the same regularities as observed in the clonal material, except the results were little more variable.

Paramecium putrinum

Two clones of *P. putrinum* were used. The experiments were carried out at room temperature, since *P. putrinum* is less thermoresintant than *P. caudatum* and cannot be adapted to 29°C. The temperature 36°C was chosen as the lethal temperature for thermoresistance tests. The average survival time of the ciliates kept under normal food conditions was at this temperature 11.67 ± 0.19 up to 11.99 ± 0.62 min. (clone 1), and from 10.87 ± 0.32 to 11.29 ± 0.26 min. (clone 2). The thermoresistance measurements were made 24—48 hours after feeding.

Paramecia kept at room temperature under poor food conditions showed a decrease of their thermoresistance all along 30—35 days of experiment, particularly in the second half of this period (Fig. 4). The lowering of thermo-

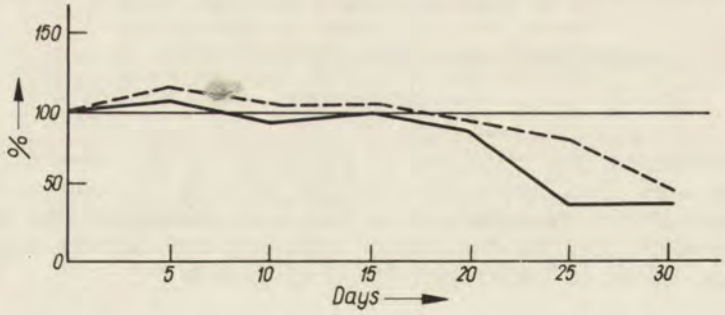

Fig. 4. Thermoresistance of *Paramecium putrinum* grown at room temperature. The curves show the changes in thermoresistance during starvation in the clone 1 (continous line) and clone 2 (the dotted line), in percentage of the resistance of well-fed animals kept in the same temperature

resistance was observed in each experiment and appeared not to be related to the environmental temperature.

Paramecium putrinum turned out to be a very suitable object for investigation of thermoresistance during the conjugation. Within this period of the life cycle the ciliates do not ingest food and undergo starvation (Poljansky 1934). Clone 1 and 2 of *P. putrinum* belong to different mating types and it was easy to get a lot of conjugation pairs when the clones were mixed in a test tube or in a Petri dish. The first test of thermoresistance was performed in both mating types just before conjugation. The average survival

time at 36°C, in both clones was 13.91 ± 0.40 min. and 13.34 ± 0.29 min. respectively. This almost ideal identicity of the average survival time in ciliates belonging to the clones 1 and 2 is very convenient for thermoresistance measurements in conjugating pairs.

The thermoresistance of mating pairs was recorded about 24 and 48 hours after the beginning of conjugation. The average survival time of conjugants was 12.83 ± 0.02 min. after 24 hours and 12.30 ± 0.20 min. after 48 hours. The average survival time of exconjugants was 11.59 ± 0.19 min.; it was recorded soon afer separation of mating individuals.

It seems that the survival of ciliates at 36°C shows some decrease during the course of conjugation, but the differences are relatively small and hardly statistically reliable. The process of conjugation in *P. putrinum* usually lasts for about 3 days, and it can be supposed that this period of time is rather too short for some essential shift in thermoresistance.

Discussion

The temperature is one of the principal environmental factors influencing all processes of protozoa metabolism. Moreover, the environmental temperature defines the thermoresistance of free-living and endoparasitic unicellular organisms. On the other hand, its is also evident that some important non-temperature factors, like feeding conditions, ionic medium and salt concentration (Irlina 1963 a, 1963 b; Grigorjan 1963), oxygen consumption, and other factors of the environment interfere with temperature and may result in very essential shift in the thermoresistance of protozoa cells. It is known that the nutrition of protozoa is closely connected with the thermic factor.

Dogiel 1927 thoroughly investigated some physiological peculiarities of feeding process in protozoa and proved the relation between the digestion and environmental temperature. Reshetnjak 1952 demonstrated in *P. caudatum* the dependence of the rate of food vacuoles formation, their number and size on the temperature regime, either at the moment of food ingestion or before it. Mühlpfordt 1960 pointed out the relationship between food conditions and thermoresistance in protozoa.

The present data confirm this supposition. The experiments have shown considerable decrease in the thermoresistance of *Paramecium caudatum* and *P. putrinum* kept under poor food conditions. The shift was observed at each experimental regime: at low (4°), room (18—22°) and high (29°) temperatures. On the other hand, the temperature of culture also defines the survival time of paramecia on heating, and — as a consequence — the fall of thermoresistance of starving ciliates proceeded on three different levels (Figs. 1, 2, 3).

Also the opposite relation may be found, i.e. the dependence of protozoa resistance to starvation on the thermic factor. The longest survival without food was characteristic of the ciliates kept at low temperature (more than 70 days), and the shortest was found at high temperature (about 10 days).

The decrease of thermoresistance in *P. caudatum* kept in poor food conditions was observed both in clone cultures and in natural population. It should be emphasized that ciliates populations in natural conditions usually undergo starvation during the Winter time. The experiments with paramecia adapted to 4°C and grown under poor food conditions were carried out in conditions very similar to those occurring in Winter. The temperature of water

in natural fresh-water basins is known to be not higher than 4°C in Winter. Besides, some deficiency of O_2 and other unfavorable factors may act during this time. Paramecia probably are living all along the period of Winter at trophic stage. Consequently, their thermoresistance should be extremely low, due to the environmental thermic and feeding factors.

A relatively high thermoresistance of paramecium in Summer should be promoted not only by high environmental temperature, but also by normal food conditions.

The data obtained on *Paramecium putrinum* also support the rule of reduced thermoresistance at poor food conditions (Fig. 4).

The decrease in thermoresistance of *P. caudatum* and *P. putrinum* kept in sterile mineral solution without food was regular and distinct in all temperature applied. Thus, the deficiency of food was the true reason for this gradual fall of thermoresistance. The temperature conditions simultaneously affected only the level of the process.

A number of morphological and physiological alterations was previously observed in starving ciliates (Wallengren 1902; Chainsky 1906; Barbarin 1937a). Barbarin found an essential change of glycogen and neutral fat content in *Paramecium caudatum* under starvation. He observed some accumulation of neutral fat in endoplasm at the beginning of starvation. Then the amount of fat drops began to decrease within the second phase of experiment. By the end of each experiment, i.e. in 15 days, only some remnants of fat drops were usually detected in the endoplasm. Simultaneously with the fat accumulation the storage of glycogen occurred at the beginning of starvation. However, by the end of starvation period only very small amount of glycogen was noticed.

Barbarin observed also a very considerable decrease in size of the ciliates under starvation. This phenomenon is rather characteristic for many protozoa and it was also noticed by the present author that *P. caudatum* and *P. putrinum* change their size under poor food conditions. For instance, the size of *P. putrinum* in well-fed cultures was as follows: the smallest individuals — 96×48 μ, the biggest — 144×72 μ. The size of *P. putrinum* in cultures without food was from 60×34 μ in the smallest individuals to 96×36 μ in biggest ones.

Poljansky 1963 b and Kovaleva 1963 observed a very essential changes in glycogen and neutral fat accumulation in *Paramecium caudatum* grown unedr low (4°C), room, and high (28—29°C) temperatures, in normal food conditions. As a rule, the low temperature results in the storage of great amount of reserve materials. At high temperature the amount of glycogen granules and fat drops is usually very small. Moreover, Poljansky 1934 and Pomrjaskinskaya 1940 stated that some changes in reserve stores and cell metabolism occure during conjugation, incystation, and in the cyst period, and that these changes are related to the starvation.

Leichsenring 1925, working with some unicellular organisms, showed a difference in the rate oxygen consumption in protozoa grown at normal food conditions and under starvation. Pace 1945 obtained some interesting data concerning the effect of cyanide on respiration of *Paramecium caudatum* and *P. aurelia* during the period of starvation.

Barbarin 1937 b proved the dependence of the survival time of starving paramecia on the temperature regime: paramecia usually dyed very quickly

at high temperature (30°C), but could survive for a long time at low temperature (5°C). Barbarin found also that the resistance of paramecium to the effects of some unfavorable environmental factors became extremely low during the period of starvation.

All these data indicate that the starvation of unicellular organisms results in very essential shift in their physiological state related to many basic processes of cell metabolism. The same is commonly stated with various species of multicellular organisms.

The thermoresistance of protozoa is an important index of their physiological state, closely connected with cell metabolism. It undergoes significant alterations with the temperature of the environment (Poljansky 1957, 1959; Sukhanova 1959, 1962, 1963; Irlina 1960; Luknitskaya 1963). However, a number of non-thermic factors acting simultaneously with the temperature are capable to decrease or increase the thermoresistance of protozoa cells. Food conditions proved to play a very important role among them.

Summary

Paramecium caudatum (clonal material and a natural population) kept in 4°C showed lower thermoresistance, and in 29°C — higher thermoresistance, than the animals grown at 18—22°C. On the other hand, the animals kept in a sterile salt solution at the same temperature show much lower thermoresistance than those grown in a food-rich medium. The same holds true also for *P. putrinum*. The gradual decrease of thermoresistance caused by the starvation proceeds very distincly in all temperatures studied. The dependence of cell thermoresistance on food conditions reflects the ecological coincidence and the metabolic linking of both factors.

РЕЗЮМЕ

Настоящая экспериментальная работа посвящена исследованию влияния условий питания на теплоустойчивость клеток простейших. Объектом исследования служили два клона и одна естественная популяция *Paramecium caudatum* и два клона *Paramecium putrinum*.

Культуры инфузорий, регулярно получавшие пищу и культуры, не получавшие пищи, содержались в условиях трех температур: низкой (4°C), комнатной (19—22°C) и высокой (29°C).

В культурах, не получавших пищи, инфузории сильно снижали свою теплоустойчивость по сравнению с таковой контроля. Особенно значительное снижение теплоустойчивости наблюдалось во второй половине каждого опыта.

Такое снижение теплоустойчивости не зависит от температуры культивирования инфузорий и причиной его является именно недостаток пищи. Но температура культивирования определяет уровень теплоустойчивости инфузорий и длительность переживания их в культурах, лишенных пищи. Поэтому процесс снижения теплоустойчивости голодающих парамеций протекал на разных ее уровнях: низком при низкой температуре, среднем при комнатной и высоком — при высокой окружающей температуре.

Полученные экспериментальные данные позволяют предположить, что среди разнообразных внешних факторов, действующих параллельно с температурой окружающей среды и сильно влияющих на теплоустойчивость клеток простейших, фактору питания принадлежит одно из основных мест.

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The culture, structure, and locomotion of *Halteria grandinella*

Die Kultur, Bau und Bewegung von *Halteria grandinella*

Research on the oligotrich ciliates of the genus *Halteria* has been hampered by lack of a good culture method, and protozoologists have differed about the morphology of the most common species, *Halteria grandinella* (O. F. Müller, 1786).

Moody 1912 first cultured *Halteria grandinella* in plain pond water. Szabó 1934 maintained a good culture for about 5 weeks in swamp water containing *Sphagnum* by replacing evaporated water daily with tap water. He described *Halteria grandinella* from Hungarian sites as invariably having 15 adoral membranelles, and possessing 7 oral membranelles and a somatic ciliature of 3 bristles in each of 7 furrows. Szabó 1934 emphasized only sausage-shaped macronuclei were present in his Hungarian specimens. On the other hand, André 1912 and Kahl 1935 respectively found Swiss and German specimens to have an ovoid macronucleus.

Fauré-Fremiet 1953 obtained abundant populations of *Halteria grandinella* from the forest of Saint Germain by daily adding a suspension of bacteria from the original biotope to boiled water. He found 16 adoral and 8 oral membranelles, and traced a row of paroral ciliature extending from the last oral membranelles. The somatic ciliature, of 4 bristles in each of 6 rows, formed an equatorial half-belt interrupted ventrally.

Frings 1948 reared many *Protozoa*, including *Halteria grandinella*, with dried skim milk powder, and the Carolina Biological Supply Company, Burlington, North Carolina, U.S.A., offers *Halteria* cultured on the regular wheat medium for amoeba. However, Mackinnon and Hawes 1961 report that no culture method is known for *Halteria*.

In this paper a satisfactory culture method and new findings on the structure and movement of *Halteria grandinella* are presented.

Materials and methods

Culture

Halteria grandinella obtained from Deming Lake, Terre Haute, Indiana, and a stock supplied by the Carolina Biological Supply Company and collected at Burlington, North Carolina, were cultured in test tubes containing a me-

dium of 20 ml. bacteriologically-filtered lake water, one house fly (*Musca domestica*), and one drop of 0.1 N HCl (to lower the pH to neutrality), and kept between 22°—28°C. These cultures contained *Halteria grandinella, Bodo edax,* and the bacteria *Aerobacter aerogenes,* a diplococcus, and spirilla, and yielded as many as 3—10 *H. grandinella* per mm.^{3} of culture fluid. By subculturing on every second or third day, the mass culture of *H. grandinella* from Deming Lake, Terre Haute, was maintained for 11 months, while the mass culture from Burlington, N. C., was subcultured for 7 months.

It was found possible to substitute approximately $^1/_3$ of a black field cricket (genus *Gryllus*) for *Musca domestica* in the culture medium.

A culture medium consisting of 14 ml. of bacteriologically-filtered lake water and one *Musca domestica,* buffered with 3 ml. of 0.02 M Na_2HPO_4 and 3 ml. of 0.02 M KH_2PO_4, and set to pH 7 with 0.1 N HCl and 0.1 N NaOH, yielded the highest numbers of *H. grandinella.*

A number of good reserve cultures for later use in subculturing were produced by adding another *Musca domestica* to 20 ml. lake water cultures in which only a few *H. grandinella* remained. In some of these cultures, a good increase in *H. grandinella* followed a fourth additional fly, and individual cultures were thus maintained for over 60 days. In 22°C and 28°C cultures, the minimum period between addition of new flies was 8 days.

By use of autoclaved lake water washes a species-pure culture of Deming Lake *Halteria grandinella* was also produced. Then 5 culture tubes, each containing one fly, 15 ml. of bacteriologically-filtered lake water, 2.5 ml. of 0.02 M Na_2HPO_4, and 2.5 ml. of 0.02 M KH_2PO_4, were autoclaved and following this set to pH 7 with 0.1 N HCl. The 5 tubes were inoculated from the species-pure culture and kept at 25°C. Eleven days after inoculation they contained only good numbers of *H. grandinella* and bacteria. Mr. McGuinness, bacteriologist of Union Hospital, Terre Haute, found only 2 species of bacteria in these cultures, a preponderant number of *Aerobacter aerogenes,* and an unidentified diplococcus.

However, in many of the best cultures of *H. grandinella* a high number of spirilla were also present.

Preliminary experiments indicate that boiling the fly or entire culture medium, unlike autoclaving, is inimical to the culture of *H. grandinella.*

Apparatus and techniques

The structure of unstained live and disintegrating specimens was observed and photographed by phase-contrast microscopy. The adoral membranelles and somatic ciliature were best studied under a cover glass in a mixture of one part culture fluid, one part 2% methyl cellulose, and one part M/50 KCl. The KCl acted on the adoral membranelles to greatly reduce the rate of swimming after one to several minutes. It may also have stimulated reproduction.

Direction of spiraling and most movement was observed in uncovered drops or deep layers of culture fluid with a binocular microscope.

A potentiometer accurate to ± 0.05 pH units was used to determine pH, the KCl solutions were accurate within 3%, and temperatures were maintained within ± 0.4°C.

Observations

Parameters of culture

Temperature

In each of 4 preliminary experiments, 3 identical groups of 5 cultures, prepared with 20 ml. bacteriologically-filtered lake water and one *Musca domestica,* were kept at different temperatures. The *Halteria grandinella* concentrated at the surface of the culture fluid in these early experiments, which was later determined to be due to oxygen insufficiency.

In 9 of 10 22°C cultures high numbers of *H. grandinella* (3—10 or more per surface mm.^{3}) were present between 3—11 days after inoculation, and all 22°C cultures contained less than 2 *H. grandinella* in any mm.^{3} of culture fluid at 16 days. Many 28°C cultures had high numbers of *H. grandinella* already by the second day, and there was a more rapid drop in numbers than at 22°C. 8 of 10 32°C cultures yielded high numbers on the second day after inoculation or before, and all 32°C cultures had less than 2 *H. grandinella* per mm.^{3} by 11 days. Only 5 of 10 35°C cultures yielded high numbers on the second day or after, but low numbers were still present in 9 35°C cultures on the sixteenth day.

Ten test tubes maintained at 37°C did not yield cultures, and it can be assumed that 37°C exceeds the thermal limit of *Halteria grandinella* culture in the described medium. Ten test tubes kept at 3°—9°C yielded no usable cultures, but in 3 of these a few *H. grandinella* were present 11 days after inoculation.

H ion concentration

In each of 2 experiments with the lake-water medium and 2 with the buffer medium, 2 identical groups of 5 cultures were maintained at different pH ranges at 25°C. The results with both media, corroborated in experiments with less restricted pH ranges, gave the following relationships in number of *H. grandinella* obtained: pH 6.8—7.2 > pH 5.8—6.2, pH 6.9—7.15 > pH 7.8—8.1, and pH 6.9—7.35 > pH 7.4—7.8. Thus *Halteria grandinella* showed the highest reproductive rate at pH's within or fairly close to the neutral range.

Oxygen concentration

To determine the causes of surface concentration of *Halteria grandinella* in the earlier cultures, bacteriologically-filtered lake water of pH 7.1 was run at that time into Kimax fermentation tubes to fill the inverted, closed arm of each tube, and the adjacent horizontal portion. In 4 Kimax tubes a house fly was placed at the surface of the culture fluid, in the horizontal portion of the tube, while in 10 others the fly was located at the top of the closed, inverted arm. All air bubbles were eliminated and the inoculating drops were placed on the surface of the culture fluid. Movement of the *H. grandinella* into the inverted arm did not require downward swimming.

In all 14 tubes, kept in darkness at 23°C, the *H. grandinella* concentrated at the surface of the culture fluid in the bottom, horizontal portion of the tube.

In 8 of the above Kimax tubes, in which the fly had later settled to the bottom, an air bubble, or a sprig of *Chara* that released an oxygen bubble,

were introduced into the closed arm. Numerous *H. grandinella* then located near the bubble in the top of the closed arm. In 2 of 4 tubes in which *Chara* had produced no oxygen bubble, all *H. grandinella* remained collected at the air surface in the horizontal portion.

The location of *Halteria grandinella* in a culture fluid column thus is not determined by negative geotropism or the position of the fly, but depends on oxygen concentration, acting either directly or through the bacteria.

Structure

No morphological differences were found between the *Halteria grandinella* from Deming Lake, Terre Haute, and those from ponds near Burlington, North Carolina. The *H. grandinella* possessed 16 adoral membranelles (Pl. I 3). In some organisms 8 oral membranelles could be distinguished, the deepest and smallest membranelle not being visible in most live phase-contrast preparations (Pl. I 4). In 2 cases the granular tract giving origin to the paroral ciliature was seen (Pl. II 5). The somatic ciliature was studied in over 25 specimens, and all but 3 were clearly observed to have 7 rows of bristles (Pl. II 6—7). In many *H. grandinella* the presence of 4 bristles in each row was established, by observing this number of bristles per row while all 7 rows were simultaneously visible and the adoral membranelles were still active (Pl. III 8). All 4 bristles in a row had the same length, more than half the diameter of the organism.

In over 45 preparations the macronucleus became clearly visible, after differing amounts of cell disintegration had taken place. In many cases the macronucleus was spherical (Pl. III 9), while in a smaller number of specimens it was oval or ovoid to varying degrees (Pl. III 10). Macronuclei visible before full disintegration of the cell, and those seen immediately after bursting the protozoan by pressure, were more frequently ovoid than macronuclei observed after full cell disintegration. In several cases the macronucleus was seen to become more spherical during continued disintegration of the specimen.

In a few cases the macronucleus had a constriction, located at different points along the macronucleus in different specimens (Pl. IV 11—12). The macronucleus of some newly-disintegrating organisms was highly constricted in the middle or after a third of the macronucleus. In 2 specimens macronuclei highly constricted in the middle became ovoid. Elongation and constrictions, especially those located beyond small portions of the macronucleus, were more frequent in preparations which had started to dry, and in which full cell disintegration had occurred. Some of these last constrictions appeared to be associated with a break in the macronuclear membrane.

A small, spherical micronucleus was observed in all suitable preparations.

The size of the specimens varied from 23 × 23 μ to 33 × 36 μ, this ranging around Szabó's 1934 and Fauré-Fremiet's 1953 measurements.

Movement

Forward spiraling

The previously reported typical motion of *Halteria grandinella* consists of forward spiraling interrupted by brusque jumps.

The direction of spiraling around the longitudinal axis during forward swimming was observed in many specimens moving directly toward or away from the binocular microscope. All spiraled to the left. Observation of this spiraling with the single-objective phase microscope created the illusion of reversals in direction of spiraling described by Bullington 1925.

Shaking of a slide bearing culture fluid stimulates a greater amount and a much higher speed of forward spiraling.

The adoral membranelles responsible for forward spiraling are more sensitive to potassium chloride (KCl) than are the somatic bristles used in jumping. If *H. grandinella* are placed in a solution of M/300 KCl in bacti-filtered lake water, normal forward spiraling is replaced by only a slow and reduced movement. When the organisms are in this last condition spiraling is not discernable at 30 × magnification with the binocular microscope, and phase contrast at 500 × magnification shows the ring of adoral membranelles to be relatively inactive, with individual membranelles visible and only slightly blurred. In M/500 KCl forward spiraling is slowed, but in most specimens a typical spiraling motion is still easily observable at 30 × binocular magnification.

The nature and rate of the jumping movements is not affected by M/300 or M/500 KCl.

Jumping

H. grandinella were observed to jump backwards in response to hitting other specimens, and on being touched with a needle. However, backwards jumping occurs normally at intervals during forward spiraling. After a jump the organisms generally circle to one side as they resume forward spiraling, and thus move forward in a new direction. As a result of intermittent jumping and subsequent changes in direction of swimming. undisturbed specimens sometimes remain in one area for some time.

Shaking of a slide on which fluid from a *H. grandinella* culture has been placed inhibits jumping.

If *H. grandinella* are placed in a bacti-filtered lake water solution of M/200 KCl, the subsequent rapid backward spiraling is followed by some inactivity on the substrait and slow movement devoid of any jumping, or interrupted only by an occasional jump or abnormal jumps. The same inhibition of jumping during slow movement occurs in M/100 KCl after the rapid backward spiraling, the spiraling in circles or in place, and then the original period of inactivity on the substrait are over. Abnormal jumps consist of rapid movement in an arc, quick movement in an arc away from encountered objects, short, noticeably slow jumps, and only turning on the animal's axis from time to time.

Rapid backward spiraling

A new method of locomotion was also observed. This consists of an extremely rapid backward spiraling, so that the posterior end now precedes, and is produced through fast beating of the ring of adoral membranelles. In rapid backward spiraling separate spirals around the longitudinal axis cannot be distinguished at 30 × binocular magnification, at which instead the organisms appear to simply be moving in a straight line at high speed. At 500 × phase magnification the spirals merge to give the impression of continuous oscilla-

tion around the longitudinal axis as the animals swiftly pass by backwards in a straight line.

The direction of spiraling in rapid backward movement was observed with the binocular microscope in specimens placed in distilled water. *H. grandinella* whose backward spiraling was slowing were seen to spiral to their left. In an equal mixture of 2% methyl cellulose and culture fluid slowed backward-spiraling specimens also spiraled to their left. Thus in both forward and backward swimming *H. grandinella* spirals to its left, or it swims backward along a right-wound spiral. In other words, if in rapid backward spiraling the posterior end is considered the temporary anterior, it leads in a right-wound spiral.

Rapid backward spiraling is clearly an escape movement. It can be elicited by shaking, and sometimes by touching specimens with a needle. If a drop of 1 molar KCl is placed in culture fluid on a slide, *H. grandinella* reached by the expanding drop do rapid backward spiraling through it and then the great majority stop just beyond the opposite periphery of the drop. *H. grandinella* placed in solutions of M/10 to M/200 KCl in lake water first show rapid backward spiraling, followed by death in M/10 KCl, and spiraling in circles or in place in M/50 KCl (M/100, M/200 KCl, see jumping).

Rapid backward spiraling can also be observed by placing a small drop of culture fluid containing *H. grandinella* into pure imported Sultana olive oil, distributed by the Great A. and P. Tea Co., N.Y., N.Y. Within half a minute of placement of the drop, the *H. grandinella* begin to hit its olive oil interface and then start rapid backward spiraling. They repeatedly impinge on the olive oil interface as they continue rapid backward spiraling in straight lines close to and around the globule's periphery, or cross the globule, and some specimens burst at the olive oil interface. Then, after approximately 4—13 minutes, the remaining *H. grandinella* start to spiral in small circles and finally burst at the olive oil interface or settle to the bottom of the globule. A similar phenomenon was observed in purified oleic acid.

Effect of surface tension

Szabó 1934 found *H. grandinella* could not be dried for staining. When the organisms came into contact with the water's surface membrane, the water ran off them and they burst under the high surface tension.

During the present research many specimens were seen to actively swim into the air — water interface of globular, deep drops and relatively deep quantities of culture fluid or pond water. They immediately burst and their contents spread out on the interface. Most of the other *H. grandinella* in the same quantities of fluid continued normal forward spiraling and jumping for long periods of time. Although *H. grandinella* shows some tendency to concentrate in the middle of a culture drop and to avoid the shallow edges of a quantity of fluid, many specimens can sometimes be seen swimming along the air — water interface of a drop without ill effect.

Similar bursting of specimens was observed at culture fluid — olive oil interfaces, sometimes after the specimens had remained at the interface without much movement for a time.

Initiation of function by the new adoral membranelles

Szabó 1934 suggested that in reproducing *H. grandinella* the new adoral membranelles initiate their activity after constriction appears. Szabó 1935 also found that in *Halteria geleiana* the adoral membranelles are still not functional before constriction becomes clearly apparent.

In the present study, the new adoral membranelles of *Halteria grandinella* reproducing in equal parts of culture fluid, M/50 KCl, and 2% methyl cellulose were seen to beat before constriction of the organism became visible (Pl. IV 13). This early beating of the new adoral membranelles was irregular, and in some cases slower than that of the old adoral membranelles. The new ring of adoral membranelles was associated with a raised area or "cap" of ectoplasm.

Discussion

There exist consistent differences in the reported number and arrangement of the rows of somatic bristles and in the reported number of adoral membranelles, as well as in the observed shape of the macronucleus of *Halteria grandinella*. These outstanding differences in regard to major structural features subject to clear observation makes it likely that there are varieties within *Halteria grandinella*.

The rounding-up of the macronucleus observed in several preparations indicates that after breakdown of the cell plasma membrane water may diffuse into the ovoid macronucleus of *H. grandinella* and cause it to swell and become spherical. The macronucleus will also appear to become spherical if one end turns toward the viewer.

The constriction of an occasional macronucleus into very unequal portions appears to be due to a degenerative change. The observation in *H. grandinella* of macronuclei constricted in the middle or after a third is of interest in view of the finding of Szabó 1934 that most of the macronuclei of *Halteria maxima* were thus constricted.

Bullington 1925 reported *H. grandinella* as rotating to the left in forward spiraling, as does the author, but the 60×50 μ dimensions he ascribed to this species suggests he observed another form. Szabó 1934, who differentiated several species of *Halteria,* observed *H. grandinella* to turn slowly from left to right by the oral membranelles alone during feeding, but did not determine the direction of spiraling in forward swimming.

The greater sensitivity of the adoral membranelles than the somatic bristles to KCl, as well as the differential effect of shaking on these 2 groups of structures, demonstrates that the adoral membranelles are functionally different from the somatic bristles. The above results are also physiological evidence for the structural autonomy of the peristomal ciliature (adoral and oral membranelles and paroral ciliature) from the somatic ciliature determined for *H. grandinella by* Fauré-Fremiet 1953.

The several types and degrees of motion available to *H. grandinella,* as well as the physiological difference between its 2 main ciliary systems, may make this species unusually suitable for physiological research.

A paper on the responses of *Halteria grandinella* to a variety of stimuli is in preparation.

Summary

A culture method for the oligotrich ciliate *Halteria grandinella* is presented, involving lake water, a house fly (*Musca domestica*) or cricket (*Gryllus*), and preferably also buffers. Temperatures between 22—32°C, a pH near neutral, and sufficient oxygen are optimal.

Halteria grandinella from 2 American sites possessed 16 adoral and 8 oral membranelles, 7 somatic rows of 4 bristles each, and an ovoid macronucleus. There may be varieties of this species.

Types of locomotion are described and a physiological difference between the adoral membranelles and the somatic ciliature is indicated. Specimens burst at interfaces. The new adoral membranelles function before constriction.

ZUSAMMENFASSUNG

Eine Kulturmethode für den oligotrichen Ciliaten *Halteria grandinella* wird gegeben. Teichwasser, eine Hausfliege (*Musca domestica*) oder eine Grille (*Gryllus*), und vorzüglich auch Puffers werden benützt. Temperaturen zwischen 22—32°C, pH nahe dem Neutralpunkt, und genügend Sauerstoff sind optimal.

Halteria grandinella von zwei amerikanischen Fundorten hatten 16 Adoral- und 8 Oralmembranellen, 7 Körperreihen von je 4 Borsten, und einen eiförmigen Makronucleus. Es mag Varietäten von dieser Art geben.

Bewegungsarten sind beschrieben und ein physiologischer Unterschied zwischen den Adoralmembranellen und den Körperborsten wird gezeigt. Die Tiere zerplatzen an Grenzschichten, und die neuen Adoralmembranellen funktionieren vor der Einschnürung.

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EXPLANATION OF PLATES I—IV

1: Portion of culture drop. Phase contrast, 125×

2: Forward spiraling *H. grandinella.* Two somatic groups of 4 bristles (S) can be seen. The anterior adoral membranelles (A) are blurred.

3: Frontal view of the anterior end, showing the 16 adoral membranelles, slowed with equal parts of culture fluid, 2% methyl cellulose, and M/50 KCl

4: Specimen flattened and distorted to expose the 8 oral membranelles originating from the floor of the vestibular depression

5: Portion of disintegrating specimen, showing macronucleus (M), micronucleus (N), bases of the oral membranelles (B), and the granular tract of the paroral ciliature (G)

6: Frontal view of a somatic bristle layer, showing one bristle from each of the 7 rows

7: Frontal view of a somatic bristle layer in another *H. grandinella*

8: Frontal view. Bristles from 6 of 7 rows are visible. All 4 bristles of one row (S) are seen under the shadows of functioning adoral membranelles

9: Spherical macronucleus in a disintegrating *H. grandinella*

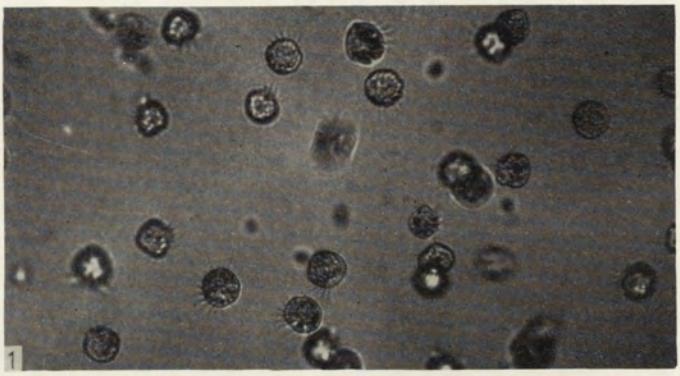
10: Oval macronucleus from a disintegrated specimen

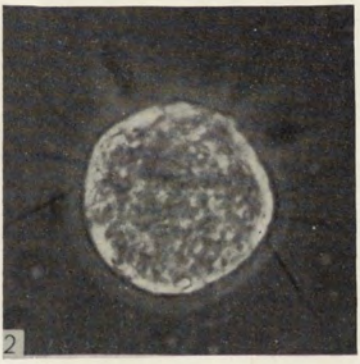
11: Macronucleus with a middle constriction in a disintegrated specimen

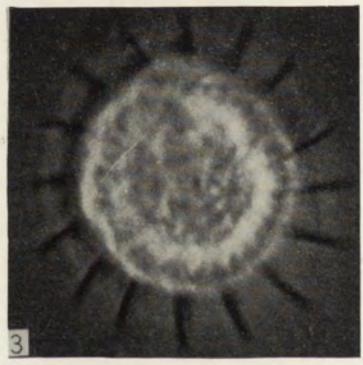
12: Macronucleus with an end constriction

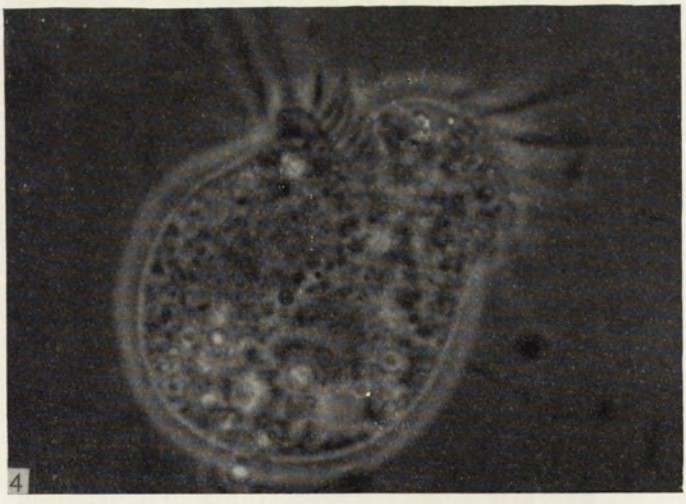
13: Functioning new adoral membranelles, to left on reproducing *H. grandinella* not yet showing a constriction. Equal parts culture fluid, 2% methyl cellulose, M/50 KCl

[All photomicrographs, except first, 500× phase contrast, Tri-X film, Diafine developer. Printed by Audio-Visual Center, Indiana State University]









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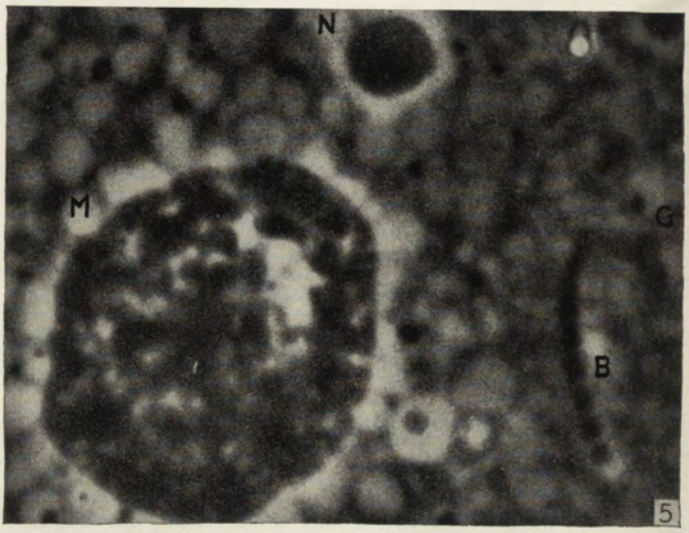

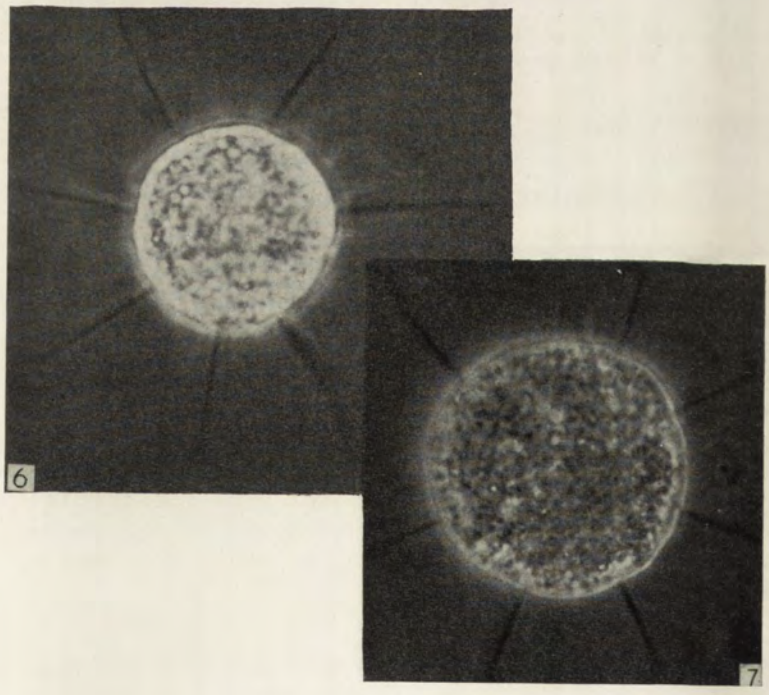

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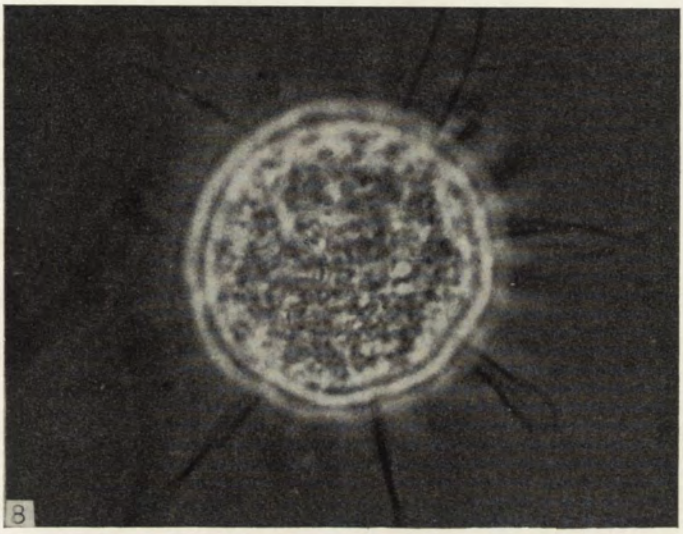

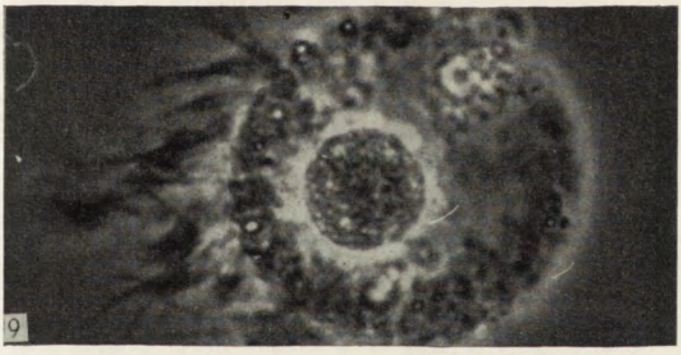

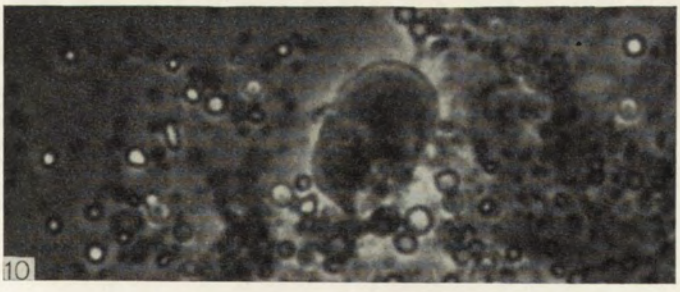

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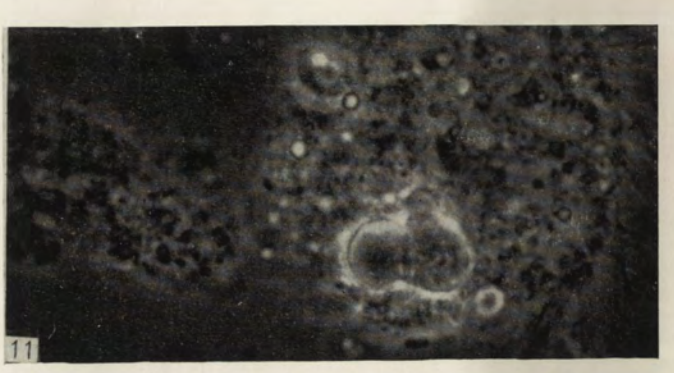

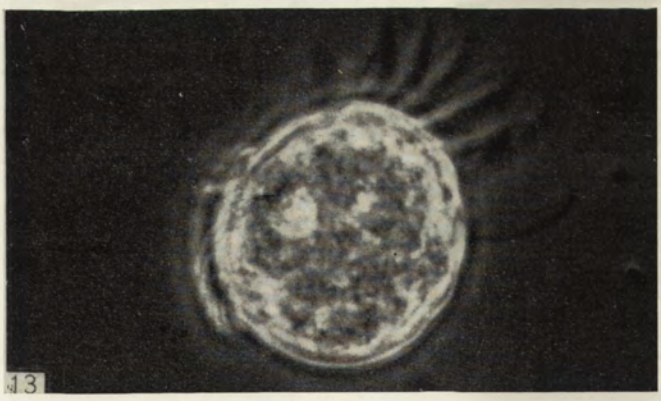

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The response of *Dileptus cygnus* to the bisection

Reakcja *Dileptus cygnus* na przecięcia

The response of *D. anser* to the puncture and bisection was described by the author in the previous papers as well as the reactions of *D. cygnus* to the puncture (Doroszewski 1961 a, b, 1962, 1963 a, b, c). Several points still remain obscure and the question arose whether the most posterior fragment after the bisection performs some active reaction or only follows it course. This problem is much easier to solve in sessile cilate *D. cygnus* as in this case the operation can be perfomed upon the resting individual. In *D. anser* the behavior of the anterior fragment after the operation was not uniform; in general it followed its course so that fragment were drawn apart from each other, but there occured short reversion and circling backwards (Doroszewski 1961 b). As it will be shown later, the approach to this question was also easier in *D. cygnus*. Finally, the relation of the area sensitive to puncture to that sensitive to the section was also worth studying. In both cases we certainly deal with some kind of the traumatic effect, but the difference consists in the fact that in the case of bisection the fragment of the ciliate is liberated from the rest of the body and may exhibit its own reactions. The role of the rotating movement in the reactions of Dileptus was also till now unexplored and the author tries to approach this question in the present study.

The author wishes to express his thanks to Dr. S. Dryl for reading the manuscript, and to Miss K. Golińska for growing *D. cygnus* and for performing the drawings.

Material and methods

As concerns the cultivation of the ciliate and the description of its life cycle the reader can be referred to the previous papers (Doroszewski 1963 a, Golińska and Doroszewski 1964), and also the extensive monograph study of this genus published by Dragesco 1963. The bisections were performed free-hand or by means of the micromanipulator. In both cases the steel spear-point needles or the glass needles were used. The results were registered by the movie camera. For the initial observations the time recorder and paper with millimeter scale was used.

In order to determine the structure of the fragments whose reactions were investigated, the vital photomicrographs and the stained preparations were

made. The Párducz staining and the osmium tetroxide impregnation technique was applied. In each series of bisections the response of ca. 200 fragments was observed.

Results

Transection through the middle part of the proboscis

Promer [1]

The behavior of the anterior fragment was rather simple since it consisted of circling backwards (Fig. 1, sect. 1). The behavior of isolated proboscis of *Dileptus* was already studied by Metzner 1932, and the author was able to confirm his finding. The fragment of proboscis decayed after some hours probably because of the lack of the nuclear apparatus.

The operation by the means of micromanipulator was illustrated on the series of movie photomicrographs in the previous work Doroszewski 1963 c (Pl. I 5).

Opimer[1]

The ciliate deprived of the half of the proboscis reacted by the cycle of avoiding reactions, in general features similar to that evoked by other stimuli (Fig. 1, sect. 1 and Pl. I 6). The individual moved backward some 3 to 5 distances of its length and then moved forward under the angle changed with respect to its starting position. Before the change of the direction of movement, the anterior part of the animal described several circles so that the whole animal described the cone. The rotatory movement in the majority of cases consisted in counter-clockwise rotation. In the experiments with sessile *D. cygnus* the reaction performed was that of starting backwards instead of the change of the movement from the forward to the backward one as in the normal reversion in ciliates. As the backward movement was followed by the forward one, the final result of the operation was that of starting forwards. When the bisection was performed (Fig. 1, sect. 2) near the base of the proboscis, the reversion of movement lasted longer.

Transection behind the cytostome

Promer

Immediately after operation the proboscis becomes spiraly coiled (Fig. 1, sect. 3). The similar reaction of proboscis was already observed by Golińska (unpublished) for tactile stimulation. The fragment was circling in the backward direction, describing several large circles with the accompanying lateral rotation. After some 6—7 sec. the direction of the movement changed and the promer started to swim forwards.

Opimer

The character of the backward movement evoked by the transection differed essentially from that observed in the normal avoiding reaction.

[1] The terms "promer" for the anterior fragment of the ciliate after the bisection, and "opimer" for the posterior one, were used in the paper of Golińska and Doroszewski 1964.

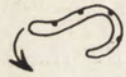
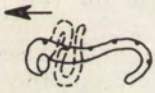
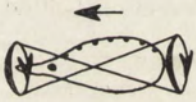
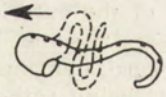
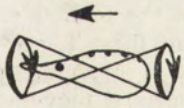
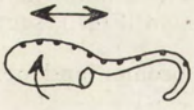
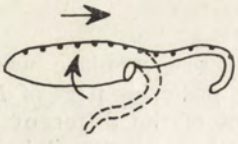
bisection	promer	reaction	opimer	reaction
1		−		−
2		−		−
3		−		−
4		−		−
5		+−		+−
6		+		+

Fig. 1. Scheme summarizing the different responses of the anterior and posterior fragments of *Dileptus cygnus* after bisection performed at different levels. Detailed explanation in the text

The duration of the reaction as well as the distance covered by animal during backward movement was remarkably longer due to its traumatic character. The nutational movements were of greater range and sometimes the characteristic lateral flections of the fragment could be clearly visible (Fig. 1, sect. 3 and Pl. I 4—5).

Transection in the middle part of the cell body

Promer

In general the behavior of the fragment was similar to that noticed in the case of the transection behind the cytostome (Fig. 1, sect. 4).

Opimer

The duration of the backward movement and the distance covered by the animal was longer than in the former case.

The lateral flections were less pronounced if the fragment was smaller; however the nutations still took place (Fig. 1, sect. 4).

Transection behind the middle part of the cell body

If the fragment was attached for a moment after the bisection to the substrate (glass plate) successive forward movement, not the reversion, could occur.

Promer

In contrast to the former cases the behavior of the anterior fragment was far from uniform (Fig. 1, sect. 5 and Pl. I 2—3). In some 42% of observed cases promer performed the reaction of backward movement but it could also start the forward movement, both reactions being rather weak.

Opimer

The reaction of posterior fragment was also not uniform i.e. forward or backward movement was observed after transection (Fig. 1, sect. 5).

In general, the four following reactions of promer and opimer were observed:

1. Promer starting forward, opimer backward.
2. Promer starting backward, opimer forward.
3. Promer and opimer starting forward.
4. Promer and opimer starting backward.

The performance of the transection in the proper place was sometimes difficult due to the lack of the markers in the posterior part of *Dileptus* and also to the varying dimensions and proportions of the different parts of the body J a n o v y 1962. The nuclear chain could be easy found in vivo but its position varied from one individual to another. It should be pointed out that not all transections were performed under the exact right angle to the body axis. With these restrictions the author could determine that the transition field was located behind the area of the greatest width of the cell between the third and fourth dorsal contractile vacuole.

Transection through the posterior part of the ciliate

Promer

In contrast to the former cases this kind of transection evoked the distinct reaction of starting forward movement. After transection the ciliate started the movement in every case. Then it can stop or continue swimming in the forward direction (Fig. 1, sect. 6 and Pl. I 1). This reaction proved similar to that which could be evoked by the water shock (Doroszewski 1963 a) and did not bear the sharply marked traumatic character as compared with the reaction in the case of transection in the middle part of the body.

Opimer

The fragment started and continued the forward motion. The reaction of starting the movement forward occurs regularly (Fig. 1, sect. 6). If the fragment contains no part of nuclear apparatus it becomes immobilized and dies in few hours. The successive reactions of such fragment were described elsewehre (Doroszewski 1963 c).

Repeated transections performed soon after the first bisection

The opimers produced by bisection behind the cytostome were sectioned once more through their posterior part. The bisection was performed while the fragment was in the state of the retrogressive motion after the first section. Both of the newly formed posterior fragments immediately started forward. The third consecutive cut caused once more the reversion. In this case the results were similar to whose obtained with *D. anser*.

The promers in the state of reversion of movement after the cut throughout the posterior part of the cell body were bisected once more near the previous location of the cut. The new fragments started forward movement.

The opimers moving backward after the cut performed behind the cytostome were bisected once more through their anterior part. The direction of the movement remained unchanged and the fragment continued its retrogressive motion.

The sections across the middle part of the proboscis were performed upon the promers moving backwards after the first transection. There was no change of the swimming direction. The reaction was evoked once more, when the cut across the proboscis was performed after the end of reversion of the movement.

The study of the reaction of individuals in the various stages of the cell division is now in progress.

Discussion

The promer of *D. cygnus* exhibits more clear and uniform reaction to transection than promer of *D. anser*. The reversion of movement occured in every case except when the cut was performed across the caudal region, and in this last case it did not occur at all. The active reaction of starting forwards of the posterior fragment in the response to transection is the most remarkable. Probably the same may occur in *D. anser* but actually it was not observed because of the greater mobility of this ciliate.

The previous experiments with puncture and the present observations of the behavior of cut fragments indicate than in *Dileptus* we have probably to do with two susceptible areas: one for the forward and one for the backward movement (Fig. 2). The bisection is a traumatic stimulus as well as the puncture. The intermediary region that is capable of both responses was larger in *Dileptus anser*. Also the backard movement of the anterior fragment after transection in *D. cygnus* occurs more regularly and lasts longer than

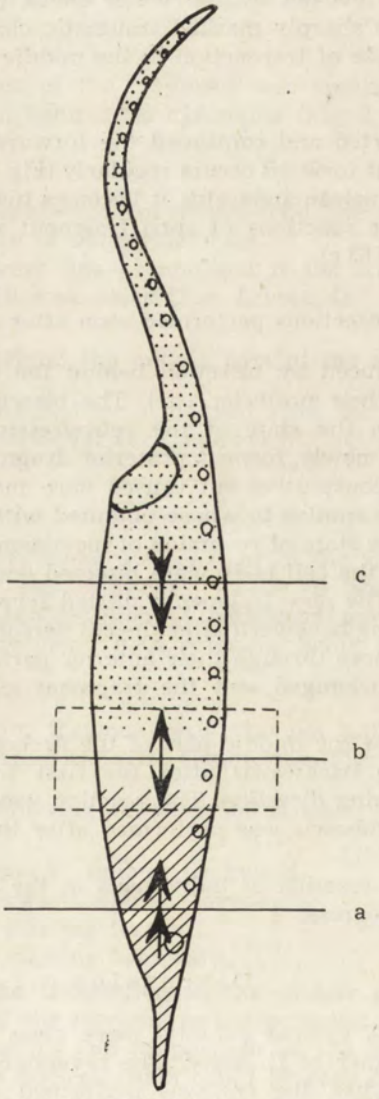


Fig. 2. Sensitive areas in *Dileptus cygnus*. The lines indicate one of the possible transections, the arrows — the direction of movement of the fragments produced (a — forward response area, b — intermediate region, c — backward response area)

in *D. anser*. In *D. cygnus* the reaction of swimming of both fragments in opposite directions was never observed, except in some cases when the transection was performed through the intermediary area. The border line between the backward and forward movement response area in D. cygnus is situated more anteriorly as it was previously stated by the author (Doroszewski 1963 c). The activity of these two areas in *D. cygnus* could be experimentally separated by bisection. Each fragment exhibited its own reactivity, if released from the dominance of the other. In the moving ciliate or in the fragment containing both areas the anterior one was the site of the active reception area which, if stimulated, can alter the direction of the whole ciliate. In the immobile ciliate the stimulus applied to the morphologically anterior area can evoke the backward movement, whereas the stimulation of the caudal one induces the forward movement.

The effect of repeated transections upon the fragments containing only one area indicate its stability, as in the case of reactions of *D. cygnus* to the puncture. In *D. anser* only the relatively large fragments were investigated containing perhaps both areas.

The described areas may correspond to the regions of the cell body in *Spirostomum* distinguished by Clark 1946 and Seravin 1962 as sensitive to the puncture. Among lower *Holotricha* the post-operational reversion of movement as a reaction to the transection was stated by Holmes 1907. Since the results described in the present study were obtained by a rather simple and not always accurate technique it would be perhaps too much to present now more precise theoretical conclusions. In general, however, they suggest the existence of clearly differentiated cytological areas of reactivity in the ciliate cell.

Summary

The immediate response to the bisection was studied in *Dileptus cygnus*, in both produced fragments. If the plane of the section was situated in the anterior part of the ciliate, both fragments reacted by backward movement accompanied by the rotation of the body around its long axis. In the case of a posterior transection, both fragments started the forward movement. Sections in the intermediate regions could avoke various responses, i.e. forward or backward movement in one or both fragments. Repeated transections caused the reversion of movement when applied to the anterior fragment, or the starting forward in the case of posterior one.

It is suggested that the forward and backward movement response areas can be identified with the areas described for the response to the puncture. Starting of forward movement in the posterior fragment of *D. cygnus* proved to be an active reaction.

STRESZCZENIE

Badano natychmiastową reakcję na przecięcie u obu fragmentów *Dileptus cygnus*. Jeśli płaszczyzna cięcia biegnie przez przednią część orzęska, oba fragmenty reagują ruchem wstecznym z rotacjami wokół długiej osi ciała. Przy przecięciu w tylnym rejonie obydwa fragmenty podejmują ruch postępowy. Cięcia w okolicy pośredniej

mogą wywoływać różne reakcje, tzn. zarówno ruch postępowy jak wsteczny w jednym lub w obydwóch fragmentach. Cięcia powtórne powodują odwrócenie kierunku ruchu, gdy dokonuje się ich na fragmentach przednich, albo wyzwalają ruch postępowy w przypadku fragmentów tylnych.

Wysunięto przypuszczenie, że okolice wyzwalające ruch postępowy lub wsteczny mogą być zidentyfikowane z analogicznymi okolicami opisanymi przy reakcji na ukłucie. Stwierdzono, że podejmowanie ruchu postępowego przez tylne fragmenty *D. cygnus* jest reakcją aktywną.

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EXPLANATION OF THE PLATE I

Living fragments of *Dileptus cygnus* after the bisection

1: *Dileptus* reacting by starting the forward movement after the transection throughout the caudal region. Note the typical position of proboscis accompanying this behaviour

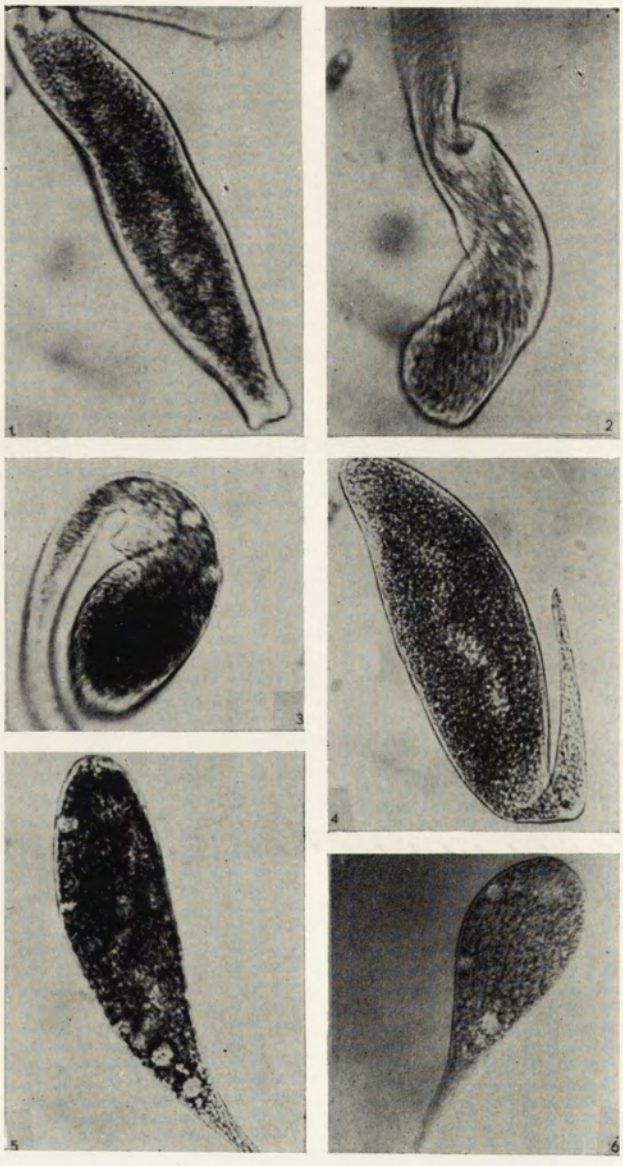
2: The anterior fragment after the bisection in the intermediate area. The proboscis in motion

3: The anterior fragment reacting with the reversion of movement

4: The posterior fragment deprived of the cytostomal area, reacting with the reversion of movement

5: The posterior half of the ciliate. This fragment reacted with reserved movement

6: The largest caudal fragment reacting by starting forward movement. Note the contractile vacuoles as the markers



M. Doroszewski auctor phot.

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Experimental studies on *Babesia divergens* in rhesus monkeys with special reference to its diagnosis by serological methods

Études expérimentales sur *Babesia divergens* chez les singes rhesus concernant en particulier la diagnose par des méthodes serologiques

Piroplasms are widely distributed in the Animal Kingdom, occurring in the blood of mammals, birds and reptiles, but are not normally found in man. In 1957, however, Skrabalo and Deanovíc reported a fatal case of human babesiasis. They found a massive infection of the peripheral red blood cells with *Babesia*, and the patient died after a few days of illness. Significantly, the patient had lost his spleen in a traffic accident several years earlier. Garnham and Bray 1959 then showed that splenectomized chimpanzees and rhesus monkeys were also susceptible to *Babesia divergens*, while intact primates fail to become infected. It may be noted that splenectomy of African monkeys frequently produces a relapse of natural infection of *Babesia pitheci* (Garnham 1950). The question arises whether primates, including man, have occult piroplasmosis, the result of being bitten by infected ticks, in nature. Such infections could be revealed by splenectomy, by inoculation of large quantities of blood into susceptible animals, or possibly by demonstrating fluorescent antibodies, and the present study was undertaken to ascertain whether a serological method was effective for the diagnosis of occult babesiasis in primates.

We are grateful to Dr. J. R. Baker and Mr. R. Killick-Kendrick and his assistants for their help in this work.

Methods and results

The strain of *Babesia divergens* was that described by Davis et al. 1958 and was kindly made available by Dr. L. P. Joyner of the Ministry of Agriculture and Fisheries. It was used in fresh blood or after storage at − 70°C, at which temperature it remained virulent for several months at least.

Experiment I

Three splenectomized rhesus monkeys, numbers 143, 253 and 262, were each given an intravenous inoculation of heparinized blood from a cow with 4% of its red blood cells infected with *Babesia divergens*. Daily thick and thin blood films were taken from the three monkeys, and were stained with Giemsa stain.

A standard of 50 oil immersion fields were examined on the thick film for parasites.

Three weeks later the monkeys were each challenged with 3.5 ml heparinized blood from a splenectomized monkey with 2.5% of its red blood cells infected with *B. divergens*. Thick films were again examined daily.

Serum was taken before infection, at the time of challenge, and after challenge.

Table 1

Splenectomized rhesus monkeys inoculated with *Babesia divergens*

Monkey:	143	253	262
Splenectomized	1 year earlier	2 years earlier	2 years earlier
Days after inoculation when *Babesia* first seen in thick films	6	7	9
Days of patency	6	6	5
Fluorescent Antibody Titres*			
a. before infection	— ve	— ve	— ve
b. 10 weeks after infection	50	25	200
c. 10 weeks after challenge	800	100	400

* F. A. Titres — reciprocal of serum dilution at the end-point.

Titrations were carried out on the sera by the indirect F. A. T. (Voller 1962, 1964) using acetone-fixed *B. divergens* as slide antigen. The slide antigen could be stored unfixed at − 70°C and did not deteriorate, even after storage for 9 months. Serial two-fold dilutions of test sera were made and the F. A. T. end point was the last dilution at which the parasites were clearly fluorescent. The results are given in Table 1. It can be seen that all the monkeys became infected on their first exposure to *B. divergens*. Parasitaemia was short, and was terminated by a crisis. On challenge,none of the monkeys developed a detectable parasitaemia. Relapses were not observed in any of the animals.

Before infection the sera gave a negative F.A.T. result, but after the infection all 3 were positive. The titres became considerably elevated after challenge.

Experiment II

A parallel series of monkeys with spleens, numbers 309, 310, 311 and 312, were each given an intravenous inoculation of 5 ml of heparinized blood from a calf with 4.1% of its red blood cells infected with *B. divergens*. Thick films were examined as in the first series.

After 24 days the spleen of monkey 309 was removed and thick films were examined for the next three weeks.

Twenty-two days after the splenectomy of monkey 309, i.e. 46 days after inoculation of monkeys 309, 310, 311 and 312 with *Babesia divergens*, all four monkeys were challenged with 3.0 ml heparinized blood (stored at −70°C

for 3 months) from a monkey with a 4.1% parasitaemia of *B. divergens*. Daily thick films were again examined.

Sera were taken from all there monkeys before infection, at the time of splenectomy of monkey 309, and three weeks after splenectomy, and 3 weeks after the challenge of all four monkeys. The results are given in Table 2.

Table 2

Intact rhesus monkeys inoculated with *Babesia divergens*

Monkey:	309	310	311	312
1st inoculation. All monkeys intact. Examination of thick film, 21 days	— ve	— ve	— ve	— ve
Splenectomy day after inoculation	22th	—	—	—
Examination of thick film, 21 days	— ve	— ve	— ve	— ve
Challenge with *B. divergens*	46	46	46	46
Days after challenge when *Babesia* first seen in thick films	9	—	—	—
Period of patency in days	16	—	—	—
Fluorescent Antibody Titres				
a. before inoculation	— ve	— ve	— ve	— ve
b. 3 weeks after inoculation	— ve	— ve	— ve	— ve
c. 6 weeks „ „	— ve	— ve	— ve	— ve
d. 9 weeks (3 weeks after challenge)	50	— ve	— ve	— ve

It can be seen that none of the intact rhesus monkeys developed *B. divergens* infections. Monkey 309 did not show any parasites in the peripheral blood, even after splenectomy, but it did become positive 9 days after challenge with *B. divergens*. The other 3 monkeys remained negative on challenge.

Antibody to *Babesia* was not detected in any of the monkeys after the initial inoculation of *B. divergens*, nor in monkey 309 after splenectomy. However, after challenge monkey 309 developed detectable antibody, whereas monkeys 310, 311 and 312 did not.

Discussion

The results given here confirm that the splenectomized rhesus monkey is susceptible to *Babesia divergens*, the causative agent of red-water fever in cattle in the United Kingdom. This is, perhaps, a little surprising as Enigk und Friedhoff 1962 found that splenectomized goats and sheep were not susceptible to this parasite, although splenectomized moufflon, red deer, fallow deer and roe deer were. It is of special interest to note that primates

are rendered susceptible even though the splenectomy is performed many years before exposure to infection; thus the case of human piroplasmosis described by Skrabalo and Deanovíc 1957 involved a man who lost his spleen over 10 years earlier, while one of the chimpanzees, infected by Garnham and Bray 1959, had been splenectomized 2½ years before infection; in the present series of experiments the rhesus monkeys had been splenectomized between 1 and 2 years earlier. It is particulary surprising that such an injury to the reticulo-endothelial system is not repaired over the long periods involved.

The present results suggest that the rhesus monkey with a spleen does not develop an occult infection which can be rendered patent by splenectomy. There is also evidence that inoculation of the intact animal with quite large amounts of parasitic material does not confer protection when it is challenged after a subsequent splenectomy, nor does it produce antibodies detectable by F. A. T. This latter finding does not necessarily mean that the parasitic material is non-antigenic, but may reflect the intracellular location of the parasite. Under these conditions it may not be able to stimulate the antibody production mechanism. It must be noted that Ingram et al. 1962 failed to elicit antibodies to malaria parasites by inoculation of infected blood into rabbits, although Zuckerman 1964 has clearly shown that fractions of the released parasites are active in this respect.

The results given in Tables 1 and 2 indicate that the fluorescent antibody test can be used as an easy serological method to give a quantitative estimation of antibodies to *Babesia*. Ristic et al. 1964 have used immunofluorescent techniques in a non-quantitative manner in the study of the morphology of piroplasmosis. The possibility that occult infections in man might be detectable by this test is highly unlikely in view of the consistently negative results we obtained on the sera from the intact monkeys.

Our results indicate that rhesus monkeys without spleens produce antibodies to *B. divergens* and that these are considerably elevated on challenge with the homologous organism. After a single infection with *B. divergens*, all our splenectomized rhesus monkeys were found to be immune to reinfection. The presence of the antibodies in high titre, and the typical crisis, would indicate that a humoral factor is involved in the subsequent immunity of infected splenectomized primates.

Summary

Splenectomized rhesus monkeys were infected with *Babesia divergens*. All developed patent parasitaemia and *Babesia* antibody detectable by immunofluorescence. On subsequent challenges, all the monkeys were refractory, but the antibody levels rose.

Intact rhesus monkeys did not show patent parasitaemia after injection of *B. divergens*. Antibodies were not detectable by immunofluorescence. Splenectomy of one monkey did not result in a patent parasitaemia. On challenge with. *B. divergens*, only the splenectomized monkey became infected, and this was the only one of this group to develop detectable antibodies.

The presence of high titre antibodies and the typical crisis would indicate that a humoral factor is involved in the subsequent immunity of infected splenectomized primates.

The detection of occult infections in man by immunofluorescence is unlikely in view of the negative results obtained here on sera of intact monkeys challenged with *B. divergens*.

RÉSUMÉ

Des singes rhesus splénectomisés ont été infectés de *Babesia divergens*. Tous ont contracté un parasitime et des anticorps *Babesia* lesquels se montraient par l'immunofluorescence. Après de subséquentes réinfections tous les singes se trouvaient réfractaires, mais les niveaux d'anticorps sont montés.

Des singes rhesus intacts n'ont montré aucun parasitime après l'inoculation de *B. divergens*. Des anticorps ne se montraient pas par l'immunofluorescence. A la suite, un de ces singes a été splénectomisé, mais aucun parasitime n'a résulté. Après la réinoculation de *B. divergens*, le signe splénectomisé était le seul de s'infecter, et le seul du groupe de développer des anticorps.

La présence d'anticorps d'un titre élevé et de la crise typique implique un agent humoral dans l'immunité subséquent chez les primats infectés et splénectomisés.

En vue des résultats négatifs obtenus sur des sérums des singes intacts, inoculés de *B. divergens*, il est peu probable que l'immunofluorescence montrerait des infections occultes chez l'homme.

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Taxocénoses des *Testacea* de quelques petits bassins de terrains inondables de la Narew

Taksocenozy *Testacea* kilku drobnych zbiorników wodnych z terenów zalewowych Narwi

En 1961—1962 des recherches furent entreprises concernant quelques petits bassins situés à l'embouchure du Bug dans la Narew. Ces bassins se trouvaient alors dans la vallée des deux rivières, mais après l'établissement du barrage de Dębno, ils furent inondés par le nouveau lac artificiel de Zegrzynek.

Le but du travail était l'examen de la faune des *Testacea* de ces quelques petits bassins qui furent la base de la faune qui peupla le nouveau lac. En outre on observa les groupements des *Testacea* des petits bassins et leur dynamisme durant ces deux années. Ce travail est la continuation des travaux entrepris par l'auteur en 1957 concernant la taxocénose des *Testacea* de différents biotopes. Il semble que la connaissance en détails et la classification de ce genre de groupements des *Testacea* n'est pas dénuée d'intérêt pour l'écologie des protozoaires et d'autres microorganismes qui constituent un des principaux maillons de la chaîne alimentaire des biocénoses aquatiques. Les observations écologiques de ce grupe sont signalées de plus en plus fréquemment par la littérature mondiale. Des recherches très intéressantes sur les taxocénoses des *Testacea* furent accomplies par Schönborn 1962 a qui développa ce sujet sur la base des matériaux recueillis sur du sphagnum (Schönborn 1962 b). Tout comme auparavant un grand intérêt éveilla la "Rhisopodenanalyse" des tourbières dont les bases furent forgées par Harnisch 1924, les recherches contemporaines sont si promettantes pour la faune des *Testacea* des sols. Bonnet 1961 et Chardez 1960—1961 publièrent dernièrement des travaux écologiques extrêmement intéressants concernant les problèmes de population chez les *Testacea* des sols.

L'écologie et surtout la microdistribution des *Testacea* est l'objet des travaux de Heal 1962.

En outre des travaux concernant l'écologie des *Testacea* beaucoup de travaux sont devoués à des recherches faunistiques ou taxonomiques ce qui contribue à la connaissance de plus en plus détaillée de la distribution des espèces.

Des travaux concernant la biologie de différentes espèces et surtout leur physiologie seraient de grande aide dans la connaissance des relations entre les populations ainsi que de leur structure. Malheureusement les travaux de ce genre sont très rares.

Méthode

Les échantillons étaient prélevés et analysés d'après la méthode décrite auparavant (Moraczewski 1962) avec cette différence que la gaze de moulin No 18 fut remplacée par la gaze No 25, plus dense. L'abondance des différentes espèces dans les échantillons fut déterminée d'après l'échelle suivante:

Classe de l'abondance (A)	Quantité d'individus (q = 2)
+	1 (a)
1	2—3 (aq)
2	4—7 (aq^2)
3	8—15 (aq^3)
4	16—31 (aq^4)
5	32—63 (aq^5)

La constance de l'espèce à l'emplacement donné durant l'année fut également estimée d'après l'échelle:

Classe de constance (K)	Présence de l'espèce dans les échantillons (en %)
I	0—20
II	20.1—40
III	40.1—60
IV	60.1—80
V	80.1—100

Description du terrain

Les échantillons étaient prélevés de 5 petits bassins emplacés dans la vallée de la Narew, dans ses terrains inondables. La vallée de la Narew possédait en cet endroit 3 km de large. La rive droite, le long de laquelle la riviére coule, est haute, et se dessine de façon distincte. La rive gauche de la vallée ne s'élève que très doucement. Le fond de la vallée est formé avant tout de sable at de remblai d'accumulation fluviale. Sur les terrains des vieux lits et des lacs de la vallée se trouvent d'assez importants gisements de tourbe.

Tous les bassins étudiés étaient inondés par la rivière durant ses débordements jusqu'à 2 m. de profondeur. Ces bassins d'après la classification de Žadin 1950 appartenaient au vieux lit du Narew. Le terrain où se trouvaient les bassins étaient couvert de près et de bois clairsemés.

Les bassins situés dans les environs du village Białobrzegi furent désigné de la lettre B:

B_0 — étang de 3 m. de profondeur avec une zone littorale développée, couverte d'une zone étroite de *Acorus* et de *Phragmites*. Les échantillons furent prélevés parmi les plantes du littoral (profondeur 10—20 cm.) ainsi que du fond (50—70 cm.).

B_1 et B_2 — bassins éphémères de près, de 30 cm. de profondeur, disparaissant au début de mai. Le fond des bassins était couvert en abondance d'herbe, d'*Equisetum* et de feuilles mortes.

Les bassins B_3, B_4 et B_5 situés près l'un de l'autre et joints par un fossé

formaient un seul groupe, malgré certaines différences morphologiques et certaines divergences dans leur flore de macrophytes.

B_3 — bassin assez grand et marécageux, abondant en plantes aquatiques et surtout en *Isoetes* sp. ses rives étaient couvertes de *Acorus calamus, Phragmites communis, Typha latifolia, Iris pseudoacorus. Hydrocharis morsus ranae* et *Lemna trisulca* s'y trouvaient en grande quantité. Le fond était couvert de vase mou sentant nettement l'acide sulfhydrique. Le bassin était entouré d'un marécage et couvert d'herbes.

B_4 — 2 m.^{2} de surface, 3 m. de profondeur, ses bords d'argile étaient donc assez abrupts. Des petites parties de ses bords étaient couvertes de *Elodea canadensis*. L'eau étaient trouble et de couleur d'argile.

B_5 — bassin à la forme de rein, de 2 m. de profondeur. L'eau de ce bassin était claire jusqu'au fond, couverte d'une façon dense d'*Elodea canadensis*. Des touffes de *Typha* ou de *Phragmites* assez espacées poussaient sur ses bords.

Aux environs du village Wierzbica, en amont de l'embouchure du Bug, il y avait un bassin dénommée. W. Il se trouvait dans un grand pré, pas loin de la rive haute de la vallée. C'était un étang en partie couvert de végétation et passant d'un côté en marécage. Les échantillons étaient prélevés du côté du marécage, dans les touffes épaisses d'*Acorus calamus* et d'une touffe bien distincte d'*Equisetum palustre* qui occupait la partie centrale du bassin. La profondeur à l'endroit du prélevement était égale à 50 cm.

Le bassin désigné de la lettre D se trouvait près du village Dzierżenin. C'était une branche de rivière peu profonde (50 cm.), située dans un pré humide et reliée constamment à la rivière.

Caractérisation écologique des espèces nouvelles pour la Pologne

Dans les bassins étudiés on trouva 77 espèces et 35 variétés des *Testacea* dont 35 espèces et 21 variétés nouvelles pour la faune de la Pologne. Leur liste est fournie par la Table 1.

La description des espèces nouvelles pour la Pologne se base sur les données de la littérature et les propres observations. Les noms des auteurs sont cités en abréviation: Awer: =Awerintzew, Bart. =Bartoš, Bon. =Bonnet, Chard. =Chardez, Decl. =Decloître, Def. =Deflandre, Fran. =Franken, Gol. =Golemansky, Gros. =Grospietsch, Kour. =Kourov, Marg. =Margalef, Opr. =Opravilova, Pen. =Pénard, Schön. =Schönborn, Štěp. =Štěpánek, Thom. =Thomas.

Cochliopodium actinoporum Auerbach

C'est une espèce assez rare parmi les plantes aquatiques et le sapropèle.

Microchlamys patella Clap. et Lach.

La présence de cette espèce dans les petites bassins fut déjà constatée (Pen. 1902, Cash 1905, Def. 1927), dans les lacs (Aver. 1906) ainsi que dans les mousses forestières aériennes ou subaériennes (Thom. 1961, Schön. 1962 b). Dans les lacs ainsi que dans les petits bassins on le trouve assez fréquemment dans le sapropèle (Bart. 1954) et sur les plantes (Cosh 1905, Pen. 1902).

Table 1

Index des espèces trouvées dans les bassins étudiés

Testacealobosa (Eulobosa)

Cochliopodiidae

* *Cochliopodium actinophorum* (Auerbach) Penard
Cochliopodium bilimbosum (Auerbach) Leidy
* *Microchlamys patella* Clap. et Lach.
* *Pyxidicula petens* Clap. et Lach

Arcellidae

* *Arcella catinus* Penard
Arcella conica (Play.) Deflandre
Arcella discoides Ehrenberg
* *Arcella discoides* var. *pseudovulgaris* Deflandre
Arcella discoides var. *scutelliformis* Playfair
Arcella gibbosa Penard
Arcella gibbosa var. *loevis* Deflandre
Arcella gibbosa var. *mitriformis* Deflandre
Arcella haemisphaerica Perty
* *Arcella haemisphaerica* var. *intermedia* Deflandre
Arcella haemisphaerica var. *undulata* Deflandre
Arcella megastoma Penard
Arcella rotundata var. *alta* Playfair
Arcella rotundata var. *aplanata* Deflandre
* *Arcella rotundata* var. *stenostoma* Deflandre
Arcella vulgaris Ehrenberg
Arcella vulgaris var. *undulata* Deflandre
Arcella vulgaris var. *penardi* Deflandre

Centropyxidae

Centropyxis aculeata Ehrenberg
Centropyxis aculeata var. *grandis* Deflandre
Centropyxis aculeata var. *oblonga* Deflandre
* *Centropyxis cassis* Wallich
* *Centropyxis cassis* var. *spinifera* Playfair
Centropyxis constricta Ehrenberg
Centropyxis discoides (Penard) Deflandre
* *Centropyxis delicatula* Penard
Centropyxis ecornis Leidy
* *Centropyxis marsupiformis* Wallich
Centropyxis minuta Deflandre
* *Centropyxis orbicularis* Deflandre
* *Centropyxis platystoma* (Penard) Deflandre
* *Centropyxis platystoma* var. *armata* Penard
Cyclopyxis arcelloides Penard
* *Cyclopyxis eurystoma* Deflandre
* *Cyclopyxis kahli* Deflandre
* *Trigonopyxis arcula* (Leidy) Penard
* *Plagiopyxis callida* Penard
* *Plagiopyxis labiata* Penard

Difflugiidae

Difflugia acuminata Ehrenberg
* *Difflugia acuminata* var. *acaulis* Perty
* *Difflugia acuminata* var. *curvata* Cash
* *Difflugia acuminata* var. *inflata* Penard
* *Difflugia acuminata* var. *inflata* f. *bicornis* Gauthier
* *Difflugia acuminata* var. *laevanderi* Playfair
* *Difflugia acuminata* var. *umbilicata* Penard
Difflugia amphora Leidy
* *Difflugia avelana* Penard
* *Difflugia bidens* Penard
Difflugia corona Wallich
Difflugia elegans Penard
Difflugia fallax Penard
Difflugia globulosa Dujardin
* *Diffugia gramen* Penard
* *Difflugia gramen* var. *globularis* Štěpánek
Difflugia hydrostatica var. *lithophila* Penard
Difflugia lanceolata Penard
Difflugia lebes Penard
Difflugia leidyi Wailes
Difflugia lobostoma Leidy
Difflugia mica Frenzel
Difflugia oblonga Ehrenberg
Difflugia oblonga var. *anguisticolis* Štěpánek
Difflugia oblonga var. *brevicolis* Gassowski
* *Difflugia oblonga* var. *caudata* Štěpánek
* *Difflugia oblonga* var. *cilindricus* Thomas
* *Difflugia oblonga* var. *claviformis* Penard
* *Difflugia oblonga* var. *kempnei* Štěpánek

Difflugia oblonga var. *lacustris* Penard
Difflugia oblonga var. *longicolis* Gassowski
* *Difflugia oblonga* var. *magna* Thomas
* *Difflugia oblonga* var. *manicata* Penard
* *Difflugia oblonga* var. *parva* Thomas
* *Difflugia oblonga* var. *schizocaulis* Štěpánek
* *Difflugia olliformis* Lagerheim
* *Difflugia oviformis* Cash
* *Difflugia penardi* (Penard) Hopkinson
Difflugia pristis Penard
Difflugia scalpellum Penard
Difflugia similion Thomas
Difflugia tuberculata Wallich
Difflugia tuberculata var. *loevis* Penard
Difflugia urceolata Carter
* *Pontigulasia bigibbosa* Penard
* *Pontigulasia incisa* Rhumbler
* *Pontigulasia spectabilis* Penard
* *Cucurbitella mespiliformis* Penard

Nebelidae

* *Lesquereusia modesta* Rhumbler
Lesquereusia spiralis Ehrenberg

Testacealobosa (Reticulobosa)

Cryptodifflugiidae

Phryganella nidulus Penard
* *Cryptodifflugia compressa* Penard
* *Wailesella eboracensis* Weiles

Testaceafilosa

Euglyphidae

Euglypha acantophora (Ehrenberg) Perty
Euglypha ciliata (Ehrenberg) Leidy
Euglypha cristata Leidy
Euglypha loevis (Ehrenberg) Perty
* *Euglypha scutigera*
* *Euglypha tuberculata* Dujardin
Pareuglypha reticulata Penard
* *Placocysta lens* Penard
* *Trinema enchelys* (Ehrenberg) Leidy
* *Trinema enchelys* var. *galeata* Penard
Trinema lineare Penard
Corythion dubium Taránek
* *Corythion pulchellum* Penard

Cyphoderiidae

Cyphoderia ampula (Ehrenberg) Leidy
* *Cyphoderia loevis* Penard
Cyphoderia trochus Penard

Gromiidae

Pseudodifflugia gracilis Schlumberger

* Le signe indique les espèces nouvelles pour la Pologne

C'est une espèce aquatique, assez répandue, liée aux milieux marécageux. Je l'ai trouvée dans le petit bassin éphémère B_1.

Pyxidicula petens Claparède

Espèce constatée avant tout dans le sapropèle sur les restes végétaux en putréfaction. Elle était très rare, je ne l'ai vue qu'au fond d'un seul bassin.

Arcella catinus Penard

Espèce répandue dans différents types des bassins: tourbières Def. 1927, Bart. 1949 Ertl 1955, Opr. 1960), petites bassins (Štěp. 1963 a), mousses (Štěp. 1959, 1963 a, Decl. 1962 b) et les lacs artificiels (Štěp. 1959). Cette espèce se trouve très souvent sur les plantes aquatiques comme les mousses aquatiques et les *Sphagnum* (Grosp. 1958). Mais sa présence est la plus fréquente parmi les mousses humides au bord des rivières (Bart 1949, Grosp. 1958). Elle se trouve également sur les mousses subaériennes (Ertl 1955, Def. 1927).

Cette espèce est donc assez commune vivant le plus souvant dans les mousses humides des tourbières ou aux alentours des bassins. Dans les autres sa présence est plutôt fortuite.

Arcella discoides var. *pseudovulgaris* Deflandre

Espèce observée dans les petits bassins et sur les mousses (Štěp. 1963 a) les tourbières (Ertl 1955, Opr. 1960), sur les plantes aquatiques (Chard. 1961 a). C'est une espèce assez rare, liée à la végétation. Elle ne se trouvait que dans les bassins riches en végétation.

Arcella haemisphaerica var. *intermedia* Deflandre

Cette variété fut constatée dans un lac eutrophique (Oye 1937), une rivière (Štěp. 1963 a), un petit bassin (Štěp. 1963 a), une tourbière (Grosp. 1958, Opr. 1960) — dans le sapropèle tout comme sur les plantes.

Elle est très probablement un eurytope associé le plus fort aux milieux eutrophiques. Cette variété fut trouvée dans un bassin fortement eutrophique.

Arcella rotundata var. *stenostoma* Deflandre

Cette variété est assez rare (Def. 1928) parmi les plantes aquatiques et le sapropèle des petits bassins (Chard. 1964), dans les rivères (Štěp. 1953 a) et la littoral (Chard. 1964). C'est très probablement une espèce aquatique.

Centropyxis cassis (Wallich) Deflandre

Espèce habitant les tourbières (Bart. 1949, Ertl 1955, Opr. 1960), les ruisseaux et les rivières (Decl. 1958 a) et les petits bassins (Decl. 1958 a, Štěp. 1959, Chard. 1961 a), sur les mousses immergées.

Cette espèce peut être sans aucun doute considérée comme moussophyle, habitant les mousses à humidité constante. Dans les petits bassins étudiés elle était assez fréquente.

Centropyxis cassis var. *spinifera* Playfair

Variété assez rare, présente dans les tourbières et les marécages (Bart. 1954, Decl. 1959). La variété a probablement les mêmes besoins écologiques que la forme type.

Centropyxis delicatula Penard

C'est une espèce très rare. Deflandre 1929 l'a observée dans un petit bassin, Chard. 1964 dans le littoral d'un lac du Congo. Dans les petits bassins elle ne se trouve que parmis les plantes aquatiques.

Centropyxis marsupiformis Wallich

Espèce observée dans les étangs (Pen. 1902, Štěp. 1963 a), les rivières (Bart. 1954, Štěp. 1963 a) et mousses forestières (Štěp. 1959).

Espèce aquatique, liée probablement au fond des bassins. Dans les bassins étudiés elle était très rare.

Centropyxis minuta Deflandre

Espèce présente dans les étangs et les petits bassins (Oye 1938, Štěp. 1963 a), les rivières (Bart. 1954, Štěp. 1963 a), dans les embouchures salines des riviéres (Chard. 1961 b) ainsi que dans les mousses aussi bien sèches qu'humides (Gol. 1963, Štěp. 1959).

On l'a trouvé fréquemment dans différents genres du sol. (Chard. 1960 a, 1962).

On voit donc que l'espèce en question appartient aux espèces eurytopiques.

Centropyxis orbicularis Deflandre

C'est également une espèce eurytopique comme le démontrent ses habitats: tourbières, puits, étangs, rivières, différents genres des mousses et des sols.

Centropyxis platystoma (Penard) Deflandre

C'est une espèce très fréquente dans différents genres de mousses, des mousses très humides jusqu'aux mousses aériennes (Bart. 1947, 1950, Chard. 1961 a, Štěp. 1963 a) mais présente également dans les rivières et les étangs (Štěp. 1963 a), dans le sol (Chard. 1960 a).

Centropyxis platystoma est une espèce probablement ubiquiste, vivant avant tout dans les mousses.

Centropyxis platystoma var. *armata* Deflandre

C'est une forme assez rare, habitant avant tout les mousses des tourbières.

Cyclopyxis eurystoma Deflandre

Cette espèce se trouve dans les tourbières (Opr. 1960, Ertl 1955), le sol (Chard. 1961 a, Schön. 1962 b) et dans différents genres de mousses (Gol. 1963, Bart 1947), elle fut également constatée dans une rivière et un étang à un pH égal à 5.5—6.0 (Štěp. 1963 a) ainsi que dans le littoral d'un lac oligotrophique (Schön. 1962 a). C'est une espèce ubiquiste.

Cyclopyxis kahli Defrandre

Cette espèce fut trouvée avant tout sur des mousses aériennes (Bart. rochers (Cach 1909, Štěp 1959, Grosp. 1958); cette espèce fut également dans une rivière et un étang (Štěp. 1963 a) et dans les sédiments de lacs oligotrophiques (Schön. 1962 a). C'est donc une espèce moussophyle mais capable de s'adapter également à d'autres milieux.

Trigonopyxis arcula (Leidy) Volz

Cette espèce fut notée le plus souvent dans les mousses humides poussant sur les arbres ou les rochers (Cash, 1908, Def. 1927, Bart. 1949, Chard. 1961 c, Gol. 1963) ou dans le sol (Bart. 1954, Chard. 1960 a, 1962, Schön. 1962 b); bien plus rare dans les lacs ou les petits bassins ou elle se trouvait alors au fond (Kour. 1925). Il semble que l'espèce est typique pour la faune des mousses surtout des tourbières et des marécages et qu'elle se trouve également assez souvent dans le sol riche en humus.

Plagiopyxis callida Penard

P. callida, tout comme tous les représentants de ce genre est un représentant typique de la faune edafique (Chard. 1960 a, 1961 a, 1962, Bon. 1961, Schön. 1962 b) et se trouve dans les mousses poussant sur les arbres et les rochers (Cash 1909, Štěp. Grosp. 1958); cette espèce fut également trouvée dans les rivières (Bart. 1954, Štěp. 1963 a) et les petits bassins (Chard. 1961 a).

C'est le représentant d'un groupe assez nombreux d'espèces des *Testacea*, carastéristiques pour les sols et les mousses humides, souvent notées dans les bassins vaseux.

Difflugia acuminata Ehrenberg

Toutes les variétés de l'espèce *Difflugia acuminata* (var. *acaulis* Perty, var. *curvata* Cash, var. *inflata* Penard, var. *inflata* f. *bicornis* Gaut. Thom., var. *laevanderi* Playfair, var. *umbilicata* Penard) vivent, d'après Chardez 1961 a, dans des miliaux identiques, notamment: parmi les plantes aquatiques, les tapis de *Lemna*, les mousses et *Sphagnum* des tourbières. Variétés nommées ci-dessus étaient assez rares.

Difflugia avelana Penard

Espèce vivant dans les petits bassins (Pen. 1962, Bart. 1954), dans les lacs oligo — trophiques, les près immergés et les sédiments (Schön. 1962 a), ainsi que dans les mousses humides (Gol. 1963).

C'est probablement une espèce typique du fond des petits bassins, habitant également assez souvent le littoral des lacs; dans les mousses elle est assez rare.

Difflugia bidens Penard

Cette espèce est très rare. Elle fut trouvée dans le sapropèle des bassins.

Difflugia gramen Penard

C'est une espèce très commune, vivant avant tout dans le sapropèle des bassins (Cash 1909, Thom. 1954, Bart. 1954, Štěp. 1959, Chard. 1961 a) et sur les plantes aquatiques (Def. 1927, Gros. 1958).

Difflugia gramen est une espèce typique pour les petits bassins.

Difflugia gramen var. *globulosa* Štěpanek

Cette variété a été décrite assez récémment sans données précises concernant l'écologie. En dehors du travail de Štěp. and Jelinek 1958 je n'ai vu aucun travail à ce sujet. Dans les petits bassins étudiés cette variété se trouvait en général au fond.

Difflugia oblonga Ehrbg.

Toutes les variétes appartenant à cette espèce (var. *caudata* Štěp., var. *cylindricus* Thom., var. *claviformis* Pen., var. *kempnei* Štěp., var. *magna* Thom., var. *manicata* Pen., var. *parva* Thom., var. *schizocaulis* Štěp.) habitent le plus souvent le sapropèle des petits bassins et du littoral des lacs; dans le profondal elles sont plus rares.

Difflugia olliformis Penard

Cette espèce fut trouvée dans les petits bassins (Thom. 1954, 1961, Decl. 1958 a), sur les plantes aquatiques et plus rarement dans le sapropèle. Cette espèce assez rare appartient à la faune des petits bassins. Elle ne fut notée que dans un petit bassin sur des plantes aquatiques.

Difflugia penardi (Penard) Hopkinson

Espèce vivant avant tout dans les petits bassins. Elle fut trouvée dans un petit bassin parmi l'*Equisetum*.

Difflugia pristis Penard

Espèce notée parmi les plantes aquatiques (C o s h 1909) dans le plankton (O y e 1937) et dans les marécages (H e a l 1961).

C'est probablement une espèce des petits bassins. Elle fut trouvée au fond de deux petits bassins.

Difflugia similion Thomas

Cette espèce fut trouvée assez récemment dans le sapropèle du fond des bassins et dans des feuilles mortes (T h o m. 1954, 1961, C h a r d. 1964).

Comme cette espèce est récente, son écologie est peu connue. Elle fut trouvée dans le sapropèle des bassins étudiés.

Pontigulasia bigibbosa Penard

C'est une espèce du profondal et du littoral des lacs (P e n. 1902, S c h ö n. 1962 a), des rivières (Š t ě p. 1963 a), des tourbières (O p r. 1960); on la trouve sur des plantes aquatiques, dans le sapropèle, sur des mousses humides (Š t ě p. 1963 b). C'est une espèce ubijuiste aquatique. Dans les bassins étudiés elle était assez fréquente.

Pontigulasia incisa Rhumbler

Cette espèce se trouve dans les petits bassins (P e n. 1902, K o u r. 1925, C h a r d. 1961 a), dans le littoral de lacs (K o u r. 1925), dans les tourbières (C a s h 1909), dans le sapropèle et sur les plantes aquatiques. Cette espèce appartient à la faune des petits bassins. Elle fut trouvée seulement dans un seul bassin parmi les plantes aquatiques.

Pontigulasia spectabilis Penard

Cette espèce fut notée dans des tourbières (B a r t. 1949, 1954), des petits bassins (T h o m. 1955), des rivières (Š t ě p. 1953 a) et surtout dans le sapropèle (Š t ě p. 1952). C'est une espèce vivant au fond des bassins. Elle fut trouvée dans quelques petits bassins.

Cucurbitella mespiliformis Penard

Cette espèce fut notée dans de petits bassins (P e n. 1902, A v e r. 1906) C a s h 1909, D e f. 1927, K o u r. 1925, T h o m. 1954), dans le sapropèle (C a s h 1909, B a r t. 1954) ainsi que sur des plantes aquatiques (D e f. 1927, B a r t. 1954, T h o m. 1954). C'est une espèce typique pour les petits bassins, trouvée fréquemment dans les bassins étudiés.

Lesquereusia modesta R h u m b l e r

C'est une espèce très répandue habitant les petits bassins (P e n. 1902, A v e r. 1906, C a s h 1909, F r a n. 1933, Š t ě p. 1952, M a r g. 1955) et les tourbières (E r t l 1955).

En accord avec la monographie du genre *Lesquereusia*, de T h o m a s et G a u t h i e r - L i è v r e 1959, on peut conclure que cette espèce est typique pour les groupements de microbentos des petits bassins et en particulier des mares tourbeuses et sphaignes. Elle fut trouvée parmi l'*Equisetum* aquatique.

Cryptodifflugia compressa Penard

Cette espèce fut observée dans des petites bassins (Pen. 1902, Cash 1909, Gol. 1963) et dans le littoral d'un lac oligotropique (Schön. 1962 a), en faveur parmi la végétation aquatique.

Wailesella eboracensis Wailes

On remarque cette espèce le plus souvent dans les tourbières (Def. 1928, Ertl 1955, Thom. 1955, Heal 1961), parmi le sphagnum et les mousses humides.

L'espèce est typique pour le sphagnum quoique dans les bassins étudiés elle fut constantée dans les milieux assez variés.

Euglypha cristata Leidy

E. cristata fut notée dans des tourbières et des marécages sur des mousses humides (Pen. 1902, Kour. 1925, Def. 1927, Gros. 1958, Decl. 1959, 1962 a), ainsi que dans un bassin artificiel et dans une rivière (Štěp. 1959, 1963 a).

C'est une espèce moussophyle. Elle ne fut trouvée que dans un bassin.

Euglypha scutigera

D'après Decloître 1962 a cette espèce vit dans le sphagnum, les plantes immergées et les mousses aériennes. Sa présence ne fut constantée que dans un seul bassin.

Euglypha tuberculata Dujardin

Elle fut observée dans des tourbières (Decl. 1962 a, Ertl 1955), des petits bassins (Decl. 1962 a, Thom. 1954, Decl. 1959, Chard. 1961 a), dans des mousses aériennes (Thom. 1954, Decl. 1962 a, Gol. 1963), dans le sol (Decl. 1962 a).

C'est probablement un eurytope. L'espèce était assez fréquente dans les bassins étudiès.

Placocysta lens Pénard

Espèce notée dans le profondal des lacs (Pen. 1902, Cash 1919), une rivière (Štěp. 1963 a), un petit bassin (Decl. 1958 a) avant tout au fond.

Les informations peu abondantes concernant l'espèce en question permettent de la considérer en tant qu'espèce habitant les lacs ou les petits bassins, en tout cas leur sapropèle. On l'a trouva dans un seul bassin.

Trinema enchelys (Ehrenberg) Leidy

Espèce très répandue, cosmopolite et ubiquiste.

Trinema enchelys var. *galeata* Penard

Cette variété assez rare a été notée dans les mousses humides et aériennes (Cash 1909, Def. 1927, Thom. 1954).

C'est une forme probablement moussophyle. Elle ne fut trouvée que dans un bassin.

Corynthion pulchellum Penard

Espèce notée dans les mousses et le sphagnum (Pen. 1902, Cash 1909, Opr. 1960, Chard. 1961 a, 1964, Thom. 1954). Elle est typique pour la

faune des mousses, présenté de façon fortuite dans des petits bassins. Constatée ici dans des mousses humides bordant les petits bassins.

Cyphoderia loevis Penard

Espèce trouvée dans les lacs dans le profondal tout comme dans le littoral (Pen. 1902, Schön. 1962 a), dans des rivières et des étangs (Štěp. 1963 a). Elle appartient à la faune des grands basisns.

Pseudodifflugia gracilis Schlumberger

L'espèce habite les petits bassin (Thom. 1955, Štěp. 1955), les sources (Grosp. 1958) et les tourbières (Bart. 1954, Gol. 1963) où elle se tient sur des plantes aquatiques.

C'est une forme du périphyton, vivant surtout dans les petits bassins. On ne la trouva, que dans un bassin à végétation abondante.

Taxocénoses

Le terme taxocénose, employé déjà dans un travail précedant (Moraczewski 1962), est une légère modification du terme taxocène introduit par Chodorowski 1960.

Dans sa description d'un groupement des *Testacea* d'un lac oligotrophique Schönborn 1962 a emploie le mot „die Taxozönose", se basant de même sur la définition de Chodorowski. Il semble qu'il serait plus correct d'employer au lieu de taxocène le mot taxocénose qui, composé de deux membres: taxo — provenant de taxonomie et cénose — groupement de plantes ou d'animaux (Odum 1963), rend mieux la signification qu'il doit exprimer.

Les taxocénoses des petits bassins étudiés furent différenciés d'après les critères décrits dans le travail précédent (Moraczewski 1962); les méthodes amployées ont été décrites ci-dessus.

Bassin B_0

Comme il a été déjà mentionné dans la description du terrain, les échantillons étaient prélevés à deux emplacements situés près l'un de l'autre. La composition en espèces des deux groupements s'avéra bien différente. Les deux groupements possédaient chacun environ 20 espèces, dont seules 9 leur étaient communes. Près du bord, en 1961 à partir de la moitié de juin jusqu'à la moitié de juillet, deux espèces dominaient: *Arcella gibbosa* Pen. et *Arcella discoides* Ehrbg., tandis qu'à partir de la moitié de juillet jusqu'à octobre la seule dominante devint *Arcella gibbosa* Pen. La domination n'était pas très prononcée, toute la structure de la biocénose n'étant d'ailleurs pas très nette, ce qui fait que l'on ne peut parler en ce cas que d'une „face" de taxocénose d'autant plus que le groupement ne possédait aucune espèce caractéristique, c'est-à-dire présente uniquement dans ce milieu.

Au fond, durant la période fin mai—fin juillet, l'espèce dominante était *Difflugia globulosa* Duj. A partir d'août jusqu'à octobre il n'y avait pas de dominant. On ne constata pas de phénomène d'adomination de la part d'une autre espèce. Aucune espèce caractéristique ne fut trouvée. La Fig. 1 illustre la dynamique du développement des *Testacea* dans les deux milieux étudiés.

Malgré que dans ces deux milieux dominent des espéces différentes et que toute la composition en espèces est loin d'être identique, les changements des quantités d'espèces tout comme des quantité d'individus démontrent un cours

très semblable. Dans ces deux milieux on constata une faune assez pauvre en *Testacea* et le rapport entre la quantité d'espèces et la quantité d'individus témoique d'un faible développement de ces groupements.

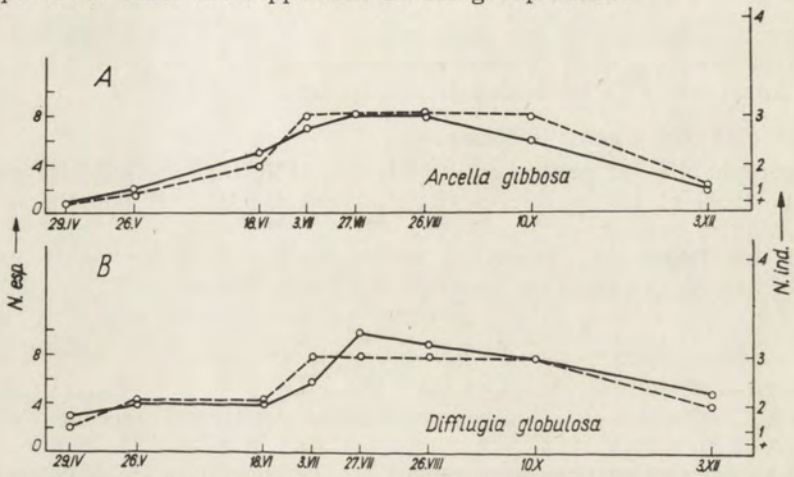

Fig. 1. Le nombre d'espèces (N. esp. — ligne continue) et le nombre d'individus (N.ind. — ligne pointillée) dans le bassin B_0, en 1961. A. Le bord du bassin. B. Le fond du bassin

En 1962 vers la moitié du mois de mai la Narew déborda une seconde fois dans l'année et ses eaux inondèrent toute la vallée. La vallée se trouva sous l'eau jusqu'au début de juin et après sa retraite, les prés où étaient situés les bassins étudiés se trouvèrent couverts d'une couche d'environ 0.5 cm de vase

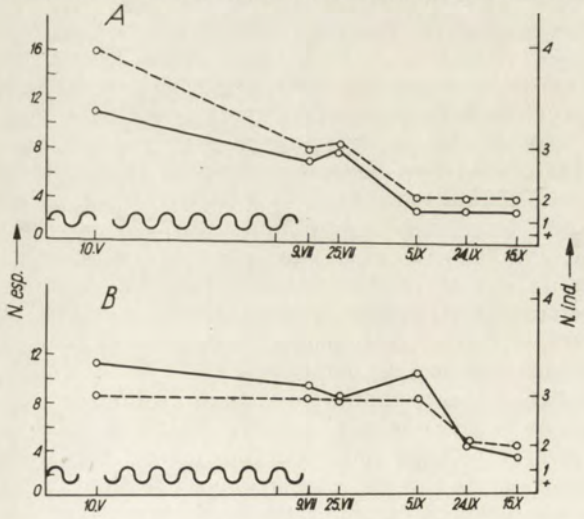

Fig. 2. Le nombre d'espèces et d'individus (pour description v. Fig. 1) dans le bassin B_0, en 1962; la sinusoïde marque la période d'inondation quand les prélèvements n'étaient pas faites. A. Le bord. B. Le fond

La Fig. 2 demontre les changements des quantités des especes et des indiwidus aux mêmes emplacements du basin B_0 que l'année précédente. Près du bord ainsi qu'au fond du bassin les taxocénoses des *Testacea* ne se sont pas formées. À aucun de ces postes on ne constata ni d'espèce dominate, ni d'espèce caractéristique. On voit donc nettement que l'inondation a détruit la première période de la vie des bassins — celle de la formation des groupements.

Bassins B_1 et B_2

Les bassins B_1 et B_2 étaient très riches en *Testacea*, mais ils desséchèrent entièrement à la fin de mai. Vers la fin d'avril — début de mai 1961 on y constata les espèces suivantes: *Microchlamys patella* Clap., *Arcella gibbosa* Pen., *Arcella rotundata* var. *alta* Play., *A. rotundata* var. *aplana* Def., *A. vulgaris* var. *penardi* Def., *Centropyxis marsupiformis* Wall., *C. aculeata* Ehrbg., *Cyclopyxis kahli* Def., *Difflugia oblonga* Ehrbg., *D. obl.* var. *brevicolis* Gass., *D. amphora* Leidy, *D. gramen* var. *globularis* Štěp., *D. gramen* Pen., *D. acuminata* Ehrbg., *D. acuminata* var. *acaulis* Perty, *Pontigulasia spectabilis* Pen., *Pareuglypha reticulata* Pen.

Bassin B_3

Ce bassin était bien plus riche en faune jue les précédents. Durant l'année on y trouva 54 espèces des *Testacea*. L'espèce *Difflugia oblonga* Ehrbg. — V, 2 (le chiffre romain indique la constance, le chiffre arabe — l'abondance); les espèces adominantes furent *Difflugia acuminata* Ehr. — V, 1 et *Arcella megastoma* Pen. — IV, 1.

Les espèces suivantes furent notées à cet emplacement: *Cochliopodium actinoporum* (Auer.) Pen. (I, +), *Arcella vulgaris* var. *penardi* Def. (I, 3), *Centropyxis discoides* (Pen.) Def. (II, +), *Centropyxis delicatula* Pen. (I, +), *Difflugia oblonga* var. *manicata* Pen. (I, +), *Difflugia similion* Thom. (I, +), *Cryptodifflugia compressa* Pen. (I, +), *Leusquereusia spiralis* Ehrbg. (I, +). Ces espèces furent considérées comme caractéristiques pour ce bassin. On peut donc sans aucun doute considérer le groupement des *Testacea* du bassin B_3 en tant que taxocénose bien formée. D'autant plus que durant la période du développement maximal des *Testacea* dans le bassin en fin de mai et au mois d'août le groupement possédait une structure nettement dessinée: 1 dominant, 2 adominants et des individus peu nombreux pour le reste des espèces. Cette structure est illustrée par la Fig. 3 A, où, au contraire du bassin précédent, la courbe de la quantité des individus surmonte nettement celle de la quantité des espèces. Au début de juillet on remarque une baisse considérable du nombre d'individus et d'espèces dans la taxocénose. La raison de ce phénomène est demeurée inconnue; il se peut jue durant cette période il se produisit une situation critique dans le milieu du bassin.

En 1962 le bassin contenait 32 espèces (Fig. 4 A). Durant la période entre les deux inondations *Difflugia globulosa* Duj. fut l'espèce dominante. Après le recul des eaux de la deuxième inondation on ne trouva dans le bassin que 4 exemplaires appartenant à 4 espèces différentes ainsi qu'une grande quantité de coquilles vides.

Dès le début de juillet jusqu'à octobre la domination était assurée par *Difflugia oblonga* Ehrbg. (V, 1) et l'adomination par *Difflugia globulosa* Duj. (V, 1). Les espèces suivantes étaient caractéristiques pour ce bassin: *Cyclopyxis eurystoma* Def., *Centropyxis cassis* var. *spinifera* Play., *Difflugia oblon-*

ga var. *magna* Thom., *Difflugia acuminata* var. *laevanderi* Play. Parmi les 28 autres espèces 70% avaient déjà été notées dans le bassin l'année précédente. La base principale de la taxocénose ne subit donc d'important changement en dépit des différences si importantes entre les milieux de ces années. En 1960 tout le bois de la contrée fut coupé. Les environs marécageux du bassin desséchèrent, les eaux du bassin devinrent beaucoup plus troubles et vers la fin de l'été sur toute la surface poussaient des *Stratiotes*.

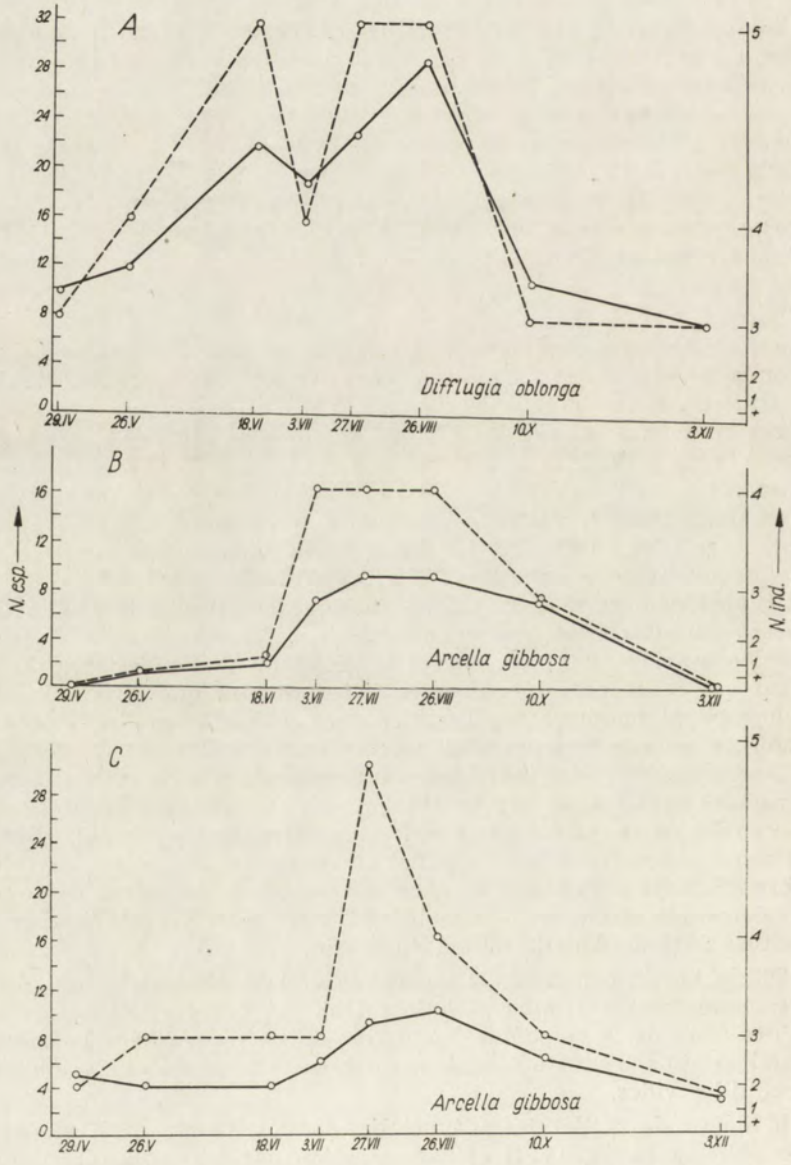

Fig. 3. Le nombre d'espèce et d'individus, en 1961, dans les bassins B_3 (A), B_4 (B) et B_5 (C) (pour description v. Fig. 1)

Même dans ce cas, malgré l'effect considérable de l'inondation, la taxocénose fut restaurée. L'absence des *Testacea* directement après la retraite des eaux et le nombre élevé de coquilles vides pourrait signifier que les *Testacea* endurent la période défavorable dans la vase du bassin, probablement sous la forme de cystes.

En 1961 aux mois de mai, juin et août (jusqu'au déssèchement) des échantillons furent prélevés du pré marécageux entourant le bassin B_3. Ces échan-

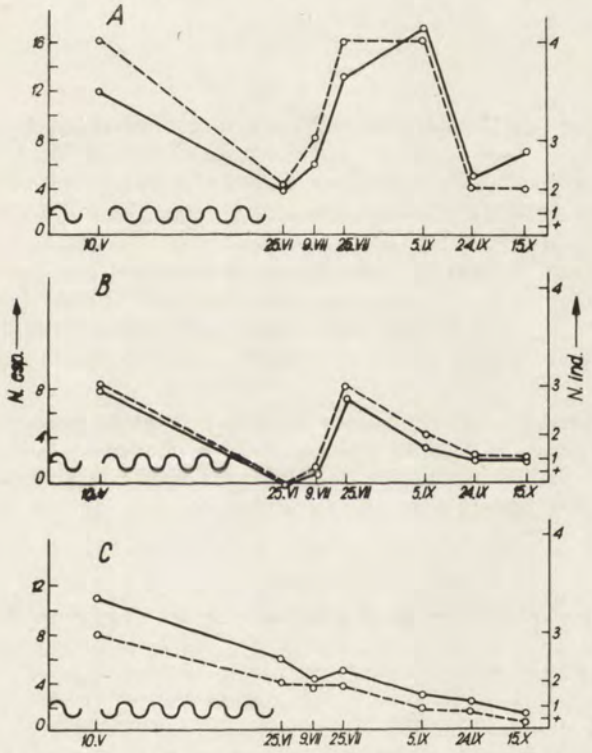

Fig. 4. Le nombre d'espèces et d'individus, en 1962, dans les bassins B_3 (A), B_4 (B) et B_5 (C) (pour description v. Fig. 1)

tillons furent prélevés en pressant l'eau du sol du pré. 34 espèces furent notées dans ces prélévements. Au mois d'août la structure du groupement s'esquisse nettement. Le dominant est *Trinema enchelys* (Ehrbg.) Leidy, l'adominant — *Cyphoderia ampula* (Ehrbg.) Leidy. Deux espèces n'étaient présentes que dans ce milieu: *Difflugia urceolata* Cort. et *Cyphoderia loevis* Pen. En septembre le niveau de l'eau était si bas que le pré déssécha entiérement.

Bassin B_4

En raison probablement du manque de végétation, de la grande turbidité de l'eau, ainsi que des mauvaises conditions alimentaires, la faune des *Testacea* de ce bassin était très pauvre. On y n'y trouva que 20 espèces durant toute l'année, dont 60% ne furent notées que dans un échantillon.

Comme on le voit de la Fig. 3 B, la taxocénose se développa dans ce bassin beaucoup plus tard que dans les autres. De juillet à octobre la dominante était *Arcella gibbosa* Pen. (IV, 2), mais en octobre *Difflugia gramen* var. *globulosa* Štěp. (III, 1) détrôna cette dernière. Les espèces adominantes changeaient plus fréquemment. Ce furent *Arcella haemisphaerica* Pen. et *Difflugia tuberculata* Wall. Seule une espèce — *Arcella conica* (Play.) Def. habitait uniquement ce milieu. En 1962 il y avait 14 espèces dans le bassin (Fig. 4 B). Toutes étaient représentées par un exemplaire dans les échantillons. La taxocénose de l'année précédente ne fut donc pas restaurée.

Bassin B_5

La taxocénose des *Testacea* de ce bassin se composait de 16 espèces. *Arcella megastoma* Pen. (V, 2) y dominait, avec *Arcella discoides* (III, 1) en tant qu'adominante. Les espèces caractéristiques étaient les suivantes: *Arcella rotundata* var. *stenostoma* Def. (I, +), *Difflugia mica* Fren. (II, +), *Difflugia olliformis* Lager. (I, +), *Difflugia bidens* Pen. (I, +).

Au mois d'août, durant le développement maximal des *Testacea* du bassin, les individus de l'espèce dominante constituaient 70% de la population, les adominants 17%. Les 7 autres espèces ne constituaient que 13% du nombre d'individus. Ses relations prouvent que la taxocénose du bassin était bien formée.

En 1962 on établit dans ce bassin la présence de 18 espèces (Fig. 4 C) dont seulement 5 figuraient l'année précédente. La coupe du bois, l'inondation et le comblement partiel du bassin par les branches des arbres coupés, furent les causes des différences entre la faune des *Testacea* de 1961 et celle de 1962.

Bassin W

Comme dans ce bassin on distinguait deux groupements différents de plantes, comme il y a déjà été mentionné, les échantillons étaient prélevés à ces deux positions. Il s'est avéré que les taxocénoses des *Testacea* des deux milieux différaient considérablement l'un de l'autre. L'*Acorus* (Fig. 5 A) se caractérisait par une abondance en espèces des *Testacea* — 47. *Difflugia oblonga* Ehrbg. (V, 2) y dominait tandis qu'en octobre ce fut *Trinema lineare* Pen., apparue dans le bassin en fin d'août, qui devint l'espèce dominante. Quelques espèces adominaient à tour de rôle, durant l'année: mai — *Arcella gibbosa* Pen., juin — début de juillet — *Difflugia acuminata* Ehrbg. et *Difflugia lobostoma* Leidy, juillent — août — *Arcella gibbosa* Pen. et ensuite *Difflugia lobostoma* Leidy, octobre — *Centropyxis constricta* Ehrbg. et *Euglypha tuberculata* Duj.

Ces changements constants d'espèces adominantes sont probablement causés par des changements continuels des conditions du milieu. La domination de *Difflugia oblonga* Ehrbg. résulte du caractère marécageux du bassin. Les importantes fluctuations du niveau de l'eau, le développement intense de la végétation durant l'année, la putréfaction des restes végétaux — tout cela causa les successions frèquentes des espèces adominantes.

Les espèces caractéristiques du bassin étaient: *Arcella haemisphaerica* var. *intermedia* Def. (I, +), *Centropyxis minuta* Def. (I, +), *Plagiopyxis callida* Pen. (I, +), *Pontigulasia incisa* Pen. (I. +).

Tout comme dans le bassin B_3 on y observa une baisse du nombre d'espèces au début de juillet. Le milieu *"Acorus"* était très différencié et c'est pour cette raison que la taxocénose qui s'y trouvait était la plus différenciée de toutes celles qui furent étudiées. Le milieu *"Equisetum"* du même bassin, par contre beaucoup plus uniforme (Fig. 5 B), n'abritait que 29 espèces dont 20

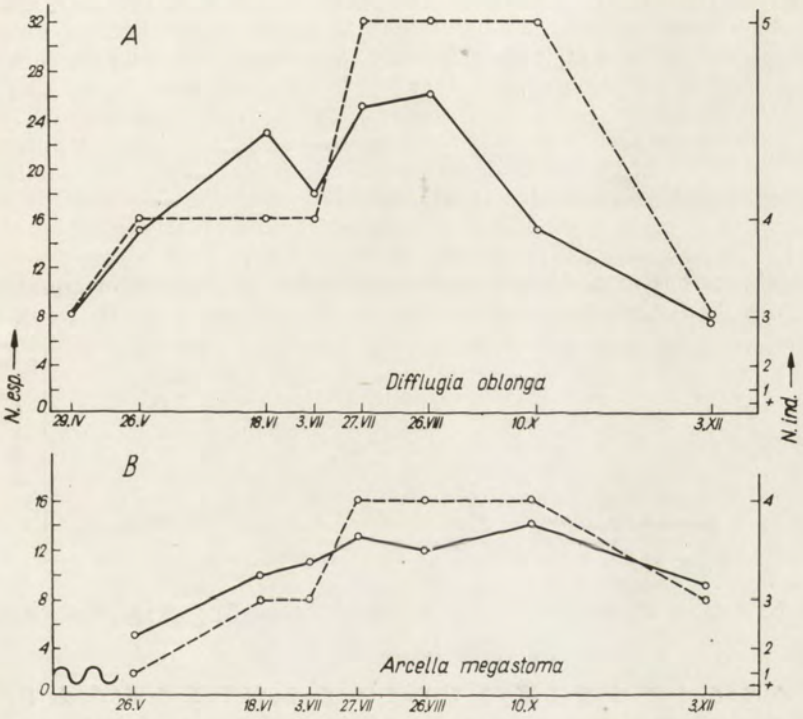

Fig. 5. Le nombre d'espèces et d'individus, en 1961, dans le bassin W. A. Le milieu d'*Acorus*. B. Le milieu d'*Equisetum* (pour description v. Fig. 1)

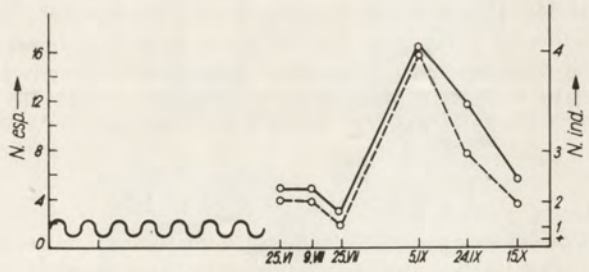

Fig. 6. Le nombre d'espèces et d'individus dans le bassin W, en 1962 (pour description v. Fig. 1)

étaient communes au milieu précédent. Trois espèces: *Lesquereusia modesta* Rhum. (II, +), *Difflugia penardi* (Pen.) Hop. (I, +) et *Pontigulasia spectabilis* Pen. (I, +), ne furent notées que dans ce milieu. À part le mois d'août, mois

de développement maximal des *Testacea*, où dominait *Difflugia globulosa* Duj., le reste de l'année ne possédait pas d'espèce dominante. On put néanmoins distinguer deux groupes d'espèces: le premier composé de 3—5 espèces représentées par 2 ou 3 exemplaires dans l'échantillon, et le deuxième, assemblant de 10 à 15 espèces, chacune représentée: par un seul exemplaire.

En 1962 les échantillons furent prélevés seulement de l'*Acorus*. On trouva 28 espèces (Fig. 6), dont 23 habitaient la même place l'année précédente. L'espèce dominante y était *Difflugia oblonga* Ehrbg. Les espèces caractéristiques y étaient constituées par: *Arcella gibbosa* var. *mitriformis* Def. (I, +), *Difflugia lebes* Pen. (I, +), et *Pseudodifflugia gracilis* Schlum. (II, +). Une telle quantité d'espèces communes démontre que ce n'est qu'une autre "face" de la même taxocénose, se développant dans des conditions partiellement changées, certainement moins favorables.

Bassin D

C'était un bassin relié à la rivière et dénué de végétation aquatique. Sa faune des *Testacea* était très modeste — 21 espèces (Fig. 7). Deux d'entre

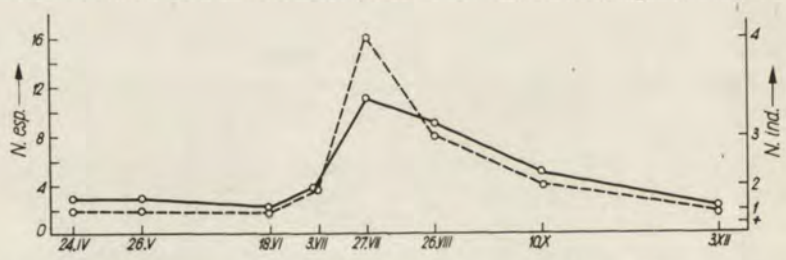

Fig. 7. Le nombre d'espèces et d'individus dans le bassin D, en 1961 pour description v. Fig. 1)

elles, *Pareuglypha reticulata* (II, +) et *Centropyxis marsupiformis* (I, +), ne furent notées que dans ce bassin. Un dominant apparut en juillet — *Arcella gibbosa* Pen., et deux adominants — *Difflugia acuminata* Ehrbg. et *Difflugia fallax* Pen.

Dans le reste des prélèvements toutes les espèces étaient représentées par des individus isolés. Il est possible que, si les prélèvements avaient été plus volumineux, il y aurait été possible de distinguer un dominant pour toute l'année. Il semble néanmoins que le matériel assemblé permet d'affirmer que la taxocénose dans ce bassin était peu développée. En 1962 le bassin se trouvait presque constamment dans le cours de la rivière et l'on n'y trouva que 5 espèces.

Remarques générales

En vue de systématiser les taxocénoses décrites et avant tout dans le but d'établir entre elles un degré de ressemblance (de parenté) sur la seule base de leur composition en espèces on a construit une dendrite selon la méthode de taxonomie de Wrocław (Faliński 1960), tout comme d'ailleurs dans un travail précédent concernant les taxocènes des *Testacea* du littoral peu profond.

La Fig. 8 A présente une dendrite, effectuée pour l'année 1961. Cette dendrite permet de distinguer des groupes de taxocénoses.

Le groupe B_0 du fond, B_4, B_5 — ce sont des taxocénoses liées au fond des bassins; dans ce groupe dominaient les espèces des petits bassins. Au centre de la dendrite se dispose la taxocénose W de l'*Acorus*. Comme il a été déjà mentionné dans sa description cette taxocénose est très riche en espèces et très bien formée. Elle contenait la majorité des espèces communes pour les taxocénoses du fond ou liées à la végétation. Un groupe en principe à part

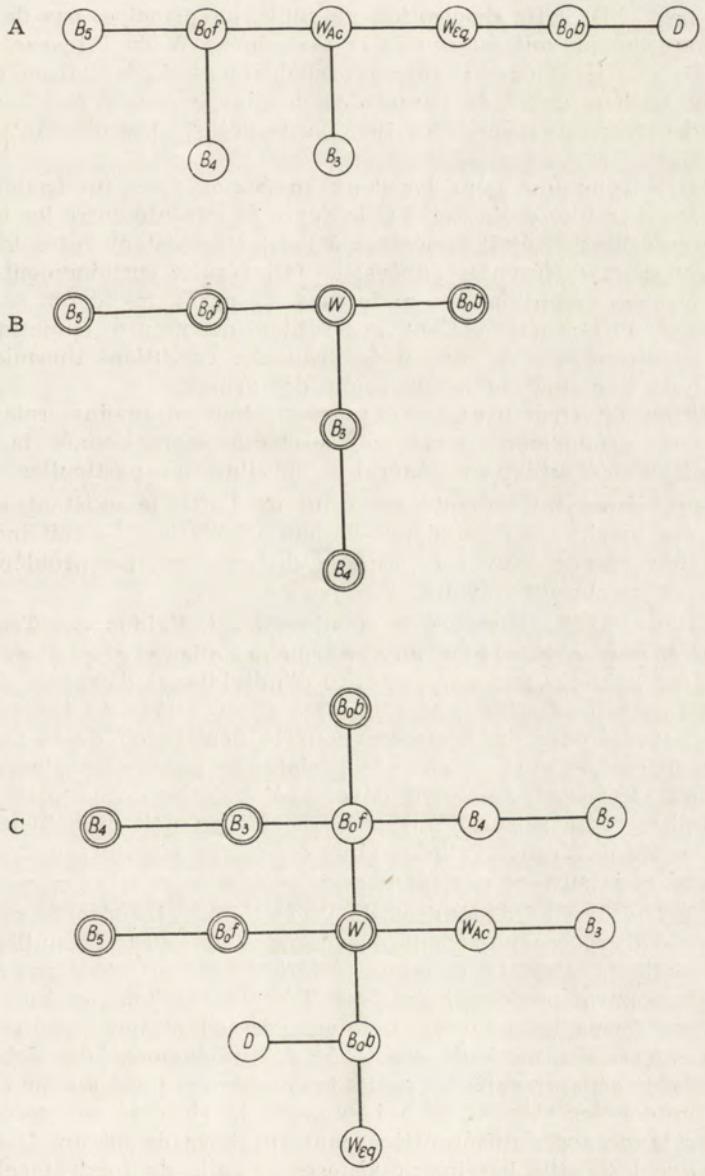

Fig. 8.Dendrites de ressemblance des taxocénoses des *Testacea* dans les bassins étudiés. A. Pour 1961. B. Pour 1962. C. Dendrite synthétique pour 1961 et 1962

est formé par la taxocénose B_3 qui, tout en étant aussi bien développée que la taxocénose W de l'*Acorus*, possede beaucoup plus d'espèces caractéristiques. Elle constituait donc un groupement bien spécifique de *Testacea*. Les trois taxocénoses suivantes (W de l'*Equisetum*, B_0 du bord, D), formant sur la dendrite une seule ligne, forment encore un groupe séparé de taxocénoses des *Testacea*, chez lesquelles les plantes aquatiques ou les plantes immergées des prés constituaient le taxotope principal.

En 1926 (Fig. 8 B) cette disposition ne subit, en principe, pas de changement. De nouveau on voit au centre la taxocénose W de l'*Acorus*, seule la taxocénose B_4 est déplacée. En raison probablement de la liaison entre les bassins B_3 et B_4 leur degré de parenté est le plus prononcé (ces bassins ont le plus grand nombre d'espèces). En 1962 on ne préleva pas d'échantillons des bassins W et D.

Une dendrite commune pour les deux années étudiées fut également esquisée. Comme il résulte de la Fig. 8 C le degré de parenté entre les taxocénoses de différents bassins était supérieur à l'affinité existant entre les mêmes bassins de ces deux différentes années; ce fait résulte certainement des immenses différences, mentionnées maintes fois, entre les deux saisons de végétation. Ces différences étaient aussi bien de nature climatique dont découlaient es différences de niveau de l'eau, des conditions thérmiques etc. que de l'activité humaine comme la coupe des arbres.

La répétition de structures identiques ou, tout au moins, relativement semblables d'un groupement paraît peu probable étant donnée la variabilité des conditions du milieu en général et du climat en particulier.

Un des problèmes intéressants est celui de l'affinité existant entre les taxocénoses des bassins en dépendance de leur végétation. Ce fait indiquerait une grande importance pour les *Testacea* du substrat. Ce problème a été discuté dans de nombreux travaux.

Schönborn 1962 a comparé le nombre des individus des *Testacea* se trouvant sur la même superficie du fond ou des plantes. Il en résulte que les plantes sont habitées par une quantité d'individus et d'espèces dépassant de quelques fois celle du fond. Mes propres observations du littoral de lac indiquent une préférence des *Testacea* pour le périphyton de la partie peu profonde du littoral et surtout pour le périphyton pauvre an algues filiformes tandis que le périphyton bien développé était beaucoup moins peuplé par ces organismes. La raison d'une telle répartition doit probablement être cherchée dans les différences alimentaires ou autres règnant entre les sédiments du fond et la surface des plantes.

Dans les petits bassins dépourvus de végétation la taxocénose des *Testacea* se compose d'espèces possédant des grandes et lourdes coquilles, faites en majeure partie de sable. La même observation a été effectuée par Schönborn 1962 b pour le profondal des lacs. Dès l'apparition des plantes dans un bassin il se forme un nouveau taxotope, possédant une population plus dense des *Testacea*. La méthode employée de prélèvement des échantillons n'a pas permis de séparer dans les petits bassins le taxotope du fond de celui des plantes immergées et c'est ce qui explique la richesse en espèces et en individus des taxocénoses différentiées dans ce genre de bassin. Il est presque certain que les petits bassins possènoses — celle du fond et celle de la végétation. La détermination des limites et des relations mutuelles exigerait des méthodes bien plus fines.

Dams les travaux concernant les *Testacea* de temps en temps apparaît le problème de l'euryvalence de ce groupe et simultanément des différentiations écologiiques qu'il est facile d'y observer. Schönborn 1962 b considère le problème d'attachement de quelques espèces aux mousses ou au sol et l'associé à un certain degré à la structure de la coquille ainsi qu'à la dépendance mentionnée maintes fois par la littérature, de la présence des *Testacea* du degré de l'humidité du milieu. Les connaissances que l'on possède sur l'écologie des *Testacea* font penser que les classifications employées en général en hydrobiologie ou écologie sont peu précises en égard à ce groupe et dans bien des cas, peuvent même être fausses. Les espèces considérées comme euryvalentes en raison de leur présence aussi bien dans les tourbières que dans les petits bassins, les mousses et les lacs, possèdent un biotope bien précis, par example la surface des feuilles. Derrière l'euryvalence ou l'ubiquisme de ce groupe se dissimulent sans doute des exigences très précises envers le milieu, des exigences difficiles à déterminer à l'aide des méthodes peu précises des recherches écologiques. La même question des microdifférentiations existe sans doute également dans les recherches écologiques sur d'autres protozoaires et elle est la source de difficultés majeures.

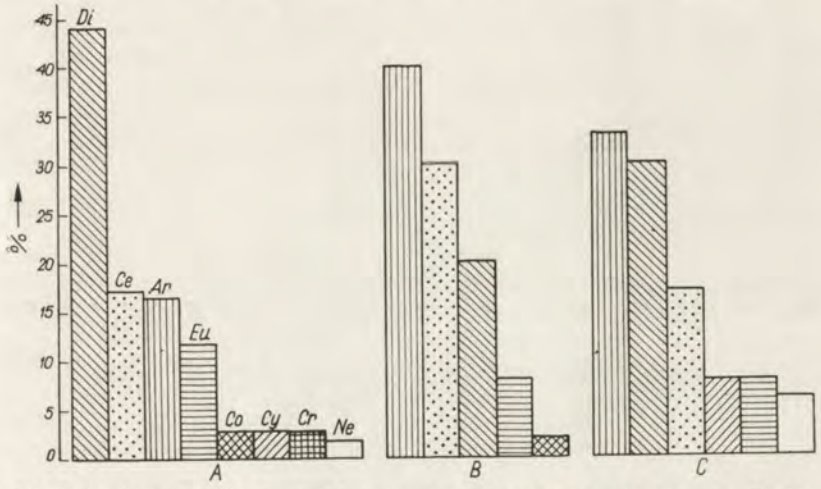

Fig. 9. Participation (en %) de différentes familles dans la faune des *Testacea* de quelques types de bassins en Pologne (*Di* — *Difflugiidae*, *Ce* — *Centropyxidae*, *Ar* — *Arcellidae*, *Eu* — *Euglyphidae*, *Co* — *Cochliopodiidae*, *Cy* — *Cyphoderiidae*, *Cr* — *Cryptodifflugiidae*, *Ne* — *Nebelidae*). A Les petits bassins. B. Le littoral d'un lac, C. Les rivières

Je pense que l'on ne peut parler de faune des *Testacea* de petits bassins, de lacs, ou de tourbières, mais qu'on doit par contre différentier les faunes des sédiments du fond, des feuilles ou tiges des plantes immergées, des eaux interphytiales du sol ou des cellules aquatiques des mousses.

La classification des *Testacea* se base sur la structure des coquilles. Les différentes familles ont été différentiées selon la forme de la coquille qui dépend à son tour en grande partie du matériel dont elle est construite. Il est vrai que Schönborn 1962 a a noté dans la vase des espèces qui construisent leur coquille à l'aide de sable tandis que dans le périphyton ces

mêmes espèces utilisaient la pseudochitine ou le détritus. On peut néanmoins constater de façon générale que par exemple *Difflugiidae* sont des habitants du fond, *Arcellidae* — du phytal etc.

Le diagramme de la Fig. 9 représente la participation en % dans différents types de bassins des espèces appartenant à différentes familles. Dans les petits bassins on trouve des représentants de 8 familles des *Testacea*, qui construisent leurs coquilles de différent matériel. *Difflugiidae* y dominent — ce sont en général des espèces à lourdes coquilles habitant le fond. Dans le littoral du lac 70% environ des espèces appartiennent aux *Arcellidae* et *Centropyxidae*. On constata (Moraczewski 1962) que dans le périphyton dominent les *Arcellidae*, au fond les *Difflugiidae*, tandis que les *Centropyxidae* se trouvaient en grande quantité dans les deux milieux. Le seston de rivière (Moraczewski 1964) est riche en *Testacea* mais sa composition est accidentelle, ne dépendant que du genre de milieu duquel le courant emporte les individus.

Résumé

Quelques petits bassins situés á l'embouchure du Bug á la Narew furent étudiés en 1961—1962. Ces bassins se trouvaient dans la vallée de la rivière et après la construction du barrage de Dębe ils se trouvèrent au fond du lac artificiel Zegrzyńskie.

In semble que l'étude et la classification des groupements des *Testacea* peut être d'un certain intérêt pour l'écologie des protozoaires et autres microorganismes constituant un des principaux maillons de la chaîne alimentaire de la biocénose aquatique.

Les échantillons ont été prélevés de 5 petits bassins. Tous les bassins étaient inondés par la Narew durant les inondations et tous peuvent être considérés d'après la classification *Žadin* en tant que vieux lit provenant du de la rivière.

Dans les bassins étudiés on nota 112 espèces et variétés des *Testacea* dont 56 étaient nouvelles pour la faune de la Pologne.

Bassin B_0 — étang de vieux lit de la Narew possèdant une zone développée de végétation cormophyte. On compara la taxocénose du fond avec celle de la végétation riveraine. Les deux taxocénoses se distinguaient par leur composition en espèces et leur structure de dominance. Les variations du nombre des individus et des espèces durant l'année suivaient à peu près le même cours.

Bassins B_3, B_4, B_5 — petits bassins de différente composition végétale et de différente morphologie mais communiquant à l'aide d'un fossé. Ils possèdaient des différentes taxocénoses des *Testacea*. La dynamique de ces groupements était très semblable.

Bassin W — petit bassin éntouré d'une centure d'*Acorus, Typha* et *Scirpus* et couvert à l'intérieur d'une touffe d'*Equisetum*. Il possédait deux différentes taxocénoses des *Testacea*. Une, celle de l'*Acorus*, était bien différentiée avec une nette structure de dominance, tandis que la taxocénose d'*Equisetum* était bien plus pauvre et plus uniforme.

Le matériel a été analysé à l'aide de la méthode de taxonomie de Wrocław et on a pu déduire que les bassins possèdant une flore bien développée

possèdent des taxocénoses des *Testacea* également bien développées. Les taxocénoses du fond du bassin constituent un deuxième groupe possédant des traits communs.

STRESZCZENIE

W latach 1961—1962 przeprowadzono badania kilku drobnych zbiorników wodnych, leżących w okolicy ujścia Bugu do Narwi. Zbiorniki te były położone w dolinie rzeki i z chwilą powstania jeziora Zegrzyńskiego znalazły się na dnie powstałego zbiornika zaporowego.

Wydaje się, że poznanie i sklasyfikowanie zbiorowisk *Testacea* może być bardzo interesujące dla ekologii pierwotniaków i innych drobnych organizmów, stanowiących jedno z ważniejszych ogniw łańcucha pokarmowego biocenozy wodnej.

Próbki pobierano z 5 drobnych zbiorników. Wszystkie zbiorniki były zalewane wodą Narwi w czasie powodzi i wszystkie według klasyfikacji Żadina należy zaliczyć do starorzeczy.

W badanych zbiornikach stwierdzono występowanie 112 gatunków i odmian *Testacea* z pośród których 56 były nowe dla fauny Polski.

Zbiornik B_0 — staw w starym korycie Narwi z wykształconą strefą przybrzeżnej roślinności naczyniowej. Porównano taksocenozy *Testacea* dna z taksocenozą roślinności strefy przybrzeżnej. Różniły się one składem gatunkowym i strukturą dominacji. Jednak zmiany ilości osobników i gatunków w ciągu roku były bardzo zbliżone.

Zbiorniki B_3, B_4, B_5 — drobne zbiorniki o różnym składzie makroflory i morfologii, połączone ze sobą rowem. Posiadały rozmaite taksocenozy *Testacea*. Dynamika tych zbiorowisk była bardzo zbliżona.

Zbiornik W — drobny zbiornik otoczony pasem tataraku, pałki, sitowia i pokryty wewnątrz zwartą kępą skrzypu. Posiadał dwie różniące się od siebie taksocenozy *Testacea*. Dobrze zróżnicowaną i wyraźną strukturę dominacji miała taksocenoza „tataraku" i znacznie uboższa bardziej jednorodna taksocenoza „skrzypu".

Materiał uporządkowano posługując się metodą taksonomii wrocławskiej i na tej podstawie stwierdzono, że zbiorniki o dobrze rozwiniętej makroflorze mają zbliżone do siebie i najlepiej zróżnicowane taksocenozy *Testacea*. Drugą grupę stanowiły taksocenozy związane z dnem zbiornika.

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