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Thécamoebiens nouveaux et peu connus

Нови и малко познати тестацеи

Le but du travail présent est de décrire les Rhizopodes thécamoebiens trouvés dans un matériel provenant de Guinée, Afrique occidentale.

Arcella multilobata sp. n.

La théque est plus ou moins en forme de calotte de sphère à bord arrondi. En vue frontale le contour circulaire, rarement elliptique. Le pseudostome est lobé: 6—10 lobes peu profonds, arrondis se raccordent par des dents plus ou moins pointues. En vue latérale, face dorsale représente un segment de cercle ou une portion de parabole sans ondulation marginale. L'angle de raccordement avec la face orale est arrondi. Face orale s'invaginant régulièrement vers le pseudostome et pourvue d'un tube buccal très court. L'invagination est égale à $^1/_3$—$^1/_2$ de l'hauteur de la théque. Membrane jaunâtre, rarement foncée à aréolations très fines, mais toujours visibles.

Dimensions de la théque: diamètre de 84—98 μ; hauteur de 28—31 μ; ouverture du pseudostome de 20—24 μ; rapport H/Dm de 0.30—0.38; invagination de 12—16 μ.

Ecologie: mousses mouillées de rochers.

Distribution géographique: Guinée, v. Labé, Carières de l'ardoise, 7.4.1963.

La seule espèce connue du genre à pseudostome lobé est *Arcella lobostoma* Deflandre, 1928. Cette dernière diffère de *A. multilobata* par sa forme sensiblement hémisphérique (H/Dm: 0.65—0.68) et son pseudostome irrègulièrement quadrilobé. Les dimensions de la théque de *A. lobostoma* sont inferieures à celles de *A. multilobata*. D'après Deflandre 1928 le diamètre de cette première espèce est de 42—54 μ.

Lesquereusia pseudonebeloides sp. n.

La théque est formée de deux parties distinctes: le col et la panse. Le col, courbé et tubulaire ressemble le col de *L. epistomium* Penard, 1893. Le pseudostome, tronqué droit est irrégulièrement lobé et bordé de petits éléments exogènes. L'ouverture paraît avoir 4—6 lobes. La conformation du pseudostome rappelle celle de *Pseudonebela africana* G.-Lièvre, 1953.

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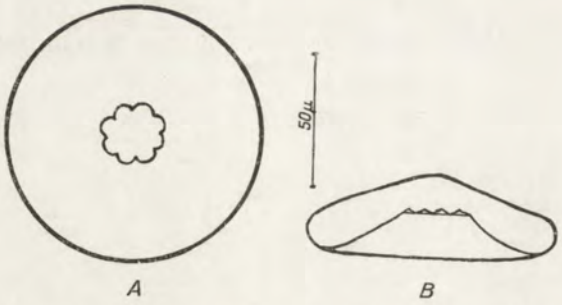

Fig. 1. *Arcella multilobata* sp. n. A. Vue du pseudostome. B. Vue latérale

La panse est ovoïde ou ronde à section transversale circulaire. Coque en général claire, hyaline est incolore. Le revêtement de nature endogène est formé de petites plaquettes de forme variable, irrégulièrement arrangées et soudées dans un ciment chitinoïde abondant.

Animal vivant non observé.

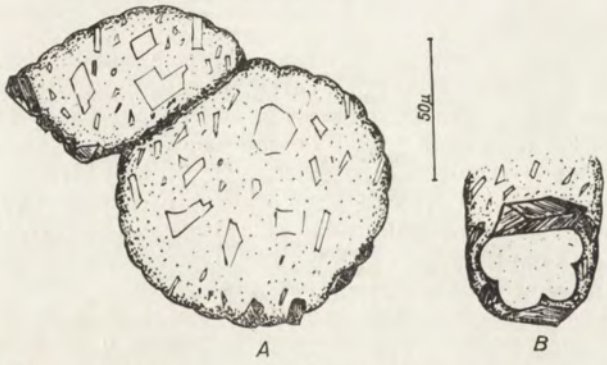

Fig. 2. *Lesquereusia pseudonebeloides* sp. n. A. Vue de profil. B. Vue du pseudostome

L'aspect général et les caractères morphologiques de la théque nous permettent de placer cette intéressante espèce à la suite de *L. epistomium* Penard, 1893.

Dimensions de la théque: longueur totale de 144—148 µ; largeur de 100 µ; ouverture du pseudostome de 34—36 µ; col de 80—88×60—64 µ.

Ecologie: *Lithotelmes* sur rivage d'une rivière.

Distribution géographique: Guinée, v. Pita, Kinkon, 17.3.1963.

Difflugiella pusilla (Playfair, 1917) comb. nov.

Syn.: *Cryptodifflugia pusilla* Playfair, 1917.

La théque est largement ovoïde, régulière, transparente, incolore et lisse à section transversale circulaire. D'une extrémité la théque est ouverte par un pseudostome tronqué droit et circulaire, bordé d'un bourrelet chitinoïde interne.

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Les formes observées au cours de nos recherches sur les Thécamoebiens de Guinée (Golemansky 1963) correspondent à la déscription de Playfair 1917. Elles diffèrent de formes trouvées en Australie seulement par ses dimensions plus élevées. Il s'agit probablement d'une espèce assez variable suivant les conditions du milieu externe.

Fig. 3. *Difflugiella pusilla* (Playfair, 1917) comb. nov., vue latérale

Dimensions de la théque d'après Playfair 1917: longueur = largeur = 10 μ. D'après nos observations les dimensions sont les suivantes: longueur de 10—22 μ; largeur de 10—20 μ; ouverture du pseudostome de 6—7 μ; rapport L/1 de 0.9—1.0.

Ecologie: mousses mouillées de rochers.

Distribution géographique: Australie, Guildford (Playfair 1917); Guinée, v. Labé, Carières de l'ardoise, 7.4.1963.

Résumé

Dans le présent travail l'auteur décrit deux espèces nouvelles de Rhizopodes thécamoebiens, trouvées pour la première fois dans un matériel provenant de Guinée, Afrique occidentale. De plus l'auteur établit la position systématique de *Difflugiella pusilla* (Playfair, 1917) comb. nov. et élargit la description de cette espèce, relativement très rare.

РЕЗЮМЕ

В предлаганата работа авторът описва два нови вида тестацеи, намерени за първи път в материал, произхождащ от Гвинея, Западна Африка. Освен това авторът изяснява систематичното положение на *D. pusilla* (Playfair, 1917) comb. nov. и разширява описанието на този сравнително рядък вид.

BIBLIOGRAPHIE

Deflandre G. 1928: Le genre *Arcella* Ehrenberg. Arch. Protistenk. 64, 152—287.

Gauthier-Lièvre L. 1953: Les genres *Nebela, Paraquadrula* et *Pseudonebela* (Rhizopodes testacés) en Afrique. Bull. Soc. Hist. Nat. Afr. Nord 44, 324—366.

Golemansky V. 1963: Matériaux sur la faune rhizopodique de Guinée. Thécamoebiens du massif Fouta Djalon. Recherches Africaines 1, 1—25.

Penard E. 1893: *Pelomyxa palustris* et quelques organismes inférieures. Arch. Sci. Phys. Nat., Génève (3), 29, 165—182.

Penard E. 1902: Faune Rhizopodique du bassin du Léman. Kündig, Génève.

Playfair G. J. 1917: Rhizopods of Sydney and Lismore. Proc. Lin. Soc., N-S Wales, 42, 633—675.

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M. V. KRYLOV

On the validity of the genus *Smithia* França

К вопросу о самостоятельности рода *Smithia* França

The publication of França 1910 on the classification of *Piroplasma* contains the description of two parasites and the diagnoses of genera *Nuttallia* and *Smithia* which are first distinguished by him. Characteristic features distinguishing *Nuttallia* França 1910 are: „Parasites ovoid or pear-shaped. The reproduction in cross-shaped form. No bacillar parasites". The diagnosis of *Smithia* França 1910 is as follows: „Parasites pear-shaped in one of their life phases. The majority of those pear-shaped parasites occupy the entire width of the erythrocyte and fail to appear in pairs. Reproduction by cross division into 4 individuals".

In the same publication França includes drawings of two species of parasites; one of them represents the genus *Nuttallia* (Fig. 1), another — *Smithia* (Fig. 2). A detailed characteristic of the species *Smithia microti* follows in which the author points out that the juvenile forms are ovoid or pear-shaped, the parasite divides into four individuals and the young forms are disposed in cross-like pattern, their thin ends diverging towards periphery (Fig. 2). This form of reproduction seems to the author appropriate for definition of the genus. It is however stated at the same place that this form is identical with those of the genus *Theileria* and *Nuttallia*.

Consequently — according to the description of França — the only feature distinguishing the genus *Nuttallia* from the genus *Smithia* is the size of the parasite. The pear-shaped forms of parasites of the genus *Smithia* occupy sometimes the entire diameter of the erythrocyte reaching up to 6—6.5 μ in length.

Observations on the genus *Smithia*

Let us analyse the constancy of the size in the parasites classed within the genus *Smithia*. In the article of França we find the description of the pear-shaped forms of *Smithia microti* occupying not the whole diameter of the erythrocyte but just a part of it. Further on França states that between the juvenile parasites and the big pear-shaped forms all the transitory types occur. In this way — according to França — the pear-shaped parasite forms may be big — reaching the diameter of erythrocyte and also small ones occupying only a part of the erythrocyte diameter.

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Fig. 1. *Nuttallia herpestedis* (after França 1910)

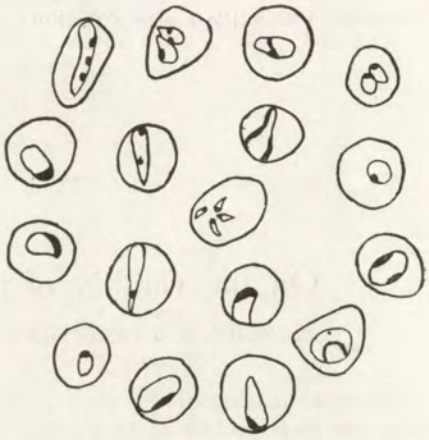

Fig. 2. *Smithia microti* (after França 1910)

The second species of the genus *Smithia: Smithia talpae* was described by Galli-Valerio 1914. This author failed to find the reproduction forms and related the parasite, first found by him in the mole *Talpa europea*, to the genus *Smithia* by reason of the size of its pear-shaped forms fluctuating from 3—4 to 4.5 μ in length. Juging by the drawings of Galli-Valerio, added to the description of *Smithia talpae*, this pear-shaped forms occupy far less than the whole diameter of the erythrocyte.

The author of the present study (Krylov i Zanina 1963) found a parasite in smears of blood of *Meriones erythrourus* and included it to the genus *Smithia* distinguishing it as a separate species *S. tadzhikistanica*.

Fig. 3. *Smithia talpae* (after Galli-Valerio 1914)

Subsequently another *Meriones* individual was found infected with parasites, and the morphology of *S. tadzhikistanica* from a vertebrate host was studied in details in the laboratory conditions (Fig. 4).

Reproduction of the parasite in the erythrocyte occurs by means of division into 4 individuals, the pattern of disposition of the dividing forms

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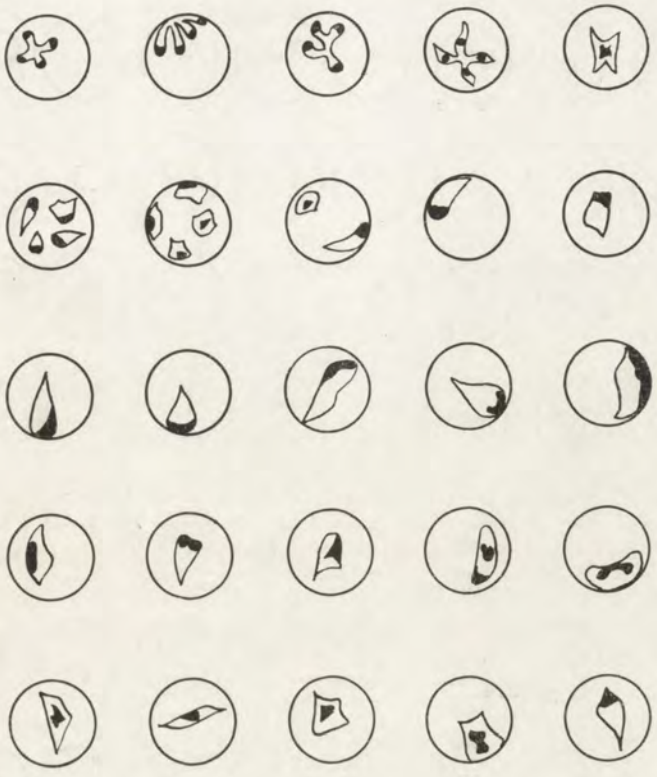

Fig. 4. *Smithia tadzhikistanica* Krylov et Zanina, 1963

being most variable. Sometimes they separate as diverging chromatin complexes, then resembling the Maltese cross, in other cases division initiates by fission of cytoplasm, while the chromatin in the centre remains undivided for some time. In the forms before division, chromatin produces rather frequently a ribbon with uneven margin. Division of nucleus into four may proceed simultaneously or asynchronically; in the latter case chromatin forms 2 or 3 agglomerations within cytoplasm.

In the case of a strong infection, up to 12 parasite specimens may be stated in one erythrocyte.

All transitory forms may be found between the juvenile individuals — just after fission — and the big pear-shaped ones. This fact speaks against the theory that two different species of parasites are present in blood. Their dimensions fluctuate within large limits, from 0.7 to 3.75 μ in width and 0.7 to 5.6 μ in length. Difference in dimensions is connected with the growth of the parasite and evidently depends on many factors (e. g. in the conclusive period of disease small forms are more abundant whereas the big ones occur at the peak of the parasitic reaction). Splenectomy accounts also for the occurrence of big forms occupying sometimes the whole diameter of the erythrocyte

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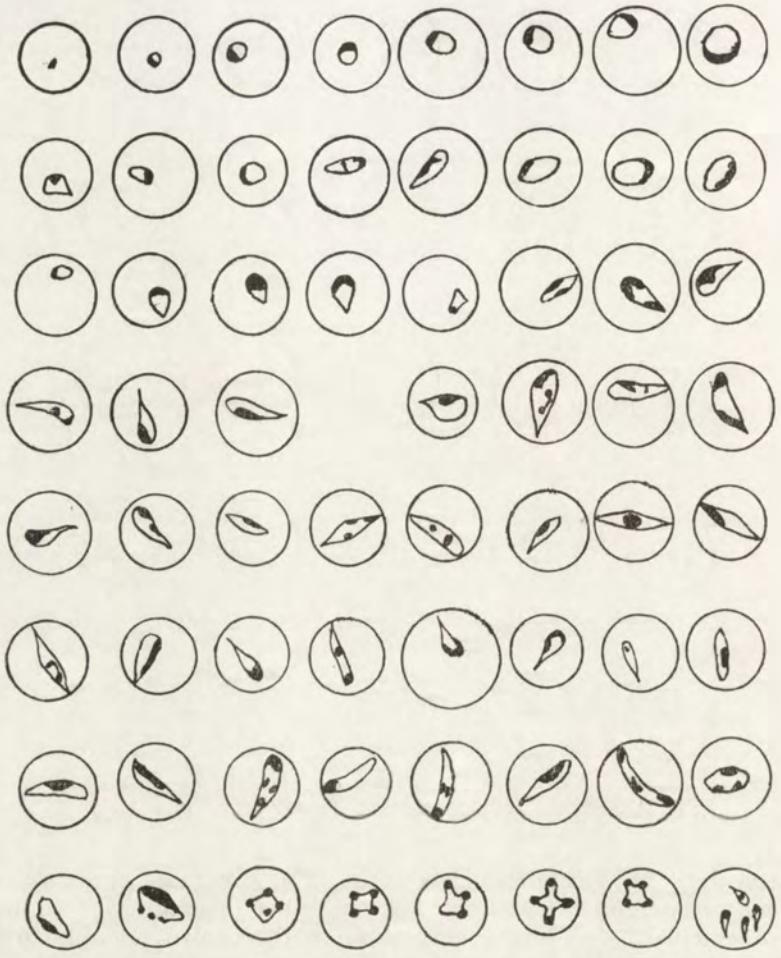

Fig. 5. *Nuttallia equi* (after Agrinskij 1936)

In conclusion, the description of the two parasites of the genus *Smithia* by Galli-Valerio, as well as the author's own observations on *Smithia tadzhikistanica* seem to indicate that pear-shaped forms may be big, occupying the whole diameter of the erythrocyte, as well as the small ones, occupying only a small part of it.

Observations on the genus *Nuttallia*

Morphology of the erythrocytal forms of the parasitic genus *Nuttallia* has been studied most in details in *Nuttallia equi*. Especially interesting are the investigations of Agrinskij 1936 who executed his study on abundant material of *Nuttallia equi*. He examined blood smears of nearly one thousand of infected horses. He found *Nuttallia* individuals occupying the whole

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erythrocyte diameter (Fig. 5); the size of big specimens reached up to 2.70—4.68 μ. When comparing those forms with the analogous forms of *Smithia microti* (Fig. 2) as described by França — their evident similitude becomes doubtless. They resemble each other so strikingly that — if there was no explanation of the figure — it would be difficult to decide to which of the two genera: *Nuttallia* or *Smithia* they should be included.

Besides the big forms of *Nuttallia* there are fairly small ones as well as all the transitory types.

The investigations of Abramov 1961 indicate that the shape and size of *Nuttallia* depend considerably on the species of tick by which they were transmitted to the vertebrate host. Besides, morphology of the parasite is changing in the course of disease. Towards the conclusion of disease, the reduction of the parasite size is observed. Nevertheless, despite the considerable polymorphism of *N. equi*, cross infection with different strains indicates the presence of a reciprocal immunity (Agrinskij 1936, Abramov 1962).

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The only character which was França's criterion of distinction between the genus *Smithia* and *Nuttallia:* the size of the erythrocytal form, fails to prove constant and typical for any of those two genera. Consequently there is no reason to separate, in the genus *Smithia*, the pear-shaped parasites of a length equal to the diameter of the erythrocyte, and dividing into four individuals. Intracellular parasites included into the genus *Smithia* are identical with *Nuttallia* and should be recognized as synonyms of the latter. According to the nomenclatoric regulations the generic name *Nuttallia* should be kept since this genus was decribed in the publication of França 1910 on the page 13, earlier than the genus *Smithia* (p. 14).

It should be kept in view that the parasites of the genus *Nuttallia* are characterized by a strongly expressed polymorphism manifested in a great variety of form and dimensions.

Summary

The author's own material of *Smithia tadzhikistanica* Krylov et Zanina, 1963 was analysed and compared with the first description of *Smithia talpae* Galli-Valerio, 1914 and *Smithia microti* França, 1910. The morphology of those parasitic species has been compared with *Nuttallia herpestedis* (França, 1908) and *Nuttallia equi*. The results of this comparative study involved the author's conclusion that the genus *Smithia* França 1910 is identical with the genus *Nuttallia* França 1910.

Keeping the generic name: *Nuttallia* França 1910 for all the analysed species is suggested.

РЕЗЮМЕ

На основании изучения собственных материалов по *Smithia tadzhikistanica*, Krylov et Zanina, 1963, первоописаний *Smithia talpae* Galli-Valerio, 1914 и *Smithia microti* França. 1910 и сравнения морфологии этих паразитов с *Nuttallia her-*

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pestedis (França, 1908) и *Nuttallia equi* автор приходит к заключению о том, что род *Smithia* França 1910 идентичен роду *Nuttallia* França 1910.

Предлагается сохранить одно родовое название *Nuttallia* França 1910.

REFERENCES

Abramov I. B. 1961: K voprosu biologii vozbuditelja nuttallioza (*Nuttallia equi* Laveran, 1901) lošadej. Sb. rab. naučn. konf. po protozool. probl. 36—40.

Abramov I. B. 1962: Osobennosti piroplazmoza i nuttallioza lošadej različnyh zon SSSR. Doctor Diss.

Agrinskij N. I. 1936: Biologičeskoe edinstvo raznyh morfologičeskih form *Nuttallia equi* v Srednej Azii. Sov. Veterin. 3, 54—63.

França C. 1910: Sur la classification des Piroplasmes et description de deux formes de ces parasites. Arch. Real. Inst. Bacteriol. Camara Pestana 3, 11—18.

Galli-Valerio B. 1914: *Smithia talpae* n. sp. *(Piroplasmidae)* chez *Talpa europaea* L. Zbl. Bakt. Parasitenk. Infektionskrank. 73, 142—143.

Krylov M. V. i Zanina Z. L. 1963: *Smithia tadzhikistanica* n. sp. iz krasnohvostoj pesčanki (*Meriones erythrourus* Gray, 1842). Tr. Inst. Zool. Parazitol. A. N. Tadjik SSR, Zool. i parazitol. 24, 169—170.

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Anna CZAPIK

La stomatogenèse du Cilié *Uronema marinum* Dujardin *(Hymenostomatida, Tetrahymenina)*

Stomatogeneza orzęska *Uronema marinum* Dujardin *(Hymenostomatida, Tetrahymenina)*

L'espèce *Uronema marinum* Duj. a attiré l'attention de plusieurs chercheurs par égard à sa morphologie aussi bien qu'à son écologie. Elle appartient aux petites formes: sa longueur ne dépasse pas 30—40 μ; le corps est ovoïde, sa partie postérieure arrondie. La partie antérieure possède une plaque frontale sans cils. Le cytostome est situé dans une dépression allongée, entourée par la zone adorale des membranelles du type tetrahymenien. Les cils somatiques rangés en 12—15 méridians sont d'une longueur d'environ 10 μ; le cil caudal est plus long. La vacuole contractile se trouve dans la partie postérieure du corps. Le protoplasme contient souvent des grains réfringeants.

Uronema marinum a été découverte pour la première fois par Dujardin dans la Mediterrannée, puis dans le Golf du Mexique, enfin on a constaté que cette forme vit aussi dans les eaux douces. A présent on sait, qu'elle est commune dans le sable et detritus marin et qu'on la rencontre souvent en grande quantité aux laboratoires dans les cultures à eau douce contenant des bactéries. Finley 1930 affirme, qu'elle peut être transportée sans dommage de l'eau douce à l'eau marine. En étudiant la biologie des eaux résiduaires on a constaté, que *Uronema marinum* est commune non seulement dans les eaux polluées mais aussi aux stations d'épuration des eaux à savoir dans la boue activée et dans les lits bactériens où elle est comptée parmi les organismes qui jouent un rôle important dans le procédé de purification biologique de l'eau. Liebmann 1951 la considère comme une des espèces-indices de la zone α-mezosaprobiotique.

Pàrducz 1939a a décrit minutieusement la morphologie et le système argentophile de cette espéce et il a attiré l'attention sur sa ressemblance frappante au genre *Cyclidium*, en étudiant *Cyclidium glaucoma* (Párducz 1939b). Il a fait une analyse très détaillée de l'anatomie des deux espèces. Cette ressemblance concerne non seulement l'anatomie, mais le mode d'alimentation et de mouvement. La forme et les dimensions du corps, la ciliature et la zone adorale des membranelles se ressemblent; les deux espèces se nourrissent de bactéries. En nageant elles s'arrêtent de temps en temps et restent pendant quelques instants immobiles. Pourtant si l'on a une certaine pratique,

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on peut bien distinguer les deux formes d'après leurs mouvements: le mouvement *d'Uronema* est plus lent, quant à *Cyclidium* il avance la plupart du temps par des sauts rapides.

Párducz a cru, entre autres en raison des petites dimensions de cet animal, que *Cyclidium* est une forme plus primitive que *Uronema* et que l'une est issue de l'autre. A présent il est hors de doute qu'il n'en est pas ainsi parce que la zone adorale des membranelles chez *Cyclidium* est beaucoup plus specialisée que chez *Uronema*. Il ne reste plus qu'à déterminer si la ressemblance morphologique est suivie d'une ressemblance dans le cours de la stomatogenèse et c'est celà qui est le sujet de ce travail.

*

J'ai trouvé l'espèce *Uronema marinum* en examinant les échantillons d'eau résiduaire pris d'un lit bacterien expérimental. C'était de l'eau résiduaire industrielle, qui contenait entre autres du phénol. Le passage du Cilié dans une décoction de foin ne présentait aucune difficulté; il se multipliait si vite dans le milieu nouveau, qu'après quelques jours j'étais en possession d'une culture très dense. Les préparations argentées ont été faites d'après la methode sèche de Klein.

La zone adorale des membranelles chez *Uronema marinum* est composée d'une membrane ondulante qui entoure du coté droit la dépression allongée où est situé le cytostome, et de 2 ou 3 membranelles localisées vis-à-vis d'elle. Les contours de toutes les membranelles sont, au contraire du contour de celles de *Cyclidium*, irréguliers, peu distincts, le cytostome est situé plus haut, au-dessus de l'équateur (Pl. I 1).

La courte cinétie qui joint le cytostome au cytopyge ("Richtungsmeridian") est ici stomatogène. Ce qui signale la division c'est l'apparition d'un petit champ obscur dans la partie inférieure ce cette cinétie. Au microscope à l'immersion on voit que ce champ est formé par des stries fines qui sortent de la cinétie et sont orientées plus ou moins obliquement par rapport à cette dernière. Ces stries se forment à cause des divisions des cinétosomes dans la cinétie. Cette première phase de stomatogenèse est très difficile aussi bien à répérer dans les preparations qu'à photographier. Elle est visible sur la Pl. I 2—4. Le péristome du proter ne subit pour le moment aucun changement.

Pendant la phase suivante l'appareil buccal du proter subit une désorganisation à partir du fragment inférieur de la membrane ondulante et de la troisième membranelle. Ses cinétosomes se divisent et forment d'irrégulières stries transversales. En même temps le champ oral formé par la cinétie stomatogène augmente (Pl. I 5).

Toute la zone adorale des membranelles se transforme successivement en d'irrégulières stries transversales et, en même temps, le champ oral s'allonge vers le haut. En conséquence un grand champ anarchique se forme qui s'étend sur presque toute la longueur du corps de l'animal. A cette phase de division la ressemblance entre *Uronema marinum* et *Cyclidium* est frappante (Pl. I 6).

Ensuite, l'uniforme champ anarchique commence à se différencier: la partie centrale disparaît et les stries des parties extrêmes s'accumulent; chez le proter elles forment de distincts grossisements aux endroits où se déve-

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lopperont les futures membranelles, chez l'opisthe elles forment trois stries épaisses transversales (Pl. I 7).

Au cours de ces changements une nouvelle phase très distincte et caractéristique apparaît, où l'on voit chez le proter les contours déjà clairs, bien qu'encore irréguliers, de la zone adorale des membranelles pendant que chez l'opisthe une épaisse strie verticale se forme à la place de la membrane ondulante future ainsi que trois semblables stries transversales (Pl. II 8—11). Donc nous avons ici de nouveau une image identique que chez *Cyclidium*. Cette phase dure probablement assez longtemps parce qu'elle se répète souvent parmi les exemplaires argentés. Les deux individus possèdent déjà le cytopyge. Ensuite la phase finale arrive: les trois stries transversales de l'opisthe se déplacent et reconstruisent les membranelles. Le corps du Cilié commence à s'amincir, les restes du champ anarchique disparaissent et enfin les deux individus reconstruisent la zone adorale des membranelles (Pl. II 12).

Pour arriver à des conclusions plus générales il faut d'abord comparer les resultats obtenus avec la morphogenèse chez des espèces plus primitives des *Tetrahymenina* d'une part et chez *Cyclidium* de l'autre. Gelei a observé la morphogenèse chez *Glaucoma scintillans* Ehrbg. et Frankel chez *Glaucoma chattoni* Corliss. Or, chez la première espèce c'est la cinétie qui joint

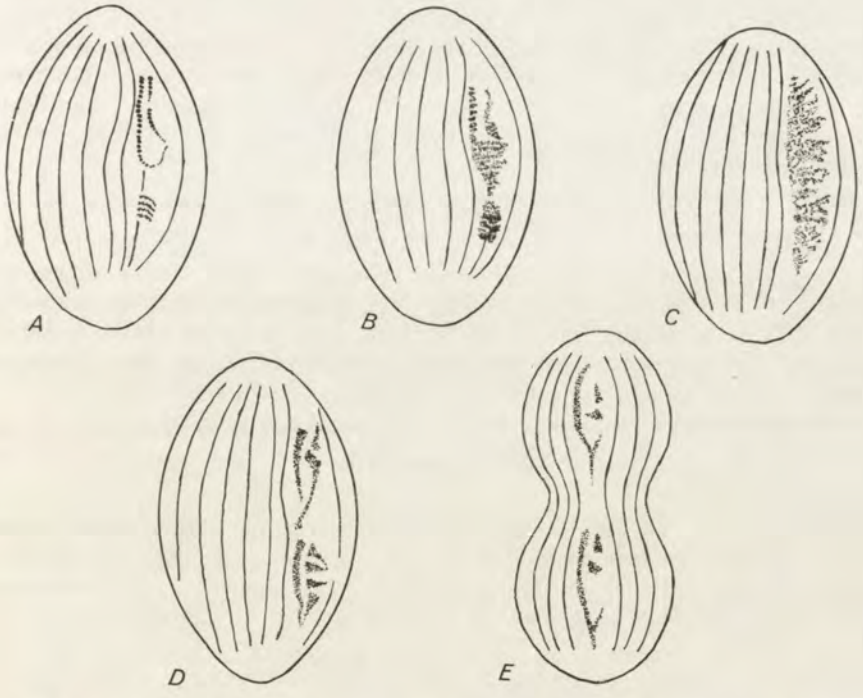

Fig. 1. Le schémat de la stomatogenèse chez *Uronema marinum*: A. La formation du champ oral, B. La désorganisation de l'AZM du proter, C. La formation du champ anarchique, D. La phase de trois stries, E. La reconstruction de l'AZM des deux individus

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le cytostome au cytopyge qui est stomatogène, chez l'autre c'est la cinétie 1 (la première à droite des cinéties orales). Dans les deux cas les cinétosomes de la cinétie stomatogène au-dessous de sa partie équatoriale commencent à se multiplier en formant de courtes rangées transversales qui, d'après F r a n k e l, se plient vers le bas. Les cinétosomes dans ces rangées continuent à se multiplier de telle façon, que les rangées transversales se transforment en longitudinales; cette transformation est continue et à aucun stade le champ oral n'est anarchique. Ensuite les rangées longitudinales reconstruisent les membranelles. Pendant toute la morphogenèse l'appareil buccal du proter ne subit aucun changement. Chez *Cyclidium* c'est la membrane ondulante (UM), qui est stomatogène; elle forme un grand champ anarchique, qui s'étend sur presque toute la longueur du corps. Ce champ passe ensuite par la phase de trois stries pour reconstruire enfin la zone adorale des membranelles chez les deux individus.

Il resulte de cette comparaison que la stomatogenèse chez *Uronema marinum* unit les élements de deux cas décrits plus haut. Elle commence comme chez *Glaucoma scintillans* à partir du court meridian situé entre le cytostome et le cytopyge qui forme le champ oral composé de stries transversales. Les cinéties dans ces stries continuent à se multiplier; en même temps l'appareil buccal du proter disparaît et enfin un grand champ anarchique se forme, analogue à celui du *Cyclidium*. Les phases finales de la stomatogenèse ont un cours identique chez les deux espèces.

Si l'on prend en considération la ressemblance anatomique très nette entre *Cyclidium* et *Uronema marinum* et le type sans doute intermédiaire de la morphogenèse chez cette dernière espèce, on en vient à conclure, que l'espèce *Uronema marinum* peut être tenue pour lien unissant les groupes *Tetrahymenina* et *Pleuronematina*.

Résumé

Le travail contient la description de la ciliature chez *Uronema marinum* ainsi que de sa stomatogenèse. L'auteur conclut que le cours de la morphogenèse chez cette espèce représente un type intermédiaire entre la morphogenèse des *Tetrahymenina* et celle des *Pleuronematina*, en considérant ainsi *Uronema* en tant que forme intermédiaire entre ces deux groupes.

STRESZCZENIE

Opisano budowę układu rzęskowego *Uronema marinum* oraz proces stomatogenezy. Autorka dochodzi do wniosku, że przebieg morfogenezy u tego gatunku reprezentuje typ pośredni między morfogenezą *Tetrahymenina* a *Pleuronematina* i zatem uznaje *Uronema* za ogniwo pośrednie między tymi grupami.

BIBLIOGRAPHIE

C z a p i k A. 1963: La morphogenèse du Cilié *Cyclidium citrullus* Cohn *(Hymenostomatida, Pleuronematina)*. Acta Protozool. 1, 5—11.

F i n l e y H. 1930: Toleration of fresh water protozoa to increased salinity. Ecology 11, 337—347.

http://rcin.org.pl

Frankel J. 1960: Morphogenesis in *Glaucoma chattoni*. J. Protozool. 7, 362—376.
Gelei J. 1935: Der Richtungsmeridian und die Neubildung des Mundes während und ausserhalb der Teilung bei den Ciliaten. Biol. Zentralbl. 55, 436—445.
Kahl A. 1930: Wimpertiere oder *Ciliata (Infusoria)*. Die Tierwelt Deutschlands.
Liebmann H. 1951: Handbuch der Frischwasser- und Abwasserbiologie.
Párducz B. 1939a: Körperbau und einige Lebenserscheinungen von *Uronema marinum* Duj. Arch. Protistenk. 92, 283—314.
Párducz B. 1939b: Verwandschaftliche Beziehungen zwischen *Uronema* und *Cyclidium*. Bau und Lebensweise von *Cyclidium glaucoma*. Arch. Protistenk. 93, 185—214.

EXPLICATION DES PLANCHES I — II

1: *Uronema marinum* Duj., vue du coté ventral
2—4: La première phase de la stomatogenèse: la formation du champ oral
5: AZM du proter commence à se désorganiser. Le champ oral augmente
6: Le champ anarchique s'étend sur presque toute la longueur du corps
7: Le champ anarchique commence à se différencier. La partie centrale disparaît. Chez l'opisthe (en haut trois stries transversales se forment.
8—11: La phase de trois stries. AZM du proter est presque reconstruite
12: Le corps s'amincit; l'AZM de l'opisthe est reconstruite

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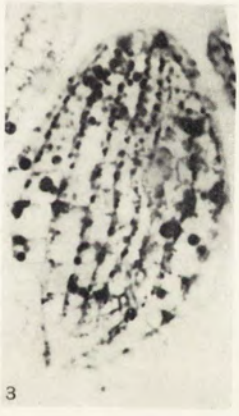
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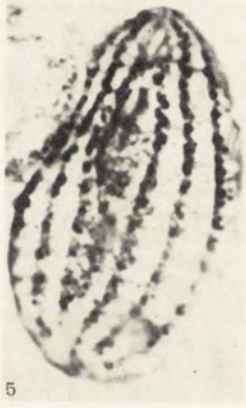
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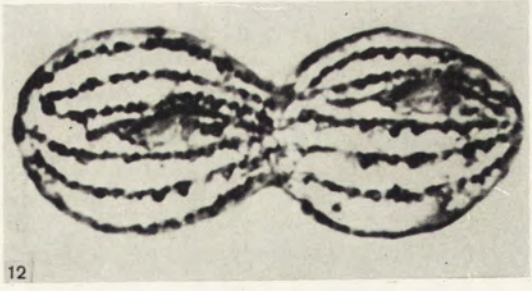
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Studies on the representatives of the family *Paraisotrichidae* Da Cunha *(Ciliata, Trichostomata).* I. Somatic infraciliature in the genus *Paraisotricha* Fior. and *Rhizotricha* g. n.

Badania nad przedstawicielami rodziny *Paraisotrichidae* (Da Cunha (*Ciliata, Trichostomata*). I. Infraciliatura somatyczna rodzaju *Paraisotricha* Fior. i *Rhizotricha* g. n.

The present information on the somatic and adoral ciliature in the representatives of the genus *Paraisotricha* Fiorentini is rather scarce. It is comprised in the publications of Fiorentini 1890, Dogiel 1929 and 1930, Hsiung 1930 a and b, Strelkov 1939. Those results might be briefly summarized as follows. In all the species of the genus *Paraisotricha* cilia run in meridional rows (their number is different in any species) more or less spiralized. The ciliary rows lie in grooves and meet at the apical pole above the oral opening and also on the antiapical pole near cytopyge. Some of those rows terminate on the ventral margin of the peristome (Hsiung 1930 b). In *Paraisotricha beckeri* Hsiung, each of the ciliary rows is accompanied laterally by rows of "bristles" (Strelkov 1939). On the apical pole, above and below the buccal opening, the cilia are longer. According to Dogiel 1929 those cilia are really disposed in single transverse rows. It might be concluded from the descriptions of Hsiung and Strelkov that the longer cilia belong to the segments of somatic kineties of the buccal region but there is no detailed information clearly proving this interpretation. Cilia prolonge their course to cytopharynx, according to some authors, or to the oesophagus, according to the others.

In the present descriptions the terminology of Corliss 1955 is followed because his terms are clear and exactly defined, excluding ambiguity and misunderstanding.

The present study of the infraciliature has been initiated for determining the character of the buccal and somatic ciliature in some species of the genus *Paraisotricha,* since the infraciliature reflects the pattern of the ciliary system.

History of the genus *Paraisotricha*

Fiorentini 1890 based the new genus *Paraisotricha* on the description of 6 species from the horse coecum, namely: *Paraisotricha colpoidea, P. oblonga, P. ovalis, P. triangularis, P. ampulla* and *P. incisa.*

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In the generic diagnosis, the following characters are pointed out by Fiorentini: the non-contractile body covered completely by delicate cilia, a tuft of long cilia present over the pigmented mass and over the transparent zone, long ciliary tufts in the peristome, presence of the anal opening.

Five of the species sygnalized by Fiorentini proved to be identical with the typical species *P. colpoidea* or were included into other genera by later authors.

Bundl 1895 recognized *P. incisa* as a synonym of *P. colpoidea*. *P. incisa* occurs exceptionally rarely, according to Fiorentini, and differs from *P. colpoidea* only in the outline of the anterior part; this character was recognized by Bundl for a deformation of *P. colpoidea*. This opinion was supported by Szwayer 1900, and subsequently, by Hsiung 1930 and Strelkov 1939.

Gassovskij 1918 stated that the species *P. ovalis* and *P. triangularis*, showing no differences in their essential characters, fail to correspond to the diagnosis of the genus *Paraisotricha* Fiorentini and he included them therefore to the genus *Holophryoides* Gassovskij. This opinion was shared by Hsiung 1930 and Strelkov 1939.

Paraisotricha oblonga differing from the typical species by the triangular posterior body part, was recognized by Hsiung 1930 as a deformed *P. colpoidea*. The reversible deformation of the posterior body end in *P. colpoidea* (occurring under the action of some not detectable factors) was observed by Hsiung in the living protozoa.

P. ampulla, not corresponding to the diagnosis of the genus, was excluded by Hsiung 1930 from the genus *Paraisotricha*. This author suggested a new genus *Ampullacula* Hsiung for this species.

Bundl 1895 described a new species *P. truncata*. Hsiung 1930 observing the living specimens of *P. colpoidea* stated that in certain conditions they may be deformed and then assume temporarily the shape of *P. truncata* Bundl. For that reason Hsiung recognized *P. truncata* for synonym of *P. colpoidea* and Strelkov 1939 supported the adequacy of this alteration.

Da Cunha 1915 described two new species of the genus *Paraisotricha* from coecum of *Hydrochaerus capibara*: *P. hydrochaeri* and *P. acuminata*. Obviously, those species should not be included into the genus *Paraisotricha* Fiorentini being both deprived of the vacuole with concretions ("massa pigmentata con zona jalina" of Fiorentini, "Konkrementenvakuole" of Dogiel 1929) characteristic for this genus. This was noticed by Buisson 1923 b. Strelkov 1939 stated that the ciliates found by Da Cunha 1915 in *Hydrochaerus capibara* and included to the genus *Paraisotricha* should be included to another genus and to another family since in one of these species the vacuole with concretions is located in the middle part of the body — whereas for the genus *Paraisotricha* its terminal position is characteristic — and in the second species it fails to occur at all. Although the names of species are not cited by Strelkov, it is clear that he means *P. hydrochaeri* and *P. acuminata*. His description of the concretion vacuole position in one of the species is rather surprising because this structure is absent in both species.

Dogiel 1929 separated a new species *P. magna* from *P. colpoidea* on the ground of its big dimensions, when compared with those reported by Bundl

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1895, however they did not surpass the dimensions stated by Fiorentini for *P. colpoidea*. This confusion was corrected by Hsiung 1930 who considered the term *P. magna* Dogiel as a synonym of *P. colpoidea* Fiorentini.

Hsiung 1930b described a new species *P. minuta* of smaller dimensions than *P. colpoidea* and of a small number of ciliary rows, being only 20, whereas in *P. colpoidea* — according to Hsiung — they occur in the number 34—40. The synonym of the term *P. minuta* Hsiung would be *P. colpoidea* Dogiel. Strelkov 1939 discussing the synonymics of the species *P. colpoidea* and *P. minuta* agreed with the opinion of Hsiung and suggested that in the material of Fiorentini both species occurred, and were recognized by this author as a single one.

Hsiung 1930a described also another species *P. beckeri*, differing from the previous species in the number and pattern of ciliary rows and in the position of the peristome. There are only 11 rows, more twisted than in the former species and the direction of their spiralization is also different. The peristome is shifted towards the apical pole. Thus, in the genus *Paraisotricha* Fiorentini 1890 only 3 species were left as valid: *Paraisotricha colpoidea* Fiorentini, 1890, *P. minuta* Hsiung, 1930 and *P. beckeri* Hsiung, 1930.

The present study concerns these 3 species. The detailed morphological analysis of their representatives inclined the present author to recognize the two first species in the genus *Paraisotricha*, and to exclude the third — *P. beckeri*, establishing for it a new genus *Rhizotricha* g. n.

Material and methods

The representatives of the genus *Paraisotricha* are abundant in the coecum and colon of the horse, together with other ciliates of the class *Holotricha* and the order *Entodiniomorpha*. Strelkov 1939 reported the occurrence of two species: *P. colpoidea* Fiorentini and *P. minuta* Hsiung in the ass as well.

The material of the present study was provided from the content of the horse coecum. The samples were taken in the slaughterhouse of Łódź in course of slaughter. After being transported in a vacuum flask to the laboratory, a part of the material was fixed at once, another part was preserved in the thermostate till next day.

In the horses of the Łódź district, mostly the genus *P. colpoidea* occurred (approx. 50% of samples), *P. minuta* was less frequent (approx. 30%); the most rare was *P. beckeri* (in two out of 60 horses examined). For examination, before all 8 samples were chosen, in which the genus *Paraisotricha* was most abundant and the dividing individuals occurred.

For revealing the infraciliature, the combination of the method of Chatton and that of Fernandez Galiano was applied. This procedure was tried previously for *Balantidium coli* (Wolska 1963). In the latter case, structures of a trichit character appeared; kinetosomes were impregnated less intensely. For *Paraisotricha* this method proved to be more effective: kinetosomes appeared very distinctly, as well as fibrous structures in the peristome. Similar good results gave the application of the Bielschowsky's reagent in the preparations coated with a film of gelatine after Chatton. Living ciliates were observed under microscope with phase contrasting optics.

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For preparations, as well as for observation of living material, carefull cleaning from the non-digested food mass and densification of the ciliates sample was essential. The fixed material was filtrated, sedimented and centrifuged. The living material could not stand those manipulations, executed in the room temperature and in aerobic conditions. Nevertheless, it was possible to filtrate the intestine content through a mill-gause put in a container, covered, and placed in the thermostate. If ciliates were numerous, such a filtrate could be observed for several hours. Despite the susceptibility of the material to temperature and to the contact with air, protozoa could be observed for about 1 hour because the coverslip protects them from air and the illumination of the microscope delays the fall of temperature.

A suitable densification and cleaning of a sample of living protozoa was succeeded several times by improving their galvanotaxis. The essential condition for this manner of densification of material was its good viability. Samples were often obtained in which the ciliates promptly died and were not fit for vital observation; such material had to be fixed immediately. Viability of ciliates depends certainly on the viability of the host; the horses brought for slaughter are in rather bad form; they are sometimes kept in slaughter-house for several days being then scarcely fed. In the rather unfrequent instances of a full vitality of ciliates, a circuit of direct current was applied joined with a small cuvette measuring 12×2×2 cm. The intestine content was poured into it, platine electrodes ware immersed near the narrow edges of the vessel and the current was made from a battery of 4.5V. The whole device was placed into a thermostate. After 4—5 min. ciliates gathered at the cathode, then they could be sampled from the upper layer of the medium. The sample was densified and free from contaminating particles which quickly fall to the bottom. Nevertheless, small suspended particles cannot be avoided in this procedure.

This method of isolating protozoa from the very abundant plant material is very effective and quick. In one case *Paraisotrichidae* had been isolated seccessfully from a sample containing also other *Holotricha* and *Entodiniomorpha* — as being more resistant than other intestine ciliates. When the sample was kept in thermostate for several hours and then the current was applied, all other dead *Holotricha* and *Entodiniomorpha* fell to the bottom and only the living *Paraisotricha* were sampled from the upper layer of medium near the cathode. A prolonged action of the current (over 10 min.) caused death of all the protozoa in the experiment.

For preparations, the purified, densified and dehydrated animals were transferred to benzene and centrifuged. Paraffin in which protozoa were to be embedded, was poured on a watch glass and frozen. In the solidified paraffin a small hollow was made. Ciliates were introduced into it with a pipette together with traces of benzene, the hollow was covered by delicate crumbs of paraffin and the watch glass was placed in the thermostate. A few hours later, paraffin was frozen together with the ciliates and a small block containing the material was cut out and put into another paraffin bath, on a wath glass, and placed in the thermostate again. This procedure permits to avoid the dispersion of ciliates over the glass; they remain collected in the centre. After the second freezing, paraffin was removed from the glass (it may be easily removed when the glass is very clean and smeared with

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glycerol) and the block ready for sectioning was cut out. Sections 5—6 μ thick were executed. A part of sections was stained with iron haematoxylin, the other one coated with a gelatine film and impregnated with silver in the same manner as the whole preparations.

Measurements were executed on silver impregnated preparations. In the present study individuals with no division changes in infraciliature were chosen for measuring.

Results

Paraisotricha colpoidea Fiorentini, 1890

The body is elliptic, more narrow at its anterior end, the broadest in the middle length (Fig. 1 A, Pl. I 1), circular in the cross section (Pl. III 11). The anterior body end is somewhat distinctive because of a slight constriction, running on the dorsal, lateral and ventral side and by the oblique course of the peristome margin (Figs. 1 B, 2 A, B and Pl. I 2, II 5—6). A similar structure in *Paranassula brunnae* Fabre-Domergue is called by Fauré-Fremiet 1962 "balanization". Balanus is occupied by the vacuole with concrements ("Konkrementenvakuole" — Dogiel 1929). A thin layer of transparent ectoplasm contrasts distinctly with the granular endoplasm containing tiny food particles and more or less numerous bodies apparently identical with the concretions in the "Konkrementenvakuole". Dogiel 1929 suggested that the concretions from the vacuole may penetrate into cytoplasm. The big ovoid macronucleus has no definite position; it mostly lies centrally, being often shifted to the anterior or posterior pole. The orientation of the main Ma axis related to the main body axis is various as well. The small round micronucleus is sometimes intruded into a depression of Ma in the median part. The contractile vacuole is near the posterior pole. The cytopyge occupies a terminal polar position. The buccal cavity initiates on the boundary of balanus and sinks down into the cytoplasm as a deep cone-shaped crooked sac, its peak reaching the middle body length or farther. The ventral margin of the peristome runs obliquely lowering from left to right (Fig. 1 B, 2 A). The body dimensions are in the limits: length 47—84 μ, width 24—53 μ. The ratio: length/width is variable; elongated slender forms occur as well as some shortened and rounded.

Nearly all the somatic meridional kineties, slightly spiralizing, initiate on the posterior pole n the region of cytopyge and run towards the anterior pole. On the dorsal side, near the anterior pole, the kineties of the left and right side meet in the suture (Fig. 1 B, 2 A, B, Pl. II 5—6). The suture passes over the peak and runs down slightly ventrally. Several kineties terminate on the ventral side before reaching the ventral margin of the peristome; those are the postoral kineties (Fig. 1 B, C, Pl. I 2—3). On the ventral side, lower than the preoral suture and over the peristomal depression, a group of short and densely set kineties covers the preoral area as a crescent-shaped structure (Fig. 1 A, B, C, Pl. I 1—3 and others). Above the ends of postoral kineties, densely set rows of short kineties run towards the margin of the peristome forming a collar which covers the marginal area (Fig. 1 B, C, Pl. I 2—3). A part of those kineties prolongate over the internal wall of the peristome, others (on the left side) terminate on the surface, on a slight convexity. On the right margin of the peristome, leftwards from the kinety 1, runs a short paroral

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kinety (Figs. 1 C, 2 A, Pl. I 3, II 5). Between the kinety 1 and 2, three short interstitial kineties reach as far as the crescent-shaped area; between the kineties 2 and 3, between 3 and 4 etc. as far as the kinety 9 or 10, single interstitial kineties are seen (Fig. 1 B, 2 A, B, and Pl. II 5—6). This highly differentiated ciliature needs a more detailed discussion.

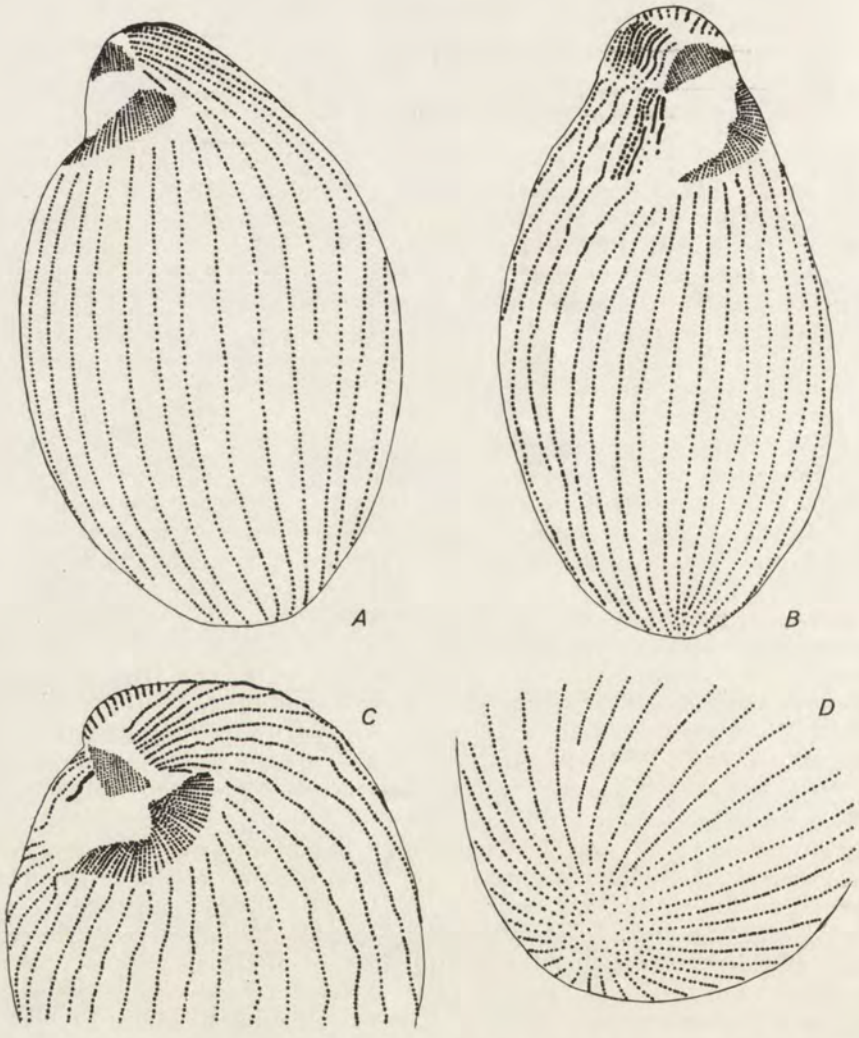

Fig. 1. *Paraisotricha colpoidea* Fior. A. General view from the left, B. From the right side, C. Anterior body part from the left side, D. Posterior part of the body (× 1500)

The number of meridional kineties fluctuates within broad limits from 28 to 50. The extreme values occur rarely, the numbers little over thirty are the most frequent. The first kinety lying to the right of the postoral kine-

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ties, running along the peristome margin, begins on the antiapical pole near the cytopyge, occasionally at a certain distance from the cytopyge. Its anterior end reaches the crescent-shaped area. The segment of the kinety 1, on the level of the peristome margin is sometimes interrupted or its kinetosomes are distinctly located more loosely. The varying aspect of this segment is represented in Fig. 1 B, 2 B, D and Pl. II 9. In the nearest vicinity of the crescent-shaped area, the kinetosomes of this kinety often closely adhere to one another. The kinety 2 is distinguished by its length: as a rule it fails to reach the posterior pole, and initiates at a certain distance from it (Fig.

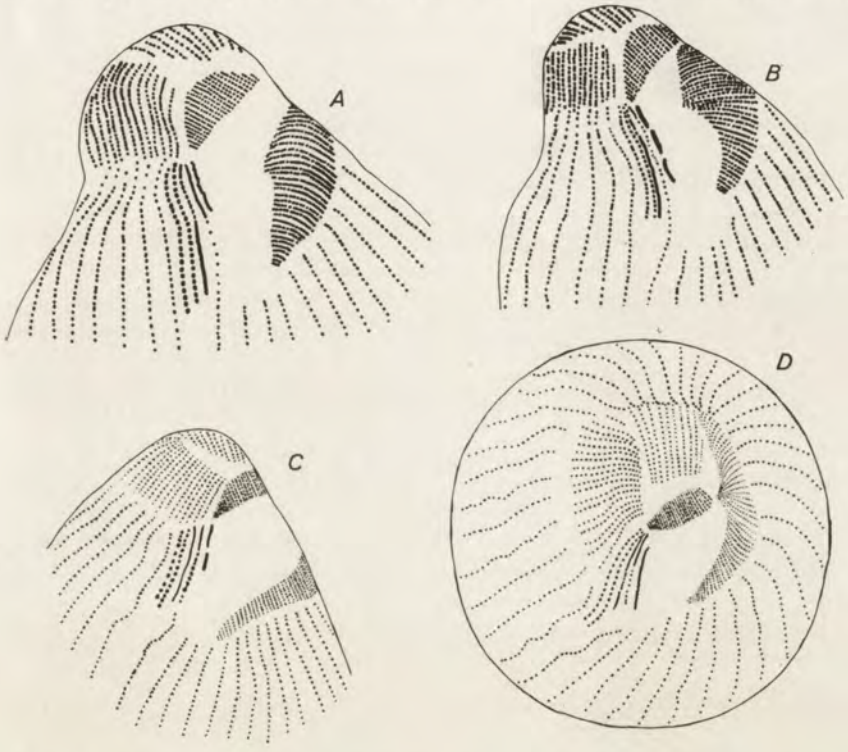

Fig. 2. *Paraisotricha colpoidea* Fior. A—C. Anterior body part from the right side, D. The view from the anterior body pole (A × 1750, B — D × 1500)

1 B, Pl. I 2). Sometimes one or two kineties more begin at a certain distance from the posterior pole but this distance is rarely so long as in the case of the kinety 2. A rare case of a very short kinety on the left body side is represented in the Fig. 1 A.

On the posterior pole, all the kineties show a certain disturbance of the regularity of kinetosomes pattern (Fig. 1 D and Pl. I 4). Between the kinety 1 and 2, three interstitial kineties are lowered to the level of the ventral margin of the peristome. The interstitial row nearest the kinety 1, in different spots along its length, has kinetosemes in different degree approximated to one another (Fig. 1 B). Sometimes the kinetosomes closely adhere to one another

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along the whole course, producing a continuous line on silver impregnated preparations (Fig. 2 C). On two other interstitial rows, kinetosomes are very big, never coalesce and are set at regular distances (Fig. 1 B, 2 A and Pl. I 2, II 5 and 9—10). The triad of short kineties on the right margin of the peristome, scarcely noticeable in living material, appears very distinctly (as prove the photomicrographs) in silver impregnated preparations and is a very characteristic structure. Interstitial rows between the kineties of the right side (from 2 to 9 or 10) are shorter and reach the suture on the right side, together with the kineties between which they are placed.

The bipolar kineties enclosing the inserted ones, are either continuous (Pl. II 7) or their continuity is lost on the level of the insertions ends or the kinetosomes pattern is rarified only behind the insertions (Fig. 1 B, 2 A, B and Pl. I 2, II 5—6). Further kineties on the dorsal side (their number is approx. 10, depending on the general number of kineties), show no gapes except for one or two nearest postoral kineties (Fig. 1 A, C and Pl. I 1 and 3). These ten kineties reach the suture on the left side. The remaining kineties (over ten) are postoral kineties. Three or four of them (from the kinety n to the kinety n−2 or n−3) terminate on the free margin of the peristome. The term "free margin of the peristome" denotes the margin without the short kineties of the collar.

The paroral kinety on the right peristome margin, shows variability: sometimes it consists of two (Fig. 1 B) or three (Fig. 2 B) segments, another time it is an uninterrupted kinety (Fig. 2 A and D). Kinetosomes of the paraoral kinety, or of the segments of the paroral kinety are very big and adhere to one another so closely that only in few cases single kinetosomes may be distinguished and in silver impregnated preparations they form a thick continuous line. This structure corresponds to the undulating membrane (UM). Kinetosomes of the frontal area and of the collar are much smaller than those of the meridional kineties and adhere to one another closely, so each of their rows corresponds to UM. They give origin to cilia which are longer than those covering the body; they had been described as a tuft or a transverse row of long cilia located above and below the buccal opening.

Paraisotricha minuta Hsiung, 1930

The body shape, position of the peristome, concretion vacuole and contractile vacuole as well as cytopyge are like those structures in *P. colpoidea*. Ma is elongated and, like in *P. colpoidea*, has no defined position. Mi adhering to one of the Ma poles. The course of kineties is essentially like in *P. colpoidea*. Body dimensions in the limits — length: 27—67 μ, width: 18—39 μ.

The most frequent number of kineties is 20, rather rarely 19 or 18 but often 21 (Fig. 3 A, Pl. IV 19), and even 24. Usually some kineties fail to reach the posterior pole (Pl. IV 18). The number of postoral kineties is 8—10. One kinety reaches the free peristome margin (Fig. 3 A, B Pl. IV 16). Interstitial rows occur between all the bipolar kineties (Fig. 3 A, Pl. IV 17). The triad of short kineties on the right side shows the same pattern as in *P. colpoidea*. The paroral kinety is of a UM character, mostly composed of two segments, The pattern of the short kineties of the frontal area and of the peristomal collar is the same as in *P. colpoidea*.

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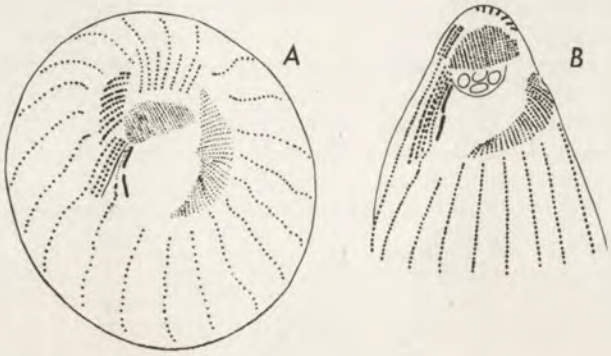

Fig. 3. *Paraisotricha minuta* Hsiung. A. The view from the anterior body pole. B. The anterior part of the body (× 1500)

Rhizotricha beckeri (Hsiung, 1930)

"Paraisotricha" beckeri differs distinctly from the preceding species. It may be recognized in vivo at once owing to its body shape, to rows of cilia which are distinctly marked, not numerous, strongly spiralized, and to very long and strong cilia of the anterior body end (Pl. V 21, VI 28). The ellipsoidal body, rounded on its posterior pole, is narrowing rather abruptly on the anterior pole. Its maximal width is at a somewhat higher level than the middle body length. The cross section of the body is circular. On the anterior pole lies the concretion vacuole. The concretion grains are bigger and not so numerous as in *P. colpoidea*. One contractile vacuole — situated in the posterior part of the body. A big ovoid Ma has no defined position; sometimes its long axis is oriented vertically in respect to the long body axis. Mi is pressed against the Ma pole. Cytopyge in terminal position in the posterior body part. Peristome located so that its upper margin crosses nearly the anterior pole (Pl. V 21—22).

Dimensions of the ciliate body are given, as derived from the measurements of two samples. In one of the two horses shorter forms occurred — length: 50—70 μ, width: 34—42 μ; in the other one, slender forms were found — length: 66—95 μ, width: 31—42 μ.

The strongly spiralized kineties lie in deep grooves with elevated margins. Grooves with kineties are rigid structures upon which the pellicle is stretched (Pl. V 25, VI 27). The number of kineties is most often 11. Hsiung 1939 b reported it as the highest. In the present investigations some individuals were found with 12 (Pl. V 25) and sometimes 10 kineties. Strelkov 1939 also cited a few cases when the number of kineties was 10. Kineties begin on the posterior pole at a variable distance from cytopyge and meet in the short suture reaching the peristome on the dorsal side. On the anterior pole, between all the bipolar kineties up from the kinety 2, two short interstitial rows are seen (Pl. VI 26, 29). Below the inserted rows the kineties loose often their continuity. The four postoral kineties approach closely to the peristome margin (Pl. V 21, VI 29). On the right superior margin of the peristome, a group of short kineties occurs in the form of a narrow zone (Fig. 4 A, B and Pl. V 23).

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Their position corresponds to that of the frontal cilia in *P. colpoidea*. Rows of delicate peristomal kineties pass over the inner left and ventral wall of the peristome. The kinety 1 is interrupted before reaching the short kineties of the superior peristome margin (Fig. 4 A, B and Pl. VI 29, 30, 31). The triad of kineties lying between the kinety 1 and kinety 2, shows a regular pattern: all these kineties are of the same length, composed of kinetosomes equally

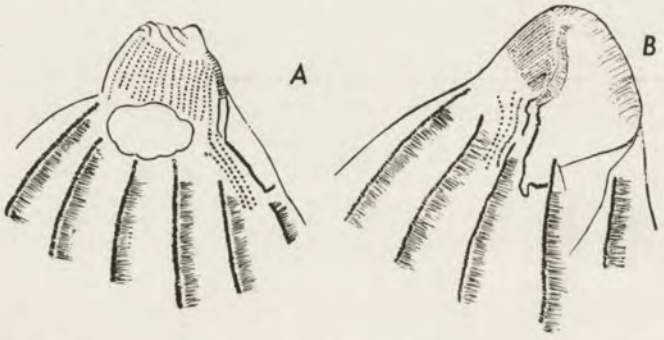

Fig. 4. *Rhizotricha beckeri* (Hsiung); the anterior part of the body (× 1500)

large; in the kinety nearest the 1st one, kinetosomes are packed more densely, The continuous paraoal kinety, of a UM-character, has a wavy course (Fig. 4 B and Pl. VI 31). "Bristles" on the sides of every kinety as described by S t r e l k o v 1939 were not stated. Strongly impregnating fibers, running sideways (left) from the kinetosomes, vertically to the direction of the kinety — were found. Fibers have an undulating course and terminate freely in cytoplasm, reaching approx. half the distance between the kineties.

Discussion

"Paraisotricha" beckeri Hsiung differs very much from the type species *Paraisotricha colpoidea* Fiorentini and from closely related species — *Paraisotricha minuta* Hsiung. The characters differing *P. beckeri* Hsiung from the typical species of the genus *Paraisotricha* are as follows: 1. shape of the body, 2. the highly reduced number of kineties and their distinct spiralization, 3. presence of two interstitial kineties between the anterior segments of the bipolar kineties, 4. the margin of the peristome with no kineties forming the so called collar, 5. the reduction (in part) and different position of the so called crescent-shaped area, 6. presence of transversal fibers accompanying the kineties on the left side.

Existence of such conspicuous differences, especially of the new element: the fibers connected with kinetosomes, justify the separation of the species *P. beckeri* Hsiung from the genus *Paraisotricha* Fiorentini and establishing a new genus for it. The generic name *Rhizotricha* gen. novum is suggested.

Genus *Rhizotricha*, genus novum

Ellipsoidal body, rounded on the posterior pole, narrowed on the anterior. On the anterior pole a vacuole with concretions, near the posterior pole the contractile vacuole. The big ovoid Ma with no constant position., Mi adhering

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to Ma. 10—12 ciliary rows in deep grooves, strongly spiralized, with fibers running leftwards. Between the superior segments of ciliary rows 2 short interstitial rows. Three interstitial rows on the right peristome margin. Superior margin of peristome passes near the anterior pole. Peristomal cilia long, protruding over the anterior body pole as a tuft. Zone of short ciliary rows above the superior right margin of peristome. On the posterior right peristome margin the paroral kinety.

The type species *Rhizotricha beckeri* (Hsiung, 1930).

Genus *Paraisotricha* Fiorentini 1890, emend.

Ellipsoidal body with the distinctive anterior part, round in cross section The anterior part is occupied by the vacuole with concretions, contractile vacuole on the posterior pole. Ovoid Ma with no stable position, Mi pressed against Ma. 18—50 rows of cilia in narrow groves, slightly spiralized. Between the superior segments of all or only some of those rows, one short interstitial row. Three interstitial rows on the right margin of the peristome. Ciliated peristome in subapical position. Above the peristome a zone of long frontal cilia. On the left and ventral margins of the peristome short rows of long cilia. On the right margin of peristome the paroral kinety.

The type-species *Paraisotricha colpoidea* Fiorentini, 1890.

Summary

A detailed description of the somatic infraciliature in the species: *Paraisotricha colpoidea* Fiorentini, *Paraisotricha minuta* Hsiung and *"Paraisotricha" beckeri* Hsiung — is reported. On the ground of distinctive characters of the infraciliature detected in the species *"Paraisotricha" beckeri* Hsiung, establishing of a new genus for this species is suggested: *Rhizotricha* gen. nov.

STRESZCZENIE

Autorka podaje szczegółowy opis infraciliatury somatycznej gatunków: *Paraisotricha colpoidea* Fiorentini, *Paraisotricha minuta* Hsiung i *"Paraisotricha" beckeri* Hsiung. Na podstawie wykrytych odrębności infraciliatury gatunku *"Paraisotricha" beckeri* Hsiung autorka proponuje utworzenie dla tego gatunku nowego rodzaju: *Rhizotricha* gen. nov.

REFERENCES

Buisson J. 1923: Sur quelques infusiores nouveaux ou peu connus, parasites des mammifères. Ann. Parasitol. 1, 209—246.

Bundle A. 1895: Ciliate Infusorien in Coecum des Pferdes. Zeitschr. Wiss. Zool. 60, 284—350.

Corliss J. O. 1955: Proposed uniformity in naming "mouth parts" in ciliates. J. Protozool. 2 (suppl), Abstr. 12.

Da Cunha A. M. 1915: Sobre os ciliados intestinaes dos mammiferos. II. Mem. Inst. Osw. Cruz. 7, 139—145.

Dogiel V. A. 1929: Die sog. "Konkrementenvakuole" der Infusorien als eine Statocyste betrachtet. Arch. Protistenk. 68, 319—348.

Dogiel V. A. 1930: Die Prospektive Potenz der Syncaryonderivate an der Konjugation von *Paraisotricha* erlautert. Arch. Protistenk. 70 497—516.

http://rcin.org.pl

Fauré-Fremiet E. 1962: Le genre *Paranassula* Kahl *(Ciliata, Cyrtophorina)*. Cahiers de Biologie Marine 3, 61—77.
Fiorentini A. 1890: Intorno ai protisti dell'intestini degli equini.. Boll. Sci. Pavia 12, 51—60.
Gassovskij G. 1918: K mikrofaunie kišečnika lošadi. Trudy Petrogr. Obšč. Estestvoispyt. 49, 20—36.
Hsiung T. S. 1930 a: Some new ciliates from the large intestine of the horse. Trans. Amer. Micr. Soc. 49, 34—41.
Hsiung T. S. 1930 b: A monograph on the *Protozoa* of the large intestine of the horse. Iowa St. Coll. J. Sci. 4, 359—423.
Strelkov A. 1939: Parazitičeskie infuzorii iz kišečnika neparnokopytnyh semejstva *Equidae*. Učen. Zap. Leningrad. Gosud. Pedagog. Inst. Gercena 17, 1—162.
Wolska M. 1963: Morphology of the buccal apparatus in *Balantidium coli* (Malmsten, 1857). Acta Protozool. 1, 147—152.

EXPLANATION OF PLATES I — VI

Paraisotricha colpoidea Fiorentini

1—2: General view from the left and right side
3: The anterior body part from the left side
4: The posterior body part
5, 6, 9, 10: The anterior body part from the right side
7—8: The anterior body part from the left side
11: The transversal section in the anterior body part
12: The view from the anterior body pole

Paraisotricha minuta Hsiung

13—14: General view from the left and right side
15: General view from the ventral side
16—17: The anterior body part from the ventral and dorsal side
18: The posterior body part
19: The view from the posterior body pole
20: The view from the anterior body pole

Rhizotricha beckeri (Hsiung)

21—22: General view from the left and right body side
23: The anterior body part from the ventral left side
24: The posterior body part
25: The view from the posterior body pole
26—27: General view from the dorsal and frontal body side
28: The anterior body part from the left side
29—31: The anterior body part from the right side
[Photomicrographs 5, 13, 18 — × 1750, all the others × 1500]

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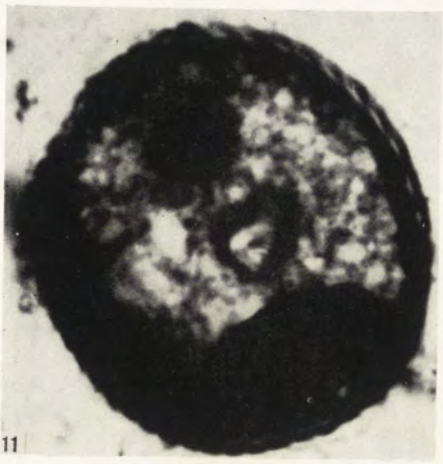
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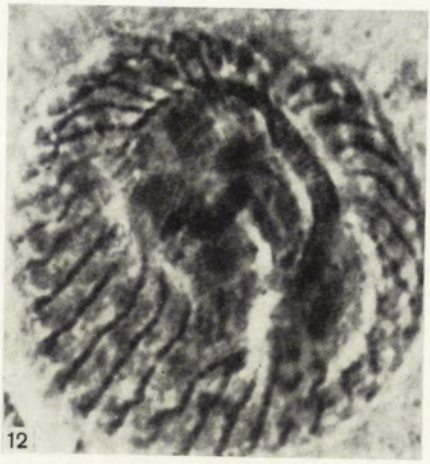
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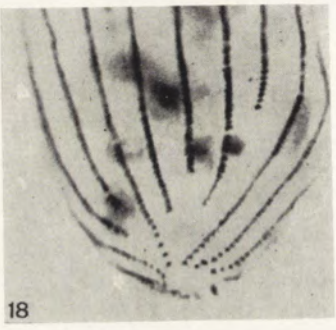
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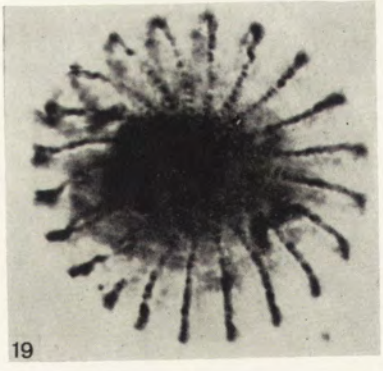
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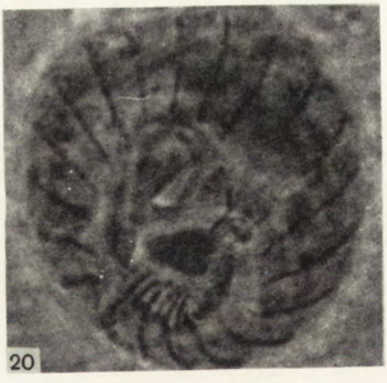
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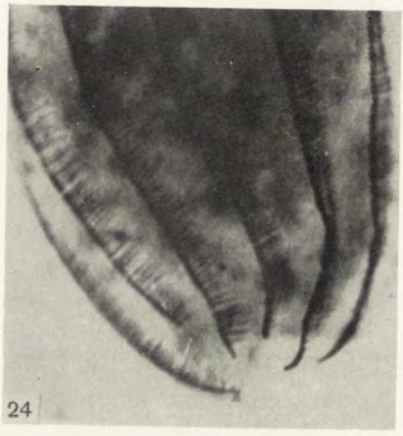
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A photometric study of DNA content in macronuclei and micronuclei of different species of *Paramecium*

Фотометрическое изучение содержания ДНК в макронуклеусах и микронуклеусах разных видов *Paramecium*

Quantitative methods of cytochemical investigations allowed to state rather precisely that macronuceus (Ma) of ciliates is a polyploid nucleus (Moses 1950, Walker and Mitchison 1957, Seshachar and Dass 1954, Woodard and oth. 1961, Ruthmann und Heckmann 1961, Dysart, Corliss and De la Torre 1962, Blanc 1962, Raikov, Cheissin and Buze 1963, Dysart 1963). Besides, ciliates were found with a very high polyploidy, as e. g. *Bursaria truncatella* (5000 n) or *Epistylis articulata* (approx. 1400 n). On the other hand it was stated that the Ma in *Loxodes magnus* is only diploid. Except these species, polyploidy of Ma was found in *Paramecium caudatum, P. aurelia, Chilodonella uncinata, Nassula ornata, Tetrahymena limacis, T. patula, T. rostrata.* In all these species Ma proved to be polyploid. In some cases polyploidy fluctuated in the limits from 20 to 100 n, in others — from 100 to 900 n.

A considerable difference is reported in the DNA content in Ma, and its polyploidy respectively, within one species (e. g. in various species of *Tetrahymena* after Dysart and oth. 1962 and Dysart 1963). The same was observed for *P. caudatum* by different authors. Some investigators stated the polyploidy of 50—60 n (Walker and Mitchison 1957), others (Moses 1950) determined it at 80 n, according to some others (Blanc 1962; Raikov, Cheissin and Buze 1963) it amounted 150—160 n.

Besides those facts, the occurrence of essential differences in the content of DNA and polyploidy of Ma may be stated in closely related ciliate species. This was reported for *Tetrahymena limacis, T. patula* and *T. rostrata* (Dysart 1962, 1963) and also for *P. caudatum* and *P. aurelia* (Raikov, Chessin and Buze 1963). Nevertheless all those informations are too scarce to draw any general conclusions about the polyploidy in different species of ciliates. In the study of Raikov, Cheissin and Buze 1963 attention was payed to the different polyploidy of Ma only in two species of *Paramecium* and it was suggested that it depends on different initial content of DNA in micronuclei (Mi). The study of polyploidy of Ma in nearly related species of ciliates was continued and in the present article results of comparative study of polyploidy in 5 species of *Paramecium* are discussed.

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Together with those investigations, the polyploidy of different strains of the same species *(Paramecium caudatum)* was studied, but this is analysed in another article (Cheissin, Ovchinnikova and Kudriavtsev 1964).

Material and methods

Strains of *Paramecium caudatum, P. woodruffi, P. calkinsi* and *P. putrinum* cultivated at 22° in the medium of Losina-Losinsky 1931 were used as material in the present study. For the subsequent examination, objects were fixed in the sublimate mixture of Nissenbaum, which sticks the ciliates to the glass. The whole ciliates were stained after the Feulgen method (hydrolysis 6 min). For the Schiff's reagent only some standard samples of basic fuchsine were used. For the subsequent photometry and comparative quantitative study of the DNA content in the nucleus, it is of an essential importance to use always the same standard samples of fuchsine.

The stained preparations were photographed under the microscope MUF-6 in the monochromatic green light (line 546 mμ). Negatives density was measured with a scanning microphotometer MF-4. This photometric methodics and the following evaluation of the results are described in details in the preceding articles (Ovchinnikova and oth. 1963, Cheissin and oth. 1963, Raikov and oth. 1963). The quantity of DNA (Q) and the area (S) are quoted in arbitrary units multiplied by 10^{-6}. In calculation of the Ma polyploidy, the quantity of DNA in Ma and Mi was determined first, and then the ratio of DNA content in both nuclei was calculated. The resulting number was multiplied by 2, presuming that the Mi are diploid. In order to get the most precise data about polyploidy, only those ciliates were used for calculation in which Ma and Mi were in the presynthetic stage of interphase. In *P. caudatum* this stage lasts approx. as long as a half of the whole interphase period. If it lasts 18 hrs. (in 22°) then during 8—10 hrs. after division no synthesis of DNA in Ma and Mi occurs (Cheissin and oth. 1963). *P.woodruffi* and *P. calkinsi* divide every 12—14 hrs., and *P. putrinum* and *P. bursaria:* 6—8 hrs. Consequently in these ciliates, the presynthetic period is much shorter than in *P. caudatum.*

No less than 25 Ma and an equal number of Mi were measured for every species. If several Mi were present in one ciliate, the content of DNA was measured in all the nuclei.

The authors express their gratitude to A. W. Jankowski who supplied the clones of *P. putrinum, P. calkinsi* and preparations of *P. bursaria* after Feulgen stained, for photometric examination.

Results

The nuclear apparatus of all the investigated species of *Paramecium* consists of one compact Ma and of one or several Mi.

The biggest of the investigated ciliates were *P. caudatum, P. woodruffi* and *P. bursaria.* As seen in the Table 1, the area of their body is slightly larger than it is in *P. putrinum* and *P. calkinsi.* Especially big were *P. caudatum* of

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the strain 135, whereas the ciliates of the strain B failed to differ by their dimensions from *P. bursaria* and *P. woodruffi*. The same may be said on the dimensions of Ma. It is in *P. caudatum* of the strain 135 about twice larger than in the strain B and in *P. woodruffi* (Table 1, Pl. I). In the three species (*P. bursaria, P. putrinum* and *P. calkinsi*) the area of Ma did not exceed in average 2.0 $cm^2 \times 10^{-6}$, whereas in *P. caudatum* and *P. woodruffi* it was over this value (Table 1). In this way, the ratio of the body area to the Ma area, compared in different species of *Paramecium* shows the highest value for *P. bursaria* (28), then the three species (*P. putrinum, P. caudatum* strain B, *P. woodruffi*) have approximately the same coefficients (18, 17, and 15), and finally *P. caudatum* strain 135 and *P. calkinsi* have the lowest value of this ratio (11 and 12).

Table 1

Degree of the Ma polyploidy (× n), quantity of DNA ($Q \times 10^{-6}$), areas of the presynthetic Ma, presynthetic Mi and the body ($S \times 10^{-6}$ cm^2) in different species of *Paramecium*

Species	×nMa	Q Ma	S Ma	Q Mi	S Mi	$\frac{S\ Ma}{S\ Mi}$	S body	$\frac{S\ body}{S\ Mi}$	$\frac{Q\ Ma}{QMi}$
P. woodruffi	882	3.53	2.60	0.008	0.02	130	38.3	1915	441
P. calkinsi	560	1.40	1.78	0.005	0.03	59	21.6	720	280
P. caudatum strain B	160	1.47	2.44	0.019	0.08	30.5	42.0	525	80
P. caudatum strain 135	88	6.10	6.0	0.14	0.32	20.0	65.6	218	44
P. putrinum	24	1.50	1.61	0.13	0.18	8.9	30.2	167	12
P. bursaria	16	0.98	1.68	0.12	0.24	7.0	45.2	188	8

As to the structure of their micronuclei the first two species shown in the Table 1, belong to the *"aurelia"* type (Wichterman 1953). Their relatively small Mi is vesicular. According to our findings the Mi area of those species is in average 0.02—0.03 $cm^2 \times 10^{-6}$ and relation of Ma to Mi area is over 50, as follows from the Table 1. This ratio is especially high in *P. woodruffi* because of the large dimensions of its Ma when compared with those in *P. calkinsi*. In the representatives of the *"caudatum"* type to which all the other species studied belong, Mi is compact and its area is approx. 10 times larger than in the ciliates of the first group. In consequence, the ratio of the area of Ma to that of Mi is in the type *"caudatum"* much lower than in the representatives of the type *"aurelia"*. The extreme position, with the lowest coefficient is occupied by *P. bursaria*, with a relatively small Ma and big Mi.

The type *"caudatum"* differs from the type *"aurelia"* not in the dimensions of Mi only but also in its content of DNA. In the first group, it is much higher than in the second (Table 1), although the strain B of *P. caudatum* occupies an intermediate position with this regard. Although the area of Mi in this strain exceeds 2.5—4 times that in the representatives of the type *"aurelia"*,

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the quantity of DNA in Mi is only twice higher, whereas in other members of this group the difference was 10 times or even more.

In determination of DNA quantity in Mi, in each species of ciliates the presence of two forms of interphase Mi was stated. One form contained a small quantity of DNA whereas the other was rich in it. The high content of DNA was nearly double when compared with the minimal one in the second form of interphase Mi. Considering that in interphase the duplication of DNA in Mi occurs, it may be assumed that those nuclei with the minimal DNA content were presynthetic, i.e. contained the not doubled quantity of DNA and — evidently — the diploid set of chromosomes (the question of heteroploidy of Mi will be discussed below). Mi with a high content of DNA was included to the cathegory of postsynthetic, i.e. with a double (or near to it) quantity of DNA. In the determination of polyploidy of Ma only the presynthetic Mi were considered.

Table 2

Quantity of DNA ($Q \times 10^{-6}$) in the presynthetic and postsynthetic Mi in different species of *Paramecium*

Species	Presynthetic Mi		Postsynthetic Mi	
	Extremes	Mean	Extremes	Mean
P. woodruffi	0.005—0.009	0.008	0.01 —0.02	0.014
P. calkinsi	0.004—0.006	0.005	0.007—0.014	0.009
P. caudatum strain B	0.013—0.022	0.019	0.024—0.038	0.033
P. caudatum strain 135	0.11 —0.19	0.14	0.20 —0.27	0.24
P. putrinum	0.11 —0.15	0.13	0.17 —0.27	0.23
P. bursaria	0.10 —0.15	0.12	0.18 —0.26	0.23

Within the same strain the content of DNA in Mi is varying only slightly (Table 2), whereas in different strains of the same species (*P. caudatum*) the content of DNA in Mi may be different; e.g. in the strain B the quantity of DNA is 7 times higher than in the strain 135.

In *P. woodruffi* which has 2 or 3 Mi, the synthesis of DNA is usually synchronic in all the nuclei. Nevertheless one or two Mi have sometimes a presynthetic quantity of DNA, whereas in the third Mi the content of DNA has already been doubled. The duplication of DNA in Mi is not connected with the increase of its area which extends only then when the Mi with the postsynthetic DNA content enters the prophase.

The DNA content in Ma is not equal in different species. It was found the highest in the presynthetic Ma of the strain 135 of *P. caudatum*. Ma of these ciliates is characterized by a considerable size ($S = 6.0\ cm^2 \times 10^{-6}$) and its DNA content was twice higher than e.g. in P. woodruffi and 4—5 times higher than in the other species and in *P. caudatum* of the strain B. In *P. calkinsi, P. putrinum* and *P. caudatum* strain B the content of DNA in the presynthetic Ma amounted strictly 1.4—1.5 arbitrary units, whereas in *P. bur-*

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saria it corresponds to 0.98. The area of Ma was in all these species more or less the same (Table 1). The DNA content in Ma of *P. woodruffi* proved to be 2—2.5 higher than in Ma of other species of *Paramecium*. It should be noted that in *P. bursaria* especially, and also in *P. putrinum, P. caudatum* of the strain B and in *P. calkinsi,* Ma contains a considerable number of nucleoli. This fact accounts for its lower content of DNA than in *P. woodruffi,* although the dimensions of nuclei in all these species are the same.

The content of DNA in Ma was measured without evaluation of the stage of the interphase period. In consequence, all the individuals segregate into two groups according the content of DNA in their Ma. In the first, Ma is in the presynthetic conditions, in the second — in the postsynthetic, at various stages of genom duplication. As shown in the Table 3, Ma of the second

Table 3

Quantity of DNA ($Q \times 10^{-6}$) in the presynthetic and postsynthetic Ma, and the degree of polyploidy ($\times n$) in different species of *Paramecium*

Species	Presynthetic Ma		Postsynthetic Ma		$\frac{Q\ Ma}{Q\ Mi}$		$\times n$
	Extremes	Mean	Extremes	Mean	Extremes	Mean	
P. woodruffi	2.8—4.1	3.53	4.8—7.2	5.6	296—670	441	882
P. calkinsi	1.3—1.8	1.4	1.9—3.1	2.4	201—397	280	560
P. caudatum strain B	1.18—1.74	1 47	1 77—2.22	2.04	58—103	79.5	159 (160)
P. caudatum strain 135	5.2—7.0	6.1	8.1—11.6	10.3	35—65	43 5	87 (88)
P. putrinum	1.2—1 7	1.5	1.8—2.7	2.02	8—17	11.5	23 (24)
P. bursaria	0.7—1.2	0.98	1 3—1.9	1.48	5.7—11	8.2	16

group fails to contain the double DNA quantity, when compared to the first group. This is accounted for by the fact that the content of substance was not measured just before the division of the nucleus when the DNA content reaches its maximal value and when its duplication in all genoms is concluding. Since the initial content of DNA may be different in the individuals within the strain (C h e i s s i n and oth. 1963), consequently the content of DNA in Ma is varying. Those deviations are not considerable but they are observed in all the species studied, in a more or less expressed degree.

Let us elucidate now the problem of ploidy degree of Ma in the studied ciliate species. For that reason the DNA coefficient in the presynthetic Ma and Mi should be compared. Basing on those coefficients, as already mentioned in the article of R a i k o v and oth. 1963, the most precise ratio of quantity of DNA in Ma and Mi may be determined. In the first group of ciliates the DNA syntheses in Ma and Mi have not yet begun. In the studied material, among *P. caudatum* 40% of individuals belonged to the first group. In *P. woodruffi* such individuals amounted 33%, in *P. calkinsi* 15%, in *P. bursaria*

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7%, and in *P. putrinum* just 5% of the whole culture. The low percentage of individuals of the first group in the last 3 species, is presumably involved by the fact that they divide much faster than the first two species and their interphase period is respectively shorter. Since the DNA synthesis in Ma begins in the middle of the interphase period (Kimball and Barca 1959, Cheissin and oth. 1963), obviously the study of ciliates with a rapid rate of division and a rather short interphase period offers more possibilities of finding individuals in which the DNA synthesis has already begun. And indeed, in *P. calkinsi, P. putrinum* and *P. bursaria* with a rapid rate of division, in 80—90% of individuals Ma was in different steps of DNA synthesis, i.e. it belonged to the group of postsynthetic Ma.

Among ciliates, in which Ma had the presynthetic DNA content, individuals might be found in which Mi had postsynthetic DNA content. Such individuals were found in all the species studied in a low percentage (5—15), except *P. calkinsi.* Existence of this group of individuals proves that DNA synthesis in Mi initiates and terminates earlier than in Ma. Nevertheless, the most frequently occurring are the representatives of the third group of ciliates with postsynthetic Ma and presynthetic Mi. In other terms, in this group of ciliates, the DNA synthesis in Mi is retarded when compared to the begining of DNA synthesis in Ma. In *P. putrinum* and *P. bursaria* those individuals amounted 10—15%, in *P. caudatum* and *P. woodruffi* 20—28% and in *P. calkinsi* 40%.

Finally, there is still another group of ciliates with postsynthetic nuclei which corresponds to the second half of interphase. The Mi contains, as a rule, a double amount of DNA and Ma is on different stages of duplication of genoms (Table 3). Such individuals are especially numerous among *P. putrinum* and *P. bursaria* (79 and 67%), somewhat less numerous in *P. calkinsi* (45%) and still more rare in *P. woodruffi* and *P. caudatum* (29 and 25%). This group of individuals cannot be used for estimation of the ploidy degree because the duplication of DNA is not full in all the genoms which involves a slight fall of ratio of DNA content in Ma and in Mi (Raikov and oth. 1963), the more so as the individuals of the second and third group are incomparable because their nuclei are at various stages of DNA synthesis.

Comparing the DNA content in Ma and Mi in the individuals of the first group, it was stated that in every species the ratio of DNA content in both nuclei proves to be different but in a sufficient degree constant. The most considerable predominance of DNA quantity in Ma over that in Mi is observed in *P. woodruffi* and *P. calkinsi.* In average, it exceeds in the first species 440 and in the second 280 times, although in single individuals this ratio is varying from 296 to 670 times in *P. woodruffi* and from 207 to 317 times in *P. calkinsi,* which may be accounted for by the lack of stability of the DNA content in Ma within the strain.

The least predominance of DNA content in Ma over that in Mi was recorded in *P. putrinum* (12 times) and in *P. bursaria* (8 times).

Both strains of *P. caudatum* are intermediate between the first and second pair of species cited above. In the strain 135, the DNA content in Ma exceeds that in Mi 43.5 times and in the strain B 79.5 times in average.

If it was assumed that Mi in all these species is diploid so it might be stated arbitrarily that the degree of polyploidy of Ma in *P. woodruffi* is in

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average 882 n, in *P. calkinsi* — 560 n, in *P. caudatum* strain B — 160 n, in *P. caudatum* strain 135 — 88 n, in *P. putrinum* — 24 n, and in *P. bursaria* — 16 n. Those average coefficients are valid for these strains which were investigated in every species.

Discussion

The above results prove that in nearly related species the polyploidy degree of Ma may be very different. In paramecia, three groups of species are distinct. The first — with a high polyploidy degree (over 500 n). This group includes the species: *P. woodruffi, P. calkinsi,* and *P. aurelia* in which, after Woodard, Gelber and Swift 1961 the polyploidy is 860 n. The second group is of a middle polyploidy degree (60—200 n); *P. caudatum* (all its strains) belongs to it. The third group are the species of a low n (16—24): *P. putrinum* and *P. bursaria.* A similar picture is observed in *Tetrahymena* (Dysart, Corliss and De la Torre 1962, Dysart 1963) in which species were stated as well with a high as a middle polyploidy.

A problem arises what factor is responsible for such a difference of Ma in nearly related species. Before all it may depend on different size of Ma and its content of DNA. Those species in which Ma is the biggest and contains the highest quantity of DNA should have the nucleus of a highest ploidy. Nevertheless dimensions of Ma — estimated on ground of its optical section — are in all species studied similar (exception is the strain 135 of *P. caudatum).* The content of DNA in them (except the strain 135) shows little difference whereas the polyploidy degree is various. This is especially striking after comparison of *P. calkinsi, P. caudatum* strain B and *P. putrinum.* Besides although Ma of *P. caudatum* strain 135 is bigger than that of *P. woodruffi* and contains more DNA, yet its polyploidy is 10 times lower of that in *P. woodruffi.* On the other hand, Ma of *P. woodruffi* contains twice more DNA than Ma of *P. caudatum* strain B and 3.5 times more than Ma of *P. bursaria.*

The difference in dimensions of Ma in those species is insignificant, but the polyploidy of *P. woodruffi* is 5 times higher than in *P. caudatum* strain B and 55 times higher than in *P. bursaria.* Consequently, although the polyploidy degree is the highest in a species containing the highest DNA quantity in Ma, and the lowest in the species with the minimal DNA quantity, yet no proportional relation exists between those magnitudes, since in *P. bursaria* the DNA content is only 3.5 times lower than in *P. woodruffi* and the degree of polyploidy is 55 times lower.

The analysis of results indicates that the differences in the polyploidy degree of Ma in different species are strongly conditioned by the uneven content of DNA in Mi as well as by their size. Indeed, the highest polyploidy is observed in those species which have the smallest Mi, the lowest content of DNA in it, and the highest value of ratio: Ma area to Mi area (Table 1). The smallest Mi of *P. woodruffi* and in *P. calkinsi* are 3—10 times smaller than the Mi of *P. caudatum,* whereas the DNA content in those nuclei is 2.5—4 times lower than in Mi of *P. caudatum* strain B and 12—20 times lower than in the strain 135 with bigger Mi. The dimensions of the latter exceed 3 times the dimensions of Mi in *P. caudatum* strain B and its DNA content is 7 times higher. Similarly the polyploidy degree of the strain B is twice as high as

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in the strain 135, although Ma of the strain B in twice smaller than in the strain 135, and its DNA content is 4 times lower than in the strain 135. In this way in *P. woodruffi* and *P. calkinsi* the high degree of polyploidy (when compared to that in other species) is determined by the small Mi size, low content of DNA in it and a high coefficient of the ratio Ma/Mi areas. Nevertheless, the difference in the polyploidy degree of Ma in these two species with similar Mi dimensions depends on different DNA content in Ma. In *P. woodruffi* it is twice higher than in *P. calkinsi.*

The lowest polyploidy degree of Ma in *P. putrinum* and *P. bursaria* is entirely determined by the high DNA content in the big Mi and by the low coefficient of the ratio: area of Ma to area of Mi (Table 1 and 3). In the case of equal DNA content in Ma of *P. putrinum* and *P. bursaria* on one side, and *P. calkinsi* and *P. caudatum* strain B on the other side — Mi of the two first species contains 24—26 times more DNA than that of *P. calkinsi,* and 6—7 times more than that of *P. caudatum* strain B. This fact accounts for the difference in the polyploidy degree of Ma in the compared ciliate species. Besides — as mentioned before for *P. woodruffi* and *P. calkinsi* — in the case of equal quantity of DNA in Mi of *P. putrinum* and *P. bursaria,* a slight difference in the polyploidy degree of their Ma is evoked by a slight difference in the DNA content in Ma.

In one of our preceding articles (Raikov and oth. 1963) the view was expressed that the polyploidy degree depends on the ratio: body dimensions to Mi dimensions. So e.g. in the very big ciliate *Bursaria truncatella* the polyploidy degree reaches 5000 n (Ruthmann und Heckmann 1961). In this species, Ma is big ribbon-shaped, and Mi is very small. In paramecia which are much smaller than *Bursaria,* the degree of Ma polyploidy is not exceeding 900 n.

Comparison of different species of *Paramecium* indicates that the highest polyploidy is observed in *P. woodruffi* in which the body area many times exceeds the Mi area. Its value is in *P. woodruffi* 1915, in *P. calkinsi* 720, in *P .caudatum* strain B 525, in *P. caudatum* strain 135 218, in *P. putrinum* 167 and in *P. bursaria* 180. In this sequence falls the degree of Ma polyploidy.

The determination of the polyploidy degree of Ma is based on the comparison of the quantity of DNA in Ma with the same in Mi considering the fact of diploidy of the latter. Nevertheless some facts prove the heteroploidy of Mi in some strains of *P. caudatum* and *P. bursaria* (Diller 1940, 1948, Chen 1940). Diller found that the difference in chromosome number in different individuals of the same species may be very considerable, exceeding the diploid number. According the early observations of Calkins and Cull 1907 in *P. caudatum* 150 chromosomes were revealed. Subsequently Penn 1937 found 36 chromosomes in the same species. In different strains of *P. bursaria* Chen 1940 revealed differences in the size of Mi and in number of chromosomes. In one of heteroploid strains, the chromosomes number in the diploid set was 80, in the other strains they were several hundreds. Chen 1940 suggested that the *P. caudatum* investigated by Calkins and Cull were polyploid as to Mi.

In the material of this study, two strains of *P. caudatum* were present differing in the dimensions of Mi and in the content of DNA in it. Nothing

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could be said about the chromosomes number in these ciliates. As to the content of DNA in Mi, one strain differs from the other 7 times. It is difficult to decide whether this difference in DNA content corresponds to the chromosomes number. Presumably the strain with a higher DNA content has more chromosomes. At any rate *P. putrinum* and *P. bursaria* have similar number of chromosomes, the first 98—100 (Jankowski personal communication), another 80 (Chen 1940) or several hundreds (depending on the strain) and also similar quantity of DNA in Mi. Chen 1940 believes that in different strains of *P. bursaria* Mi dimensions correlate with the number of chromosomes. Dimensions of Mi in *P. bursaria* investigated in this study corresponded to this strain of *P. bursaria*, according the information of Chen, which had 80 chromosomes. If this is the diploid number, then Mi of our *P. bursaria* is also diploid. According to Diller 1936 *P. aurelia* has 20—30 chromosomes, and to Sonneborn 1947 their number is 30—40. Mi of this species is very small, like that of *P. woodruffi* and probably its content of DNA is lower than in those two species. Unfortunately we failed to count the chromosomes number in *P. woodruffi* and *P. calkinsi*. If the content of DNA in Mi is really proportional to the number of chromosomes, it might be asumed that in *P. woodruffi* and *P. calkinsi* a small number of chromosomes is present. The considerable difference in the DNA content in Mi of *P. caudatum* strain B and strain 135 suggests that this fact is connected with a different n number of Mi. If the strain B is considered as diploid so the strain 135 might be polyploid in its Mi and consequently the ploidy of Ma in this strain may prove to be different. To avoid the error in the determination of ploidy, it would be more correct to compare those strains considering the degree of predominance of DNA content in its pre-synthetic Ma over the pre-synthetic Mi.

It was stated in the preceding article (Raikov and oth. 1963) that the degree of polyploidy is rather little dependent on the systematical position of the ciliate. Just in the lowest ciliates, e.g. *Loxodes*, Ma is diploid which reflects the primitive state of the nuclear apparatus of those forms. In other ciliates which have been investigated with photometric methods (they are rather few: no more than 10—12 species) the n coefficients are most various but in all cases they surpass diploidy. The information concerning the polyploidy degree of nearly related species, found by the authors of the present study, indicate that species of a very high and very low polyploidy may occur even within the same genus. In the type *"aurelia"* the high polyploidy and in the type *"caudatum"* — the low polyploidy is characteristic as a rule. *P. caudatum* occupies the median position with this regard.

Since Ma is a vegetative nucleus, the differences in the polyploidy degree in nearly related species of paramecium should, as it seems, influence the physiological activity of the ciliates. Nevertheless there are no special investigations concerning this problem. It may be only noted that the same rate of division may occur as well in the species of high, as of low polyploidy. *P. aurelia* e.g. divides twice faster than *P. caudatum* and at the same rate as *P. putrinum*, although the polyploidy of the latter is much lower than that of the former.

Possibly the physiological study in this field would make the functional role of different polyploidy of the vegetative nucleus in ciliates more comprehensible.

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Summary

DNA in Ma and Mi of *P. woodruffi, P. calkinsi, P. caudatum* (strains B and 135), *P. putrinum* and *P. bursaria* was determined by photometry of Feulgen stained preparations. The size of Ma is similar in the 5 species, with the exception of the strain 135 of *P. caudatum*. DNA content is the greatest in this strain, in *P. woodruffi* it is lower, and the lowest in *P. bursaria* (the others occupy an intermediate position). The *"aurelia"* type of Mi *(P. woodruffi, P. calkinsi)* differs from the "caudatum" type not only by the structure and smaller size but also by a lower amount of DNA and a greater value of the ratio of Ma/Mi areas. Postsynthetic Mi contain about twice as much DNA as the presynthetic ones. Ma also can be presynthetic and postsynthetic. Postsynthetic Ma are found at different stages of genomic duplication. Most frequently DNA synthesis begins in Ma and in Mi it is somewhat delayed. Degree of Ma polyploidy may be estimated in the following series, since the presynthetic DNA content in Ma exceeds that in Mi 440× in *P. woodruffi* (880 n), 280× in *P. calkinsi* (560 n), 80× in *P. caudatum* strain B (160 n) and 44× in the strain 135 (88 n), 12× in *P. putrinum* (24 n), 8× in *P. bursaria* (16 n). The above calculation was made considering Mi to be diploid, but in some cases its heteroploidy seems possible. The relation of the degree of polyploidy to the differences of DNA contents in Mi, to the various size of Mi, to the ratio of body area vs. Mi area and Ma/Mi areas, are discussed in detail.

РЕЗЮМЕ

Количество ДНК в Ма и Ми 5 видов парамеций (*Paramecium woodruffi, P. calkinsi, P. caudatum* линия В и 135, *P. putrinum, P. bursaria*) определялось фотометрически на препаратах окрашенных по Фельгену.

Размеры Ма (площадь) у всех видов более или менее сходны (кроме линии 135 *P. caudatum*, в которой размеры Ма превышали таковые остальных видов примерно в 3 раза). Наибольшее содержание ДНК в Ма отмечено в той же линии *P. caudatum*, затем у *P. woodruffi*, а наименьшее у *P. bursaria*.

Остальные виды занимают промежуточное положение и имеют примерно одинаковое количество ДНК. По характеру строения Ми одна группа видов (*P. caudatum, P. putrinum, P. bursaria*) относящаяся к типу *„caudatum"* отличается от другой группы, относящейся к типу *„aurelia"* (*P. woodruffi, P. calkinsi*) не только строением и более крупными размерами Ми, но и большим содержанием в них ДНК (табл. 1). Для первой группы видов характерна низкая величина (до 30) отношения площади Ма и площади Ми, тогда как для второй группы свойственна высокая величина, превышающая 50.

У каждого вида выявлены два сорта Ми, один — пресинтетический, другой — постсинтетический, содержащий примерно двойное количество ДНК. Синтез ДНК в Ми осуществляется во время интерфазы; при наличии нескольких Ми (*P. woodruffi*) этот процесс обычно происходит синхронно во всех Ми одной особи.

По содержанию ДНК Ма также распадаются на две группы, пре- и постсинтетическую. Последние находятся на разных этапах дупликации геномов. У всех

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исследованных видов не более чем у 30% особей синтез ДНК в Ми начинается и заканчивается ранее чем наступает синтез ДНК в отдельных геномах Ма. У большинства особей всех видов парамеций сначала начинается синтез ДНК в Ма, тогда как начало синтеза ДНК в Ми запаздывает.

Для определения степени плоидности Ма были использованы показатели содержания ДНК только пресинтетических Ма и Ми.

В среднем, содержание ДНК в Ма превосходит таковое в Ми у *P. woodruffi* в 440 раз, у *P. calkinsi* в 280 раз, у *P. caudatum* линия В в 80 раз, у *P. caudatum* линия 135 в 44 раза, у *P. putrinum* в 12 раз и у *P. bursaria* в 8 раз. Если признать что у всех исследованных видов Ми является условно диплоидным, то степень плоидности Ма у *P. woodruffi* будет 880 n, у *P. calkinsi* 560 n, у *P. caudatum* линия В — 160 n, у *P. caudatum* линия 135 — 88 n, у *P. putrinum* 24 n, и у *P. bursaria* 16 n. Первые два вида (а также *P. aurelia*) являются высокоплоидными, *P. caudatum* среднеплоидная и последние два вида — низкоплоидные. Различие в превышени содержания ДНК в Ма над таковым в Ми у разных видов (вероятно соответственно и плоидность) определяется в основном неодинаковым содержанием ДНК в Ми и их разными размерами. Наибольшая величина превышения содержания ДНК в Ма над Ми выявлена у видов (*P. woodruffi, P. calkinsi*) имеющих самый маленький Ми с наименьшим содержанием в нем ДНК, тогда как наименьшая величина выявлена у *P. putrinum* и *P. bursaria*, с наиболее крупными Ми содержащими наибольшее количество ДНК (в 25 раз больше, чем у первых двух видов). Наибольшая величина превышения содержания ДНК в Ма над Ми наблюдается у того вида, для которого характерно наивысшее отношение площади тела к площади Ми и площади Ма к площади Ми. По этим показателям все виды парамеций располагаются в той же последовательности, как и показатели отношений количества ДНК в Ма и Ми, от *P. woodruffi* до *P. bursaria* (табл. 1). *P. putrinum* и *P. bursaria* с одной стороны и *P. caudatum* линия В и *P. calkinsi* с другой, имеют сходное количество ДНК в Ма, но по содержанию ДНК в Ми первые два вида превосходят вторую пару в 6—20 раз, чем и определяется различие в степени плоидности их Ма. При наличии сходных по содержанию ДНК Ми, различие в плоидности Ма может быть обусловлено так же и некоторым различием в содержании ДНК в Ма (сравнить *P. woodruffi* и *P. calkinsi* или *P. putrinum* и *P. bursaria*).

Сравнение между собой двух линий *P. caudatum* показывает, что они могут отличаться по содержанию ДНК как в Ма так и в Ми. Можно думать, что одна из линий (135) является полиплоидной по Ми или имеет политенные хромосомы. Вследствие этого, показатель степени плоидности у этого вида может быть не точным. Возможность наличия гетероплоидии Ми у *P. putrinum* и *P. bursaria* позволяет думать, что степень плоидности Ма у этих видов, определяемая нами, может быть частично заниженной, вследствие большей плоидности Ми, чем мы предполагали в отношении исследованных нами линий, считая их диплоидными.

REFERENCES

Blanc J. 1962: Observations sur la teneur en acide déoxyribonucléique de l'appareil nucléaire du Cilié *Paramecium caudatum* Ehrb. C. R. Ac. Sc. 254, 2822—2824.

Calkins G. N. and Cull S. W. 1907: The conjugation of *Paramecium (caudatum) aurelia*. Arch. Protistenk. 10, 375—415.

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Cheissin E. M., Ovchinnikova L. P. and Kudriavtsev B. N. 1964: A photometric study of DNA content in macronuclei and micronuclei of different strains of *Paramecium caudatum*. Acta Protozool. 2, 237—245.
Cheissin E. M., Ovchinnikova L. P., Selivanova G. V. and Buze E. G. 1963: Izmenenie količestva DNK v makronukleuse *Paramecium caudatum* v period ot delenija do delenija. Acta Protozool. 1, 63—69.
Chen T. 1940: Polyploidy in *Paramecium bursaria*. Proc. Nat. Acad. Sci. 26, 231—240.
Chen T. 1940: Polyploidy and its origin in *Paramecium*. J. Heredity 31, 175—184.
Diller W. F. 1936: Nuclear reorganization process in *Paramecium aurelia* with descriptions of autogamy and "hemixis". J. Morphol. 59, 11—67.
Diller W. F. 1948: Nuclear behavior of *Paramecium trichium* during conjugation. J. Morphol. 82, 1—52.
Dysart M. P. 1963: Cytochemical and quantitative DNA analyses of the macronucleus and its extrusion body in species of *Tetrahymena*. J. Protozool. 10 (Suppl.), 8—9.
Dysart M. P. Corliss J. O. and De la Torre L. 1962: Comparative DNA measurements in two species of *Tetrahymena*. J. Protozool. 9 (Suppl.), 17.
Kimball R. F. and Barka T. 1959: Quantitative cytochemical studies on *Paramecium aurelia*. II. Feulgen microspectrophotometry of the macronucleus during exponential growth. Exp. Cell Res. 17, 173—182.
Losina-Losinsky L. 1931: Zur Ernährungsphysiologie der Infusorie: Untersuchungen über die Nahrungsauswahl and Vermehrung bei *Paramecium caudatum*. Arch. Protistenk. 74, 18—120.
Moses M. J. 1950: Nucleic acids and proteins of the nuclei of *Paramecium*. J. Morphol. 87, 493—536.
Ovchinnikova L. P., Selivanova G. V. i Cheissin E. M. 1963: Issledovanije metodom ultrafioletovoj citofotometrii vlijanija golodanija na količestvo RNK i DNK u *Paramecium caudatum*. Sb. Morfol. Fiziol. Prostejših, 44—53.
Penn A. B. K. 1937: Reinvestigation into the cytology of conjugation of *Paramecium caudatum*. Arch. Protistenk. 89, 45—54.
Raikov I. B., Cheissin E. M. und Buze E. G. 1963: A photometric study of DNA content of macro- and micronuclei in *Paramecium caudatum*, *Nassula ornata* and *Loxodes magnus*. Acta Protozool. 1, 285—300.
Ruthmann A. und Heckmann K. 1961: Formwechsel und Struktur des Makronucleus von *Bursaria truncatella*. Arch. Protistenk. 105, 313—340.
Seshachar B. R. and Dass C. M. S. 1954: Photometric study of desoxyribonucleic acid (DNA) synthesis in regenerating macronucleus of *Epistylis articulata* From. Proceed. Nat. Inst. Sci. India 20 656—659.
Sonneborn T. M. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Adv. in Genetics 1, 263—358.
Walker P. and Mitchison J. 1957: DNA synthesis in two Ciliates. Exp. Cell Res. 13, 167—170.
Wichterman R. 1953: The biology of *Paramecium*. New York.
Woodard J., Gelber B. and Swift H. 1961: Nucleoprotein changes during the mitotic cycle in *Paramecium aurelia*. Exp. Cell Res. 23, 258—264.

EXPLANATION OF THE PLATE I

Macronuclei and micronuclei of *Paramecium bursaria* (1), *P. calkinsi* (2), *P. putrinum* (3), *P. caudatum* strain B (4), *P. caudatum* strain 135 (5), and *P. woodruffi* (6). All the photomicrographs taken with the microscopical magnification 50×0.65 and reproduced with the photographic magnification of about 3×.

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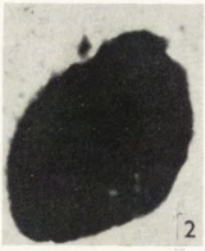

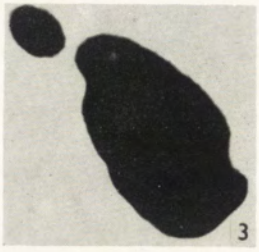

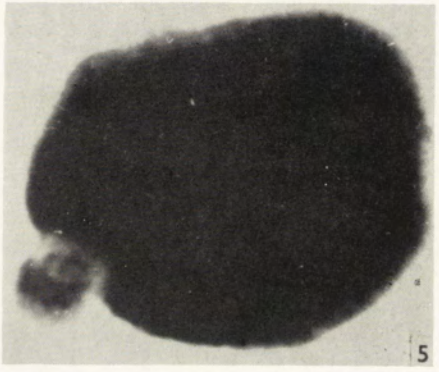

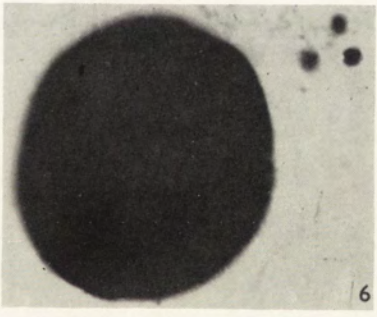

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A photometric study of DNA content in macronuclei and micronuclei of different strains of *Paramecium caudatum*

Фотометрическое изучение содержания ДНК в макронуклеусах и микронуклеусах различных линий *Paramecium caudatum*

The study of five species of *Paramecium (P. woodruffi, P. calkinsi, P. putrinum, P. caudatum, P. bursaria)* revealed a different DNA content as well in macronuclei (Ma) as in micronuclei (Mi) of these species (Cheissin and Ovchinikova 1964). Besides, it was stated that two strains of *P. caudatum* differ as well by the DNA content in their both nuclei, and have a different degree of polyploidy, if their Mi was considered as diploid. The study carried out by different authors revealed different coefficients of the ratio: DNA of Ma to DNA of Mi, and consequently, a different polyploidy in *P. caudatum*. So according to Moses 1950 the ploidy degree of Ma is 80 n, after the findings of Walker and Mitchison 1957: 50—60 n. Raikov, Cheissin and Buze 1963 stated for one of the Leningrad strains — and Blanc 1962 for one of the French ones — the polyploidy degree for *P. caudatum* 160 and 150 respectively. Those findings evoked surprise and interest in attempts to elucidate the cause of such diverse results of different authors. In *Tetrahymena patula* after Dysart 1963 a considerable fluctuation of polyploidy degree of Ma is also observed.

The authors of the present study suggested that the uneven degree of Ma polyploidy in different species of paramecia is rather determined by the different DNA content in Mi than by the difference in the DNA content in Ma. It was also revealed by the comparison of two strains of *P. caudatum* that they highly differ in the DNA content in Mi. Considering the possibility of heteroploidy of Mi in *Paramecium*, the supposition was expressed that one of the *P. caudatum* strains either has a doubled set of chromosomes which lowers the n-coefficient of Ma in this strain, or the difference in the DNA content in Mi of two strains of *P. caudatum* may be due to the polyteny of chromosomes (Cheissin and Ovchinnikova 1964). No doubt, the solution of the problem of difference in the DNA content in the nuclei of ciliates of one species, and indication of the factors responsible for this phenomenon, requires examination of more than two strains. In the present study material is reported explaining to some extent the problems concerning the polyploidy in ciliates.

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Material and methods

For the photometric study 10 strains of *P. caudatum* were used. They all were clones isolated at different time, from 1961 till 1963. The ciliates were cultivated in the medium of Losina-Losinsky 1931. They were fed on yeast. The cultures were kept in the temperature 22°C.

The strain B was obtained in 1962 from a small basin in Leningrad. It was not determined whether as to the variety and mating type. All the other strains were supplied by D. Ossipov (University of Leningrad, Department of Invertebrates Zoology), to whom we express our most vivid thanks for offering the cultures to our disposition. The strains 124 and 127 originated also from a pool in the environment of Leningrad (1962). Both strains belong to the variety 1, I and II mating type, as determined by Ossipov 1963[1]. To the same variety belongs the strain Ps isolated from a basin in Pskov, in 1961. It is a clone of the I mating type. The strain Lab-6 was received from Prof. Vivier from France. According to his information it belonged to the variety 3 and the mating type V. Ossipov 1963 identified this strain with Leningrad standard lines. It proved to belong to the mating type I, variety 1.

The strains 131 and 135 isolated from pools of the Leningrad environment in 1962 were ranged among the mating type III and IV, variety 2. In the same locality the strain 117 was found, belonging to the mating type VI, variety 3 (after Ossipov 1963). The strains Nor-4b and Jap-4 supplied to us by Ossipov originate from the cultures of Dr. Gilman, USA. The first strain is native from Norway, the second from Japan. The strain Nor-4b has not been identified, and the second belongs to the variety 12, mating type XXIV (after Gilman).

For photometry 25—30 individuals of each strain were taken. Examination was carried out on whole preparations fixed after Nissenbaum's and stained after Feulgen's procedure (hydrolized 6 min.). Preparations were photographed under a special microscope MUF-6 and the negatives density was measured with the scanning microphotometer MF-4. The detailed description of the photometric method has been reported in our previous publications (Cheissin and oth. 1963, Raikov and oth. 1963, Ovchinnikova and oth. 1963).

The DNA content and area of Ma and Mi was determined in non-dividing ciliates, being in the presynthetic and postsynthetic phase. However, for the analysis of the polyploidy degree of Ma only those coefficients were utilized which corresponded to the presynthetic Ma and Mi. Reasons for this detail of method are developed in the article of Raikov and oth. 1963 and

[1] Ossipov 1963 uses Cyrillic lettering to determine the varieties, and the arabic numerals for mating types. For terminological reasons in the present article the varieties are denoted by arabic numerals, and the mating types by the latin numerals, which system is concurrent to that commonly used for *P. aurelia*, and is also applied for *P. caudatum* by Gilman. So e. g., the terms: variety 1, mating types I and II, are used, meaning in the terminology of Ossipov: variety A, mating types 1 and 2. It should be, however, kept in mind that the majority of strains isolated by Ossipov had not been identified with those with international designation. It follows from it that the symbols used in this paper are not corresponding to those of American authors, exception done for the strain Jap-4 denoted as variety 12

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Cheissin and oth. 1963. All the quantitative data reported in the text and in the Tables correspond to the presynthetic nuclei. The quantity of DNA (Q) is expressed in arbitrary units $\times 10^{-6}$. Ratio of DNA quantity in Ma and Mi is defined as Q Ma/Q Mi.

Results and discussion

The 10 strains investigated differed from one another by the DNA content as well in their Ma as in Mi (Table 1). In different strains the DNA quantity[2] in Ma varies approx. from 1.47 (strain B) to 6.1 (strain 135). Between those strains with the extreme coefficients of DNA content in Ma, other strains with median coefficients are ranged. In the strain Jap-4 the DNA content is in average 2.94, consequently in the four strains (117, Lab-6, Ps, 124) the DNA quantity is approx. equal and amounts 3.2—3.8. Next the strain 131 may be placed with its DNA content amounting 4.6, and finally two strains follow (Nor-4b and 127) with their DNA content in Ma amounting 5.1—5.5. Consequently, the highest content of DNA in Ma of the strain 135 exceeds that in the strains B and Jap-4 2—3 times.

The Ma of the strain B with the lowest DNA content has the smallest area. In the other strains, ranged in the following sequence from Jap-4, 117, 124, to Ps the area[3] of Ma enlarges from 4.7 to 5.5 (Table 1); concurrently increases the DNA content. Only in the strains Lab-6 and Nor-4b a certain lack of correlation between the dimensions of Ma and the DNA content in it is observed. Ma in those strains are the biggest whereas the DNA content in them is relatively low although a much higher quantity of DNA in them could be expected. On the other hand in the strain 135 the dimensions of Ma are smaller than in the strains Lab-6 and Nor-4b whereas the DNA quantity in them is higher than in those strains. Nevertheless in the majority of strains, there is a proportional relation between the size of Ma and their content of DNA.

All the strains studied may be ranged according the increase of the Mi size and of the corresponding DNA content in them. The lowest content of DNA (0.019) in Mi and its smallest size (0.08) were stated for the strain B. The highest content of DNA (0.17) was found in the biggest Mi (0.53) of the strain Nor-4b. The DNA quantity in Mi of this strain exceeds that of the strain B, 8 times. In the strain Jap-4, Mi contains the double DNA quantity of the strain B. In the strains 131, 117 and Lab-6 the DNA content exceeds 4 times that in the strain B and 6 times that in the strains 127, 135, Ps and 124. Increase of the DNA content in Mi corresponds to the increase of their size. Mi with a double or 4-fold quantity of DNA have approx. the same area amounting 0.2—0.3. Mi with a 6-fold quantity of DNA have a somewhat larger area amounting 0.31—0.39. An exception presents only the strain Lab-6; its Mi contains 4 times more DNA than in the strain B, however its area is considerably larger than in the strains with the same DNA content in Mi. The dimensions of Mi in the strain Lab-6 are in agreement with those of the strain Nor-4b although the DNA content in Mi in the latter strain is double.

The data concerning the DNA content and dimensions of Ma and Mi allow to segregate the ciliates under study into 3 groups according to the value

[2] Here and subsequently further on the coefficient $\times 10^{-6}$ is omitted.
[3] Coefficient $\times 10^{-6}$ cm^2 here and further on is omitted.

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of the ratio: Q Ma/Q Mi. The first group, i.e. the strains B, Jap-4, 131 is characterized by a high coefficient Q Ma/Q Mi amounting 66—80. The second group with the mean coefficient Q Ma/Q Mi amounting 42—50, includes the strains 117, Lab-6, 127, 135. To the third group with a low coefficient amounting 26—30 belong the strains Ps, 124 and Nor-4b.

The different coefficients in these strains are determined by two varying magnitudes and their different ratio: 1. different DNA content in Mi, 2. different DNA content in Ma. The highest Q Ma/Q Mi coefficient is observed in the ciliates with the lowest DNA content in Mi (0.019—0.07). On the other hand, the lowest Q Ma/Q Mi (22—30) occurs in ciliates with a higher DNA content in Mi (0.13—0.17). The representatives of the second group with the moderate Q Ma/Q Mi coefficient (42—50) have the Mi with an intermediate DNA content (0.07—0.14).

Table 1

Characteristics of different strains of *Paramecium caudatum* as to the DNA content ($Q \times 10^{-6}$) in their Ma and Mi, the area ($S \times 10^{-6}$ cm^2) of their body. Ma and Mi, and the degree of polyploidy ($\times n$) in their macronuclei

Strain	Variety	Mating type	S body	Q Ma	S Ma	Q Mi	S Mi	Q Ma / Q Mi	×n	S Ma / S Mi	S body / S Mi	Q Mi str. B
B	?	?	42	1.47	2.44	0.019	0.08	79.5	160	30.5	525	1
Jap-4	12	XXIV	40	2.94	4.7	0.04	0.2	73.5	147	23.5	200	2
131	2	III	66	4.60	5.7	0.07	0.2	65.7	132	28.1	330	4
117	3	VI	63	3.22	4.8	0.07	0.28	46	92	17	225	4
Lab-6	3* / 1	V* / I	63	3.8	6.4	0.09	0.52	42	84	12	120	4
127	1	II	54	5.5	5.9	0.11	0.31	50	100	19	180	6
135	2	IV	66	6.1	6.0	0.14	0.32	44	88	20	218	6
Ps	1	I	59	3.6	5.5	0.13	0.39	27	54	14	151	6
124	1	I	69	3.4	5.3	0.13	0.31	26	52	17	220	6
Nor-4b	?	?	58	5.1	7.3	0.17	0.53	30	60	14	108	8

* After Vivier.

Although in the first group Mi has a lowest DNA content, its relative quantity in three strains belonging to this group is different. If the DNA content in Mi of the strain B was considered as minimal so in the strain Jap-4 it is twice higher and then in the strain 131, 4 times higher. In the second group with the moderate coefficient Q Ma/Q Mi (42—50) the DNA content is 4 and 6 times higher than in the strain B. At last in the strains of the third group the DNA content in Mi is 6 and 8 times higher than in the strain B. Consequently as follows from the presented evidences, one of the varying factors (DNA content in Mi), determinates before all the differences in coefficients Q Ma/Q Mi. Nevertheless this coefficient may be different in two strains even in this case when Mi of both strains show the same DNA content (e. g. the strain 131 and 117). The coefficient Q Ma/Q Mi is higher in the strain with a higher DNA content in Ma.

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It is an essential fact that the DNA content in Ma of *Paramecium caudatum* remains in a definite relation with the DNA content in Mi. If Mi are small and with a minimal content of DNA (strains B and Jap-4), a relatively small DNA content in Ma is always observed. With the rise of DNA content in Mi, the DNA content in Ma increases proportionally. Ciliates with a low content of DNA in Mi (e.g. 0.019) and a very high DNA content in Ma (e.g. over 5.0) were never observed. Presumably this may be involved by the fact that the degree of Ma polyploidy in *Paramecium caudatum* is determined by the more or less constant number of endomitotic cycles which undergo the Ma-anlagen in the exconjugants in the phase of formation of the final Ma of vegetative individual. In the case of a constant number of the endomitotic cycles, the DNA content in Ma would vary depending on the initial quantity of DNA in the Ma primordium.

A presumable number of endomitoses responsible for the definite level of DNA content in Ma was calculated for the strains under study. The moderate DNA content in Ma characteristic for the strains 117, Lab-6, 127, Ps, 124, 135 and Nor-4b is conditioned by approx. 5 endomitotic cycles, wheres in the strains: B, Jap-4 and 131, occur 6 such cycles. We failed to obtain perfectly exact numerical coefficients of endomitotic cycles number because evidently endomitoses occur not synchronically in all the genoms. However, the number of endomitotic cycles may be constant for every ciliate species, or — at any rate — sufficiently constant in definite conditions of the exconjugant development. The data supplied by the previous study (C h e i s s i n and oth. 1964), concerning the DNA quantity in Ma and Mi of different species of *Paramecium*, indicate that the level of DNA in Ma of every species is determined by the definite number of endomitoses. In *P. woodruffi* 8—9 endomitoses should occur for the comparatively high DNA content, specific for this species, might be formed in the presynthetic Ma. In *P. calkinsi* 8 endomitoses should occur, in *P. putrinum* and *P. bursaria* only 3—4 endomitotic cycles bring the DNA quantity in Ma characteristic of these species.

Consequently, the ratio of the DNA content in Ma to that in Mi may be different in various strains as a result of the differences in the exit quantity of DNA in Mi; in such case, the relatively constant number of endomitotic cycles involves the formation of uneven quantity of DNA in Ma in various strains.

Considerable differences in DNA content of Ma in different strains within the same species complicates the problem of the ploidy degree in Ma. As shown above, ratio of the coefficients of DNA content in Mi in different strains are — 8:1, 6:1, 4:1 and 2:1. If the strain B with its lowest DNA content in Mi (0.019) was diploid, consequently the other strains would be 4-, 8-, 12- and 16-ploid as to their DNA content in Mi.

What is determining those differences in the DNA content in Mi? A long time before the photometric investigation method was introduced, C h e n 1940 found different races in *Paramecium bursaria*, which differ from one another in the Mi size and chromosomes number. In one of these races Mi contained about 80 chromosomes, other races were polyploid and contained up to several hundreds of chromosomes. The same author reported also the occurrence of a race with numerous chromosomes, in *P. caudatum*, which was considered (C h e n 1940) to be polyploid. In C h e n's opinion, a possibility

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exists that Calkins and Cull 1907 having stated in *P. caudatum* about 150 chromosomes, had to do namely with such a polyploid race, because Penn 1937 revealed only 36 chromosomes in a race of *Paramecium caudatum* which he investigated.

Is the difference in the DNA content of Mi, in the *P. caudatum* strains under study, connected with polyploidy (number of chromosomes) or is it involved by another factors? Since we had strains differing considerably by the content of DNA in Mi (4—8 times), so it could be expected that Mi in those strains would strongly differ in chromosomes number. Much more chromosomes were expected to be revealed in Mi of the strain Nor-4b or 135, 124, Ps than in the Mi of strain B and Jap-4. Nevertheless, this seems not to be true, however, the exact count of chromosomes could not be done. At any rate, in the big Mi of the strain Nor-4b and 135 the 6- or 8-fold predominance of the chromosomes number, when compared with the rather small Mi in the strain B and Jap-4, was not observed. Consequently, the polyploidy of Mi in the strains of *P. caudatum* under study cannot be stated for a certainty.

However, in the big Mi of the strains Nor-4b, 135, 124 and 127, chromosomes were found thicker than in the small Mi of the strains B and Jap-4. It is not impossible that here their polyteny occurs, but proving polyteny of chromosomes requires more precise study of their ultrastructure. Polytenic chromosomes, among ciliates, were found in Mi of *Tetrahymena pyriformis* (Alfert and Balamuth 1957). It may be supposed that the differences in DNA quantity in Mi of different strains, as reported in this study, may be due to a different degree of polyteny of chromosomes and not of their polyploidy. This suggestion does not exclude the existence of polyploid races (strains) in *P. caudatum* as stated by Diller 1940 and Chen 1940. The latter author held an opinion that polyploidy of Mi in *P. bursaria* and *P. caudatum* is a frequent phenomenon. So it may be assumed that the polyteny of chromosomes exists in *P. caudatum* besides the polyploidy, and is responsible for the difference of DNA content in Mi of different strains.

If we arbitrarily suppose that the manyfold rise of DNA content in Mi of different strains fails to reflect the corresponding increase of chromosomes number, it should be assumed that Mi of all the strains is diploid or, at any rate, equal as to its ploidy. The count of chromosomes pairs in meiosis and stating the individuality of bivalents is indispensable for ascertaining diploidy. There was no possibility to perform this in the present investigation. Chen 1940 could not define precisely whether in the race Fd of *P. bursaria* with the very small Mi the chromosomes number is diploid or not.

Assuming arbitrarily the diploidy of Mi in the material under study, the authors succeeded in distinguishing three groups of strains according the degree of their polyploidy — the first with the highest degree of ploidy: 132—160 n, the second with a moderate degree of polyploidy: 88—100 n, and the third with a low polyploidy degree: 50—60 n. Those results are in full agreement with the determination of polyploidy degree in *Paramecium caudatum* as reported by several earlier authors working in different places and — surely — with different strains. So, Blanc 1962 in France found the coefficient Q Ma/Q Mi = 75 (ploidy 150 n), Moses 1950 in USA stated the 40-fold predominance of DNA content in Ma, as compared to that in Mi (80 n), Walker and Mitchison 1957 found a strain of *P. caudatum* with 25—

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30-fold predominance of DNA content in Ma over that in Mi which corresponds to 50—60 n. That striking coincidence with the data of the present study speaks in favour of the view that *P. caudatum* in different spots of its extensive area, and even within one locality of occurrence (like some strains in the present study), appears in the form of 3 similar variations as to the degree of their polyploidy (or of the coefficient Q Ma/Q Mi). Of course, further findings of other strains characterized by different coefficients Q Ma/Q Mi seem not improbable. In this case, the number of endomitotic cycles should be increased, amounting not 5—6, as it was characteristic for the strains under study, but much more.

Occurrence of groups differing in their DNA content in Mi and in Ma as well as in their degree of polyploidy, within the same ciliate species, has evidently a definite biological meaning. Nanney 1953 suggested that development of a definite mating type might depend on a different degree of polyploidy of Ma.

The results of the present investigation support to some extent this supposition. Several strains (124, Ps, 127 and Lab-6) belong to the same variety 1 (A—according the terminology of Ossipov 1963), and among them, the strains 124, Ps and Lab-6 belong to the I mating type and the strain 127 to the II mating type. The strains 124 and Ps have the polyploidy 52—54 n (all coefficients indicate that they are nearly related to each other — Table 1), whereas the polyploidy of the strain 127 is 100 n. An exception presents only the strain Lab-6 (I type) with the ploidy 84 n, although a lower polyploidy could be expected. Strains 131 and 135 belong to the variety 2 and to the III and IV mating types respectively. The first of those strains has the ploidy 132, — the second 88. These evidences are surely unsufficient for drawing any conclusions, but it seems quite possible that the differentiation of mating type within a species is in some way connected with a different DNA content in the nuclei, in different individuals always present in every population of any ciliate species.

Summary

DNA contents in macronuclei (Ma) and micronuclei (Mi) of 10 strains of *Paramecium caudatum* were measured photometrically. A comparison of DNA contents was carried out only on presynthetic nuclei. The strains differ from each other both by Mi dimensions and DNA contents in the Mi, the least values for the two features being found for the strain B. The other strains appeared to show their DNA contents as much as 2, 4, 6, or 8 times higher, if compared with that of the strain B. Different DNA contents in Mi do not correspond to their respective chromosomes number. This finding allows to suggest that the higher DNA contents in larger Mi would not depend upon polyploidy degree of the latter but might be related with the formation of polytene chromosomes, however, for a decisive conclusion the chromosomes fine structure should be studied. DNA content in Ma of different strains, represents a value depending on the number of endomitotic cycles during Ma development in a vegetative specimen. In the examined strains the number of endomitotic cycles was 5 or 6. The final DNA content in Ma depends upon its initial content in Mi.

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According to values of the ratio of DNA content in Ma to that in Mi, the strains studied are ranged into three groups: with high (66—80), moderate (42—50) and low (25—30) values. Considering Mi to be diploid, one can distinguish in *P. caudatum* three groups of strains differing from each other by Ma polyploidy degree: 136—160, 84—100 and 25—30 n, resp. It can be noted that the strains belonging to complementary mating types possess different polyploidy of Ma.

РЕЗЮМЕ

Количество ДНК в Ма и Ми 10 линий *P. caudatum* определялось фотометрически. Для сравнения использовались данные по содержанию ДНК только в пресинтетических Ма и Ми (таблица 1). Исследованные линии различаются по размерам Ми и содержанию в них ДНК. Наименьшие размеры и наименьшее количество ДНК в них обнаружены в линии В. В остальных линиях количество ДНК в 2, 4, 6, 8 раз больше, чем в линии В. Различное содержание ДНК в Ми не соответствует изменению количества хромосом в них и поэтому можно думать, что большее содержание ДНК в крупных Ми не определяется их полиплоидией, а скорее зависит от образования политенных хромосом. Однако, для окончательного вывода необходимо более точное изучение тонкой структуры таких хромосом. Количество ДНК в Ма неодинаковое в разных линиях представляет собой величину определяемую числом эндомитотических циклов при формировании Ма нейтральной особи. В разных линиях число таких эндомитотических циклов равно 5 или 6. В зависимости от различий в исходном содержании ДНК в Ми, содержание ДНК в Ма будет также различным. По показателям отношения ДНК в Ма к Ми все линии распределяются на три группы: 1. с высоким показателем (66—80), 2. со средним показателем (42—50) и 3. с низким показателем (25—30). Если признать Ми всех линий за диплоидные, то соответственно у *P. caudatum* можно выделить три группы линий, отличающихся по степени плоидности Ма: 1. с плоидностью 136—160 n, 2. с плоидностью 84—100 n и 3. с плоидностью 25—30 n. Можно отметить, что линии относящиеся к комплементарным типам спаривания имеют разную плоидность Ма.

REFERENCES

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

Blanc J. 1962: Observations sur la teneur en acide déoxyribonucléique de l'appareil nucléaire du Cilié *Paramecium caudatum* Ehrb. C. R. Ac. Sc., 254, 2822—2824.

Calkins G. N. and Cull S. W. 1907: The conjugation of *Paramecium (caudatum) aurelia*. Arch. Protistenk. 10, 375—415.

Chen T. 1940: Polyploidy in *Paramecium bursaria*. Proc. Nat. Acad. Sci. 26. 231—240.

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

Cheissin E. M. and Ovchinnikova L. P. 1964: A photometric study of DNA content in macronuclei and micronuclei of different species of *Paramecium*. Acta Protozool. 2, 225—236.

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

http://rcin.org.pl

Diller N. F. 1940: Nuclear variation in *Paramecium caudatum*. J. Morphol. 66, 605—633.
Dysart M. P. 1963: Cytochemical and quantitative DNA analyses of the macronucleus and its extrusion body in species of *Tetrahymena*. J. Protozool. 10 (Suppl.), 8—9.
Moses M. J. 1950: Nucleic acids and proteins of the nuclei of *Paramecium caudatum*. Arch. Protistenk. 74, 18—20.
Nanney D. 1953: Mating type determination in *Paramecium aurelia* a model of nucleo-cytoplasmic interaction. Proc. Nat. Acad. Sci. USA 39, 113—118.
Ossipov D. V. 1963: Tipy sparivanija klonov *Paramecium caudatum* iz vodoemov nekotoryh rajonov Sovetskogo Sojuza. Vestn. Leningrad. Universiteta, S. Biol. 21, 106—117.
Ovchinnikova L. P. Selivanova G. V. i Chessin E. M. 1963: Issledovanie metodom ultrafioletovoj citofotometrii vlijanija golodanija na količestvo DNK i RNK u *Paramecium caudatum*. Sb. morfol. fiziol. prostejših 3, 44—53.
Penn A. V. K. 1937: Reinvestigation into the cytology of conjugation in *Paramecium caudatum*. Arch Protistenk. 89, 45—54.
Raikov I. B., Cheissin E. M., Buze E. G. 1963: A photometric study of DNA content of macro- and micronuclei in *Paramecium caudatum*, *Nassula ornata* and *Loxodes magnus*. Acta Protozool. 1, 285—300.
Walker P. and Mitchison J. 1959: DNA synthesis in two Ciliates. Exp. Cell Res. 13, 167—170.

EXPLANATION OF THE PLATE I

Photomicrographs of the Feulgen stained nuclei of *Paramecium caudatum*. strain B (1), strain Jap-4 (2), strain 131 (3), strain 117 (4), strain Lab-6 (5), strain 127 (6), strain 135 (7), strain 124 (8), strain Ps (9) and strain Nor-4b (10). All ×1350.

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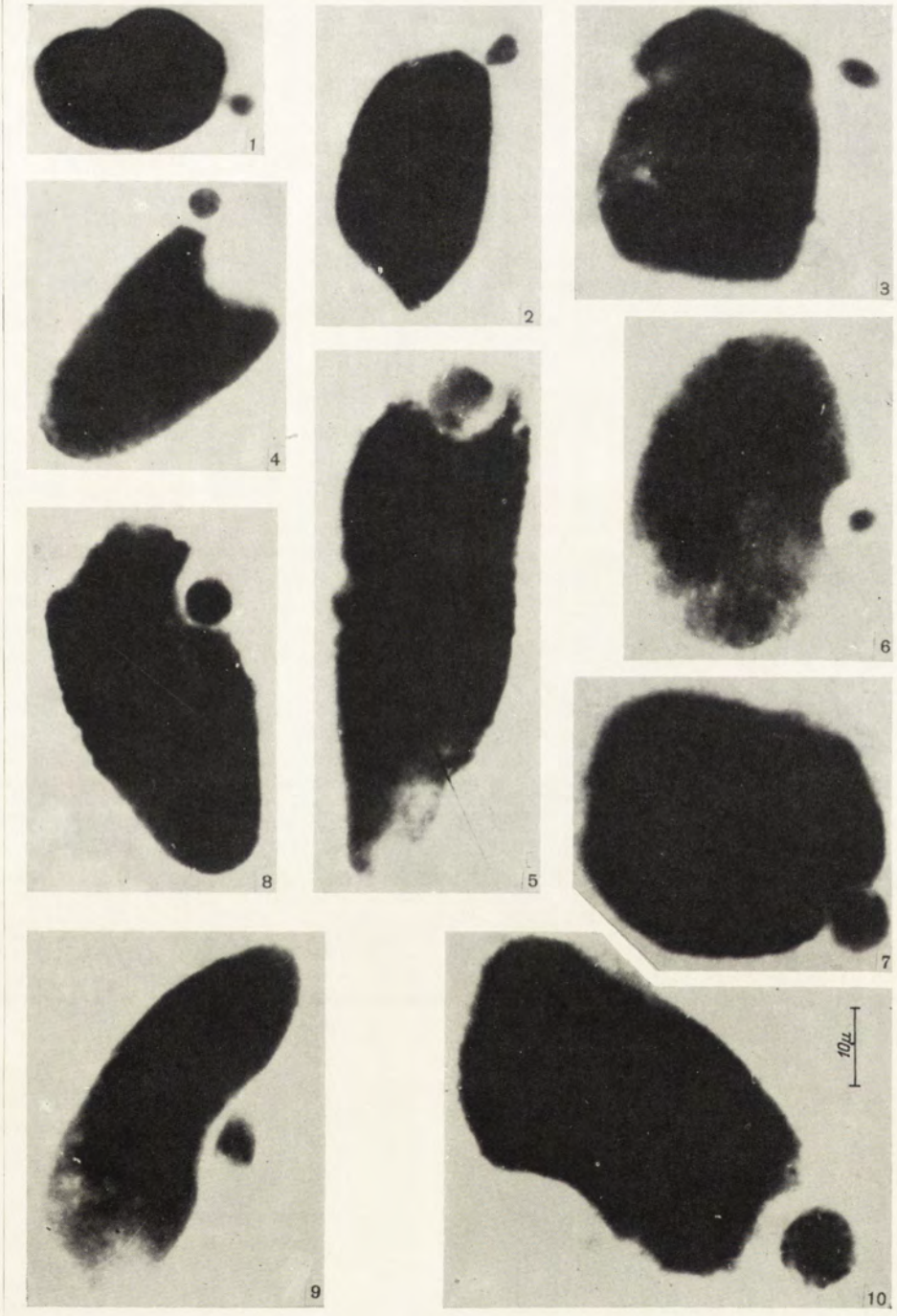

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Cytology of *Frontonia elliptica* (Beardsley)

Cytologie der *Frontonia elliptica* (Beardsley)

Frontonia elliptica is a holotrichous ciliate belonging to the sub-class *Holotricha* order *Hymenostomatida,* family *Frontoniidae* (Corliss 1961), first described by Beardsley 1902. Kahl 1930 lists species of *Frontonia* of which the best known is *F. leucas. F. elliptica* differs from *F. leucas* in body size, macronuclear size, number and size of micronuclei and in certain aspects of binary fission cycle. Cytology of binary fission and autogamy in *F. leucas* has been studied by one of us (Vimala Devi 1960, 1961). The present paper gives an account of the cytology of *F. elliptica.*

Material and methods

Specimens were collected from fresh water ponds in Srinagar, Kashmir. The animals were cultured in the laboratory in hay infusion in which a good supply of bacterial growth was maintained by the addition, once in fortnight, of baked cabbage leaves.

For cytological studies, animals were fixed in Schaudinn's fluid and stained with Feulgen's reagent. Light green was used as counter-stain. Throughout the study, living animals were observed with the aid of a roto-compressor, to determine the nature of the oral apparatus, contractile vacuole and other parts of the anatomy of the animal. The Figures (1—5) are camera lucida drawings of Feulgen — light green preparations of whole mounts.

Observations

A fully grown vegetative animal has a length of from 175 μ to 200 μ and a breadth of from 50 μ to 75 μ. Pattern of body ciliature is the same as that found in most holotrichous ciliates. Body cilia measure about 2.5 μ in length. Towards the posterior tip of the ciliate there is a set of longer cilia, measuring about 4 μ in length. Trichocysts are arranged uniformly below the pellicle. They measure about 5 μ in length in the non-ejected condition, but when ejected, attain a length of 40 μ to 50 μ. Variation in length of ejected trichocysts is due to the different degree of ejection.

Contractile vacuole is single and is in the centre of the cell. From five to six collecting canals lead into it.

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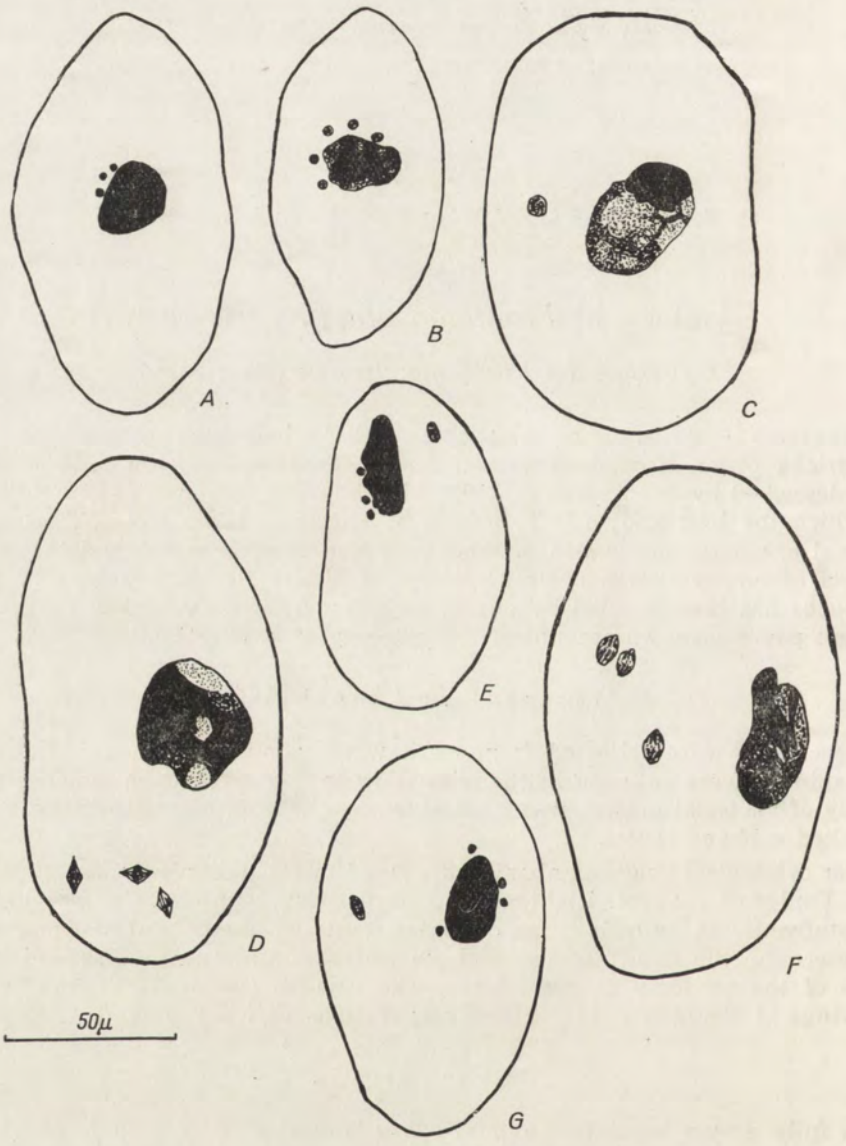

Fig. 1. Course of division in *Frontonia elliptica:* A. Vegetative animal with single Ma and three Mi. B. Animal with Ma showing differential staining with Feulgen's reagent; five Mi in early prophase. C. Ma showing differential staining with Feulgen's reagent; single Mi in late prophase. D .Ma showing differential staining with Feulgen's reagent; the Mi in metaphase. E. Ma shows undulations; the three Mi lying close to it are in resting phase; the fourth, lying away from the Ma, is in metaphase. F. Ma shows out-pushings; also, exhibits differential staining with Feulgen's reagent; Mi in anaphase. G. Four Mi are in resting phase and one Mi in anaphase

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Peristome is in the anterior third of the body on the ventral surface and a peristomial groove runs down to the posterior end of the animal. An undulating membrane is present on the right side of the oral fossa. This structure is made up of closely matted cilia. These cilia are quite long and measure from 6 μ to 7 μ.

The nuclear apparatus consists of a single oval Ma and from 1 to 6, occasionally 8, Mi arranged around and very close to the Ma (Fig. 1 A). Some Mi are even embedded on the surface of the Ma The Ma is 15 μ to 20 μ long and 10 μ to 16 μ broad. It is strongly Feulgen positive. The Mi have a diameter of 3.5 μ to 4 μ (Figs. 1 A and 5 A). They are also strongly Feulgen positive.

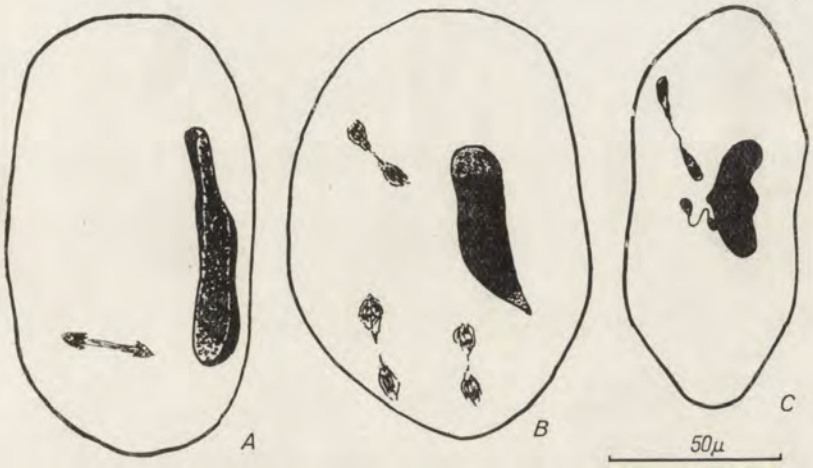

Fig. 2. Course of division in *Frontonia elliptica* (continued): A. Elongated Ma exhibiting differential Feulgen staining; the single Mi in telophase. B. Elongated Ma showing differential Feulgen staining; three Mi in telophase. C. Slightly elongated Ma; two Mi in late telophase

Binary fision is marked by the Mi moving away from the Ma and their swelling to a diameter of about 4.5 μ to 5 μ (Figs. 1 B, C and 5 B). Marked changes are also seen in the Ma (Figs. 1 B and C). It shows wavy and folded edges and also exhibits differential staining with Feulgen's reagent. During metaphase of the Mi, the chromosomes are arranged on the spindle at its equator (Figs. 1 D and 5 C). Anaphase spindles are quite large. They measure from 4.5 μ to 5.5 μ in length and are only slightly less in breadth (Figs. 1 F and 5 D). By telophase the spindles elongate greatly and attain a length of from 22 μ to 32 μ (Figs. 2 and 5 F, G). Soon after this, the daughter Mi separate, with the broken ends trailing off (Figs. 3 A, C, D, E and 5 H). The segregation of the daughter Mi during binary fission is generally such as to ensure an equal distribution of their number between the two cells (Figs. 3 F and G).

While the Mi are in prophase, the Ma exhibits differential staining, as already mentioned (Figs. 1 B and C), with Feulgen's reagent. By the time the Mi reach anaphase, the Ma shows highly characteristic out-pushings (Fig.

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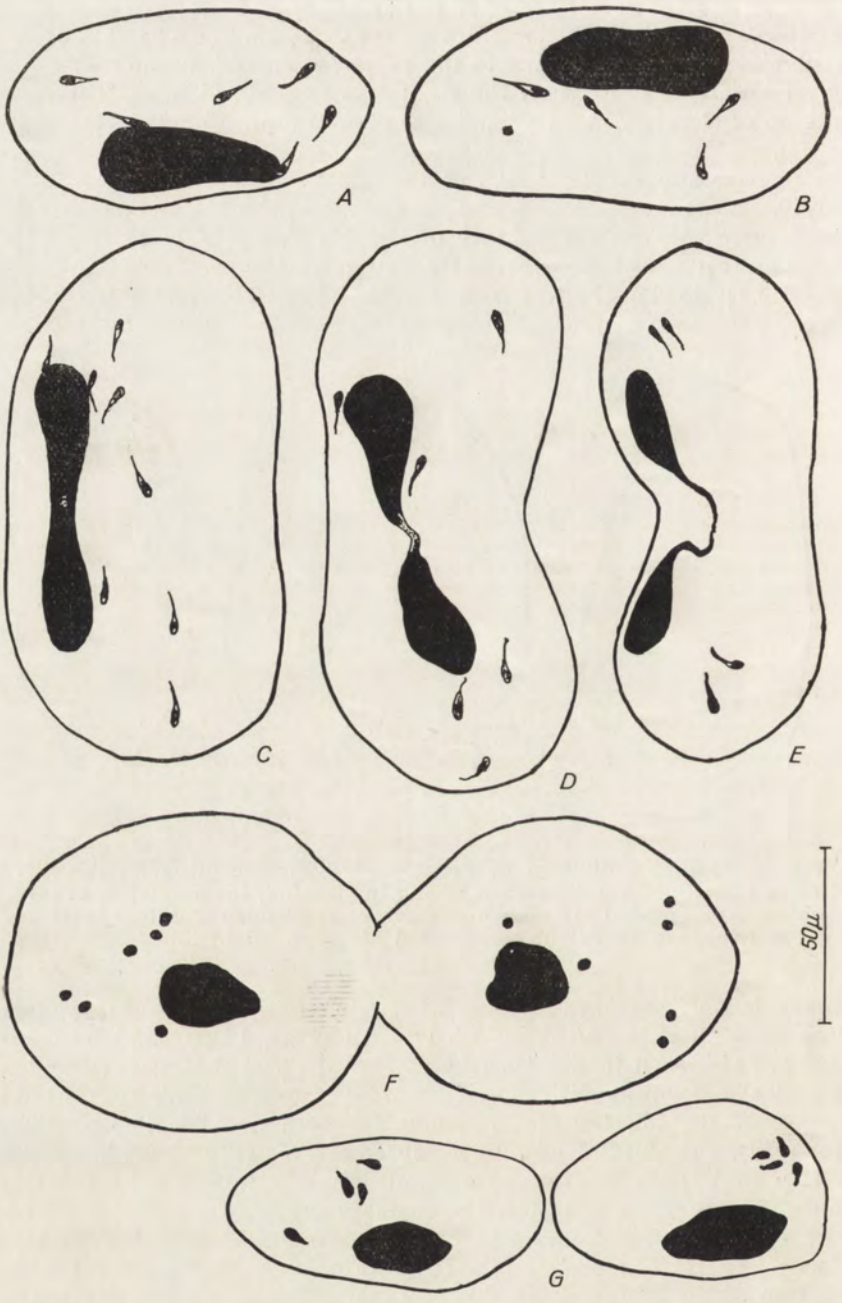

Fig. 3. Course of division in *Frontonia elliptica* (continued): A. Elongated Ma and six Mi (division products). B. Elongated Ma, four Mi (division products) and one Mi in resting phase. C. Highly elongated Ma, with a slight constriction in the middle and eight Mi (division products). D. Elongated Ma with a deep constriction in the middle; three Mi have segregated to either daughters. E. Animal with dividing Ma; the two daughters are about to separate; two Mi have segregated to either side of the cell. F. Daughters about to separate; the Ma halves have already separated; each daughter has six Mi. G. The daughters have just separated; each of them has one Ma and four Mi

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1 F) and later elongates (Figs. 2 A and B). This process of elongation continues even after daughter Mi have completely separated (Figs. 3 A, B). Occasionally, slight variations in this synchrony of division of the two components of the nuclear apparatus are observed. While in some cases, before the Ma has reached its highest elongation, the Mi would have already separated (Figs. 3 A and B), in others, the latter are still in early telophase at a correspond-

Fig. 4. Animal with the Ma in two unequal parts and four Mi in resting phase (the same magnification as in preceding Figs.)

ing stage (Figs. 2 A and B). One can also see some animals with the Ma only slightly elongated but with the Mi in late telophase (Fig. 2 C). The greatest length attained by the Ma in our material was about 60 μ. A constriction soon appears in the middle of this elongated Ma (Figs. 3 C and D). When the cytoplasmic furrow appears, the two halves of the Ma separate (Fig. 3 E). The two daughter Ma go to either daughter as the cytoplasmic furrow deepens (Fig. 3 F), getting condensed in the meantime. Here again, slight variations

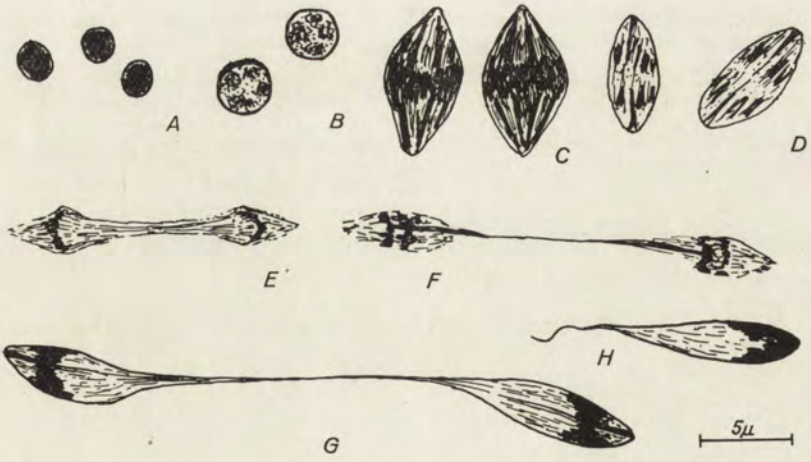

Fig. 5. Micronuclear cycle: A. Vegetative Mi. B. Mi in prophase. C. Mi in metaphase. D. Mi in anaphase. E. — F. Mi in early telophase. G. Mi in late telophase. H. Just separated Mi

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are noticed among different individuals, in that in some of them, when daughter cells are yet to separate, Ma and Mi have started condensation after division (Fig. 3 F). In others, the daughter Mi still have the tail-like remnants of their spindles, when the daughter cells have already separated (Fig. 3 G). The two daughter cells separate about three quarters to one hour after the appearance of the cytoplasmic furrow.

A phenomenon of interest seen in connection with binary fission of *F. elliptica* concerns the origin of inequality in the number of Mi noticed in the vegetative animals. Occasionally, during fission some of the Mi do not move away from the Ma as the others and continue to be in the resting condition (Figs. 1 E and G). Such resting nuclei are noticed in early stages of binary fission but usually not in later ones. Apparently they disintegrate. However, in a few animals, resting Mi were observed during later stages (Fig. 3 B). Probably in these individuals they persist and go to one of the daughter cells at the time of segregation.

During the present study, a few individuals have been observed where the Ma was in two unequal pieces and the Mi in interphase (Fig. 4). They are believed to be undergoing some reorganization process like "hemixis" described by Diller 1936 in *Paramecium*.

Discussion

There are a few interesting differences between *Frontonia elliptica* and the better known *F. leucas*. Those that relate to the nuclear apparatus are the most important.

The Mi in species of *Frontonia* are subject to variation both in number and size (Okada 1956, Vimala Devi 1960, Roque 1961). They would appear to be species specific as shown by Seshachar and Padmavathi 1956 in *Spirostomum* and Seshachar et al. 1959 in *Blepharisma*.

The phenomenon of multimicronuclearity is of much interest in ciliates. Closely related to it is the problem of variation in Mi number noticed among many species of ciliates.

A correlation between micronuclear count and body size has been attempted by Bullington 1939. Manwell 1928 attributed variation in Mi number to metabolic activity in *Pleurotricha*. Woodruff 1931 regarded it as due to environmental conditions, while Beers 1946 considered that variation is due to unequal distribution during binary fission. Diller 1940 basing his studies on *Paramecium* concluded that unequal distribution, senility, environmental and nutritional conditions or failure to reorganize properly after conjugation lead to changes in Mi number. Wichterman 1954 suggested that the great fluidity of the protoplasm, due to the presence of foreign, deleterious micro-organisms, led to unequal distribution of daughter Mi and hence to variation in Mi number in a race of *P. caudatum*. Vimala Devi 1960 in *Frontonia leucas* and Seshachar and Kasturi Bai 1963 in a species of *Oxytricha* noticed unequal distribution of daughter Mi during binary fission.

Difference in micronuclear number in many ciliates is also brought about by failure of some Mi to divide at the time of binary fission and their later degeneration. This occurs in a species of *Spirostomum* (Seshachar and

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Padmavathi 1956), *Frontonia leucas* (Vimala Devi 1960) and *Oxytricha* sp. (Seshachar and Kasturi Bai 1963).

In *Frontonia elliptica* also, variation in micronuclear number is brought about by some of the Mi not dividing during binary fission. This is particularly noticeable in Mi lying close to the Ma and would lead to the view that nearness to the Ma is inhibitory to micronuclear mitosis. Usually, such non-dividing Mi are noticed only during early binary fission. But occasionally, they were observed when the others had reached telophase. Probably these are stragglers which did not move away from the Ma earlier and thus have missed the initiation of division. The conclusion is that whatever the factor responsible for inducing mitosis, it appears to act only for a limited

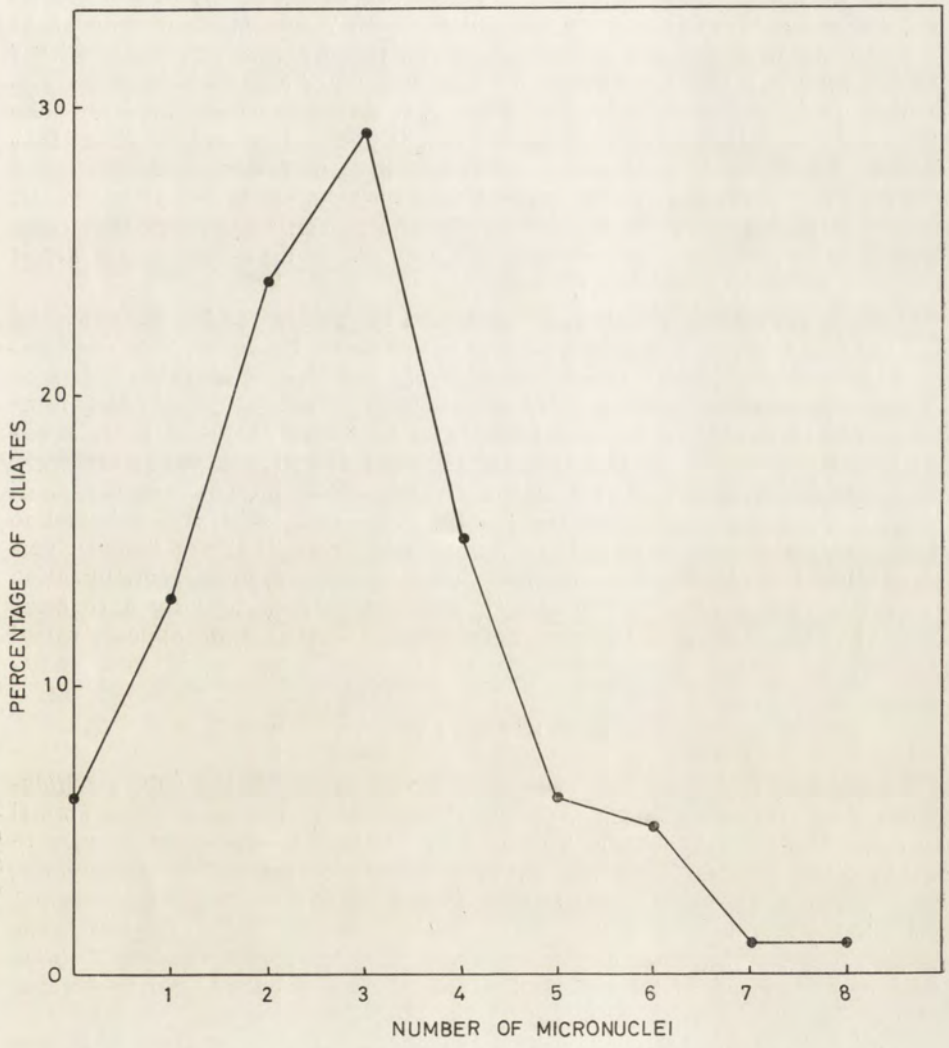

Fig. 6. Graph showing variation of Mi in a population of *F. elliptica*

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period of time and once the Mi have started on mitosis, this factor disappears or becomes inoperative, permitting no more mitotic divisions until it reappears at the next binary fission. Had this factor been operative over a longer period, it would have been expected that Mi in all stages of mitosis would be present in a single cell. That such a condition does not exist and that all Mi are in the same state of division, with a few Mi which show no division whatsoever, appears indicative of the mitosis — initiating factor being present only for a short period.

It is apparent that arrest of mitosis, operative over a period, would lead to a lessening of Mi number in the species. That this is so is shown by studies on *F. elliptica*. In a population, about 70% of the ciliates have Mi number between 2 and 4. About 15% have a single Mi, in about 5% to 6%, no Mi were observed, 10% have 5 or 6 Mi. Ciliates with 7 or 8 Mi form only about 1% of the total population (Fig. 6). However, the presence of ciliates with 7 or 8 Mi, even in a small percentage in a population of ciliates, where the major Mi number is from 2 to 4 would show that arrest of mitosis does not take place in all the ciliates. A study showed that it takes place only in about 10% of them. Moreover, it is observed that more than half the population has 2 or 3 Mi. It appears that there is some factor operating to maintain the Mi number at this level. Probably this is also the reason why we do not come across a large number of amicronucleate forms as would be the case if arrest of mitosis occurred in higher frequency.

Striking pre-fission changes are exhibited in foldings and undulations by the Ma of *F. elliptica*. Their significance is unknown. However, in other ciliates, elimination of chromatin material from the Ma is a fairly common phenomenon and has been regarded as indicative of some type of reorganization (Calkins 1930, Kidder 1933, Kidder and Diller 1934, Kidder and Summers 1935, Vimala Devi 1960). Alterations in nucleolar and chromatin structure (Roth and Minick 1961) and condensation, elongation and skein formation by the Ma (Weisz 1949, 1954) are believed to be reorganization phenomena. Apart from a suggestion that pre-fission changes exhibited by *F. elliptica* also may indicate some type of reorganization process, we are unable to offer any other explanation. Actually a renewed effort at understanding the amitotic division of the ciliate Ma appears called for.

Summary

Frontonia elliptica is a fresh-water ciliate belonging to the subclass *Holotricha*, order *Hymenostomatida*, family *Frontoniidae*. The vegetative animal has a length of 175 μ to 200 μ. The somatic ciliature is essentially similar to that in other species of *Frontonia*. Trichocysts, located below the pellicle, measure about 5 μ in situ, but have a length of 40 μ to 50 μ when ejected. Peristome is found at the anterior third of the body. An undulating membrane is present on the right side of the oral fossa. The contractile vacuole is single and is in the centre of the cell. It has from 5 to 6 collecting canals leading into it.

F. elliptica has a single compact Ma and from 1 to 8 Mi. During binary fission, only such Mi that move away from the Ma divide by mitosis. Others

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that remain in close proximity with it appear to be arrested. In *F. elliptica*, this is believed to account for the variation in number of Mi in different individuals. The possibility of some kind of chemodifferentiation in the cytoplasm is suggested with reference to this phenomenon of selective mitosis of Mi. The Ma shows striking prefission changes in the form of foldings and undulations and exhibits differential staining with Feulgen's reagent. The possible significance of this has been discussed in the light of earlier work done in this field.

ZUSAMMENFASSUNG

Frontonia elliptica ist ein süsswasser Infusorium, das zur Unterklasse *Holotricha*, Ordnung *Hymenostomatida* und Familie *Frontoniidae* angehört. Die Länge der vegetativen Form beträgt von 175 μ bis 200 μ. Die somatische Bewimperung ist grundsätzlich ähnlich solcher der anderen Arten von *Frontonia*. Die, unter der Pellikula steckenden Trichocysten messen engefähr 5 μ in situ, doch wenn ausgeschleudert, sind sie von 40 μ bis 50 μ lang. In dem vorderen, $^1/_3$ der ganzen Länge messenden Körperteil, befindet sich das Peristom. An der rechten Seite der Peristomalgrube treffen wir die undulierende Membran. In der Mitte der Zelle steckt die einzelne kontraktile Vakuole, ausgerüstet mit 5 bis 6 zuführende Kanäle.

F. elliptica besitzt einen einzelnen, dichten Makronukleus und von 1 bis 8 Mikronuklei. Während der Doppelteilung, werden durch die Mitose nur solche Mi zerteilt, die sich vom Ma zur Seite schieben. Vermutlich, alle die eng neben Ma bleiben, werden festgehalten. Voraussichtlich ist diese Erscheinung bei *F. elliptica* verantwortlich für das Variieren der Zahl der Mi bei verschiedenen Individuen. Die Erscheinung solcher selektiver Mitose der Mi spricht einigermassen für die Möglichkeit, einer Art von Chemodifferenzierung des Cytoplasma. Der Ma zeigt erstaunliche Vorteilungsveränderungen der Faltenform wie auch der Undulation, und mit Feulgen behandelt, zeigt er differenzierte Färbung. Auf Grund der bisherigen Arbeiten, die sich damit beschäftigten, wird die Wichtigkeit dieser Erscheinung diskutiert.

REFERENCES

Beardsley A. E. 1902: Note on Colorado Protozoa with description of new species. Trans. Amer. micr. Soc. 23, 49—59.

Beers C. D. 1946: *Tillina magna:* micronuclear number, encystment and vitality in diverse clones: capabilities of amicronucleate races. Biol. Bull. 91, 256—271.

Bullington W. E. 1939: A study of spiraling in the ciliate *Frontonia* with a review of the genus and a description of two new species. Arch. Protistenk. 92, 10—66.

Calkins G. N. 1930: *Uroleptus halseyi* Calkins II. The origin and fate of the macronuclear chromatin. Arch. Protistenk. 69, 151—174.

Corliss J. O. 1961: The Ciliated Protozoa. Pergamon Press. New York.

Diller W. F. 1936: Nuclear reorganization processes in *Paramecium aurelia*, with descriptions of autogamy and "hemixis". J. Morphol. 59, 11—67.

Diller W. F. 1940: Nuclear variations in *Paramecium caudatum*. J. Morphol. 66, 605—633.

Kahl A. 1930: Urtiere oder *Protozoa* 1. Wimpertiere oder *Ciliata (Infusoria)*. G. Fischer. Jena.

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

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Kidder G. W. and Diller W. F. 1934: Observations on the binary fission of four species of common free-living ciliates, with special reference to the macronuclear chromatin. Biol. Bull. 67, 201—219.

Kidder G. W. and Summers F. M. 1935: Taxonomic and cytological studies on the ciliates associated with the amphipod family *Orchestiidae* from the Woods Hole district. 1. The stomatous holotrichous ectocommensals. Biol. Bull. 68, 51—68.

Manwell R. D. 1928: The occurrence of nuclear variation in *Pleurotricha lanceolata* (Stein). Biol. Bull. 55, 433—438.

Okada T. A. 1956: Cytological study of *Frontonia leucas*, with a note on the variation of the number of micronuclei. Annot. Zool. Jap. 29, 213—218.

Roque M. 1961: Recherches sur les hyménostomes péniculiens. Bull. Biol. France Belgique 95, 431—519.

Roth L. E. and Minick O. T 1961: Electron microscopy of nuclear and cytoplasmic events during division in *Tetrahymena pyriformis* strains W and HAM 3. J. Protozool. 8, 12—21.

Seshachar B. R. and Padmavathi P. B. 1956: The cytology of a new species of *Spirostomum*. J. Protozool. 3, 145—150.

Seshachar B. R. and Kasturi Bai A. R. 1963: Cytology of a new species of *Oxytricha*. Arch. Protistenk. 106, 456—464.

Seshachar B. R., Prabhakara Rao A.V.S. and Bhandary A.V '1959: Micronuclear variation in races of *Blepharisma undulans* Stein. Current Science 28, 369—370.

Vimala Devi R. 1960: The nuclear apparatus of *Frontonia leucas* (Ehrbg). Proc. Natl. Inst. Sci. India 26, 269—277.

Vimala Devi R. 1961: Autogamy in *Frontonia leucas* (Ehrbg). J. Protozool. 8, 277—283.

Weisz P. B. 1949: The role of the macronucleus in the differentiation of *Blepharisma undulans*. J. Morphol. 85, 503—518.

Weisz P. B. 1954: Morphogenesis in protozoa. Quart. Rev. Biol. 29, 207—229.

Wichterman R. 1954: The common occurrence of micronuclear variation dufing binary fission in an unusual race of *Paramecium caudatum*. J. Protozool. 1, 54—59.

Woodruff L. L. 1931: Micronuclear variation in *Paramecium bursaria*. Quart. J. Micr. Sci. 74, 537—545.

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Observations on *Spirostomum teres* Clap.

Beobachtungen über *Spirostomum teres* Clap.

Kahl 1930 described two species of *Spirostomum* with oval macronuleus. *Spirostomum teres* is one of them. Bhatia 1936 reported this species from Kashmir. Villeneuve-Brachon 1940 studied binary fission with special reference to stomatogenesis and contractile vacuole formation. Packard 1948 reported the effects of certain chemicals on the macronucleus of this ciliate. The interest in this unimicronucleate species of *Spirostomum* arose out of the studies on this genus in this laboratory as a part of a programme of investigations into the cytology of ciliate protozoa. The present paper reports studies on the cytology as well as volume changes in macronucleus and cell during the division cycle of *S. teres*.

Material and methods

Spirostomum teres has never been taken in peninsular India. Bhatia 1936 himself obtained it from Srinagar, Kashmir. Our collections were also made there. In the Laboratory, the animals were cultured in a medium of hay infusion, inoculated twice a week with Horlick's malted milk to maintain a good growth of bacteria. Under favourable conditions of temperature (around 25°C) and nutritional supply, the animal divides once every 36 hours.

For morphological studies, dry silver preparations were made according to the method of Klein 1928. Observations were also made on living animals both in the vegetative and dividing stages. Cytological studies of the nuclear apparatus were made by fixing animals in Schaudinn's fixative which were stained with Feulgen's reagent. Light green was used as counterstain.

A study of volume relationships of the cell and the Ma during vegetative and dividing stages was also made. For volume studies, the life-cycle was divided into 5 stages as follows:

Stage 1: Vegetative stage — The Ma is ovoid. The Mi is in interphase.

Stage 2: Primary fission stage — The Ma shows changes in the form of undulations and flattening. The Mi is in prophase.

Stage 3: Mid-fission stage — The Ma slightly elongated and Mi is in late telophase or has finished mitosis.

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Stage 4: Pre-fission stage — The Ma is highly elongated, with a constriction in the middle. The oral organelles and the contractile vacuoles of the daughter cells are formed. The fission furrow is also formed.

Stage 5: Binary fission stage — The Ma halves have separated. The fission furrow has deepened. The two daughter cells are connected by a thin strand of cytoplasm.

These stages are represented in Fig. 1. The histogram (Fig. 7) provides information about the Ma volume and the cell volume during different stages of the life cycle.

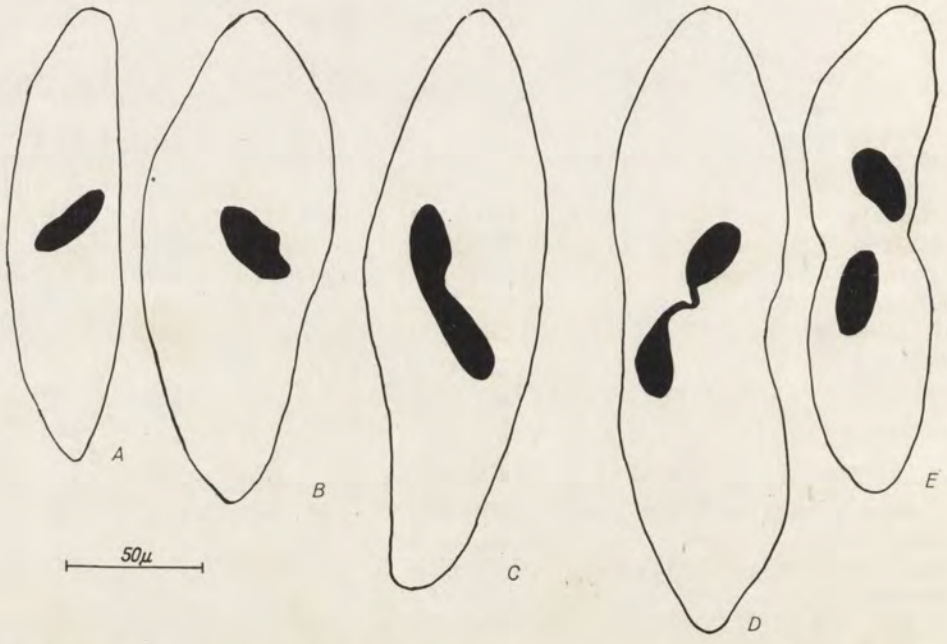

Fig. 1. Different stages of binary fission of *Spirostomum teres:* A. Vegetative stage. B.Primary fission stage. C. Mid-fission stage. D. Pre-fission stage. E. Binary fission stage.

The outlines of the Ma and the cell (Figs. 2—4) at different stages were drawn from the Feulgen — light green preparations fixed in Schaudinn's fluid, with the aid of a camera lucida at known magnification. The area was measured by an Albert's planimeter. It was divided by the known magnification to obtain the actual area. This was multiplied by the thickness, which was determined by using the calibrated fine adjustment screw of a Leitz Ortholux microscope. Errors in measurements of thickness of this nature were not believed to play a great part in the overall pattern of the growth curve as this is a study of the relative volume of the cell and the Ma from stage to stage. Statistical analysis was made after Dixon and Massey 1951 and Fisher and Yates 1953.

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Observations

Spirostomum teres has a length of 250 μ to 400 μ. Its breadth varies from 50 μ to 75 μ. The peristomial groove extends to about half the length of the animal. Contractile vacuole is at the posterior end occupying about 150 μ in length in an animal which measures 400 μ. A narrow canal extends forwards to nearly the anterior tip of the animal. The somatic infraciliary rows follow a spiral course as in other spirotrichous ciliates (Pl. I 1—2). They run about 4 μ apart as seen in dry silver preparations. These infraciliary rows converge at the anterior and posterior ends of the animal (Pl. I 1, 2, 4, 5). To the left of the peristomial groove is a ridge of long cilia (Pl. I 3). The peristomial groove starts at the anterior dorsal tip of the cell and bends ventrally, extending to about half the length of the animal (Pl. I 3).

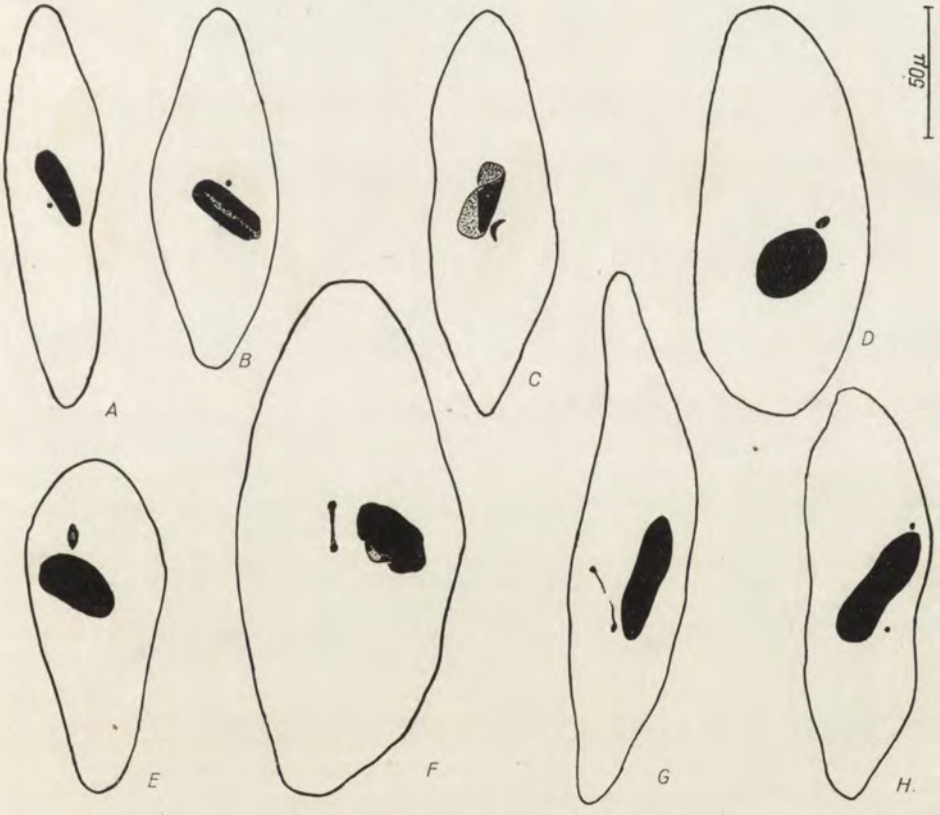

Fig. 2. Course of division in *Spirostomum teres:* A. Vegetative animal with single Ma and Mi. B. Ma shows foldings and differential staining with Feulgen's reagent; Mi in early prophase. C. Ma shows foldings and differential staining with Feulgen's reagent; Mi in crescent phase. D. Ma flattened out; Mi in metaphase. E. Ma in same stage as in D; Mi in anaphase. F. Ma shows foldings and undulations and also exhibits differential staining with Feulgen's reagent; Mi in telophase. G. Ma slightly elongated; Mi has divided into two. H. Ma almost in same stage as in G; the two Mi have come to either end of the elongating Ma; they have condensed into compact bodies.

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The vegetative Ma is oval and measures from 25 μ to 35 μ (Fig. 2 A) in length and 9 μ to 12 μ in breadth. The single Mi is rather small and lies close to the Ma, often embedded on its surface. It has a diameter of 1 μ and stains very lightly with Feulgen's reagent (Figs. 2 A and 4 A).

The onset of binary fission is marked by changes in the Ma as well as in the Mi (Figs. 2 B and C). The Mi moves away from the Ma and mitosis begins (Figs. 2 B and 4 B). Contrary to its poor staining during interphase the Mi stains intensely once mitosis begins and also throughout division.

The crescent stage is highly characteristic of micronuclear prophase (Figs. 2 C and 4 C). The metaphase (Figs. 2 D and 4 D), and anaphase follow rapidly. The anaphase spindle is quite conspicuous and reaches a length of from 4 μ to 4.5 μ (Figs. 2 E and 4 E). The spindle elongates further at telophase and measures from 10 μ to 13.5 μ in length (Fig. 2 F).

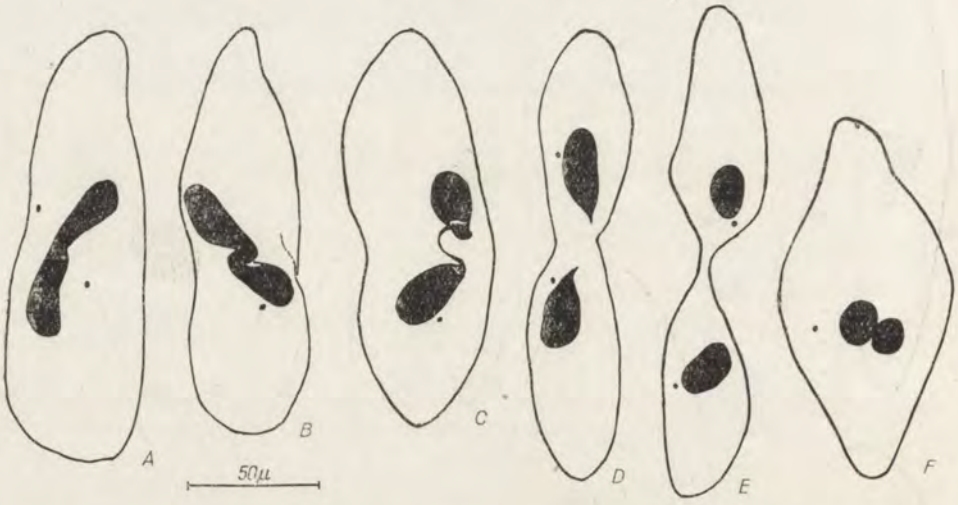

Fig. 3. Course of division in *Spirostomum teres* (continued): A. Ma is at its highest elongation with constriction in the middle; Mi as in Fig. 2H. B. Ma shows a deeper constriction; Mi as in Figs. 2H and 3A; the cell shows constriction at the fission plane. C. Ma is about to separate into two; only one Mi is visible; the cell shows constriction at the fission plane. D. Ma has divided into two; Mi lying close to the daughter Ma; constriction of the cell is deeper. E. The Ma and Mi of each daughter have reached vegetative phase; the daughters are about to separate. F. Ciliate with the Ma in two unequal pieces; single Mi

Macronuclear changes start simultaneously with the onset of micronuclear division. Even as the latter is in prophase, the Ma flattens out and its edges become wavy and folded. When telophase of Mi is completed and and the two daughter nuclei draw apart and become reorganized, the Ma elongates (Figs. 2 G and H) into a cylindrical body, often 55 μ to 65 μ long (Figs. 3 A and B). A constriction appears in the middle and gradually deepens. Later the two halves are connected by a thin strand (Fig. 3 C) and these separate (Figs. 3 D and E) about an hour after the appearance of the cytoplasmic furrow.

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During fission, the new contractile vacuole is formed as an expansion of the canal of the contractile vacuole of the dividing animal, at its middle. It is formed before the appearance of the cytoplasmic furrow. The separation of the two daughter cells takes about 80 to 90 minutes from this time.

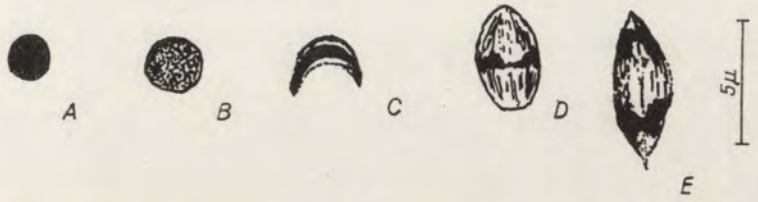

Fig. 4. Micronuclear cycle: A. Mi in vegetative phase. B. Mi in prophase. C. Mi in crescent phase. D. Mi in metaphase. E. Mi in anaphase

A few animals were encountered in our stocks where the Ma was in two unequal pieces. The Mi in these forms was in interphase and displayed no signs of mitosis (Fig. 3 F). It is possible that in these animals, some type of reorganization process like „hemixis" is taking place.

Volume changes during binary fission

Change in cell volume

The cell has a mean volume of 0.1252 mm^3 with a standard error of 0.0111 mm^3 in the vegetative stage and 0.1318 mm^3 with a standard error of 0.0146 mm^3 in the primary fission stage. The increase in mean volume from stage 1 to stage 2 is not statistically significant ($t=1.12$, $d.f.=18$). In stage 3, the cell attains a mean volume of 0.1722 mm^3 with a standard error of 0.0158 mm^3 which is significantly greater than the mean volume in stage 2

Table 1

Mean volume and standard error of mean volume of the cell and the macronucleus in *Spirostomum teres**

Stages	Cell		Macronucleus	
	Mean Volume	Standard error	Mean Volume	Standard error
1. Vegetative	0.1252	0.0111	0.0058	0.0002
2. Primary fission	0.1318	0.0146	0.0083	0.0008
3. Mid-fission	0.1722	0.0158	0.0103	0.0008
4. Pre-fission	0.2035	0 0102	0.0110	0.0013
5. Binary fission	0.0833	0 0066	0.0053	0.0003

* The number of observations used in the calculation of mean and standard error of mean is 10 for all stages except last (binary-fission stage) for which it is 20 (daughters from ten dividing animals). All values are given in cubic millimetres.

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($t = 5.86$, d.f. $= 18$). The cell has its largest mean volume during the binary fission cycle, in stage 4 i.e., 0.2035 mm^3 with a standard error of 0.0102 mm^3, which again, is significantly greater than the mean volume in stage 3 ($t = 5.13$, d.f. $= 18$).

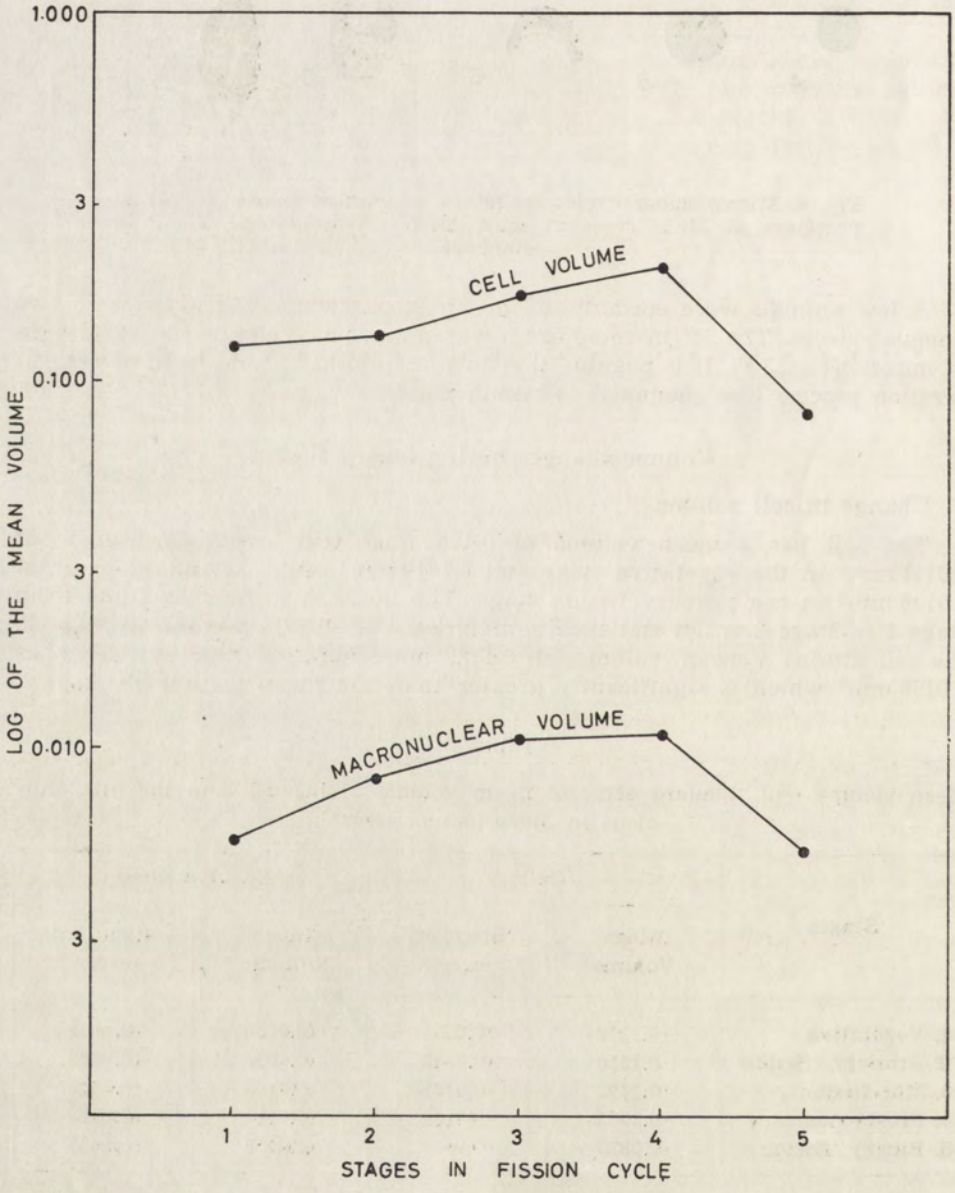

Fig. 5. Graph showing cell volume and Ma volume during different stages of binary fission cycle

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In stage 5 or the binary fission stage, the daughter cell has a mean volume of 0.0833 mm^3 with a standard error of 0.0066 mm^3 and has an upper 95% confidence limit of 0.0971 mm^3 and an upper 99% confidence limit of 0.1022 mm^3. Considering the mean volume of the cell in the stage 4 animal, we may expect the daughter cell to possess, on the average, a mean volume of 0.1017 mm^3 which lies outside the 95 % confidence limit but within the 99 % confidence limit of the mean volume of the daughter cells. Thus, there is a barely significant reduction in the cell volume. However, the difference in mean volume of the cell between stage 5 and stage 1 is highly significant ($t = 33.27$, d.f. = 28). Apparently, after fission the daughter cell grows in volume. Fig. 5 and Table 1 represent the changes in Ma and cell volumes during the binary fission cycle

Change in macronucleus volume

In the vegetative stage (stage 1), the Ma has a mean volume of 0.0058 mm^3, with a standard error of 0.0002 mm^3. In stage 2, i. e., in the primary fission stage, the mean volume is 0.0083 mm^3 with a standard error of 0.0008 mm^3. The increase in the mean volume from stage 1 to stage 2 is statistically highly significant ($t = 3.06$, d. f. = 0.2). In stage 3, the animal has a mean macronuclear volume of 0.0103 mm^3 with a standard error of 0.0008 mm^3. The increase in the mean Ma volume from stage 2 to stage 3 is not statistically significant ($t = 1.81$, d.f. = 8). In stage 4 the Ma has a mean volume of 0.0110 mm^3 with a standard error of 0.0013 mm^3, and this increase again is not statistically significant ($t = 0.46$, d.f. =18).

In the binary fission stage (stage 5), the Ma has a mean volume of 0.0053 mm^3 with a standard error of 0.0003 mm^3 which gives an upper 95 % confidence limit of 0.0060 mm^3. The volume of each daughter Ma is about half that of the Ma at stage 4, which is what one would expect. Further, the mean Ma volumes in the just divided animals and those in the vegetative stages do not show statistically significant differences ($t = 1.19$, d.f. = 29.2).

Volume relationship of cell and macronucleus

The vegetative animal has a mean cell/Ma volume ratio of 22.194 with a standard error of 1.432 which is reduced in the primary fission stage to 16.261 with a corresponding standard error of 1.231. The reduction in the mean ratio is statistically highly significant ($t = 3.14$, d.f. = 18). This is reflected also in a greater increase in the mean volume of Ma (from 0.0058 mm^3 to 0.0083 mm^3) than that of the cell (from 0.1252 mm^3 to 0.1318 mm^3) in the corresponding stage. Cytologically, the Ma shows significant prefission reorganization changes. The mean ratio is seen to increase in stage 3 to 16.996 with a standard error of 1.398 which is not significantly different from the ratio in stage 2 ($t = 0.39$, d.f. = 18). The mean ratio increases further to 20.273 in stage 4, with a standard error of 2.138, but is not statistically significant when the standard error of the mean ratio is considered ($t = 1.28$, d.f. = 18). At this stage, the increase in mean volume of the cell is greater than that of the Ma.

In the binary fission stage, the ratio falls to 15.785 with a standard error of 0.959, which is the minimum ratio obtained during the binary fission cycle, and is significantly lower than the ratio in stage 4 ($t = 2.22$, d.f. = 28). This

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Table 2

Mean and standard error of the ratio of volume of cell to macronucleus in *Spirostomum teres*

Stages	Mean	Standard error
1. Vegetative	22.194	1.432
2. Primary fission	16.261	1.231
3. Mid-fission	16.996	1 398
4. Pre-fission	20.273	2.138
5. Binary fission	15.785	0 959

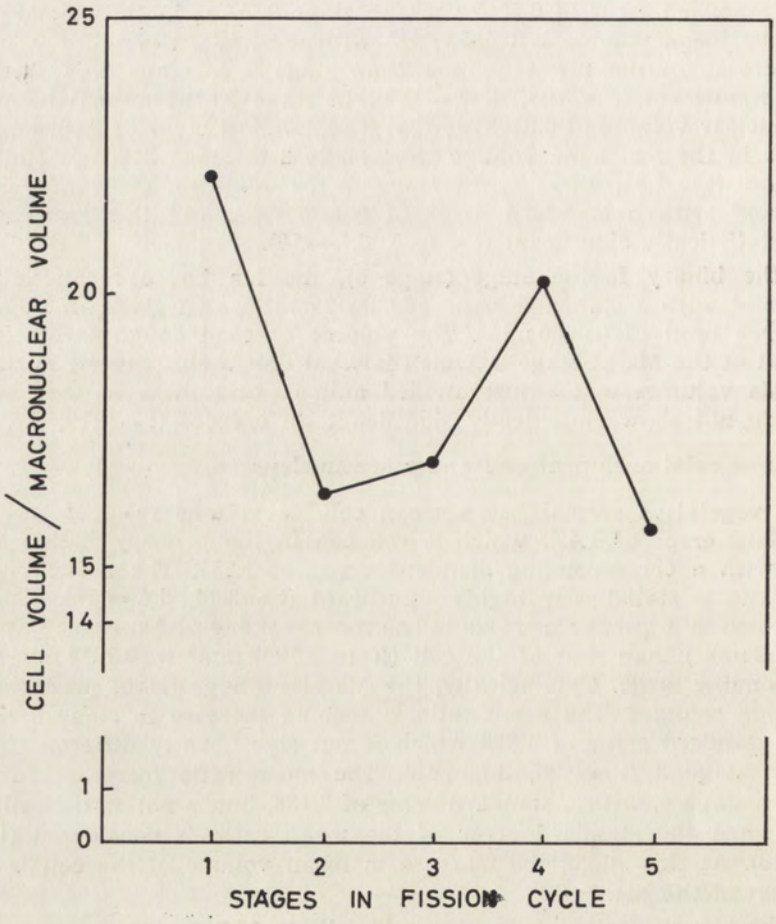

Fig. 6. Graph showing cell/Ma volume relationship during different stages of binary fission cycle

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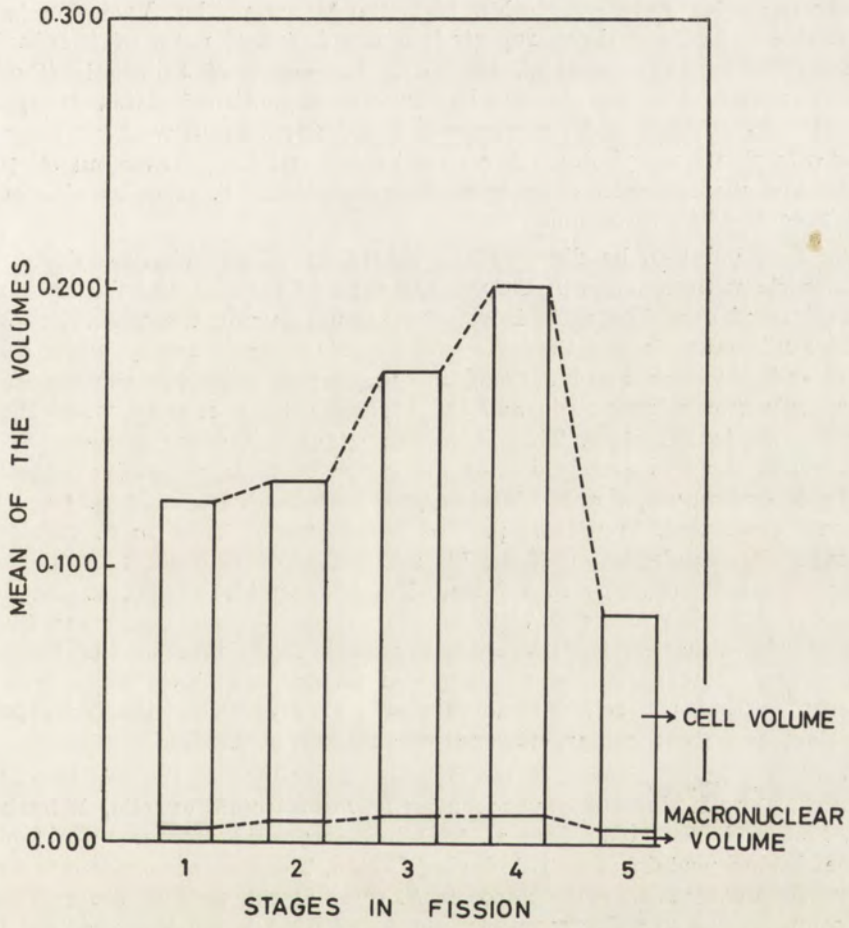

Fig. 7. Histogram showing cell volume and Ma volume during different stages of binary fission cycle

is due to a reduction in cytoplasmic volume at the time of binary fission. After binary fission, the cell grows significantly in volume but the Ma does not, as its volume in stage 5 is not very different from that in stage 1. Fig. 6 and Table 2 show the relationship between cell and Ma volumes during the different stages of binary fission.

Discussion

The interest in *Spirostomum teres* relates primarily to the structure of the nuclear apparatus and the mode of division of its nuclei. While the majority of species of *Spirostomum* exhibit a beaded or banded Ma and multiple Mi (Bishop 1923, Finley and Wanza 1950, Padmavathi 1955a, b, Seshachar and Padmavathi 1956), *S. teres* has a consolidated, re-

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latively small Ma and a single Mi. Also, the Mi is smaller. Variation in size and number of Mi has been reported in the different races of *Blepharisma undulans* (Seshachar et al. 1959) and in species of *Frontonia* (Roque 1961, Seshachar and Vimala Devi, unpublished data). It appears that size and number of Mi are species specific and could well be diagnostic features in ciliate morphology. A crescent stage and long drawn out telophase spindle are characteristic of micronuclear mitosis in *S. teres* as also of the other species of *Spirostomum*.

The first point to be discussed in regard to volume changes during the fission cycle is the change in the volume ratio of the cell and the Ma during the various phases. The ratio is at a maximum during the vegetative stage and at a minimum during the binary fission stage. In *S. ambiguum* (Seshachar and Padmavathi 1959), the vegetative stage has the lowest nucleo-cytoplasmic volume ratio and the highest ratio is noticed when the Ma is in the condensed state. This is an important difference between *S. ambiguum* and *S. teres* and is due to the shape of their respective vegetative Ma. In *S. ambiguum*, the Ma is elongated and beaded and in *S. teres* it is oval and condensed. The latter part of binary fission-cycle in *S. ambiguum*, the stage after condensation of the Ma and before cytokinesis, could be called „growth period" (Seshachar and Padmavathi 1959), as major increases in both Ma and cell volumes take place during this period. But in *S. teres*, the situation is different, as growth in volume of the Ma is at a higher rate towards the early stages of fission and slows down later on. Contrary to this, the cell volume increases slowly during the early period of fission, and more rapidly towards the middle of fission.

Another point of interest is the decrease in volume of the cell in *S. teres* at the time the fission furrow is formed. In *Paramecium aurelia*, Kimball et al. 1959 and Woodard et al. 1961 have reported decrease in cytoplasm and net loss of protein and RNA during fission. There are two possible explanations for decrease in cell volume in *S. teres* at the time of binary fission: 1. It could be due to a decrease in protein and RNA which are used for building new organelles. 2. Alternatively, it could be due to a loss of water from the cytoplasm.

In *P. aurelia*, Kimball et al. 1960 found that most of the dry mass increase of the Ma occurred during the last half of interphase. Also DNA increase at a corresponding stage in *P. aurelia* is reported by Kimball and Barka 1959. But in *Tetrahymena*, observations differ as to the time of DNA synthesis. Prescott 1960 and McDonald 1958 reported its occurrence in the first half of interphase and Walker and Mitchison 1957 found a linear synthesis of DNA throughout interphase. According to Cameron and Prescott 1961 increase in Ma volume in *Tetrahymena* is due to an uptake of materials from the cytoplasm rather than a rapid synthesis within the nucleus. From a study of the volume changes alone it is not possible to say whether the increase in Ma volume in *S. teres* is an indication of DNA synthesis. However, at the time of major increase in its volume, the Ma exhibits characteristic changes — foldings and undulations accompanied by differential Feulgen staining.

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Summary

Spirostomum teres is a fresh water ciliate belonging to order *Heterotrichida*. This paper reports studies on the cytology of this ciliate. A study of the volume changes of the cell and the Ma during the binary fission cycle is also included.

A fully grown ciliate is 250 μ to 400 μ in length and 50 μ to 75 μ in breadth. Contractile vacuole is found at the posterior end of the cell. The somatic infraciliary rows run a spiral course, converge and meet at the anterior and posterior tips of the ciliate. On the left side of the peristomial groove is a ridge of long cilia.

The Ma is oval and measures 25 μ—35 μ × 9 μ—12 μ. The single Mi is rather small, measuring 1 μ in diameter and lies close to the Ma. Crescent stage is characteristic of micronuclear prophase. Anaphase spindle is quite conspicuous and attains a length of 4 μ to 4.5 μ and at telophase the spindle measures 10 μ to 13.5 μ in length. The Ma exhibits characteristic prefission changes. A few animals, where the Ma is in two unequal pieces, were observed. Probably they were undergoing "hemixis".

In *S. teres* volume ratio between cell and Ma is at its maximum during the vegetative stage and at its minimum immediately prior to fission. Growth in volume of the Ma is at a higher rate towards the early stages of fission and slows down later on. The cell volume increases slowly during the early period of fission and more rapidly towards the middle of fission. There is decrease in volume of the cell in this ciliate at the time of fission, when the fission furrow has formed.

ZUSAMMENFASSUNG

Spirostomum teres ist ein süsswasser Infusorium, welches zur Ordnung *Heterotrichida* angehört. Die vorliegende Arbeit betrifft Forschungen über Cytologie dieses Infusorium. Sie umfasst ebenso Forschungen über Volumenveränderung der Zelle und des Makronukleus während des Ziklus der Doppelteilung.

Die Länge des voll ausgewachsenes Infusorium beträgt von 250 μ bis 400 μ, die Breite von 50 μ bis 75 μ. In dem hinteren Teil der Zelle befindet sich die kontraktile Vakuole. Das Silberliniensistem ist in spiralen Reihen angeordnet, die nach dem vorderen und hinteren Ende des Infusorium zusammenlaufen, dort miteinander treffen. An der linken Seite der Peristomalgrube befindet sich ein Kamm langer Wimper.

Der Ma ist oval und messt 25 μ — 35 μ × 9 μ — 12 μ. Der einzelne Mi ist eher klein, er hat 1 μ in Diameter nud liegt eng am Ma. Für die Prophase des Mikronukleus ist eine Sichelform charakteristisch. Der Anaphasespindel ist sehr deutlich und erreicht die Länge von 4 μ bis 5 μ. In der Telophase ist dieser Spindel von 10 μ bis 13,5 μ lang. Vor seiner Teilung zeigt der Ma charakteristische Veränderungen. Es wurden einige Tiere gesehen mit Ma, die in zwei, ungleich grosse Stücke verteilt waren. Vermutlich unterlagen sie der "Hemixis".

Bei *S. teres* erreicht das Volumenverhältnis zwischen der Zelle und dem Ma sein Maximum während des vegetativen Zustand, und sein Minimum unmittelbar vor der Teilung. Die Grössezunahme des Makronukleusvolumen steigert in der

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früherer Periode der Teilung und nimmt naher ab. Das Volumen der Zelle nimmt langsam zu während des früheren Zeitabschnitt der Teilung; doch in dem mittleren Abschnitt der Teilung steigert die Grössezunahme heftiger. Nach der Bildung der Teilungsfurche findet, bei diesem Infusorium, eine Verminderung des Zellvolumens statt.

REFERENCES

Bhatia B. L. 1936: *Protozoa: Ciliophora*. Ed. Sewell R.B.S. — The Fauna of British India, including Ceylon and Burma. Taylor and Francis, London.

Bishop A. 1923: Some observations upon *Spirostomum ambiguum* (Ehrenberg). Quart. J. Micr. Sci. 67, 391—434.

Cameron I. L. and Prescott D. M. 1961: Relation between cell growth and cell division. V. Cell and macronuclear volumes of *Tetrahymena pyriformis* HSM during the cell life-cycle. Exp. Cell Res. 23, 354—360.

Dixon W. J. and Massey F. J., Jr. 1951: Introduction to Statistical Analysis. McGraw-Hill, New York — Toronto — London.

Finley H. E. and Wanza J. W. 1950: Effects of allyl isothyocyanate upon *Spirostomum ambiguum*. Trans. Amer. Micr. Soc. 68, 110—117.

Fisher R. A. and Yates F. 1953: Statistical Tables for Biological, Agricultural and Medical Research. 4th ed. Oliver and Boyd. Edinburgh — London.

Kahl A. 1930: Urtiere oder *Protozoa*. I. Wimpertiere oder *Ciliata (Infusoria)*. G. Fischer, Jena.

Kimball R. F. and Barka T. 1959: Quantitative cytochemical studies on *Paramecium aurelia*. II. Feulgen microspectophotometry of the macronucleus during exponential growth. Exp. Cell Res. 17, 173—182.

Kimball R. F., Caspersson T. O., Svensson G and Carlson L. 1959: Quantitative cytochemical studies on *Paramecium aurelia*. I. Growth in total dry weight measured by the scanning interference microscope and X-ray absorption methods. Exp. Cell Res. 17, 160—172.

Kimball R. F., Vogt-Köhn L. and Caspersson T. O. 1960: Quantitative cytochemical studies on *Paramecium aurelia*. III. Dry weight and ultraviolet absorption of isolated macronuclei during various stages of the interdivision interval. Exp. Cell Res. 20, 368—377.

Klein B. M. 1928: Die Silverliniensysteme der Ciliaten. Weitere Resultate. Arch. Protistenk. 62, 177—260.

McDonald B. B. 1958: Quantitative aspects of deoxyribose nucleic acid (DNA) metabolism in an amicronucleate strain of *Tetrahymena*. Biol. Bull. 114, 71—94.

Packard C. E. 1948: The effects of certain chemicals on the macronucleus of *Spirostomum teres*, with notes on the genus. Trans. Amer. Micr. Soc. 67, 275—279.

Padmavathi P. B. 1955 a: The micronuclei of *Spirostomum ambiguum*. Current Science (India) 24, 24.

Padmavathi P. B. 1955 b: Observations on the nuclear apparatus of *Spirostomum ambiguum*. J. Zool. Soc. India 7, 91—101.

Prescott D. M. 1960: Relation between cell growth and cell division. IV. The synthesis of DNA, RNA and protein from division to division in *Tetrahymena*. Exp. Cell Res. 19, 228—238.

Roque M. 1961: Recherches sur les hyménostomes péniculiens. Bull. Biol. France Belgique 95, 431—519.

Seshachar B. R. and Padmavathi P. B. 1956: The cytology of new species of *Spirostomum*. J. Protozool. 3, 145—150.

Seshachar B. R. and Padmavathi P. B. 1959: A study of the volume changes in *Spirostomum ambiguum* Ehrbg., during various phases of life history. Arch. Protistenk. 104, 492—502.

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Seshachar B. R., Prabhakara Rao A. V. S. and Bhandary A. V. 1959: Micronuclear variations in races of *Blepharisma undulans* Stein. Current Science (India) 28, 369—370.
Villeneuve-Brachon S. 1940: Recherches sur les Ciliés hétérotriches. Arch. Zool. exp. gén. 82, 1—180.
Walker P. M. B. and Mitchison J. M. 1957: DNA synthesis in two ciliates. Exp. Cell Res. 13, 167—170.
Woodard J., Beatrice G. and Swift H. 1961: Nucleoprotein changes during the mitotic cycle in *Paramecium aurelia*. Exp. Cell Res. 23, 258—264.

EXPLANATION OF THE PLATE I

Dry silver preparations of *Spirostomum teres*

1—2: Animals showing spiral arrangement of somatic kineties
3: Part of an animal showing the oral ciliature
4—5: The posterior and anterior ends respectively of the animal showing meeting of kineties

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G. I. POLJANSKY and T. M. POSNANSKAJA

Длительное культивирование *Paramecium caudatum* при 0°

A long-lasting culture of *Paramecium caudatum* at 0°

Вопрос о возможности инфузории *Paramecium caudatum* длительное время существовать при 0°, на границе замерзания, долгое время оставался неясным. В литературе имелись указания (Ефимов 1922, Лозина-Лозинский 1948), что 0° для парамеций является летальной температурой. Гибель инфузорий при 0° сопровождается комплексом характерных изменений, связанных с набуханием.

В наших исследованиях по холодоустойчивости *P. caudatum* (Полянский 1958, 1959, 1963 а) было показано, что способность парамеций переносить 0°, а также отрицательные температуры (переохлаждение), определяется предшествующей температурой культивирования. Например для линий инфузорий, культивируемых в 28—29°, температура 0° летальна — они погибают в ней в течение 2—3 часов. Инфузории того же клона, но предварительно культивируемые при 4—5° могли жить в 0° длительное время. Возник вопрос, имеющий несомненный цитофизиологический интерес, представляет ли собою существование инфузорий при температуре пограничной с замерзанием лишь более или менее длительное переживание или же при этом температурном режиме возможно нормальное осуществление всех жизненных функций (обмен веществ, движение, размножение)? Решению этого вопроса и посвящено настоящее исследование.

Материал и методика

Работа велась на клонах *Paramecium caudatum*, культивируемых на среде Лозина-Лозинского и питавшихся смесью *Saccharomyces cerevisiae* и *Bacillus subtilis* (подробнее о методике культивирования см. Полянский 1957). Клоны велись в пробирках, а кроме того для учета темпа деления в микроаквариумах (солонках) ставились индивидуальные линии. Культуры в пробирках пересевались в свежую среду через 4—6 недель. Свежая пища добавлялась один раз в 7—10 дней.

Для длительного сохранения температуры 0° использовался холодильный шкаф в котором температура поддерживалась в пределах 4—5°. Культуры со-

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держались в шкафу в термосах (в пробирках или солонках) со льдом. В этих условиях таяние льда происходит очень медленно и во все время опыта температура сохраняется на уровне 0°.

Результаты опытов и обсуждение

Культуру клона *Paramecium caudatum* велись при 0° непрерывно с мая 1958 г. по март 1963 г., что составляет 4 года 10 месяцев. За этот период не наблюдалось вымирания инфузорий. Эти опыты доказывают, что *P. caudatum* могут жить при 0° неограниченно долгое время, при условии если они охлаждались постепенно. Наблюдение за живыми парамециями, живущими при 0° показывает, что темп движения их чрезвычайно замедлен.

Темп деления при 0°

Темп деления изучался на индивидуальных линиях инфузорий. В ноябре 1958 г. — феврале 1959 г. он был определен на 10 линиях, в марте — июле 1962 на 30 линиях. При 0° темп деления парамеций чрезвычайно замедлен. В среднем в 1958—1959 г. промежуток между двумя делениями равнялся 19.2 суток, в 1962 г. — 19.8 суток. Таким образом за три с половиной года темп деления практически не изменился. Казалось можно было ожидать, что длительное культивирование при 0° приведет к постепенной адаптации парамеций к этим крайним температурным условиям, лежащим на границе замерзания и темп деления повысится. Однако опыт показал, что это не имело места.

Следует отметить некоторые особенности, характеризующие темп деления парамеций при 0°. Сравнение между собою отдельных линий показывает широкую изменчивость темпа деления. В опытах 1958—1959 г. для отдельных линий величина промежутка между двумя делениями варьировала от 12.5 до 31.6 суток, в опытах 1962 г. — от 11.0 до 32.5 суток. Эта изменчивость еще более бросается в глаза, если учитывать не средний темп деления для данной линии, а промежутки между последовательными делениями в пределах линии. При этом изменчивость в темпе деления оказывается чрезвычайно широкой. Так в опытах 1958—59 г. она колебалась от 5 до 65 суток, в 1962 г. от 4 до 37 суток. Приведем несколько конкретных примеров по отдельным линиям. Например последовательные промежутки между делениями в линии № 2 (1958 г.) равнялась (в сутках): 29, 7, 23, 11, 26, 16, 7, 18, 14, 14; в линии № 9: 65, 31, 18, 18, 16, 17, 11, 17, 8; в линии № 14 (1962 г.): 16, 19, 5, 5 и т. д.

Таким образом за несколько лет культивирования парамеций при 0° не произошло „выравнивания" темпа агамного размножения и промежутки между делениями варьировали в очень широких пределах.

Известно, что при культивировании парамеций в стандартных условиях при температурах выше 0° темп деления всегда характеризуется высоким постоянством и варьирует в очень незначительных пределах.

В этом отношении инфузории, живущие при 0° существенно отличаются от инфузорий, культивируемых при более высоких температурах.

Размеры

Хорошо известен отмеченный многими авторами факт увеличения размеров инфузорий (в том числе и *Paramecium*) при понижении температуры. Мы провели измерения длины тела в пределах клона S *Paramecium caudatum*, длительно культивировавшихся при различном термическом режиме. Измерения произво-

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дились на материале фиксированном жидкостью Люголя. Результаты их представлены на Таблице 1.

Эта таблица подтверждает известную уже из литературы закономерность о зависимости величины парамеций от температуры. Обращают на себя внимание некоторые особенности изменчивости размеров инфузорий, культивируемых при 0°. Средняя длина тела их несколько меньше, чем у парамеций, живущих при 4°. Эти различия статистически вполне оправданы, t_d равняется 8.4. Таким образом для 0° общая закономерность об увеличении размеров тела с понижением температуры оказывается не приложимой. Некоторое уменьшение

Таблица 1

Зависимость длины тела *Paramecium caudatum* (клон S) от температуры культивирования (измерения в микронах)
Relation of the body length in *Paramecium caudatum* (strain S) on the temperature of the culture medium (data in microns)

Температура Temperature	Длина тела Body length		Среднее квадратическое уклонение Mean square deviation	Коэффициент вариации Variance coeff.	Число измерений Number of measurements
	Средняя Mean	Границы Extremes			
0°	248.6±2.9	186.2—323.4	28.71±2.06	11.6±0.8	100
4—5°	268.1±2.1	215.6—323.4	20.58±1.47	7.6±0.5	100
14—15°	219.7±1.9	186.2—264.6	18.33±1.27	8.3±0.6	100
28—29°	185.2±1.3	147.0—215.6	12.54±0.78	6.8±0.6	100

средней величины связано в данном случае повидимому с тем, что наряду с крупными инфузориями встречаются и довольно мелкие и минимальная длина при 0° соответствует таковой при 14—15° (Таблица 1). Вариационный ряд длины тела при 0° оказывается сильно растянутым, что находит свое выражение в значительном повышении изменчивости. Последнее особенно наглядно видно при сравнении коэффициентов вариации в линиях парамеций, культивируемых при разных температурах. Таким образом в отношении размеров парамеций, культивируемых при 0° наблюдается то же явление, что и в отношении темпа деления: довольно резкое повышение изменчивости.

Фагоцитоз

Металь ников 1911, 1912, впервые исследовавший влияние температуры на образование и циклоз пищеварительных вакуолей у парамеций, указывает, что при 0° пищеварительные вакуоли не формируются. Это утверждение представляется мало вероятным, поскольку при 0° возможно длительное существование и размножение парамеций. В работе Решетняк 1952 было показано, что у *Paramecium caudatum* в условиях переохлаждения до —1° образование пищеварительных вакуолей возможно, но при условии, что предварительно инфузории культивировались при 4°. Это делает понятным результаты полученные Металь никовым, который не прибегал к предварительному длительному охлаждению парамеций.

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Проведенное нами специальное исследование этого вопроса вопроса показало, что при 0° фагоцитоз не прекращается. Происходит формирование пищеварительных вакуолей и их циклоз, но все эти процессы протекают чрезвычайно замедленно.

В Таблице 2 приведены результаты опытов по кормлению парамеций тушью (клон S) длительно живших при 0°.

Таблица 2

Результат кормления *Paramecium caudatum* тушью при 0°. Продолжительность каждого опыта 45 минут

Effects of feeding of *Paramecium caudatum* on Indian ink at 0°. Duration of the experiment 45 min. in each case

N° опыта Nr. of experiment	Среднее количество образовавшихся пищеварительных вакуолей Mean number of the food vacuoles formed	Средний объем вакуолей (в μ^3) Mean volume of a vacuole (in μ^3)	Количество инфузорий в опыте Number of ciliates in the experiment
1	1.6	673.6	45
2	1.8	915.9	50
3	1.1	485.3	31
4	2.5	1600.6	50

Если инфузорий из 0° перевести в более высокую температуру, то темп фагоцитоза сразу же возрастает, а объем пищеварительных вакуолей резко увеличивается. Например через час после перевода парамеций из 0° в 14° за 15 минут (а не 45, как в опытах при 0°) образовалось в среднем 5.7 вакуолей (среднее из 35 наблюдений) при среднем объеме 4690.5 μ^3, в другом опыте соответственно 3.3 вакуоли при объеме 3147.0 μ^3. Эти цифры близки к тем, которые для 15° приводит Решетняк.

Изменчивость темпа фагоцитоза при 0°, также как темпа деления и размеров, очень высока. Вот, например, результаты просчета числа вакуолей у отдельных особей для приведенного в таблице 2 опыта № 1: 3, 2, 3, 3, 4, 3, 3, 1, 3, 1, 3, 3, 1, 1, 2, 2, 0, 0, 0, 0, 3, 2, 2, 3, 2, 0, 3, 0, 2, 2, 3, 0, 1, 1, 0, 0, 2, 0, 0, 4, 0, 4, 2. Очень широко варьирует и объем вакуолей. В том же опыте № 1 он колебался от 38.5 до 5615.3 μ^3.

Столь же замедленно, как образование пищеварительных вакуолей, происходит и их циклоз в цитоплазме. Согласно наблюдениям Металь никова 1911 продолжительность пребывания пищеварительной вакуоли с кармином в теле инфузории при комнатной температуре (точного измерения температуры автор не производил) составляет 45—60 минут. Совершенно иную картину обнаруживают парамеции, культивируемые при 0°. При кормлении их тушью первые вакуоли начинают выводиться наружу через 3—4 часа. В дальнейшем процесс этот очень растягивается. Через 24 часа в цитоплазме остается еще 15—20% невыведенных пищеварительных вакуолей. Полное очищение инфузорий от тушевых вакуолей завершается лишь через 36—48 часов.

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Еще более медленно осуществляется циклоз пищеварительных вакуолей при 0°, если они заполнены не тушью, а усвояемой пищей (дрожжи, бактерии). При этом отдельные вакуоли задерживаются в теле инфузорий до двух суток и более.

Приведенные выше факты показываеют, что при 0° процессы фагоцитоза у *Paramecium* не прекращаются, но протекают чрезвычайно замедленно. Циклоз вакуолей в эндоплазме растянут во времени и обнаруживает очень большую изменчивость. В широких пределах варьирует также объем пищеварительных вакуолей.

Гликоген и жир

Количество гликогена и нейтрального жира, откладывающегося в эндоплазме *Paramecium*, как это показано работами Ковалевой 1962 и Полянского 1963 б в большой степени зависит от температурного режима. При низких тем-

Таблица 3

Количество гликогена у *Paramecium caudatum* (клон S) при различных температурах культивирования
Glycogen content in *Paramecium caudatum* (strain S) in different temperatures of the culture medium

Температура культивирования Temperature of the culture medium	Классы накопления гликогена Glycogen content classes					Число инфузорий Number of ciliates
	I	II	III	IV	V	
0°	—	8	28	43	21	100
4°	—	—	31	53	16	100
28—29°	51	49	—	—	—	100

пературах (4—5°) происходит накопление значительных количеств этих веществ. При высоких температурах (28—29°), напротив, отложения гликогена и жира у парамеций почти отсутствуют. Как ведут себя в этом отношении инфузории длительно культивируемые при 0°? Ответ на этот вопрос дают материалы, суммированные в таблицах 3 и 4.

Таблица 4

Количество нейтрального жира у *Paramecium caudatum* (клон S) при различных температурах культивирования
Neutral fat content in *Paramecium caudatum* (strain S) in different temperatures of the culture medium

Температура культивирования Temperature of the culture medium	Классы накопления жира Fat content classes					Число инфузорий Number of ciliates
	I	II	III	IV	V	
0°	15	29	6	—	—	50
4°	—	—	9	42	49	100
28—29°	51	43	6	—	—	100

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В этих таблицах по количеству гликогена и жира в эндоплазме инфузории условно разбиты на 5 классов. К классу 1 относятся инфузории у которых обычными цитохимическими методами эти вещества не обнаруживаются, к 5 классу — инфузории с максимальным отложением гликогена и жира.

В нашей цитированной выше работе (Полянский 1963 б) на стр. 104 приведены рисунки парамеций, относящихся к разным классам по содержанию гликогена и жира.

Из таблицы 3 видно, что парамеции, культивируемые при 0° богаты гликогеном и напоминают в этом отношении инфузорий того же клона из 4° и резко отличаются от линий, культивируемых при 28—29°.

Несколько иная картина наблюдается в отношении жира. В то время, как парамеции, культивируемые при низких положительных температурах (4—5°) всегда очень богаты жировыми включениями (Ковалева 1962, Полянский 1963 б), что подтверждается и наблюдениями над клоном S (Таблица 3), инфузории, живущие при 0° оказались бедны жиром. Лишь небольшое число их относится к классу III, большинство же принадлежит к первым двум классам.

Подводя итог нашим наблюдениям над длительно культивируемыми при 0° *Paramecium caudatum* можно констатировать следующие факты. Температура 0° не является летальной для парамеций. При этой температуре инфузории могут жить годами, не обнаруживая депрессии. Однако физиологические функции их в этих условиях (темп деления, фагоцитоз, движение) очень замедлены. При 0° наблюдается повышенная (по сравнению с другими температурами) изменчивость, затрагивающая как морфолгические (размеры), так и физиологические (темп деления, фагоцитоз) признаки.

Резюме

При 0° возможно длительное культивирование *Paramecium caudatum* при условии постепенного приучения их к этому температурному режиму. Непосредственный перевод инфузорий в 0° из относительно высоких температур (28—29°) приводит к их быстрой гибели.

Темп деления парамеций при 0° чрезвычайно замедлен (в среднем одно деление за 19—20 суток). Многолетнее культивирование инфузорий в этих температурных условиях не привело к изменению темпа деления.

P. caudatum при 0° характеризуются очень крупными размерами.

Фагоцитоз в этих условиях протекает чрезвычайно замедленно. В среднем образуется от 1 до 2,5 пищеварительных вакуолей за 45 минут. Объем этих вакуолей по сравнению с таковыми, формирующимися при более высоких температурах, очень мал (Таблица 2). Циклоз их в эндоплазме также очень замедлен. Некоторые вакуоли остаются в теле инфузорий свыше 2 суток.

Культивирование парамеций при 0° приводит к накоплению в цитоплазме большого количества гликогена. Параллельного накопления нейтрального жира не происходит. В этом заключается существенное их отличие от парамеций, культивируемых при 4°, которые всегда очень богаты жировыми включениями.

Характерной особенностью культур парамеции при 0° является высокий уровень их модификационной изменчивости, затрагивающей как морфологические признаки (размеры), так и физиологические функции (темп деления, фагоцитоз, накопление в эндоплазме гликогена).

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Способность *Paramecium caudatum* длительно жить при 0° демонстрирует широкие адаптационные возможности этого вида к термическому режиму среды. На изученнном нами клоне парамеций температурные границы жизни лежат в пределах от 0° до 30°С. Широкая индивидуальная приспособляемость инфузорий к термическому фактору играет, очевидно, существенную роль в их экологии.

SUMMARY

It is quite possible to cultivate *Paramecium caudatum* at 0° during several years if the ciliates were gradually adapted to such a low temperature. However, if the ciliates cultivated at relatively high temperatures (28—29°) are very quickly transferred to 0°, they die immediately.

The division rate of *P. caudatum* cultivated at 0° is extremely slow (on the average one division within 19—20 days). This division rate does not change if the ciliates live under these conditions during several years.

P. caudatum living at 0° are characterized by a very large body size.

The processes of phagocytosis are also extremely slow at 0°: on the average 1—2.5 food vacuole is formed within 45 minutes. The volume of each food vacuole is very small as compared with that of the ciliates living under higher termic conditions (for instance at room temperature). The cyclosis of the food vacuoles is also very slow and some of the vacuoles remain in the endoplasm for more than 2 days.

A great amount of glycogen is stored up in the endoplasm of the ciliates cultivated at 0°. However, no storage of neutral fat was observed in the endoplasm and this is the essential difference as comparing to the ciliates cultivated at 4°C.

A high level of variability is characteristic of *P. caudatum* living at 0°. The high variability is one of the principle peculiarities of the ciliates under study, touching upon some morphological features (the body size, for example) and physiological functions (the division rate, the phagocytosis rate and the process of glycogen accumulation).

The ability of *P. caudatum* to live and multiply at 0° reflexes its wide adaptibility to different environmental temperatures. The *Paramecium* clone in question can live from 0° till 30°C. A wide individual adaptibility of ciliates to the termic factor plays an important part in their adaptations to ecological factors of the environment in natural conditions.

ЛИТЕРАТУРА

Ефимов В. В. 1922: Вымерзание и переохлаждение простейших. Арх. Русск. протистол. общ. 1, 153—168.

Ковалева Н. Е. 1962: Влияние температуры культивирования на чувствительность инфузорий к повреждающему действию рентгеновых лучей. Цитология 4, 306—317.

Лозина-Лозинский Л. К. 1948: Влияние солей на холодоустойчивость инфузорий и причины смерти при охлаждении. Журн. общ. биол. 9, 441—453.

Метальников С. И. 1911: К физиологии внутриклеточного пищеварения. Извест. С. Петерб. биол .лаб. 11, 3—121.

http://rcin.org.pl

(Метальников С. И.) Metalnikov S. I. 1912: Contributions à l'étude de la digestion intracellulaire chez les Protozoaires. Arch. Zool. exp. gen., Ser. 5, 9, 373—499.

Полянский Ю. И. 1957: Температурные адаптации инфузорий. I. Зависимость теплоустойчивости *Parameciиm caudatum* от температурных условий существования. Зоол. журн. 36, 1630—1646.

(Полянский Ю. И.) Poljansky G. I. 1958: Experimental investigation of temperature adaptations of Infusoria. XV Int. Congr. Zoology, Sect IX, Paper 26, 1—3.

Полянский Ю. И. 1959: Температурные адаптации у инфузорий. II Изменение теплоустойчивости и холодоустойчивости *Parameciиm caudatum* при культивирвании при низких температурах. Цитология 1, 714—727.

Полянский Ю. И. 1963 а: О способности инфузорий туфельки *(Parameciиm caudatum)* переносить отрицательные температуры. Acta Protozool. 1, 165—175.

Полянский Ю. И. 1963 б: Зависимость содержания гликогена и жира в цитоплазме *Parameciиm caudatum* от температуры. Морфол. физиол. простейших 3, 102—110.

Решетняк В. В. 1952: Влияние температуры на фагоцитоз у *Parameciиm*. Уч. Зап. Ленингр. Гос. Унив. 141, Серия биол. 28, 252—264.

http://rcin.org.pl

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Влияние различных условий культивирования зеленых и бесцветных клеток *Euglena gracilis* на их выживаемость после рентгеновского облучения

Influence of different culture conditions in green and colourless cells of *Euglena gracilis* on their survival after X-ray irradiation

Одноклеточный организм *Euglena gracilis* широко используется в цитофизиологии при изучении изменений, происходящих в нём при разрушении и потере хлорофилла и хлоропластов под действием некоторых субоптимальных факторов. В обесцвеченных клетках *Euglena gracilis* нарушается синтез хлорофилла и ксантофилла (Brawerman and Chargaff 1959); уменьшается содержание РНК и увеличивается содержание ДНК (Neff 1960), а их устойчивость к действию неблагоприятных факторов снижается. Так, бесцветные штаммы оказываются более чувствительными по сравнению с зелёными к действию повышенной температуры (Gross and Jahn 1956, 1958), и рентгеновских лучей (Касинова 1963). По нашим данным, при облучении мягкими рентгеновскими лучами средняя летальная доза для бесцветных клеток равна приблизительно 60 кр., а для зелёных — 120 кр. В этих опытах зеленые и бесцветные клетки как до, так и после облучения содержались при неодинаковых условиях освещения (бесцветные — в темноте, а зеленые — в специальной световой камере), тогда как остальные условия содержания — состав и кислотность среды, температура культивирования и т. д. были одинаковыми для обоих штаммов. Между тем имеются указания, что условия культивирования после экспозиции к рентгеновским лучам в сильной степени влияют на выживаемость эвглен. Так, в опытах Wichterman 1955 зелёные эвглены обычно погибали после дозы в 16.5 кр при содержании в темноте, но выдерживали дозы в 55 кр при культивировании после облучения на свету. Можно полагать, что не только свет или темнота, но также наличие в среде органических веществ, или их отсутствие окажут влияние на выживаетмость эвглен, которые, как известно, при росте на органической среде в условиях освещения, имеют смешанный тип питания (миксотрофный).

Целью настоящей работы мы ставили изучение выживаемости эвглен при содержании их в различных условиях после рентгеновского облучения.

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Материал и методика

Материалом служили зелёный и бесцветные штаммы *Euglena gracilis*. Использованный в опытах стойкий бесцветный штамм, не зеленеющий на свету до 6-ти месяцев, был получен из зелёного путем выращивания в течение 8 дней в среде со стрептомицином (Заар и Касинова 1963). У эвглен, обесцвеченных стрептомицином, не удаётся обнаружить хлорофилла и хлоропластов ни при наблюдении in vivo (исследование под люминесцентным микроскопом), ни на фиксированном материале. Кроме того были использованы клетки старого, полученного 10 месяцев тому назад бесцветного штамма, в которых хлорофилл продолжает отсутствовать только при содержании их в темноте. При перенесении на свет клетки такого штамма зеленеют в течение двух недель. Все штаммы до облучения выращивались на органической среде — отваре из колосков тимофеевки (*Phleum pratense*) при pH 7.0. При этом зелёный штамм находился в специальной световой камере (Заар 1964) со следующим световым режимом: освещенность 1500 люкс, соотношение света к темноте 16 к 8 (в часах), а бесцветный — в темноте, в термостате. В камере и термостате поддерживалась постоянная температура + 22°C.

Перед облучением десятидневные культуры всех штаммов, находившиеся в логарифмической фазе роста, разводились свежей органической средой так, чтобы в 1 мл суспензии содержалось 30 000 эвглен. 10 мл суспензии эвглен помещались в стеклянные сосуды (диаметр 45 мм; высота 15 мм) и облучались на установке РУМ-7. Условия облучения: напряжение 50 кв, сила тока 20 ма, без фильтра на расстоянии 25 мм от трубки мощность дозы 30.2 кр/мин. Были применены следующие дозы рентгеновских лучей: 30.2; 151 и 302 кр.

Таблица 1

Различные комбинации условий, в которых содержались штаммы *Euglena gracilis* Klebs после рентгеновского облучения. Цифры означают варианты опыта

Different combined conditions in which the strains of *Euglena gracilis* were maintained following the X-ray irradiation. Figures mean the type of experiment

Штамм Strain / Среда для культивирования Culture medium	Зелёный Green		Бесцветный (нестойкий) Colourless (facultatively)		Бесцветный (стойкий) Colourless (constantly)	
	На свету Light	В темноте Darkness	На свету Light	В темноте Darkness	На свету Light	В темноте Darkness
Органическая Organic	1	2	4	5	6	7
Минеральная Inorganic	3	—	—	—	—	—

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Сразу после облучения при помощи капилляра клетки эвглен переносились поодиночке в пробирки с 1 см³ свежей среды. Отсаживалось по 30—35 клеток каждого штамма после облучения соответствующей дозы.

Были поставлены следующие варианты опыта (Таблица 1).

Через 14 суток после начала опыта пробирки с отсаженными клетками просматривались и определялось число клеток, давших клональные культуры. Второй просмотр производился через 30 суток после облучения. Тем самым определялся процент клеток, выживших и давших клоны к 30 дню после облучения.

Наличие или отсутствие хлорофилла определялось под люминесцентным микроскопом при рассмотрении в котором хлорофилл даёт красное свечение.

Результаты исследования

Данные по выживаемости эвглен после облучения в различных вариантах опыта приведены в таблице 2.

Как видно из данных таблицы 2, зелёные клетки, помещенные после облучения на органическую среду в темноту (вариант 2) и лишенные возможности фотосинтезировать, выживают значительно хуже зелёных эвглен, находящихся на той же среде, но в условиях освещения (вариант 1). При исследовании под

Таблица 2

Процент клеток, давших клоны к 30-му дню после облучения дозами 30.2, 151 и 302 кр в различных вариантах опыта, указанных в Таблице 1

Percentage of cells still producing clonal lines on the 30th day following the irradiation with 30.2, 151 and 302 kr., in the different types of experiment, as characterized in the Table 1

Вариант опыта Type of experiment	Доза в кр — Dosis in kr.		
	30.2	151	302
1	94.0	33.0	19.0
2	63.3	20.8	3.1
3	9.7	—	—
4	86.6	37.5	16.6
5	72.0	18.0	10 0
6	67.0	16.6	10.0
7	73.0	21.9	10.0

люминесцентным микроскопом эвглен, развивавшихся из клеток, помещенных в темноту (2 вариант опыта) не удаётся обнаружить хлорофилла после воздействия любой из применённых доз. Следовательно, рентгеновские лучи, также как и ультрафиолетовое излучение, субоптимальные температуры и другие неблагоприятные факторы вызывают разрушение хлорофилла в зелёных клетках.

В третьем варианте опыта зелёные клетки, отсаженные в минеральную среду и выставленные на свет, практически не выживают после облучения. Так, в этом

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варианте опыта после обучения дозой в 30.2 кр только 9.7% клеток дали клональные культуры, а при облучении дозами в 151 и 302 кр не выжила ни одна клетка.

Поскольку в третьем варианте опыта эвглены могли питаться только автотрофно, то их высокая смертность свидетельствует о явной недостаточности фотосинтеза в процессе восстановления их пострадиационных повреждений. При дозах в 151 кр и выше клетки вообще не выживают, если они лишены возможности питаться гетеротрофно.

Интересно отметить, что при дозе в 30.2 кр выживаемость клеток, питающихся исключительно гетеротрофно (второй вариант) составила 63.3%, и если к этой величине прибавить 9,7% выживаемости, „полученной за счет фотосинтеза", то выживаемость должна была бы повыситься до 63.3 + 9.7 = 73%. Однако, в действительности предоставление возможности к миксотрофному питанию (первый вариант) приводит к значительно большему повышению выживаемости (до 94.0% при дозе 30.2 кр). Вероятно, в этих условиях происходит большее восстановление фотосинтетической функции, чем в третьем варианте опыта, когда возможность питаться гетеготрофно для клеток исключена.

К таким же выводам о значении типа питания для выживаемости эвглен приводит анализ и других вариантов опыта, приведенных в таблице 2.

Выживаемость стойко обецвеченных клеток как на свету, так и в темноте (варианты 6 и 7) оказалась, примерно, одинаковой для каждой из примененных доз. Между прочим, это свидетельствует о том, что свет не оказывает на бесцветных эвглен фотореактивирующего действия. Выживаемость бесцветных, но способных к позеленению клеток в темноте (вариант 5) такая же, как в вариантах 6 и 7. Зато на свету бесцветные, зеленеющие клетки (вариант 4) показывают примерно такую же выживаемость, как и зелёные клетки в первом варианте опыта. Все выжившие и давшие клоны клетки 4-го варианта к 30-му дню после облучения содержали хлорофилл, за исключением двух, в которых он появился на 45-е сутки после облучения.

Сравнение зелёных и обесцвеченных эвглен, растущих в темноте (варианты 2, 5 и 7) и питающихся гетеротрофно, показывает, что выживаемость зелёных и бесцветных клеток сходна после облучения дозами в 30.2 и 151 кр. После дозы в 302 кр выживаемость зелёных эвглен 2-го варианта опыта ниже выживаемости бесцветных клеток, облучённых той же дозой.

Обсуждение результатов

Полученные результаты показывают, что зелёные клетки *Euglena gracilis* не во всех условиях выживают лучше, чем бесцветные. Так, при содержании клеток после облучения на органической среде в темноте, выживаемость зелёных клеток равна или даже ниже (вариант 2, доза 302 кр), чем у бесцветных. Поэтому, сравнивая зелёный и бесцветный штаммы эвглен по выживаемости, следует непременно указывать условия, в которых они содержались после облучения.

Проведённые под люминесцентным микроскопом исследования зелёных эвглен, содержащихся после облучения в темноте, показали, что при рентгеновском облучении, так же как при действии других неблагоприятных факторов, происходит исчезновение хлорофилла и обесцвечивание клеток. Повреждением хлорофилла при облучении, по-видимому, можно объяснить крайне низкую выживаемость клеток в третьем варианте опыта (при содержании эвглен на минеральной среде). Очевидно, фотосинтетическая функция резко ослабевает у облу-

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ченных клеток, которые при достаточно высокой дозе могут выжить только при предоставлении им возможности гетеротрофного питания. Означают ли эти результаты, что облученные достаточно высокой дозой зелёные клетки становятся всецело гетеротрофами и фотосинтез в них не имеет места? Этот вопрос можно решить лишь путем непосредственного измерения интенсивности фотосинтеза в облученных клетках. Однако, полученные данные о резком увеличении выживаемости зелёных и зеленеющих клеток при их освещении (варианты 1 и 6) позволяют сделать предположение, что фотосинтетическая функция не утрачивается полностью в результате облучения даже высокими дозами и что она особенно быстро восстанавливается при наличии двух условий: света и гетеротрофного питания.

Итак, условия содержания эвглен после облучения отчетливо влияют на их выживаемость. Насколько можно судить, это влияние связано (в условиях наших опытов) с функцией питания эвглен. Гетеротрофное питание после облучения в большей мере, чем фотосинтез, определяет выживаемость клеток и потому условия культивирования, исключающие возможность такого питания (вариант 3), приводят к 100% гибели клеток при дозах в 151 и 302 кр.

Выживаемость зелёных, зеленеющих и бесцветных эвглен при содержании их в условиях, исключающих фотосинтез (варианты 2, 5 и 7) оказалась, в общем, сходной. Отсюда следует, что различия в выживаемости между зелёными и бесцветными клетками, при развитии их на свету, всецело обусловлены наличием системы фотосинтеза у первых и отсутствием её у вторых.

Проведенные исследования приводят к следующим заключительным выводам:

1. Зелёные клетки *Euglena gracilis* Klebs лишенные по условиям опыта возможности фотосинтезировать, не отличаются по выживаемости от бесцветных клеток, находящихся после облучения в темноте.

2. Зелёные клетки, лишённые возможности питаться гетеротрофно, погибают полностью при дозах 151 кр и выше.

3. Зелёные клетки, имеющие миксотрофный тип питания, обнаруживает наиболее высокую выживаемость после рентгеновского облучения.

4. Облученные бесцветные клетки, ресинтезирующие хлорофилл, при помещении на свет, не отличаются по выживаемости от зелёных клеток при росте на свету в органической среде.

5. Предполагается, что бóльшая выживаемость облученных зелёных эвглен по сравнению с бесцветными, не способными к ресинтезу хлорофилла при развитии их на свету в органической среде, всецело обусловлена наличием системы фотосинтеза у первых и отсутствием её у вторых.

6. Рентгеновские лучи, также как и другие субоптимальные факторы, вызывают обесцвечивание зелёных клеток *Euglena gracilis* Klebs при выращивании их после облучения в темноте.

Резюме

Изучалось влияние условий культивирования после рентгеновского облучения на выживаемость клеток трёх штаммов *Euglena gracilis* Klebs: 1. содержащих хлорофилл, 2. не содержащих хлорофилла, но зеленеющих при помещении клеток на свет и 3. не содержащих хлорофилла и не зеленеющих на свету. Содержание эвглен после облучения на органической или минеральной среде на

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свету или в темноте позволило получить такие комбинации условий, при которых клетки могли питаться: а. миксотрофно (т. е. гетеро- и автотрофно), б. только гетеротрофно, в. только автотрофно. Выживаемость клеток всех штаммов при исключении фотосинтеза (т. е. при культивировании в темноте) оказалась одинаковой при дозах 30.2 и 151 кр, а при дозах 302 кр зелёные клетки обнаруживали меньшую выживаемость, чем бесцветные. При культивировании на свету (на органической среде) выживаемость штаммов 1 и 2 значительно превысила выживаемость штамма 3.

Наконец, при культивировании на свету на минеральной среде клетки штамма 1 погибали полностью при дозах 151 кр и выше. Это показывает, что одно лишь автотрофное питание не обеспечивает выживаемости зелёных эвглен после облучения дозами 151 кр и выше, но что, тем не менее, наличие фотосинтетического аппарата у зелёных клеток обеспечивает их лучшую выживаемость по сравнению с бесцветными (штамм 3) при культивировании на органической среде на свету.

Найдено также, что рентгеновское облучение вызывает разрушение хлорофилла в клетках *Euglena gracilis*.

SUMMARY

The influence of culture conditions after X-ray irradiation on survival of cells belonging to three strains of *Euglena gracilis* Klebs: 1. chlorophyllous, 2. colourless, but becoming chlorophyllous in the light, and 3. colourless and remaining the same in the light, was investigated. The maintenance of the *Euglena* cells, after such exposure, on the organic vs. inorganic medium as well as in the light or in the darkness, allowed to obtain such combinations of the culture conditions which were permitting: a. both hetero- and autotrophic feeding, b. heterotrophic feeding only, and c. autotrophic feeding only. After excluding the photosynthesis (i.e. in the darkness) the number of surviving cells, after exposure to the dosage of 30.2 kr and 151 kr, proved to be the same for all the three strains, while at the dosage of 302 kr the percentage of survival was less in chlorophyllous cells than in the colourless ones. The maintenance of the *Euglena* cells under light + organic food culture conditions brought to the survival of the strains 1 and 2, much higher than that in the strain 3. Finally, the maintenance under light + inorganic food culture conditions resulted in the complete lethality of the strain 1 at the dosage of 151 kr or more. The results suggest that autotrophic feeding alone is not sufficient to ensure the survival of coloured *Euglena*, exposed to 151 kr or more but nevertheless, the presence of development (after exposure) of the photosynthetic apparatus in a cell makes its survival better under light + organic food culture conditions. In addition, desintegration of chloroplasts and disappearing of chlorophyll have been found in *Euglena* green cells after X-ray treatment.

ЛИТЕРАТУРА

Brawerman G. and Chargaff E. 1959: Relation of ribonucleic acid to the photosynthetic apparatus in *Euglena gracilis*. Biochem. Biophys. Acta 51, 172—173.

Gross J. A. and Jahn T. L. 1958 a: Temperature tolerance of some bleached strains of *Euglena* J. Protozool. 5 (Suppl.) 7.

http://rcin.org.pl

Gross J. A. and Jahn T. L. 1958 b: Some biological characteristics of chlorotic substrains of *Euglena gracilis*. J. Protozool. 5, 126—135.

Касинова Г. В. 1963: Чувствительность к рентгеновским лучам зелёных и бесцветных клеток *Euglena gracilis* Klebs. Радиобиология (В печати).

Лозина-Лозинский Л. К. 1929: К физиологии питания *Paramecium caudatum*. Изв. Научн. инст. им. Лесгафта 15, 91—136.

Neff R. N. 1960: Volume nucleic acid and nitrogen contents of strains of green and colorless *E. gracilis* and of *Astasia longa*. J. Protozool. 7, 69—174.

Wichterman R. 1955: Survival and other effects following X-irradiation of the flagelatte *Euglena gracilis*. Biol. Bull. 109, 371.

Заар Э. И. 1964: Комбинированные термостаты для физиологического изучения микроорганизмов. Ботан. журн. 49.

Заар Э. И. и Касинова Г. В. 1963: Влияние некоторых физических и химических агентов на ресинтез хлорофилла в апохлоротических клетках *Euglena gracilis* Klebs. Ботан. журн. 48, 896—898.

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Role of the K^+ and Ca^{2+} ions in the excitability of protozoan cell. Chemical and electric stimulation of contractile vacuoles

Znaczenie jonów K^+ i Ca^{2+} w pobudliwości komórki pierwotniaczej. Drażnienie chemiczne i elektryczne wodniczek kurczliwych

The experiments described on the following pages were undertaken in the aim to continue the study of the part played by K and Ca in the electrotonic excitation processes in *Protozoa*, which was initiated just recently in this laboratory by the article of Grębecki 1964.

Osmoregulation is considered presently the most important function of the contractile vacuole. Considerable differences of the osmotic pressure in the ciliate organism and in its external medium exist. As result, a constant inflow of water into the cell occurs. To keep the balance, this water should be pumpt out to the medium at a suitable rate. Removing water is performed by the contractile vacuoles.

The hydrostatic pressure in the vacuole should exceed the hydrostatic pressure of the medium that water might be discharged from the vacuole during the systole. The excess of the hydrostatic pressure in the vacuole might be evoked either by the general turgor of the body or by the tension exerted upon the vacuolar membrane and upon the content of the vacuole. Since the systole takes place in *Paramecium* still flattened by exoosmosis, it should be postulated that the body turgor is not the only and sufficient factor responsible for the systole, playing however a certain role in its mechanism.

Systole itself should be mainly ascribed to the local pressure upon the vacuolar membrane. The most important factor seems to be the contraction of the fibrils coating the central receptacle and the excretory canal.

It is commonly known that the osmotic pressure of the medium exerts an influence upon the rate of the contractile vacuole activity. Nevertheless if the vacuoles are contractile structures, then the factors acting in a specific way on other contractile structures — e.g. cilia — should influence the vacuoles activity in a similar way. To those factors belong potassium and calcium as antagonistic ions and in a similar way — the cathode and anode when the ciliate is stimulated by the direct current (Grębecki 1963).

A comparative study of the osmotic action of non-electrolytes on one — and of the influence of the above mentioned factors on the other side —

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seems to be appropriate, the more so as neither the action of the electric current nor differences in the reaction of the contractile vacuoles to the chemical stimuli were as yet comparatively investigated. Besides the results may be one evidence more in favour of the results of Grębecki obtained by the study of cilia.

I wish to express my thanks to Doc. A. Grębecki for initiating this research and for the constant care during the work. My sincere aknowledgements are also due to Prof. Z. Raabe and Doc. S. Dryl for reading the manuscript.

Material and methods

As experimental material *Paramecium caudatum* from the culture maintained in the Department of the General Biology of the M. Nencki Institute of Experimental Biology was used. Ciliates were fed with the monobacterial Brussel sprout nutrient with *Aerobacter aerogenes*, following the Sonneborn's method.

Paramecia were collected geotactically and rinsed several times in 1 mM $CaCl_2$ + 1 mM KCl in distilled water (which is subsequently called "control solution" in the present article) then placed in 5 ml of chloral hydrate, and after 48 hrs. rinsed in the control solution again. Observations begun 1 h. after rinsing. The method of immobilization (deciliation) by chloral hydrate was worked out by Grębecki and Kuźnicki 1961. According the more detailed study of Kuźnicki 1963 cilia regenerate: 7 hrs. after rinsing more than $^1/_2$ of individuals are fully ciliated. After regeneration of cilia, as well the movement as pulsation of vacuoles become normal. The observation lasted 1—5 hrs. after rinsing and then the ciliates started moving which made the observation impossible.

The rate of pulsation of the vacuoles in *Paramecium* immobilized, by chloral hydrate and rinsed was 2—3 times slower than in the non-treated individuals. Although the cycle was prolonged, the frequency of contractions in the sample remained at the same level.

After rinsing the ciliates were placed in experimental solution of defined concentrations and the time of 10 consecutive contractions of the anterior and posterior vacuoles was measured in 10 individuals. The experimental solutions contained substances diluted in the control solution i.e. in the presence of 1 mM $CaCl_2$ and 1 mM KCl. The observation begun 1—3 min. after placing the ciliates in the solution.

During the experiment the control measurements were made, i.e. the time of 10 consecutive contractions of the anterior and posterior vacuoles of individuals in control solution was measured. Results are given in percentage related to the control.

In the study of the influence of chemical stimuli upon the activity of vacuoles, the mean of control measurements executed in the beginning and in conclusion of the experiments was calculated. The change of the evacuation rate of the vacuole in the solutions under experiment was calculated as related to this mean value. The control measurements were performed at the beginning and at the conclusion of the experimental series because at this time ciliates gradually return to their norm, i.e. the rate of their vacuoles function is slightly accelerated.

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For measurement of the influence of current upon the rate of the contractile vacuoles activity, 0.1 ml of paramecia culture rinsed in the control solution were placed in a cuvette of a construction applied by Grębecki and Dryl (Grębecki 1962), containing 2 ml of control solution; this was performed while the electric circuit was open, at an equal distance from both electrodes. Then the time of 3 successive contractions of vacuole was measured. After the 3 rd contraction the circuit of the direct current was closed (the density of current in the cuvette had been adjusted previously) and the time of 3 successive contractions of the same vacuole was measured. For every current density 25 measurements of the activity rate of the anterior and posterior vacuole in the homodrome and antidrome position was executed, in different specimens.

The time of 3 successive contractions of the vacuole in the open circuit was the control. The result obtained after closure of the circuit was calculated in percentage, related to the above measurement.

Effect of chemical stimuli

The influence of different concentrations of electrolytes and non-electrolytes solutions upon the rate of work of the contractile vacuole in *Paramecium* was compared. This was experimented in order to ascertain whether the time of contractions depends only on the osmotic pressure of medium or is influenced by some other factors. On the other hand, the effect of the osmotic factors is the deciding control in the study of other chemical stimuli, of a more specific action. Such stimuli may be the ions influencing the contractile structures in cilia in a characteristic manner. This was kept in view when the experimental solutions were chosen.

Osmotic action

For the study of non-electrolytes only slightly penetrating into the cell (i.e. of the osmotic influence of the medium upon the rate of vacuoles contractions) solutions of urea and glucose were used in rising concentrations: 10 mM, 20 mM, 30 mM, 40 mM, 50 mM. Both urea and glucose evoke inhibition of the vacuoles work proportionally to the rise of molarity of the solutions used and — consequently — of the osmotic pressure in the medium. A very regular course of the curves in the diagram (Fig. 1) proves the adequacy of the method applied.

Action of the antagonistic ions

For experimenting the action of electrolytes upon the work rate of the contractile vacuoles, solutions of potassium, calcium and barium chlorides were used. The action of potassium and calcium upon the contractile structures is antagonistic similarly as that of the cathode and anode. Potassium applied externally — like the cathode — depolarizes the cell membrane of the ciliate, while calcium and anode act as hyperpolarizers (Kamada 1934, Yamaguchi 1960, Ueda 1961). In consequence, Grębecki 1963 suggested the determination of K action as the chemical cathelectrotonus and Ca action as anelectrotonus.

The motory manifestation of both antagonistic effects are as follows: the external potassium and the cathodal current evoke the ciliary reversal, cal-

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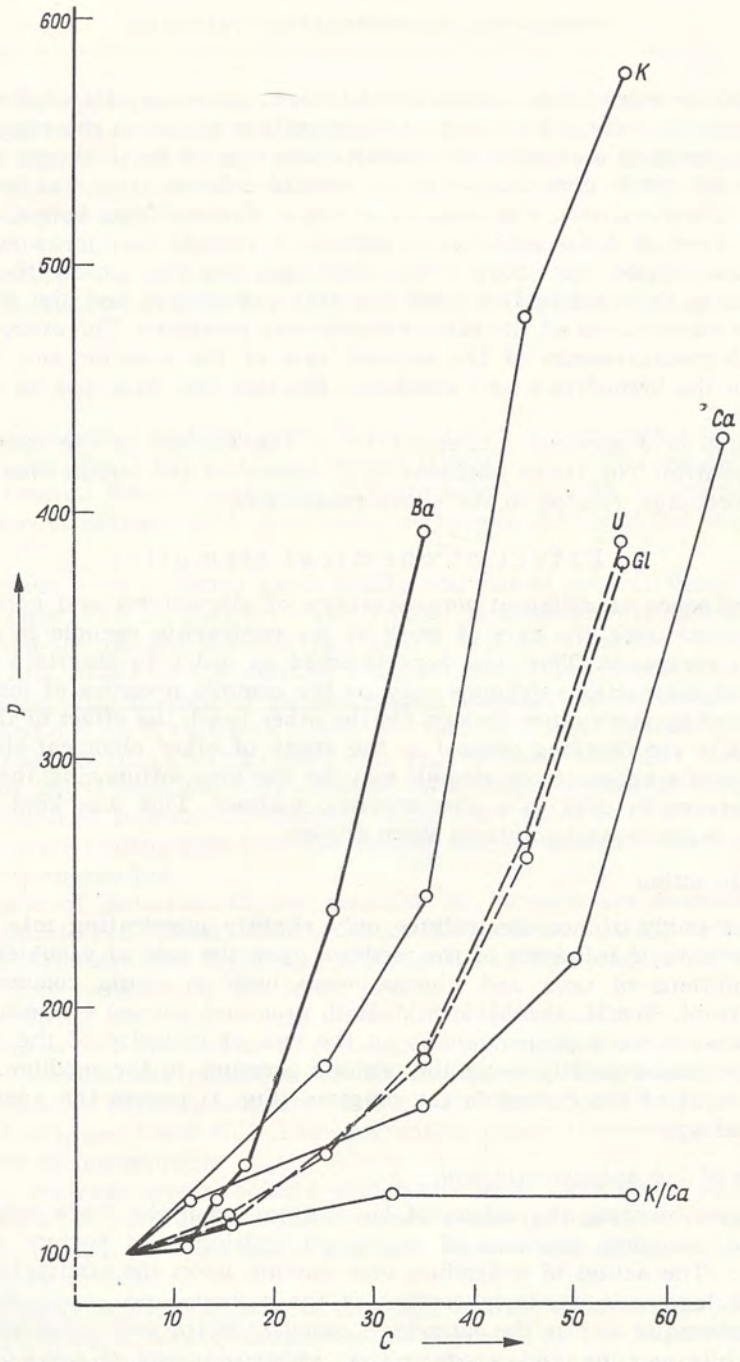

Fig. 1. Effect of chemical agents upon the rate of pulsation of contractile vacuoles in *Paramecium caudatum*. Concentrations (C) are given in ionic or molar equivalents, pulsation rate (P) in the duration of vacuolar cycle calculated in percents related to the control medium. The curves are labelled as follows: U — urea, Gl — glucose, Ba — barium chloride, K — potassium chloride, Ca — calcium chloride (all diluted in the control solution), and K/Ca — potassium and calcium chlorides mixtures in the Donnan ratio amounting 1.

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cium and the anodal current — an acceleration of the normal work (or renormalization). The potassium reversal is inhibited by the excess of calcium ions in the medium, similarly the external calcium inhibits the cathodal reversion. On the other hand, addition of potassium to the medium annihilates the acceleration of movement evoked by calcium ions and complicates the renormalization effect of the anode (Grębecki 1963).

For determining the action of potassium and calcium upon the vacuoles, solutions of following concentrations were used: KCl — 10 mM, 15 mM, 20 mM, 25 mM, and 30 mM, while $CaCl_2$ — 2 mM, 5 mM, 10 mM, 15 mM and 20 mM

In the diagram (Fig. 1) the effects of non-electrolytes and electrolytes are confronted. In the first case molar equivalents were applied, in the second — the ion equivalents, i.e. the isoosmotic solutions are compared.

As follows from the diagram, effects of potassium and calcium differ considerably from the osmotic effect of urea and glucose. The curves of urea and glucose run between those of potassium and calcium. It means that potassium and calcium show antagonism in their action upon the contractile vacuoles. Potassium diminishes the rate of work of the vacuoles in a higher degree than urea and glucose do (i.e. than the osmotic pressure), while calcium acts weaker than those non-electrolytes. The stimulating action of calcium upon the activity of contractile vacuoles in *Paramecium* was mentioned by Kamada 1935.

The action of potassium and calcium ions in the experiments reported in this article are in harmony with their influence upon the movement of the protozoon. Calcium — as known — accelerates the movement, and potassium slows it down as to cause reversal, i.e. its action is inhibitory. Consequently a strong fall of the work rate of the contractile vacuoles characteristic for potassium proves to be analogous whith slowing the activity of the ciliary contractile structures, and a slight fall (when compared to that of urea and glucose) in the solutions of calcium salts is characteristic for its capability to increase the activity of those structures.

Barium was the third electrolyte which action upon the contractile vacuoles was experimented. It was chosen as the only bivalent cation evoking — like potassium — the ciliary reversal (Mast and Nadler 1926, nad recently Dryl 1961). As follows from the experiments, the action of barium upon the contractile vacuoles is really analogous to that of potassium and even is still stronger, i.e. barium diminishes the rate of their work still more than potassium (Fig. 1).

The above experiments prove that the rate of vacuoles work depends not only on the osmotic conditions of the medium but the responses of the vacuoles to external potassium and calcium are also strictly comparable with the effects of those ions upon the typical motory activity.

Compensation of the antagonistic ions

As known, potassium and calcium are antagonistic ions. The excess of potassium ions causes reversal and the excess of calcium — acceleration of movement. If the concentrations of potasium and calcium in solution fullfill the postulation of Donnan, i.e. if the ratio $[K^+]/\sqrt{[Ca^{2+}]}$ = const., ciliates behave in a similar manner and show the same excitability, independently from the absolute value of concentration. This view was put forward by Jahn 1962.

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It was postulated by him theoretically basing on the results of K a m a d a and K i n o s i t a 1940 and was supported recently by experimental findings (G r ę b e c k i 1964).

In the present study, solutions containing potassium and calcium in the ratio $[K^+]/\sqrt{[Ca^{2+}]} = 1$ were applied, equally as in the control solution. They were: 2 mM KCl + 4 mM $CaCl_2$, 3 mM KCl + 9 mM $CaCl_2$, and 4 mM KCl + 16 mM $CaCl_2$.

Those solutions containing potassium and calcium in a constant Donnan ratio, cause only insignificant fall of the work rate of the vacuoles with no tendency to fall together with the rise of the absolute value of concentration. So the stability of the vacuole rythm was attained in spite of very high changes of the osmotic pressure in the medium. The curve of the compensated mixed solutions of potassium chloride and calcium chloride lies in the diagram (Fig. 1) nearly concurrently to the level of the control.

So the potassium/calcium ratio compensating the behaviour of cilia compensates also the work of contractile vacuoles. Since the curve under discussion agrees not with the curve of the osmotic effect (glucose and urea) but with the level of control, it might be concluded that not only the compensation of the contraction activity was attained but even that of the osmotic conditions.

A similar result was reported by E i s e n b e r g 1924, however this author neither realized the specificity nor the antagonism of ions action upon the rythm of the contractile vacuoles in *Paramecium*. She drew a general conclusion from her results that "two electrolytes in their optimal quantitative ratio are able to keep the intensity of water flow through the cell in the hypertonic solution at the level near the norm". E i s e n b e r g failed to report (and this is not to be concluded from her research) what should the optimal quantitative ratio be in the pairs of cations applied by her.

Effect of electric stimuli

Since some chemical stimuli (potassium and calcium) act upon the ciliary contractile structures similarly as the cathode and anode in the electric stimulation do — it seemed advisable to experiment the influence of the direct current of various density upon the work of the vacuoles. The time of the full cycle of the anterior and posterior vacuoles was measured in the homodrome and antidrome positions applying the current of the density: 0.66 mA/cm², 1.32 mA/cm², 2.65 mA/cm² and 5.30 mA/cm². The mean values from measurements (in percentage of the control) are shown in the Table 1.

General contraction effect

The rate of the contractile vacuoles work increases beginning with the current density 2.65 mA/cm² for the antidrome and 1.32 mA/cm² for homodrome position, as well from the side of the cathode as of the anode. At 5.30 mA/cm² the accelerating action of the electric field is expressed by the fall of the time of the vacuolar cycle down to 83.72% (Table 1, section 1+2+3+4).

This result is clear in the light of the study of K a m a d a and K i n o s i t a 1936 and H i s a d a 1952 on the current action upon the ectoplasm of *Para-*

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Table 1

Effect of direct current upon the activity of contractile vacuoles in *Paramecium caudatum* (cycle duration in percents of the control)

Current density (mA/cm²)			0.66	1.32	2.65	5.30
Homodrome position	cathode	(1)	104.80	98.60	90.15	84.10
	anode	(2)	96.30	96.40	82.73	80 90
Antidrome position	cathode	(3)	102.80	104.30	96.40	85.40
	anode	(4)	93.70	98.30	86.75	84.50
Polar effect	cathode	(1+3)	103.80	101.23	93.27	84.75
	anode	(2+4)	95.00	97:30	84 74	82.70
Vacuole	anterior	(1+4)	99.25	98.23	88.45	84.30
	posterior	(2+3)	99.55	100.35	89.56	83.15
Position effect	homodrome	(1+2)	100.55	97.80	86.44	82.50
	antidrome	(3+4)	98 25	101.30	91.57	89.00
General contraction effect		(1+2+3+4)	99.40	99 29	89.00	83.72

mecium caudatum. Hisada reported that the action of current 1.06 volt/mm for 1 sec. causes the body contraction. This accounts for the tendency to accelerate the vacuoles work of the ciliate in the electric field. The body contraction evokes the rise of the body turgor which, as known, may be one of the factors accelerating the systole.

Role of the body orientation

Beginning from the current density 1.32 mA/cm² a difference is noticeable between the effect of the electrodes action upon the vacuoles depending on the homodrome or antidrome positions of the ciliate. In the homodrome position, at the current density 1.32 mA/cm² the general acceleration of the vacuoles work occurs, whereas in the antidrome position it is observed only beginning with 2.65 mA/cm². At the density 2.65 mA/cm² the acceleration of 86.44% was stated for the homodrome and only 91.57% for antidrome position. As shown in the Table 1 (sections 1+2 and 3+4) the effect of electrodes upon the vacuoles is always stronger when *Paramecium* is in the homodrome than in the antidrome position.

Similar differences of the current effects depending on the body orientation of *Paramecium* were stated by Hisada 1952 in his study on the contractibility of the ectoplasm, and it was even earlier stated (Kinosita 1936) that the ciliary system responds to the electric current with the same pattern.

Especially the results of Hisada might help to understand the present findings. According to Hisada the ectoplasm in the homodrome position contracts more than in the antidrome, then the body turgor with this position will increase more, although the current of the same density is applied. As effect of increase of the turgor, the rate of vacuoles discharge will rise similarly, more in the homodrome than in the antidrome position.

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Polar effect of the current

The cathode affects the work of the vacuoles inversely as the anode. The antagonistic action of the both electrodes is manifested in different manner in the weak currents and in the strong ones.

In the ciliates subjected to the action of the current of a density 0.66 mA/cm^2 (homodrome and antidrome positions) as well as of the current of a density 1.32 mA/cm^2 (antidrome position) — the cathode slightly inhibits the work of the vacuoles while the anode accelerates it. The general contraction effect is still absent, i.e. the summarized effects of the cathode and anode action upon the vacuoles in the homodrome and antidrome positions (Table 1, section 1+2+3+4) are expressed by the figures approximate to 100% for both first values of the current density. Consequently we have yet to do just with the purely polar action of the electric field. The differences observed in the action of electrodes are rather slight, which is accounted for by the low intensity of stimulus.

In the higher densities of the current the picture is different. The cathodal and the anodal effects are manifested against the background produced by the general acceleration of the rate of vacuoles work, presumably evoked by contraction of ectoplasm and by rise of the body turgor. Consequently, at those current densities, the differences in the cathode and anode action are manifested as a much stronger acceleration at the side of the anode than at the cathode, under the current of the same density and at the same position of the ciliate related to the electrodes.

As shown in the Table 1, the regular differences in the action of the anode and of the cathode upon the work of vacuoles may be determined by the statement that the time of the cycle of the vacuoles oriented towards the cathode (section 1+3) is always longer than the total mean (section 1+2+3+4) and in those turned towards the anode (section 2+4) — is always shorter. As already stated in the present study, the time of the vacuole cycle is longer in solutions rich in potassium than that which results of only osmotic conditions, and in excess of calcium it is always shorter. It should be concluded that the analogy of the potassium — cathode and calcium — anode action, when related to other contractile structures, holds true in the case of action of those factors upon the contractile vacuoles of *Paramecium*.

Excitability of the anterior and posterior vacuole

The work rate of the anterior and posterior contractile vacuole is different. In the majority of individuals studied, the anterior vacuole works more rapidly than the posterior. The difference is however not important and the inverse situation occurs frequently, i.e. the rate of discharge of the posterior vacuole may be somewhat higher than that of the anterior (Kuźnicki 1952).

No differences in the general irritability of both vacuoles were stated in this study. The changes of the work rate under the action of electric stimuli are in both vacuoles the same (Table 1, sections 1+4 and 2+3). As well, the anterior and the posterior vacuoles respond similarly to the rise of osmotic pressure of medium and to the presence of various concentrations of such ions as potassium, calcium and barium in it.

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The reaction of the anterior and posterior vacuoles to potassium and calcium introduced into the medium in the ratio $[K^+]/\sqrt{[Ca^{2}]} = \text{const.}$ is also similar.

Structure of the contractile vacuoles in the light of their behavior

Some information about the fine structure of contractile vacuole has been provided by the electron microscope observations. According to Rudzińska 1957, Puytorac 1960 and Schneider 1960 one of the elements composing the vacuolar system in *Protozoa* are the contractile fibrils. They twine around the central terminations of the canals spreading over the central vesicle surface (Schneider 1960 and Puytorac 1960) on one side, and embrace the excretory canal and the adjacent part of the central vesicle of the vacuole (Rudzińska 1957).

It follows from the present study that the contractile vacuoles in *Paramecium caudatum* react to certain stimuli in a pattern characteristic for the superficial contractile structures (cilia). Together with the information of the vacuole structures those results seem to indicate a rather large applicability of conclusions put forward by Grębecki 1963 concerning the cilia.

Moreover, the results seem to be an indirect evidence of the fact that the vacuole is a contractile structure. Kitching 1956 is right suggesting that injection of such substances as ATP, adrenaline and acetylcholine might introduce essential information about those structures.

Summary

Reactions to the osmotic pressure, chemical stimuli and action of direct current are similar in the anterior and posterior vacuole. Application of the electric current of a sufficiently high density, evokes the acceleration of the work in both vacuoles of the ciliate being in the electric field. Action of the cathode and anode, in spite of the general acceleration, is specific: cathode causes a prolongation of the time of the vacuolar cycle (when compared to the total mean) and anode shortens it. Potassium and calcium effect is analogous to that of cathode and anode. Potassium slows the vacuole work more than the non-electrolytes of the same osmotic pressure do, while calcium accelerates it. Compensation of the potassium/calcium concentrations, as determined by the Donnan ratio, brings about not only the compensation of the contraction activity of vacuoles but also that of the osmotic effect. The results of the above investigation may be looked upon as an indirect evidence of the view that the vacuoles are contractile structures in the strict sense.

STRESZCZENIE

Reakcje na ciśnienie osmotyczne, bodźce chemiczne i działanie prądu stałego są jednakowe u wodniczki przedniej i tylnej. Działając prądem elektrycznym o wystarczająco wysokim natężeniu powodujemy przyspieszenie pracy obu wodniczek kurczliwych pierwotniaka znajdującego się w polu działania prądu. Na tle ogólnego przyspieszenia katoda i anoda działają specyficznie: katoda zwiększa czas trwania cyklu wodniczek w porównaniu ze średnią globalną, a anoda go zmniejsza. Analogicznie do katody i anody działają potas i wapń. Potas zwalnia pracę wod-

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niczek bardziej niż nieelektrolity o tym samym ciśnieniu osmotycznym, a wapń w porównaniu z nimi przyspiesza. Zrównoważenie potas/wapń określone warunkami stosunku Donnana powoduje nie tylko zrównoważenie aktywności skurczowej wodniczek, lecz także zrównoważenie efektów osmotycznych. Wyniki niniejszej pracy mogą być uważane za dowód pośredni, że wodniczki tętniące są strukturami kurczliwymi sensu stricte.

REFERENCES

Dryl S. 1961: The ciliary reversal in *Paramecium caudatum* induced by simultaneous action of barium and calcium ions. J. Protozool. 8 (Suppl.), 55.

Eisenberg E. 1924: Działanie wodniczka tętniącego u wymoczków *Paramecium caudatum* Stein. Przyczynek do badań nad przepuszczalnością komórki. Trav. Inst. Nencki 37, 1—30.

Grębecki A. 1962: Phénomènes électrocinétiques dans le galvanotropisme de *Paramecium caudatum*. Bull. Biol. France Belgique 96, 723—754.

Grębecki A. 1963: Rebroussement ciliaire et galvanotaxie chez *Paramecium caudatum*. Acta Protozool. 1, 99—112.

Grębecki A. 1964: Rôle des ions K^+ et Ca^{2+} dans l'excitabilité de la cellule protozoaire. I. Équilibrement des ions antagonistes. Acta Protozool. 2, 69—79.

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

Hisada M. 1952: Induction of contraction in *Paramecium* by electric current. Annot. Zool. Japon. 25, 415—419.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ciliary reversal and ion antagonism. J. Cell. Comp. Physiol. 60, 217—228.

Kamada T. 1934: Some observations on potential differences across the endoplasm membrane of *Paramecium*. J. Exp. Biol. 11, 94—102.

Kamada T. 1935: Contractile vacuole of *Paramecium*. J. Fac. Sci. Imp. Univ. Tokyo 4, 49—62.

Kamada T. and Kinosita H. 1936: Protoplasmic contraction in *Paramecium*. Fac. Sci. Imp. Univ. Tokyo 4, 349—358.

Kamada T. and Kinosita H. 1940: Calcium-potassium factor in ciliary reversal of *Paramecium*. Proc. Imp. Acad. Tokyo 16, 125—130.

Kinosita H. 1936: Effect of change in orientation on the electrical excitability in *Paramecium* J. Fac. Sci. Imp. Univ. Tokyo 4, 189—194.

Kitching J. A. 1956: Contractile vacuoles of *Protozoa* Protoplasmatologia 3, 1—45.

Kuźnicki L. 1952: Działanie wodniczków kurczliwych *Paramecium caudatum* w płynach hodowli i roztworach niektórych soli. (unpublished).

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

Mast S. O. and Nadler J. E. 1926: Reversal of ciliary action in *Paramecium caudatum*. J. Morphol. Physiol. 43, 105—117.

Puytorac P. de 1960: Observations en microscope electronique de l'appareil vacuolaire pulsatile chez quelques Ciliés astomes. Arch. Anat. microsc. Morph. exp. 49, 241—256.

Rudzińska M. 1957: Mechanisms involved in the function of the contractile vacuole by electron microscopy. J. Protozool. 4 (Suppl.), 9.

Schneider L. 1960: Elektronenmikroskopische Untersuchungen über das Nephridialsystem von *Paramecium*. J. Protozool. 7, 75—90.

Ueda K. 1961: Electrical properties of *Opalina*. I. Factors affecting the membrane potential. Annot. Zool. Japon. 34, 99—110.

Yamaguchi T. 1960: Studies on the modes of ionic behavior across the ectoplasmic membrane of *Paramecium*. I. Electric potential differences measured by the intracellular microelectrode. J. Fac. Sci. Univ. Tokyo 8, 573—591.

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