ACTA PROTOZOOLOGICA

REDACTORUM CONSILIUM

E. M. CHEISSIN (LENINGRAD), S. DRYL (WARSZAWA), A. GRĘBECKI (WARSZAWA), O. JIROVEC (PRAHA), G. I. POLJANSKY (LENINGRAD), Z. RAABE (WARSZAWA), K. M. SUKHANOVA (LENINGRAD)

50 YEARS OF THE NENCKI INSTITUTE

VOLUMEN VI

Fasciculi: 1—11

W A R S Z A W A 1 9 6 8

http://rcin.org.pl

INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO
POLSKIEJ AKADEMII NAUK

ACTA PROTOZOOLOGICA

Redaktor Naczelny: ZDZISŁAW RAABE
Zastępca Redaktora Naczelnego: STANISŁAW DRYL
Sekretarz Redakcji: STANISŁAW L. KAZUBSKI

NOTICE TO AUTHORS

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

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

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

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

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

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

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

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

Galley proofs are sent to the authors. Authors receive 100 reprints without covers.

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

http://rcin.org.pl

http://rcin.org.pl

ПРОФЕССОР ЕВГЕНИЙ МИНЕЕВИЧ ХЕЙСИН

(1907—1968)

26 июня 1968 г. скончался выдающийся протозоолог и биолог Евгений Минеевич Хейсин, член редакционной коллегии Acta Protozoologica.

Биологическое образование Е. М. Хейсин получил в Ленинградском университете, который он окончил в 1930 году по кафедре профессора В. А. Догеля. От своего учителя Е. М. Хейсин воспринял глубокий интерес к протозоологии и широкий биологический подход к изучению простейших.

Научная деятельность его началась ещё на студенческой скамье и продолжалась до последних дней жизни. Е. М. Хейсиным опубликовано свыше 150 работ, большинство которых посвящены разным группам паразитических простейших.

Первые крупные цитолого-протистологические работы его были опубликованы ещё в начале 30-х годов и посвящены морфологии и систематике инфузорий *Astomata* из олигохет Байкала. Эти исследования и до наших дней пользуются широкой известностью у специалистов. Ряд интересных статей Е. М. Хейсина посвящён паразитическим амёбам, у которых особенно подробно была им изучена изменчивость дизентерийной амёбы.

Излюбленным объектом исследований Е. М. Хейсина на протяжении почти всей его научной деятельности являлись кокцидии кролика. На этом материале им выполнен ряд крупных работ. Особенно подробно изучен жизненный цикл кокцидий с детальной цитологической и цитохимической характеристикой всех его стадий. За последние годы Е. М. Хейсин опубликовал несколько статей по электронной микроскопии кокцидий. Им изучен на электронномикроскопическом уровне процесс формирования гамет и мерозоитов, открыта особая ультраструктура — ультрацитостом, по-видимому, участвующий во внутриклеточном питании паразита. Кроме большого числа специальных работ, Е. М. Хейсиным опубликованы две больших монографии по кокцидиям, из которых одна („Жизненные циклы кокцидий домашних животных") вышла в 1967 году.

Много внимания в своих исследованиях Е. М. Хейсин уделял изучению жизненного цикла пироплазмид. Ему удалось убедительно доказать, что в клеще-переносчике паразит не размножается половым путём, а имеет лишь бесполое размножение. Это заставляет в корне пересмотреть весь вопрос о положении пироплазмид в системе и исключить их из класса споровиков, куда их причисляли ранее.

Ряд работ Е. М. Хейсина посвящён свободноживущим простейшим. В частности, очень интересны выполненные им вместе с рядом сотрудников исследования по фотометрии ядерного аппарата инфузорий, при этом получены очень интересные данные о полиморфизме микронуклеуса в пределах одного вида инфузорий.

http://rcin.org.pl

Е. М. Хейсин всегда интересовался общими проблемами протистологии. Он обсуждал построение системы простейших. Е. М. Хейсин — активный участник составления обзорно-критических работ по протозоологии. Он — один из трёх авторов большого руководства „Общая протозоология", вышедшего в СССР в 1962 году, а затем переведённого и напечатанного в Англии.

Главные научные интересы Е. М. Хейсина всегда лежали в области протозоологии. Но, будучи зоологом очень широкого профиля, он выполнил ряд ценных исследований в области экспериментальной гельминтологии и акарологии.

Наряду с научной деятельностью, Е. М. Хейсин отдал немало сил педагогической работе в качестве профессора высшей школы. Многие годы он преподавал в Педагогическом институте им. Герцена в Ленинграде, в Петрозаводском и в Ленинградском университетах. В Институте цитологии Академии Наук СССР в Ленинграде Е. М. Хейсин работал с самого основания Института в 1957 г. Им подготовлен большой отряд молодых учёных-протозоологов.

Е. М. Хейсин — активный участник международных научных мероприятий. Он участвовал во многих зоологических и паразитологических конгрессах, выступая с докладами и сообщениями. Особенно активно участвовал Е. М. Хейсин в работе протозоологических конференций в Праге в 1961 году и в Лондоне в 1965 году.

За последние два года в качестве вице-председателя Оргкомитета Е. М. Хейсин включился в работу по подготовке 3-го Международного Протозоологического Конгресса, который состоится в Ленинграде в июле 1969 г.

Умер Е. М. Хейсин внезапно, в полном расцвете творческих сил. Последние полгода своей жизни он работал над большой монографией по электронной микроскопии простейших, которая осталась незавершённой. Его смерть — большая потеря для протозоологии и большое горе для всех тех, кто близко знал этого замечательного человека и учёного и работал вместе с ним.

http://rcin.org.pl

PROFESSOR EUGENE M. CHEISSIN

(1907—1968)

On June 26th 1968 died suddenly the prominent protozoologist Eugene Mineevitch Cheissin, the member of the Editorial Board of Acta Protozoologica.

E. M. Cheissin received his biological education at the Leningrad University. He accomplished it in 1930 in the Laboratory of Prof. W. A. Dogiel. E. M. Cheissin took from his master a profund interest in protozoology as well as a broad biological approach to the research of this animal group.

His scientific activity had begun already at his student time and continued till the last days of his life. E. M. Cheissin published over 150 papers, the majority of them being devoted to various groups of parasitic protozoa.

His first more extensive cyto-protozoological works had been published already at the beginning of the thirties and concerned the morphology and systematics of the ciliates *Astomata* from the Baikal oligochaeta. Those studies are broadly known among specialists up to our days. A number of interesting publications of E. M. Cheissin concern the parasitic amoebae, the variability of the dysenteric amoeba being investigated especially exactly.

The favourite investigation object along nearly the whole scientific activity of E. M. Cheissin were the rabbit coccidia. On this material he executed a number of extensive researches. The most detailed was the study of the coccidian life cycle with an accurate cytological and cytochemical characteristic of all its stages. In the recent years, E. M. Cheissin has published several articles concerning the electron-microscopy of coccidia. The process of formation of gamets and of merozoits was investigated by him on the electronmicroscopic level and a peculiar ultrastructure — the ultracytostome — was discovered. It participates presumably in the intra-cellular nutrition of the parasite. Besides the great number of special works, E. M. Cheissin published two extensive monographs on coccidia. One of them ("The life cycles of coccidians of the domestic animals") appeared in 1967.

In his investigations E. M. Cheissin payed much attention to the study of life cycle of piroplasmids. He succeeded in demonstrating convincingly that in this parasite no sexual reproduction occurs in its vector — tick, the reproduction being asexual. This fact caused a fundamental revision of the whole problem of the taxonomic position of piroplasmids and their exclusion of the class *Sporozoa* wherein they were included previously.

A number of works of E. M. Cheissin were devoted to the free-living ciliates. Especially interesting are his investigations executed, together with his co-workers, on photometry of the nuclear apparatus in ciliates. Interesting facts on polymor-phism of micronucleus within the same species of ciliates were revealed.

E. M. Cheissin was always interested in the general problems of protistology. He discussed the structure of the protozoan system and was an active collaborator of the critical review works in protozoology. He was one of the three authors of

http://rcin.org.pl

the great treaty "General protozoology" published in USSR in 1962, subsequently translated and published in England.

The main scientific interests of E. M. Cheissin were always in the field of protozoology. Being however a zoologist of a broad profile, he performed a number of valuable studies in the field of experimental helminthology and acarology.

Besides his scientific activity E. M. Cheissin devoted much of his forces to the pedagogical work as a University professor. For many years he was a lecturer at the Herzen Pedagogical Institute in Leningrad, in the University of Petrozawodsk and of Leningrad.

E. M. Cheissin worked in the Institute of Cytology of the Academy of Sciences of USSR in Leningrad since the very formation of the Institute in 1957. He educated a considerable group of young protozoologists.

E. M. Cheissin was an active contributor of the international Scientific enterprises. He participated in many zoological and parasitological scientific congresses, presenting his communications. His share was especially active in the works of the protozoological conferences in Prague (1961) and in London (1965).

In the last two years he was active as the vice-president of the Organization Commitee of the 3-rd International Congress on Protozoology planned in July 1969 in Leningrad.

E. M. Cheissin died suddenly in the full expansion of his creative forces. In the last six months of his life he worked on an extensive monography of electron-microscopy of protozoa. This work remains unfinished. His death is a great loss for protozoologists and a great sorrow for all those who knew closely this remarkable man and scientist and who worked with him.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 1

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

E. M. CHEISSIN

On the distinctness of the species *Eimeria neoleporis* Carvalho, 1942 from the cottontail rabbit *Sylvilagus floridanus mearnsii* and *Eimeria coecicola* Cheissin, 1947 from the tame rabbit *Oryctolagus cuniculus*

О самостоятельности видов *Eimeria neoleporis* Carvalho, 1942 из белохвостового кролика *Sylvilagus floridanus mearnsii* и *Eimeria coecicola* Cheissin, 1947 из домашнего кролика *Oryctolagus cuniculus*

Carvalho 1942 described a new species *Eimeria neoleporis* from the cottontail rabbit *Sylvilagus floridanus mearnsii* in USA. The oocysts of this species (Fig. 1) are of a subcylindrical or elongated ellipsoidal form usually tapering slightly towards the micropyle which is seen distinctly. The walls of the oocyst are smooth, their thickness being even on the whole surface

Fig. 1. Oocyst of *E. neoleporis* after Carvalho 1942

except for the micropyle region. Around it, a thickening of the oocyst envelope is observable. The extra-residual body of the oocyst consists of 4 or less granules at the sporoblast stage only. They usually disappear after the completion of sporulation. The intra-residual body of spores is large. Sporulation lasts 50—75 hrs. The oocyst length is 32.8—44.3 μ (mean length 38.8 μ), its breadth — 15.7—22.8 μ (mean 19.8 μ). The mean shape index — 1.95. The spores with the Stieda body are elliptical, their dimensions are 17.1×8—9.

http://rcin.org.pl

Sporozoits are banana-shaped. The prepatent period lasts 11—14 days, in average 12 days. The patent period — 8—16 days, in average 10 days. The development of the endogenous stages in the vermiform process and in the coecum as well as in the area of the ileo-coecal valve.

In Soviet Union Cheissin 1947 revealed in the tame rabitt *Oryctolagus cuniculus* oocysts which differed in their shape and size from the oocysts of other coccidia species from the same host, and established the new species *E. coecicola*. The oocysts of this species have a cylindrical form, they are sometimes ovoid or ellipsoidal, slightly asymmetrical. On their more narrow end, micropyle is seen well. It is surrounded by an external, slightly thickened envelope. The oocyst length fluctuates from 25.3 to 39.9 μ (in average 31.1—35.5 μ). Their breadth fluctuates from 14.6 to 21.3 μ (in average 16.9—19.6 μ). The mean shape index is 1.8—1.96 (Fig. 2). The prepatent period — 9—10 days, the patent period — 7—9 days. The localization of the endogenous stages is in the vermiform appendix and in coecum. Schizonts may also develop in the lower segments of the small intestine.

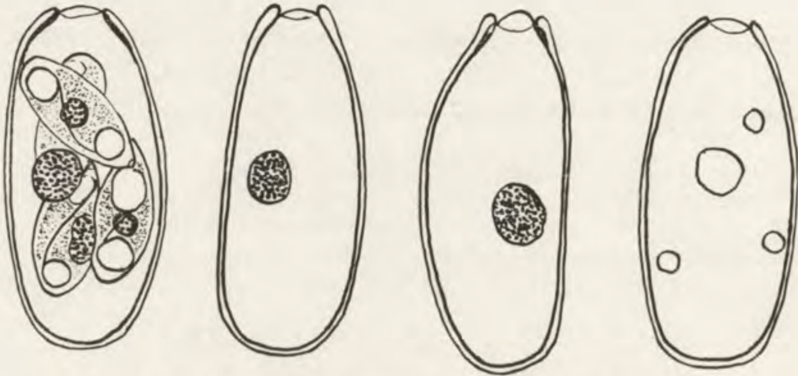


Fig. 2. Different forms of oocysts of *E. coecicola*

Pellerdy 1954 compared the description of both species and came to the conclusion that *E. coecicola* is identical with *E. neoleporis*. Since the first species has been decribed several years later than the second one, consequently it should be considered as the synonim of *E. neoleporis* (Pellerdy 1965). Pellerdy mentioned that he revealed oocysts very similar to that of *E. neoleporis* in wild European rabbits (*Oryctolagus cuniculus*) in Hungary. They were of a cylindrical form with a distinct micropyle on their thinner end. The dimensions of oocysts fluctuated in the limits: 30—40×16—23 μ. In some oocysts a small residual body was observable. In the oocysts found after the infection of the tame rabbit, the extra-residual body was constituted only of a small number of granules and had no character of a compact body as it was observed e.g. in *E. media* (Pellerdy 1954). After an experimental infection of the tame rabbit with the oocysts of *E. neoleporis* from the wild rabbit, Pellerdy revealed that the endogenous stages are localized in the vermiform appendix and in the coecum, and the prepatent period amounts 10—11 days.

In this way, according to Pellerdy, the similitude of *E. coecicola* and *E. neoleporis* is expressed in the structure of oocysts, in the localization of

http://rcin.org.pl

the endogenous stages and in the duration of the endogenous development. Nevertheless Pellerdy failed to consider some essential differences between those two species which should be evaluated when the question of their distinction is to be solved.

We would consider the comparison of oocysts in the first place (Table 1). My own data, gained after the measurements of a great number of oocysts indicate that their length does not exceed 40 μ. Oocysts measuring 42—43 μ occur very rarely. It should be remarked that the clone of *E. coecicola* was obtained after the infection of a rabbit with one oocyst (Cheissin 1947 a). The mean length of the oocysts did not exceed 35.5 μ. As seen in the table, Gill and Ray 1960 revealed even shorter oocysts in the tame rabbit in India.

Table 1

Comparison of *Eimeria coecicola* and *E. neoleporis* according to the data of different authors (dimensions in microns; mean values in parantheses)

Species	*E. coecocola*		*E. neoleporis*		
Authors	Cheissin 1947	Gill and Ray 1960	Carvalho 1942	Pellerdy 1954	Gill and Ray 1960
Shape of oocysts	ovoid, cylindrical, ellipsoidal	ovoid	subcylindrical, elongated elliptical	cylindrical	elongated ellipsoidal
Length of oocysts	25.3—39.9 (31—35.9)	27.5—33 (30.25)	32.8—44.3 (38.8)	30—40	30—54 (37.5)
Breadth of oocysts	14.6—21.3 (16.9—19.6)	14—19.5 (16.5)	15.7—22.8 (19.8)	16—23	16—22 (19.0)
Extra-residual body	1.3—6.7 (3.9—5.3)	6	4 granules disappearing after sporulation	small	none
Micropyle	+	+	+	+	+
Spores	16×9	—	17.1×8—9	—	—
Time of sporulation	46—72 hrs.	—	50—75 hrs.	—	—
Prepatent period	9—10 days	—	11—14 days	10—11 days	—
First gamonts	7th day	—	10th day	—	—
Patent period	7—9 days	—	8—16 days	—	
Localization	vermiform process, coecum, schizonts till the 7th day in small intestine, all stages in crypt epithelium, oocysts in connective tissue	—	vermiform proc. coecum, ileo-coecal valve, schizonts in connective tissue	vermiform append., coecum, ileo-coecal valve	
Host	*Oryctolagus cuniculus*	*Oryctolagus cuniculus*	*Sylvilagus floridanus mearnsi*	*Oryctolagus cuniculus*	*Oryctolagus cuniculus*
Country of investigation	U.S.S.R.	India	U.S.A.	Hungary	India

http://rcin.org.pl

The length of the oocysts of *E. neoleporis* fluctuates approximately in the same limits but its mean value reported by Carvalho is never lower than 39 μ. Gill and Ray mention a great variability of length of the oocysts of *E. neoleporis* frcm 30—54 μ, mean value being 37.5 μ. Consequently the oocysts of *E. neoleporis* are longer than these of *E. coecicola*. Their length differs approx. by 2.5—3 μ (in average). The width of the oocysts is approximately the same in both species, as follows from the Table 1.

As to the oocysts found by Pellerdy in the wild rabbit in Hungary, it may be stated that they approach by their dimensions rather *E. coecicola* than *E. neoleporis*. The most essential difference between both species has been revealed in the structure of the extra-residual body of the cocyst. Carvalho remarked that the extra-residual body of *E. neoleporis* consists of 4 or less grains which are seen at the stage of sporoblast and are absent in the oocysts which have concluded their sporulation. Gill and Ray mentioned that in the oocysts of *E. neoleporis* found in the tame rabbit in India, the extra-residual body is absent. In the oocysts of *E. coecicola* however a fully formed extra-residual body is present (Fig. 2) with the diameter 4—5 μ in average (Cheissin 1947 a, b) or — according to the data of Gill and Ray — 6 μ. It is nearly the same as in *E. media*. In the oocysts produced toward the conclusion of the patent period, the extra-residual body is sometimes not formed. In some cases the number of such oocysts was 1—2%, in other ones their number reached even 30% (Cheissin 1947 a, b). In the subsequent examination of oocysts of *E. coecicola* it has been stated that the formed extra-residual body is characteristic of them, and its absence in some oocysts is an exception of the rule. Absence of the formed extra-residual body is characteristic of the oocysts of *E. neoleporis*. As to the findings of Pellerdy concerning the extra-residual body of the oocysts of *E. neoleporis* from the wild rabbit — they are sometimes controversive. In some oocysts belonging to the species *E. neoleporis* from the wild rabbit, according to Pellerdy, a small residual body was found ("In etlichen Oocysten war ein kleiner äusserer Restkörper zu sehen" 483 p.). Simultaneously in the oocysts excreted by the tame rabbit after their infection with *E. neoleporis* from the wild rabbit, a few grains were seen which formed the extra-residual body after sporulation. It cannot be excluded that Pellerdy had to do not with *E. neoleporis* but with *E. coecicola*.

Considering the difference in the structure of the extra-residual body and in the length of the oocyst, the validity of the species under study should be looked upon as sufficiently grounded. Their oocysts differ as distinctly as the cocysts of *E. intestinalis* and *E. piriformis*. The first ones have an extra--residual body in their oocyst, the second ones have not (Cheissin 1948, 1967).

Endogenous development of both species. Their localization in the intestine is the same. However the agamic generation of *E. coecicola* develops not only in the vermiform appendix but in the small intestine as well whereas that of *E. neoleporis* develops in the vermiform appendix and in the coecum only. Schizonts of the first generation begin to develop in the epithelium of the crypts and subsequently the infection with the parasite penetrates into the tunica propria of the mucous membrane in which the development is concluded (Carvalho 1944). Schizonts of *E. coecicola* are developing in the epithelium beneath the nucleus and do not migrate into the tunica propria.

http://rcin.org.pl

Such a case is observed only in the oocysts which after their formation may penerate from the epithelium into the tunica propria and remain there for a prolonged time after conclusion of their endogenous development.

Any differences in the structure of the agamic generations and in gamonts can scarcely be detected because those forms have not been sufficiently investigated. In *E. coecicola*, on the 9th day after infection, only small schizonts are seen, 12—15 μ of size with merozoits 10 μ long and 0.8 μ wide (Fig. 3). According to the data of Carvalho, in *E. neoleporis* on the 9th day not only such schizonts as described above are seen but also some much bigger with 60—86 merozoits. The structure and dimensions of macrogametes and of microgametocytes are approximately the same in both species. More distinct differences are observed in the duration of the development of endogenous stages. The agamic generations of *E. neoleporis* — as reported by Carvalho occur up to the 12th day whereas in *E. coecicola* already on the 10th day the agamic generations fail to appear. The last mature schizonts occur on the 9th day. The gamogony of *E. neoleporis* begins on the 10th day whereas in *E. coecicola* the gamonts begin to form on the 7th day. In consequence the prepatent period in *E. neoleporis* lasts 11—14 days and in *E. coecicola* — less

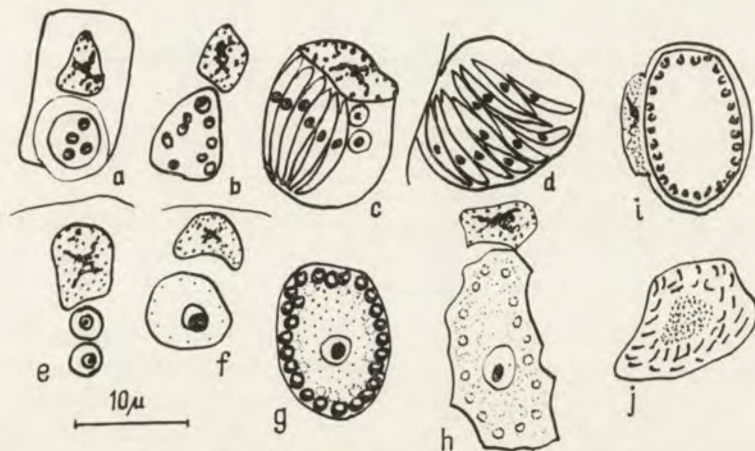


Fig. 3. Development stages of *E. coecicola* on the 8th—9th day after infection in the vermiform appendix: a, b — the last generation of schizonts; c, d — mature schizonts of the last generation; e, f, g — different stages of development of macrogametes; h — oocyst in the course of formation; i, j — immature and mature microgametocyst

than 11 days and the first oocysts appear usually on the 9th—10th day. Corresponding differences are marked in the terms of the prepatent period (Table 1). Pellerdy however remarked that the prepatent period amounted 10 days in the case of infection of the tame rabbit with the oocysts which he recognized as *E. neoleporis*. Oocysts appeared one day earlier which was observed by Carvalho for *E. neoleporis* from the cottontail rabbit. This difference may serve as evidence that Pellerdy had to do not with *E. neoleporis* but with the oocysts of *E. coecicola*.

E. neoleporis was found by Carvalho in *Sylvilagus floridanus mearnsii* whereas *E. coecicola* occurs in *Oryctolagus cuniculus* only. According to

http://rcin.org.pl

Carvalho a spontaneous infection of the tame rabbit with the coccidia *E. neoleporis* has never occurred in the state Iowa (USA). However the tame rabbit was successfully infected with this species by Carvalho. Oocysts isolated from the tame rabbit after this infection fluctuated in average length from 38.5 to 39.5 μ and breadth from 18.2 to 19.8 μ. Consequently the dimensions of oocysts were not altered when the parasite developed in another host. Nevertheless the behaviour of *E. neoleporis* is somewhat different in the tame rabbit than in *S. floridanus mearnsii*. In the first place after infection of tame rabbits and of the cottontail rabbits with equal doses of oocysts a different productivity of endogenous stages of development was observed. In the tame rabbit, a smaller number of oocysts was produced after the infection with a dose of 150 000 oocysts of *E. neoleporis*, than in the cottontail rabbit. Besides, in the tame rabbit the oocysts of *E. neoleporis* are excreted unable to sporulation, toward the conclusion of the patent period which never takes place after the infection of the cottontail rabbit. Besides, *S. floridanus mearnsii* proved to be more susceptible to the desease than the tame rabbit. The same invasion dose of oocysts evokes in *S. floridanus mearnsii* a more acute pathogenic effect than in the tame rabbit. All the above statements speak in favour of the existence of some protective mechanisms against *E. neoleporis* in the tame rabbit. Carvalho observed in his experiments with tame rabbit, a full immunity from infection with *E. neoleporis* in one 3 months old individual. The mature tame rabbits over 4 months of age possessed a clearly expressed age resistance and were not infected with *E. neoleporis*. In contrast to this in *S. floridanus mearnsii* the age immunity from *E. neoleporis* fails to develop and the adult individuals as well as the young ones are equally sensitive to infection (Carvalho 1944).

The above observations indicate that the tame rabbit is a normal host for *E. neoleporis* and — in the opinion of Carvalho 1944 — this species of coccidia "is a parasite with the potentiality of gaining a new host in the future" (p. 187).

At the same time *E. neoleporis* is fully adapted to *S. floridanus mearnsii* which may be accepted as a normal or specific host of this parasite.

E. coecicola presents another case. This species is well adapted to *Oryctolagus cuniculus* and widely distributed among the tame rabbits in all the parts of the world. Evidently Pellerdy found exactly this species in the wild rabbit in Hungary and not *E. neoleporis* as reported by the author himself. The dimensions of oocysts found by Pellerdy as well as the duration of the prepatent period and the behaviour of the parasite in the tame rabbit correspond more to those factors in *E. coecicola* than in *E. neoleporis*. The occurrence of *E. neoleporis* in the tame rabbit in India needs some experimental support.

E. coecicola develops in the tame rabbit at any age of the host. The latter shows no age insensitivity to this parasite. The pathogenic effect is very strong even in the case of infection with moderate doses which has also been indicated by Pellerdy 1954. Therefore the tame rabbit is a normal or specific host for *E. coecicola*.

In this way *E. coecicola* and *E. neoleporis* present two nearly related but yet separate species. They differ in the structure of oocysts, in the length of the endogenous development and in adaptation to different hosts.

It is quite possible that both species have a common ancestor which was

http://rcin.org.pl

the parasite of the representatives of the family *Oryctolagidae* in Pliocen prior to the separation of the genera *Oryctolagus* and *Sylvilagus* (Cheissin 1957). Subsequently the divergence to two host species led to the divergence of the one common ancestor of coccidia into two contemporary species of the genus *Eimeria*. One species became adapted to *Oryctolagus* which developed in the Old World, another one proved to be adapted to the genus *Sylvilagus* which is distributed in the New World. Subsequently specific species of coccidia had been formed in each of those hosts. The geographical separateness of the areals of *Oryctolagus* and *Sylvilagus* species restrained the exchange of parasites. Out of 5 species of coccidia from the cottontail rabbit from USA, only *E. neoleporis* was able to develop in the tame rabbit. Evidently many other species from the tame rabbit may develop in the cottontail species (Carvalho 1945) which has not yet been proved by an exact experimentation.

Summary

Eimeria coecicola and *E. neoleporis* present separate species which differ in the length of the oocysts, presence or absence of the extra-residual body of the oocyst, localization of schizonts, time of the endogenous development, length of the prepatent and patent period and in the adaptation to different hosts as well. The first species is specific to *Oryctolagus*, the second one to *Sylvilagus*. *E. neoleporis* is able to develop in the tame rabbit.

РЕЗЮМЕ

Eimeria coecicola и *E. neoleporis* представляют собой самостоятельные виды, которые различаются по длине ооцист, наличию или отсутствию остаточного тела ооцисты, локализацией шизонтов, длительностью эндогенного развития, препатентного и патентного периодов, а так же приспособленностью к разным хозяевам. Первый вид специфичен для *Orystolagus*, второй вид для *Silvilagus*. *E. neoleporis* способна развиваться у домашнего кролика.

REFERENCES

Carvalho J. 1942: *Eimeria neoleporis* n. sp. occurring naturally in the cottontail and transmissible to the tame rabbit. Iowa St. Coll. J. Sci., 16, 409—410.

Carvalho J. 1943: The Coccidia of wild rabbits of Iowa. I. Taxonomy and host-specificity. Iowa St. Coll. J. Sci., 18, 103—135.

Carvalho J. 1944: The Coccidia of wild rabbits of Iowa. II. Experimental studies with *Eimeria neoleporis*. Iowa St. Coll. J. Sci., 18, 177—189.

Cheissin E. M. 1947 a: Kokcidii kišečnika krolika. Uchen. Zap. leningr. gos. pedag. Inst., 51, 1—229.

Cheissin E. M. 1947 b: Novyj vid kišečnoj kokcidii krolika *Eimeria coecicola*. Dokl. Akad. Nauk SSSR, 55, 181—183.

Cheissin E. M. 1948: Razvitie dvuh kišečnyh kokcidij krolika *Eimeria piriformis* i *E. intestinalis*. Uchen. Zap. karel.-finsk. Univ., 3, 179—187.

http://rcin.org.pl

Cheissin E. M. 1957: Topologičeskie različija soprjažennyh vidov kokcidij domašnego krolika. Trudy leningr. Obšč. Estest., 73, 150—158.
Cheissin E. M. 1967: Žiznennye cikly kokcidij domašnyh životnyh. Izd. Nauka, Leningrad 1—192.
Gill B. and Ray H. 1960: The Coccidia of domestic rabbit and the common field hare of India. Proc. zool. Soc. Calcutta, 13, 129—142.
Pellerdy L. 1954: Beiträge zur Specifität der Coccidien des Hasen und Kaninchens. Acta vet. hung., 4, 481—487.
Pellerdy L. 1965: Coccidia and coccidiosis. Budapest, 1—657.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 2

Shanklin Laboratory of Biology
Wesleyan University, Middletown, Conn., USA

Concettina G. GILLIES and Earl D. HANSON

Morphogenesis of *Paramecium trichium*[1]

Morphogénèse de *Paramecium trichium*

There are at least two modes of stomatogenesis in the genus *Paramecium*: a budding process and a surface invaginating process. However, recent descriptions of stomatogenesis in various species of *Paramecium*, using silver impregnation studies, have grouped all the species observed under one type of development, i.e. the budding process. Yusa 1957 states, "No detectable differences with respect to stomatogenesis... were found among the species of the genus." His paper refers to *P. aurelia*, *P. caudatum*, *P. multimicronucleatum*, *P. bursaria*, *P. trichium*, *P. calkinsi*, and *P. polycaryum*. Roque 1961 similarly refers to a budding process and comments on the same species used by Yusa except for the last two. Porter 1960 also describes initial proliferation near the endoral kinety with subsequent development of a bud in *P. aurelia*. Ehret and Powers 1959, however, using electron microscopic studies of *P. bursaria*, describe a different stomatogenesis for *Paramecium*. They state, "This food in-take system is replicated at cell division not by budding a new gullet from the old one, but by the laying down of three rows of nonciliated 'microsomes' in the pellicle surface between the ribbed wall and pellicle organelles."

It is apparent that, basically, only two modes of development have been proposed but there is no clear picture yet as to the distribution of these modes among the species of *Paramecium* nor have the differences and similarities of these modes been adequately compared.

In our laboratory we have been studying both *P. trichium* and *P. aurelia*. Preliminary reports on *P. trichium* (Hanson 1963) presented evidence for a surface invaginating process in this species, and various other studies (Hanson 1955, 1962; Hanson and Gillies 1966) report a budding process for *P. aurelia*. It is the purpose of this paper to present a detailed account of morphogenesis in *P. trichium*. This account will stress development of oral structures and the pattern of proliferation of cortical ciliary units. The discussion presents comparative comments on oral development within the genus *Paramecium* and some broader comments on the phylogenetic position of *P. trichium*. Also the significance of the new findings on development of the cortical units is analyzed.

---

[1] This work has been supported by a grant from the National Science Foundation

http://rcin.org.pl

Materials and methods

The strain of *P. trichium* used here was isolated from a collection taken locally, and identified by its characteristic vacuoles, micronucleus, and small truncated shape (Wichterman 1953). Cultures were maintained on Cerophyl inoculated with *Aerobacter cloacae* (Hanson 1962). Although *P. trichium* is maintained on this medium it does not divide at a constant rate. The interfission period varies between 6 to 12 hours at room temperature.

The Chatton-Lwoff silver impregnation technique was used as described by Corliss 1953 and modified by us. Fixation with Champy's followed by DeFano's was routinely employed. A rinse in distilled water preceded embedding the animal in gelatin. A small paraffin oven proved useful in keeping slides, pipettes and gelatin at 50°C. After embedding the animal in gelatin on a glass slide and orienting it ventral side up with fine glass probes drawn from glass rods, the slide was chilled on a tray of ice. A staining dish with a removable slide rack was also kept on the tray of ice (Wise 1965). Chilled slides were transferred to the slide rack and the full rack — 10 to 20 slides — was immersed in the silver solution in the refrigerator for 20 to 30 minutes. After several rinses with cold distilled water the slides were put into 70% alcohol. The slides were often left overnight in the refrigerator in 70% alcohol. With the slides still in the rack, immersed in 70% alcohol, the whole staining assembly was placed in an ice bath under a desk lamp with a 100 watt bulb. Thirty minutes to an hour exposure was found to be adequate. The slides were then dehydrated and mounted with Permount.

Observations were made under high dry (40×) and oil (100×) objectives using brightfield illumination as well as phase contrast. Phase contrast was especially useful in following the changes in the nuclei and in observing the ribbed wall.

Photographs of live animals were taken by immobilizing the animals in a rotocompressor (Biological Institute, 2018 N. Broad St., Philadelphia, Pa.). A high contrast copy film was used to photograph the silver impregnated animals.

To find when in the interfission period proliferation of oral structures began and how development proceeded, two methods were used. First, individual paramecia were fixed at known times in their interfission cycle, as follows. A dividing animal was isolated into fresh culture medium in a depression of a 3-spot depression slide. When the proter and opisthe separated, the time was recorded. One animal was fixed and the sister cell observed until the next division. On the assumption that sister cells divide at nearly the same time (Kimball et al 1959) the time of division of the unfixed cell can, therefore, provide a reference point for determining the relative position in the cell cycle of the fixed cell. To test this assumption the interfission intervals were measured for 23 pairs of sister cells growing in the presence of excess food. These data show a correlation coefficient of 0.58, indicating that there is significant agreement between the interfission intervals of sister cells. Using this information the interfission age of fixed cells was determined by first estimating the interfission interval of the cell, $\hat{T}$, had it not been fixed. This is given by the formula (Kimball et al 1959) $\hat{T} = rt+(1-r)\,\overline{T}$ where t is the interfission period of the sister cell, $\overline{T}$ is the

http://rcin.org.pl

average interfission interval for all control cells, and r is the correlation coefficient. The actual time between the prior division and fixation, divided by $\hat{T}$, gives a decimal value, called the interfission age. This defines the time in the interfission interval when fixation occurred. For example if $\hat{T}$ is 6 hours and the cell was fixed 3 hours after fission, the interfission age is 0.5. Cells divided more consistently between 6—7 hours than at any other time, therefore only fixed cells whose sister cell divided within 7 hours were used.

The second method for determining the course of events in oral morphogenesis was to fix and stain a sample from a rapidly growing culture. Each animal was then arbitrarily scored as to stage of proliferation and morphogenesis and the number of animals in each of these stages was recorded and used to determine the relative duration of each stage (Frankel 1960). Using the formula $F = \frac{\ln(R+1)}{\ln 2}$ given by Frankel for a mass culture, we are able to arrive at a value for F, the fraction of the generation time spent in a given phase of the cell cycle. In the formula, R is the proportion of cells seen in a certain phase of the cycle. The difficulty in getting consistently good growth in *P. trichium* indicates that we were probably unable to get good log phase cultures, which is a prerequisite for applying the method just described. Hence it was all the more important to have an independent check on these results using a different method. This is provided, of course, by the method of studying individually timed sister cells — the first procedure outlined above.

In our description of the morphogenetic events associated with stomatogenesis and development of the fission plane, we have two categories of observations to analyze. One of these is the appearance, placement and behavior of visibly complex organelles such as the gullet or nucleus. The other is to follow the development of structures associated with the ciliary corpuscle (Ehret and Powers 1959), especially the cilia and parasomal sac which, in our material, appear simply as dots of deposited silver. Our interpretations of these data, in particular the position of the parasomal sac relative to cilia, rely on conclusions presented by electron microscopists. Ehret and Powers 1959 showed in their studies of the cell surface of *P. bursaria* that the parasomal "... sac opening is next to the base of the membrane of the posterior cilium." Their diagrams of the ciliary corpuscle clearly indicate that the parasomal sac is always positioned to the right (the animal's right) of the cilium. The accumulation of silver grains to mark the position of these structures was shown by Dippell 1962 to be "... throughout the parasomal sac, at the juncture of cilium and basal plate, and at the juncture of the trichocyst and body surface..."

The terms doublet, triplet and quadruplet, when used in this report, refer, respectively, to one cilium and its parasomal sac, to two cilia and one parasomal sac, and to three cilia and one parasomal sac. The cilia are always in a straight line, forming part of a kinety, and the parasomal sac is to the right of this line. In referring to "proliferating units" and "units in a kinety," as we will later on, we are following Ehret and Powers 1959 and Dippell 1962 and are using "unit" as an all-inclusive term for the complex of fibers, basal bodies, cilium or cilia, parasomal sac and membranes

http://rcin.org.pl

as seen with the electron microscope and otherwise referred to as a ciliary corpuscle (Ehret and Powers 1959; Pitelka 1963). Although we see only the position of the cilia, parasomal sac, trichocyst, and some outer limits of the units, these clearly represent essential parts of that structure permitting its identification and enumeration.

Results

The results will be described in five sections: morphology of the non--dividing animal; stomatogenesis; karyokinesis; proliferation of cortical units and fission; and the timing of the fission cycle.

Morphology of the non-dividing animal

The surface of non-dividing *P. trichium* has been described by Roque 1961 using silver impregnation. However, one feature of the kinety pattern, very pertinent for stomatogenesis, was not described in that report. This is a group of 6—8 kineties to the right of the endoral kinety. These kineties curve around the buccal opening starting at the anterior suture line and terminating posterior to the buccal opening where they abut on a kinety which extends posteriorly from the left side of the buccal opening (Pl. I 1). As will be described later it is within this group of kineties that unit proliferation begins and much of the development of the anlage takes place. It should be noted, that the vestibule of *P. trichium* is not as deep as that in the aurelia group, as described by Yusa 1957 and others. Only the first 2 or 3 kineties are in the vestibule to the right of the buccal opening and the anterior end of the dorsal peniculus lies next to the first kinety on the left which is not in the vestibule.

The total number of surface kineties, about 55, agrees with Roque 1961. The number of cortical units per kinety varies according to the length of the kinety. The largest variations exist within 8 kineties on either side of the buccal opening. For the most part, in the other kineties there are 45 to 50 units in a kinety. The units at the posterior tip are difficult to count, as all kineties converge in a small area.

The buccal opening is situated in the anterior third of the animal. The components of the oral structure were clearly observed in our material and are essentially as described by Roque 1961. Contrary to Yusa's 1957 report of 5 and 4 ciliary rows in the dorsal and ventral peniculi, respectively, we find 4 and 4 in the peniculi and also 4 rows in the quadrulus (Pl. I 2 A.B). The granules in the endoral kinety (Pl. II 3) varied between 18—26. Yusa 1957 reports 9 kinetosomes in the endoral kinety of *P. trichium* but Roque 1961 reports 20.

The ribbed wall mentioned by Roque 1961, as seen in silver preparations, and clearly observed by Ehret and Powers 1959 and Pitelka 1964 using electron microscopy, was noted in most of the preparations in this study. The use of phase contrast made it much clearer than brightfield since it does not appear to have any accumulation of silver. Observations indicate

http://rcin.org.pl

a close association and spatial relationship of the ribbed wall and endoral membranelle. The ribs appear to have their origin at the sites of the silver deposits of the endoral kinety — similar to Roque's 1961 drawing. The ribbed wall apparently forms the right wall of the buccal cavity and extends down into the food vacuole forming region (Pl. II 4). Pitelka 1964 observed the termination of the ribbed wall at the food vacuole forming region in electron micrographs of serially sectioned *P. multimicronucleatum*.

A structure which has not been reported in either stained preparations or electron microscopic studies of *Paramecium* was observed in *P. trichium*. Just inside the buccal opening the size of the buccal cavity is much reduced by the presence of a structure extending right and left and arising from the posterior wall of the buccal cavity (Pl. II 5). The cavity, at this point, appears to be about 1/3 the size of the buccal opening. It was observed in many animals at different stages in the fission cycle and, hence, appears to be a constant feature of the gullet. It does not stain but can be seen with phase contrast.

The contractile vacoule of *P. trichium*, a vesicle-fed rather than a canal--fed structure (Wichterman 1953), is distinctive in the live state. It appears in fixed preparations as a tube-like structure with a circular opening to the exterior and is attached to vacuoles internally. There are two such structures per cell, positioned on the dorsal side, one towards the anterior and one towards the posterior end of the animal.

The macronucleus and micronucleus of a non-dividing animal are very characteristic as to shape and position. The macronucleus is more or less centrally located and twisted or folded over slightly into a kidney-like form. The micronucleus is of the compact type (Wichterman 1953), is tear drop shaped, and lies tucked against the inner curve of the macronucleus.

An interesting observation on the cortical pattern bears special mention here since it will be important when division of the cell is discussed. The typical triplet configuration of two kinetosomes and a parasomal sac and occasionally a doublet of one kinetosome and one parasomal sac, obeying Lwoff's law of desmodexy (1950), was assumed to be the overall pattern of the cortex. Careful checking, however, showed a consistent variation. The posterior end of the animal from the posterior tip to almost the anterior end of the cytoproct consists of doublets, i.e. one kinetosome and one parasomal sac — as indicated by the placement of the silver granules (Pl. II 6). The rest of the cell is covered by triplets. We will return later to the problem of this curious distribution of two types of units.

Stomatogenesis

The stages of stomatogenesis, from the earliest proliferation of structures which are visible with silver impregnation to a complete gullet, have been divided into five stages. The series of events begins at the lower right side of the buccal overture.

Stage I

Proliferation of an anarchic area. This proliferation first appears along the right posterior edge of the buccal overture between the endoral membra-

http://rcin.org.pl

nelle and the first vestibular kinety, as a cluster of smaller, lighter staining dots. (Pl. III 7).

Stage II

Oral anlage. A band of kinetosomes now extends from a point midway on the right edge of the buccal opening beyond the posterior edge of the buccal overture. (Pl. III 8 A). Late Stage II figures begin to show two rows of granules in each of the three bands new apearing. (Pl. III 8 B).

Stage III

Differentiation of the oral anlage. Three distinct bands of essentially longitudinal rows can be seen as the gullet anlage becomes nearly as long as the mature gullet. Each band consists of three rows of granules. (Pl. III 9). The first evidence of the fission line appears now, at the left posterior end of the anlage and during this stage develops around the cell, passing first dorsally and then returning ventrally to the right anterior side of the anlage.

Stage IV

Invagination. The oral anlage, now posterior to the old gullet, has started to invaginate and the fission furrow is clearly indented. (Pl. IV 10).

Stage V

Completion of proter and opisthe (Pl. IV 11). The new gullet is complete and appears to be functional before final separation of the fission products. The kinetosomes of approximately nine so-called latitudinal rows or paratenes (Ehret et al 1964 b) above and below the fission furrow have proliferated and each fission product has a full complement of cortical units. These are now organized into characteristic patterns. As the posterior, ventral kineties of the proter meet, they form postoral suture and the cytoproct. The anterior kineties of the opisthe gradually curve inward anteriorly and abut to form the preoral suture.

Two points require further comment: the endoral kinety and proliferation within the oral anlage. Although the anarchic field to the right of the endoral kinety of Stage I is the most easily distinguished feature of the beginning of stomatogenesis, several observations indicate that there is doubling of the endoral kinety prior to the appearance of the anarchic field. Pl. IV 12 clearly shows several granules on the kinety that appear double. Several authors have indicated involvement of the endoral membranelle in stomatogenesis. Pl. IV 12 appears to support this view. The animal photographed was fixed at that period of its fission cycle which comes within the time of onset of stomatogenesis.

The anarchic area is gradually organized into rows by subsequent proliferation in early Stage II. In Pl. III 8 B three groups of double rows can be seen. By late Stage III the groups are quite distinct and in Pl. III 9 each appears to have 3 rows of granules. Proliferation of these rows seems to be lateral to rather than anterior to preexisting granules.

Karyokinesis

There is a correlation of growth and differentation of a new gullet and cortical units with division of the micro- and macronucleus. The macronuc-

http://rcin.org.pl

leus and micronucleus were observed in the silver preparations, and the stages of mitotic division were therefore observed along with development of a new gullet. Since the micronucleus of *P. trichium* is of the large compact type, its division was easily followed. Phase contrast photographs were taken of live animals to illustrate these changes. In this material it was easier to get the macronucleus and micronucleus in the same focal plane to illustrate their correlated behavior. In the following description of what occurs, we will refer to those stages already described for gullet development.

Throughout Stage 0 the micronucleus lies against the macronucleus (Pl. V 13 A). In Stage I, the micronucleus begins to move away from the inner curve of the macronucleus. At Stage II of the oral anlage, the micronucleus is slightly enlarged and the macronucleus has rounded out somewhat (Pl. V 13 B). Stage III shows a slightly elongated macronucleus and clear chromosomes in the micronucleus. A late Stage III (Pl. V 13 C) shows, a micronucleus in anaphase. Telophase is observed in Stage IV, (Pl. 13 D) with the macronucleus now elongated to extend below the fission line. At completion of telophase, which occurs in Stage V, the micronuclear spindle is greatly elongated with its poles lying at the extreme ends of proter and opisthe. The macronucleus soon separates (see Pl. IV 11). After fission, the micronucleus gradually returns to the macronucleus which bends slightly to form the curve where the micronucleus will lie until the next fission. The appearance and disappearance of the nucleoli in the macronucleus (see dark spots in Pl. V 13 A) also coincide with morphogenetic events. These changes within the macronucleus are presently being studied further.

Unit proliferation and fission

There are three related problems here. The first is to define the area of proliferation, for only those units proliferate which are adjacent to the fission line. Next, there is the nature of proliferation itself, as determined by our techniques. And, third, there is the fission process and the transforming of a cellular midsection into two new ends for each fission product.

The development of the fission line begins in Stage III, sometime before the fission furrow is seen in the live animal. The quadruplet groups of dots, which represent proliferation of kinetosomes, is first observed to the left of the posterior end of the anlage. First one, then several. on up to approximately 18 units in a kinety seem to be reproducing. But the number of units per kinety that appear to be replicating at a given time, is not consistent. At an early stage, for example, there can be two replicating in one kinety and as many as four in an adjacent one. However, the final number of units replicating per kinety does seem to be quite constant.

This proliferation extends in a band around the mid-third of the cell until it terminates at or near the right sides of the old and new buccal openings. Also, it shows two very regular features: a) in proceeding around the cell from left to right, proliferation appears in successive kineties, only occasionally skipping one and that one subsequently always proliferates sooner or later; and b) in proceeding along a kinety, anteriorly or posteriorly from the fission plane, proliferation is again orderly, always moving along

http://rcin.org.pl

unit by unit, until the last one which will show new parts is reached. Irregularity is also evident: a) as already stated, the individual kineties, once they have started to proliferate, do not always do so in phase — some forming new structures faster than others; and b) the final number of newly proliferated units in a kinety is largely constant (ca. 18) but can vary a bit from one to the next. Finally there are some differences to be noted between kineties on opposite sides of the fission line, which at Stage IV is a visible discontuity or separation between previously adjacent units. As can be seen from Pl. VI 14, 15 there is a series of doublets in the opisthe, while quadruplet groups are still seen in the proter. This becomes more evident at a later stage when the new units in the anterior end of the opisthe are all triplets and those in the posterior end of the proter are still doublets. As was noted earlier these remain doublets after separation and apparently through the life of the cell. As the fission constriction proceeds to completion, the typical kinety patterns of the new posterior and anterior ends, of proter and opisthe, respectively, become apparent. (See comments under Stage V, above). At this point the essential developmental problem, in terms of cortical units, is to understand how the cell remolds a surface, originally built to cover its midsection, into two apical surfaces. A priori the mechanical problem would appear to involve a change in number and/or size of units. Preliminary observations confirm this, but the problem is more complicated than originally anticipated and only the general direction of the analysis can be indicated. Briefly, it involves the following points. The kineties closest to the oral area remain the shortest and curve around the oral area and abut on each other, forming suture lines. This is more pronounced in the preoral suture. Kineties further removed from the oral area elongate, but there is no evidence of any more proliferation and we are, therefore, forced to conclude that the units themselves become at least longer and thus achieve lengthening of kineties. Kineties on the right, left and dorsal sides elongate sufficiently to reach the cell apex and are somewhat compressed in the limited space available. Thus, overall unit number is reduced at the apex as a result of a limited number of kineties extending that far, but there is also some difference in unit size as the units are adjusted to the topographic constraints imposed on them.

An interesting abnormality was observed in several animals in which certain kinetosomes were oriented counter to Chatton and Lwoff's law of desmodexy. Under low power a difference in the spacing of the kineties was noted, and, when checked under higher power, several kinetosomes were seen to be reversed; in two cases the entire kinety was reversed (Pl. VI 16). While checking the 800 animals of the mass culture for stages of stomatogenesis, incidental observations revealed 18 cases of reversed kinety. Most of the 18 cases consisted of 5 to 10 reversed units in a kinety, usually in the 6 to 8 rows concerned with stomatogenesis. The initial expression of this abnormality in any given cell was not observed. However, the position of the reversed doublets at the posterior end of a reversed kinety, indicate that the inverted pattern is probably the same as that produced experimentally by Beisson and Sonneborn 1964. They were able to show by digitonin preparation that the kinetodesmal fibers emerge to the left of the kinetosome and extend posteriorly. Normally the fibers emerge to the right and extend anteriorly. Although we were unable to see kinetodesmal fibers

http://rcin.org.pl

in our examples of reversed kinety, the position of the parasomal sac in the doublets of a reversed row is indicative of a reversed pattern. If the units were merely transposed from a right to left orientation, then the doublet would appear with the parasomal sac anterior and to the left of the kinetosomes. However, the parasomal sac is to the left and posterior to its kinetosome. We would expect kinetodesmal fibers to extend posteriorly in this pattern.

In sum, these data on proliferation of cortical units tell us that proliferation is indeed limited to a restricted area of the cell and we have some idea of the regularities, irregularities and anomalies associated with the proliferation and organization of kineties. These facts are the basis for several new ideas on cortical development which will be brought out in the Discussion.

Timing of morphogenetic events

Two different methods were used to estimate the duration of the various development stages. It will be seen that both approaches have their limitations, and therefore both are needed as independent checks on each other. The method using samples drawn from a mass culture is valid when four conditions are met: 1) there is log phase growth, 2) the interfission period is constant for all cells, 3) there is no synchrony of cell divisions, 4) the samples are taken at random. Only the last two conditions were fully met in this study. The first condition is partially realized in that the cells were grown in the presence of excess food but the erratic growth of *P. trichium* could not guarantee a log phase culture. This inconsistent growth also eliminates uniformity of interfission periods.

The results in Table 1 A show that the sum of the F values — the total of the portions of the interfission period spent in each of the developmental stages — is 1.17. This value is greater than 1.0 for reasons alluded to above,

Table 1

The determination of the duration of the developmental stages of *Paramecium trichium*. A. Analysis of samples taken from a mass culture. B. Study of single cells fixed at a known time after fission.

Stages	0	I	II	III	IV	V	Totals
A. Mass culture							
Number of cells	256	136	54	33	35	16	809
F	0.69	0.23	0.10	0.06	0.06	0.03	1.17
Duration in minutes	256	72	28	17	17	8	398
Average time (min.) since previous fission	128	293	342	365	382	394	
B. Individual cells							
Number of cells	19	20	9	9	13	11*	81
Average time (min.) since previous fission	**	288	338	358	368	384	

* This figure includes observations of 8 live animals

** See text

http://rcin.org.pl

namely, the absence of 100% log phase growth. Cells not in log phase grow more slowly than those in it. This causes heterogeneity of interfission periods and of course increases the average length of time spent in the cell cycle, which in turn distorts *F*. It is impossible to say whether a few cells which are greatly delayed or many cells slightly delayed are the cause of the trouble. And, also, from these results we cannot decide whether the delays are spread proportionately throughout the fission cycle or effect one or few stages disproportionately. Regarding the latter alternatives we tend to the view that one stage is especially effected, for reasons given below.

The other method of estimating the duration of the developmental stages used individual cells of known interfission ages which were stained and their developmental phase determined. This direct method of correlating age and stage had the drawback of providing relatively small samples of data. Furthermore, the cells were very variable. This latter was ameliorated by discarding all cells whose interfission cycle was longer than seven hours. (See Materials and methods). Table 1 B summarizes the results obtained by this second approach. To compare Tables 1 A and 1 B, the horizontal rows labeled "Average time since previous fission" should be examined. It can be seen that there is close agreement for Stages I, II and III, with lessening agreement for Stages IV and V. Stages 0 cannot be compared because a random sample of animals was not obtained in working with the single cells, for we were concerned with finding the terminus of that stage, knowing full well that cells a few minutes after fission would be Stage 0. The cells in this stage were selected to lie towards the end of it and the average calculated from them would be meaningless.

There remains only to comment on the cells which were excluded from this analysis, i.e. those with excessively long interfission periods. These were stained at a time when other cells normally show morphogenesis but none of them gave evidence of such development. This indicates that all stages are not proportionately lengthened when the cell cycle is longer. Rather, it seems likely that morphogenetic activity, once it starts, runs to completion at a constant rate — note the agreement in Table 1 A and 1 B; it is the preparation for morphogenesis which is variable. In other words it appears that Stages I—V proceed at a regular pace once initiated, but Stage 0 can be quite variable in length.

Discussion

The preceding account of development in the cell of *P. trichium* has emphasized the formation of new organelles; it is an account of morphogenesis. It says nothing explicitly of the biochemical events which precede differentation and little regarding differentation itself, which supplies the components that are molded and integrated by morphogenetic processes into whole new cells. However, since in our view morphogenesis represents the final steps in development, a careful analysis of it will pose problems and, hopefully, new insights into the processes which underlie it — in this case, differentiation and various biochemical activities. With this in mind we turn to a discussion of the formation of major organelle systems in this ciliate. In order, we shall examine morphogenesis and nuclear activity, the proli-

http://rcin.org.pl

feration of cortical units, and the formation of oral structures including comparisons with other ciliates.

Two questions can be raised regarding the correlated behavior of nuclear and morphogenetic events. First, what is the situation in other ciliates? Second, do nuclear events control cortical or stomatogenetic phenomena, or vice versa, or does the endoplasm control both?

The relationships between nuclear activity, stomatogenesis and cell division which were observed in this study have also been noted in *P. bursaria* (Ehret and de Haller 1963). Three different structural states of the macronucleus were observed during the last 40 to 50 minutes preceding cell division, these were correlated with stages of development of the new gullet as well as stages of micronuclear division.

A relationship between nuclear events and morphogenetic events has been shown to exist in other organisms. The first signs of oral primordium formation in *Euplotes eurystomus* were observed to be preceded by the initiation of replication bands in the macronucleus (Wise 1965). After a survey of the literature on ciliates with replication bands, Wise made the folowing generalization "... primordium formation during division and regeneration is invariably accompanied or preceded by the appearance of reorganization bands ... in the macronucleus or macronuclei ..." The fission line has also been related to nuclear events. In *Stentor*, development of the fission line is keyed to mitosis and macronuclear splitting and not to stomatogenesis (Weisz 1951). But it is also true in this form that whenever new oral structures are formed, during fission or regeneration, the macronucleus undergoes a rounding up and then subsequent elongation (Tartar 1961).

As a start on the analysis of these precisely correlated events, it can be asked whether the blockage of nuclear division, or of cytokinesis, or of stomatogenesis block any or all of the other events? In *Glaucoma chattoni*, Frankel 1960 reports cell division occurring without stomatogenesis. But these are admittedly abnormal lines, since eventually the already existing gullet degenerates and the whole line starves to death. At least it does show that there is not an unbreakable connection between division and oral structure formation. Perhaps the least likely events to be separated are karyo- and cytokinesis. Natural or spontaneous occurrences of this seem to be unreported in the literature.

Finally, one practical use of the close temporal occurrence of nuclear activity with morphogenetic events, is to permit accurate determination of the interfission stage of live cells. Details of stomatogenesis or unit proliferation are difficult to determine in the living state, but the state of nuclear events is easily observed with phase microscopy, for example, and can thus define the developmental stage of the cell.

The question of the origin of new kinetosomes, whether "de novo" as reported by Ehret and de Haller 1963, or by division of the preexisting unit as described by Dippell 1963, cannot be resolved with the silver techniques. However, certain observations suggest the possibility that both methods may be in operation. The anarchic area of the gullet anlage in *P. trichium* (Pl. III 7) first appears as smaller, lighter, randomly arranged granules. This appearance might suggest "de novo" development especially when compared with the ordered replication of kinetosomes on the cortex

http://rcin.org.pl

in the fission line area. Indeed, Dippell 1964 reports that the polygonal units which make up the surface of *P. aurelia*, are not present in the anarchic field which persists throughout interfission nor in the gullet anlage and newly cut surface. She further states "... the concept of the ciliary corpuscle may not be applicable here." Yet the pattern of replication of kinetosomes described by Dippell, i.e. a new kinetosome formed anterior to a preexisting one is clear in the fission line area. The differentiation of the gullet anlage could be a unique process quite distinct from development of the pellicular kinetosomes. Frankel 1960 suggests that in *Glaucoma* the two phases of stomatogenesis — organization of oral membranes and lateral proliferation of kinetosomes — "... might be controlled in two different ways." (p. 375). The observation of reversed units also points to the possibility of "de novo" formation. As was mentioned earlier, many of those observed were in the 6—8 kineties concerned with stomatogenesis. It seems significant that these kineties are formed as the gullet anlage begins to invaginate and appear to form in the same manner as the gullet kinetosomes. Once formed they would then act as a template and future division of units during cell-division would result in more reversed kineties. That they are found in other areas on the cell surface is consistent with Sonneborn's 1964 finding that the inverted kineties seemed to move around the cell-body, which is a consequence of subsequent growth.

The two types of cortical units, i.e. one kinetosome with one parasomal sac and two kinetosomes with one parasomal sac, must be reproduced at each fission to maintain a constant cortical pattern. Our observations show that unit proliferation occurs in an area above and below the fission plane for approximately 18 units in each kinety. The following method of replication of the cortical unit at least on the surfaces lateral to the oral area, has been described by Dippell 1963. Where there is one kinetosome and a parasomal sac, a new kinetosome is formed anterior to the existing one and then the new one forms its own parasomal sac. When the unit has two kinetosomes with a parasomal sac, each existing kinetosome forms a new one anteriorly and the anterior old kinetosome develops a new parasomal sac. The units in each case are then partitioned. Figure 1 is a diagrammatic representation of our interpretation of Dippell's report. However, our silver preparation reveals a somewhat different pattern. The quadruplet group which appears at the initiation of the fission line is seen in Pl. VI 17 of a Stage III animal. Later stage III or early stage IV do not show two new units as would be expected from Dippell's proposal, but a series of doublets. In trying to devise a model of kinetosome replication to account for our observations, a combination of Dippell's proposals for proliferation of doublet and triplet units seems applicable. One or both existing kinetosomes in a unit produces a new kinetosome anteriorly; then each kinetosome, except the most posterior one which retains the original parasomal sac, produces a parasomal sac. This is the group of doublets seen in our material. The units are then completed by the production of a new kinetosome anterior to each one already present. In this way the components for three or four cortical units are formed, (Fig. 2).

An electron micrograph of *P. bursaria* (Ehret and de Haller 1963 Fig. 48, p. 23) appears to show the quadruplet and quintuplet (four kinetosomes and one parasomal sac) configuration in adjacent kineties. The figure

http://rcin.org.pl

legend identifies this as the region of the new fission furrow on the left side of the cell.

The conclusion that cortical units on only part of the cell surface, i.e. the area of the fission plane, proliferate at each division has been reached by several authors (Sonneborn 1963, Ehret 1960, Ehret et al 1964 a, J. Sonneborn and Plaut 1967). Some experimental support is provided by two different labeling experiments for this conclusion. Tritiated thymidine incorporation and fluorescent staining by J. Sonneborn and Plaut done on *P. aurelia* indicated the presence of DNA in the pellicle and more specifically in the kinetosomes, and also showed a concentration of label in particular regions of the cell. They observed an asymmetrical pattern of the label after division with heavier concentration at the posterior end of the proter and the anterior end of the opisthe which suggests incorporation of the label in the mid-region of the parent cell, a possible consequence of synthesis of new kinetosomal DNA.

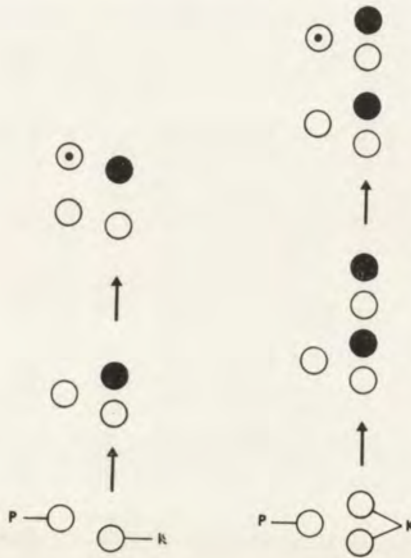


Fig. 1. Unit proliferation. A unit with one cilium (left) and a two cilia unit (right), showing subsequent steps in proliferation based on Dippell 1963. K — kinetosome, P — parasomal sac, ○ — existing organelle; ● — new kinetosome; ⊙ — new parasomal sac

Also coinciding with the pattern of development we see with silver impregnation is the work of Ehret et al 1964 a. Using tritiated leucine to follow trichocyst formation of *P. bursaria* they observed a stability of the anteriormost and posteriormost ends of the dividing animal where little or no proliferation occurs.

There are approximately 40—50 units per kinety on the surface of *P. trichium*. If all the units divided then the full complement would be restored for each fission product. But as we have just indicated only part of the cell surface undergoes proliferation, therefore, it is necessary for one unit to

http://rcin.org.pl

divide more than once or for one unit to give rise to two or more units. The pattern of proliferation which we have proposed (Pl. VI 16) allows us to use kinetosome increase on only part of the cell and still explain the appearance of a full complement of units in each fission product. For example, if the cell has 40 units per kinety, then sometime during fission it will have to produce 40 more units — to provide a total of 80 units. We indicated earlier that about 18 units in a kinety were observed to be proliferating and also that at least

P K P K

Fig. 2. Unit proliferation of triplet units. A model of proliferation resulting in three or four units from the original one. This model also explains the appearance of doublets as an intermediate step. Symbols as in Fig. 1. ⊗ — second new kinetosome to complete a triplet

http://rcin.org.pl

three or four of these appeared to quadruple and the others triple. This can provide two animals with 40 units each, for example as follows: 18 of the 40 units proliferate, of these 18, 14 triplicate and 4 quadruplicate; adding these to the 22 which do not divide results in a total of 80 units.

The intermediate doublet stage appears essential to the explanation of the doublets in the posterior end of the non-dividing animal. We suggest that there is simply a premature block of the last step necessary to complete the triplet configuration in that area of the cell. But this seems inconsistent with the gradient which Sonneborn 1963 describes and the pattern of development we have described. Sonneborn suggests that there is "... a growth gradient which is high near the equator and which decreases toward both ends." (1963 p. 206). This is also borne out by our observations in the fission area, in that proliferation and completion of triplets seems to move from the equator out toward the ends of the animal. However, if this were so, the posterior-most units in the proter would be triplets and a group half way to the middle would still be doublets. To yet avoid this problem and achieve the appearance of the doublets at the posterior end we suggest a shift in the gradient. Once the dividing animal has reached a determinative stage of development, it might then act as two single cells although the cells are still attached. If there is a gradient which moves from the fission line out to the ends in the dividing animal perhaps at a late Stage IV, for example, it now shifts to the equatorial plane of the proter and opisthe. Since we have indicated that the surface development of the opisthe seems to be out of phase with that of the proter, i.e. completing its triplets earlier, it would not be affected by this shift, but the proter would be affected in that those units in the posterior end would now be at the weak end of the gradient and would not complete triplet formation, thus remaining as doublets.

Lastly, regarding stomatogenesis, four points need examination. These include, first, some comment on the ribbed wall and its possible relations to other oral structures. Next, timing of developmental events deserves comments relative to the apparent constancy in duration of stomatogenesis despite variation of the whole interfission period. Third, comparative comments on stomatogenesis in the genus *Paramecium* will lead, finally, to some speculations on the phylogenetic position of *P. trichium*.

The ribbed wall, which is an integral part of the oral apparatus was observed in the silver preparations, but the actual time in the fission cycle when it appears is not clear. Ehret and Powers 1959, however, using electron microscopy on *P. bursaria* reported that the ribbed wall along with the endoral membranelle are the final stages of development of the new oral apparatus. Ehret and de Haller 1963 later reported "... the posterior migration associated with synthesis of the new ribbed wall, is complete ..." It has also been suggested that the endoral kinety may produce the ribbed wall Roque 1961.

Observations of the ribbed wall in the preparations used here may help to resolve further the relationship between the ribbed wall and the endoral membranelle. As was mentioned earlier, there seems to be an intimate spatial relationship between the endoral kinety granules and the ribs of the ribbed wall. In several cases the ribs were counted and in each case matched the number of granules in the endoral kinety. This is clear in Pl. II 4 where at least 18 ribs are countable. Whether or not the ribbed wall is indeed formed

http://rcin.org.pl

in some way from the endoral kinety and in fact forms the food vacuole forming region is still conjecture. However, experiments using ultraviolet microbeam irradiation might help in this regard. Hanson 1962 reported that ultraviolet irradiation at the "critical buccal area" (which coincides with the early anlage area) produced damage that ranged from complete loss of the gullet through a shortened food vacoule forming region to a distorted gullet. As Sonneborn 1963 pointed out, irradiation of this area followed by silver impregnation or electron microscopy might help to elucidate the origin and morphogenetic role of the endoral membranelle.

Initially, stomatogenesis in the various species of *Paramecium* is the same, i.e. there is an anarchic area of proliferation at the right, posterior end of the buccal overture between the endoral membranelle and the first kinety of the vestibulum. After this initial proliferation, the two types of stomatogenesis proceed in distinctly different ways but by similar stages. That is, one type of development, as seen in *P. bursaria* (Ehret and Powers 1959) and *P. trichium* (Hanson 1963), occurs completely on the surface and the other, seen in *P. aurelia* (Roque 1961, Yusa 1957, Porter 1960) below the surface of the cell-body, but the steps from anarchic field to complete oral apparatus are similar.

The real possibility that Stage 0 can be of variable length but that morphogenesis proceeds at a constant rate deserves comment for what it suggests of the underlying mechanisms of development. In that morphogenesis, as we use the term in this paper, is the terminal step in development, we can propose that it is initiated when there are accumulated those precursors necessary to assembly of the organelles appropriate for the appearance of two daughter cells. Assembly, however, is not a problem of synthesis in the sense of forming new covalent bonds but is rather the specific association of already formed and folded macromolecules as a result of myriad weak bonds. On this view morphogenesis is triggered when there are conditions appropriate to the interaction of specific macromolecules which may well depend on the numbers and states of the molecules themselves (Hanson 1967). This view receives support from Frankel's 1962 observation that the only factors known to upset morphogenesis of oral structures in *Tetrahymena pyriformis* is heat — a physical factor capable of inhibiting the proper alignment of weak bonds. Chemicals do not have this effect. They act in an all or none fashion as to whether development occurs or not (Frankel 1965). A further consequence of this view is a simple explanation of the universally observed fact that cells which have limited food rarely start division unless they can finish it, or that cells in the stationary phase of growth do not show partial development. These cells have not completed preparations for the morphogenesis which leads to fission. Until that preparation is sufficient nothing in the way of assembly happens; when it is sufficient it goes all the way. This is in effect a control device protecting cells from abortive development.

A comparison of the structure of the vestibule in the "*aurelia*" group and in at least *P. trichium* of the "*bursaria*" group may account for the differences between internal bud versus surface development of the new gullet. In *P. aurelia* the vestibule is at least 8 kineties deep (Yusa 1957, Fig. 1), whereas in *P. trichium* it is very shallow — 2 or 3 kineties deep. Since development in both groups begins at essentially the same point, i.e. to the right of the endoral kinety, the growth which progresses posteriorly on the surface in

http://rcin.org.pl

P. trichium would, in *P. aurelia*, have to move internally, giving the appearance of what some authors have called a bud. Thus, early invagination may simply be the result of vestibular topography rather than a different stomatogenic process.

It has been suggested (Hanson 1963) that *P. trichium* holds a phylogenetic position intermediate to the *Tetrahymena* and the "*aurelia*" group of *Paramecium*. There are certain similarities that are observed in stomatogenesis which support this idea. For example, in *Glaucoma chattoni* (Frankel 1960) and in *Tetrahymena pyriformis* (Frankel 1962) the earliest proliferation is on the surface to the animal's left of kinety no. 1. This kinety is numbered as the first to the right of the undulating membrane (UM). Proliferation begins below the existing oral area but follows a pattern similar to the stages seen in *P. trichium*. If the UM is equated with the endoral kinety in *Paramecium* as has been suggested (von Gelei 1934, Furgason 1940, Yusa 1957, Corliss 1961) then the similarities are clearer.

Similarity of stomatogenesis is also seen in *Disematostoma tetraedrica* as described by Roque 1961. Proliferation is on the surface between the first vestibular kinety and the "cinétie parorale." As the anlage elongates posteriorly it differentiates into distinct rows — all of which are restricted within the vestibular kinety area. Organization of the buccal organelles and invagination follow. The absence of a vestibule in the above examples may also account for similarities closer to *P. trichium*.

The v-shaped area in which the gullet anlage develops is also present in spirotrichs. A v-shaped area where stomatogenesis takes place in *Stentor* was termed the "... ramifying zone..." by Weisz 1951. Tartar's figure (1961 fig. 14) of division stages in *Stentor* clearly show the oral anlage in this v-shaped area. The earliest evidence of stomatogenesis in *Blepharisma* (Suzuki 1957) was an anarchic field in the "V-area." The "V-area" is formed by the boundaries of the juncture of the left and right kineties. Another v-shaped area was observed by Yagiu 1956 in *Condylostoma*, composed of 6—9 kineties. It is clear as Corliss 1961 has stressed, that an analysis of morphogenetic patterns are an aid in taxonomy, especially in determining the position of a species whose other traits do not clearly specify its possible evolutionary relationships.

Summary

Development during the cell cycle in *Paramecium trichium* is described using phase microscopy and silver impregnation. Formation of the oral apparatus was found to be a surface invaginating process similar to that described in *P. bursaria* and unlike that found in *P. aurelia*. Proliferation of cortical structures as well as nuclear events are also presented. The phylogenetic position of *P. trichium* is discussed.

RÉSUMÉ

La développement pendant le cycle évolutif de la cellule est décrit en *Paramecium trichium* en employant la microscopie en contraste de phase et l'imprégnation argentique. La formation de l'appareil buccal est un procès d'invagination de

http://rcin.org.pl

la surface comme pour *P. bursaria*, pas comme le procès décrit en *P. aurelia*. La prolifèration des structures corticales et les événements nucléaires sont aussi presentés. La position phylogénetique de *P. trichium* est discutée.

REFERENCES

Beisson J. and Sonneborn T. M. 1964: Cytoplasmic Inheritance of the Organization of the Cell Cortex in *Paramecium aurelia*. Proc. natn. Acad. Sci. U.S.A., 53, 275—282.

Corliss J. O. 1953: Silver impregnation of ciliated protozoa by the Chatton--Lwoff technique. Stain tech., 28, 97—100.

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

Dippell R. 1962: The site of silver impregnation in *Paramecium aurelia*. J. Protozool., 9 (Suppl.) 84.

Dippell R. 1963: Reproduction of surface structure in *Paramecium*. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. Int. Congr. ser. No 91, 65.

Dippell R. 1964: Perpetuation of cortical structure and pattern in *P. aurelia*. J. Protozool. 9 (Suppl.).

Ehret C. F. 1960: Organelle Systems and Biological Organization. Science, 132, 115—123.

Ehret C. F. and Powers E. L. 1959: In: International Review of Cytology VIII (G. H. Bourne and J. F. Danielli, eds.) Academic Press, 97—133.

Ehret C. F., Savage N. and Albinger J. 1964 a: Patterns of segregation of Organelles and Organelle Systems of the Cell Surface of *Paramecium*. J. Ultrastruct. Res., Suppl. 6, 1—42.

Ehret C. F., Savage N. and Albinger J. 1964a: Patterns of segregation of structural elements during cell division. Z. Zellforsch., 64, 129—139.

Ehret C. F., Savage N. and Albinger J. 1964 b: Developmental and ultrastructural studies of cell organelles. Argonne nat. Lab. Ann. Report biol. and med. Res. Div., 63—70.

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

Frankel J. 1962: The effects of heat, cold and p-flurophenylalanine on morphogenesis in synchronized *Tetrahymena pyriformis* GL. C. R. Trav. Lab. Carlsberg, 33, 1—52.

Frankel J. 1965: The effect of nucleic acid antagonist on cell division and oral organelle development in *Tetrahymena pyriformis*. J. expt. Zool., 159. 113—148.

Furgason W. H. 1940: The significant cytcstomal pattern of the "*Glaucoma--Colpidium*" group and a proposed new genus and species, *Tetrahymena geleii*. Arch. Protistenk., 94, 224—266.

Gelei J. von. 1934: Der feinere Bau des Cytopharynx von *Paramecium* und seine systematische Bedeutung. Arch. Protistenk., 82, 331—362.

Hanson E. D. 1955: Inheritance and regeneration of cytoplasmic damage in *Paramecium aurelia*. Proc. natn. Acad. Sci., U.S.A., 41, 783—786.

Hanson E. D. 1962: Morphogenesis and regeneration of oral structures in *Paramecium aurelia*: An analysis of intracellular development. J. exp. Zool., 150, 45—68.

Hanson E. D. 1963: Morphogenesis of oral structures in *Paramecium trichium*. J. Protozool., (suppl.) 10, 44.

Hanson, E. D. 1967: Protozoan Development. In: Chemical Zoology (M. Florkin and B. T. Scheer, eds.) Vol. I. Protozoa (G. W. Kidder, ed.) Academic Press. 395—539.

Hanson, E. D. and Gillies C. G. 1966: Oral structures during conjugation in *Paramecium aurelia*. J. Protozool., (suppl.) 13.

Kimball R. F., Caspersson T. O., Svenson G. and Carlson L. 1959: Quantitative cytochemical studies on *Paramecium aurelia*. Exp. Cell Res., 17, 160—172.

Lwoff A. 1950: Problems of Morphogenesis in Ciliates. Wiley and Sons, Inc. N.Y.

Pitelka D. 1963: Electron Microscopic Structure of Protozoa. Pergamon Press.

http://rcin.org.pl

Pitelka D. 1964: The morphology of the cytostomal area in *Paramecium*. J. Protozool., (suppl.) 11, 23.
Pitelka D. 1965: New observations on cortical ultrastructure in *Paramecium*. J. Microscopie, 4, 373—394.
Porter E. D. 1960: The buccal organelles in *Paramecium aurelia* during fission and conjugation, with special reference to kinetosomes. J. Protozool., 7, 211—217.
Roque M. 1961: Recherches sur les infusoires ciliés: des hymenostomes peniculiens. Bull. Biol., 45, 433—519.
Sonneborn T. M. 1963: In: The Nature of Biological Diversity. (Ed. J.M. Allen.) McGraw-Hill, 165—221.
Sonneborn T. M. 1964: Differentiation of cells. Proc. natn. Acad. Sci., U.S.A., 51, 915—929.
Smith-Sonneborn J. and Plaut W. 1967: Evidence for the presence of DNA in the pellicle of *Paramecium*. J. Cell Sci., 2, 225—234.
Suzuki S. 1957: Morphogenesis in the regeneration of *Blephorisma undulans japonicus* Suzuki. Bull. Yamagata Univ. 4, 85—191.
Tartar V. 1961: The Biology of Stentor. Pergamon Press.
Weisz P. B. 1951: An experimental analysis of morphogenesis in *Stentor coeruleus*. J. exp. Zool., 116, 231—258.
Wichterman R. 1953: Biology of *Paramecium*. The Blakiston Co. Inc. New York.
Wise B. 1965: The morphogenetic cycle in *Euplotes eurystomus* and its bearing on problems of ciliate morphogenesis. J. Protozool., 12, 626—648.
Yagiu R. 1956: Studies on morphogenesis in ciliates. 1. The regulation of the silverline systems. J. Sci. Hiroshima Univ., Series B, 16, 53—62.
Yusa A. 1957: The morphology and morphogenesis of the buccal organelles in *Paramecium* with particular reference to their systematic significance. J. Protozool., 4, 128—142.

http://rcin.org.pl

EXPLANATION OF PLATES I—VI

1: Ventral surface of *Paramecium trichium* — note the group of 6—8 kineties to the right (animal's right) of the buccal opening. Seven kineties can be seen in this animal between the endoral kinety (EK) and the arrow

2: Details of the gullet. A. The quadrulus consisting of four rows of granules. B. Dorsal and ventral peniculi, each with four rows of granules

3: The endoral kinety (EK). Note that this kinety extends up to a point near the top edge of the quadrulus. Faint ribs of the ribbed wall can be seen

4: Ribbed wall. Approximately 15 ribs can be seen

5: The oral membrane which appears to limit the opening to the buccal cavity to an ovoid area one-third the size of the buccal opening. Arrow indicates membrane

6: Doublets at the posterior end of the animal. In several kineties 11 or 12 doublet units can be counted between the cytoproct (left) and a more anterior triplet unit. Markers indicate anterior limits of doublets

7: Stage I of oral morphogenesis. Note the small irregularly spaced dots which constitute the earliest anarchic field

8: A. Stage II. The anarchic field is larger, extending anteriorly and posteriorly. B. Stage II. later than A

9: Stage III. Three distinct bands, each now composed of at least three rows. (Anoptral phase contrast.)

10: Stage IV. Separation of the two gullets with slight invagination of the new gullet. Clear discontinuity of surface kineties where the fission furrow will form

11: Stage V. Nuclei and cortical elements have separated, gullets are complete

12: Endoral kinety (anoptral phase contrast) showing apparent lateral proliferation of granules

13 A—D: Phase contrast of live animals showing the mitotic phases in the micronucleus that correspond to stages of gullet development. The changes in shape and internal structure of the macronucleus are also apparent. M — macronucleus; m — micronucleus

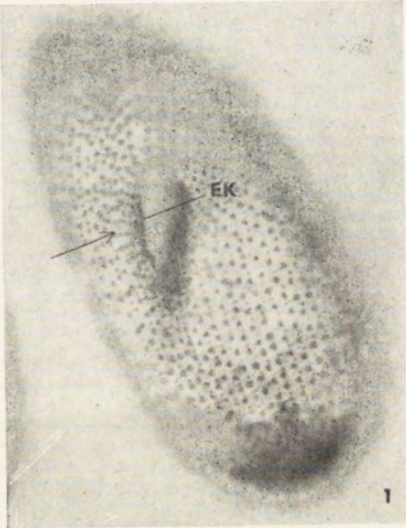
14: Stage IV. Doublet units in opisthe, quadruplet groups in proter. See Fig. 15 for details

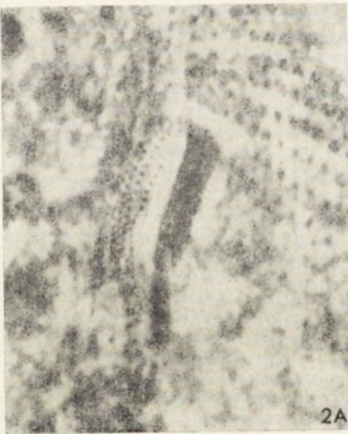
15: Stage IV. Doublet units in opisthe

16: Reversed kinety. Marker on triplet unit

17: Quadruplet groups just anterior to fission line. See markers

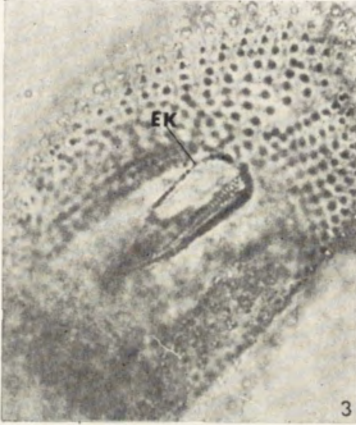
http://rcin.org.pl

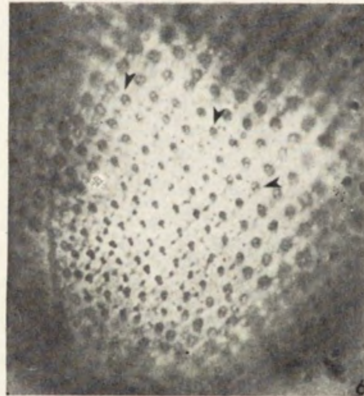




C. G. Gilles et E. D. Hanson auctores phot.

http://rcin.org.pl



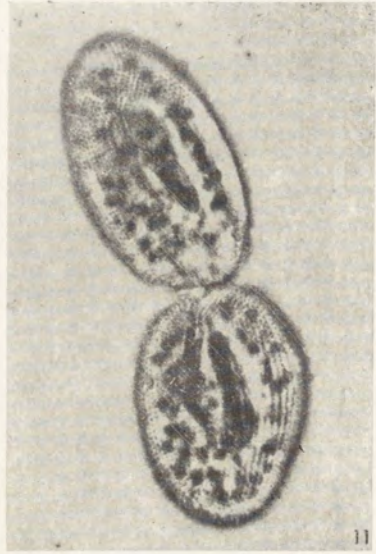


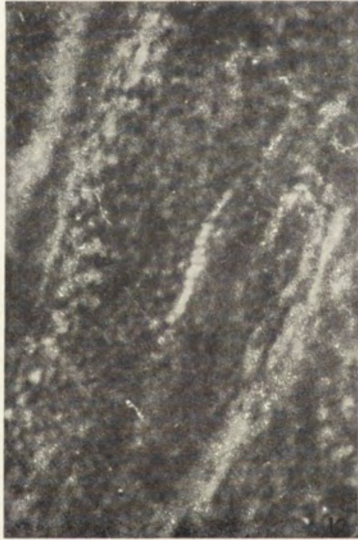
C. G. Gilles et E. D. Hanson auctores phot.

http://rcin.org.pl

C. G. Gilles et E. D. Hanson auctores phot.

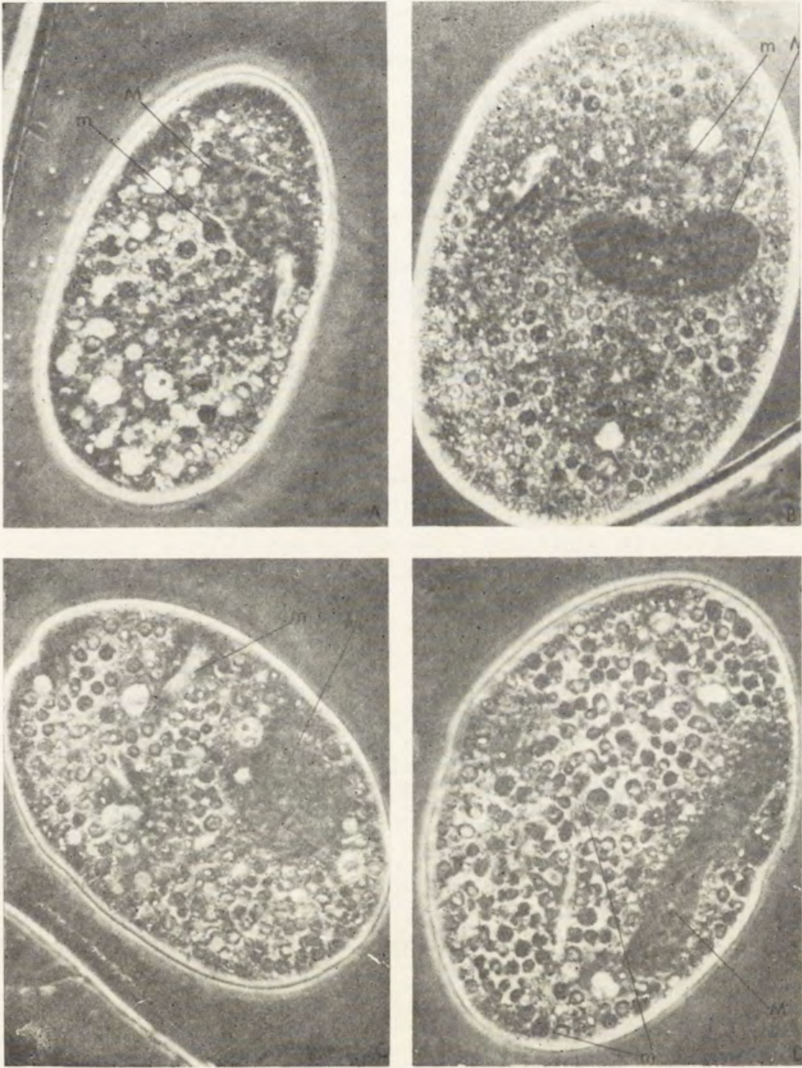
http://rcin.org.pl





C. G. Gilles et E. D. Hanson auctores phot.

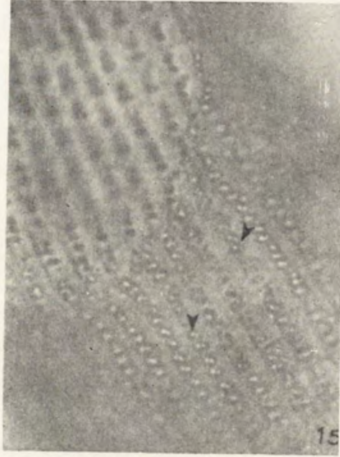
http://rcin.org.pl

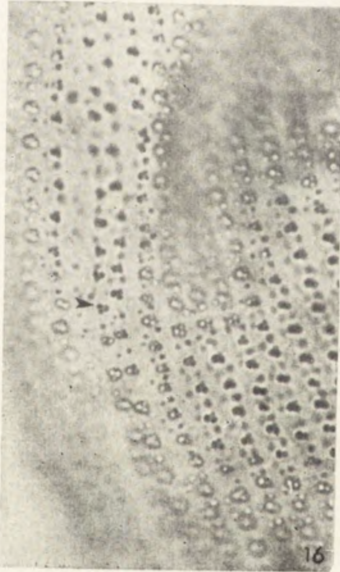


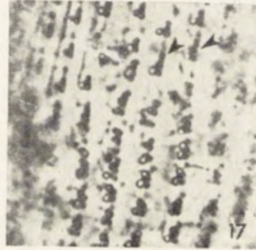
13

C. G. Gilles et E. D. Hanson auctores phot.

http://rcin.org.pl







C. G. Gilles et E. D. Hanson auctores phot.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 3

Laboratory of Invertebrate Animals, Biological Research Institute of Leningrad State University, Leningrad B-164, USSR

D. V. OSSIPOV and I. I. SKOBLO

The autogamy during conjugation in *Paramecium caudatum* Ehrbg.

II. The ex-autogamont stages of nuclear reorganization

Автогамия при конъюгации *Paramecium caudatum* Ehrbg.
II. Экс-автогамонтный цикл ядерной реорганизации

In ciliates, the genetic consequence of autogamy is the self-fertilization and — owing to the specifity of the nuclear reorganization — a full homozygotization of the ex-autogamonts (Sonneborn 1942, 1947, Beale 1952). According to Diller 1940, Gilman 1959, Skoblo and Ossipov 1968 the natural autogamy does not occur in *P. caudatum*.

In the previous communication (Skoblo and Ossipov 1968) it was ascertained that the nuclear reorganization of the autogamy type is observed in single cells of *P. caudatum* after a temporary union of conjugating pairs. A cytological analysis of autogamy up to the stage of the third metagamic division of synkaryon has been reported by the authors. It presents an interesting problem to study the whole course of karyological consequences of autogamy evoked by the precocious separation of the partners of conjugation pairs of *P. caudatum*. In the present communication, the description of the ex-autogamont cycle of *P. caudatum* is reported as well as the results of the study of variability in ex-autogamonts.

Methods

The same clones of *P. caudatum* Ehrbg. as in the previous study (Skoblo and Ossipov 1968) were used: M-13 (amicronuclear) and M-17 (normal) of the complementary mating types. The conjugating pairs — which have arisen after crossing of those two clones — separate precociously. A part of the paramecia of the clone M-17 resist autogamy whereas in the amicronuclear partner (M-13) the nuclear reorganization fails to proceed and partners behave like the normal vegetative cells.

All the methodical part of the present study has remained unchanged. The mass autogamy was evoked by feeding with china ink. Single autogamonts were placed by one in separate microaquaria. For investigation only those ciliates were used in which the nuclear reorganization had been initiated.

For preparing the material of the ex-autogamonts being at any stage of

http://rcin.org.pl

division fit for our study, up to the first metagamic division — ciliates were catched out from several tens of microaquaria and fixed on one slide. The ex-autogamonts which were after the first, second and third metagamic divisions respectively were taken out of the microaquarium and fixed on one slide in this way that it was possible to ascertain the generation sequence of the cells. This procedure permitted to define the character of the distribution of the new macronucleus (Ma) primordia and of the fragments of the old Ma among the division products of ex-autogamonts. The latter ones and the products of their division were fixed at definite intervals (30, 35, 49, 56 hrs. and 4, 5, 6 and 10 days) since the momemt of the sexual process.

The rate of division and death of ex-autogamonts were recorded every day after an examination of the microaquarium content under a low stereoscopic magnification. In sum, viability was proved in 800 ex-autogamonts. The viable clones (karyonides) were obtained from the products of the second division of ex-autogamonts. One of the 8 cells resulting from the second metagamic division was stained after the method of Dippell 1955 for elucidating the conditions of the nuclear apparatus. This served as a precise cytological control of the fact that the nuclear reorganization has really taken place in the autogamont which had given origin to the cell.

In several series of experiments, the method of separating single conjugating partners M-13×M-17 was applied. Each partner of the pair, was placed after their separation in individual microaquaria. In the case when a M-17 cell produced a viable ex-autogamont clone, the progeny of its partner in conjugating pair — which up to this moment succeeded to divide many times — were stained after the Dippell's method. As a rule those cells proved to be amicronuclear i.e. they originated of the clone M-13. Staining both members of the pair fully excludes the possibility of "contamination" of ex--autogamonts by the ex-conjugants from the pair M-17×M-17 which appear with the frequency of about 9% when the clones M-13×M-17 are crossed.

The cells were fixed with the Bouin's fluid, stained after Feulgen followed by light green. Only the whole preparations were considered. Over 500 autogamonts and products of their division were examined and drawn by means of the drawing apparatus (lens ×40, eye piece ×7). Microphotographs were executed by means of the set MFN-3 under the microscope MBI-3 (lens ×10, eye piece ×20).

Results

After the third metagamic division of synkaryon, in the cytoplasm of ex--conjugants, 8 undifferentiated nuclei are present and the old Ma begins to fragmentate. The streaks of the old Ma desintegrate into rod-shaped fragments which become ovoid, and subsequently spheroid particles. Soon after the desintegration of the old Ma, the dimensions of its fragments are various. The number of fragments fluctuates from 30 to 70 at different development stages of the Ma primordia within the confines of the paramecium cell. No essential deviations from the normal ex-conjugants M-17 were observed in the character of fragmentation of the old Ma and in the number of arising fragments in the ex-autogamonts of the clone M-17.

http://rcin.org.pl

Several hours after the conclusion of Ma fragmentation it is impossible to follow the fate of nuclei because the derivatives of synkaryon are practically undistinguishable among the numerous fragments. Only 45—50 hrs. after the onset of the nuclear reorganization, the developing primordia of Ma may be distinguished. At this stage they present spheroid, slightly Feulgen--positive bodies. The primordia of Ma gradually increase in size and stain more intensely. Feulgen-positive grains appear in them. In the present communication no special attention has been paid to the structural changes in the chromatin elements in the process of development of the Ma primordia in the ex-autogamonts of *P. caudatum*. This problem will be the subject of a special study.

The count of the developing Ma primordia in ex-autogamonts at the stage of 51—60 hrs. proved that it fluctuates in a large range from 0 to 10 (Table 1). The ex-autogamonts with 2, 3, 4, 5, 6 and 10 Ma primordia are represented in Pl. I 1—7. The precise number of Ma primordia at more advanced stages can scarcely be stated because of pycnosis. As follows from the data represented in the Table 1, only in 27.6% (24 cells) of ex-autogamonts, the number of Ma primordia proved to be the same as in the normal ex-conjugants of *P. caudatum* being 4 (Pl. I 4). The set of Ma primordia was abnormal in the remaining 72.4% of ciliates. Primordia were absent in 20.7% (18 cells) of ex-autogamonts whereas an unusually high number of Ma primordia (9—10) was found in nearly 10% of ex-autogamonts (Pl. I 7).

Table 1

Number of primordia of macronuclei in ex-autogamonts of *Paramecium caudatum* and their distribution after the first metagamic division of ex-autogamont

Number of macronucleus primordia in ex-autogamont	0	1	2	3	4	5	6	7	8	9	10
Number of ex-autogamonts studied. Total number 115	18	19	12	7	24	8	12	6	1	4	4
Number of ex-autogamonts divided within 5—6 days. Total number 29	2	5	4	1	8	3	0	3	0	1	2
Distribution of macronucleus primordia after the first metagamic division of ex-autogamont	0:0	0:1	1:1	1:2	2:2	2:3		3:4		3:6	5:5

This first metagamic division of the ex-autogamonts occurs no earlier than after 4—5 days whereas in the ex-conjugants it takes place after 2 days. We studied the character of the Ma primordia distribution in the ciliates no later than 5—6 days after the conclusion of their first metagamic division. The data concerning 29 ex-autogamonts are presented in Table 1. The results indicate that the first metagamic division may take place not only in the

http://rcin.org.pl

paramecia containing the normal number of Ma primordia (4) but also in those with an abnormal set of primordia. The great variability of the initial number of Ma primordia is reflected in the following count of primordia distribution: 0:0, 0:1, 1:1, 1:2, 2:2, 2:3, 3:4, 3:6, 5:5. Some types of the Ma primordia distribution in the daughter cells arisen after the first metagamic division of ex-autogamonts are represented in the Pl. I 8 A, B. Pl. II 9 A—12 B. It should be noted that the most frequent is the normal distribution of primordia — 2:2 (in 27.6% of cases) being characteristic for the ex-conjugants of *P. caudatum* (Pl. II 10 A, B).

The absence of cells with 8 Ma primordia among the ex-autogamonts after the first metagamic division may be accounted for by their low selectivity and their simultaneous low frequency of appearance (1.2%). Their low selectivity cannot however explain the fact that we failed to find even one divided ex--autogamont containing 6 Ma primordia whereas paramecia with such a set of primordia appear with a frequency 10.4%. Another striking fact is the low percentage (6.9%) of separated ex-autogamonts with no Ma primordia although the frequency of occurrence of those cells is rather high (25.8%). The regular distribution of Ma primordia among the daughter cells in the first metagamic division of ex-autogamonts should be stressed. An exception is only one cell with the initial content of 9 primordia which are distributed at the ratio 3:6 in the daughter cells.

The variations in the number of Ma primordia in the ex-autogamonts are reflected in the size of primordia as well. A decrease of size of the primordia is observed in proportion as their number in the ex-autogamonts rises. The regularity observed remains at the stage following the first metagamic division of ex-autogamont. The differences in the size of the Ma primordia has evidently no influence on the dimensions of the developing Ma since in the ex-autogamont clones, the Ma dimensions in the vegetative cells do not differ essentially. It may be presumed that the variations in the Ma primordia dimensions reflect the asynchrony of their development but not the differences in the ploidy of the derivatives of synkaryon from which they originate.

Sometimes in the ex-autogamonts containing a superfluous number of Ma primordia, micronucleus could not be revealed. In a number of cases this was connected with the difficulty of distinguishing the tiny micronucleus among the numerous similar fragments of the old Ma. It seems quite possible that in some ex-autogamonts micronucleus is absent indeed which is associated with the disturbance of the process of differentiation of the synkaryon derivatives. On the other hand, the supplementary divisions of synkaryon (besides the usual 3 divisions) may possibly impair the normal ratio of Ma and micronuclei number in the ex-autogamont.

As a result of our study of the distribution of primordia and of Ma fragments among the sister cells arising in the first metagamic division of the ex-autogamont of *P. caudatum,* the phenomenon of regeneration of Ma has been revealed. It was demonstrated in some ciliates that the new Ma develops not from the products of synkaryon division but from the fragments of the old Ma (Sonneborn 1940). This phenomenon was determined by Sonneborn as regeneration of Ma. It has been shown in our previous publication that in *P. caudatum,* the normal fragmentation of the old Ma (the indispensable but not sufficient condition of occurrence of the Ma regeneration)

http://rcin.org.pl

takes place only in this case when a synkaryon with the ability of metagamic division has been formed. We succeeded to find a cytological evidence of the growth of the old Ma fragments, to examine the character of their distribution in the first, second and third metagamic divisions of the ex-autogamonts, and to follow their gradual transformation into the final Ma (Pl. III 13—16 D, IV 14 A—H).

Among the ex-autogamonts of *P. caudatum,* cells were found in which no nuclei could be revealed except the fragments of the old Ma (Pl. III 13—14). The dimensions and shape of those fragments differed no more from one another than those in the ex-autogamonts M-17 with the normal type of nuclear reorganization. In some individuals, besides the normal number of Ma fragments, some pycnotic Ma primordia were observed, different in size from one another. It has been stated that the derivatives of synkaryon and especially the Ma primordia may undergo pycnosis in the course of differentiation at the most different stages of development (Pl. VI 31—33).

In the ex-autogamonts with no functional Ma primordia, after 4—5 days of growth, the old Ma fragments show larger dimensions than in the ex--autogamonts of a normal type of development of the nuclear apparatus (Pl. I 1—7). However not all the fragments of the old Ma show an increased size. Approximately a half of their total number in a cell start staining more intensely and are gradually resorbed in cytoplasm. The other fragments begin to increase in size and assume sometimes an irregular shape. Their structure does not differ from that of the normal Ma in the vegetative cell (Pl. III 14).

Till the moment of the first metagamic division, only 30—40 fragments remain in the cytoplasm of the ex-autogamont. The differences in the size of the fragments become still more conspicuous. Among the fragments of the old Ma, those which have set on the regeneration of the new Ma may be distinguished with no error although the differences in shape and size are marked among themselves as well (Pl. III 15 A, B).

After the second metagamic division of the ex-autogamont, the number of fragments in the daughter cells considerably diminishes. In the cytoplasm, only 10—15 big fragments stain intensely, the remaining 10—20 fragments become quite small and are soon resorbed (Pl. III 16 A—D). The biggest of the fragments reach about this time the size of 1/4—1/3 of the normal Ma (Pl. III 16 B, D). The preserved fragments of the old Ma become localized in the central part of the cell as a compact group and show a tendency of a definitive Ma. It should be reminded that in the earlier stages of the nuclear reorganization, the fragments are regularly distributed over the whole cytoplasm (Pl. III 13, 14).

In the cytoplasm of cells which arose as a result of the third metagamic division of ex-autogamont, only the regenerating fragments of the old Ma persist in a number of 1 up to 4 (Pl. IV 17 A—H). In the case when only one fragment remains in the cell (Pl. IV 17 C, H), its size is similar to that of Ma in the normal vegetative individual. In paramecia containing several fragments, the nuclei become smaller in proportion as their number increases. Nevertheless their total mass approaches in all the cases that of the definitive Ma.

It follows from the above description of the Ma regeneration that the Ma fragments gradually increase in size whereas the intensity of their staining and their structure remain essentially unchanged and fully correspond to

http://rcin.org.pl

those in the normal Ma. Consequently the nuclear fragments in the development process — in contrast to the Ma primordia — fail to pass the achromatic stage in which DNA in the nucleus is diffuse and cannot be revealed by the Feulgen test. It should be stressed that not all the fragments of the old Ma become the normal Ma. Some of them undergo the same degeneration process as in the usual process of nuclear reorganization.

An important peculiarity of ex-autogamonts at the onset of their Ma regeneration is the regular absence of micronuclei in them. Presently it is rather difficult to decide whether the micronuclei in such ex-autogamonts are pycnotic or fail to develop themselves from the derivatives of synkaryon. In a number of cases in the ex-autogamonts at the stage of 51—60 hrs., such a multitude of pycnotic bodies may be revealed that the onset of pycnosis prior to the moment of their differentiation into Ma and micronucleus seems very possible.

The study of dynamics of the old Ma degeneration process and of regeneration of their fragments indicate that the determination of one of the two possible paths of the fragment development occurs at a period which is rather restricted in time. One of the conditions of the Ma regeneration is the pycnosis of the synkaryon derivatives at rather early stages of differentiation. In the case when the Ma primordia reach considerable dimensions and the ex-autogamont has passed the first metagamic division, the fragment loses irreversibly the capability to regenerate the whole Ma. We failed to observe the Ma regeneration in the cells into which the developing Ma primordia did not penetrate after the first metagamic division of ex-autogamont, e.g. in the case of distribution of primordia 0:1 (Pl. I 8 A, B). All those facts indicate that the possibility of the development of fragments into the definitive Ma may occur in the period of nuclear reorganization particularly. This period is strictly limited in time.

As it has been stated in our previous publication, for the normal fragmentation of the old Ma, the development of synkaryon capable for metagamic division is indispensable in the autogamont (Skoblo and Ossipov 1968). Among the autogamonts, cases of abnormal condition of the old Ma were observed, when its fragmentation fails to occur (Pl. V 18—20). In such ex-autogamonts, Ma presents for 6—10 days a compact nucleus with numerous foldings and nucleoli, the cytoplasm is quite homogenous and transparent, food vacuoles are absent, the body dimensions are much smaller than in the vegetative individuals of the clone M-17. Presumably in the ex-autogamonts with the persisting compact Ma, disturbance of the stomatogenic process occurs. The individuals with this nuclear anomaly perish and we failed to find any evidence of a normal functioning of the non-fragmented Ma.

Disturbance of the stomatogenic process occurs in a number of ex-autogamonts in which Ma had desintegrated into the normal number of fragments, and the primordia of a new Ma and of the micronucleus had been formed. The disturbance of the stomatogenic process which leads to absence of food vacuoles, evokes the reduction of the body dimensions. In proportion as the body dimensions diminish, the Ma primordia gradually undergo pycnosis. The ex-autogamonts lose the capability to metagamic divisions and perish within 8—10 days.

Among several hundreds of the ex-autogamonts examined, we succeeded to find only two cells with the following coinciding features: the non-frag-

http://rcin.org.pl

mented old Ma and a small number of food vacuoles (Pl. V 24, 25). No doubt, in those ciliates occurred the nuclear reorganization since pycnotic bodies were marked in them. It should be pointed out that in no one case a normal ex-autogamont division was found in which Ma had not been fragmented. Evidently the cells with the above anomaly proved to be not viable.

Among the ex-autogamonts which failed to perform the first metagamic division within the normal period of time (on the 4th—6th day after the onset of reorganization), arise sometimes giant cells with a body volume exceeding many times the normal one (Pl. VI 26, 27). In all the giant cells, the fragmentation of the old Ma and the development of primordia proceed with no essential deviations although the number of primordia in a cell is abnormal as a rule. The cytoplasm of those cells is filled with a great number of food vacuoles. Only a few of the giant cells are capable to conclude the first metagamic division. They all perish by lysis within 9—10 days. Possibly the gigantism of ex-autogamonts is associated with some impairment of the mechanism which controls the onset of the metagamic divisions, whereas the other processes (fragmentation of the old Ma, differentiation of the new nuclear apparatus, stomatogenesis etc.) proceed with no perceptible changes.

In a number of cases, in each of the two daughter cells after the first metagamic division, the dimensions and the structure of the fragments of the old Ma are essentially different (Pl. VI 28 A, B). In one of those cells the fragments are of a normal size, homogenous and stain intensely (Pl. VI 28 A) whereas in the daughter cells the fragments are much bigger and have a loose structure (Pl. VI 28 B). Since all the fragments are similar within the confines of one cell, it might be postulated that the changes evoking the different characters of nuclei arise already after the first metagamic division of the ex-autogamont.

Subsequently we will analyse the character and the localization of the old Ma fragments as far as it might provide some information as to the functional activity of nuclei. In the normal type of nuclear reorganization in the ex-autogamonts and ex-conjugants M-17, till the stage of the first metagamic division and immediately after its conclusion, the Ma fragments are regularly dispersed over the whole cell and their segregation in division has a fortuitous character. This secures their even distribution to the daughter cells (Pl. I and II). After the second metagamic division of ex-autogamont, the Ma fragments become disposed around the developing primordium of the new Ma which occupies the central position in the cell (Pl. VI 29). After the second division of ex-autogamont in the case of regeneration, the Ma fragments tend also to accumulate in the central region of the cell (Pl. III 16 A—D). Around the regenerating fragments of Ma, a few fragments in the course of degeneration accumulate. In this way, the regenerating fragments show an inclination similar to that of developing Ma primordia in their localization within the cell. An interesting fact is that in the ex-autogamonts with only one Ma primordium, the fragments accumulate sometimes around it much earlier namely prior to the first metagamic division of ex-autogamont (Pl. VI 30).

For comparing the peculiarities of the nuclear reorganization in autogamy with that in conjugation, the ex-conjugant cycle in paramecia of the clone M-17 was followed. It was mentioned above, that the character of fragmentation of the old Ma and the number of arising fragments in ex-autogamont and ex-conjugant of the clone M-17 are quite identical. However the count of

http://rcin.org.pl

the number of primordia in the ex-conjugants prior to the first metagamic division (Table 2) has demonstrated that they are much less variable than in the ex-autogamonts (Table 1). In 87.2% of ex-conjugant individuals of *P. caudatum*, 4 primordia arise usually in each of them. Very rarely cells containing 3, 5 or 6 Ma primordia in each cell are observed among the ex-autogamonts. No other deviation of the usual scheme of the course of nuclear reorganization in ex-conjugations has been observed.

Table 2

Number of macronucleus primordia in ex-conjugants of the clone M-17 of *Paramecium caudatum* prior to the first metagamic division

Number of macronucleus primordia in ex-conjugant	3	4	5	6
Number of ex-conjugants studied. Total number 47	2	41	3	1

The examination of viability of ex-conjugants M-17 obtained after the intra-clonal conjugation M-17×M-17 was carried out on 60 conjugating pairs. The partners of each pair were placed in separate microaquaria after their disjoining. It was stated that the viable ex-conjugants arise with a frequency of 53.5%.

Table 3

Nuclear apparatus and mating type in ex-autogamont clones of *Paramecium caudatum*

Mating type \ Nuclear apparatus	Amicronuclear	Normal
M-17	1	3
M-13	41	9
No mating reaction	1	11
Total number 66	43 (65%)	23 (35%)

Further on we will analyse the general characteristic of the ex-autogamont clones obtained in our experiments. The Table 3 presents the data provided by the analysis of the nuclear apparatus and of the mating types of clones obtained from the products of the second division of viable ex-autogamonts. Karyonids from 68 ex-autogamonts were examined although not in all the cases 4 karyonids could be obtained from every autogamont. As a rule, the sister karyonids did not differ from each other in their nuclear apparatus (only the presence or absence of micronucleus was considered), and

http://rcin.org.pl

in the type of mating. An exception presented the karyonids from two ex--autogamonts: 11-a2 (with micronucleus, mating type M-13), 11-a3 (amicronuclear, mating type M-13) and 23-a3 (with micronucleus, mating type M-13), 23-a4 (with micronucleus, mating type M-17).

The fission of karyotype in sister karyonids proves the possibility of arising of amicronuclear clones as result of an abnormal distribution of micronuclei to the sister cells in the metagamic division of the ex-autogamont in which a normal differentiation of synkaryon derivatives into Ma and micronucleus had occurred.

Attention should be called to the fact that 65% of ex-autogamonts gave origin to amicronuclear clones, possibly as a result of the Ma regeneration process whereas 35% of clones proved to be micronuclear. Out of 12 ex-autogamont lines which failed to show the mating reaction after a repeated mixing with every one of the "paternal" clones (M-13 and M-17) in the course of two months following the nuclear reorganization — only one line proved to be amicronuclear whereas the paramecia of 11 other lines contained micronuclei.

In the present study we do not intend to analyse the problem of inheritance of the mating types in autogamonts of *P. caudatum*. We just mention that it evidently differs from the "cytoplasmic" type (Beale 1954) since the majority of the autogamont lines (74%) exhibit a different mating type than that of their cytoplasmic predecessor — the clone M-17. In one of the ex-autogamont lines (karyonids 23-a3 and 23-a4) the karyonidic inheritance was observed: the karyonid 23-a3 showed the mating type M-13 whereas its sister-karyonid 23-a4 — the mating type M-17. It should be mentioned that the low viability of the ex-autogamont clones complicates the analysis of the inheritance character of the mating types.

The study of the nuclear apparatus of the ex-autogamont clones of *P. caudatum* revealed essential differences in the shape, size and in the affinity of micronuclei for stain. No doubt, this is associated with a various DNA content in different types of micronuclei. Sometimes this difference was observed even in sister-karyonids. The study of the nature of differences between the types of micronuclei presents the subject of a special investigation.

It should be remarked in conclusion that the viable ex-autogamont clones were obtained by us from the clone KT-20 (originating from another population than the clone M-17). Paramecia of the clone KT-20 similarly as those of M-17 when crossed with the M-13 cells, produce conjugating pairs which separate precociously. The cells of the clone KT-20 underwent the nuclear reorganization of the autogamy type.

Discussion

The results presented in the above study indicate that the differences in the course of the nuclear reorganization of ex-autogamonts — when compared with that in the ex-conjugants of *P. caudatum* — concern essentially the number and the character of various anomalies. In the previous publication we analyzed the paths of formation of the fundamental types of nuclear aberrations in the progamic part of the autogamic cycle in *P. caudatum*

http://rcin.org.pl

(Skoblo and Ossipov 1968). The data accumulated in the present study permit to understand the fate of those as well as of the new arising nuclear disturbances in the metagamic part of the cycle.

It was demonstrated by us previously that in 31% of autogamonts, the nuclear processes undergo profound disturbances already at the stage prior to the second progamic division of micronucleus (the pycnotic crescent, pycnotic anaphase of the first division, blocking of the second division of micronucleus). It has been stated that in the autogamonts of this collective group, the fragmentation of the old Ma is disturbed subsequently as well as the stomatogenic processes which makes the ciliate not viable.

In 18% of autogamonts, all the four nuclei produced after the second progamic division of micronucleus, initiate the third maturation division which leads to formation of multiple pronuclei and of synkaryon. It has been demonstrated previously that in the case when two synkaryons are formed in the autogamont then only one of them is capable to start the metagamic division. Nevertheless in the more advanced stages it is difficult to follow the picture of the nuclear reorganization in the ex-autogamonts with the observed anomaly because it is practically impossible to determine the origin of nuclei and their subsequent fate. Besides, we tried to answer the question whether — in the case of a multiple formation of pronuclei — synkaryon arises always as a result of fusion of the sister nuclei. The answer to the above question would permit to establish the fact whether all the ex-autogamont clones of *P. caudatum* are really homozygotic or perhaps some of them (when synkaryon arises as a result of fusion of non-sister pronuclei) would prove to be heterozygotic. Solution of this problem needs some further study.

The process of the nuclear reorganization reaches the metagamic part of the cycle only in 51% of autogamonts. As a rule, synkaryon divides three times, giving origin to 8 nuclei. About this moment, the old Ma begins to fragmentate. According to the generally accepted view (Wichterman 1953) after the third synkaryon division, in the ex-conjugant of *P. caudatum* 3 nuclei degenerate, one gives origin to micronucleus, and the 4 remaining nuclei develop Ma. Deviations of this usual scheme of the metagamic part of cycle in the ex-conjugants of *P. caudatum* have been reported very rarely in the literature (Diller 1940, Wichterman 1953).

Our findings indicate that in the ex-autogamonts of *P. caudatum* the nuclear reorganization undergoes significant changes. This is reflected in the first place in the great variability of the number of the new Ma primordia (Table 1). The normal number of Ma primordia (4) appears only in 27% of cases whereas in 72% of ex-autogamonts their number fluctuates from 0 to 10. The data about formation of more than 8 primordia in one cell suggest that at least a part of the products of the third synkaryon division conclude their fourth division. Autogamonts with a just beginning fragmentation of Ma and with 10 non-differentiated nuclei have been detected by us previously. Presumably such autogamonts produce cells with a supplementary number of Ma primordia. In the ex-conjugants of *P. caudatum* cases are known of a supplementary metagamic division of synkaryon. Diller 1950 observed in one of the many races investigated by him, four metagamic divisions after the formation of synkaryon although after the first division only one nucleus has started the subsequent divisions. In conclusion of four metagamic divisions of synkaryon, only 8 nuclei arise, 4 of them being the

http://rcin.org.pl

Ma primordia. Diller 1940 in his 10 years long study of a great number of cultures found a variability of the number of arising Ma primordia in ex--autogamonts. Although Diller failed to record the percentage of the anomaly observed in separate cultures (mixed cultures and populations have been studied) — it appears very interesting to compare the spectrum of deviation in the ex-autogamonts and in ex-conjugants of *P. caudatum*. Diller observed the ex-conjugants containing 14, 16, 20 and even 22 non--differentiated nuclei and also some cases of formation of supplementary Ma primordia (5, 6, 7 and 9). Some indications about the formation of an abnormal number of primordia in the ex-conjugants of *P. caudatum* may be found in nearly every publication concerning the nuclear reorganization (Maupas 1889, Klitzke 1914, Penn 1937, Diller 1940).

Occurrence of cells with a number of primordia lower than the normal one in the ex-autogamonts of *P. caudatum* — as reported in the present study — may be accounted for by the degeneration of a part of products of the precocious synkaryon division as well as by disturbance of differentiation of nuclei into Ma and the micronucleus.

As known, the character of differentiation of the derivatives of synkaryon is influenced as well by the external as by the intra cellular factors.

Centrifuging the ex-conjugants of ciliates with a central position of nuclei considerably alters the correlation of arising Ma and micronuclei (Nanney 1953, Sonneborn 1954). Artificial separation of conjugating pairs of ciliates prior to the stage of their pronuclei exchange evokes considerable fluctuations of the number of Ma primordia (Poliansky 1938). It should be noted that in this case the nuclear reorganization in the single ciliates follows the type of autogamy. The character of nuclear reorganization is considerably disturbed in re-conjugation of the ciliates when the ex-conjugant begins a new mating with a vegetative cell prior to the conclusion of the full reorganization of its nuclear apparatus. So in the re-conjugation of *P. putrinum*, the following sets of Ma primordia: 0, 3, 4, 5, 6 and 8 have been observed (Jankowski 1966) whereas in the normal ex-conjugants, formation of 4 primordia is characteristic.

In our experiments, the nuclear reorganization of ex-autogamonts of *P. caudatum* occurs in quite normal external conditions. It may be however presumed that the alterations of the character of differentiation in the synkaryon derivatives may be evoked by the disturbance of the mechanism which controls the separate stages of the sexual process. The precocious separation of the partners of conjugating pairs M-13×M-17 which induces autogamy, proved to influence not only the peculiarities of the nuclear reorganization in the progamic sector of the cycle (Skoblo and Ossipov 1968) but — at a high degree — the ex-autogamont cycle as well.

An important peculiarity of the ex-autogamont cycle of *P. caudatum* is the formation of a considerable number of cells in which an unusual nuclear reorganization takes place i.e. the regeneration of the new Ma from the fragment of the old one.

The phenomenon of the Ma regeneration in *P. caudatum* has not been observed till now. One of the authors (Ossipov 1966) made an attempt to evaluate the frequency of the Ma regeneration in conjugation of two selected clones of *P. caudatum*. The ex-conjugants arising after crossing of those two clones proved not to be viable in 100% of cases. In conditions favorable

http://rcin.org.pl

for Ma regeneration (raised temperature after the stage of synkaryon formation), the percentage of viable ex-conjugants fails to increase. If Ma regeneration has taken place, the occurrence of viable cells after the action of raised temperature should be expected. This gave reason to postulate that Ma regeneration does not occur with an observable frequency in conjugation of *P. caudatum* clones which were studied previously. Although a detailed cytological examination of the conjugants of those two clones has not been performed, it was stated that the nuclear processes reach at least the second metagamic division of synkaryon. It should be mentioned at last that regeneration has not been revealed by us in the ex-conjugants M-17. Consequently the possibility of development of the new Ma in ex-autogamonts of *P. caudatum* becomes determined by a certain disturbance in the course of the nuclear reorganization evoked by the precocious separation of the partners of the conjugating pairs. Possibly the abnormal development of the new nuclear apparatus may be due to pycnosis of all the derivatives of synkaryon as well prior to the stage of formation of 8 nuclei as during the subsequent prolonged period of nuclear differentiation into the macro- and micronucleus.

As proved by our study, in all the ex-autogamonts with regenerating Ma, micronuclei are absent. It should be remarked that in *P. aurelia,* the regeneration of Ma does not affect the possibility of the micronuclei development from the derivatives of synkaryon. As a result, two nuclei of different genotypes develop in the cell: Ma of a genotype identical with that of the old Ma whereas micronucleus has the genotype of synkaryon from which it has originated (Sonneborn 1940, 1947). The absence of micronuclei in the ex-autogamonts with regenerating Ma may be explained by the fact that the regeneration of Ma occurs only in those cells of *P. caudatum* in which only the non-differentiated synkaryon derivatives became pycnotic, or in the cells in which micronucleus has not been formed — as results of disturbances of nuclear differentiation — and all the Ma primordia became pycnotic.

Another peculiar feature of the Ma regeneration in the ex-autogamonts of *P. caudatum* consists in the fact that not all the 30—70 fragments of the old Ma become the definitive Ma. Only 10—15 fragments regenerate to form the new Ma whereas all the remaining ones become resorbed in cytoplasm.

In the case when the sexual process of paramecia (conjugation or autogamy) proceeds in the optimal conditions, the regeneration of Ma is very rarely observed (Wichterman 1953). Its frequency however rises considerably under an extreme action upon the cell at the moment when the formation of synkaryon has already occurred and the old Ma has fragmented normally (raised temperature — Sonneborn 1940, Jankowski 1961, 1962, x-rays — Kovaleva and Jankowski 1965).

A phenomenon resembling to Ma regeneration was observed by Diller 1954 in autogamy of *Paramecium polycarium.* However a comparison of those findings with our own results is rather complicated since the nuclear reorganization in *P. polycarium* was followed only in mass cultures, and the course of the nuclear processes in the progeny of single autogamonts was not examined in the study of the ex-autogamont cycle.

It should be pointed out when comparing the general characteristic of the cytological differences of the nuclear reorganization during autogamy and that in conjugation within one species — that those differences were often

http://rcin.org.pl

not revealed, or were found only at some stages. So in the ciliate *Paramecium calkinsi*, the natural autogamy does not exist but it may be artificially evoked in the single ciliates after the application of the method of Metz 1947. Diller 1948 observed that the process of nuclear reorganization in single individuals of *P. calkinsi* is consistent in details with the phenomenon of conjugation. In the conjugation autogamy of *Paramecium putrinum* induced by the method of multiagglutination of reactive clones of several mating types, it is impossible to distinguish the ex-autogamonts from the ex-conjugants on preparations (Jankowski 1965). However in the progamic part of the autogamont cycle of *P. putrinum*, special nuclear aberration has been observed.

Dispite the numerous aberrations in the progamic and metagamic part of the cycle of nuclear reorganization in the autogamonts of the clone M-17 of *P. caudatum*, about 23% of viable ex-autogamonts arise as result of the sexual process whereas in the intra-clonal conjugation M-17×M-17, the percentage of viable ex-conjugants is much higher amounting 52.5%. The fall of viability in ex-autogamonts is surely associated with the occurrence of a number of nuclear aberrations which are absent in conjugation. Such anomalies in the autogamont as: pycnotic crescent, pycnotic anaphase, blocking of the second micronucleus division, pycnosis of all the pronuclei of synkaryon and of the products of its first divisions, lead to impairment of the course of the sexual process and to a full inability of reorganizing the new nuclear apparatus and to the inevitable death of the cell. All those disturbances are accompanied by the loss of ability of the old Ma to a normal fragmentation.

In numerous ex-autogamonts of *P. caudatum* pycnosis of the new nuclear apparatus takes place at the stage when nuclei are already differentiated into Ma and micronuclei and the old Ma has normally fragmented. In this case, a part of ex-autogamonts may prove to be viable in result of regeneration process of Ma which has been studied in details in the present publication.

The disturbance of the stomatogenic process and of the ability of ex-autogamonts to enter into the first metagamic division involve the formation of non-viable cells as well. In the first case ciliates arise in which no food vacuoles are present in cytoplasm, and the size of the individuals is several times smaller than the normal one. In the second case — on the contrary — giant cells appear with a great number of food vacuoles in their cytoplasm. It should be mentioned that the death of ex-autogamonts or of the products of their division is not always accompanied by visible nuclear aberrations.

It is accepted that the sequence and single details of reorganization in conjugation of ciliates are consistent with those in autogamy, nevertheless the data provided by the literature permit not always to establish which is the degree of similitude, or inversely, which are the differences within the same clone. The cytological examination of autogamy in *P. aurelia* had been carried out at this period of development of cytology when the system of mating types was not yet discovered (Diller 1936). The description of autogamy in *P. polycarium* reported by the same author cannot be compared to the nuclear changes in conjugation — if in the culture studied by him only autogamy took place — and conjugation in this species has not yet been described. Jankowski 1965 selected several clones of *P. putrinum* for his study of the conjugation autogamy. It was however impossible to establish to which

http://rcin.org.pl

of the clones studied each autogamont belongs, and consequently the comparison of the nuclear anomalies in conjugation with those in autogamy of the same clone could not be performed.

The comparison of the nuclear reorganization in autogamy with that in conjugation carried out on different clones and races within the same species, may present some difficulties involved by some clonal and racial peculiarities of the nuclear reorganization which have been often observed (Wichterman 1953, Jankowski 1960, 1962).

Summary

The precocious separation of the conjugating pairs which have arisen after crossing of clones M-13 × M-17 of *Paramecium caudatum*, evokes a nuclear reorganization of the autogamy type in a part of individuals of the clone M-17. The process of the nuclear reorganization in ex-autogamonts does not differ essentially from that in the intraclonal conjugation of M-17 × M-17. The principal difference consists in the occurrence of anomalies in the ex-autogamonts. Those anomalies were not revealed in ex-conjugants M-17. A great variability of the number of Ma primordia (0—10) was stated. Only in 27.6% of ex-autogamonts arise 4 primordia in one cell. In the majority of ex-autogamonts, the Ma regeneration has been revealed. Not all of the old Ma fragments became the new Ma. Paramecia with regenerating Ma proved always to be amicronuclear. Formation of a synkaryon capable to metagamic divisions of the cell is indispensable for completion of the process of fragmentation of the old Ma. Ex-autogamonts containing a non-fragmented Ma are not capable to metagamic divisions. They all perish. Disturbance of the stomatogenic process was observed in all the ex-autogamonts with a compact non-desintegrated Ma and in a certain number of cells in which the nuclear reorganization has proceeded without observable deviations. The percentage of viable ex-autogamonts amounts 22.8%, wheres in the intraclonal conjugation of the same clone (M-17 × M-17) the viable clones appear with the frequency 52.5%. Viable clones (karyonids) were gained of 68 ex-autogamonts, 65% them proved to be amicronuclear and only 35% were normal. An essential disturbance of the nuclear reorganization in ex-autogamonts of *P. caudatum* is evidently associated with the full homozygotization and with the appearance of all the recessive lethals at the earliest stages of development of the synkaryon derivatives as well as with the disturbance of the interconnection between the separate links of the sexual process.

РЕЗЮМЕ

Преждевременное расхождение конъюгирующих пар, образующихся при скрещивании клонов М-13 и М-17 *Paramecium caudatum*, вызывает ядерную реорганизацию по типу автогамии в части парамеций клона М-17. Процесс ядерной реорганизации в экс-автогамонтах принципиально не отличается от такового при внутриклональной конъюгации М-17 × М-17. Основные различия заключаются в появлении у экс-автогамонтов аномалий, которые не были обнаружены у экс-конъюгантов М-17. Отмечена большая вариабильность количества

http://rcin.org.pl

образовавшихся в клетке зачатков Ма (от 0 до 10). Только в 27.6% экс-автогамонтов возникает по 4 зачатка. У значительной части экс-автогамонтов обнаружено явление регенерации Ма. Не все фрагменты старого Ма превращаются в новые Ма. Парамеции с регенерирующими Ма всегда оказываются амикронуклеарными. Для завершения процесса нормальной фрагментации старого Ма необходимо образование в клетке синкариона, способного к метагамным делениям. Экс-автогамонты, содержащие нефрагментированный Ма, не способны к метагамным делениям и все без исключения погибают. Нарушение процесса стоматогенеза отмечено у всех экс-автогамонтов с кампактным, нераспавшимся Ма и в незначительной части клеток, ядерная реорганизация в которых протекает без заметных отклонений. Процент жизнеспособных экс-автогамонтов составляет всего 22.8%, тогда как при внутриклональной конъюгации того же клона (M-17 × M-17) жизнеспособные клоны возникают с частотой 52.5%. От 68 экс-автогамонтов получены жизнеспособные клоны (карионидыы), из них 65% оказалось амикронуклеарными и только 35% нормальными. Существенные нарушения ядерной реорганизации в экс-автогамонтах *P. caudatum*, по-видимому, связаны как с полной гомозиготизацией и проявлением всех рецессивных леталей на самых ранних этапах развития дериватов синкариона, так и с нарушением взаимосвязи между отдельными звеньями полового процесса.

REFERENCES

Beale G. H. 1952: Antigen variation in *Paramecium aurelia*, variety I. Genetics, 37, 62—74.

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

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

Diller W. F. 1948: Induction of autogamy in single animals of *Paramecium caudatum* following mixture of two mating types. Biol. Bull., 95, 265.

Diller W. F. 1950: An extra postzygotic nuclear division in *Paramecium caudatum*. Trans. Amer. micr. Soc., 69, 309—316.

Diller W. F. 1954: Autogamy in *Paramecium polycaryum*. J. Protozool., 1 (suppl.), 60—70.

Dippell R. V. 1955: A temporary stain for *Paramecium* and other ciliate Protozoa. Stain tech., 30, 69—71.

Gilman L. C. 1959: Nuclear reorganization in *Paramecium caudatum*. J. Protozool., 6 (suppl.), 19.

Jankowski A. W. 1960: Processy konjugacii *Paramecium trichium* Stokes. 1. Amfimiksis i avtogamija. Citologija, 2, 581—588.

Jankowski A. W. 1961: Processy konjugacii u redkoj solonovatovodnoj paramecii *Paramecium woodruffi*. Dokl. Akad. Nauk SSSR, 137, 989—992.

Jankowski A. W. 1962: Processy konjugacii *Paramecium putrinum* Clap. et Lachm. II. Apomiktičeskie reorganizacionnye cikly i sistema miksotipov. Citologija, 4, 434—444.

Jankowski A. W. 1965: Processy konjugacii *Paramecium putrinum* Clap. et Lachm. VII. Jadernye processy pri vnekonjugacionnoj avtogamii, inducirovannoj novym metodom „množestvennogo sparivanija". Acta Protozool., 3, 239—262.

Jankowski A. W. 1966: Processy konjugacii *Paramecium putrinum* Clap. et Lachm. VIII. Jadernye processy pri povtornom sparivanii — rekonjugacii. Zool. Zh., 45, 818—828.

Klitzke M. 1914: Über Wiederkonjuganten bei *Paramecium caudatum*. Arch. Protistenk., 33, 1—20.

Kovaleva N. E. i Jankowski A. W. 1965: Vlijanie ionizirujuščego izluče-

http://rcin.org.pl

nija na processy jadernoj reorganizacii *Paramecium putrinum*. 1. Jadernye processy u oblučennyh konjugantov. Zh. obsc. Biol., 36, 176—189.

Maupas E. 1889: La rajeunissement karyogamique chez les cilies. Arch. Zool. exp. gen., 7, 149—517.

Metz C. B. 1947: Induction of "pseudoselfing" and meiosis in *Paramecium aurelia* by formalin-killed animals of the opposite mating type. J. exp. Zool., 105, 115—139.

Nanney D. L. 1953: Nucleo-cytoplasmic interaction during the conjugation in *Tetrahymena*. Biol. Bull., 105, 133—148.

Ossipov D. V. 1966: K voprosu o regeneracii makronukleusa *Paramecium caudatum*. Citologija, 8, 108—110.

Penn A. B. 1937: Reinvestigation into the cytology of conjugation in *Paramecium caudatum*. Arch. Protistenk., 89, 45—54.

Poljansky Ju. I. 1938: Rekonstrukcija jadernogo apparata u *Bursaria truncatella* pri eksperimentalnom razedinenii konjugirujuščih paroček. Biol. zh., 7, 123—131.

Skoblo I. I. and Ossipov D. V. 1968: The autogamy during conjugation in *Paramecium caudatum* Ehrb. I. Study on the nuclear reorganization. Acta Protozool., 5, 273—290.

Sonneborn T. M. 1940: The relation of macronuclear regeneration in *Paramecium aurelia* to macronuclear structure, amitosis and genetic determination. Anat. Rec., 78, 53—54.

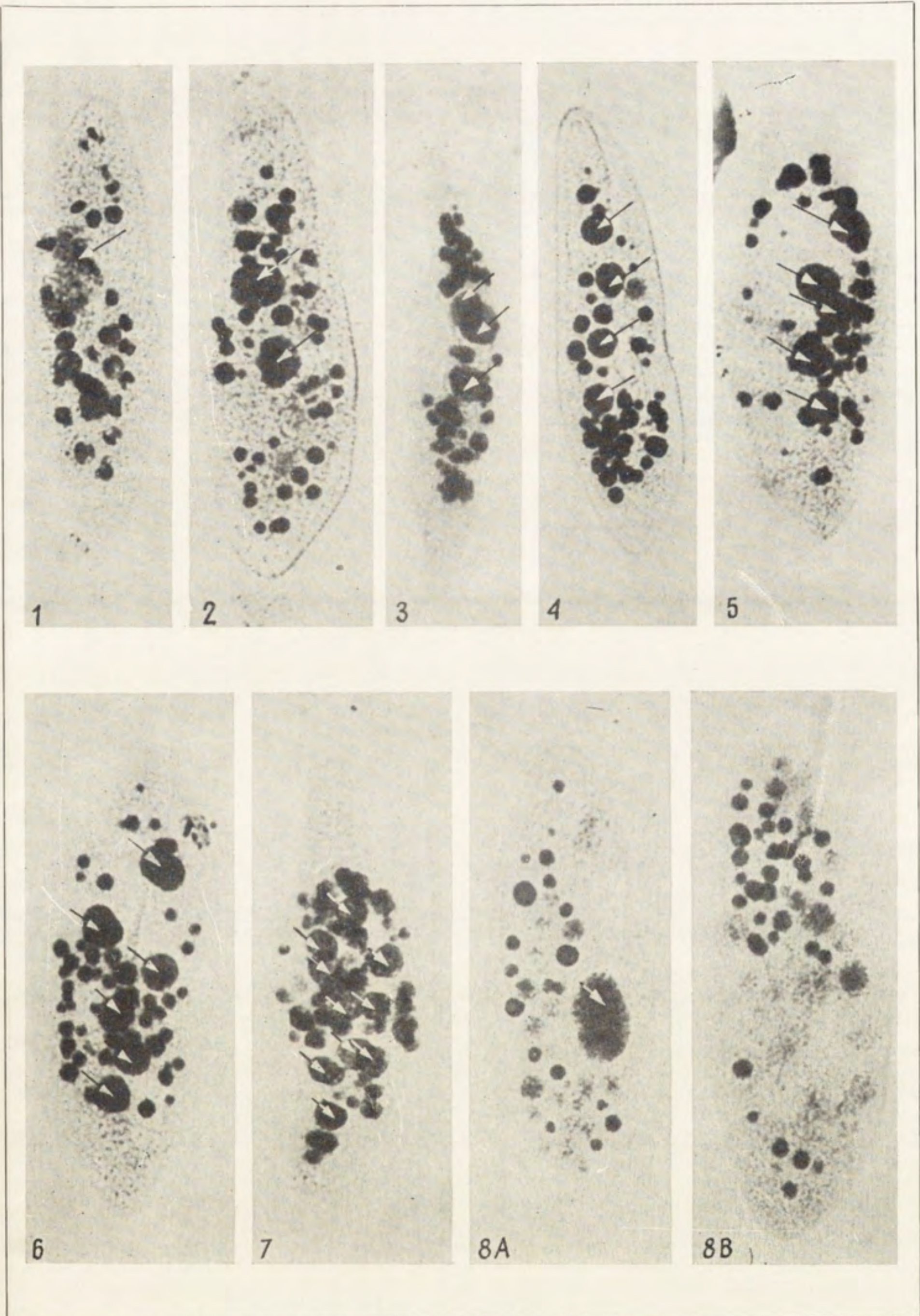
Sonneborn T. M. 1942: Inheritance in ciliate Protozoa. Amer. Nat., 76, 46—62.

Sonneborn T. M. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Advanc. Genet., I, 264—358.

Sonneborn T. M. 1954: Patterns of nucleocytoplasmic integration in *Paramecium*. Caryologia, suppl., 307—325.

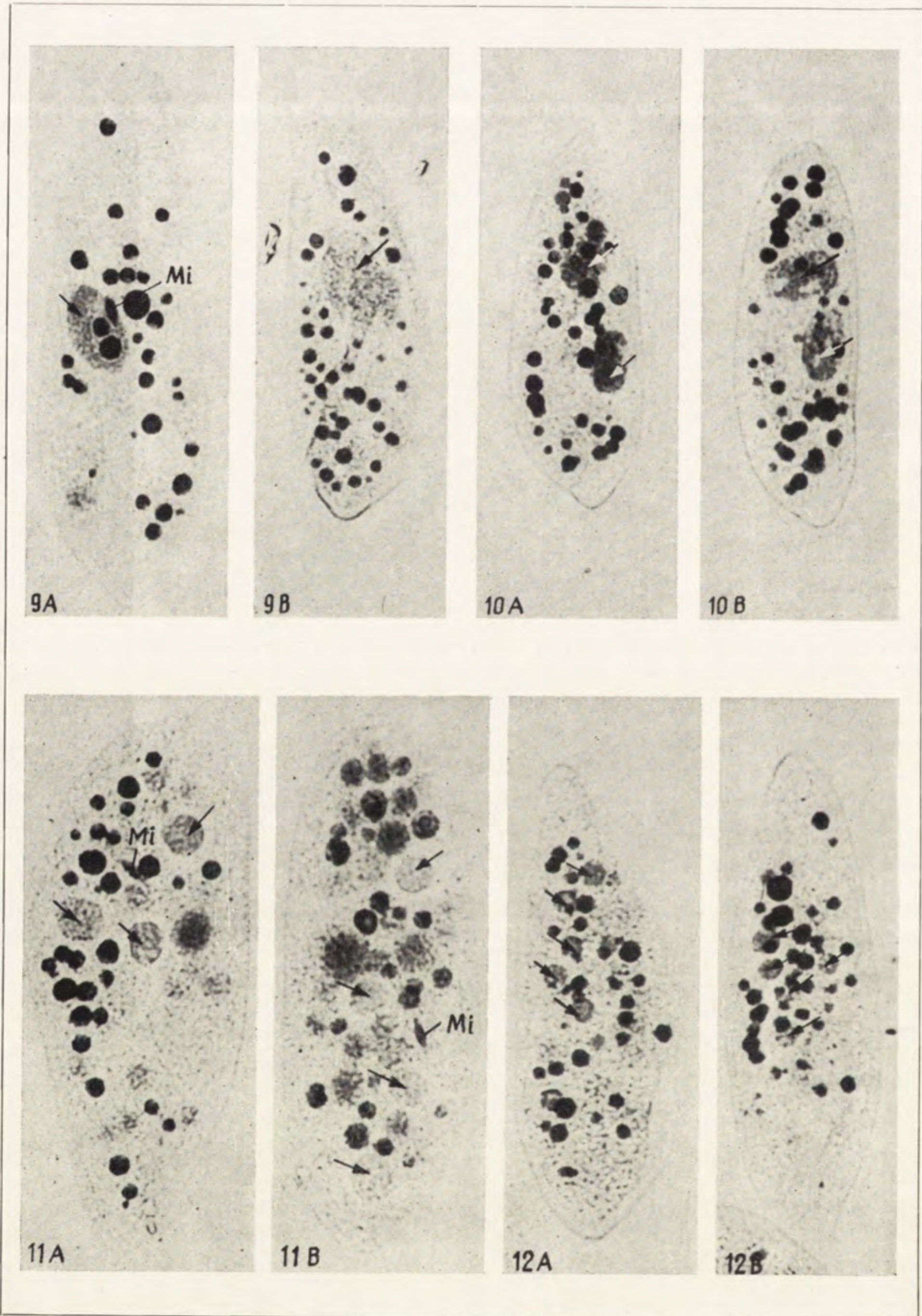
Wichterman R. 1953: The biology of *Paramecium*. New York.

http://rcin.org.pl



D. V. Ossipov et I. I. Skoblo auctores phot.

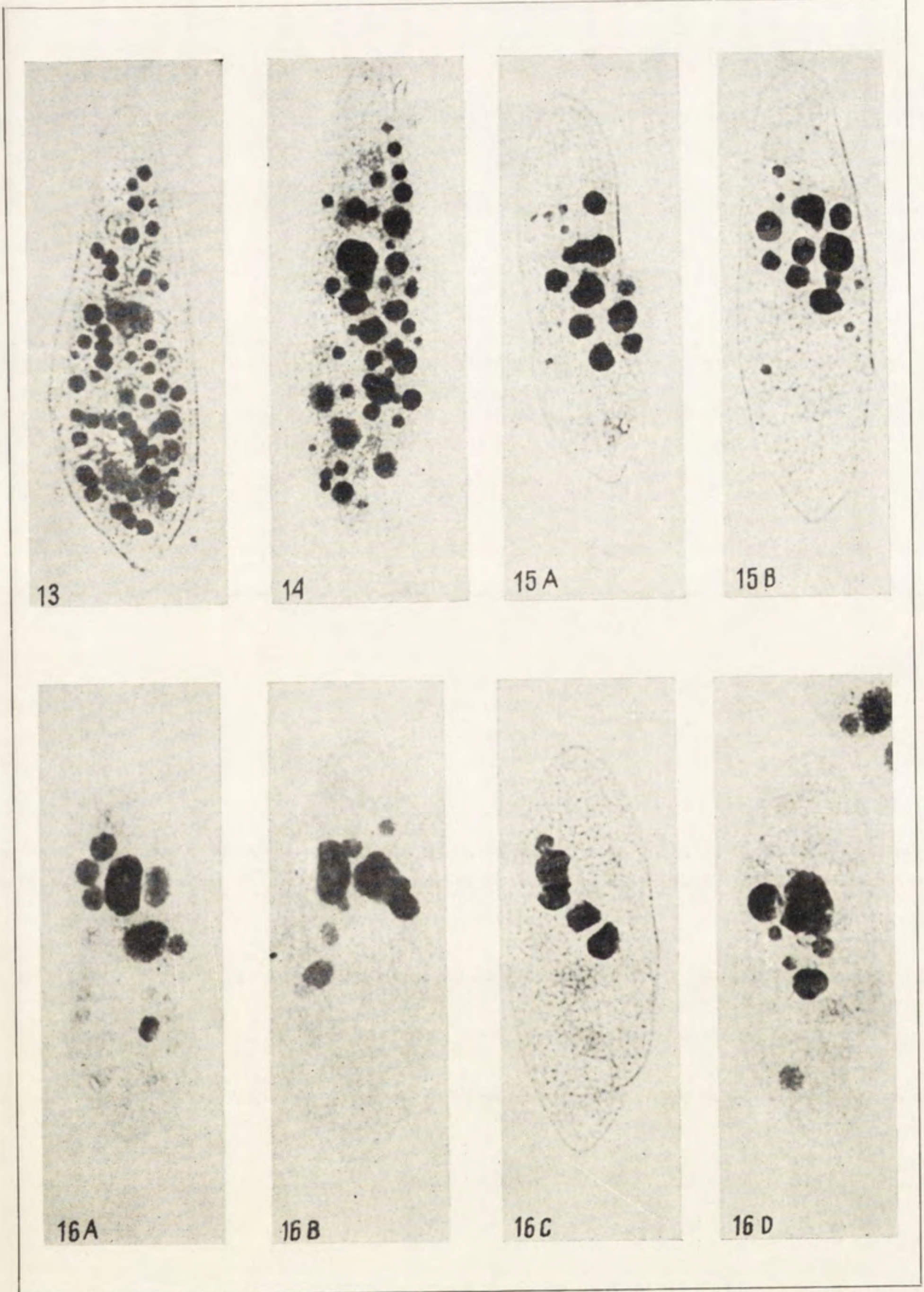
http://rcin.org.pl



D. V. Ossipov et I. I. Skoblo

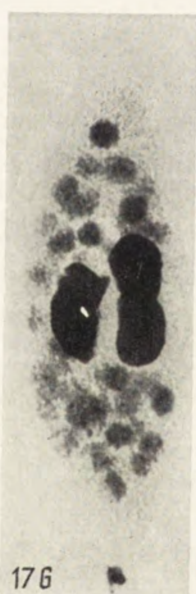
auctores phot.

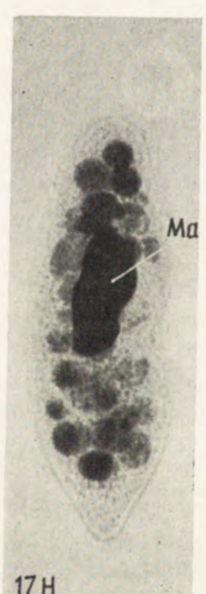
http://rcin.org.pl



D. V. Ossipov et I. I. Skoblo auctores phot.

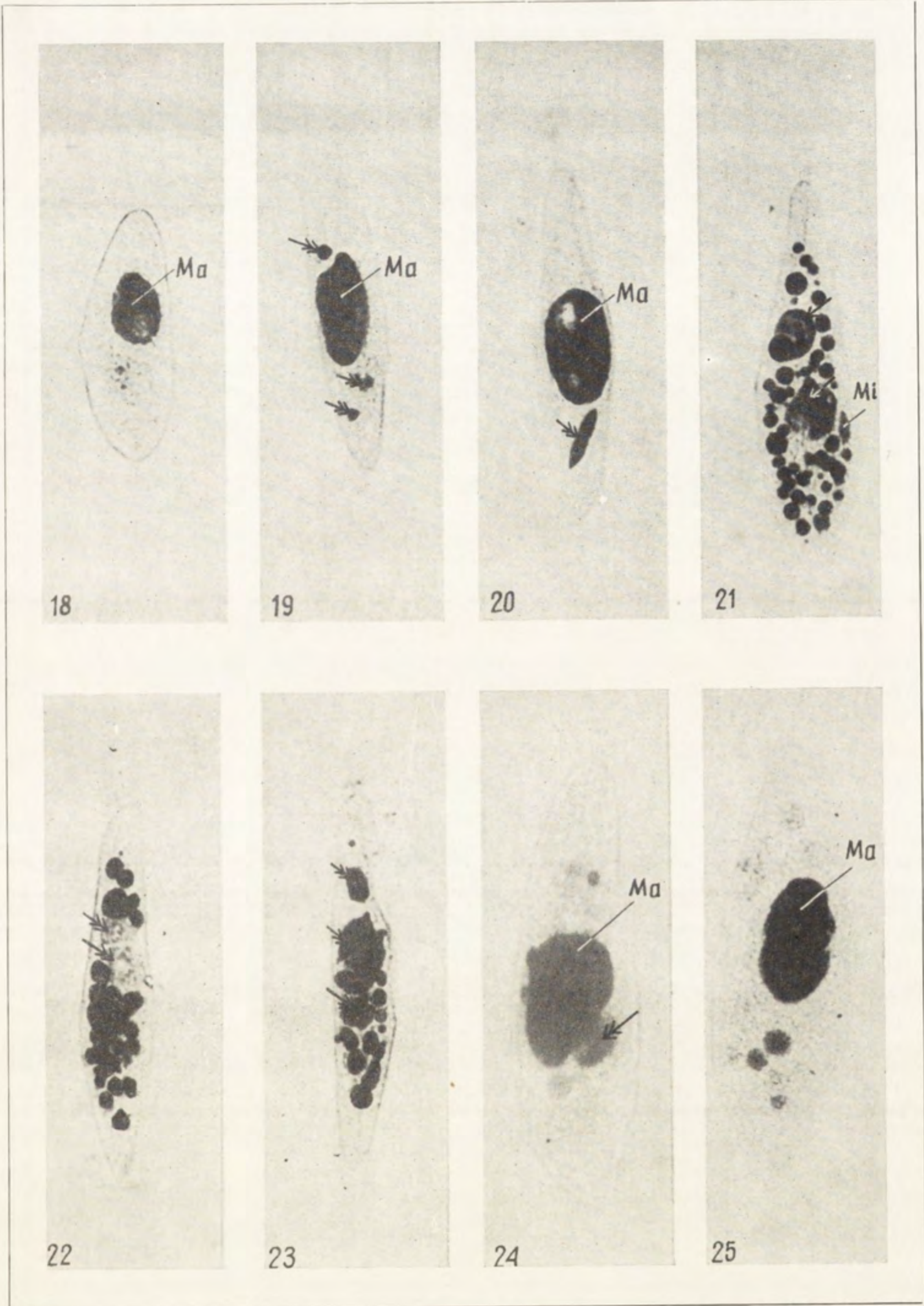
http://rcin.org.pl





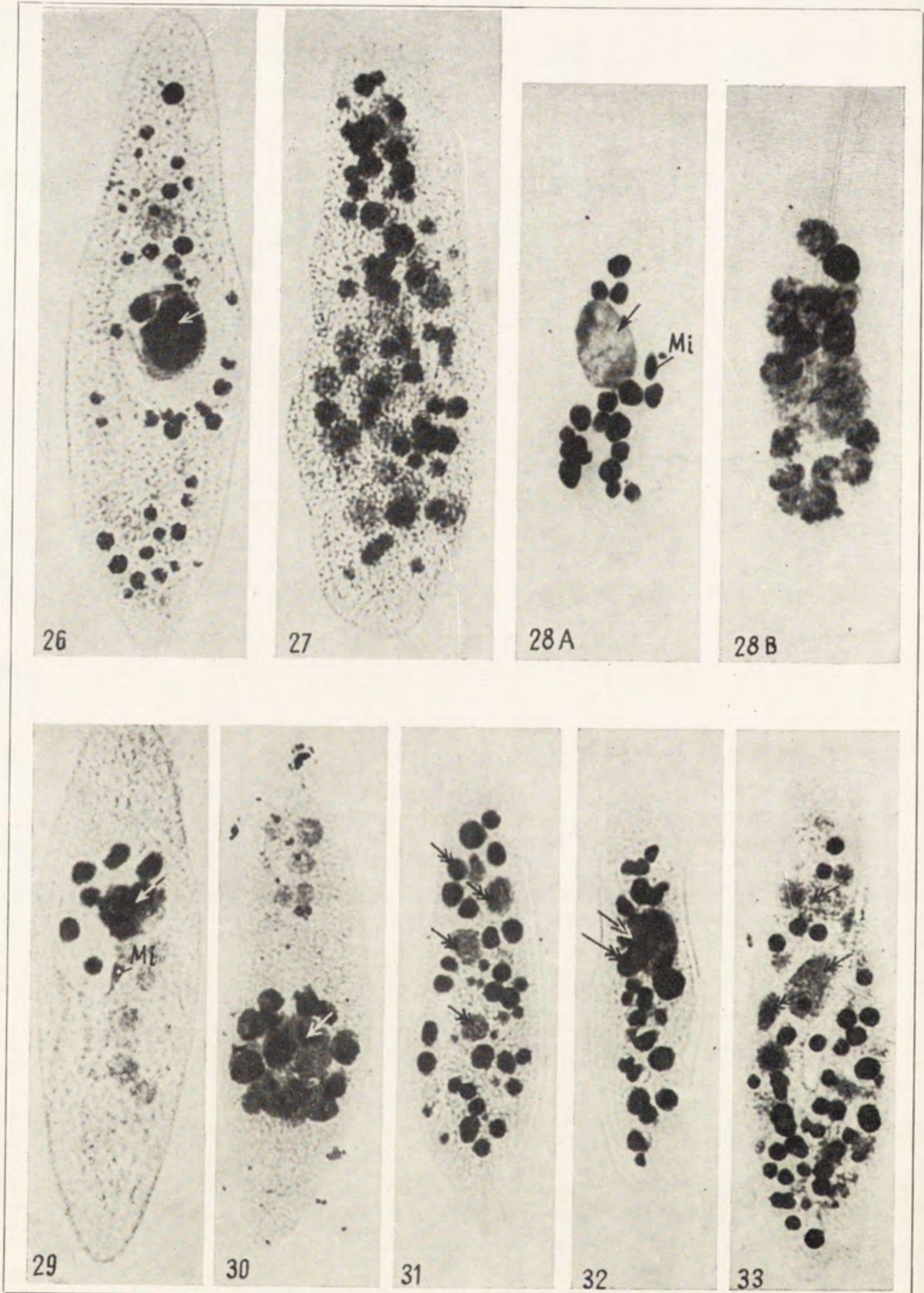
D. V. Ossipov et I. I. Skoblo auctores phot.

http://rcin.org.pl



D. V. Ossipov et I. I. Skoblo auctores phot

http://rcin.org.pl



D. V. Ossipov et I. I. Skoblo auctores phot

http://rcin.org.pl

EXPLANATION OF PLATES I—VI

Nuclear reorganization in ex-autogamonts of *Paramecium caudatum* Ehrbg. Feulgen reaction. Total preparations. Lens 10×, eye piece 20×

1—7: Ex-autogamonts prior to the first metagamic division, various number of Ma primordia (arrows), old Ma fragments. 1 — one Ma primordium, 2 — two Ma primordia, 3 — three Ma primordia, 4 — four Ma primordia, 5 — five Ma primordia, 6 — six Ma primordia, 7 — ten Ma primordia

8—12: Pairs of the daughter cells after the first metagamic division of ex-autogamonts with various number of primordia

8 A, B: Offsprings of ex-autogamont with one primordium, 8 A — cell with one Ma primordium (arrow), 8 B — cell with fragments of old Ma only

9 A, B: Offsprings of ex-autogamont with 2 Ma primordia, in each cell one Ma primordium (arrows), in cell 9 A micronucleus is distinctly seen

10 A, B: Offsprings of ex-autogamont with 4 Ma primordia, in each cell 2 Ma primordia (arrows)

11 A, B: Offsprings of ex-autogamont with 7 Ma primordia, in the cell 11 A three Ma primordia (arrows), in the cell 11 B four primordia (arrows), in every cell the micronucleus is seen

12 A, B: Offsprings of ex-autogamont with 10 Ma primordia, in every cell 5 Ma primordia are present (arrows)

13, 14: Ex-autogamonts containing no Ma primordia prior to the first metagamic division

15 A, B: Two daughter cells after the first metagamic division of ex-autogamont with no Ma primordia, a part of the old Ma fragments become small, others increase in size

16 A—D: Four daughter cells after the second metagamic division of ex-autogamonts with no Ma primordia, some fragments of the old Ma increase in size considerably, the others undergo pycnosis

17 A—H: Eight daughter cells after the second metagamic division of ex-autogamont not containing Ma primordia, the remaining old Ma fragments become the definitive Ma (in cytoplasm numerous spherical structures with indistinct outline — food vacuoles)

18—20: Ex-autogamonts at a stage prior to the first metagamic division, old Ma not fragmented, cytoplasm fully free of food vacuoles, in cells 19 and 20 pycnotic bodies are seen (double arrows)

21—23: Ex-autogamonts at a stage prior to the first metagamic division, old Ma fragmented normally, in every cell Ma primordia are seen, in the cell 21 — two normal Ma primordia (arrows), in the cell 22 — two Ma primordia at various stages of pycnosis (double arrows), in the cell 23 — three pycnotic Ma primordia (double arrows), cytoplasm free of food vacuoles

24—25: Ex-autogamonts at a stage prior to first metagamic division, old Ma not fragmented, in cytoplasm several pycnotic bodies (double arrows) and food vacuoles

26—27: Ex-autogamonts of giant dimensions at a stage prior to the first metagamic division, old Ma fragmented normally, in the cell 26 one Ma primordium (arrow), in the cell 27 primordia fail to differ from the food vacuoles

28 A, B: Daughter cells after the first metagamic division of ex-autogamont with two Ma primordia, in each cell one Ma primordium (arrows), dimensions and structure of the old Ma fragments in daughter cells are various

29: Cell after the second metagamic division of ex-autogamont containing four Ma primordia, in cytoplasm one developing Ma primordium (arrow), one micronucleus and several fragments of old Ma disposition of fragments around Ma primordium is characteristic

30: Ex-autogamonts prior to the first metagamic division, in cytoplasm one Ma primordium (arrow), surrounded by fragments of the old Ma

31—33: Ex-autogamonts prior to the first metagamic division, old Ma normally fragmented, Ma primordia are at various stages of pycnosis (double arrows)

http://rcin.org.pl

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 4

Department of Zoology, University of Delhi-7, and Department of Zoology, Central College, Bangalore, India

B. R. SESHACHAR and A. R. KASTURI BAI

Conjugation in *Spirostomum ambiguum* Ehrbg.

Konjugation von *Spirostomum ambiguum* Ehrbg.

Conjugation in *Spirostomum ambiguum* is very rare. Few accounts of it exist. The last one, to our knowledge, is that by Bishop as long ago as in 1923. We have had strains of this species for some years and have observed the process occasionally. And every time, it has been in the form of an epidemic with as many as 40% of the animals conjugating. It has not been possible clearly to determine the conditions under which conjugation occurs: it has been sporadic and unpredictable. However, its occurrence was made use of to study the cytological changes during conjugation and these are reported here.

Material and methods

As soon as conjugation was observed, the conjugating pairs were isolated and kept under observation. Some of them were fixed at regular and fixed intervals of time. The fixatives used were Carnoy's and Schaudinn's fluids.

Table 1

Days	Events
0	Conjugants become attached to each other
1	Ma breakdown
	1st and 2nd Mi divisions
2	3rd division
3	Fusion of pronuclei
	Separation of conjugants
4	Post-zygotic divisions
5	„Bag-of-Beads" stage of Ma anlagen
	Elongation of Ma anlagen and appearance of nucleoli
6	Fusion of Ma anlagen
7	Attainment of vegetative condition
8	Prefission condensation of Ma followed by fission

http://rcin.org.pl

Fixation was done on slides. The stains used were haematoxylin, toluidine blue, pyronin-methyl green, Feulgen and aceto-carmine. Soon after the conjugating animals separated, they were picked up and were fixed at fixed intervals and stained. It was thus possible to determine the time sequence of the changes taking place in the conjugating animals as well as the exconjugants. This is given in Table 1.

Observations

There is no agglutination of animals preparatory to conjugation. However, two animals which will ultimately conjugate, isolate themselves, keep moving together, twist and turn about each other for some time before they become attached at the anterior end. This initial attachment later extends over a greater part of the length of the animals (Pl. I 1).

The first visible changes in the nuclear apparatus occur in the macronuclear chain which breaks and leaves the individual nodes dispersed in the cytoplasm. The large number of micronuclei which also are scattered in the cytoplasm start their first division. They divide synchronously (Pl. I 2). A few micronuclei however, do not divide.

The second pregamic division follows soon after and is characterized by an aggregation together of division figures in one area near the region of fusion of the conjugants (Pl. I 3).

The third division affects only one nucleus (Pl. I 4). It occurs in a special region of each conjugant sharply marked off from the rest of the cytoplasm by its intense basophilia, specially clear in toluidine blue preparations as well as those stained in haematoxylin and carmine (Pl. I 5). It is a hemispherical area applied to the contact membrane and very characteristically seen in the conjugants where the third division is in progress (Pl. I 6). It first appears soon after the second division as a clearly marked zone running across the conjugant at the level of the future fusion point and later shrinks and consolidates into a special region on either side of the contact membrane in which one nucleus comes to be included.

The plane of this third division figure is at right angles to the line of fusion of conjugants and the daughter nucleus nearer the line in each case is the migrating pronucleus (Pl. II 7). Moving towards the line of fusion, each nucleus enters a small protuberance on the surface, the equivalent of the paroral cone of *Paramecium*. The two cones appear to be placed dorsoventral to each other and occur as outpushings of the pellicle. The cytoplasmic bridge is established and the nuclei migrate into opposite partners. The bridge is of short duration and the gap is healed soon after. Fusion of pronuclei occurs and the conjugants then separate.

The synkaryon in each exconjugant undergoes the first postzygotic mitosis. The two resulting nuclei already exhibit differences. The second postzygotic division starts in both but while one of them goes through mitosis, the other lags in metaphase. A very characteristic picture at this stage is the long telophase figure of one lying alongside the metaphase figure of the other (Pl. II 8). Another division quickly follows in the former, resulting in 4 nuclei. These become the four micronuclei. The lagging division is also eventually completed and the two products become the macronuclei. The exconjugant

http://rcin.org.pl

now possesses four micronuclei and two macronuclear anlagen. Occasionally we have noticed in some exconjugants 3 or even 4 macronuclear anlagen instead of two. This however is extremely rare. There are also the fragments of the old macronucleus in various stages of degeneration.

The later history of the macronuclear anlagen is interesting. Each anlage enlarges and accumulates within its membrane an increasing number of spherical bodies, each of the size of a micronucleus. At first few, these Feulgen positive bodies increase in size until it appears at this stage like a bag of beads (Pl. II 9). We have counted more than 200 bodies in some anlagen.

However, soon, the two anlagen lose this characteristic "bag-of-beads" appearance. Each bead appears to give off fine filaments which become intercalated with filaments from other beads. But these are light microscope observations and it is obvious that the light microscope can hardly be regarded as an adequate instrument to determine the changes taking place inside the anlage.

Other changes follow soon. The two macronuclear anlagen become elongated and nucleoli appear (Pl. II 10). They approach each other and accommodate themselves in interesting positions and relationships (Pl. II 11). Fusion takes place between them and a single macronuclear body results. This becomes elongated and pinched off into beads (Pl. II 12). At first the beads are few, but they increase in number until, by the time the first fission occurs, the macronucleus has attained the form characteristic of that of the vegetative animal.

Discussion

There are two points in this narrative of conjugation in *Spirostomum*, which appear to be of some interest. The first concerns the special region in each conjugant where the third pregamic division takes place. In all accounts of conjugation in ciliates, the choice of one nucleus among many for the third and final division has been a matter of interest. In *Paramecium*, Sonneborn 1951 felt that the cytoplasm in the paroral region was in some manner differentiated and the one nucleus that happened to be included in this area was able to pass through the third division. He said, "this region of the cytoplasm probably differs from the rest in a way decisive for nuclear survival" (p. 491), and cited this as an instance of the action of cytoplasm on the nucleus. Similar observations were made by Nanney 1953 who, in *Tetrahymena*, reported that one of the products of the second pregamic division in each conjugant attaches to the contact membrane anteriorly and it is this nucleus that is able to pass through the third division. Here again, it was a specific cytoplasm location of one nucleus that caused it to divide and a cytoplasmic influence on the nucleus for the initiation of division was postulated, though no visible proof for it was provided. To our knowledge such a detectable differentiation of the cytoplasm has not been reported in any ciliate.

In *Spirostomum* we have found a demonstrable difference. In the early stages of conjugation, the cytoplasm of the conjugant shows no differentiation. Towards the end of the second pregamic division, a dark zone exhibi-

http://rcin.org.pl

ting intense basophilia appears acrcss each conjugant. This zone gradually shrinks and is drawn towards the contact membranes of the conjugants as a highly granular, intensely basophilic region sharply marked off from the rest of the cytoplasm. In the centre of this region is the single nucleus which goes through mitosis to produce the two gametic nuclei. The intense basophilia would lead us to believe that an accumulation of RNA occurs here, but other possibilities exist.

It does not seem possible to say at present how this region exerts its influence on the nucleus included in it. It would largely depend on the nature of the accumulated substance. It would also depend on the role it is to perform. It is clear that this specialised region of the cytoplasm should discharge either or both functions: (a) help initiate mitosis in the one nucleus included in it, and (b) afford protection to this nucleus from the cytoplasm outside the area where the relic nuclei are absorbed. We are examining these aspects of the problem.

The second feature on which we wish to comment refers to the appearance and accumulation of beadlike structures in the early macronuclear anlagen of *Spirostomum*. These are discrete, Feulgen positive bodies of more or less uniform size, strikingly similar to micronuclei. It will be recalled that the structure of the ciliate macronucleus in relation to its origin and function has been a challenging problem and many attempts have been made to solve it. Its large size has been accounted for on the basis of its being compound, made of many subnuclei (Sonneborn 1947) or merely polyploid (Kimball 1953). Measurements of its DNA content have been made in several ciliates and amounts varying from 40 to 2500 times as much DNA as in the micronucleus have been reported. A recent report in *Spirostomum* (Ovchinnikova et al. 1965) puts it at the enormous figure of 13 150 n. This is not surprising since the macronucleus of this protozoan is very large as compared with the small micronucleus.

But the arrangement of this large amount of DNA in the macronucleus is not clear. Chromosomes of the conventional form have been reported in the early development of the macronucleus in several ciliates but how they become organized later to constitute the vegetative macronucleus is not known. Recently Grell 1949 has developed the view that the chromosomal complex is in the form of a long greatly coiled compound chromosome (Sammelchromosomen), at least in its early stages of development. Grell is not clear how the chromosomes are organized in the vegetative macronucleus. Meanwhile, reports of polytene-like chromosomes in the early macronucleus of some hypotrichous ciliates have come in (Silva and Alonso 1966) but even here, the appearance and arrangement of chromosomes in later stages are not known. Recent electron microscope studies of *Spirostomum* macronucleus have shown that in this animal (Seshachar 1965) as well as in the allied *Blepharisma* (Seshachar 1964) the chromosomes are in the form of greatly convoluted and extremely long filaments.

Whatever the nature of chromosomes in the macronucleus, that they have been derived from those of the micronucleus is clear and the interest in the present findings is that the initial stages of development of the macronucleus of *Spirostomum* consist of a multiplication of micronuclei which remain as distinct bodies within it. The manner in which the micronuclei multiply in number is at present obscure.

http://rcin.org.pl

Summary

Conjugation in *Spirostomum ambiguum* is described. Among the first changes is the breakdown of the macronuclear chain. The micronuclei go through three pregamic divisions; the third occurs only in one nucleus which is placed in the centre of a highly basophilic area close to the fusion zone in each conjugant. Syngamy results in a synkaryon in each conjugant which, by the first post-zygotic mitosis, produces two nuclei. One of these two divides twice to give rise to four micronuclei and the other only once to form the two macronuclear anlagen. The macronuclear anlagen accumulate a large number of, as many as 200, Feulgen positive bodies. Later, they disappear and a mass of fine filaments are produced. Nucleoli appear. The two anlagen fuse to give rise to the macronucleus of the adult vegetative animal.

ZUSAMMENFASSUNG

Es wird die Konjugation von *Spirostomum ambiguum* beschrieben. Am Anfang beobachtet man den Zerfall der Makronuklearkette. Die Mikronuklei unterliegen drei pregamischen Teilungen, wobei die dritte nur in einem Kern stattfindet; dieser befindet sich in der Mitte einer stark basophieler Zone, dicht an der Verschmelzungszone in jedem Konjuganten. In der Folge der Syngamie entsteht ein Synkaryon in jedem Konjuganten, der bei der ersten postzygotischer Mitose, zwei Kerne erzeugt. Einer von diesen macht zwei Teilungen durch, und erzeugt auf diesem Wege vier Mikronuklei, der andere, sich nur ein Mal teilend, erzeugt zwei Makronukleusanlagen. In diesen werden viele, bis 200, Feulgen positive Körperchen angehäuft. Später verschwinden sie, und es wird eine Menge von feinen Filamenten erzeugt. Die Nukleoli erscheinen. Die beide Anlagen verschmelzen miteinander und erzeugen den Makronukleus des reifen, vegetativen Tieres.

REFERENCES

Bishop A. 1923: Some observations upon *Spirostomum ambiguum*. Quart. J. micr. Sci., 67, 391—434.

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

Kimball R. F. 1953: The structure of macronucleus of *Paramecium aurelia*. Proc. nat. Acad. Sci. U.S.A., 39, 345—347

Nanney D. L. 1953: Nucleocytoplasmic interactions during conjugation in *Tetrahymena*. Biol. Bull., 105, 115—139.

Ovchinnikova L. P., Selivanova G. V. and Cheissin E. M. 1965: Photometric study of DNA content in the nuclei of *Spirostomum ambiguum* (*Ciliata, Heterotricha*). Acta Protozool., 3, 69—78.

Silva J. P. and Alonso P. 1966: Demonstration of polytene chromosomes in the macronuclear anlage of Oxytrichous ciliates. Arch. Protistenk., 109, 65—70.

Seshachar B. R. 1964: Observations on the fine structure of the nuclear apparatus of *Blepharisma intermedium* Bhandary (*Ciliata: Spirotricha*). J. Protozool., 11, 402—409.

Seshachar B. R. 1965: The fine structure of nuclear apparatus and the chromosomes of *Spirostomum ambiguum* Ehrbg. Acta Protozool., 3, 337—343.

Sonneborn T. M. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Adv. Genet., 1, 264—358.

http://rcin.org.pl

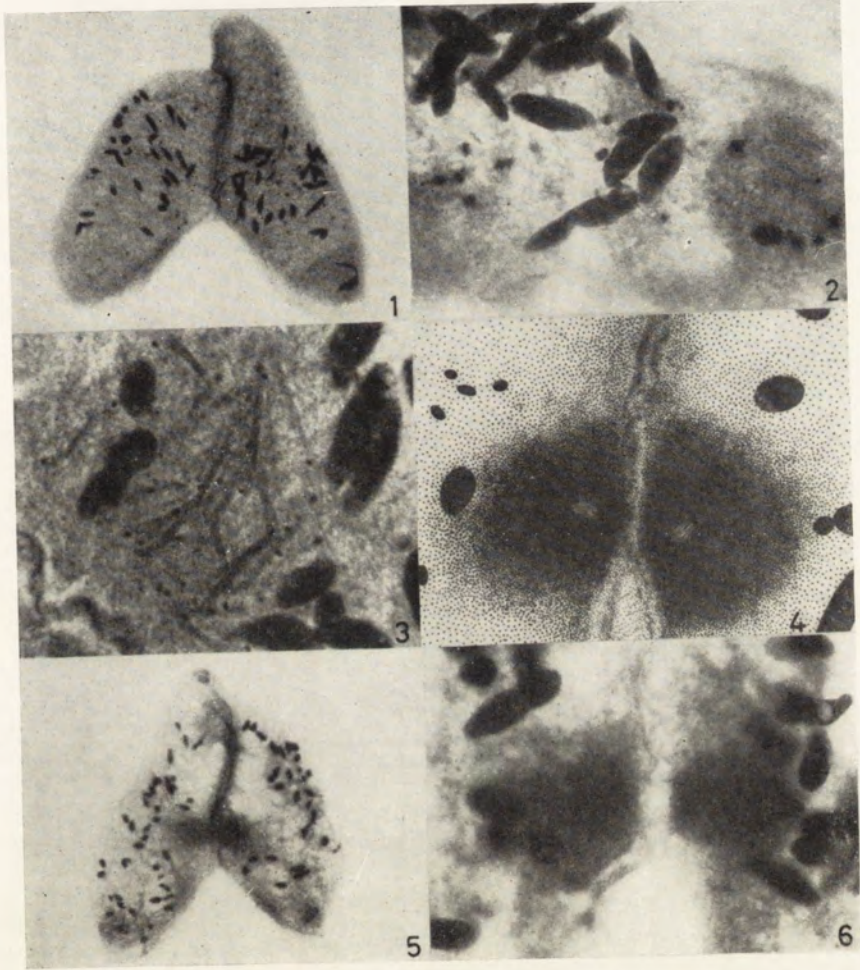
Sonneborn T. M. 1951: Some current problems of genetics in the light of investigations in *Chlamydomonas* and *Paramecium*. Cold spring Harb. Symp. quant. Biol., 11, 483 –503.

EXPLANATION OF PLATES I—II

Conjugation in *Spirostomum ambiguum* Ehrbg.

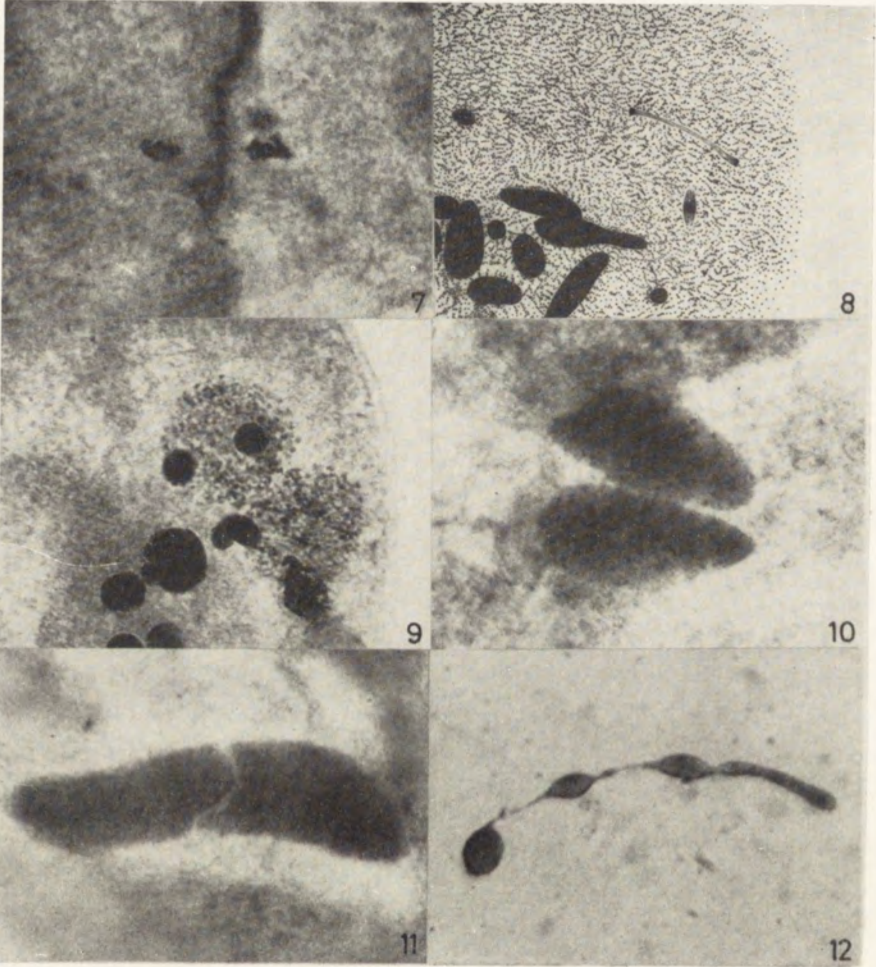
1: Early stage in conjugation showing two animals attached to each other. The macronucleus has broken up into its component beads. A number of micronuclei are visible (Acetocarmine) — 150×
2: Enlarged view of one of the conjugants showing macronuclear fragments and synchronously dividing micronuclei in first pregamic division (Acetocarmine) — 500×
3: Micronuclei in telophase of second pregamic division. They tend to aggregate in one small area in each conjugant close to the fusion line (Acetocarmine) — 500×
4: Telophase of third pregamic division. Only one nucleus passes through the third division and this is in the centre of a highly basophilic area on either side of the fusion line — 1500×
5: Conjugating animals showing basophilic area (Acetocarmine) — 150×
6: The basophilic area at higher magnification. The nuclei inside are not visible on account of intense basophilia (Acetocarmine) — 500×
7: Migratory pronuclei near the fusion line. Note their polymorphic nature (Acetocarmine) — 1300×
8: Exconjugant showing two nuclei in second post-zygotic division. One nucleus is in metaphase and the other in telophase — 1500×
9: Exconjugant with two macronuclear anlagen in "bag of beads" stage (Feulgen) — 500×
10: Macronuclear anlagen: later stage showing nucleoli (Haematoxylin) — 500×
11: Fusion of macronuclear anlagen (Haematoxylin) — 500×
12: Early stage in the formation of vegetative macronucleus (Feulgen) — 500×

http://rcin.org.pl



B. R. Seshachar et A. R. Kasturi Bai auctores phot.

http://rcin.org.pl



B. R. Seshachar et A. R. Kasturi Bai auctores phot.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 5

Institut Zoologique de l'Université Jagiellone, Kraków, Krupnicza 50, Pologne

Anna CZAPIK

Remarques sur *Deltopylum rhabdoides* Fauré-Frémiet et Mugard *(Ciliata, Hymenostomata)*[1]

Uwagi o *Deltopylum rhabdoides* Fauré-Frémiet et Mugard (*Ciliata, Hymenostomata*)

Deltopylum rhabdoides, un histophage appartenant à la famille *Tetrahymenidae* a été décrit par Fauré-Frémiet et Mugard 1946 qui ont crée un genre nouveau pour cet animal. Sa morphogénèse a été étudiée par Mugard et Lorsignol 1956 qui ont constaté que le macronucleus subit de remarquables changements au cours de la division. Dès ce temps cette espèce n'a été retrouvée nulle part ce qui prouve qu'elle n'est pas fréquente. Je l'ai trouvée en novembre dans une petite rivière aux environs de Cracovie. Comme les circonstances dans lesquelles *Deltopylum rhabdoides* a été rencontrée par ses auteurs étaient semblables (dans une rivière en hiver) on peut supposer que cet animal préfère des temperatures basses. Il se laisse cultiver très facilement sur des tissus frais (je le nourrissais d'Enchytraeides).

Pour faire les préparations j'ai appliqué la méthode de Chatton modifiée par Corliss. Comme cette espèce est difficile à imprégner je me suis servie d'une solution plus forte (10%) de nitrate d'argent dans laquelle je laissais les lames pendant 2—3 heures.

Comme l'aspect général de l'animal, sa ciliature somatique ainsi que son cycle vital et sa division correspondent à la description des auteurs, ils n'ont pas besoin d'être redécrits ici. Cependant en ce qui concerne la structure de la bouche je veux y ajouter quelques détails. Le péristome a une forme plutôt ovale que triangulaire (Pl. I 1). Chez un théronte mesurant 120 μ de long la bouche a 27×6 μ. La membrane vibratile (UM) contourne le côté droit de la bouche formant un faible arc. Les trois membranelles situées parallèment à la bouche inclinent légérement leurs extrémités antérieures vers le côté droit. M_1 et M_2 sont de la même longueur, M_3 est plus courte. Chaque membranelle consiste de deux rangées de cinétosomes.

Les divisions ont lieu dans un rythme suivant: le théronte devient tomonte 12 heures après avoir trouvé la nourriture et subit sa première division. Après quelques heures une deuxième division s'accomplit et à leur tour les petits animaux deviennent thérontes au bout de 24 heures. Ainsi en 24 heures le

[1] La présente note n'est qu'un supplément à mon ouvrage sur *Tetrahymenidae* (Czapik 1968) et concerne une espèce qui n'a été trouvée qu'après l'envoi du manuscript à l'imprimerie.

http://rcin.org.pl

nombre des animaux se quadruple. Une seul fois j'ai observé une conjugaison entre des individus formés après la deuxième division.

Deltopylum rhabdoides ne forme pas de kystes; les individus privés de nourriture meurent.

Resumé

L'auteur rapporte avoir trouvé le cilié *Deltopylum rhabdoides* Fauré-Frémiet et Mugard dans une petite rivière près de Cracovie. Le travail contient une description de la ciliature orale en particulier, ainsi que certains détails concernant la division.

STRESZCZENIE

Autorka donosi o znalezieniu orzęska *Deltopylum rhabdoides* Fauré-Frémiet et Mugard w niewielkiej rzeczce w okolicach Krakowa. Zostały podane: opis urzęsienia gębowego oraz pewne szczegóły dotyczące podziału.

BIBLIOGRAPHIE

Czapik A. 1968: La famille *Tetrahymenidae* et son importance dans la systématique et l'évolution des Ciliés. Acta Protozool., 5, 315—367.

Fauré-Frémiet E. et Mugard H. 1946: Sur un infusoire holotriche histophage *Deltopylum rhabdoides* n. gen., n. sp. Bull. Soc. zool. Fr., 71, 161—164.

Mugard H. et Lorsignol L. 1956: Étude de la division et la regénération chez deux *Ophryoglenidae* d'eau douce: *Ophryoglena pectans* et *Deltopylum rhabdoides*. Bull. biol. Fr. Belg., 90, 446—464.

EXPLICATION DE PLANCHE I

1: *Deltopylum rhabdoides* Fauré-Frémiet et Mugard, l'aspect general

http://rcin.org.pl

A. Czapik auctor phot.

http://rcin.org.pl

http://rcin.org.pl

ACTA PROTOZOOLOGICA

VOL. VI WARSZAWA, 31.VIII.1968 FASC. 6

Laboratory of Biological Control, Institute of Plant Protection, Grunwaldzka 189, Poznań 8, Poland

Jerzy J. LIPA

Some observations on flagellate parasites of hemipterans *Corimelaena, Euschistus, Gerris, Leptocoris* and *Oncopeltus* in the United States

Obserwacje nad wiciowcami pasożytującymi w pluskwiakach *Corimelaena, Euschistus, Gerris, Leptocoris* i *Oncopeltus* w Stanach Zjednoczonych

Flagellates are rather frequently observed in the organism of insects belonging to the suborder *Hemiptera*. Although they inhabit the gut or live in the body cavity, only some species can be regarded as parasites and a great majority of them seem to be simply commensals (Lipa 1963, 1965). However, with the progress of research and use of more precise methods, some new and important data can be obtained as to their effect on insect hosts.

While working in 1958—59 under the fellowship of the Rockefeller Foundation, at the Division of Invertebrate Pathology, University of California at Berkeley, I had the opportunity to collect some material and to conduct some studies on flagellates of hemipterans. The results of these studies, completed at our laboratory in Poznań, are included in this paper.

Sincere thanks are expressed to Professor E. A. Steinhaus, Dean of Biological Sciences, University of California, Irvine, USA, for allowing me to use his material for this study.

Materials and methods

Ten insect species were collected in various places in California and the eleventh *Leptocoris trivitattus* was collected at Salt Lake City, Utah. The list of examined insects belonging to nine families is as follows:

Aradidae: Mezira sp.
Coreidae: Anasa tristis (DeGeer)
Chelinidea vittiger Uhler
Gerridae: Gerris remigis Say
Lygaeidae: Oncopeltus fasciatus Dallas
Corizidae: Leptocoris trivitattus Say
Neididae: Acanthophysa echinata Uhler
Pentatomidae: Corimelaena extensa Uhler
Euschistus conspersus Uhler
Pyrrhocoridiae: Europhthalmus convivus (Stal)

http://rcin.org.pl

Specimens of *Oncopeltus fasciatus, Gerris remigis* and *Leptocoris trivitattus* were collected by the author. Other insects were examined as smeared preparations kindly given to me by Professor E. A. Steinhaus, University of California, Irvine (formerly Berkeley Campus). These slides were previously used for studies on symbionts of insects and were already fixed and stained.

The specimens of insects collected by the author were dissected and salivary glands and hemolymph were checked on flagellates.

The morphology of flagellates was studied on smeared preparations stained with 1% Giemsa's solution for 24 hours.

Results

Out of ten examined hemipterans five were found to be hosts for various flagellates (Table 1).

Table 1

Type of material and number of examined and infected specimens of *Hemiptera* harbouring flagellates

Insect	Number of specimens		Flagellate
	examined	infected	
Corimelaena extensa	slides	+	*Crithidia corimelaenae* sp.n.
Euschistus conspersus	slides	+	*Crithidia euschisti* sp.n.
Gerris remigis	3	2	*Blastocrithidia gerridis* (Patton)
Leptocoris trivitattus	6	2	*Blastocrithidia leptocoridis* (McCulloch)
Oncopeltus fasciatus	24	3	*Leptomonas oncopelti* Noguchi et Tilden

1. *Blastocrithidia leptocoridis* (McCulloch) comb. nov.

Synonyms: *Crithidia leptocoridis* McCulloch, 1915, *Herpetomonas leptocoridis* (McCulloch) Kay, 1942.
Host insect: *Leptocoris trivitattus* Say.
Habitat: alimentary tract.
Locality record: Salt Lake City, December 3, 1958.
Infection level: Out of six specimens 3 adults were infected.

Morphology and development

The series of forms observed in the host insect are shown in Figures 1—8. These may be divided into three groups: blastocrithidial (having an undulating membrane), leptomonad and crithidial.

Predominant in the insect gut is the blastocrithidial form having a short undulating membrane (Pl. I 1—2). The form varies in length from 20 to 37 μ (Table 2). The nucleus is oval or slightly elongate, 1—2 μ in diameter, and is located in the middle of the body. The kinetoplast is located in the anterior part of the body.

Leptomonad forms are slender (Pl. I 3), while crithidial are short or oval with the kinetoplast located closely to the nucleus, and they have a short flagellum.

http://rcin.org.pl

Table 2

Results of measuring of *Blastocrithidia leptocoridis*

Form	1	2	3	4	5	6	7	8	9	10
pe-mn	8.1	6.1	3.5	6.1	15.3	5.1	5.8	13	5	2
mn-k	2.3	4.1	1.6	2.5	3.1	1.5	4.5	5.5	3	3.1
k-tff	15.3	16.3	16.3	10.3	1.9	14.1	21.4	19	25	15.1
lff	8.1	7.1	11.1	4.5	10.2	7.5	8.1	10	18	11
gb	3.1	3.0	3.1	3.1	2.3	4.5	2.3	1.5	2.1	2.9
oal	25.7	26.5	21.4	18.9	37.4	21.7	31.7	31.5	33	20.2

pe-mn — posterior extremity to middle of nucleus
mn-k — middle of nucleus to kinetoplast
k-tff — kinetoplast to tip of free flagellum
lff — length of free flagellum
gb — greatest breadth
oal — over-all length

The most typical type of development is binary fission. It can start from various organellas; from the kinetoplast and flagellum (Pl. I 5) or the nucleus (Pl. I 6). Some forms were without a nucleus (Pl. I 4). There are also cases of multiple fission in which an agregation rosette is produced (Pl. I 8).

Taxonomic position

This flagellate was originally described by McCulloch 1915 as *Crithidia leptocoridis* from *Leptocoris trivitattus*. Kay 1942 transferred this species to the genus *Herpetomonas* as *H. leptocoridis*.

Since the time of McCulloch's and Kay's papers the generic criteria of the insect trypanosomatids have greatly changed (Wallace 1963; Lipa 1963, 1965). At present all forms having a short undulating membrane and infecting insects belong to the genus *Blastocrithidia* Laird, 1959.

In the original description of this flagellate, McCulloch 1915 described and gave drawings of forms with the undulating membrane. In my studies I observed that the blastocrithidial form was predominant. Therefore, I consider that the transfer of the flagellate described by McCulloch, from *Leptocoris trivitattus*, to the genus *Blastocrithidia* as *Blastocrithidia leptocoridis* (McCulloch) comb. nov. is justified.

Host records

The original host insect of *B. leptocoridis* was *Leptocoris trivitattus* collected at the Campus of Kansas State University. The same insect species served as a source of flagellates in the studies of Kay 1942 and reported in this paper. However, I found in Prof. Steinhaus' reprint collection a paper by Bonnestal 1933 in which infected insects were collected in California.

Barber 1955 determined that there were two species of *Leptocoris: L. trivittatus* Say distributed in Eastern United States and *L. rubrolineatus* Barber occuring in California and neighbouring states.

In the light of Barber's paper one must accept that McCulloch's,

http://rcin.org.pl

K a y's and my records of *B. leptocoridis* refer to *L. trivitattus*, while B o n n e s t a l's record refers to *L. rubrolineatus*. Accordingly, *B. leptocoridis* parasitizes two insect species *L. trivittatus* and *L. rubrolineatus*.

Distribution

Recorded from Kansas, California and Utah.

2. *Blastocrithidia gerridis* (Patton)

Synonym: *Crithidia gerridis* Patton, 1908.
Host insect: *Gerris remigis* Say.
Habitat: alimentary tract.
Locality record: Putah creek close to Winters, California, June 7, 1959.
Infection level: Out of two adults and one larva, only one adult was infected.

Morphology and development

The predominant form is blastocrithidial (Pl. II 9—10); these forms are elongated and have a short undulating membrane at the anterior end of the body. The body length is from 15 do 39 μ long and 2.0 to 3.5 μ wide (Table 3). The anterior and posterior ends of the elongated forms are sharply pointed. The nucleus is 1 to 3 μ in diameter and is located in the half of the length of the body or slightly close to the posterior end.

Table 3

Results of measuring of blastocrithidial and leptomonad forms of *Blastocrithidia gerridis*

Form		1	2	3	4	5	6	7	8	9	10
Blastocrithidial	pe-mn	11.2	9.2	15	11.2	10.2	6	9.1	13	16.3	8.1
	mn-k	3.6	3	2.1	2.3	1.5	11	1.5	1.3	1.5	1.1
	k-tff	22.4	20.4	15.3	19.4	29.6	21	16	20.4	11.2	19
	lff	7	15.3	6	14.2	6	7	4	6	5	8.1
	gb	2	2	3	2.6	2.2	31	3.5	3	2.5	2.1
	oal	31.2	32.6	32.4	32.9	41.3	33.5	26.6	32.7	29.0	28.2
Leptomonad	pe-mn	6	7.1	9.1	7.5	9.2	6.1	8.1	4.1		
	mn-k	1	1	1.2	1	1	0.9	1.1	0.8		
	k-tff	14	15.3	15	12	18	14	11	13		
	lff	12	13	12.2	11	16	12.5	9.5	11.5		
	gb	1	1	1.1	1	1	1.1	1.1	1.2		
	oal	21	23.4	25.3	20.5	28.2	21	20.2	17.9		

The kinetoplast is oval or bean-shaped and has 0.5 to 1.0 μ in diameter.

The leptomonad forms are elongated and slender being 6.4 to 12.2 μ long and 0.8 to 1.2 μ wide. The anterior end is rounded and sharply cut. The nucleus is located at one fourth of the body length toward the anterior end. The nucleus is elipsoidal and 2 μ in diameter. The kinetoplast is oval and 0.5 μ in diameter. The free flagellum is 9.5 to 16 μ long.

http://rcin.org.pl

Taxonomic position

This species was originally described by Patton 1908 as *Crithidia gerridis* from the alimentary tract of *Gerris fossarum* Fabr. and later reported from *Gerris* spp. and *Microvelia americana* (Uhler) (Porter 1909, Becker 1923). Laird 1959 created a new genus *Blastocrithidia* to include forms having the undulating membrane and exclusively parasitizing insects. The type species for the new genus was *Blastocrithidia gerridis* (Patton).

The flagellate discovered in *Gerris remigis* in California is identified as *Blastocrithidia gerridis* (Patton) because of their close resemblance.

Host records

Blastocrithidia gerridis was recorded, so far, from *Gerris palustris* L., *G. remigis* Say, *G. marginatus* Say, *G. rufoscutellatus* Latr., and *Microvelia americana* (Uhler).

Distribution

B. gerridis was reported from India, England, Canada and United States (New York and Maryland). This is the first record in California.

3. *Crithidia corimelaenae* sp.n.

Host: *Corimelaena extensa* Uhler
Habitat: alimentary tract
Locality record: vicinity of Berkeley, California.

Morphology and development

The predominant form was crithidial (Pl. II 11—12). They measure from 8 to 18 μ (Table 4) and are heart-like in shape with straight cut anterior end

Table 4

Results of measuring of leptomonad and crithidial forms of *Critihidia corimelaenae* sp.n.

Form		1	2	3	4	5	6	7	8	9	10
Crithidial	pe-mn	2.5	2	2	4	2.5	2	2	3	1.5	1.8
	mn-k	2.5	2.6	3	2.5	1.5	2	2.6	1.5	2.5	3
	k-tff	6	5	1	12	15	5	4.5	3.5	12	8
	lff	4.5	3	5	10.5	14	4	1.5	1.5	11	6
	gb	4	2	3	4.5	4	3	4	5	5	3
	cal	11	9.6	12	18.5	19	9	9.1	8	16	12.8
Leptomonad	pe-mn	4	6	6	5	3	3.5	1.2	5	3	4.5
	mn-k	2.1	2	3.5	2.8	3.1	4.1	5	2	2	2
	k-tff	11	16	10	8	15	11	17	13	6	13
	lff	8	12	6	4	11	7	15	10	1.5	9
	gb	1.1	2.5	2	2	2	2	2	2	1	2
	oal	17.1	24	19.5	15.8	21.1	18.6	23.2	20	11	19.5

http://rcin.org.pl

of the body. The flagellum arises from a widely opened reservoir. The nucleus is oval, 3 μ in diameter and the kinetoplast is located closely to the nucleus.

The leptomonad forms are up to 24 μ long. The nucleus is elongate with a width equal to the body length. The maximum length of the free flagellum is 15 μ.

Development is by binary fission.

Taxonomic position

As the predominant form is crithidial and besides only leptomonads occur this flagellate belongs to the genus *Crithidia* as defined by Wallace 1963. This is the first record of flagellate infection of *Corimelaena extensa* and evidently a new flagellate is involved. I propose, therefore, the name *Crithidia corimelaenae* sp.n. for it.

4. *Crithidia euschisti* sp.n.

Host insect: *Euschistus conspersus* Uhler.
Habitat: alimentary tract.
Locality record: vicinity of Berkeley, California.

Morphology and development

The predominant form is crithidial (Pl. II 13, 14). These forms are rather short being up to 20 μ long (Table 5). The nucleus is up to 2 μ in diameter. The kinetoplast is oval or bean-shaped 1×0.5 μ. The flagellum is very short.

Table 5

Results of measuring of critihdial form of *Crithidia euschisti* sp.n.

Form	1	2	3	4	5	6	7	8	9	10
pe-mn	3.2	3	4	5	4	4	3	3	2	3
mn-k	2	3	3	2.5	2.5	2.0	2.5	1.5	2.5	2.9
k-tff	6	5	13	8	6	8	6	6	5	6
lff	3	4	10	5	3.5	5	4	3	3	3
gb	3	2.9	1.2	2	1.3	3	2	1.8	2	3
oal	11.2	11	20	15.5	12.5	14	11.5	10.5	9.5	11.9

The leptomonads are rare and are up to 20 μ long. Incysted forms are very prevalent and are 2 to 3 μ in diameter (Pl. II 14). Their nuclei are dense and stain deeply red, the same applies to the kinetoplast. The flagellum is not seen at that stage.

The development is by binary fission.

Taxonomic position

Steinhaus et al. 1956 was the first to notice flagellate in *Euschistus conspersus* Uhler but did not name it. Hanson and McGhee 1962 described *Blastocrithidia euschisti* from *Euschistus servus* (Say). However, as the flagellate studied by me in *E. conspersus* does not possess the undulating

http://rcin.org.pl

membrane it cannot be identified with *B. euschisti*. Its morphological characters clearly indicate that it belongs to the genus *Crithidia* sensu Wallace 1963. Therefore I propose the name *Crithidia euschisti* sp.n. for it.

The same specific name of *C. euschisti* as of *B. euschisti* is given with purpose in case they were synonymized. Hanson and McGhee 1962 gave rather poor drawings of *B. euschisti* and no photographs were included in their paper which would definitely prove the generic position of the flagellate from *E. servus*.

On the other hand, *Crithidia euschisti* sp.n. is described on a single microscopic slide and therefore it might happen that not all developmental stages were taken under consideration by me in the description of this species.

I hope that further research on flagellates of *Euschistus servus* and *E. conspersus* will bring data indicating whether *Blastocrithidia euschisti* and *Crithidia euschisti* sp.n. are identic or separate species.

5. *Leptomonas oncopelti* (Noguchi et Tilden)

Synonyms: *Herpetomonas oncopelti* Noguchi et Tilden, 1926, *Leptomonas oncopelti* (Noguchi et Tilden) Lwoff et Lwoff, 1931.
Host insect: *Oncopeltus fasciatus* Dallas.
Habitat: salivary glands and alimentary tract.
Locality record: Tracy, California, October 15, 1958.
Infection level: out of 24 examined specimens 3 adults were infected.

Morphology and development

The predominant forms were typical leptomonads (Pl. II 15). They are slender and elongate, being 20 to 54 μ long (Table 6). The kinetoplast is oval or bean shaped 0.5 to 1.0 μ in diameter, and is located in the anterior part of the body. The nucleus is about 2 μ in diameter, and is located in the middle of the body.

Table 6

Results of measuring of *Leptomonas oncopelti*

Form		1	2	3	4	5	6	7	8	9	10
Leptomonad	pe-mn	8	6	7	11	8	7	14	17	7	10
	mn-k	4	3.8	3.5	5	4	3	6	5	3.5	5
	k-tff	20	12	10	18	21	21	24	32	34	35
	lff	17	10	7.5	16	18	19	21	29	31	32
	gb	2	2	2	2	2	2	1.8	3	2	2.1
	oal	32	21.8	20.5	33	33	31	44	54	44.5	50

Incysted or so called leishmanial forms were also observed. However, no crithidial forms were observed.

Taxonomic position

The flagellate was originally described by Noguchi and Tilden 1926 from the milkweed bug *Oncopeltus fasciatus* as *Herpetomonas oncopelti*.

http://rcin.org.pl

Later Lwoff et Lwoff 1931 designated the species as *Leptomons oncopelti* (Noguchi et Tilden). However, McGhee and Hanson 1962 stated that "The forms figured by the latter authors do not confirm with those figured by Noguchi and Tilden 1926 as occuring in the hemipteran gut and which were obviously leptomonads". In their later paper, McGhee and Hanson 1963 mention *Crithidia (Strigomonas) oncopelti* Lwoff et Lwoff, 1931. Besides, *O. fasciatus* is suspected as vector of *Phytomonas elmassiani* Migone, a flagellate very similar to *Leptomonas* but living in the sap of milkweeds e.g. *Asclepiadaceae* (Holmes 1925).

Summarizing, there are three flagellate species reported from *Oncopeltus fasciatus*, namely *Leptomonas oncopelti*, *Crithidia (Strigomonas) oncopelti* and *Phytomonas elmassiani*. Therefore, it would be very useful to make a detailed study on the relationship of all three species.

As in the material collected by me I have observed only leptomonad forms in the gut and salivary glands of *Oncopeltus fasciatus* I determine the observed flagellate as *Leptomonas oncopelti* (Noguchi et Tilden).

Discussion

Flagellates of the family *Trypanosomatidae* are rather common inhabitants of the gut of hemipterans and the number of strictly entomophilic species reported in literature is close to fifty species. Unfortunately we do not know how many of them are really valid species.

As seen in this paper and pointed out by Lipa 1963, 1965 many previously recognized species as *Herpetomonas* and *Crithidia* should be now assigned to the genus *Leptomonas* or *Blastocrithidia*. Many of these species are incorrectly described and systematic revision of entomophilic *Trypanosomatidae* seems to be rather urgent and the complex criteria for a new taxonomy proposed by Wallace 1963 should be used.

An extensive survey and studies on entomophilic flagellates are important for many reasons. These flagellates are an useful object for biochemical studies; many flagellates live in insects that serve as vectors; the parasitism of entomophilic flagellates is not clear. These and other questions may be answered only after detailed investigations on this group of protozoans.

Summary

Out of ten examined hemipteran species collected in the USA, the following five were found to be infected with flagellates: *Corimelaena extensa* with *Crithidia corimelaenae* sp.n., *Euschistus conspersus* with *Crithidia euschisti* sp.n., *Gerris remigis* with *Blastocrithidia gerridis* (Patton); *Leptocoris trivittatus* with *Blastocrithidia leptocoridis* (McCulloch); and *Oncopeltus fasciatus* with *Leptomonas oncopelti* (Noguchi et Tilden). The morphology, measurements, development and taxonomic position of the studied species are discussed. Some flagellates are reclassified according to the present taxonomic concept.

http://rcin.org.pl

STRESZCZENIE

Z dziesięciu gatunków pluskwiaków zbieranych w USA pięć było zarażone wiciowcami: *Corimelaena extensa* przez *Crithidia corimelaenae* sp.n., *Euschistus conspersus* przez *Crithidia euschisti* sp.n., *Gerris remigis* przez *Blastocrithidia gerridis* (Patton), *Leptocoris trivitatus* przez *Blastocrithidia leptocoridis* (McCulloch) i *Oncopeltus fasciatus* przez *Leptomonas oncopelti* (Noguchi et Tilden). Omówiono morfologię, wymiary, rozwój i stanowisko systematyczne badanych gatunków. Niektóre gatunki zostały sklasyfikowane zgodnie z aktualnymi poglądami taksonomicznymi.

REFERENCES

Barber H. G. 1956: A new species of *Leptocoris*. The Pan-Pacific Entomol., 31, 9—11.

Becker E. R. 1923: Observations on the morphology and life cycle of *Crithidia gerridis* Patton in the water strider, *Gerris remigis* Say. J. Parasitol., 9, 141—152.

Bonestell A. 1933: Studies on the intestinal parasites of *Leptocoris*. Term paper, unpublished. (Manuscript in Prof. E. A. Steinhaus' reprint Collection, University of California, Irvine, California).

Hanson W. L., McGhee, R. B. 1961: The biology and morphology of *Crithidia acanthocephali* n. sp., *Leptomonas leptoglossi* n. sp., and *Blastocrithidia euschisti* n. sp. J. Protozool., 8, 200—204.

Holmes F. O. 1925: The relation of *Herpetomonas elmassiani* (Migone) to its plant and insect hosts. Biol. Bull., 49, 323—337.

Kay M. W. 1924: A study of *Herpetomonas leptocoridis* (McCulloch) of the alimentary canal of the box elder bug, *Leptocoris trivittatus* (Say). Trans. Am. micr. Soc., 61, 120—130.

Laird M. 1959: *Blastocrithidia* n.g. (*Mastigophora: Protomonadina*) for *Crithidia* (in part), with a subarctic record for *B. gerridis* (Patton) Can. J. Zool., 37, 749—752.

Lipa J. J. 1963: Protozoan infections other than sporozoan. pp. 335—361. In "Insect Pathology: An Advanced Treatise" (E. A. Steinhaus, ed). Academic Press, New York, vol. 2.

Lipa J. J. 1965: Trypanosomes of insects. Przegląd zool., 9, 17—28.

Lwoff M. et Lwoff A. 1931: Recherches sur la morphologie de *Leptomonas oncopelti* Noguchi et Tilden, et *Leptomonas fasciculata* Novy, McNeal et Torrey. Arch. Zool. exp. gen., Notes et Rev., 71, 21—37.

McCulloch I. 1915: An outline of the morphology and life history of *Crithidia leptocoridis*, sp. nov. Univ. Calif. Publ. Zool., 16, 1—22.

McGhee R. B. and Hanson W. L. 1962: Growth and reproduction of *Leptomonas oncopelti* in the milkweed bug, *Oncopeltus fasciatus*. J. Protozool., 9, 488—493.

McGhee R. B. and Hanson W. L. 1963: The relationship of various crithidias as determined by serological reactions. J. Protozool., 10, 239—243.

Noguchi H. and Tilden E. B. 1926: I. Cultivation of herpetomonads from insects and plants. J. exp. Med., 44, 307—325.

Patton W. S. 1908: The life cycle of a species of *Crithidia* parasitic in the intestinal tract of *Gerris fossarum* Fabr. Arch. Protistenk., 12, 131—136.

Porter A. 1909: The morphology and life-history of *Crithidia gerridis*, as found in the British water-bug *Gerris paludum*. Parasitology, 2, 348—366.

Steinhaus E. A., Batey M. M., Boerke C. L. 1956: Bacterial symbiotes from the caeca of certain *Heteroptera*. Hilgardia, 24, 495—518.

Wallace F. G. 1963: Criteria for the differentiation of genera among trypanosomatid parasites of insects. pp. 70—74. Progress in Protozoology, Proc. First int. Congr. Protozool., Prague 1961, Publ. House Czechoslov. Acad. Sci. Prague 1963.

http://rcin.org.pl

EXPLANATION OF PLATES I—II

Blastocrithidia leptocoridis (McCulloch)

1—2: Blastocrithidial forms
3: Leptomonad forms
4: A flagellate without nucleus
5: A flagellate under division with double flagellum
6: A flagellate under division with double nucleus
7: Crithidial and blastocrithidial forms
8: A rosette in multiple division

Blastocrithidia gerridis (Patton)

9—10: Blastocrithidial forms

Crithidia corimelaenae sp. n.

11—12: Crithidial and leptomonad forms

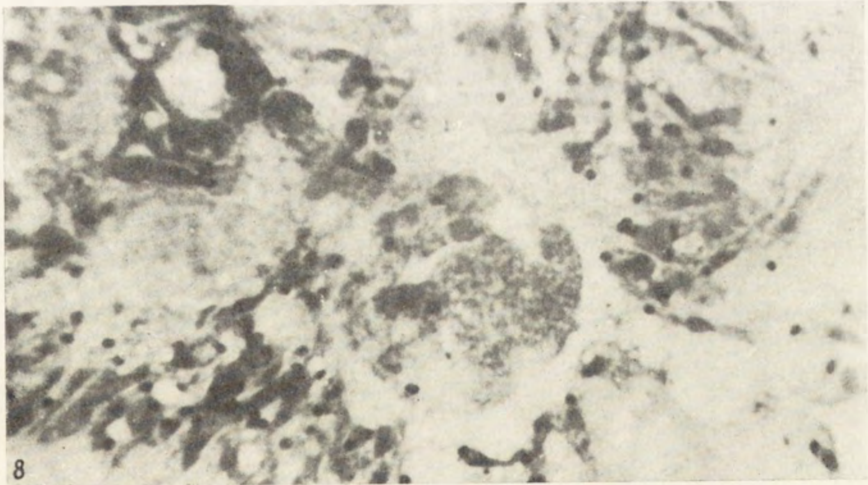
Crithidia euschisti sp. n.

13—14: Crithidial and leishmanial forms

Leptomonas oncopelti (Noguchi et Tilden)

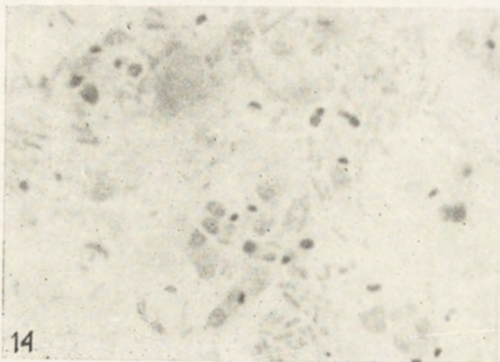
15: Leptomonad forms under division

http://rcin.org.pl



J. J. Lipa auctor phot.

http://rcin.org.pl



J. J. Lipa auctor phot.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 7

Laboratory of Biological Control, Institute of Plant Protection, Grunwaldzka 189, Poznań 8, Poland

Jerzy J. LIPA

Stempellia scolyti (Weiser) comb. nov. and *Nosema scolyti* sp. n. microsporidian parasites of four species of *Scolytus (Coleoptera)*

Mikrosporidia *Stempellia scolyti* (Weiser) comb. nov. i *Nosema scolyti* sp. n. pasożytujące w czterech gatunkach *Scolytus* (*Coleoptera*)

Members of the genus *Scolytus*, (*Coleoptera, Scolytidae*) are considered to be the most serious pests of elm trees (*Ulmus* spp.) all over the world. It is so not only because of their direct damage to trees but mainly because they are vectors (carriers) of the dreadful Dutch elm disease. In fact, this disease caused by the fungus *Ceratocystis ulmi* (Buism.) kills thousands of trees of various *Ulmus* species in Europe, North America and Asia (Leach 1940, Carter 1963, Sauve 1964).

Several species of *Scolytus* are known to be vectors of *C. ulmi*. The most important are *Scolytus scolytus* Fabr., *S. multistriatus* Marsh., *S. pygmaeus* Fabr., *S. ensifer* Eichh. and others. Therefore the study of the mortality factors of these insects is of great practical interest, as it may lead to a biological control program of these pests.

While conducting studies on infections processes caused by *Protozoa* in insects,[1] I found, in 1964, that various species of *Scolytus* are parasitized in Poland with two microsporidian which evidently were new for science. In 1966 I had the opportunity to collect some infected *Scolytus* spp. in the Soviet Union (at Voronezh) and in the German Democratic Republic (at Leipzig).

While preparing results of this study for publication I found that Weiser 1966 mentioned *Plistophora scolyti* Weiser from *Scolytus scolytus* Fabr. in his book "Nemoci hmyzu" (Diseases of Insects). Evidently, having an information from Dr. J. Weiser, Evlachova and Švecova 1965 included in the list of parasites of *S. scolytus* the microsporidian *Plistophora scolyti* Weiser (they misspelled the specific name referring to it as "*scolytii*"). The proper taxonomic description of this species has not been published so far.

I wish to thank some people who offered their generous help in collecting the material during this study: Dr. Jacek Michalski, College of Agriculture,

[1] This investigation was supported in part, by a grant from the United States Department of Agriculture, Agricultural Research Service FG—Po—112—61.

http://rcin.org.pl

Poznań, who also identified insects; Prof. P. A. Położencev, College of Forestry at Voronezh, Soviet Union; and Dr. H. Fankhanel, Director, Deutsches Entomologisches Institut, Eberswalde, German Democratic Republic.

Results

Stempellia scolyti (Weiser) comb. nov.

Synonyms: *Plistophora scolytii* Weiser: Evlachova et Shvecova, 1965, *P. scolyti* Weiser, 1966.
Host insects: *Scolytus ensifer* Eichh., *S. multistriatus* Marsh., *S. pygmaeus* Fabr., *S. scolytus* Fabr.
Locality records: Rogalin (Poznań voivodeship, Poland) 26.XII.1964 and in the following years; Voronezh (Soviet Union) 25.III.1966; Leipzig (German Democratic Republic) 22.IX.1966.
Habitat: intestine, Malpighian tubules, hemocytes.

Development and morphology

Schizogony: Young schizonts are uninucleate and are about 3 μ in diameter (Pl. I 1—3). Their nuclei are deep red and the cytoplasm is deep blue. Older schizonts are binucleate, tetranucleate or more and their size increases significantly, as their maximum size is up to 8 μ in diameter. When stained with Giemsa's solution the cytoplasm of schizonts is uniformly blue.

Sporogony: The mature schizonts undergo sporogony which differs greatly among various schizonts. The twin nuclei of some schizonts divide once and four sporonts are produced which turn into sporoblasts and later into spores. In such case four spores are produced from one sporont (Pl. I 4, II 6). Other sporonts may produce six or eight spores (Pl. I 4, II 6), while still others sixteen or more (Pl. II 5 and 7). This type of sporogony clearly indicates that the investigated species belongs to the genus *Stempellia* which is characterized by producing 2, 4, 8, and 16 or more spores from one sporont.

Spores: The type of sporogony, that is the number of produced spores, has some, but little, effect on the size of spores (Pl. II 5). In case when 16 spores are produced from one sporont, spores are somewhat smaller than when they are produced in a smaller number. These differences, however, are insignificant as seen in Table 1 and in several figures (Pl. II 5, 6, and 7).

All spores are ellipsoidal and occur in groups 2, 4, 8, 16 or more (Pl. II 5, 6, 7). Fresh spores are from 2.7 to 4.0 μ long and from 1.2 to 2.2 μ wide. Stained spores are from 1.5 to 2.5 μ long, and 1.0 to 1.5 μ wide (Table 1). The length of polar filament, extruded by pressure applied to cover glass, is about 35 μ.

Taxonomic position

The type of sporogony clearly indicates that the investigated species belongs to the genus *Stempellia*. Weiser 1966, in his textbook "Nemoci hmyzu" (Diseases of Insects), mentioned *Plistophora scolyti* Weiser from *Scolytus scolytus* and indicated that a taxonomic description was being prepared by him. Evlachova and Švecova 1965 mentioned *Plistophora scolyti* Weiser, evidently having a direct information from Dr. Weiser.

The microsporidian recognized by Weiser as *Plistophora scolyti* in-

http://rcin.org.pl

Table 1

Frequency distribution of the length of spores of *Stempellia scolyti* (Weiser) comb.nov. and *Nosema scolyti* sp.n.

Microsporidian	Dimensionable groups (microns)										
	1.1—1.5	1.6—2.0	2.1—2.5	2.6—3.0	3.1—3.5	3.6—4.0	4.1—4.5	4.6—5.0	5.1—5.5	5.6—6.0	6.1—6.5
Stempellia scolyti (Weiser) fresh spores:			5	33	11	1					
stained spores:	6	29	15								
Nosema scolyti sp.n. fresh spores:						3	52	45	15	1	1
stained spores:				1	33	10	6				

Table 2

A list and characteristic of microsporidians recorded in bark beetles *Scolytidae*

Microsporidian	Host insect	Tissue attacked	Length and width of spores in microns	References
Nosema curvidentis Weiser	*Pityocteines curvidens* Germ.	fat body	2.5—3.6 by 1.5—2.0	Weiser 1961
Nosema scolyti sp.n.	*Scolytus ensifer* Eichh. *S. multistriatus* Marsh. *S. pygmaeus* Fabr. *S. scolytus* Fabr.	midgut, Malpighian tubules, hemocytes	3.6—6.2 by 2.0—3.0	Lipa this paper
Nosema typographi Weiser	*Ips typographus* L.	fat body	3.6—5.3 by 2.0—3.5	Weiser 1955
Stempellia scolyti (Weiser)	*Scolytus ensifer* Eichh. *S. multistriatus* Marsh. *S. pygmaeus* Fabr. *S. scolytus* Fabr.	midgut, hemocytes	2.5—3.6 by 1.1—2.2	Weiser 1966, Lipa this paper

http://rcin.org.pl

fected the intestine of *Scolytus scolytus* and its spores were 3 by 2 μ. Without doubt this microsporidian is identical with the species observed by me in *Scolytus* spp. and identified as *Stempellia*. This view is strongly supported by the data on size of spores and host tissue attacked. Therefore, I propose to transfer the species named by Weiser 1966 to the genus *Stempellia* as *Stempellia scolyti* (Weiser) comb. nov.

Pathogenicity

The gut epithelium is most heavily infected with *Stempellia scolyti*. The damage of the intestine depends on the stage of infection. In the newly infected insects the infection is focal (Pl. II 8, III 9), but it quickly spreads over the whole gut. At advanced infection practically all cells of the midgut and the gut ampules are destroyed by the parasite (Pl. III 10).

Figures 8, 9 and 10 clearly indicate that *Stempellia scolyti* is a highly destructive parasite of various species of *Scolytus*.

It is interesting to notice that mixed infections caused by *Nosema scolyti* sp.n. and *Stempellia scolyti* (Weiser) were frequently noticed (Pl. VIII 23—24).

Distribution

Stempellia scolyti (Weiser) was recorded by Weiser 1966 in Czechoslovakia and by the author in Poland, the Soviet Union, and the German Democratic Republic.

Nosema scolyti sp. n.

Host insects: *Scolytus ensifer* Eichh., *S. multistriatus* Marsh., *S. pygmaeus* Fabr., *S. scolytus* Fabr.
Habitat: Malpighian tubules, intestine, hemocytes.
Locality record: Rogalin (Poznań voivodeship, Poland) 6.XII.1964 and in the following years; Voronezh (Soviet Union) 25.III.1966; Leipzig (German Democratic Republic) 22.IX.1966.

Development and morphology

Schizogony: The schizonts of *Nosema scolyti* sp.n. are distinctivelly larger than those of *Stempellia scolyti* (Weiser). They are oval and are up to 8 μ in diameter (Pl. IV 11 and 12). Their nuclei are deep red while the cytoplasm is deep blue. Quadrinucleate schizonts have been also observed and some of them appear as short chains (Pl. IV 12, VIII 25).

Sporogony: The sporogony is typical of the genus *Nosema* as one spore is produced from each sporont (Pl. IV 12). The latter is slightly elongate and is up to 6 μ long.

Spores: Spores are oval but a few spores have an irregular shape. Fresh spores are: 3.6 to 6.2 μ long and 2.0 to 3.3 μ wide (Pl. IV 12 and 14). Stained spores are 2.7 to 4.0 μ long and 1.8 to 3.2 μ wide (Table 1). As seen in Pl. IV 13, 14, and Pl. V 15, 16 spores of *Nosema scolyti* sp.n. are clearly larger than spores of *Stempellia scolyti* (Weiser).

Taxonomic position

As one spore is produced from each sporont the investigated species is placed among the genus *Nosema*. Therefore this species cannot be identifed

http://rcin.org.pl

with *Stempellia scolyti* (Weiser) as it differs greatly in the type of sporogony, size of spores and host tissue attacked.

There are two *Nosema* species recorded in bark beetles: *Nosema curvidentis* Weiser from *Pityocteines curvidens* Germ. and *Nosema typographi* Weiser from *Ips typographus* L. However, as seen in Table 2 they attack other hosts, different tissues and differ in size of spores. Therefore, I consider that *Nosema* recorded in *Scolytus* spp. is a new species and I propose for it the name *Nosema scolyti* sp.n.

Pathogenicity

Nosema scolyti sp. n. attacks Malpighiam tubules and hemocytes of four species of *Scolytus*. Besides, it is noticed in the gut epithelium. In Pl. IV 11 one can see a number of uni- and binucleate schizonts developing inside the hemocytes. In Pl. IV 12 and VIII 25 schizonts are observed in smeared preparation of gut intestine.

The intensity of infection caused by *Nosema scolyti* sp. n. is very heavy. In many cases Malpighian tubules (Pl. VI 18 and 19) and epithelium and gut ampules (Pl. VII 20 and 22) are completely destroyed by *Nosema scolyti* sp. n. Frequently pseudocysts, that is groups of spores are seen in gut epithelium (Pl. V 17).

It is interesting to notice that mixed infections caused by *Nosema scolyti* sp. n. and *Stempellia scolyti* (Weiser) were frequently noticed (Pl. VIII 23—25).

Distribution

Nosema scolyti sp.n. was recorded first in Poland and later in the Soviet Union, and the German Democratic Republic.

Microsporidians as mortality factors in populations of *Scolytus* spp.

Since the discovery of microsporidian infections among *Scolytus* species in Rogalin, Poland, the level of parasitization was periodically checked to obtain data on the role of various pathogens in the mortality of *Scolytus* spp. As seen in Table 2 the highest parasitization was among populations of *S. multistriatus* and then in *S. ensifer, S. pygmaeus* and *S. scolytus*. The lowest rate of infection in populations of *S. scolytus* is rather a rule and

Table 3

Cumulative results of diagnosis of microsporidian infections caused by *Nosema scolyti* sp.n. and *Stempellia scolyti* (Weiser) in four species of *Scolytus*

Scolytus species	Number of specimens		Per cent of infection
	examined	infected	
S. ensifer Eichh.	39	10	25.6
S. multistriatus Marsh.	427	136	31.8
S. pygmaeus Fabr.	265	49	18.4
S. scolytus Fabr.	249	31	12.4

http://rcin.org.pl

Table 4

Occurrence of microsporidian infestions among *Scolytus* spp. in some localities in the Soviet Union and the German Democractic Republic

Country, locality and date	*Scolytus* species	Number of examined insects	Number of insects infected with	
			Nosema scolyti sp.n.	*Stempellia scolyti* (Weiser)
Soviet Union: Voronezh 25.III.1967	*S. multistriatus* Marsh.	28	6	3
	S. pygmaeus Fabr.	13	—	—
	S. scolytus Fabr.	58	—	—
German Democratic Republic: Leipzig 29.IX.1966	*S. multistriatus* Marsh.	95	11	2
	S. scolytus Fabr.	40	—	—

Table 5

Infection level and mortality of *Scolytus* spp. caused by microsporidians and fungi in Rogalin (January to March of 1965)

Pathogen	*Scolytus multistriatus* Marsh.				*Scolytus pygmaeus* Fabr.				*Scolytus scolytus* Fabr.			
	L	P	I	Total L+P+I	L	P	I	Total L+P+I	L	P	I	Total L+P+I
Nosema scolyti sp.n.	6	—	3	9	1	6	10	17	—	8	5	13
Stempellia scolyti (Weiser)	2	—	17	19	9	—	9	18	5	1	2	8
Microsporidians*	90	3	2	95	12	—	2	14	10	—	—	10
Fungi**	2	—	—	2	1	—	1	2	—	—	—	—
Healthy	139	3	22	164	103	36	74	213	111	24	42	177

Abbreviations: L — larvae; P — pupae; I — imagoes

* At the beginning of this study *Stempellia scolyti* (Weiser) and *Nosema scolyti* sp.n. were regarded as one species and examined insects were diagnosed only as infected with a microsporidian

** The primary fungus observed on larvae, pupae and adults of *Scolytus* spp. was *Beauveria bassiana* (Bals.)

http://rcin.org.pl

specimens of this species examined in other countries (Table 4) were free from infection. As seen in Table 4, in Leipzig *S. scolytus* was healthy while *S. multistriatus* was infected in 14.7%. At Voronezh three species were examined and only *S. multistriatus* was found to be infected. The infection level of *S. multistriatus* at Voronezh was very low: *N. scolyti* sp. n. was found in 21.4% of insects and *S. scolyti* (Weiser) in 10.7% of insects.

From January to March of 1965 a few hundred of various developmental stages of three species of *Scolytus* were collected at Rogalin and examined macro- and microscopically. During that study we recorded microsporidians, fungi and a mite *Pyemotes scolyti* Oud. The quantitative results of this study are given in Table 5. Observations on mite infestation will be published elsewhere.

The data in Table 5 show that microsporidians are more frequent parasites of *S. multistriatus* and *S. pygmaeus* than of *S. scolytus*. This conclusion was already pointed out above when discussing data in Table 3.

Data on the frequency of infection of *Scolytus* spp. with *Stempellia scolyti* (Weiser) and *Nosema scolyti* sp. n. are inconclusive. In Leipzig, as seen in Table 4, *N. scolyti* sp. n. was found in eleven specimens of *S. multistriatus* while *S. scolyti* (Weiser) parasitized only two. In Rogalin (see Table 5) *S. scolyti* (Weiser) was more frequently observed in *S. multistriatus* while *N. scolyti* sp. n. was prevalent in populations of *S. scolytus*. Both microsporidians occurred at the same level in populations of *S. pygmaeus*.

Both microsporidians were observed in larvae pupae and adult insects; eggs were not checked on the presence of spores or other stages of parasites.

Discussion

Mortality factors affecting the population of *Scolytus* species are intensively studied throughout the world as these insects are known as vectors of the Dutch elm disease responsible for decline of elm trees. Beaver 1967 and Stark et Borden 1965, Michalski 1963, and others have examined various biotic factors and analyzed their importance in populations of *Scolytus*. In none of these papers protozoa were mentioned. Dr. Beaver (letter communication) has informed me that during his study in England he did not check insects on protozoan infection. Therefore, the lack of data in papers is simply a result of special studies.

Our extensive studies carried out in Poland have shown that microsporidians play a very important role in the mortality of four species of *Scolytus*. Both investigated microsporidians *Nosema scolyti* sp. n. and *Stempellia scolyti* (Weiser) comb. nov. were also recorded in Czechoslovakia (Weiser 1966) and by me in the Soviet Union and the German Democratic Republic. It seems quite sure that the investigated microsporidians have a wide distribution and with time they will be recorded in other countries.

It is very important that both species have a wide spectrum of hosts and so far, were found to infect four host species namely *Scolytus ensifer, S. multistriatus, S. pygmaeus* and *S. scolytus*. Further investigations should reveal other hosts of the genus *Scolytus*. At any rate the investigated microsporidians seem to be very promising as agents in the biological control of *Scolytus* species, and studies on their introductions to other countries seem to be worthwhile.

http://rcin.org.pl

Summary

The life cycle and pathogenicity of two microsporidians *Nosema scolyti* sp. n. and *Stempellia scolyti* (Weiser) comb. nov. are discussed. These parasites infects Malpigian tubules, the intestine and hemocytes of their hosts. Fresh spores of *N. scolyti,* sp. n. are 3.6—6.2 μ long and 2.2—3.0 μ wide; fresh spores of *S. scolyti* (Weiser) are 2.7—4.0 μ long and 1.2—2.2 μ wide. Both species are common parasites of four species of bark beetles: *Scolytus ensifer, S. multistriatus, S. pygmaeus* and *S. scolytus*. The highest percentage of microsporidian infection was observed in populations of *S. multistriatus* (31.8% of infected insects) followed by *S. ensifer* (25.6%), *S. pygmaeus* (18.4%), and *S. scolytus* (12.4%). *Scolytus* spp. infected with microsporidians were also collected in the Soviet Union and the German Democratic Republic. Because of their high pathogenicity, broad host specificity and wide distribution both parasites are very promising agents in the biological control of *Scolytus* spp. well known vectors of a fungus *Ceratocystis ulmi* (Buism.) causing the Dutch elm disease.

STRESZCZENIE

Omówiono cykl rozwojowy i chorobotwórczość dwóch gatunków mikrosporidiów *Nosema scolyti* sp.n. i *Stempellia scolyti* (Weiser) comb. nov. Mikrosporidia zarażają cewki Malpighiego, nabłonek jelita i hemocyty swych żywicieli. Świeże spory *N. scolyti* sp.n. mają 3.6—6.2 μ długości i 2.2—3.0 μ szerokości; świeże spory *S. scolyti* (Weiser) mają 2.7—4.0 μ długości i 1.2—2.2 μ szerokości. Oba pasożyty zarażają cztery gatunki korników: *Scolytus ensifer, S. multistriatus, S. pygmaeus* i *S. scolytus*. Najwyższe zarażenie mikrosporidiami obserwowano w populacjach *S. multistriatus* (31.8% zarażonych owadów), oraz kolejno *S. ensifer* (25.6%), *S. pygmaeus* (18.4%) i *S. scolytus* (12.4%). Pasożyty stwierdzono również w populacjach *Scolytus multistriatus* w Związku Radzieckim i Niemieckiej Republice Demokratycznej. Z uwagi na wysoką chorobotwórczość i zarażanie kilku żywicieli oba gatunki mikrosporidiów mogą mieć duże zastosowanie w biologicznym zwalczaniu *Scolytus* spp. znanych wektorów grzyba *Ceratocystis ulmi* (Buism.) — sprawcy holenderskiej choroby wiązów.

REFERENCES

Beaver R. A. 1964 (1965): Some mortality factors affecting a population of *Scolytus scolytus* (F.). (*Coleoptera*: *Scolytidae*). Proc. XII Int. Congr. Ent., London 1964, pp. 694.

Carter W. 1962: Insects in Relation to Plant Diseases. Interscience Publi., New York, 705 pp.

Evlachova A. A., Švecova O. I. 1965: Bolezni vrednych nasekomych. Izdat. Kolos, Moskva 50 pp.

Leach J. G. 1940: Insect Transmission of Plant Diseases. McGraw-Hill Book Co. New York, 615 pp.

Michalski J. 1963: Biologia *Scolytus* (*Scolytochelus*) *ensifer* Eichh. (*Coleoptera, Scolytidae*). PTPN Prace Komisji Nauk Roln. i Prace Komisji Nauk Leśnych 16 (3), 31—79.

http://rcin.org.pl

S a u v e M. 1964: A review of the Dutch elm disease. Canada Dept. Forestry, Bi-Monthly Progress Rept. 20 (4), 1—8.
S t a r k R. W., B o r d e n J. H. 1965: Observations on mortality factors of the fir engraver beetle, *Scolytus ventralis* (*Coleoptera: Scolytidae*). J. econ. Entomol. 58, 1162—1163.
W e i s e r J. 1966: Nemoci Hmyzu. Academia. Praha 554 pp.

http://rcin.org.pl

EXPLANATION OF PLATES I—VIII

Stempellia scolyti (Weiser) comb. nov.

1—3: Schizonts and sporonts (sporoblasts) (×600)
4: Schizogonic and sporogonic stages, and spores in pansporoblasts (×1600)
5: Pansporoblasts with variable number of spores (×640)
6: Two pansporoblasts having four and six spores (×1600)
7: A pansporoblast having twelve spores (×1600)
8: Stained spores in microtome section of the body of larvae of *Scolytus multistriatus* Marsh.; notice that only the gut cells are infected and the fat body is free from infection (×1600)
9—10: An early and an advanced stage of infection of the gut of *Scolytus multistriatus* Marsh. (×320)

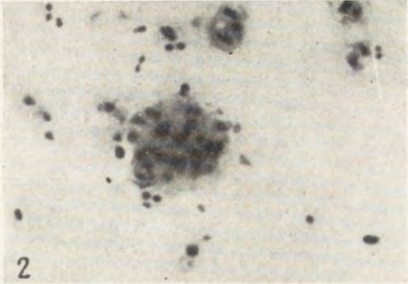
Nosema scolyti sp.n.

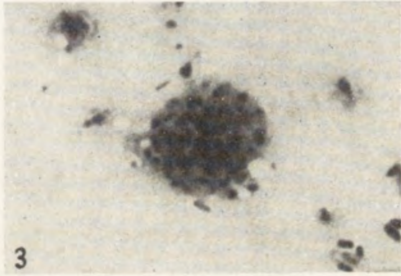
11: Schizonts in the cytoplasm of a hemocyte (×1600)
12: Schizonts in cells of smeared epithelium of the gut of *S. multistriatus* (×1600)
13: Spores seen in fresh smeared preparation of the gut epithelium of *S. multistriatus* (×1440)
14: Spores in water seen under phase contrast (×1440)
15: Stained spores in the cytoplasm of cells from the epithelium of the gut of *S. multistriatus* (×1600)
16: Group of stained spores (×1600)
17: Pseudocysts (groups of spores) in a piece of gut wall of *Scolytus pygmaeus* Fabr. (×256)
18—19: Spores in a heavily infected Malpighian tubules of *S. multistriatus* (×250 and ×640)
20—21: Spores in infected gut ampules of *S. multistriatus* (×250 and ×640)
22: Completely destroyed gut wall of *S. multistriatus* by the parasite: notice the great hyperthrophy of epithelial cells and presence of pseudocysts (×640)

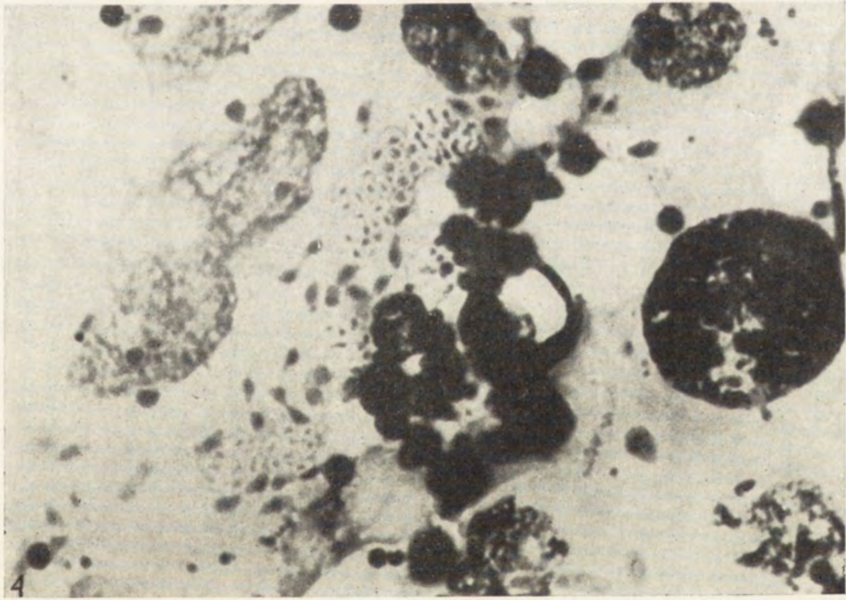
Mixed infections with *N. scolyti* sp. n. and *S. scolyti* (Weiser)

23—24: Spores of *S. scolyti* (Weiser) and *N. scolyti* sp. n. as seen in a fresh smeared preparation of the gut of *S. multistriatus;* notice great difference in the size of spores (phase contrast) (×440)
25: Schizogonic stages of *N. scolyti* sp. n. and pansporoblasts of *S. scolyti* (Weiser) in smeared preparations of tissues of *S. multistriatus* (×1600)

http://rcin.org.pl

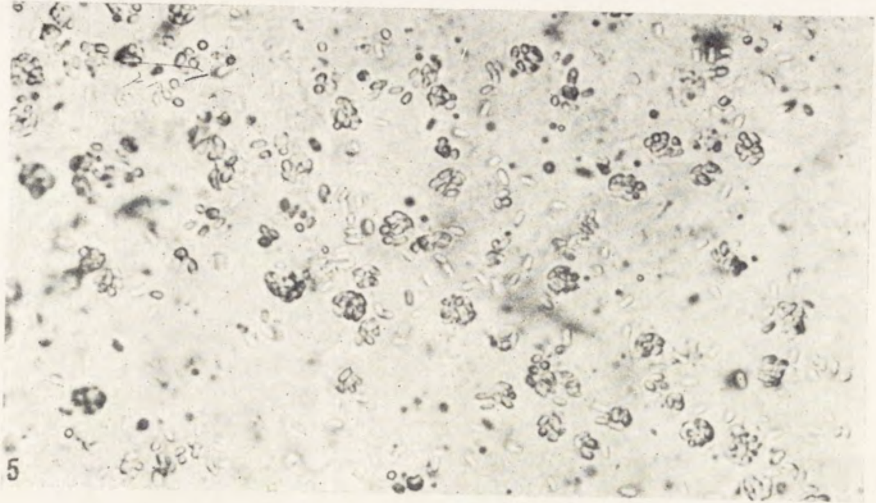


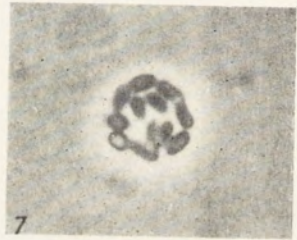


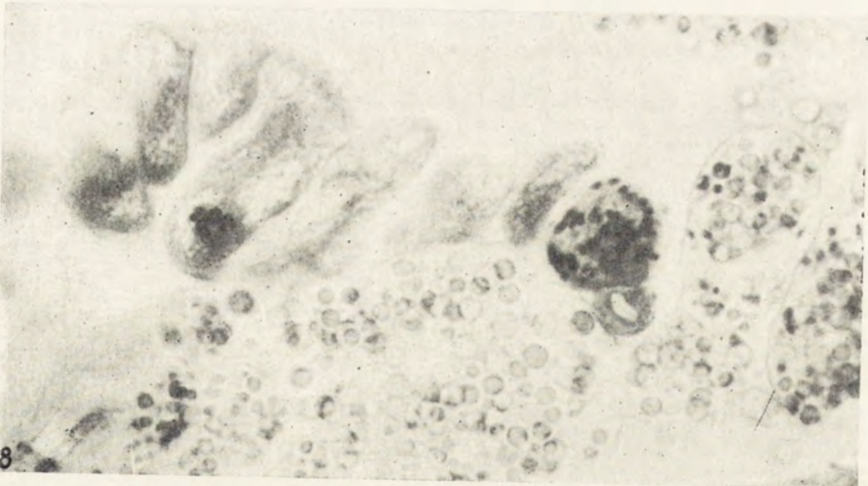


J. J. Lipa auctor phot.

http://rcin.org.pl



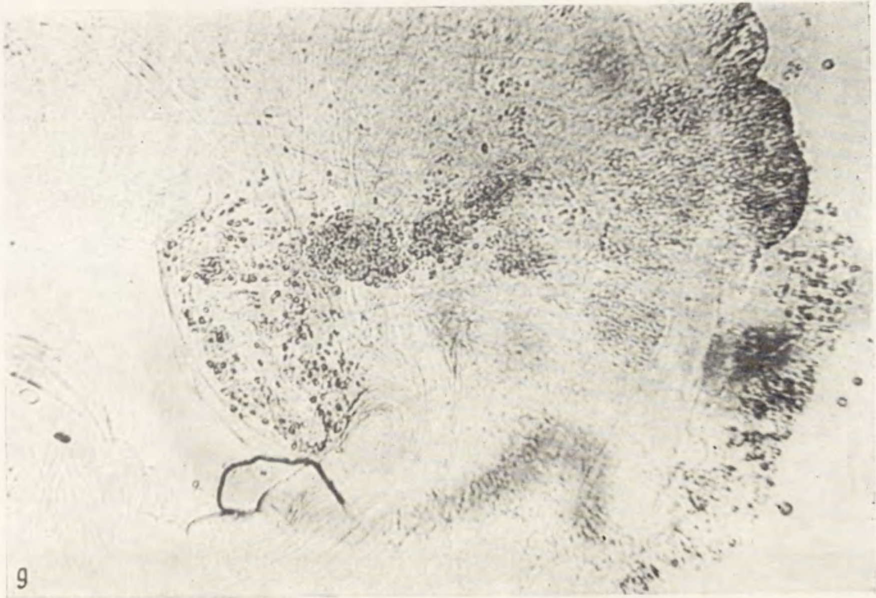


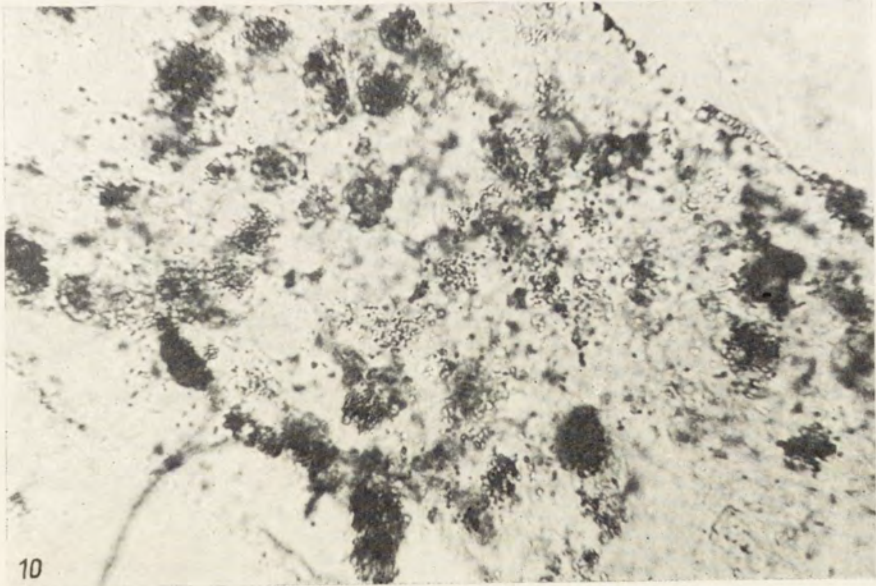


J. J. Lipa

auctor phot.

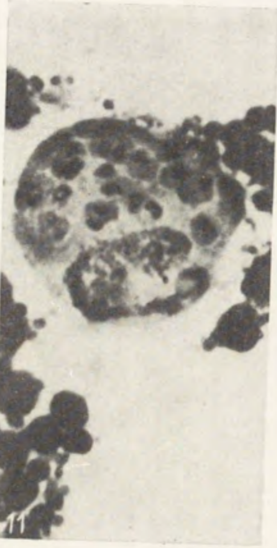
http://rcin.org.pl

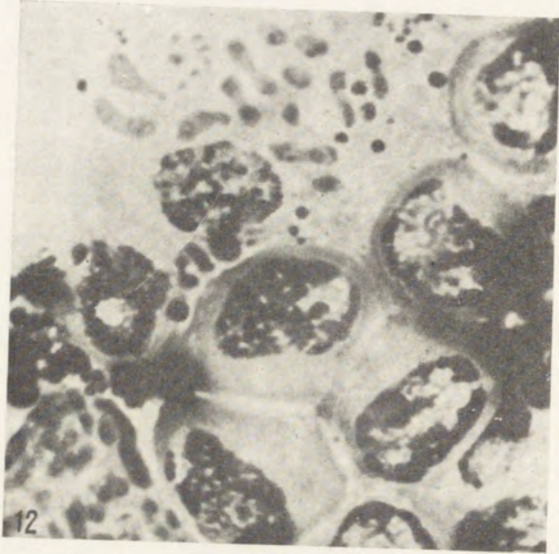


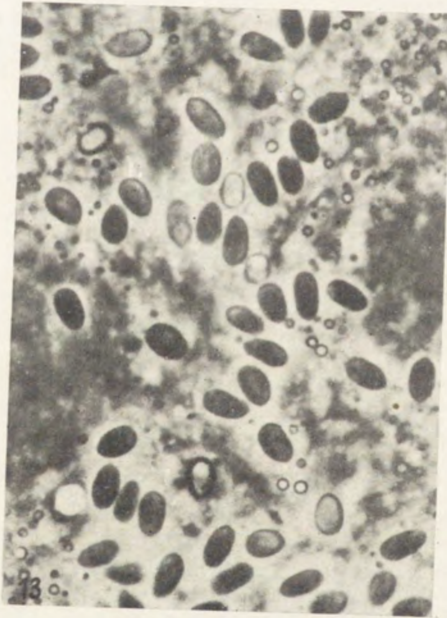


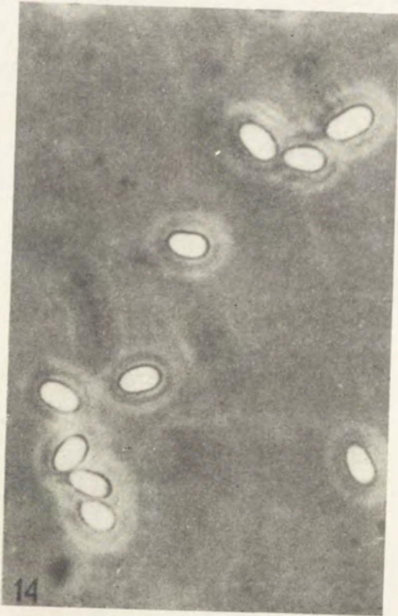
J. J. Lipa auctor phot.

http://rcin.org.pl





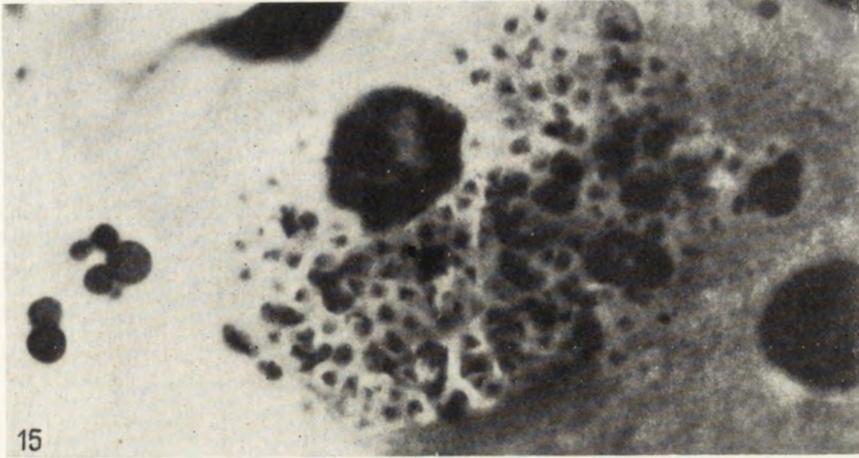




J. J. Lipa

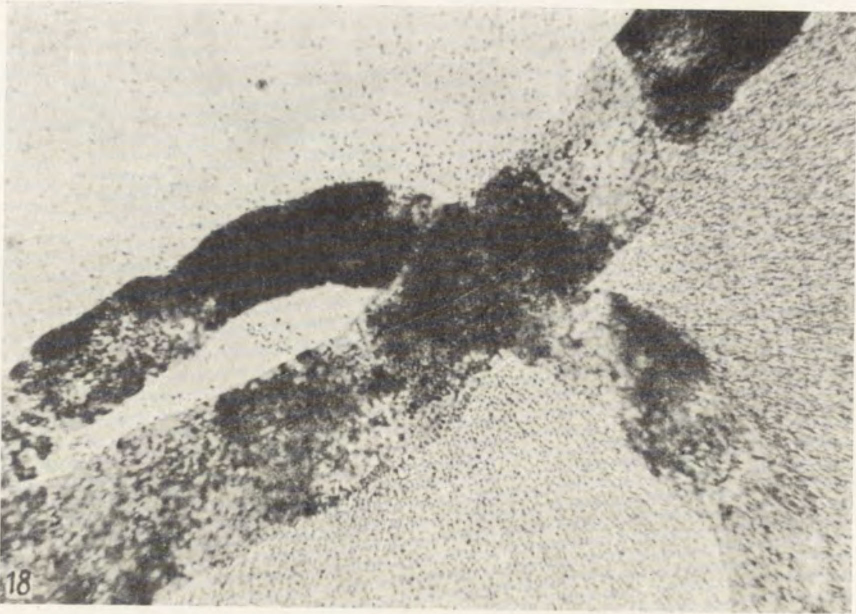
auctor phot.

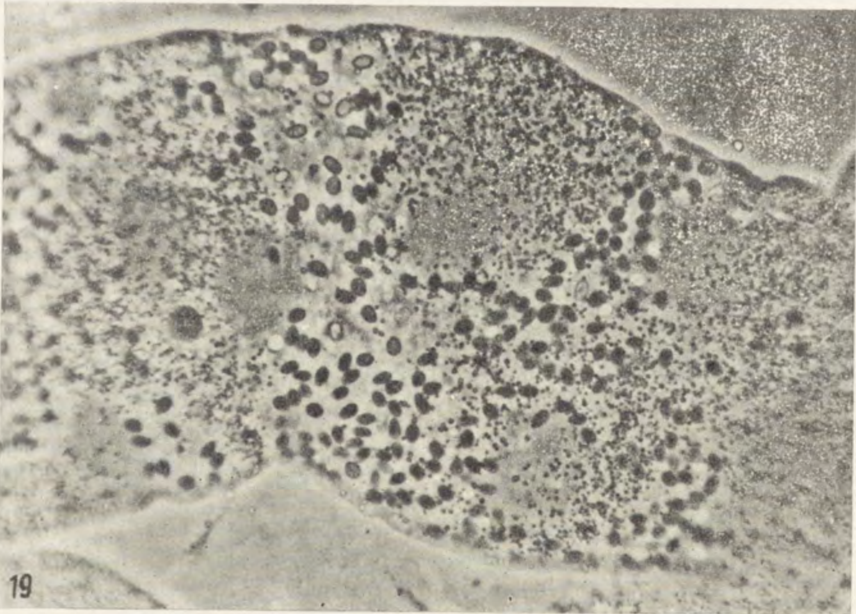
http://rcin.org.pl



J. J. Lipa auctor phot.

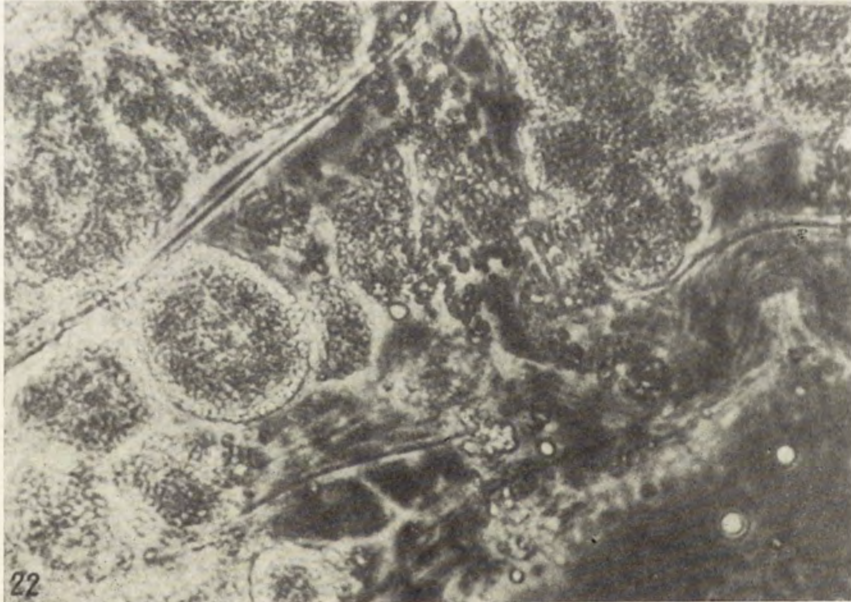
http://rcin.org.pl





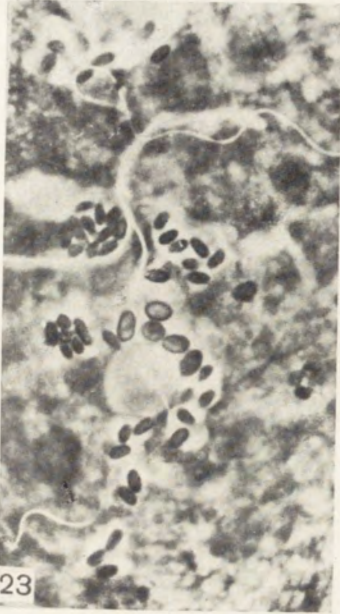
J. J. Lipa auctor phot.

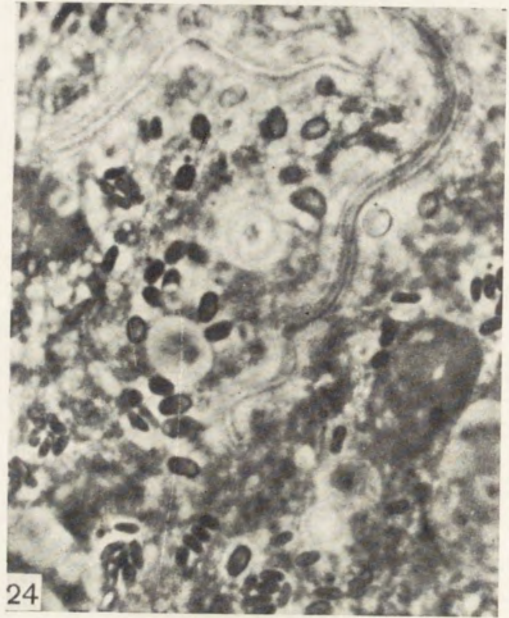
http://rcin.org.pl

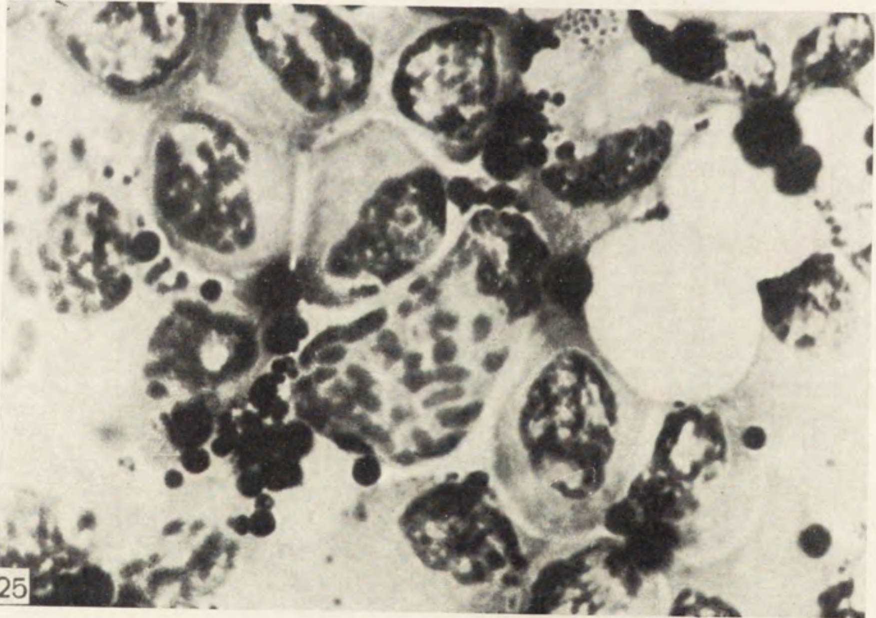


J. J. Lipa auctor phot.

http://rcin.org.pl







J. J. Lipa

auctor phot.

http://rcin.org.pl

ACTA PROTOZOOLOGICA

VOL. VI WARSZAWA, 31.VIII.1968 FASC. 8

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

T. V. BEYER

Cytochemical studies on the haemogregarines of Armenian reptiles. I. DNA in the nuclei of some *Karyolysus* species from the rock-lizards *Lacerta armenica* and *L. saxicola*

Цитохимическое исследование гемогрегарин из рептилий Армении. I. ДНК в ядрах некоторых видов *Karyolysus* из скальных ящериц — *Lacerta armenica* и *L. saxicola*

The nucleus of different species of *Sporoza* has called for many years the attention of scientists. A cytochemical analysis of nucleic acids involved the Unna procedure with methyl green-pyronin, as well as toluidine blue and gallocianine staining (Grell 1953, Pattillo and Becker 1955, Sen and Ray 1955, Cheissin 1958, Beyer 1963 and others). Moreover, almost all the studies included the Feulgen nuclear reaction for DNA. In result, this substance was demonstrated throughout the whole life cycle of the sporozoans examined except the gamont stage which nucleus in some objects revealed a negative Feulgen reaction.

Within the subclass *Coccidiomorpha* [1] one can found a transition from Feulgen positive to Feulgen negative nuclei of gamonts. Dasgupta 1959 reported on the positive Feulgen reaction revealed in the nuclei of both kinds of the *Hepatozoon* gamonts *(Adeleida, Adeleidae)*. Similar results were obtained for another member of the same order — *Haemogregarina (Haemogregarinidae)* (Sen and Ray 1955). At the same time the corresponding stages of *Plasmodium* and *Hepatocystis (Coccidiidea, Haemosporidiidea)* appeared to have the Feulgen negative nuclei (Dasgupta 1959). The intermediate position in this respect was revealed for coccidians belonging to *Eimeria* genus *(Coccidiida, Eimeriidae)*. The nuclei of their macrogamonts are Feulgen negative, while those of microgamonts are Feulgen positive.

The cause of these variations in the nuclear reaction within a group of rather related organisms seems not yet clear. It is not unlikely, however, that some methodical factors may account for the differences observed which was first discovered by Deane 1945 in studies on the Feulgen reaction in the malaria parasite.

The present paper deals with cytochemical studies of DNA carried out in gamonts of the blood parasites of the rock-lizards from the *Lacerta saxicola*

[1] The taxonomy of the class *Sporozoa* is given according to Cheissin 1967.

http://rcin.org.pl

group that might be thought to belong to the genus *Karyolysus* (*Adeleida, Haemogregarinidae*). The attempt is made to understand the cause of divergency in results obtained with the Feulgen procedure in gamont nuclei.

Materials and methods

Rock-reptiles belonging to two species *Lacerta armenica* Méhely and *L. saxicola nairensis* Darevsky were obtained from the Sevan Lake region (Armenia) in the autumn 1966. [2] Blood parasites were examined on blood films (over 220 slides) and on smears made of internal organs (liver, kidney) from 26 lizards.

Fresh films of parasitized blood and smears of internal organs were fixed with methyl alcohol. For morphological study some slides were stained with Giemsa-Romanovsky. DNA in the nuclei was detected by the Feulgen procedure and by the Unna staining with methyl green. The reliability of the two stainings was checked by a preliminary DNA-se digestion.

The staining intensity was found to be much higher, if the staining procedure was preceded by at least an 1 hour's socking of the slide in water. The Unna staining was carried out for 20—30 minutes. If the colour was not bright enough, no alcohol differentiation followed. The period of acid hydrolysis, at the Feulgen procedure, was 7 to 9 minutes after which the slides were treated with the Schiff reagent for 2—3 hours.

Sometimes, we were met with the necessity to carry out the cytochemical reactions on the slides earlier stained after Giemsa. In this case, the slides to be investigated were placed in 70° alcohol until colourless, afterwards they were left socking in water for at least 1—2 hours. The cytochemical pictures obtained on these slides were practically identical to those seen of freshly stained ones.

A limiting amount of material we had at our disposal as well as some other purposes made it necessary sometimes to perform different cytochemical reactions on one and the same slide. In this case, the field of the unstained slide is divided into two parts and only one part is stained after Giemsa-Romanovsky. After examining this part of the slide, the other one may be submitted, if necessary, an appropiate cytochemical procedure, thus allowing to have on the same slide both "morphological" and "cytochemical" picture of parasites. Unfortunately, acid hydrolysis, at the Feulgen reaction, may spoil a "morphological picture" which can be, however, restored after a repeated staining.

Results

At least five different forms of blood parasites were discovered on the slides and smears. Some similar forms, found in the Caucasian lizards *Eremias* sp., were earlier described by Breindl 1914. Besides, the blood

---

[2] Living animals as well as some blood smears were kindly given at our disposal by Prof. B. P. Ushakov and Dr. I. M. Pashkova from the Institute of Cytology, for which I have a pleasure to express my gratitude to them.

http://rcin.org.pl

parasites of the caucasian rock-lizards were investigated by Krasilnikov 1967.

The description of our findings is given below.

Form I (Figs 1 and 6)

The crescent shape parasites ranging $14.4 \pm 0.20 \times 2.33 \pm 0.07$ μ are laid along a longer axis of a blood red cell, sometimes a bit bent. The cytoplasm of parasite is coloured light blue or light violet after Giemsa-Romanovsky staining, coinciding with the tone of the erythrocyte cytoplasm and thus making the body of parasite contours hardly seen. A number of vacuoles in the cytoplasms makes a most characteristic feature of this form. The nucleus of the parasite takes a central position.

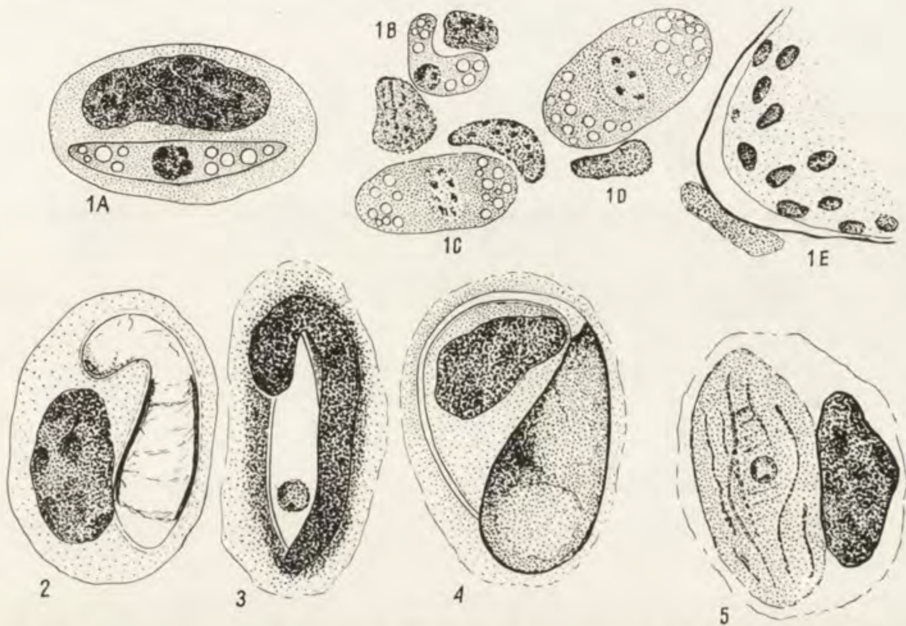


Fig. 1—5. Giemsa-Romanovsky staining of different forms of *Karyolysus*. 1. Form I A — a schizont stage in the peripheral blood. B—E — stages of development in the kidney. 2. Form II. 3. Form III. 4. Form IV. 5. Form V. Magnification 1800×

In the peripheral blood red cells, long and narrow forms are met (Fig. 1). On the smears of kidney one can follow a growth and segmentation of this forms occurring mainly within blood cells of this organ (Fig. 1 B—E). No segmented parasites were seen in the host blood cells from the peripheral flow.

The positive Feulgen reaction was detected in the nuclei of all the steps of development of this form. Chromatin grains were easily seen along the edges of the nucleus in the stage from the peripheral blood (Fig. 6). The Unna staining revealed an even distribution of methyl green throughout the whole nucleus.

http://rcin.org.pl

Form II (Figs 2 and 7)

The body of parasite is elongated, sometimes strongly curved, ranging $16.07 \pm 0.20 \times 3.52 \pm 0.09\ \mu$. On one end the body is rather enlarged, the other one is narrow, frequently bent at an angle of 90—180° toward the long axis of the body. This bent part sometimes resembles a separate head connected with the other mass of the parasite with a narrow constriction. The parasite is separated from the host cell cytoplasm with a clearly seen capsule. The nucleus of the parasite is elongated, laying mainly closer to the enlarged body part. It looks like two thick threads with thinner transversal fibres connecting the former. The Feulgen reaction is positive in the nuclei, the lateral thick threads staining especially intensive (Fig. 7).

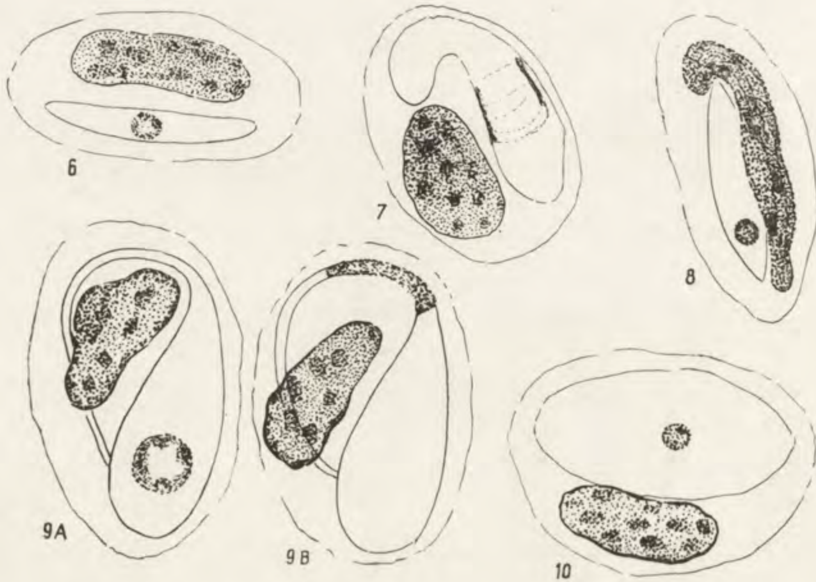


Fig. 6—10. The Feulgen reaction in different forms of *Karyolysus*. 6. Form I. 7. Form II. 8. Form III. 9. A. Form IV — the nucleus in the widened body part, B — the nucleus in the "tail" part. 10. Form V. Magnification 1800×

Form III (Figs 3 and 8)

The body of parasite is elongated, spindle-like, both the ends being sharpened. The dimensions are $12.90 \pm 0.15 \times 4.50 \pm 0.28\ \mu$. This form differs from the two above by its rather peculiar position about the host cell nucleus. The cytoplasm of parasite looks colourless except brightly stained grains on the either end of the body. The nucleus is situated a bit closer to one of the body ends and is round in shape. Not rarely the nucleus of parasite is overlaid by the nucleus of the host that is in a most close contact with the parasite cell despite the presence of a distinct capsule separating the parasite from the cytoplasm of host. Among the forms examined, this one demonstrates the highest influence upon the host. The changes concern both the nucleus and the cytoplasm. The host red cell nucleus is frequently seen elongated, often

http://rcin.org.pl

exceeding the size of the parasite body. Moreover, the erythrocyte nucleus is often seen fragmentated or winding round the parasite body, thus masking the parasite nucleus.

The region of the red cell cytoplasm near the parasite has a more intensive Giemsa-Romanovsky stain compared to the remote cytoplasm, the contours of the erythrocyte vanishing. The stronger staining of the neighbouring cytoplasm of the erythrocyte may be expected at the expense of certain nuclear substances released into the cytoplasm. The ability of haemogregarines to deforming the host red cell nucleus was stressed by the first investigators of these and found its reflection in the name of one genus — *Karyolysus*. However, neither the Feulgen reaction, not the staining with methyl green revealed any appropriate substance in the neighbouring part of the host cytoplasm.

The nucleus of parasite reveals a distint Feulgen reaction, chromatin grains being distributed on the periphery of the nucleus (Fig. 8).

Form IV (Figs 4 and 9)

The body shape is very peculiar. The parasite is easily seen devided into a wide retort-like part and a long "tail" making aproximately as much as a half of the total lenght of the parasite. The size of the parasite (without the "tail") is $22.03 \pm 0.30 \times 5.0 \pm 0.13\ \mu$. On describing a similar form from the Caucasian lizard *Lacerta muralis* (which is very likely one of the *Lacerta saxicola* — rock-lizards) Breindl 1914 suggested it should have another systematic position, not within the Sporozoa group.

With Giemsa-Romanovsky staining, the parasite body is stained almost completely reddish, except the terminal part of the "tail". On the slides it is difficult to identify the nucleus of the parasite (Fig. 9 B). Such a continuous staining may be accounted for by the presence of a capsule around the parasite adsorbing the dye.

On Feulgen slides, the nucleus of parasite can be easily seen. The position of the nucleus is not stable, ranging within a widened part of the body (Fig. 9 A) and the "tail" (Fig. 9 B). Chromatin grains are distinctly seen on the periphery of the nucleus with its central part remaining light. The methyl green staining reveals a pink nucleolus in this light zone.

Form V (Figs 5 and 10)

The oval parasite with the size $12.55 \pm 0.14 \times 5.88 \pm 0.07\ \mu$. On Giemsa stained slides the surface of the parasite body is easily seen adsorbing the dye, this being arranged in raised lines connecting the poles of the body. The nucleus has a central position within the cell (Fig. 10) and shows a positive Feulgen reaction.

Discussion

Since our findings were restricted by stages of the life cycle in the vertebrate host only, we cannot pretend to name the parasites examined correctly, meaning their precise systematic position. According to the Wenyon scheme (Wenyon 1926, Doflein und Rechenow 1953) we suppose that the parasites in question may belong to the genus *Karyolysus* since in no

http://rcin.org.pl

instances we managed to find any dividing parasite in the peripheral blood erythrocytes which is known as a characteristic feature of the genus *Haemogregarina*.

We observed some dividing stages of form I in the internal organs. In this respect, form I may be considered to be a trophosoite that undergoes schizogony in the kidney (Fig. 1 B—E). All the rest forms (II—V) we attribute to the gamont category without pointing out to any visible morphological distinctions between the gamonts of different sexes. As a rule, in all the cases examined the gamont nucleus demonstrated DNA, the Feulgen reaction being positive. It may be said a priori that the possibility of meeting female gamonts on the blood films is equal or close to that of meeting male gamonts which allows the following conclusion: the gamonts of both sexes of *Karyolysus* show a positive Feulgen reaction.

This is in accord with what has been reported for *Hepatozoon* (Dasgupta 1959) and for *Haemogregarina* (Sen and Ray 1955), which belong to the same order *Adeleida* as *Karyolysus*.

The Feulgen negativity of the nuclei of macro- and microgamonts is described for the malaria parasite and *Hepatocystis*, both belonging to the order *Coccidiida* (suborder *Haemosporidiidea)*. Actually the coincidence in results obtained for these species by various authors (see the reference list in Dasgupta's paper, 1959) does not seem accidental. This fact was often mentioned but never explained. Deane 1955 was the first who succeeded in demonstrating that a weak or negative Feulgen reaction might be due to technical peculiarities involving the presence of the malarial pigment haematine. It was shown that the latter inhibited the Feulgen reaction. After its removal by alkaline alcohol, staining was obtained in the nuclear material of all the developmental stages earlier considered as negative. The most intensive reaction was followed in the trophozoites and schizonts, and a very faint in the gametocytes. The fact of a very faint staining of the *Plasmodium* gamonts after the Feulgen method may suggest that this phenomenon may be not accidental, but connected with some changes occurring in the nuclei at this stage of development.

It is known that the Feulgen negativity of the nucleus is not connected with the absence of DNA but with its state of depolimerization (Kurnic 1950; see: Pearse 1960), when it stops staining while the Feulgen procedure is employed. On the other hand, a vast increase of the nucleus volume may lead to the dilution of DNA and in consequence its too low concentration appears beyond the possibilities of this method of detection (Swift 1955). A good illustration of this thesis may be the growth and development of macrogamonts of some other species of the subclass *Coccidiomorpha* — *Eucoccidium* (Grell 1953) and *Eimeria* (Pattillo and Becker 1955, Cheissin 1958, 1960, Beyer 1963). The macrogamont nucleus grows, its volume increases tremendously compared with that of the microgamont nucleus. The concentration of DNA in the nucleus gets fainter and at last it stops showing a positive Feulgen reaction. This phenomenon is known also for the growing oocytes of metazoans.

In *Eucoccidium* and *Eimeria* a sexual dimorphism of gamonts is very well pronounced which corresponds to different staining when the Feulgen procedure is employed. No such dimorphism is found either in haemogregarines, or in haemosporidians met in the host blood cells. Therefore the very

http://rcin.org.pl

fact of coincidence of the Feulgen reaction in both the gamonts of either group does not seem puzzling. However, the cause of the Feulgen negativity in the nuclei of haemosporidian gamonts needs itself a further explanation which may reveal some specific peculiarities, yet unknown, of the sexual cycle in comparison with those of the asexual development of the parasite.

Summary

The DNA distribution was studied in the nuclei of *Karyolysus* gamonts from the peripheral blood of two rock-lizards of the group *Lacerta saxicola*. It was ascertained that as well the macro- as microgamonts of *Karyolysus* demonstrate the positive Feulgen reaction which is consistent with the results obtained previously on *Haemogregarina* and *Hepatozoon* which belong to the same order *Adeleida* (Sen and Ray 1955, Dasgupta 1959).

The factors evoking the Feulgen-negative result in the nuclei of both gamont types of *Plasmodium* and *Hepatocystis* are discussed, as well as the Feulgen-negative reaction in the macrogamonts of *Eucoccidium* and *Eimeria*.

РЕЗЮМЕ

Изучено распределение ДНК в ядрах гамонтов *Karyolysus* из периферической крови двух скальных ящериц из группы *Lacerta saxicola*. Оказалось, что как макро-, так и микрогамонты *Karyolysus* демонстрируют положительную реакцию Фельгена, что согласуется с данными, ранее полученными на *Haemogregarina* и *Hepatozoon*, относящимися к тому же отряду *Adeleida* (Sen and Ray 1955, Dasgupta 1959).

Обсуждается вопрос о причинах Фельген-негативности ядер обоих типов гамонтов *Plasmodium* и *Hepatocystis*, а также Фельген-негативности макрогамонтов *Eucoccidium* и *Eimeria*.

REFERENCES

Beyer T. V. 1963: Citohimičeskoe issledovanie kišečnyh ciklov kokcidij krolika pri raznyh uslovijah ih suščestvovanija v hozjaine. Thesis of cand. diss. Leningrad; unpublished.

Breindl V. 1914: Ueber die Haemocytozoen einiger kaukasischen Reptilien. Sber. K. böhm. Ges. Wiss., 1—11.

Cheissin E. M. 1958: Cytologische Untersuchungen verschiedener Stadien des Lebenszyklus der Kaninchencoccidien. I. *Eimeria intestinalis* E. Cheissin, 1948. Arch. Protistenk., 192, 265—290.

Cheissin E. M. 1960: Citologičeskoe issledovanie žiznennogo cikla kokcidij krolika. 2. *Eimeria magna*. In: Voprosy citologii i protistologii. Sbornik rabot. Izd. Akad. Nauk SSSR, Moskva-Leningrad, 258—276.

Cheissin E. M. 1967: Žiznennye cikli kokcidij domašnih životnyh. Izd. Nauka, Leningrad-Moskva.

Darevsky I. S. 1967: Skalnye jaščericy Kavkaza. Izd. Nauka, Leningrad-Moskva.

Dasgupta B. 1959: The Feulgen reaction in the different stages of the life-cycle of certain Sporozoa. Qaurt J. micr. Sci., 100, 241—255.

Deane H. W. 1954: Studies on malaria parasites. 2. The staining of two primate parasites by the Feulgen technique. J. cell. comp. Physiol., 26, 139—144.

http://rcin.org.pl

Doflein F. und Reichenow E. 1953: Lehrbuch der Protozoenkunde. 6 Aufl. G. Fisher Verlag, Jena, 89.
Grell K. G. 1953: Entwicklung und Geschlechtsbestimmung von *Tucoccidium dinophili*. Arch. Protistenk., 99, 3—13.
Krasilnikov E. N. 1967: Parasity skalnyh jaščeric Kavkaza. Učen. Zap. Volgogr. gosud. Pedag. Inst., 17, 12—15.
Pattillo W. H. and Becker E. R. 1955: Cytochemistry of *Eimeria brunetti* and *E. acervulina* of chicken. J. Morphol., 96, 61—96.
Pearse A. G. 1960: Histochemistry. Theoretical and Applied. 2nd. ed. London.
Swift H. 1955: Cytochemical studies of nucleic acids. In: The Nucleic Acids. Acad. Press. Inc., New. York.
Wenyon C. M. 1926: Protozoology. London, I vol.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 9

Department of Cytology and Histology of Moscow University, Moscow, USSR

V. D. KALLINIKOVA

Cytochemical study of enzymes of the respiratory chain in the life cycle of *Tryponosoma cruzi* Chagas, 1909. II. Respiratory enzymes of crithidial forms in the course of cultivation and some observations on the growth of the culture

Цитохимическое исследование ферментов дыхательной цепи в жизненном цикле *Trypanosoma cruzi* Chagas, 1909. II. Дыхательные ферменты критидиальных форм в процессе культивирования *Trypanosoma cruzi*, наряду с другими показателями роста культуры

The present study is a continuation of our previous research (Kallinikova 1968) and is devoted to a more detailed examination of the cultivated forms i.e. mostly of crithidia of *T. cruzi*. Since the dynamic of the culture growth is associated with regular changes of pH of the medium as well as with those of the parasite morphology, those indices have been studied simultaneously.

Material and methods

The dynamic of cell accumulation, changes in their morphology, activity of peroxidase, cytochromoxidase, NADH- NADPH-diaphorases, as well as of indices of the medium pH were studied in the course of 25—26 days of growth of the *T. cruzi* culture.

Cell accumulation was counted in the chamber of Gorjaev, pH was determined by the LPU-01 potentiometer. For the morphological characteristic of the culture, the percentage of leptomonad, metacyclic and crithidial forms was counted, and among the latter, the percentage of deformed individuals as well.

The activity of respiratory enzymes was studied by means of cytochemical methods (Kallinikova 1968). The level of enzyme activity was evaluated quantitatively either by the percentage of cells which showed a positive reaction (for peroxidase) or by the mean number of grains in a cell which indicate the places of activity of the enzyme (for cytochromoxidase and diaphorases). In both cases, 100 cells were examined. *T. cruzi* was cultivated in the pepton-yeast medium of Romanov with a 1% content of glucose.

Four experiments had been carried out, in each of them 3—4 samples of *T. cruzi* culture were used. In the experimental material no difference

http://rcin.org.pl

between the samples were found. Therefore it was possible to determine the mean values for each experiment. Those values are presented in the results of the study.

Results

Dynamic of accumulation of *T. cruzi* in the culture

The dynamic of cell accumulation failed to differ essentiatially in the experiments carried out, however certain differences in duration of particular growth phases were observed (Fig. 1 A—D).

The lag-phase did not occur practically. The phase of logarithmic growth lasted during the first 8—16 days. The stationary phase with a maximal accumulation of 20—40 millions/ml lasted for 10—14 following days, later on the number of cells diminished gradually.

In one of the first three experiments (experiment No. 2), the curve of accumulation had a two-peaked character, the second peak occurring on the 25th day. Since according to the data reported in the litterature the appearance of the second peak is associated with the conditions of feeding with carbohydrates, we executed a special experiment in which the culture was kept in the media with various content of glucose: 1% — like in all the previous experiments and 0.1% (experiment No. 4). Only at a lowered glucose content the curve of accumulation had a two-peaked character. The second peak of accumulation occurred on the 29th day of growth (Fig. 1 D, dotted line).

Changes of pH of the medium

In proportion as the accumulation of cells in the culture took place a regular acidification of medium occurred (Fig. 1 E—H). pH being 7.5—7.2 on the first two days, gradually fell in the course of the logarithmic and the first half of the stationary phases reaching a minimum of 5.5—5.6 on the 16—21 day. However subsequently i.e. usually towards the end of the stationary growth phase in all the experiments, a temporary rise of pH was observed which was expressed more or less distinctly (0.2—0.8) and lasted for a various period of time (in the experiment No. 4 — up to 10 days). This rise of pH was particularly significant at a low glucose content in the medium (Fig. 1 H, dotted line). In this case pH rose from 5.8 to 6.9 and remained at this level for 16 days. The rise was twice as high as at a usual glucose content in the same experiment.

In those cases when the curve of cell accumulation had two peaks, the second one coincided with the rise of pH or followed it immediately (Fig. 1 B, F and D, H).

The temporary rise of pH was followed by its subsequent inevitable fall which coincided with the phase of senescence of the culture.

Changes of the morphology of the culture

As to the morphological aspect of the culture, it was represented almost exclusively by crithidia with a small percentage of metacycylic forms. In the course of 25—36 days of growth of the culture, two essential changes in this morphological picture were observed: a gradual deformation of crithidia

http://rcin.org.pl

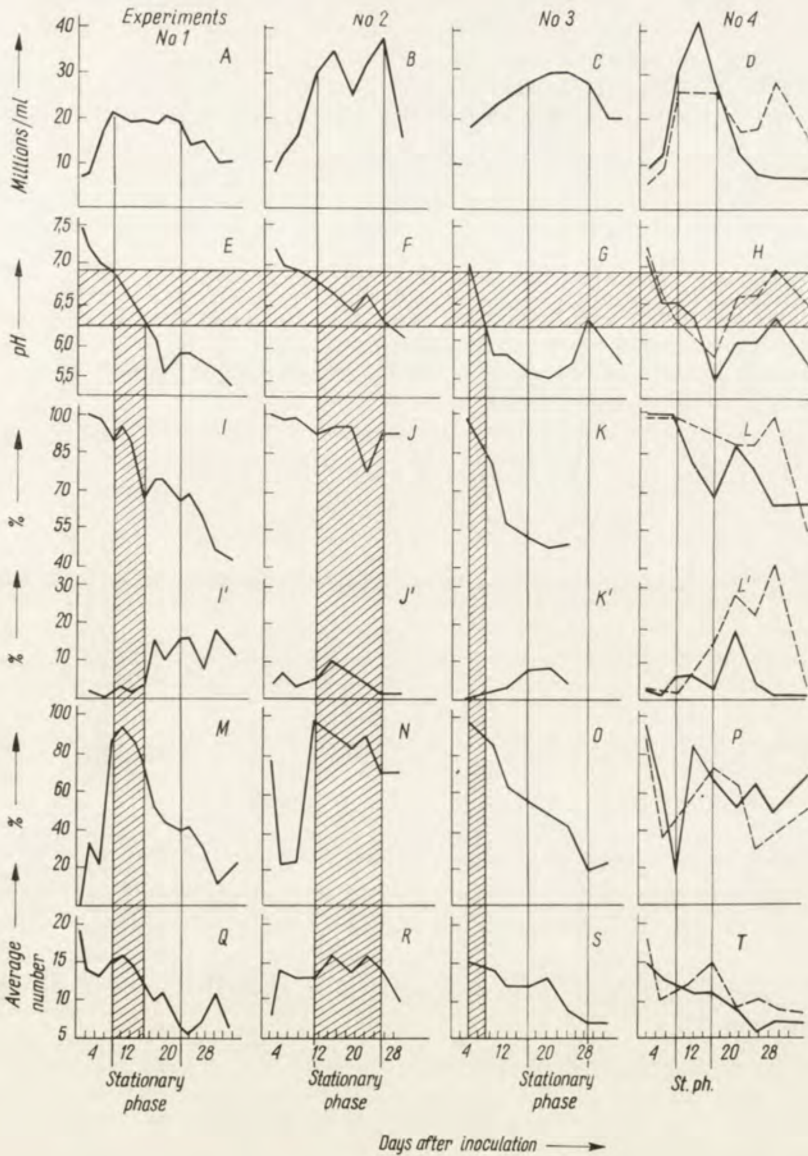


Fig. 1. General results of investigations of *T. cruzi* in the culture. Experiments No 1—4 medium with 1 per cent and experiment No 4 (dotted line) medium with 0.1 per cent content of glucose

A—D — the cell accumulation (millions/ml.), E—H — pH of the medium (zone pH 6.2—6.9 is striped), I—L — and I′—L′ — the morphology of the culture, I—L — the percentage of the normal crithidia, I′—L′ — the percentage of the metacyclic forms, M—P — the peroxidase activity of the crithidial forms (the percentage of crithidia with the positive reaction), Q—T — the cytochromoxidase activity (average number of the grains of indophenol blue in a cell)

http://rcin.org.pl

and an increase of percentage of metacyclic forms (Fig. 1 I—L and I′—L′). The deformed — so called "bulb-shaped" crithidia — present spherical cells with a flagellum and with a highly reduced membrane.

Deformation of crithidia was closely connected with the changes of pH of the medium. It became significant (20—30% of deformed individuals) with the fall of pH down to 6.2 which usually coincided with the stationary phase and continued to increase subsequently. The curve of the number of crithidia which remained normal coincided distinctly with the curve of the pH fall. However the temporary rise of pH towards the conclusion of the stationary phase was accompanied by a certain normalization of crithidia only then when their deformation was not too far advanced (no more than 20% of deformed individuals). In those cases the rise of pH involved the second peak of cell accumulation (Fig. 1 I, L and B, D).

Reducing the glucose content in the medium brought about a considerable inhibition of deformation of crithidia.

In proportion as the number of normal crithidia falls, the number of metacyclic forms increases from a few percentage in the first days of growth up to 10—20% in 16—30 days (Fig. 1 I′—L′). A specially high number of metacyclic forms appeared after the fall of glucose content in the medium reaching in this case 27—35% (Fig. 1 L′ dotted line). The increase of number of metacyclic individuals is however sometimes temporary. The rise of pH either occurs at a period of a high content of metacyclic individuals or follows their maximum or coincides with it at a certain degree (compare Fig. 1 E—H and I′—L′). In the case of fall of glucose content, the curves of cell accumulation and of pH changes in the second half of the period of the culture growth under study, clearly coincide with the dynamic of the content of those forms (experiment No. 4).

Changes of the peroxidase activity in crithidial forms of *T. cruzi*

These changes were considerable and regular (Fig. 1 M—P). In each experiment, a definite peak of activity of this enzyme was observed when nearly all the cells (70—100%) showed a positive reaction. In three experiments out of four the maximal peroxidase activity of crithidia coincided with the transition of the culture to the conditions of the acidic medium. The maximal activity bagen at pH 6.9—6.8 and remained at this level till pH had fallen down to 6.2. In the majority of cases, the rise of peroxidase activity coincides with the passage of the culture to its stationary phase. However this was associated not so much with the dynamic of cell accumulation as with the changes of pH. If at pH 6.9—6.8 the stationary phase was reached earlier, then the maximal activity of the enzyme was observed at an earlier stage of growth (experiment No. 3, Fig. 1 O). Nevertheless the rise of peroxidase activity in crithidia was always connected with the onset of deformation, even in the logarithmic phase of growth.

The persistence of peroxidase activity depended on the fact how rapidly the culture achieved pH 6.9 and low long it kept the level of 6.9—6.2. In the case of a rapid disappearing of the enzyme, the curves had various character which complicated the revealing of any regularity. A further fall of pH evoked the fall of peroxidase activity despite the temporary rise of pH at the conclusion of the stationary phase even if its level was high and reached the interval 6.2—6.9 (Fig. 1 P). Not only the percentage of crithidia with

http://rcin.org.pl

a positive reaction became lower but also the number of cellular granules which condition this reaction.

The peroxidase activity of crithidia provides a less clear picture at the phase of logarithmic growth. Although the activity of the enzyme is generally insignificant at this stage, in two of our experiments the culture growth began in the first two days with a high peroxidase activity which subsequently fell abruptly and rose again when the culture passed to the stationary phase as described above (Fig. 1 N, P). Subsequently those experiments differed from the others in several respects namely: they were characterized by two-peaked curves of cell accumulation by their insignificant deformation and by an even temporary and incomplete normalization of crithidia as a response to the rise of pH at the conclusion of the stationary phase.

It should be reminded that the metacyclic forms fail to posess the peroxidase activity and the above statements concern only the enzyme of crithidia.

Changes of the cytochromoxidase activity

The activity of cytochromoxidase in *T. cruzi* changed in an insignificant degree (Fig. 1 Q—T). In the majority of cases, a regular and gradual fall of the enzyme activity occurred with the growth of the culture (from 15—18 to 5—7 grains of indophenol blue in a cell) through all the growth phases. At the onset of the logarithmic growth phase, the changes of the cytochromoxidase activity are sometimes inverse to the changes of peroxidase activity (Fig. 1 Q, R).

Changes of the diaphorase activity

Any regularity in the dynamic of activity of those enzymes in *T. cruzi* was not found mostly because of the difficulty of their cytochemical detection.

Most frequently the activity of diaphorases was found at more advanced terms of growth of the stationary phase, at its conclusion or even after it. In one experiment, a high activity of diaphorases was observed also in the first three days of the culture growth. The curves of diaphorase activity and that of peroxidase showed an inverse character.

The activity of NADPH-diaphorase was higher in the majority of cases than that of NADH-diaphorase.

Discussion

The dynamic of growth of *T. cruzi* culture as followed in this study — including some differences in single experiments — remains in full conformity with the results on this subject reported in the litterature (Nemakova-Sukhareva 1963, Chang and Nagherbon 1947, Warren 1960). This concerns also the rise of pH of the medium at the conclusion of the stationary phase (Brand and al. 1946, Chang and Nagherbon 1947, Chang 1948). Nevertheless the results of this study permit to supplement the picture of the culture growth in *T. cruzi* with some cytological and partly with cytochemical data.

The phase of logarithmic growth is characterized not only by an intense increase of the cell number and by an inevitable fall of pH of the medium

http://rcin.org.pl

but also by an normal morphology of the culture (normal crithidia and some percentage of metacyclic forms), by a high activity of cytochromoxidase in *T. cruzi*, as well as by an insignificant activity of peroxidase and its variability in some cases which is reflected in the subsequent behaviour of the culture.

The passage to the stationary phase of growth involves not only a new interrelation between the cell accumulation and the changes of medium pH, when despite the interruption of the increase of cell number — pH continues to fall. The onset of the stationary phase marks an important crisis in the physiology of the culture which is expressed in very essential cytological and cytochemical alterations of the forms in culture: in the just beginning deformation of crithidia and in the onset of their peroxidase activity with the simultaneous fall of the cytochromoxidase activity.

The transient rise of pH of the medium at the conclusion of the stationary phase is far not always accompanied by the rise of the accumulation curve. This takes place only then, when the deformation of crithidia has proceeded not too far and is in some degree reversible. This has been observed at a high peroxidase activity of crithidia in the first two days of the culture growth.

The transient rise of pH is correlated with the increase of number of metacyclic forms in the culture which is particularly evident when the glucose content is low in the medium.

The culture forms of *T. cruzi* fail to respond to the rise of pH in the medium by an intensification of respiratory enzymes (cytochromoxidase and peroxidase) activity which has been already considerably lowered about this moment. Nevertheless the second peak of the RNA content, of nucleotides, of organic phosphorus and glutathione in the cells of *T. cruzi* — as described previously — coincides with the rise of pH of the medium (Kallinikova i Roskin 1963, Balicheva i Roskin 1963, Roskin i Kolomina 1964, Roskin i Kozhukhova 1964). Being not accompanied by stimulation of respiratory enzymes, this phenomenon proves a very temporary vitalization of the culture.

The stationary phase is inevitably followed by the phase of senescence which is accompanied by a further acidification of the medium, by an extreme fall of the activity of respiratory enzymes and of the content of the most important biochemical components of the culture forms as well as by the death of cells.

The cytochemical investigation of the enzymes under study proves that the respiratory possibilities of *T. cruzi* are different at particular phases of the culture growth. For the phase of logarithmic growth, the cytochromic path of oxidation is most characteristic whereas peroxidase represents the residual peroxidasic activity of the inoculum. The critical moment in the development of the culture, connected with the deformation of crithidia, is characterized by the ability to follow as well the cytochromic as non-cytochromic path of oxidation.

Peroxidase not only appears exclusively at the crithidial stage of the life cycle of *T. cruzi* but has a definite dynamic of activity at this stage, being associated with a decisive moment of the culture life. The rise of peroxidase activity marks a critical moment in the development of the culture, namely the onset of deformation of crithidia. The level of peroxidase activity on the two first days of growth defines the fate of the culture in the subsequent

http://rcin.org.pl

phases of development: the level of deformation of crithidia and appearing of the second peak of cell accumulation as repsonse to the rise of pH.

All this proves once more that the visible morphological changes of cells precede considerable cytochemical shifts and that the cytochemical characteristic of the cell population may at a certain degree define its development.

In conformity with the results of Brand and al. 1949 we found that the rise of pH and of the accumulation curve at the conclusion of the stationary phase are connected — before all — with the deficiency of carbohydrate food. The tenfold fall of the glucose content in the medium — when compared to the normal one — led in our experiments to a more significant and more stable rise of pH of the medium, to a second peak of cell accumulation, diminished of the deformation of crithidia and increased the increment of metacyclic forms. The fall of glucose content influenced only the advanced stages of the culture growth and was not reflected in the development of the culture at the logarithmic phase of growth.

The association of pH rise in the late culture with the deficiency of glucose in the medium indicates the necessity of passage of *T. cruzi* culture to another paths of metabolism after the exhaustion of carbohydrate store in the medium. However this does not permit to decide which are the new paths: passage to the protein nourishment (Brand and al. 1946) or using the accumulated non-fully oxidized transitory acids of the Krebs cycle (Chang and Nagherbon 1947, Chang 1948). It should be not omitted that the second theory has found some support in a number of biochemical results concerning a better utilization of Krebs cycle acids by trypanosomides at a lower pH (5.0—5.6) and at a low glucose content i.e. in conditions which arise in the culture of a more advanced age (Brand and Agosin 1955, Zeledon 1960, Ryley 1962). It follows from the results of the present study that the rise of pH i.e. the passage to a new metabolism, is not associated with a stimulation of such respiratory enzymes in crithidia as cytochromoxidase and peroxidase.

Since the growth of *T. cruzi* culture and changes in its metabolism are accompanied by distinct morphological changes, the problem under discussion has a cytological aspect as well. The problem concerns also the elucidation of the question which of the culture forms of *T. cruzi* are responsible for the changes in the metabolism of the more aged cultures.

Chang ascribes the ability of utilization of the Krebs cycle acids to the metacyclic forms and to the so called "narrow" — i.e. presumably to the normal crithidia.

The results of the present study induce to discriminate between the rise of pH and the rise (the second peak) of cell accumulation — which do not coincide always. The second peak of accumulation is evidently connected with the normal ("narrow") crithidia because it may be observed only in the case of a slight deformation of crithidia and is accompanied by a certain temporary normalization of them. On the contrary, the rise of pH correlates more distinctly with the content of metacyclic forms in the culture. This allows to postulate that the metacyclic forms are responsible for pH rise and this secures some "vitalization" and multiplication of crithidia if their deformation has not been too significant and might be to a certain degree reversible. This postulation is also supported by the fact that the increase of number of

http://rcin.org.pl

metacyclic forms either coincides or somewhat preceedes the normalization of crithidia if such one is observed.

The examination of metabolism of metacyclic forms would possibly permit to answer the question: which biochemical mechanisms are responsible for the rise of pH in the *T. cruzi* cultures of a more advanced age.

Conclusions

The logarithmic growth phase of the culture of *T. cruzi* is characterized by normal morphology (crithidia and some percentage of metacyclic forms), by a high activity of cytochromoxidase and an insignificant — rather variable — peroxidase activity of crithidia. The last one is reflected on the subsequent development of the culture.

The onset of deformation of the culture coincides in the majority of cases with the passage to the stationary phase of growth and is accompanied by a rise of peroxidase activity in crithidia with a simultaneous fall of cytochromoxidase activity.

At the conclusion of the stationary phase of growth, a temporary rise of the medium pH is observable accompanied by a fall of peroxidase and cytochromoxidase activity, by a raised content of metacyclic forms of *T. cruzi* as well as by the second peak of cell accumulation if the deformation of crithidia has not proceeded too far.

The rise of pH at the conclusion of the stationary phase and the changes connected with it, are involved — in the first place — by the deficiency of carbohydrate nutrition.

Possibly the temporary rise of pH, conditioned by an increase of the number of metacyclic forms, secures a certain "vitalization" of crithidia which is connected with the second peak of cell accumulation.

Peroxidase activity of crithidia plays an essential role in the development of the culture of *T. cruzi*, being connected with its deformation.

Summary

The peroxydase activity of crithidial forms plays an essential part in the development of the *T. cruzi* culture. The enzyme is most active in the medium pH 6.9—6.2 that always indicates the beginning of deformation of the crithidia and usually coincides with passing of the culture into the stationary phase. The degree of peroxydase activity during the first two days of the culture growth (the residual activity of the inoculum) effects in some extent its further development: the degree of deformation and the second peak of cell accumulation.

The cytochromoxydase activity gradually decreases in the course of growth of the culture.

Temporary increase of pH at the end of the stationary phase is connected with the glucose deficiency in the medium and is accompanied by the decrease of cytochromoxydase and peroxydase activity by the increase of the

http://rcin.org.pl

number of metacyclic forms and by second peak of cell accumulation, if the deformation of crithidia was still reversible. The increase of pH might be connected with metacyclic forms and the second peak of cell accumulation with normal crithidia.

РЕЗЮМЕ

В развитии культуры *T. cruzi* существенную роль играет пероксидазная активность критидиальных форм. Максимум активности фермента наблюдается в интервале pH среды 6.9—6.2, что всегда означает начало деформации критидий и обычно совпадает с переходом культуры в стационарную фазу. Степень пероксидазной активности в первые два дня роста культуры (т.е. остаточная активность посевного материала) в известной мере определяет ее дальнейшее развитие: степень деформации и второй пик накопления.

Активность цитохромоксидазы постепенно снижается по мере роста культуры.

Связанный с недостатком глюкозы в среде временный подъем pH в конце стационарной фазы происходит на фоне снижения активности цитохромоксидазы и пероксидазы, повышенного числа метациклических форм и сопровождается вторым пиком накопления, если деформация критидий еще обратима. Видимо, подъём pH связан с метациклическими формами, а второй пик накопления — с нормальными критидиями.

REFERENCES

Balicheva L. V. i Roskin G. I. 1963: Citohimija svobodnyh purinovyx ribonukleotidov v žiznennom cikle *Schizotrypanum cruzi*. Citologija, 5, 630—638.

Brand Th. and Agosin M. 1955: The utilization of Krebs cycle intermediates by the culture forms of *Trypanosoma cruzi* and *Leshmania tropica*. J. infect. Dis., 97, 274—279.

Brand Th., Johnson E. M., Rees Ch. 1946: Observation on the respiration of *Trypanosoma cruzi* in culture. J. Physiol., 30, 163—175.

Brand Th., Tobie E. J., Kissling R. E., Adams G. 1949: Physiological and pathological observations on four strains of *Trypanosoma cruzi*. J. infect. Dis., 85, 5—16.

Chang S. L. 1948: Studies on hemoflagellates. 4. Observations concerning some biochemical activities in culture and respiration of three species of *Leishmanies* and *Trypanosoma cruzi*. J. infect. Dis., 82, 109—118.

Chang S. L. and Nagherbon 1947: Studies on hemoflagellates. 2. A study of the growth rates of *Leishmania donovani, L. brasiliensis, L. tropica* and *Trypanosoma cruzi*. J. infect. Dis., 80, 172—184.

Kallinikova V. D. 1968: Cytochemical study of enzymes of the respiratory chain in the life cycle of *Trypanosoma cruzi* Chagas, 1909. I. Enzymes of the respiratory chain at all stages of the life cycle, in vivo and in culture. Acta Protozool., 5, 395—403.

Kallinikova V. D. i Roskin G. I. 1963: Ribonukleinovaja kislota v žiznennom cikle *Schizotrypanum cruzi*. Citologija, 5, 303—310.

Nemakova-Sukhareva N. N. 1963: Okislitelno-vosstanovitelnyj potencial v kulture *Schizotrypanum cruzi*. Izw. Akad. Nauk SSSR, Ser. biol., 2, 287—292.

Roskin G. I. i Kolomina S. M. 1964: Citohimičeskoe issledovanie kalija i svjazannyh s organičeskimi veščestvami fosfora i železa u *Schizotrypanum cruzi*. Citologija, 6, 175—183.

http://rcin.org.pl

Roskin G. I. i Kozhukhova S. B. 1964: Citohimija tiolovyh grupp u *Schizotrypanum cruzi* i ih izmenenija v cikle žizni protozojnoj kletki. Citologija, 6, 35—43.
Ryley J. F. 1962: Studies on the metabolism of the Protozoa. 9. Comparative metabolism of blood-stream and culture forms of *Trypanosoma rhodesiense*.
Warren L. G. 1960: Metabolism of *Schizotrypanum cruzi* Chagas. I. Effect of culture age and substrate concentration on respiratory rate. J. Parasit., 46, Biochem. J., 1, 211—223.
529—539.
Zeledon R. 1960: Comparative physiological studies on four species of hemoflagellates in culture. 3. Effect of the Krebs cycle intermediates on the respiration. Revta Biol. trop., Univ. Costa Rica, 8, 25—34.

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 10

Лаборатория зоологии беспозвоночных, Биологический Институт Ленинградского Государственного Университета, Ленинград В-164, СССР

Laboratory of invertebrate Animals, Biological Research Institute of Leningrad State University, Leningrad B-164, USSR

Л. Н. СЕРАВИН
L. N. SERAVIN

Роль механических и химических раздражителей в индуцировании фагоцитарной реакции у *Amoeba proteus* и *A. dubia*

The role of mechanical and chemical stimulators on the induction of phagocytic reactions in *Amoeba proteus* and *A. dubia*

В естественных условиях полиподиальные амебы типа *Amoeba proteus* (сюда же можно отнести *A. dubia*, *A. discoides* и *Chaos chaos*) питаются главным образом живой пищей. Добычей этих амеб становятся простейшие (инфузории и жгутиконосцы), водоросли и даже мелкие многоклеточные животные, например коловратки. Некоторые исследователи (Gibbs and Dellinger 1908, Mast and Hahnert 1935) прямо утверждают, что *Amoeba proteus* не захватывает ничего мертвого. Имеются экспериментальные данные, показывающие, что амебы могут заглатывать и несъедобные частицы (например, кусочки стекла), если они тем или иным способом были приведены в движение (Schaeffer 1914, Christiansen and Marshall 1965). Факты такого рода дают, казалось бы, основание сделать вывод, что захват пищи у *A. proteus* и других полиподиальных амеб определяется главным образом механическим раздражением, которое оказывают на этих простейших живые движущиеся объекты. Однако уже Дженнингс (Jennings 1904) отметил, что реакция *A. proteus* на пищу хотя бы частично обусловлена ее химическим действием. Шаффер (Schaeffer 1912, 1914, 1916 a, b, 1917) в целом ряде тщательно поставленных экспериментов показал, что полипоидиальные амебы могут захватывать неподвижные частички некоторых белков, изолированных из тканей животных и растений (глобулин, фибрин и др.), а также частички кармина и мочевой кислоты. Белл (Bell 1963) вызывал пищевую реакцию у *A. proteus* с помощью капилляра, наполненного гомогенатом, который был приготовлен из живых гидр (*Hydra*).

Шаффер (1914, 1916 a, b) отмечает, что нередко амебы чувствуют твердые частички различных веществ на расстоянии 50—60 μ, а по данным Джеана и Белла (Jean and Bell 1965), — и до 200 μ. Однако, по мнению Христиансена и Маршала (Christiansen and Marshall 1965), для того, чтобы возникла фагоцитарная реакция у *Chaos chaos*, необходим прямой контакт тела простейшего с пищей.

По данным Шаффера (1915 a, 1917), на неподвижные частички белковых веществ реагируют *Amoeba proteus* и *A. discoides*, тогда как *A. dubia* заглаты-

http://rcin.org.pl

вают обычно лишь подвижные объекты. Он отмечает при этом, что и для первых двух видов амеб захватывание пищи может определяться механическими раздражениями, поэтому и *A. proteus* и *A. discoides* поглощают частички стекла, угля и других несъедобных веществ, в том случае, если эти частички приведены в движение.

По-видимому, вопрос об относительной роли механического и химического факторов в возникновении фагоцитарной реакции у полиподиальных амеб нуждается в дальнейшем изучении.

Процесс захватывания пищи полиподиальной амебой описан многими авторами (Rhumbler 1898, Mast and Root 1916, Kitching 1956, Bell 1963, Christiansen and Marshall 1965). При сближении с пищевым объектом амеба образует в его направлении несколько псевдоподий, которые вскоре сливаются, давая начало пищевой чашечке. Внутренний конец этой чашечки оказывается замкнутым, а наружный открыт и ведет в полость чашечки, величина которой обычно соответствует размерам заглатываемой добычи. Когда наружное отверстие пищевой чашечки замыкается (благодаря притекающей сюда цитоплазме), образуется пищеварительная вакуоль, внутри которой оказывается захваченный объект. Вакуоль ограничена мембраной, которая происходит из плазмалеммы простейшего.

Основная цель настоящей работы заключается в том, чтобы уточнить роль механических и химических раздражителей в индуцировании пищевой чашечки у полипоидальных амеб.

Материал и методика

Опыты проводились на *Amoeba proteus*[1] и *Amoeba dubia*[2]. Амеб культивировали на среде Прескота общепринятым способом (Prescott and James 1955, Chapman-Andersen 1962, Юдин 1963), пищей служили тетрахимены (*Tetrahymena pyriformis*). Для опытов использовались амебы, голодавшие 1—3 дня.

В экспериментах по кормлению амеб твердыми частичками исходное вещество расстирали в ступке, затем полученный порошок помещали в сосуд со средой Прескота, откуда необходимое количество этих частичек брали с помощью стеклянной пипетки. В опытах по исследованию пищевой реакции амеб на растворы различных веществ использовали капилляры с оттянутыми кончиками (с внутренним диаметром кончика 10—30 μ). Капилляр, заполненный исследуемым раствором, укреплялся под нужным углом пластилином на стеклянной трубочке держателя микроманипулятора ММ-1, с помощью которого производились все дальнейшие операции: кончик капилляра подводился к амебе на то или иное расстояние. Наблюдения за фагоцитарной реакцией амебы осуществлялось в темном поле под бинокулярным микроскопом МБС-2. Растворы всех веществ приготовлялись на среде Прескота.

---

[1] Пользуясь случаем, приношу искреннюю благодарность сотруднику института Цитологии АН СССР А. Л. Юдину за предоставленные культуры *A. proteus*.

[2] Культуры *A. dubia* были получены благодаря любезности сотрудницы Карлсбергской лаборатории (Копенгаген, Дания) д-ра Cecil Chapman-Andresen с помощью д-ра M. Müller; приношу этим исследователям самую глубокую благодарность.

http://rcin.org.pl

Для получения однозначных результатов следует соблюдать ряд предосторожностей при заполнении капилляров и во время работы с ними. Для того, чтобы заполнить капилляр, его широким концом опускали в микроаквариум с испутуемым раствором; под влиянием капиллярных сил жидкость полностью заполняет капилляр. После этого его укрепляли на держателе микроманипулятора и оттянутым кончиком опускали в микроаквариум с испытуемым раствором. Вслед за этим широкий конец запаивали на пламени спиртовки. При нагревании часть жидкости, естественно, выталкивается из капилляра, но по мере остывания он вновь заполняется через узкий конец. Капилляр пригоден для работы только тогда, когда движение жидкости в нем полностью прекратится. Даже небольшие колебания внешней температуры вызывают истечение жидкости из капилляра, поэтому после его заполнения экспериментатор не должен касаться капилляра руками.

Вибрацию кончика капилляра (или стеклянной иглы) для механического раздражения амебы вызывали частым постукиваением ножкой маленького пинцета по стеклянному держателю микроманипулятора или же ритмическими движениями микровинта точной наводки микроманипулятора.

Каждый опыт повторяли не менее чем на 20—50 амебах.

Результаты исследования

Если вблизи от движущейся *Amoeba proteus* поместить небольшие (10—40 μ в длину) частички таких веществ, как уголь, крахмал, стекло, кварц, селикогель, казеин, лецитин, холестерин или полистерол, простейшее к некоторым из них останется индифферентным (стекло, кварц, селикогель, полистерол, крахмал), на другие же реагирует положительно, начиная двигаться в их направлении [3]. Особенно четким привлекающим действием обладают желатин, холестерин и лецитин. К кусочкам этих веществ амеба направляется, изменяя первоначальное направление своего движения. Достигнув частички холестерина, простейшее обычно вновь меняет направление движения и уходит прочь. Частички желатина или лецитина амеба обхватывает псевдоподиями, однако образование пищевой чашечки в этих случаях не происходит, и обхваченные кусочки не заглатываются. Это показывает, что не всякое вещество, которое оказывает привлекающее действие, может вызвать у амебы фагоцитоз. Частички веществ, к которым амебы индифферентны, не захватываются даже при прямом контакте *A. proteus* с ними.

Крупинки желтка вареного куриного яйца не только вызывают у амеб положительный хемотаксис, но и охотно поедаются этими простейшими. У *A. proteus*, двигающейся к такой крупинке, уже до соприкосновения с ней (на расстоянии 30—50 μ) начинает формироваться пищевая чашечка. Если около амебы оказывается сразу несколько крупинок желтка, она может начать образование 3—4 пищевых чашечек одновременно, без контакта с пищевыми частичками. В том случае, когда крупинки желтка расположены так, что одна оказывается вблизи переднего, а другая — вблизи заднего конца тела *A. proteus*, простейшее начинает двигаться сразу в двух противоположных направлениях. В некоторых случаях при этом наблюдается разрывание тела амебы на две части. Поскольку это происходит еще до контакта простейшего с частичками, можно сделать вы-

[3] В этой части работы принимала участие Л. В. Славашевская.

http://rcin.org.pl

вод, что химическое пищевое раздражение оказывает чрезвычайно сильное воздействие на *A proteus*, и им никак нельзя пренебрегать при анализе поведения этих одноклеточных организмов (см. также Bell 1963).

Частички активированного угля не вызывают образование пищевой чашечки у амеб и не заглатываются этими простейшими. Однако такое утверждение справедливо лишь при условии, что уголь предварительно не находится в контакте с пищевыми веществами. Мы помещали кусок вареного куринного желтка в пробирку со средой Прескота, встряхиванием приготовляли суспензию этого белка. Затем фильтрованием отделяли частицы. В отфильтрованную жидкость на 30 мин. помещали частички активированного угля, который, как известно, является прекрасным адсорбентом. Затем угольные частички фильтрованием отделялись от жидкости, тщательно промывались средой Прескота и после этого использовались в опытах по кормлению амеб.

Частички угля, бывшие в контакте с раствором желтка, вызывают у *A. proteus* образование пищевой чашечки и поедаются этими простейшими столь же охотно, как и зерна желтка. Однако в этом случае фагоцитарную реакцию вызывает уже не сам желток, а его „запах", перенесенный на уголь.

Как показали опыты, *A. proteus* охотно поедают свежеубитых простейших. Мы убивали парамеций (*Paramecium caudatum*), помещенных в раствор Прескота, трехминутным нагреванием при температуре 60°. Если каплю умервщленных таким образом, совершенно неподвижных туфелек добавить в микроаквариум с голодными амебами, последние моментально начинают образовывать пищевые чашечки, обычно еще до контакта с парамециями. Фагоцитарная реакция выражена столь сильно, что у некоторых *A proteus* пищевые чашечки полностью замыкаются, так что образуются настоящие (хотя и пустые) пищеварительные вакуоли прежде, чем амебы коснутся поверхности тела убитой парамеции.

Сколь долгое время мертвые парамеции способны вызывать фагоцитоз у *A. proteus*? Чтобы ответить на этот вопрос, мы помещали убитых нагреванием инфузорий в микроаквариум, в котором среда Прескота сменялась через каждый час. После очередной смены часть парамеций изымалась из микроаквариума и использовалась для кормления амеб. Через 4 часа после начала такого вымачивания реакция амеб на инфузорий оказалась сильно ослабленной, а через 6 час *A. proteus* перестали образовывать пищевые чашечки даже при прямом контакте с мертвыми парамециями. Однако, если инфузорию, которая перестала быть пищевым раздражителем для амебы, насадить на стеклянную иглу микроманипулятора, а кончик иглы заставить вибрировать, амеба, оказавшаяся в контакте с парамецией, начинает образовывать пищевую чашечку, а в конечном итоге полностью заглатывает инфузорию. Механическое раздражение (вибрация), по-видимому, повышает чувствительность *A. proteus* к химическому пищевому радражению.

Мертвая парамеция, которая вымачивалась в растворе Прескота 8 час и более, не вызывает у амебы образование пищевой чашечки даже в том случае, если парамеция будет насажена на вибрирующую стекляную иглу. Следовательно, одного механического раздражения недостаточно, чтобы *A. proteus* реагировала на пищевой объект, нужен еще „запах" пищи. Это особенно четко было выявлено в следующих опытах.

К переднему концу тела *A. proteus* подводили стеклянную иглу (на расстояние 15—25 μ от плазмалеммы). Простейшее не реагировало на нее. Тогда постукиванием пинцета по держателю иглы вызывали слабую вибрацию. Амеба моментально реагировала на это механическое раздражение, направлялась к его

http://rcin.org.pl

источнику и начинала обхватывать псевдоподиями кончик иглы. Однако образование пищевой чашечки не происходило. Мы повторяли эту операцию на 120 особях *A. proteus* и всегда получали один и тот же результат.

Если кончик стеклянной иглы 3—4 раза погрузить в тело только что убитой сильным нагреванием парамеции, а затем поднести его к переднему концу тела амебы, моментально возникает пищевая реакция. Еще до контакта с иглой *A proteus* начинает образовывать пищевую чашечку. Такой иглой можно вызвать фагоцитарную реакцию у 3—4 амеб подряд. Но затем „запах", по-видимому, ослабевает, и простейшие перестают образовывать пищевую чашечку даже при прямом контакте с кончиком иглы. Если вызвать вибрацию такой иглы, пищевая чашечка у амебы возникает. Это еще раз подтверждает, что механическое раздражение снижает порог чувствительности *A. proteus* к химическому раздражению.

Контрольные опыты показали, что если конец иглы, погружавшийся в цитоплазму убитой парамеции, сразу же обмыть кипящей водой, то он не вызывает пищевую реакцию у амеб.

Как уже отмечалось, вареный желток куриного яйца индуцирует у *A. proteus* образование пищевой чашечки. Этим же свойством, по-видимому, обладает и водная вытяжка из этого белка, поскольку активированный уголь, находившийся в ней некоторое время, начинает вызывать у простейших четко выраженную фагоцитарную реакцию. Однако, если *A. proteus* поместить в водную вытяжку желтка, образование пищевой чашечки не наблюдается. Амебы явно возбуждены, двигаются быстрее, чем в среде Прескота, но пищевой реакции у них не возникает. Отсутствие фагоцитарной реакции у простейших в том случае, когда они со всех сторон окружены веществом, которое способно индуцировать такую реакцию, можно объяснить одной из двух следующих причин: а) амебы образуют пищевую чашечку только в ответ на локальное химическое действие пищевого раздражителя, б) пищевая чашечка может возникать только лишь в том случае, когда в среде присутствуют твердые частицы. Какое из двух этих предположений является правильным, — было проверено экспериментально.

A. proteus помещали в микроаквариум с водной вытяжкой из желтка куриного яйца, туда же добавляли мелкие частички стекла, кварца или полистерола, которые в обычных условиях не захватываются престейшими. Однако и в этом опыте амебы не образовывали пищевые чашечки даже при прямом контакте с твердыми частичками. Если же в экспериментальный раствор добавить частички активированного угля, *A. proteus* начинает фагоцитировать их. Это объясняется тем, что уголь является адсорбентом и благодаря этому его частички вскоре оказываются более сильным пищевым раздражителем, чем окружающая среда (содержащая вытяжку из желтка). Имеея небольшие линейные размеры (10—30 μ), частички угля, адсорбировавшие „запах" пищи, могут индуцировать у амеб фагоцитоз. Когда частичка имеет довольно большие размеры, *A. proteus* реагирует на нее положительным хемотаксисом, иногда распластывается по ее поверхности, но пищевой чашечки не образуется.

Уже основываясь на этих опытах, можно сделать предварительный вывод, что пищевая чашечка возникает у амебы только под влиянием локально действующего пищевого раздражителя. Если этот вывод верен, то, по-видимому, типичную пищевую реакцию можно вызвать у амебы не только с помощью твердых частиц, но и при действии на этих простейших растворами тех или иных химических агентов, если подводить их к какому-то определенному

http://rcin.org.pl

участку тела амебы, например с помощью капилляра. Дальнейшие эксперименты подтвердили правильность этого предположения.

Прежде всего мы исследовали с помощью капилляров влияние на *A. proteus* гомогената, приготовленного из парамеций, которые были убиты высокой температурой, а также влияние водной вытяжки из вареного желтка куриного яйца. В обоих случаях кончик капилляра подводили так, что он оказывается на расстоянии 50—60 μ от переднего конца тела амебы. *A. proteus* сразу же начинала двигаться в сторону капилляра, если даже для этого ей приходилось изменять первоначальное направление движения. Уже на расстоянии 20—30 μ от кончика капилляра амеба начинала образовывать пищевую чашечку. Если до того как *A. proteus* коснулась кончика капилляра, его начать медленно оттягивать прочь от простейшего, амеба ползет за капилляром, непрерывно наращивая чашечку на переднем конце. В результате все тело амебы превращается в большую пищевую чашечку, которая замыкается. Внутри простейшего оказывается большая пустая пищеварительная вакуоль. Весь этот процесс протекает без прямого контакта *A. proteus* с капилляром. Гранулы гомогената ярко светятся в темном поле микроскопа, поэтому во время опыта хорошо видно, что жидкость в капилляре остается неподвижной, т.е. не происходит ее истечение наружу.

С помощью одного и того же капилляра, наполненного гомогенатом парамеций или водной вытяжкой из желтка, можно вызвать образование пищевой чашечки у большого числа амеб (у 14—18 особей подряд).

В контрольных опытах использовались капилляры, заполненные чистой средой Прескота. В этом случае вызвать фагоцитарную реакцию у *A. proteus* не удается ни на расстоянии, ни при прямом контакте капилляра с простейшим, ни при раздражении амебы вибрирующим капилляром.

Безусловно, было бы весьма интересно выявить круг веществ, которые могут индуцировать пищевую реакцию у амебы. Поэтому были поставлены соответствующие опыты. Они показали, что хорошими индукторами фагоцитоза являются растворы очищенных белков, например, ферментов (Табл. 1). Капилляры, наполненные 2%-ми растворами гиалуронидазы, пепсина, трипсина или цитохрома С, вызывают у *A. proteus* образование пищевых чашечек на расстоянии, т.е. без прямого контакта. Когда в опыте используется натуральный яичный белок, амебы образуют пищевую чашечку только в том случае, если они касаются кончика капилляра с этим веществом. 1—2%-е растворы желатина вообще не вызывают пищевую реакцию у *A. proteus*. Чем определяются эти различия, — пока еще трудно сказать. Возможно, что диффузия молекул различных белков из капилляра в окружающую среду происходит с неодинаковой скоростью. Не исключена возможность, что имеет значение и природа белка. Например, известно, что в желатине, в отличие от многих других белков, практически отсутствует цистеин (Jirgensons 1962). Только дальнейшие исследования могут дать ответ на этот вопрос.

Как показали наши опыты, кроме белков образование пищевой чашечки у *A. proteus* вызывают твины (Твин 40, 60 и 80) и кармин [4]. С помощью капиллярного метода было установлено, что нуклеиновые кислоты, аминокислоты, дипептиды, полисахариды, неорганические соли и некоторые другие вещества не индуцируют фагоцитоз (Табл. 2), хотя большей частью растворы применявшихся веществ испытывались в широком диапазоне концентраций.

[4] Шаффер 1917 сообщает, что продажный кармин всегда содержит примеси различных органических веществ, например лецитина.

http://rcin.org.pl

Таблица 1

Table 1

Список веществ, растворы которых вызывают образование пищевой чашечки у *Amoeba proteus*

Substances evoking the formation of food cup in *Amoeba proteus*

1. Гомогенат, приготовленный из *Paramecium caudatum*, убитых трехминутным нагреванием при 60°.
   Homogenate prepared of *Paramecium caudatum* killed by temp. of 60° applied for 3 min.
2. Натуральный белок куриного яйца
   Natural protein of hen egg
3. Натуральный желток куриного яйца
   Natural yolk of hen egg
4. Водная вытяжка из вареного желтка куриного яйца
   Aqueous extract of boiled hen yolk
5. Пептон
   Peptone
6. Гиалуронидаза
   Hyaluronidase
7. Липаза
   Lipase
8. Эластаза
   Elastase
9. Алкогольдегидрогеназа
   Alkohol dehydrogenase
10. Цитохром С
    Cytochrome C
11. Пепсин
    Pepsin
12. Трипсин
    Trypsin
13. Сывороточный альбумин
    Serum albumin
14. Твин 40
    Tween 40
15. Твин 60
    Tween 60
16. Твин 80
    Tween 80
17. Водная суспензия кармина
    Aqueous suspension of carmin

Примечание: No 5 — No 13 применялись в виде 2%-ых растворов, No 14 — No 16 применялись в виде 5%-ых растворов

Remark: Nrs 5—13 were applied as 2% solutions, 14—16 as 5% solutions

Проведенные исследования показывают, что пищевая реакция *A. proteus* зависит от концентрации белка или твина в капилляре. Амебы индифферентны по отношению к капилляру, заполненному 0.001%-ым раствором пепсина. Более высокие концентрации этого белка вызывают у простейших позитивную двигательную реакцию. Когда капилляр содержит 0.5%-ый раствор пепсина, амебы четко положительно реагируют на него, хотя образования пищевой чашечки не наблюдается. Правда, пищевая реакция у *A. proteus* возникает, если химическое раздражение сочетать с механическим (вызывая вибрацию капилляра, наполненного 0,5%-ым раствором пепсина).

Если к переднему концу тела ползущей *A. proteus* подвести кончик капилляра, наполненного 1%-ым раствором пепсина, простейшее быстро образует пищевую чашечку. Этот же капилляр не индуцирует пищевую реакцию, если он поднесен к средней или задней части тела *A. proteus*. Однако пищевая чашечка может образоваться и здесь, если кончик капилляра будет слегка вибрировать. 2 или 5%-ые растворы пепсина вызывают пищевую реакцию, к какой бы части тела простейшего не был поднесен капилляр.

Аналогичные результаты были получены также в опытах с Твином 80. Капилляр, наполненный 10%-ым раствором этого вещества, вызывает образование пищевой чашечки в любой части тела *A. dubia*. 3%-ый раствор Твин 80 индуцирует образование пищевой чашечки только в области переднего конца тела простейшего; 1%-ый раствор вызывает фагоцитарную реакцию только в том случае, если кончик капилляра вибрирует.

http://rcin.org.pl

Таблица 2

Table 2

Список веществ, растворы которых не вызывают образование пищевой чашечки у *Amoeba proteus*

Substances not evoking the formation of food cup in *Amoeba proteus*

Вещество Substance	Концентрация Concentration of solution
1—3. NaCl, KCl, $CaCl_2$	0.01 — 0.125 M
4. АТФ ATP	1.10^{-4} — 5.10^{-3} M
5. Глютаминовая к-та Glutamic acid	0.01 — 0.1 M
6. Глицин Glycine	0.01 — 0.1 M
7. α-Аланин α-Alanine	0.01 — 0.1 M
8. Аргинин Arginine	0.01 — 0.1 M
9. Аспарагиновая к-та Aspartic acid	0.02 — 0.05 M
10. Гистидин Histidine	0.1 M
11. Лизин Lysine	0.1 M
12. Метионин Methionine	0.1 — 0.05 M
13. Серин Serine	0.01 — 0.1 M
14. Аланил-аланин Alanine-alanine	0.01 — 0.1 M
15. Глицил-лейцин Glycine-leucine	0.01 — 0.1 M
16. Желатин Gelatine	1 — 2%
17. РНК RNA	0.1 — 1%
18. ДНК DNA	0.01 — 0.2%
19. Холестерин Cholesterin	0.01 — 0.05%
20. Лецитин Lecitine	0.1 — 0.5%
21. Водорастворимый крахмал Water soluble starch	0.5 — 2%
22. Гуммиарабик Gum arabic	0.5 — 2%
23. Трагант Tragant	0.5 — 2%
24. Окси-метилцеллюлоза Oxy-methylcellulose	0.5 — 2%
25. Инулин Inulin	1 — 5%
26. Глюкоза Glucose	1 — 5%
27. Сахароза Saccharosa	1 — 5%

http://rcin.org.pl

Эти данны говорят о том, что вдоль тела ползущей *A. proteus* существует передне-задний градиент чувствительности к пищевым раздражителям. Они также доказывают, что механическое раздражение повышает чувствительность амебы к химическим пищевым раздражениям.

Капилляры, наполненные 2%-ми растворами пепсина, трипсина, гиалуронидазы или цитохрома С, уже на расстоянии, т.е. еще до контакта с плазмалеммой, вызывают образование пищевой чашечки у другой полиподиальной амебы — *A. dubia*. В этом отношении она ничем не отличается от *A. proteus*. Однако, если к переднему концу тела *A. dubia* подвести вибрирующий кончик стерильной стеклянной иглы, у этой амебы моментально возникает фагоцитарная реакция; медленно убирая вибрирующую иглу, можно вызвать полное замыкание образовавшейся пищевой чашечки. В результате образуется пустая пищеварительная вакуоль, индуцированная действием механического раздражителя. Та же самая игла в контрольных опытах не вызывает пищевую реакцию у *A. proteus*.

Обсуждение результатов

Полученные данные, на наш взгляд, доказывают, что химические раздражители играют весьма большую роль в возникновении пищевой реакции у полиподиальных амеб. В самом деле, убитая нагреванием парамеция, частичка угля, находившаяся в контакте с пищевым веществом, стеклянная игла, несущая следы цитоплазмы инфузории, наконец капилляр, наполненный раствором белка, — все эти неживые, неподвижные объекты легко вызывают образование пищевой чашечки у *A. proteus*. Пищевая чашечка может возникать и даже замыкаться в пищеварительную вакуоль еще в тот период, когда амеба не находится в непосредственном контакте с объектом, обладающим „запахом" пищи. Следовательно, фагоцитарная реакция может возникать под влиянием одного химического пищевого раздражения. С помощью одного лишь механического раздражения вызвать пищевую реакцию у *A. proteus* невозможно; простейшее реагирует на вибрирующий кончик стеклянной иглы положительной двигательной реакцией, но пищевую чашечку не образует. Это не означает, однако, что механический фактор не играет никакой роли в фагоцитарной реакции *A. proteus*. Механическое раздражение вызывает повышение чувствительности амебы к химическому действию пищи. Благодаря этому *A. proteus* охотнее всего и в первую очередь захватывает и заглатывает живые подвижные объекты, более редко ее добычей становятся неподвижные водоросли и т.п. Неживые частички, которые не обладают „запахом" пищи, *A proteus* практически никогда не поедает. У *A. dubia* пищевую реакцию может вызвать как „запах" пищи, так и механическое раздражение. В результате это простейшее заглатывает не только пищевые объекты, но и несъедобные частички, которые приведены в движение случайными причинами (например, током воды). Некоторым исследователям удавалось накормить *A. dubia* шариками ртути и золота (Allen 1961). Однако и эти амебы легче и быстрее реагируют на двигающиеся объекты, обладающие „запахом" пищи.

Имеющиеся данные позволяют сделать вывод, что физиологические механизмы, контролирующие возникновение пищевой реакции, не одинаковы у разных видов полиподиальных амеб. Поэтому и способность выбирать пищу не одинакова у разных форм этих простейших.

http://rcin.org.pl

Резюме

Капилляры, наполненные раствором какого-нибудь белка (гиалуронидазы, липазы, эластазы, алкогольдегидрогеназы, цитохрома С, пепсина, трипсина и др.) вызывают фагоцитарную реакцию у *Amoeba proteus* и *A. dubia*. Сначала образуется пищевая чашечка, а затем и пищеварительная вакуоль. Для этого зачастую не нужно никакого контакта амебы с капилляром. Кроме белков четкую пищевую реакцию у *A. proteus* вызывают растворы твинов (Твин 40, 60 и 80). Все остальные использованные химические агенты: нуклеиновые кислоты, аминокислоты, дисахариды, полисахариды, неорганические соли и др. — в этом отношении оказались совершенно неэффективными. Механическое раздражение (вибрация кончика капилляра) увеличивает чувствительность *A. proteus* к химическому действию пищи. *A. proteus* в норме не захватывает такие несъедобные вещества, как стекло, кварц, полистерол, уголь и др. Если же частички активированного угля предварительно выдержать в растворе, содержащем водный экстракт желтка куриного яйца, они охотно поедаются этими простейшими. Следовательно, „запах" пищи делает частички угля съедобными для *A. proteus*.

У *A. dubia* фагоцитарную реакцию (образование пищевой чашечки) можно вызвать не только с помощью одного химического раздражителя (капилляром, наполненным белковым раствором), но также и одним механическим раздражителем (вибрацией кончика стеклянной иглы). На основании этих и других опытов делаются выводы, что а) химические пищевые раздражения играют большую роль в выборе пищи у *A proteus* и *A. dubia* и б) роль механических раздражений в выборе пищи различна у двух этих видов амеб.

SUMMARY

Glass capillaries filled with a solution of any protein (hyaluronidase, lipase, elastinase, alcohol dehydrogenase, cytochrome C, pepsin, trypsin and others) evoke a phagocytic reaction in *Amoeba proteus* and *Amoeba dubia*. Initially a food cup is formed and subsequently a food vacuole. For this, no any contact of amoeba with the capillary is necessary. Besides the proteins, a distinct alimentary reaction in *A. proteus* is evoked by tween solutions (tween 40, 60, and 80). All the other chemical agents applied by us: nucleic acids, amino acids, disaccharides, polysaccharides, inorganic salts and others proved to be fully uneffective in this regard. The mechanical agents (vibration of the capillary tip) augment the sensivity for chemical action of food in *A. proteus*. Normally it fails to retain the inedible substances as glass, quartz, polysterol, carbon etc. However the particles of activated carbon which had been kept in a solution containing water extract of hen yolk, are readily ingested by amoeba. Consequently, the "smell" of food makes the carbon particles edible for this protozoon.

In *A. dubia*, the phagocytic reaction (formation of the food cup) may be evoked not only by means of chemical stimulation (capillar filled with protein solution) but also by mechanical stimulation only (vibration of the tip of a glass needle). The above experiments — as well as some others — permit to conclude that: a. chemical alimentary stimulations play an important role in food selection in *A. proteus* and *A.dubia*, b. the role of mechanical stimulations in food selection is different in those two amoeba species.

http://rcin.org.pl

ЛИТЕРАТУРА

Allen R. D. 1961: Ameboid movement. In "The cell", 2, 135—216, N. Y.

Bell L. G. E. 1963: Some observations concerning cell movement and cell clevage. Int. soc. for cell biol., 2, 215—228.

Chapman-Andresen C. 1962: Studies on pinocytosis in amoebae. C. r. Trav. Lab. Carlsberg., 33, 73—264.

Christiansen R. G. and Marshall J. M. 1965: A study of fagocytosis in ameba *Chaos chaos*. J. Cell Biol., 25, 443—458.

Gibbs D. and Dellinger A. P. 1908: The daily life of *Amoeba proteus*. Am. J. Psychol., 19, 230—241.

Jeon K. W. and Bell L. G. E. 1965: Chemotaxis in large, free-living amoebae. Expl. Cell Res., 38, 536—555.

Jennings H. S. 1904: Behavior of the lower organisms. N. Y., Columbia Univ.

Jirgensons B. 1962: Natural organic macromolecules. Pergamon Press, Oxford. L., N. Y., Paris.

Юдин А. Л. 1963: Сравнительная чувствительность ядра и цитоплазмы *Amoeba proteus* к поврежденному действию акрифлавина и уретана (опыты по трансплантации ядер). Цитология злокачественного роста. Сб. 7, 128—144, Изд. Академии Наук СССР, М.-Л.

Kitching J. A. 1965: Food vacuoles. Protoplasmalogia. III D 3b, I-57.

Mast S. O. and Hahnert W. 1935: Feeding, digestion and starvation in amoeba. Physiol. Zool., 8, 255—272.

Mast S. O. and Root F. M. 1916: Observation on ameba feeding on Rotifers, Nematodes and Ciliates, and their bearing on the surface-tension theory. J. exp. Zool., 21, 33—49.

Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus* on *Tetrahymena*. Expl. Cell Res., 8, 256—258.

Rhumbler L. 1898: Die verschiendenartigen Nahrungsaufnahmen bei Amoben als Folge verschiendener Colloidalzustände ihrer Oberflächen. Arch. Entw. Mech. Org., 30, 194—223.

Schaeffer A. A. 1912: Selection of food among lower animals. Trans. Tenn. Acad. Sci., I, 35—43.

Schaeffer A. A. 1914: Feeding habits of amoeba. Science. 39, 272.

Schaeffer A. A. 1916 a: On the behavior of amoeba toward fragments of glass and carbon and other indigestible substances, and toward some very soluble substances. Biol. Bull. 31. 303—328.

Schaeffer A. A. 1916 b: On the feeding habits of amoeba. J. exp. Zool., 20, 529—584.

Schaeffer A. A. 1917: Choice of food in amoeba. J. Anim. Behav., 7, 220—258.

http://rcin.org.pl

http://rcin.org.pl

ACTA PROTOZOOLOGICA
VOL. VI WARSZAWA, 31.VIII.1968 FASC. 11

Department of Zoology, University of California Los Angeles, California 90024, United States of America and Department of General Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa 22, Pasteura 3, Poland

Leszek KUŹNICKI

Behavior of *Paramecium* in gravity fields. I. Sinking of immobilized specimens[1]

Zachowanie się *Paramecium* w polach grawitacyjnych I. Opadanie immobilizowanych orzęsków

The effects of gravity on protozoa were first described at the end of the 19th century. In mass cultures in flasks or tubes, specimes often showed a tendency to swim toward the top and to collect in the upper region of a container. This phenomenon was called "negative geotaxis" ("negative geotropisms"). The first observations of geotaxis were made on *Chlamydomonas* and *Euglena* (Schwarz 1884, Aderhold 1888, Massart 1891), but the majority of later research concetrated on *Paramecium*. Four hypotheses have been proposed for the mechanism of space orientation of protozoa:

1. Jensen's 1893 "pressure hypothesis." *Protozoa* were thought to have a difference in hydrostatic pressure between the upper and lower surfaces of their body. In a horizontally swimming *Paramecium*, greater hydrostatic pressure accelerates ciliary beating, and in consequence, the animal changes the direction of movement vertically.

2. Davenport's 1897 "resistance hypothesis." *Protozoa* are heavier than water. Swimming upward increased the resistance more than swimming downward, bringing about their vertical orientation.

3. The "Statocyst hypothesis," proposed independently by Loeb 1897 and Lyon 1905 and developed by Kanda 1914, 1918, Lyon 1918, and Koehler 1922, 1930. This implies an analogy between balance sensory organs of metazoans and the protozoan cell. *Paramecium* contains many inclusions (granules, crystals, food vacoules) and nuclei of greater specific gravity than the cytoplasm. According to the statocyst hypothesis the entire protozoan cell acts as a statocyst, since it contains substances of greater specific gravity called statoliths. Statoliths press down on the cytoplasm, in the direction of gravity, tranferring a stimulus to the cilia resulting in augmentation of ciliary stroke. Thus, stimulated paramecia are oriented parallel and opposite to the action of gravity.

4. The "Mechanical hypothesis," proposed by Verworn 1889, supported by Harper 1911, 1912 and fully developed by Dembowski 1929 a,

[1] Supported by grant 5T1 AI 70-09 from N. I. H.

http://rcin.org.pl

1929 b, 1931. Space orientation of protozoa is a purely physical, or passive, effect. It depends upon the position of the center of gravity in the animal's body. The behavior of *Paramecium caudatum* in gravity fields was explained as follows by Dembowski. The center of gravity in all ciliates is located posteriorly. Negative geotaxis occurs as an effect of agitation of paramecia by many different kinds of (chemical, mechanical) stimuli. In the normal culture conditions this could be observed. The first response of *Paramecium* on stimulation is stopping, or a weakened ciliary beat for a few seconds. It lasts as long as the position of sinking specimens is exactly vertical. Then, *Paramecium* begins to swim forward in a straight line, until it reaches the top of the tube. When approaching upper surface, the ciliate slows its speed of movement or comes to a stop for a few seconds and then swims downwards. Swimming velocity downward and upward is the same. At the bottom, animals do not change speed of movement but rebound and swim upward. The origin of the geotactic aggregation is statistical in nature. In the first phase, after stimulation, most specimens swim upward on a straight path. In the second phase even with the same physical properties at the upper and lower surface of the tube, aggregation occurred by means of the difference in motile behavior of the ciliate on those levels.

Besides those mentioned above, many papers have been published about geotaxis; however, in the most recent review Jahn and Bovee 1967 stated: "Little is known for protozoa." That opinion is correct since:

1. Very little evidence concerning geotaxis is unquestioned.

2. At the present time no hypothesis of the mechanisms of space orientation of protozoa seems to be satisfactory. Jennings 1904 and Lyon 1904 showed Jensen's pressure hypothesis to be inadequate. Animals can not differentiate hydrostatic pressures below 10^{-6} atm. existent between anterior and posterior end of the ciliates body. Schaefer 1922 also showed, that the swimming velocity of *P. aurelia* is equal at different hydrostatic pressures.

Davenport's resistance hypothesis was examined by Platt 1899 and Lyon 1905. Ciliates were immersed in the medium of the same density as themselves and negative geotactis occurred. Paramecia placed in gum arabic solutions with a specific gravity higher than their specific weight, did not show positive geotaxis, as it should have according to Davenport's explanation.

Recently the statocyst hypothesis has not been accepted from any point of view. As a matter of fact the only experimental basis was the centrotactic reaction. After centrifuging all paramecia swim centripetally irrespective of the tube position in space (Koehler 1922). However, Dembowski 1931 demonstrated that this is not an exact observation. Centrotaxis is really a normal negative geotactic reaction. After centrifuging in the tube placed at horizontal position, paramecia swim centripetally but always rebound off the upper surface of the tube. If the tube has a branch tube directed vertically, animals show typical negative geotaxis. On the other hand, in light of recent research it is improbable that *Paramecium* have sensory structures in the endoplasm which influence the ciliary beat.

The mechanical hypothesis also is in considerable doubt. Koehler 1939 recorded on film the response to stimuli of fragments of *Paramecium*. All merozoa showed negative geotaxis in the same general manner as the

http://rcin.org.pl

entire animal. Śmiechowska (unpublished), observed in *P. caudatum* vertical movement of filial cells after division and in specimens with cut posterior ends. In this same condition dividing and injured specimens showed typical negative geotactic reactions.

Irrespective of that contradictory evidence, the basis of the mechanical hypothesis, i.e., the opinion that the posterior end of *Paramecium caudatum* is heavier than the anterior end, has not been experimentally established. Merton 1935 examined that problem once more. Like his predecessors he observed sinking of dead and fixed specimens. He concluded that Jensen's and Lyon's (heavier anterior end), and Koehler's (state of the labile balance) idea that the center of gravity of *Paramecium caudatum* may randomly shift anteriorly-posteriorly, and Dembowski's (heavier posterior end) opinion were erroneous. Merton differentiated paramecia between those from the bottom, with large numbers of food vacuoles (gorged) and those freely swimming in upper level of the culture with smaller numbers of food vacuoles (hungry). The former have heavier posterior ends, whereas the latter either have heavier anterior ends or are in state of labile balance. Merton 1935 confirmed the earlier Koehler's 1922 and Dembowski's 1929 b data that CO_2 bubbled through media or immersion in acetic acid solutions (pH ranges 4.9—6.4), involved negative geotaxis. According to Merton this is "artificial geotaxis" — response to stimuli of an injurious or deleterious nature. Paramecia accumulated in the upper region, because at the top the lowest concentration of harmful agents was encountered. "Natural geotaxis" — without negative stimulation, showed only bottom specimens. This opinion completely conflicts with Fedecka's 1956 quantitative analysis of the vertical movements of *Paramecium caudatum*. In culture media diluted with tap water 1:1, all specimens respond alike to gravity fields. Under these conditions, swimming time along the tube is the same in both directions, and the shorter stopping time at the top is constant for all specimens. As Dembowski earlier stated, geotactic aggregation was brought about by the motile behavior of single ciliates at the top and at the bottom.

In summary it is clear that:

1. During nearly 80 years of investigation on geotaxis in protozoa, establishing the position of the center of gravity in the *P. caudatum* body has been a key problem.

2. Until the present time only dead and fixed specimens or models (Dembowski 1929 a) were used in observations on the sinking of ciliates.

3. Even using this same killed-fixed technique, authors obtained divergent results as to the position of the center of gravity in *Paramecium*.

The meaning of those data may be also questioned from another point of view. Killed-fixed paramecia show significant alterations of physical constitution (size, body shape, specific gravity) as compared to living ones.

Since the 1930's important progress has been made in the immobilizing technique of *Paramecium*. This is the basis for estimating the position of the center of gravity in ciliate bodies by observations of the sinking of motionless but living specimens. There are three known ways to achieve the immobilizing effect (by homologous anstiserum, chloral hydrate and nickel ions), the latter being the most preferred. Nickel ions are universal immobilizing agents

http://rcin.org.pl

for ciliates and neither agglutinate cilia as homologous antiserum does, nor destroy the ciliary apparatus as chloral hydrate does. In this connection the physical status of an animal immobilized by nickel ions is the most similar to motile ones.

Methods

The organisms studied were *P. caudatum, P. aurelia, P. multimicronucleatum* and *P. bursaria*. The experiments on the sinking of the immobilized specimens were performed in the following way:

1. Ciliates were rinsed in 1 mM $CaCl_2$ solutions until the initial culture medium was diluted 100 times.

2. For the experiment specimens which collected in the upper part of flasks (i.e., negatively geotactic) were used.

3. Nickel was applied in the chloride form. Solutions of $NiCl_2$ were added to the paramecium samples in the volume ratio 1:1. In each case, the final concentration of nickel chloride was adjusted, so that the "immobilization phase" Kuźnicki 1963 or "physiologic immobilization stage" Grębecki, Kuźnicki, Mikołajczyk 1967 occurred within about 10—18 minutes. Generally 0.1—0.4 $NiCl_2$ solutions were applied.

4. Sinking immobilized specimens were examined in capillary tubes (100 mm long, 1.8 mm dia.). When ciliates treated by $NiCl_2$ sank to the bottom of the vessel, tubes were filled with solutions in which immobilization occurred, to within about 20 mm from the top.

5. Specimens were transferred from vessels to tubes in "the physiological immobilization stage." In this stage work of the ciliature is so ineffective that most ciliates sink as a physical body, without any ciliary disturbances.

6. On the tubes a point 40 mm from the bottom was marked. Behavior of the sinking paramecia was examined for the full length of the tube held in a vertical position. The velocity of sinking was measured (Table 1), when the specimens crossed the marked point (first level) and close to the bottom (second level). For quantitative data, space orientatation in sinking ciliates (presented in Tables 3 and 4) were examined at the second level. In all cases, only specimens without any significant alteration in size and body shape, and sinking without any ciliary disturbances were considered. The whole experimental procedure was performed at $24 \pm 1°C$.

Results

The results of experiments on the velocity of sinking of immobilized ciliates are summarized in Table 1. In each species examined, specimens showed a narrow range of variability. Marked differences, however, exist between range and mean values of *P. multimicronucleatum* and *P. bursaria* which are different from the similar values of *P. caudatum* and *P. aurelia*. This relation presented in another way, i.e., relative to the average time of sinking one body length in seconds, is presented in Table 2. In both absolute and relative values, the mean velocity of sinking is lowest in *P. caudatum* and highest in *P. bursaria*.

http://rcin.org.pl

Immediately after transfer to the experimental tubes, Ni- immobilized ciliates take a random position in space. This is most apparent when large numbers of specimens are introduced into a tube. Close to the bottom after approximately 80 mm of free sinking, paramecia also showed different spatial positions (either vertical, horizontal, or oblique).

Table 1

The velocity of sinking immobilized ciliates

Species	Total number of specimens tested	Velocity in μ/sec	
		range	mean value
P. caudatum	132	15—94	61.5±17.3
P. aurelia	110	18—96	64.7±15.0
P. multimicronucleatum	124	49—176	104.3±27.9
P. bursaria	116	75—221	156.6±31.3

Quantitative estimation of this phenomenon presents some difficulty. Establishing that sinking specimens take exactly vertical positions is impossible, because it requires observation at right angles from two sides of each individual at the same time. But the biggest problem is the determination of the perpendicular plane of the ciliates. In any one species examined,

Table 2

The relative values of velocity of sinking immobilized ciliates

Species	Typical length in μ	Average time of sinking one body length in seconds
P. caudatum	220	3.6
P. aurelia	150	2.3
P. multimicronucleatum	300	2.9
P. bursaria	120	0.8

there is no case that the perpendicular plane crosses exactly between two points farthest away from each other in *Paramecium* body. From the optical point of view, the glass tubes prevented making precise measurement of angular orientation. In this connection, however four classes of positions can be distinguished: posterior end downward, anterior end downward, horizontal and oblique (different inclinations), with an assumed probable deviation ±15° for first three positions.

http://rcin.org.pl

Position data presented in Table 3 originated from the study of standardized experimental conditions. Specimens of each species were taken from one culture and treated with the same concentration of $NiCl_2$ solutions. The only ciliates examined were those whose time sinking for the full length of the tubes was no longer than: *P. caudatum* — 30 min., *P. aurelia* — 30 min., *P. multimicronucleatum* — 18 min., *P. bursaria* — 12 min.

Table 3

Positions of sinking immobilized ciliates

Species	Total number of specimens tested	Positions after approx. 80 mm. free sinking			
		Vertical		Horizontal	Different kind obliques
		posterior downard	anterior downard		
P. caudatum	257	34.2%	16.7%	16.0%	33.1%
P. aurelia	224	12.0%	13.8%	20.0%	54.0%
P. multimicronucleatum	280	9.3%	37.5%	18.2%	35.0%
P. bursaria	273	17.0%	13.9%	47.6%	21.6%

According to Merton 1935, position of the center of gravity in *P. caudatum* body was altered so much by the degree of feeding, that he distinguished two separate categories of ciliates. Food concentration in the culture medium is a factor which undoubtedly influences the size and shape of specimens. Nevertheless, experiments with Ni-immobilized ciliates did not confirm Merton's results, which were obtained with killed-fixed ones. *P. caudatum* taken from the thigmotactic ring, 24 hours after food was added were compared with specimens taken 9 days later from this same flask, when medium was transparent and ciliates free swimming on all levels of the medium (Table 4). Slight differences between the two groups exist, but the ciliates could not be divided into two classes "gorged" — having a heavier posterior end and "hungry" — having a heavier anterior end, as Merton 1935 postulated.

Table 4

Position of sinking immobilized *P. caudatum* in relation to degree of feeding

Time after feeding	Total number of specimens tested	Position after approx. 80 mm. free sinking			
		Vertical		Horizontal	Different kind obliques
		posterior downward	anterior downward		
24 hours	125	42.4%	17.6%	11.2%	28.8%
10 days	131	31.3%	25.2%	13.7%	29.8%

http://rcin.org.pl

The velocity of angular orientation of free sinking ciliates has never been accurately examined. Dembowski 1929 a, 1929 b dismissed it with the statement that, in a few seconds after the ciliary beat stopped, paramecia assumes the exact vertical position, posterior end downward. Behavior of Ni-immobilized specimens in relation to this angular orientation is extremely variable. Some ciliates may even be oblique, for the full length (80 mm) without any detectable change in position. Others change but with different speeds. For reasons mentioned above, precise measurement of this phenomenon is impossible. The fastest sinking specimens, however, take a vertical position, either posterior or anterior downward; this allows an approximate measurement of the velocity of angular orientation. The shortest time in changing position from the horizontal to the vertical posterior end downward was: *P. caudatum* — 90 sec., *P. aurelia* — 120 sec., *P. multimicronucleatum* — 75 sec., *P. bursaria* — 65 sec.

Concluding remarks

Until the present time the mechanical hypothesis was the one logical explanation of the negative geotaxis in *Paramecium*, in spite of contradictory evidence. The data presented above do not support the mechanical hypothesis. Four species (*P. caudatum, P. aurelia, P. multimicronucleatum, P. bursaria*) were studied, which differ in size and shape as well as in other morphological characters. For determination of the orientation during sinking of ciliates, only negative geotactic specimens were used. However, in all species the assumption of the mechanical hypothesis, that differential buoyancy of the two ends of the *Paramecium* body could direct the ciliate vertically, anterior end upwards, has not been confirmed. Ni- immobilized specimens oriented in various positions with the slight majority: *P. caudatum* — posterior end downward; *P. multimicronucleatum* — anterior end downward; *P. bursaria* — horizontal positions; and *P. aurelia* — random. In those cases when ciliates take vertical positions (posterior end downward), the velocity of angle orientation is so low, that it is improbable that it is the mechanism of space orientation.

Irrespective of the results discussed above the mechanical hypothesis is not a coherent conception. It is based on the morphology of the shape. By the assumption of identical specific density of the entire *Paramecium* body, the posterior end, because of its thickness, must be heavier than the anterior end. From this same point of view, free sinking ciliates should be passively oriented, not exactly vertical with anterior end upwards, but tilted on the dorsal side. All authors have expressed the opinion that negative geotaxis occurs as an effect of stimulation of *Paramecium* by many different kinds of stimuli. Particularly effective is acetic acid at pH 5.1—5.3, Dembowski 1929 b or pH 5.3—5.5, Merton 1935. At this range of pH the high swimming velocity (Dryl 1961) and the highest beat frequency of cilia (Kinosita, Dryl, Naitoh 1964) in *P. caudatum* has been found. It is impossible that paramecia become motionless by stimuli which simultaneously extremely increase the beat frequency of cilia. As a matter of fact, the stopping of cilia has been observed only under the thigmotactic reaction of *Paramecium*.

http://rcin.org.pl

At present, it is not possible to state more; the question of the mechanism of space orientation of protozoa in gravity fields remains still open. Further experimental research and more evidence is required for the explanation of the geotaxis phenomenon [2].

Summary

The Ni-immobilizing technique was used to establish velocity of and orientation during sinking, in living but motionless specimens of four species of *Paramecium* (*P. caudatum, P. aurelia, P. multimicronucleatum,* and *P. bursaria*). Only individuals which collected in the upper region of the flasks after rinsing in $CaCl_2$ solutions were axamined. In all species, the mechanical theory that negative geotaxis of *Paramecium* is an effect of posterior center of gravity has not been confirmed. Ni-immobilized specimens oriented in various positions with the slight majority: *P. caudatum* — posterior end downward, *P. multimicronucleatum* — anterior end downward, *P. bursaria* — horizontal and *P. aurelia* — random. The velocity of sinking and angle of orientation also showed that it is improbable that the differential buoyancy of the two ends of the *Paramecium* body could have been the mechanism of space orientation.

STRESZCZENIE

Technika immobilizacji niklowej została wykorzystana w celu ustalenia szybkości i pozycji swobodnie opadających, żywych orzęsków. Doświadczenia prowadzono na czterech gatunkach *Paramecium* (*P. caudatum, P. aurelia, P. multimicronucleatum, P. bursaria*). Immobilizowane były wyłącznie orzęski, które po przepłukaniu roztworami $CaCl_2$, zbierały się w górnych obszarach naczynia. U żadnego z badanych gatunków nie potwierdzono założeń teorii mechanicznej, zgodnie z którą geotaksja ujemna wywołana jest przewagą ciężaru tylnej połowy *Paramecium*. Immobilizowane orzęski, opadając przyjmują różne pozycje przy nieznacznej przewadze: *P. caudatum* — tylny koniec ciała w dół, *P. multimicronucleatum* — przedni koniec w dół, *P. bursaria* — położenie horyzontalne, *P. aurelia* — rozkład przypadkowy. Szybkość opadania, jak i szybkość orientacji kątowej wskazują również, że mechanizmem orientacji przestrzennej *Paramecium* nie może być różnica ciężarów obu połów ciała.

REFERENCES

Aderhold R. 1888: Beiträge zur Kenntnis richtender Kräfte bei der Bewegungen niederer Organismen. Jena. Z. Naturwiss., 22, 310—342.

Davenport Ch. B. 1897: Experimental Morphology. I. The Macmillan Company, New York.

Dembowski J. 1929 a: Die Vertikalbewegungen von *Paramecium caudatum*. I. Die Lage des Gleichgewichtscentrums im Körper des Infusors. Arch. Protistenk., 66, 104—132.

[2] Author thanks Professor Theodore L. Jahn and James R. C. Fonseca of the University of California, Los Angeles, for reading and editing of manuscript.

http://rcin.org.pl

Dembowski J. 1929 b: Die Vertikalbewegungen von *Paramecium caudatum*. II. Einfluss einiger Aussenfaktoren. Arch. Protistenk., 68, 215—260.
Dembowski J. 1931: Die Vertikalbewegungen von *Paramecium caudatum*. III. Polemisches und Experimentelles. Arch. Protistenk., 74, 153—187.
Dryl S. 1961: The velocity of forward movement of *Paramecium caudatum* in relation to pH of medium. Bull. Acad. pol. Sci., Cl. II, 9, 71—74.
Fedecka B. 1956: Badania ilościowe nad geotropizmem *Paramecium caudatum*. Folia Biol., 4, 65—76.
Grębecki A., Kuźnicki L., Mikołajczyk E. 1967: Right spiralling induced in *Paramecium* by Ni ions and the hydrodynamics of the spiral movement. Acta Protozool., 5, 389—408.
Harper E. H. 1911: The geotropism of *Paramecium*. J. Morphol., 22, 993—1000.
Harper E. H. 1912: Magnetic control of geotropism in *Paramecium*. J. Anim. Beh., 2, 181—189.
Jahn T. L. and Bovee E. C. 1967: Motile behavior of protozoa. 40—198. In T. T. Chen: Research in protozoology, Vol. I, Pergamon Press, Oxford, England.
Jennings H. S. 1904: The behavior of *Paramecium*. J. comp. neurol. Pschol., 14, 441—510.
Jensen P. 1893: Über den Geotropismus niederer Organismen. Pflüger's Arch. ges. Physiol., 53, 428—480.
Kanda S. 1914: On the geotropism of *Paramecium* and *Spirostomum*. Biol. Bull., 26, 1—24.
Kanda S. 1918: Further studies on the geotropism of *Paramecium caudatum*. Biol. Bull., 34, 108—120.
Kinosita H., Dryl S. and Naitoh Y. 1964: Relation between the magnitude of membrane potential and ciliary activity in *Paramecium*. J. Fasc. Sci. Tokyo, Univ. Sec. IV, 10, 303—309.
Koehler O. 1922: Über die Geotaxis von *Paramecium*. Arch. Protistenk., 45, 1—94.
Koehler O. 1930: Über die Geotaxis von *Paramecium*. II. Arch. Protistenk., 70, 279—306.
Koehler O. 1939: Ein Filmprotokoll zum Reizverhalten querzertrennter Paramecien. Zool. Anz. Suppl., 12, 132—142.
Kuźnicki L. 1963: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 1, 301—312.
Loeb J. 1897: Zur Theorie der physiologischen Licht und Schwerkraftwirkungen. Pflüger's Arch. Ges. Physiol., 66, 439—466.
Lyon E. P. 1904: On Jensen's theory of geotropism in *Paramecium*. Am. J. Physiol., 13, 15—16.
Lyon E. P. 1905: On the theory of geotropism in *Paramecium*. Am. J. Physiol., 14, 421—432.
Lyon E. P. 1918: Note on geotropism of *Paramecium*. Biol. Bull., 34, 120.
Massart J. 1891: Recherches sur les organismes inférieurs. La sensibilité à la gravitation. Bull. Acad. r. Belg., Cl. Sci. 22, 158—167.
Merton H. 1935: Versuche zur Geotaxis von *Paramecium*. Arch. Protistenk., 85, 33—60.
Platt J. B. 1899: On the specific gravity of *Spirostomum*, *Paramecium*, and the tadpole in relation to the problem of geotaxis. Amer. Nat., 33, 31—38.
Schaefer I. G. 1922: Studien über den Geotropismus von *Paramecium aurelia*. Pflüger's Arch. ges. Physiol., 195, 227—244.
Schwarz F. 1884: Der Einfluss der Schwerkraft auf die Bewegungsrichtung von *Chlamydomonas* und *Euglena*. S. B. dtsch. Bot. Ges., 2, 51—72.
Śmiechowska Z. (unpublished)
Verworn M. 1889: Psychophysiologische Protistenstudien. G. Fischer Ver., Jena.

http://rcin.org.pl

http://rcin.org.pl

ARCHIV FÜR PROTISTENKUNDE

110 Band

Erstes Heft

Ettl H. — Die Gattung *Pedinomonas* Korschikoff. 1
Beinbrech G. — Zur Morphologie, Fortpflanzung und Übertragung von *Urceolaria paradoxa (Peritricha, Mobilia)* 12
Ammermann D. — Beobachtungen zur Paarbildung bei *Stylonychia mytilus* 54
Ablin R. and Ritter Jr. H. — Immunological Characterization of Protozoa Symbiotic to a Roach and a Termite 58

Zweites Heft

Gisler R. — Über Protozoen im Darm höherer Termiten (Fam. *Termitidae)* der Elfenbeinküste 77
König K. — Wirkung von Lithium- und Rhodanid-Ionen auf die polare Differenzierung und die Morphogenese von *Stentor coeruleus* Ehrbg. 179

Drittes Heft

Geus A. — Gregarinen aus dem Ohrid-See 231
Heynig H. — Beiträge zur Taxonomie und Ökologie der Gattung *Chrysococcus* Klebs *(Chrysophyceae)*. (Zur Kenntnis des Planktons mitteldeutscher Gewässer IV) 259
Arnold, Z. M. — Biological observations on the Foraminifer *Calcituba polymorpha* Roboz. 280

Viertes Heft

Lenzenweger R. — Beobachtungen zu einer besonderen Form von Missbildungen bei Desmidiaceenzellen 305
Throm G. — Untersuchungen zum Reaktionsmechanismus von Phobotaxis und Kinesis an *Rhodospirillum rubrum* 313
Reiff I. — Die genetische Determination multipler Paarungstypen bei dem Ciliaten *Uronychia transfuga (Hypotricha, Euplotidae)* 372
Ettl H. — *Tetratoma schussnigii* nov. sp., eine neue Volvocale mit getrennter Geisselinsertion. 398
Bardele C. — *Acineta tuberosa* I. Der Feinbau des adulten Suktors . . 403
Jennings J. — A new astomatous ciliate from the entocommensal rhynchocoelan *Malacobdella grossa* (O. F. Müller) 422
Narasimhamurti C. and Kalavati C. — Observation on the morphology and sporogony of a new coccidian *Chagasella ganapatii* n. sp. from the gut of a xylophagous termite, *Odontotermes obesus* (Rambur) 426
Ammermann D. — Die Kernverhältnisse des Ciliaten *Protocrucia depressa* n. sp. 434
Geus A. — Die Gregarinen der Cisiden *(Coleopt.)* 439
Rieth A. — Beiträge zur Kenntnis der Vaucheriaceen XII. *Vaucheria undulata* Jao 1963 in Europa 452
Kessler E., Czygan F., Fott B. und Nováková M. — Über *Halochlorella rubescens* Dangeard 462

http://rcin.org.pl

F a s c i c u l i p r a e p a r a t i:

I. V. I s s i and S. S. S h u l m a n: The systematic position of *Microsporidia* [О систематическом положении микроспоридии] — S. L. K a z u b s k i and K. M i g a ł a: *Urceolariidae* from breeding carp — *Cyprinus carpio* L. in Żabieniec and remarks on the seasonal variability of trichodinids [*Urceolariidae* karpi hodowlanych — *Cyprinus carpio* L. w Żabieńcu i uwagi o zmienności sezonowej trichodin] — O. N. B o r c h s e n i u s and D. V. O s s i p o v: Heteroploidy of micronuclei of *Paramecium caudatum*. II Mitotical cycles of micronuclei of different morphological types [Гетероплоидия микронуклеусов *Paramecium caudatum*. II. Митотические цикли микронуклеусов разных морфологических типов] — Z. R a a b e: Two new species of *Thigmotricha (Ciliata, Holotricha)* from *Theodoxus fluviatilis* [O dwu nowych gatunkach *Thigmotricha (Ciliata, Holotricha)* z *Theodoxus fluviatilis*] — H. T a m a r: Observations on *Halteria bifurcata* sp. n. and *Halteria grandinella* [Beobachtungen an *Halteria bifurcata* sp. n. und *Halteria grandinella*] — И. В. К у л е м и н а: Паразитические инфузории (*Peritricha, Urceolariidae*) с молоди некоторых рыб оз. Селигер [Parasitic ciliates (*Peritricha, Urceolariidae*) from the fry and young fishes of lake Seliger] — M. A n w a r: Cytochemical investigations of *Isospora lacazei* and *I. chloridis* of the sparrow (*Passer domesticus*) and the grenfinch (*Chloris chloris*) [Цитохимическое исследование *Isospora lacazei* и *I. chloridis* из воробья (*Passer domesticus*) и зелёного вьюрка (*Chloris chloris*)] — B. V. G r o m o v and K. A. M a m k a e v a: *Amoeboaphelidium protococcarum* sp. nov. and *Amoeboaphelidium chlorellavorum* sp. nov. — endoparasites of protococcus algae [*Amoeboaphelidium protococcarum* sp. nov. и *Amoeboaphelidium chlorellavorum* sp. nov. — ендопаразиты протококковых водорослей].

SUBSCRIPTION

price is $ 7.50 for one volume consisting of four parts appearing quarterly
Place your order with your bookseller or directly with:

Export and Import Enterprise „RUCH"
Warszawa, Wronia 23, Poland

Cable: Exprimruch, Warszawa
Bank Account: Bank Handlowy S.A. Warszawa

In the East-European countries the subscription orders are to be placed with the local agencies for press distribution.

Państwowe Wydawnictwo Naukowe
(PWN — Polish Scientific Publishers)
Warszawa

http://rcin.org.pl

Cena zł 42.—

Fasciculi:

Профессор Евгений Минеевич Хейсин (1907—1968) 1

Professor Eugene M. Cheissin (1907—1968) 3

1. E. M. Cheissin: On the distinctness of the species *Eimeria neoleporis* Carvalho, 1942 from the cottontail rabbit *Sylvilagus floridanus mearnsii* and *Eimeria coecicola* Cheissin, 1947 from the tame rabbit *Oryctolagus cuniculus* [О самостоятельности видов *Eimeria neoleporis* Carvalho, 1942 из белохвостового кролика *Sylvilagus floridanus mearnsii* и *Eimeria coecicola* Cheissin, 1947 из домашнего кролика *Oryctolagus cuniculus*] . . 5
2. C. G. Gillies and E. D. Hanson: Morphogenesis of *Paramecium trichium* [Morphogénèse de *Paramecium trichium*] 13
3. D. V. Ossipov and I. I. Skoblo: The autogamy during conjugation in *Paramecium caudatum* Ehrbg. II. The ex-autogamont stages of the nuclear reorganization [Автогамия при конъюгации *Paramecium caudatum* Ehrbg. II. Экс-автогамонтный цикл ядерной реорганизации] . . 33
4. B. R. Seshachar and A. R. Kasturi Bai: Conjugation in *Spirostomum ambiguum* Ehrbg. [Konjugation von *Spirostomum ambiguum* Ehrbg.] 51
5. A. Czapik: Remarques sur *Deltopylum rhabdoides* Fauré-Frémiet et Mugard (*Ciliata, Hymenostomata*) [Uwagi o *Deltopylum rhabdoides* Fauré-Frémiet et Mugard (*Ciliata, Hymenostomata*)] 57
6. J. J. Lipa: Some observations on flagellate parasites of hemipterans *Corimelaena, Euschistus, Gerris, Leptocoris* and *Oncopeltus* in the United States [Obserwacje nad wiciowcami pasożytującymi w pluskwiakach *Corimelaena, Euschistus, Gerris, Leptocoris* i *Oncopeltus* w Stanach Zjednoczonych] 59
7. J. J. Lipa: *Stempellia scolyti* (Weiser) comb. nov. and *Nosema scolyti* sp. n. microsporidian parasites of four species of *Scolytus* (*Coleoptera*) [Mikrosporidia *Stempellia scolyti* (Weiser) comb. nov. i *Nosema scolyti* sp. n. pasożytujące w czterech gatunkach *Scolytus* (*Coleoptera*)] . . 69
8. T. V. Beyer: Cytochemical studies on the haemogregarines of Armenian reptiles. I. DNA in the nuclei of some *Karyolysus* species from the rock-lizards *Lacerta armenica* and *L. saxicola* [Цитохимическое исследование гемогрегарин из рептилий Армении. I. ДНК в ядрах некоторых видов *Karyolysus* из скальных ящериц — *Lacerta armenica* и *L. saxicola*] . . 79
9. V. D. Kallinikova: Cytochemical study of enzymes of the respiratory chain in the life cycle of *Trypanosoma cruzi* Chagas, 1909. II. Respiratory enzymes of crithidial forms in the course of cultivation and some observations on the growth of the culture [Цитохимическое исследование ферментов дыхательной цепи в жизненном цикле *Trypanosoma cruzi Chagas, 1909.* II. Дыхательные ферменты критидиальных форм в процессе культивирования *Trypanosoma cruzi*, наряду с другими показателями роста культуры] 87
10. Л. Н. Серавин: Роль механических и химических раздражителей в индуцировании фагоцитарной реакции у *Amoeba proteus* и *A. dubia* [The role of mechanical and chemical stimulators on the induction of phagocytic reactions in *Amoeba proteus* and *A. dubia*] 97
11. L. Kuźnicki: Behavior of *Paramecium* in gravity fields. I. Sinking of immobilized specimens [Zachowanie się *Paramecium* w polach grawitacyjnych. I. Opadanie immobilizowanych orzęsków] 109

http://rcin.org.pl