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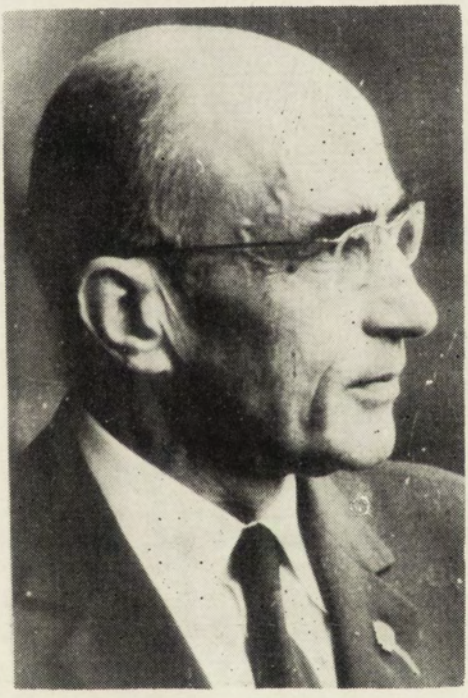

ZDZISŁAW RAABE
1909–1972

ZDZISŁAW RAABE

(1909—1972)

Zdzisław RAABE, the editor of "Acta Protozoologica", the ordinary member of the Polish Academy of Sciences, Professor of Zoology at the University of Warsaw, died suddenly on February 12-th, 1972, leaving in great sorrow his family, friends, colleagues and students.

Zdzisław RAABE was born on October 19-th 1909 in Cracow. He received training at the University of Warsaw, being inspired by his father a distinguished Polish protozoologist Henryk Raabe and by the world-famous Polish zoologist and parasitologist, Konstanty Janicki. He started his scientific career in the Zoological Museum (at present: the Institute of Zoology, Polish Academy of Sciences). He was fighting during the second world-war as an officer of the Polish Army, being imprisoned for more than five years in Germany. Soon after the war he resumed his scientific work in the Zoological Museum of Warsaw. He got Ph. D. (1945) and Docent D. (1947) for his dissertation on "The ways of morphological adaptations to parasitic life in the ciliate" (Annls Univ. Mariae Curie-Skłodowska 2, 299—411, 1947). His first academic position was a chairman of the Department of Zoology and Parasitology of the Veterinary Division of Maria Curie-Skłodowska University in Lublin. He came back to Warsaw in 1953 and he became a chairman of the Zoological Institute at the University of Warsaw. He remained on this position until his death on February 1972.

Professor Zdzisław RAABE studied mainly the parasitic ciliate protozoa in the group *Thigmotricha* and parasitic *Peritricha*, the family *Urceolariidae*, being a world authority in this field. He described a number of new species and contributed to the knowledge of morphology of many other species. He was interested in the division morphogenesis, taxonomy and ecology of various ciliates. The extensive monograph on Ordo *Thigmotricha* was the last work of his life and parts of it are still published in Acta Protozoologica. Zdzisław RAABE contributed very much to discussions of general importance for protozoology as it was evident from his "Remarks on the principles and outline of the system of *Protozoa*" (Acta Protozool., 2, 1—18, 1964) and his views about the development of various systems by polimerisation and differentiation and resulting integration. He expressed also very interesting concept of somatisation in protozoa and phylogenetic tendencies in the development of parasitic way of life in unicellular organisms ("The morphogenetic principles of Sewertzoff, their extension and application to protozoa", Acta Protozool., 9, 1—22, 1971).

Professor Z. RAABE devoted much of his time to the teaching as a professor of Zoology at the University of Warsaw. Most of his graduated students were educated by him from the ground up. More than twenty students successfully completed Ph. D. under his direction while six additional ones got the degree of docent. He contributed much to the international cooperation among protozoologists. He belonged to the group of scientists who initiated the organisation of the First International Conference in Prague in August 1961, although he was not able to attend that Conference because of his progressing illness. He was

the member of the International Commission on Protozoology, taking part in organization of the II-nd International Conference on Protozoology in London (1965) and of the III-rd one in Leningrad (1969).

As concerns his editorial activities, Zdzisław RAABE established the new international journal on protozoology "Acta Protozoologica" in 1962. He was also the editor of "Annales of the University Maria Curie-Skłodowska" and "Acta Parasitologica Polonica".

He was a member of the Society of Protozoologists and a correspondent-member of Groupment des Protostologues de Langue Francaise. In appreciation of his great contribution to the development of science Zdzisław RAABE received many high Polish State distinctions and Polish Government Awards.

As a man he was devoted to his family; he is survived by his wife Janina and son Zbigniew. It would be not exaggerated to say that most of his students were feeling like a part of his family and all of them will be missing him very much.

On behalf of Editorial Board

Stanisław DRYL and Stanisław L. KAZUBSKI

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Institute of Inland Fishery, Department of Fish Culture, Żabieniec near Warszawa, and Laboratory of General Parasitology, Department of Parasitology, Polish Academy of Sciences, Warszawa 22, Pasteura 3, Poland

Kazimierz MIGAŁA and Stanisław L. KAZUBSKI

Occurrence of nonspecific ciliates on carps (*Cyprinus carpio* L.) in winter ponds

Występowanie niespecyficznych orzęsków u karpi (*Cyprinus carpio* L.) w zimochowach

In the middle of March 1970 the possibility arose to collect fishes from several ponds in Żabieniec and Wilga (environs of Warszawa). The fishes appeared to be heavily infected with ciliates. Particular attention has been paid to mass incidence of nonspecific ciliates on examined fishes. The same phenomenon has been observed in the following year, however, fishes were infected to a lesser degree. The present paper deals with this problem.

The end of winter, when ponds are covered with ice, is not convenient for parasitological examination of breeding fishes. For breeding purposes, to avoid the disturbance of fishes (by removing ice, pulling the net etc.), fishing is not commended. At the end of winter fishes are in bad health condition due to usual overcrowding of winter ponds, bad oxygen conditions under the ice, long staying in low temperatures without feeding, and also due to the action of parasites, fungi, bacteriae and viruses.

There are only few papers in the literature concerning the occurrence of parasites on fishes during winter time (Ljajman 1951, Ivasik 1957, Čečina 1960); they contain only a rough survey of protozoan species found on fishes.

The period of winter had not been also taken into account by the present authors in their former papers (Kazubski and Migała 1968, Migała 1969, 1970, 1971), dealing with qualitative and quantitative studies on protozoans occurring on carps.

The data described in this paper had been presented briefly in a research note (Migała and Kazubski 1971). In this note the occurrence of nonspecific ciliates on phytophagous cyprinids (*Aristichthys nobilis*, *Hypophthalmichthys molitrixi* and *Ctenopharyngodon idella*) had been also reported.

Material and methods

Examined carps (*Cyprinus carpio* L.): fingerling (K_1) and two year fishes (K_2), were collected from several ponds in Żabieniec and Wilga (environs of Warszawa), belonging to the Department of Fish Culture of the Institute of Inland Fishery.

Most part of the material was collected in Żabieniec near Piaseczno. Description of this territory and establishment was given in the previous paper (Migała 1971). The remaining material was collected in fish farm Wilga near Garwolin. Both farms, in spite of similar structure and management, differ in ecological as well as hydrological conditions. Situation of ponds in Wilga in a depression with peaty ground, near the Wisła river, is one of the most important differences. Moreover, the ponds in this locality are supplied with water from the Wilga river, polluted with sewage from a dairy and a sloughterhouse.

The first fishing was undertaken on 16th March 1970, when the ice covering the winter ponds began to melt. The last fishing was accomplished in April, after complete melting of ice. The accurate dates of fishing are presented in Table 2. The examined fishes were heavily infected with protozoans. Moreover, the carps from Żabieniec suffered from the sickness of nostrils (Staff 1925). This disease was probably caused by low temperature (Table 1) and too strong current of water in winter ponds at the beginning of 1970.

Table 1

Temperature of the water in winter ponds in Żabieniec in the years 1964–1971 (mean value monthly)

Months	Years							
	1964	1965	1966	1967	1968	1969	1970	1971
January	0.6	1.6	0.7	0.6	0.6	0.3	0.2	0.5
February	0.6	0.9	0.9	0.7	1.8	0.4	0.2	1.6
March	0.7	2.0	3.7	4.8	3.3	0.4	0.6	2.1
April	6.8	7.8	10.7	10.2	11.9	5.4	4.6	10.8

A total number of 60 carps collected from 7 ponds in Żabieniec and Wilga was examined in March and April 1970. Of this number 45 specimens of K_1 and 15 K_2 were examined. Rough microscopical survey of fresh mucus from gills and skin showed the infection of 19 fishes with the ciliate species which were not the usual parasites of fishes. These ciliates were examined further on permanent preparations.

Similiar investigations were carried in February and March 1971 on fishes from Żabieniec. The nonspecific ciliates were found on 3 out of 10 examined fishes (K_2). It is worthy noting that the fishes examined in 1971 originated from the same stock as the fishes examined in the former year. They were in bad health condition and showed also traces of branchionecrosis from which they had suffered a year before.

Fishes were brought alive into the laboratory in 10 l tanks in which they stayed not longer than one hour in well aerated water. Immediately after killing the random samples of mucus from skin and gills were taken and smears about 10 cm^2 were prepared. Dried smears were silver impregnated after Klein. They served as a basis for counts as well as for preparing the descriptions of found species. These preparations gave good picture of argentophilic system of ciliates, however the structure of buccal apparatus and nuclei was usually invisible.

In Table 2, for comparative purposes, the relative numbers of particular ciliates calculated for one preparation are given.

Results

The examination of carps revealed the occurrence of a series of species of parasitic ciliates on skin and gills. They were as follows: *Chilodonella cyprini* Moroff, *Ch. hexasticha* Kiernik, *Ichthyophthirius multifiliis* Fouquet, *Trichodina pediculus* Ehrbg., *T. nigra* Lom, *T. mutabilis* Kazubski et Migała, *Trichodinella subtilis* Lom and *Sessilia* sp. sp. These species, except *Ch. hexasticha*, had been found on carps from Żabieniec also in previous years (Kazubski and Migała 1968, Migała 1971).

Besides the species mentioned above, some normally free-living species of ciliates were found on the examined carps. The number of these nonspecific ciliates on some fish specimens was very high. In all the species, especially in these occurring in great numbers, the dividing specimens were found. It witness for the normal course of life processes in these ciliates.

The following species of nonspecific ciliates were found: *Chilodonella cucullulus* (O. F. M.), *Chilodonella uncinata* Ehrbg., *Dexiostoma campylum* (Stokes), *Glaucoma scintillans* Ehrbg., *Colpidium colpoda* (Ehrbg.), *Frontonia acuminata* Ehrbg. and *Frontonia leucas* Ehrbg. Short descriptions of these species are included.

Chilodonella cucullulus (O. F. M.) (Pl. I 1)

Flattened ciliate with body shape characteristic of the genus (Pl. I 1). Measurements on silver impregnated preparations after Klein are as follows: body length 90–130 μ (most frequently about 110 μ), body width 60–78 μ. Ciliature pattern typical of the species: 17 to 20 longitudinal rows (most frequently 18 and 19) including two postoral rows. Buccal ciliature normally formed. Contractile vacuole pores 3–4 in number. Dorsal kinety, normally formed, situated at the dorsal side.

Chilodonella uncinata Ehrbg. (Pl. I 2)

Flattened ciliate with the typical body shape (Pl. I 2). Posterior part of the body somewhat broadened and rounded. Dimensions: body length 42 μ, width 24–33 μ. Typical ciliature pattern. There are 5 rows in the right and 5 rows in the left part of the ciliature on the ventral side of the body.

Dexiostoma campylum (Stokes, 1886) Jankowski, 1967 (Pl. I 3–6)

syn.: *Colpidium campylum* (Stokes, 1886) Breslau, 1922

Body egg-shaped but proportionally slender. Anterior end slightly tappered, the posterior one rounded (Pl. I 3–6). Dimensions: body length 40–70 μ, (mean 51.9 μ), width 23–52 μ (mean 35.7 μ). Body width to length proportion most frequently about 0.7. Large oral cavity, up to 10 μ long, is situated in the first quarter

of the body (Pl. I 3). UM well visible. Ciliature uniform. The number of primary meridional rows usually 24–26 (there may be 12–13 rows at one side). The kineties of the right body side, at the level of the anterior margin of the oral cavity, strongly bent toward the suture forming a characteristic breakdown (Pl. I 3, 5). All rows, together with two postoral ones, are accompanied by well formed secondary rows. Kineties of the right and left body sides, nearest to the buccal cavity form a characteristic suture. The kineties run at an acute angle toward the suture (Pl. I 4). One pore of the contractile vacuole is situated at the end of 5th row near the posterior end of the body (Pl. I 5).

Glaucoma scintillans Ehrbg. (Pl. II 7–10)

Slightly egg-shaped, dumpy body with broadly rounded ends (Pl. II 7, 9, 10). Dimensions: body length 42–75 μ (mean 59.9 μ), width 30–60 μ (mean 47.9 μ). Body width to length proportion about 0.8. Fairly large oral cavity, 10–12 μ long, is situated in the first quarter of the body (Pl. II 7). UM visible. Ciliature fairly uniform. The number of primary rows about 40 or somewhat less (there are 17 to 23 rows at one side of the body). Nearly all these rows are accompanied by secondary rows. Only the postoral rows, 7–8 in number, are without secondary rows.

The kineties of the right body side are characteristically bent in front of the oral cavity forming the anterior suture, running from the right upper corner of the buccal cavity to the top of the body (Pl. II 7, 8, 10). The kineties of both sides are nearly perpendicular to the suture. Contractile vacuole pore at the end of 7th row.

Colpidium colpoda (Ehrbg., 1831) (Pl. II 11–12, III 13–15)

Egg-shaped body of the ciliate rounded at both ends (Pl. II 11–12). Dimensions: body length 70–120 μ (mean 98.9 μ), width 40–85 μ (mean 68.4 μ). Body width to length proportion somewhat overpassing 0.7. Sometimes slightly smaller specimens were found with more slender body, probably young ones (Pl. III 13). Triangular buccal cavity situated in the distance of about 1/4 of the body length from the anterior end.

There are about 60 meridional rows (27–32, most frequently 30 rows at one side, equatorially). Arrangement of the rows characteristic of the genus. The anterior suture runs from the upper margin of the buccal cavity to the left, spiralling to the top of the body (Pl. III 14–15). Due to this structure the rows of the left part of the ciliature elongate becoming more distant from the buccal cavity. Several rows of the right part of the ciliature bend and, running meridionally over the buccal cavity, reach the anterior suture. The first row is shortest, further rows become more elongated (Pl. II 12). There is one postoral row (rarely 2). Posterior suture slightly shifted to the ventral side. Pore of the contractile vacuole is situated equatorially, near to 16th row.

Table 2

Ciliates occurring on carps (*Cyprinus carpio* L.) in winter ponds

No. of fish	Locality and number of pond	Age and mean weight of fishes	Date of examination	Localization	Nonspecific ciliates							Parasites							
					Chilodonella		*Dexiostoma campylum*	*Glaucoma scintillans*	*Colpidium colpoda*	*Frontonia*		*Chilodonella*		*Ichthyophthirius multifiliis*	*Trichodina*			*Trichodinella subtilis*	*Sessilia* sp. sp.
					cucullulus	*uncinata*				*acuminata*	*leucas*	*cyprini*	*hexasticha*		*pediculus*	*mutabilis*	*nigra*		
1	Żab. 6	K_1 79 g	16 Mar. 1970	gills	+++		+++	++	++	+		+						+	
2			26 Mar. 1970	,,	+		+		+			+++						+++	
3	Żab. 10	K_1 28 g	16 Mar. 1970	,,	++		+	+	+			+++	+		+	+	+	++++	
4			21 Mar. 1970	,,	++				+			++++						+++	
5					+							++++						++++	+
6					+							++						++	++
7					+				+			+++						+++	
8	Żab. 5	K_1 30 g	26 Mar. 1970	,,	++					+		+++						++++	+
9					++				+			+++	+				+	+++	
10	Żab. 9	K_1 19 g	26 Mar. 1970	,,	++		+					+++				+	++	++	
11			9 Apr. 1970		+++		++					++	++	+?		++	++	+++	+
12	Żab. "Mł."	K_1 64 g	9 Apr. 1970	,,	++		++	+	+			++	+		+		+	+++	+
13					++					+		+++	+		+	+	+	++	+
14	Żab. 12	K_2 269 g	9 Apr. 1970	,,	++		+		+				++		++		+		++
15					+		++	++	+			+	+++		++			++	+
16	Wilga 5	K_2 290 g	8 Apr. 1970	,,	+					+++	+		+		+			++	+
17										++		+	++		++	+		++	+
18										+		++		+	+	+	+	+++	
19					+					+			+++						
20	Żab. 9	K_2 287 g	20 Feb. 1971	skin					+								+++		+
21				,,					+								++		
				gills	+	+	+		+	+		+	+	+		+	++	+++	+
22		K_2 287 g	26 Mar. 1971	,,		+	++	+	++				++++	+		+	+	++	+

Explanation and abbreviation: +1–4, ++5–16, +++17–64, ++++65–256, number of ciliates is calculated for one preparation. Żab. – Żabiniec, "Mł" – pond "Młyński".

Frontonia acuminata Ehrbg., 1833 (Pl. III 16)

Body of the ciliate spindle-shaped, frequently pear-shaped, then the greatest width being about 2/3 of the body length from the anterior end. Body length 80–117 μ (mean 97.5 μ), width 41–66 μ (mean 54.5 μ). Body width to length proportion 0.56. Fairly large buccal cavity is situated in the distance of 1/3 of the length between the anterior end and mid-length of the body (Pl. III 16).

The ciliature is composed of about 80 rows, or somewhat less (34–44 rows, mean 38.2 at one side, equatorially), forming a characteristic suture in front of the buccal cavity. The rows loosely arranged become more dense near the ventral line. Argyrome characteristic of the genus. Kinetosomes occurring in triplets are fairly large.

Details of the structure of buccal acvity and its region as well as the nuclear apparatus were not examined.

Frontonia leucas Ehrbg., 1838 (Pl. III 17)

Single specimen, strongly elongated. Body length 335 μ, maximum width 135 μ, in the distance of about 2/3 from the anterior end (Pl. III 17). Number of rows 61 on the right side of the body. The rows are densely arranged and the kinetosome triplets smaller than in *F. acuminata* described above.

Buccal cavity, excretory pore and nuclear apparatus were not observed.

The incidence of particular species of ciliates and their relative mean intensity, counted for one slide, are given in Table 2. The most frequent and numerous species appeared to be *Chilodonella cucullulus*. Other species, *Dexiostoma campylum*, was also numerous but occurred on smaller number of fishes. The remaining species occurred rather rarely and usually not numerously except *Colpidium colpoda*. The last mentioned species was found on more than half of examined fishes from Żabieniec.

It is noteworthy that carps from Wilga were frequently infected by *Frontonia acuminata*. On one fish numerous specimens of this species were found. In the same farm another species of this genus *F. leucas* was found also, but only a single specimen. On the other hand *Chilodonella cucullulus* in Wilga was less frequent and numerous.

Beside the described species in the same preparations there were found also some other species of ciliates but their specific identification was not possible. One of these species was fairly frequent, the remaining ones occurred only in single specimens.

Particular attention was attracted to one fish specimen collected on 16th March 1970 from the pond No. 6 (Table 2, fish No. 1). This fish, weighing about 80 g, showed significantly lowered reaction to stimuli and symptoms of dyspnoea. It swam at the surface of water, in an air hole in the ice, near the outflow from the pond. It was easy to catch this fish with the aid of a hand net. Examination of this fish alive, just after catching, showed the unnatural appearance of gill lamelli, which

were dilated and coloured brown at the ends. Microscopical examination revealed mass incidence of free-living ciliates; their number overpassed one thousand on four branchial arcs of one body side. Weakness and change of the colour of gill lamelli were not so strongly marked in another specimen (Table 2, fish No. 3) on which about 500 nonspecific ciliates were found. On remaining fishes the number of nonspecific ciliates was lower and only small pathological changes, resulting from the illness of nostrils in previous year, were observed.

Discussion

Nonspecific ciliates found on fishes are typical in structure and do not differ in ciliature pattern from free-living representatives of the species. On can find only some quantitative differences concerning body dimensions and number of cortical elements (mainly the number of kineties). The specimens of *Chilodonella cucullulus*, occurring on fishes, had somewhat smaller dimensions than the dimensions given for this species by various authors, among others by Radzikowski 1966. The specimens of *Dexiostoma campylum* were also smaller but the number of kineties was greater than that described by Jankowski 1967. The body dimensions as well as the number of kineties in *Glaucoma scintillans* oscillated near the upper range known for this species (Czapik 1968, Klug 1968).

Similar situation was observed in the case of *Colpidium colpoda* (Czapik 1968). *Frontonia acuminata* having the body dimensions similar to the values given by Roque 1961 had greater number of kineties. The remaining two species, *Chilodonella uncinata* and *Frontonia leucas* were too few in number to be used for such comparisons.

At present it is difficult to explain the differences observed by us in body dimensions and number of kineties between the specimens found on fishes and free-living representatives of the species described by other authors. Possibly it is a result of different conditions found by these ciliates on the fish body in comparison with normal habitat of free-living specimens. This problem is additionally complicated by the fact that the free-living forms used for comparison originated from cultures bred on various media. In general the influence of parasitic mode of life on the variability of ciliates is known. Among others Kozloff 1956 infecting experimentally the mollusc *Deroceras reticulatum* by ciliates *Tetrahymena pyriformis* obtained the specimens with the same number of kineties but smaller body in parasitic phase. Similar results were obtained by Seaman and Tosney 1967 with *T. pyriformis* from experimentally infected cockroaches *Periplaneta americana*. In the case of our material similar influence may be looked for in *Chilodonella cucullulus* and *Dexiostoma campylum*.

On the other hand the factors of the environment, especially the temperature, may play an important role. Increasing of the the body dimensions and of the

number of cortical elements in ciliates in winter and early spring connected with low temperature was demonstrated by Kazubski and Migała 1968 and Kazubski 1969. In the described case it would be the effect of lower temperature of water in ponds from which the material was collected in comparison with the temperatures used in laboratory cultures of these ciliate species. It would be the case of *Glaucoma scintillans*, *Colpidium colpoda*, and *Frontonia acuminata*.

Of course it is only a possible interpretation of observed differences and this problem requires further study.

The species composition of nonspecific ciliates found on gills of fishes is also interesting. They all are ubiquitous species which may occur in high oxygen deficiency, in polluted water and even in sewage. *Chilodonella cucullulus*, *Ch. uncinata*, *Dexiostoma campylum*, *Glaucoma scintillans* and *Colpidium colpoda* are mentioned by Curds 1969 in his key to ciliated protozoa commonly found in activated sludge as the species occurring in polysaprobic, α-mezosaprobic and β-mezosaprobic conditions. Roque 1961 writes about both species of *Frontonia* that they occur in putrefying water. All the species occur in small numbers nearly in each water reservoir, quickly multiplying in favourable conditions. Some of them (*Chilodonella cucullulus*, *Ch. uncinata*, *Glaucoma scintillans*, *Frontonia acuminata* and *F. leucas*) were observed in microbenthos of fish ponds in Poland (Czapik 1959, Grabacka 1971).

Probably in ponds, especially in winter ponds from which the fishes infected with nonspecific ciliates were collected, developed conditions similar to those in reservoirs with strongly polluted water. These conditions favoured multiplying of saprobiontic ciliates and their settling in branchial cavity and gills of fishes. In winter ponds such conditions develop easily due to accumulation of great number of fishes in proportionally small space, pollution of the water with organic substances, accumulation of shed off epidermis (Staff 1925) and lowering of oxygen content in the water under long lasting covering by ice and weak current of water. During the examined period, in March 1970, in ponds No. 5, 6, 9, 10 and 12 the pH value was 6.5 and oxygen content was very low. In the extreme case, in pond No. 10 the oxygen content attained only to 5.1 mg/l it is only 35.1% of saturation the water with oxygen in the water temperature 0.1°C noted on this day (19th March 1970). Toward the spring the quantity of nitrates and phosphates in ponds increased also.

That the occurrence of nonspecific saprobiontic ciliates on fishes is connected with described conditions may prove the fact that in spring, after melting away of the ice, improvement of water current and increasing of oxygen content in the water (in the middle of the April 1970 the content of oxygen increased up to 100–115%) these ciliates almost completely disappear from gills of fishes. Migała 1970, 1971 carried out the research some years earlier in this territory during the vegetative season and found only few specimens of *Chilodonella uncinata* on carps.

In general we have probably to do with the following course of the events. Usually free-living ciliates displaying great possibilities to live in various habitats (poly-

saprobionts and mesobionts) in specific conditions of winter ponds at the end of winter (dense population of fishes, slow current of water, accumulation of great amount of organic matter and low oxygen content) may quickly multiply and settle on gills and skin of fishes. The most favourable conditions they find probably on weakened or ill fishes, especially when the gills and skin are pathologically changed. Further multiplication of the ciliates on fishes can not be harmless for their hosts, worsening their sickness. In such a way nonspecific ciliates may be a factor accelerating the death of fishes in spite of the fact that they are not a primary cause. And with such case we had to do in our research. In spite of rather low infection rate there appeared single specimens of carps heavily infected. They were in very bad health conditions and their death was merely a question of time. It would be interesting to search in the future whether these nonspecific ciliates do harm or do not harm directly to their fish hosts.

There is also another cause of dangerous action of nonspecific ciliates on fishes. These ciliates are a common, impossible to eliminate, component of protozoon fauna is most water reservoirs. In specific conditions they begin to act as pathogens. Of course, the list of such species, given in the present paper, is not complete. Even in our material some more species had been found, however they were not identified. After all, the pathogenic action of nonspecific ciliates on fishes depends on special conditions in water reservoir. It may be also a suggestion how to counteract such infection preventing the development of circumstances in which these ciliates would multiply and settle on fishes. It would consist upon preventing to accumulate organic matter and development of putrefying processes in winter ponds. It is also necessary to maintain oxygen content in the water at defined level.

Summary

The authors described several species of ciliates: *Chilodonella cucullulus* (O. F. M.), *Ch. uncinata* (Ehrbg.), *Dexiostoma campylum* (Stokes), *Glaucoma scintillans* Ehrbg., *Colpidium colpoda* (Ehrbg.), *Frontonia acuminata* Ehrbg., and *F. leucas* Ehrbg. found on gills and skin of carps (*Cyprinus carpio* L.) form winter ponds in the end of winter and in early spring. It was found that these ciliates infest mainly weakened fishes and in favourable conditions may multiply massively leading to worsening fish condition and even to the death. The ways of development of such infestations and the remedy for them are discussed.

STRESZCZENIE

Opisano szereg gatunków orzęsków: *Chilodonella cucullulus* (O. F. M.), *Ch. uncinata* (Ehrbg.), *Dexiostoma campylum* (Stokes), *Glaucoma scintillans* Ehrbg., *Colpidium colpoda* (Ehrbg.), *Frontonia acuminata* Ehrbg. i *F. leucas* Ehrbg. stwierdzonych w końcu zimy i wczesną wiosną na skrzelach

i skórze karpi (*Cyprinus carpio* L.) w zimochowach. Stwierdzono, że te niespecyficzne orzęski atakują głównie ryby osłabione i w sprzyjających warunkach mogą rozmnożyć się masowo, co może prowadzić do dalszego osłabienia a nawet śmierci ryb. Dyskutowane są drogi powstawania takiej inwazji i ewentualne środki zaradcze.

REFERENCES

Curds C. R. 1969: An illustrated key to the British freshwater ciliated *Protozoa* commonly found in activated sludge. Water Polution Research Technical Paper No. 12, H. M. Stationary Office London, pp. 90.

Czapik A. 1959: Microbenthos of fish-ponds in Mydlniki. Acta Hydrobiol., 1, 187–196.

Czapik A. 1968: La famille *Tetrahymenidae* et son importance dans la systématique et l'évolution des ciliés. Acta Protozool., 5, 315–358.

Čečina A. S. 1960: Sezonnye i vozrastnye izmenenija parazitofauny karpov v prudovyh hozjajstvach BSSR. Trudy belorussk. naučno-issled. Inst. rybn. hoz., 3, 107–118.

Grabacka E. 1971: Ciliata of the bottom of rearing fish-ponds in the Gołysz Complex. Acta Hydrobiol., 13, 5–28.

Ivasik V. M. 1957: Parazitofauna karpa v zimnij period. Zool. Zh., 36, 1571–1573.

Jankowski A. W. 1967: Granicy i sostav rodov *Tetrahymena* i *Colpidium*. Zool. Zh., 46, 17–23.

Kazubski S. L. 1969: Seasonal variability in ciliates and its consequences. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, 369–370.

Kazubski S. L. and Migała K. 1968: *Urceolariidae* from breeding carp — *Cyprinus carpio* L. in Żabieniec and remarks on the seasonal variability of trichodinids. Acta Protozool., 6, 137–160.

Klug S. 1968: Cortical studies on *Glaucoma*. J. Protozool., 15, 321–327.

Kozloff E. N. 1956: Experimental infection of the gray garden slug *Deroceros reticulatum* (Müller) by the holotrichous ciliate *Tetrahymena pyriformis* (Ehrenberg). J. Protozool., 3, 17–19.

Ljajman E. M. 1951: Sezonnaja zaražennost karpa parazitami. Trudy mosk. tehn. Inst. rybn. promyšl. i hoz., Tom IV.

Migała K. 1969: Succesional appearance of parasitic *Protozoa* on carps in ponds. Progress in Protozoology, Third int. Congr. Protozool., Leningrad 1969, Nauka, 215–217.

Migała K. 1970: Badania zewnętrznych pasożytów karpia (*Cyprinus carpio* L.) w warunkach intensyfikowania produkcji stawowej. *Protozoa*. Roczn. Nauk roln., 92-H-4, 57–79.

Migała K. 1971: Studies on natural populations of parasitic protozoa on *Cyprinus carpio* L. in pond culture. Carps in the first year of life. Acta Protozool., 8, 309–339.

Migała K. and Kazubski S. L. 1971: Nonspecific ciliates on cyprinid fishes in winter ponds. J. Protozool., 18, suppl, in press.

Radzikowski S. 1966: Study on morphology, division and postconjugation morphogenesis in *Chilodonella cucullulus* (O. F. Müller). Acta Protozool., 4, 89–95.

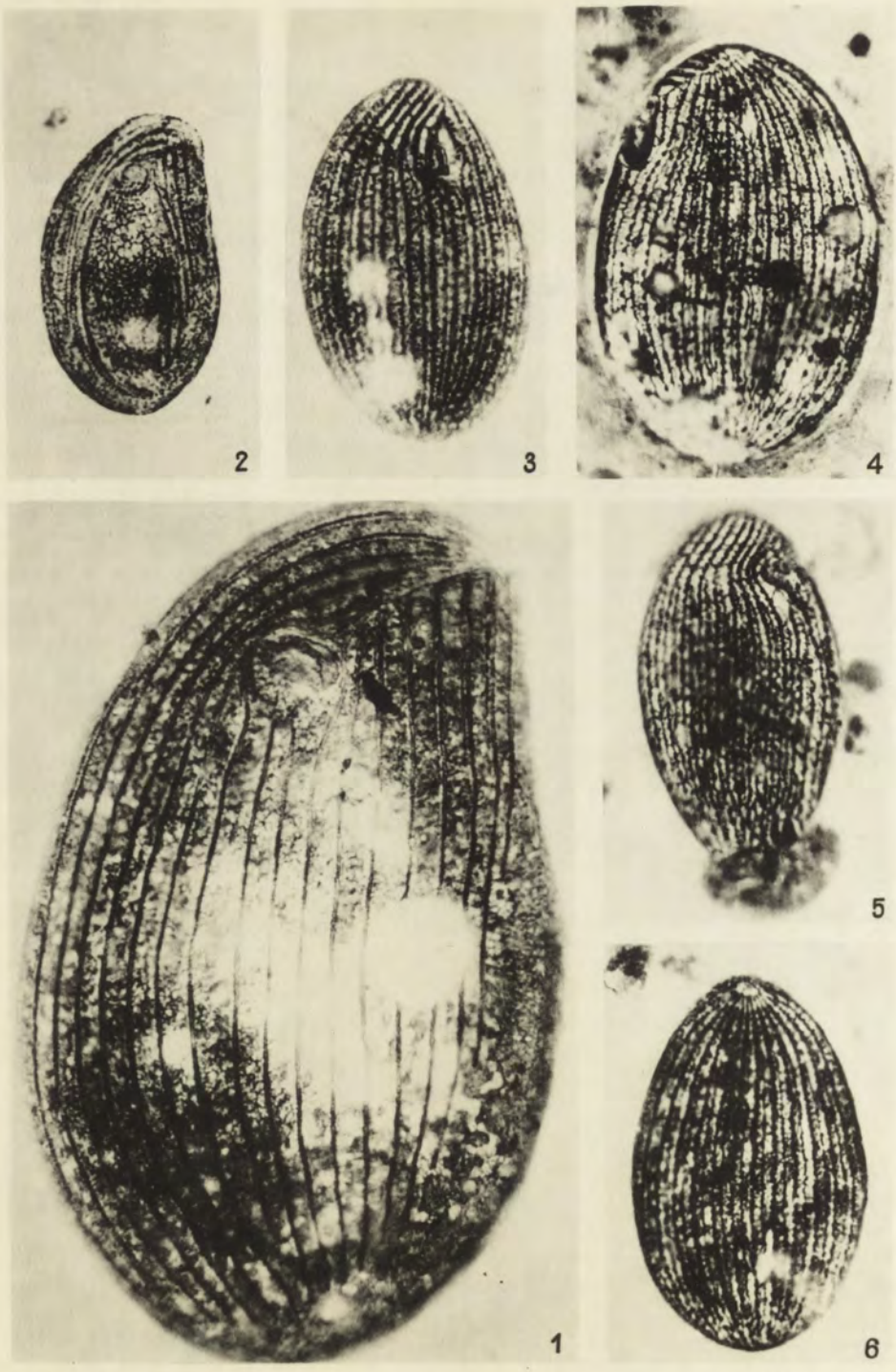
Roque M. 1961: Recherches sur les Infusoires Ciliés: les Hyménostomes péniculiens. Bull. biol. Fr. Belg., 95, 431–519.

Seaman G. R. and Tosney T. J. 1967: Alternations in morphology of *Tetrahymena pyriformis* S. after facultative parasitism in the cockroach, *Periplaneta americana*. J. Protozool., 14, suppl. 23–24.

Staff F. 1925: Choroba nozdrzy u karpi jako przyczynek do fizjologii i patologii snu zimowego ryb. Roczn. Ichtiobiol., 4, 1–47.

EXPLANATION OF PLATES I–III

1: *Chilodonella cucullulus* (O. F. M.), ventral viev
2: *Chilodonella uncinata* Ehrbg., ventral viev
3–6: *Dexiostoma campylum* (Stokes), 3 — general viev of the ventral side, 4 — general view of the left side, posterior suture is visible, 5 — right side, breakdown of kineties and single pore of the contractile are visible, 6 — dorsal view of ciliate
7–10: *Glaucoma scintillans* Ehrbg., 7 — ventral view, posterior suture and postoral kineties are visible, 8 — right side, 9 — ventral side, postoral kineties are visible, 10 — left side of ciliate
11–15: *Colpidium colpoda* (Ehrbg.). 11 — right view, 12 — left view, 13 — slender specimen, 14–15 — posterior suture and apex of ciliate
16: *Frontonia acuminata* Ehrbg., general view of the ventral side
17: *Frontonia leucas* Ehrbg., general view
Photographs 1–16 magnification 1000×, 17 — magnification 250×



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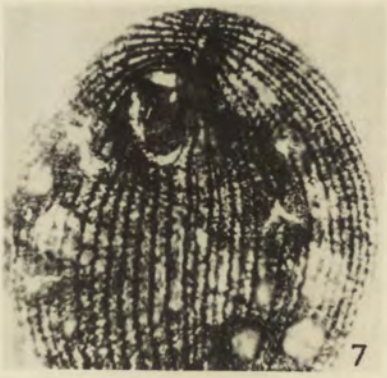

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A comparative cytophotometrical investigation of the dynamics of cytoplasmic RNA in the endogenous development of *Eimeria tenella* grown in vivo, in tissue culture and in chick embryo. I. Schizogonous development

Сравнительное цитофотометрическое исследование динамики цитоплазматической РНК в эндогенном развитии *Eimeria tenella* in vivo, в культуре ткани и в курином эмбрионе. I. Бесполая фаза жизненного цикла

In recent years, much progress has been achieved in cultivation of *Eimeria* species outside the host body. Thus far, six species have been succesfully observed to complete their life cycles in the chorioallantoic membrane (C. A. M.) of chick embryo (Long 1965, 1966, 1969, Shibalova 1968 b, Shibalova et al. 1969, Shibalova and Korolev 1971). Beginning from the pioneer study of Patton 1965, the growth of *Eimeria*, involving the asexual stages of the parasite, was followed in tissue culture for several chick and bovine eimerians (Strout et al. 1965, Doran and Vetterling 1967 a, b, 1968, Fayer and Hammond 1967, Hammond and Fayer 1968, Matsuoka et al. 1969). The complete life cycle has been so far followed for *E. tenella* only (Doran and Vetterling 1967 b, Bedrnik 1967, 1969, Strout and Ouellette 1969, Shibalova 1968 b, 1969, 1970, Doran 1970).

The propagation of *Coccidia* in cultured cells and chick embryo offers a new approach to study the host-parasite relationships, to examine peculiarities of the required environmental conditions both favoring and limiting the normal development of the parasite. Thus, it would seem of great importance to follow the patterns of similarity and of dissimilarity in morphology and metabolic behaviour of the cultured and naturally growing parasites, both at species and strain levels. Some liimited amount of evidence so far available suggests that, in essence, the ultrastructure of *E. tenella* asexual stages cultivated in vitro appears similar to that reported from in vivo material (Scholtyseck and Strout 1968, Strout and Scholtyseck 1970).

A comparative analysis of the above problems would seem to contribute much to the solution of some questions yet unsolved. For example, we are not fully aware of the food or environmental requirements of the cultured or embryo grown eimerians. This may account for the failure in the attempts to obtain the whole life cycles of

Eimeria, other than *E. tenella*, in tissue culture. On the other hand, *E. maxima* and *E. acervulina* fail to grow in the chorioallantoic membrane (C. A. M.) of the chick embryo, however, this substrate is known to be easily utilized by other chicken *Eimeria* (Long 1966, Shibalova et al. 1969). How does the parasite behave in the non-specific environment? Are the patterns of its metabolism similar while in the host body and outside it? To answer these and other related questions would be of interest from both theoretical and practical standpoint.

The present communication reports an attempt of such a comparative analysis of the metabolic activity of *E. tenella* grown in vivo (chick caeca) as well as in tissue culture and the C. A. M. Using methods of cytophotometry, the dynamics of the cytoplasmic RNA was chosen for the estimation of metabolic activity of the asexual stages in corresponding life conditions.

Material and methods

In vivo experiments

A pure culture of oocysts was employed originated from feeding coccidia-free chickens with one oocyst of *E. tenella*. The strain was continuously passed through 14–22 day old "Russkaya Belaya" chickens kept in the Laboratory of Protozoology of the All-Union Institute for Poultry Diseases in Leningrad. The birds were hatched in the laboratory incubator or obtained from the poultry farm in the age of one day. The chickens were kept under conditions precluding coccidiosis or any other infection.

The oocysts used for the per os administration were obtained from the caeca of chickens, treated with sodium hypochloride for desinfection (Jackson 1964) and then set for sporulation in sterile conditions with a permanent aeration.

Due to some peculiarities of the cytophotometrical technique used (see below), the birds were fed fractionated doses of sporulated oocysts, administered at least during two days, which enabled us to have one generation schizonts of various maturity on the same preparation.

Sporozoite suspension

Sporozoites were obtained in vitro by excystation of sporulated oocysts, mechanically destroyed, using a technique similar to that described by Doran and Farr 1961 in our modification (Shibalova 1968 a)

Inoculation of tissue culture

A primary cell culture of fibroblasts derived from 10–11-day chick embryos was employed. Suspensions of fibroblast cells were introduced into test-tubes, containing cover slips, 600 000 cells into each tube. The cultures were incubated at 40–41°C for 2–3 days, until the cover slips were covered with monolayered cells, and then sporozoites were inoculated. For this, sporozoites, in concentration of 100 000 per 1 ml, were suspended in a proper medium. The pH of the latter being adjusted to 7.0–7.2, 2 ml of the medium was placed into each test-tube from which the growth medium had been removed. The inoculated cell cultures were incubated at 41°C.

Inoculation of chick embryo

8 day old chick embryos served for the experiments. These were incubated at 37° and 40°C before and after sporozoite injection, resp. The C.A.M of each embryo was inoculated with 100 000–300 000 sporozoites. The number of sporozoites was counted in the Goryaev count chamber.

Histological examination

The caeca of infected birds were removed 3–4 days after oocyst administration, when the first generation schizogony of *E. tenella* occurs. As the fixative, the Carnoy fluid was used. Paraffin 5 μ sections were stained with gallocyanin-chromalum for 48 h (Pearse 1960); sections treated with crystalline ribonuclease were used as a control.

Infected chick embryos were sacrificed 48–60 h following sporozoite injection when first generation schizogony of *E. tenella* is in progress. The site of the C.A.M. near the injection place, easily distinguished with a nacked eye, was removed and fixed with the Carnoy fixative, the following treatment being similar to that described for the caeca.

At 48–55 h, the cover slips with inoculated cell cultures were taken off the test-tubes and fixed with methanol on the Carnoy fixative. The material was stained with gallocyanin-chromalum for 48 h.

Cytophotometrical examination

Due to some technical difficulties encountered throughout the investigation of multinucleated schizonts, two methods of cytophotometry were employed, whose identity, as to the eventual results, was earlier reported (Ovchinnikova et al. 1963). RNA measurements on tissue culture and C.A.M. preparations was performed using the microspectrophotometer MUV-4 by scanning in $\lambda = 579$ mμ. For the sections of infected caeca of chickens, photographical method was applied using the cytophotometer MUV-6 in $\lambda = 579$ mμ.

The cell squares were measured planimetrically, both the square of the whole growing trophozoite (protoplasm) and that of the anucleated cytoplasm being taken into account. Correspondingly, the cytoplasmic RNA was calculated for the total and fractional squares. The quantity of the RNA was calculated in realtive units according to the formula (Brodsky 1956, Ovchinnikova et al. 1963):

$$Q = DS,$$

where Q is the quantity of cytoplasmic RNA, D — optimal density, and S — square.

All the cells measured were then distributed into three "age" classes according to the values of their areas and the number of the nuclei, for either experiment group separately. It must be stressed that the absolute cell body spacings do not coincide while studied on cross-sections and total preparations, thus precluding any reasonable comparison of cell sizes between the different groups. Anyhow, the comparison of the eventual results obtained for all the three groups (in the chick caeca, in tissue culture, and in C. A. M.) seemed admissible because it was performed within the same asexual generation, and because it was the general tendency of RNA changes (the dynamics), rather than absolute amounts of RNA, that was of primary interest of the present communication.

The cytophotometric technique employed for studies of the cytoplasmic RNA throughout schizogonic development of *E. tenella* appeared to have some limitations when compared with its similar application for studies on the coccidian macrogametogenesis (Beyer and Ovchinnikova 1964). The fact is that the nuclei in growing schizonts occupy some definite space which must be taken into account at measuring. Thus, two definite events occur simultaneously in the growing trophozoite, the augmentation of the cytoplasmic share along with the increase of the total nuclear amount. In large mature schizonts (yet non-segmented) the nuclei are so numerous that cytoplasmic segments, free of nuclei, can be found with difficulty. In this situation there is a danger to measure accidentally not only the cytoplasmic RNA, but also the nuclear RNA and DNA. This made us confine our measurements to those multinucleate cells whose cytoplasmic areas were easily available to scanning (Pl. I 1c, 3, 4 c).

As has been pointed out above, the cytoplasmic RNA was calculated both for the whole trophozoite square and for the square of the "anucleated" cytoplasm. Subtracting the squares of the nuclei, the total result of measurement would appear to be less than a theoretical one by the amount of RNA adjoining above and below the nuclei. On the other hand, however, the total count

of RNA for the whole protoplasm square would inevitably give a much higher result than the theoretically expected one. But since the aim of our investigation was to compare the dynamical tendency of RNA in parasites growing under different life conditions, rather than to obtain the absolute values of the RNA, the mode of counting involving the highest and the lowest results seems to be quite acceptable. It goes without saying that the true result may be found somewhere between.

Another difficulty encountered throughout the measurements involved impossibility to compare the absolute counts obtained for cells even within one experimental group, if these were examined from different coverslips or glasses. To make the material uniform, only cells sharing the same preparation were measured. To obtain sufficient number of in vivo material, the repeated inoculation of the host was performed.

The data obtained are treated statistically.

Results

The results obtained from the cytophotometry of trophozoites of *E. tenella* are represented in Table 1.

As seen from the Table 1, the average density (D) has a tendency to increase in all the three classes of trophozoites. However, the differences between the neighbouring classes and between classes I and III in the in vivo material appeared statistically non-significant (Pl. I 1). In the in vitro material, t_{dif} is less than 3 between classes I and II in tissue culture (Pl. I 2), and between classes I and II, and between II and III in C. A. M. material (Pl. 1 4). However, the differences between classes I and III, both in tissue culture and C. A. M. material, appeared to be statistically significant.

The cell spacings calculated from the cell squares are seen to increase progressively in all the three experimental groups, the differences between the neighbouring classes being statistically significant.

The quantity of the cytoplasmic RNA is found to increase as the parasite grows, whatever the life conditions provided may be: the chick caeca, tissue culture, or chorioallantoic membrane of chick embryo. The differences between the neighbouring "age" classes of trophozoites are significant, with one exception being found between classes I and II in C. A. M. material where t_{dif} is less than 3.

Thus, both the cytophotometric techniques employed have revealed that the main tendency of cytoplasmic RNA in the growing parasite is a progressive increase throughout asexual generation of *E. tenella*.

Discussion

The applicability of gallocyanin-chromalum method for the aims of quantitative histochemistry of nucleic acids was reported elsewhere (Einarson 1951, Pearse 1960, Sandritter et al. 1954, Oram 1955, Weber 1958, Ovchinnikova and Selivanova 1964). Some years ago, we succeeded in using this technique for studies

Table 1

Results of the cytophotometric estimation of the cytoplasmic RNA in the first asexual generation of *Eimeria tenella* developing in the chick caeca, tissue culture and in the chorioallantoic membrane of chick embryo (in relative units)

Locality	"Age" classes of trophozoites	Average optical density (D)	Cell square (S)		RNA quantity (Q) calculated for	
			total (protoplasm)	"anucleated" cytoplasm	total cell square	square of the anucleated "cytoplasm"
Chick caecal epithelium	I	0.1845±0.015	78.90±4.51	27.10±1.09	14.34±0.63	4.84±0.41
	II	0.2265±0.010	164.80±3.22	40.87±1.35	36.51±1.85	8.69±0.60
	III	0.2384±0.050	264.01±8.42	54.20±2.40	62.42±2.51	12.28±0.62
Tissue culture	I	0.2507±0.010	29.64±1.50	17.01±0.87	7.41±0.80	4.22±0.51
	II	0.2725±0.027	148.61±7.12	40.95±2.00	40.76±2.53	10.96±0.81
	III	0.3806±0.017	373.80±18.42	50.05±2.01	144.50±9.60	18.67±1.83
C. A. M.	I	0.2035±0.012	20.44±1.31	11.85±0.80	4.32±0.32	2.27±0.30
	II	0.2105±0.014	38.00±1.80	15.75±1.00	7.95±0.61	3.21±0.30
	III	0.2385±0.012	69.51±3.61	26.80±1.80	16.07±1.40	6.09±0.89

of the cytoplasmic RNA in the macrogametogenesis of two rabbit intestinal coccidia — *E. intestinalis* and *E. magna* (Beyer and Ovchinnikova 1964, 1966). According to the authors' knowledge, no other studies have been made on cytophotometry of RNA in coccidia or other parasitic protozoa. The technique in question has the advantage of accuracy, precluding any accidental assumptions of the substrate amount, and in addition, providing the possibility to follow the process in dynamics.

Of special interest is the application of the quantitative technique for growing cells whose volume (square) progressively changes during the cell cycle. With these cells, an erroneous determination by sight is most easily achieved, because the rapidly decreasing concentration of the substance in the increasing cell volume can produce a false impression of a falling amount of the substance. Actually, young cells, either mammalian oocytes or coccidian macrogametocytes, stained with pyronine for revealing RNA, look much more basophilic than the mature forms (Brachet 1947, 1952, Caspersson 1950, Pattillo and Becker 1955, Cheissin 1958, 1960, Beyer 1963). Thus, judging by RNA stainability, one may suggests that RNA decreases as the cell grows.

Beginning from pioneer discoveries of Brachet who in 1942 applied quantitative technique for cytological studies, some evidence has been provided for metazoan cells showing definetely that the quantity of RNA increases as the oocyte grows (Eisenstadt et al. 1964). No similar observations were reported for parasitic protozoa until 1964 when Beyer and Ovchinnikova demonstrated a progressive increase of RNA in the cytoplasm of growing macrogametes of *E. intestinalis* and *E. magna*.

The present communication summarizes the results of the cytophotometrical study of cytoplasmic RNA within one asexual generation of *E. tenella*. These results are discussed in terms of their possible significance in elucidating the mode of metabolic activity of the parasite grown in different conditions.

The quantity of RNA was calculated for the total amount of the cell protoplasm and for the "anucleated" cytoplasm, both the results being higher and lower, resp., than the true value for the cytoplasmic RNA which may be somewhere between. Of the two results, we consider the "minimal" one more reliable for further speculations. The square of the "anucleated" cytoplasm is seen to increase, with t_{dif} between the neighbouring classes being more than 3. The optical density, examined in the in vivo material, changes insignificantly not only between the neighbouring classes, but also between classes I and III. This may suggest its slow and even increase throughout the whole asexual generation. However, the resulting increase of the RNA quantity, calculated for the "anucleated" cytoplasm only, has appeared statistically reliable.

Somewhat different picture was observed on in vitro material. The optical density changes between classes I and III appeared to be statistically non-reliable, whereas those between classes II and III were statistically significant ($t>3$) or non-

significant ($3 > t > 2$) for the tissue culture and C. A. M. material, resp. The resulting increase of RNA quantity in both the cases was found to be statistically significant, as in the in vivo material. The differences observed in the dynamics of the average optical density between the "normal" development of the parasite (chick caeca) and that outside the host body seem to be explained like this.

The chicken caeca serve as a natural medium for the *E. tenella* schizonts, and therefore the growth of the latter begins immediately after the penetration of the host cell, proceeding evenly throughout the further development of the parasite. Unlike, both the cultured and C. A. M. cells provide an unnatural habitat for *E. tenella*. It does not seem unlikely, therefore, that before the parasite begins to grow, some period of adaptation to the immediate environment, i.e. cultured or C. A. M. cell, may be required. The duration of the period may be different for individual parasites. A well-known asynchrony in coccidian development outside the host body is to be mentioned as having a bearing on the problem. The maturation from an inoculated sporozoite to a schizont is not synchronous with all parasites on the same preparation. Thus, 48–55 h following sporozoite inoculation of the tissue culture, early trophozoites and schizonts with different numbers of nuclei may be easily found along with mature unsegmented schizonts. However, to have similar diversity in asexual stages on the same in vivo preparation, we had to administer the oocysts to the chickens repeatedly. The step-like character of the optical density changes may thus be thought to reflect, to a certain degree, the parasite's adaptation to the environment.

The correlation between RNA and protein synthesis is a well established phenomenon. Proteins produced during schizogony are required for building the bodies of the numerous merozoites of *E. tenella*. In addition, some portion of this RNA becomes what appears to be a residual body of the schizont. Thus, the intensive production of cytoplasmic RNA and protein throughout the whole schizogonic development is easily understood from the metabolic requirements of the trophozoite. This assumption is in keeping with recent electron microscope discoveries of many ribosomes in the cytoplasm of early segmenting first generation schizonts of *E. tenella* grown in tissue culture (Strout and Scholtyseck 1970).

Although the present study has been so far made on one species only, we are inclined to think that the similar phenomenon may also be the case in the schizogonic development of other eimerians. However, a study on cytochemistry of *E. labbebeana* (Srivastava 1967) seems to oppose this view. The author seems to identify a decreased pyroninophilia of mature schizonts with the diminishing of RNA content in these. He claims that because the trophozoite (schizont) is a short-living stage a lesser amount of RNA and proteins in it is not surprising. We cannot agree with the author's speculations, because the life-span alone is not determinative for estimating the physiological responsibility of the stage. Srivastava used qualitative cytochemistry only and was likely to overestimate a well known (and well documented) statement that young growing cells are more basophilic than mature ones, and to

take, presumably, as synonyms two different notions — "the level of basophilia" and "the quantity of RNA".

The study performed has substantial that within the life cycle of *E. tenella* the trophozoite (schizont) stage fulfils an important physiological function — production of RNA and proteins necessary for building merozoites. One of the main conclusions of this work is that the patterns of fulfilling this function are similar, in essence, whatever the locality of the parasite may be: chicken caecal epithelium, tissue culture of chick embryo fibroblasts, or chorioallantoic membrane of the chick embryo.

Summary

A comparative quantitative study of the cytoplasmic RNA content during the schizogonous development of *Eimeria tenella* was performed using the microspectrophotometers MUV-4 and MUV-6 by scanning in λ 579 mμ. Growing first generation schizonts were compared from chick caecal epithelial cells, primary cell culture of fibroblasts derived from 10–11 day chick embryos, and from the chorioallantoic membrane of chick embryo. It has been shown that as the trophozoite (schizont) grows the RNA content in its cytoplasm progressively increases, the increase being similar, in essence, in all the three cases examined. The average optical density of the cytoplasmic RNA grows evenly, whereas in the in vitro material its increase bears a step-like character. The latter fact may be explained in terms of a gradual adaptation of the parasite to the unusual environment provided by cultured and chorioallantoic cells.

РЕЗЮМЕ

Методом сканирования в длине волны 579 ММК на микроспектрофотометре МУФ-4, а также фотографическим методом в длине волны 579 ММК на цитофотометре МУФ-6 проведено сравнительное исследование динамики цитоплазматичестой РНК в процессе развития 1-ой генерации шизонтов *Eimeria tenella* в процессе развития в эпителии слепых отростков кишечника, в культуре фибробластов, а также в хорионаллантоисной оболочке куриного эмбриода. Показано, что по мере развития трофозоита (шизонта) количество РНК в exo цитоплазме неуклонно возрастает, причем это возрастание происходит в основном одинаково во всех трех изученных случаях. Средняя оптическая плотностъ РНК в цитоплазме шизонтов, растущих в кишечнике цыпленка возрастает постепенно, тогла как при развитии вне организма наблюдастся ступенчатое возрастание плотности. Предполагается, что это может бытъ связано с постепеиной адаптацией паразита к непривычноц среде обитания – клетке кулътуры или хорионаллантоисной оболочки.

REFERENCES

Bedrnik P. 1967: Further development of the second generation of *Eimeria tenella* merozoites in tissue culture. Folia Parasit., 14, 361–364.

Bedrnik P. 1969: Cultivation of *Eimeria tenella* in tissue cultures. 1. Further development of second generation merozoites in tissue cultures. Acta Protozool., 7, 87–98.

Beyer T. V. 1963: Citohimičeskoe issledovanie kišečnyh kokcidii krolika pri raznych uslovijach ih suščestvovanija v hozjaine. Avtoreferat kand. diss. L. (A cytochemical study of rabbit intestinal coccidia under different life conditions in the host. Abstract of cand. diss. L.).

Beyer T. V. and Ovchinnikova L. P. 1964: Cytophotometric study of RNA content in the macrogametogenesis of two rabbit intestinal coccidia *Eimeria magna* and *E. intestinalis*. Acta Protozool., 2, 33, 329–337.

Beyer T. V. and Ovchinnikova L. P. 1966: A cytophotometrical investigation of the cytoplasmic RNA content in the course of oocyst formation in the intestinal rabbit coccidia *Eimeria intestinalis* Cheissin, 1948. Acta Protozool., 4, 9, 75–80.

Brachet J. 1947: The metabolism of nucleic acids during embryonic development. Cold Spring Harb. Symp. Quant. Biol. 12, 18–21.

Brachet J. 1952: The role of nucleus and the cytoplasm in synthesis and morphogenesis. Symp. Soc. exp. Biol., 6, 173–200.

Brodsky V. Ya. 1956: Citofotometry. Usp. sovrem. Biol., 42, 87–107.

Caspersson T. 1950: Cell growth and cell function. A cytochemical study. Norton and Co. New York.

Cheissin E. M. 1958: Cytologische Untersuchungen verschiedener Stadien des Lebenszyklus der Kaninchencoccidien. I. *Eimeria intestinalis*. E. Cheissin, 1948. Arch. Protistenk., 102, 265–290.

Cheissin E. M. 1960: Citologičeskoe issledovanie žiznennogo cikla kokcidij krolika. 2. *Eimeria magna* Pérard, 1924. In: Voprosy citologii i protistologii, 258–276, Moscow–Leningrad.

Doran D. 1970: *Eimeria tenella*: From sporozoites to oocysts in cell culture. Proc. helminth. Soc. Wash., 37, 1, 84–92.

Doran D. and Farr M. M. 1961: In vitro excystation of *Eimeria acervulina*. J. Parasit., 4 (suppl) 45.

Doran D. J. and Vetterling J. M. 1967 a: Cultivation of the turkey coccidium, *Eimeria meleagrimitis* Tyzzer, 1929, in mammalian kidney cell cultures. Proc. helminth. Soc. Wash., 34, 59–65.

Doran D. J. and Vetterling J. M. 1967 b: Comparative cultivation of poultry coccidia in mammalian kidney cell cultures. J. Protozool., 14, 657–662.

Doran D. J. and Vetterling J. M. 1968: Survival and development of *Eimeria meleagrimitis* Tyzzer, 1929, in bovine kidney and in turkey intestine cell cultures. J. Protozool., 15, 796–802.

Einarson L. 1951: On the theory of gallocyanin-chromalum staining and its application for quantitative estimation of basophilia. Acta path. microbiol. scand., 28, 82–102.

Eisenstadt T. B., Brodsky V. Ya. and Ivanova S. N. 1964: Citologičeskoe issledovanie ovogeneza. II. Citohimičeskoe issledovanie rosta ovocitov ulitkovoj pijavki po dannym ul'trafioletovoj citofotometrii i interferencionnoj mikroskopii. Citologija, 6, 1, 77–88.

Fayer R. and Hammond D. M. 1967: Development of first generation schizonts of *Eimeria bovis* in cultured bovine cells. J. Protozool., 14, 764–772.

Hammond D. M. and Fayer R. 1968: Cultivation of *Eimeria bovis* in three established cell lines and in bovine tracheal cell line cultures. J. Parasit., 54, 559–567.

Jackson A. R. S. 1964: The isolation of viable coccidial sporozoites. J. Parasit., 34, 87–93.

Long P. L. 1965: Development of *Eimeria tenella* in avian embryos. Nature, 208, 5009, 509–510.

Long P. L. 1966: The growth of some species of *Eimeria* in avian embryos. J. Parasit., 56, 575–581.

Long P. L. 1969: Observations on the growth of *Eimeria tenella* in cultured cells from the parasitized chorioallantoic membranes of the developing chick embryo. J. Parasit., 59, 757–765.

Matsuoka T., Callender M. and Shumard R. 1969: Embryonic bovine tracheal cell line for in vitro cultivation of *Eimeria tenella*. J. Vet. Res., 30, 1119–1122.

Oram V. 1955: The cytoplasmic basophilic substance of the exocrine pancreatic cells. Acta anat., Suppl. 23, 34–41.

Ovchinnikova L. P. and Selivanova G. V. 1964: Proverka primenimosti okraski gallocianinomhromovymi kvascami dlja fotometričeskogo izmerenija količestva RNK v citoplazme. Citologija, 6, 3, 387–388.

Ovchinnikova L. P., Selivanova G. V. and Cheissin E. M. 1963: Issledovanie metodom ul'trafioletovoj citofotometrii vlijanija golodanija na količestvo DNK i RNK u *Paramecium caudatum*. In: Morfologija i fiziologija prostejših, 44–53, Leningrad.

Pattillo W. H. and Becker E. R. 1955: Cytochemistry of *Eimeria brunetti* and *E. acervulina* of the chicken. J. Morph., 96, 61–96.

Patton W. 1965: *Eimeria tenella*: Cultivation of the asexual stages in cultured animal cells. Science, 150, 767–769.

Pearse A. E. G. 1960: Histochemistry. Theoretical and applied. J. and A. Churchill LTD.

Sandritter W., Diefenbach H. und Krantz F. 1954: Uber die quantitative Bindung von Ribonucleinsäure mit Gallocyaninchromalum. Experientia, 10, 210–212.

Scholtyseck E. und Strout R. 1968: Feinstrukturuntersuchungen über die Nahrungsaufnahme bei Coccidien in Gewebenkulturen (*Eimeria tenella*). Z. ParasitKde., 30, 291–300.

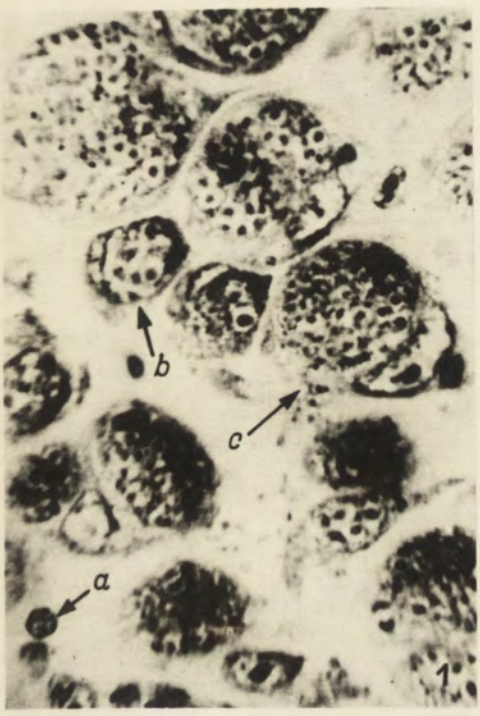
Shibalova T. A. 1968 a: Ob ekscistirovanii sporozoitov *Eimeria tenella* in vitro. Parasitologija, 11, 4, 372–374.
Shibalova T. A. 1968 b: Kultivirovanie *Eimeria tenella* na kurinyh embrionah i v kulturah tkanei. Parasitologija, 11, 5, 483–484.
Shibalova T. A. 1969: Kultivirovanie stadij bespoloj fazy žiznennogo cikla *Eimeria tenella* v kulturah kletok. Citologija, 11, 6, 707–713.
Shibalova T. A. 1970: Cultivation of the endogenous stages of chicken *Coccidia* in embryos and tissue culture. J. Parasit., 56, 4, sect. 2, part 1, 315–316.
Shibalova T. A. and Korolev A. M. 1971: Cultivation of chicken *Coccidia* in chick embryo. I. The development of *Eimeria tenella*. Acta Protozool., 8, 349–354.
Shibalova T. A., Korolev A. M. and Sobchak I. A. 1969: Kultivirovanie kokcidii kur na kurinyh embrionah. Veterinarija, 11, 68–71.
Srivastava H. K. 1967: Role of nucleic acids and proteins in the life cycle of *Eimeria labbeana* Pinto with a review of the opinions regarding the DNA in macrogametocytes. Cellule, 66, 3, 363–376.
Strout R. and Ouellette C. 1969: Gametogony of *Eimeria tenella* (*Coccidia*) in cell cultures. Science, 163, 695–696.
Strout R. G. and Scholtyseck E. 1970: The ultrastructure of first generation development of *Eimeria tenella* (Railliet and Lucet, 1891) Fantham, 1909 in cell cultures. Z. Parasit., 35, 87–96.
Strout R. G., Solis J., Smith S. and Dunlop W. 1965: In vitro cultivation of *Eimeria acervulina* (*Coccidia*). Expl. Parasit., 17, 241–46.
Weber J. 1958: The basophilic substance of the gastric chiff cells and its relation to the process of secretion. Acta anat., Suppl. 31, 29–36.

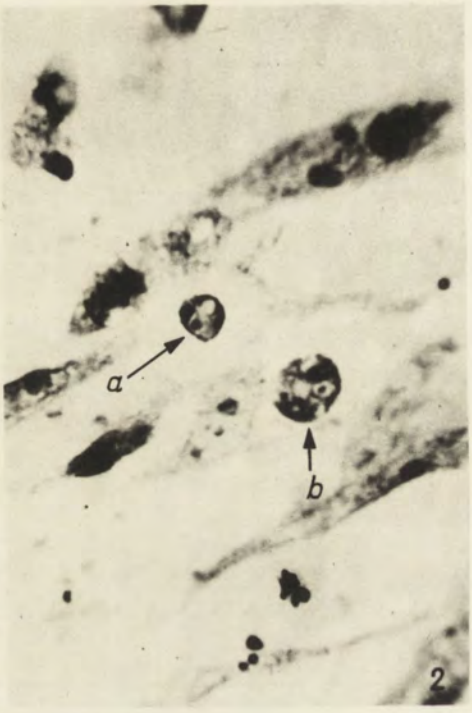
EXPLANATION OF PLATE I

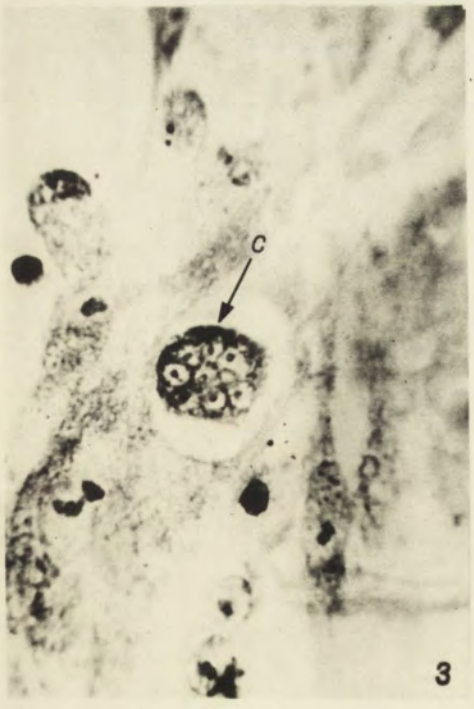
Photomicrographs of *Eimeria tenella* first generation trophozoites (schizonts) belonging to different "age" classes;

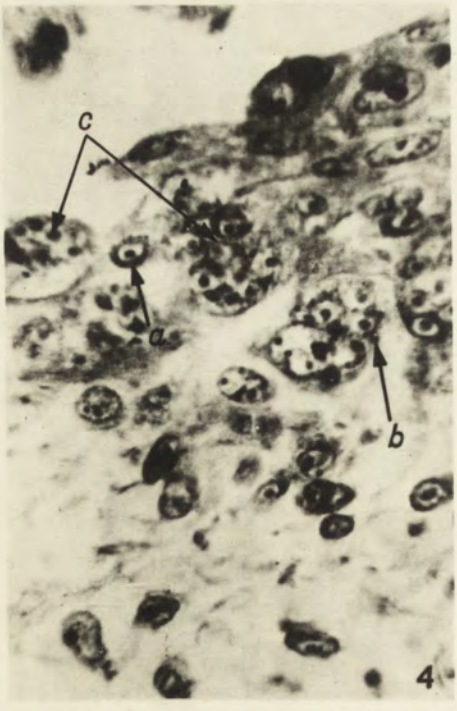
1: Cross-section of the infected chick caecal epithelium (a, b, c, — I, II, and III classes, resp.)
2: Trophozoites of I and II class (a and b, resp.) in fibroblast cell culture
3: Trophozoite of III class in fibroblast cell culture
4: Cross-section of the infected chorioallantoic membrane of chick embryo (a, b and c — I, II and III classes, resp.)

All stained with gallocyanin-chromalum 90×, 7×









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The potassium-calcium equilibrium and chemotactic sensitivity in *Stentor coeruleus**

Równowaga potasowo-wapniowa a wrażliwość chemotaktyczna *Stentor coeruleus*

Basing on the experiments on *Paramecium*, a hypothesis was put forward that all the motoric reactions evoked by chemical stimulation are the result of desorption of Ca^{++} ions from the ciliate membrane (Jahn 1962). It should be expected therefore that similar processes may occur in the ciliate *S. coeruleus* as well. It was known from the results of Kamada and Kinosita 1940 that potassium is a strong antagonist of calcium. Jahn 1962 stated on the base of analysis of the results of Kamada and Kinosita, that the competition of those two ions for the adsorption places on membrane, persists in the state of equilibrium if the principle of Donnan i.e. $[K^+]/\sqrt{[Ca^{++}]}=$const. is fulfilled, whereas other values of this ratio determine various states of excitability of the protozoan cell. This conclusion is also supported by the studies of Grębecki 1964 on the duration of ciliary reversal in *Paramecium* at different levels of Ca^{++} and K^+ ions in medium. It was shown also by analysis of the results of experiments concerning the influence of adaptation to ions on chemotaxis in *Stentor coeruleus* (Pietrowicz-Kosmynka 1971 b), that the most considerable fall of chemotactic sensitivity to the presence of potassium ions in medium occurs at a constant Ca^{++} concentration. These observations inclined the author of the present paper to carry out studies on the model of *S. coeruleus* in order to detect a possible dependence of chemotactic reaction on the value of $[K^+]/\sqrt{[Ca^{++}]}$ in the medium.

The next stage of the study has been to apply the value $[K^+]/\sqrt{[Ca^{++}]}$, called in this article the value Eq_{G-D} (Gibbs–Donnan equilibrium), as the index for determining the state of the cell sensitivity in chemotactic reactions.

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Material and methods

All the experiments had been carried out on a monoculture of *Stentor coeruleus*. Description of the culture method and of the treatment of protozoa prior to experiments, was described by the author previously (Pietrowicz-Kosmynka 1971 a).

The registration methods of the ciliate motile behaviour and of chemotactic reactions were analogous to those applied on *Paramecium* (Dryl 1958, 1959) and are described in detail in the previous articles (Pietrowicz-Kosmynka 1971 a, b).

All the experiments were carried out in different conditions as summarized in Table 1. Experiments and observations were performed in seven solutions marked by the letters a, b, c, d, e, f, g,

Table 1

Characteristic of solutions applied in experiments

Successive solutions	Value of Eq_{G-D}											
	0.5		1		1.5		2		4		8	
	Concentrations of ions in mM/l											
	$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$
a	0.16	0.1	0.32	0.10	0.48	0.1	0.64	0.1	—	—	—	—
b	0.27	0.3	0.54	0.3	0.81	0.3	1.08	0.3	—	—	—	—
c	0.35	0.5	0.7	0.5	0.05	0.5	1.4	0.5	—	—	—	—
d	—	—	1.0	1	—	—	2	1	4	1	8	1
e	—	—	2	4	—	—	4	4	8	4	16	4
f	—	—	3	9	—	—	6	9	12	9	24	9
g	—	—	4	16	—	—	8	16	16	16	32	16

each one of a different concentration of K^+ and Ca^{++} ions, using 1 mM/l Tris/HCl solution of pH 7.2 as a solvent. In each solution, the concentration of K^+ and Ca^{++} was adjusted so, that the value of Eq_{G-D} amounted successively: 0.5, 1, 1.5, 2, 4, 8, at the constant level of calcium. Initially the studies were carried out in the solutions d–g in the conditions: $Eq_{G-D} = 1, 2, 4, 8$. However the results obtained suggested the necessity to verify the work thesis on solutions of lower absolute concentrations of K^+ and Ca^{++}, as well as to perform additional experiments in the conditions of $Eq_{G-D} = 0.5$ and 1.5.

The experiments in conditions $Eq_{G-D}=1$, as well as those in solutions a–c (at lower absolute concentrations of potassium and calcium), as in d–g (at higher absolute concentrations of potassium and calcium), present a link between those two complexes. Similar results obtained in the series $Eq_{G-D}=1$ for the solutions: a–g, prove that the absolute concentrations of K^+ and Ca^{++} play no role in this case.

The photograms of ciliates in movement, necessary for the determination of the path character, of the movement rate and of the measurements of length have been carried out twice: in the course of the first 3 min of the ciliate stay in the solution studied, and after 45 min. The observation on the chemotactic reaction were performed within the first 3 min after introduction into the solution studied.

Experiments

Analysis of behaviour of *S. coeruleus* at different values of Eq_{G-D} in medium

Behaviour in $Eq_{G-D}=0.5$

At 3 solutions studied a–c, at different absolute concentrations of potassium and calcium, the ciliate behave normally. They move forwards with a normal movement, with a rate of 1009–1026 μ/sec. The length of protozoa body remains within the limits 248–280 μ (Fig. 1A).

Behaviour in $Eq_{G-D}=1$

The observations of *S. coeruleus* behaviour at these conditions were carried out in 7 solutions (a–g) at various values of absolute concentrations of potassium and calcium. In all those cases, ciliates showed a similar character of movement. It was a normal forward movement (NFM) which — after 45 min — passed into a typical periodic ciliary reversal (PCR) i.e. periods of backward movement began to appear. Consequently the movement rate fell distinctly after 45 min long stay in this medium. A certain rise of length of the ciliates body was also observed (Fig. 1B).

Behaviour in $Eq_{G-D}=1.5$

In these conditions the measurements and observations were carried out in 3 solutions (a–c) of low absolute concentrations of potassium and calcium. Within the first minutes of stay in solution, the ciliates manifested a typical PCR, which gradually passed into a partial ciliary reversal (PaCR) of type A and B (see Fig. 3 and description at the end of this chapter, p. 353). Together with the change of the movement character occurs its considerable slowing. The mesaurements of length indicate simultaneously a gradual elongation of individuals remaining in these conditions (Fig. 1 C).

Behaviour in $Eq_{G-D}=2$

In these conditions the observations were carried out in seven solutions (a–g). Within the first minutes of stay in these media continuous ciliary reversal (CCR) of type B and partial reversal of type A and B appeared in all cases. The continuous and partial reversals are difficult to be distinguished from each other. However the photographic registration and precise observations reveal distinct differences in them. CCR of type B reveals circles or loops with empty centres, or commas (Pietrowicz-Kosmynka 1971 b, Fig. 8). This depends on the rate of movement. In contrast to this, PaCR of type A and B may be distinguished on photograms by circles which are full or incised, when the movement is slower. This type of movement character persisted for the whole time of observation and passed in many cases into the continuous reversal of type A. Its rate was initially low and rose after 45

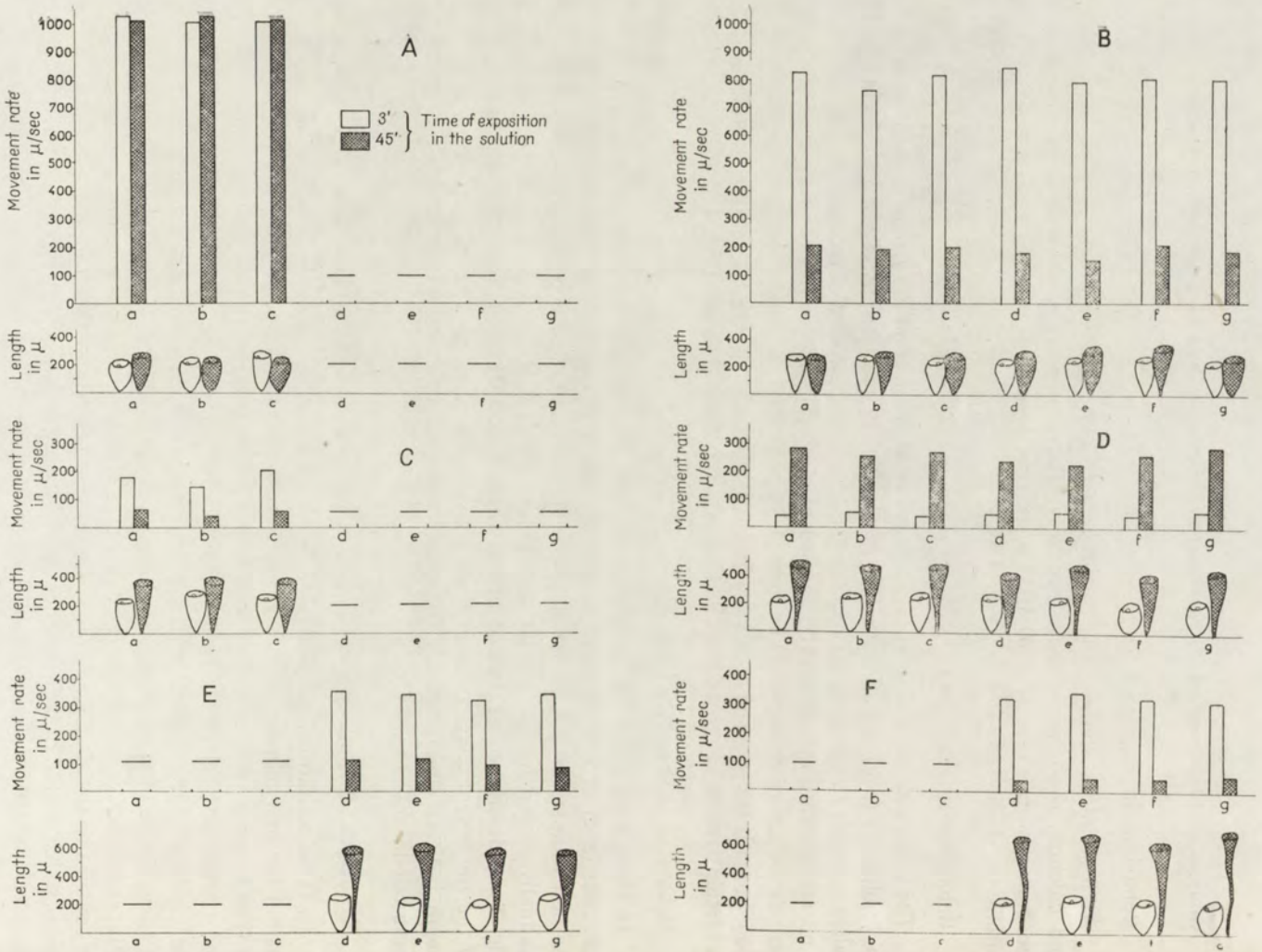

Fig. 1. Behaviour of *Stentor coeruleus* at various conditions of Eq_{G-D} A — $Eq_{G-D} = 0.5$: B — $Eq_{G-D} = 1$; C — $Eq_{G-D} = 1.5$; D — $Eq_{G-D} = 2$; E — $Eq_{G-D} = 4$; F — $Eq_{G-D} = 8$

min. This was evoked by appearing of CCR type A. The length of protozoa showed also perceptible changes. Ciliates elongated distinctly (Fig. 1 D).

Behaviour in $Eq_{G-D}=4$

Observations in these conditions concern the solutions d–g. The continuous ciliary reversal occurred during the whole experiment. It passed from the type A (in the first minutes of observation) into the type B (after 45 min of observation). So the rate of movement falls respectively, and the protozoan length increases (Fig. 1 E). After 45 min protozoa reached their maximal length, the holdfast became twisted, and desintegration followed after 90 min.

Behaviour in $Eq_{G-D}=8$

The last group of observations and measurements in the solutions d–g concludes the cycle of studies on the behaviour of *S. coeruleus* at different conditions of Eq_{G-D} value. In the media of a composition conditioned by the value $Eq_{G-D}=8$, the ciliates remained in reversal during the whole time of observation. Initially CCR of type A appeared and it soon passed into the reversal of type B. This transit was manifested by relaxation of movement, extinction of rotation, later on — of spiralization, and — at last — by describing circles at a very slow rate. Of course it is a swimming pattern with the posterior body part moving forwards. The changes of the movement rate could be observed together with the changes of its character. Ciliates elongated attaining simultaneously their maximal length of 600–700 μ (Fig. 1 F). After one hour, spontaneous contractions and desintegration occurred.

Consequently the behaviour of ciliates proved to be dependent on the definite values of Eq_{G-D} of medium. The results gained indicate the existence of the following interdependences: (a) behaviour i.e. the character of movement, its rate and degree of contracture of *S. coeruleus* are similar, and even the same, in the conditions defined by the constant value of Eq_{G-D} independently of different absolute concentrations of K^+ and Ca^{++}, (b) the rate of movement depends on its character, (c) in proportion as the value of Eq_{G-D} rises the character of movement changes as it is shown in Fig. 2, with the inclination to elongate the body, (d) the duration of adaptation exerts an influence on the changes of the character and on the rate of movement and on the length of ciliates.

Besides, in the conditions of external medium determined by the value $Eq_{G-D}=1.5$ and 2, the typical PaCR was observed. *S. coeruleus* moving in this manner describes circles (PaCR type A) or loops (PaCR type B). A characteristic feature of the partial ciliary reversal is the fact that a part of the oral ciliary apparatus beats in one — and the remaining cilia in another direction. Then the posterior body part remains almost immobilized. The effect — is a sideway movement (Fig. 3).

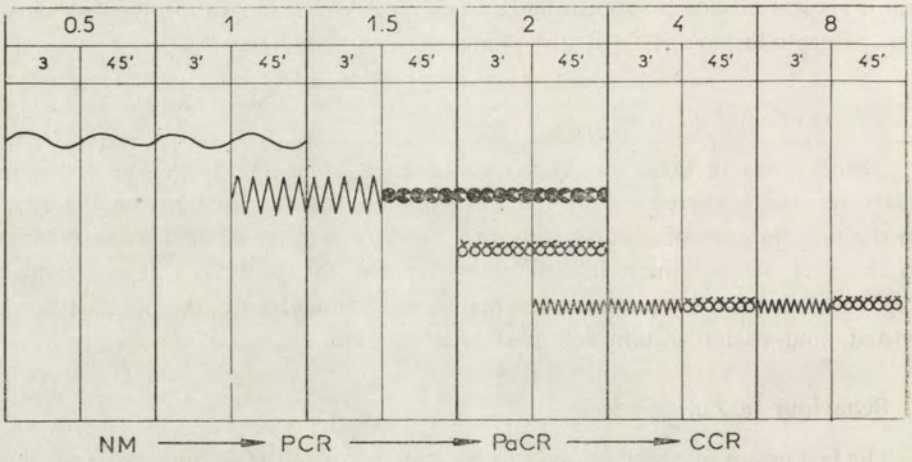

Fig. 2. The pattern of movement of *Stentor coeruleus* at various conditions of Eq_{G-D}

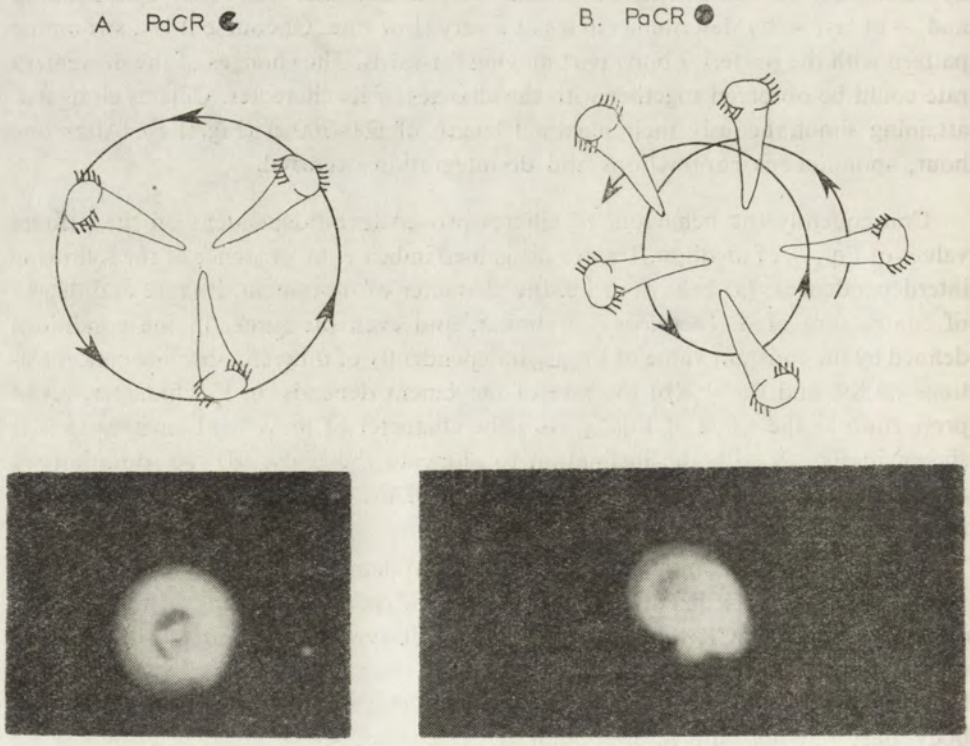

Fig. 3. The partial ciliary reversal (PaCR)

Character of the chemotactic reaction in the media of different Eq_{G-D} values

Solutions were applied of equal absolute concentrations of potassium and calcium marked in the same manner as in the preceding part and described in Table 1. The sequence of experiments was also the same. As the substance evoking the chemotactic reaction, the 0.007% solution of quinine chlorhydroxide was applied.

Behaviour in $Eq_{G-D}=0.5$

Experiments were carried out with 3 different combinations of solutions (a–c) of values of absolute concentrations of K^+ and Ca^{++}. The percentage of individuals which failed to response to the chemotactic stimulus was very low in all three solutions. Statistically significant differences between the number in T and in C indicate the occurrence of a very strong negative chemotactic reaction (Table 2).

Table 2

Influence of value $Eq_{G-D} = 0.5$ on negative chemotactic response

Successive solutions	$m^C \delta$	$m^T \delta$	%nR	Significance of difference in T and C
a	132.9±5.2	4.5±0.1	3.4	sign.
b	74.7±2.6	1.3±0.0	1.7	sign.
c	66.3±3.2	1.1±0.3	1.6	sign.

C – control square, T – test square, m – medium number of individuals σ – standard deviation, %nR – percentage of individuals non-responding by escape related to the control assumed as 100%.

Behaviour in $Eq_{G-D}=1$

A cycle of experiments was executed in all solutions (a–g). A high chemotactic sensitivity to 0.007% quinine solution was ascertained in all cases. A low percent of individuals non-responding (nR) in all cases indicates the typical negative chemotaxis response. Very low absolute values of K^+ and Ca^{++} concentrations in solution a–c and rather high values of absolute concentrations of K^+ and Ca^{++} in solutions d–g, failed to evoke any differentiation of chemotactic sensitivity to quinine (Table 3).

Behaviour in $Eq_{G-D}=1.5$

In all the three test solutions (a–c) a rather high % of nR individuals fails to show the negative chemotaxis response. In general, the value of $Eq_{G-D}=1.5$ compared with the results of former experiments, involves the extinction of chemotactic sensitivity in a rather high number of individuals $\sqrt{nR}$ although the character of reaction has still remained negative (Table 4).

Table 3

Influence of value of $Eq_{G-D} = 1$ on negative chemotactic response

Successive solutions	$m^{C} \delta$	$m^{T} \delta$	%nR	Significance of difference in T and C
a	48±5.2	3.1±0.1	6.4	sign.
b	25.5±4.1	1.1±0.05	4.3	sign.
c	59.3±2.2	2.9±0.3	4.5	sign.
d	43.2±3.8	2.4±0.1	5.6	sign.
e	27.3±1.8	1.6±0.7	6.1	sign.
f	62.3±2.4	2.9±0.04	4.8	sign.
g	35.6±1.8	1.6±0.2	4.7	sign.

Explanations – see Table 2.

Table 4

Influence of value $Eq_{G-D} = 1.5$ on negative chemotactic response

Successive solutions	$m^{C} \delta$	$m^{T} \delta$	%nR	Signi cance of difference in T and C
a	56.3±3.6	18.6±3.0	33.0	sign.
b	45.3±1.4	12.1±1.1	26.4	sign.
c	45.7±1.3	13.7±0.9	29.9	sign.

Explanations – see Table 2.

Behaviour in $Eq_{G-D}=2$

In the conditions determined by the value of $Eq_{G-D}=2$, the experiments were carried out in seven solutions (a–g). However in no case the quantitative evaluation of the chemotactic phenomenon was attained. According to the introductory renarks, the photographic registration should be executed within the first 3 min of the ciliate stay in the given solution. However a considerable slowing of movement made impossible the return of ciliates to the normal exit number in the control square, and consequently made impossible the adequate quantitative evaluation of ciliate number as related to the stimulating substance introduced on a test square

Behaviour in $Eq_{G-D}=4$

The quantitative data of this group of experiments indicate a turn in the character of chemotactic reaction uniform in all the four test solutions (d–g). Really the negative chemotaxis to 0.007% solution of quinine ceased to be manifested in these conditions. This is indicated by the statistically non-significant differences between the number of individuals in T and C. It seems that in these conditions the ciliates became

completely insensitive to quinine as a powerful chemotactic stimulus (Table 5).

Table 5

Influence of value $Eq_{G-D} = 4$ on negative chemotactic response

Successive solutions	m C δ	m T δ	%nR	Significance of difference in T and C
d	42.3±1.3	43.6±2.3	103.2	non-sign.
e	35.2±1.5	36.1±1.2	102.6	non-sign.
f	26.8±1.2	28.8±1.7	107.5	non-sign.
g	22.9±1.1	23.9±1.9	10.40	non-sign.

Explanation – see Table 2.

Behaviour in $Eq_{G-D}=8$

Also in this case, the extinction of chemotactic sensitivity to quinine solution appeared in all the four test solutions (d–g). The % of non-responding individuals and the statistically non-significant differences between the individuals in C and in T, indicate the absence of any motoric response to this stimulus and consequently, lack of negative chemotaxis (Table 6).

Table 6

Influence of value $Eq_{G-D} = 8$ on negative chemotactic response

Successive solutions	m C δ	m T δ	%nR	Significance of difference in T and C
d	32.8±1.2	37.1±1.3	113.2	non-sign
e	47.3±1.8	59.5±2.3	108.2	non-sign.
f	24.0±0.7	24.1±0.1	100.8	non-sign.
g	33.5±1.2	35.5±1.3	106.0	non-sign.

Explanation – see Table 2.

The results of all experiments from this part of studies are assembled in the diagram (Fig. 4). They visualize a distinct interdependence of chemotaxis on the value of Eq_{G-D} in medium. This interdependence in inversely proportional. The rise of Eq_{G-D} value involves a gradually higher number of ciliates which fail to manifest the negative chemotaxis response to the strong chemical stimulus. This should be accounted for by a complete extinction of chemotactic sensitivity in those individuals.

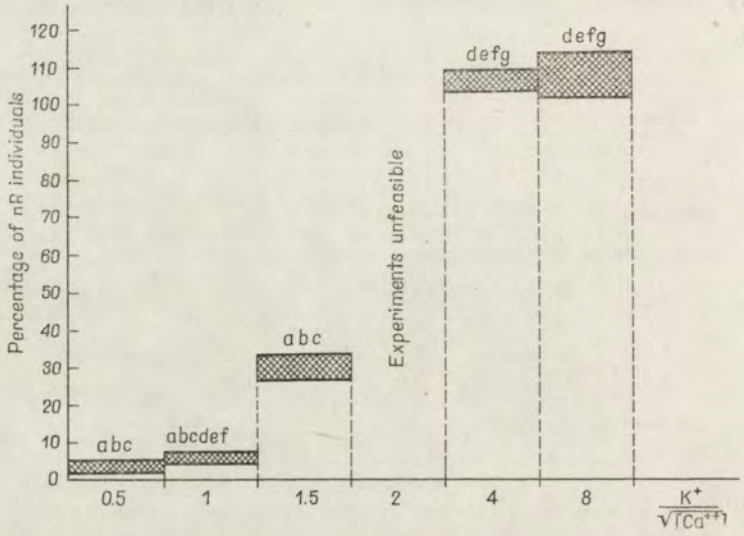

Fig. 4. Chemotactic response at various Eq_{G-D} values in external medium

Discussion

It was observed in the first part of experiments of the present study that the changes of the movement character run according to the general scheme from NFM→PCR→PaCR→CCR and are closely dependent on the values of Eq_{G-D} in medium. The types of movement defined above appear in the observed sequence together with the rise of Eq_{G-D} value. The first symptom of ciliary reversal (PCR) takes place after a prolonged stay in $Eq_{G-D}=1$. Later on, reversal transits gradually in $Eq_{G-D}=1.5$ into the stage PaCR, and reaches the stage of continuous reversal at higher values of Eq_{G-D}.

The phenomenon of transit of motoric responses to all the consecutive stages has been described first in *Paramecium* (Grębecki 1965, Kuźnicki 1966 a). The changes of motoric responses depend presumably in this case on the level of calcium ions adsorbed on the membrane. Application of precipitating and chelating agents (Grębecki 1965) or the action of inorganic cations (Kuźnicki 1966 a), involved a fall of the calcium ion level in medium and — consequently — appearing of different types of motor responses in a different sequence. In this way, the modification of response sequence depended on the amount of Ca^{++} in medium.

Recently Naitoh 1968 proved that the liberation of Ca^{+} from its adsorption place on the membrane of *Paramecium* is involved by the reaction of exchange with the competing ions. This is the first phase of action of the stimulating factor in the physiological mechanism which evokes the ciliary reversal after the contact with

chemical stimuli. Liberation of Ca^{++} ions activates directly the mechanism which is responsible for the ciliary reversal.

It should be presumed therefore that in the case of experiments on *Stentor coeruleus*, discussed in the present study potassium is the agent influencing the behaviour of Ca^{++} ions in the membrane. If it is so, the rise of Eq_{G-D} value would be correlated with the fall of Ca^{++} level bound by adsorption on the membrane. This is supported by the results of Naitoh and Yasamasu 1967 who put forward the postulation that ciliary reversal occurs as a consequence of ion exchange being in accordance to with the principle of Gibbs–Donan. The Japanese authors established a formula which defines the quantity of Ca^{++} adsorbed by the membrane of *Paramecium* in the case when besides Ca^{++} other cations occur in medium. The quantity of Ca^{++} would be correlated with the value $[K^+]/\sqrt{[Ca^{++}]}$ if K^+ ions were present in medium.

In this way, an additional support was gained on the model of *Stentor coeruleus* for the hypothesis put forward by Jahn 1962 and Kuźnicki 1966 b, that all the motor responses evoked by the chemical stimulation are a result of description of Ca ions from the membrane of ciliates.

As it has been mentioned previously the ciliary reversal presents an external manifestation of excitability of the protozoan cell, and its different forms (PCR, PaCR, CCR) reflect different states of its excitation, it may be assumed therefore that the state of cell excitability changes proportionally to the fall of level of calcium bound at the places of adsorption on the membrane. The transit of periodic reversal (PCR) into the partial one (PaCR) occurs in *S. coeruleus* distinctly in dependence on the time of stay at definite conditions of Eq_{G-D} (1.5) value. The transit from PaCR to CCR, and the prolonged duration of CCR (till the ciliate death), is typical for *S. coeruleus*. In *Paramecium*, CCR may last several to over ten minutes, and disappears then independently of the presence of the factor evoking it in the medium (Dryl 1959) PCR however may last for tens of hours. In *S. coeruleus*, the time of stay in the medium of a definite value of Eq_{G-D} beginning with $Eq_{G-D}=1$, acts in the first place on the character of movement. This is always associated with the change of the movement rate. Basing on this fact, two forms of continuous reversal CCR — type A and type B have been distinguished. Similar reversal phases were also observed in *Stylonychia* (Dryl 1965).

The observations of Sleigh 1969, ascertaining that the reversal in *S. coeruleus* is always connected with the inhibition of coordination of beat of AZM cilia, finds in some degree a support in the observation of the continuous form of ciliary reversal. A conclusion follows, that although the general rules of evaluating the state of excitability of the protozoan cell on the ground of its behaviour are similar in *Stentor* and in *Paramecium*, yet the above differences indicate a distinct dissimilarity in behaviour of those two protozoan species.

There exists still one measurable factor accompanying the definite stages of excitability of *S. coeruleus* cell depending on the value of Eq_{G-D}. This is the degree

of contraction of the cell. Especially distinct changes (elongation) accompany the late phase of continuous ciliary reversal ($Eq_{G-D}=2$). It appears usually after 20–30 min and reaches its maximum at the moment of appearing of CCR of type B.

It follows from the recent studies of Jahn and Bovee 1964, Seravin et al. 1965, Jones et al. 1965, Sleigh 1969 that the contractile systems of *Protozoa* act according a similar rule as does the muscle of *Metazoa*. In the *Stentor* species many investigators noticed the similitude of endoplasmatic microfibrilles to the fibrils of smooth muscles of cells in vertebrates and of the membrane system of protozoan cell to the sarcoplasmic reticulum of myoneme. As it is postulated for muscle, those membranes might conduct the stimulus to all parts of the fiber, or liberate the metabolites necessary for a rapid contraction or evoke both effects.

Allen and Eckert 1969 gained a reorientation of cilia in glycerined cells of *Paramecium* in presence of ATP, as response to the change of Ca^{++} concentration. More detailed studies on cortex proved in it the presence of a system which accumulates Ca^{++} within alveolae which lie closely under the membrane. Those alveolae lie between the kineties, near kinetosomes. They have apertures in all their walls. This system resembles to the surface of endoplasmic reticulum of striated muscles of *Metazoa* and may — similarly as reticulum — accumulate Ca^{++}. Consequently, the main role in the excitation of contractile structures is performed by the presence of calcium in the medium surrounding the myofibrils.

As known, *S. coeruleus* belongs to the ciliates which posses — besides the ciliary contractile system (tubular fibrils — Pitelka and Child 1964) — another double system responsible for contraction: microtubules in ectoplasm, and microfibrils lying more profoundly in endoplasm. Bannister and Tatchell 1968 and Grain 1968 postulate that all those systems are connected with one another in some way. There exists in this way a direct contact between the kinetosome region of cilium and the remaining contractile systems. Kuźnicki 1969 suggested that the reversal of the ciliary beat takes place according to the rule of contraction or sliding of the myofilaments of cilia, in the presence of Ca^{++} liberated from the system of reticular membranes of alveoli surrounding the kinetosome. The trigger for this reaction would be the fall of Ca^{++} level adsorbed on the cell surface. Since the ciliary reversel state at higher values of Eq_{G-D} is associated with the elongation of ciliate, so the relaxation of contractile systems in *S. coeruleus* should be assumed as specific. The same phenomenon observed after the adaptation to potassium, is associated with the long-lasting action of K^+ ions in medium. It may be presumed that the K^+ ions removing the increasingly high quantities of Ca^{++} ions from the membrane surface evoke indirectly gradual changes in the ion equilibrium on the surface of the membraneous structures lying more profound under the pellicle. Those structures embrace also the contractile elements. The recent studies of Grain 1968 on *S. coeruleus* confirm the presence of vesicles surrounding the microfibrils in endoplasm, however, a distinct reticular system has not been observed around them. At any rate, this relaxation is — no doubt — connected with the gradual

fall of level of free calcium around them. Dryl 1969 maintains that potassium ions may block a mechanism which is of a physiological importance being necessary for a normal conduction of external impulse of the cell to the apparatus of contractile myonemes. In which way it occurs, and by which processes it is evoked — remains a problem for further studies.

The results of studies on the changes of contractibility of *S. coeruleus* at high values of Eq_{G-D} suggest that such form of internal excitability acts here and is connected simultaneously with stiffening of AZM i.e. with inhibition of the rotatory movement of the whole cell. Possibly in the analysis of these processes, the results of Sleigh on metachronism of AZM should be considered. This author put forward the neuroidal mechanism for explaining the ciliary coordination of the oral apparatus in *Stentor* species.

The changes of paths, of movement rate and of contractibility in *S. coeruleus* are a support of the adequacy of the postulations of Jahn concerning the ciliate membrane functioning according to the principle of Gibbs–Donnan. Really in all the cases studied, at a constant value of EQ_{G-D} the results were almost the same despite the different absolute values of Ca^{++} and K^{+} concentrations in medium.

The quantitative data concerning the chemotactic sensitivity to quinine have been collected on the ground of the reaction to this stimulus after a 5 min stay of the ciliate in the medium of a definite value of Eq_{G-D}. These results are in this way — connected with the data concerning the behaviour at the initial moments of stay in a solution of different values of Eq_{G-D}. The first manifestations of extinction of sensitivity to quinine which take place at $Eq_{G-D}=1.5$, are distinctly correlated with the onset of periodic reversal PCR at a rather high number of individuals which fail to response to this stimulus.

Occurrence of the partial reversal of type B at the initial period of stay in $Eq_{G-D}=2$ is associated — as mentioned — with a considerable slowing of movement. This slowing was the reason why the experiments were unfeasible in all the cases studied. The ciliates dispersed by the water shake of the falling drop could not attempt to assume a new directional orientation related to the stimulus because of the slowed, circular character of their movement involving the lack of progressive movement. It should be presumed however that the ciliary reversal (PaCR and CCR) indicate the change of excitability of the cell and are a distinct evidence of a lowered — and may be completely abolished — ability of response to any next chemical stimulus.

Those facts caused a complete extinction of the negative chemotactic reaction to quinine at higher values of $Eq_{G-D}=4$ and 8. It is just then, at the initial phase of stay in solution, ciliates performed a backward swimming movement (CCR type A). Occurrence of this type of movement indicates that the shake of balance in equilibrium: potassium–calcium attains its maximum in the membrane of ciliate at such high values of Eq_{G-D}. Any further rise of Eq_{G-D} value would not change the situation concerning the excitability of membrane. It may only accelerate the desintegration process of the cell.

Consequently the value of Eq_{G-D} proved to be an excelent standard of sensitivity to the chemical stimulus in protozoa. The $Eq_{G-D}=1.5$ value is a crucial point, above it the sensitivity to the chemical stimulus extincts completely. This is proved by the statistically significant results which speak in favour of lack of the negative chemotactic reaction. The connection of the extinction of chemotactic sensitivity with the state of excitability of the protozoan cell is also supported by the data concerning the behaviour of this ciliate species at the definite values of Eq_{G-D} in medium.

The results of the experiments on the influence of $[K^+]/\sqrt{[Ca^{++}]}$ — determined here as Eq_{G-D} value — upon the chemotactic reaction of *S. coeruleus*, provide a support and extension of the thesis of Jahn of assuming the cell memebrane of protozoa as a specific cation exchanger. The ionic equilibrium on the membrane, determined by the value of Gibbs–Donnan, may serve as evidence of the state of excitation of the cell, i.e. of its chemotactic sensitivity.

Summary

In *Stentor coeruleus* the sensitivity to chemotactic stimuli is decreased or abolished in external medium containing potassium and calcium ions in ratio $[K^+]/\sqrt{[Ca^{++}]}$ (Eq_{G-D}) higher than 1.5.

The extinction of chemotactic sensitivity in different ion media was always correlated with the appearing of a definite type of ciliary reversal, and the changes in the manner of behaviour evoked by the rise of Eq_{G-D} value in medium followed in a stabilized sequence: normal forward movement, periodic ciliary reversal, partial ciliary reversal and continuous ciliary reversal.

The achieved results support and extend the thesis of Jahn that the cell membrane of ciliate protozoa possess the properties of specific cation exchanger.

STRESZCZENIE

Wrażliwość chemotaktyczna u *Stentor coeruleus* jest zmniejszona lub zniesiona w środowisku zewnętrznym zawierającym jony potasu i wapnia w stosunku $[K^+]/\sqrt{Ca^{++}]}$ (Eq_{G-D}) wyższym niż 1.5.

Zanik wrażliwości chemotaktycznej w różnych środowiskach jonowych był zawsze skorelowany z pojawieniem się określonego typu rewersji ruchu rzęskowego, przy czym zmiany w zachowaniu orzęsków powodowane przez wzrost wartości Eq_{G-D} zachodziły w ustalonej kolejności: ruch normalny do przodu, periodyczna rewersja rzęskowa, częściowa rewersja rzęskowa i ciągła rewersja rzęskowa.

Uzyskane rezultaty popierają i rozwijają pogląd Jahna, że błona komórkowa orzęskowa posiada właściwości specyficznego wymieniacza kationowego.

REFERENCES

Allen R. D. and Eckert R. T. 1969: A morphological system in ciliates comparable to the sarcoplasmic reticulum transverse tubular system in striated muscle. J. Cell. Biol., 43, 5.

Bannister L. H. and Tatchell C. 1968: Contractility and the fibre systems of *Stentor coeruleus*. J. Cell Sci., 3, 295–308.

Dryl S. 1958: Photographic registration of movement of *Protozoa*. Bull. Acad. pol. Sci., Cl. II, Ser. Sci. biol., 6, 426–430.

Dryl S. 1959: Effects of adaptation to environment on chemotaxis of *Paramecium*. Acta Biol. Exp., 19, 83–93.

Dryl S. 1965: Motor response of *Stylonychia mytilus* to chemical electric and mechanical stimuli. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, "Nauka".

Dryl S. 1969: The role of potassium in control of contraction in *Stentor coeruleus*. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, "Nauka".

Grain J. 1968: Les systemes fibrillaires chez *Stentor igneus* Ehrenberg et *Spirostomum ambiguum* Ehrenberg. Protistologica, 4, 27–35.

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

Grębecki A. 1965: Role of Ca^{2+} ions in the excitability of protozoan cell. Decalcification, recalcification and the ciliary reversal in *Paramecium caudatum*. Acta Protozool., 3, 275–289.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ion antagonism and ciliary reversal. J. cell comp. Physiol., 60, 217–228.

Jahn T. L. and Bovee E. C. 1964: Protoplasmic movements and locomotion of *Protozoa*. In: Biochemistry and Physiology of Protozoa, ed. S. H. Hutner, Academic Press, New York–London, 3, 112.

Jones A. R., Jahn T. L. and Foneseca J. R. 1965: Cinematographic analysis of the anodally stimulated contraction of *Spirostomum ambiguum*. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. int. Congr. ser. No. 91.

Kamada T. and Kinosita H. 1940: Calcium–potassium factor in ciliary reversal of *Paramecium*. Comm. by N. Yatsu, M. I. A., 12.

Kuźnicki L. 1966 a: Role of Ca^{2+} ions in the excitability of protozoan cell. Calcium factor in the ciliary reversal induced by inorganic cations in *Paramecium caudatum*. Acta Protozool., 4, 241–256.

Kuźnicki L. 1966 b: Ciliary reversal in *Paramecium caudatum* in relation to external pH. Acta Protozool., 4, 257–261.

Kuźnicki L. 1969: Mechanizmy reakcji ruchowych *Paramecium*. Kosmos A, 5, 473–505.

Naitoh Y. 1968: Ionic control of the reversal response of cilia in *Paramecium caudatum*. A calcium hypothesis. J. gen. Physiol., 51, 1, 85.

Naitoh Y. and Yasamasu I. 1967: Binding of Ca ions by *Paramecium caudatum*. J. gen Physiol., 50, 1303.

Pietrowicz-Kosmynka D. 1971 a, b: Chemotactic effects of cations and of pH on *Stentor coeruleus*. Acta Protozool., 9, 235–244.

Pitelka D. R. and Child F. M. 1964: The locomotor apparatus of ciliates and flagellates: relations between structure and function. In: Biochemistry and Physiology of Protozoa ed. S. H. Hutner, Academic Press, New York–London, 3, 131.

Seravin L. N., Skobło I. I. and Bagniuk I. G. 1965: Mechanizm sokraščenija mieniem infuzorii *Spirostomum ambiguum*. Acta Protozool., 3, 327–335.

Sleigh M. A. 1969: The excitation of contractile organelles in ciliate *Protozoa*. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, "Nauka", 165.

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The use of *Protozoa* as test organisms for studies of toxic effects of human serums from schizophrenic patients

A schizophreniás betegek serumának toxicus hatása a protozoonokra, mint test-objektumokra

Some authors began to deal with the biology of single-celled cultures at the turn of the century. The referring literature data have been rapidly increasing since 1930. This growing interest may be explained by the fact that a number of issues may be studied on cellular level and tissue relations do not interfere with the results. Results gained with single-cells may often be referred to metazoan organisms. The significance of protozoa, besides theoretical references, is not indifferent in practice either. Apart from its medical and veterinarian use it is an important fact that when their demand of nutrition is known, certain substances of biological importance can be determined. On this ground suggestions have been made to determine substances of the greatest variety. Certain species are very sensitive towards toxins thus being very suitable for establishing the toxicity of pharmacological preparates Müller 1959. In several cases protozoa have been used for test-investigations — Harrison et al. 1948, Gellért et al. 1961, Huszák et al. 1958, Hutner et al. 1952, Nastiukova 1944, Roskin 1946, Vargha et al. 1955 especially when the authors wanted to detect the harmful or extraneous matters that appeared in the organism during the illness. But in these cases the lack of an adequate method caused a lot of problems. As a rule paramecia were used with these tests and the cultivation of the protozoa is agnotobiotic. This fact might have decisive effect on the obtained results.

The serum of schizophrenic patients was used for our presently reported investigations. Our aim was, partly to verify the results of the earlier investigations and partly to gain more accurate results by using the most appropriate method and *Tetrahymena* cells. Thus the optimal concentration and dilution grade of the control serum was established, the temperature fluctuations were eliminated and the results gained with *Paramecium* and *Tetrahymena* were compared.

Methods

Paramecium caudatum cultivated in hay infusion at 25°C in thermostat was used for the investigations. The *Tetrahymena* cultures were kept at similar temperature in 0.85% aqueous pepton culture media under sterile conditions. The paramecia cultures were freshened up with some drops of milk every week. In case of *Tetrahymena* we used them 5 days after inoculation.

At the early stage of the investigations the effect of human serum and the multiplication of the animals were observed. For informative aims dilution seria was prepared. The serum with 1.8/100 and 3.5/100 dilution grade appeared to be most suited. Having investigated them again in 100–100 cases the 1.8/100 dilution was used for our test.

When using *Tetrahymena* the following factors had to be observed: (1) These animals are multiplying more rapidly than the paramecia and can be cultivated under sterile conditions only. (2) When using the dilution series the 0.5/100 dilution serum proved best. (3) The dilution was made with 0.85% pepton.

The control serums were obtained from undergraduates after having undergone internal examination. The serum of the patients were preserved by the Department of Neurology of the Szeged Medical School. Within one and half year 92 patients had been examined. In 15 cases it was possible to make three test-investigations on the patients. Firstly when the patient was admitted to the hospital, secondly between the 14th and 21st day after admittance and thirdly after the 4th week. *Tetrahymena* was used in all these cases.

To perform the tests 0.2 ml of diluted serum was weighed onto slides then 2 paramecia were isolated in this micro-culture-media. *Tetrahymena* was dealt with in the same way but under sterile conditions. The slides were kept in dark wet chambers at 25°C temperature. After 24 h incubation the number of animals was determined at low magnification, while in the *Tetrahymena* it was counted with microscope after fixation. With the control and unknown serum 10–10 slides were tested. In all tests controls cultivated on normal culture-media were also used to be able to consider the eventual climatic factors too. The basis of the estimation was the mean-value of 10 parallel slides and the mean-value was used to calculate the Relative Vitalis Index (RVI). It was done by the:

$$\text{RVI} = \frac{N_x - N_c}{N_c} \times 100 \text{ formula}$$

where N_x = the number of animals in the patients' serum and N_c = the number of animals in control culture-medium.

Results

The results of our investigations are summarized in Tables and Figures. Table I shows how well 1.8/100 diluted control serums can be applied for the tests. The 3.5/100 diluted serum is inhibiting the multiplication. Fig. 1 is illustrating the same values in column diagrams expressed in RVI. It is evident that the normal serum with 3.5/100 dilution is not suitable for the investigations. Table 2 shows the results of 92 schizophrenic patients in successive order of the investigation. The RVI values of Fig. 2 show that the multiplication of paramecia is inhibited in the patients' serum nearly in all cases, their values being under 0. The cases where the patients' serum could be tested at three different times are shown in Table 3. These data illustrate clearly that when the patient is admitted (to the hospital) the inhibition of

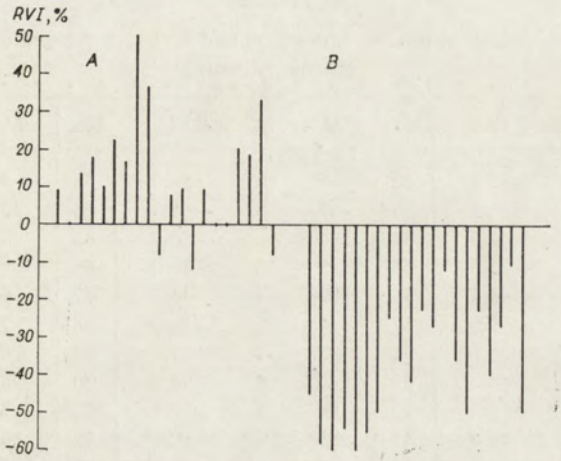

Fig. 1. RVI of *Paramecium* in normal serum. A — 1.8/100 dilution and B — 3.5/100 dilution of serum

Table 1

Effect of normal sera on the multiplication of paramecia

No.	Number of paramecia after 24 h			RVI% 1.8/100	RVI% 3.5/100
	Normal serum 1,8/100	Normal serum 3.5/100	Control media		
1	12	6	11	+9.1	—45.4
2	12	5	12	0.0	—58.3
3	17	6	15	+13.3	—60.0
4	13	5	11	+18.2	—54.6
5	11	4	10	+10.0	—60.0
6	11	4	9	+22.2	—55.6
7	14	6	12	+16.7	—50.0
8	12	6	8	+50.0	—25.0
9	15	7	11	+36.4	—36.4
10	11	7	12	— 8.3	—41.7
11	14	10	13	+7.7	—23.1
12	12	8	11	+9.1	—27.3
13	7	7	8	—12.5	—12.5
14	12	7	11	+9.1	—36.4
15	12	6	12	0.0	—50.0
16	13	10	13	0.0	—23.1
17	12	6	10	+20.0	—40.0
18	13	8	11	+18.2	—27.3
19	12	8	9	+33.3	—11.1
20	11	6	12	—8.3	—50.0

Table 2

Data showing the multiplication rate of *Paramecium* and RVI% in the sera of normal and schizophrenic patients

No.	N_x	N_c	RVI%	No.	N_x	N_c	RVI%	No.	N_x	N_c	RVI%
1	12	18	—33.3	32	5	10	—50.0	63	9	10	—10.0
2	15	16	—6.2	33	7	15	—53.3	64	10	9	+11.1
3	14	16	—12.5	34	6	12	—50.0	65	8	11	—27.3
4	13	17	—23.5	35	5	10	—50.0	66	6	9	—33.3
5	10	18	—44.4	36	9	14	—35.7	67	5	6	—16.6
6	6	12	—50.0	37	4	8	—50.0	68	7	7	0.0
7	8	12	—33.3	38	3	7	—57.1	69	6	7	—14.3
8	9	11	—18.2	39	2	8	—75.0	70	8	12	—33.3
9	13	12	+8.3	40	10	9	+11.1	71	7	15	—53.3
10	10	12	—16.6	41	11	10	+10.0	72	6	12	—50.0
11	11	12	—8.3	42	7	9	—22.2	73	7	11	—36.4
12	12	14	—14.3	43	6	7	—14.3	74	6	10	—40.0
13	6	16	—62.5	44	10	11	—9.1	75	10	16	—37.3
14	10	17	—41.2	45	8	15	—46.6	76	6	11	—45.4
15	11	15	—26.7	46	7	13	—46.2	77	7	11	—36.4
16	15	16	—6.2	47	9	12	—25.0	78	9	9	0.0
17	10	17	—41.2	48	8	12	—33.3	79	8	9	—11.1
18	8	15	—46.6	49	7	11	—36.3	80	2	6	—66.7
19	2	8	—75.0	50	10	11	—9.1	81	3	7	—57.1
20	10	11	—9.1	51	7	8	—12.5	82	5	9	—44.4
21	11	13	—15.4	52	6	7	—14.3	83	6	11	—45.4
22	10	16	—37.5	53	7	10	—30.0	84	7	10	—30.0
23	8	19	—57.8	54	8	9	—11.1	85	8	10	—20.0
24	13	14	—7.1	55	7	14	—50.0	86	9	13	—30.7
25	14	15	—6.6	56	9	12	—25.0	87	10	15	—33.3
26	6	10	—40.0	57	10	11	—9.1	88	6	11	—45.5
27	5	11	—54.5	58	8	7	+14.3	89	5	7	—28.5
28	4	11	—63.6	59	7	8	—12.5	90	10	9	+11.1
29	9	12	—25.0	60	3	6	—50.0	91	8	9	—11.1
30	12	11	+9.1	61	4	7	—42.8	92	5	11	—54.5
31	11	13	—15.3	62	7	8	—12.5				

multiplication appears uniformly, between the 14th and 21st day the inhibiting effect is diminishing and after the 4th week in about 50%+RVI values can also be found. Table 4 shows the effect of the serums of schizophrenic patients exerted on *Tetrahymena pyriformis* cells cultivated axenically. Comparing the results with the ones gained with *Paramecium* it can be seen that the differences are not considerable. The diagrams of Fig. 3 provide comparison about the RVI values obtained with *Paramecium* and *Tetrahymena*. In latter case the data are more homogeneous and it is especially conspicuous in the third case after the fourth week where too many values near "0" can be observed. This shows the decrease of inhibiting factors in the serum.

Table 3

Multiplication of *Paramecium*. Results (I) at the admission of the patients. (II) between the 14th and 21st days. (III) after the 4th week in the hospital

No.	I			II			III		
	Number of *Paramecium*		RVI%	Number of *Paramecium*		RVI%	Number of *Paramecium*		RVI%
	Serum	Control		Serum	Control		Serum	Control	
1	5	9	—44.4	7	11	—36.4	12	11	+9.1
2	10	15	—33.3	9	13	—30.8	12	13	—7.7
3	6	12	—50.0	7	11	—36.4	8	9	—11.1
4	9	14	—35.7	9	13	—30.8	13	12	+8.3
5	7	13	—46.2	7	12	—41.7	8	11	—27.3
6	8	11	—27.3	10	10	0.0	11	13	—15.4
7	4	7	—42.8	6	8	—25.0	13	16	—18.7
8	5	10	—50.0	8	10	—20.0	9	11	—18.2
9	8	15	—46.6	9	14	—35.7	12	14	—14.3
10	9	12	—25.0	11	12	—8.3	11	13	—15.4
11	6	9	—33.3	13	11	+18.2	13	10	+30.0
12	7	15	—53.3	10	9	+11.1	12	11	+9.1
13	7	10	—30,0	12	10	+20.0	15	13	+15.4
14	5	11	—54.5	7	10	—30.0	11	12	— 8.3
15	7	11	—36.4	12	11	—9.1	12	10	+20.0

Table 4

Multiplication of *Tetrahymena*. Results (I) at the admission of the patients. (II) between the 14th and 21st days. (III) after the 4th week in the hospital

No.	I			II			III		
	Number of *Tetrahymena*		RVI%	Number of *Tetrahymena*		RVI%	Number of *Tetrahymena*		RVI%
	Serum	Control		Serum	Control		Serum	Control	
1	23	45	—48.9	38	47	—19.1	48	52	—7.7
2	22	47	—53.2	41	48	—14.6	53	55	— 3.6
3	26	52	—50.0	39	46	—15.2	50	49	+2.0
4	17	38	—55.3	30	44	—31.8	49	50	—2.0
5	25	43	—41.8	31	45	—31.1	48	47	+2.1
6	24	46	—47.8	40	48	—16.7	47	51	—7.8
7	25	48	—47.9	41	49	—16.3	46	43	+6.9
8	32	42	—23.8	39	45	—13.3	45	46	—2.2
9	20	41	—51.2	32	47	—31.9	48	49	—2.0
10	21	46	—54.4	36	38	—5.3	50	48	+4.2
11	17	40	—57.5	38	39	—2.6	47	41	+14.6
12	18	40	—55.0	39	43	—9.3	51	54	—5.5
13	25	45	—44.4	35	41	—14.6	49	46	+6.5
14	27	50	—46.0	45	45	0.0	38	43	—11.6
15	18	54	—66.6	37	42	—11.9	46	47	—2.1

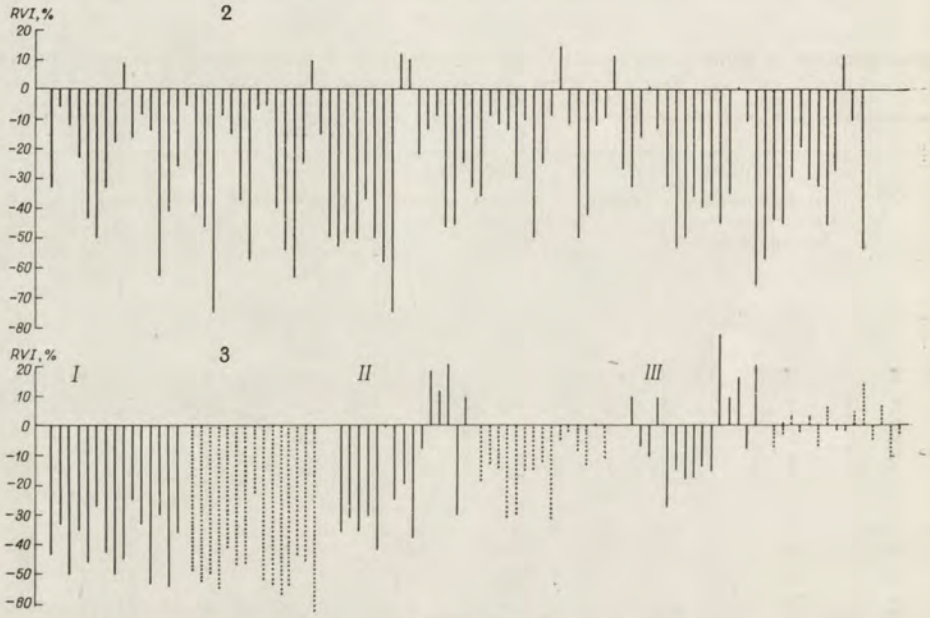

Fig. 2 RVI of *Paramecium* in sera of schizophrenic patients

Fig. 3. RVI of *Paramecium* (continuous line) and *Tetrahymena* (dotted line) in sera. Results (I) at the admission of the patients to the hospital, (II) between the 14th and 21st days, and (III) after the 4th week in the hospital

Discussion

In 1944 Nastiukova and in 1946 Roskin were using the serum of tumorous patients as test-liquids. In serums with various dilution the multiplication of animals was unanimous at few concentrations only. For test animals paramecia were selected. The results were given in RVI. The value of RVI fluctuated between (+) and (—). Its value shows the percentage of multiplication stimulation, i.e. inhibition in the unknown serum in relation with the control. Using the previous serums Egan 1950 applied *Paramecium* for the test-investigations. Huszák et al. 1958 used similar method to examine the urine and liquor of schizophrenic patients, later Gellért et al. 1961 repeatedly examined the serum of cancerous patients using the same method. All the authors observed that substances inhibiting the multiplication of paramecia are present in the patients' serum urine and liquore notwithstanding the smaller or greater deviation of the results from the mean-value.

The primary aim of our investigation was to eliminate possibly most of the factors disturbing the results. It has also been proved that the results became more exact when using appropriate protozoa. For such investigations *Tetrahymena* is very suitable, because we need not reckon with other organism and the other factors

may also be controlled. The obtained data confirm the finding that in the serum of schizophrenic patients there are factors having an effect both upon the multiplication of paramecia and of the *Tetrahymena*. With both animals the inhibition of multiplication will appear in the serum of the patients, but it is more homogeneous and distinct in *Tetrahymena*. Alpatov et al. 1961 are using this method in their routine work for suplementary diagnosis of cancerous patients in Moscow. The recognition of the nature of inhibiting factors was not in plans of the present study, we wanted to elucidate the problem from biological point of view only.

Summary

The inhibiting effects of serums from the schizophrenic patients on the multiplication rate in *Paramecium caudatum* and *Tetrahymena pyriformis* is increased or decreased due to progressive or regressive phase of the illness. The action of serum is not specific since it was proved that serums from patients with cancerous disease showed similar inhibiting effects. The diagnostic value of serum tests is discussed.

ÖSSZEFOGLALÁS

Vizsgálatainkban a teszteléshez nemcsak Parameciumokat, hanem *Tetrahymena* egyedeket is felhasználtunk. Korábbi vizsgálatok eredményeit óhajtottok kisérleteinkben továbbfejleszteni, mert a Parameciumok tenyészete agnotobiotikus és igy a mellette lévő sok ismeretlen faj, különösen ilyen vizsgálatok esetén nagyban befolyásolhatja a kisérleti eredményeket. A Tetrahymenák esetében ezek a tényezők kiesnek, mert csak steril körülmények között tenyészthető meghatározott összetételü táptalajon. A teszt-vizsgálatokhoz schizophreniás betegek serumát használtuk fel és adott higitásu savóban megfigyeltük az állatok szaporodását. A vizsgálatok meggyőzően tanuskodnak arról, hogy a betegek serumában vannak szaporodást gátló tényezők. A gátló hatás a betegség progressziv vagy regressziv szakaszától függően növekszik, vagy csökken. Specifikus hatása nincs, mert rákos betegek seruma is gátló hatásu. A vizsgálatokat pontosabba tehetjük ha helyes módszert alkalmazunk és megfelelő állatot választunk ki. A betegek serumának protozoonokkal történő test-vizsgálata mind elméleti, mind gyakorlati szempontból érdeklödésre tarthat számot továbbfejlesztve a differenciáldiagnosztika területén, de mellette a tiszta tenyészetek felhasználásának a lehetőségét is kibőviti.

REFERENCES

Alpatov et al. 1961: In: Gellért — Rákos betegek vérszérumának hatása *Paramecium caudatum* Ehrbg.-re. Ann. Biol., Tihany, 28, 3–10.

Egan R. W. 1950: Investigation of the *Paramecium* toxicity test for the detection of human malignant tumors. Cancer, 3, 26–31.

Hamilton L. D., Hutner S. M. and Provasoli L. 1952: The use of *Protozoa* in analysis. The Analyst, 77, 618–628.

Harrison I. A., Sane M. E., Fowler E. H., Shellhamer R. M. and Bocher R. H. 1948: Toxicity for *Paramecium* of sera from cancerous and noncancerous persons. Fedn. Proc., 7, 306.

Gellért J., Penkov I., Kamarás L. and Józsa G. 1961: Rákos betegek vérszérumának hatása *Paramecium caudatum* Ehrb. -re. Annls biol. Univ. szeged, 28, 3–10.
Huszák I., Vargha M. and Gellért J. 1958: Die Wirkung des Blutes Liquors, und Tumors Schizophrener auf Protozoen. Wiener Zeitsch. f. Nervenheilk. u. d. Grenzgebiete, 14, 356–362.
Hutner S. H., Provasoli L., Stockstadt E. L. R., Hoffman C. E., Belt M., Franklin A. L., Jukes T. H. 1952: Assay of antipernicious anaemia factor with *Euglena*. Proc. Soc. exp. Biol. Med., 4, 115–119.
Müller M. 1959: A protozoonok tiszta tenyészetének néhány kérdése. Biol. Közl., 7, 83–96.
Nastiukova O. K. 1944: Malignant growth in man as diagnosticated by blood serum toxicity for paramecia. C. r. Acad. Sci. d. sc. USSR, 43, 360–364.
Roskin G. 1946 a: Cytotoxic factor in blood of cancer patients. Am. Rev. Soviet. Med., 4, 115–117.
Roskin G. 1946 b: The toxicity of blood in cancer, serodiagnosis of malignant tumors. Am. Rev. Soviet. Med., 4, 118–127.
Vargha M., Gellért J., Huszák I. 1955: Schizophreniások vérének, liquorának, vizeletének hatása az egysejtüekre. Fájdalom. Az Ideggyógy. Szemle 1955, évi melléklete, 286–287.

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Destruction of cellular components in pigmented protozoa by laser microbeam

Niszczenie struktur cytoplazmatycznych za pomocą mikrowiązki laserowej w barwnych pierwotniakach

Selective destruction is one of the methods used in functional studies on cellular organelles. UV microbeam of about 100 μm in diameter was used for this purpose already in 1912 by Tschachotin. UV light is in current use until now, a high pressure mercury lamp serving as a light source and the microbeam diameter being in modern microscopes about 0.1 μm (Bessis et Nomarski 1960). In this method use is made of the intensive biological effects of UV irradiation. Nevertheless, because of technical difficulties in obtaining sufficiently high microbeam power densities, long exposition times up to several minutes are needed. As laser light sources became available, the use of microbeams of coherent light of very high energy density was made possible. Destructive effects may be obtained after exposition even to single laser pulses, i.e., the necessary irradiation times being of the order of mili-, micro-, or nanoseconds. The obvious advantage is that microsurgery on living, moving cells may be easily performed.

The rapidly developing laser technique permits to obtain coherent light of many wavelengths; up to now, however, mainly ruby (694.3 nm), neodymium doped-glass (1060 nm) and argon (514.5 nm and 488.0 nm) lasers were applied in cellular microsurgery. Recently the use of UV laser microbeam (265 nm) obtained as the IV harmonic by quadrupling the frequency of neodymium-doped-glass laser was reported (Moreno et al. 1969).

Laser microbeam irradiation of living cells was first introduced by Bessis in 1962. A ruby laser beam (5 mm in diameter) was focused to a spot of calculated diameter 2.5 μm through a 6× ocular and 100× phase contrast objective. Vital staining with Janus green B was also used in these experiments. This dye, which absorbs the ruby wavelength, was specifically bound to mitochondria and thus permitted selective destruction of these organelles (Amy et al. 1967, Storb et al.

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1966 and 1967, Wertz et al. 1967). This technique was useful in studying the structural and functional correlations between this organelle and cell metabolism.

A similar equipment with a ruby laser source was used for microsurgery on protozoa and plant cells containing chlorophyll (Saks et al. 1965, Jenkins and Sawyer 1970), as well as fertilized frog eggs (McKinnel et al. 1969).

During the last three years cytological investigations on the application of argon laser with principal wavelengths of 514.5 nm and 488.0 nm, with a peak power output of 1 W and a pulse repetition rate of 60 pulse per second were carried out in the USA. The beam was focused through a Zeiss phase-contrast microscope with a 100× oil-immersion objective. Lesions less than 1 μm in diameter could be obtained with this system. Selected regions of chromosomes, nucleoli and mitochondria in mamalian and amphibian cells in tissue culture were destroyed. To obtain lesions in chromosomes photosensitization with nontoxic concentrations of acridine orange was necessary. Nucleolar lesions were produced following photosensitization with quinacrine hydrochloride (Berns et al. 1969 a, b, 1970).

Because of the presence of respiratory enzymes, cytochrome C and C_1, mitochondria absorb the green-blue part of the spectrum. Berns and Rounds 1970 evoked mitochondrial lesions in unstained frog heart muscle cells using argon laser. In a previous work Rounds et al. 1968 observed a reduced oxygen consumption of cells in culture, following irradiation from a frequency doubled Q-switched neodymium laser (530.0 nm).

Coherent UV-light (265 nm) focused by quartz condenser was used to damage selected regions of cell nuclei, taking advantage of high absorption of this wavelength in nucleic acids (Moreno et al. 1969).

Only a few attempts at quantitation of laser microbeam effects in dependence on radiation dose were made. Amy et al. 1967 demonstrated that the severity and extent of injury caused by ruby laser microbeam in vitally stained cells is proportional to absorbed energy dose, as a function of incident energy and dye concentration.

The present work concerns the use of ruby and neodymium laser microbeams for obtaining lesions in selected limited areas of the cell in naturally pigmented protozoa. Attempts at determination of the dependence of effects obtained from exposition parameters were made. Viability and ability for cell division following irradiation of various cellular components were also determined.

Material and methods

Equipment

Laser microbeam equipment designed in the Institute of Quantum Electronics was used (Pl. I 1). Laser ruby rod head ($\lambda = 694.3$ nm) or neodymium doped glass rod head ($\lambda = 1060$ nm) optically pumped by a flash lamp and cooled by a closed water circuit were used as a coherent light source. In both instances the pulse duration was about 0.2 msec. The beam at the source was 7 mm in diameter, maximal output was 2.5 J. The pulse energy could be controlled by changing the voltage

in the circuit of the flash lamp (Fig. 1 and 2). The beam passed through the ocular and the objective and was focused in the object plane of the microscope. Microscopes NfpK or Ergaval (Zeiss-Jena) with a microphototrinocular were used. The direct pathway of the trinocular served for directing the laser beam, the prism deflected pathways served either for visual observation or for microphotography. The visual observation ocular was equipped with a hair-cross for adjusting the laser beam and the laser spot. Between the laser source and the ocular a pinhole diaphragm with

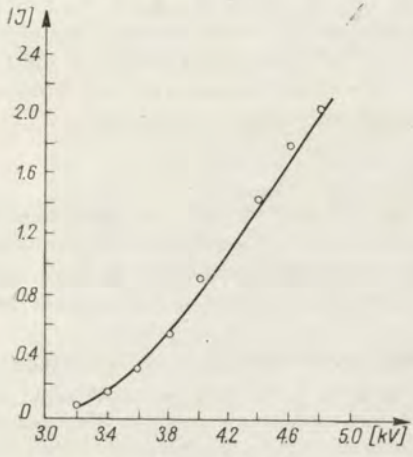


Fig. 1

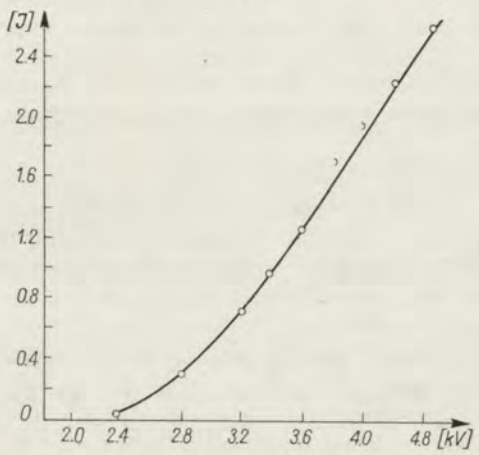


Fig. 2

Fig. 1. Output energy of the ruby rod laser head in function of the voltage in the pumping flash lamp circuit

Fig. 2. Output energy of the neodymium doped glass laser head in function of the voltage in the pumping flash lamp circuit

a diameter of 0.3 or 0.5 mm or a colour glass filter could be introduced. A colour glass filter set with graded transmission of 1, 2, 6, 12, 25, 50 or 70% was used together with the ruby rod laser head.

For precise adjustment of the laser beam with the optic axis of the microscope test preparations were used. Slides covered by aqueous solution of a dye and subsequently air-dried served for this purpose, light green was used for the ruby beam (Saks et al. 1965) and neutral red for the neodymium laser. Following irradiation with the laser beam a sharply delineated light spots appear in the dye layer. The laser head was adjusted in such a manner that the spot appeared in the point corresponding to the center of the hair-cross in the ocular. Comparison of the size of the spots obtained in the dye layer demonstrated that the laser beam was focused exactly on its surface, when it was focused sharply for visual observation by the prism deflected pathway of the trinocular.

The size of the spots obtained in the dye layer depends not only from exact focussing but also from the energy of the incident beam. The diameter of spots obtained after ruby laser irradiation using filters with a light transmission of 1 to 50% or pinhole diaphragms with a diameter of 0.3 or 0.5 mm was measured and compared with the spot-size obtained without filters or diaphragms. Means from the measurements of five spots obtained in set conditions were compared. The laser beam in these experiments was focussed using a 16× or 40× objective with a wide-angle ocular 12.5×. The voltage in the circuit of the flash lamp was constant 3.6 kV, the condenser capacity being 140 μF. The size of the spots obtained in the dye layer was measured with a measuring ocular with a bisector with accuracy to 1 μm.

No direct measurements of the incident energy at the level of the object under the microscope were made and the loss of energy in the optical system was not determined. It may be, however, evaluated at 98–99%, i.e., of the same order as in the equipment used by Daniel and Takahashi 1965.

Material

Naturally pigmented protozoa, *Stentor coeruleus*, *Urostyla* sp. sp., *Keronopsis rubra*, *S. polymorphus*, *Blepharisma* sp. and *Paramecium bursaria* were used in these experiments. The protozoa were obtained from the collection of the Nencki Institute of Experimental Biology of the Polish Academy of Sciences. Standard cultures were held in Pringsheim's medium and fed *Tetrahymena pyriformis*, in the case of *P. bursaria* — with egg yolk suspension.

Technique of cell irradiation

Single protozoa were immobilized in a rotocompressor and placed in the microscope field, the region selected for irradiation centered with the intersections of the arms of the hair-cross in the ocular. Single pulses were used for irradiation. In certain instances the same individual was irradiated several times in different body regions. In all 120 individuals, belonging mostly to *S. coeruleus* species, were irradiated.

Using ruby laser in particular series of experiments constant working conditions of the source were kept. The energy incident on the object was controlled by the introduction of colour filters in pathway of the laser beam. The attenuation of the filter used being known, the relative incident energy at the subject level could be evaluated. To obtain destruction of small areas of the cell low transmission filters or pinhole diaphragms were used. In experiments with neodymium laser irradiation neither filters nor diaphragms were used, the pulse energy being changed within a wide range by regulating the voltage in the feeding circuit.

The laser effects obtained in protozoan cells were evaluated by direct observations in a conventional light or phase contrast microscope and registered photographically. In two series of experiments the protozoa were recovered from the rotocompressor for further observation or fixation and staining. In these instances the duration of compression was kept below 5 min. A longer time caused often death. Nonirradiated individuals compressed for similar time periods, served as controls. To examine surface laser effects the protozoa were fixed in Bouin's fluid and stained with protargol according to the method described earlier (Jerka-Dziadosz and Frankel 1969). The irradiation effects on the macronucleus were evaluated in Feulgen-stained preparations.

Results

Efficacy of equipment

The results of measurement of size of the spots obtained in test preparations using ruby laser beam are presented in Table 1. At very low energy levels (transmission of the filters applied 1% or 2%) spots less than 10 μm in diameter were obtained both with a 16× and 40× objective. The smallest size of spots obtained with the 40× objective were 2.5 μm in diameter. Table 2 presents the mean diameters of spots obtained on test preparation using pinhole diaphragms of 0.3 and 0.5 mm. Both the use of filters or of pinhole diaphragms permit smaller areas of interaction of the laser beam and the irradiated object to be obtained.

Table 1

Relationship between the diameter of the laser microbeam spot and filter transmission. Diameters are given in micrometers. Output energy equal to 0.4 J, in parentheses diameters obtained at energy of 0.6 J

Objective	1%	2%	6%	12%	25%	50%	100% — without filter
40×	2.5	8.1	11	11.5	11.5	11	12 (35)
16×	4	8.3	13	13.7	16	20	22.5 (70)

Table 2

Relationship between the diameter of the laser microbeam spot and size of the pinhole diaphragm. Diameters are given in micrometers. Output energy equal to 0.4 J, in parentheses diameters obtained at energy of 0.6 J

Objective	Pinhole size 0.3 mm	Pinhole size 0.5 mm	Without pinhole diaphragm
40×	7 (10)	8.3	12 (35)
16×	11.0	13.4	22.5 (70)

The morphology of laser microbeam effects in the cytoplasm of different species of protozoa

The effects of laser microbeam irradiation in the cytoplasm of different ciliate species in dependence of the incident energy are shown in Table 3. The experiments on *S. coeruleus* were carried out using energy output of 0.7 J, ocular 12.5×, objective 40× and colour filters. If the transmission of the filter used was more than 50%, immediately after the pulse a gaseous vesicle 10 μm in diameter appeared in the irradiated part of cytoplasm (Pl. I 2). During the following 5–8 sec the vesicle was displaced to the cell surface, decreased in size and finally disappeared. An area of coagulated cytoplasm, darker than its surroundings, remained in the irradiated region. After a period of about 40 sec the coagulated part of cytoplasm was extruded from the cell (Pl. 3). In the surface structures of *S. coeruleus* containing longitunal stripes of pigment granules a light area without these granules appeared as a sign of injury (Pl. I 3). Following the irradiation with a single laser pulse without any filter both the ventral and dorsal pellicles were injured. The gaseous vesicle appeared inside the cytoplasm (Pl. I 4). As filters of decreasing transmission were used the gaseous vesicle appeared nearer the upper cell surface; it was smaller and disappeared sooner. When filter of 50% transmission was used the vesicle was not always observed and with decreasing transmission it did not appear. Using a filter of 12% transmission only a coagulated dark area of cytoplasm was seen. At lower transmission levels of 2% only a small area of immobile coagulated cytoplasm not differing in colour from its surroundings could be observed.

Table 3

The relationship between effects obtained in various protozoan species and the output pulse energy and the transmission of the filters used

Species	Pulse energy J	Effect	% of filter transmission						
			2	6	12	25	50	70	100
Stentor coeruleus	0.7	Gaseous vesicle	—	—	—	—	+—	+.	—
		Cytoplasm coagulation	+	+	+	+	+	+	+
		Change of colour of the coagulated area	—	—	+—	+	++	++	+
Keronopsis rubra	0.9	Gaseous vesicle	—	—	—	—	—	—	—
		Cytoplasm coagulation	—	—	—	+—	+	+	+
		Change of colour of the coagulated area	—	—	—	+	+	+	+
Paramecium bursaria	0.7–0.9	Pellicle rupture and streaming out of cytoplasm	—	+	+	+	+		
		Cytoplasm coagulation	+	—	—	—			
Blepharisma sp.	0.9–1.2	Gaseous vesicle	—	—	—	—	—	—	—
		Change of colour of the coagulated area	—	—	—	—	—	—	—
		Cytoplasm coagulation	⎫ effects irreproducible after						
		Paling of the whole cytoplasm	⎭ first exposition						

In other examined species gaseous vesicle could not be obtained. In *Keronopsis rubra* a higher energy (0.9 J filter transmission more than 25%) was necessary for obtaining coagulation of the cytoplasm. The coagulated area was also darker (Pl. II 6). In *P. bursaria* similarly as in *S. coeruleus* a comparatively low energy (0.7–0.9 J, filter transmission 2%) was sufficient to obtain coagulation of a small area of cytoplasm.

Irradiating *Blepharisma* a great variability of results was noted. In certain instances higher energy doses did not cause any effects, while exposition to lower doses led to cytoplasm coagulation. Some individuals were exposed to many repeated pulses. This inconsistency of results may be perhaps explained by the observation that in *Blepharisma* besides local effects, exposition to the first pulse caused loss of intensity of colour of the whole cytoplasm, following which further exposition to pulses of even higher energy did not provoke any visible local effects.

Because of the rapid sequence of events in the above experiments the diameter of the lesions obtained could not be measured. Both subjective evaluation and comparison of microphotographs indicate that the size of the injured area was proportional to pulse energy. The size of the lesion changed also with the objective magnification power. In the zone of adoral membranelles of *S. coeruleus* using 16× objective at 0.7 J output energy about 10 membranelles were destroyed (Pl. I 5) and using 40× objective — only 3 (Pl. II 7). In *K. rubra* stained with protargol

following irradiation with 40× objective, output energy 0.9 J the loss of 5 membranelles and traces of injury to a few of neighbouring ones may be seen in the AZM region (Pl. III 14).

Besides strictly localized effects, observed in protozoa with a dense cytoplasm, such as *Stentor* and *Keronopsis*, bursting of the pellicle and streaming out of the cytoplasm was seen in *Paramecium bursaria*, the cytoplasm of which is highly fluid. This phenomenon was observed at output energy of 0.7–0.9 J with filters of a transmission higher than 6%. Following irradiation of the cell margin, in this species a small indentation, containing a small cytoplasmatic vesicle, appeared (Pl. II 11). In the neighbouring region extrusion of trichocyts was observed (Pl. II 10). The cell remained deformed up to 30 min.

The following sums up shortly the above described experiments. Laser irradiation effects differ in various species of protozoa. To constant effects belong:

(1) coagulation of circumscribed area of cytoplasm,

(2) the coagulated part is extruded,

(3) surface structure lesions demonstrable both by vital observation and in fixed and stained preparations.

Inconstant effects are:

(1) the appearance of a gaseous vesicle,

(2) change of optic desnity of the coagulated area of the cytoplasm — its darker appearance,

(3) loss of colour of the whole cytoplasm — its paling.

In various species different doses of energy are necessary to obtain a definite local lesion.

Neodymium doped glass laser microbeam was used for irradiation of *S. coeruleus* cells. Pulse energy ranged from 0.15 to 1.75 J, a 40× objective and 12.5× ocular were used for focusing. Neither filters nor diaphragms were introduced into the beam's pathway. In a part of experiments effects, similar to those after ruby laser irradiation, were observed. A gaseous vesicle and coagulated area of cytoplasm, subsequently extruded from the cell, appeared. Lesions in surface structures were seen (Pl. II 9). The coagulated part of the cytoplasm was in this case lighter than surroundings. The reproducibility of results was low. Because of this the ruby laser microbeam was chosen for further experiments. *Stentor coeruleus* proved to be the most convenient object for irradiation because of its pigmentation and size. Attempts of obtaining a smallest possible diameter of the lesion were made, decreasing the diameter of laser beam at the source and lowering pulse energy. Using a pinhole diaphragm 0.3 mm, pulse energy output 0.8 J, 40× objective and 12.5× ocular a lesion of about 8 μm in diameter was obtained, i.e., 3 membranelles of the AZM region were injured. In similar conditions, differing only by a lower pulse energy of 0.28 J only one AZM membranelle was destroyed. Using a pinhole diaphragm 0.5 mm in diameter and pulse energy of 0.45 J two membranelles were destroyed

(Pl. II 7, 8). Comparison of these results with the data given in Table 2 demonstrates that the size of lesions obtained is within the range of the size of spots obtained in test slides using a diaphragmed laser beam.

Injury to the nuclear apparatus

The laser microbeam, at the same output energies which provoked definte cytoplasmic lesions, directed and focused at the level of Ma interior did not cause any visible effects. Even in these instances when gaseous vesicles appeared in the cytoplasm above the nucleus and the surface structures were injured both below and above the nucleus, the shape and appearance of the irradiates nodes and of the whole nuclear apparatus did not change. Attempts at injuring the nucleus were made on several individuals belonging to the *Stentor*, *Paramecium bursaria* and *Urostyla weissei* species, using both ruby and neodymium lasers. The presence of surface and cytoplasmic lesions both above and below the nucleus proves that it was within the pathway of the beam and was irradiated.

Additionally the first or the first and second nodes of the macronucleus of 10 *S. coeruleus* specimens were irradiated with single ruby laser pulses at output energy of 1.05 J using a 40× objective and a filter with 70% transmission. The protozoa were subsequently fixed and Feulgen-stained. No differences in the chromatin structure, shape and general appearance of irradiated and unirradiated nodes were seen (Pl. II 12, 13).

Viability and ability to cell division of irradiated *S. coeruleus* cells

During the above described experiments a part of the examined cells was lost due to a too high degree of compression or rapid decompresssion, while in certain instances breakage of the pellicle and outflow of the cytoplasm following laser irradiation was also the cause of cell death. A part of individuals following freeing from the rotocompressor showed a rapid return to norm and motility. *S. coeruleus* individuals proved to be most resistant to the whole experimental procedure, i.e., compression, irradiation and decompression. Even the eversion of the gullet observed during compression regressed rapidly. Seven *S. coeruleus* individuals following irradiation of the frontal region or of Ma by neodymium laser microbeam were freed from the rotocompressor and observed during 10 days. One individual died during the 5th day, the remaining six survived during the whole observation period, while four divided.

In another experiment 21 irradiated *S. coeruleus* were observed during 4 days, 10 individuals compressed in the rotocompressor for 4 min and 10 individuals not subjected to any experimental procedure were serving as controls. The experimental group was irradiated with single pulses at output energy of 0.4 J through a 12.5× ocular and a 40× objective, 11 individuals at the peristomal region and 10 at the first anterior node of the macronucleus. Following each irradiation characteristic changes in the cytoplasm were observed — injury at the peristomial region or to

a few AZM membranelles, the appearance of a gaseous vesicle and coagulation of a circumscribed area of the cytoplasm. A gaseous vesicle appearing in the cytoplasm above the macronucleus was taken as a proof of its irradiation.

All cells were kept isolated in single cultures containing 1 ml of medium and fed for the first time six hours after isolation. The results of this series are presented in the Table 4 and Fig. 3. In the first group (irradiation of the AZM region) of 11

Table 4

The influence of ruby laser microbeam irradiation on the division rate of *Stentor coeruleus*

Group	Hours following irradiation or compression					Number of division	Number of cells dead before division
	0	24	48	72	96		
AZM region irradiated	11	—1 11 +1	—1 15 +4	—1 24 +10	—2 40 +18	33	2*
Ma region irradiated	10	—0 11 +1	—1 10 +0	—1 16 +7	—0 28 +12	20	2
Control-compressed in the rotocompressor and freed	10	—0 11 +1	—0 18 +7	—1 23 +6	—0 30 +7	21	—
Control	10	—0 13 +3	—0 13 +3	—1 28 +10		19	—

* One cell survived four days without division.

individuals 2 died before the first division, 8 cells were capable to divide and 5 of these divided during the first 48 h following irradiation, the total number of divisions being 33. In the second group (irradiation of the Ma) of 10 individuals 8 cells were capable to divide, one cell divided during the first 48 h following irradiation and the total number of divisions was 20.

In the control group not subjected to any experimental procedure, all cells were capable of division, 8 cells divided during the first 48 h after isolation, the total number of divisions being 21. The observation of the compressed and decompressed cells did not demonstrate any differences with the foregoing group.

The differences between the both irradiated groups and the controls consisted in:

(1) a lower number of cells capable to divide,

(2) differences in time and division rate, experimental group I and II differing also among themselves.

Among the cells irradiated at the AZM region a slightly lower number divided during the first 48 h of observation, later on the division rate was, however, higher,

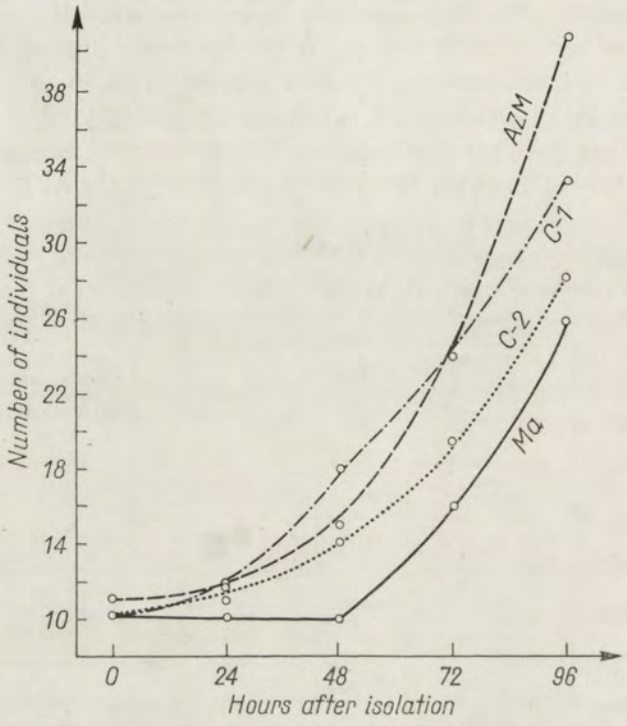


Fig. 3. Increase of number of *Stentor coeruleus* cells following AZM region irradiation, anterior Ma segment irradiation and in a control group. See text

as in the control groups. In the group irradiated at the Ma the first division was retarded, later the division rate being similar as in controls. Nevertheless the small number of observed individuals does not permit to draw conclusions concerning the differences in the time of the first division.

It may be suggested, however, that in the above described conditions irradiation of selected areas of the *S. coeruleus* cell does not provoke lethal changes and the cells cultivated after the exposition are usually capable to form a normal clone. In a few instances the exposition caused cell death or influenced the division rate.

Discussion

The above presented results demonstrate that the described equipment may be used to obtain reproducible sharphy delineated lesions in selected areas of a cell, the size of these lesions being predetermined by exposure conditions. The smallest obtainable size of the lesion was about 2 μm in diameter. Laser microbeam cellular surgery is an easy and convenient experimental procedure.

The mechanism of injury in the living cell merits discussion. Coherent light causes in living matter thermal effects and what is called nonlinear effects. Thermal effects consist in a temperature rise in the place of interaction proportional to the dose of absorbed energy. In consequence thermal denaturation of proteins may occur and after a certain critical temperature is reached the evaporation takes place. Different regions of the spectrum are absorbed in a different degree by the cytoplasm, in the visible light region the deciding factor being the pigmentation of the cell. Melanins, natural or artificial green and blue pigments absorb specially the red ruby laser light of 694.3 μm wavelength (Bessis and Ter-Pergossian 1965, Rounds et al. 1965). The magnitude of the temperature rise depends on the absorption, which is related to pigmentation and the does of incident energy.

Among nonlinear effects in living objects mechanical interaction, ionization and generation of secondary radiation may play a role. These effects should be considered in such instances, in which the pulse duration is very short, for shorter than that used in our experiments (Wilkening 1970). Nevertheless pressure changes caused by thermal dilatation, specially in the case of evaporation of irradiated material should be considered.

The localized lesions seen in the pigmented protozoa in our experiments may be explained satisfactorily by thermal action. The effects seen in different species depended on the specific natural pigmentation in the ectoplasm and pigment granula (*Stentor* — bluish, *Keronopsis* and *Blepharisma* — dark red). *Paramecium bursaria* and *Stentor polymorphus* show green pigmentation due to the presence of chlorophyll in the symbiotic algae present in the cytoplasm.

The same dose of incident energy of the ruby laser microbeam caused more profound changes in the cytoplasm of green or blue pigmented protozoa than in red ones. Within a single species the extent and the severity of the lesion depended on the dose of incident energy. At lower doses denaturation of the irradiated area was observed, at higher doses a gaseous vesicle appeared indicating evaporation of the cytoplasm.

No simple correlation between the pigmentation of the cell and energy absorption was observed during irradiation with neodymium laser. It may be supposed that absorption of this wavelength does not depend on substances causing differences in colouring in the visible light spectrum. This explains also the poor reproducibility of results obtained by irradiation of protozoa with neodymium laser microbeam.

The above described direct irradiation effects cause permanent lesions of certain cell areas. The extrusion of the coagulated part of the cytoplasm is caused probably by high contractility of the ectoplasm. Elimination of the denatured part causes decrements. These are seen easily in surface structures. Loss of AZM membranelles, pigment granules, kinetosomes and extrusion of trichocysts were observed. It follows that not only naturally pigmented structures, but also their neighbourhood (kinetosome with cilium) could be injured. The appearance of a gaseous vesicle within the cytoplasm and lesions of both dorsal and ventral pellicles seen in certain instances,

indicate that the microbeam penetrates the cell interior and may be absorbed by the pigment dispersed in the cytoplasm. It follows that lesions at the focal point of the microbeam were obtained and not selective destruction of certain specific organelles.

Easily visible decrements in AZM membranelles served for evaluation of the smallest obtainable size of the lesion, which was compared with the size of spots obtained on test slides in similar irradiation conditions. The smallest spots had a diameter of 2.5 μm. The smallest lesion involved 1 AZM membranelle and an area of about 2 μm diameter in size.

Local or generalized changes in colour following irradiation are of some interest. The coagulated area in the cytoplasm of *Stentor coeruleus* or *Keronopsis rubra* sp. was darker following irradiation with 694.3 nm wavelength and lighter after 1060 nm. In *Blepharisma* sp. paling of the whole cytoplasm was noted after exposition to a single laser pulse. This phenomenon remains unexplained. Rapid changes in the chemical structure of the pigment may occur, possibly connected with changes in hydrogen ion concentration. Among other generalized reactions the ease with which the external pellicle of irradiated *Paramecium* cells is ruptured should be mentioned. Probably in such a small cell the generated pressure wave cannot be sufficiently attenuated and causes the rupture.

In our experiment no direct affects were demonstrable in the nuclear apparatus. Even in those instances when in the near neighbourhood of the Ma a gaseous vesicle was formed by cytoplasm evaparation no lesions in the nuclear membrane or chromatin structure were seen by light microscopy.

The influence of limited lesions in a selected area of the cell on its functioning must be also considered. According to Storb et al. 1966 KB cells irradiated with ruby laser microbeam did not show any traces of injury after a period of 4 hours following exposition. Jenkins and Sawyer 1970 observed in situ regeneration of AZM membranelles in *Blepharisma japonicum* occurring during 8 h following exposition to ruby laser microbeam. Saks et al. 1965 and Storb 1967 reported that amoebae and animal cells in tissue culture show a decreased division rate following irradiation with ruby laser microbeam. Individual observations of *Stentor* irradiated at the Ma or AZM region demonstrate that in the majority of cases no lethal changes occurred and the cells retain the ability to form normal clones. The sublethal changes consisting in retardation of the first division following irradiation of the Ma region nead confirmation on a larger material.

Conclusions

It is the authors view that the above presented results allow the following conclusions to be drawn:

(1) the described laser microbeam equipment is a convenient tool for performing

delicate microsurgical experiments on protozoan cells, ruby laser head being specially suited for this purpose,

(2) controlling the pulse energy, using filters of various transmission and diaphragms limiting the beam diameter, the extent and severity of the obtained lesion may be varied within a wide range,

(3) laser microbeam surgery does not cause a general impairment of vital cell functions and may be used conveniently for the study of cellular regeneration following destruction of limited cell areas.

Acknowledgements

Sincere thanks are due to the constructors of the equipment for laser microbeam surgery used in this investigation — prof dr Z. Puzewicz, dr eng. T. Machowski and engs T. Rutkowski and J. Pszenicki from the Institute of Quantum Electronic WAT, Warszawa-Bemowo both for the permission to use this equipment as well as for constant advice and many profitable discussions during the experiments and preparation of the manuscript.

Summary

A laser microbeam microscopic equipment with ruby rod or neodymium doped glass head was used for the study of effects obtainable on living protozoan cells and proved to be a convenient tool for microsurgery. Controlling the pulse energy and using filter of various transmission and diaphragms, the extent and severity of the lesions obtained may be varied within wide limits, the smallest obtainable lesion being about 2.5 μm in diameter. The relationship between the extent and severity of the lesion and incident energy was studied and the influence of absorption characteristics of the cell considered. Laser microbeam surgery does not cause a general impairment of vital cell functions and may be used conveniently for the study of regeneration or functional disturbances following destruction or limited cell areas.

STRESZCZENIE

Za pomocą mikrowiązki laserowej wywoływano uszkodzenia w naturalnie zabarwionych pierwotniakach. Stosowano prototypowe urządzenie do mikropunkcji z głowicą laserową rubinową (694.3 nm) i neodymową (1060 nm). Za pomocą mikrowiązki lasera rubinowego udawało się z łatwością wywoływać uszkodzenia w różnych okolicach cytoplazmy pierwotniaków. Rozległość i stopień uszkodzenia komórki zależała od ilości energii padającej na komórkę, zmieniano zatem energię impulsu laserowego, względnie stosowano filtry tłumiące czy przesłony. Wywoływane w komórkach uszkodzenia oceniano przeżyciowo lub w preparatach utrwalonych i barwionych w obrazie zwykłego mikroskopu świetlnego lub mikroskopu kontrastowo-fazowego. Najmniejsze wywołane mikrowiązką uszkodzenia miały średnicę ok. 2.5 μm. Naświetlanie mikrowiązką lasera rubinowego czy neodymowego aparatu jądrowego nie powodowało w nim zmian morfologicznych. Obserwacja kilkudnio-

wa komórek wykazała, że większość komórek przeżywa naświetlanie i jest zdolna do podziałów. Laserowe urządzenie do mikropunkcji jest wygodnym narzędziem do wykonywania zabiegów mikrochirurgicznych w pierwotniakach. Opisana metoda może być przydatna w badaniach regeneracji lub zaburzeń czynnościowych związanych z uszkodzeniem określonych struktur komórkowych.

REFERENCES

Amy R. L., Storb R., Fauconier B. and Wertz R. K. 1967: Ruby laser microirradiation of single tissue culture cells vitally stained with Janus green B. I. Effects observed with the phase-contrast microscope. Exp. Cell Res., 45, 361–373.

Berns M. W., Olson R. S. and Rounds D. E. 1969 a: In vitro production of chromosomal lesions with an argon laser microbeam. Nature, 221, 74–75.

Berns M. W., Rounds D. E. and Olson R. S. 1969 b: Effects of laser microirradiation on chromosomes. Exp. Cell Res., 56, 292–298.

Berns M. W., Ohnuki Y. and Rounds D. E. 1970: Modification of nucleolar expression following laser microirradiation of chromosomes. Exp. Cell Res., 60, 133–138.

Berns M. W. and Rounds D. E. 1970: Laser microbeam studies on tissue culture cells. Ann. N. Y. Acad. Sci., 168, 550–563.

Bessis M. 1967: Irradiation of cell organelles by laser microbeam: Problems and techniques. In: Formation and Fate of Cell Organelles ed. K. B. Warren New York–London Academic Press.

Bessis M. and Nomarski G. 1960: Irradiation ultra-violette des organites cellulaires avec observation continue en contraste de phase. J. biophys. biochem. Cytol., 8, 777–792.

Bessis M. and Ter-Pergosian M. M. 1965: Micropuncture of cells by means of laser beam. Ann. N. Y. Acad. Sci, 122, 689–694.

Daniel J. C. and Takahashi K. 1965: Selective laser destruction of rabbit blastomeres and continued cleavage of survivors in vitro. Exp. Cell Res., 39, 475–482.

Jenkins R. A. and Sawyer S. R. 1970: Selective extirpation of oral ciliature of *Blepharisma* by laser microbeam. Exp. Cell Res., 63, 192–195.

Jerka-Dziadosz M. and Frankel J. 1969: An analysis of the formation of ciliary primordia in the hypotrich ciliate *Urostyla weissei*. J. Protozool., 16, 612–637.

McKinnel R. G., Mims M. F. and Reed L. A. 1969: Laser ablation of maternal chromosomes in eggs of *Rana pipiens*. Z. Zellforsch. mikrosk. Anat., 93, 30–35.

Moreno G., Salet C. and Bessis M. 1969: Microirradiation of nuclei by ultraviolet laser. Compt. rend. Soc. Biol., Paris, 269, 781–782.

Rounds D. E., Chamberlain E. C. and Okigaki T. 1965: Laser radiation of tissue cultures. Ann. N. Y. Acad. Sci., 122, 713–727.

Rounds D. E., Olson R. S. and Johnson F. M. 1968: The effect of the laser on cellular respiration. Z. Zellforsch. mikrosk. Anat., 87, 193–198.

Saks M. M., Zuzolo R. C. and Kopac M. J. 1965: Microsurgery of living cells by ruby-laser irradiation. Ann. N. Y. Acad. Sci., 122, 695–712.

Storb R., Amy R. L., Wertz R. K., Fauconier B. and Bessis M. 1966: An electron microscope study of vitally stained single cells irradiated with a ruby laser microbeam. J. Cell Biol., 31, 11–29.

Storb R., Wertz R. K. and Amy R. L. 1967: Ruby laser microirradiation of single tissue cells vitally stained with Janus green B. II. Effects on dehydrogenase acivities. Exp. Cell Res., 45, 374–384.

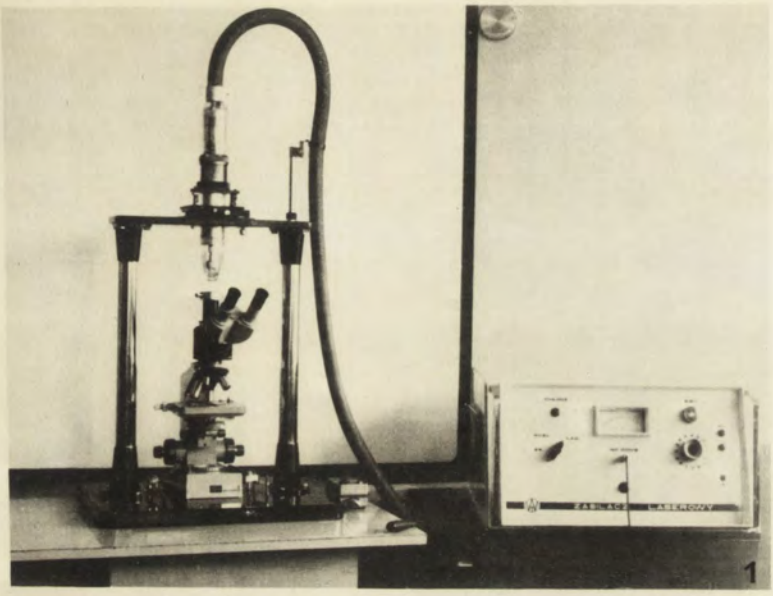
Wertz R. K., Storb R. and Amy R. L. 1967: Ruby laser microirradiation of single tissue cultures cells vitally stained with Janus green B. III. Effects on the incorporation of an RNA pyrimidine precursor. Exp. Cell Res., 45, 61–71.

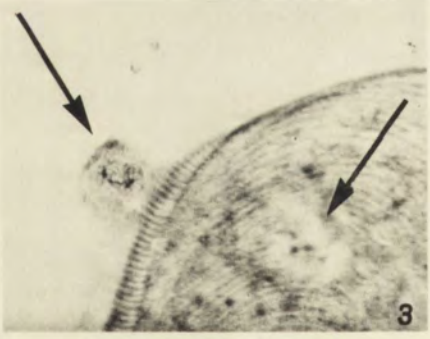
Wilkening G. M. 1970: A commentary on laser induces biological effects and protective measures. Ann. N. Y. Acad. Sci., 168, 621–633.

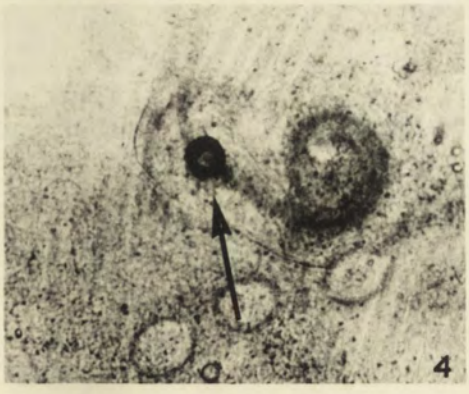
EXPLANATIONS OF PLATES I–III

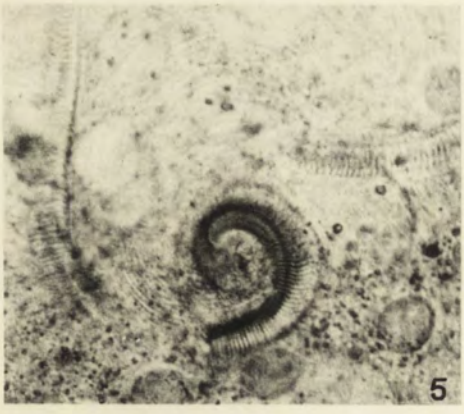
1: Laser microbeam equipment with the ruby rod head. Microscope with head on the left, feeding equipment on the right
2: *Stentor coeruleus* after irradiation of the frontal region. Objective 16×, ocular 12.5×, filter transmission 70%. Arrow shows gaseous vesicle in the irradiated region
3: *S. coeruleus* same individual as phot. 2, after 40 sec. Arrow on the left shows a darker, coagulated cytoplasmic clot extruded out side the cell. Arrow on the right shows surface structure lesion
4: *S. coeruleus* irradiated through objective 16×, ocular 12.5, filter transmission 60%. Arrow shows gaseous vesicle in the interior of the cell
5: *S. coeruleus* irradiated in the AZM region through objective 16×, ocular 12.5×. Arrow show lesion in the AZM
6: *Keronopsis rubra* irradiated in the frontal region through objective 40×, ocular 12.5×. Arrow shows a darker coagulated part of cytoplasm
7: *S. coeruleus* irradiated in the AZM region through objective 40×, ocular 12.5×, pinhole diaphragm 0.3 mm. Loss of 3 membranelles should be noted. Phase-contrast photograph
8: *S. coeruleus* irradiated in the AZM region through objective 40×, ocular 12.5×, pinhole diaphragm 0.3 mm. Lack of one membranelle. Phase-contrast photograph
9: *S. coeruleus* irradiated in the AZM region through objective 40×, ocular 12.5×. Lack of 2 membranelles
10: *Paramecium bursaria* irradiated through objective 40×, ocular 12.5, filter transmission 50%. The photograph was made 12 sec following irradiation, note extrusion of trichocysts and outstreaming cytoplasm
11: *P. bursaria*, vesicle on the cell border in the neighbourhood of the exposed region. The photograph was made 17 min after irradiation through objective 40×, ocular 12.5×, filter transmission 6%
12: *S. coeruleus*. Two anterior nodes of the Ma were irradiated (arrows). Following irradiation through objective 40×, ocular 12.5×, without filter the cell was fixed and Feulgen-stained.
13: *S. coeruleus*, nonirradiated. Feulgen-stained
14: *Keronopsis rubra* irradiated in the AZM region through objective 40×, ocular 12.5×. Following irradiation, fixed and Protargol-stained

The protozoa on phot. 2–8 and 10–14 were irradiated using ruby rod laser head, voltage in the pumping flash lamp circuit 4 kV. Phot 9 irradiation using neodymium doped glass laser head, voltage 4 kV









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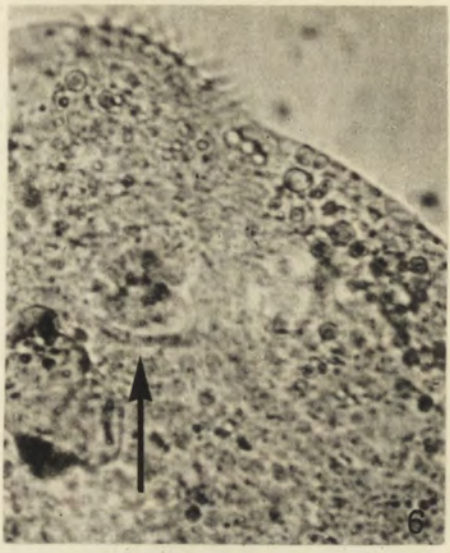

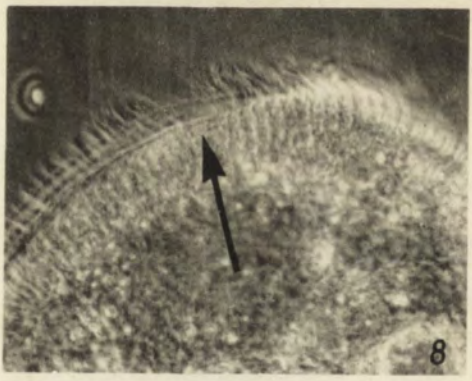

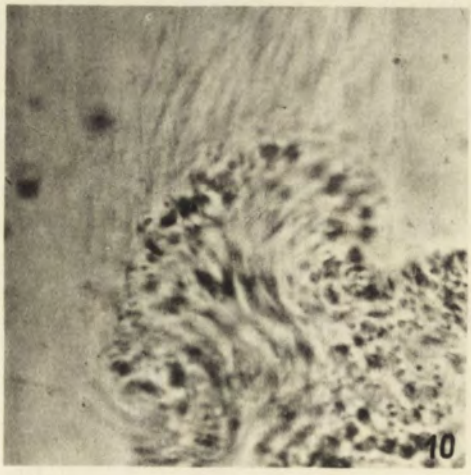

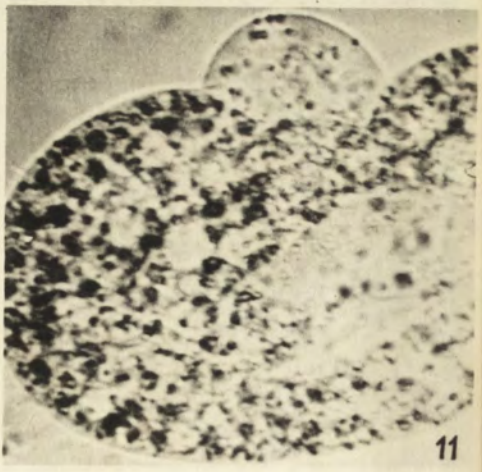

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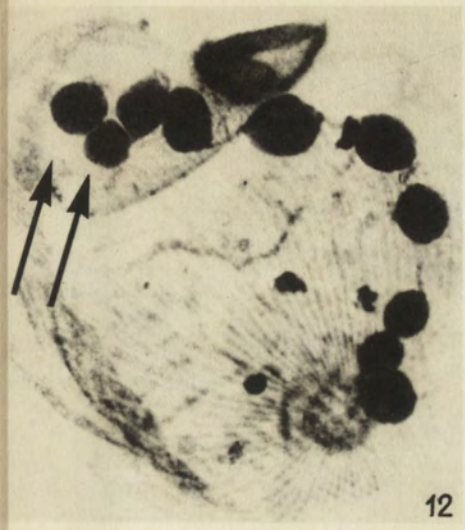

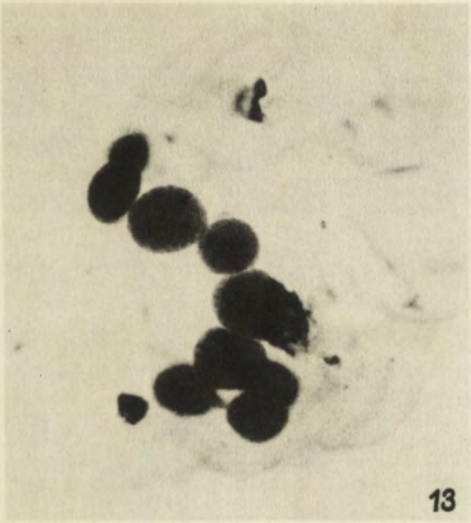

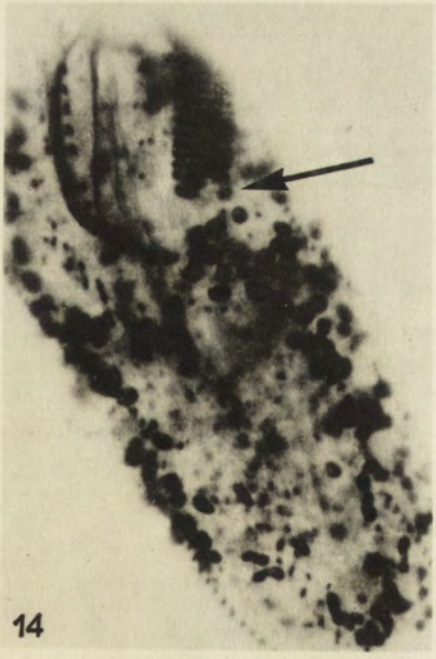

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ЧЕТВЕРТЫЙ МЕЖДУНАРОДНЫЙ ПРОТОЗООЛОГИЧЕСКИЙ КОНГРЕСС (1973)

Четвертый Международный Протозоологический Конгресс будет иметь место в Клермон-Ферране (Франция) со 2-го по 9 сентября 1973 г. включительно.

Первое извещение касающееся принципов организации Конгресса, разработанных Международным протозоологическим Комитетом будет направлено всем протистологам в конце 1971 года. Только тем, которые обнаружат заинтересованность, будут посланы последующие извещения.

Корреспонденцию направлять по адресу:

Международный Протозоологический Конгресс, Зоология, п/я 45, 63, Обиер, Франция.

Международным протозоологическим Комитетом принято следующее предложение:

"В соответствии с духом Международного комитета научных обществ Международная протозоологическая комиссия, собранная 9–11 сентября 1971 г. в Клермон-Ферране, единогласно рекомендует:

(а) направить правительствам разных стран настоятельное обращение, цель которого сделать возможным присутствие всех национальностей на международных заседаниях во всех странах без национальной дискриминации и

(б) просить, чтобы при наличии трудностей такого рода и в каждом конкретном случае, существующие международные организации брали на себя труд по осуществлению международных связей.

Подписи: **T. Crippa-Franceschi, H. Finley, P. C. C. Garnham, J. Grain, A. Grębecki, B. M. Honigberg, J. Jadin, B. A. Newton, T. R. Nilsson, Ю. И. Полянский, P. de Puytorac, W. Trager, A. Zuckerman.**

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CATALOGUS FAUNAE PARASITICAE POLONIAE

Editors: Eugeniusz GRABDA, Tadeusz JACZEWSKI and Stanisław L. KAZUBSKI

Part II

Pasożyty krągłoustych i ryb

Parasiti cyclostomatorum et piscium

by Jadwiga GRABDA

The parasites, including *Protozoa*, found in one species of lamprey (*Cyclostomata*) and 74 species of fishes (*Pisces*) in Poland are accounted in the catalogue. For each host species the parasites are encountered in systematic order. Their distribution in Poland and in the world, incidence and intensity of infestation, location in the host body biology and pathogenicity are treated. In the catalogue 51 species of parasitic *Protozoa* occurring on fishes in Poland are recorded. A survey of literature on fish parasites as well as indexes and a polyglot vacabulary of expressions used in text are included (main text in Polish).

Part III

Pasożyty płazów i gadów

Parasiti amphibiorum et reptilium

by Bożena GRABDA-KAZUBSKA

The parasites of 16 species of amphibians and 6 species of reptiles in Poland are recorded in this part of Catalogue. In all 14 species of parasitic *Protozoa* are noted.

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David E. GREEN und Robert F. GOLDBERGER. **Molekulare Prozesse des Lebens** aus dem Englischen übersetzt von L. und R. Träger. Mit 98 Abb. 240 Seiten. 1971. Berlin–Heidelberg–New York: Springer-Verlag ISBN 3-540-05109-0.

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