POLISH ACADEMY OF SCIENCES

ACTA PROTOZOOLOGICA

VOLUME 14

Number 3/4

W A R S Z A W A 1 9 7 5

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

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ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 253–262

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Updating the Tetrahymenids III. Natural Variation in *Tetrahymena setosa* nov. comb.

Synopsis. Tetrahymena setosa nov. comb. results from the transfer of *Glaucoma setosa* Schewiakoff, 1893, and the suppression of *T. setifera* Holz and Corliss, 1956. Strains of this species have been collected from throughout the United States and established in axenic medium. Extensive polymorphism for cortical and enzymatic traits has been found, but there is no evidence of cryptic speciation. The caudal cilium arises in the proter by the modification of the last basal body of the kinety directly to the left of the oral apparatus.

Tetrahymena setifera Holz and Corliss, 1956, was described with due consideration of all other species then included in the genus. However, possible synonymy outside the genus was not discussed. Since *T. pyriformis* was long known as *Glaucoma pyriformis* Ehrenberg, we want to draw attention to Schewiakoff's description (1893) of *G. setosa*, which differs from his own figures of "*G. pyriformis*" only in the possession of a caudal cilium. The size range ("about 37 μm long and 16 μm wide") agrees nicely with the data of Holz and Corliss. Even the contractile vacuole pore (CVP) is positioned near the correct meridians — and this is truly remarkable, considering the haphazard treatment of these structures in the early literature. The location of the CVP allows us to eliminate from consideration a number of superficially similar forms, such as *Sathrophilus*. Kahl (1930–1935) repeats Schewiakoff's description. However, there is very little experimental work pertaining to this species, and it is mentioned mainly in reviews. By recognizing this synonymy, *T. setifera* becomes *T. setosa* nov. comb.

Corliss (1971) established a lectotype for *T. setifera* which would, under appropriate sections of the International Code of Zoological Nomenclature, become the neotype for *T. setosa* nov. comb. The neotype strain HZ-1 was isolated from a reservoir only 64 km from the source of most of our New York strains, and is plausibly of the same genetic species as our strains. It would be desirable to examine the electrophoretic patterns of the reported amicronucleate strains to see if they indicate an origin directly from the known micronucleate species, *T. setosa*. One strain

which seemed to be amicronucleate was recovered from the Chicago River (!) but was lost before permanent preparations could be made. Another is reported to have arisen in the laboratory (Holz 1955), but the continued presence of the caudal cilium, which would distinguish a *setosa*-like strain from a mislabeled *T. pyriformis* GL, was not documented.

The strains studied (Table 1) were collected from locations ranging from 72° west longitude to 122° west longitude, from 37° north latitude to 50° north latitude, and from near sea level to over 3000 m elevation. From its overall occurrence in our collections, we calculate it is the most common (biological, genetic) species of

Table 1

Strains of *T. setosa* nov. comb. Mentioned in This Paper

Strain No.	Habitat and collector
UI-7103	Stewart Park, Ithaca, New York (Cayuga L.)
UI-7104	Ithaca Yacht Club, Ithaca, New York (Cayuga L.)
UI-7135*	Montezuma Wildlife Refuge, New York (canal between Seneca L. and Cayuga L.)
UI-7116	Presque Isle, New York (L. Erie)
UI-7105	Mill R., Connecticut (Dr. G. S. Whitt)
UI-7125	St. Alban's Bay, L. Minnetonka, Minnesota (Dr. D. Nyberg)
UI- 7172	L. Rawson, near Bureau, Illinois (Dr. D. Borden)
UI-7174	Bureau Creek, near Interstate Route 108, Illinois (Dr. D. Borden)
UI-7177*	pond, junction of routes 71 and 51, Illinois (Dr. D. Borden)
UI-7211b	Salt Fork, Vermillion R., near Urbana, Illinois (G. Dearlove)
UI-7151i	Poudre L., Rocky Mountain National Park, Colorado (on continental divide, elevation over 3000 m.) (Dr. F. P. Doerder)
UI-7208f	L. Samish, near Bellingham, Washington (J. W. McCoy)

The first four collections in this Table were made by D. Borden, F. P. Doerder, D. Nyberg and B. Perlman, during a collecting trip in August, 1971

* Now extinct

Tetrahymena in temperate North America, approached in frequency only by syngen *2* of "*T. pyriformis*". *T. setosa* has been recovered from snow-melt runoff, alpine ponds, large lakes, bogs, free-flowing streams, polluted rivers, and irrigation ditches. Our studies reported here demonstrate that this species makes use of extensive polymorphism in cortical as well as biochemical parameters, some of which may eventually be correlated with geographical factors.

Materials and Methods

Collections of about 100 ml each were examined for *Tetrahymena*, and cells of the appropriate size, shape, and behavior were isolated into bacterized medium. Alternately, 5 ml portions were added aseptically to tubes containing 10 ml of 1% proteose peptone plus penicillin G (1000 units/ml)

and streptomycin sulfate (1 mg/ml). This medium permits survival of most tetrahymenas (including those that grow poorly in cerophyl) but kills bacteria and most predators. However, molds are not affected, and cells must be washed individually before axenic cultures can be established. For axenic cultures, individual cells are transferred through sterile depressions containing 0.5 ml antibiotic peptone, as described above. All strains of *T. pyriformis* multiply in this medium. *T. setosa* rarely does, although it will feed on any fungal contaminants which may be present. Washed cells of *T. setosa* grow vigorously when transferred to skim milk peptone (McCoy 1974 a). This species does poorly in proteose peptone. Cultures are maintained in skim milk peptone at 15° C with monthly transfers. Methods for protargol preparations and starch gel electrophoresis have been given in earlier papers (McCoy 1974 a, b, Borden et al. 1974 b, after Shaw and Prasad 1970).

Cortical Features

Twelve strains were studied cytologically (Table 1). Cells were scored for corticotype and CVP position. The CVP's occur slightly to the left of the nearest kinety, so that a CVP next to kinety *6* is considered to occupy a position of 5.75. Next the "central angles" were calculated, i.e., the dihedral angle between the mean CVP position and kinety *1* (Nanney 1966). This value is most conveniently calculated as $360 \times (\text{mean CVP position} - 1) / (\text{number of kineties on cell})$. All these values are listed in Table 2. The range of corticotypes and CVP positions is of the same magnitude as that shown by "*T. pyriformis*". However, the latter is a species complex whose individual component species show a much more restricted variation (Nanney 1967 a). The variability of *T. setosa* (central angles from 89° to 114°) therefore raised the possibility that we were dealing with another species complex. Several lines of evidence lead us to a different conclusion, however. First, antiserum against strain UI-7105 cross-reacts noticeably with other strains of *T. setosa*. For *T. pyriformis*, cross-reaction is only within the biological species, and never between species of this species complex, to the extent that this has been tested (unpublished studies, Nanney and Gross unpublished, Loefer et al. 1958). Further, pairing reactions have also been observed, and these are similar to mating reactions of other tetrahymenids under suboptimal conditions (see below). Finally, electrophoretic analysis as described below reveals substantial homogeneity among the *T. setosa* strains with one exception.

Our protargol slides show that the caudal cilium which is present in every strain of this species, although not in every cell, arises in the proter by modification of the last basal body in the first non-postoral meridian to the left of the mouth (Pl. I). This is the first time a morphogenetic event has been associated with that meridian in *Tetrahymena*.

The oral apparatus (Pl. II) is indistinguishable from that of *T. pyriformis* (McCoy 1974 a) and arises in the same way. The third membranelle again consists of only 17 kinetosomes, while the corresponding anlage is much larger.

Doublets occur frequently in some strains and are comparable with doublets of *T. pyriformis*. One or two caudal cilia may be present. Doublets are more common in axenic cultures and generally disappear in cerophyl.

Table 2
Mean CVP Positions of *T. setosa* Strains

Strain	7103, 7104, 7135 (combined data)	7105	7116	7125	7151i	7172	7174	7177	7208f	7211b
Sample size	178	63	37	117	50	67	50	64	63	74
Corticotype										
18	5.973(3)		5.416(9)							
19	6.750(2)		6.072(15)						6.250(1)	
20	6.250(2)	5.750(1)	6.125(12)				7.250(1)	7.250(2)	6.650(5)	6.250(1)
21	6.287(30)	6.350(5)	6.250(1)	7.753(20)		6.250(1)	6.750(1)	7.293(19)	7.150(5)	7.075(20)
22	6.511(43)	6.091(21)		8.014(34)	8.125(4)	6.450(5)	7.750(8)	7.610(25)	7.143(14)	7.250(33)
23	7.019(40)	6.187(24)		8.338(43)	8.250(8)	6.757(24)	8.094(14)	8.222(18)	7.750(19)	7.250(17)
24	7.145(37)	6.528(9)		8.471(18)	8.250(30)	7.201(31)	8.288(13)		8.000(18)	7.583(3)
25	7.379(9)	7.083(3)		8.500(1)	8.250(8)	7.250(5)	8.500(10)		8.250(1)	
26	7.458(10)						8.250(2)			
27	7.750(1)					8.250(1)	8.750(1)			
28	7.250(1)									
Mean central angle	92°	89°	93°	114°	109°	92°	109°	110°	104°	102°

Sample size for each corticotype is given in parentheses.

Electrophoretic Analysis

Zymograms were prepared from stationary phase cultures grown with aeration in skim milk peptone at temperatures between 15° and 25°C. In preliminary experiments various strains of *T. pyriformis*, syngen *1* were included on the same gels with several strains of *T. setosa*, but since the definitive interstrain comparisons of the ten surviving *T. setosa* strains (Table 3) involve several systems not examined earlier, as well as different lots of starch, different buffer concentrations,

Table 3

Classification of Zymographic Patterns for *T. setosa* Strains

Strain	7103	7104	7105	7116	7125	7151i	7172	7174	7208f	7211b
Enzyme										
IDH	L	L	L	L	L	H	L	L	L	L
E-1	1	1	4	4	3	5	3	2	3	4
E-2	3	2	2	1	2	4	2	2	1	2
P-1	1	3	3	2	5	5	3	1	2	4
P-2	B	A	C	A	B	A	A	B	A	A
LDH	A	A	A	A	A	A	A	A	A	A
NAD-MDH-1	A	A	A	A	A	A	A	A	A	A
NAD-MDH-2	H	H	H	H	H	L	H	H	H	H
NAD-MDH-3	M	L	L	L	L	H	L	L	L	M
LAP	4	1	2	4	2	5	3	4	2	2
TAT	H	H	H	L	H	L	L	H	H	H
GDH	A	A	A	A	A	A	A	A	A	A
TO	L	L	L	L	L	H	L	L	L	L
AP	L	*	M	L	H	*	H	H	*	*
α-GPDH	H	L	L	H	L	L′	H	L	L	H
Transaminase	H	L	H	H	H	H	H	H	L	L

Enzyme bands have been arbitrarily designated for convenience. * indicates no activity seen. For each enzyme activity, bands of the same mobility are represented by the same symbol. Abbreviations not listed in text: TO – tetrazolium oxidase; α-GPDH – α-glycerophosphate dehydrogenase.

etc., we have not attempted to calculate R_f values, which would be misleading in many cases. In several enzyme systems, there is no good reference band in syngen *1*, for NAD-dependent malate dehydrogenase (NAD-MDH), leucine amino peptidase (LAP), and alkaline phosphatase (AP) activities do not give discrete bands under conditions which focus the corresponding enzyme activities of other tetrahymenids.

A single mobility was found for the following enzymes: lactate dehydrogenase (LDH), cytoplasmic NAD-MDH, glutamate dehydrogenase (GDH). The other enzymes showed at least one variant, and three esterases (LAP), a butyryl esterase [E-1], and an acid phosphatase [P-1] had five variants each. The "E-3" activity which is of identical mobility in all tetrahymenids we have examined (see McCoy 1974 b) was constant for *T. setosa* also, and was therefore excluded from the calculation of similarity coefficients.

Several systems had unusual patterns. LAP activity has not been described before in *Tetrahymena.* A survey of selected "*T. pyriformis*" strains shows that it sometimes corresponds to one or more acid phosphatase bands, but in *T. setosa* it appears as a single sharp band not correlated with any of the phosphatase bands. The mitochondrial form of isocitrate dehydrogenase (IDH) was seen too infrequently to include in the analysis. Esterase and acid phosphatase activities could be subdivided into at least two systems which vary independently. The esterases split proprionate and butyrate esters, and each system consists of several bands. Phosphatases are similarly complex. Three NAD–MDH systems appeared instead of the usual two. The cytological source of the third band has not been determined. No NADP–MDH activity could be demonstrated.

When tyrosine amino transaminase (TAT) was inadvertently stained on a starch gel containing Allen's tris-borate buffer (Allen and Weremiuk 1971) instead of the usual tris-borate-EDTA mixture (Borden et al. 1974 b), the TAT bands were all of identical mobility, but a new system was seen. This transaminase is stained faintly under normal conditions. Its substrate specificity has not been determined.

The fraction of identical enzyme mobilities was calculated for each pair of strains (Table 4). Every strain except UI-7151i shares at least 2/3 of its enzyme mobilities with at least one other strain. However, no two strains are identical. Strain UI-7151i,

Table 4

Similarity Matrix of Zymographic Data for Strains of *T. setosa*

	7103	7104	7105	7116	7125	7151i	7172	7174	7208f	7211b
7103	—	.53	.50	.63	.56	.27	.50	.69	.44	.60
7104		—	.73	.53	.67	.27	.67	.67	.73	.67
7105			—	.56	.75	.27	.63	.63	.63	.67
7116				—	.50	.40	.69	.56	.67	.60
7125					—	.33	.69	.81	.73	.60
7151i						—	.40	.27	.27	.27
7172							—	.63	.60	.60
7174								—	.60	.53
7208f									—	.67
7211b										—

Numbers are the fraction of enzyme systems having identical mobilities for a given pair of strains, for all enzyme systems where data is available in both members of a pair.

from an alpine habitat, has a unique variant for 8 of 15 enzyme systems. But before concluding that this strain represents a new species, it will be necessary to obtain a better understanding of the breeding structure and clinal variation of *T. setosa.* Sampling of intermediate elevations might uncover strains which link UI-7151i with the other strains.

Conjugation

On several occasions pairing has been observed in mixtures of two or more strains of *T. setosa* (Table 5). Pairs isolated into separate depressions separated within several hours, however. Since the pairing is seen only in mixtures and only under very special circumstances, we believe it represents a typical ciliate mating

Table 5

Known Pairing Reactions of *T. setosa* Strains

	7103	7104	7105	7116	7125	7151i	7172	7174	7208f	7211b
7103	−	+	+	−	−	−	−	−	+	−
7104		−	+	?	+	−	?	+	+	−
7105			−	−	−	−	−	−	+	−
7116				−	−	−	−	−	−	−
7125					−	−	+	+	+	+
7151f						−	−	−	−	−
7172							−	−	−	+
7174								−	+	−
7208f									−	−
7211b										−

reaction observed under suboptimal conditions. Cells grown in cerophyl or skim milk peptone must be washed in distilled water or Dryl's solution (Dryl 1959) before pairing can occur. Pairs have formed only when cells were washed at the end of the day, which may indicate a photoperiodism of sexuality like that of some other ciliates (Sonneborn 1957). A large number of variables can influence mating behavior. Until these are tested, it may be noted that the formation of pairs only in mixtures lacks only the exchange of nuclei to constitute a system of mating types. If the pairing reaction can be controlled, the *T. setosa* strains might provide an excellent system for studies in ciliate genetics, since there is extensive cortical and enzymatic variation, a situation which has not been encountered before in *Tetrahymena*. In *T. pyriformis* syngen *1*, cortical variation is extremely limited (but see Nanney and Doerder 1972), while in the other syngens, in which a number of cortical variants have been noted (see Nanney 1967 b and 1971, also McCoy unpublished), enzymatic variants are rare or lacking (Borden et al. 1974 a).

Discussion

Speciation in the genus *Tetrahymena* was discussed recently by Corliss (1972), who unfortunately chose to leave unexpressed any notion of what a *Tetrahymena* species should be. Especially disturbing is his statement that "*T. setifera*" might be only a variety of "*T. pyriformis*". Yet we have found only one enzyme band which, by its identical mobility, clearly links *T. setosa* with any of the known species in the *T. pyriformis* complex. According to current theories of molecular evolution,

we must suppose that the divergence of *T. setosa* and the *T. pyriformis* complex greatly predates the divergence of the described breeding groups within "*T. pyriformis*", which are more closely related at the molecular level (Borden et al. 1974 a). One of the most interesting puzzles embedded in the present results is that *T. setosa* has remained apparently a monolithic species, while *T. pyriformis* has undergone an extreme diversification at the genetic level. Further, *T. setosa* seems to use extensive polymorphism in enzymatic and cortical traits as a means of adaptation, while the individual species of *T. pyriformis* vary only slightly by comparison.

T. pyriformis is the only documented species complex of the genus, although several authors have raised the possibility of cryptic speciation in *T. rostrata* and *T. limacis*. In particular, Roque et al. (1971) report a strain which they call *T. bergeri* which differs in some details from *T. rostrata*. With only a few exceptions (Brooks 1968, Michelson 1971), the distribution and geographical variation of *Tetrahymena* species have received little attention in recent years, and there has been a pronounced tendency to suppose that any natural variants not already catalogued will be unimportant anyway. The idea that any of the known variants "may be an incipient species evolving before our very eyes" (Corliss 1972) will unfortunately be completely sterile unless we have at hand the whole range of natural variation in which to make comparative observations. There is no substitute for the collection, maintenance, and analysis of wild strains, as it was practiced by Gruchy (1955). We must know the phenotypic, genetic, and geographical limits of species and populations in order to perceive the significance of breeding strategies, genetic adaptations, competition, and particular evolutionary changes.

The undeniable success of *T. setosa* cannot be easily explained. Its odd nutritional requirements (Holz et al. 1962) and even the caudal cilium itself only increase our puzzlement! At this point, it is not even clear whether *T. setosa* occupies a niche similar to that of any "*T. pyriformis*". The frequent simultaneous occurrence of both species in wild collections might only result from a locally abundant food supply, for instance, since the collector usually selects a location which seems to him auspicious for the occurrence of ciliates. It remains possible, then, that the two species play very different roles in the economy of nature. Whatever the reason, *T. setosa* is common throughout the United States, and a reasonable estimate of its diversity is now available, although the alpine and sub-alpine habitats still need to be investigated.

RÉSUMÉ

T. setosa nov. comb. résulte du déplacement de *Glaucoma setosa* Schewiakoff, 1893, et de la suppression de *T. setifera* Holz et Corliss, 1956. Souches de cette espèce ont été recueillies des habitats diverses aux États Unis et établies en milieu axénique. Quoique la polymorphisme des traits enzymatiques et corticales est très étendue, il manque d'évidence des espèces cryptiques. Le cil caudal provient au proter par modification de la dernière cinétosome de la cinétie immédiatement à la gauche de l'appareil oral.

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Received 16 October 1974

EXPLANATION OF PLATES I–II

1: Origin of the caudal cilium in a proter of strain UI-7105. Modified basal body bearing the nnascent caudal cilium appears as a triangular "arrowhead" just above the fission furrow. Protarggol, 5400 ×

2: Fine structure of the oral apparatus, from the inside, looking out. Strain UI-7177. Protarggol, 7500 ×

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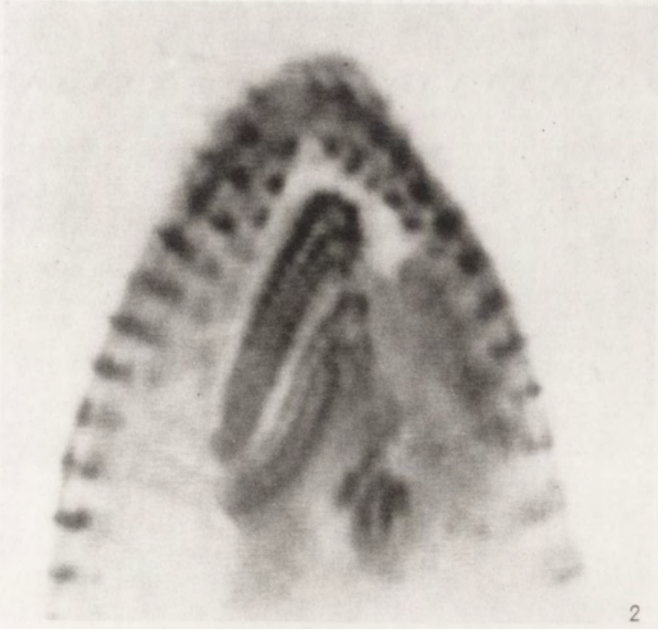

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ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4 WARSZAWA, 31. XII. 1975 pp. 263–280

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Iota-particles, Macronuclear Symbiotic Bacteria of Ciliate *Paramecium caudatum* Clone M-115

Synopsis. In ciliates *Paramecium caudatum* clone M-115 the symbiotic gram negative bacteria were found, denoted as iota-particles. The symbionts are localized exclusively in the macronucleus, being totally absent from the micronucleus and the cytoplasm. The presence of symbionts in the macronucleus evokes changes in the morphological structure of chromatin and a volume increase of the nucleus as compared to "clean" macronuclei. In the life cycle of the symbionts two morphological forms are interchanging: fusiform particles fast dividing by transversal narrowing, and rod-shaped particles. The iota-particles of paramecium fail to elicit any killer effect in cells of all examined *P. caudatum* clones free from these symbionts.

The capacity of iota-particles M-115 was investigated to infect cells of 41 *P. caudatum* clones, belonging to syngens *1* and *2*.

The investigated biological features of symbionts reveal broad potentialities in studying the mechanisms of nuclear differentiation of ciliates.

Various problems of the ciliate nuclear apparatus attract nowadays the attention of numerous authors. Particularly interesting and of wide comprehension are the investigations on mechanism of differentiation of both types of nuclei (micro- and macronucleus). The present authors (Ossipov and Ivakhnyuk 1972, Ossipov 1973, 1974, Ossipov et al. 1973 a, b) submitted an original method of studying these processes, based on the investigation of symbiotic organims occurring in the nuclei of ciliates. Such symbiotic bacteria and related microorganisms are usually referred to as particles and denoted by letters of greek alphabet. Some of these particles are characterized by rigorously specific localization in particular elements of nuclear apparatus of ciliates. For example the alpha-particles were found exclusively in the macronucleus of *Paramecium aurelia* (Preer 1969), the epsilon-particles in the perinuclear space of nuclear envelope of *P. multimicronucleatum* macro- and micro-nucleus (Jenkins 1970), and the omega-particles (Pl. I 7) in the micronucleus of *P. caudatum* (Ossipov and Ivakhnyuk 1972, Ossipov et al. 1973 a, b).

The presence of endosymbionts is connected with general or local morphological

changes of the nuclei. An example is to be quoted not directly related to endonucleosymbionts of ciliates. Stevenson (1970) found that kappa-like particles, localized in the cytoplasm of *P. aurelia* stock A37, enter in a peculiar interrelationship with the nuclear envelope of macronucleus. The somatic nucleus of infected *Paramecium* exhibits characteristic invaginations, so that the individual symbiont cells prove to be enclosed round by parts of the macronucleus. Epsilon-particles bring about a hypertrophy of perinuclear space of nuclear envelope of macro- and micronucleus. Omega-particles induce a dozens of times volume increase of the infected micronucleus and the accompanying disappearance of electron absorbing streaks, that correspond to condensed chromatin of normal generative nuclei. As the result of spontaneous loss of omega-particles by the cell, the nuclei recover essentially their characteristic features typical to ciliate micronuclei. The alterations induced by symbionts are then reversible (Borchsenius and Ossipov 1973, Ossipov et al. 1973 a). In ciliate *Neobursaridium gigas* (Nilsson 1969) the nucleoplasma of macronucleus in zones of agglomeration of symbiotic bacteria is deprived of chromatin granules (0.1 μm) typical for somatic nuclei. The mentioned examples prove that the nuclei of ciliate infected by endonucleosymbionts exhibit characteristic ultrastructural changes (despiralization and decondensation of chromatin). These changes can be interpreted as those of functional state of chromatin. Thus endonucleosymbionts of ciliates are able to induce a broad range of structural and ultrastructural changes of nuclei, acting as a sort of genetic inductors. On the other hand it is important to underline that the presence of symbionts in the nucleus has usually no effect on the dividing capacity of cells, although several papers of the beginning of the 20th century related forms of symbiotic microorganisms (often qualified as parasitic) causing pathological changes in ciliates, and even their death, especially in hyperinfection of cells (see reviews by Kirby 1941, Wichterman 1953, Ball 1969).

The analysis of interrelation of endonucleosymbionts and the nuclei may then appear as one of the effective methods of studying the structure and functional activity of nuclei. However, investigations devoted to symbiosis of bacteria in the nuclear apparatus of ciliates show so far an accidental and non-systematic character. It is reasonable to presume that the higher will be the number — within the same protozoan species — of investigated forms of symbionts, characterized by various localization and effects, the more effective will prove the "symbiotic" method. These reasons justify and stimulate further investigations on various forms of endonucleosymbionts.

The present paper aims at characterizing the symbiotic microorganisms found in the macronucleus of *P. caudatum* clone M-115 and at experimental investigating some of their biological properties. There is a high probability that earlier scientists met already a similar or identical type of symbionts, which even have been given a species name — *Holospora obtusa* (see Wichterman 1953). Analogically to the examined micronuclear symbionts of *P. caudatum* (Ossipov and Ivakhnyuk

1972), the determination of taxonomic position of symbionts was not the aim of the present paper. Hence the symbiotic macronuclear microorganisms of *P. caudatum* clone M-115 will be denoted by the Greek letter iota (iota-particles). A preliminary report on macronuclear symbionts of *P. caudatum* was presented at the IV International Congress of Protozoologists at Clermont-Ferrand (Ossipov et al. 1973 a)

Material and Methods

Symbionts were found in macronuclei in only one clone M-115 iota among over 500 clones of *Paramecium caudatum* isolated from 52 populations from water bodies of various regions of the Soviet Union (a collection of the Laboratory of Invertebrate Zoology, Petergof Biological Institute). This clone has been is lated in summer 1972 from a natural water body (Province of Leningrad, Staryi Petergof, the garden of the Biological Institute). In summer 1973 four *P. caudatum* clones with identical endosymbionts were, moreover, obtained from the same natural population.

The applied methods of cultivation, cloning of cells, determination of killer-effect and of infective activity of symbionts as well as all other methods were in accordance with those being in common use for paramecia (Sonneborn 1950, 1970, Preer 1969). Paramecia were cultivated in 12 ml tubes at 25°C on standard buffered (pH 6.9) lettuce medium, previously seeded with *Aerobacter aerogenes*.

The 41 iota-particle free clones of *P. caudatum* were infected using the non-purified homogenates of M-115 iota cells, after Preer (1969). In 12 ml tubes three drops of fresh homogenate were combined with three drops of dense culture of each examined clone of *Paramecium*. The experiments were carried out using two independently prepared homogenate samples of M-115 iota cells, all clones being infected twice. After 10–20 days since the addition of homogenate of *Paramecium* M-115 iota into "pure" cultures, a sample of some hundreds cells was taken from each examined clone to make slides, on which cytological observations were carried out. On each slide 50–100 cells were examined and the percent of infected ones was established.

Beside the above method of infecting, experiments with infecting individual cells were carried out with four clones (8 M-15, M-116, MI-48-56 omega and MI-48-67-20 omega$^-$). Twenty cells were taken of each clone and each cell was placed in a microaquarium with a drop of culture fluid, to which one drop of fresh homogenate was added. After 5–6 divisions from each initial cell a test-tube culture of cell line (subclone) was led out and past 13–18 days after the moment of addition of homogenate the subclones were examined for infection by iota-particles. This series of experiments was accompanied by a control one, where from each of the indicated four clones ten subclones were cultivated without addition of homogenate of M-115 iota cells.

To establish the degree of stability of occurrence of iota-particles in generation of cells of experimentally infected clones, in 22 days after addition of homogenate, cloning of paramecia was performed of one of repeated variants, ten cells being taken from each examined clone. After five days since the beginning of cloning the presence of iota-particles in cells was determined and test tube cultures of infected subclones were obtained. There was no considerable difference in frequency of divisions of the cells infected by iota-particles and "pure" ones of the same *Paramecium* clone.

The intracellular localization of iota-particles was examined using a light microscope in transmitted light, on slides stained after Feulgen or with acetocarmine and fast green, after Dippell (1955). The morphology of symbionts was surveyed by means of a phase contrast microscope on non-fixed pressed specimens of M-115 iota paramecia or on particles isolated from cells in Triton X-100 solution, diluted 1:2000 with distilled water and containing 100 mg spermidin per 1000 ml (Prescott et al. 1966). This solution is normally used to isolate the intact nuclei from

protozoans. The macronuclei infected with iota-particles liberated from the *Paramecium* cytoplasm either underg lysis after some time, or are destroyed by means of gentle pipetting.

The capacity of paramecia of the clone M-115 iota to induce the killer-effect was tested in two ways: samples of "pure" cultures of about 0.5 cm^3 were mixed with the same volume of intact live infected cells or with fresh homogenate of M-115 iota cells.

All clones investigated in the present experiments belong to two independent syngens, i.e., reproductively isolated intraspecific groups (Sonneborn 1957). Unfortunately we did not succeed so far to identify the clones of our two syngens with cultures, for which the generally accepted denotations of mating types and syngens were established (Sonneborn 1957, 1970). Therefore in the present paper, analogically as before (Ossipov and Skoblo 1968, 1973, Skoblo and Ossipov 1968) numbers of syngens and of mating types (m.t.) are introduced that differ from those generally accepted: syngen *1* with m.t. I and m.t. II; syngen 2 with m.t. III and m.t. IV. Further the peculiarities of *P. caudatum* cultures, utilized in the present paper for infecting with iota-particles, are discussed (Table 1, 2 and 3).

All five amicronuclear clones represent syngen *1* m.t. I (Ossipov and Skoblo 1973), two of them (8 M-15 and 8 M-23) being isolated from a natural population (Province of Leningrad, Staryi Petergof, the garden of the Biological Institute) in summer 1968, and three other caryonidic clones (MG-43a2, MG-32a3 and MG-19a1), obtained from exautogamonts of clone M-17 (Ossipov and Skoblo 1968). The indices of 22 micronuclear clones, their mating types and identity with a definite syngen are shown in Table 1.

Apart from the initial cultures with a "clean" nuclear apparatus, cultures were used infected with omega-particles, in order to obtain cells and cell lines with double infection (simultaneously omega + iota) and to examine the interrelationship between two kinds of intranuclear symbionts. These were five subclones of the clone MI-48 omega, isolated from a natural population in summer 1971 (Ossipov and Ivakhnyuk 1972) and three other clones (M-137 omega, M-142 omega and M-144 omega) from the same source (Table 2). In this part of experiments 6 subclones of MI-48 omega, obtained from cells that spontaneously and completely lost the omega-particles, were used as a control (Table 3). Clones containing symbionts in their nuclear apparatus are denoted by an index with added name of Greek letter of the particle type, e.g., MI-48 omega, M-115 iota. Clones that lost their symbionts are marked by "−", e.g., MI-48-66 omega−.

The comparison of the dimensions of macronuclei of "clean" and infected cells was carried out by measuring the surface of nuclei. In order to obtain uniform data (influenced by fixation time and cell dyeing and other factors in slide preparation and measurement), an approximately even number of live paramecia from a "clean" culture of each clone and from an infected one with iota-particles (with 100% extensiveness) were mixed and the mixture fixed with Bouin-liquid. The preparations were stained after Feulgen. The infected and the "clean" macronuclei were clearly distinguishable. The contours of projected "clean" and infected macronuclei were outlined on plotting paper using a drawing apparatus RA-4. The surface of nuclei was determined in relative units by weighing the projections (Chesin 1967). The normal distribution of the obtained variational series was checked using the χ^2 test, the Student test was used to prove the importance of the difference of means (Plochinskij 1970).

Results and Discussion

The macronuclear symbionts of clone M-115 iota and the omega-particles, micronuclear symbiotic bacteria of *Paramecium caudatum* clone MI-48 omega (Pl. I 7) as described earlier (Ossipov and Ivakhnyuk 1972, Ossipov 1973), have many morphological and biological features in common.

The nuclear apparatus of M-115 iota cells consists of a "clean" micronucleus and a macronucleus infected with iota-particles (Pl. I 2,5). In contrast with bright and homogenous staining after Feulgen of non-infected macronuclei of "clean" clones (Pl. I 1), the somatic nuclei of paramecia M-115 iota remain pale (Pl. I 2, 5), due to numerous clear zones corresponding to agglomerations of symbionts. The symbionts are not evenly distributed over the nuclear space, but locally concentrated, forming irregularly orientated crystal (Pl. I 5).

The iota particles of clone M-115 iota survive in paramecia at least for 17 months, that corresponds to 1000 cellular fissions. Despite of regular preparation of slides of the clone M-115 iota no cell was observed that would spontaneuosly lose the iota-particles from the macronucleus. These data indicate that the infected macronuclei maintain the reproductive capacity through divisions of paramecia M-115 iota. The unequal fission of macronucleus — a fairly common phenomenon in these highly polyploid nuclei (Cheissin et al. 1963, Raikov 1968) — was observed also in the infected nuclei. Nevertheless the observed high stability of maintenance of symbionts throughout several hundreds of cell generations proves that in the case of uneven division of infected macronuclei the transference of symbionts to daughter nuclei is likewise secured. This seems to be primarily an effect of a great number of particles, attaining several hundreds per nucleus.

Another feature distinguishing the nuclei containing symbionts from the "clean" ones concerns the zone of narrowing between the daughter macronuclei of dividing paramecia M-115 iota. In the "clean" paramecia the chromatin strand between separated daughter nuclei disappears soon, sometimes it changes into one or a few small chromatin bodies situated in the cytoplasm independently of the macronucleus; after some time they undergo resorption (Raikov 1968). In the infected paramecia a large agglomeration of iota-particles remains in the zone of narrowing between the daughter macronuclei (Pl. I 3, 4); however, the concentration of chromatin fragments is much lower here, than in newly separated daughter nuclei. The further fate of the residual body with iota-particles in separated paramecia drew so far no particular attention. One can yet presume that it does not differ from the fate of the residual body of the separated micronuclei of clone MI-48 omega infected with omega-particles (Ivakhnyuk 1973). In the latter case the residual body remains in the *Paramecium* cytoplasm no longer than 2.5 h (the generation time of *Paramecium* being 10–12 h) and at least some symbionts are liberated from the cell into cultural liquid.

The morphology and developmental stages of iota-particles resemble in many points those of micronuclear symbionts of *P. caudatum* MI-48 omega. In the life cycle of macronuclear symbionts an interchanging of two morphological forms takes place: intensely dividing by transverse narrowing fusiform particles measuring 2.0–2.5 μm (Pl. I 8, 9) and rod-shaped ones 15–18 μm long (Pl. I 12, 13). All possible transitory stages are observed between these two forms (Pl. I 10, 12). The rod-shaped iota-particles are formed from the fusiform ones by gradual lengthening of the body.

The further transformations of the rod-shaped iota-particles and their role in the symbiont life-cycle remain so far unexplained. Certain particles of the rod-shaped stage are much stronger light-refracting (Pl. I 13), sometimes the refracting zone being situated only at one particle end. The examined developmental stages of iota-particles are deprived of active motion. The character of reproduction of the symbiont, its dimensions and morphology as well as the properties of its life cycle enable to consider the iota-particles to be bacteria. The particles isolated from the nuclei proved gram-negative, analogically to other numerous symbiotic bacteria of paramecia (Beale et al. 1969).

Negative results were obtained in experiments that aimed at revealing the capacity of M-115 iota cells to elicit the killer-effect in paramecia of other clones. In none of the "clean" cells of all 41 clones used in the experiment pathological changes were observed on mixing with cells M-115 iota. Apparently the cells bearing the iota-particles fail to elicit the killer-effect in particle-free paramecia. On the other hand it does not seem possible to explain the observed negative results by a most unlikely genotype grounded resistance of cells of all 41 clones. All so far known killer-lines of *P. aurelia* (Sonneborn 1959) and some other ciliates, e.g., *Euplotes minuta* (Heckmann et al. 1967) are active towards numerous syngens and even other species.

The perspectives of using iota-particles for the "symbiotic" method of analysis of structure and function of *Paramecium* macronucleus are primarily limited by the extent to which these symbionts are able to infect "clean" nuclei and by particular experimental conditions securing reproducible positive results. In the present paper an attempt was made of experimental infecting the 41 "clean" paramecium clones with iota-particles from M-115 iota cells homogenate. Clones isolated from the same natural water body as the clone M-115 iota represented the major group within the "clean" cultures utilized in testing the infective power of iota-particles.

The positive results of infecting "clean" cultures of *P. caudatum* with iota-particles from homogenized cells M-115 iota (Table 1–4) prove, that symbionts are able to penetrate through the cultural liquid and infect "clean" macronuclei of other clones. So far it remains unknown in what way and what developmental stage of iota-particles reaches the "clean" cells, although regarding similar nuclear symbionts of *P. caudatum* it is presumed that symbionts enter the cell through food vacuole (Wichterman 1953).

The following consistent results were obtained from two independent experiments, where each clone was ultimately infected four times. The attempts to infect with iota-particles were successful with all five amicronuclear and 22 normal clones of *P. caudatum* of syngens *1* and *2* (Table 1). Nevertheless between these clones certain differences were observed, concerning the percent of infected cells (extensiveness of infection), since almost in all clones a definite part of cells did remain "clean". Such clones as M-84, M-114, M-125, in all repeated variants exhibited the lowest extensiveness of infection, whereas a considerable number of other clones (M-82, M-66,

Table 1

The Results of Experiments on Infecting in Test Tube Culture Conditions of 27 *Paramecium caudatum* Clones with Iota-particles from M-115 Cell Homogenate

Clone index, syngen and mating type, peculiarities of nuclear apparatus	Total number of cells examined in all repeated variants	Average per cent of cells infected with iota-particles	Number of obtained subclones with 100% infection extensiveness
Amicronuclear clones syngen *1* mating gype I			
8 M-15	200	95.5	1
8 M-23	300	92.7	—*
MG-43a2	171	99.4	—*
MG-32a3	492	94.1	3
MG-19a1	112	99.1	3
Clones with normal nuclear apparatus syngen *1* mating type I			
KG-11a2	526	96.4	3
M-31	413	73.8	3
M-71	379	92.3	3
M-84	325	16.5	—
M-104	397	50.8	3**
M-114	354	28.6	2
mating type II			
M-24	247	96.4	3
M-26	319	97.8	3
M-55	391	98.5	3
M-82	403	100	3
M-93	371	93.6	3
M-116	222	85.6	3
M-125	367	38.6	3
M-122	222	93.7	3
syngen *2* mating type III			
M-36	377	64.6	3
M-43	286	95.8	3
M-72	482	98.0	—*
M-78	374	97.4	3
mating type IV			
M-62	441	89.8	3
M-56	95	86.3	3
M-66	92	100	3
M-81	210 *ś*	99.5	3

Notes * — no vital subclone was successfully obtained.

** — one of three subclones (M-104-2 iota) contained a fraction of cells that liberated themselves from iota-particles.

M-81, MG-43a2, MG-19a1, M-55 etc.) were infected in 100 per cent. It is to be pointed out that in counting the infected paramecia the cell was assumed as infected, if even only one particle of rod-shaped stage was found in the macronucleus. Still it was practically impossible to find other developmental stages of iota-particles on total preparations obtained by the given methods, at such a low infection intensity. In this connection one should stress that the differences between clones with respect to their ability of being infected by iota-particles consisted as a rule not in the intensity of infection, but in its extensiveness, i.e., the per cent of infected cells in cell population. A great majority of infected cells contained in the macronucleus a large number of iota-particles. The question remains still unsolved, whether the observed differences between clones in the extensiveness of infection are due to genotype peculiarities of individual clones or by some other stable features of paramecia cultures and experimental conditions.

The results of experiments with infecting the subclones of the initial clone MI-48 omega should be discussed separately. These experiments were set in order to study the effect of presence of omega-particles in cells on their capacity to be infected by another type of endonucleosymbionts — the iota-particles (Table 2 and 3). In the control variant of this series of experiments all the six subclones, derived

Table 2

The Results of Experiments on Infecting in Test Tube Culture Conditions of *Paramecium caudatum* Containing Omega-particles in the Micronucleus, with Iota-particles from M-115 Cell Homogenate

Subclone index, syngen and mating type	Total number of cells examined in all repeated variants	Average per cent of cells with double infection: in the micronucleus — omega, in the macronucleus — iota	Number of obtained subclones with 100% infection extensiveness
Syngen *2* mating type III			
MI-48-81-1 omega	218	0	
MI-48-88 omega	182	0.5	
MI-48-56 omega	287	1.0	
MI-48-89 omega	569	2.8	4*
MI-48-60-1 omega	372	0.5	
		On the average for subclones MI-48.... — 1.0%	
M-137 omega	152	0	
M-142 omega	339	2.9	
M-144 omega	175	0	

Note * — All cells of these 4 subclones were free of omega-particles. Only subclone MI-48-89 omega underwent additional cloning.

Table 3

The Results of Experiments on Infection in Test Tube Culture Conditions of 6 MI-48 Omega *Paramecium caudatum* Subclones, Obtained from Cells that Spontaneously Lost Omega, with Iota-particles from M-115 Cell Homogenate

Subclones index, syngen and mating type	Total number of cells examined in all repeated variants	Average percent of cells infected with iota-particles	Number of obtained subclones with 100% infection extensiveness
Syngen *2*, mating type III			
MI-48-66-5 omega	344	36.6	
MI-48-67-4 omega	90	26.7	2
MI-48-70-1 omega	261	31.0	3
MI-48-71 omega	349	13.2	
MI-48-79 omega	90	7.8	
MI-48-79-2 omega	201	42.8	2
		On the average 26.4%	

Note: The indices of subclones, which were additionally cloned after the experiment, are underlined.

from cells that spontaneously lost the omega-particles from the micronucleus, were capable of infection by iota-particles (Table 3), although, as with the mentioned 27 "clean" clones, with various extensiveness (from 7.8 to 42.8%). In this case the extensiveness of infection is actually lower than in most clones listed in Table 1. Yet these results are not sufficiently convicing, that there does occur any remote consequence of residence of omega-particles in the micronucleus which would cause a lower infection extensiveness in subclones omega. At the same time among the mentioned 27 clones (Table 1) that did not bear any symbionts in their nuclear apparatus, clones are found with a similarly low per cent of cells infected with iota-particles (clones M-84, M-114, M-125).

Entirely different results were obtained with five subclones of MI-48 omega and three other clones containing omega-particles in the micronucleus. Some of them (MI-48-81-1 omega, M-137 omega and M-144 omega) did not display any capability for sustaining iota-particles at all, whereas in five other clones the extensiveness of infection was as low as 0.5–2.9%. The cell nuclear apparatus with double infection in macronucleus — iota, and in macronucleus — omega, comprises the features of structural changes of the nuclei characteristic for both symbiont types (Pl. I 6) All subclones of MI-48 omega should be considered as genotypically identical, since they derive from vegetative cells and the MI-48 omega paramecia are deprived of the power of entering the sexual process due to the presence of omega-particles in the micronuclei (Ossipov et al. 1973 a, Rautian 1973). On this basis one can summarize the data obtained for subclones and compare mean values of infection extensiveness. The comparison of these values for omega–subclones (Table 3 — 26.4%) and omega bearing subclones (Table 2 — 1%) supplies evidence that experimental combination of two particle types: iota and omega, in a cell of clone MI-48

genotype leads to antagonistic (or competitive) interrelationship of micro- and macronuclear symbionts, expressing itself in these experiments by an exceptionally low per cent of cells found with double infection, the latter being entirely absent in three subclones.

It may be interesting to mention here, that rare cases are known of simultaneous cell infection in infusoria with different forms of endosymbionts. Preer (1969) was first to observe this phenomenon in paramecia cultures isolated from natural populations, namely in *Paramecium aurelia* stock 562. In the macronucleus of this line alpha-particles were present, and in the cytoplasm the kappa-particles. In this paper the unpublished experiments of Schneller are quoted, where mixed populations of endosymbionts were obtained by artificially induced cytoplasmic exchange between conjugants containing each a single symbiont type in their cytoplasm: either kappa and sigma or kappa and lambda. After Preer (1969) the coexistence in the same cell of two types of symbionts may be possible when one of them — alpha — prefers the macronucleus as its ecological niche, whereas the second — kappa — the cytoplasm, and consequently in the reproducing itself symbiotic system none of the vital biochemical processes is disturbed.

After 22 days since the addition of homogenate ten individual cells were transplanted from each clone used in the experiment on infecting with iota-particles. This was done to obtain subclones where each cell would bear symbionts in its macronucleus (extensiveness 100%). Five days after the beginning of cloning on depression slides, the presence of iota-particles in cell lines was established by staining after Dippell (1955) and the infected subclones were led out into test tube cultures. A positive result of our experiment required no more than 2–3 subclones with 100% extensiveness, deriving from clones exposed to infection with iota-particles, although the number of infected subclones was considerably higher for most clones. The cloning was successful with the exception of two amicronuclear clones (8 M-23 and MG-43a2) and one normal (M-72), where no vital subclone was obtained, although these clones keep easily in test tube cultures. This is usually an effect of progressive senescence of cultures (Sonneborn 1957, 1960). As a rule the cells of such clones gradually lose completely the capacity to multiply also in test tube cultures and finally die. Subclones of other 24 clones (Table 1, right column) were successfully obtained, where in 100% cells the macronuclei contained a high number of iota-particles. In this way the present results prove, that iota-particles are maintained not only in cells of clone M-115 iota, but also in cells of other genotypes and even other syngens. The mating type and syngen of the clone M-115 iota was not determined yet, since, according to our data (Ossipov et al. 1973, Rautian 1973), the presence of symbionts (iota and omega as well) in paramecia is followed by complete loss of mating capacity. The clone M-84 constituted an exception, all ten derived subclones being "clean". This was probably connected with a very low extensiveness of infection with iota-particles of this particular clone (16.5%) and the selection of cells proved insufficient for the imposed purpose.

Finally cloning was performed (after addition of iota-particles to the cultures) of some subclones of the initial clone MI-48 omega, both containing or having spontaneously lost the omega-particles (Table 2 and 3). Among 20 subclones obtained from the MI-48-89 omega culture, exhibiting a highest cell per cent with experimental double infection (Table 2), only four contained iota-particles in the macronucleus, still no subclone cells were found with double infection of nuclear apparatus (omega + iota). At present it is not yet possible to attribute the priority to one of two likely explanations: either iota-particles infect only cells having spontaneously lost their omega-particles (according to our data the per cent of such cells in subclones MI-48 omega was 1–10), or in cells in which the double infection lasted for a short time (such cells are observed on slides), the state of double infection proves unsteady. It is also possible that both processes occur simultaneously. The data indicating the occurrence of antagonistic interrelationship between iota- and omega-particles were already discussed (Table 2 and 3, third column from the left). This view would be corroborated by the absence of cultures with double infection of nuclear apparatus among all subclones of MI-48-89 omega, obtained upon treating of paramecia with iota-particles.

The results of experiments on infecting with iota-particles single cells of clones 8 M-15, M-116, MI-48-56 omega and MI-48-67-2 omega$^-$ (Table 4), will be discussed in turn. The effectiveness of cloning of these four cultures in a control series (single cells, no homogenate M-115 iota added) was variable — from ten isolated cells of each clone the following numbers of vital subclones (in brackets) were obtained: 8 M-15 (9), M-116 (10), MI-48-56 omega (7), and MI-48-67-2 omega$^-$ (5). In an experimental series the effectiveness of cloning was somewhat lower with three clones, whereas no vital subclone was received from MI-48-67 omega$^-$. Since in a variant of experimental infecting in test tube cultures of MI-48-67 omega$^-$ the vital subclones were obtained infected with iota-particles, it should be admitted that

Table 4

Results of Experiments on Infecting Single Cells of 4 *Paramecium caudatum* Clones with Iota-particles from M-115 Cell Homogenate

Clone index	Number of cells utilized for further cloning and infecting	Number of the obtained vital subclones	Number of subclones infected with iota-particles	Average percent of cells with iota-particles, in all subclones
8 M-15	20	12	12	63.9 (N = 864)
M-116	20	15	15	72.3 (N = 1881)
M-46-67-2 omega	20	0		
MI-48-56 omega	20	9	1*	0 (N = 241)

Note: * — In subclone (MI-48-56-7 omega) about 1% of cell infected with iota-particles was found, but not all of them contained omega-particles.

negative results of attempts to infect single cells do not follow from some specific action of iota-particles on cells of this culture. Further it is noteworthy that in subclones received from 8 M-15 and M-116 (Table 4) the per cent of cells infected with iota-particles is lower as compared to test tube cultures (Table 1). This is undoubtedly a result of different physiological situation of paramecia and a higher rate of cell proliferation in conditions of individual culture (Sonneborn 1970).

It has been already mentioned that the nuclei of infusoria infected with endonucleosymbionts exhibit certain characteristic ultrastructural changes. No such data are available as yet concerning the macronucleus infected with iota-particles. However, comparing the dimensions of nuclei of "clean" and infected cultures of some examined clones, it has been observed that the volume of the infected macronucleus clearly tends to increase. In Table 5 there are summarized the results of karyometric

Table 5

Surface of Macronuclei of 5 *Paramecium caudatum* Clones "Clean", and Infected with Iota-particles M-115

Clone index	$\bar{X}\pm S_{\bar{X}}$ (arbitrary units	N	t_d	t_{st} p 0.001	$\frac{\bar{X}\text{ infected}}{\bar{X}\text{ "clean"}} \times 100\%$
M-55	36.6±1.41	22	7.6	3.4	170
M-55-3 iota	62.1±3.02	55			
M-62	32.0±0.98	51	6.9	3.4	157
M-62-2 iota	50.3±2.37	49			
M-56	33.0±0.89	54	16.2	3.4	199
M-56-2 iota	65.5±1.80	50			
M-125	34.7±0.78	52	5.6	3.4	168
M-12-5-1 iota	58.2±2.89	52			
M-24	48.2±2.11	27	4.2	3.5	250
M-24-2 iota	120.7±7.46	28			

measurements of five *P. caudatum* clones before and after their infection with iota-particles, the increase of dimensions of infected macronuclei is apparent in all five examined clones. This increase in comparison to "clean" nuclei attains considerable values, e.g., 250% for clone M-24. These results cannot substantiate, however, an answer, whether the volume increase of the infected macronucleus is a simple result of summing up the volume of "clean" nucleus and the mass of symbionts, or whether it indicates a change of nuclear chromatin (its state or quantity), induced by endonucleosymbionts.

To summarize shortly the main results of the present experiments, it is to be noticed that the iota-particles show a rigorously specific localization in the infusorian nuclear apparatus, i.e., exclusively in the macronucleus. In simple experimental

conditions the macronuclei of "clean" clones are readily infected with high effectiveness. The iota-particles can be stably maintained in the nuclear apparatus of ciliates for more than 1000 agamic generations, without inducing any appreciable pathological alterations in cells. All these biological peculiarities of iota particles along with the features of omega-particles as described earlier (Ossipov and Ivakhnyuk 1972, Ossipov et al. 1973 a, b, Ossipov 1973, 1974), when parallelly utilized in experimental conditions, reveal broad capabilities for investigating the mechanisms of nuclear differentiation of ciliates and particularly the structure and function of macronucleus.

In the course of the last five years the opinions of karyologists about the structural organization of macronucleus underwent a considerable evolution (Raikov 1967, 1968, Nilsson 1970, Woodard et al. 1972, Prescott et al. 1973).

If all investigators share at present the polyploid theory of macronucleus in ciliates (see Raikov 1967, 1968), then no uniform opinion exists so far as regards the pattern of structural organization of chromosomal units in the actual macronucleus. Till the recent times most investigators upheld a so called "subnuclear" hypothesis of macronuclear organization in ciliates (Sonneborn 1947, Raikov 1967, 1968, Nilsson 1970). According to this hypothesis the macro- and micronucleus of a cell both bear an identical genetical information. In a polygenome macronucleus the chromosomes of individual genomes would be structurally isolated into diploid subnuclei. The subnuclei would be randomly distributed between daughter macronuclei in the course of division. According to another hypothesis "of collective chromosomes" (see Raikov 1967, 1968) the chromosomes within each genome are interconnected end to end over whole mitotic cycle and thus form a collective chromosome. After Raikov (1967, 1968) there are no fundamental differences between the theory of collective chromosomes and the one of subnuclei, if the diploid collective chromosomes would be considered as a morphological formulation of subnuclei. Finally some convincing observations were gathered recently, that in various ciliate species the organization of genetical material of the actual macronucleus may differ considerably. In some species the macronuclei do not contain chromosomes brought together into blocks (subnuclei or complex chromosomes), but contrariwise fragments of chromosomes. The most utter form of fragmentation of genetical material in the actual macronucleus was recently detected and examined in some infusoria of the order *Hypotricha* and there are no analogical examples so far among other eukaryotic nuclei, capable of division (Ammermann 1971, Prescott et al. 1973). Thus in *Stylonychia mytilus* it appears that in the macronucleus the structurally split functional subunits, analogical to transcriptones and seemingly to replicones, are identical with small fragments of DNA, that correspond to one or several genes of mean "size" as regards the number of nucleotide pairs (Prescott et al. 1973). Prescott et al. suggested the term "sack with separate genes" to denote the pattern of organization of the actual macronucleus. After a recent hypothesis, the macronuclear DNA does not contain the whole genetical information in compar-

ison to the genome of micronucleus (Allen and Gibson 1972, Prescott et al. 1973).

A further progress in unravelling the structural organization of macronucleus in various ciliate species is trammelled to a high extent by the situation, that in these investigations the specific genetical methods cannot be applied, as being based on hybridological analysis of macronuclear genome, since the macronucleus is a somatical nucleus and it is completely resorbed in the course of sexual process. It follows therefore for investigators the necessity of searching for new approach in solving the problem of structural macronuclear organization, one of them being possibly the "symbiotic" method. The endonucleosymbionts of macronucleus were found in nuclei of most various ciliate groups (Ball 1969), though experimental research was carried out so far on a rather limited number of forms. One may then hope, that the symbiotic method would prove applicable not only to investigations on *Paramecium*, but also on other ciliate groups with a different macronuclear structural organization of genetical material and the nucleosymbionts themselves would serve as precise tools in the hands of tenacious experimentators.

Summary

In ciliates *Paramecium caudatum* clone M-115 the symbiotic gramnegative bacteria were found, denoted as iota-particles. The symbionts are localized exclusively in the macronucleus, being totally absent from the micronucleus and the cytoplasm. The iota-particles persist steadily over more than a thousand of cellular division of the clone M-115 iota, inducing no perceptible pathological changes in paramecia. The presence of symbionts in the macronucleus evokes changes in the morphological structure of chromatin and a volume increase of the nucleus as compared to "clean" macronuclei. In the life cycle of the symbionts two morphological forms are interchanging: fusiform particles fast dividing by transversal narrowing, 2.0–2.5 μm in size, and rod-shaped particles of the size up to 18 μm. The rod-shaped particles originate from the fusiform ones, however, their role in the symbiont life cycle remains as yet not elucidated. The iota-particles of paramecium fail to elicit any killer-effect in cells of all examined *P. caudatum* clones free from these symbionts.

The capacity of iota-particles M-115 was investigated to infect cells of 41 *P. caudatum* clones, belonging to syngens *1* and *2*. These were 5 amicronuclear clones, 22 clones with normal nuclear apparatus, 3 clones containing omega-particles, isolated out of a natural population, and 11 subclones derived from the culture MI-48 omega (Ossipov and Ivakhnyuk 1972). The latter consisted of 5 subclones containing in the micronucleus the symbiotic bacteria — omega-particles, and 6 other obtained from cells that spontaneously lost omega-particles. The results of experiments proved a high effectiveness of infecting with iota-particles. All clones, with the exception of five bearing omega-particles in the micronucleus, undergo

infection with iota-particles and steadily keep them in the macronucleus, that is the iota-particles are maintained in cell lines other than those deriving from M-115. On the other hand clones already containing symbionts — the omega-particles — in the micronucleus reveal a considerably lower capacity to be infected with iota-particles. In this case as little as 1% of cells were found to bear simultaneously both symbiont types. An antagonistic interrelationship was stated between different types of symbionts.

The investigated biological features of symbionts reveal broad potentialities in studying the mechanisms of nuclear differentiation of ciliates.

РЕЗЮМЕ

В инфузориях *Paramecium caudatum* клона М-115 обнаружены симбиотические грамотрицательные бактерии, обозначенные нами как йота-частицы. Симбионты локализуются только в макронуклеусе, тогда как в микронуклеусе и цитоплазме они не обнаруживаются. Йота-частицы стабильно поддерживаются вот уже более 1000 клеточных поколений клона М-115 йота, не приводя к заметным патологическим изменениям парамеций. Присутствие симбионтов в макронуклеусе вызывает изменения морфологической структуры хроматина и увеличение объема ядра, по сравнению с "чистыми" макронуклеусами. В жизненном цикле симбионтов имеет место смена двух морфологических форм: быстро делящихся поперечными перетяжками веретеновидных частиц размером 2.0–2.5 μм и палочковидных частиц размером до 18 μм. Веретеновидные дают начало палочковидным частицам, роль последних в жизненном цикле симбионта пока остается невыясненной. Йота-частицы парамеций М-115 не вызывают никакого killer-эффекта у клеток всех исследованных клонов *P. caudatum*, лишенных этих симбионтов.

Изучена способность йота-частиц М-115 инфицировать клетки других 41 клонов *P. caudatum*, относящихся к двум независимым сингенам *1* и *2*. Среди них: 5 амикронуклеарных, 22 с нормальным ядерным аппаратом и 11 субклонов от исходной культуры МІ-48 омега (Ossipov and Ivakhnyuk 1972), из которых 5 содержат в микронуклеусе симбиотических бактерий — омега-частицы — а 6 других получены от клеток, спонтанно утративших омега, 3 омегасодержащих клона, выделенных из природной популяции. Результаты проведенных опытов выявили высокую эффективность заражения йота-частицами. Все клоны, кроме 5, содержащих омега-частицы в микронуклеусе, заражаются йота-частицами и стабильно поддерживают их в макронуклеусах, т.е. йота-частицы могут поддерживаться в клеточных линиях иного происхождения, чем М-115. Напротив, клоны уже содержащие в микронуклеусе симбионтов — омега-частицы — имеют заметно более сниженную способность инфицироваться йота-частицами. В этом случае обнаружено только около 1% клеток, поддерживающих одновременно оба типа симбионтов. Между разными видами симбионтов обнаружены антагонистические взаимоотношения.

Изученные биологические особенности симбионтов открывают широкие возможности для изучения механизмов ядерной дифференцировки инфузорий.

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Received 10 October 1974

EXPLANATION OF PLATE I

Light microphotographs of *Paramecium caudatum* cells, infected with intranuclear symbionts, and the iota-particles at various developmental stages, isolated from the macronucleus. Denotations ma — macronucleus, mi — micronucleus

1–7: *P. caudatum* cells of "clean" and infected with endonucleosymbionts clones. Feulgen reaction

1: "clean" — uninfected cell, normal macro- and micronucleus. 400 ×

2: cell with macronucleus infected with iota-particles, a normal micronucleus is situated above the infected nucleus. 400 ×

3: dividing cell with the macronucleus infected with iota-particles. "Residual body" indicated by an arrow. In the paramecium cytoplasm normally divided daughter micronuclei are visible. 400 ×

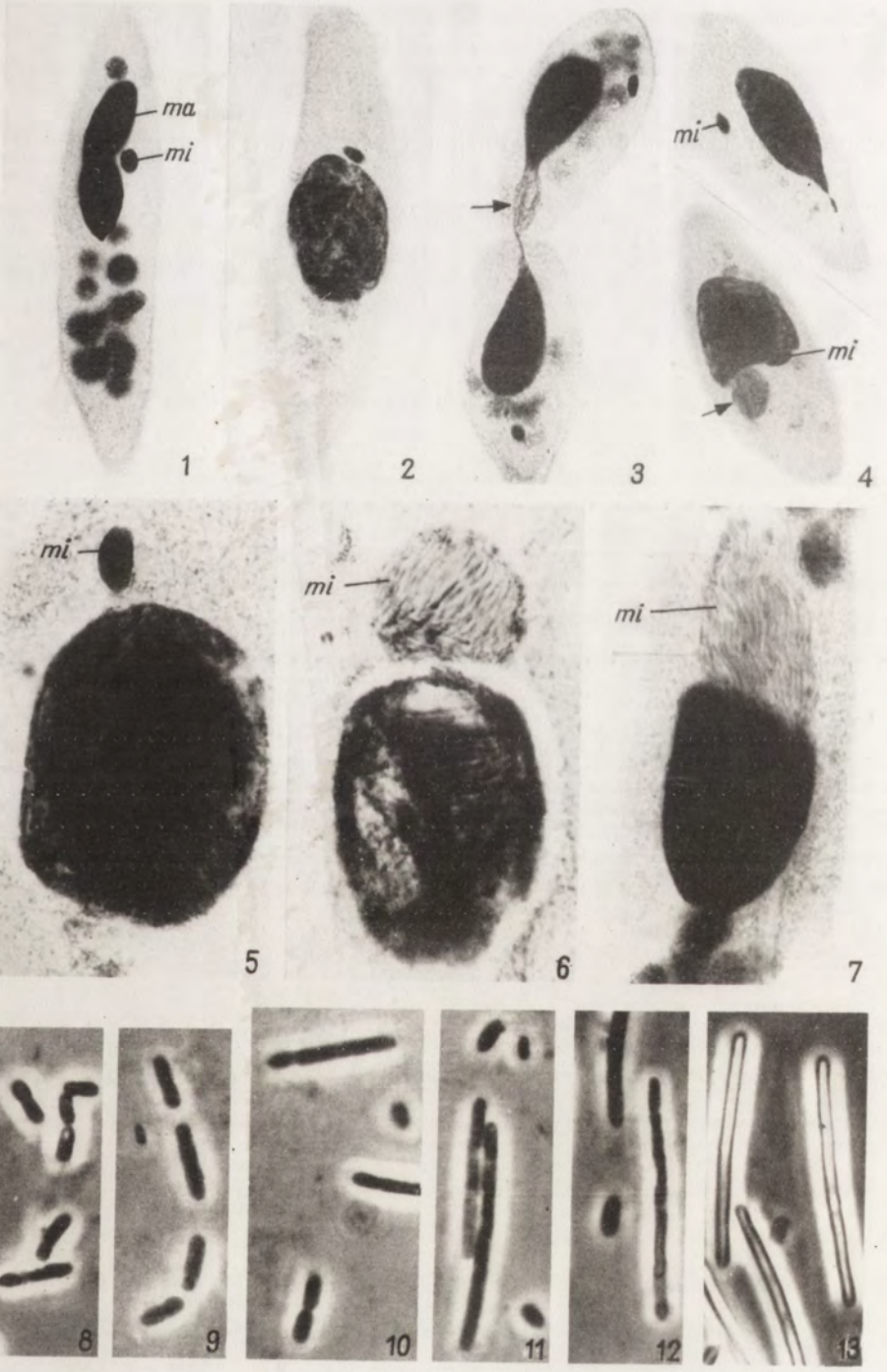
4: recently divided two sister cells with macronuclei infected with iota-particles. The "residual body" (indicated by an arrow) is found only in one of two daughter individuals. 400 ×

5: the nuclear apparatus of a cell infected with iota-particles. In the macronucleus numerous iota-particles and faintly stained chromatin zones are visible. 1000 ×

6: nuclear apparatus of a cell with double infection: in the macronucleus the iota-particles, in micronucleus — omega. 1000 ×

7: nuclear apparatus of a cell infected with omega-particles. A normal macronucleus, the micronucleus filled with a great number of tightly packed omega-particles and considerably enlarged. 000 ×

–13: iota-particles isolated from the macronucleus of clone M-115 iota cells at various stages of development. Phase contrast. 2500 ×



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ACTA PROTOZOOLOGICA
VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 281–290

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Differentiation of the Macronuclear Anlagen of *Paramecium aurelia* (Syng. *1*) in the Presence of Calcium Chloride: Analysis of the Variability of the Disturbances

Synopsis. This paper is devoted to the analysis of a $CaCl_2$ induced delay in the differentiation of macronuclear anlage, a consequence of which is the appearance of selfing-caryonides (up to 25 per cent). This phenomenon occurs during cytogamy as well as during true conjugation. The sister-caryonides show some tendency to be either both selfers or both non-selfers; this correlation is particularly strong when the conjugants possess exactly the same strictly homozygous genotype. In addition, an increase of the frequency of mating-type I caryonides is observed.

Introduction

In *Paramecium aurelia*, syngen *1*, the mating type is determined by the macronucleus. This macronucleus acquires its specificity at the end of a sexual phenomenon (conjugation or autogamy) when it differentiates from a micronucleus. The asexual progeny of the daughter-cell of an exconjugant or an autogamous individual is called a caryonide. The macronuclei of all Paramecia belonging to the same caryonide are the products, by a series of amitotic divisions, of a single macronucleus: all these Paramecia possess, with very unusual exceptions in normal conditions, the same mating type (Sonneborn 1937).

However, if conjugants are put into $CaCl_2$[1] at a non-lethal concentration during the phase of reconstitution of the nuclear apparatus, the following phenomena are observed (Hallet 1972):

— from a cytological point of view, the postzygotic nuclear reorganization is qualitatively the same that in untreated individuals;

— about 20% of the caryonides derived from treated individuals are selfers;

[1] The adaptation and loss of adaptation of populations of *Paramecium aurelia* to this salt were studied by Génermont (1966–1969).

these caryonides are composed of stable subcaryonides: the stabilization occurs approximatively at the 5th fission following conjugation.

We concluded from these two observations that $CaCl_2$ induces a delay in macronuclear differentiation.

— in addition, the two sister-caryonides derived from one exconjugant, proved to exhibit the same character (either two selfers or two non-selfers) in most cases.

These results call a more detailed analysis upon three points[2]:

(1) In the presence of $CaCl_2$, some pairs dissociate prematurely. As in the culture conditions it is observed that the following macronuclear reorganization is normal for quite all the individuals, we conclude that macronuclear regeneration and complete lack of nuclear reorganization are very unusual; it is likely that for these pairs, cytogamy[3] occurs instead of conjugation. Sonneborn (1941) suggested an action of the calcium on the frequency of pairs which failed to achieve cross fertilization. Using marker genes it is possible to check if the effects of $CaCl_2$ are identical on caryonides obtained after cross-fertilization or after self-fertilization.

(2) The frequency of selfing-caryonides differs considerably from one mating to another. When it is very low, the effect of $CaCl_2$ results in a deviation of the relative frequencies of both mating types with regard to their usual values. It is thus interesting to approach, more generally, the question of the effect of $CaCl_2$ upon the frequencies of mating types I and II.

(3) From the point of view of the alternative selfer vs. non-selfer a strong positive correlation appears between caryonides derived from the same exconjugant. It is thus necessary to find out its determining factors through experimentation.

Material and Methods

Culture Conditions

The culture medium is an extract of dried lettuce bacterized with *Klebsiella aerogenes* (Sonneborn 1970). The experiments are performed at 27° C.

Exposure to $CaCl_2$

Two sexually reactive populations, of opposite mating type are brought together. Four hours later, conjugating pairs are isolated (only those which do not separate after three or four sudden sucking and rejecting by means of the micropipette are retained) they are put into a medium enriched in $CaCl_2$ (concentration 0.01 M). The exconjugants and later their daughter-cells, are isolated. The Paramecia are maintained in the presence of $CaCl_2$ until all the exconjugants have divided at least once. They are then transferred to normal culture medium where the caryonides begin exponential growth.

[2] Some results developed in the points 1 and 3 were reported in a paper read in the Fourth International Congress on Protozoology (Hallet 1973).

[3] Self-fertilization process described by Diller (1936) and Wichterman (1939).

Strains Used

We used the stock 60, upon which our previous experiments had already reported (Hallet 1972). We also used the strain 60 θ_1[4] homozygous for a recessive θ_1 gene determining thermosensitivity: it is an F_2 line originally derived from a cross between stock 60 and a thermosensitive mutant obtained from stock 168. The stock P, which belongs to mating type I, allowed us to identify the mating type.

Detection of Cytogamy

It is possible to detect the pairs which undergo cytogamy, and not conjugation, during matings between the strains 60 and 60 θ_1. In the case of conjugation, each of the partners breeds a thermoresistant clone, possessing the recessive θ_1 gene in the heterozygous state; in the case of cytogamy, on the contrary, among the two partners of a pair, one begets a thermosensitive clone while the other a thermoresistant one, respectively homozygous for θ_1 and for its wild allele.

We test the thermoresistance of each caryonide in the following manner: two individuals are set apart when the caryonide has undergone at least five cellular divisions; they are cultivated at 37° C for 48 h. The only cultures which develop are those derived from thermoresistant caryonides. At the same time some cultures from the two strains 60 and 60 θ_1 are set up as controls.

As may be expected, two kinds of pairs are found:

(a) — those which underwent conjugation give rise to four thermoresistant caryonides;

(b) — those which underwent cytogamy give rise to two thermoresistant caryonides (derived from one partner) and two thermosensitive caryonides (derived from the other partner).

Remarks upon the Genotype of the Population Taking Part in the Crosses

The strains used in this experiment, kept for a long time in the laboratory are much inbred; therefore nearly all loci are homozygous.

In some crosses, we used populations derived from Paramecia taken from the choosen strain. In these crosses between clones, very few genes of the parents are heterozygous; if clones of the same strain are crossed, the descendants of the pairs which really underwent conjugation most probably have some homozygous loci.

Other crosses were carried out between pure lines. Each of the population concerned comes, by way of a vegetative multiplication, from an individual which underwent autogamy, being then homozygous for all its genes. When such crosses occur the parents are homozygous for all their genes, except for mutations. In the case of crosses between different pure lines, some loci of the descendants are certainly heterozygous.

Finally, it is possible to carry out matings between caryonides of the same pure line. These caryonides are isolated after induction of autogamy in a pure line. Such crosses lead, except for mutations, to a perfectly homozygous state.

Induction of Selfing during Conjugation and Cytogamy

Two matings were carried out between the strain 60 and the strain 60 θ_1. The first one is a mating between clones, the second one is a mating between pure lines.

During the first mating, among the 35 pairs studied, 24 have undergone conjugation and 11 cytogamy. In the second mating, 26 have undergone conjugation and only one cytogamy.

[4] We thank Dr Beisson who provided us with this strain obtained in her laboratory (Centre de Génétique Moléculaire, Gif-sur-Yvette).

Table 1

Induction of Selfing during Conjugation and Cytogamy

$60 \times 60\ \Theta_1$	Conjugation		Cytogamy		Total
	non-selfing caryonides	selfing caryonides	non-selfing caryonides	selfing caryonides	
1st cross (between clones)	79	8	29	8	124
2nd cross (between pure lines)	95	8	2	2	107
Total	174	16	31	10	231

The mating type was determined for all the caryonides which reached sexual reactivity. The results bearing upon the number of selfing-caryonides obtained at the time of each mating after conjugation and cytogamy, are presented in Table 1. It is considered that these matings are homogenous from one mating to another; on the whole, the frequency of selfing-caryonides is 0.084 after conjugation, and 0.244 after cytogamy.

Thus, the induction of selfing by action of $CaCl_2$ upon the differentiation of the macronuclear anlagen occurs under two circumstances: true conjugation or cytogamy. The efficiency of this induction seems to be higher in the case of cytogamy ($\chi^2 = 8.61$ for one degree of freedom).

Distribution of the Mating Types of the Caryonides in the Different Crosses

The following analysis is based upon the results of all the crosses carried out until now. The crossed populations belong to the strains 60 and $60\,\theta_1$; they are either clones, or caryonides belonging to one pure line.

(1) Relative Frequencies of Type I and II among Non-Selfing-Caryonides

If only non-selfing-caryonides are considered, the relative frequencies of both mating types do not vary significantly from one experiment to another ($\chi^2 = 17.43$ for 13 degrees of freedom). Among all the 984 studied caryonides, 453 belong to type I and 531 belong to type II, giving thus a frequency of 0.540 for type II.

We compared these frequencies with those obtained in two control experiments, i.e., crosses within stock 60, without action of $CaCl_2$. In the first one, 26 caryonides exhibited the mating type I and 68 the type II, while in the second, the corresponding numbers were 33 and 81. Pooling these results which do not differ significantly ($\chi^2 = 0.041$ for one degree of freedom), we estimate the type II frequency as 0.716, a value which agrees with the formula established by Sonneborn (1939)[5]; this

[5] According to this formula, the probability of a type II caryonide dependent on the temperature t is: $(18.4+1.96\,t)\ 10^{-2}$; which gives at 27°C, 0.713.

Table 2

Distribution of the Different Kinds of Caryonides

	Cross	Type I caryonides	Type II caryonides	Selfing caryonides	Total	Confidence interval
Between clones	60×60	33 f = 0.54	28 f = 0.46	0 f = 0.00	61	0–0.06
	60 θ×60 θ_1	21 f = 0.323	34 f = 0.523	10 f = 0.154	65	0.066–0.24
	60×60 θ_1	22 f = 0.297	45 f = 0.608	7 f = 0.095	74	0.028–0.162
	60×60 θ_1	54 f = 0.439	53 f = 0.431	16 f = 0.130	123	0.071–0.189
Between pure lines	60×60	49 f = 0.52	42 f = 0.45	3 f = 0.03	94	0.007–0.09
	60 θ_1×60θ_1	37 f = 0.398	50 f = 0.538	6 f = 0.0645	93	0.015–0.114
	60×60 θ_1	45 f = 0.42	52 f = 0.49	10 f = 0.09	107	0.036–0.144
	60×60 θ_1	21 f = 0.32	42 f = 0.64	3 f = 0.045	66	0.009–0.13
	60×60 θ_1	35 f = 0.49	34 f = 0.48	2 f = 0.03	71	0.003–0.10
Between caryonides of the same pure line	60×60	12 f = 0.37	12 f = 0.37	8 f = 0.25	32	0.10–0.40
	60×60	37 f = 0.43	36 f = 0.42	13 f = 0.15	86	0.075–0.225
	60×60	29 f = 0.46	34 f = 0.54	0 f = 0.00	63	0–0.06
	60×60	19 f = 0.41	27 f = 0.57	1 f = 0.02	47	0.0005–0.11
	60 θ_1×60 θ_1	39 f = 0.42	42 f = 0.46	11 f = 0.12	92	0.054–0.186

value is significantly higher than the frequency obtained for the caryonides developed after $CaCl_2$ treatment ($\chi^2 = 21.1$ for one degree of freedom).

(2) Frequencies of the Selfing-Caryonides

The frequency of the selfing-caryonides and its confidence interval ($\alpha = 5\%$) are calculated for each cross. The results of these calculations are given in 3rd and 5th figure columns of Table 2.

The data were obtained from 14 experiments; though the numbers of selfing-caryonides obtained in some crosses are low or null, a χ^2 test for homogeneity was carried out. The heterogeneity shown by this test is certainly overestimated ($\chi^2 = 47.54$ for 13 degrees of freedom), but its existence seems to be unquestionable.

Correlations between Anlagen Developed in the Same Cytoplasm

In certain crosses, it seems that, very often, the selfing induced by $CaCl_2$ affects both caryonides of one clone, while in other crosses such correlation does not seem obvious. It is possible that the presence of $CaCl_2$ during the macronuclear differentiation induces a correlation, which does not exist in normal culture conditions, between the mating types of sister-caryonides. To analyze these results, the observed frequencies of the different kinds of clones must be compared with those which would be expected according to hypothesis that sister-caryonides are independent.

(1) Mating types I and II

Three classes of clones are considered:
- those formed of two type I caryonides;
- those formed of two type I caryonides and one type II caryonide;
- those formed of two type II caryonides.

Table 3

Test of Conformity to the Hypothesis of Independence for the Mating Type of Sister-Caryonides, after Action of $CaCl_2$ during Macronuclear Differentiation

Clones formed of non-selfing caryonides			χ^2 (1 d. f.)
2 type I caryonides	1 type I caryonide 1 type II caryonide	2 type II caryonides	
8	17	5	0.63
5	17	8	0.63
4	10	7	0.016
4	10	13	0.75
14	19	11	0.77
9	15	10	0.465
1	15	8	3.47
4	10	15	1.10
10	26	10	0.79
7	19	14	0.016
11	19	14	0.77
2	8	2	1.33
10	14	9	0.75
9	19	11	0.021
		Total: (χ^2 14 d.f.)	11.51

The results obtained from all crosses carried out until now are shown in Table 3. They agree with the statistical hypothesis that sister-caryonides are independent ($\chi^2 = 11.51$ for 14 degrees of freedom).

(2) The selfing character

Some crosses were systematically carried out within the strain 60 θ_1, within the strain 60 and between these two strains. The crossed populations are either clones, pure lines, or caryonides derived from one pure line. The results were interpreted by considering as a whole the descendants of those pairs which really conjugated, and of those which underwent cytogamy[6]. Only the crosses which gave more than five selfing-caryonides in their descent were kept for this study.

The three following classes of clones were considered:

— those formed of two non-selfing-caryonides;

— those formed of one non-selfing-caryonide and one selfing-caryonide;

— those formed of two selfing-caryonides;

The results which are then obtained from three crosses between clones may be considered as homogenous[7] ($\chi^2 = 1.28$ for four degrees of freedom). The same conclusion is valid for the two crosses between pure lines ($\chi^2 = 0.88$ for two degrees of freedom) and for the three crosses carried out between caryonides of one pure line ($\chi^2 = 3.83$ for four degrees of freedom). It is thus possible to pool the results of each kind of cross (Table 4).

Table 4

Correlation between Sister-Caryonides for the Selfer Character

Cross	2 non-selfing caryonides	1 selfing caryonide 1 non-selfing caryonide	2 selfing caryonides	χ^2 (1 d. f.)
Between clones	98	23	5	4.95
Between pure lines	84	12	2	3.26
Between caryonides of the same pure line	84	6	13	62.69

There is no significant difference between clonal or pure lines crosses ($\chi^2 = 2.31$ for two degrees of freedom). On the contrary, the results obtained with the third type of cross are significantly different from the others ($\chi^2 = 15.86$ for two degrees of freedom).

The results of the test of conformity to the hypothesis that the two sister-caryonides are independent, are presented in the last column of Table 4:

— in the two first classes of crosses, the probability corresponding to the value of the calculated χ^2 approaches 5%. The significance of the test is thus debatable;

— in the third case, the difference is highly significant;

— in any case, the difference sides with a positive correlation.

[6] It is only possible to distinguish these two types of descent for the crosses 60 × 60 θ_1.

[7] The χ^2 tests of homogeneity presented in this paragraph were carried out upon classes the size of which were sometimes low; the heterogeneity shown is then certainly overestimated

The existence of a correlation between sister-caryonides is then questionable in the case when new macronuclear anlagen are at least partly heterozygous, or when their genomes are at least slightly different from the parental one.

When the macronuclear anlagen are homozygous and when their genes are identical to those of the old nuclei, the positive correlation between sister-caryonides is most clear. It can then be the result of either the homozygosity of the differentiating macronucleus, or the non-perturbation of the interactions between the cytoplasm and this macronucleus, the genotype of which is the same as that of the conjugants.

General Discussion and Conclusions

The results presented in this paper lead to the consideration of three kinds of effects produced by the presence of calcium chloride in the culture medium;

— an induction of cytogamy;

— an orientation of the macronuclear differentiation different from the one which can be observed in normal conditions;

— a delay in the macronuclear differentiation of a varying fraction of the population; in certain kinds of crosses, this delay most frequently affects both anlagen differentiating in one cytoplasm.

Those various effects are introduced and discussed from the point of view of their mode of expression, of their determining factors and of the interactions they can exercise upon each other.

(1) Induction of Cytogamy

The presence of $CaCl_2$ in the culture medium leads to a quick dissociation of some pairs, of which the partners generally undergo cytogamy. The frequency of this phenomenon differs from one experiment to another, and seems to depend upon the state of sexual reactivity of the mating populations.

The two kinds of pairs generate selfing-caryonides; which lead us to think that:

— this effect of $CaCl_2$ is not related to the biparental nature of the process of sexual reproduction;

— the early separation of the partners results from a surface phenomenon without any direct link with the delay in macronuclear differentiation.

Nevertheless, cytogamy systematically leads to the formation of homozygous macronuclear anlagen. When the partners are homozygous but genotypically different from each other, it allows the maintenance of nucleoplasmic relationships which conjugation would destroy. Then, the frequency of clones formed of two selfing-caryonides might be increased in a sensitive way when a high enough number of pairs undergo a sexual process of uniparental nature.

(2) Direction of the Macronuclaer Differentiation

The results concerning the relative frequencies of the type I and II caryonides when the macronuclear differentiation occurs in the presence of $CaCl_2$ are similar; whatever be the kind of cross considered, an increase of the frequency of type I caryonides is observed.

This direction of the macronuclear differentiation by $CaCl_2$ is similar to the effect induced by a fall of temperature (Sonneborn 1939). It is then possible to consider that, in both cases, it results in a non-specific slowing-down of the synthetic processes of the cell. In fact, it has been proved that in *Paramecium aurelia* (syngen *4*), $CaCl_2$ induces *in vitro* the dissociation of the ribosome-messenger RNA complex in the absence of monovalent cations (Reisner and Bucholtz 1972); this might explain a slowing down of the synthesis in the presence of the salt.

The presence of $CaCl_2$ does not alter the independence of the mating types of sister-caryonides. On the contrary, the selfing phenomenon may prove the existence of a correlation between sister-caryonides. Then, the process which leads to the preferential acquiring of type I is different from the one which slows down the macronuclear differentiation.

Finally, the mating type of Paramecia set apart about 72 h after conjugation (i.e., after the disappearance of the macronuclear heterogeneity) from the lineage of pairs put together into a medium containing $CaCl_2$, at 27°C, was determined (Hallet 1972). The frequency of type I and II is not then significantly different from what is found in normal culture conditions. Two hypotheses can explain what may appear as a contradiction:

— either there has been a selection regarding the rate of multiplication, to the benefit of the type I individuals. That is unlikely since a difference of growth between type I and II populations has never been observed;

— or the macronuclei the differentiation of which was delayed by $CaCl_2$ gave in their lineage more type I than type II; which allowed the equilibrium to set up. It will be possible to test this hypothesis experimentally later on.

(3) Expression of the Delay in the Macronuclear Differentiation

The presence of $CaCl_2$ during the macronuclear differentiation leads in a number of cases to an intracaryonidal heterogeneity which particularly appears through the formation of selfing-caryonides. The frequencies of selfing found till now are variable, but always under 25%. The determinism of this character should be specified in a further experiment.

Besides, each time a cross has been carried out between two caryonides of one pure line, a strong positive correlation had been observed between sister-caryonides for the selfing phenomenon. This result leads to consider two hypotheses:

— either a consequence of the homozygous state of the anlagen differentiating inside one cytoplasm is that they are affected in the same way by $CaCl_2$;

— or a number of nucleoplasmic relationships previously established are not disturbed and subsist for both anlagen.

To decide which one of the two hypotheses is right, it is necessary to know whether the clonal effect results from the homozygous state of the anlagen or from a genotypic identity of those with the old macronuclei. An answer will be provided through the study of the effect of $CaCl_2$ upon the macronuclear differentiation after a sexual autogamic process (autogamy or cytogamy).

RÉSUMÉ

Ce travail est destiné à l'analyse du retard induit par $CaCl_2$ dans la différenciation des ébauches macronucléaires et dont l'apparition de caryonides selfers (jusqu'à 25%) est l'une des conséquences. Ce phénomène apparait lors de la cytogamie aussi bien que lors de la conjugaison. Les caryonides frères présentent quelque tendance à être tous deux selfers ou tous deux non-selfers: cette corrélation est particulièrement forte quand les conjugants possèdent exactement le même génotype strictement homozygote. De plus, on observe une augmentation de la fréquence des caryonides de type I.

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Received 4 October 1974

ACTA PROTOZOOLOGICA
VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 291–296

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Observations des oocystes et des spores libres de *Sarcocystis* sp. (*Protozoa*: *Coccidia*) dans le gros intestin du renard commun (*Vulpes vulpes* L.) en Bulgarie

Synopsis. Chez 14 des 146 exemplaires étudiés de renard commun (*Vulpes vulpes* L.) de Bulgarie, l'auteur a trouvé des oocystes et des spores libres de *Sarcocystis* sp. qui ressemblent, par leurs caractères morphologiques, aux oocystes et aux spores de *S. tenella* et *S. fusiformis*, ilsolées des chiens, des chats et des coyotes americaines. Cela permet à l'auteur de conclure que le renard commun est aussi l'un des vecteurs dans la distribution de la sarcosporidiose des animaux dans la nature.

Les parasites unicellulaires du genre *Sarcocystis*, découverts encore au milieu du siècle passé, sont largement répandus et trouvés dans plus de 200 espèces de vertébrés des groupes des reptiles, des oiseaux et des mammiferes. Pourtant leur biologie, et surtout leur cycle évolutif, ne sont pas tout à fait connus jusqu'à présent. Les premieres hypothèses de Perrin (1907), Sergent (1921) etc., admettant la transmission des parasites et la contamnation des animaux et de l'homme par des hôtes intermédiaires — des insectes, n'étaient pas confirmées. Pour la première fois Rommel et al. (1972) ont trouvé que le développement de *Sarcocystis tenella* était de type coccidial et que la sporogonie du parasite se produisait dans l'appareil digestif des chats. Après la contamination expérimentale de 16 chats avec des cystes de *S. tenella* de brebis, données per os, ils ont observé que, entre le 12^{e} et le 16^{e} jour de la contamination, les animaux commencent à dégager des oocystes mûres. Les seconds expériments de Heydorn und Rommel (1972) avec des cystes de *S. fusiformis* de vaches, données per os aux chiens et aux chats, ont montré encore une fois que le développement des parasites du genre *Sarcocystis* se fait dans les cellules épithéliales de l'intestin grêle des carnassiers sus-mentionnés. Les premières informations des auteurs cités ont été confirmées plus tard et par Mahrt (1973), Fayer and Johnson (1973) etc. qui ont prouvé définitivement le rôle des chiens et des chats en tant que hôtes intermédiaires des sarcosporidioses des animaux domestiques. Très recemment Fayer (1974) a trouvé des spores libres de *Sarco-*

cystis fusiformis et dans les coyotes americains (*Canis latrans*), ainsi que dans les chiens domestiques en Amerique du Nord. Les spores observés par cet auteur avaient des dimensions de 16.9×10.9 μm. (N = 50).

Lors de nos études sur les coccidies des renards communs de Bulgarie, faites en 1973 et 1974, nous avons observé plusieurs fois des oocystes mûres et des spores libres de *Sarcocystis* sp. qui, par leurs caractères morphologiques, ressemblent bien aux spores de *S. tenella* et de *S. fusiformis*, décrites par les auteurs susmentionnés. Dans le présent travail nous allons exposer les résultats de nos observations présentes sur les oocystes et les spores de *Sarcocystis* sp., trouvées dans le renard commun (*Vulpes vulpes* L.) en Bulgarie, lesquelles, bien que préalables, nous permettent de montrer que ce carnassier de la famille des Canides est aussi un des vecteurs de la sarcosporidiose des animaux dans la nature.

Objet de nos études étaient 146 exemplaires de renards, recueillis du Nord-Ouest de la Bulgarie entre le 1. IV. 1973 et le 30. V. 1974. Une partie du gros intestin des animaux tués, avec le contenu fécal, était conservée jusqu'au moment de l'étude dans une solution 3% de bichromate de potasse ($K_2Cr_2O_7$). L'examen est fait selon la méthode de Fülleborn.

Je profite de l'occasion pour exprimer mes remerciements les plus sincères à M. Nikola Ridjakov, ingénieur du forêt à la ville de Berkovitza, avec l'aide active duquel a été recueilli le matériel étudié.

En résultat de nos études nous avons trouvé des oocystes mûres, ainsi que des spores libres de *Sarcocystis* sp. chez 14 exemplaires des renards communs de deux sexes (9.59%). Des animaux invasés, 7 étaient à l'âge de 2 à 5 mois et 7 avaient plus d'une année. L'intensité de l'invasion varie en grand diapason, mais en général, chez les exemplaires adultes elle est relativement petite: 1 à 25 spores à 100 champs du microscope, oculaire 10× et objectif 16×. Chez trois animaux jeunes à l'âge de 2 à 3 mois, on a observé une grande quantité de spores, dont le nombre était de 10 à 50 à un champ du microscope.

Les oocystes de *Sarcocystis* sp. étaient observées en général rarement dans les animaux étudiés. Elles sont incolores, ellipsoïdales et leur enveloppe est très mince (environs 0.5 μm), souvent à peine visible à un agrandissement moyen du microscope. (Planche I 1–3). Légèrement préssé ou mise dans une solution hypertonique, l'enveloppe dégage facilement les spores. Reliquat cytoplasmique, granule polaire et micropyle absents. Les dimensions des oocystes varient de 17–20.2×10.2–17.7 μm. (En moyenne: 18.6×14.0 μm). Dans tous les cas observés les oocystes étaient mûres, avec des sporozoïtes bien formés, ce qui prouve que la sporogonie se produit dans l'appareil digestif de l'hôte.

Les spores sont ellipsoïdales ou ovoïdes allongées et incolores. Leur enveloppe est plus solide et plus épaisse, environs 1 μm. Dans les spores il y a un reliquat cytoplasmique à grains grossiers, souvent dispersé parmi les sporozoïtes. Granule

polaire et micropyle absents (Planche I 4,5). Les dimensions des spores varient de 12.8–17.8×9.5–11.0 μm. (En moyenne: 15.3×10.2 μm).

Les sporozoïtes sont allongés et légèrement rétrécis à l'une des extrémités. Leur vacuole est bien visible. Dimensions: 7–8×3–3.5 μm. (En moyenne: 7.5×3.2 μm).

L'un des caractères généraux des oocystes du genre *Sarcocystis*, c'est qu'elles commencent et achèvent leur sporulation dans l'appareil digestif des hôtes et dans les féces se dégagent des oocystes mûres et des spores libres du parasite. Cette particularité a été observée aussi par d'autres auteurs qui ont étudié les coccidies des carnassiers des fam. *Canidae* et *Felidae*, mais qui avaient pensé qu'il s'agissait dans ce cas de spores libres des autres espèces du genre *Isospora*, connues jusqu'à ce moment-la. Ainsi par exemple, Gassner (1940) a observé relativement souvent des spores libres des coccidies dans les chiens qu'il a étudiés, mais il suppose que ce sont des spores des espèces *I. bigemina* et *I. rivolta*. Mahleureusement il ne donne pas les dimensions des spores observées. Kotlan (1953), signale aussi la prèsence des spores libres des coccidies dans les renard qu'il suppose être de l'espèce *I. canivelocis*. Mais en réalité les spores libres qu'il a observé diffèrent sensiblement des spores de *I. canivelocis* par leur forme et leurs dimensions, le caractère du reliquat cytoplasmique et par d'autres caractères morphologiques. A cause de ces différences Pellerdy (1965) suppose que "the sporocysts passing freely from the fox can by no means be *I. canivelicis* sporocysts but derive from some other species". Bearup (1954) a trouvé aussi des spores libres dans les féces de *Canis dingo* en Australie qu'il rapporte, avec un certain doute, au genre *Cryptosporidium*, décrit par Tyzzer (1910). Pourtant il suppose qu'il est bien possible que les spores trouvées appartiennent aussi à *I. rivolta*, trouvé dans *C. dingo* qu'il a étudié. Les spores observées par Bearup (1954) ont les dimensions 17×11 μm et un reliquat cytoplasmique dont le diamètre est environs 7 μm. Les quatres sporozoïtes en forme de boudin atteignent 11 à 12 μm de longueur.

Les observations de Levine and Ivens (1965) sont aussi d'un intérêt particulier. Chez 4 de 139 chiens étudiés (3%) ils ont trouvé des spores mûres libres avec des dimensions de 15–17×10–11 μm, contenant un reliquat cytoplasmique compact ou dispersé. Les sporozoïtes ont la forme de banane, légèrement rétrécis à l'une des extrémités et atteignent 11×2–3 μm. Mais en conclusions de leur travail, les auteurs pensent qu'il s'agit en réalité de spores libres de l'espèce *I. rivolta*.

En réalité, les spores libres observées par Bearup (1954), et Levine and Ivens (1965), ressemblent beaucoup par leur caractères morphologiques aux spores de *S. tenella* et *S. fusiformis*, décrites par Rommel et al. (1972), Heydorn und Rommel (1972) et Mahrt (1973). Cela nous permet de conclure que les spores libres observées par les auteurs cités, quoique identifiées avec un certain doute comme *I. rivolta*, sont en réalité des stades exogènes du genre *Sarcocystis*.

Les oocystes et les spores de *Sarcocystis* sp. des renards, que nous venons de decrire plus haut, présentent de fortes ressemblances morphologiques avec les oocystes et les spores déjà decrites des espèces *S. tenella* et *S. fusiformis* (Tableau 1).

Tableau 1

Comparaison des caractères morphologiques des oocystes

Caractères morphologiques	*Sarcocystis tenella* H.: *Felis domestica* L. Rommel et al. (1972)	*Sarcocystis fusiformis* H.: *Felis domestica* L. Heydorn und Rommel (1972)
(a) Oocyste		
forme	–	–
couleur	incolore	incolore
enveloppe	mince	mince
Mi	non	non
reliquat cytoplasmique	non	non
dimension	–	–
moyenne	–	–
(b) Spore		
forme	ellipsoïdale, parfois non symétrique	ellipsoïdale
couleur	incolore	incolore
reliquat cytoplasmique	+	+
dimensions	10.8–13.9×7.7–9.3	10.7–13.9×6.9–9.3
moyenne	12.4±0.8×8.1±0.5	12.5±0.8×7.8±0.6
(c) Sporozoïtes		
nombre	4	4
dimensions	–	–
(d) Localisation	–	intestin grêle

Mais, à cet étape de nos études, il est difficile de dire s'il s'agit de quelque — une de mêmes espèces, car on sait que le renard est un carnassier avec un spectre alimentaire assez large et dans la nature il se nourrit de différentes espèces d'oiseaux, de rongeurs, d'insectivores etc., et parfois aussi de la viande de gros animaux domestiques et sauvages. Mais l'observation des oocystes et des spores de *Sarcocystis* sp. dans les renard, souvent avec une haute intensité d'invasion, montre que ce carnassier aussi joue un rôle dans la distribution de la sarcosporidiose des animaux dans la nature.

SUMMARY

The author has found sporulated oocysts and free spores of *Sarcocystis* sp., parasitizing in the common red fox (*Vulpes vulpes* L.) from Bulgaria. Out of 146 specimens examined, 14 were infected (9.59%). The oocyst and spore morphological features are similar to these *S. tenella* and *S. fusiformis*, parasites of the dogs, cats and american coyotes. Oocyst dimensions: 17–20.2 × × 10.2–17.7 μm (with a mean of 18.6 × 14 μm) and spore dimensions: 12.8–17.8 × 9.5–11.0 μm (with a mean of 15.3 × 10.2 μm). The author concludes that the European common red fox is one of the vectors in the maintenance and distribution of sarcosporidiosis in nature.

et des spores de *S. tenella*, *S. fusiformis* et *Sarcocystis* sp.

Sarcocystis fusiformis H.: *Canis familiaris* L. Heydorn und Rommel (1972)	*Sarcocystis fusiformis* H.: *Canis familiaris* L. Mahrt (1973)	*Sarcocystis* sp. H.: *Vulpes vulpes* L. Observations personnelles
–	–	ellipsoïdale
incolore	incolore	incolore
mince	–	mince, 0.5 μm
non	non	non
non	non	non
–	–	17.0–20.2×10.2–17.7
–	–	18.6×14.0
ellipsoïdale	ellipsoïdale	ellipsoïdale
incolore	incolore	incolore
+	+	+
13.9–17.0×6.2–10.8	14.8–17.3×8.7–9.9	12.8–17.8×9.5–11.0
15.9±1.0×8.3±1.1	16.0×9.8	15.3×10.2
4	4	4
–	–	7–8×3.0–3.5
–	–	–

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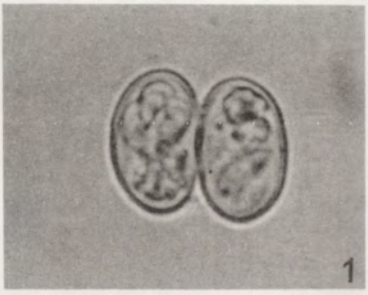
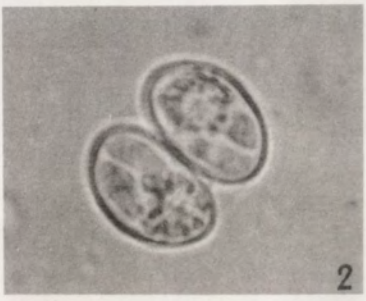
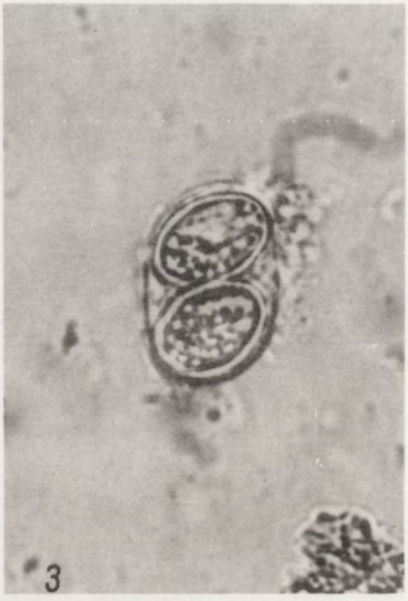
Received 9 November 1974

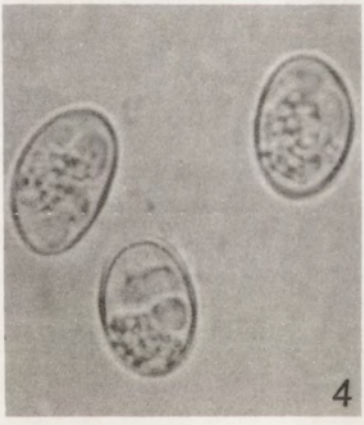
EXPLICATION DE PLANCHE I

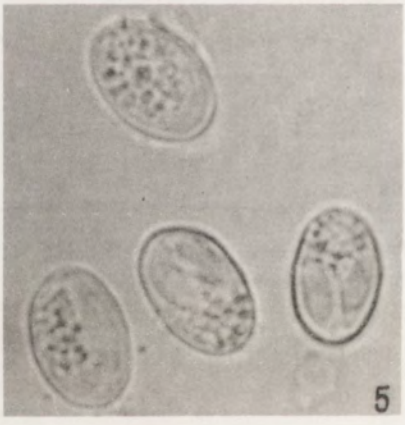
1–3: Oocystes de *Sarcocystis* sp., observées dans le gros intestin du renard commun. × 1200
4–5: Spores libres de *Sarcocystis* sp., trouvées dans le même hote. × 1200











V. Golemansky auctor phot.

ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 297–311

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Формирование таксоценозов раковинных амеб (*Rhizopoda*: *Testacea*) в Каховском водохранилище

Formation of *Rhizopoda*: *Testacea* Taxocenoses in the Kachovka Reservoir

Синопсис. Изучались состав и количественное развитие раковинных корненожек (*Rhizopoda*: *Testacea*) Каховского водохранилища в первые годы его существования (1955–57) и спустя 9 и 14 лет. На грунтах различного типа собрано 300 количественных проб. Найдено 89 видов раковинных корненожек, относящихся к 11 родам. Наибольшее число видов принадлежит к родам *Diffugia* – 49 и *Centropyxis* 16 видов. Наиболее бедны по видовому разнообразию и количественному развитию таксоценозы корненожек глин и песков, наиболее богат таксоценоз илов – 48 видов, средняя численность 1219.9 тыс. экз/m². Грунты различного типа отличаются между собой характером обитающей на них фауны ризопод, характером их ценозов. Эти различя обуславливаются количественным развитием корненожек и частотой встречаемости видов, а не их видовым составом вообще. Формирование таксоценозов корненожек происходило по мере формирования донных отложений водохранилища. За 9 лет существования водохранилища на сильно заиленных песках, заиленных почвах и илах сформировался однотипный комплекс доминирующих видов: *Diffugia oblonga oblonga*, *Diffugia oblonga acuminata*, *Centropyxis aculeata*, *Diffugia bidens* и некоторые другие. Эти виды послужили "основой" для дальнейшего формирования едного пелофильного таксоценоза. За 14 лет существования водохранилища на указанных 3-х биотопах сформировался едный пелофильный таксоценоз раковидных корненожек, который является доминирующим в водохранилище и занимает почти всю площадь дна.

В пресноводных водоемах различного типа раковинные корненожки, как правило, развиваются в больших количествах, часто являясь доминирующей группой микробентических ценозов. О значительном видовом разнообразии и количественном развитии корненожек в водохранилищах говорится в работах Гурвича (1961, 1967, 1969, 1972 a, b), Бузаковой (1966), Фатовенко

(1971), Stepanek (1967) и некоторых других авторов. Питаясь водорослями, детритом, а иногда и другими простейшими, корненожки несомненно играют немаловажную роль в круговороте органического вещества в водоемах. Однако в литературе содержится еще мало данных по биологии этих организмов. Особенно мало данных по количественному развитию ризопод и по формированию ценозов раковинных амеб.

В настоящей работе описывается формирование таксоценозов раковинных амеб в большом искусственно созданном водоеме — Каховском водохранилище.

Материал и методика

Материалом для настоящей работы послужили анализы результатов обработки 300 количественных проб микрозообентоса, собранных на Каховском водохранилище. Пробы собирались в мае и августе 1956 года, ежемесячно с мая по октябрь в 1957 г., в сентябре 1963 и августе 1968 года — по сетке постоянных "станций", охватывающих всю акваторию водохранилища — с учетом экологических особенностей его отдельных участков. Пробы — монолиты грунта высотой 10–12 cm с сантиметровым слоем придонной воды над ними — с глубин до 3-х метров отбирались при помощи трубки Владимировой (Владимирова 1961), а с глубин свыше 3-х метров микробентометром Гурвича–Цееба (1958). Обработка проб проводилась по принятой нами методике (Гурвич 1969 a, b).

Каховское водохранилище расположено на нижнем течении Днепра между г.г. Запорожье и Новая Каховка. Заполнение водой его происходило с сентября 1955 по июль 1965 г. Площадь водохранилища 2150 km^2, объем — 18.2 km^3, длина по оси — 230 km средняя ширина — 9 km, максимальная — 26 km. Глубины до 2 m составляют 5% всей акватории, 2–5 m — 18% и свыше 5 m — 77%. Водохранилище разделяется на три части: верхнюю расширенную (шириной до 25 km), среднюю, шириной до 18 km и нижнюю, наиболее узкую (4–8 km) и удлиненную. Нижняя часть водохранилища характеризуется большими глубинами (12–16 m), увеличивающимися к плотине до 25–30 m. Водохранилище характеризуется малой проточностью. В маловодные и средние по водности годы водообмен в водохранилище происходит 2.5 раза, в многоводные — 3.5 раза; это водоем озерного типа. Максимальная скорость течения 0.8–1.8 m/s наблюдается в узком русловом участке вдоль правого берега от Запорожья до Верхней Тарасовки. В верхней части скорость течения не превышает 0.05–0.09 m/s. В средней части водохранилища она равна 0.25–0.6 m/s, нижней — 0.01–0.08 m/s летом и 0.16–0.19 m/s весной.

Ложем Каховского водохранилища явилась хорошо сформированная долина с большим количеством водоемов, которые занимали около 13–20% ее площади. Кроме Днепра с развитой сетью водоемов придаточной системы (заливы, протоки, рукава и т.д.) в зоне подтопления было большое количество других водоемов: притоки Днепра — речки Конка, Базавлук, Рогачик и др., лиманы — Великие Воды, Рогачикский, пойменные озера и разнообразные по размерам временные пересыхающие водоемы.

Результаты

Таксоценозы различных биотопов

Различные по своему происхождению и экологическим условиям исходные водоемы ложа Каховского водохранилища обусловили богатство и раз-

нообразие фауны раковинных корненожек в первые же годы существования водоема.

За весь период исследования в Каховском водохранилище найдено 89 видов раковинных корненожек, относящихся к 11 родам. По числу видов резко выделяются род *Diffugia* — 49 и род *Centropyxis* — 16 видов. К роду *Arcella* относится 6 видов, к роду *Pontigulasia* — 5; р.р. *Lesquereusia* и *Cyclopyxis* — по 4 вида. Роды *Bullinula*, *Cucurbitella*, *Plagiopyxis*, *Phryganella* и *Nebela* представлены одним видом каждый.

Естественно, все 89 видов не были найдены одновременно; видовое разнообразие корненожек изменялось по мере становления водохранилища. В первые годы существования водоема (1956–57) в нем было найдено всего 37 видов корненожек. Спустя 6 лет число видов возросло до 61, а еще через 5 лет — т.е. на 13-й год существования водохранилища было найдено 75 видов этих простейших.

Данные о видовом составе и о разнообразии корненожек в 1956–57, 1963 и 1968 г.г. позволяют сделать вывод об относительной стабилизации фауны ризопод, т.е. о сформировавшихся таксоценозах.

В Каховском водохранилище можно выделить 5 основных типов грунта (биотопов): глина, песок, заиленный песок, залитые (заиляемые) почвы и ил, на каждом из которых формируется соответствующий таксоценоз раковинных амеб.

Естественно, что грунты различного типа отличаются между собой по механическому составу, водонасыщенности, плотности, содержанию органических и биогенных веществ, микроэлементов. Расчеты коэффициентов корреляции между численностью корненожек и некоторыми инградиентами химического состава грунтов показывают наличие прямолинейной зависимости между численностью корненожек, гранулометрическим составом грунтов, содержанием в них органических веществ и некоторых биоэлементов: "r"[1] = 0.7–0.9 (Гурвич и др. 1972). В этой же работе приведен гранулометрический и химический состав донных отложений Каховского водохранилища.

В Таблице 1 приведено число видов и средняя численность корненожек, найденных на различных грунтах. В Таблице 2 — проценты встречаемости и средняя численность корненожек, преобладающих на различных биотопах Каховского водохранилища.

Таксоценоз глинистых грунтов

Глинистые отложения, а вернее продукт размыва лессовых берегов, занимают лишь 0.1% площади всего водохранилища. В основном это узкие полоски вдоль лессовых с суглинистыми прослойками абразионных берегов среднего

[1] "r" — выборочный коэффициент корреляции для $n = 12$.

Таблица 1

Число видов и средняя численность (в тыс. экз/м²) раковинных корненожек на различных грунтах Каховского водохранилища

Table 1

Quantity of the Species and an Average Number (Thousands of Specimen/m²) of the *Rhizopoda testacea* on Various Bottoms of the Kachovka Reservoir

Тип грунта (биотоп) Kind of the bottom (biotope)	Май и август 1956; май, июль и октябрь 1957 May and August 1956; May, July and October 1957		Сентябрь 1963 September 1963		Август 1968 August 1968	
	Число видов Quantity of species	Численность Number	Число видов Quantity of species	Численность Number	Число видов Quantity of species	Численность Number
Глины Clays	10	33.8	11	18.6	2	3.4
Пески Sands	18	17.3	13	6.6	19	20.8
Заиленные пески Silted sands	20	259.1	46	481.1	41	193.1
Залитые (заиляемые) почвы Flooded soils	19	95.0	30	126.8	51	639.6
Илы Silts	22	168.3	50	433.4	48	1219.9

участка водохранилища. Глинистые отложения как правило содержат то или иное количество примеси песка.

По видовому разнообразию и количественному развитию это самый бедный таксоценоз Каховского водохранилища. На глинистых грунтах ни один вид не достигал своего максимального развития. Наибольшая численность корненожек на этом грунте наблюдалась в первые годы существования водоема. Несмотря на то, что в отдельных случаях численность некоторых видов была довольно высокая (*Centropyxis aculeata* — до 25 тыс. экз/m², *Difflugia bidens* — до 14 тыс. экз/m²), встречались эти виды редко.

Очевидно, в первые годы существования водохранилища на глинистое прибрежье приносилось довольно много корненожек, вымытых из близлежащих биотопов (грунтов) более обильно населенных раковинными амебами.

В сентябре 1963 года численность корненожек уменьшилась в 1.8 раза. В августе 1968 г., т.е. на 14-й год существования водохранилища, в пробах найдено всего 2 вида, численность резко упала.

Характер самого грунта и волны, постоянно размывающие лессово-суглинистые берега делают глинистый грунт весьма неблагоприятным биотопом для развития корненожек.

Таксоценоз песчаных грунтов

Песчаные грунты в соответствии с режимом течения (у дна не ниже 0.15 m/s) расположены в основном вдоль бывшего русла Днепра, главным образом в верхнем участке водохранилища и в районе островов Большие Кучугуры, а также на месте размытого песчаного массива Малые Кучугуры. Чистые пески встречаются редко, большей частью они содержат небольшое количество ила или растительных остатков. По видовому составу и количественному развитию раковинных амеб это бедный таксоценоз.

Основную численность корненожек в течение всего периода исследований обуславливали *Centropyxis aculeata*, *Difflugia oblonga oblonga*, *Difflugia oblonga acuminata*, *Difflugia corona*. Чистые пески, особенно крупнозернистые и с примесью мелкого гравия являются весьма неблагоприятным биотопом для развития корненожек. Они бедны органическим веществом (т.е. мало трофичны), а их верхние слои движутся под влиянием стоковых и волновых течений. Поэтому населяющий их таксоценоз беден как по видовому составу, так и по количественному развитию.

Таксоценоз заиленных песков

Заиленные пески являются довольно широко распространенным типом грунта, они встречаются во всех участках водохранилища. К заиленным пескам в данном случае отнесены слабо и сильно заиленные пески, заиленные пески с примесью растительных остатков. Слабо заиленные пески расположены в верхнем участке водохранилища, у берегов песчаных островов. Сильно

Таблица 2

Встречаемость и средняя численность (в тыс. экз/м²) корненожек, преобладающих на различных грунтах Каховского водохранилища

Table 2

Occurrence and Average Number (Thousands of Specimen/m²) of *Rhizopoda testacea* Dominating on Various Soils of the Kachovka Reservoir

Грунты Bottoms	Май и август 1956; май, июль и октябрь 1957 May and August 1956; May, July and October 1957			Снтябрь 1963 September 1963			Август 1968 August 1968		
	Виды Species	Процент встречаемости Occurrence percentage	Средняя численность Average number	Виды Species	Процент встречаемости Occurrence percentage	Средняя численность Average number	Виды Species	Процент встречаемости Occurrence percentage	Средняя численность Average number
1	2	3	4	5	6	7	8	9	10
Заиленные пески Silted sands	Наиболее часто встречающиеся Most frequent			Руководящие Predominating			Руководящие Predominating		
	Centropyxis aculeata	81	20.8	*Centropyxis aculeata*	86	28.6	*Centropyxis aculeata*	89	27.7
	Difflugia oblonga acuminata	63	20.8	*Difflugia oblonga oblonga*	79	23.8	*Difflugia oblonga oblonga*	67	25.0
	Difflugia corona	36	9.9	*Difflugia urceolata*	73	18.8	*Difflugia oblonga acuminata*	78	12.7
	Difflugia oblonga oblonga	54	7.7	*Difflugia oblonga acuminata*	86	16.5	*Difflugia bidens*	78	9.7
	Difflugia amphora	36	4.6	Характерные Typical			Характерные Typical		
	Centropyxis constricta	27	3.3						
	Difflugia urceolata	27	3.0	*Difflugia bidens*	59	14.5	*Difflugia viscidula*	56	29.4
				Difflugia oblonga curvicaulis	53	10.9	*Difflugia globulosa*	34	18.3

1	2	3	4	5	6	7	8	9	10
				Difflugia amphora	46	7.4	*Difflugia corona*	56	6.5
				Centropyxis constricta	53	4.6	*Difflugia hydrostatica lithophila*	44	6.8
				Difflugia viscidula	59	4.7	*Centropyxis constricta*	44	6.7
				Lesquereusia modesta	52	4.5	*Lesquereusia modesta*	34	4.8
Залитые (заилленные)почвы	Наиболее часто встречающиеся			Руководящие			Руководящие		
	Most frequent			Predominating			Predominating		
Flooded soils	*Difflugia oblonga oblonga*	64	6.8	*Difflugia oblonga oblonga*	80	32.4	*Difflugia oblonga oblonga*	93	138.3
	Centropyxis aculeata	24	2.7	*Difflugia oblonga acuminata*	90	23.1	*Difflugia oblonga acuminata*	87	110.2
	Difflugia oblonga acuminata	4	0.2	*Centropyxis aculeata*	80	7.1	*Difflugia bidens*	87	26.3
							Lesquereusia modesta	75	19.8
				Характерные			Характерные		
				Typical			Typical		
				Pontigulasia bigibbosa	50	7.1	*Difflugia viscidula*	68	20.2
				Difflugia urceolata	50	6.3	*Pontigulasia bigibbosa*	68	18.6
				Difflugia bidens	50	5.7	*Difflugia oblonga curvicaulis*	50	14.7
							Pontigulasia incisa	68	12.8
							Difflugia oblonga kempnyi	56	10.0
							Centropyxis aculeata	60	21.1
Илы	Наиболее часто встречающиеся			Руководящие			Руководящие		
Silts	Most frequent			Predominating			Predominating		
	Difflugia oblonga oblonga	90	57.6	*Difflugia oblonga oblonga*	96	80.8	*Difflugia oblonga acuminata*	100	226.2
	Centropyxis aculeata	78	32.2	*Centropyxis aculeata*	100	56.8		100	188.9
	Difflugia oblonga acuminata	72	31.7	*Difflugia oblonga acuminata*	100	49.0	*Difflugia oblonga oblonga* *Centropyxis aculeata*	92	129.5

1	2	3	4	5	6	7	8	9	10
	Difflugia corona	60	13.1	*Pontigulasia bigibbosa*	78	12.2	*Difflugia oblonga kempnyi*	85	124.1
	Difflugia bidens	54	7.5						
				Характерные			Характерные		
	Difflugia urceolata	36	6.7	Typical			Typical		
	Difflugia amphora	30	3.7						
				Difflugia oblonga curvicaulis	66	25.6	*Difflugia hydrostatica lithophila*	71	63.1
				Difflugia viscidula	72	13.4	*Difflugia oblonga curvicaulis*	78	60.8
				Difflugia corona	54	12.6	*Lesquereusia modesta*	78	40.3
				Difflugia urceolata	68	12.2	*Difflugia bidens*	100	36.9
				Difflugia bidens	60	10.0	*Difflugia viscidula*	85	35.8
				Lesquereusia modesta	66	8.9	*Centropyxis platystoma*	50	30.8
				Difflugia amphora	54	8.5	*Centropyxis constricta*	71	27.1
							Pontigulasia bigibbosa	57	25.6
							Difflugia corona	70	19.7
							Difflugia urceolata	57	18.3
							Difflugia oblonga angusticollis	30	28.8
							Centropyxis marsupiformis (var. *obesa*)	50	26.3

заиленные пески расположены в районе бывших протоков, рукавов и пойменных водоемов Днепра, в заливах. С возрастом водохранилища почти на всех участках степень заиления песка возрастает.

По видовому разнообразию и количественному развитию это богатый таксоценоз (см. Табл. 1). В первые 8 лет существования водохранилища это был самый богатый по количественному развитию ценоз: заиленные пески образовывали ложе и берега протоков, рукавов, стариц и пойменных водоемов Днепра в зоне затопления и их заселяла уже сформировавшаяся богатая фауна раковинных корненожек. По сравнению с первыми годами существования водохранилища видовое разнообразие корненожек в 1963 и 1968 г.г. увеличилось больше чем в 2 раза, однако состав "руководящих" видов и их средние численности почти не изменялись в течение всего периода исследований (см. Табл. 2). То же в значительной степени относится и к "характерным" видам.

Для сообщества корненожек, обитающих на заиленных песках характерно постепенное увеличение числа и численности пелофильных видов, что говорит об интенсивном процессе заиливания ложа водохранилища, т.е. о расширении зоны илов.

Таксоценоз залитых (заиленных) почв

Залитые почвы являлись сложным и варьирующим биотопом, что обусловлено разнообразием почв, затопленных при заполнении чаши водохранилища. Этот тип грунта в процессе формирования донных отложений водохранилища естественно подвергся наибольшей — по сравнению с другими грунтами — переработке. По мере становления водохранилища залитые почвы постепенно заиливаясь, превращались в тот или иной Тип илов. К 1968 году, т.е. за 13 лет существования водохранилища, верхние 3–5 cm грунта уже представляли собой иловые отложения, чего было совершенно достаточно для развития разнообразной и богатой фауны ризопод, характерной чертой которых является пелофилия.

По видовому составу и количественному развитию корненожек это довольно богатый ценоз. Разнообразие и количественное развитие корненожек увеличивалось по мере переработки и заиления почв и превращения их в илистые грунты (см. Таблицы 1 и 2). Интересно отметить, что из 3-х видов корненожек, которые в первые годы существования водохранилища начали заселять залитые почвы и являлись доминирующими формами в 1956–57 гг., два вида *Difflugia oblonga oblonga* и *Difflugia oblonga acuminata* в 1963 и 1968 г.г. остались в группе "руководящих" видов. Изменение в видовом составе корненожек залитых (заиленных) почв характеризуется появлением в группе "характерных" видов форм, типичных для илов: *Difflugia viscidula*, *Difflugia oblonga angusticollis*, *Pontigulasia bigibbosa*, *Pontigulasia incisa*, *Difflugia oblonga kempnyi*.

Изменения в видовом составе и количественном развитии корненожек свидетельствуют о том, что за 13 лет верхние слои залитых почв превратились в илистые донные образования, "соединившись" с ранее имеющимися в водохранилище илами.

Таксоценоз илов

Илы являются наиболее широко распространенным типом грунта в Каховском водохранилище. В первые годы существования водоема илы залегали в участках, где ранее были пойменные водоемы и илистые пойменные болота. Характер илов был весьма разнообразный: цвет варьировал от серого до черного, нередко они содержали примесь песка или растительных остатков. По мере становления гидрологического и биологического режимов водохранилища происходит сильное заиление ложа водоема, накопление на дне илистых отложений. К 1963 и 1968 г.г. илы стали доминирующим типом грунта. По видовому составу и количественному развитию таксоценоз корненожек, развивающийся на илах, является самым богатым таксоценозом раковинных амеб Каховского водохранилища. Илы являются настоящим "царством" корненожек.

На илах число видов кореножек в 1963 г. г. и 1968 возросло по сравнению с 1956–57 г.г. в 2.3 раза. Очень сильно увеличилась средняя численность этих организмов: в 1963 году она возросла в 2.6 раза, а в 1968 году в 7.3 раза — по сравнению с 1956–57 г.г. (см. Табл. 1). Интересно отметить, что так же, как и на залитых (заиленных) почвах, на илах с момента образования водохранилища доминировали 3 вида: *Difflugia oblonga oblonga*, *Difflugia oblonga acuminata* и *Centropyxis aculeata*. В 1963 г. к этим видам добавилась *Pontigulasia bigibbosa*, а к 1968 г. — еще несколько видов, в том числе *Difflugia oblonga kempnyi* средняя численность которой составляла 124.1 тыс. экз/м^2 (см. Табл. 2).

Обсуждение

В первые же годы существования водохранилища на грунтах различного типа начали формироваться различные по характеру таксоценозы раковинных амеб. В течение нескольких лет эти формирующиеся ценозы по видовому составу не сильно отличались друг от друга. Большинство видов корненожек было встречено почти на всех типах грунта; однако процент встречаемости и средняя численность почти каждого вида были весьма различны на грунтах различного типа. По мере переработки и заиливания ложа водохранилища в значительной степени нивелировались различия в характере грунтов.

Вместе с характером грунта менялась и населяющая их фауна раковинных корненожек.

Таксоценозы раковинных амеб различных грунтов на 9-й год существования

Таблица 3

Состав пелофильного таксоценоза раковинных корненожек (*Rhizopoda, Testacea*) Каховского водохранилища (август 1968 г.)

Table 3

Composition of the Pelophilous Taxocenosis of *Rhizopoda, Testacea* in the Kachovka Reservoir (August, 1968)

Виды Species	Процент встречаемости Occurrence percentage	Численность в тыс. экз/м² Number (thousands of specimen per m²)	
		Максимальная Maximum	Средняя Average
1	2	3	4
Руководящие Predominating			
1. *Difflugia oblonga acuminata* Ehrenberg	100	1058.7	228.2
2. *Difflugia oblonga oblonga* Ehrenberg	100	1150.3	189.9
3. *Centropyxis aculeata* (Ehrenberg) Stein	92	600.6	130.5
4. *Difflugia oblonga kempnyi* Stepanek	85	1170.7	125.1
Характерные Typical			
5. *Difflugia hydrostatica lithophila* Penard	71	427.6	64.1
6. *Difflugia oblonga curvicaulis* Penard	78	478.5	62.8
7. *Difflugia bidens* Penard	100	112.0	46.0
8. *Lesquereusia modesta* Rhumbler	78	224.0	42.3
9. *Difflugia viscidula* Penard	85	224.0	36.8
10. *Centropyxis platystoma* Penard	50	305.4	31.8
11. *Centropyxis constricta* Ehrenberg	71	162.9	28.1
12. *Pontigulasia bigibbosa* Penard	57	142.5	26.6
13. *Difflugia corona* Wallich	70	42.8	20.7
14. *Difflugia urceolata* Carter	57	71.3	19.3
15. *Difflugia oblonga angusticollis* Stepanek	30	325.8	29.8
16. *Centropyxis marsupiformis* var. *obesa* Deflandre	50	203.6	26.3
Второстепенные Minor			
17. *Difflugia lebes* Penard	28	203.6	16.7
18. *Centropyxis minuta* Deflandre	10	192.6	13.7
19. *Difflugia amphora* Leidy	57	50.9	9.5
20. *Pontigulasia incisa* Rhumbler	50	50.9	8.2
21. *Difflugia gramen* Penard	50	20.4	6.2
22. *Centropyxis marsupiformis* Wallich	35	58.9	6.0

1	2	3	4
23. *Difflugia hydrostatica liphothila* Penard	9	79.7	5.7
24. *Centropyxis platystoma armata* Penard	28	40.7	5.0
25. *Cucurbitella mespilliformis* Penard	27	30.7	5.2
26. *Difflugia avellana* Penard	43	20.4	3.4
27. *Difflugia oblonga schizocaulis* Stepanek	29	20.4	39
28. *Difflugia lobostoma* Leidy	15	20.2	2.9
29. *Difflugia globulosa* Dujardin	35	10.2	2.8
30. *Lesquereusia combinata* Stepanek	15	20.4	2.3
31. *Difflugia elegans* Penard	21	20.4	2.2
32. *Difflugia oblonga nodosa* Leidy	14	20.4	2.0
33. *Difflugia limnetica* Levander	21	10.2	1.7
34. *Centropyxis spinosa* Cash	14	10.2	1.5
35. *Difflugia capreolata* Penard	14	10.2	1.5
36. *Centropyxis aerophila* Deflandre	21	8.0	1.4
37. *Centropyxis gibba inermis* Bartos	20	8.0	1.3
38. *Plagiopyxis callida* Wailes	16	10.2	1.1
Случайные (редкие) Casual (rare)			
39. *Difflugia oblonga brevicolla* Cash	14	6.6	0 .8
40. *Difflugia fallax* Penard	9	10.2	0.7
41. *Cyclopyxis kahli* Deflandre	9	10.2	0.7
42. *Arcella discoides* Ehrenberg	7	8.0	0.6
43. *Difflugia oblonga* var. Stepanek	7	5.6	0.5
44. *Cyclopyxis eurystoma* Deflandre	7	5.1	0.4
45. *Difflugia oblonga bryophila* Penard	7	5.1	0.4
46. *Difflugia oblonga claviformis* Penard	7	5.1	0.4
47. *Difflugia elongata* Penard	7	5.1	0.4
48. *Pontigulasia spiralis* Rhumbler	7	4.0	0.3
Difflugia sp. sp.	12	8.0	2.2

водохранилища еще имели свои специфические особенности в видовом составе, структуре и количественном развитии. Но уже к этому времени на сильно заиленных песках, заиленных почвах и илах сформировался довольно однотипный комплекс "руководящих" и "характерных" видов. Эта группа организмов послужила "основой" для дальнейшего формирования единого пелофильного таксоценоза раковинных корненожек.

В 1968 году еще сохранились некоторые отличия в видовом составе, структуре и главное — количественном развитии таксоценозов раковинных амеб, формирование которых началось в 1956–57 г.г. на заиленных песках, залитых (заиленных) почвах и илах. Однако эти отличия настолько незначительны, что можно говорить о том, что за 14 лет существования Каховского водохранилища в нем на указанных 3-х биотопах сформировался единый пелофильный таксоценоз раковинных корненожек. Этот таксоценоз занимает почти всю площадь дна и является доминирующим в водохранилище. Характеристика его приведена в Таблице 3.

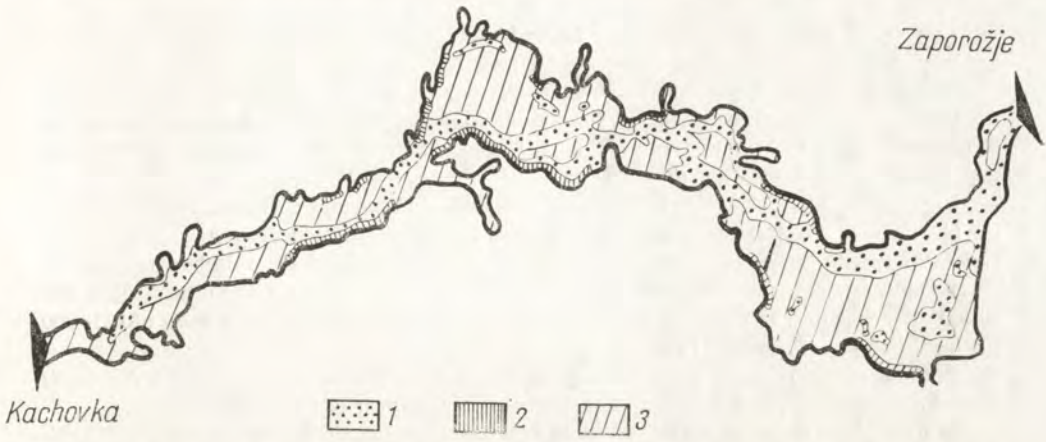

Рис. 1. Схема размещения таксоценозов раковинных амеб в Каховском водохранилище (Август 1968), 1 — псамофильный и псамопелофильный таксоценозы, 2 — аргиллофильный таксоценоз, 3 — пелофильный таксоценоз

Fig. 1. The scheme of distribution of *Rhizopoda* : *Testacea* taxocenoses in the Kachovka reservoir (August 1968), 1 — psamophilous and psammopelophilous taxocenoses, 2 — argillophilous taxocenosis, 3 — pelophilous taxocenosis

Следует согласиться с мнением ряда исследователей Аверинцев (1906), Bartoš (1940), Moraczewski (1961, 1962), Schonborn (1962), Heal (1964) и др. о том, что несмотря на значительную эвритопность — экологическую пластичность — раковинных корненожек, существуют определенные эколоичегские границы их обитания.

В Каховском водохранилище как уже указывалось большинство видов было найдено почти на всех биотопах. Но частота встречаемости и численность одного и того же вида на грунтах различного типа не одинакова. Это естественно говорит о различных оптимальных условиях, необходимых для различных видов. Таким образом грунты различного типа будут всегда отличаться между собой характером обитающей на них фауны ризопод, характером их ценозов. В ряде случаев эти различия будут обуславливаться в первую очередь количественным развитием раковинных корненожек (как величиной их общей численности на m^2, так и численностью отдельных видов) и частотой встречаемости, а не их видовым составом.

Очевидно для полной характеристики фауны ризопод (ценотических группировок любого биотопа), как и для любой группы гидробионтов, необходимы по крайней мере четыре широко употребляемые в гидробиологии показателя: видовой состав, частота встречаемости, средняя численность каждого вида и общая средняя численность раковинных корненожек, обитающих на данном биотопе. И, конечно, весьма необходимы работы по определению индивидуальных весов (величины биомассы) хотя бы наиболее широко распространенных видов корненожек.

SUMMARY

The species composition and the quantitative development of *Rhizopoda*: *Testacea* fauna from Kachovka[2] reservoir were studied, the considered period including the first years of existence of the reservoir (1955–57) and 9–14 years later. During this period 300 quantitative samples were taken from bottoms of different types.

Eighty nine species of *Rhizopoda*: *Testacea* belonging to 11 genera were found. Most of the species (49) are the representatives of the genus *Difflugia*, 16 species belong to *Centropyxis*. The poorest variety of species and quantitative development are characteristic of the rhizopod taxocenoses on clay and sands: number of species 2 and 19 (respectively), an average quantity — 3400 and 20 800 specimens /m². The taxocenosis of silt is the most abundant: an average number of 1 219 900 specimens/m² for 48 species (Table 1).

Bottoms of various types differ between themselves according to the nature of the *Rhizopoda* fauna inhabiting them and also according to the nature of their cenoses (Table 2).

In many cases these differences first of all depend on the quantitative development of the rhizopods (on their total number per m² and on the number of particular species) and on the occurrence rate of certain species, not on their specific composition in general.

Formation of *Rhizopoda* taxocenoses occurred as bottom sediments of the reservoir appeared. For the 9 year period of existence of the reservoir a uniform complex of the predominating species: *Difflugia oblonga oblonga*, *Difflugia oblonga acuminata*, *Centropyxis aculeata*, *Difflugia bidens* and some others have been formed on silt, sands and soils containing much silt. These species have been the basis for further formation of the common pelophilous taxocenosis (Table 3). For the 14 year period of existence of the reservoir the pelophilous taxocenosis of *Rhizopoda*: *Testacea* has been formed in 3 above mentioned biotopes and this taxocenosis predominates in the reservoir and covers almost the whole bottom square.

ЛИТЕРАТУРА

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Received 10 December 1974

ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 313–320

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Endocytosis in *Paramecium*. I. Effect of Trypsine and Pronase

Synopsis. The treatment of *Paramecium caudatum* and *Paramecium aurelia* with proteolytic enzymes: pronase and trypsine causes at first the inhibition of accumulation of particles in food vacuole, secondly the interruption of food vacuole formation. The surface coat in cytostome is thinner after this treatment.

It has been stated within last years, that the surface coat forms the most external part of the membrane in many kinds of animal cells (Rambourg 1971). It was mainly demonstrated in mammalian cells (Leblond 1950, Rambourg 1966) and, among *Protozoa*, in amoebae (Marshall and Nachmias 1956, Szubinska and Luft 1971, Hausmann and Stockem 1972, Kommnick et al. 1972). Many observations indicate that in these cells the surface coat plays a very important role in the process of pinocytosis and phagocytosis. First of all the contact of the food material with the external part of cell surface is needed for a stimulation of engulfment (Chapman-Andresen 1965, Christiansen and Marshall 1965, Holter 1965, Braatz-Schade and Stockem 1973), secondly the accumulation of ingested particles or substances occurs at the cell surface (Chapman-Andresen and Holter 1964, Korn and Weisman 1967, Stockem and Wolfarth-Bottermann 1969, Hendil 1971, Braatz-Schade and Stockem 1973).

The existence of the cell coat has been also shown in *Tetrahymena* (Nilsson and Behnke 1971), and *Paramecium aurelia* (Wyroba and Przełęcka 1973). For many years it has been well known that the concentration of dyes and particles before the ingestion takes place in *Paramecium* (Mast 1947, Grębecki and Kuźnicki 1956). Nilsson (1971) stated that the concentration of nutrient material also must occur at the coat which covers the cytostome of *Tetrahymena*. The necessity of mechanical (Müller and Töro 1962, Müller et al. 1965) or chemical (Seaman 1961, Seaman and Mancilla 1963, Ricketts 1971, 1972) induction of phagocytosis in Ciliata was also postulated.

Recently it is clear that in all endocytosis processes of cell the surface coat must play an important role. Thus it was interesting to study, how the enzymes which change the structure of the coat influence the process of phagocytosis in two *Paramecium* species.

Material and Methods

Paramecium caudatum and *Paramecium aurelia* growing in lettuce infusion medium were used in this study. The cells were collected by centrifugation and then washed twice with 0.005 M Tris-HCl buffer at pH 7.6. The experiments were carried out 18 h later in room temperature (20–22° C). In order to study the mortality of paramecia in used enzymes the 2 ml samples containing about 400 cells were treated with pronase (Serva) and trypsine (Serva) in the concentration 0.05–0.5 mg/ml during 1 min to 24 h. Enzymes were diluted in 0.005 M Tris-HCl buffer pH 7.6. The moment of the death of 50% of cells was recorded. The experiment was repeated three times. The same procedure was applied to study the phagocytosis — but the suspension of carmine particles was added after 1, 2, 3, 4, 5, 15, 30, 60 min of incubation in enzyme for 10 min. The number of carmine containing vacuoles was counted in the cells fixed with 10% neutralized formaline. Observations of living cells which locomotion was arrested by method introduced by Sikora (1975) were carried out. Preparation for electron microscopy.

Ruthenium red staining (Luft 1971) was applied in 0.15 M cacodylate buffer pH 7.4. The cells were dehydrated through increasing ethanol series and two changes of propylene oxide and then embeded in Epon. The material was sectioned on LKB ultramicrotome. Contrasted with uranyl acetate and lead citrate and non-contrasted sections were examined in the JEM 100 B electron microscope.

Results

The observations of the mortality of *P. aurelia* and *P. caudatum* in various concentrations of trypsine and pronase indicate that these two species are sensitive in different degrees (Fig. 1 and Fig. 2). *P. aurelia* is much more resistant to pronase than to trypsine (Fig. 2). The cells in 0.05 mg/ml pronase live till 24 h, whereas 50% of *Paramecium caudatum* die in this solution after about 45 min, but they live until the next day in 0.05 mg/ml trypsine (Fig. 1).

The formation of food vacuoles in *P. caudatum* is interrupted almost immediately in 0.5 and 0.25 mg/ml trypsine (Table 1). Only a small number of ciliates forms few vacuoles during 10 min exposition to carmine. The treatment with 0.1 and 0.05 mg/ml trypsine causes only slight decrease of the food vacuole formation rate. Almost complete interruption of phagocytosis in *P. caudatum* is caused by all used concentrations of pronase. Only the cells in 0.05 mg/ml pronase solution are able to form the vacuoles during first five minutes of treatment.

In *P. aurelia* all concentrations of trypsine stop the uptake of suspension after

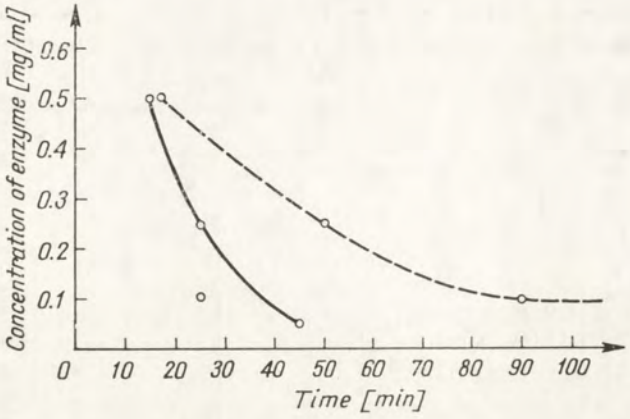

Fig . 1. Mortality of *Paramecium caudatum*. Full line — in pronase, dotted line — in trypsin

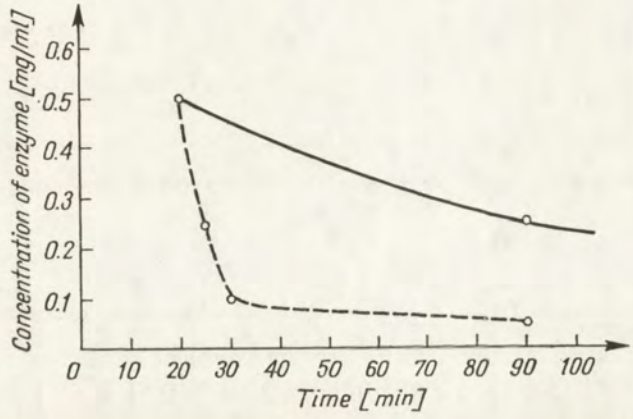

Fig. 2. Mortality of *Paramecium aurelia*. Full line — in pronase, dotted line — in trypsine

5 min treatment (Table 2) almost completely. The vacuole formation in pronase decreases rapidly within 15 min treatment. Even in 0.05 mg/ml pronase, where cells remain alive over 24 h, the paramecia are not able to ingest carmine particles after 30 min exposition to enzyme.

The observations of living cells indicate that in enzyme treated paramecia at first no aggregation of carmine particles (as it is clearly visible in control ciliates) takes place. The paramecia are able to form few vacuoles without suspension, and only later the food vacuole formation is stopped completely. No enlargement of the lower part of cytostome is visible in this stage.

The examination of paramecia in electron microscope reveals the existence of surface coat covering the cytostome. This layer is thinner in cells which are not able to form vacuoles (Plate I).

Table 1

Mean Number of Food Vacuoles Formed in *P. caudatum* after Treatment with Trypsine and Pronase*

Time min	Enzyme								Control
	Trypsine				Pronase				
	0.5	0.25	Concentration mg/ml						
			0.1	0.05	0.5	0.25	0.1	0.05	
1	0	0	5	—	—	—	—	3.93	
2	0	1.66	4.3	—	—	—	—	4.78	
3	0	0.5	3.5	—	—	—	—	3.3	
4	0	0	4.14	—	—	—	—	1.92	
5	0.37	0.62	3.2	1.33	0.04	0.14	0	1.13	5.46
15	1	0.6	3.5	5.25	0	0	0.33	0	4.14
30	2	1	2.6	0.8	0	0	0		
60	—	—	2.4	4	—	—	0	0.1	4.26

* Each average number was calculated from about 100 cells.

Table 2

Mean Number of Food Vacuoles Formed in *P. aurelia* after Treatment with Trypsine and Pronase*

Time min	Enzyme								
	Trypsine				Pronase				
	Concentration mg/ml								
	0.5	0.25	0.1	0.05	0.5	0.25	0.1	0.05	Control
5	0	0	0.1	0.45	2	3.33	2.19	4.14	7.15
15	0	0	0.21	0.13	0	0	0.27	2.17	6.45
30	0	0	—	0	0	0	0.21	0.3	3.03
60	—	—	—	—	—	0.14	0.04	0.1	5.03

* Each average number was calculated from about 100 cells.

Discussion

Nilsson and Behnke (1971) prooved the existence of the surface coat in cytostome of *Tetrahymena pyriformis*. This study indicates that mucous covers also the cytostome of *Paramecium*. The enzymatic treatment has revealed the importance of the surface coat in both phenomena involved in phagocytosis of *Paramecium*: (1) Concentration of particles within food vacuoles. (2) Food vacuole formation.

Trypsine acts on the basic bonds which link the carboxyl group of a basic amino acid (arginine and lysine) to the amino group of another amino acid or to hydroxyl group of an alcohol. Pronase is less specific in action. It contains several proteinases and peptidases (Nomoto et al. 1960 a, b, Trop and Birk 1970) — so it also acts on the glycoproteins. Both enzymes remove the external part of the cell coat. where anionic sites exist. Many authors have stated that the substances which induce pinocytosis in amoebae must be previously bound to the anionic sites (Brandt 1958, Schumaker 1958, Marshall et al. 1959, Chapmann-Andresen 1965, Stockem and Wolfarth-Bottermann 1969, Braatz-Schade and Stockem 1973). Some authors state, that mechanical stimulus is sufficient for promoting endocytosis in Ciliata (Müller et al. 1965, Müller and Töro 1962). Carmine particles ingestion belongs rather to this kind of phenomena. But in the cases of endocytosis where no chemical binding is involved the surface coat plays also an important role (Christiansen and Marshall 1965, Korn and Weisman 1967).

The data from electron microscope indicate that even the removing of the very thin, most external part of the surface coat inhibits the phagocytosis. It means that the interaction between ingested material and the cell takes place in this region. The results of Braatz-Schade and Stockem (1973) showing that only filamentous part of the coat binds different pinocytosis inducing substances, are in agreement with this findings. The mechanical contact may also occur only at the most external part of surface coat. After the enzyme treatment it cannot take place. It may cause that the stimulus needed for vacuole formation is not produced. Another possible explanation is that in first stage no contact and no aggregation of carmine particles can take place and then the vacuoles without the suspension are formed. Later on the structure of the surface coat or even unit membrane is changed to such extent that the membrane of cytostome is unable to "expand" (in spite of the existence of the stimulus).

Observations of the author on *Paramecium caudatum* treated with cytochalasin B indicate that the effect of pronase and trypsine is not due to the influence of this enzymes on filaments involved in the food vacuole formation. In cytochalasin B treated *P. caudatum* the aggregation of carmine particles and enlargement of esophageal sac take place but the separation of food vacuole is impossible.

The differences in the mortality of *P. caudatum* and *P. aurelia* in pronase and trypsine can be explained by different protein components of pellicular complex in this two species.

ZUSAMMENFASSUNG

Die Behandlung von *Paramecium caudatum* und *Paramecium aurelia* mit proteolitischen Enzymen ergab erst eine Anhaltung von Akkumulation der Partikeln in Nahrungsvakuolen, dann eine Unterbrechung der Ausbildung von Nahrungsvakuolen. Mucoidschicht in Cytostome wird dünner nach der Behandlung.

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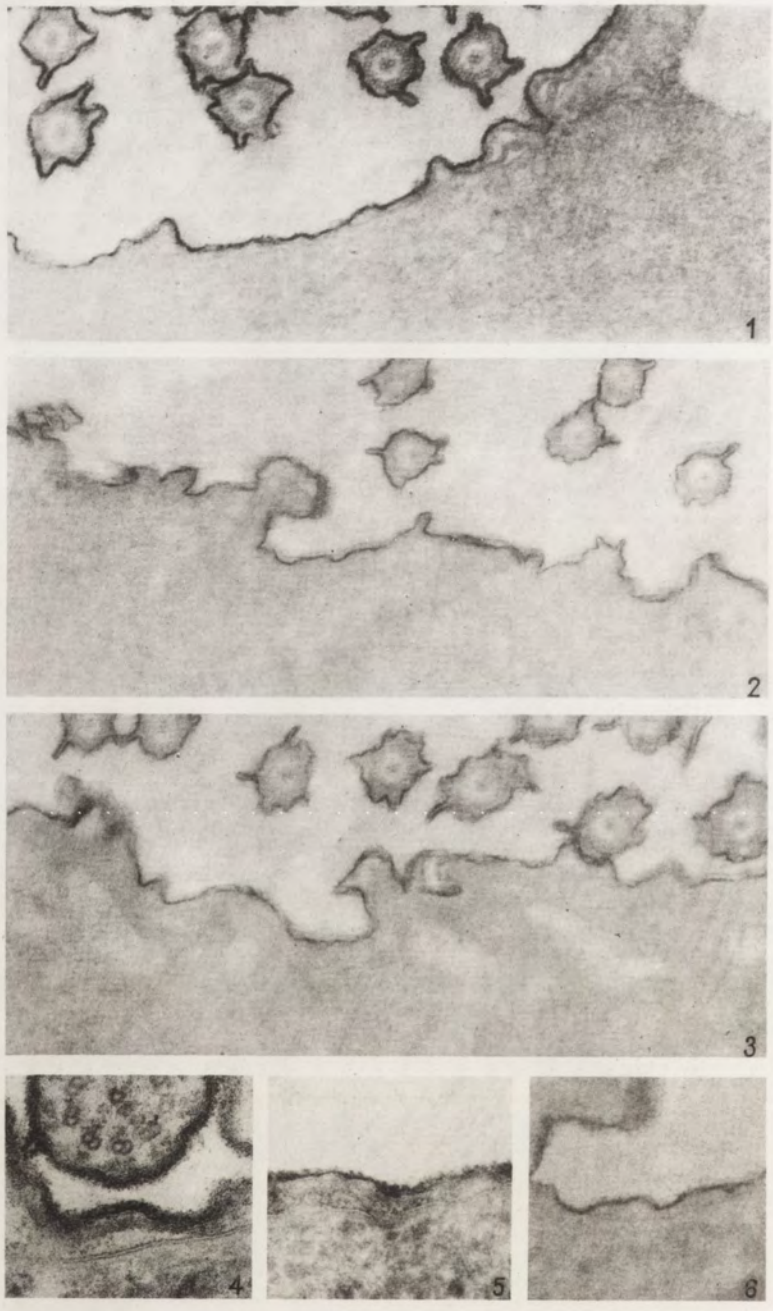
Received 5 December 1974

EXPLANATION OF PLATE I

Fragments of membrane in cytostome of *Paramecium caudatum*:

1: No enzyme treatment. Ruthenium red, no counterstain 48000 ×
2: After pronase treatment (0.05 mg/ml, 5 min). Ruthenium red, no counterstain 48000 ×
3: After trypsine treatment (0.25 mg/ml, 15 min). Ruthenium red, no counterstain 48000 ×
4: No enzyme treatment. Ruthenium red, counterstained with uranyl acetate and lead citrate 99000 ×
5: After trypsine treatment (0.25 mg/ml, 15 min). Ruthenium red, counterstained with uranyl acetate and lead citrate 99000 ×
6: After pronase treatment (0.05 mg/ml, 5 min). Ruthenium red, no counterstain 99000 ×



B. Tołłoczko auctor phot.

ACTA PROTOZOOLOGICA
VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 321–326

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Lipid Composition of the Gregarine, *Stylocephalus mesomorphi*

Synopsis. The gregarine, *Stylocephalus mesomorphi* Devdhar, parasitic in the gut of the coleoptera, *Mesomorphus velliger*, consists of approximately 28.5% total lipid on fresh weight basis. The neutral lipids and phospholipids form about 75% and 25% respectively of the total lipid. The amount of cholesterol is 3.5% of the total lipid. The chief neutral lipids are: monoglycerides, 1,2 diglycerides, 1,3 diglycerides, free fatty acids and triglycerides. The phospholipids are comprised of phosphatidic acid and cardiolipin, phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, lysophosphatidylethanolamine, lysophosphatidylcholine, phosphatidylserine and phosphatidylinositol.

Several earlier studies (Daniels 1938, Dutta 1962, Stein 1963, Vivier et al. 1969, Loubes and Bouix 1970 and Schrevel 1971) on various gregarines have reported the occurrence of lipids. However, it appears that no attempt has been made so far to elucidate the nature of this lipid material (Von Brand 1952 and Dewey 1967). This may be due to difficulties encountered in obtaining sufficient material. Because of the relatively large size (900 μm × 145 μm) of the gregarine, *Stylocephalus mesomorphi*, (Devdhar 1962), which produces heavy infection in the beetle host, *Mesomorphus velliger* it was possible to undertake the present qualitative and quantitative investigation of gregarine lipids.

Materials and Methods

Host specimens of *Mesomorphus velliger* were collected around Dharwar, Karnataka State, India. These were sacrificed on the same day for their gregarine parasites which were collected in insect Ringer solution. After pooling sufficient number of gregarines, they were washed repeatedly with the same solution, centrifuged and weighed after draining.

For total lipid, weighed samples were extracted with chloroform–methanol (2:1, v/v) following the method of Folch et al. (1957). The combined lipid extract was dried over anhydrous sodium sulphate, and the solvent evaporated under reduced pressure on water bath at 40° C. The lipid thus obtained was weighed and dissolved in redistilled chloroform. The neutral lipids and

phospholipids were separated on silicic acid columns with chloroform and methanol respectively, as described by Entenmann (1957). The elutes of the two fractions were again evaporated to dryness, weighed and redissolved in a known volume of pure chloroform. For the separation of different components of neutral and phospholipids, aliquots of each sample were run on silica gel G coated thin layer chromatograms using the solvent system recommended by Katyal et al. (1969). To identify neutral lipids, known standards were run along with samples. Phospholipids were identified by reference to standards prepared from albino rat (Holtzman strain) liver extracts following the procedure of Katyal et al. (1969). Glycerides of neutral lipids were estimated by the modified method of Raghavan and Ganguly (1967). Phosphorus content of phospholipids was determined by the modified procedure of Marinetti (1962). Total cholesterol was estimated in lipid extracts according to the method of Stadtman (1957).

Results

Table 1 indicates that the total lipid content of the gregarine is about 29% on a fresh weight basis, equivalent to 85% on a dry weight basis. Neutral lipids and phospholipids constituted 75% and 25% respectively of the total lipid. As concerns the five components of the neutral lipid, free fatty acids (calculated by difference) formed the major fraction (over 1/3), followed by triglycerides (28.86%). The remaining glycerides were found in relatively small quantities. Cholesterol accounted for about 3.5% of the total lipid or 16.5% of the neutral lipids. Among the components of the phospholipid fraction, phosphatidic acid and cardiolipin were found in large amounts (23.88%) compared with other forms; sphingomyelin was found in negligible amounts (2.31%).

Table 1

Lipid Composition of *Stylocephalus mesomorphi*

(1) Total lipids	(mg per cent fresh wt.)	28.45
(2) Neutral lipids	(mg per cent lipids)	75.01
(i) Monoglycerides	(μg per cent neutral lipids)	13.26
(ii) 1,2 diglycerides	(– – – – ditto – – – –)	11.73
(iii) 1,3 diglycerides	(– – – – – – – – –)	14.28
(iv) Free fatty acids	(– – – – – – – – – –)	34.86*
(v) Triglycerides	(– – – – – – – – – –)	25.86
(3) Cholesterol	(mg per cent lipids)	03.49
(4) Phospholipids	(– – – ditto – – –)	24.99
(i) Phosphatidic acid and Cardiolipin	(μg per cent phospholipids)	23.88
(ii) Phosphatidylethanolamine	(– – – – ditto – – – –)	15.35
(iii) Phosphatidylcholine	(– – – – – – – – – –)	17.69
(iv) Sphingomyelin	(– – – – – – – – – –)	02.31
(v) Lysophosphatidylethanolamine	(– – – – ditto – – – –)	12.94
(vi) Lysophosphatidylcholine	(– – – – – – – – – –)	06.35
(vii) Phosphatidylserine	(– – – – – – – – – –)	08.74
(viii) Phosphatidylinositol and Origin	(– – – – – – – – – –)	13.87

* Approximate quantity calculated on the basis of difference.

Discussion

The present study reveals that unlike carbohydrates (3.1%) and proteins (1.83%) (unpublished), lipids (28.45%) form the major constituent of the gregarine, *Stylocephalus mesomorphi*, comprising such components as neutral and phospholipids, and cholesterol. Since no tangible data is available either on qualitative or on quantitative aspects of the lipids and their components in gregarines, it is felt essential to discuss our findings with those of earlier workers on other parasitic protozoa. The lipid content (28.45%) of this gregarine is much higher than that reported for other parasitic flagellates, rumen ciliates and sporozoa. It is relevant here to mention that whereas in other parasitic protozoa the phospholipids are the major constituents, in this gregarine the neutral lipids constitute the principal lipid (75%) group.

The occurrence of neutral lipids such as: diglycerides, sterols (ergosterol), free fatty acids and triglycerides in *Trypanosoma ranarum* (Halevy and Gisry 1964); monoglycerides, diglycerides and sterols in *Crithidia fasciculata* (Dewey and Kidder 1963); sterols (cholesterol), free fatty acids and triglycerides in *Plasmodium knowlesi* (Morrison and Jeskey 1947, 1948 and Ball et al. 1948), *P. lophurae* and *P. berghei* (Wallace et al. 1965); sterols (cholesterol) and free fatty acids in *Goussia gadi* (Panzer 1913); and sterols (cholesterol/ergosterol) in *T. cruzi* (Von Brand et al. 1959), *T. rhodesiense* (Threlfall et al. 1965 and Williams et al. 1966),

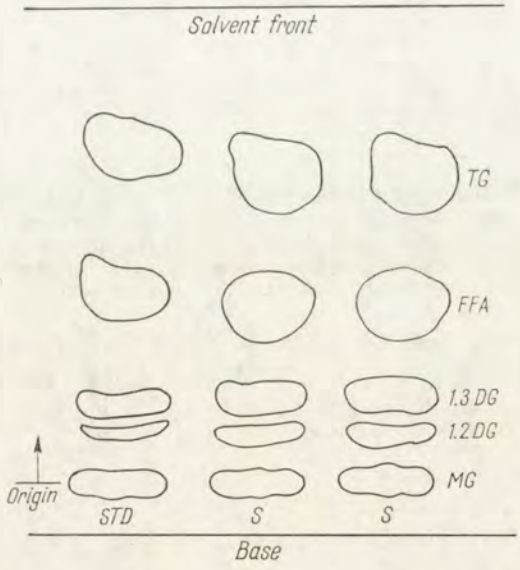

Fig. 1. Thin layer chromatography of the neutral lipids of *Stylocephalus mesomorphi*. Solvent system: n-hexane-diethyl ether–glacial acetic acid (90 : 10 : 1, v/v). S, sample; STD, standard; MG, monoglycerides; 1, 2 DG, 1, 2 diglycerides; 1, 3 DG, 1, 3 diglycerides; FFA, free fatty acids; TG, triglycerides

T. mega (Williams et al. 1966), *Leishmania tropica* (Halevy 1962), *L. donovani* (Ghosh 1963), *C. culicidarum*, *C.* (*Herpetomonas*) *muscidarum* (Halevy 1962) and in *Trichomonas foetus* (Halevy 1963) has been reported. However, the present study indicates the presence of five neutral lipid components viz., monoglycerides, 1,2 diglycerides, 1,3 diglycerides, free fatty acids and triglycerides (Fig. 1) in this gregarine. The cholesterol level is almost the same as reported by Panzer (1913) in *G. gadi*, a coccidium.

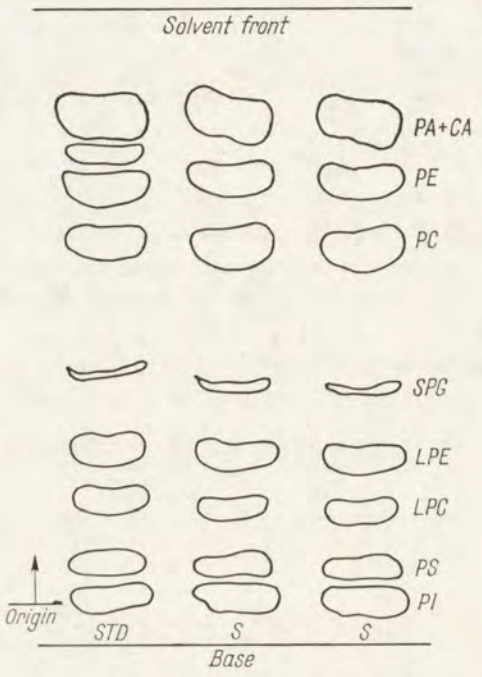

Fig. 2. Thin layer chromatography of the phospholipids of *Stylocephalus* mesomorphi. Solvent system: chroloform–methanol—conc. ammonium hydroxide (25 per cent, w/v)—water (70 : 30 : 4 : 1, v/v). S, sample; STD, standard; PI, phosphatidylinositol; PS, physophatidylserine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanol-amine; SPG, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA + CA, phosphatidic acid + cardiolipin

The only information available on the phospholipid components of flagellates by chromatographic method is from Hack et al. (1962). They reported the occurrence of phophatidylethanolamine, phosphatidylcholine and phosphatidylinositol in *C. fasciculata*, *C. luciliae*, *C.* (*Herpetomonas*) *culicis*, *T. cruzi* and *L. donovani*. The crithidians were found to posses cardiolipin but not trypanosomes and *Leishmania*. Of the eight components of phospholipids (Fig. 2), the occurrence of cardiolipin in this gregarine could not be ascertained with certainity due to the similar Rf values of both phosphatidic acid and cardiolipin. The sphingomyelin which is reported to be absent in flagellates and ciliates (Hack et al. 1962) is present in this gregarine.

The significance of the occurrence of various phospholipids in the gregarine is presumably attributed to the formation of intracellular membrane as well as to that of mitochondrial configuration (Petrushka et al. 1959 and Green and Fleischer 1963). They are also known to be actively involved in the transport of sodium ions through cell membrane (Wolfe 1964). The presence of high amount (28.86%) of triglycerides in this gregarine suggests their probable function as reserves for biosynthetic and energetic purposes.

RESUMÉ

Stylocephalus mesomorphi Devdhar, grégarine parasitaire de l'intestin du coléoptere *Mesomorphus velliger*, contient au total 28.5% de lipides par rapport au poids du matériel frais. Les lipides simples constituent environ 75% du contenu total des lipides, et les phospholipides 25%. La part du cholésterol s'elève à 3.5% du contenu total des lipides. Les lipides simples sont réprésentés en prémier lieu par les monoglycérides, 1,2-diglycérides, 1,3-diglycérides, acides gras libres, et les triglycérides. Parmi les phospholipides on trouve làcide phosphatidique et la cardiolipine, phosphatidylethanolamine, phosphatidylcholine, sphinogomyeline, lysophosphatidylethanolamine, lysophosphatidylcholine, phosphatidylserine et le phosphatidylinositol.

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Received 26 September 1974

ACTA PROTOZOOLOGICA
VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 327–336

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Effects of Ultraviolet Irradiation on the Carbohydrate Metabolism of *Paramecium aurelia*

Synopsis. The chromatographic analysis of the polysaccharide purified from *Paramecium aurelia* showed the presence of galactose and arabinose as monomers in 1 : 3.14 ratio. The total polysaccharide level increased in first five minutes exposure to UV irradiation of 7.829×10^{-12} ergs at 2537 Å. Later on, the level gradually depleted with duration of irradiation. The total free sugar levels in the ciliates showed an opposite trend. When the active paramecia were incubated in a medium consisting of glucose, galactose and arabinose, it is demonstrated that the sugar is incorporated into the polysaccharide. This *in vitro* synthesis is affected by irradiation time. Five minutes irradiation inhibited the synthesis with all substrates. Irradiation for longer periods inhibited incorporation of glucose but not the other monomers. The amylase activity at 5.2 pH increased substantially during 10 min irradiation. Later on, the original activity was restored. Irradiation affected the oxygen consumption of the organisms and resulted in cytolysis. It is discussed that UV irradiation disturbs the carbohydrate metabolism of the paramecia causing alteration in the amylase molecule and ultimately denatures the enzymic activity.

Introduction

The effects of UV rays on the growth and metabolism of protozoans are multifarious (Giese 1941). UV irradiation increases the vacuolar activity of *Amoeba proteus* (Rinaldi 1959 a), induces pinocytosis in *Amoeba* (Rinaldi 1959 b), causes cytolysis (Weinstein 1930) and plasmolysis in *Paramecia* (Hutchinson and Ashton 1931) and alters the sodium and potassium ion movements in *Tetrahymena* (Andrus 1961). However, the physiological state of the protozoan determines these UV irradiation effects (Giese 1967). For example, starvation or nutritional status (Giese and Reed 1940), species-specific resistance to irradiation (Alpatov and Nastjukova 1934) and the state of hydration (Shepard 1956) influence and determine the extent of cellular or metabolic damage by irradiation. All these reports have been oriented to demonstrate the effects of UV irradiation localized in the cytoplasm of ciliates. Some of these effects are sensitization to heat, changes in ciliary activity,

immobilization etc. (Giese and Crossman 1946). All these changes are known to occur due to molecular lesions leading to changes in the structural or enzymatic proteins caused by UV irradiation. In spite of these a few attempts have been made to study the effects of irradiation on enzymatic activity and respiratory metabolism (Herbert and Roth 1953). In this paper, a few changes in the carbohydrate metabolism of *Paramecium aurelia* on time course of exposure to UV rays have been described.

Materials and Methods

Stock Culture

Stocks of the ciliate, *Paramecium aurelia* were grown in autoclaved Dryl's solution (Dryl 1970). These were fed once in three days with *Aerobacter aerogenes* grown separately on agar slants. Once a month, the culture medium was tested for bacterial contamination. The culture was maintained purely monaxenic. The ciliates were fed 48 h prior to experimentation.

UV Source

The UV source consisted of a Philips UV tube mounted inside a chamber. This emitted UV rays of wavelength 2537 Å (7.829×10^{-12} ergs). The samples were placed at a constant distance of 54 cm from the source. For studying the various effects, the exposure time was altered.

Estimation of Total Polysaccharides

After UV irradiation for 5, 10, 15, 20 and 25 min, the ciliates were collected into pellets by centrifugation for 5 min at 2000 rpm. The pellet was dissolved in 2 ml of 1 N sodium hydroxide and heated for 10 min. After cooling, 3 ml of ethanol was added for precipitating the polysaccharide. The precipitate was dissolved in 5 ml of anthrone reagent (0.1% anthrone in 72% sulphuric acid) and boiled for 10 min, cooled and read at 630 nm in Beckman DU_2 spectrophotometer (Carrol et al. 1956). Pure glucose was used as standard.

Estimation of Proteins

The proteins were estimated by the method of Lowry et al. (1951). The samples were prepared by deproteinizing with 10% trichloroacetic acid and the precipitate was dissolved in 0.1 sodium hydroxide.

Chromatographic Analysis

The polysaccharides were precipitated with ethanol as described previously and hydrolysed in 6 N hydrochloric acid and spotted for chromatography. Using Whatman No. 1 filter paper, two dimensional descending chromatograms were run using n-butanol: acetic acid: water (4 : 1 : 1, v/v/v) as the first solvent and ethyl acetate : acetic acid : water (3 : 3 : 1, v/v/v) as the second solvent. Aniline hydrogen oxalate was used as spray (Charalampous and Mueller 1953). Glucose, galactose and arabinose standards were purchased from the British Drug Houses, Private Ltd.

For quantitative studies, parallel samples were run using the same amount of samples (25 iambda). One was sprayed and in the other the area was identified. The cut strips were refluxed In 2 ml of distilled water for two hours. The colour was developed with anthrone reagent and read in Beckman DU_2 spectrophotometer at 630 nm.

Estimation of Total Free Sugars

The procedure adapted was similar to that of total polysaccharides, but the pellet obtained after centrifugation of the culture was directly homogenized in absolute alcohol and the free sugars in the supernatant fluid was determined using anthrone reagent.

In vitro Synthesis of the Polysaccharide

The ciliates were irradiated and immediately carbon dioxide was bubbled into the medium for 2–3 min. After 30 min, one ml aliquots were taken. With one aliquot, protein was estimated by the method of Lowry et al. (1951). With the second aliquot, the immediate level of polysaccharide was determined. This served as the control. The third aliquot was incubated at 25° C with glucose or galactose or arabinose for 30 min and the amount of polysaccharide was determined. To the fourth aliquot, 1 ml of epinephrine was added (0.001 g/ml) and after 30 min incubation at 25° C, the total polysaccharide content was determined.

Amylase Activity

After irradiation, the pellet of the ciliates was obtained and homogenized in distilled water. The supernatant was used as the enzyme sample and the amylase activity was determined as described by Bernfeld (1955). The substrate consisted of 1% starch solution buffered with citrate-phosphate buffer from pH range 5.0 to 6.0 and 0.0067 M sodium chloride. Proteins were estimated in the enzyme sample by the method of Lowry et al. (1951).

Respirometry

About 12 000 ciliates were concentrated in two ml of medium and introduced into a Warburg flask. 0.5 ml of freshly prepared 10% potassium hydroxide was placed in the centre well and the rate of oxygen consumption was determined using Warburg apparatus (Precision Instruments, Chicago) at 25° C. The thermobarometer consisted of 2 ml of distilled water. For irradiation studies, the ciliates were exposed to UV rays in the Warburg flask prior to respirometry.

Results

48 000 organisms yielded one mg of protein as estimated by the method of Lowry et al. (1951). Active paramecia show 730 ± 36 µg of polysaccharide per mg protein (Fig. 1).

Paper chromatographic analysis (Fig. 2) showed that the polysaccharide is a complex carbohydrate with galactose and arabinose polymers.

On irradiation, the levels of the carbohydrate expressed per unit protein showed variations (Fig. 1). Five minutes exposure increased the carbohydrate or polysaccharide level. Later on, the level gradually depleted with time of irradiation. It is evident from Fig. 1 that the total free sugars per unit protein decreased initially and showed a slight increase upto 10 min UV irradiation. After 10 min irradiation, the total free sugars gradually depleted to a lower value.

The monomer composition is not affected by UV irradiation. (Table 1) since the ratio of arabinose to galactose were maintained constant despite the irradiation time.

When the active paramecia were incubated in a medium consisting of glucose,

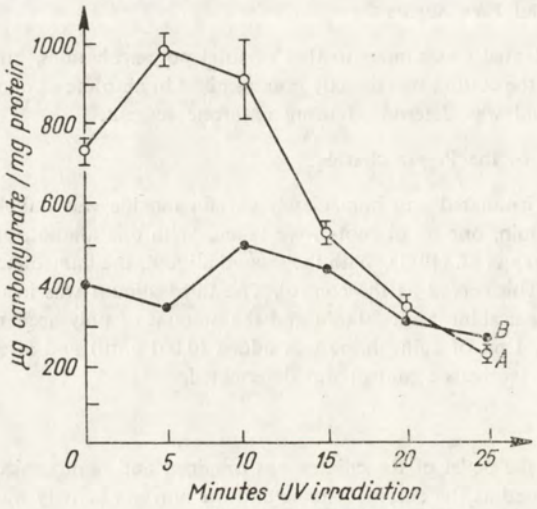

Fig. 1. Changes in the levels of carbohydrate content of *Paramecium aurelia* due to UV irradiation. A — Total polysaccharide content, B — Total free sugar level

Table 1

Changes in the Monomer Component Levels of the Polysaccharide of *Paramecium aurelia* Due to UV Irradiation

Irradiation time (minutes)	Ratio of Arabinose/Galactose
control	3.14
5	3.14
10	3.18
15	3.22
20	3.19
25	3.17

Table 2

In vitro Synthesis of Polysaccharide in *Paramecium aurelia*

Irradiation time (minutes)	Rate: µg polysaccharide/h/mg/protein Substrate		
	Glucose	Galactose	Arabinose
Control	62.5±5.2	28.7±9.2	18.45±2.31
5	Inhibited	Inhibited	Inhibited
10	Inhibited	3.53	0.45
15	Inhibited	4.87	3.41
20	Inhibited	—	—
25	Inhibited	—	—

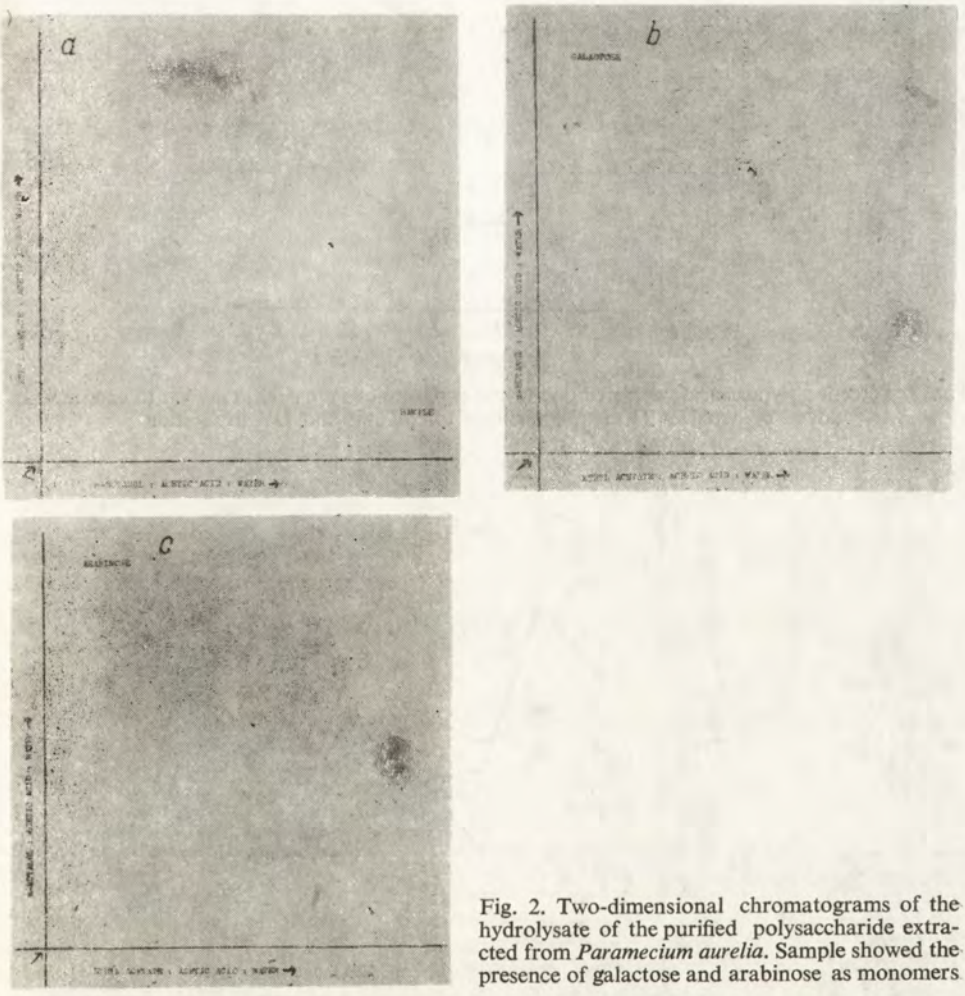

Fig. 2. Two-dimensional chromatograms of the hydrolysate of the purified polysaccharide extracted from *Paramecium aurelia*. Sample showed the presence of galactose and arabinose as monomers

galactose or arabinose, it is shown that the sugar is incorporated into a polysaccharide. Among these sugars, glucose seems to be efficiently incorporated than galactose and arabinose. *In vitro* synthesis of polysaccharide is affected by irradiation time (Table 2). Five minutes irradiation completely inhibits the polysaccharide synthesis with all substrates. Irradiation for longer periods inhibits glucose incorporation completely, but galactose and arabinose are utilized by the organisms for synthesis.

Figure 3 illustrates the changes in the level of the total polysaccharide due to exposure of organisms to UV irradiation and epinephrine. Epinephrine is known to induce glycogenolysis in higher animals. Under epinephrine *in vitro* stress, the paramecia deplete 30% of the total polysaccharide content after 5 min UV irradiation. Later on, the polysaccharide is restored to about a level of 10% depletion of

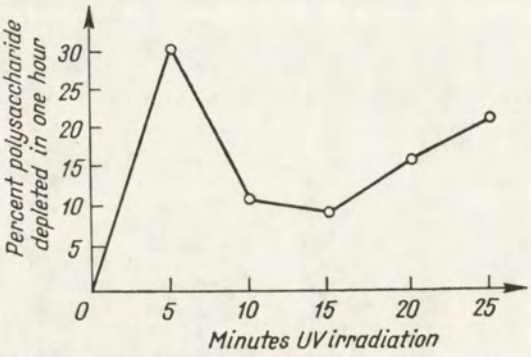

Fig. 3. Percent polysaccharide depleted due to the exposure of *Paramecium aurelia* to exogenously added epinephrine (1 mg/ml) medium for 30 min and UV irradiation

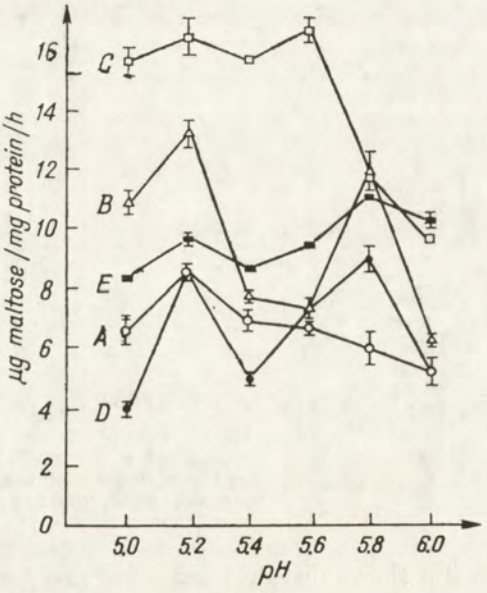

Fig. 4. Specific activity of amylase in the neutral homogenates of *Paramecium aurelia*. A — Control homogenate, B, C, D and E — Homogenates of 5, 10, 15 and 20 min UV irradiated *Paramecium aurelia* respectively

the total polysaccharide. Irradiation for longer duration tends to increase the depletion of the polysaccharide.

The amylase activity (Fig. 4) is drastically affected by UV irradiation. The specific activity at 5.2 pH increases substantially during 10 min irradiation. Later on, the original activity is restored. The influence of pH on the enzyme activity is affected by irradiation. The control paramecia showed only one activity peak in the pH activity profile. Irradiation increased the number of peaks, the second one appearing at 5.8 pH.

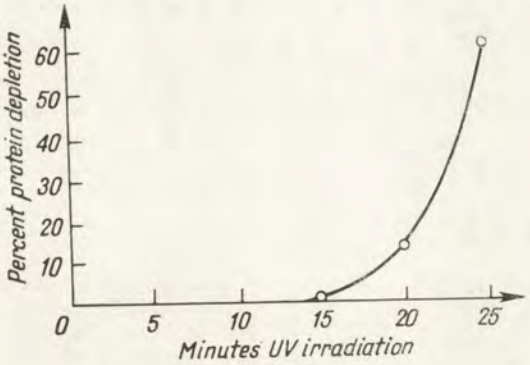

Fig. 5. Percent depletion of endogenous protein of *Paramecium aurelia* due to UV irradiation

Figure 5 illustrates the depletion of the total protein content. There was no change in the total protein content of the organisms up to 10 min duration. 25 minutes exposure seems to be lethal as the organisms lose 60% of proteins.

Irradiation affects oxygen consumption of the organisms. Five min exposure increased the rate of oxygen consumption. The consumption decreased rapidly on further exposures. After 25 min irradiation, there was practically no respiration (Fig. 6).

Discussion

The occurrence of a polysaccharide with galactose and arabinose monomers in *Paramecium aurelia* is an interesting observation since typical animal glycogen as in *Tetrahymena pyriformis* (Manners and Ryley 1952), starches as in flagellates (Bourne et al. 1950), amylopectin in rumen ciliates and paraglycogen as in gregarines (Ryley 1967) are known to occur as storage carbohydrates in protozoa. The polysaccharide of the present study maintains a monomer ratio of arabinose: galactose as 3.14 irrespective of irradiation effects. *In vitro* the animals absorb glucose to synthesize this polymer more efficiently than galactose or arabinose (Table 2). It is presumed that there must be some enzymatic mechanism existing in the organism *in vivo* to convert absorbed glucose into arabinose and galactose as the latter sugars are not present in the medium.

The results presented clearly demonstrate that the effects of UV irradiation are time dependent. The time course of changes observed in specific amylase activity (Fig. 4), epinephrine sensitive glycogenolysis (Fig. 3), oxygen consumption (Fig. 6) and catabolism of total polysaccharide and free sugars (Fig. 1) illustrate the typical "shock reactions" described by Grainger (1958). There are overshoot responses as seen in amylase activity, QO_2 and polysaccharide level due to the irradiation of animals for 5 min contrary to an "undershoot" response as described in free sugar level (Fig. 1) and *in vitro* polysaccharide synthesis (Table 2).

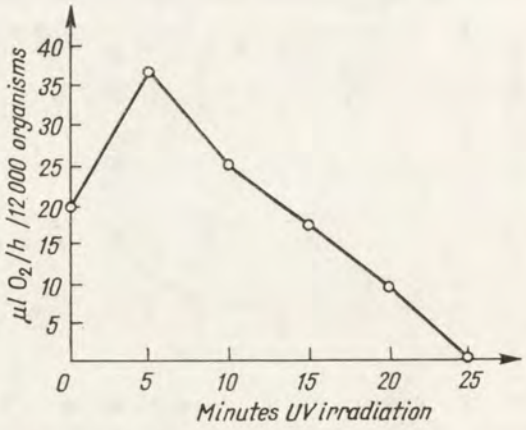

Fig. 6. Oxygen consumption (µl O_2/h/12000 *Paramecium aurelia*) measured in Warburg flasks at 25°C in Dryl's solution

The organism seems to show certain adaptation for the irradiation phenomenon, although the latter causes appreciable amount of damage in the form of cytolysis (Fig. 5) on longer exposures. This adaptation is noticeable between 5 and 15 min period of irradiation. Upto this period, the UV irradiation is not manifested in lethal effects (Fig. 5). Longer exposures resulted in permanent impairments like cytolysis (Fig. 5) and death (Fig. 6) probably due to the effects of UV rays causing molecular lesions leading to structural and enzymatic changes.

Another interesting observation from the present data is the impairment of carbohydrate metabolism in the organism due to UV irradiation. Synthesis of polysaccharide (Table 2) is inhibited by the exposure. Longer exposures increase the catabolization (Fig. 1) of glycogen as well as free sugars, inhibit the oxygen consumption (Fig. 6) and amylase activity (Fig. 4). UV irradiation also brings forth an 'anomaly' in the pH profile curves of amylase activity. Normal organisms inhibited only one peak activity at pH 5.2, but on either longer of shorter exposure to UV rays, the enzyme inhibited another pH optimum around pH 5.8. The appearance of new peak could be attributed to the release of SH groups of the enzyme due to UV irradiation as has been shown in many enzymes subjected to specific agents releasing the SH groups (Katz 1970). It is presumed that the UV irradiation causes an alteration in the amylase molecule and ultimately denatures it so that the specific activity is lowered.

ACKNOWLEDGEMENTS

Our grateful thanks are due to Dr. R. V. Krishnamoorthy, Associate Professor and Head of the Department of Zoology, University of Agricultural Sciences, Bangalore for his valuable suggestions and to the Bangalore University for the award of a scholarship to one of us.

ZUSAMMENFASSUNG

Die chromatographische Analyse von Polysaccharid, das aus *Paramaecium aurelia* in reiner Form erhalten wurde, zeigte die Anwesenheit von Galaktose and Arabinose als Monomere im Verhältnis 1:3.14. Die Gesamthöhe des Polysaccharids nahm zu in den ersten 5 Minuten bei UV-Bestrahlung von 7.829×10^{-12} erg bei 2537 Å. Mit weiterer Bestrahlung nahm die Polysaccharidhöhe allmählich ab. Die Gesamthöhen von freiem Zucker bei den Ziliaten weisen dagegen den umgekehrten Verlauf auf. Als die aktiven Paramecien in einem aus Glukose, Galaktose und Arabinose bestehenden Medium inkubiert wurden, wurde nachgewiesen dass der Zucker in das Polysaccharide inkorporiert wurde. Diese *in vitro*-Synthese wird von der Bestrahlungsdauer Beeinflusst. Bestrahlung über 5 Minuten verhinderte die Synthese bei allen Substraten. Bestrahlung über längere Perioden verhinderte den Einbau von Glukose, aber nicht den Einbau der anderen Monomere. Die Amylasenaktivität nahm bei 5.2 pH während einer Bestrahlungsdauer von 10 Minuten wesentlich zu. Danach wurde die anfängliche Aktivität wiederhergestellt. Die Bestrahlung beeinflusste auch die sauerstoffaufnahme der Organismen und führte zur Zytolyse. Es wird auch gezeigt, dass die UV-Bestrahlung den kohlehydratenabbau der Paramaicien stört und dedurch zur Veränderung des Amylasemoleküls und schliesslich zur Denaturierung der Enzymaktivität führt.

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Received 2 December 1974

ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 337–361

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Morphometric Study of Moving *Amoeba proteus*

Synopsis. Relative sizes of the uroid, trunk, contracting and advancing pseudopodia, and of the subdivisions of trunk into its posterior, middle, and anterior segments, were evaluated from the area of profiles (optical cross-sections) of amoebae recorded with time-lapse cinematography. Comparison of successive stages of locomotion at 10 s intervals permits to calculate the specific contraction or expansion rates of each body region, and its contribution to the general shape evolution in moving amoeba, and to reveal a decreasing contraction/increasing expansion gradient from the uroid up to the bases of advancing pseudopodia. The bearing of these qualitative data on the elucidation of the modalities of amoeba locomotion and of the direction of the motive force action is discussed.

Introduction

The total volume of *Amoeba proteus* measured first by Brachet (1955) is the only morphometric parameter known from the abundant literature on free-living amoebae. There is no information at all concerning the size of distinct regions of amoeba's body and their changes during locomotion. Andresen (1973) in his most recent review of morphology of amoebae gives only a general definition of their basic body regions: "An amoeba normally shows three distinct regions during locomotion: pseudopodia, a central zone from which the pseudopodia originate, and the uroid, which forms the posterior part of amoeba". The changes in size of different body regions in a moving amoeba are usually described only in very general terms and descriptions given by various authors do not always corroborate each other. The reason is that they are all based on rough visual estimations. We can quote a few examples of such approximative statements: "The gel in the tail liquefies after it has contracted to about a quarter of its original length" (Goldacre and Lorch 1950), and "Tail ectoplasm shortens as cytoplasm flows out of it" (Allen 1973), or "The cell uroid... underwent only limited changes in shape; the shortenings were most marked in the cell region just in front of the uroid and the constriction ring..." (Korohoda 1970). All such descriptions are not supported by any

quantitative records, nevertheless we are forced to use them, because the evolution in size of distinct regions of amoeba during locomotion is essential for the elucidation of mechanisms of amoeboid movement. The purpose of the present study is to fill this gap in our knowledge about the morphometry of moving *Amoeba proteus*.

General Techniques

Amoebae were cultured in Chalkley medium and fed on *Colpidium* sp. Prior to the experiments, samples containing about 20 specimens each, were washed and purified from the detritus occurring at the bottom of culture, by means of a few transfers through small containers filled with the clear fluid collected from the upper layer of the same culture. This way of proceeding to wash amoebae excludes any changes of the physical or chemical properties of their medium. For observation and filming, the washed amoebae were transferred with a few drops of surrounding fluid on a microscopic slide and covered with a cover-glass supported with glass rods of about 0.5 mm in diameter.

The interference contrast device PZO MPI 3 was used with the microscope PZO MB 30. Pictures were taken with Bolex H 16 Reflex movie camera controlled by the time-lapse equipment Variotimer Paillard-Wild. The locomotion of amoebae was recorded with the frequency of 1 frame per second, which with a standard frequency of projection results in a 24-fold acceleration of movement. With total microscope power equal to 50 ×, amoebae remain in the field for 1–2 min (or longer if they change the direction of locomotion during recording) and the number of pictures taken in that time is largely sufficient to analyse a sequence of stages of locomotion.

The exposure time was 0.2 s. The movement is so slow that the cell profile is still sharp on the pictures, even after such a long exposure, which on the other hand makes possible to reduce considerably the light intensity and to restrain its influence on the motory behaviour of amoebae.

For analysis, the profile of a moving amoeba, and some reference points on the substrate were redrawn on the tracing paper from the projections of each 10th frame. So eventually, the locomotion was represented by a sequence of successive stages spaced at 10 s intervals.

In total the locomotion of about 100 individuals has been recorded for this study. The number of individuals, or of the pairs of locomotion stages matched to analyse an interstage period, which were used for specific measurements, will be given further in the text.

Definition of the Body Regions in Amoeba

It is evident that, for identification of the particular body regions in a moving amoeba, we cannot refer to the static marking points on the substrate which were used in all earlier cinematographic studies of amoeboid movement, but we have

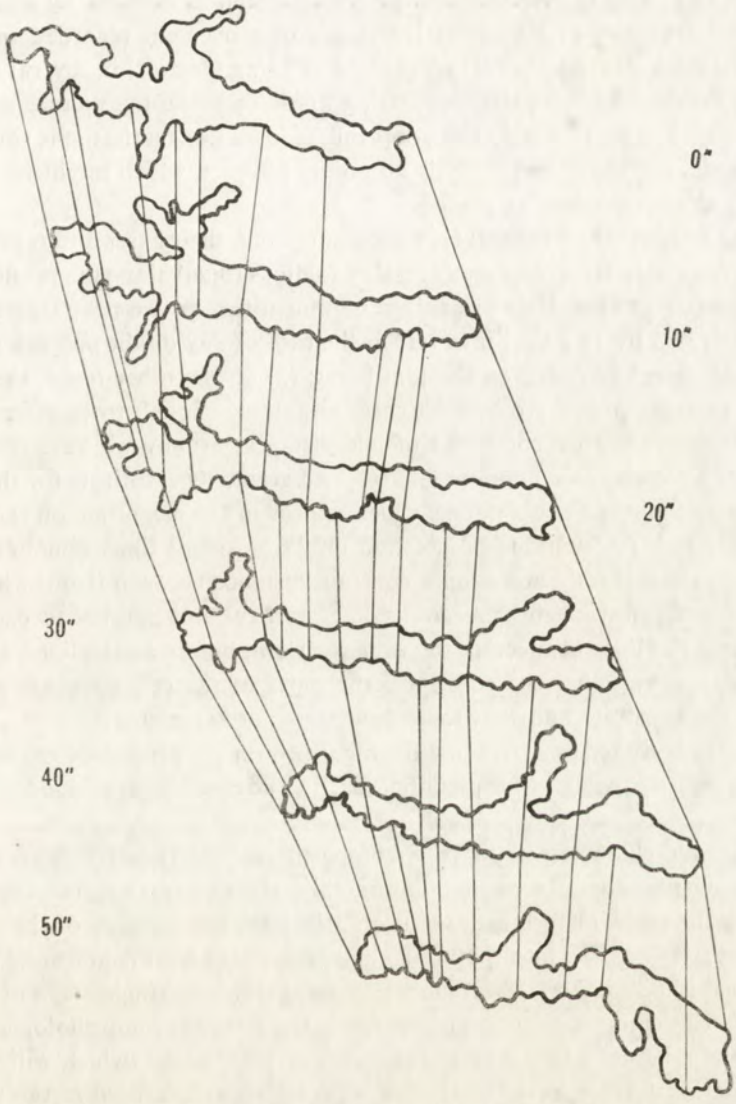

Fig. 1. Identification of moving marking points on the outline of the profiles of an amoeba recorded in six successive stages of its locomotion (individual No. 1)

to rely on some moving reference points to be chosen on the cell surface itself. This is a main difficulty which probably hampered in the past any attempt to analyse the amoeboid movement along this line, because it is perfectly true that amoeba cannot be described in any terms of static morphology but, as Abé (1961) said, only in terms of a "dynamic structure".

Nevertheless, when following the stages of locomotion recorded with the time-lapse technique, we can find on the outline of profiles of moving cells some reliable

marking points. An example of identifying such points is brought by Fig. 1 which presents the sequence of six successive stages of movement recorded in a single individual during 50 s. The points recognized as being identical in several stages are shown on the drawing with thin connecting lines. To make the picture more clear only the points lying on the right sides of profiles (down side on the figure) are marked but, of course, for the actual analysis all points are used which might be identified all along the contour line of profiles.

Figure 1 puts in evidence that: (1) the pseudopodia, their bases and ramifications, the uroid constriction ring, and many smaller foldings found along the profile outline, are stable enough to be safely recognized and identified in two near stages of locomotion separated by 10 s time interval; in the case of any doubt one can refer to 9 intermediary stages recorded on the film band; (2) on the other hand, the foldings chosen as marking points are in most cases not stable enough to be referred to all along the whole sequence composed of many stages; usually we have to abandon some points which become no more reliable, and gradually substitute for them other points corresponding to new foldings which appear in the meantime on the contour line, (3) only very few marking points, and only for a short time, remain immobile in respect to the substrate and keep a constant distance between them, which is represented on the drawing by connecting lines vertical and parallel to each other; in most cases the lines connecting the same marking points are inclined at various angles, which proves that the corresponding parts of the cell body are shifted in respect to the substrate and that their dimensions are changing.

Four main body regions were first distinguished on the profiles of moving amoebae: the uroid, the contracting pseudopodia, the advancing pseudopodia, and the trunk.

The dynamic character of the morphology of amoeba (which means, e.g., that the advancing pseudopodia become contracting, the contracting pseudopodia are resorbed by the trunk or fuse with the uroid, the posterior sections of the trunk are incorporated step by step into the uroid) makes impossible to delimit and to determine the main body regions in an amoeba recorded in one single stage of its locomotion. We can define without ambiguity only the transitory morphological condition of amoeba between two subsequent stages of locomotion (which will be called interstage period further in the text), i.e., with the technique used in this study we can decide what a given body part of amoeba it actually is, judging from its further fate and shape transformation after 10 s.

Figure 2 explains this principle in the case of pseudopodia. The same pseudopodium at the stage 20″ is recognized as advancing when compared with the stage 10″ (10 s earlier), but it must be considered as contracting relative to the stage 30″ (10 s later). So, a body part may be unequivocally defined on the dynamic way in relation to two successive stages of locomotion (i.e., for an interstage period), but in one single stage of movement, statically, it cannot be defined at all.

Usually there is no difficulty to find the marking points permitting to trace the

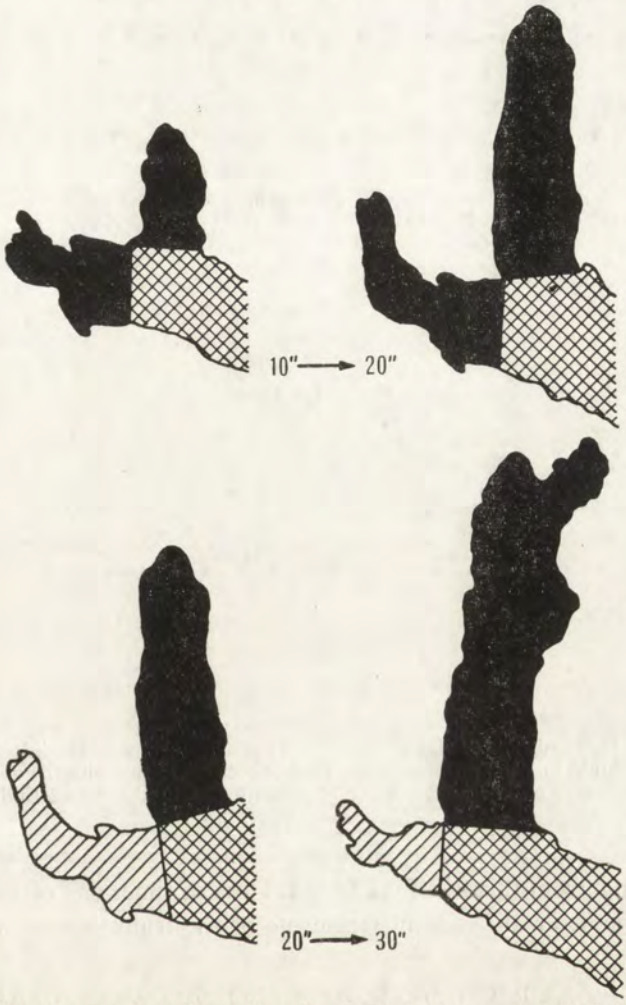

Fig. 2. Conversion of a pseudopodium advancing in the interstage period 10″—20″, into a contracting pseudopodium in the period 20″—30″; the advancing pseudopodia black, the contracting pseudopodia dotted with oblique strokes, the trunk hatched (individual No. 13)

line separating a lateral pseudopodium from the trunk. It becomes more difficult to lay out a borderline between the trunk and the uroid. The ectoplasm constriction ring at the base of the uroid described by some authors (e.g. Korohoda 1970) is not always very distinct; moreover, this constriction appears shifted more and more forwards at each next stage of locomotion because more and more segments of trunk, and occasionally some lateral contracting pseudopodia, are incorporated into the uroid. In the case of three stages of movement of an individual represented in Fig. 3, according to the principle of dynamic definition of body regions in amoeba, it is necessary to trace the borderline separating the uroid from the trunk in one way for the interstage period 0″–10″, and in another way for the interstage period

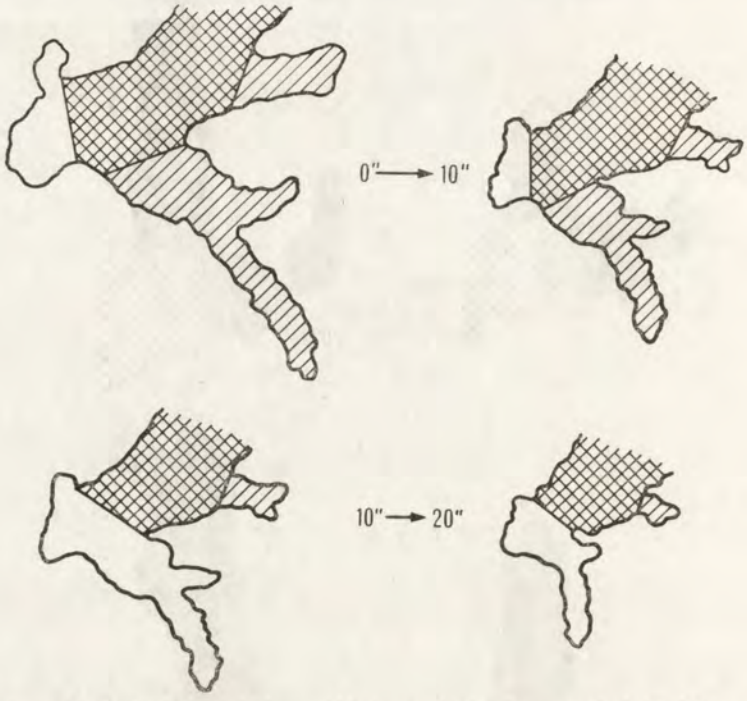

Fig. 3. Change of the borderline of the uroid between the interstage periods 0″–10″ and 10″—20″ due to the incorporation into the uroid of a contracting pseudopodium with the adjacent posterior portion of the trunk; the uroid white, other regions dotted as in the Fig. 2 (individual No. 25)

10″–20″. It results from this fact that, in that lapse of time, one of the contracting pseudopodia together with adjacent fragments of the trunk became a part of the uroid.

A similar, although in some way opposite problem is arising when the borderline between the trunk and the advancing pseudopodia is to be traced. In fact, in the anterior part of amoeba, the areas which earlier were advancing pseudopodia, constitute later the frontal portion of the trunk.

Definition of the trunk results directly from the definitions of other body regions: the profile of the trunk is constituted of what remains after delimitation of the profiles of the uroid and of all contracting and advancing pseudopodia.

Figure 4 gives an example of a sequence of five stages of locomotion of amoeba in which all body regions were defined and separately marked for all four transient interstage periods. It demonstrates that only if a long sequence composed of many stages is considered as a whole, our judgments might be in some extense subjective, because we must base on qualitative estimations, when we gradually shift the borderlines between body regions, to follow the gradual changes in the dynamic structure of amoeba. On the contrary, when we take into consideration just two succes-

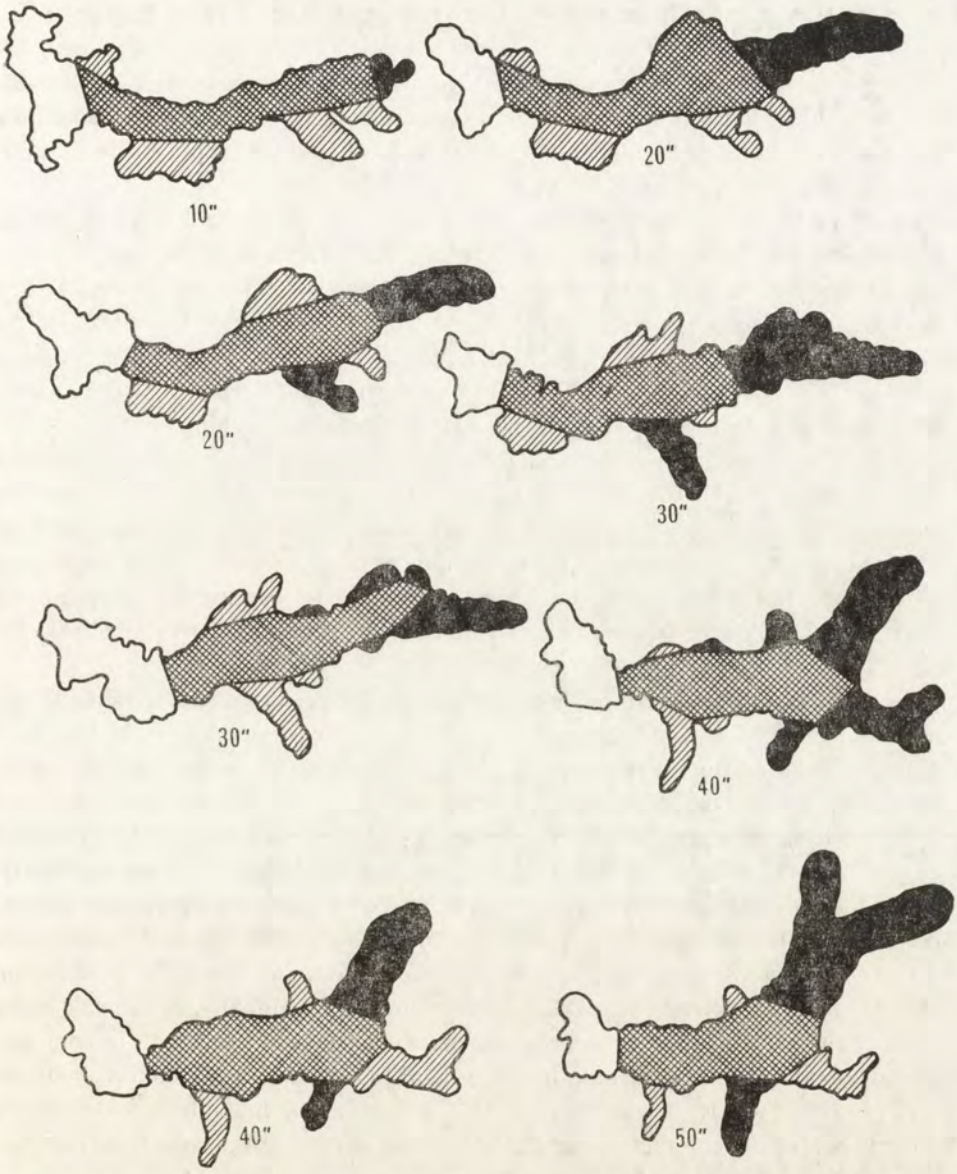

Fig. 4. Delimitation of the uroid, the trunk, the contracting and the advancing pseudopodia in four interstage periods formed by five successive stages of locomotion of a single amoeba (individual No. 5); all regions are dotted as in the Figs. 2 and 3

sive stages of locomotion, i.e., when we are describing the transitory morphological state of amoeba and its evolution in one single interstage period, all body regions proove to be delimited unequivocally and are strictly comparable between two coupled stages.

Quantitative Assessment of the Changing Size of Body Regions

First measurements were done on 12 moving amoebae registered during 20–70 s, i.e., 12 individual series were used containing each 3–8 stages of locomotion recorded at 10 s intervals. All this material in total could be arranged in 65 pairs of successive stages of locomotion (or interstage periods).

All profiles were redrawn on the tracing paper, cut out, and divided along the lines separating four main body regions (which were traced as in the case shown in Fig. 4). The dimensions of the uroid, the contracting pseudopodia, the trunk, and the advancing pseudopodia, were calculated from the weight of their profiles cut out from the complete profile of amoeba. With the average weight of a complete profile of amoeba amounting 137.15 mg and the readout accuracy of 0.05 mg of the analytical balance used, we can neglect the error in weighing.

It is to be reminded that the changes in size of distinct body regions of amoeba calculated with this method are expressed as the evolution of area of their profiles (their optical projections)[1], and not as their volume evolution. It is impossible to obtain volumetric data neither directly because we have no stereometric techniques of recording the movement of amoebae, nor indirectly because the irregular cell shape excludes a possibility to find any formula for calculating the volume from the area.

For that reason it seems more justifiable, instead of presenting the absolute values of area of the profiles and their parts, to determine and to quantify only the most important relationships between them. First, the relative dimensions of the uroid, the trunk, and of the contracting and advancing pseudopodia, were calculated in respect to the total area of complete profile of each amoeba (assumed to be equal to 100). Secondly, the decrease or increase in size of all body regions separately, during the 10 s of each interstage period, were found and expressed in per cent of the respective initial sizes. These per cent values show the specific contraction rates of the body regions reduced in size during locomotion, and the specific expansion rates of regions which are increasing in their dimensions. Finally, it was calculated what are the per cent shares of the decreasing dimensions of the uroid, the contracting pseudopodia, and the trunk, in the general loss of size in all reduced body regions; in other words, it was an atempt to determine the individual contributions of the uroid, of the contracting pseudopodia, and of the trunk, to the total contractile activity of a moving cell.

It should not be forgotten that the values of the specific contraction rates of distinct body regions and of their share in the general cell contraction, as calculated from the changing areas of their profiles, are not equivalent to the values which would

[1] Evidence will be given below, that the error due to the absence on the recorded profiles, of some pseudopodia lying in other planes, is statistically balanced with a sufficiently large number of profiles examined.

be based on the volume relations. However, there are two good reasons for which the quantitative approach to the size evolution of different regions of moving amoeba may be relevant, even when based on the area of profiles. It is to be noted first that, although the profile area is not directly proportional to the volume, we can reasonably expect it to be proportional to the area of the outer surface, which is a crucial point because the cell materials directly involved in the locomotion in amoeba, in particular the cell membrane and the contractile structures (see for review Wohlfarth-Bottermann 1974) have a peripheric distribution in the cell, and for that reason their resources probably are more proportional to the surface area than to the volume of specific regions of amoeba. Secondly, all we are able in fact to observe directly in a moving amoeba is limited to its optical cross-section (the profile), it seems therefore worth to describe exactly and to quantify at least that what is actually seen and what provides the background to draw conclusions.

Size Evolution of the Trunk, Uroid and Pseudopodia

Table 1 gives the dimensions of four main body regions in 12 specimens relative to the total profile area of each individual respectively. Following the dynamic principle of defining the body regions, as formulated above, the data presented in Table 1 are the means calculated only from the initial size of each region in each interstage period (e.g., in the case presented in Fig. 4 the mean would be calculated from all profiles situated at the left side of the drawing).

Table 1

Relative Size of the Uroid, Trunk, Contracting and Advancing Pseudopodia, Expressed for each Amoeba as Per cent of the Total Area of its Profile, in 12 Individuals (Calculated from 4–8 Stages of Locomotion per Individual)

Individual No.	Uroid	Trunk	Contracting pseudopodia	Advancing pseudopodia
1	18.4	48.4	16.8	16.4
2	5.7	51.0	19.0	24.4
5	22.4	41.1	19.3	17.2
7	12.5	42.2	12.3	33.0
8	14.8	26.5	24.0	34.7
10	11.7	48.9	12.9	26.6
11	16.4	45.1	19.0	19.5
12	14.2	34.0	24.2	27.6
13	16.8	42.4	20.6	20.2
18	16.6	46.1	20.9	16.4
25	8.0	47.5	30.9	13.6
26	16.1	42.7	24.0	17.2
Average	14.6 ±4.3	43.3 ±6.6	21.3 ± 5.0	20.8 ± 6.8

The results put in evidence some morphometric properties of the dynamic structure of amoeba which appear fairly regular in spite of clearly pronounced individual variation. The trunk always is the largest region of the body, and the uroid is in a great majority of cases the smallest one. Usually the uroid is 2–3 times smaller than the trunk. The total dimensions of all contracting pseudopodia are, in all individuals, much lower than the dimensions of trunk, and mostly a little higher than those of the uroid. The same size relations apply to the advancing pseudopodia. In average in all amoebae, the uroid represented 1/7, the trunk a little more than 2/5, and the

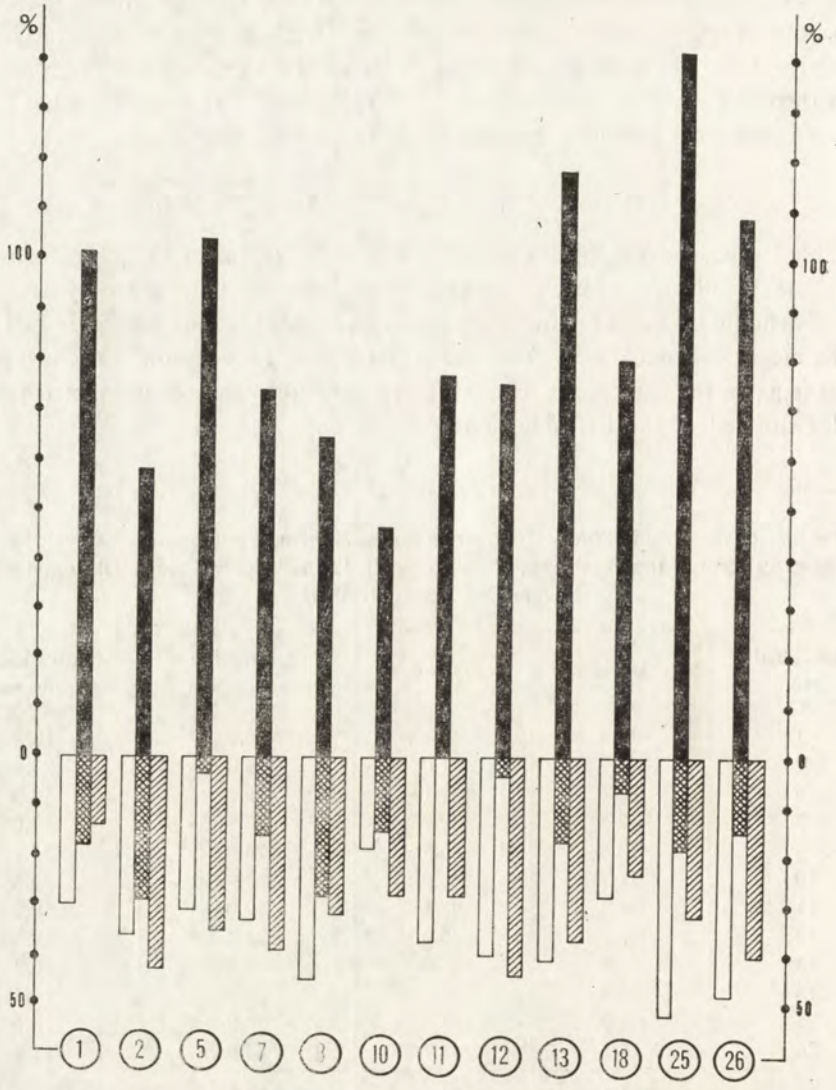

Fig. 5. Graphic representation of the individual means of the specific contraction rates of the uroid, the trunk and the contracting pseupodia, and of the specific expansion rate of the advancing pseudopodia, in 12 specimens; all body regions are dotted as before

Table 2

Specific Contraction Rates of the Uroid (U), the Trunk (T), and the Contracting Pseudopodia (CP), their Combined Contraction Rate (U+T+CP), and the Expansion Rate of the Advancing Pseudopodia (AP), in All Recorded Interstage Periods of Locomotion of 12 Individuals

Ind. No.	Region	0″–10″	10″–20″	20″–30″	30″–40″	40″–50″	50″–60″	60″–70″
	U	−15.4	−26.6	−10.3	−35.7	−50.9	−15.6	
	T	−15.0	−19.4	−2.0	0	−32.8	−35.9	
1	CP	−6.7	−23.2	−8.0	0	0	−34.5	
	U+T+CP	−13.2	−21.4	−5.8	−17.3	−38.2	−33.1	
	AP	+104.6	+147.9	0	+50.6	+78.7	+185.0	
	U	−40.0	−25.0	−25.0	−24.2	−49.5	−32.6	
	T	−8.7	−12.8	−15.6	−22.1	−32.9	−38.1	
2	CP	−36.7	−45.4	−59.4	−51.4	−41.6	−32.3	
	U+T+CP	−20.3	−21.7	−29.4	−25.8	−37.6	−35.9	
	AP	+144.1	+95.9	+55.8	+32.0	+48.9	+29.4	
	U	−25.7	−27.9	−38.9	−26.3	−40.7	−43.6	−28.0
	T	−4.5	+8.6	−7.2	+14.4	−11.8	−1.4	−17.3
5	CP	−7.9	−43.2	−13.6	−44.9	−39.4	−48.7	−19.5
	U+T+CP	−16.1	− 17.4	−17.1	−10.2	−25.0	−22.0	−19.8
	AP	+120.7	+287.1	+64.3	+117.7	+118.5	+47.5	+89.1
	U	−18.6	0	−11.8	−53.3	−31.9		
	T	−3.1	−11.0	−31.7	−17.7	−19.5		
7	CP	−23.9	−63.5	−26.0	−42.6	−57.3		
	U+T+CP	−7.5	−15.6	−27.7	−32.3	−28.3		
	AP	+86.4	+65.3	+67.6	+76.3	+75.7		
	U	−40.2	−51.7	−45.5	−47.0			
	T	−8.7	−33.2	−39.8	−37.7			
8	CP	−44.4	−49.7	−9.6	−46.0			
	U+T+CP	−25.6	−41.8	−24.0	−43.7			
	AP	+131.1	+77.8	+40.1	+42.8			
	U	−19.9	−15.8	−12.0	−16.9	−17.7	−31.5	
	T	−8.3	−22.7	+6.7	−13.9	−25.1	−27.0	
10	CP	−28.4	0	−23.0	−25.9	−30.4	−35.2	
	U+T+CP	−13.9	−20.1	−2.5	−17.1	−25.2	−29.1	
	AP	+31.6	+10.0	+71.3	+116.1	+95.3	+41.3	
	U	−40.7	−40.5	−29.2				
	T	−6.6	+3.1	+3.4				
11	CP	−54.9	−35.3	−14.2				
	U+T+CP	−24.1	−12.4	−7.7				
	AP	+58.0	+49.2	+229.7				
	U	−41.1	−23.8	−61.4				
	T	−18.1	+25.7	−10.6				
12	CP	−37.6	−33.7	−56.1				
	U+T+CP	−24.3	−9.9	−38.8				
	AP	+66.0	+77.5	+82.9				

Table 2 (continued)

Ind. No.	Region	0″–10″	10″–20″	20″–30″	30″–40″	40″–50″	50″–60″	60″–70″
	U	−43.1	−46.3	−38.0	−31.2	−43.5	−41.5	
	T	−1.7	−17.6	−19.9	−25.2	−15.7	−13.6	
13	CP	−33.3	−36.3	−55.9	−50.8	−10.7	−43.4	
	U+T+CP	−23.6	−28.6	−35.0	−28.4	−22.7	−25.7	
	AP	+120.1	+275.7	+73.1	+143.2	+123.0	+87.9	
	U	−42.3	−13.7	−30.9	−33.4	−11.0	−33.8	−41.3
	T	−8.9	−7.8	−9.1	−7.3	−2.8	−1.8	−9.4
18	CP	−25.0	−11.6	−13.2	−26.6	−20.2	−42.5	−31.9
	U+T+CP	−19.7	−9.9	−14.4	−15.7	−9.4	−19.6	−19.6
	AP	+18.7	+89.9	+66.0	+68.1	+168.2	+85.2	+96.0
	U	−57.3	−23.7	−7.5	−42.9	−71.1		
	T	−17.0	−20.6	−10.1	−23.3	−19.3		
25	CP	−43.1	−43.9	−9.2	−63.1	−45.6		
	U+T+CP	−29.8	−28.7	−9.5	−32.7	−36.3		
	AP	+103.4	+210.0	0	+178.4	+105.6		
	U	−39.0	−29.0	−55.2	−56.5	−62.1	−47.1	−37.5
	T	−10.4	−7.6	−15.4	−14.3	−22.5	−2.4	−28.1
26	CP	−10.6	−37.6	−34.7	−40.8	−50.6	−100.0	0
	U+T+CP	−16.5	−24.2	−28.4	−32.0	−37.4	−32.5	−29.8
	AP	+106.1	+90.7	+194.2	+154.5	+55.8	+170.1	+98.8

contracting and advancing pseudopodia each slightly more than 1/5 of the total size, as expressed by the profile area.

Table 2 brings the complete data on the specific contraction rates of the uroid, the trunk, and the contracting pseudopodia, as well as the specific expansion rate of the advancing pseudopodia, in all 12 individuals and in all recorded interstage periods of their locomotion. Means calculated for each individual from all its interstage periods, are graphically represented in Fig. 5.

The data demonstrate that the increase in size of the advancing pseudopodia is counterbalanced by a combined size decreasing of three other body regions: not only of the uroid and of the contracting pseudopodia but of the trunk as well. The specific contraction rate of the uroid is in most individuals slightly higher, and in some of them a little lower, than the contraction rate of contracting pseudopodia. The contraction rates of both these body regions may also change one in respect to another in the same individual, in different interstage periods of its locomotion. The contraction of the uroid is more regular, while the contracting pseudopodia may be temporarily inactive or sometimes absent (contraction rate 0 in Table 2). However, in general, the specific contraction rates of both these regions are close to one another. Total mean values calculated from all interstage periods of all individuals

(Table 4) show that during 10 s the uroid is loosing in average a little more than 1/3, and the contracting pseudopodia exactly 1/3, of their initial size.

The specific contraction rate of the trunk is considerably lower in all individuals and in all periods of their locomotion. In some few cases it has been even found to reach positive values, which means that occasionally, during a 10 s interstage period, the trunk may not contract at all, but instead it may slightly expand. However, such situations are rather exceptional, and in average (Table 4) the trunk loses in 10 s almost 1/7 of its size.

All three decreasing body regions may be considered as a whole and their combined contraction rate calculated. The result (Table 4) shows that the uroid, the trunk and the contracting pseudopodia taken together, lose in 10 s almost 1/4 of their total original size.

In the Fig. 6 two examples are shown of the specific contraction rates of the uroid, of the trunk, and of the contracting pseudopodia, as changing during 50 s in one individual and during 70 s in another. Variations in time of the specific contractions of three regions taken separately, and of their combined contraction rate, are perfectly seen but one could hardly find any regularity in them. There is no alternation clearly pronounced between the activities of the uroid and the trunk, neither between the uroid and the contracting pseudopodia. Changes of the specific contraction rates of these three body regions seem rather to coincide and to follow together the changes of the combined general contraction. It should be concluded therefore that in *Amoeba proteus* there is no distinct rythmicity in the general contraction, and no rythmic alternations of partial contractions, or that such rythms, if they do exist, cannot be detected with the frequency of recording the cell movement which has been chosen in this study.

The rate of increase in size of the advancing pseudopodia prooves to be much higher than the rate of the size decreasing in the uroid, the trunk, and the contracting pseudopodia, either calculated separately or combined together. Many exam-

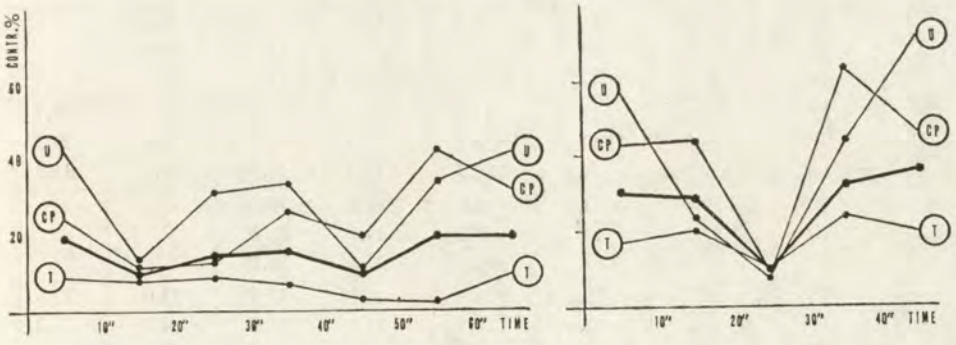

Fig. 6. Specific contraction rates of the uroid (U), the trunk (T) and the contracting pseudopodia (CP), and their combined contraction rate (thick line), as changing during the time of recording of two amoebae in their movement (individuals No. 18 and No. 25)

Table 3

Share of the Uroid (U), the Trunk (T), and the Contracting Pseudopodia (CP), in the Total Loss of Size in all Contracting Regions of *Amoeba* (=100), in All Recorded Interstage Periods of Locomotion of 12 Individuals

Ind. No.	Region	0″–10″	10″–20″	20″–30″	30″–40″	40″–50″	50″–60″	60″–70″
1	U	23.6	21.7	19.0	100.0	39.7	5.9	
	T	65.0	56.6	13.9	0	60.3	76.6	
	CP	11.4	21.7	67.1	0	0	17.5	
2	U	13.4	8.1	5.2	7.2	19.9	5.9	
	T	25.6	40.5	32.4	68.4	51.7	65.7	
	CP	61.0	51.4	62.4	24.4	28.4	28.4	
5	U	83.2	52.3	64.0	85.1	32.1	25.4	25.9
	T	8.9	−19.8	23.8	−68.2	25.1	3.5	51.8
	CP	7.9	67.5	12.2	83.1	42.8	71.1	22.3
7	U	29.0	0	4.7	44.3	34.0		
	T	31.2	55.3	65.6	29.1	38.7		
	CP	39.8	44.7	29.7	26.6	27.3		
8	U	67.5	32.7	22.2	8.6			
	T	16.1	40.6	56.0	24.7			
	CP	16.4	26.7	21.8	66.7			
10	U	32.1	20.4	62.8	13.3	8.4	13.2	
	T	37.2	79.6	−72.1	51.4	70.0	64.7	
	CP	30.7	0	109.3	35.3	21.6	22.1	
11	U	42.9	70.5	59.6				
	T	15.4	−15.7	−22.0				
	CP	41.7	45.2	62.4				
12	U	15.2	67.5	31.1				
	T	52.0	−92.6	11.0				
	CP	32.8	125.1	57.8				
13	U	48.5	22.7	26.4	13.4	56.6	36.6	
	T	2.8	29.9	26.3	69.1	32.2	30.9	
	CP	48.6	47.4	47.3	17.5	11.2	32.5	
18	U	36.8	28.8	43.1	34.0	30.0	39.3	35.7
	T	23.3	43.1	36.2	28.5	14.6	4.8	29.5
	CP	39.9	28.1	20.7	37.5	55.4	55.9	34.8
25	U	20.8	3.7	2.0	16.1	35.7		
	T	32.2	43.7	41.0	49.8	28.2		
	CP	47.0	52.6	57.0	34.1	36.1		
26	U	50.1	15.7	29.6	46.3	32.5	33.0	22.8
	T	27.3	12.8	26.2	21.8	33.1	4.2	77.2
	CP	22.6	71.5	44·2	31.9	34.4	62.8	0

ples may be found in Table 2 of advancing pseudopodia which expand in area twice or more during 10 s. The mean calculated from all cases studied (Table 4) shows that in average they are increasing during that time by 6/7 of their initial size. This result is explained by the difference of the original size of the advancing pseudopodia in respect to the size of other body regions: as it may be calculated from the data shown in Table 1, nearly 4/5 of the body are contracting to produce expansion in only 1/5 of the total area.

It is evident that not only for the advancing pseudopodia but in all cases it is necessary to take account of the initial size of a body region, in addition to its specific contraction rate, when estimating its role in cell locomotion. The question is what fraction of the total loss of size in all contracting regions together is the particular share of the uroid, of the trunk, and of the contracting pseudopodia, separately, i.e., what per cent value will describe the particular contribution of each contracting region to locomotion. These values could be derived by computation of data on the size (Table 1) and data on the specific contraction rate (Table 2) of each body region, however they were calculated here directly again from the primary measurements, and are presented in the Table 3.

We can conclude from the figures given in all three tables that the contribution of the uroid to the general cell contraction is much smaller than it could be expected from its highest contraction rate, because this body region is of the smallest size. The trunk on the contrary, being the biggest region, eventually contributes to the general contraction in the same degree as the uroid, in spite of its much lower specific contraction rate (with exception of a few cases mentioned above, when it could

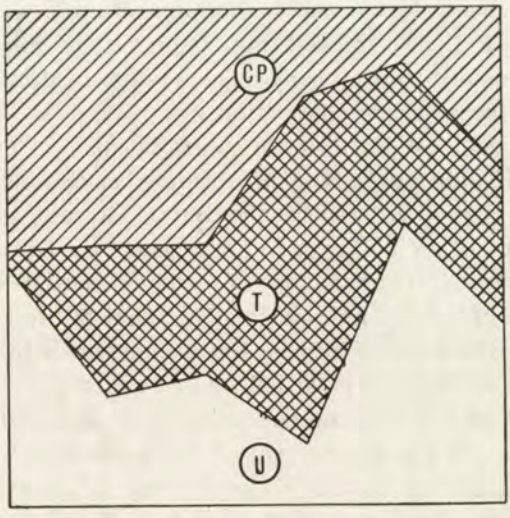

Fig. 7. Changes of contributions of the uroid, the trunk and the contracting pseudopodia to the general contraction, in a single specimen (individual No. 13) during the time of recording its locomotion; dotting and labelling as in former drawings

Table 4

Mean Values of the Relative Size and its Changes, Contractility of Expansion Rat., and the share in General Contraction, as Calculated for the Uroid (U), the Trunk (T), the Contracting Pseudopodia (CP), for All Contracting Regions Combined (U+T+CP), and for the Advancing Pseudopodia (AP)

Region	Relative size			Contraction or expansion rate	Share in general contraction
	Initial	After 10″	Difference		
U	14.64 ±4.33	9.24 ±3.21	−5.40 ±1.80	−36.9 ±8.9	29.6 ±11.9
T	43.30 ±6.59	37.58 ±6.41	−5.72 ±3.93	−13.2 ±8.9	31.4 ±18.8
CP	21.25 ±5.02	14.14 ±3.40	−7.11 ±2.53	−33.5 ±8.3	39.0 ±11.1
U+T+CP	79.19 ±6.35	60.96 ±7.94	−18.23 ±3.02	−23.0 ±6.6	100
AP	20.81 ±6.79	38.84 ±7.06	+18.03 ±3.92	+86.6 ±26.6	–
Total	100	99.80	−0.20	−0.2	–

occasionally not contract at all in one 10 s interstage period). In the contracting pseudopodia their high specific contraction rate results in equally high contribution to the general contraction. Mean values given in Table 4 show that the uroid contributes nearly in 30% to the total contraction in a moving amoeba, the trunk also in 30%, and the remaining 40% represent the share of contracting pseudopodia.

As it is demonstrated in Fig. 7, the contributions of uroid, of trunk, and of contracting pseudopodia to the general cell contraction vary in time in one single individual, but it is impossible to detect any regular rythm of their changes, as it was also impossible for their specific contraction rates.

Table 4 brings together different mean values calculated from all 65 interstage periods recorded in 12 individuals. Besides providing background to the conclusions drawn above on the relative size, the specific contraction rate, and the contribution to general contraction, of each body region of amoeba, the figures in Table 4 bring also some evidence more of the reliability of methods used in this study.

First argument is that the total area of the complete profile of amoeba after 10 s of locomotion, calculated as average for all individuals in their all interstage periods, amounts to 99.80 which is virtually equal to its original total area which was assumed to amount 100. This means that there is statistically no change in the area of profiles of amoebae at the interval of 10 s between two recorded stages of locomotion. Such changes in fact can be found very frequently in the individual cases, when some contracting or advancing pseudopodia are out of focus (i.e., out of the plane of the profile), but with a large number of cases studied the difference prooves to be statistically balanced.

Another evidence of the authenticity of the data obtained is that the total mean of the area of all the contracting pseudopodia (21.25) is almost exactly equal to that of the advancing pseudopodia (20.81). It is well known that in each individual amoeba, at any particular stage of its locomotion, these areas actually may differ one from another, but this difference should be expected in fact to become statistically balanced if a sufficient number of specimens is investigated during a sufficiently long period of time. This is an obvious result of the fact that all contracting pseudopodia are exclusively and necessarily formed from all pseudopodia which earlier were advancing.

Finally, it seems also significant that the average rate of expansion of advancing pseudopodia exceeds the combined rate of contraction of other body regions, exactly in the proportion needed to balance the inferiority in size of the expanding part of the body.

It may be concluded in general, that the method used in this study prooves to be reliable, because all accounts of profit and loss in the size evolution of particular body regions of moving amoebae, based on total means, result in a null balance.

Longitudinal Contraction/Expansion Gradient

A hypothesis may be put forward, basing on the contraction rate being about one half lower in the trunk than in the uroid, that in amoeba there may exist a longitudinal gradient of the contraction rate decreasing from the uroid towards the more anterior body regions. It could be even expected that the trunk might actually expand in its anterior portion. One could predict that the behaviour of the trunk is not uniform also for the reason that the standard deviation from the mean value of the specific contraction rate is proportionally much higher in the trunk than in any other body region (c. f. Table 4). Therefore, in the next series of experiments, the fragment of each profile corresponding to the trunk of amoeba has been further subdivided into sub-regions, and the changes in the areas of sub-regions were calculated in the same manner as before.

In this series 24 pairs of locomotion stages separated by 10 s interval were analysed. They were chosen at random from all stages of locomotion recorded in different amoebae, however, with two restrictions: (1) only the profiles with at least two advancing and two contracting pseudopodia were allotted to the sample, and (2) only amoebae moving straight forwards were positively selected, because in such cells all advancing psudopodia, as a rule, are located more frontally than the contracting ones (in animals which change the direction of locomotion the pseudopodia of both types may alternate in distribution).

In Fig. 8 one interstage period actually used for analysis is shown, as an example explaining how in this series the borderlines were traced to divide the profile into regions and sub-regions. The uroid was delimited from the trunk in the same manner

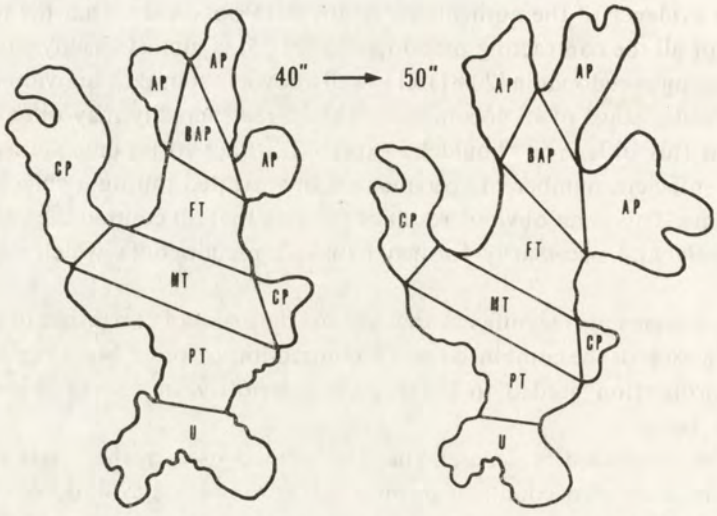

Fig. 8. Delimitation of the uroid (U), the posterior segment of the trunk (PT), its middle part (MT), its frontal part (FT), the base of advancing pseudopodia (BAP), their free parts (AP), and the contracting pseudopodia (CP), in one of the interstage periods chosen for analysis (individual No. 18)

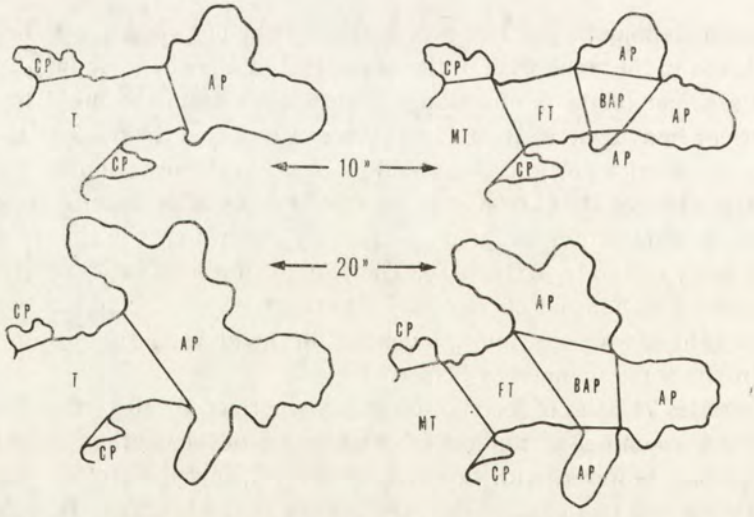

Fig. 9. Difference in the manner of delimitation of regions and sub-regions in the anterior part of amoeba in the first series (left) and the second one (right); example taken from the record of the individual No. 26

as in the former series. The region which before was generally defined as trunk became now divided in three sub-regions: posterior segment — from the borderline of the uroid up to the last (i.e., most posteriorly located) contracting pseudopodia, middle segment — between the contracting pseudopodia, frontal segment — from

the most anteriorly located contracting pseudopodia forwards up to the last advancing pseudopodia. Moreover, the area lying between the bases of advancing pseudopodia was considered separately, as the most anterior sub-region. As it is shown in the Fig. 9, this last sub-region has been usually considered in the former series as a part of the advancing pseudopodia.

So eventually, an attempt was made to detect a contraction/expansion gradient in five segments of amoeba's body which are roughly linear in the posterio-anterior direction: the uroid (defined as in the former series), the sequence of three sub-regions of trunk (formerly considered all together as a whole), and the base of advancing pseudopodia (before partly included into the pseudopodia). The size changes of the free parts of all pseudopodia were not taken into consideration this time, because they represent lateral protrusions which deviate from the investigated posterio-anterior axis.

Table 5

Mean Values of the Relative Size and its Changes, and of the Contraction or Expansion Rate, as Calculated for the Uroid (U), the Posterior Part of the Trunk (PT), its Middle Part (MT), its Frontal Part (FT), and the Base of Advancing Pseudopodia (BAP)

Region	Relative size			Contraction or expansion rate
	Initial	After 10″	Difference	
U	14.90 ±4.31	9.08 ±2.70	−5.82 ±1.64	−39.0 ±8.0
PT	13.94 ±4.08	9.11 ±3.07	−4.83 ±1.79	−34.6 ±11.7
MT	17.91 ±4.72	14.56 ±3.92	−3.35 ±1.75	−18.7 ±4.5
FT	12.47 ±3.50	13.61 ±3.80	+1.14 ±1.61	+9.1 ±5.0
BAP	7.58 ±2.25	9.94 ±2.87	+2.36 ±1.30	+31.1 ±8.5

In Table 5 the mean values are brought together, of the relative initial size of all five regions and sub-regions, their size after 10 s. of locomotion, the difference between both these values, and the specific rate of contraction or expansion. The results indicate that the contraction in the posterior segment of trunk, next to the uroid, is only slightly weaker than in the uroid itself. In the middle segment of trunk the contraction rate is falling to about half of the value found in the uroid. In its anterior part the trunk is slightly expanding, instead of contracting, and the profile area of this sub-region shows an increase of 1/10 of its original value, during 10 s of locomotion. At the base of the advancing pseudopodia the increase of area at the same time reaches as much as 1/3 of its former value.

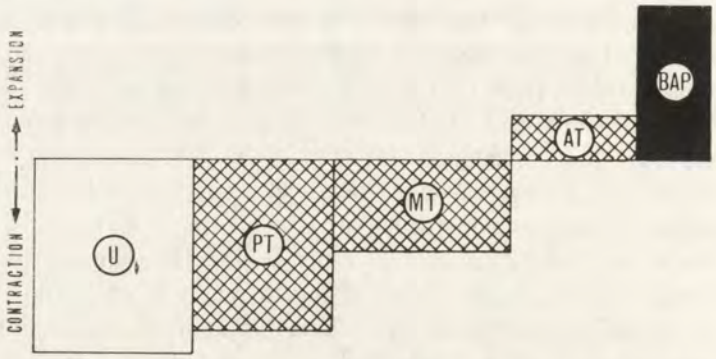

Fig. 10. Gradient of the contraction/expansion rates in the successive regions and sub-regions from the uroid up to the base of advancing pseudopodia (dotted and labelled as before), further explanation in the text

The results put in evidence the existence in amoeba of a gradient of contraction/expansion which follows the posterio-anterior axis from the uroid to the base of advancing pseudopodia. The gradient is actually so well pronounced, that it is possible to discern even visually its existence when comparing the changing size of different regions and sub-regions in the amoeba redrawn in Fig. 8. The means from the Table 5 have been used to represent this gradient graphically in Fig. 10. In this scheme each body region or sub-region is shown in the form of a rectangle with the basis representing its initial size and the height (negative or positive) corresponding to its rate of contraction or expansion.

It is important to compare in Tables 4 and 5 these mean values which express the size of the same regions of amoeba in both series of experiments. They are in fact very close to one another. For example, the relative size of the uroid is 14.64 in the first series and 14.90 in the second. The size of the trunk in Table 4 is shown to amount to 43.30 and the sizes of all three sub-regions of trunk from Table 5 give a sum equal to 44.32. The specific contraction rate of the uroid in the second series (−39.0) and the combined contraction rate of three sub-regions of trunk (which may be calculated from Table 5 as equal to −15.8) are higher but not much deviated from the corresponding values found for the uroid and the trunk in the first series (−36.9 and −13.2 respectively). Moreover, it should be noted that the very high standard deviation from the mean value of the trunk contraction rate in Table 4, which has been discussed above as a possible result of heterogeneity of this body region, becomes in fact considerably lower in Table 5, i.e., after the trunk has been divided into three separate sub-regions.

Reconstruction of a Synthetic Model of Amoeba

All data collected in Tables 4 and 5 were used to design a model of an "ideal amoeba" in its original form and after 10 s of locomotion, i.e., a graphical recon-

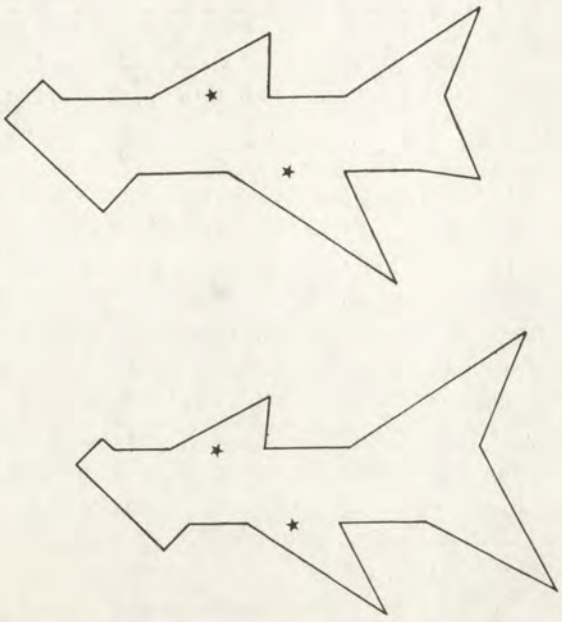

Fig. 11. Two successive stages of locomotion, separated by a 10 s time interval, of a model of "ideal amoeba" graphically constructed from the mean values of all morphometric parameters measured in this study; points assumed to remain immobile in respect to the substrate during this interstage period, are marked with asterisks; detailed description in the text

struction of amoeba's profile with all parameters strictly corresponding to the mean values obtained in this study. The result of this exercise is shown in Fig. 11.

It is evident that for the construction of such a model some assumptions are to be made to simplify and to standardize the pattern of the profiles. It has been assumed that: (1) the model of "ideal amoeba" is to be designed with two contracting and two advancing pseudopodia, (2) only changes in size, and not in character of pseudopodia, intervene during 10 s interval between two stages of locomotion, which means that at that lapse of time, in the model, no new advancing pseudopodia are produced, there is no conversion of an advancing pseudopodium into a contracting one, no accomplished resorption of a contracting pseudopodium by the trunk neither its completed fusion with the uroid, (3) the changing size of the trunk and of its sub-regions is expressed in the model only in the longitudinal dimension, (4) the uroid and the trunk with its sub-regions are represented as quadrangles, the base of advancing pseudopodia, the advancing pseudopodia themselves, and the contracting pseudopodia, are shown as triangles, (5) all lateral pseudopodia in the scheme deviate from the main body axis at the angle of 45°.

When representing two stages of locomotion actually recorded in an individual living amoeba, it is easy to show, besides the evolution in shape and in size of all body regions, the actual change of position of the cell and its parts in respect to the

static marking points on the substrate. In the case of two stages of locomotion of the artificial "ideal amoeba" we can only assume *a priori* that some points on the profile are immobile in respect to the substrate. In Fig. 11 two stages of locomotion in the model are drawn with the assumption that the points on the axis of both contracting pseudopodia (marked with asterisks on the scheme) do not change their position.

The model shown in Fig. 11 permits a visual comparison of the average contraction of all regions and sub-regions from the rear edge of amoeba up to the anterior limit of contracting pseudopodia, and the average expansion of all parts located forward from this borderline, up to the front of amoeba. On the other hand it makes evident that, if the points lying on the axis of contracting pseudopodia are assumed to keep a constant position in respect to the substrate, all other points on the profile, as well those more posteriorly as those more anteriorly located, cannot be immobile but must be displaced forwards, in the direction of cell locomotion. In this respect the behaviour of the model of "ideal amoeba" is very close to the behaviour of a living individual, as shown in Fig. 1. Of course, the "behaviour of the model" as well as the behaviour of living amoebae, should be different if other points on the body outline would be immobile (i.e., under normal conditions, with changing sites of amoeba's attachment to the substrate), and the analysis of such situations is now under way.

Conclusions and Discussion

To summarize, a generalized picture of locomotion of amoeba may be drawn as follows:

The contracting regions and sub-regions (the uroid, the contracting pseudopodia, and the posterior and middle segments of the trunk) constitute about 67% of amoeba's body (as related to the profile area), and the expanding parts (the advancing pseudopodia with their base, and the frontal segment of the trunk) represent the remaining 33% of the cell size.

The uroid contracts most vigorously, but it contributes to the general contraction in amoeba only in 30%, being the smallest region of the body. Higher contribution, reaching nearly 40%, is brought by the contracting pseudopodia which contract at a rate which is only slightly inferior to that of the uroid. The remaining 30% of the total contraction is due to the trunk which shows in its different sub-regions a distinctly pronounced gradient of contraction decreasing in the posterio-anterior direction.

This pattern of distribution of the contractile activity in moving amoeba has some strong implications on its mode of locomotion. The posterior parts of the body do not only contract by themselves, but they are also passively pulled in the direction of locomotion by the combined effect of contractions effected in all other seg-

ments located more anteriorly: between them and the temporary immobile site of attachment to the substrate. For example the uroid, undergoing its own contraction, is simultaneously dragged forwards by the contraction in the trunk. The frontal portions of amoeba are not only growing on at the tips of advancing pseudopodia, but are also passively pushed in the direction of locomotion by the expansion of all body segments lying behind, between them and the attachment sites.

If the attachment points are located at the borderline which separates the contracting posterior 2/3 of amoeba, from its expanding anterior 1/3, exclusively this zone may be immobile in respect to the substrate. All other parts of the body, those lying in front of the attachment sites as well as those situated behind them, must move forwards according to the direction of locomotion. If so, one of the basic assumptions common to all recent theories of amoeboid movement, that the intracellular streaming of endoplasm is responsible for all transfer of mass in moving amoeba, turns out to be not in full extent true. In fact, the endoplasm streaming carries forward the material in a cell which simultaneously crawls forwards itself as a whole.

From this last conclusion three new questions arise. First problem is, what is the share of the crawling component and what of the intracellular transportation of material, in producing the total effect of cell locomotion in amoeba. Secondly, what different forms of crawling movement are produced by changing the temporary sites of attachment of amoeba to the substrate. Third question is, to what extent the displacement forward of the cell as a whole, might be related to the symmetrical forward sliding of the cell membrane which has been demonstrated earlier by the present authors (Czarska and Grębecki 1966) and further investigated by Haberey et al. (1969), Stockem et al. (1969), and Haberey (1972), and also whether it can be related to the forward movement of small granules in the hyaline ectoplasm as described by Rinaldi and Jahn (1963). All these three problems are under further investigation.

Even the terminology adopted in this paper prooves that the present authors, as the majority of other students of amoeboid movement (Goldacre 1961, 1964, Grębecki 1964, Jahn 1964, Bovee and Jahn 1973, Wohlfarth-Bottermann 1964 a, b, 1974, Komnick et al. 1973), support the view that the motive force of amoeboid movement is a contraction in the rear portions of amoeba's body. At first sight it would seem also possible to base on the hypothesis of frontal contraction (Allen 1961 a, b) and do not describe the reduction in size of the posterior parts of amoeba as a product of local active contraction, but instead to assert that: "The shortening in the tail may very well be a passive phenomenon: collapse of the ectoplasm and solation when the endoplasm is pulled out" (Allen 1973). However, this second interpretation of facts which were established in this study would be hardly admissible for two separate reasons. First reason is, that the decreasing in size of the successive regions and sub-regions of amoeba's body follows a gradient, being most effective in the uroid and sloping gradually in the forward direction. Existence of such a gradient may be easily explained on the ground of local differ-

ence in the contractile activity which is supposed to be the highest in the rear. On the contrary, if basing on the hypothesis of frontal contraction, we would face the very puzzling necessity to admit that the effects of the pulling force become better pronounced with the growing distance from its source. Second argument is provided by the size distribution of the parts of amoeba which decrease or increase during locomotion. It seems obvious that a system which contracts in 2/3 of its size to push the other 1/3, needs to develop much less force to be effective, than a system which would contract in 1/3 only to pull the remaining 2/3.

It seems therefore, that the results reported here provide not only the information, lacking until now, on the morphometric parameters of moving *Amoeba proteus*, and put in evidence some modalities of amoeba locomotion which were neglected before, but on the other hand, they offer some new arguments against the view looking for the motive force of amoeboid movement in the frontal part of the cell.

RÉSUMÉ

En partant de la superficie des profils (sections optiques) des amibes enrégistrés en cinématographie à cadence ralentie, on a calculé les dimensions rélatives de l'uroïde, du tronc, des pseudopodes qui regressent et qui progressent, et des subdivisions du tronc en parties postérieure, médiane et antérieure. La comparaison des stades du mouvement consécutifs éspacés de 10 s permet de calculer le degré de la contraction ou de l'expansion spécifique pour chaque région de la cellule, et la contribution qu'elle apporte à la transformation générale de forme que l'amibe subit pendant sa locomotion, ainsi que d'établir la présence d'un gradient de contraction qui diminue et d'expansion qui augmente en partant de l'uroïde jusqu'à la base des pseudopodes en progression. On discute l'importance de ces résultats quantitatifs pour la compréhension des modalités de la locomotion et de la direction dans laquelle la force motrice agit dans la cellule de l'amibe.

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Received 14 October 1974

ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 363–369

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Some Effects of Externally Applied Pressure upon Cytoplasmic Movements in the Ameba *Chaos chaos*

Synopsis. Glass and agar microcapillaries were used to study the effect of positive and negative pressures (1 to 120 cm H_2O) upon cytoplasmic movements in the ameba *Chaos chaos*. The reaction to the applied positive and negative (suction) pressures was recorded on films and analyzed by a cinematographic single frame analyzer.

The observations confirm that small differences of hydrostatic pressure can cause the mass transport of endoplasm as predicted by the ectoplasmic tube contraction theory. Some experimentally induced streaming phenomena are not adequately explained by the front zone contraction theory. It is concluded that at the present time, pressure experiments cannot serve as a "direct test" of the contemporary theories of ameboid locomotion.

It is generally accepted that the motive force responsible for ameboid locomotion results from mechanochemical processes of actin- and myosin-like proteins in the cytoplasm (see review Komnick et al. 1973). However, it remains controversial where precisely the contractile events are localized in the cell during ameboid motion. According to the most generally accepted model of ameboid motion, which may be termed the "ectoplasmic tube contraction theory" formulated by Pantin (1923) and advanced principally by Mast (1926, 1931), contraction and related volume changes of the ectoplasmic tube produce a pressure which can move endoplasm. Ancillary to this process is the capacity of the endoplasm to form ("gelate") and dissolve ("solate") the ectoplasmic tube. The above model was challenged upon observing that amebic cytoplasm released from its membrane can undergo directed movements (see reviews Allen 1961, 1973). Thus a new hypothesis, "fountain zone contraction" (FZC) was postulated, requiring contraction of the axial endoplasm as it forms its ectoplasmic tube to pull the endoplasm forward. Recently, Allen et al. (1971) report that suction pressures as high as 35 cm H_2O when applied by microcapillary pipettes onto or inserted into an ameba do not cause endoplasmic

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streaming to reverse relative to the imposed pressure. Allen (1972) interpreted these results as invalidating the ectoplasmic tube contraction theory and supporting FZC. However, the above results do not agree with classical investigations which noted that pressure applied to amebae cause inhibition, reversal and acceleration of endoplasmic movements relative to the pressures magnitude and direction (Mast 1931, Allen and Roslansky 1959, Kanno 1964). Moreover, Kamiya (1964) reported, upon adapting his double chamber technique to amebae, the motive force for endoplasmic motion is from 0.5 to 15 mm H_2O column pressure. This investigation seeks to determine the basis for the above discrepancy.

Materials and Methods

Chaos chaos was grown in Chalkley's solution with *Paramecium* used as its principal food source.

Glass Capillaries and High Pressure (5 to 120 cm H_2O column pressure). The glass capillaries were made with a NAKAGISHI Microelectrode Puller and were soaked in 0.1 M EGTA for several hours and then rinsed before use. A vacuum pump capable of pressures from 5 to 120 cm H_2O column was used. A LEITZ micromanipulator aided in controlling the placement of the microcapillary pipettes relative to the subject. The viewing microscope was a LEITZ Ortholux equipped with 10 and 35 × long working distance objectives. Cine recordings were made with a BEAULIEUX 16 mm camera on black-white and color films.

Agar Capillaries and Low Pressures (ca. 1 cm H_2O).
Generally the methods were as above but with two differences:

(a) Smaller manometers, corresponding to the lower pressure, and a hypodermic syringe as a pressure source were used.
(b) Agar capillaries were used to prevent the amebae from contacting glass during the application of pressure.

It was observed that contact with glass during our experiments tended to stop normal ameboid motion. However, in the agar capillaries the amebae appeared to behave normally. In constructing the agar capillaries we utilized the techniques developed by Kamiya (1964). The only difference was to use a glass capillary as a support for the agar capillary. A glass pipette (1 mm in diameter) was filled with warm agar. Into the cooling agar were placed glass microcapillaries (which were later removed) of two sizes: (1) one which can contain a whole ameba and (2) another which can accomodate only a single pseudopodium of the ameba. The supporting glass pipette allowed manipulation of the contained agar capillaries without the use of a micromanipulator. There was a technical limitation as an ameba does not adhere strongly to agar and there occurred a sliding of the ameba or pseudopodium in the agar capillary during application of pressures near 1 cm H_2O making it difficult to assess the effects on the cytoplasm. Sliding of the subject was reduced by using pressures below 5 mm H_2O.

Cytoplasmic reactions and related phenomena were cine recorded and analyzed by a cinematographic single frame analyzer.

Results

Glass Capillaries and High Pressures

Glass capillaries of various diameters are used either for direct suction of endo-

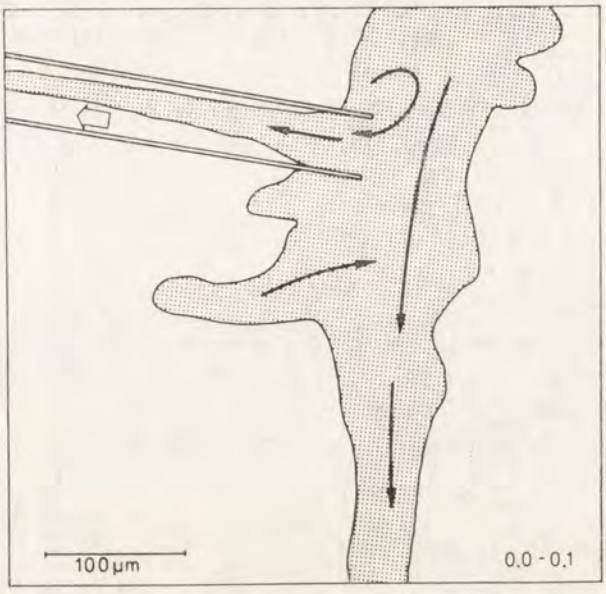

Fig. 1. Rotatory motion upon application of high external suction pressure (ca. 70 cm H_2O) with a glass microcapillary. Note the flow into the microcapillary and the flow adjacent but separate towards an extending pseudopodium. Black arrows within the ameba represent flow patterns. The white arrow outside indicates the direction of the imposed external pressure. All movement phenomena drawn from cine recordings and analyzed by a cinematographic single frame analyzer

plasm into the capillary or for applying pressure to a pseudopodium. When a capillary is inserted into the endoplasm of the ameba and pressure applied, one can observe rapid circulatory motions of the endoplasm. This motion is not limited to the vicinity of the capillary orifice but extends into adjacent pseudopodia (Fig. 1). After 0.5 to 2 s the pseudopodia begin to "escape" from this suction pressure and extend in an opposite direction. Flow of the endoplasm into the capillary is sometimes intermittent. Often cytoplasm in the capillary is torn from the ameba indicating that the endoplasm has become "solid-like". Frequently a gelated plug is formed near the capillary orifice or along the entire pseudopodium, which prevents transmission of the imposed pressure to the remaining cytoplasm. In the case of smaller capillaries (ca. 10 µm) the gel plug resisted constant suction pressure of 120 cm H_2O blocking the influx of the endoplasm. By gentle tapping of the micromanipulator or by alternating application of low and high pressures the plug can be disrupted and the endoplasm then flows into the capillary. Generally, our results (Fig. 1) are similar to those of Allen et al. (1971) in that high suction pressures, when locally applied with a glass micropipette, did not prevent the extension of pseudopodia in an opposite direction.

Agar Capillaries and Low Pressures

Upon application of both positive and negative (suction) pressures on an entire ameba, one could observe a reversal of endoplasmic streaming direction with the application of low oppositely directed pressures (5 mm H_2O). Suction pressure applied in the same direction as normal endoplasmic streaming accelerates the flow. Analysis of the cine records demonstrates that the reversals caused by very low positive pressures (5 mm H_2O) begins at the pseudopodial tip and then spreads posteriorly. Figure 2 illustrates that the endoplasm could be forced to flow backward by positive pressure (0.5 to 1.5 s) while the previous (at t_0) counter-directed streaming is slowed considerably, but continues to move against the pressure from the left side. Thus the endoplasm is streaming from its two ends simultaneously towards the middle of the ameba (0.5 to 1.5 s) before the applied external pressure accomplishes the complete reversal of endoplasmic flow direction (3.0 s).

Application of slightly higher pressures, 5 to 15 mm H_2O, produces an immediate reversal of endoplasmic stream direction within the entire ameba. Further, this reversal is accompanied by the formation of a new hyaline cap at the original uroid, confirming Mast's (1931) and Chambers' investigations.

Upon rapid application of alternating positive and negative pressures of ca. 10 mm H_2O there is a reversible and definitive change in shape of the two ends of the ameba.

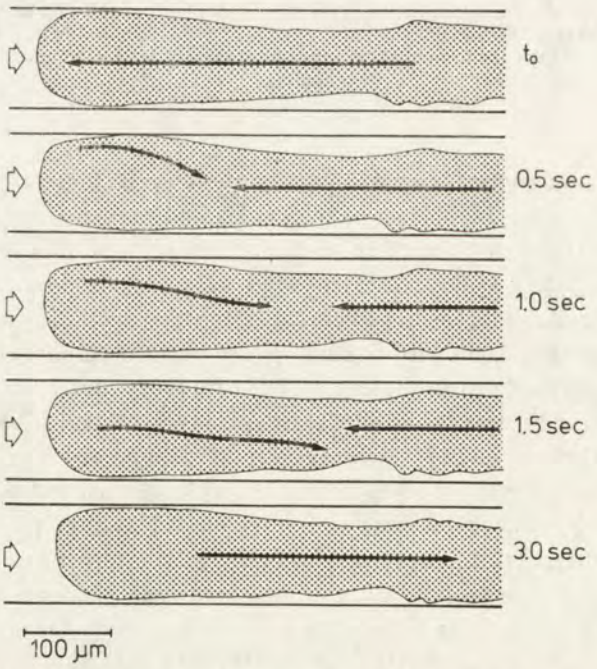

Fig. 2. Whole ameba within an agar capillary. Pressure 5 mm H_2O. Note the reversal of the flow by positive pressure from the left side (white arrow). Endoplasm can be forced to flow backward while the previous streaming (0 sec) is slowed down, but continued (0.5 to 1.5 sec), before the imposed pressure accomplishes the complete reversal (3.0 sec)

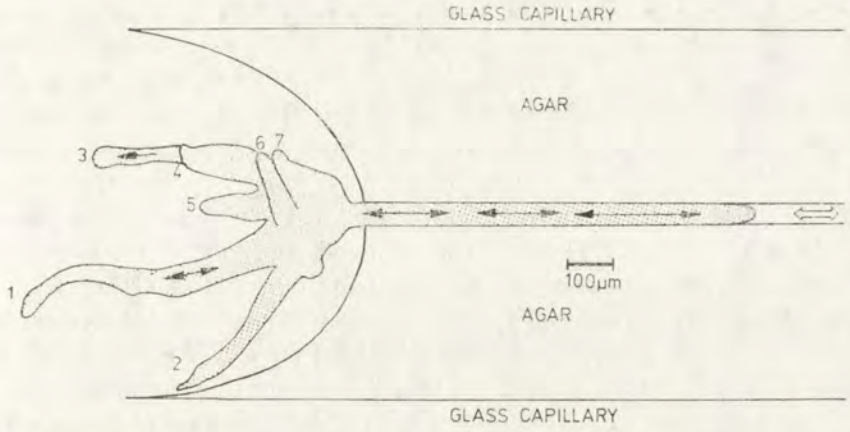

Fig. 3. Effects of low pressure (less than 10 mm H_2O) on one pseudopodium inside of the agar capillary. Note that some pseudopodia (*1*) outside of the agar capillary respond to the external imposed pressure whereas another (*3*) does advance independently of the imposed pressure

Endoplasmic movements are in direct relationship to the magnitude and direction of the applied pressure. Noteworthy is the fact that the endoplasm appeared to react independently of the ectoplasmic tube relative to which it is forcibly being displaced. There is no apparent transformation of the ectoplasmic tube into endoplasm. In one ameba which had small pseudopodia at one end during the application of pressures less than 10 mm H_2O, these small extending pseudopodia collapsed as the endoplasm is sucked out, while the uroid end ballooned from the influx of the endoplasm.

When one pseudopodium of a polypodial ameba is sucked into a narrow capillary one can study the effect of low pressures (less than 10 mm H_2O) on the endoplasm (Fig. 3). Endoplasm within the pseudopodium sucked into the agar capillary reacts in accordance with the magnitude and direction of the applied external pressures. Some displacements of the ectoplasm occur, probably due to the low adherence of the ameba to the agar. Moreover, one could observe stretching and elastic return of the ectoplasmic tube of the pseudopodium. However, reversal of the direction of the original endoplasmic streams is produced by externally applied pressure. This reversal occurs in the entire pseudopodium within the agar capillary and is also exhibited in another pseudopodium within the immediate region of the capillary. However, other pseudopodia extend or retract independently of the applied pressure.

Discussion

Our results using high suction pressures and glass capillaries are in general agreement with the investigations of Allen et al. (1971). However, since the suction pressure applied locally does not prevent pseudopodia from extending even when al-

most ten times greater than the forces required for endoplasmic movement (Kamiya 1964), we are uncertain whether the external forces (particularly at high pressure) are transmitted to all of the endoplasm. Injury of the cell membrane, contact of the cytoplasm with glass capillaries and the application of large torsion forces to the cytoplasm are expected to cause changes in the consistency of the endoplasm. Recall that in our experiments gelated endoplasm is able to resist very high suction pressures of 120 cm H_2O. Heilbrunn (1958, p. 20) noted that "any uneven pressure such as might be produced by the centrifuge would tend to raise the viscosity of the protoplasm rather than lower it". Further, upon centrifuging *Chaos chaos*, he wrote: "after these amebae are centrifuged so that they become rounded up, there may be several streams of protoplasm separated by inert masses of protoplasm". The conditions of suction may be more severe than during centrifugation. Since there is no real information concerning the transmission of high external pressures within endoplasm which evidently can compartmentalize itself into gelated and solated regions, we are unable to determine how extensive a "pressure sink" develops within an ameba upon application of high external pressures by means of glass capillaries. Therefore, we submit that these suction experiments, though interesting, are presently inconclusive for a "direct test" of contemporary theories of ameboid locomotion.

Observations conducted on amebae within agar capillaries confirm previous reports (Kamiya 1964) that small differences of applied hydrostatic pressure can cause the mass transport of endoplasm within the ectoplasmic tube as predicted by the ectoplasmic tube contraction theory. Also, the pattern of endoplasmic streaming reversal induced by externally applied hydrostatic pressure corresponds to the postulates of the latter theory. Moreover, the pattern of endoplasmic flow observed in Fig. 2 (i.e., streaming from both ends towards the center of the ameboid cell, as in the 0.5, 1.0 and 1.5 s recordings) is not adequately explained by FZC but is predictable from considerations of pressure flow mechanisms.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Dr. W. Stockem and Prof. Dr. M. Haberey for valuable discussions, Dr. N. Hülsmann and Dr. R. Stiemerling for the preparation of the films and drawings and Miss M. F. Go for cultures. Dr. W. Korohoda thanks the Alexander von Humboldt Stiftung for a scholarship which supported this work.

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Received 5 October 1974

ACTA PROTOZOOLOGICA

VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 371–377

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Measurement of Aggregate Sedimentation Rate as a Chemical Bioassay

Synopsis. Sedimentation rate of *Euglena gracilis* and *Tetrahymena pyriformis* aggregates is affected by formaldehyde and glutaraldehyde at concentrations as low as 1×10^{-4} mM. The following conclusions have been reached in regard to measurement of aggregate sedimentation rate as a chemical bioassay: (1) the aggregate system is capable of detecting very low sublethal concentrations of toxic chemicals, (2) the aggregate system may be used to differentiate between chemicals and (3) the aggregate systems of different species respond differently to the same chemical.

The aggregation of free-swimming protozoa and algae has been studied by describing the patterns as they appear over the surface of a culture (Baker 1953, Gittleson and Resler 1972, Gittleson and Jahn 1968 a, b, Jahn et al. 1961, Jennings 1906, 1944, Jones and Baker 1946, Lloyd 1946, Loefer and Mefferd 1952, Nettleton et al. 1953, Platt 1961, Robbins 1952, Wager 1910, Wille and Ehret 1968) and by describing the vertical displacement of aggregates (Gittleson and Jahn 1968 a, Jahn et al. 1962, Jahn and Brown 1961, Rose 1964, Wager 1911). The horizontal configurations vary considerably with species but vertical activity is similar. Vertical aggregation consists of four phases of activity (Gittleson and Rogers 1972): (1) accumulation-aggregation — formation of aggregates among cells that accumulate at the culture surface: (2) aggregate sedimentation — falling of aggregates from the surface; (3) dispersal — breakup of aggregates at bottom of culture; and (4) negative geotaxis — upward movement of individual cells. Activity during phase (2) is expressed as sedimentation rate, e.g., the average for *Polytomella agilis* is 860 μm/sec (Gittleson and Jahn 1968 a). A 10% increment in viscosity of the medium was found to decrease sedimentation rate of *P. agilis* by 140 μm/sec (Gittleson and Rogers 1972). *Tetrahymena* patterns underwent a marked change in configuration when subjected to CO_2 and NH_3 (Winet and Jahn 1972 a, b).

Thus, changes in the environment of the organisms are reflected in the behavior of the macroscopic aggregates. In the case of viscosity the effect is directly upon

the aggregate but any parameter that effects cell motility as in the case of *Tetrahymena* exposed to CO_2 and NH_3 would be expected to influence aggregation. An increase in light intensity from 2 to 32 ft-c decreased pattern formation time of *P. agilis* by 50% (Gittleson and Jahn 1968 b).

The objective of this study is to evaluate the effect of two well-known toxic substances — formaldehyde and glutaraldehyde on sedimentation rate of *Tetrahymena pyriformis* and *Euglena gracilis* aggregates specifically to understand (1) if the aggregate system is capable of detecting low concentrations of chemicals and (2) if the aggregate system may be used to differentiate between chemicals; and (3) if species variability to chemical influence may be assayed by analysis of aggregation activity.

Materials and Methods

Tetrahymena pyriformis and *Euglena gracilis* were both cultured axenically in a medium composed of 0.1 g sodium acetate, 0.2 g yeast extract (Difco), and 0.1 g bacto-tryptone (Difco) per 100 ml of glass distilled water. Experiments were carried out on six day cultures maintained in 250 ml Erlenmeyer flasks at 28°C. Portions of these cultures were transferred to 25 × 200 mm test tubes. Sedimentation rate of vertical aggregates was measured as the aggregates fell from the 100 to 90 mm levels. Experiments were run at 28°C over a period of 6 h.

All glassware was washed in Alconox laboratory soap, rinsed 10× in tap water, 3× in dilute nitric acid to remove soap residue and 3× in glass distilled water. New test tubes were used for each separate experiment with glutaraldehyde and formaldehyde and then discarded. The small volumes of glutaraldehyde and formaldehyde needed to obtain concentrations of 0.0001, 0.001, 0.01, and 0.1 mM were added to the cultures in the test tubes which were immediately shaken. Equal volumes of sterile media added as a control did not produce any significant change and so all control measurements at each time interval are reported below as one average.

Results

Tables 1 and 2 show the changes in sedimentation rate of *T. pyriformis* and *E. gracilis* aggregates when glutaraldehyde and formaldehyde were added to the cultures. In 0.0001 mM of glutaraldehyde and formaldehyde both species within 15 min exhibited a significant decrease in sedimentation rate of about 20%. Generally, as concentration of these chemicals increased there was a decrease in sedimentation rate. *T. pyriformis* ceased to form aggregates at 0.1 mM in both glutaraldehyde and formaldehyde while *E. gracilis* continued to form aggregates until a concentration of 1.0 mM was reached. There was a trend for sedimentation rate to decrease with time in any of the concentrations tested for both species. There was no significant change in control values with time.

Table 1

Average Sedimentation Rates ± Standard Deviation of *Tetrahymena pyriformis* Aggregates in Response to Glutaradehyde and Formaldehyde. Sedimentation Rate is Expressed in μm/sec. Values in Parentheses are Ranges of 20 Measurements

Glutaraldehyde				
Time in hour	Control	0.0001 mM	0.001 mM	0.01 mM
0.25	1075±114 (1250 – 1000)	849±117 (1000 – 625)	801±86 (1000 – 714)	678±43 (714 – 625)
1	1075±114 (1250 – 1000)	766±83 (830 – 625)	719±65 (830 – 625)	597±34 (625 – 555)
2	–	704±156 (1000 – 555)	645±117 (830 – 500)	584±94 (714 – 454)
4	1041±151 (1250 – 830)	604±72 (714 – 500)	643±35 (714 – 625)	576±72 (714 – 500)
6	1050±140 (1250 – 830)	719±65 (830 – 625)	611±95 (714 – 500)	576±72 (714 – 500)
Formaldehyde				
0.25		858±102 (1000 – 714)	837±124 (1000 – 625)	719±65 (830 – 625)
1		650±77 (714 – 555)	656±104 (830 – 555)	530±70 (625 – 413)
2		677±109 (830 – 500)	699±184 (1000 – 555)	590±73 (714 – 500)
4		642±112 (830 – 555)	604±72 (714 – 500)	547±46 (625 – 500)
6		652±93 (830 – 555)	593±82 (714 – 500)	600±62 (714 – 555)

Table 2

Average Sedimentation Rates ± Standard Deviation of *Euglena gracilis* Aggregates in Response to Glutaraldehyde and Formaldehyde. Sedimentation Rate is Expressed in μm/sec. Values in Parentheses are Ranges of 20 Measurements

	Glutaraldehyde				
Time in hour	Control	0.0001 mM	0.001 mM	0.01 mM	0.1 mM
0.25	953±173 (1250 – 714)	794±145 (1000 – 625)	763±71 (830 – 625)	598±77 (714 – 500)	521±78 (625 – 413)
1	1012±180 (1250 – 714)	669±103 (830 – 413)	606±103 (714 – 413)	525± (714 – 454)	573±116 (694 – 406)
2	1012±180 (1250 – 714)	649±137 (1000 – 454)	655±119 (830 – 454)	558±73 (714 – 454)	547±81 (625 – 360)
6	1041±151 (1250 – 830)	708±99 (830 – 555)	644±86 (830 – 500)	590±35 (625 – 555)	553±106 (625 – 413)
	Formaldehyde				
0.25		736±90 (830 – 555)	769±156 (1000 – 500)	663±117 (830 – 454)	629±117 (830 – 500)
1		784±56 (830 – 714)	661± (714 – 625)	655±95 (830 – 500)	614±110 (830 – 454)
2		648±105 (830 – 500)	607±103 (714 – 454)	634±75 (714 – 500)	610±103 (830 – 500)
6		719±96 (830 – 500)	624±99 (830 – 500)	618±68 (714 – 500)	585±75 (714 – 454)

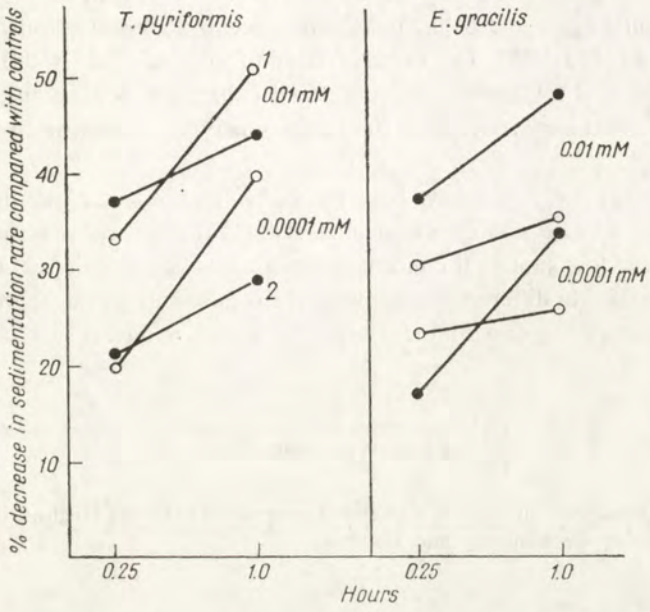

Fig. 1. A comparison of the sedimentation rate of *Tetrahymena pyriformis* and *Euglena gracilis* aggregates in glutaraldehyde (*1*) and formaldehyde (*2*) at concentrations of 0.01 and 0.0001 mM

Figure 1 contrasts the sedimentation rates in glutaraldehyde and formaldehyde for both species at 0.0001 and 0.01 mM by comparison with control values. The % decrease in sedimentation rate differs for the two chemicals initially and after 1 h. Formaldehyde produces the greatest effect on *T. pyriformis* at each concentration while glutaraldehyde is generally more effective on *E. gracilis*.

Discussion

The classic assay for determination of the effect of chemicals on cells is survival time but this method is not very discriminating as aptly pointed out by Fenn (1969). Measurement of rate of locomotion is a sensitive indicator which, however, requires a more elaborate technique. The use of aggregate activity as at chemical bioassay combines the simplicity of the survival time technique and the sensitivity of cell locomotion measurements.

Bringmann and Kühn (1959 a, b) have shown that survival of *Microregma*, *Daphnia*, *Escherischia coli*, and *Scenedesmus* is affected by formaldehyde at concentrations of 1.7, 0.7, 0.03, and 0.01 mM, respectively. Using rate and extent of oxidation as indices of toxicity, the toxic concentration of formaldehyde in domestic sewage

was found to lie between 4.3 and 5.8 mM (Gellman 1952). In contrast, *E. gracilis* and *T. pyriformis* aggregate behavior is influenced by concentrations of formaldehyde as low as 1×10^{-4}mM. This concentration of formaldehyde is 300× lower than the limit established for sewage and industrial effluents (McKee and Wolf 1963) which indicates that important biological effects may be occurring at levels heretofore unresolved.

The following conclusions have been reached in regard to measurement of aggregate sedimentation rate as a chemical bioassay: (1) the aggregate system is capable of detecting very low sublethal concentrations of toxic chemicals; (2) the aggregate system may be used to differentiate between chemicals; and (3) the aggregate systems of different species respond differently to the same chemical.

ACKNOWLEDGEMENTS

For valuable assistance in these experiments I am grateful to Gloria Hughs. I thank Mr. Tautscher for translating the summary into German.

ZUSAMMENFASSUNG

Die Ablagerungsgeschwindigkeit von *Euglena gracilis* und *Terahymena pyriformis* zu einer Anhaeufung wird durch Formaldehyde und Glutaraldehyde in Konzentrationen von weniger als 1×10^{-4} mM beeinflusst. Die folgenden Rueckschluesse wurden durch Messungen der Geschwindigkeit erreich, in welcher angehaeufte Ablagerungen von chemisch-biologischen Proben erreicht wurden: (1) Das "Anhaeufungs-System" kann angewendet werden, um sehr geringe, nicht toedliche, Konzentrationen von giftigen Chemikalien aufzuzeigen. (2) Das "Anhaeufungs-System" kann verwendet werden, um einzelne Chemikalien voneinander zu unterscheiden. (3) Das System reagiert unterschiedlich auf gleiche Chemikalien und haengt von der Art der Spezie ab.

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Received 27 September 1974

ACTA PROTOZOOLOGICA
VOL. 14 (No. 3/4) WARSZAWA, 31. XII. 1975 pp. 379–383

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A Maintenance Medium for the Axenic Culture of *Paramecium aurelia*

Synopsis. A medium containing an ethyl ether extract from Semen lini (linseed) instead of TEM-4T supports the growth of *Paramecium aurelia* for a long period, up to 27 days. This medium does not support a large population, but maintains the cells for a long period, thereby eliminating the need for frequent transfers to fresh medium.

Paramecium aurelia can be cultivated axenically in various media. Nutritional studies by van Wagtendonk and associates (1974) revealed that *Paramecium* has an absolute requirement for a steroid and for fatty acids. The steroid requirement is satisfied by stigmasterol and the fatty acid requirement by TEM-4T (diacetyl tartaric esters of tallow monoglycerides) or by a mixture of fatty acids based on the fatty acid composition of TEM-4T and a phospholipid. Since TEM-4T is not readily available a search was made for an easily obtainable source of fatty acids essential for the growth of paramecium. Anethyl ether extract of Semen lini (linseed) could substitute for TEM-4T, although the medium did not support a high population of paramecium. However, in this medium paramecium survived much longer and the cells did not have to be transferred to fresh medium every seven days.

Materials and Methods

Organism and Maintenance of Stock Cultures. *Paramecium aurelia*, syngen 8, stock 299 (endosymbiote-free) was maintained in the medium described by Soldo et. al. (1966). The stock cultures were transferred on the 7th day.

Preparation of Inocula. For the experiments described in this communication large cultures were grown in 5 ml tubes as described by van Wagtendonk and Soldo (1970). Every week two drops of culture were transferred to next 5 ml of fresh medium.

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Semen Lini Extract. Linseeds were finely ground and 75 g of the powder were extracted for 24 h with etyl-ether in a Soxhlet apparatus. The ether was removed under vacuum and the resulting oil, about 20 g, stored in a refrigerator.

Test Media. The test media were prepared according to the methods described by van Wagtendonk and Soldo (1970). Five diffferent media (10 times concentrated), containing the standard concentrations of proteose peptone, trypticase, yeast nucleic acid, vitamins and $MgCl_2$, but differing in composition with respect to stigmasterol and lipids were constructed. These media are given in Table 1. The concentrated media were stored in a freezer.

Table 1

Stigmasterol and Lipid Composition of the Test Media

Medium	Stigmasterol	TEM-4T	Semen lini extract
	mg/100 ml of final medium		
M–I	0.5	10	0
M–II	0	0	0
M–III	0.5	0	0
M–IV	0	0	25
M–V	0.5	0	25

Assay for Growth. Every two weeks 5 ml of each concentrated medium was diluted to its final volume and distributed in 5 ml quantities over 10 culture tubes. The tubes were autoclaved at 1.5 atm, 30 min. All tubes were inoculated with two drops of the previously prepared paramecium suspension. The number of cells per ml of culture was determined by counting two samples of 25 or 100 µl on day 0, 3, 10, 13, 17, 20, 24 and 30. The experiments using media M-I, M-II and M-V were repeated five times and the experiments utilizing media M-III and M-IV were repeated seven times. The results were statistically analyzed.

Results

It is evident from Fig. 1 that only the medium containing both stigmasterol and TEM-4T will support growth of paramecium to a maximum density above 20 000 cells per ml. However, the 10th to 14th day of culture in this medium appeared to be the critical time limit of maintaining these cultures. Unless the organisms were transferred to fresh culture medium before the 10th day, the population density would decline very rapidly to zero during the 10th–14th day. Transfers to fresh medium during this period failed to develop into large cultures. In the medium containing the Semen lini extract the population density never exceeded 2500 cells per ml, but after 20 days the population density was still above the initial inoculum. Even though the population density had decreased considerably on the 27th day of culture, the cells were still alive on that day. When the 20 day old culture in medium M-V was transferred to medium M-I the cells grew very well after a short time lag and reached

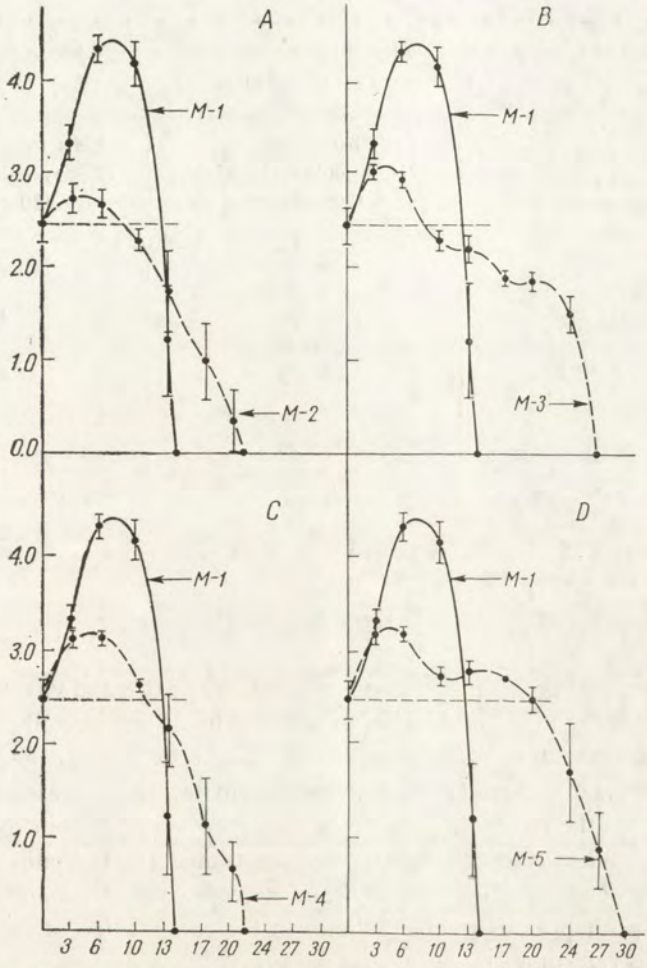

Fig. 1. Influence of stigmasterol and lipids composition in axenic medium on growth and maintenance of *P. aurelia* mass cultures. M-1 — control curve of full standard medium with stigmasterol and TEM-4T, M-2 — medium without stigmasterol and lipids, M-3 — medium with stigmasterol, without lipids, M-4 — medium without stigmasterol, with Semen lini extr., M-5 — medium with stigmasterol, with Semen lini extr.

the maximum population density of ±20 000 cells per ml in the second transfer (Table 2). Cells from medium M-V could be as well transferred to fresh medium M-V every three weeks. In this medium cultures were on the low population density level for several months. Besides, *P. aurelia* syng. 4, stock 51 in medium M-V were mailed from U.S.A. to Poland and survived three weeks journey in very good condition. Cells grown in medium M-II, M-III, or cells in medium M-IV could also be maintained for about 20 days, but no growth would ensue when these cells were transferred to the M-I medium.

Table 2

The Growth Rate of *P. aurelia* 299 S Cultivated three weeks on the M–V Medium, and Transferred into the M-I medium

Days after transfer to the M–I		Cells from Exp. No. 2 (21-day culture in M–V, 120 cells/ml)	Cells from Exp. No. 4 (21-day culture in M–V, 120 cells/ml)
		cells/ml	
First transfer	3	50	20
	6	1200	1200
	7	4800	—
	10	16000	6000
	14		
Second transfer	3	3200	—
	4	—	1500
	6	19200	—
	8	—	22400

Discussion

These experiments confirm earlier observations by Soldo and van Wagtendonk (1967, 1968) that the kind and balance of supplied lipids and phospholipids is essential for the growth and maintenance of life of ciliate cells. Similar observations were made by Soldo and Merlin (1972) with marine ciliates. In the presence of cephaline and asolectine as the only source of lipids they grew very fast, but died off rapidly after ten days. When cerophyl extract was substituted for cephalin and asolectin the population density declined rapidly, but the cells could be maintained for 3–4 weeks. There probably exists some balance in the composition of the lipids, which determines the rate of metabolism and growth.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Miss Danuta Kucharczyk for excellent technical assistance.

RÉSUMÉ

Le milieu contenant l'extrait du Semen lini (semence du lin) prepare á l'ether ethylique, au lieu du TEM-4T, soutient la croissance de *Paramecium aurelia* très longtemps, jusqu'a a 27 jours. Ce milieu ne peut pas alimenter une population très nombreuse, mais il maintient les cellules en vie pour une période assez longue pour éviter la nécessité des passages trop fréquents.

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Received 22 October 1974

ERRATA

Page	Instead of	Read
272_1	cells	ten cells
281_8	$CaCl\frac{1}{2}$	$CaCl_2$
308^5	3.4	3.9
308^6	39	3.4

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