ACTA PROTOZOO-LOGICA

VOLUME 21

Number 2

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Studies on Interpopulational Variation in Trichodinas (Ciliata)

Synopsis. A comparative analysis has been made on interpopulational variation in seven species of ciliates of the family *Urceolariidae*, namely: *Trichodina vesicularum, T. faurefremieti, T. ranae, T. urinaria, T. nigra, T. reticulata*, and *Semitrichodina sphaeronuclea* inhabiting inner organs, gills and skin of fish, amphibians, and land snails. For this study the multilevel nested analysis of variance was used. It has been found that in the species of trichodinas forming "closed" populations, characterized by small exchange of individuals and inhabiting inner organs, e.g., urinary bladder of fish and amphibians, interpopulational variation, most probably genetic in the character, is dominant. In trichodina species forming "open" populations, characterized by great exchange of individuals and occurring on the body surface or in the gill cavity of fish, significance of interpopulational variation diminishes in favor of variation conditioned by ecological factors of the outer environment. The meaning of host induced variation and of geographical variation in trichodina populations has been also considered.

Recognition of the structure of species, including its variability is one of the principal tasks of contemporary zoology. Investigations on intra- and interpopulational variation and their sources may be regarded as a keystone for such consideration. According to recent concept of the species the population is a fundamental unit of each biological species. This unit is genetically uniform to some extent, ecologically integrated, and occupying a defined area. Studies on the range of inter- and intrapopulational variation, and on factors bearing on them, allow to recognize and to describe more precisely the species under investigation, as well as to elucidate some evolutionary processes involved in their formation.

Such studies ought to be based on large series of the material, originating from various environments and evolving under the influence

of various factors. So, it is difficult to perform such a study on larger animals. In contrast, the protozoans, and especially the parasitic ciliates, give a good model fulfilling all purposes essential for such consideration. Within their host body they form aggregations of individuals which intensively multiply and may perform sexual processes with the exchange of genetic material. Such aggregations are delimited in space and variously isolated from other similar groups of the same species. Thus, such groups may be treated as true populations. The fact, not without meaning, is that many species of parasitic ciliates occur in great number on their hosts, so it is not difficult to collect appropriately representative, great samples, the more so, the methods of preparation and elaboration of the material for morphological purposes are quite simple and time-saving.

The present paper is an attempt to such study undertaken on trichodinas, parasitic ciliates of the order *Peritrichida*, family *Urceolariidae*. They parasitize the body surface and the lumen of some inner organs of invertebrates and vertebrates living in the aquatic environment or, at least, in humid habitats (e.g., frogs, land snails). The protozoans belonging to this family have the same pattern of the body structure giving an opportunity to compare the results of investigations on a fairly great number of species. Moreover, these ciliates are easy to collect, fairly well known, and, the more so, some elements of their structure, important for taxonomy, do not undergo more pronounced deformations during technical procedure.

In the present paper results of investigations on the following species of trichodinas have been used: *Trichodina vesicularum* Fauré-Fremiet and *T. faurefremieti* Kazubski from the urinary bladder of newts (K a z u b s k i 1979), *T. ranae* da Cunha from the urinary bladder of frogs (K a z u b s k i 1980), *T. urinaria* Dogiel from the urinary bladder of perch (unpublished), *T. nigra* Lom from the gills and the skin of perch-pike (K a z u b s k i and P i l e c k a - R a p a c z 1981), *T. reticulata* Hirschmann et Partsch from the body surface of crucian carp (K a z u b s k i 1982) and *Semitrichodina sphaeronuclea* f. *macrodentata* Lom from the mantle cavity of slugs (K a z u b s k i 1981 a). Detailed results of these investigations have been already presented in papers concerning particular species of examined trichodinas.

In the study on morphological variation in trichodinas the following characters were used: the adhesive disc diameter, the denticulate-ring diameter and the number of denticles. The first two characters are highly correlated one with the other while the correlation between the second and the third one is not so high. Such choice of characters has been considered as representative for the group of ciliates under investigation (K a z u b s k i 1979).

In order to recognize the variation within particular examined species the multilevel nested analysis of variance after S o k a l and R h o l f (1969) has been used. This analysis allows to distinguish the variation conditioned by particular factors in the total range of variation characteristic of the species. The examined populations, (aggregations of ciliates from single host individuals, named sub-populations in previous papers), grouped in a defined way have been tested for particular factors, as a rule, in each analysis the interpopula-tional variation has been taken into account. A care has been taken to select such groups of populations which would be the most uniform, e.g., the host dependent variation being tested on the material from the same territory in the case of *T. ranae* and *S. sphaeronuclea* and the geographical variation on trichodinas from the same host species (in *Semitrichodina sphaeronuclea*) or after having found that host induced variation was not important (in *Trichodina ranae*). The aim of the present paper is to compare the results obtained in particular examined cases and to draw some more general conclusions. The primary conclusions have been already presented at the VIth International Congress of Protozoology in Warszawa (K a z u b s k i 1981 b).

It has been decided to base the investigation on variation in trichodinas on the meaning of defined factors for particular species. In order to facilitate and to make objective such a comparison the following criteria were used. The comparison was based on previously counted significance of differences characterizing the influence of particular factors. The value of the risk of error was determined in per cents by interpolation when the significance of differences ranged within 5–1% level of error. When the risk of error was smaller that 1% than the calculation was made how many times the obtained F_o value overpassed the critical value at 1% level. These values were used in further considerations. It is supposed that such a procedure allows to compare variations conditioned by diverse factors and characteristic of particular species in the most objective way.

Variations in Trichodinas

The results of the analysis of variance and their estimates in all the examined species are presented in Table 1. As in some species of trichodinas the populations were examined in various configurations the results of each analysis of variance are given separately. In Table 2 the summaric estimation of variation in particular examined species is given. In this estimation somewhat smaller meaning is attributed to the results of the analysis presented in the columns two and three for *Trichodina vesicularum* because in both cases only population from lawlands of

Table 1

Results of the analysis of variance (F_0-values and their significance) in examined species of trichodinas

Species	Examined material	Variation						References
		Interpopulational	Local	Ecological	Seasonal	Host induced	Geographical	
	total material from Poland	25.716[a] 15.0×	1.084 ns	0.313 ns	—	1.316 ns	—	—
		19.530[b] 11.4×	1.750 ns	0.844 ns		1.854 ns		
		13.176[c] 7.7×	0.837 ns	3.551 ns		2.746 ns		
		1.71**	2.51*	5.79*		18.51*		
	trichodinas from T. helveticus from France	57.488 16.7×	—	4.363 ns	—	—	—	—
		23.033 6.7×		9.178 4%				
		3.669 1.1×		9.840 3%				
		3.44**		7.71* 21.20**				
Trichodina vesicularum	trichodinas from lowlands of Poland and France	15.063 4.8×	—	—	—	—	0.282 ns	Kazubski 1979
		17.047 5.5×					0.493 ns	
		18.268 5.9×					0.475 ns	
		3.11**					6.61*	
	trichodinas from mountain of Poland and France	31.912 14.5×	—	—	—	—	16.918 1.8×	—
		20.816 9.5×					40.645 4.4×	
		7.552 3.4×					0.501 ns	
		2.20**					4.75* 9.33**	
T. faurefremieti	total material	3.989 2.5%	—	5.055 ns	—	—	—	—
		6.850 1.4×		1.525 ns				
		6.988 1.5×		14.947 ns				
		3.09* 4.82**		200*				

total material	14.872	9.0×	1.014 ns	—	—	—	—	2.170 ns	Kazubski 1980
	15.548	9.4×	1.104 ns					0.870 ns	
	18.688	11.3×	0.385 ns					3.262 ns	
		1.66**	2.28*					4.74*	
T. ranae	11.883	6.9×	—	—	—	—	1.620 ns	—	
trichodinas from Konin lakes	13.099	7.6×					1.958 ns		
	8.611	5.0×					0.329 ns		
		1.72**					3.32*		
	12.926	7.6×	—	—	0.237 ns	—	—	—	
	14.112	8.3×			0.784 ns				
	8.159	4.8×			1.208 ns				
		1.71**			3.32*				
T. urinaria	16.531	7.4×	4.710 2%	—	—	—	—	—	unpublished
trichodinas from lakes Dagł, Warniak and others	17.104	7.7×	3.100 ns						
	5.897	2.6×	5.226 1.5%						
		2.23**	3.49* 5.95**						

Table 1, cont.

Species	Examined material	Variation						References
		Interpopulational	Local	Ecological	Seasonal	Host induced	Geographical	
	total material	6.692 3.3× 7.282 3.6× 6.545 3.3× 2.00**	4.776 1.1× 4.119 1.2% 8.139 1.9× 2.81* 4.34**	—	—	8.405 3.5% 20.587 1.3× 11.206 2% 6.61* 16.26**	—	Kazubski 1981a
Semitrichodina sphaeronuclea	trichodinas from *Lehmannia marginata* from Poland and France	1.428 ns 2.559 1% 7.589 3.0× 1.97* 2.57*	—	—	—	—	26.332 2.3× 10.267 1.5% 2.313 ns 5.32* 11.26**	
	trichodinas from *Bielzia coeruleus* from Babia Góra Mt.	9.570 3.9× 10.394 4.2× 7.457 3.0× 2.45**	—	5.667 2.5% 5.559 3% 13.737 1.7× 4.26* 8.02**	—	—	—	
Trichodina nigra	trichodinas from *L. lucioperca* from Szczecin Gulf	3.147 1% 3.712 1.2× 2.284 5% 2.29* 3.17	—	—	3.672 ns 4.672 ns 0.232 ns 6.61*	—	—	Kazubski and Pilecka-Rapacz 1981
T. reticulata	trichodinas from *C. carassius* from pond in Kortowo	3.385 1.8× 3.704 1.9× 6.705 3.5× 1.90**	—	—	18.301 3.1× 24.677 4.2× 12.679 2.2× 5.85**	—	—	Kazubski 1982

Explanations: F_0 — value of a diameter of adhesive disc, [b] diameter of denticulate ring, [c] number of denticles, * critical value of F at 5% risk of error, ** critical value of F at 1% risk of error.

Table 2

Character of variation in trichodinas (*Ciliata, Peritrichida*)

Species	Host	Localization	Variation					
			Interpopulational	Local	Ecological	Seasonal	Host induced	Geographical
Trichodina vesicularum	newts		×××, ×××, ×××	ns	ns	—	ns	××, ×××, ns
T. faurefremieti		urinary bladder	×, ××, ××	—	ns	—	—	—
T. ranae	frogs		×××, ×××, ×××	ns	—	ns	ns	ns
T. urinaria	perch		×××, ×××, ××	×, ns, ×	—	—	—	—
Semitrichodina sphaeronuclea	land snails	mantle cavity	××, ××, ××	××, ×, ××	×, ×, ××	—	×, ××, ×	××, ×, ns
Trichodina nigra	perch-pike	gills	×, ××, ×	—	—	ns	—	—
T. reticulata	crucian carp	body surface	××, ××, ××	—	—	××, ×××, ××	—	—

Explanations: × — significance of differences ranged within 5—1% risk of error, ×× — significance of differences when F_0 overpassed 1—4 times the critical value at 1% risk of error, ××× — significance of differences when F_0 overpassed 4—16 times the critical value at 1% risk of error.

France was analyzed. Also the results concerning *T. faurefremieti* are regarded as rough ones due to small (only four) number of examined populations.

These tabularized data allow to conclude that statistically significant interpopulational variation occurs in all the examined species of trichodinas and the differences manifest themselves in the range of this variation and its relation to variation caused by other agents. These data show also that the variation depends on the type of population formed by the species under examination. The highest interpopulational variation occurs in trichodinas inhabiting inner organs of their hosts and forming there closed populations, well isolated from each other. In such conditions particular populations correspond, the most probably, to single clones or to a mixture of a few clones. The essential role seems to be played by a founder effect or genetic drift. Such kind of interpopulational variation has been observed in trichodinas parasitizing the urinary bladder of amphibians. These hosts the most part of their life spend on land where the possibility of exchange of their parasites is rather difficult. As the examples may serve *T. vesicularum* parasitizing the urinary bladder of newts and *T. ranae* from the urinary bladder of frogs. It is characteristic that in both cases other factors, such as occurrence in representatives of the same local population of hosts, ecological and seasonal factors and even occurrence in different host species have no significant influence on morphological variation in these trichodinas. Only in the case of *T. vesicularum* the geographical variation seems to have some bearing. Slightly lower interpopulational variation noted in *T. faurefremieti* is probably due to too small number of examined populations (four) of this species, originating from two localities.

Similar type of variation occurs in trichodinas parasitizing the urinary bladder of fish, e.g., in *T. urinaria* from the urinary bladder of perch. But in this case the interpopulational variation, being always highly significant, is smaller than in preceeding species. Probably it is due to the occurrence of these protozoans in hosts inhabiting aquatic environment, and the more so, in fish living in small shoals. This reduces barriers between particular populations of trichodinas and enables to some extent a flow of these parasites among particular host individuals. Statistically significant, although not to high degree, appeared to be the differences observed between the trichodinas from local groups of hosts. This was observed in fish originating from two lakes. Thus, such isolation appeared to be sufficient to produce defined morphological variation in examined ciliates.

Quite different type of variation is presented by the species of trichodinas parasitizing the skin and the gills of their fish hosts. In

such case localization of the parasite on the surface of their host and direct communication with surrounding environment (aquatic environment) facilitate contacts among particular populations of parasites. As a result, in both examined species, *T. nigra* from the gills and skin of perch-pike and *T. reticulata* from the skin of crucian carp, the interpopulational variation, although statistically significant, is maintained at fairly low level, while the importance of external factors increases. True enough, high variation dependent on the season of the year has been noted only in one of these species, namely in *T. reticulata*, originating from small ponds showing high seasonal changes of temperature. While in the other species, *T. nigra*, examined till now only from the perch-pike from Szczecin Gulf, the seasonal variation has not been ascertained. In fact, these trichodinas are not submitted to greater changes of temperature, occurring in large water reservoir with fairly low oscillation of temperature during the year, and on fairly large fish host having the possibility to find out the optimum temperature of the water.

An intermediate position between two types of variation mentioned above occupies *Semitrichodina sphaeronuclea*. In this species of ciliate the interpopulational variation maintains at fairly high level, although lower than in trichodinas inhabiting the urinary bladder of amphibians and fish. Simultaneously, other factors, such as the occurrence in local populations of hosts, various host species and other ecological factors, play important role in their variation. Also the variation connected with geographical distribution of the host snail has been noted in this species of ciliate.

A comparison of the types of variation discussed above supports the previous opinion of the author that the kind of population formed by the species under discussion is the most important for variation in trichodinas. The main role plays the character of isolation between particular populations. In the case of "closed" populations of ciliates inhabiting inner organs of their host body and characterized by a very small flow of individuals, being thus strongly isolated from each other, the genetically conditioned interpopulational variation is dominant. While in the case of "open" populations of ciliates, occurring on the body surface of their hosts and characterized by large exchange of individuals, the interpopulational variation diminishes and, simultaneously, grows the meaning of variation induced by ecological factors.

Two sources of variation are worthy of being mentioned: the variation conditioned by the host species and geographical variation. In the case of "closed" populations the host induced variation seems to be unimportant. Such situation is advantageous for the species enlarging its ecological niche and enabling its existence even in conditions difficult

for spreading on new hosts. While in the case of "open" populations a defined variation conditioned by the host species probably occurs. It is true that in the present material it may be demonstrated only in the case of *Semitrichodina sphaeronuclea* but the up to date descriptions of numerous forms connected with host species in trichodinas parasitizing fish and invertebrates (being, however, not analyzed from the point of view of the population) seems to ascertain such opinion. In such cases, however, great care must be taken in forming some conclusions because the differences, one can meet with, may be uphold by quite another isolating mechanism, probably of ecological origin. It may be illustrated by two forms of *S. sphaeronuclea* f. *macrodentata* and f. *microdentata*, differing by the number, dimensions and even by the shape of denticles, and occurring in the representatives of two different families of land snals, living in completely different ecological conditions, probably without any contact (K a z u b s k i 1971). Also the distribution areas of both forms of *S. sphaeronuclea* do not overlap. Thus, if we consider any variation as host induced one we must take into account that in this place the intraspecific variation achieves its boundary and that the observed differences may be due to different species involved.

The geographic variation showed in trichodinas seems to be a result of spatial isolation. In analysed cases, in *Trichodina vesicularum* and *Semitrichodina sphaeronuclea*, there is a lack of substantial differences in the variation of the number of denticles while the differences in the dimensions of the adhesive disc and of the denticulate ring are opposite in the direction. In the case of *T. vesicularum*, the trichodinas with larger elements of the adhesive disc occurred in the Central Massif in France while those with smaller elements in Beskidy Mts in Poland. The opposite situation was observed in the case of *S. sphaeronuclea*. At present it is difficult to distinguish a factor or a group of factors responsible for such distribution of characters. It seems that too small number of observations collected till now makes impossible somewhat more advanced conclusions.

Discussion and Conclusions

The papers on intraspecific variation in protozoans have been published since long. At first they concerned mainly the morphology being based on searching for and describing specimens outstanding by their body structure and dimensions. Such papers beared rather a casuistic character. Such attitude to the problem of variation was connected with widely expanded typological species concept. Recently the more and more frequent are the papers based on large material

showing variation within greater groups of individuals under an influence of any defined factor. Most papers deal with ciliates due to their fairly complicated structure and rather small deformations of the body during preparation procedure.

Some of these papers are devoted to variation in *Peritrichida*. The paper by R e y n o l d s o n (1950) on seasonal variation of the body dimensions in *Urceolaria mitra* from triclade turbellarians may be mentioned here as well as the paper by K a z u b s k i and M i g a ł a (1968) on seasonal variation of the dimensions of the body, of the adhesive disc and of the number of denticles in trichodinas occurring on carp. The seasonal variation in the body length of *Thigmocoma acuminata* (*Scuticociliatida*) (K a z u b s k i 1968) was also noted as well as the variation in the length of lorica in representatives of *Tintinnida* from the New York Bight (G o l d and M o r a l e s 1975). In all mentioned cases the observed variation was related to seasonal change in temperature.

Variation in the body shape and the dimensions of ciliates of the family *Ophryoscolecidae* (*Entodiniomorphida*) from ruminants was observed by D o g i e l (1927) and by K o f o i d and M a c L e n n a n (1932). P o l j a n s k y and S t r e l k o v (1938), who studied this phenomenon in experiments on clones bred in hosts, related it to the diet of hosts. But E a d i e (1979) in his study on *Polyplastrom multivesicularum* and *Euplodinium maggii* supposed that the source of this variation was not obvious as the change of diet did not affect the morphology of ciliates.

In last years the studies on variation more and more acquire quantitative character being supported by statistical methods using distinct populations as subjects for such analyses. It is connected with widespreading of the biological species concept and the development of computation methods. For such studies single and multilevel analysis of variance as well as numeric methods have been used.

As far studies on morphological variation were performed on *Paramecium aurelia* complex (P o w e l s o n et al. 1975 and G a t e s and B e r g e r 1976), *Tetrahymena pyriformis* complex (G a t e s and B e r g e r 1974), and within the genera *Blepharisma* (R e p a k et al. 1977) and *Euplotes* (G a t e s 1977, 1978 a, b and G a t e s and C u r d s 1979). The variation among monodemes (groups of individuals from single host specimen) was studied in ciliates parasites or endocomensals of aquatic invertebrates: in *Plagiopyliella pacifica* occurring in echinoderms (L y n n and B e r g e r 1972) and in *Ancistrum mytili* parasitizing marine bivalves (B e r g e r and H a t z i d i m i t r i o u 1978). These studies have shown that within each of examined species great and statistically significant differences exist between particular populations.

Especially interesting are the results of the two last mentioned papers in which the differences analogous to those observed among populations of trichodinas have been shown. In these papers, however, the sources of variation were not studied except the ciliate *Ancistrum mytili* in which some variation was noted between xenodemes (groups of ciliates originating from different hosts) e.g., from *Mytilus edulis* and *Modiolus modiolus,* after all living in various ecological conditions. Such observations are concordant with the results presented for "open" populations of trichodinas inhabiting gills and body surface of fish. Investigations on *Plagiopyliella pacifica* were also the subject of analysis by K l u g e and K e r f o o t (1973) and by S o k a l (1976) who counted quantitative relationships between intra- and interpopulational variation. The same analysis was made by B e r g e r and H a t z i d i m i t r i o u (1978) for *Ancistrum mytili.* In this respect the data discussed in the present paper will be analyzed separately.

Summing up the above considered data it may be ascertained that the primary hypothesis assuming that the groups of trichodinas from single host individual correspond well to true populations is right and that the multilevel nested ANOVA is a fruitful method of statistical analysis, enabling examination of variation and its sources in many aspects.

As concerns the variation in trichodinas the following conclusions may be drawn:

— interpopulational variation is a common phenomenon in these ciliates, being observed in all examined species,

— in "closed" populations with very small or no exchange of individuals the interpopulational variation, probably genetic in character, is dominant while the influence of any environmental factors is inconspicuous,

— in "open" populations with easy exchange of individuals the significance of interpopulational variation relatively diminishes in favour of the influence of various factors of outer environment,

— intermediate systems, characterized by various relations of both types of variation, may also exist,

This concept is in agreement with the fundamental thesis of populational genetic that the level of variation depends on richness and diversity of gene pool and a degree of isolation between populations.

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R o b e r t   C H I O V E T T I [1], Jr. and E u g e n e   C.   B O V E E [2]

Initiation of Encystment by Multiple Contacts among Starving Amebas, *Naegleria gruberi (Schardinger, 1899)* [3]

Synopsis. Encystment of the ameboflagellate, *Naegleria gruberi* (Schardinger, 1899) was studied, relative to the roles of starvation and/or crowding for initiating encystment. It was found that the amebas needed to be b o t h starving and crowded to begin encystment. Feeding amebas did not encyst, no matter how crowded. Starving amebas did not encyst, u n l e s s they were critically crowded. A filtrate from a maximally encysting population of amebas had no effect on the rate of encystment of other populations. The rate of encystment of starving amebas was found by arc-sine plot to be a linear function of the number of amebas per sq mm of surface (agar) on which they were dispersed or crowded during encystment. A role for contact-sites at the surface of amebas as initiators of encystment is suggested, since encystment is inhibited among starving, crowded amebas by exposure of them for 48 h to 0.1% trypsin. Since trypsin breaks peptide bonds, the potential initiator-sites are tentatively assumed to be the terminices or side-chains of protein molecules.

Many protozoa undergo, due to stresses in their natural environments, periods of dedifferentiation and encapsulation (encystment). This well-known phenomenon occurs among both freeliving and parasitic protozoa, in many of the latter being an important part of the regular life-cycle. However, for only a few species is anything known as to how the formation of the cyst is initiated or how the organism is caused to excyst, redifferentiate and become active (C o r l i s s and E s s e r 1974).

For the small-but-hardy, bactivorous amebas that constitute the great majority (up to 90%) of active, bactivorous protozoa in moist

soils (B a m f o r t h 1970), it is assumed that encystment in moist conditions is triggered by lack of food (S c h u s t e r and S v i h l a 1968), or in drying (C h a n g 1958) as critical cationic conditions develop (N e f f et al. 1970). However, B o v e e (1965) found that slow starvation of small amebas as scattered isolates in small numbers did not trigger encystment, although the same species encysted quickly and in dense numbers in crowded populations shortly after food was exhausted.

We chose to investigate this apparent discrepancy more critically, using the well-known, easily cultured and ubiquitous ameboflagellate, *Naegleria gruberi* (Schardinger, 1899).

Materials and Methods

Stock cultures of *Naegleria gruberi* strain EG [4] were raised on 1.5% proteose-peptone-yeast-extract-glucose (PYG) agar with *Enterobacter aerogenes* as food. Bacterial "lawns" were prepared by spreading an 0.1 ml aliquot of PYG broth-grown culture of *E. aerogenes* on a PYG-agar plate. Live or encysted amebas were inoculated at one edge of the plate. These grew, divided and then encysted behind the advancing edge of the colony, producing agar plates covered with cysts as the bacteria were cleared from the plate. These cysts served as stock cultures for future experiments.

Amebas were obtained for experiments by flooding a growing PYG-plate culture with 3 ml of sterile PYG-broth. Gentle agitation for 90 s released amebas and bacteria, leaving cysts attached to the agar. These amebas were transferred to a 12 ml centrifuge-tube by sterile pipette and were centrifuged at 3000 rpm ($\sim$4G) for 2 min. The supernatant, with bacteria, was discarded and 5 ml of sterile broth was added and centrifuging was repeated. The amebas were thus washed four times.

After a final resuspension, amebas were counted in triplicate in a Nebauer hemocytometer and density of amebas was adjusted to 7×10^5 trophozoites by serial dilution with added broth. Enough aliquots to yield 7×10^4 trophozoites were pipetted onto fresh PYG-agar plates, 0.1 ml of broth culture of *E. aerogenes* was added and the inocula were mixed and spread evenly on the plate and allowed to grow at 33° C for 18 h, producing logarithmically growing cultures.

These were harvested and washed four times in 2 mm of sterile TRIS [5] buffer, fortified with 1000 U/ml sodium-penicillin-G and 0.001 g/ml streptomycin-sulfate (PS-TRIS solution) to retard bacterial growth. The washing procedure took less than 10 min.

To test effects of density and starvation on the amebas relative to encystment, the amebas, suspended in PS-TRIS, were pooled, counted in triplicate and density adjusted by dilution with more PS-TRIS to produce when spread on 1.5% non-nutrient agar (that was mixed with PS-TRIS) densities of 2000, 1000, 500, 300, 200, or less than 100 trophozoites per sq mm of agar surface.

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[4] Kindly supplied by the late Dr. William Balamuth, Department of Zoology, University of California, Berkeley, California.
[5] Tris (hydroxymethylaminomethane hydrochloride) at pH 7.4.

To determine if some substance secreted by the amebas might be involved in encystment, stock plates on which maximal encystment had occurred (90%) in 48 h were flooded with 5 ml of sterile broth and agitated for 30 s at each 2 min for a 10 min period. The broth was removed by sterile pipette and was centrifuged at 4G for 15 min. The supernatant was then passed through a 0.45 μm millipore filter and transferred to a 10 ml cotton-plugged test-tube. Amebas were harvested as above and washed in sterile PYG-broth, then were divided into control groups and experimental groups. The control groups were plated in triplicate on PYG-agar fortified with PS-TRIS solution. To minimize cell-contacts, a final density of 200 trophs/mm² were plated for both control and experimental groups.

Since cysts clump (F u l t o n 1970), the randomness of the sampling technique (number of cysts per microscopis field viewed at 400×) was verified by a G-test for goodness of fit to the Poisson distribution (S o k a l and R o h l f 1969). Either Chi-square contingency tables (R e m i n g t o n and S c h o r k 1970) or R × C tests of association (S o k a l and R o h l f 1969) were used to analyze the effects of experimental manipulations. Mean values and standard errors of the means of replicates were computed on a Hewlett-Packard-35 calculator.

Results and Observations

Effects of Starvation, Alone: When amebas were starved on non-nutrient agar, scattered at less than 100 trophozoites/mm² of surface, amebas survived, moving about, for about 48 h — then died and disintegrated between 48 and 72 h. No amebas survived beyond 72 h. More importantly, n o n e e n c y s t e d.

Effects of Population-densities, Alone: Amebas feeding in logarthmic growth do not encyst so long as food is available. Teeming numbers in densely packed groups feed on the bacterial lawn, without encystment.

Effects of Population Densities and Starvation, Together: The percentage of starving amebas that encyst varies directly with the density of the population — the greater the number of amebas per mm² of surface, the greater the percentage of the amebas that encyst. At less than 100/mm², none encyst in 48 h. At 200/mm², less than 40% encyst, at 300/mm², less than 50% encyst. At denser populations, however, greater numbers encyst: At 500/mm², over 55% encyst, at 1000/mm², over 80% encyst, and at 2000/mm², over 90% encyst (Fig. 1). An arcsine plot shows the relationship is linear (Fig. 2).

Effects of a Filtrate from a Maximally Encysted Population: Populations of amebas at 200/mm² of agar surface that encyst at less than 30% in 48 h showed no change of numbers encysting in a filtrate from a maximally encysted culture. The percentage of those encysting in the filtrate was almost identical to the numbers encysting in the control (Fig. 3). The filtrate had no effect on the rate of encystment.
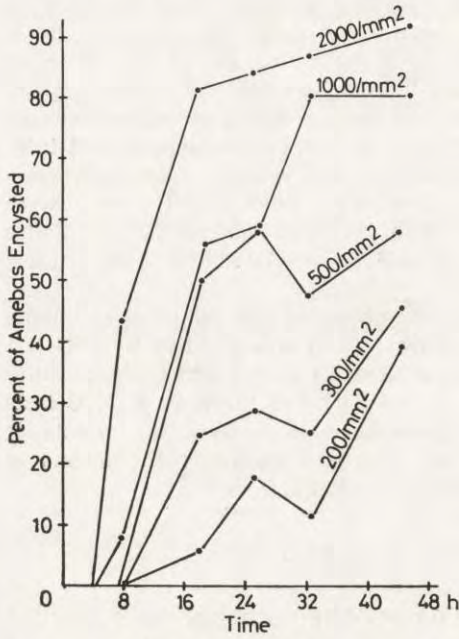
http://rcin.org.pl

Fig. 1. Encystment of *Naegleria* trophozoites related to numbers of trophozoites per sq. mm. of agar surface on which they are distributed
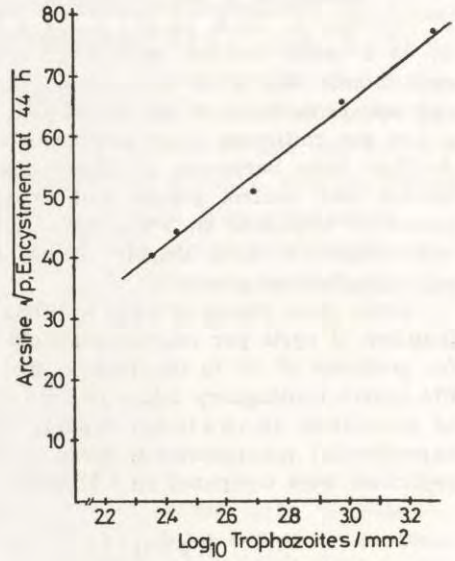
Fig. 2. An arc-sine plot of the relationship of numbers of trophozoites encysted as a factor of the concentration of numbers per sq. mm. of surface on which they were distributed (i.e., more or less crowded)
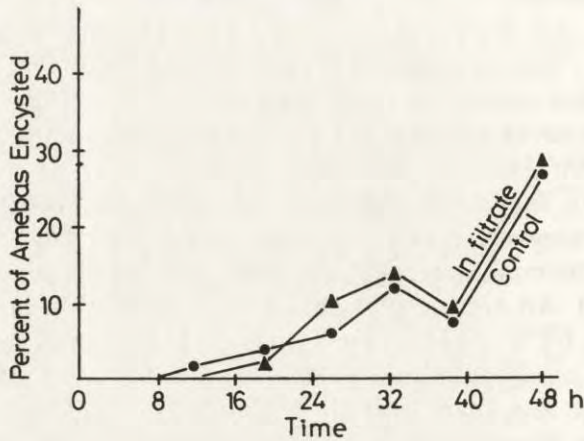

Fig. 3. Encystment of trophozoites placed in filtrate of a medium in which a previous group of amoebae had maximally encysted compared to the encystment of a control-group. There is no difference

Discussion

It is clear from the above experiments that starvation, alone, does not initiate encystment of the amebas, nor does any substance the amebas may secrete into the solution. It is also clear that crowding and multiple contacts among the amebas do not, alone, initiate encystment since densely crowded masses of amebas do not encyst while feeding.

The results diagrammed in Fig. 1, however, show clearly that starving amebas in a crowded population encyst quickly and in maximal numbers. This strongly indicates that they transfer some signal to one another, while starving, that initiates the encystment. Amebas that are feeding and not starving either do not generate, or receive the signal, or ignore it, since crowded, feeding amebas do not encyst; yet crowded non-feeding amebas behind them where all bacteria have been cleared from the agar, do encyst in large numbers.

The signal that is exchanged is not secreted into the surrounding fluid, since scattered amebas do not encyst in a filtrate in which maximal encystment of amebas had previously occurred, and amebas dispersed on the agar (less that 100/mm^2), do not encyst (Fig. 3).

Therefore, the signal most probably is exchanged upon contact by one starving ameba with another or others. Since maximal encystment occurs in the most crowded population, those amebas that more quickly make numerous contacts with one another also more quickly encyst.

Four requisites are necessary for the encystment of the ameba: that (a) it be starving; (b) it therefore be able to generate the signal; (c) it therefore be able to receive the signal; and (d) it make sufficient contacts in a limited time with other starving amebas to both transfer and receive the signal. The more contacts that are made in a short time, the more effective are signals and the sooner encystment is begun.

Since the signal is contact-generated, molecules at the surface of the ameba must be involved, acting on complementary sites on another ameba to initiate encystment. One or both sites may be absent from or inactive at the surface of crowded but non-starving, feeding amebas, or the signal is blocked, since such amebas do not encyst, while their starving, crowded neighbors do so.

Where such contact-active sites have been identified on other cells in other interactions, they have been shown to be the exposed terminices of protein-side-chains (e.g., contact-inhibition receptors on embryonic hamster fibroblasts, M a s t r o et al. 1974; prey-identifying receptors on *Chaos carolinense*, Lindberg, 1974; L i n d b e r g and B o v e e 1975). We have some evidence that these activator-sites for encystment of *Naegleria gruberi* are proteinaceous, since some preliminary experiments wherein we have exposed dense, starving populations of amebas to

0.1% trypsin (which breaks peptide bonds) for 48 h show significant delay of encystment, whereas a 15 min exposure to the same amount of trypsin does not (Figs. 4, 5).
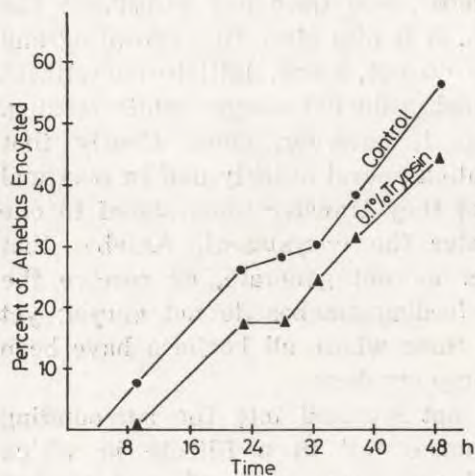
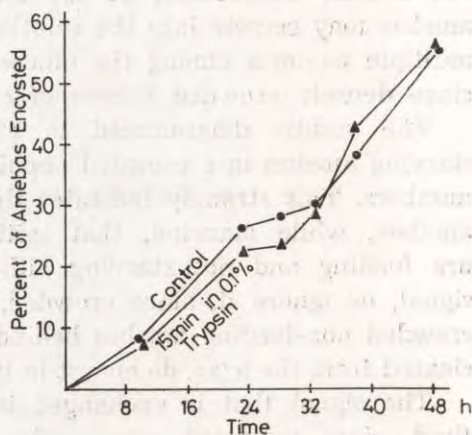Fig. 4. Delay of encystment caused by continuous exposure to 0.1% trypsin, compared to a control-group

Fig. 5. Lack of delay of encystment of trophozoites exposed to a limited (15 min) exposure to 0.1% trypsin

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Ewa PERKOWSKA

Growth of Dense Populations of *Acanthamoeba castellanii* in Small Volumes of Nutrient and the Influence of DAPI (a DNA Ligand) on Such Populations

Synopsis. It is signalled that a freshly inoculated population of *Acanthamoeba castellanii* started with a high number of cells growing in small volumes of nutrient can undergo a marked fall in cell number before it begins proliferate in the new environment. Our experiments confirmed also the general outlines of DAPI influence on cell proliferation inhibiting the growth of the populations.

DAPI a phenyl-indole derivative reduces the accessibility of DNA for nucleic acid polymerases. In this way DAPI hampers transcription on DNA. DAPI inhibits growth in cell populations such as paramecium. DAPI forms also intensely fluorizing complexes with the DNA double helix, both in nuclei and in other cell structures which possess DNA (Kapuściński and Skoczylas 1977, 1978, Skoczylas 1980).

These properties of DAPI should bear on the growth pattern of developing populations of cells such as *Acanthamoeba castellanii*.

Material and Methods

Acanthamoeba castellanii, starting with at least 2×10^5 cell/ml, were cultured axenically in 5 ml, 2.5 ml or 1.2 ml culture medium (Neff 1957) without shaking or aeration, in darkness at 27°C. Cells were counted immediately after inoculation, and later counts were made at intervals of 24 or 48 h. Samples were taken aseptically and counted in a Thomas Zeiss chamber. DAPI (4′6-diamidine-2-phenyl indole 2 HCl) was applied aseptically at the beginning of the exponential growth of the population. DAPI was added in three different concentrations per experiment — 1 µg/ml, 2 µg/ml and 5 µg/ml final concentration.

Growth of the population was expressed as duration difference between two cell division during 24 h. In fluorescence investigations a drop of *Acanthamoeba* suspension was spread on a microscope slide, fixed in Carnoy for 10–15 min and DAPI was added in final concentration of 0.1 to 1.5 µg/ml. These cells were observed under a PZO fluorescence microscope. DNA-ase (Schuchardt) was used in a solution of 1 µg/ml for digestion of DAPI treated cells for 2 h at 37°C.

Results and Discussion

Fall of Number of Cells at the Beginning of the New Growth Cycle in Freshly Prepared Cultures of *Acanthamoeba castellanii*

Before starting the experiments with DAPI, tests were made to examine the behaviour of cells in small volumes of culture media — 5 ml, 2.5 and 1.2 ml. The use of such small volume cultures was vital because of the necessity of economizing on DAPI.

In conditions of reduced volume of nutrient the growth curves of *Acanthamoeba castellanii* populations have shown a considerable depression at the region of the growth lag-period.

In ten experiments consisting of 38 batches, 27 batches (i.e., 71% of the total) have shown a drop in number of cells from the initial cell number during the first 48 h of the lag-period. This phenomenon seemed independent of the volume of nutrient in which the cells were growing. In Fig. 1 such decrease of cell number is shown in seven experiments where they were most conspicuous. More details shows Table 1. The fall in cell number in populations, named here "reducing populations" can be expressed as a percentage of survival, or percentage of missing cells. One should note as much as 13 batches out of the 27 in which the percentage of missing cells oscillated between 37 to 76% of the initial number of cells (Table 1).

Why the rise of the percentage of missing cells seems to be greater in 5 ml cultures than in 2.5 ml and 1.2 ml (except in two cases) is not clear. In more experiments the values should level out. Since this paper is concerned only with the pointing out of frequent (71%) drop of cell number in fresh cultures, a greater or lesser statistical validity inside this 71% is of no avail.

The remaining 29% "nonreducing populations" (those which had not undergone reduction of their initial cell number) grew slowly at a rate of 0.5 cell divisions p.d. in the first and second 24 h.

After the "reducing populations" reached their lowest cell number, they started growing at a rate similar to that of "nonreducing populations" and both reached afterwards the level of about 1–2 cell divisions in 24 h (Fig. 1).

It seems that the lag period in small volume cultures does not represent a stationary state in which the initial number of cells remains constant. Our results indicate that in case of *Acanthamoeba castellanii*, in many instances a considerable drop in this initial cell number can occur. This should be taken into account in order to avoid false conclusions in experiments in which drugs or other agents are used for testing growth rate of populations.

Table 1

The number of missing cells in *Acanthamoeba castellani* cultures
expressed as percentage of the initial number of cells during
first 24–48 h after inoculation

Experiment	Batch	Percentage of missing cells	Volume of culture medium [ml]
(1)	I	53.2	5
	III	5	
	IV	50	
(2)	II	9	5
(3)	I	52	5
	II	37	
	III	26.2	
(4)	I	56	5
	II	55.8	
	III	55.4	
(5)	I	44.2	5
	II	49.2	
	III	39	
(6)	I	49	5
	II	26	
	III	10	
(7)	I	10	2.5
(8)	I	6	2.5
	III	13	
(9)	I	16	1.2
	III	13	
	IV	20	
	V	4	
	VI	20	
(10)	II	7	1.2
	V	74	
	VI	76	

DAPI Influence of Number of Cell Divisions in Populations of *Acantha-moeba castellanii* During the First 48 h after DAPI Administration

As *Acanthameoba* develops readily and rapidly, the small volume of culture medium leading to overcrowding and chocking of the populations was a handicap not allowing for a long maintenance of batches. In view of these conditions attention was payed chiefly to the first 48 h after addition of DAPI to the cultures, and this addition was done as near as possible at the onset of the exponential growth of the population.

Diagram II shows the number of cell divisions which the DAPI treated populations and their corresponding controls underwent in 48 h
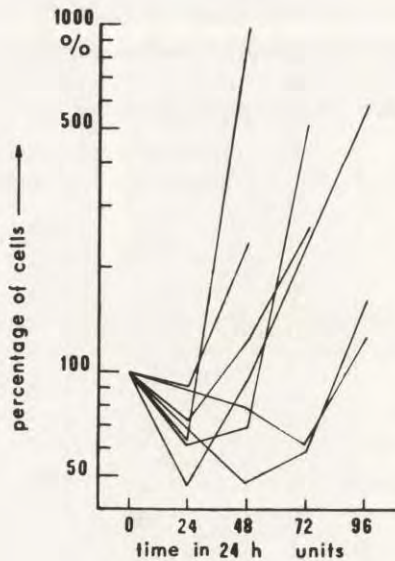
Fig. 1. Changes in cell number during the lag-period in *Acanthamoeba castellanii* populations inoculated into small volumes of medium, expressed as percentage of surviving cells counted at intervals of 24 h. Each curve represents the mean percentage value of number of cells in one experiment. Volume of culture medium — 5 ml. *Ordinate* — percentage of cells, *Abscissa* — time in 24 h units

from the moment of DAPI administration. The concentration of DAPI seems (at applied dosage) to play not an essential role in inhibiting the cell divisions. In all instances the cell division rate in DAPI treated cultures turned out to be markedly lower than in controls. (Fig. 2).
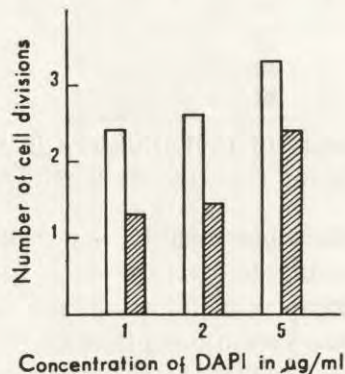
Fig. 2. Number of cell divisions undergone by populations of *Acanthamoeba castellanii* treated with DAPI and their respective controls in the first 48 h after DAPI administration. Volume of culture medium 1.2 ml in all experiments. Mean values obtained from 3 batches in each of the three experiments. Controls — plain columns, DAPI administered experimentals — striped columns

The ratio between the mean cell division values of treated and untreated cells is about 1 : 2 in 48 h which means that the treated cells divided approximately two times slower than the controls.

Although for clarity's sake only a growth period of 48 h is shown in Fig. 2, observations were carried out on several populations of treated and untreated cells till their overcrowding. In all of them the cell divisions went on, and DAPI treated cells divided as well as the controls although little slower.

Parallel to the above experiments some observations were carried out under the fluorescence microscope, because, as stated before, DAPI produces strong fluorescence when bound to DNA.

In our experiments was shown that after DAPI treatment the fluorescence was not confined only to the nuclei but was diffused throughout the whole cytoplasm entering even the alveoli. When these cells were digested with 1 µg/ml DNA-ase at 37° for 2 h the enzyme wiped out the fluorescence completely. This action of DNA-ase points to two facts: (1) that the binding between DNA and DAPI does not hamper DNA-ase to react with nuclear DNA, and (2) that, as reported by other authors (B y e r s 1979) the *Acanthamoeba* cells possess extranuclear DNA. This extranuclear DNA seems to be removed by the DNA-ase if one can assume that the vanished cytoplasmic fluorescence was caused by a binding of DAPI with non nuclear DNA.

In a radio assay (So b o t a, unpublished) it was shown that in course of 13 h the incorporation of ^{3}H-thymidine into DAPI treated cells was lower by 46% than in the controls. This trial test points to a possibility that in *Acanthamoeba castellanii* DAPI as a DNA-ligant affects the replication of DNA.

Our results coincide with known behaviour of DAPI in other organisms (C h a n d r a et al. 1977). However, an intriguing question remains — why DAPI does not inhibit completely the growth of a population, and why does it allow the population to carry out the cell divisions although at a slower rate than in the control populations.

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Leszek SZABLEWSKI

The Effect of Penicillin G (Sodium Salt) on *Tetrahymena pyriformis*

Synopsis. The effect of Penicillin G (sodium salt) on the multiplication dynamics, moving speed and morphology of *Tetrahymena pyriformis* has been studied. In effect, it has been ascertained that this antibiotic. in each of experimental concentrations, changes the examined morphophysiological properties. Morphological changes usually consist in decreased dimensions of *Tetrahymena* with the exception of cystostome which, under the effect of the antibiotic, is elongated. Also a lowered multiplication dynamics of ciliates under the effect of penicillin, as well as restraining effect of this drug on the moving speed of cells, have been noted.

The wide application of antibiotics in medicine has necessitated the learning of the mechanism of their action. For this reason, the studies have concentrated mainly on the effect of these drugs on bacteria. Due to the observed side-effects after administering these drugs, the studies have also included changes caused by these antibiotics in tissue cells (I n g a l l and S h e r m a n 1970, N a k a z a n o et. al. 1970, K u r y- ł o w i c z 1979). Many data show that these compounds can accumulate in the organism. It is little known, however, what are the effects of a longlasting contact of organisms with this group of drugs. The obtained results shown that, in the case of an enduring effect of high concentrations of antibiotics, morphophysiological properties of protozoa are changed (S z a b l e w s k i 1981). The aim of the experiments has been to examine the effects of a longlasting contact of protozoa with Penicillin G (sodium salt) in subtoxic and toxic concentrations.

Materials and Methods

Experiments have been carried out on ciliates of *Tetrahymena pyriformis* GL. Cells have been cultivated, at dark, in a medium containing 1.5% proteose-peptone and 0.1% of yeast extract, at 28°C.

Owing to the character of experiments, it has been necessary to slow down the metabolism rate in *Tetrahymena*. This condition has been met by applying proper cultivation methods of ciliates (S z a b l e w s k i 1981). The experiment has been deemed as started at the moment of administering the antibiotic (0 h time). The culture density has been about 30,000 cells/ml. Samples for testing have been taken every 24 h. The experiment has been assumed to take 144 h. The concentration range of the antibiotic has been esablished through experiments (S z a b l e w s k i 1981). The antibiotic used in the experiments has been produced by the Warsaw Pharmaceutic Works "Polfa". Its molecular weight (in grammes) has been 356.4, the strength — 1656 units/mg. In order to check the multiplication rate, the cells have been counted by the dillution method in each examined point (0 h, 24 h, etc.). For study of results protozoa density at 0 h of each culture has been assumed as a unit (Fig. 1).
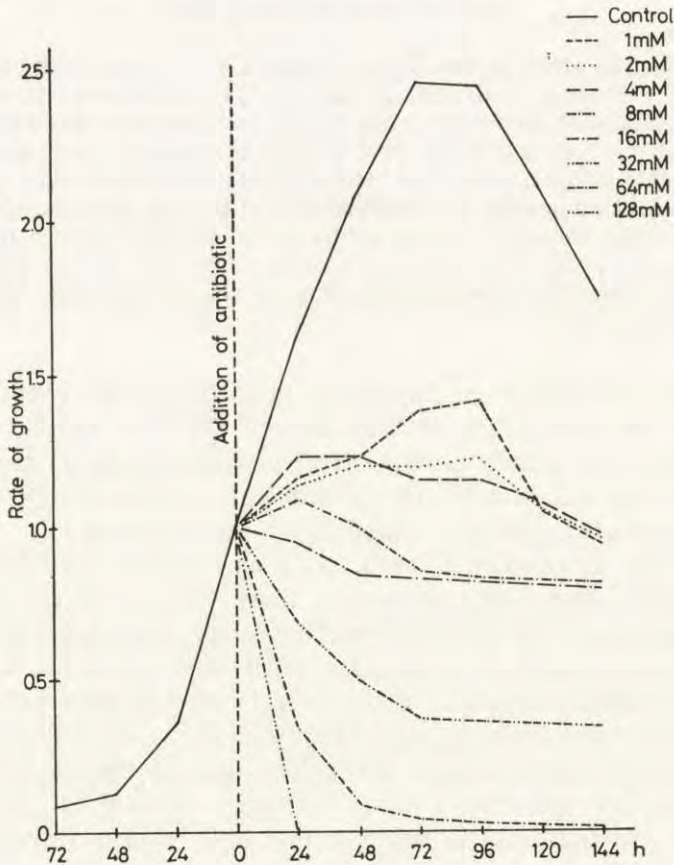
Fig. 1. Change of *Tetrahymena pyriformis* cells density depending on the concentration of Penicillin G. (sodium salt)

The moving speed of ciliates has been examined in a camera specially made for this purpose (S z a b l e w s k i 1981). Ciliate movements have been recorded by the photographic method according to D r y l (1958). The length of the path

covered by *Tetrahymena* has been measured on the screen of the microfilm negative reader.

Also morphological properties of *Tetrahymena pyriformis* incubated in different antibiotic concentrations for 144 h have been examined. They have been compared in ciliates at the beginning stage of the experiment and after 144 h of control culture (without administering the antibiotic). Preparations have been made by the Chatton-Lwoff method, modified by F r a n k e l and H e c k m a n n (1968). Measurements have been taken in accordance with the system and nomenclature offered by T a y l o r et al. (1976). Moreover, the number of kinetics on one side of the cell has been counted. Control experiments were carried out with NaCl solutions (devoid of Penicillin G) on dynamics of cell density and morphology of *Tetrahymena* (Fig. 3, Table 2).

Results

Changes in the Multiplication Dynamics

Changes in the multiplication dynamics caused by the effect of the antibiotic are presented in the Fig. 1.

With an increase of penicillin concentration the number of ciliates, as compared to the control culture, decreases. By administering lower concentrations of the antibiotic (from 1 to 8 mM), we observe a slight quantitative growth of the population during the first 24 h of the experiment. The performed analysis of variance has shown that all the used penicillin concentrations decrease the multiplication dynamics of cells as compared to the control culture in the statistically essential way.

Also the effect of particular antibiotic concentrations on the multiplication rate has been tested. Upon the variance analysis — it has been ascertained that there are no statistically significant differences in the multiplication dynamics between particular concentrations from 1 to 16 mM. In each of the remaining concentrations, however, these differences are statistically significant.

Changes in the Moving Speed of Cells

Changes in the moving speed of cells after administering the antibiotic are illustrated in Fig. 2. Generally, the moving speed of ciliates from the culture with the addition of the antibiotic is lowered, as compared to that of the control culture.

The exception here is 2 and 4 mM/l concentration after 120 h and 1, 4 and 8 mM/l after 144 h. Each of the used penicillin concentrations slows down the moving speed of cells, as compared to the control cells, in statistically significant way. Upon the variance analysis it has been
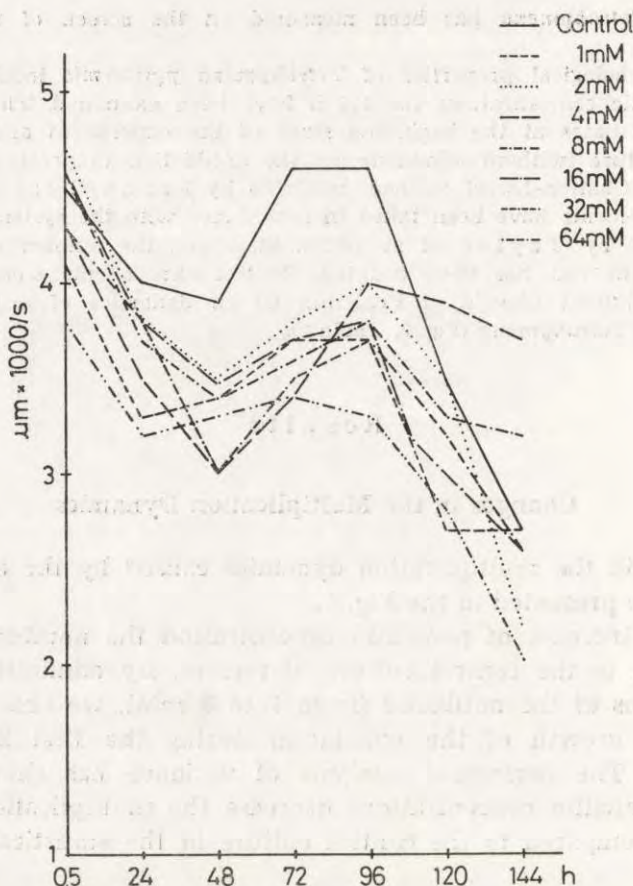
Fig. 2. Change of the moving speed of *Tetrahymena pyriformis* cells depending on the concentration of Penicillin G (sodium salt)

ascertained that the moving speed of ciliates in 64 mM/l antibiotic concentration differs, in statistically significant way, from the moving speed of the incubated cells in the remaining penicillin concentrations. For 128 mM/l concentration, the measurement could have been taken only at 0.5 h, as this concentration causes death of about 100% of cells after 24 h.

The Effect of Penicillin on Morphological Parameters

Morphological and morphometric changes caused by adding the antibiotic to the medium are shown in Table 1. The effect of the antibiotic on the length of cells is made evident here. In each of the examined penicillin concentrations this dimension is smaller than that of control cells after 144 h of the experiment. Only in 2 mM/l concen-

Table 1

Morphologic and morphometric data on *Tetrahymena pyriformis* cells incubated for 144 h in different concentrations of Penicillin G (sodium salt),

Concentration in mM/l	LENG [µm]	BRED [µm]	BUCL [µm]	A-M1 [µm]	A-UM [µm]	WLEN [µm]	BDM1 [µm]	BDUM [µm]	% AWD	% PWI	% A-M1	% A-UM	% WLN	% BUC	% BRD	% BDM	% BD1	Number of kinet-ies
Control 0 h	45.6	23.8	8.7	4.4	13.0	27.4	9.4	18.0	51.4	137.5	9.5	28.7	60.2	19.1	52.1	38.6	20.4	17.6
Control 144 h	55.7	29.2	9.1	4.6	13.8	33.9	12.0	22.8	51.8	139.5	8.1	24.4	60.6	16.2	54.2	39.9	20.6	18.0
1	44.6	21.1	9.2	4.2	13.5	21.7	9.6	17.9	54.2	120.2	10.1	32.2	51.7	21.6	50.4	42.5	22.9	17.5
2	48.5	21.8	9.3	4.3	13.6	24.8	10.1	16.9	60.3	124.1	8.9	28.2	51.2	19.3	42.8	35.1	20.8	18.0
4	43.3	19.8	9.4	3.4	12.9	23.3	8.54	15.6	54.4	130.7	7.9	30.0	53.7	22.0	45.3	36.1	19.7	18.1
8	43.0	18.2	9.4	3.7	13.3	22.8	8.6	14.4	61.1	126.0	9.0	31.1	53.1	22.0	42.6	33.2	20.0	18.0
16	45.1	18.3	9.6	4.6	14.0	24.2	9.5	14.0	68.0	132.8	10.3	31.8	54.2	21.4	40.9	31.1	22.2	18.3
32	39.8	19.2	8.7	4.0	12.7	22.0	9.4	15.2	61.6	131.9	9.5	31.4	54.1	21.7	48.2	37.2	23.0	17.6
64	41.0	22.6	9.0	3.8	12.9	21.9	10.5	18.0	57.9	128.4	9.3	31.2	53.4	21.9	55.1	43.4	25.3	17.5

A-M1 — anterior to start of M1, A-UM — anterior to end of UM, WLEN — anterior to widest point, LENG — length, BUCL — buccal length, BRED — maximal breadth, BDM1 — breadth at top of mouth, BDUM — breadth at bottom of mouth, %AWD — %BDM1/BDUM, %PWI — %BRED/BDUM, %A-M1 — %A-M1/LENG, %AUM — %A-UM/LENG, %WLN — %WLEN/LENG, %WLN/LENG, %BUC — %BUC/LENG, %BRD — %BRED/LENG, %BDM — %BDUM/LENG, %BD1 — %BDM1/LENG. All abbreviations explained in text (see chapter Results)

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tration the length of cells is bigger after completion of the experiment than at the beginning. In the remaining concentrations, ciliates are little smaller after 144 h of the experiment than at 0 h. Distinct changes are noted, as far as ciliate width is concerned. In each experimental concentration, this dimension is smaller than that of control cells after 144 h and at 0 h. *Tetrahymena* cytostome incubated with the addition of the antibiotic is usually bigger than that of control cells. The exception are cells incubated in higher penicillin concentrations (32 and 64 mM). In this case, cytostome is bigger than in the cells at 0 h, yet it is smaller than that in the control cells after 144 h of the experiment. Alike width, also the A-M1 value (distance between anterior and M1) changes. With the exception of cells incubated with the addition of 16 mM of penicillin, in each case this dimension is smaller than at 0 h, and than in control cells after 144 h. Depending on the drug concentration, also the A-UM value (the distance between anterior and UM) is connected with the size of the cytostome and A-M1 changes respectively. With the exception of cells incubated in 16 mM penicillin medium, this value does not exceed the value A-UM of control cells. In 4, 32 and 64 mM concentrations it is even smaller than in *Tetrahymena* at 0 h. The A-M1 and A-UM lengths besides shortening also show a tendency towards narrowing (see BDM1 = breadth at top of mouth and BDUM = breadth at bottom of mouth, values). Similarly, the WLEN (anterior to widest point) length undergo shortening. All the changes shown out that the examined antibiotic decreases the size of *Tetrahymena*, both in their length and width, and increases the cytostome length.

In effect, the cell looses its characteristic shape, becoming more "slim". Depending on the direction of changes of the discussed values (decrease or increase), the parameters connected with them change similarly.

The decrease of the width of cells causes the same change in %PWI (%BRED/BDUM), %BRD (%BRED/LENG), etc.

The %BUC (%BUCL/LENG), however, increases this is connected with the elongation of the cytostome. The number of kinetics does not undergo basic changes there is any interdependence between the antibiotic concentration and this corticotype parameter.

Discussion

Summing up the effect of Penicillin G (sodium salt) on *Tetrahymena pyriformis*, it can be said that this antibiotic affects all the examined morphophysiological properties. It lowers down the rate of quantitative

increase of the population, moving speed of cells and hinders their growth. As in the case of bacteria, antibiotics from this group act most strongly at the initial stage of ciliate culture growth (see results of multiplication dynamics of cells and their moving speed).

On the other hand, no stimulating effect of penicillin on *Tetrahymena* multiplication observed by N é m e t h and C s i k (1961) has been noted. These authors have also shown a proportional growth in length and width of *Tetrahymena* with the increase of crystalline penicillin concentrations.

These dimensions, however, have always been smaller than in the case of control cells, similarly as in the presented study. The observed morphophysiological changes are also a result of the effect of sodium ion introduced together with the antibiotic (see the results presented in Tables 1 and 2). It is also known that sodium ions affect the moving speed of *Tetrahymena*, especially in higher concentrations (Fig. 3). The introduced sodium ion also changes the osmotic pressure of the medium. The observed morphophysiological changes are probably the result of this.
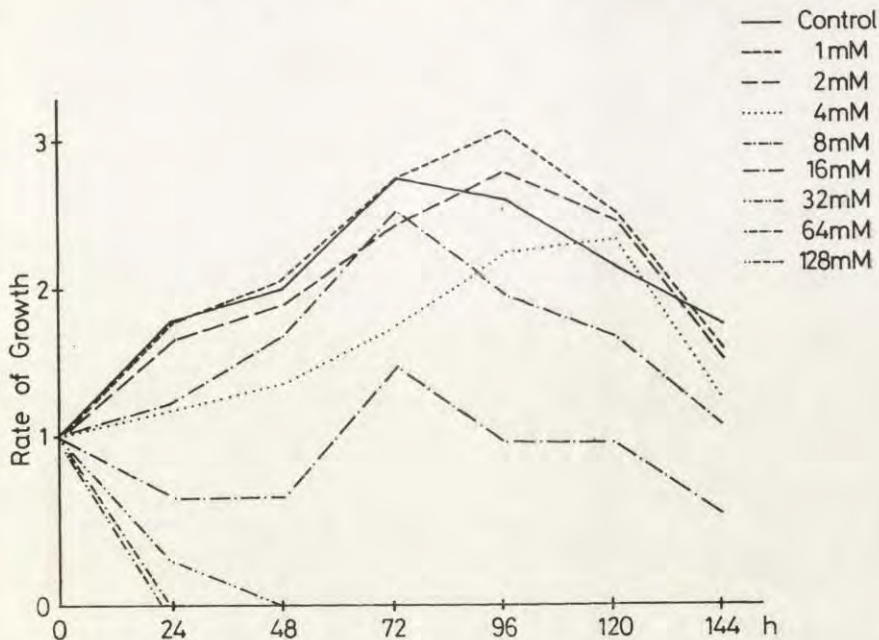
Fig. 3. Change of *Tetrahymena pyriformis* cells density depending on the concentration of NaCl

D u n h a m (1964) has shown that *Tetrahymena pyriformis* GL adapt to high NaCl concentrations. He has furthermore concluded that dimensions of the tested cells are smaller than those of control cells. Earlier studies (S z a b l e w s k i 1981) have proved that in lower antibiotic

Table 2

Morphologic and morphometric data on *Tetrahymena pyriformis* cells incubated for 144 h in different concentrations of NaCl

Conc. of NaCl [mM/1]	LENG [μm]	BRED [μm]	BUCL [μm]	A-M1 [μm]	A-UM [μm]	WLEN [μm]	BDM1 [μm]	BDUM [μm]	AWD %	%PWI	AM1 %	AUM %	WLN %	BUC %	BRED %	BDM %	BD1 %
1	55.5	25.6	9.3	3.6	12.9	27.1	10.4	21.4	48.6	119.6	6.5	23.3	48.8	16.7	46.1	38.5	18.7
2	55.4	24.7	8.7	4.1	12.9	32.3	11.3	19.6	57.5	126.0	7.5	23.3	58.3	15.7	44.7	35.4	20.4
4	50.5	22.9	7.6	4.1	11.8	30.8	10.3	18.3	56.3	125.1	8.1	23.5	61.1	15.1	45.4	36.3	20.4
8	56.7	26.2	8.5	3.8	12.4	33.1	11.0	21.7	50.8	120.6	6.7	21.8	58.2	15.0	46.1	38.2	19.4
16	54.2	28.1	8.5	4.1	12.7	26.3	12.6	24.5	51.6	114.6	7.2	22.8	47.4	15.3	50.6	44.1	22.7

All abbreviations see Table 1.

concentrations (1 and 2 mM) the observed changes are caused by the potassium ion introduced to the medium together with the antibiotic. In higher concentrations, however, the observed morphophysiological changes are the results of the ion and antibiotic effect. The results presented in this study moreover show out that the sodium ion effect on the examined morphophysiological cells is lower than of potassium ion. Consequently, penicillin G in the form of sodium salt causes less expressed morphophysiological changes in the cell than in the form of potassium salt.

However, the change in pH of the medium caused by the introduction of the drug does not affect the tested morphophysiological parameters of *Tetrahymena pyriformis* GL (S z a b l e w s k i 1981).

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DDT Uptake and Metabolism in *Blepharisma intermedium*

Synopsis. *Blepharisma intermedium* accumulated very high amounts of DDT from the medium initially containing 1 ppm DDT. The rate of accumulation was rapid during the initial period of incubation. The ciliate was also able to metabolize DDT to trace levels of p,p'-DDE and o,p'-DDT.

Microorganisms are known to accumulate DDT and other chlorinated hydrocarbon insecticides from the aquatic environments. Among these microorganisms, the role of bacteria and algae in biomagnification and metabolism has been extensively studied (R u p L a l and S a x e n a 1981). However, very little attention has been given to protozoans which are abundant in aquatic environments and also form the broad base of aquatic food chain. Our earlier studies on accumulation and metabolism of DDT in two ciliate protozoans *Stylonychia notophora* and *Tetrahymena pyriformis* have revealed that these organisms accumulate DDT rapidly from the medium and metabolize it to many different products which have also been detected from natural aquatic systems (R u p L a l et al. 1981, A g g a r w a l et al. 1981). This paper deals with the accumulation and metabolism of DDT in another ciliate protozoan *Blepharisma intermedium*.

Materials and Methods

Blepharisma intermedium used in this study was collected in 1967 from fresh water ponds in Delhi. The culture was maintained in the laboratory at $24 \pm 1°C$ in sterilized hay infusion inoculated with bacteria, *Klebsiella aerogenes*. The cultures were subcultured once in a week.

p,p'-DDT used in this study was about 99⁰/o pure as determined by gas liquid chromatography (GLC). Stock solution of 1000 ppm of DDT was prepared in acetone and added to the medium so as to have a final concentration of 1 ppm of DDT and 0.1⁰/o acetone. At this concentration of acetone and DDT, the

growth of *Blepharisma* was not affected (R u p L a l and S a x e n a 1981).
Cultures before treatment were transferred from hay infusion to Chalkley's
medium and were kept in this medium for 24 h.

For studies relating to uptake and metabolism of DDT in *Blepharisma*,
100 ml of the culture was taken and treated with 1 ppm DDT in conical flasks.
After regular intervals of time, cultures were centrifuged and pellets obtained
were washed with toxicant-free medium. DDT and its metabolites were then
extracted from the pellet and analysed by GLC as described elsewhere (R u p
L a l et al. 1981, A g g a r w a l et al. 1981). The amounts of DDT and its metabolites
were calculated on dry weight basis.

R e s u l t s a n d D i s c u s s i o n

Blepharisma intermedium accumulated very high concentration of
DDT from the medium (Table 1). Like many other microorganisms,
the rate of accumulation was rapid during the initial stages. Such an

Table 1

Concentration of DDT and its metabolites (ppm) at different time intervals in *Blepharisma inter-
medium* exposed to 1 ppm of DDT for 10 days*

Exposure time [h]	p,p'-DDE	o,p'-DDT	p,p'-DDT	Total DDT/ Accumulation factor
1	152.70 (0.17)	173.38 (0.200)	535.50 (0.620)	861.6
6	109.55 (0.007)	519.40 (0.030)	14451.00 (0.950)	15080.0
12	287.12 (0.009)	397.19 (0.013)	29173.00 (0.970)	29857.3
24	362.40 (0.012)	1379.60 (0.040)	26759.00 (0.930)	28501.0
48	174.70 (0.006)	517.20 (0.016)	28350.00 (0.970)	29042.0
72	193.40 (0.005)	612.50 (0.016)	35404.00 (0.970)	36210.0
120	228.40 (0.006)	448.90 (0.013)	32930.00 (0.970)	33607.3
240	672.70 (0.112)	453.30 (0.007)	58873.00 (0.980)	59999.0

* Values in parentheses indicate the quotient for each compound
with respect to total DDT.

accumulation of insecticides is mainly attributed to adsorption (R i c e
and S i k k a 1973), and it depends also upon the physiological state of
cells (H a n s e n 1979). During initial stages of experiment young cells

were present in large numbers which contributed to higher accumulation of DDT residues. These cells later entered into stationary phase and thus the accumulation rate declined. Unlike in *Stylonychia notophora* (R u p L a l et al. 1981) and *Tetrahymena pyriformis* (A g g a r w a l et al. 1981), the accumulation of DDT by *Blepharisma* continued throughout the experimental period of 10 days. Such an accumulation rate may be attributed to low excretion rate and metabolism of DDT in this organism. The biomagnification factor in *Blepharisma intermedium* for DDT was very high (60 000) as compared to 295 in *Stylonychia notophora* (R u p L a l et al. 1981) and 24 in *Tetrahymena pyriformis* (A g g a r w a l et al. 1981). Thus *Blepharisma intermedium* by virtue of its higher capacity to accumulate DDT residues and its wide spread distribution in the aquatic environments can serve as a better indicator of DDT contamination in aquatic environments. Further, *Blepharisma* serves as a food source for filter feeding organisms and so the organisms feeding on it would receive very high amounts of DDT from the DDT contaminated environments. In this way DDT can be biomagnified to very high levels at a single step transfer of the residues in the food web.

Blepharisma intermedium metabolized DDT to DDE and o,p'-DDT up to trace levels only. o,p'-DDT, a metabolite of DDT, has not been reported from other organisms so far. Also the p,p'-DDT which was 99% pure contained o,p'-DDT as one of the major impurities. It is possible that o,p'-DDD which was detected in samples of metabolites of *Blepharisma*, may not be a metobolic product of DDT but only a case of selective adsorption of this compound from the original DDT.

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Ndu UMECHE[1]

The Distribution of *Naegleria* spp. by Strata in Michigan Soils

Synopsis. Core samples were taken from organic soils (Houghton muck, Rifle peat, Carlisle muck) and mineral soils (Ottokee loamy sand, Sebewa loam, Fox loam, Bellefontaine sandy loam, Miami loam, Hillsdale sandy loam, Sloan Loam) in Michigan to study the distribution of various strains of *Naegleria* spp. The absence of pathogenic strains of *Naegleria* in these soils is noteworthy due to availability of bodies of freshwater for recreational purposes and the potential for contamination of these waters by the pathogen. High temperature (37°C) non-pathogenic strains of *Naegleria* were also lacking, while four strains of the low temperature (23°C) *Naegleria* were isolated from various layers of the soils. The advantage of core sampling over surface soil sampling is noted.

Strains of non-pathogenic *Naegleria gruberi* have been isolated from a variety of soil, water and air samples (R a f a l k o 1947, S i n g h 1952, C h a n g 1958, K i n g s t o n and W a r h u r s t 1969). The pathogenic *N. fowleri* which causes primary amoebic meningo-encephalitis has been isolated from a swimming pool (K a d l e c et al. 1978), from thermally polluted lakes (D e J o n c k h e e r e and V o n de V o o r d e 1977) and from the soil (A n d e r s o n and J a m i e s o n 1972, D a s 1972, L a w a n d e et al. 1979 a). These amoebae exist freely in the soil from where they may contaminate freshwater lakes and ponds.

Michigan has a variety of organic and mineral soils and the prevalence of these amoebae in these soils has not been established.

The present study was therefore undertaken to look for pathogenic and non-pathogenic strains of *Naegleria* spp. in Michigan soils and to outline their distribution by strata in these soils.

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Materials and Methods

Sampling Sites and Sampling Methods

The soil samples were obtained from Rose Lake Wildlife Research Area in Michigan. A total of 12 sites were selected with the aid of a Michigan Department of Natural Resources (1969) map. The 10 types of soil were indentified using soil survey maps (Johnsgard et al. 1942, Threlkeld and Feenstra 1974). Samples were taken twice monthly throughout the year with modified core samplers made of metal pipes 45 cm long by 5 cm diameter, A small opening was cut along the core sampler such that the soil layers could be seen and removed easily without mixing different layers.

To obtain a sample from each site, the vegetation cover and litter were removed carefully, the sampler was held vertically and driven with a hammer into the ground down to a depth of 30 cm. The samplers were pulled out gently, wrapped with polyethylene sheets, and transported to the laboratory for analysis the same day.

Culture Methods

All soil and litter samples were cultured on Dilute Stock Agar Glucose — DSAG (Balamuth, W. personal communication) which was composed of $MgCl_2 \times 6H_2O$ 2.13 g, Na_2HPO_4 0.136 g, Na_2HPO_4 0.568 g, trypticase 1 g, yeast extract 1 g, glucose 1 g, agar 15 g, and distilled water 1 l. The DSAG was autoclaved in a flask and poured into sterile Falcon multiwell tissue culture plates, and allowed to cool.

A small quantity of each soil sample was removed carefully with sterilized spatulas from the 2.5 cm and 20 cm markings of the core samplers through the opening cut along the sampler. Then 1 g of each soil sample was weighed out, immersed into 5 ml of Low salt (0.05 M NaCl, 3.2 mM $MgCl_2 \times H_2O$ and 0.36 mM $CaCl_2$) and mixed by shaking; 1 g of each litter sample was treated similarly. A few drops of each sample mixture was pipetted into the Falcon wells and *Escherichia coli* K12 was added as food. These plates were incubated at 37°C and 23°C for 48 h.

Identification Methods

After incubation, each well was examined under low power by turning the plates upside down. Specimens from positive wells were transferred to glass slides with a loop and identified under high power. Subculturing of the cysts and amoebae was carried out on fresh DSAG Petri dishes with *E. coli*, until pure isolates were obtained.

The transformation of the amoebae into flagellates was done by harvesting freshly grown amoebae with a loop, washing them three times in Low salt by centrifugation to remove the *E. coli*, and immersing them into a rotary shaker maintained at 28°C.

Pathogenicity test was carried out by counting washed amoebae on a hemacytometer and inoculating about 25 000 amoebae intranasally into each mouse. Five white mice were used for each *Naegleria* isolate. The test animals were observed for 10 days.

Results

The distribution of *Naegleria* spp. in the layers of the three organic soils (Houghton muck, Rifle peat and Carlisle muck) and the seven

mineral soils (Ottokee loamy sand, Sebewa loam, Fox loam, Bellefontaine sandy loam, Miami loam, Hillsdale sandy loam and Sloan loam) is shown in Table 1. Pathogenic *N. fowleri* or high temperature (37 °C) strains of *N. gruberi* were not seen in all the soils studied. However, low temperature (23°C) strains of *N. gruberi* were obtained from four sites, namely Rifle peat, Ottokee loamy sand, Fox loam and Miami loam. The Miami loam isolate was found in the litter layer, while the others were found in the 2.5 cm and 20 cm layers of the soils. All layers contained predominantly *Acanthamoeba* spp.

Flagellation test carried out on the four isolates indicated that all of them transformed into flagellates within 60 min of immersion into the rotary shaker.

Pathogenicity test of the isolates with white mice did not result in death of the mice within 10 days, and symptoms of amoebic meningo-encephalitis were not observed in the test animals.

Discussion

Naegleria spp. have been established as free-living soil amoebae. The availability of hundreds of freshwater lakes and ponds for recreational purposes in Michigan and the potential for contamination of these waters by the pathogenic *Naegleria* necessitated the present study. However, *N. fowleri* was not observed from the soils sampled.

Judging from the variety of soils and the number of samples studied, non-pathogenic *Naegleria* did not appear to be widespread in these soils, since only four isolates of the low temperature (23°C) strains were obtained and the high temperature (37°C) strains were lacking during more than a year of continuous sampling. The cool climate of Michigan appears to favour the existence of only the low temperature strains in these soils.

Recent studies in which *N. fowleri* and *N. gruberi* isolated from nasal passages of children were traced to dust particles carried by the wind (L a w a n d e et. al. 1979 b) seem to indicate that in future *Naegleria* spp. may be found in some sites where they are presently lacking. Hence, it becomes increasingly necessary to screen many areas for the presence of these organisms.

Some factors that govern the existence of *Naegleria* spp. and other amoebae in the soil have been discussed (S i n g h 1975). Such factors include the nature of the soil, availability of edible bacteria, oxygen and moisture. Most of the work on *Naegleria* isolation from the soil have been carried out with samples collected from surface soils (A n d e r s o n and J a m i e s o n 1972, D a s 1972, L a w a n d e et al.

Table 1

Distribution of *Naegleria* spp. by strata in Michigan soils

Soil layers	Organic soils					Mineral soils				
	Houghton muck	Rifle peat	Carlisle muck	Ottokee loamy sand	Sebewa loam	Fox loam	Bellefontaine sandy loam	Miami loam	Hillsdale sandy loam	Sloan loam
Litter	o	o	o	o	o	o	o	o	o	o
2.5 cm	o	+	o	+	o	+	o	+	o	o
20 cm	o	+	o	+	o	+	o	o	o	o

+ — *N. gruberi* present
o — *N. gruberi* and *N. fowleri* absent

1979 a). The present study utilizing core sampling method shows that isolation of *Naegleria* from the soil should not only be restricted to the soil surface, but should be extended beneath the surface into the soil layers.

The use of Falcon multiwell tissue culture plates in this study provided a convenient way for culturing the soil samples for *Naegleria* spp. In some cases, it was observed that a given well contained only one type of cysts or trophozoites. The desired organism could then be subcultured from the specific well.

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Henry TAMAR

On the Distribution of *Halteria bifurcata*

Synopsis. *Halteria bifurcata* specimens were obtained in eastern Ohio (USA) and near Los Angeles, California. The wide separation between these sites suggests that *H. bifurcata* may be extensively distributed in North America. This species may further be cosmopolitan.

Structural peculiarities of the examined specimens are described. These include a barb on a bristle number 1, bristles number 1 with unusually slight curves, and the absence cf a bifurcation on the bristles number 4.

The oligotrich ciliate *Halteria bifurcata* Tamar, 1968 has been extensively described from the environs of Terre Haute, Indiana (lat. 39° 28.0′ N, long. 87° 24.5′ W) by T a m a r (1968 and 1974). It is characterized structurally by the differentiation of the 4 bristles (Pl. I 2) of each of the 7 equatorial bristle groups, by each of the 16 adoral membranelles consisting of 2 major portions, and by a noticeable constriction of the anterior 1/4 to 1/3 of the body. T a m a r (1979) reported that a culture obtained near Terre Haute contained specimens in which one, and perhaps more, of the most anterior circle of bristles had an aberrant structure. In these specimens at least one of the 7 bristles number 1 exhibited a short barb at the end of its straight, proximal portion. This barb had the appearance of a distal continuation of the uncurved, proximal part of the bristle.

G r i m (1974) described a *Halteria* form collected near Tuba City, Arizona (lat. 36° 08.2′ N, long. 111° 14.4′ W) as similar to *H. bifurcata*, with the exception of an absence of bifurcation in bristles number 2 and 4. However, *H. bifurcata* has not heretofore been definitively identified from locales other than the vicinity of Terre Haute. This may partly be due to the need for phase contrast optics to clearly observe, in living specimens, the curves of the anterior bristles number 1, the smaller terminal bifurcation of the bristles number 2 and the large terminal bifurcation of the bristles number 4. The other outstanding

identifying structural feature of *H. bifurcata*, the division of each adoral membranelle into 2 main portions, can also best be seen with phase contrast.

Materials and Methods

A water sample was collected on June 21, 1980 from a pond at the side of interstate highway 70, at a location (lat. 40° 3′ N, long. 81° 13′ W) about 75 miles east of Columbus, Ohio.

Another water sample was obtained on May 16, 1981 from a large artificial lake in Yorba Regional Park, near the Riverside Freeway (California highway 91), in the town of Yorba Linda (lat. 33° 53.4′ N, long. 117° 48.8′ W), near Los Angeles, California. The artificial lake was surrounded by a concrete embankment, and was inhabited by a number of ducks. Algae and an aquatic plant of the genus *Sagittaria* were present in this water sample.

A third water sample was taken on June 5, 1981 from close to the surface of the water lily basin at the Los Angeles Port Authority in Wilmington (lat. 33° 46′ N, long. 118° 15′ W), in the port area of Los Angeles, California.

Two additional water samples were collected in the western United States, one from a bog-like area along the north fork of the Virgin River in Zion National Park, and the other from a catch basin for irrigation water located 26 miles east of Great Salt Lake via interstate 80. However, the first sample contained no *Halteria* and the second only *Halteria grandinella*.

Following a delay of usually not more than 55 h after collection, a small quantity of skimmed milk powder was added to the samples either once or at intervals (T a m a r 1974). Several days after the addition of skimmed milk powder, volumes of a sample were placed on slides and searched for *Halteria* with a stereoscopic microscope. Apparent *Halteria* were then picked up with a micropipette and released onto another slide until a drop of fluid had been accumulated. Threads were now placed near the drop, a coverglass was applied, and the specimens were examined with the phase contrast microscope.

The 3 Yorba Linda specimens were measured by use of a micrometer, and additional measurements of one of these specimens were made from photographs.

Results

East of Columbus

Two specimens of *H. bifurcata* were identified from the sample drawn along interstate highway 70 about 75 miles east of Columbus, Ohio and approximately 315 miles (505 km) east and slightly north of Terre Haute.

Bristles

In the first specimen the 4 bristles of each of the 7 groups of equatorial bristles were "typical", with the exception of a single bristle number 1. Only this one bristle number 1 had a barb at the base of the first curve, just beyond the straight proximal part of the bristle. The second specimen exhibited the described barb on at least a single bristle number 1.

Adoral Membranelles

The first specimen was determined to have 16 "typical" adoral membranelles, each composed of 2 main parts.

Yorba Regional Park

Three specimens of *H. bifurcata* were found in the sample taken from the artificial lake in Yorba Regional Park, about 1730 miles (2785 km) west and a bit south of Terre Haute.

Bristles

All 3 organisms (specimens A, B and C) possessed 7 groups of equatorial bristles.

In specimen A the bristles number 1 were not curved as strongly in the "long-horn shape" as were the bristles number 1 of the *H. bifurcata* from the Terre Haute region. Its bristles number 2 each showed a relatively large, clearcut bifurcation, and the bristles number 3 were "normally" straight and not bifurcated. Surprisingly, however, no bifurcation was observed in the bristles number 4.

Specimen B was indistinguishable in appearance from the average *H. bifurcata* specimen of the Terre Haute region. Its bristles number 1 had the "normal" curves, its bristles number 2 showed a small terminal bifurcation, its bristles number 3 were straight and unbifurcated, and its bristles number 4 exhibited a large bifurcation (Pl. I 1).

Measurements from photographs indicate that specimen B's bristles extended outward from the body for about 25–27 µm. The straight, proximal portion of each of two bristles number 1 was approximately 13 µm long. The longer terminal branch of several bristles number 2 was between 5 and 6 µm long. The longer terminal branch of a bristle number 4 was on the order of 14 µm long and the shorter terminal branch was about 11 µm long.

In specimen C the bristles number 1 were "normal" in appearance and the other bristles appeared to be "normal" (i.e., appeared to be of

the Terre Haute type) also. Bristles number 4 could not be clearly identified later in the preparation.

Adoral Membranelles

In the 3 specimens the side view of the membranelles and body was completely "normal" for *H. bifurcata*. In specimens A and C it was possible to establish the presence of 16 "typical" adoral membranelles, each composed of 2 main portions of differing diameters. A photograph (Pl. I 1) of specimen B yielded a natural, unstraightened membranelle length of about 14–15 µm and in 3 photographs the total diameter of specimen B's adoral wreath ranged around 42–44 µm.

Body Size

Specimens A and C had maximal body widths, respectively, of very approximately 25 µm and approx. 26 µm (direct measurement). Specimen B yielded a maximum width of about 22.6 µm by direct measurement and an estimated maximum width and length of close to 25 µm from a photograph (Pl. I 1).

Algal Matter

Algal material was present in the body of specimen C.

Los Angeles Port Authority

By means of the 10 × phase contrast objective one *H. bifurcata* specimen was seen among numerous cyclidiums in water from the water lily basin at the L.A. Port Authority.

Discussion

The results show that *H. bifurcata* is present at a pond about 75 miles east of Columbus, Ohio, and at Yorba Linda and the port area of Los Angeles, on the Pacific Coast of the United States. Yorba Linda lies some 1980 miles (3190 km) to the west, and slightly south, of the Ohio location. The Rocky Mountain chain, large arid areas and the Mojave Desert intervene between the 2 sites. The results thus suggest that *H. bifurcata* may be widely distributed in North America. This species could well be cosmopolitan.

In all investigated aspects, the *H. bifurcata* specimens from the given sites, with the exception of Yorba Regional Park specimen A, fall within, or close to, the range of variation noted for *H. bifurcata*

specimens from the Terre Haute region. Thus almost all the measurements obtained from Yorba Regional Park specimen B are average for specimens from the Terre Haute area. Only the approximate length of the longer terminal branch of a bristle number 4, 14 µm is not average; it slightly exceeds the range of 11.3–13.7 µm for the length of this part of Terre Haute specimens.

Yorba Regional Park specimen A differed significantly from the other specimens in lacking a bifurcation on the bristles number 4, and in the bristles number 1 showing less pronounced curves. These aberrations in one specimen, as well as the presence of a barb on at least one bristle number 1 in both the 2 Ohio specimens and some Terre Haute specimens, suggest that structural peculiarities may be widespread among stocks of *H. bifurcata*. The structural variations in this species could be worthy of more extensive study.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATE I

1: Side view of *Halteria bifurcata* specimen B from Yorba Regional Park. Three bristles number 4 can be seen extending from the posterior end. The scale is 10 μm long. (Photomicrograph at 500 × with dark phase contrast. Zeiss Microflash Device (60 watt/s, Kodak Tri- × 35 mm film)

2: Line drawing of a dorsal view of a typical *Halteria bifurcata* specimen from the Terre Haute region. Shown are: 2 of the 16 adoral membranelles (A), which each consist of 2 major portions, 2 of the 7 equatorial bristle groups, each composed of 4 differentiated bristles (1–4), the ventral contractile vacuole (CV), which lies to the left of the buccal cavity and its contained 7 oral membranelles, and the macronucleus (M)
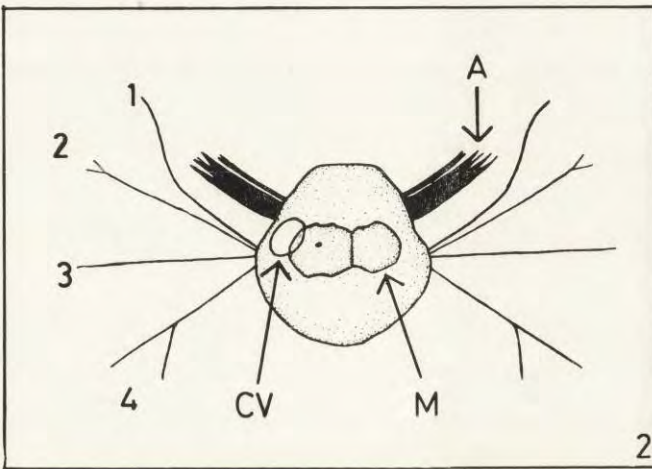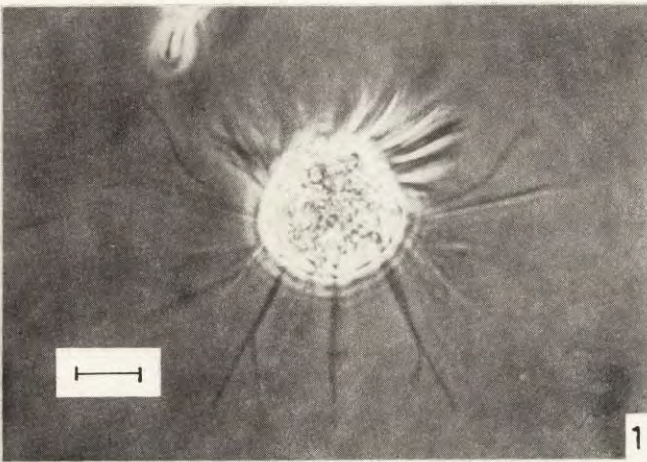
H. Tamar   auctor phot.

S. BANDYOPADHYAY, R. RAY and B. DASGUPTA[1]

A new species of *Trypanosoma* from an Indian Insectivorous Bat, *Rhinopoma hardwickei* Gray

Synopsis. A new trypanosome, *Trypanosoma rhinopoma* sp. n. is described from an Indian insectivorous bat, *Rhinopoma hardwickei* Gray. It is monomorphic and attenuated at both the ends, measuring 31.61 μm in total length. Its affinities with the allied species have been discussed and incorporated in this paper.

According to H o a r e (1964), the bat trypanosomes are divided into two subgenera: (a) the subgenus *Schizotrypanum* — representing small cruzi-like forms and (b) the subgenus *Megatrypanum* — representing large forms. The first bat trypanosome of latter type to be recorded was *Trypanosoma megadermae*, described by W e n y o n (1909) in the bat, *Lavia frons* (Syn. *Megaderma frons*) from Sudan. The same type of trypanosome had been previously encountered in the bat *Pipistrellus kuhli* (Syn. *Vespertilio kuhli*) in North Africa by S e r g e n t and S e r g e n t (1905) and N i c o l l e and C o m t e (1906, 1908) but they regarded these as large forms of *Trypanosoma (Schizotrypanum) vespertilionis*. A few more trypanosomes of bats belonging to the subgenus *Megatrypanum* have been described by eminent workers like L é g e r and B a u r y (1923), R o d h a i n (1923, 1951) R e i c h é n o w (1940), H e i s c h and G a r n h a m (1953), L i p s and R o d h a i n (1956), v a n d e n B e r g h e et al. (1963), D e a n e and S u g a y (1963), M a r i n k e l- l e and D u a r t e (1968) and Z e l e d o n and R o s a b a l (1969). Keymer (1971) reported *Trypanosoma megadermae* — like forms from two horse shoe bats (*Rhinolophus fumigatus* and *Rhinolophus hilderbrandti*) of Central Africa, which H o a r e (1972) mentioned in his Zoological Monograph, "The Trypanosomes of Mammals". B a k e r (1973) observed a species of *Megatrypanum* in England, for the first time in an European bat, *Pipistrellus pipistrellus*. He identified this parasite provisionally as *Trypanosoma incertum* Pittaluga, 1905. P i e r c e and N e a l (1974)

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reported the occurrence of *Trypanosoma pestanai* in the host *Meles meles* from British Isles. E w e r s (1974) described *Trypanosoma aunawa* in *Miniopterus tristis* from New Guinea.

In India, however, there is no such report of bat trypanosomes which leads to the present communication.

The blood smears were received through the kind courtesy of Shri R. K. Ghose, Assistant Zoologist, Mammal and Osteology Section, Zoological Survey of India, Calcutta.

The type slide will be deposited to the National collection of the Zoological Survey of India, Calcutta.

Material and Methods

The bats were collected during Rajasthan survey, 1980. Blood smears were taken from the veins of the wings and directly from the heart also.

All smears were air-dried, fixed in 100% Methanol, stained with Romanowsky stain and differentiated with neutral distilled water. The smears were examined under low dry magnification (450 ×) and subsequently under oil immersion (1000 ×). Measurements were obtained from the camera-lucida drawings. The morphometric parameters were measured (after H o a r e 1972).

Out of 25 examples of *Rhinopoma hardwickei* Gray, examined, only one was found to be infected with this trypanosome.

Description

Trypanosoma (Megatrypanum) rhinopoma sp. n.

The trypanosomes are monomorphic, elongated and attenuated at both the ends (Fig. 1 *1–3*). In fixed slides the configuration of the body is "C" or "S" shaped, with the anterior end more pointed than the posterior (Fig. 1 *1–3*). The body measures 26.18 μm (25.0–39.7 μm) in length (excluding free flagellum) and 2.8 μm (2.0–3.8 μm) in width.

C y t o p l a s m: The cytoplasm stains deep blue with Leishman's stain. It is granulated and vacuolated: more granular at the anterior region while posteriorly it is more vacuolated. No striations or myonemes were recorded.

N u c l e u s: It is oval or rounded and situated mostly at the posterior part of the body. It stains purple or red with Leishman's stain and does not occupy the entire width of the body. It measures 2.8×1.95 μm (NI = 0.63).

K i n e t o p l a s t: The kinetoplast is small, dot or rod-like, measuring 0.8×0.5 μm. It stains deep blue-black with Leishman's stain and lies always in contact with a large vacuole of 2.5 μm in diameter at the posterior end. The kinetoplast is situated nearer to the posterior end than to the nucleus (KI = 1.82).
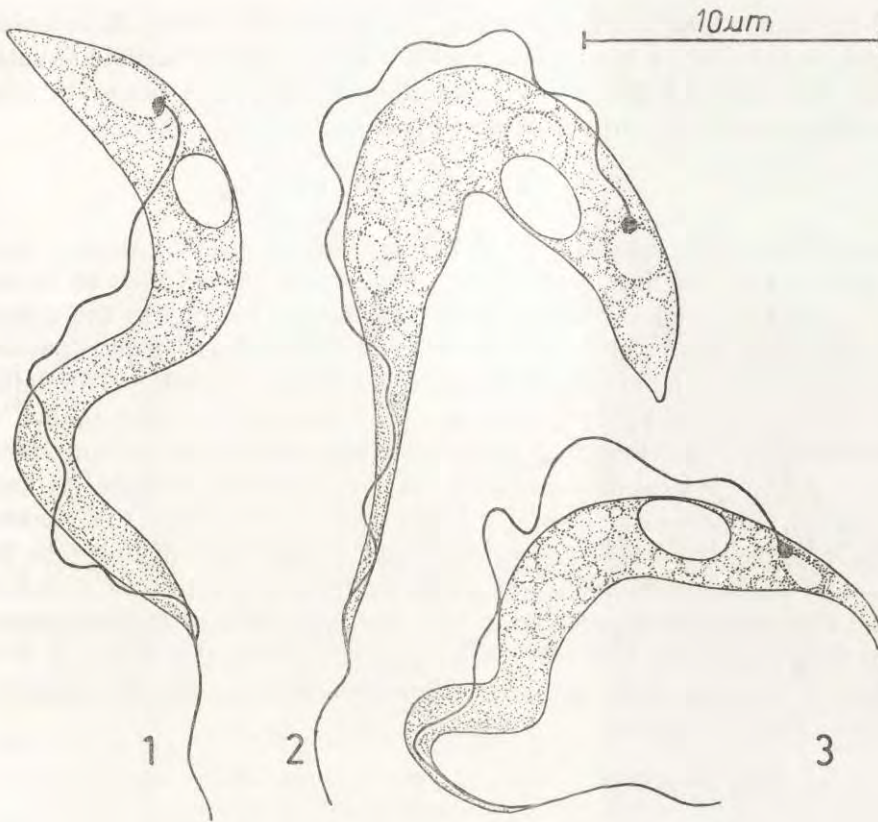
Fig. 1. *1–3. Trypanosoma rhinopoma* sp. nov. in *Rhinopoma hardwickei* Gray

F l a g e l l u m   a n d   u n d u l a t i n g   m e m b r a n e: The flagellum arises from the anterior end of the kinetoplast and trails anteriorly bordering the undulating membrane. It extends beyond the body as a distinct free flagellum of 5.44 μm in length, which is one-fifth the length of the body. The undulating membrane is very prominent having 5–7 folds and can easily be separated from body cytoplasm by its light colour bordered by thick flagellum.

Type host: A Lesser Rat-tailed Bat, *Rhinopoma hardwickei* Gray.
Type locality: Nokh, Jaishalmer Dist., Rajasthan, India.
Site of infection: Blood.
Vector and life-cycle: Unknown.

Diagnosis

Trypanosoma (Megatrypanum) rhinopoma sp. n.

The trypanosome is monomorphic, measuring 31.61 μm in total length. The cytoplasm is vacuolated and granulated. The nucleus is

oval or rounded, situated at the posterior part of the body. Kinetoplast is dot-like and always in association with a clear rounded vacuole situated at the posterior end. The free flagellum is very short and the undulating membrane is very much prominent with 5–7 folds.

Remarks

There are two main types of bat trypanosomes, one ranges from 14 to 20 μm in length and the other ranges from 25 to 40 μm in length (M a r i n k e l l e and D u a r t e 1968). The former belongs to the subgenus *Schizotrypanum* and the latter to the subgenus *Megatrypanum*, according to the nomenclatural system of H o a r e (1964). The latter is usually referred to as trypanosomes of megadermae group, but *T. megadermae* as described by W e n y o n (1909), should not be considered as a typical representative of large bat trypanosomes because its body is slender, kinetoplast lies far behind the nucleus and the nucleus extends across the body. The present species has been included in the subgenus *Megatrypanum* due to its large size, a characteristic feature of the subgenus. A comparative list of different species of *Megatrypanum* alongwith the present one has been represented in the Table 2 from which it is clear that the present species belongs to the subgenus

Table 1

Morphometric measurements of *Trypanosoma rhinopoma* sp. nov.
(all measurements in μm)

Dimension	Range	Mean
(1) Posterior end to the kineto-plast (PK)	4.0–9.5	5.79
(2) Kinetoplast to the centre of the nucleus (KN)	2.0–6.5	3.69
(3) Posterior end to the centre of the nucleus (PN)	6.5–12.7	9.48
(4) Centre of the nucleus to the anterior end (NA)	11.0–18.0	15.70
(5) Posterior to anterior end (PA)	20.5–33.7	26.18
(6) Free flagellum (FF)	4.5–6.0	5.44
(7) Total length (including free flagellum) (TL)	25.0–39.7	31.61
(8) Width of the body (BW)	2.0–4.0	2.60
(9) Length of the nucleus	2.0–3.8	2.80
(10) Width of the nucleus	1.7–2.5	1.95
(11) Nuclear index (NI)	0.41–1.15	0.63
(12) Length of the kinetoplast	0.5–1.0	0.8
(13) Width of the kinetoplast	—	0.5
(14) Kinetoplastic index (KI)	0.83–4.75	1.82
(15) Undulating membrane	5–7	5

Table 2

"Broad" trypanosomes of insectivorous bats

Host	Locality	Trypanosomes	Length range (μm)	Mean total length (μm)
(1) *Megaderma frons*	Sudan	*T. megadermae* Wenyon, 1909	—	40.0
(2) *Hipposideros tridens*	Senegal	*T. morinorum* Lèger and Baury, 1923	37.0–45.7	38.0
(3) *Nycteris hispida*	Congo	*T. heybergi* Rodhain, 1923	25.2–30.0	27.0
(4) *Nycteris aothiopica*	Tanzania (Tanganyika)	*T. mpapuense* Reichenow, 1940	28.5–35.0	—
(5) *Hipposideros caffar*	Congo	*T. leleupi* Rodhain, 1951	35.5–45.0	—
(6) *Hipposideros caffar*	Burundi	*Trypanosoma* sp. van den Berghe, et al., 1963	—	31.6
(7) *Nycteris thebaica*	Kenya	*Trypanosoma* sp. Heisch and Garnham, 1953	34.0–38.0	—
(8) *Nycteris macrotis*	Congo	*T. thomasi* Lips and Rodhain, 1956	—	45.4
(9) *Nycteris thebaica*	Zambia	*Trypanosoma* sp. Keymer, 1971	26.7–33.8	30.6
(10) *Nycteris thebaica capensis*	Zambia	*Trypanosoma* sp. Keymer, 1971	29.5–42.1	35.8
(11) *Nycteris grandis*	Zambia	*Trypanosoma* sp. Keymer, 1971	29.9–36.9	31.9
(12) *Rhinolophus fumigatus*	Zambia	*Trypanosoma* sp. Keymer, 1971	37.0–40.5	38.7
(13) *Rhinolophus hilderbrandti*	Zambia	*Trypanosoma* sp. Keymer, 1971	30.6–39.3	33.8
(14) *Artibeus lituratus* and *Phillostomus hastaut*	Colombia	*T. pifanoi* Marinkelle and Duarte, 1968	30.8–53.6	39.3
(15) *Meles meles*	British Isles	*T. pestanai* Pierce and Neal, 1974	25.6–41.4	35.7
(16) *Desmodus rotundus*	Brasil	*T. pessoai* Deane and Sugay, 1963	24.2–37.0	—
(17) *Saccopterix bilineata*	Costa Rica	*T. leonidasdeanei* Zeledon and Rosabal, 1969	33.7–53.7	—
(18) *Miniopterus tristris*	New Guinea	*T. aunawa* Ewers, 1974	24–39	30.6
(19) *Rhinopoma hardwickei*	Rajasthan, India	*Trypanosoma rhinopoma* sp. nov.	25.0–39.7	31.6

Megatrypanum. (The detailed morphometric measurements of the present trypanosome has been given in Table 1). H o a r e (1964) suggested that the shape and position of the kinetoplast may be successfully used in grouping trypanosomes of mammals.

The present species viz. *Trypanosoma rhinopoma* sp. n. has got certain resemblances with *T. pestanai* Pierce and Neal, 1974 and *T. megadermae* Wenyon, 1909 in having kinetoplast situated nearer to the posterior end than to the nucleus. But it differs in the following aspects:

Site of differences	*T. rhinopoma* sp. n.	*T. pestanai* Pierce and Neal	*T. megadermae* Wenyon
Body shape (Posterior part)	Posterior end bluntly pointed	Posterior end pointed and elongated	Posterior end drawn to a whip-like structure
Kinetoplast	Always in association with a rounded vacuole	Vacuole absent	Vacuole absent
Nucleus	Does not cover width of the body	Extends across the body	Extends across the body
Free flagellum	Measures 5.4 μm in length	Measures 7.0 μm in length	Measures 8.4 μm in length

In the remaining species the kinetoplast lies typically very close to the nucleus.

The most important feature of the present species is the presence of a clear rounded vacuole in close association with the posterior end of the kinetoplast which is absent in other species of large bat trypanosomes.

Moreover, this trypanosome has been recorded for the first time in India from a new host, *Rhinopoma hardwickei* Gray, belonging to the family *Rhinopomatidae.*

Considering the morphometric parameters and a new insectivorous host from a new locality, the present haemoflagellate is considered as new to science and the name *Trypanosoma rhinopoma* sp. n. is given after its host's generic name.

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N. K. SARKAR

Myxosporidan *Myxidium haldari* sp.n. (*Myxozoa: Myxidiidae*) from Indian Tree Frog *Hyla arborea*

Synopsis. A myxosporidan parasite *Myxidium haldari* sp. n. is recorded from the gallbladder of Indian tree frog *Hyla arborea* from West Bengal, India. Its fusiform spore with 8 to 10 longitudinal striations and a pair of round polar capsules are not found in any of the *Myxidium* spp. described from the amphibian hosts so far.

During the survey of the amphibian parasites of rural Bengal, a myxosporidan species of the genus *Myxidium* Butschli was found in the gallbladder of Indian tree frog *Hyla arborea* in September, 1981. It has been described here as *Myxidium haldari* sp. n. for many diagnostic features.

Materials and Methods

The detailed autopsy was made on live animals collected from Chakdaha (about 60 km north of Calcutta). The parasite was studied from the fresh wet materials as well as from the dry smears stained with Giemsa after fixation in absolute methanol. 2.5% KOH solution was used for the extrusion of the polar filament. All the measurements are given in micrometer (µm). Standard deviation (S.D.) of the various dimensions has also been calculated along with the range and mean. The figures have been drawn with the aid of Camera lucida.

Observations

Myxidium haldari sp. n.

Description

Trophozoite — coelozoic, large, oval to ellipsoidal in shape, cytoplasm very thin, highly alveolated, disporoblastic, polysporous (Fig. 1 *1*). Cyst — not found.
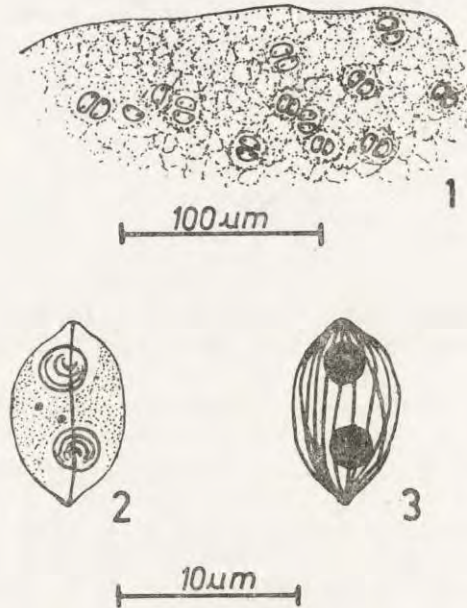
Fig. 1. *1–3. Myxidium haldari* sp. n. from the gallbladder of *Hyla arborea. 1 —*
A part of the disporoblastic, polysporous trophozoite, *2 —* A fresh spore —
sutural view, *3 —* A spore — stained with Giemsa. Note the spherical polar
capsules and many longitudinal striations

S p o r e (Fig. 1 *2–3*) — coelozoic, fusiform or cylindriobiconical in
sutural view, circular in polar view, slightly bent in the middle, suture
almost straight, 8 to 10 longitudinal (end to end) striations on the spore
wall, polar capsule 2, round, one at each end, open vertically to the
sutural line, sporoplasm finely granular, binucleated. Dimensions (based
on 30 fresh spores):

Spore length (sutural diameter) — 10.0 to 12.0 (10.82 ± 0.524)
Spore breadth — 6.5 to 7.0 (6.67 ± 0.489)
Polar capsule diameter — 3.0 to 4.0 (3.61 ± 0.309)
Polar filament length — 38.0 to 65.0 (47.00 ± 0.957)
(based on 5 filaments)
Host. *Hyla arborea*

Infection locus: Gallbladder (trophozoites and spores were found
floating in the bile)

Incidence: 1/5
Locality: Chakdaha, West Bengal, India
Pathogenicity: not apparent.
Material: Syntype specimens on slide No. H-M 6, stained with Giemsa, will
soon be deposited to the National Collection of the Zoological Survey of
India, Calcutta.

Discussion

The present species resembles *Myxidium immersuni* Lutz (from Kudo 1971) and *M. serotinum* Kudo and Sprague (from Clark and Shoemaker 1973) in the structure of the trophozoite. However, it differs from the above myxosporidans in having 8 to 10 longitudinal (end to end) striations on its spores which are 2 in *M. immersuni* and 2 to 4 in *M. serotinum* and also in the absence of transverse striations which are 7 to 9 and 10 to 13 in *M. immersuni* and *M. serotinum* respectively. Furthermore, its spherical polar capsules also differ from the pyriform polar capsules of *M. serotinum*. The present myxosporidan is, therefore, considered as new species and is described as *Myxidium haldari* sp. n. The specific name *"haldari"* is given after Dr. D. P. Haldar of the University of Kalyani, West Bengal, India.

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INFORMATION

SCHEDULED DATES

Sunday, 26 August, 1984
Registration, Opening Ceremony and Reception
Monday, August 27, 1984 to Friday, August 31, 1984
Scientific Sessions, Social Activities, Get Together
Party and Closing Ceremony

PRE AND POST CONFERENCE SATELLITE MEETINGS

There will be ample dates for holding satellite or small meetings featuring special topics in depth before and after the conference. Those of you who wish to organize satellites, please contact the Congress Secretariat.

SOCIAL ACTIVITIES

A complete program of social events will be arranged for participants, their families and guests. The Opening Ceremony will be open to all participants followed by a Get Together Party at the Congress site, Keio Plaza Hotel.

REGISTRATION AND HOTEL ACCOMMODATION

Congress registration and hotel accommodation forms will be distributed to you in the middle of 1983 as the second circular to members of IFCB. If you do not belong to your national or federal society and wish to participate the Congress, you should notify the Congress Secretariat.
Accommodation will be reserved in advance at advantageous rates at a number of hotels in various price categories within easy walking distance to the Keio Plaza Hotel. Arrangement to make more economical accommodations especially for young scientists is under preparation. Full information will be circulated later.

SCIENTIFIC PROGRAM (TENTATIVE)

GENERAL

The scientific program will be a mixture of plenary lectures, symposia and workshops with invited speakers, poster demonstrations and oral presentation sessions. While maintaining the customary breadth of subject matter, we hope to give special attention to the growth areas in Cell Biology.
Participants wishing to contribute a paper will be asked to submit an abstract on the official form, copies of which will be distributed in 1983. The official language of the Congress will be English.

PUBLICATION

Each delegate will receive a Book of Abstracts on registering at the Congress. The Proceedings of the Congress will be published in both hard and soft cover form about 5 months after the end of the Congress.

LOCATION

ABOUT TOKYO

The most populated capital city in the world with a mixture of oriental and occidental tastes, Tokyo had been misunderstood as the most expensive town

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to live or stay in. This is not true anymore. You now find hotel accommodations and food prices are rather less expensive than in many American and European cities. In fact, organizers of recent international congresses held in Tokyo were surprised by the number of participants from abroad that far exceeded their expectation. In this world's largest city, you will still discover small shops, narrow ally and typical crafts and professions where they maintain the influence of the Shogun period.

ABOUT SHINJUKU DISTRICT

Tokyo as usual of metropolis has several downtowns, each with its own unique flavor. Shinjuku is one of the latest development with skyscrapers, department stores, and a variety of economical spots for shopping and dining. Since the district is new, and attracts the younger generation, Shinjuku is called "A town for youth."

ABOUT KEIO PLAZA HOTEL

The hotel is located in the new metropolitan center Shinjuku, an area of towering buildings with open green spaces. Occupying an entire block of the center, the 47 story Keio Plaza Hotel has 1,500 deluxe rooms, 30 restaurants and bars. The combination of Main Building and Annex, Conference facilities are available for over 4,000 participants. A Plaza for people to meet, to talk, it is a City within a City.

In preparation:

V. A. S o p i n a, S. Yu. A f o n' k i n and B. N. K u d r y a v t s e v: Nuclear DNA
Content in *Amoeba proteus* Cultured at different Temperatures — R. C h i o v e t t i,
Jr. and E. C. B o v e e: Effects of Dinitrophenol, Actinomycin-D and Puromycin
on Encystment of the Amoeboflagellate, *Naegleria gruberi* — J. J a n i s z e w s k i:
Effect of Various Levels of External pCa on the Barium-induced Motor Responses of
Paramecium caudatum — I. F e r n a n d e z: Nouvelles observations sur uné gregarine
du genre *Selenidium* Giard, 1884, parasite de *Glossobalanus minutus* Kow. (*Entérop-
neusta, Hemichordata*) — N. K. S a r k a r: On Three New Myxosporidian Parasites
(*Myxozoa*) of the Ophicephalid Fishes of West Bengal, India — N. K. S a r k a r,
B. H a l d a r and R. C h a k r a b a r t i: *Myxosoma gangulii* sp. n. (*Myxozoa:
Myxosomatidae*) from the Head Cartilage of *Sillago maculata* Quoy and Gaimard
(*Sillaginidae*) — C. K a l a v a t i and D. L a k s h m i n a r a y a n a: A New Micro-
sporidian *Nosema valamugili* from the Gut Epitelium of an Estuarine Fish,
Valamugil sp. — C. K a l a v a t i and C. C. N a r a s i m h a m u r t i: Histopathologi-
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a Microsporidian Parasite of *Odontotermes horni* (Desn.)

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