ACTA PROTOZOOLOGICA

VOLUMEN XIII

Fasciculi: 21–27

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

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

Editor:
STANISŁAW DRYL

Associate Editor:
STANISŁAW L. KAZUBSKI

NOTICE TO AUTHORS

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

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

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

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

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

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

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

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

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

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

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 21

Department of Zoology, University of Illinois, Urbana, Illinois 61801, USA

J. Wynne McCOY

Updating the Tetrahymenids. II. Domestic and Natural Variation of Amicronucleate Species of the *Tetrahymena pyriformis* Complex

Modernisant les Tetrahyméniens. II. Variation domestique et naturelle des espèces sans micronoyau du complexe d'espèce *Tetrahymena pyriformis*

"*Tetrahymena pyriformis*" has long been known to consist of several genetically isolated species ("syngens") in addition to various selfing or amicronucleate strains (Gruchy 1955). In lumping these together, the precedent of *Paramecium aurelia* was used. Sonneborn 1957 objected to giving taxonomic specific status to the syngens of *Paramecium* on the grounds that it would be impossible to make accurate identifications without living reference strains, which were then in a rather precarious state of mortality. Several things have changed since that time, however. First, reference strains have been dispersed throughout the world. It has become possible to preserve strains in liquid N_2. A large body of dependable information is now available regarding the specific peculiarities of the known genetic species in *Tetrahymena* and *Paramecium* (a large portion of this information remains unpublished). Especially important are the studies of Tait 1970 and Borden et al. 1975 which define the zymographic properties of many enzymes on a comparative basis in these two genera.

In a personal communication to the author (1971), Sonneborn enumerates some of the objections which might be raised against the elevation of syngens as species. But, he writes, "None of these objections seems to me sufficient to warrant delay in taxonomic adventures when practical criteria are at hand for defining a particular assemblage that now seems to be a natural syngen or species". For the particular instance of "*T. pyriformis*", the practical criteria exist, and, as we demonstrate below, they have proved their effectiveness in separating the asexual strains into natural groups of the same dimensions as the known biological species or syngens.

While the origin of the amicronucleate *Tetrahymena* has long been assumed to be from a micronucleate form, we have not yet found an amicronucleate strain which matches the isozymic phenotype of any micronucleate strain. However,

the frequency of collection of hitherto unknown micronucleate phenotypes has been about 50% in our experience, and we doubt that the natural abundance of species of "*T. pyriformis*" will soon be exhausted.

It is the purpose of this paper to report on the degree of variation exhibited by particular amicronucleate species in nature and in the laboratory.

Materials and Methods

The strains used for this study include ten strains identified previously (Borden et al. 1973) by starch gel electrophoresis as identical to E. Zeuthen's *T. pyriformis* GL. These strains include: GL-C (E. Zeuthen via J. Frankel), GL-I (N. Williams), GL (G. Thompson, Jr.), GL (Virginia Dewey), W (D. Buetow), S (H. Eichel), S. (D. Lee), E (M. Levy), E2 (Virginia Dewey), and H (Cambridge Culture Collection). Borden et al. 1973 designate these strains as "phenoset A". Straint of a different amicronucleate species of the *T. pyriformis* complex, which exhibits totally differen: electrophoretic patterns, and which is designated "phenoset B" by Borden et al. 1973, include GL (H. Eichel, and hereafter referred to as GL-E), E (Cambridge Culture Collection), and GL (Cambridge Culture Collection). In addition, two other amicronucleates were used: strain UI-7146 was collected by Dr. F. Paul Doerder in August, 1971, from a small pond near Hyannis, Nebraska, and strain UI-7171 was collected by Dr. D. Borden in September, 1971, from Lake Arispie, Bureau County, Illinois.

Methods for cytological and electrophoretic analysis follow those described previously (McCoy 1974; Borden et al. 1973, after Shaw and Prasad 1970). Cortical data were obtained by methods similar to those of Frankel (1972), and our methods give results identical to his when the same strains are examined.

Results

Corticotypic Variation in "Phenoset A"

The ten strains of phenoset A which have been examined cytologically were found to have varied enormously in contractile vacuole pore (CVP) positions. These strains have been in laboratory culture for over 50 years and have acquired diverse labels. The summary of cortical data (Table 1) demonstrates that certain of these once identical strains have, by grace or accident, become distinguishable by their cortical patterns. For example, strain E2 consistently places its CVP's nearer to meridian *1* than the other strains. The "central angle" (Nanney 1966) is a measure of this tendency. Frankel (1972) has shown that the central angle varies over time, within a limited range. For the GL-C strain, 13 samples at different times in the history of the culture provide an estimate of this variation. By way of comparison, variation of the central angle in the individual micronucleate species of *T. pyriformis* has been found to be extremely small, usually limited to a range of about $\pm 2°$ (un-

Table 1

Cortical Characteristics of *Tetrahymena pyriformis* (Phenoset A) (Mean CVP Positions)

	GL Thompson	GL Dewey	GL-C Frankel	W Buetow	S Eichel	S Lee	E Levy	E2 Dewey	H Cambridge	GL-I Williams	GL-I Williams (restained)
Corticotype:								3.750(1)		(data of	
13	4.250(1)		(note 1)							Frankel 1972)	
14	4.250(1)			4.250(1)				4.250(6)		4.25(1)	
15	4.231(36)			4.250(1)				4.183(10)		4.39(18)	
16	4.300(37)	4.194(3)		4.667(4)	4.583(3)	4.250(2)	2.750(3)	4.296(18)		4.77(58)	
17	4.358(17)	5.161(30)		4.917(10)	4.602(9)	5.178(34)	4.667(6)	4.301(23)	4.750(2)	5.22(101)	5.150(25)
18	5.211(17)	5.285(37)		5.250(24)	5.038(26)	5.201(90)	4.895(22)	4.654(36)	5.250(1)	5.40(22)	5.261(42)
19	5.250(5)	5.399(20)		5.633(30)	5.261(30)	5.518(84)	5.183(30)	5.213(9)	5.330(25)	5.50(2)	5.328(32)
20	5.250(2)	5.437(8)		5.666(6)	5.437(8)	5.702(21)	5.483(28)	5.472(3)	5.588(37)	6.08(3)	5.892(7)
21				5.750(1)	5.500(4)		5.917(15)		6.021(24)		
22	6.250(1)						6.108(7)		6.050(5)		
23							7.250(1)				
N	116	98	650	77	80	231	112	106	94	205	106
Central angle:	77.5°	85.1°	80.03°	85.5°	80.0°	84.8°	(note 2)	74.3°	83.3°	87.2°	85.1°

Note 1: The data of Frankel 1972 give 13 samples of the central angle: 83.43°, 78.55°, 83.86°, 80.18°, 79.89° ,78.07° 79.75°, 77.00°, 78.29°, 81.25°, 79.42°, 80.84°, 80.12°, for samples of 50 cells each.

Note 2: As described in the text, this strain exhibits severe anomalies which affect the placement of the CVP's. The unadjusted angle is 79.3°.

published observations, also see Nanney 1967 a, 1971 a). The distribution of the variation in strain GL-C, and the central angles calculated for the remaining strains are given in Fig. 1.

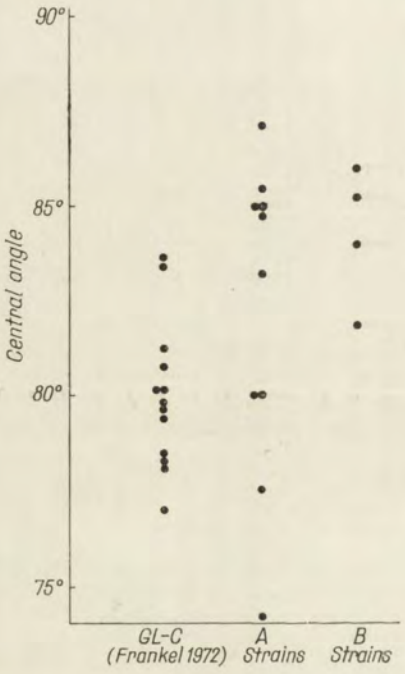


Fig. 1. Distribution of central angles for diverse amicronucleate strains

Strain E (M. Levy) requires special consideration. Many cells were found with extreme cytological anomalies: frequent, repeated oral replacement, leading to buccal organelles displaced toward the equator of the cell, cells without membranelles but with pharyngeal fibrils, cells with two sets of CVP's, or extra CVP's at unexpected places, disorganized mouthparts, disorganized anlagen, multiple anlagen on adjacent kineties, and oral replacement in developing proters and opisthes. In spite of these irregularities, the growth rate of the strain remains very high.

The CVP's and oral anlagen also tend to be misplaced in precisely the fashion described by Nanney 1967 b in his classic study of "cortical slippage". He argued that the systematic misplacement of such organelles could be rationalized in terms of field phenomena by supposing that the axis of reference for development, which normally coincides with the first or "stomatogenic" meridian, had become misaligned with respect to the cortex, and now ran from meridian *1* at the anterior end of the cell, to near meridian *n* at the posterior end of the cell.

It is possible to calculate the extent of this misalignment for the data of E (Levy). The calculation depends on the fact that, as the number of kineties increases,

the angular distance between them decreases. Thus an axis of morphogenesis shifted by a fixed number of degrees will maintain a constant relationship of central angle to corticotype, while an axis shifted by a fixed number of kineties (i.e., one to the left) will seem to have CVP' s too close to kinety *1* at low corticotypes and too far from kinety *1* at high corticotypes. This anomaly is demonstrated by E (Levy. A statistical analysis demonstrates that this deviation is highly significant, but a full explanation will be deferred to a separate paper to permit a more extended treatment.

If the CVP's of strain E (Levy) are positioned at a fixed angle from kinety *n*' that angle is about 99°, which is much higher than the central angle of any of the other strains of phenoset A. This implies either that the central angle of this strain has varied independently of the orienting axis, or that the position of that axis in some way determines the value of the angle.

A second point may be made concerning the significance of the shifted axis. If the axis were shifted by a fixed angle away from its normal position, there would be no effect on the standard deviation as the hypothetical reference point was displaced around the cell. The central angle would be in error by a fixed number of degrees instead of a fixed number of kineties. As a corollary, in the normal cell the actual axis could be located anywhere in the cell, so long as it maintained a fixed angle with kinety *1*. An analysis of cytogeometry locates only points fixed with relation to the ultimate axis used by the cell; it does not locate axis itself. That an effect on the standard deviation is seen is therefore evidence that the kineties themselves, and not only "cytogeometric axes", in some way participate in the orientation of morphogenetic fields. To produce the observed effect requires that the axis become fixed with relation to the wrong kinety. Alternatively, the regulation of the central angle might depend on the corticotype in such a way that the axis then assumed a fortuitous relationship with kinety *n*. It is difficult to imagine how this might be accomplished, but the fact that cells with more meridians tend to have proportionately fewer basal bodies per kinety (Nanney 1971 b) may be a clue,

Repeated Collection and Natural Variation in "Phenoset B"

Among the amicronucleate strains recently domesticated from collections taken from throughout the United States, strains UI-7146 and UI-7171 proved to be virtually indistinguishable by starch gel electrophoresis from the pattern described by Borden et al. 1973 for the amicronucleate species "phenoset B". Figure 2 shows the only differences which have been found between these strains and the standard phenoset B pattern shown by strain GL-E. Each of the wild strains shows differences for the complex acid phosphateses (P-1). UI-7146 is identical to GL-E for proprionyl esterase (E-1), while UI-7171 shows the same number of bands, but with increased mobility. All strains share one tetrazolium oxidase band (TO), while UI-7146 has an extra band, and a fast-moving band appears in UI-7171.

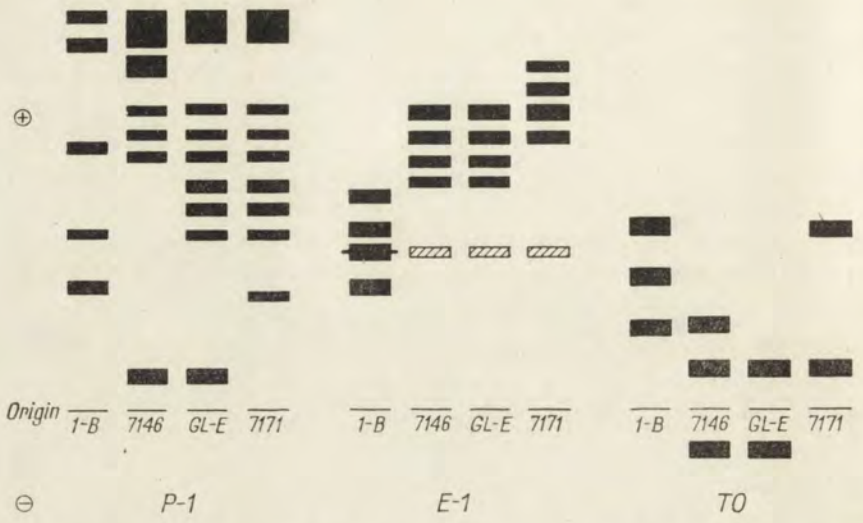


Fig. 2. Electrophoretic differences among strains of phenoset B. Strains UI-7146 and UI-7171 are compared with strains GL-E (phenoset B) and B (syngen *1*) for acid phosphatase (P-1), proprionyl esterase (E-1), and tetrazolium oxidase (TO). The E-1 pattern of syngen *1* conceals the E-3 esterase band, indicated by a line across the E-1 band, and corresponding to the E-3 band of the phenoset B strains, indicated by the shaded bands

Because of the relatively minor nature of these differences, and because all other enzymes examined, including alkaline phosphatase as well as the enzymes examined by Borden et al. 1973, were identical to the phenoset B pattern, we conclude that strains UI-7146 and UI-7171 are natural representatives of phenoset B, which heretofore contained only "classical" strains presumably derived by mislabeling accidents from a single collection. The identity of that original collection is not known, but we find no record in the literature of collections made at the sources from which our two new strains were recovered. Phenoset B has thus been collected on three separate occasions. The enzymatic variants described above guarantee that we have not merely reisolated an escaped cell of GL-E.

The cortical properties of the phenoset B strains are given in Table 2. The two new strains are indistinguishable from GL-E, but GL from the Cambridge Culture Collection has lowered its central angle appreciably. In view of the large variation shown by phenoset A when allowed to experiment freely in peptone during the course of 50 years, we suggest that the uniformity of the recent isolates of phenoset B (examined after approximately one year in the laboratory) is a residual effect of selection operating on or reflected by their cortical properties in nature.

Strains GL-E, GL (Cambridge), and UI-7146 produce soluble, dark brown pigments in proteose peptone cultures at the end of log phase, in the absence of aeration. Various kinds of pigmentation are characteristic of particular genetic species of *Tetrahymena*. Syngens 3, 6, 8, and 10 of *T. pyriformis* produce strong

Table 2

Cortical Characteristics of *Tetrahymena pyriformis* (Phenoset B)

Corticotype	GL-E	GL (Cambridge)	UI-7146	UI-7171
	Mean CVP Positions:			
15			4.250(2)	
16	4.250(4)	4.250(1)	5.250(1)	
17	5.174(46)	4.416(21)	5.107(7)	5.250(7)
18	5.233(60)	5.244(28)	5.198(34)	5.196(37)
19	5.577(28)	5.292(63)	5.205(22)	5.514(37)
20		5.653(81)	5.916(9)	6.250(1)
21		5.916(9)		
N	138	203	75	82
Central angle:	85.99°	81.94°	84.02°	85.29°

(Sample sizes for each corticotype are given in parentheses.)

pigmentation with characteristic brown, pink, orange, green, blue, or even purple tints. Some strains pigment only with aeration. These pigments are due to a family of compounds closely related to orcein (unpublished studies). Different colors arise by the substitution and conjugation of the α-amino-oxyorcein nucleus, in addition to a number of interrelated brown compounds which are probably products of tryptophan catabolism. For the present, it is important that all the strains of phenoset B are characterized by the same type of pigmentation, with the exception of UI-7171, which has never pigmented. None of the other described "phenosets" produces pigments in standing cultures.

Discussion

The concept of asexual syngens was first announced by Sonneborn 1957, but techniques of sufficient power to discriminate between asexual species of identical morphology were not then available. Borden et al. 1973 showed that a large sample of amicronucleate strains could be reduced to a small number of mutually exclusive phenotypic sets which showed differences from each other at least as great as those separating the known biological species of the *T. pyriformis* complex. This study proved the power of the techniques and the existence of discontinous variation among the domestic amicronucleates. Our own results with phenoset A demonstrate the limiting weakness of cortical techniques for interspecific discrimination, as opposed to molecular methods. The practicability of species discrimination depends not only on the ability to detect differences in phenotype, but also on the finite and discontinuous character of natural variation. It must be shown that not every isolate from nature is different from all others. By the

repeated collection of phenoset B from three different sources (one unknown) we have fulfilled this requirement and have demonstrated in principle the possibility of identifying morphologically identical asexual species. This result was obtained by a survey of only nine newly-isolated amicronucleates, with two clearly belonging to a described "phenoset". In comparison, 11 of 29 wild micronucleate strains of the *T. pyriformis* complex have been identified as members of the 12 described syngens. While identification of wild strains must continue to be laborious, we believe it will eventually be of major significance in our understanding of the ecological position of the tetrahymenids.

The three separate isolates of phenoset B are not quite identical, but strain differences in esterases and phosphatases are fairly common in the described syngens, and one other tetrazolium oxidase variant is known, in syngen *1* (Allen and Weremiuk 1971, Borden et al. 1973 a). Variation within an asexual phenoset is expected to occur through the accumulation of mutational alterations of the macronucleus. The degree of variation observed must be a function of the mutation rate and of natural selection. It should therefore be found that some asexual species are more polymorphic than others. The occurrence of polymorphism in enzymes of the several cultures of phenoset A is not expected to be common because the enzymes which can be conveniently visualized on starch gels are in general indispensable to the organism. Selection for these enzymes should not be relaxed merely by turning the organism loose in a perpetual supply of sterile peptone. In fact, only a single variant enzyme was found by Borden et al. 1973 among 21 samples of phenoset A: in GL, (Dewey), the E-l esterases were shifted slightly away from the origin. The cortical variation of phenoset A may or may not result from the relaxation of selection in domestication. Perphaps the cortex of phenoset B is simply more stable or crucial than that of phenoset A, or perhaps the "central angle" is actually of some importance in a natural situation. These possibilities can best be examined by the collection of more natural representatives of each phenoset.

Acknowledgements

This investigation was supported by National Institutes of Health grant GM-07779 to Dr. D. L. Nanney. Mrs. Margaret Chow assisted in counting kineties on hundreds of cells.

Summary

The cortex, but not the enzymatic phenotype of an amicronucleate form, "phenoset A" of *T. pyriformis* has varied during 50 years of domestication. Central angles from 74° to 87° were observed among 10 samples separated for many years. One sample has developed morphogenetic anomalies which raise interesting questions on cortical regulation. Two strains have been isolated from nature which match

almost exactly the cortical and enzymatic phenotypes of "phenoset B", another *T. pyriformis* amicronucleate. These strains demonstrate the feasibility of classifying the amicronucleate *T. pyriformis* into a finite number of asexual "syngens" or cryptic species.

RÉSUMÉ

Les structures corticales, mais pas le phénotype enzymatique d'un type sans micronoyau, "phénoset A" de *T. pyriformis*, ont variées pendant 50 années en domestication. Angles centrales de 74 à 87° étaient observés entre 10 souches séparées depuis quelques années. Une souche montre des anomalies morphogénétiques qui soulèvent des questions intéressantes sur le règlement cortical. Deux souches isolées de nature correspondent presque exactement aux phénotypes corticales et enzymatiques de "phénoset B", autre type sans micronoyau de *T. pyriformis*. Ces souches démontrent la possibilité de classifier les souches sans micronoyau dans un nombre fini des "syngens" ou espèces cryptiques asexuées.

REFERENCES

Allen S. L. and Weremiuk S. L. 1971: Intersyngenic variations in the esterases and phosphatases of *Tetrahymena pyriformis*. Biochem. Genet., 5, 119–133.

Borden D., Whitt G. S. and Nanney D. L. 1973: Electrophoretic characterization of classical *Tetrahymena pyriformis* strains. J. Protozool. 20, 693–700 .

Borden D., Miller E. T., Whitt G. S. and Nanney D. L. 1975: Electrophoretic analysis of evolutionary relationships in *Tetrahymena*. Evolution, (in press).

Frankel J. 1972: The stability of cortical phenotypes in continuously growing cultures of *Tetrahymena pyriformis*. J. Protozool., 19, 648–654.

Gruchy D. F. 1955: The breeding system and distribution of *Tetrahymena pyriformis*. J. Protozool., 2, 178–185.

McCoy J. W. 1974: New features of the tetrahymenid cortex revealed by protargol staining. Acta Protozool., 13, 155–160.

Nanney D. L. 1966: Cortical integration in *Tetrahymena*: an exercise in cytogeometry. J. exp. Zool., 161, 307–317.

Nanney D. L. 1967 a: Comparative corticotype analyses in *Tetrahymena*. J. Protozool., 14, 690–697.

Nanney D. L. 1967 b: Cortical slippage in *Tetrahymena*. J. exp. Zool., 166, 163–170.

Nanney D. L. 1971 a: Cortical characteristics of syngens 10, 11 and 12 of *Tetrahymena pyriformis*. J. Protozool., 18, 33–37.

Nanney D. L. 1971 b: The constancy of cortical units in *Tetrahymena* with varying numbers of ciliary rows. J. exp. Zool., 178, 177–182.

Shaw C. R. and Prasad R. 1970: Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genet., 4, 297–320.

Sonneborn T. M. 1957: Breeding systems, reproductive methods and species problems in *Protozoa*. In: The Species Problem, (ed. Mayr E.) AAAS Publications, Washington, D. C., pp. 155–324.

Tait A. 1970: Enzyme variation between syngens in *Paramecium aurelia*. Biochem. Genet., 4, 461–470.

Received 20 September 1973

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 22

Institute of Zoophysiology, University of Lund, S-223 62 Lund, Sweden

Erik G. CHRISTENSSON

The Effect of Temperature and Actinomycin D on the Labelling Pattern of RNA in Synchronized *Tetrahymena pyriformis* GL

Der Effekt der Temperatur und Actinomycin D auf das Muster der Inkorporation in RNS aus synchronisierten *Tetrahymena pyriformis* GL

The introduction in 1954 by Scherbaum and Zeuthen of a method for synchronizing the eukaryotic protozoan *Tetrahymena pyriformis*, by cyclic temperature shifts between 28°C and 34°C, opened a new approach to the investigation of the processes immediately concerned with cell division and to the analysis of the life cell cycle with conventional biochemical methods. Nevertheless, in spite of the rapid progress made in this field, our knowledge of the mechanism by which synchronization of cell division is induced is still far from complete.

The observations that heat shocks change the character of the RNA synthesis (Christensson 1962, 1968) and decrease the incorporation of precursors (Bernstein and Zeuthen 1966, Byfield and Scherbaum 1966 a, Christensson 1968, 1971 a) and that actinomycin D stops cell division (Lazarus, Levy and Scherbaum 1964, Moner 1965, 1967, Frankel 1965, Christensson 1968) have drawn attention to the effect of heat shocks on RNA metabolism. RNA synthesis might be affected by heat in several ways, such as impaired function of RNA polymerase, altered uptake and movement of RNA precursors into the macronucleus and enhanced decay of RNA. Moner (1965, 1967) suggested that temperature reduces the activity of RNA polymerase. On the other hand, Byfield and Lee (1970) presented evidence that decreased incorporation in RNA at 34°C is a consequence of pool effects caused by a quite random decay of existing RNA at 34°C. In a series of papers Byfield and Scherbaum (1966 b, 1967 a, 1967 b, 1968) and Byfield and Lee 1970 have reported hydrolysis of RNA at 34°C. The stability of RNA was studied by prelabelling the RNA at 29°C and then studying its decay at 29°C and 34°C respectively in the presence of actinomycin D (20 – 100 μg/ml). In a recent review, Zeuthen and Rasmussen (1971) discuss the risk of using actinomycin D at such high doses as a specific inhibitor of RNA synthesis in RNA decay experiments. Although a dose-related response to actinomycin D in the synthesis of different RNA species has been shown in higher animal cells by Perry

and Kelly (1971), the doses used in *Tetrahymena* are much higher and other effects may occur. Furthermore, analysis of temperature dependent actinomycin D effects on RNA synthesis and RNA degradation has been neglected. The aim of the present investigation has been to elucidate the labelling pattern on polyacrylamide after electrophoresis of labelled RNA, isolated from cells maintained at 28°C and 34°C, both with and without actinomycin D, in order to ascertain to what extent heat shocks and actinomycin D, respectively, are responsible for the postulated decay of RNA. The experiments showed that an increase of temperature from 28°C to 34°C did not cause any further decay of prelabelled RNA, although at a heat shock in the presence of actinomycin D, some RNA was partially degraded. On the other hand, RNA synthesized at the shock temperature (34°C) decomposed rapidly. Furthermore, double labelling experiments have been made with ^{32}P-orthophosphate and ^{3}H-uridine to obtain some information about uptake and reutilization of different RNA precursors at different temperatures. Preliminary reports have been published in the author's Ph. Dr. thesis (1971 b) and VIIth Nordic Cell Congr. (1971 c).

Material and Methods

Cell Cultivation and Sampling

Growth conditions and sampling have been described previously (Christensson 1968). The autoclaved growth medium consisted of 2% proteose peptone (Difco) with 0.3% Difco yeast extract in a balanced salt solution according to Kidder and Dewey (1951). Stock cultures were kept in 3 ml medium in test tubes stored in the dark at room temperature and were inocculated every third day with a loop. Each Fernbach flask (2.5 l) contained 500 ml medium and was inocculated with 1.5 ml of a three day-old stock culture. The stirred cultures (30 strokes/min) were kept at 28.5°C until the density was about 2×10^4 cells/ml. They were then synchronized by heat treatment, the temperature being shifted every thirty minutes between 28.5° and 34°C according to Scherbaum and Zeuthen (1954), with the modification that only 7 shocks were given. The ribonucleic acid was labelled with carrier-free ^{32}P-orthophosphate (1 μCi/ml) and/or ^{3}H-uridine (1 μCi/ml, specific activity 3.5 Ci/mmol) from the Radiochemical Centre, Amersham. In some series the cells were incubated with actinomycin D (Sigma), which was added directly to the growth media. Labelled samples were removed, chilled with crushed ice and the cells were spun down by centrifugation at 300 g for 3 min in 250 ml plastic tubes. The cells were washed four times with isotonic saline solution (0.4% NaCl). In series where RNA from free ribosomes and from other structures was studied the cells were lysed by indole according to Lyttleton (1963) except that the lysis was performed in 12 mM Tris buffer saturated with indole at pH 8 instead of pH 7.3. The unlysed structures were spun down at 10 000 g for 15 min and washed twice with the buffer. The "structure-bound" RNA was isolated from the pellet by the phenol method. The supernatant was centrifuged at 105 000 g for 60 min. The pellet containing the free ribosomes was suspended in the buffer and its RNA also isolated by the phenol method.

Preparation of RNA

RNA was extracted according to Kirby (1965) by the cresol-phenol method. The cells or cellular fractions, frozen in solid carbon ice, were homogenized directly in the cresol-phenol mix-

ture. RNA obtained by this method can be fractionated by extraction in 3M sodium acetate (Kirby 1965). Unlike ribosomal RNA (rRNA) soluble RNA (4S RNA) is extracted by sodium acetate. In a series of experiments in which the temperature dependent actinomycin D effect on RNA was studied the cell cultures, kept under different experimental conditions, were denoted as A, B, C, D, E and F. The labelled RNA isolated from them was fractionated in 3 M sodium acetate (NaAc) before the electrophoresis on polyacrylamide gels. The ribosomal RNA fractions, insoluble in 3 M NaAc, from the different cultures were denoted as AR, BR, CR, DR, ER and FR and the corresponding soluble RNA fractions as AS, BS, CS, DS, ES and FS.

Gel Electrophoresis and Measurement of Radioactivity

The different RNA classes were separated on polyacrylamide gels according to Peacock and Dingman (1967). As described earlier (Christensson 1971 a), the ribosomal RNA (25S and 17S RNA) has a complex labelling profile on polyacrylamide. In this investigation the 25S RNA peak is denoted as a, and the 17S RNA complex as a triple peak designated b, c and d. After electrophoresis the gels were cut into slices 1.8 mm in thickness. The gel slices were dissolved in the vials with 300 μl H_2O_2 for 1 h at 60°C. The activity was read in a Packard Tri-carb 3320 scintillation spectrometer, as described earlier (Christensson 1970).

Results

Incorporation of ^{32}P and ^{3}H-uridine in RNA at 30 min pulses given during the last heat shock, during the recovery period (the time from last heat shock to division) and in the cell cycle between the lst and 2nd synchronous division is recorded in Fig. 1 for RNA from free ribosomes and in Fig. 2 for RNA from cell structures not lysed in indole. At all pulses, with the exception of those at 34°C, the count of ^{3}H-uridine is about twice that of ^{32}P. From Fig. 1 it is clear that incorporation of both ^{32}P and ^{3}H-uridine in RNA from free ribosomes is most extensive close to the lst synchronous division. The labelling of RNA declines successively at pulses given in the cell cycle between the 1st and 2nd divisions. Thus synthesis of stable RNA in synchronous *Tetrahymena* cell cultures is phased. Even incorporation into structure-bound RNA is phased, but prominent labelling starts earlier in the recovery period.

In some experiments the effects of temperature on labelled ribosomal RNA was studied in cells kept at 34°C for 30 min in the presence (AR in Fig. 3) or absence of actinomycin (BR in Fig. 3). The RNA was in both cases prelabelled with ^{3}H-uridine at a pulse of 10 min, in the intermittent period at 28°C after the 6th heat shock, but at the end of the pulse (at the time for shift to the 7th heat shock) actinomycin D (10 μg/ml) was added to the A cell culture. The decay of RNA at 34°C during the 7th heat shock was then studied by inspection, at the end of the 7th shock, of the labelling pattern of ^{3}H-uridine in RNA fractionated on polyacrylamide. The basic pattern of activity in the two series was the same, but there was an important quantitative difference. The 25S RNA in AR (Fig. 3) showed a drastic decrease in label compared to 25S RNA of BR (Fig. 3). On the other hand, the radioactivity of 17S RNA in AR seemed to increase, which suggests that in the

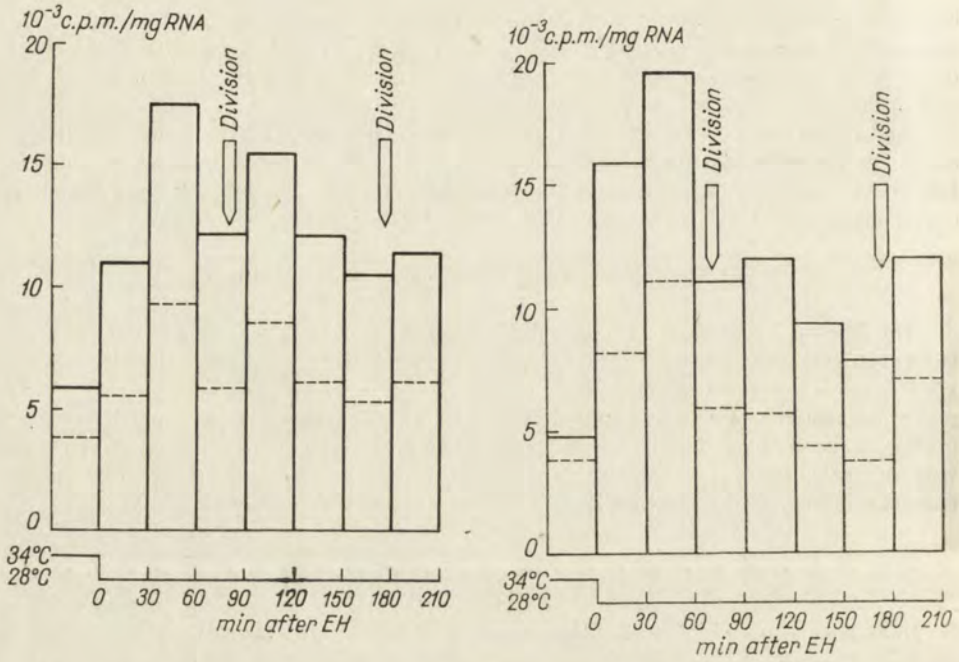


Fig. 1. The labelling of RNA isolated from free ribosomes after indole lysis. The cells were labelled with 30 min pulses of both ^{32}P (1 μCi/ml) and ^{3}H-uridine (1 μCi/ml) during the last heat shock, during the recovery period up to division, and during the cell cycle between the lst and 2nd synchronous divisions. The level of labelling with ^{32}P is represented by a dashed line and that of ^{3}H-uridine by a continuous line. The time is noted on the abscissa in min since the last heat shock (EH = end of heat treatment)

Fig. 2. The labelling of RNA isolated from unlysed by indol treatment cell structures. The cells were labelled and sampled as in Fig. 1. The level of labelling with ^{32}P is represented by a dotted line and that of ^{3}H-uridine by a continuous line. The time is noted on the abscissa in min since the last heat shock (EH = end of heat treatment)

presence of actinomycin D 25S RNA decays into 17S RNA. The labelling pattern of 4S RNA (soluble in 3M NaAc) was the same in the two cultures (A and B) and is represented for culture A in AS Fig. 4.

In two other cell cultures, C and D, Fig. 4, RNA was also prelabelled by ^{3}H-uridine pulse of 10 min after 6th heat shock, but the cells were then kept at 28°C for 30 min before isolation of RNA. In C the cells were pretreated with actinomycin D (10 μg/ml). The presence of actinomycin D strongly reduced the incorporation of ^{3}H-uridine into RNA. Most decreased was the labelling of ribosomal RNA (28S and 17 S RNA). Less reduction of labelling at actinomycin D treatment was shown by low molecular RNA in the range 3–5S RNA, slices 65–70 in CR Fig. 4.

In another two cultures E and F the incorporation at 34°C was studied in cells exposed to 34°C for a varied length of time. In one of the series represented in ER in Fig. 4 a pulse of labelling, including both ^{32}P and ^{3}H-uridine, was given during the last 10 min of the 7th heat shock. At 34°C the incorporation of both ^{32}P and

^{3}H-uridine was remarkably reduced. But, as seen in Figs. 1, 2 and in ER in Fig. 4, the incorporation of ^{3}H-uridine at 34°C is reduced more than of ^{32}P. While existing prelabelled RNA, AR in Fig. 3 and AS in Fig. 4, was affected very little by a heat shock, RNA synthesized at the shock temperature (34°) decays almost

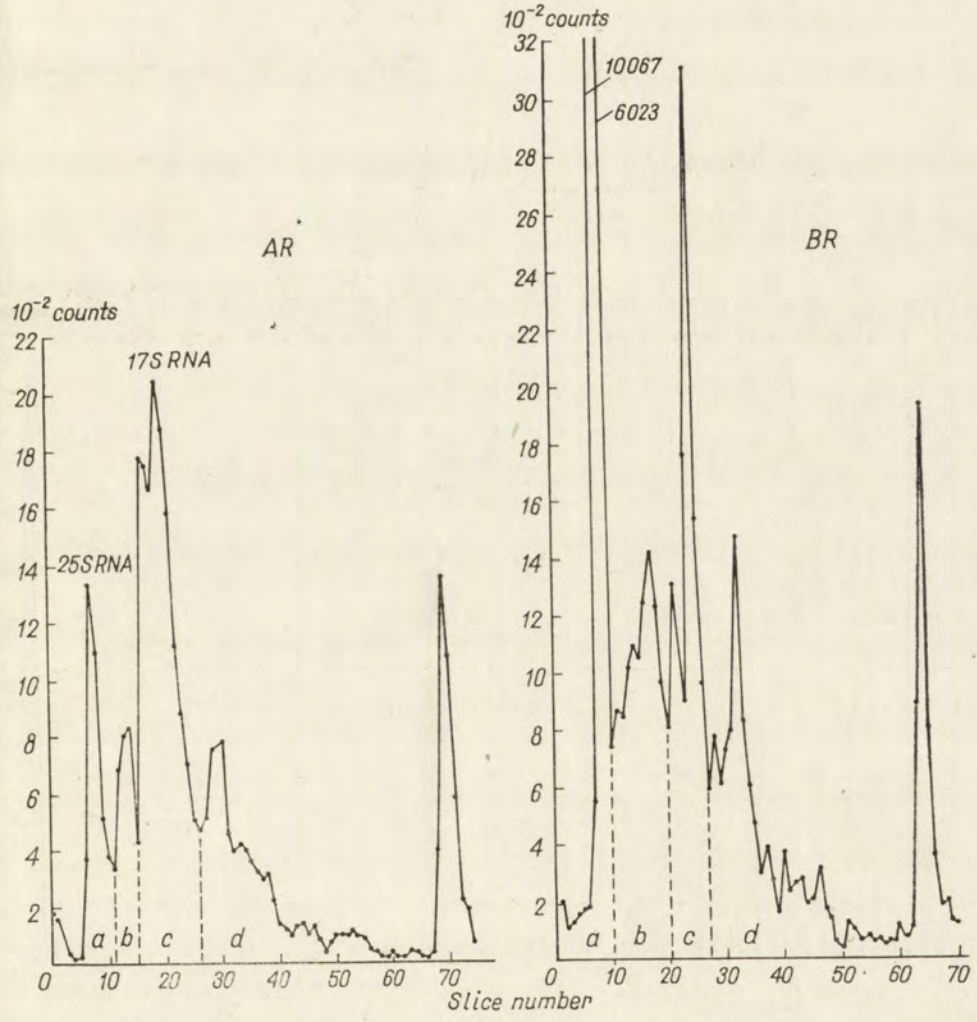


Fig. 3. AR shows the labelling pattern of RNA insoluble in 3M NaAc (rRNA) from cells in culture A prelabelled with ^{3}H-uridine at 28°C (1 μCi/ml), 20–30 min after the 6th heat shock) and given the last heat shock in the presence of actinomycin D (10 μg/ml). BR shows the labelling pattern of RNA insoluble in 3M NaAc from cells in culture B prelabelled at 28°C as in culture A and given the last heat shock without the antibiotic. Isolation of RNA was made by the cresol-phenol method of Kirby, followed by extraction of RNA soluble in 3M NaAc. The electrophoresis of RNA was carried out for 100 min in 2.6% gels (approx. 100 μg RNA/gel). In the labelling profile 25S RNA is equal to peak *a*, peak *b* represents a prefraction to 17S RNA (peak *c*) and peak *d* a postfraction to 17S RNA

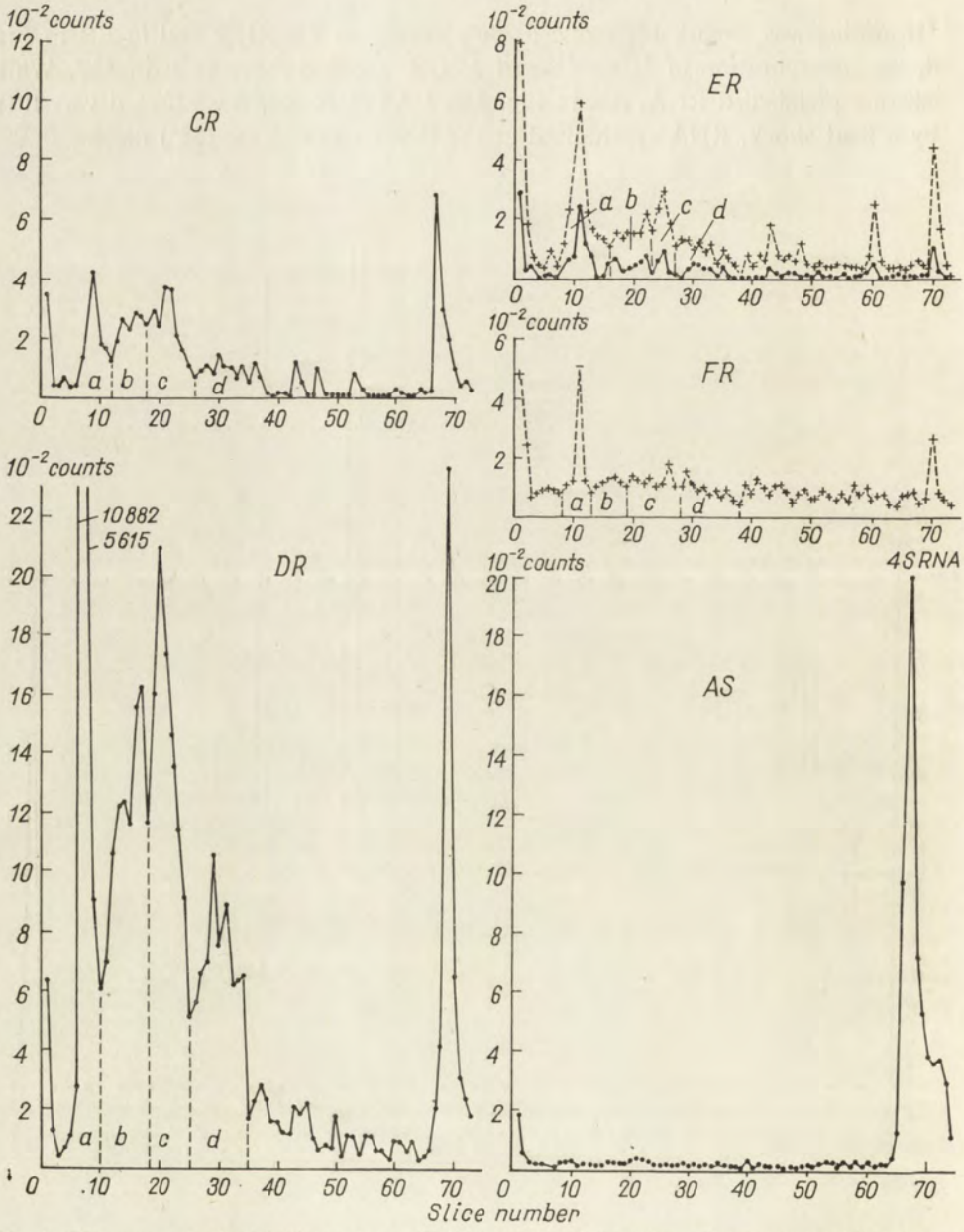


Fig. 4. CR represents the labelling pattern of RNA from cells in culture C pretreated with actinomycin D (10 μg/ml) added 0 min after the 6th heat shock. After labelling the cells were kept for 30 min at 28°C before RNA was isolated. DR shows the labelling pattern of RNA prelabelled at 28°C in culture D at the same time as in culture A, B and C. After the labelling the cells were kept at 28°C for 30 min as in culture C. In ER the cells in culture E were double-labelled with ^{32}P (1μCi/ml) and ^{3}H-uridine (1 μ Ci/ml) at 34°C during the last 10 min of the last heat shock. The labelling of ^{32}P is represented by a dashed line. In FR the RNA was labelled with ^{32}P by a 10 min pulse at 34°C in cells from culture F kept at 34°C for one hour. AS shows the labelling pattern of RNA soluble in 3M NaAc (4S RNA) from cells grown in culture A

at once and the spare labelling found seems to be distributed in many intermediary decay products, ER in Fig. 4. After 1 h at 34°C hardly any labelled stable RNA products could be isolated, as seen in FR in Fig. 4. The present results clearly show that nascent RNA is more sensible to heat than already formed RNA.

Discussion

The double labelling experiments on *Tetrahymena*, Figs. 1 and 2, using ^{32}P-orthophosphate and ^{3}H-uridine are remarkable in two respects. First, in contrast to what might be expected from labelling experiments of RNA, the ^{32}P labelling is not heavier than that of ^{3}H-uridine. But exceptional conditions prevail in *Tetrahymena*. There is no de novo synthesis of purine or pyrimidine bases in *Tetrahymena* (Conner and Linden 1970) and the intracellular nucleotide pool is therefore probably small. Furthermore, there is evidence that the ^{32}P taken up from the media is diluted by a large pool of pyrophosphate (Munk and Rosenberg 1969). Second, after a short time at 34°C there is no incorporation at all of ^{3}H-uridine while ^{32}P continues to be incorporated for some time, although at reduced rate. This lends support to the opinion of Byfield and Lee (1970) that the uptake of bases in *Tetrahymena* is active and very precisely regulated by their intracellular concentrations. At 34°C the rate of degradation of newly synthesized RNA is very high (ER in Fig. 4) and uptake from exogenous sources is unnecessary. The orthophosphate, on the other hand, is not regulated so precisely as the bases and the uptake might proceed for a considerable time, though at a steadily decreasing rate. From Figs. 1 and 2 it is also seen that in the heat synchronized cells RNA synthesis is phased. The formation of ribosomes is highest in the late phase of the recovery period and early in the cell cycle proceeding after the lst synchronous division. There is evidence for that during the late recovery period many cells have made a cycle without fission and are again in G_1 phase, as most cells start DNA synthesis immediately after the synchronous division (Hjelm and Zeuthen 1967). Addition of actinomycin D in this phase of RNA synthesis does not stop the lst division but inhibits the 2nd division (Christensson 1968).

Inspection of the labelling pattern of RNA on polyacrylamide showed that no further degradation of prelabelled RNA beyond that present at optimal temperature (28–29°C) occurred when cells were exposed to a heat shock (BR in Fig. 3 and DR in Fig. 4). Such results are consistent with conclusions drawn from earlier, quantitative registrations of different RNA fractions separated on polyacrylamide gels at the two temperatures (Christensson 1971). Although an ordered degradation of isolated and pre-labelled RNA could be followed, when homologous RNases were used in degradation experiments in vitro (Christensson 1971), no such degradation of prelabelled RNA could be traced in this investigation at a heat

shock (34°C). A degradation has, however, been reported by Byfield and Scherbaum (1966 b, 1967 a, 1967 b, 1968) and Byfield and Lee (1970) from decay experiments in the presence of actinomycin D (20–100 μg/ml). Further evidence for a decomposition of mRNA at 34°C comes from hybrid experiments (Christensson 1970). The results described in this paper suggest, however, that the degradation of prelabelled RNA noted at 34°C in the presence of actinomycin D is due to the combined effects of heat and the antibiotic treatment. The conclusion drawn is that actinomycin D increases the sensibility of RNA to heat. With actinomycin D doses with the range of 5 to 10 μg/ml, the 25S RNA is split at one or two particular linkages susceptible both to actinomycin D (at the shock temperature, 34°C, Ar in Fig. 3) and to RNases (Christensson 1971). However, with the doses used for actinomycin D no degradation products between 17S RNA and 3–5S RNA could be traced. In contrast with RNA prelabelled at 28–29°C, RNA formed at 34°C seems to be very labile and to decay rapidly (ER in Fig. 4). Any stable RNA formed at 34°C is therefore hard to trace by labelling, partly because very little ^{3}H-uridine of exogenous origin is taken up by the cells during the heat shock. As suggested by Byfield and Lee (1970), RNA precursors seem to be reutilized to a high extent in the synthesis of RNA at 34°C. The present study supports the view of Byfield et al. that changed pool conditions caused by increased decay of RNA at 34°C may drastically decrease the uptake of extracellular RNA precursors at this temperature, but it provides no evidence that a random decay of RNA already exists in the cell on the application of the heat shock. Instead the decomposition of RNA is restricted to nascent RNA formed in macronuclei at the shock temperature.

In the presence of actinomycin D the incorporation of labelled ^{3}H-uridine is drastically decreased in preribosomal and ribosomal RNA, as can be seen in C in Fig. 4 and has been shown earlier by using ^{32}P (Christensson, 1971 a), but as much as 5 μg/ml of actinomycin D has to be used. The lower sensibility in *Tetrahymena* compared with other cell materials (Perry and Kelly 1971) might depend on the high degree of polyploidy. As seen in CR in Fig. 4, the incorporation of ^{3}H-uridine into 25S RNA (a) in the presence of actinomycin D was affected more than the incorporation into 17S RNA complex (b, c and d). This might be because 25S RNA and 17S RNA are not contained in the same cluster of ribosomal genes, but it is more probable that labelled mRNA occurs among the 17S RNA, as it is well known that mRNA is usually not as sensible to actinomycin D as rRNA, even when mRNA is of the same molecular size as rRNA (Perry and Kelly 1971).

According to Zeuthen and Rasmussen 1971 the primary effect of heat shocks is the breaking of hydrogen bonds in dynamic and highly labile structures. Such heat-labile structure are contained in macronuclei and nucleoli (Elliott, Kennedy and Bak 1962; Cameron, Padilla and Miller 1966, Nilsson and Leick 1971), in microtubuli (Zeuthen and Rasmussen 1971) and maybe also in membranes, as permeability conditions are reported to change in heat-treated cells (Cann 1968)

If nucleosides are taken up by an active transport mechanism, as suggested by Byfield and Lee 1970, this could be affected by the heat shock, which might damage structures participating in the transport mechanism or decrease the supply of energy. A thermolabile mechanism associated with phosphorylation has been reported for rat liver cells (Christiansen and Kvamme 1969). RNA synthesis may also be affected by changed uptake of amino acids and/or proteins. Changes in uptake of amino acids with temperature have also been shown in *Tetrahymena* (Christensson 1959) but the changes seem hardly sufficient to disturb seriously the RNA synthesis (Byfield and Scherbaum 1968). A transport mechanism, until now overlooked, but perhaps of vital importance for stabilization and selection of nuclear RNA intended for the cytoplasm, is migration of particular proteins from the cytoplasm into the macronucleus. Movement of cytoplasmic proteins into the nucleus has been studied by Merriam (1969) and their importance in "engaging" nuclear RNA for transfer to the cytoplasm has been underlined by Harris (1968). If the interaction between cytoplasm and macronucleus is disturbed, unstabilized RNA in the macronucleus may decay very rapidly, the nucleotides become reutilized, and the need for exogenous RNA precursors thereby minimized.

The changes in RNA synthesis on a heat shock or when cells are kept at 34°C seem very similar to those reported in other eukaryotic cells during aging and starvation. In protein-deprived rat liver cells the amount of membrane-bound ribosomes decreased in relation to those free in the cytoplasm (Stenram and Nordgren 1970). In *Tetrahymena* cells the proportion of membrane-associated ribosomes, which is already low in the cells under optimal conditions, decreased further in heat-treated cells (Christensson 1971). Protein-deprived rats showed hypertrophy of their nucleoli in the liver cells (Stenram 1956). Also heat-treated *Tetrahymena* cells show structural changes of their nucleoli by formation of large fusion bodies (Elliott, Kennedy and Bak 1962, Cameron, Padilla and Miller 1966). Liver cells of rats starved for protein showed no decrease in the intensity of their RNA synthesis (Stenram and Nordgren 1970), but the half-life seemed shortened owing to a change in the stability of RNA and increased RNase activity. Heat-treated cells of *Tetrahymena* also showed increased hydrolysis of mRNA (Byfield and Scherbaum 1966 b, 1967 a, 1967 b, 1968, Christensson 1970, Byfield and Lee 1970). Contrary to suggestions by Moner (1965, 1967), Byfield and Lee (1970) found the synthetic activity of the RNA polymerases to be stimulated rather than depressed at 34°C. All known studies of thermal effect in nucleoli suggests that supraoptimal heat causes aggregation of nucleoli not only in *Tetrahymena* but also in other cell materials and in this way raduces formation of stable ribosomal RNA because 45S precursor rRNA, still formed, cannot be transformed to functional rRNA (Warocquier and Scherrer 1969, Simard et al. 1969). The directed flow of labelled RNA precursors through the nucleotide pool is thereby stopped. Instead nucleotides are rapidly brought back to the pool by decay of labile RNA and can be reutilized and the need for an exogenous supply of RNA precur-

sors is drastically minimized. In the cells of protein-deprived rats, complex changes occur in both free and membrane-bound polysomes, including an increase of free polysomes heavier than pentamers and in the amount of free monosomes (Stenram and Nordgren 1970). Such changes are very similar to those reported heat-treated cells of *Tetrahymena* (Hartman and Dowden 1970).

From these experiments and many others it is evident that the effect of heat is multifold, as suggested by Zeuthen and Rasmussen (1971). Temporary damage of labile structures essential for compartmentalization, transport mechanisms, maturation and stabilization of formed macromolecules are, however, those primarily affected, which results in increased hydrolysis of labile RNA and thereby changed nucleotide pool conditions. The heat-treated cells seem to be transformed into a new physiological state, in many respects very similar to that of aged and starved cells. The state created is very probably due to lack of some mRNA fraction necessary for further advancement towards division.

Acknowledgements

I wish to express my gratitude to Professor Ivar Agrell, the Head of the Zoophysiological Institute, University of Lund, for his support and stimulating criticism.

Valuable technical assistance was provided by Mrs Eva Dahlberg.

Grateful acknowledgements for financial support is made to The Royal Physiographic Society of Lund, The foundation of Director Albert Påhlsson, Malmö and The Foundation of Bergwall, Stockholm.

Summary

The susceptibility of RNA towards heat and actinomycin D was studied during the last heat shock in a synchronization program of cell division in mass cultures of the ciliate *Tetrahymena pyriformis* GL, in which the temperature was shifted every 30 min between 28°C and 34°C. The labelling profile of RNA on polyacrylamide showed that a heat shock alone did not degrade RNA prelabelled at 28°C. Nor did actinomycin D in doses of the range 5 – 10 μg/ml degrade RNA. When actinomycin D and heat were combined, however, the prelabelled 25S RNA partially decayed into 17S RNA components. In contrast to RNA prelabelled at 28 – 29°C, RNA formed at 34°C decomposed very rapidly. By using labelling experiments with ^{32}P and ^{3}H-uridine changed pool conditions were demonstrated at 34°C. The ribosomal RNA was most susceptible to actinomycin D. Somewhat decreased sensitivity of the 17S RNA complex to actinomycin D suggested the presence of messenger-RNA in this fraction. Pulses with ^{32}P and ^{3}H-uridine showed a phased RNA synthesis of both ribosomal RNA and RNA associated with other cell structures during the cell cycle which followed when synchronized cells were kept at 28°C.

ZUSSAMENFASSUNG

Die Empfindlichkeit in RNS für Hitze und Actinomycin D wurde während des letzen Hitzeschock eines Synchronisierungsprogramms für Zellteilung in Mass-Kulturen mit Ciliaten *Tetrahymena pyriformis* GL studiert, wo die Temperatur jede 30: e Minute zwischen 28° und 34°C gewechselt wurde. Das Profil der Inkorporation für RNS auf Polyacrylamide zeigte, dass allein durch einen Hitzeschock, RNS, die früher bei 28°C markiert wurde nicht abgebaut werden konnte. RNS wurde auch nicht abgebaut durch Actinomycin D in einer Konzentration von 5—10 μg/ml. Wenn Actinomycin D und Hitze kombiniert wurden, wurdė früher markierte 25S RNS teilweise zu 17S RNS abgebaut. Im Gegensatz zu RNS markiert bei 28—29°C, wurde RNS bei 34°C gebildet schnell abgebaut. Mit ^{32}P und ^{3}H-uridine markierte RNS konnte eine Veränderung der Pool-Verhältnisse demonstriert werden. Die Ribosomale RNS zeigte sich am meisten empfindlich für Actinomycin D. Herabgesetzte Empfindlichkeit in 17S in RNS-Komplex für Actinomycin D deutete die Gegenwart von Messenger-RNS in dieser Fraktion an. Pulse mit^{32} P und ^{3}H-Uridine während des Zellcyclus zeigten, dass eine phasische Synthese für sowohl Ribosomal wie auch für RNS, assoziert mit anderen Zellstrukturen, existierte, wenn die Zelltemperatur bei 28°C gehalten wurde.

REFERENCES

Bernstein E. and Zeuthen E. 1966: The relationship of RNA synthesis to temperature as inducer of synchronous division. C. r. Trav. Lab. Carlsberg, 35, 501–517.

Byfield J. E. and Scherbaum O. H. 1966 a: Suppression of RNA and protein accumulation by temperature shifts in a heat-synchronized protozoan. Life Sci., 5, 2263–2269.

Byfield J. E. and Scherbaum O. H. 1966 b: Temperature dependent RNA decay in *Tetrahymena*. J. cell Physiol., 68, 203–206.

Byfield J. E. and Scherbaum O. H. 1967 a: Stability of division-related protein and nucleic acid fractions in synchronized *Tetrahymena*. J. cell Physiol., 70, 265–274.

Byfield J. E. and Scherbaum O. H. 1967 b: Temperature-dependent decay of RNA and of protein synthesis in a heat-synchronized protozoan. Proc. natn. Acad. Sci., 57, 602–606.

Byfield J. E. and Scherbaum O. H. 1968: Amino acid control of RNA synthesis in an animal cell (*Tetrahymena*) and its relation to some aspects of gene expression. Expl Cell Res., 49, 202–206.

Byfield J. E. and Lee Y. 1970: Do synchronizing temperature shifts inhibit RNA synthesis in *Tetrahymena pyriformis*. J. Protozool., 17, 445–453.

Cameron I. L., Padilla G. M. and Miller O. L. Jr. 1966: Macronuclear cytology of synchronized *Tetrahymena pyriformis*. J. Protozool., 13, 336–341.

Cann J. R. 1968: On the leakage of ultraviolet absorbing materials and alanine. C. r. Trav. Lab. Carlsberg, 36, 319-325.

Christensson E. 1959: Changes in free amino acids and proteins during cell growth and synchronous division in mass cultures of *Tetrahymena pyriformis*. Acta physiol. scand., 45, 339–349.

Christensson E. 1962: Different RNA fractions during cell growth and synchronous division in mass cultures of *Tetrahymena pyriformis* GL. Acta physiol. scand., 54, 1–8.

Christensson E. G. 1968: Evidence of a synchronized RNA metabolism during cell growth and cell division in heat treated mass cultures of *Tetrahymena pyriforms* GL. Z. Biol., 116, 143–158.

Christensson E. G. 1970: Studies of messenger RNA during cell cycle in synchronized mass cultures of *Tetrahymena pyriformis* GL by hybridization. J. Protozool., 17, 496–501.

Christensson E. G. 1971 a: The heterogeneity of RNA in cellular fractions from synchronized mass cultures of *Tetrahymena pyriformis* GL. Z. Biol., 116, 413–428.

Christensson E. G. 1971 b: RNA metabolism during cell growth and cell division in synchronized mass cultures of *Tetrahymena pyriformis* GL. (Ph. Dr. Thesis).

Christensson E. G. 1971 c: VIIth Meeting of the Nordic Soc. for Cell Biol. Expl Cell Res., 67, 244.

Christiansen E. N. and Kvamme E. 1969: Effects of thermal treatment on mitochondria of brain, liver and ascites cells. Acta physiol. scand., 76, 472–484.

Conner R. L. and Linden C. 1970: Purine and pyrimidine 5'-nucleotide catabolism in *Tetrahymena pyriforms* W. J. Protozool., 17, 659–662.
Elliot A. M., Kennedy J. R. and Bak J. L. 1962: Macronuclear events in synchronously dividing *Tetrahymena pyriformis*. J. Cell Biol., 515–531.
Frankel J. 1965: The effects of anatgonists of nucleic acid and protein synthesis on cell division and oral morphogenesis in synchronized *Tetrahymena pyriformis* GL. J. exp. Zool., 159, 113–148.
Harris H. 1968: Nucleus and Cytoplasm, (ed. Harris H.) Clarendon Press, Oxford.
Hartman H. and Dowden R. M. 1970: Polyribosome profiles from heat synchronized *Tetrahymena*. Biochem. biophys. Res. Commun., 40, 964–967.
Hjelm K. K. and Zeuthen E. 1967: Synchronous DNA-synthesis induced by synchronous cell division in *Tetrahymena*. C. r. Trav. Lab. Carlsberg, 36, 127–160.
Kidder G. W. and Dewey V. C. 1951: The biochemistry of ciliates in pure culture. In: Biochemistry and Physiology of *Protozoa*, (ed. Lwoff A.) 1, New York.
Kirby K. S. 1965: Isolation and characterization of ribosomal acid. Biochem. J., 96, 266–269.
Lazarus L. H., Levy M. R. and Scherbaum O. H. 1964: Interaction of synchronized cell division in *Tetrahymena* by actinomycin D. Expl Cell Res., 36, 672–675.
Lyttleton J. W. 1963: A simple method of isolating ribosomes from *Tetrahymena pyriformis*. Expl Cell Res., 31. 385–389,
Merriam R. M. 1969: Movement of cytoplasmic proteins into nuclei induced to enlarge and initiate DNA or RNA synthesis. J. Cell Sci., 333–349.
Moner J. G. 1965: RNA synthesis and cell division in heat-synchronized populations of *Tetrahymena pyriformis*. J. Protozool., 12, 505–509.
Moner J. G. 1967: Temperature RNA synthesis and cell division in heat-synchronized cells of *Tetrahymena*. Expl Cell Res., 45, 618–630.
Munk N. and Rosenberg H. 1969: On the deposition and utilization of inorganic pyrophosphate in *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 177, 629–640.
Nilsson J. R. and Leick V. 1971: Nucleolar organization and ribosome formation in *Tetrahymena pyriformis* GL. Expl Cell Res., 60, 361–372.
Perry R. P. and Kelly D. E. 1971: Inhibition of RNA synthesis by actinomycin D characteristic dose-response of different RNA species. J. cell Physiol., 76, 127–140.
Peacock A. C. and Dingman C. W. 1967: Resolution of multiple ribonucleic acid species by polyacrylamide gel electrophoresis. Biochemistry, 6, 1818–1827.
Simard R., Amalric F. and Zalta J. P. 1969: Effect de la temperature supra-optimale sur les ribonucleoproteins et le RNA nucleolaire. Expl Cell Res., 55, 359-377.
Scherbaum O. and Zeuthen E. 1954: Induction of synchronous cell division in mass cultures of *Tetrahymena pyriformis*. Expl Cell Res., 6, 221–227.
Stenram U. 1956: Nucleolar size in the liver of rats fed diets deficient in essential amino acids. Acta path. microbiol. scand., 38, 364–374.
Stenram U. and Nordgren H. 1970: Ultrastructural organization and RNA turnover of hepatic ribosomes in protein-fed and protein-deprived rats. Cytobios., 2, 265–275.
Warocquier R. and Scherer K. 1969: RNA metabolism in mammalian cells at elevated temperature. Eur. J. Biochem., 10, 362–370.
Zeuthen E. and Rasmussen L. 1971: Synchronized cell division in *Protozoa*. In: Research in Protozoology, (ed. Chen T. T./, 4, Pergamon Press, New York.

Received 3 December 1973

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA 15. X. 1975 FASC. 23

Department of Biological Sciences, Fairleigh Dickinson University, Teaneck, New Jersey, USA

Bonnie K. LUSTIGMAN and Irwin R. ISQUITH

The Enhanced Lethality of *Paramecium* in Dyes under the Influence of Magnetic Fields

Steigende Sterblichkeit der *Paramecium* in Farbstoffen unter den Einfluss von magnetischen Feldern

The alteration of cell permeability in a magnetic field has been the subject of recent research. Dubrov (1971) suggested that the permeability of biological membranes is controlled by geomagnetic fields. Pumper and Barnothy (1969) found that cell cultures of rabbit myocardium and mouse lung fibroblast stained more intensely than non-magnetically treated controls. Using electromagnetic fields up to 15 000 Oe, Rostkowska and Moskwa (1968) found increased susceptibility to cytotoxins in *Spirostomum*. On the other hand, Isquith and Bobrow (1973) found decreased permeability to water and alkane alcohols in *Paramecium caudatum*. This decrease was greatest for the most polar molecule. Brandsch and Jitariu (1970) treated *Tubifex* eggs with colchicine and theophylline and found that an electromagnetic field caused an increase in the first divisions of these eggs. This would indicate a decreased entrance of the chemicals into the eggs. There thus appears to be a diversity of results in the effect of magnetic fields on cellular permeability.

The current work was undertaken to clarify the possible alteration in permeability brought about by a magnetic field. Since prior studies had indicated that pretreatment could have an influence on organisms (Isquith and Bobrow 1973), it was hoped that applying a magnetic pretreatment could elucidate the type of alteration incurred. As paramecia have membranes comparable to metazoans, yet allow for the entire organism to be exposed to the magnetic field, they were selected as the experimental organisms. Vital dye penetrance was selected as it would yield two distinct endpoints: (1) an alteration in the rate of lethal effects and (2) differences in the amount of cytoplasmic staining.

Materials and Methods

Paramecium multimicronucleatum was cultured in 0.1% sterilized and filtered cerophyl, enriched with two wheat grains per 30 ml culture vessel. Subcultures were made every five to seven days. Cultures were grown at a temperature of 21°C ± 2. Chalkley's solution (Chalkley 1930) was used as an experimental solution. Listed below is the final concentration of the dyes employed:

Brilliant Cresyl Blue (BCB) 1 : 2000, Basic Fuchsin (BF) 1 : 20 000, Safranin (S) 1 : 8000, Janus Green B (JGB) 1 : 1000 and 1 : 10 000.

These particular vital stains were selected as they are toxic to non-magnetically treated controls in 20–30 min.

Fifteen to thirty paramecia were removed from the cerophyl cultures, washed in Chalkley's solution and transferred to a depression plate. This was placed at varying distances from the center of the magnetic field (maximum strength 5900 gauss) on the same plane as the face of the south pole. The desired distances were determined by measuring the field strength with a gauss meter at the center of the depression, which was used as the experimental vessel. For each dye the following field strengths were employed: 4800, 1700, 1000 or 750 gauss. In addition 2400, 1200 or 400 gauss was utilized with BCB. Depending on the experiment, the slide was kept in the magnetic field for 5 to 90 min. The magnetic field was produced by a permanent magnet of a rams-horn configuration.

Control specimens were washed and placed in another three depression plate in the exact same manner for the same period of time. They were at ambient magnetic field, approximately 0.5 gauss for the entire experiment.

Immediately after the prescribed time, the organisms were removed from the magnet and mixed with a volume of dye solution equal to the volume of Chalkley's solution in which they were transferred. The specimens were microscopically observed until lethality was achieved. The controls were also given the dye regimen and similarly observed. Comparison was made between the color intensity of cytoplasm of control and experimental organisms, as well as lethality times.

The data are presented in terms of gauss-minutes. This represents a unit of dosage achieved by multiplying the magnetic treatment time by the gauss employed. The minimum effective dosage (m.e.d.) is the level needed to be reached for physiological effect to become apparent.

In recovery studies, the paramecia were subjected to a 1700 gauss magnetic field for thirty minutes, and then removed from the field. At 12 h, 18 h, 24 h, or 48 h, they were transferred to the BCB regimen and the above protocol followed. Controls were similarly treated except that they did not have the magnetic treatment.

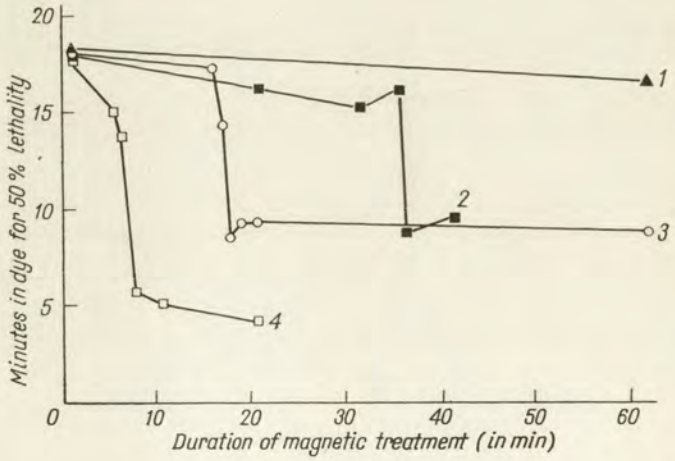


Fig. 1. The effect of increase in magnetic treatment on the time for 50% lethality in Basic Fuchsin. 1 : 20 000. Similar graphs are obtained for BCB and Safranin. 1 — 750 gauss, 2 — 1000 gauss, 3 — 1700 gauss, 4 — 4800 gauss

Results

There is enhanced lethality of *Paramecium multimicronucleatum* under the influence of magnetic fields in the basic dyes Brilliant Cresyl Blue, Basic Fuchsin and Safranin, but not Janus Green B.

Paramecia exposed to a field of sufficient magnetic intensity for the necessary time span, show a marked decrease in the time for 50% lethality in the first three dyes (Fig. 1, Tables 1–3). This occurs abruptly at a value (gauss × minutes) which is similar for each dye (the gauss-minute m.e.d.). Comparable mean m.e.d.'s for the three dyes are: ca. 35 000, 38 000 and 33 000 for BCB, S and BF respectively. If the dosage is above the m.e.d., a sharp alteration occurs in lethality time, within the range of 1–2 min in each field strength and each dye.

A minimum level above 750 gauss is necessary in order to achieve a m.e.d., e.g., 90 min exposure time at 750 gauss shows no marked decrease in 50% lethality time in the experimental as compared with the control group, even though the gauss-minute value is above the expected m.e.d. At higher field strengths, once the m.e.d. is attained, longer exposure does not cause increased alteration, e.g., a 20 min magnetic exposure at 1700 gauss produced the same 50% lethality time as 60 min exposure with Basic Fuchsin. See Fig. 1 and Tables 1–3.

The relationship between the gauss and the treatment time necessary to produce 50% lethality is presented in Fig. 2. The shape of the curve achieved is similar in each dye and represents a hyperbola, indicating the presence of a constant value.

Recovery studies were undertaken to determine if the magnetically induced alteration was permanent or temporary. After 12 h there was no recovery while after 18 h partial recovery had occurred and was completed by 24 (Table 4).

In order to rule out the possibility of magnetically increased phagocytosis causing increased dye entrance, as proposed by Kogan et al. (1968), the number and size of food vacuoles in a BCB regimen of both magnetically treated and non-treated organisms was compared. The results show a mean of 4.6 vacuoles per organism in the control group of 50 organisms and 4.5 vacuoles per organism in the experimental group of 54 organisms. The range in number of vacuoles was between 1–9 vacuoles in the experimental and 2–8 in the controls. The size of the vacuoles was the same in both groups. This indicates no significant alteration in phagocytosis in the experimental protists.

In the above three dyes, there is pronounced swelling, fixation of the contractile vacuoles and deep cytoplasmic staining. This is followed by disruption of the pellicle. In the case of JGB, the above alterations do not occur. There is no deep staining of the cytoplasm, only a slow cessation of movement followed by death. Janus Green B does not produce any significant difference in the time for 50% lethality between non-treated controls and magnetically treated paramecia, e.g., a treatment of 192 000 gauss-minutes produces 50% lethality in 31 min as occurs with non-treated controls when the concentration of dye is 1 : 10 000. Similarly when the

concentration of dye 1 : 1000, 50% lethality is produced in 28.5 min after a 51 000 gauss-minute dosage and 29 min at 106 000 gauss-minutes.

Table 1

Effect of Magnetic Treatment on the Time for 50% Lethality with Brilliant Cresyl Blue, 1 : 2000

Field Strength (Gauss)	Magnet Treatment time (min)	Gauss-Minutes (10^3)	Average time for 50% lethality (min)	No. of organisms
0	0	0	17±1.1	109
400	20	8.0	14.5±1.6	84
	60	24.0	14.75±1.1	63
750	20	15.0	15.5±1.2	54
	45	33.7	15.0±1.6	57
	60	45.0	14.3±1.7	59
1000	30	30.0	14.0±1.2	79
	35	35.0	13.0±1.4	65
	37*	37.0	9.3±1.3	63
	40	40.0	9.0±0.7	69
	45	45.0	9.25±0.9	78
1200	20	24.0	13.0±0.7	72
	25	30.0	13.25±0.9	70
	28	33.6	12.67±0.9	60
	30*	36.0	7.66±1.5	62
	35	42.0	8.0±0.8	56
1700	10	17.0	14.60±1.4	80
	15	25.5	13.67±1.4	107
	17	28.9	13.8±1.6	115
	18*	30.6	7.67±0.9	99
	20	34.0	8.2±1.0	109
	30	51.0	7.3±1.3	84
2400	10	24.0	14.25±1.1	68
	13	31.2	13.75±0.8	72
	15*	36.0	9.0±0.7	71
	30	72.0	7.5±0.7	65
4800	5	24.0	10.8±1.0	69
	7*	33.6	8.5±0.7	84
	10	48.0	6.05±0.6	78
	20	96.0	6.25±1.1	64
	25	120.0	5.6±0.4	79

* Represents minimum effective dosage (gauss-minutes).

Table 2

Effect of Magnetic Treatment on the Time for 50% Lethality with Safranin, 1 : 8000

Field strength (Gauss)	Magnet treatment time (min)	Gauss-minutes (10^3)	Average time for 50% lethality (min)	No. of organisms
0	0	0	18.5±2.2	92
750	90	67.5	16.5±1.2	69
1000	30	30.0	15.3±.47	68
	40	40.0	13.66±.47	100
	41	41.0	10.66±.94	67
	42*	42.0	8.5±0.7	68
	45	45.0	7.3±0.8	77
1700	20	34.0	15.0±0.8	64
	22	37.4	15.0±1.2	70
	23*	39.1	7.0±0.8	61
	25	42.5	7.66±.47	69
4800	5	24.0	13.33±.43	64
	7*	33.6	7.66±.47	68
	10	48.0	7.66±.97	75
	20	96.0	6.00±.82	78

* Represents minimum effective dosage (gauss-minutes).

Discussion

The cytoplasm of living cells is stained by the vital dyes Brilliant Cresyl Blue, Basic Fuchsin and Safranin. If organisms are magnetically treated and then exposed to a dye, they develop a more intense color than do controls. It therefore seems that the magnetic field produces an alteration in dye penetrance. This would be in agreement with the findings of Rostkowska and Moskwa (1968) and Pumper and Barnothy (1969) who also found increased entrance of certain molecules in magnetically treated cells.

Although 50% lethality of a paramecium population in the dye regimen was the end point employed in these experiments, this was presumably a secondary effect brought about by increased entrance of the dyes; it was used as an assay for the magnetic effect. Gross' model (1964) of bond angle alteration may in part explain the observed results. The bond angles of the molecules involved in transporting or binding with the dye were altered so that the molecules' affinity for and combination with the dye were increased.

Table 3

Effect of Magnetic Treatment on the Time for 50% Lethality with Basic Fuchsin, 1 : 20 000

Field strength (Gauss)	Magnet treeatment tim (min)	Gauss-minutes (10^3)	Average time for 50% Lethality (min)	No. of organisms
0	0	0	18.5±1.5	69
750	60	45.0	16.66±1.3	66
1000	20	20.0	17.0±1.4	68
	30	30.0	16.0±.47	85
	34	34.0	17.0±.81	81
	35*	35.0	9.66±.82	97
	40	40.0	10.33±.47	79
1700	15	25.5	18.0±1.3	63
	16	27.2	15.3±0.5	94
	17*	28.9	9.6±0.5	69
	18	30.6	10.2±1.2	74
	20	34.0	10.3±1.2	68
	60	102.0	10.0±.82	62
4800	5	24.0	16.0±.81	76
	6	28.8	14.75±1.3	70
	7*	33.6	6.66±0.5	68
	10	48.0	6.1±.93	85
	20	96.0	5.1±.58	72

* Represents minimum effective dosage (gauss-minutes).

The possibility that the magnetic field produced an alteration in selected enzymes of the protoplasm, which in turn caused death, regardless of the amount of dye was considered. However, the darker coloration of the cytoplasm in the treated organisms eliminates this possibility, since clearly more dye entered the treated organisms.

A pretreatment protocol was employed, so there could not be an alteration of the dye molecules in the magnetic field. The organisms were exposed to the magnetic field before they were transferred to the dye solution. Paramecia which were exposed to the magnetic field but not to the dye remained viable. Therefore, the magnetic force itself did not bring about lethality.

The type of death found in organisms exposed to the dyes BCB, BF and S is also caused by many other treatments (Wichterman 1953). There is vacuolization and decrease in ciliary activity, followed by pellicular destruction. It is indicative of total organismic disintegration.

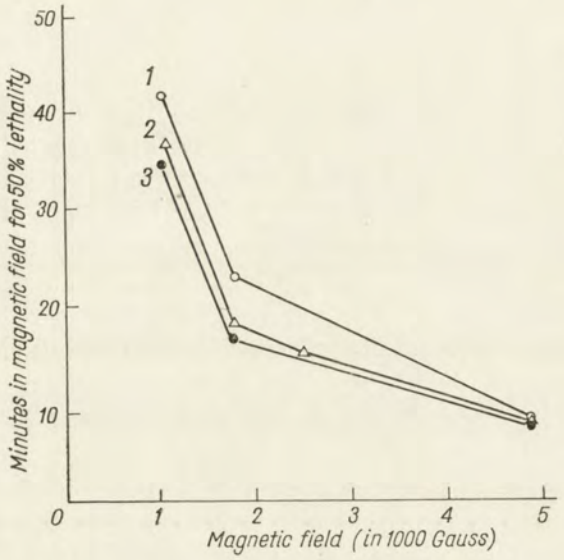


Fig. 2. Comparison of the minimum effective dosage in BCB, BF, and S in terms of treatment time and magnetic field strength. 1 — BF, 2 — BCB, 3 — S

Table 4

Recovery from 30 min Magnetic Treatment at 1700 Gauss As Determined by Brilliant Cresyl Blue Regimen

Post magnetic time (h)	Time for 50% lethality (min)	No. of organisms
0	8±1.1	109
12	8.5±.5	53
18	11.0±1.0	54
24	14.5±1.0	46
48	16.5±0.5	78

There is considerable conjecture as to the mode of molecular penetration into a cellular system. One group of investigators gives primary importance to the semi-permeable nature of the plasma membrane. Chapman-Andresen (1967 a, 1967 b, 1970) feels that dyes bind to the outer surface of a membrane and then enter pinocytotically. Troshin (1966), however, downgrades the significance of the membrane as a determinant for what enters into the cytoplasm. In this opinion, the membrane is freely permeable to molecules such as dyes; the quantity of the substance that enters is determined by the "physico-chemical state" of the protoplasm. Ling et al. (1973) claim importance for cytoplasmic adsorption in his association-induction hypothesis. Green (1972) presents an integrated approach. He feels that there is an interaction between membrane and its contained cytoplasm.

There is a mutual feedback between the two; alteration of one produces a concommitant change in the other. The net entrance of molecules into the system is determined by the physiological state of both cellular components. It is perhaps the combination of adsorption to endoplasmic protein and the transport through the membrane which causes the slight decrease in time for 50% lethality prior to attainment of the m.e.d. that is found in Fig. 1. Since there is such controversy as to the mechanism of dye entrance into a cell, it is beyond the scope of this investigation to state specifically what is the magnetic mechanism of penetrance alteration. Any of the above described penetrance mechanisms could be affected by some of the molecular alterations that have previously been attributed to magnetic fields.

Magnetically treated paramecia exposed to Janus Green B did not show a magnetic effect. This dye selectively binds with mitochondria and destroys their activity (Korde et al. 1971). The failure of mitochondria to respond to the magnetic field might be due to a lack of magnetic effects of the inner mitochondrial membranes which differ in structure from plasma membrane (Crane and Hall 1972). It is therefore conceivable that while more JGB molecules may have entered the cytoplasm, no additional dye was bound to the mitochondria and therefore there was no observable effect.

From this study, it is apparent that there is a m.e.d. which can be expressed in gauss-minutes below which there is no major effect and above which little additional change occurs. The mean m.e.d. necessary was found to be comparable in the three dyes. Since they all were between 30 000–40 000 gauss-minutes, it would indicate a similar lethal mechanism affecting the same subcellular component in each case. It would also indicate that the three dyes enter the cell by a similar mechanism.

The recovery studies with paramecia and BCB indicate that while the magnetically induced alteration persists after removal from the magnetic field, within 18 h appreciable recovery occurs and is complete by 24 h. Perhaps, the most logical view of the alteration involved is adapted from Green (1972). His proposal that any change in the membrane would then cause a change in the associated protein network. Both membranes and cytoplasm would be influenced by the magnetic field. The negative charge on the receptor sites could be increased and the basophilic apparatus of the cell also enhanced. The amount of dye entering the organism would therefore be determined by both the membrane which transports the dye and the endoplasmic protein with which it binds.

Summary

Paramecium multimicronucleatum was subjected to magnetic fields of 750, 1000, 1700, or 4800 gauss, for varying periods of time. After the magnetic treatment, the organisms were transferred to a solution of the one of the following basic dyes:

1 : 2000 Brilliant Cresyl Blue, 1 : 20 000 Basic Fucshin, 1 : 8000 Safranin Red, or 1 : 1000 or 1 : 10 000 Janus Green B.

For the first three dyes, it was observed that the time for 50% lethality of a population was significantly reduced, if the magnetic treatment dosages (gauss × minutes) exceeded specific values. The minimum effective values were: Brilliant Cresyl Tlue, 35 000; Basic Fuchsin, 33 000; and Safranin Red, 38 000 gauss-minutes. Bhere was no increased effect, if the applied magnetic regimen was greater than these minimum dosages. To obtain these values a minimum field strength above 750 gauss had to be employed. From these results it must be assumed that the enhanced lethality is due to increased entrance of dye into the organism brought about by the magnetic field.

Janus Green B did not display enhanced lethality to organisms that had been magnetically treated. This may be due to its specific affinity for mitochondria and contrasted to the more general chemical binding of the other dyes. For a magnetic field to influence the lethality of Janus Green B, there might have to be an alteration of mitochondrial membranes. If no such alteration was induced magnetically, the field would not change the lethal qualities of the dye. This seems to be the case.

ZUSAMMENFASSUNG

Paramecium multimicronucleatum ist magnetischen Feldern von 750, 1000, 1700 oder 4800 Gauss über verschiedene Zeitleangen ausgesetzt worden. Nach der magnetischen Behandlung sind die Organismen in eine Loesung einer der folgenden basischen Farbstoffe übertragen worden: 1 : 2000 Glaenzendes Cresyl Blau, 1 : 20 000 basisches Fuchsin, 1 : 8000 Safran Rot, oder 1 : 1000 oder 1 : 10 000 Janus Grün B.

Im Falle der ersten drei Farbstoffe konnte man beobachten dass die abgelaufene Zeit für 50% der Todesfaelle einer Population bedeutend verringert wurde, wenn die magnetischen Behandlungsmengen (Gauss × Minuten) bestimmte Werte überschritt. Die wirkbaren Mindestwerte waren: Galenzendes Cresyl Blau, 35 000; Basisches Fuchsin, 33 000, und Safran Rot, 38 000 Gauss-Minuten. Die Einwirkung war nicht vergroessert wenn die angewandte magnetische Quantitaet groesser als diese Mindestwerte war. Eine Mindestfeldstaerke von über 750 Gauss musste benuetzt werden um obige Werte zu erzielen. Von diesen Ergebnissen muss angenommen werden, dass die erhoehte Todesanzahl durch vergroessertes Eindringen von Farbstoffen in den Organismus zufolge des magnetischen Feldes angebracht wird.

Janus Grün B zeigte keine vergroesserte Todeszahl in Organismen die magnetisch behandelt wurden. Das kann verursacht sein durch die spezifische Affinitaet für mitochondria, im Gegensatz zu der mehr allgemeinen chemischen Binding der anderen Farben Molekuele. Man müsste die mitochondrial Membranen aendern, sodass ein magnetisches Feld die Todeswirkung von Janus Grün B beeinflusst. Wenn keine solche Aenderung magnetisch vorgenommen wurde, hat das Feld die toetlichen Qualitaeten der Farbe nicht geaendert. Das scheint der Fall zu sein.

REFERENCES

Brandsch R. and Jitariu P. 1970: Influences of colchicine and theophylline combined with an electromagnetic field on the first divisions of tubifex eggs. Rev. roum. Biol. Ser. Zool., 16, 215–220.

Chalkley H. 1930: Resistance of *Paramecium* to heat as affected by changes in hydrogen ion concentration and in inorganic salt balance in surrounding medium. US Public Health Serv. Public Health Dept., 45, 481–489.

Chapman-Andresen C. 1967 a: Studies on endocytosis in *Amoeba*. The distribution of pinocytotically ingested dyes in relation to food vacuoles in *Chaos chaos* I. Light microscope observation. C. r. Trav. Lab. Carlsberg, 36, 161–187.

Chapman-Andresen C. 1967 b: Studies on endocytosis in *Amoeba*. The distribution of pinocytotically ingested dyes in relation to food vacuoles in *Chaos chaos* II. E. M. observations using Alcian Blue. C. r. Trav. Lab. Carlsberg. 36, 189–207,

Chapman Andresen C. 1970: Uptake of ferritin by *Chaos chaos*. C. r. Trav. Lab. Carlsberg, 38, 19–57.

Crane F. and Hall J. 1972: Diversity in membrane structure from unit to binary. In: Membrane Structure and its Biological Applications, (ed. Green D.), Ann. N. Y. Acad. Sci., 195, 24–35.

Dubrov A. 1971: Relationship between the radiosensitivity of animals and the geomagnetic field. Radiobiologia, 11, 613–619.

Green D. 1972: Membrane proteins: A perspective. In: Membrane Structure and its Biological Applications, (ed. Green D.), Ann. N. Y. Acad. Sci., 195, 150–179.

Gross L. 1964: Distortion of the bond angle in a magnetic field and its possible magnetobiologicas implications. In: Biological Effects of Magnetic Fields, (ed. Barnothy M.), Plenum Press New York, 74–80.

Isquith I. and Bobrow A. 1973: The effect of a high intensity magnetic field on the entrance of various molecules into *Paramecium caudatum*. Acta Protozool., 12, 125–131.

Kogan A., Dorozhkina L. and Volynskay E. 1968: The influence of static magnetic field on the phagocytic activity of *Paramecia*. Cytologia, 10, 1342–1348.

Korde T., Baba K., Watanabe K., Ikeda M. and Shimosato Y. 1971: Mitochondrial injury, produced by Janus Green B, Enzyme, morphological and ultrastructural observations. Acta histochem. cytochem., 4, 137–152.

Ling G., Miller C. and Ochsenfeld M. 1973: The physical state of solutes and water in living cells according to the association induction hypothesis. In: Physicochemical State of Ions and Water in Living Tissues and Model Systems, (ed. Hazlewood C.) Ann. N. Y. Acad. Sci., 204, 6–51.

Pumper R. and Barnothy J. 1969: The effect of strong inhomogeneous magnetic fields on serum-free cell cultures. In: Biological Effects of Magnetic Fields. II, (ed. Barnothy M.) Plenum Press, New York, 61–67.

Rostkowska J. and Moskwa W. 1968: The influence of magnetic fields on susceptibility for toxic compounds in *Spirostomum ambiguum*. Acta Protozool., 5, 305–313.

Troschin A. 1966: Problems of Cell Permeability. Pergamon Press, London, 181–218.

Wichterman R. 1953: The Biology of *Paramecium*, Blakiston Co. Inc., New York.

Received 4 October 1973

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 24

Department of Cell Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093 Warszawa Pasteura 3, Poland

Krystyna BUJWID-ĆWIK and Stanisław DRYL

Effect of Sodium Dodecyl Sulphate on Motor Responses of *Paramecium caudatum* Induced by K^+Ions

Efekt działania siarczanu dodecylo-sodowego na reakcje ruchowe *Paramecium caudatum* pod wpływem jonów potasu

Among the few papers devoted to the effect of detergents on protozoa most concern their toxic action. Materials concerning motor responses of protozoa, caused or modified by surface active substances are fragmentary and most frequently refer to other species than *Paramecium*.

According to Chaix and Baud (1947), in *Tetrahymena gelei*, the lysis induced by 10^{-4} mM sodium linolate is preceded by gradual decrease of motile activity of the protozoan, rotation of the body around its axis and immobilization. The shape of the body is first pear-like, then spherical. In spite of the immobilization cilia work in normal way, even in fragments formed after burst of the membrane.

Rockstroh (1967) too, has observed a gradual slackening of movement in the ciliate *Cyrtolophosis* sp. treated with toxic concentrations of 3.5×10^{-2} mM sodium dodecyl sulphate (SDS) and spherical transformation accompanied by rotation around the longitudinal axis of the body. Later there occurred detachment of cilia and visible damages of the cell membrane.

Bailenger and Troadec (1953) and Bailenger et al. 1953 have determined the concentration of a number of cationic and anionic detergents causing the immobilization of some protozoa like *Tetrahymena pyriformis*, *Euglena gracilis*, *Balantidium coli* and *Entamoeba invadens*. Those authors have pointed out that cationic detergents acting in lower concentration had a higher biological activity causing immobilization, inhibition of fission, cell lysis. The authors' opinion was that this activity is mainly due to the positive charge of the substances. A great part is also played by the value of the adsorption constant of the particular detergents. Among the investigated anionic detergents SDS displayed gretaest activity and showed highest adsorption constant. It caused the immobilization of *Tetrahymena pyriformis* in concentration 1 : 20 000 g/ml.

Differences in effect of catonic and anionic detergents on *Amoeba proteus* have

been also established by Brewer and Bell (1969 a, b). CTAB lowered the excitability threshold of *Paramecium caudatum* to stimulation with electric current while SDS was inefficient (Butzel et al. 1960).

In their previous papers the present authors showed that not only lethal but also sublethal concentration of detergents STAN, SDS, Triton x 100, Tween 80 induced in protozoa motor responses other than slackening of motion. *Paramecium caudatum*, *Paramecium aurelia*, *Stylonychia mytilus*, *Spirostomum ambiguum*, *Stentor coeruleus* and *Stentor polymorphus*, placed in a detergent solution, display either short continuous ciliary reversal or periodic ciliary reversal. At the same time the authors proved that in *Paramecium caudatum*, the cationic detergent CTAB caused, depending on the concentration, complete or partly suppression of ciliary reversal response to strong chemotactic and chemokinetic stimuli in the external environment. The phenomenon of cell excitability changes caused by CTAB is reversible. It disappears gradually when the animals were washed off in a solution without detergent (Bujwid-Ćwik and Dryl 1971, Dryl and Bujwid-Ćwik 1972 a, b).

Preliminary observation has shown that anionic detergents SDS sodium dodecyl benzenosulphonate produce an opposite effect, i. e., they prolongate the duration of ciliary reversal induced by K^+ ions.

The aim of the present paper was a more detailed investigation of motor responses of *Paramecium caudatum* caused by various concentrations of sodium dodecyl sulphate and the effect of the detergent on the excitability of this ciliate revealed in the duration and character of the ciliary reversal induced by K^+ ions.

Material and Methods

The experiments were carried out on *Paramecium caudatum* which was cultivated in lettuce medium with *Aerobacter aerogenes*, according to Sonneborn's (1950) method. 18–24 h before starting the experiment paramecia were geotactically concentrated and washed with 1 mM Tris-HCl+1 mM$CaCl_2$ buffer solution of pH 7.4 so that the original medium was diluted over 1000 times. All chemical agents used in the experiments (KCl, $BaCl_2$, SDS, KCl+SDS) were diluted with the same buffer solution. Only the initial 35 mM (1% w/v) solution of sodium dodecyl sulphate (SDS) was prepared with distilled water. The solutions were prepared in concentration twice as high as necessary and during the experiment they were mixed in proportion 1 : 1 with a dense sample of protozoa from the equilibration medium.

Observation of the behaviour of *Paramecium* in the tested solutions, measurements of the duration of continuous ciliary reversal (CCR), partial ciliary reversal (PaCR) and periodic ciliary reversal (PCR) and washing off of the animals treated with SDS were performed in the way described earlier (Dryl and Bujwid-Ćwik 1972 a, b). Observations and calculations were based on 10 series of experiments. Usually each sample contained the same number of protozoa: 30–40 animals and in the samples collected during washing 15–20 animals. The experiments were carried out at room temperature — ca. 20°C.

Results and Discussion

Treated with the anionic detergent — sodium dodecyl sulphate (SDS) — *Paramecium caudatum* shows changes in the character of its movement; there also occurs a deformation of body shape and in higher concentrations — a fast lysis of the whole cell.

It results from the data presented in Table 1 that the kind of motor response

Table 1

Effect of Various Concentrations of Sodium Dodecyl Sulphate (SDS) on *Paramecium caudatum*

SDS concentration (mM)	Motile behaviour of *Paramecium caudatum*		Shape of body
	during 1 min of exposure	after 1 min to 25 min of exposure	
3.5	instant immobilization		cigar-like
1.8	,, ,,		,, ,,
7×10^{-2}	30 sec CCR or turning	immobilization after 1–3 min	pear-like; after 2–3 min cigar-like
35×10^{-2}	30 sec fast normal movement, spinning, slow movement	after 90–100 sec very slow CCR then turning and immobilization in 4–8 min. Some become normal before immobilization	,, ,,
18×10^{-2}	10 sec CCR then fast turning onward	after 2–3 min "spinning", slow movement passing in some to CCR, immobilization after 7–13 min	pear-like, after 2–3 min cigar-like
10×10^{-2}	5–10 sec CCR	after 6–8 min slowing down movement and "spinning", immobilization after 13–15 min	
3.5×10^{-2}	60–80 sec PCR	"Sporadic reversals" up to 3 min after 20 min slowing down and "spinning"	after 10 min slight swelling of front part of body after 20 min pear-like deformation of body
1.8×10^{-2}	20–60 sec PCR	"Sporadic reversals" up to 3 min	no change
3.5×10^{-3}	15–20 sec PCR	"Sporadic reversals" up to 1.5 min	,, ,,
1.8×10^{-3}	"Sporadic reversals" up to 60 sec	no change	,, ,,
3.5×10^{-4}	"Sporadic reversals" up to 40 sec	no change	,, ,,

depends on the concentration of the detergent. As the animals are kept longer in the SDS solution further changes in their movements occur.

In toxic concentration (over 1.8 mM SDS) there occurs instant cessation of movement due to the stopping of work of cilia. The length of the animal is shortened and the transverse axis is elongated so that the ciliate becomes cigar-shaped. Fast dissolution of the cell membrane (after 60–180 sec) and complete disintegration of the cell follow.

In lower toxic concentration (between 70×10^{-2} and 10×10^{-2} mM SDS) there appears, more and more distinctly, the first most response of very short CCR type occurring immediately after dipping the animal in the detergent solution, and further changes of movement: slackening of the normal quick forward movement and the appearance of "spinning"; cessation of forward movement — the animal only pivots around its longitudinal axis; the appearance of continuous reversal. Stopping of the cilia work and complete immobilization (death) of the animal occurs after several or less than 20 min, depending on the concentration of SDS. *Paramecium caudatum* treated with 3.5×10^{-2}–3.5×10^{-3} mM SDS shows PCR during the first ten seconds and up to several minutes; then the phase of forward movement becomes ever longer, the backward movement lasts shorter and the animal shows a response called "sporadic ciliary response" — the normal forward movement is interrupted, the animal stops, turns around once or several times, then swims on in the same direction.

Sporadic reversals lasting 30 to 60 sec are the only visible response of the ciliate to 3.5×10^{-4}–1.8×10^{-3} mM SDS.

In concentrations over 1.8×10^{-2} mM SDS causes deformation of the *Paramecium* body. The character of this deformation and the time of its appearance depend on the concentration of the detergent; in lower concentration there only occurs, after some time, a contraction of the ectoplasm which causes shortening of the longitudinal axis of the body, narrowing of the back part and swelling of the front part of the animal.

On the basis of the above observations it might be assumed that SDS causes two kinds of motor responses in *Paramecium caudatum*: CCR, or PCR occurring immediately after treating the animals with the detergent solution, and secondary changes of movement, preceding lysis, caused by damage of cell structures — occurring only in toxic concentrations.

Subtoxic concentration of SDS induce, in *Paramecium caudatum*, not only definite motor responses but, according to previous preliminary observations of the authors, this detergent prolongates the duration of reversal induced by KCl in the external medium. According to Kamada and Kinosita (1940) and Jahn (1962) — the duration of the reversal may be the measure of the excitability of this ciliate.

In order to investigate the influence of SDS on the excitability of *Paramecium caudatum* the authors measured the duration of CCR and PCR induced by 5 mM KCl + 1mM $CaCl_2$ in animals adapted during 3 min to different concentration

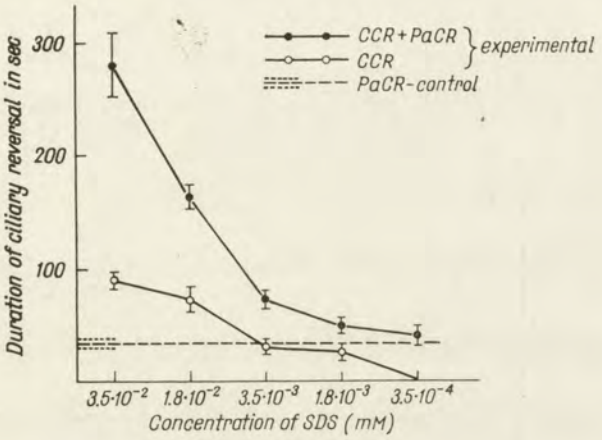


Fig. 1. Responses of *Paramecium caudatum* adapted during 3 min to various concentrations of SDS added to 5 mM KCl in 1 mM Tris-HCl + mM $CaCl_2$ (pH 7.4) buffer

of SDS and in control animals not treated with the detergent (Fig. 1). The KCl solution used to induce reversal in the tested animals contained the same concentration of SDS as in the adaptation medium.

The control animals reacted to 5 mM KCl + 1mM $CaCl_2$ only by a PaCR lasting $\pm$ 34 sec. Ciliates adapted to SDS concentration over 3.5×10^{-4} mM displayed CCR, also the duration of the reversal was longer when the SDS concentration rose in the medium.

In the next series of experiments it was established what was the duration of increased excitability in *Paramecium* caused by SDS after this substance had been removed from the outer medium. A method previously elaborated by the authors was used to this purpose. It consisted in washing off the animals with buffer 1 mM Tris HCl + 1 mM $CaCl_2$, pH 7.4

The three-minutes adaptation of *Paramecium caudatum* was carried out in $3.5 \times \times 10^{-2}$ mM solution of SDS. A toxic concentration of SDS was used after 30–50 min in order to create such conditions in which the recovery by the animals of their normal state would exclude accounting for the change of excitability level by the irreversible destruction of the membrane or of other cell structures. It results from the data obtained that the increase of cell excitability caused by SDS is a thoroughly reversible phenomenon (Fig. 2). As the animals are dipped for some time in a buffer solution without SDS, the duration of the reversal induced by 5 mM KCl decreased gradually and after 32 min reached control values. After being washed off, Paramecia did not display morphological or physiological changes, so all pathological changes could be excluded and it could be assumed that in this case the action of the detergent on the cell membrane facilitated — in accordance with Eckert's hypothesis (1972) — the panage of external Ca^{2+} through the membrane

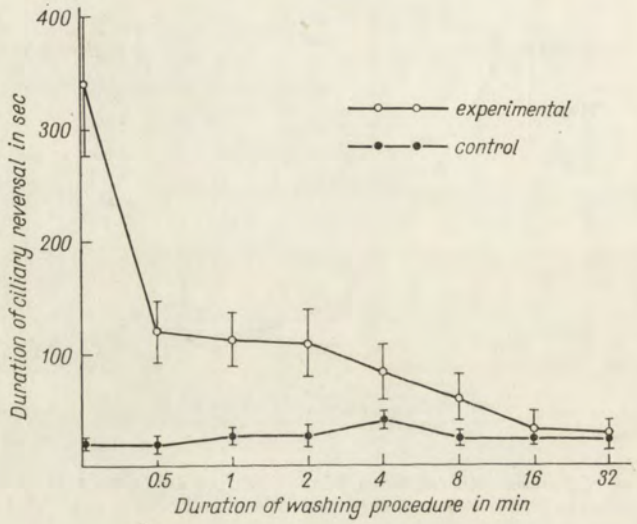


Fig. 2. Response of *Paramecium caudatum* to 5 mM KCl during washing off the animals of the solution containing 3.5 mM SDS

and its effect on the motor component of the cilia which was manifested in a longer lasting reversal of the ciliary movement.

Thus, in *Paramecium*, the cationic detergent CTAB has an inhibiting effect on the ciliary reversal induced by K^+ ions (Dryl and Bujwid-Ćwik 1972 a, b) whili SDS stimulates it as has been proved by the data obtained and presented in thəs paper. The blocking action of CTAB and SDS on the conduction of stimuli in the axons of the siatic nerve of *Rana pipiens* (Walsh and Lee 1962) was completelʎ

Table 2

Response of *Paramecium caudatum* to K/Ca and SDS

(A) Response of *P. caudatum* exposed to different KCl concentrations			(B) Response of *P. caudatum* exposed simultaneously to KCl and 3.5×10^{-3} mM SDS		(C) Response of *P. caudatum* to 3.5×10^{-3} mM SDS added after 15 min of exposure to KCl	
KCl concentration	Duration of reversal		Duration of reversal		Duration of reversal	
	CCR	CCR±PaCR	CCR	CCR±PaCR	CCR	CCR±PaCR
20	149±11	312±11	228±19	1746±307	–	7200*
10	46±5	88± 5	78± 5	146± 12	–	108±35
5	–	23± 4	10± 2	37± 6	–	24± 4
2.5	–	**	–	**	–	**

* Death.
** PaCR only.

or partly irreversible, hence it might be the result of damage in the nervous cell membrane.

Another proof of the rising sensitiveness of *Paramecium* to K^+ ions caused by SDS are the results of observations of protozoa treated with 5–15 mM KCl during 15 min and then additionally exposed to 3.5×10^{-3} mM concentration of SDS. In those conditions after exposure KCl there appeared a brief partial reversal of ciliary movement (lasting about 30 sec) followed by a gradual normalization of the movement so that after 15 min of treatment with KCl the ciliates were completely adapted to the medium containing KCl and manifested no disturbance of movement. As soon as SDS was added PaCR reappeared and its duration depended on the concentration of KCl in the medium as well as on that of the added SDS (Table 2).

Summary

Anionic detergent-sodium dodecyl sulphate (SDS), in concentrations over 3.5×10^{-2} mM causes in *Paramecium caudatum*, immobilization and cell lysis preceded by reversal of ciliary movement and pear-like or cigar-like deformation of body.

In sublethal concentration SDS induces short-lasting periodical ciliary reversal and causes prolongated duration of the reversal induced by K^+ ions in the external medium.

The phenomenon of increased excitability of *Paramecium caudatum* induced by SDS is completely reversible as was proved in experiments with animals washed off in a buffer solution without SDS.

In animals adapted to 5–20 mM KCl and exposed, after stopping of K^+ reversal, to 3.5×10^{-3} mM SDS, partial ciliary reversal (PaCR) reappears and its duration depends both on the concentration of K^+ ions and that of SDS.

STRESZCZENIE

Detergent anionowy – siarczan dodecylosodowy (SDS) w stężeniach powyżej 3.5×10^{-2} mM wywołuje u *Paramecium caudatum* immobilizację i lizę komórkową, poprzedzoną rewersją ruchu rzęskowego oraz zniekształceniem gruszkowatym lub cygarowatym ciała.

W stężeniach subletalnych SDS powoduje wystąpienie krótkotrwałej periodycznej rewersji rzęskowej oraz wpływa na wydłużenie czasu trwania rewersji wywołanej jonami K^+ w środowisku zewnętrznym.

Wywołane przez SDS zjawisko wzrostu pobudliwości *Paramecium caudatum* jest całkowicie odwracalne, jak wykazały eksperymenty z odpłukiwaniem pierwotniaków w roztworze buforowym bez SDS.

U pierwotniaków adaptowanych do 5–20 mM KCl podanie po zaniku rewersji potasowej 3.5×10^{-3} mM SDS powoduje ponowne pojawienie się parcjalnej rewersji rzęskowej (PaCR), której czas trwania zależy zarówno od stężenia jonów potasu, jak i SDS.

REFERENCES

Bailenger J. and Troadec J. 1953: Contribution a l'étude de la sensibilité des protozoaires á l'action des detergents. Étude cytologique. Archs. Sci. physiol., 7, 355.

Bailenger J., Canellas J. and Troadec J. 1953: Contribution á l'etude de la sensibilite des protozoaires à l'action des detergents. II. Essais d'interpretation. Archs Sci. physiol., 7/4, 365.

Brewer J. E. and Bell L. G. E. 1969 a: Reaction of detergents with *Amoeba proteus*. Nature, 22, 891.

Brewer J. E. and Bell L. G. 1969 b: Pseudopodium induction by the action of quaternary Ammonium ions on *Amoeba proteus*. J. Cell Sci., 4, 17–34.

Bujwid-Ćwik K. and Dryl S. 1971: The effects of detergents on motile behaviour of protozoa. J. Protozool., 18, 92.

Butzel H. M. Jr, Brown L. H. and Martin B. Jr. 1960: Effects of detergents upon electromigration of *Paramecium aurelia*. Physiol. Zool., 33, 39.

Chaix P. and Baud C. A. 1947: Etude de la lyse de *Glaucoma pyriformis* (*Tetrahymena gelei*) par l'acide linoleique et quelques autores acides gras. Archs Sci. physiol., 1, 3–9.

Dryl S. and Bujwid-Ćwik K. 1972 a: Effects of detergent cetyl trimethyl ammonium bromide on motor reactions of *Paramecium* to potasium/calcium factor in external medium. Bull. Acad. Pol. Sci. Cl. II.

Dryl S. and Bujwid-Ćwik K. 1972 b: Effects of detergents on excitability and motor response in protozoa. Acta Protozool., 11, 367–372.

Eckert R. 1972: Bioelectric control of ciliary activity in *Paramecium*. Science, 176, 472–481.

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

Kamada T. and Kinosita H. 1940: Calcium-potassium factor in ciliary reversal of *Paramecium*. Proc. imp. acad. Japan., 16, 125–130.

Kishimoto U. and Adelman W. J. 1964: Effect of detergent on electrical properties of squid axon membrane. J. Gen. Physiol., 47, 975–986.

Rockstroh T. 1967: Uber die Wirkung von Detergentien auf die Morphologie einer Ziliatenzelle. Acta biol. med. germ., 19, 161.

Walsh R. R. and Lee R. P. 1962: Action on surface-active agents on axonal conduction. Am. J. Physiol., 202, 1241–1243.

Wassano T., Ogata M. and Goto M. 1956: The effect of surface-active agents on the resting potential of muscle. Jap. J. Physiol., 6, 137–149.

Webb G. D. 1964: Effects of surfactants on the twitch tension of frog sartorius muscle. Am. J. Physiol., 207, 397.

Received 1 December 1973

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 25

Laboratory of Cell Adaptations and Exobiology, Institute of Cytology, Academy of Sciences of the USSR, Leningrad 190-121, Prospekt Maklina 32, USSR

V. N. BYCHENKOVA and L. K. LOZINA-LOZINSKY

Accumulation of Reserve Lipids in *Colpoda maupasi* in Normal Atmosphere, at Hypoxia and Increased Concentration of CO_2

Накопление резервных липидов у *Colpoda maupasi* в нормальной атмосфере, при гипоксии и повышенной концентрации CO_2

Alteration in the content of free lipids in the cytoplasm of protozoa are connected with changes in the intensity and direction of metabolic processes. Therefore cytochemical investigations of the distribution and amount of lipids in cells gives an indication of the nature of their metabolic processes.

The storage of neutral lipids in *Protozoa* has been studied by a number of investigators in relation to the physiological state of the organisms in different periods of the life cycle, and variation in some environmental conditions, such as temperature, aeration, UV-and X-rays effects etc. (Zweibaum 1922, Žinkin 1929, Poljansky 1934, 1963, Barbarin 1937, 1938, Manusova 1939, Sukhanova 1953, 1960, Engemann 1958, Kovaleva 1962, 1968, Dogel et al. 1962, Uspenskaya 1964, Frenkel 1965, Leighten, Everhart and Ronkin 1966).

Some authors explain the increase of resistance to certain environmental factors by accumulation of lipids and glycogen (Kovaleva 1962, Žinkin 1929, Barbarin 1938, Poljansky 1963).

Some authors explain the increase of resistance to certain environmental factors by accumulation of lipids and glycogen (Kovaleva 1962, Žinkin 1929, Barbarin 1938, Poljansky 1963).

The ciliates *Colpoda maupasi* are resistant to such extreme factor as drying, deep cooling, low atmospheric pressure and high CO_2 content in atmosphere (Bychenkova and Lozina-Lozinsky 1970, 1973, Lozina-Lozinsky et al. 1971).

In connection with studies on the resistance and adaptation of *Protozoa* (*Colpoda maupasi* among them) to low atmospheric pressure and increased CO_2 concentration it was of interest to follow changes of the lipid metabolism.

Materials and Methods

The quantity of reserve lipids was determined in the soil ciliates *Colpoda maupasi* during their life cycle: in active feeding forms (trophonts), before and during reproduction (reproduction cysts),

in stationary phase of the culture growth, in resting cysts and after excystment. The ciliates were kept under normal conditions, in the atmosphere rarified down to 10 mm of mercury and at high CO_2 concentration.

The experiments were performed on subclones and individual lines of isolated ciliates. The cultures was grown in Petri dishes or microaquariums in the mineral medium of Lozina-Lozinsky 1948 with addition of lettuce-infusion and *Bacillus subtilis*, under diffused light, at 22°C. The ciliates were placed in 2 l anaerostats where air has been pumped out down to 10 mm of mercury or displaced by a certain amount of CO_2 after pumping. CO_2 was used in 20 and 40% concentrations. In these concentrations the ciliates were kept from 5-66 h in microaquariums containing 0.3 ml medium.

Free lipids of *C. maupasi* were studied by cytochemical methods on total preparations. For this purpose the ciliates were stained with a mixture of red sudan and black sudan B after fixation in 8 and 10% neutral formalin and after that cleared in glycerol. For controlling the preparations were treated with chlorophorm and water heated to 60°C during 4 days. The quantitative estimation of lipids was made in accordance with the accepted method of division of ciliates into classes depending on the number and size of lipid drops. The percent of ciliates belonging to a definite class was calculated from 100 specimens examined in each preparation.

Results

The mixture of red sudans reveals orange drops of neutral fat in the cytoplasm of the ciliates. Black sudan B stains neutral fat phospho- and glycolipids intense dark blue. Lipid drops of *Colpoda maupasi* vary in size and arise initially at the front dorsal part. Lipid substances can be found in the cell membrane as well, since

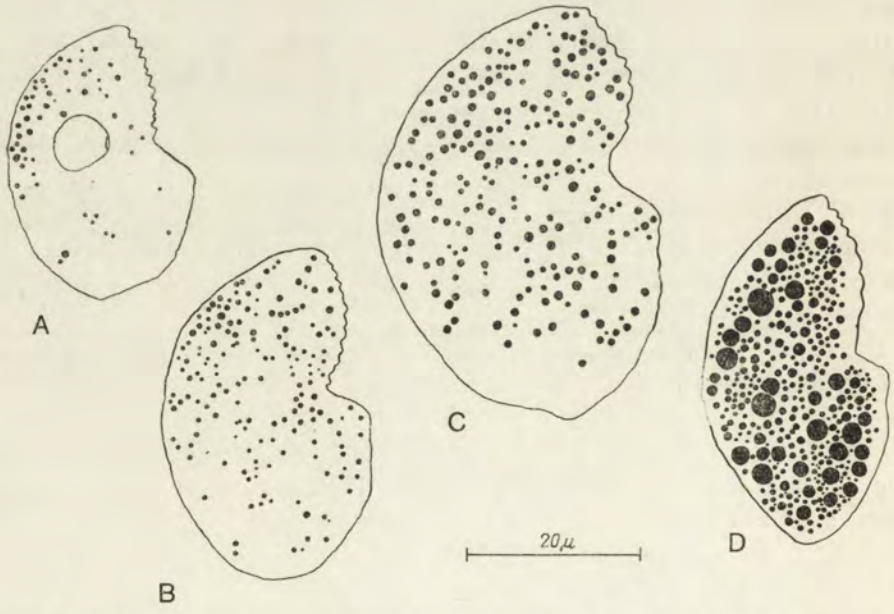


Fig. 1. The classes of accumulation of lipids in *Colpoda maupasi*: A – I, B – II, C – III, D – IV classes

it stains with sudans, at certain periods of the life cycle lipids are spread diffuse within the cytoplasm. Ciliates treated with chlorophorm and water heated to 60°C have no stained drops.

The following classes were established for quantitative determination of lipids in *C. maupasi*:

I class. Few drops in front at the dorsal side or single drops spread along the whole cell. The drops are small (Fig. 1 a).

II class. Drops occupy half of the cell (The whole anterior part to the oral cavity), single drops in the remaining part of the cell. The drops are larger (Fig. 1 b).

III class. Drops are densely packed along the whole cell leaving free a small region in the rear end and of the site of the nucleus (Fig. 1 c).

IV class. Drops occupy the whole cell leaving no gaps as a rule. The drops are large, the cytoplasm is seldom stained diffuse (Fig. 1 d).

Accumulation of Reserve Lipids in Ciliates in Normal Atmosphere

The data on free lipid content in *Colpoda maupasi* at different stages in normal atmosphere are presented in Table 1.

These results show that young ciliates belong mostly to I and partly to II class. It should be noted that lipids spread unevenly between daughter cells in the process of division. Thus out of four daughter ciliates originating from one cyst three were found to belong to I class and one to II class (Pl. I 1).

Table 1

Reserve Lipids of *Colpoda maupasi* at Different Stages of the Life Cycle in the Normal Atmosphere

Stages of life cycles	Classes*					Number of ciliates examined
	0	I	II	III	IV	
Trophonts	0	82.3±5.6**	16.0±3.2	1.7±0.4	0	900
Reproductive precysts and cysts	0	8.4±2.6	55.1±7.8	34.0±10.0	0	800
Stationary phase	0	3.7±1.4	23.7±4.3	57.8±3.9	0	900
Old resting cysts	100	0	0	0	0	300
Excysting active forms	0	11.2±4.9	28.3±5.9	58.5±11.0	2.0±1.3	600

* Classes of lipids content see above.

** In per cent.

During 3–5 h after division the content of free lipids in ciliates (trophonts) alters insignificantly. A notable increase the number of lipid drops is observed before formation of reproductive cysts and in reproductive cysts themselves the majority of which belong to II class and part to III class of reserve lipids (Pl. I 2, 3). In precysts of reproduction the drops are very large. The cytoplasm of reproductive cysts is stained dark blue with black sudan, which shows diffuse distribution of reserve lipids. Trophonts at the stage of logarithmic growth contain few reserve lipids, whereas in the stationary phase of 4–5 day culture, before resting cysts formation a great amount of free lipids accumulated in the cytoplasm of ciliates. At this time the ciliates may belong largely to II, III and IV classes (Pl. I 4). However, young ciliates arising from reproductive cysts in the stationary phase are poor in reserve lipids and probably form so-called unstable resting cysts (Pigon and Edstrom 1961). In the old resting cysts drops of free lipids are absent but in excysting active ciliates the whole cytoplasm is occupied by large drops intensely stained with black sudan.

Thus in *C. maupasi*, as in some other protozoans, the amount of reserve lipids varies at different periods of their life cycle. Our evidence support the results of other investigators obtained from experiments on *Bursaria truncatella*, *Paramecium caudatum*, *Oxytricha hymenostoma*, *Opalina ranarum*, *Balantidium*, *Tillina magna* etc.

Accumulation of Reserve Lipids in Ciliates under Low Atmospheric Pressure

It is known that the ciliates *Colpoda maupasi* can exist under low atmospheric pressure as resting cysts and also in active feeding form (Bychenkova and Lozina-Lozinsky 1970, Lozina-Lozinsky et al. 1971). They can live and reproduct in rarified (down to 15 mm of mercury) atmosphere in hermetically sealed chambers and under even lower pressure of flowing atmosphere. In the atmosphere rarified down to 10 mm of mercury *C. maupasi* survive, but usually do not reproduct probably due to oxygen deficiency.

We compared the accumulation of free lipids in the cytoplasm of active ciliates maintained for the same periods of time under pressure 10 mm of mercury and in normal atmosphere. As compared to the control, the number of lipid drops increased during 3–5 h after the ciliates had been put under low pressure (Table 2). Thus in normal atmosphere predominant the ciliates of I and II classes while under the pressure of 10 mm of mercury the ciliates been belonged to II and III classes accumulation of lipids. 18–23 h after in the control there were mostly young ciliates of I class and in the experimental sample those of II and III classes. The experimental specimens were larger, more elongated and showed slower movement.

Consequently, under the pressure of 10 mm of mercury during hypoxia in *C. maupasi* the storage of lipids increases in much the same way as was observed by Žinkin in *Stentor polymorphus*, by Barbarin in *Paramecium* under conditions of oxygen deficiency.

Table 2

Reserve Lipids of *Colpoda maupasi* at Different Stages of the Life Cycle in Atmosphere under the Pressure 10 mm of Mercury and under the Normal Pressure

Time of exposition (in h)	Conditions of experiment	Classes*				Number of ciliates examined
		I	II	III	IV	
5	Pressure 10 mm of mercury	1.3±0.5**	40.1±13.2	56.1±12.6	2.5±1.6	700
5	Pressure 760 mm of mercury	41.9±12.9	40.5±13.4	16.3±7.9	1.1±1.0	700
23	Pressure 10 mm of mercury	7.5±2.9	26.1±4.9	45.8±6.1	20.6±7.3	1200
23	Pressure 760 mm of mercury	69.5±9.6	19.2±5.9	10.0±6.9	1.3±1.0	1100

* Classes of lipids content see p. 283.
** In per cent.

Accumulation of Lipids at Increased Concentrations of CO_2

As has been mentioned the ciliates *Colpoda maupasi* are most resistant to high concentrations of CO_2. Up to 95% of active ciliates survive even at the concentration of CO_2 in the atmosphere about 20% such concentration is subtoxic for ciliates (Bychenkova and Lozina-Lozinsky 1973).

The comparison of the quantity of reserve lipids in trophonts kept in the atmosphere of CO_2 with the concentration of 20% and that in control ciliates shows that 5 h after the transfer of ciliates to atmosphere with high CO_2 content among experimental specimens III class is predominant whereas the controls mostly belong to I and partly to II class.

After a more durable incubation of the ciliates in condition of subtoxic CO_2 concentration in some experiments the reserves of lipids increased as compared to control. This is accounted for by the fact that the ciliates multiplicated during the experiment and by the end of it there arose young trophonts containing a few free lipids. Moreover, after a 3rd day of exposure control ciliates passed into the stationary growth phase and accumulated considerable amount of lipids, while the control ciliates kept on reproducting, retained the logaritmic stage and contained in some cases even lower amount reserve lipids.

CO_2 in concentrations over 20% exerts toxic effects on *C. maupasi*. In these

conditions the survival time of the ciliates is short. Thus in the atmosphere, containing 40% of CO_2, *C. maupasi* survive only for 5–8 h, their bodies became rounded and they are moving at slow rate. However, if they are transferred from 40% CO_2 concentration to the normal atmosphere in a few hours they begin to move normally, assume their usual shape and even reproduct. It is of interest to know how lipid exchange alters at the action of the toxic doses of CO_2. There are no relevant literature evidences.

We studied the effect of 40% concentration of CO_2 on the content of reserve lipids in the cytoplasm of trophonts *C. maupasi*, kept in this condition for 5 h. The results presented in Table 3 show that the toxic concentrations of CO_2 stimulate intense fattening of the ciliates. Very large drops fill up the whole cell. They stain dark blue with black sudan B (Pl. I 5–7).

Table 3

Reserve Lipids of *Colpoda maupasi* of Different Stages of the Life Cycle, under the Normal Pressure in the Normal and High CO_2 Content Atmosphere

Time of exposition (in h)	Conditions of experiments	Classes*				Number of ciliates examined
		I	II	III	IV	
5	20% CO_2 in atmosphere	2.6±1.0**	20.4±4.0	72.0±4.4	5.0±2.0	500
5	The normal atmosphere	47.0±9.5	48.4±7.4	4.6±2.9	0	500
18	20% CO_2 in atmosphere	6.2±3.3	37.8±10.9	54.0±13.2	2.0±1.7	400
18	The normal atmosphere	47.8±9.7	49.8±14.3	7.5±3.9	0	400
5	40% CO_2 in atmosphere	0	0.7±0.62	9.7±11.7	69.7±11.7	300
5	The normal atmosphere	6.7±5.0	35.3±12.7	54.7±16.6	3.3±0.9	300

* Classes of lipids content see p. 283

** In percent.

It must be pointed out that in this experimental series we used ciliates in the stationary phase of culture growth which possess reserves of free lipids and are most resistant to unfavourable environmental factors.

It must be concluded that high CO_2 content in the atmosphere causes disturbances in the lipid metabolism of *C. maupasi* due to which there is an excess of free lipids in the cytoplasm.

Discussion

Increase of free lipids in the cytoplasm of *Colpoda maupasi* at precyst stage in reproduction cysts, before formation of resting cysts, during excysting of resting cysts and the absence of lipids in formed resting cysts was demonstrated.

In the process of excystment free lipids are formed as a result of the splitting of glycogen the large amounts of which are contained in resting cysts.

Our result support the literature evidence of metabolic changes at various stages of the life cycle in other *Protozoa*. Thus there are indications about the increase in the amount of lipids during conjugation in *Bursaria* (Poljansky 1934), in precyst formation of *Tillina* (Frenkel 1965), in *Oxytricha* (Pomryaskinskaya 1940), in some protozoans in the stationary phase of culture growth (Engemann 1958, Kovaleva 1962, Leighten, Everhardt and Ronkin 1966). Changes in the amount of lipids caused by seasonal variations at different periods of the life cycle in some protozoans were observed by Žinkin 1929, Manusova 1939, Sukhanova 1960 and others.

Most of the authors suggest that at definite periods of the life cycle the increase in the amount of free lipids in the cytoplasm is related to the slowing down of metabolic reactions. Oxidative metabolism diminishes at that time (Barbarin 1938, Dogel et al. 1962). It is evidenced by the decrease in the amount of oxygen consumed before conjugation (Zweibaum 1922) and in the stationary phase of the culture growth (Pace 1945). Such reactions must be regarded as an adaptive response, connected with changes in the enzymatic activity. There is evidence about decreasing activity of oxidative enzymes and increasing activity of glycolytic enzymes in *Paramecia* in the stationary phase (Kovaleva 1968). The lowering of the glycogen amount upon the increase of lipids in *Stentor*, *Paramecium*, *Opalina* may be considered to be indirect evidence.

Nikoljuk et al. 1970 have shown that the succinic dehydrogenase activity changes at different periods in *C. maupasi* isolated from the soil. It is low in young trophonts and in resting cysts and higher in reproduction cysts before division. This is in agreement with the decrease in oxygen consumption at that time.

Thus the storage or expenditure of reserve lipids in *C. maupasi* is related to metabolic changes at different growth stages. The exchange in turn depends on the phy-

siological condition of protozoa and alterations in the environment during growth and reproduction.

The increase in the amount of free lipids in *C. maupasi* under low atmospheric pressure is induced by oxygen deficiency in the medium. According to the literature the lack of O_2 lowers the activity of oxydative metabolic reactions. It is not unlikely that in such conditions aerobic splitting metabolism is prevalent due to which the reserves of glycogen decrease and the amount of free lipids grows. It is probable that under low atomospheric pressure *C. maupasi* also shift to the glycolytic type metabolism. The results of Mavlyanova 1970 indicate, that *C. maupasi* in the stationary phase exhibit high glycolytic enzymic activity.

The storage of free lipids in *C. maupasi* in the atmosphere containing 20% of CO_2 seems to be the results of a depression inducing decrease in reproduction rate, prolongation of logarithmic growth period, inhibition of endogenic respiration enzymes (Golodov 1946).

In the atmosphere containing 40% of CO_2 there occurs a deeper depression induced by the toxic effect of CO_2 (change in the body shape of the ciliates, slackened movements). As a result pathological fattening of the cytoplasm is observed. The similar phenomenon was noted in some degenerating protozoans after prolonged fasting (Barbarin 1938, Dogel et al. 1962), at the action of X-rays (Kovaleva 1962) and ultraviolet radiation (Heillbrum and Daugherty 1938, Samoilova 1963).

Summary

The storage of lipids in the soil ciliates *Colpoda maupasi* is changing in dependence on the stage of their life cycle. The largest amount of reserve lipids is formed in precysts and reproduction cysts, in ciliates in stationary phase of culture growth and those excysting from resting cysts. Formed resting cysts contain no stores of lipids. Hypoxia in the static atmosphere at 100 mm of mercury favourable for accumulation of free lipids in the ciliate cytoplasm. The number of lipid drops in the cytoplasm is increased in the atmosphere containing 20% of CO_2. Deeper pathological disturbances are observed in lipid exchange in the atmosphere containing 40% of CO_2.

РЕЗЮМЕ

Запасы липидов у почвенных инфузорий *Colpoda manupasi* изменяются в зависимости от стадии их жизненного цикла. Наибольшее количество запасных липидов содержат предцисты и цисты размножения, инфузории в стационарной фазе роста культуры и эксцистировавшиеся из цист покоя. В сформировавшихся цистах покоя запасные липиды отсутствуют. Условия

гипоксии при 10 мм рт ст воздуха непроточной атмосферы способствуют накоплению свободных липидов в цитоплазме инфузорий. 20% концентрация CO_2 вызывает увеличение количества капель липидов в цитоплазме. 40% концентрация CO_2 ведет к более глубоким нарушениям в жировом обмене патологического характера.

REFERENCES

Barbarin V. V. 1937: Faktory, opredeljajuščie balans žira i glikogena u *Paramecium caudatum*. I. Vlijanie goloda. Biol. Zh., 6, 699–708.

Barbarin V. V. 1938: Faktory, opredeljajuščie balans žira i glikogena u *Paramecium caudatum*. III. Vlijanie uduš'ja na nakaplenie žira i glikogena. Bic . Zh., 7, 391–398.

Bychenkova V. N. and Lozina-Lozinsky L. K. 1970: Ustojčivost' infuzorij *Colpoda maupasi* k niskomu davleniju, anoksii i glubokomu ohlaždeniju. Sbornik: Žizn vne zemli i metody eje obnaruženija 81–96.

Bychenkova V. N. and Lozina-Lozinsky L. K. 1973: Ustoičivost' infuzorij *Colpoda maupasi* k dejstviju dvuokisi ugleroda. Acta Protozool., 12, 225–233.

Dogel V. A., Poljansky G. I. and Cheissin E. M. 1962. Obščaja Protozoologija. Izdat. Ak. Nauk SSSR. Moskva-Leningrad.

Engemann I. C. 1958: Lipids in *Tetrahymena*. J. Protozool., 5 (suppl.), 13.

Frenkel M. A. 1965: Žir i glikogen v žiznennom cikle infuzorij *Tillina magna* Gruber. Vest. Leningr. Univ., 15, 60–66.

Golodov I. I. 1946: Vlijanie vysokih koncentracij uglekisloty na organizm, Moscov–Leningrad.

Heilbrunn L. V. and Daugherty K. 1938: Fat release in amoeba after irradiation. Physiol. Zool., 11, 383–388.

Kovaleva N. E. 1962: Vlijanie temperatury kultivirovanija na čuvstvitelnost' infuzorij k povreždajuščemu dejstviju rentgenovyh lučej. Citologija, 4, 306–317.

Kovaleva N. E. 1968: Zavisimost aktivnosti nekotoryh degidrogenaz u *Paramecium caudatum*, adaptirovannyh k povyšennym i ponižennym temperaturam, ot vozrasta massovoj kultury. Citologija, 10, 1171–1179.

Leighten P., Everhart L. P. Jr. and Ronkin R. R. 1966: Changes in lipids of cells from aging populations of *Tetrahymena pyriformis*. J. Protozool., 13, 646–650.

Lozina-Lozinsky L. K., Bychenkova V. N. and Zaar E. I. 1971: Vlijanie na infuzorij fizičeskih uslovij, imitirujuščih sredu na poverhnosti planety Mars. Sbornik: Problemy kosmičeskoi biologii, 16, 281–292.

Manusova M. V. 1939: Sezonnye izmenenija žira i glikogena v prirodnyh uslovijah u infuzorij *Condilostoma vorticella*, *Frontonia leucas i Loxodes striatus*. Zool. Zh., 18, 451–460.

Mavljanova M. V. 1970: K voprosu izučenija aktivnosti nekotorych degidrogenez u *Colpoda maupasi*. Sbornik: Fizjologija mikroorganizmov, Taškent, 236–242.

Nikoljuk V. F., Mavljanova M. I. and Razmaev I. I. 1970: K voprosu ob osobennostjah dyhanija u počvennyh prosteiših. Sbornik: Fizjologija mikroorganismov. Taškent, 245–248.

Pace D. M. 1945: The effect of cyanide on respiration in *Paramecium caudatum* and *Paramecium aurelia*. Biol. bull., 89, 76–83.

Pigoń A. and Edström V. E. 1961: Excystment ability, respiratory metabolism and ribonucleic acid content in two types of resting cysts of *Colpoda cucullus*, O. F. Müller. J. Protozool., 8, 257–260.

Poljansky G. I. 1934: Geschlechtprocesse bei *Bursaria truncatella*, O. F. M. Arch. Protistenk., 81, 420–436.

Poljansky G. I. 1963: Zavisimost' soderžanija glikogena i žira v citoplazme u *Paramecium caudatum* ot temperatury. Sbornik: Morfologija i fiziologija prosteiših. Izd. AN SSSR. Moskwa–Leningrad, 102–110.

Pomrjaskinskaja N. A. 1940: Nabludenija nad cistami brujuhoresničnyh infuzorij *Oxytricha hymenostoma* Stein. Uč. zap. Leningr. gos. ped. in.-ta im. Gercena, 30, 93–132.

Samoilova K. A. 1963: Izmenenija v soderžanii žira i glikogena u *Paramecium caudatum* posle ultrafioletovogo oblučenija. Citologija, 5, 546–553.

Sukhanova K. M. 1953: Vlijanie faktorov vnešnej sredy na žiznennyj cikl *Opalina ranarum*. Uč. zap. Leningr. gos. ped. in.-ta im. Gercena, 91, 31–69.

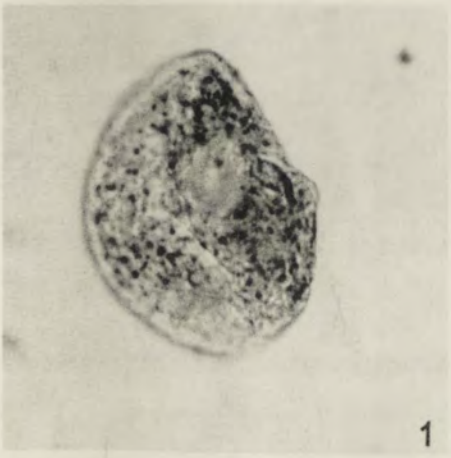
Sukhanova K. M. 1960: Citofiziologičeskaja harakteristika žiznennyh ciklov infuzorij roda *Balantidium* iz amfibij. Sbornik: Voprosy citologii i protistologii. Izdat. AN SSSR, 285–312.

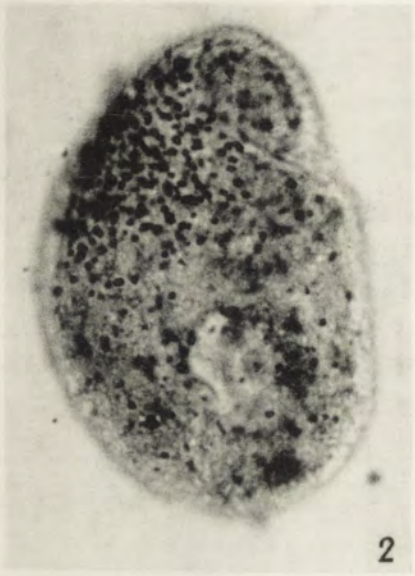
Uspenskaja A. V. 1964: Zapasnye pitatelnye veščestva, RNK, DNK i dyhatel'nye fermenty na raznyh stadijah žiznennogo cikla *Ichtiophtirius multifiliis*. Acta Protozool., 2, 175–195.
Žinkin L. N. 1929: Žir i pričiny ego obrazovanija u infuzorij *Stentor polimorfus*. Tr. Petergof. est. nauč. in.-ta, 6, 199–215.
Zweibaum J. 1922: Ricerchi sperimentali sulla conjugazione degli infusori. II. L influenca della conjugazione sulla produzione dei materiali di reserva nel *Paramecium caudatum*. Arch. Protistenk., 44, 375–396.

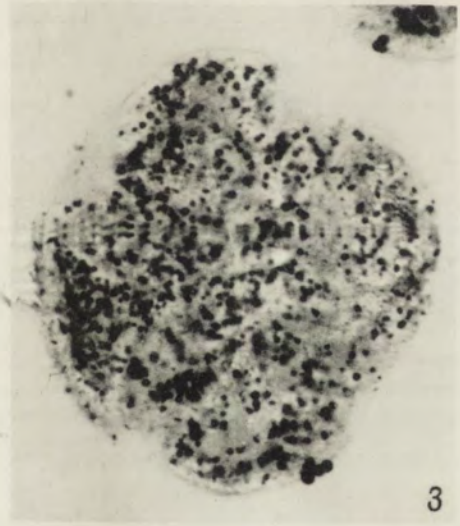
Received 21 October 1973

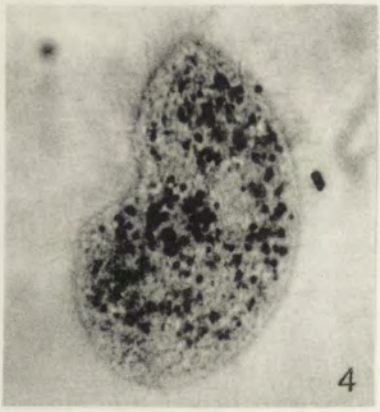
EXPLANATION OF PLATE

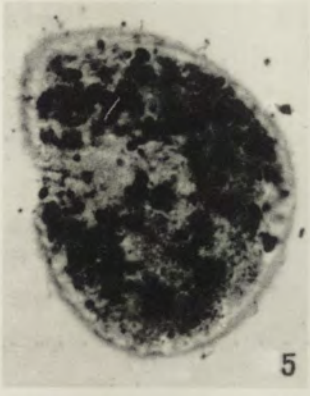
1–4: The amount of free lipids in cytoplasm of *Colpoda maupasi*: 1 — immediately after excysting from reproducting cysts, 2 — before formation of reproducting cysts, 3 — the reproducting cysts, 4 — the ciliates in the stationary phase
5–7: Drops of lipids in the cytoplasm of *Colpoda maupasi*, kept 5 h in the atmosphere with 40% of CO_2

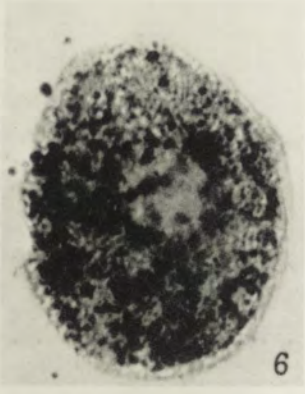


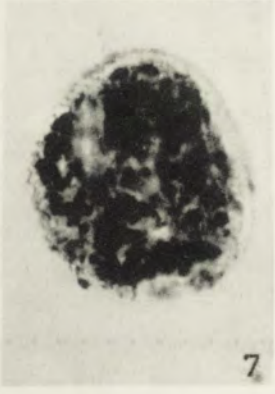












V. N. Bychenkova
et L. K. Lozina-Lozinsky

auctor phot.

ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 26

Department of Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 00-973, Warszawa, Pasteura 3, Poland

Michał OPAS

Studies on the Locomotion of *Amoeba proteus*. I. Responses to Hydrogen Ion Concentration in the Medium

Badania nad ruchem *Amoeba proteus*.
I. Wpływ stężenia jonów wodorowych w środowisku

It has long been known, that the hydrogen ion concentration of the surrounding medium affects behaviour and locomotion of large free-living amoebae of the proteus type (Jahn and Bovee 1967, Bovee and Jahn 1973).

Hopkins (1926, 1929) was first to state that there are two pH values (pH 6.7 and 7.6) optimal for *Amoeba proteus* locomotion. In addition he mentioned, however, that the locomotive activity was also strongly dependent on the kind of substratum. Mast and Prosser (1932) confirmed this observation. They found that in salt solution the speed of locomotion of *A. proteus* reached the first maximum at pH 6.8, then fell down to mediate minimum at pH 7.0 afterwards increased and reached the secondary maximum at 7.4 pH and fell down to zero at pH about 8.0. Further investigations brought only a slight change to those data. In sodium, potassium and calcium salts solutions there is the mediate minimum of the *A. proteus* speed of locomotion at pH 7.0 and two maxima — at pH 6.2 and 7.5 (Pitts and Mast 1934 b). The optimal pH for *Chaos chaos* locomotion is 6.6. (Käppner 1961). Käppner found as well, that with decreasing pH values the number of created pseudopods decreased too. According to the recent investigation (Stockem et al. 1973) the *A. proteus* locomotion pH range is between 5.0 and 8.0 and — in this range — pseudopodial activity grows with pH increase.

There is no satisfactory explanation of the relation between the locomotive activity of amoebae and the pH of the medium as for now. The decrease of locomotive activity in the neutral solutions is especially difficult to explain. It seems the most probable that the changes of the cell membrane, namely its electrostatic properties, may play a role in the response to the hydrogen ion concentration in the medium.

Some functions of the membrane as endocytosis, formation and retraction of pseudopods might be controlled by the mechanism involving surface and bioelectric potentials of the membrane (Wolpert and Gingell 1968). According to this hy-

pothesis it seems possible to explain another phenomena — as the relation between amoeboid movement dynamics and the pH of the medium.

The aim of this work was to find out the character of the relation between medium pH and locomotive activity of *Amoeba proteus* in conditions of reduced number of factors acting. To eliminate the influence of metal cations the experiments were performed in solutions containing hydrogen ions solely. Hydronium and hydroxyl ions and electrostatic forces were the only factors acting.

Material and Methods

Amoeba proteus S strain was used in the experiment. Mass cultures were grown on five different media: Chalkley solution without calcium ions (pH. 6.3–6.4), Pringsheim A solution (pH 6.3–6.5), Pringsheim B solution (pH 5.6-5.8), MES/Tris buffer (pH. 6.3–6.5) and HEPES/Tris buffer (pH 7.3–7.5). MES/Tris and HEPES/Tris buffers were obtained by mixing MES with Tris and HEPES with Tris in 3 : 2 and 1 : 1 ratios respectively. Amoebae were fed with *Colpidium* sp. grown in yolk cultures.

Before experiments amoebae were washed in MES/Tris buffer and transferred to a chamber with MES/Tris buffer of required pH. MES/Tris solutions at ratios from 9 : 1 (pH 5.2) to 3 : 7 (pH 8.2) were used as experimental solutions. The traces of amoeba routes were recorded on a low speed film in a dark field illumination by means of long time exposure photography using Robot camera recorder. The rate of locomotion of the given amoeba at different values of pH of the mediu m was estimated by means of measuring the length of recorded traces at standard duration. The pattern of traces depends on the number of pseudopodium formed at the time. Arbitral four point scale was applied to describe pseudopodial activity (Pl. I A-D). Glass, plexiglass, glass covered with SILICLAD(Clay-Adams Inc.) or carbon were used as substratum during measurements. The polypodial amoebae were used exclusively. During measurements temperature 23–24°C was kept.

Results

The relation between the rate of locomotion of amoebae and pH of medium is presented on Fig. 1 and 2. In all cases the rate of locomotion decreases to mediate minimum in neutral solutions. Maximal increase in the rate of locomotion can be observed in 6.2–6.8 pH range, with exception of amoebae from Pringsheim B culture which have the maximal rate of locomotion at pH 5.6. In the 7.3–7.5 pH range there are secondary maxima of the rate of locomotion of amoebae from all cultures. The distribution of maximal values of the rate of locomotion — that means the most advantageous pH values of the medium for the locomotion of amoebae — indicates, that they are related to hydrogen ion level in particular culture. It is also visible that the average rate of locomotion is the lowest for the amoebae from HEPES/Tris culture. The rate of locomotion of amoebae from Pringsheim B culture decrease as the pH increases.

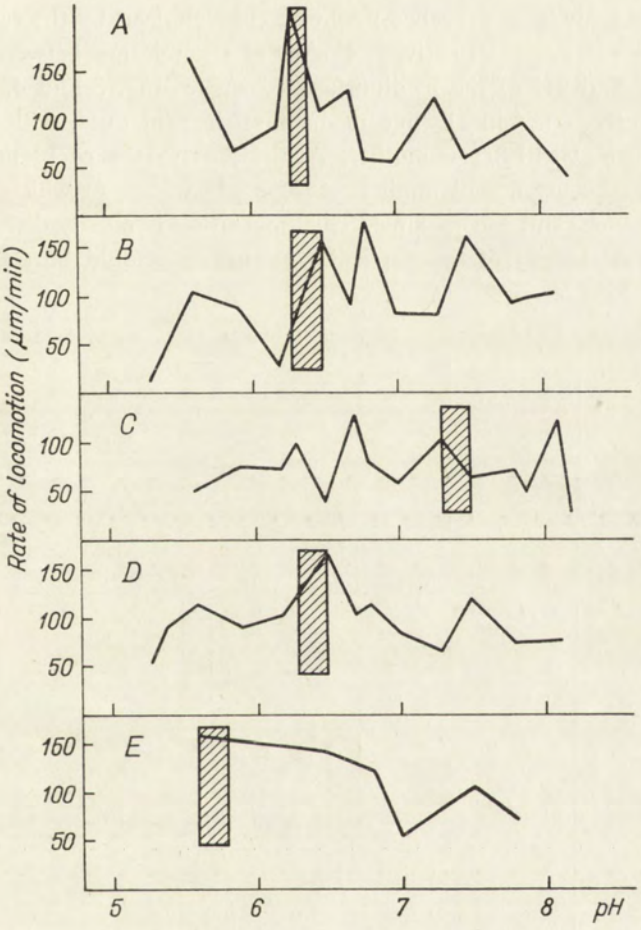


Fig. 1. Relation between the rate of locomotion of *Amoeba proteus* and pH of the medium. Amoebae taken from: A — Chalkley, B — MES/Tris, C — HEPES/Tris, D — Pringsheim A, E — Pringsheim B culture. Cultures pH values are marked by the dark fields

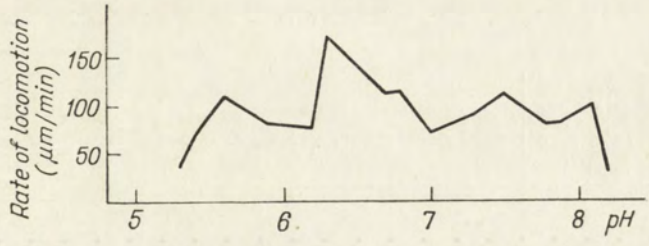


Fig. 2. Relation between the rate of locomotion of *Amoeba proteus* and pH of the medium presented in total

Locomotive activity of amoebae might be characterized by the rate of locomotion or by the pseudopodial activity. Curves of the relation between the pseudopodial activity and pH of the medium are shown on Fig. 3 and 4. Pseudopodial activity is slightly higher in alkaline media than in acid ones, with the exception of amoebae from HEPES/Tris culture. Analysis of curves of pseudopodial activity showed the occurrence of minima at these pH values, at which curves of the rate of locomotion exhibited maxima. This opposition is mostly distinct at the pH of medium equal to pH of the particular culture. It should be stated, however,

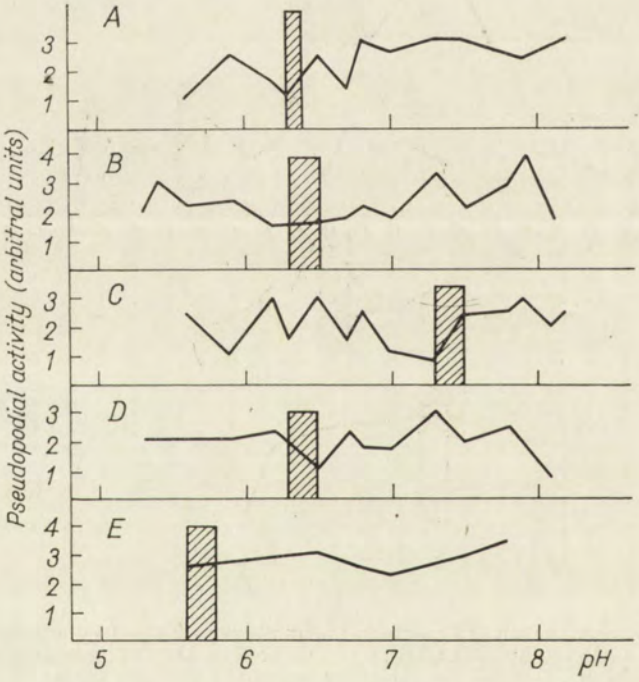


Fig. 3. Relation between the pseudopodial activity of *Amoeba proteus* and pH of the medium. Amoebae taken from: A — Chalkley, B — MES/Tris, C — HEPES/Tris, D — Pringsheim A, E — Pringsheim B culture. Cultures pH values are marked by the dark fields

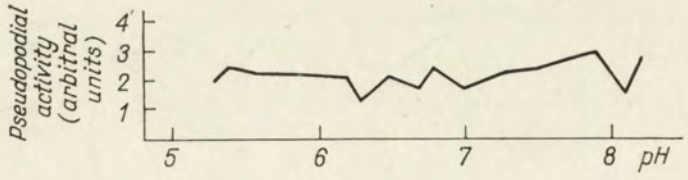


Fig. 4. Relation between the pseudopodial activity of *Amoeba proteus* and pH of the medium presented in total

that all the curves of pseudopodial activity have more or less distinct mediate minimum in neutral solution.

To find relationship between the rate of locomotion and pseudopodial activity comparison of the sets of curves was done (Fig. 5). Almost all the curves indicate that the rate of locomotion decrease as the pseudopodial activity increases. Moreover, distances of the curves from abscissae indicate that the most active amoebae were from MES/Tris culture and the least active — amoebae from HEPES/Tris.

The relation between the rate of locomotion and pseudopodial activity of amoebae is presented in total on Fig. 6. Curve, with almost linear character indicates that there exists an inverse relation between the rate of locomotion and pseudopodial activity, and it probably fits $x + y = \text{const.}$ equation.

In all the cases of 3.5 to 5 h adaptation of amoebae in MES/Tris solutions at pH different from the culture pH, before measurements, results were not univocal. So, there is no evidence that an adaptation of amoebae to pH occurs in the time shorter than 5 h.

During the experiments media with different pH values were changed in three ways: from acid to alkaline ones or vice versa or irregularly. Comaprison of data

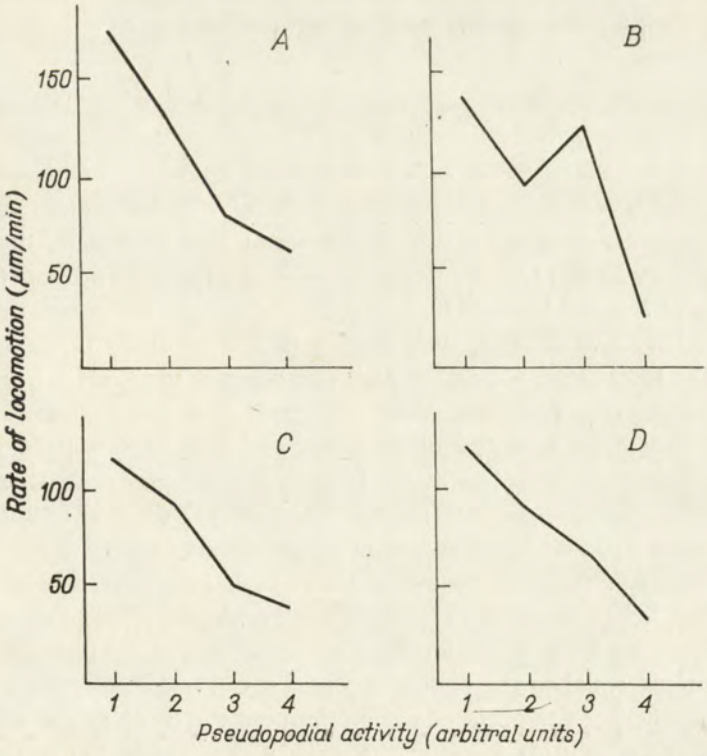


Fig. 5. Relation between the rate of locomotion and pseudopodial activity of *Amoeba proteus*. Amoebae taken from: A — MES/Tris, B — Pringsheim, C — HEPES/Tris, D — Chalkley culture

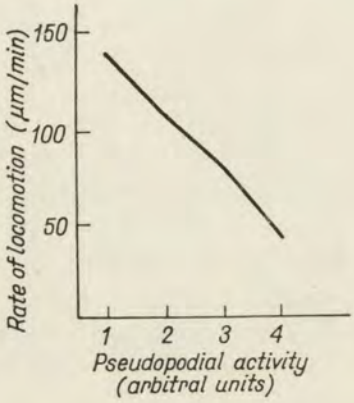


Fig. 6. Relation between the rate of locomotion and pseudopodial activity of *Amoeba proteus* presented in total

obtained in particular series allows to draw a conclusion that the direction of pH changes has no influence on the dynamics of amoeboid movement in relation to pH changes in the medium. Experiments with plexiglass and glass covered by Silicad or carbon revealed that there is no difference in shape of curves when compare with normal glass. This means that the kind of substratum has no influence on dynamics of locomotive activity of amoebae within the investigated pH range, in spite of affecting the average rate of locomotion.

Discussion

The relation between the locomotive activity of *Amoeba proteus* and the pH of the medium was investigated in two ways. Some authors tried to find out the correlation between the speed of locomotion of amoebae and pH of the medium (Mast and Prosser 1932, Pitts and Mast 1933, 1934 a, b), the others — the correlation between the pseudopodial activity and pH of the medium (Käppner 1961). General agreement exists that the locomotive activity of amoebae depends upon pH of the medium what was confirmed in presented paper. Moreover, it seems, that there exists the general distribution of velocities of amoebae within the investigated pH range, independent from the culture medium. Movement connected with locomotion of protozoa begins near pH 5.0, reaches maximum at pH 6.7–6.8, falls to mediate minimum at pH 7.0, rises again to secondary maximum at pH 7.3–7.5 and finally stops below pH 8.5. This distribution of velocities is in agreement with results of Taylor (1924), Mast and Posser (1932), Pitts and Mast (1934 b) and Käppner (1961). In the range of this distribution appears the additional mediate maximum of velocity at pH equal to the culture medium pH. The effect of the culture pH on the relation between the rate of locomotion and the medium pH was first described by Hopkins (1926). It seems that this effect is closely connected with influence of a kind of culture medium on a membrane state (Bingley and Thom-

pson 1962, Josefsson 1966, Prusch and Dunham 1972). Experiments presented in this paper indicate that culture pH affects amoebae velocity distribution in the whole pH range, that means between 5.0 and 8.5. Amoebae from acid cultures have the maximal rates of locomotion in acid range of pH too, and have relatively high rates of locomotion. Amoebae from alkaline culture have lower maximal rates in acid range of pH, and their maximal rates in alkaline range are strongly displaced to higher values of pH. Amoebae from HEPS/Tris culture (alkaline) have the lowest average rate of locomotion.

Lack of anomalies in behaviour of amoebae from Chalkley medium (without calcium ions) suggests that the amount of calcium ions served with food was sufficient.

It is known that the kind of substratum influences, fibroblasts and amoebae movement and on the other hand, that pH changes evoke charge density alteration on the cell surface (Hopkins 1929, Carter 1967, Ambrose and Forrester 1968, Wolpert and Gingell 1968, Braatz-Schade and Stockem 1972). Therefore, one can suggest that changes of cell velocity can be connected with the alteration of its adhesiveness. An amoebae velocity modifying factor could be the relation between surface charge densites of the cell and substratum. This simple interpretation cannot explain the existence of bi- or tri modal curves of velocity, however. Existence of the local maximum of the rate of locomotion at pH 6.8 can be interpreted as connected with itracellular pH which is about 6.8 (Wierciński 1955). Existence of local maxima of the rate of locomotion at pH equal to culture pH may be interpreted in this way: Cell buffering system will adjust to the culture pH, so during pH changes the buffering system will spend the least energy at pH 6.8(pH of the cytoplasm — Wierciński 1955), slightly more — at pH of the culture and the most at another pH values. This interpretation is based on the connection which exists between the energy expenditure and the motility of amoebae (Korohoda and Kalisz 1970).

From the experiments with different kinds of substratum one may conclude that — in spite of influencing the average rate of locomotion — the kind of substratum does not affect the shape of the rate of locomotion curve.

Besides the rate of locomotion, pseudopodial activity is the second parameter characterizing the locomotive behaviour of *A. proteus*. Results presented in this paper are in agreement with results of Käppner (1961), Braatz-Schade and Stockem (1972) and Stockem et al. (1973). Pseudopodial activity increases with medium alkalization what can be clearly seen above pH 6.5–6.7. Recently Braatz-Schade and Stockem (1972) proposed the hypothesis concerning the way in which medium pH can influence the pseudopodial activity.

Wolpert and Gingell (1968) obtained the expansion of *Xenopus* egg surface complex by reduction of negative surface potential and the contraction — by elevation of negative surface potential. Similar results were obtained by Korohoda et al. (1968) in experiments with isolated nuclei. Contraction or expansion of sur-

face complex evokes changes of plasma-membrane ratio, what in case of amoebae is triggering an alteration in pseudopodial number (Czarska and Grębecki 1966). Hence, it is possible that pH alterations by changing negative surface potential evoke variation in plasma-membrane ratio. Increase of medium pH value evokes the increase of negative surface potential, altering in this way bioelectrical potential profile. This change, together with membrane permeability decrease could reduce plasma–membrane ratio and cause an increase in pseudopodial number.

Experiments presented in this paper suggest that pseudopodial activity and the speed of locomotion of amoebae are connected phenomena. When one of activities increase another one decreases, and on the base of almost linear curse of curve from Fig. 6, the relation between them can be described as $x+y = \text{const}$. Relation between the rate of locomotion and pseudopodial activity allows to state that only these two parameters together characterize the movement of amoeba, that means its locomotive activity. Inverse relation between the rate of locomotion and pseudopodial activity suggests that locomotive activity of amoebae is constant at all the pH range in which their locomotion occurs. However, this hypothesis is difficult to prove because of lack of common units to describe these two parameters.

Summary

The aim of this work was to examine the effect of pH of the medium on amoebae locomotion. The number of experimental factors was limited (by use of the experimental media with hydrogen ion as the only cation) to hydrogen and hydroxyl ions and electrostatic forces.

(1). As the pH of the medium increases the rate of locomotion of amoebae starts from zero at pH about 5.0, rises to maximum at pH 6.7–6.8, next falls to the mediate minimum at pH 7.0, reaches secondary maximum at pH 7.4–7.5 and finally falls to zero at pH about 8.5. There is an additional mediate maximum of the rate of locomotion at pH near to pH values of the given amoebae culture.

(2). The kind of substratum has no influence on the rate of locomotion curve shape, but affects the average rate of locomotion.

(3). Adaptation to pH of the medium is a long lasting process — it lasts more than 5 h.

(4). As the pH of the medium increases the pseudopodial activity of amoebae increases too.

(5). Existence of the inverse relationship between pseudopodial activity and the speed of locomotion was stated, and described as $x+y = \text{const}$. Constancy of *A. proteus* locomotive activity — understood as a result of the speed of locomotion and the pseudopodial activity — within pH 5.0–8.5 range was suggested.

STRESZCZENIE

Celem niniejszej pracy było zbadanie wpływu pH środowiska na ruch ameb w warunkach ograniczonej liczby czynników doświadczalnych. Wykazano, że:

(1). Wraz ze wzrostem pH środowiska szybkość ruchu ameb rośnie od zera w okolicy pH 5.0 do maksimum pH 6.7–6.8, następnie spada do minimum pośredniego przy 7.0, rośnie do drugiego maksimum przy pH 7.4–7.5 i spada do zera w okolicy pH 8.5. Dodatkowo występuje maksimum pośrednie szybkości ruchu w okolicy pH hodowli, z której pochodził badany pierwotniak.

(2). Rodzaj podłoża pomimo, że wpływa na przeciętną szybkość ruchu nie ma wpływu na kształt krzywej zależności szybkości ruchu ameb od pH środowiska.

(3). Proces przystosowania do pH środowiska jest względnie długotrwały, to jest wykraczający poza 5 godzin.

(4). Wraz ze wzrostem pH środowiska rośnie aktywność pseudopodialna ameb.

(5). Istnieje odwrotna zależność między aktywnością pseudopodialną a szybkością ruchu ameb, najprawdopodobniej o charakterze $x+y = \text{const}$.

Postawiona została hipoteza o stałości aktywności ruchowej *A. proteus* (rozumianej jako łączny wynik szybkości ruchu i aktywności pseudopodialnej) w całym badanym zakresie pH.

REFERENCES

Ambrose E. J. and Forrester J. A. 1968: Electrical phenomena associated with cell movements. In: Aspects of Cell Motility, University Press, Cambridge, 22, 237–248.

Bingley M. S. and Thompson C. M. 1962: Bioelectric potentials in relation to movement in amoebae. J. Theor. Biol., 2, 16–32.

Bovee E. C. and Jahn T. L. 1973: Locomotion and behaviour. In: The Biology of *Amoeba*, (ed. Jeon K. W.), Academic Press, 250–290.

Braatz-Schade K. and Stockem W. 1972: Pinocytosis and locomotion of amoebae. IX. The influence of different cations on the moving behaviour of *Amoeba proteus*. Arch. Protistenk., 114, 272–290.

Carter S. B. 1967: Hyptotaxis and the mechanism of cell motility. Nature, 213, 256–260.

Czarska L. and Grębecki A. 1966: Membrane folding and plasma–membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Hopkins D. L. 1926: The effect of hydrogen ion concentration on locomotion and other life-processes in *Amoeba proteus*. Proc. natn. Acad. Sci., 12, 311–315.

Hopkins D. L. 1929: The effect of substratum, divalent and monovalent ions on locomotion in *Amoeba proteus*. J. Morph. Physiol., 48, 371–383.

Jahn T. L. and Bovee E. C. 1967: Motile behaviour of *Protozoa*. In: Research in Protozoology, (ed. Chen T.), Pergamon Press, Oxford, 1, 41–200.

Josefsson J. O. 1966: Some biochemical properties of *Amoeba proteus*. Acta physiol. scand., 66, 395–405.

Käppner W. 1961: Bewegungsphysiologische Untersuchungen an der Amoebe *Chaos chaos* L. I. — Der Einfluss des pH des Medium auf das bewegungsphysiologische Verhalten von *Chaos chaos* L. Protoplasma, 53, 81–105.

Korohoda W. and Kalisz B. 1970: Correlation of respiratory and motile activities in *Amoeba proteus*. Folia biol. (Kraków), 18, 137–144.

Korohoda W., Forrester I. A., Moreman K. G. and Ambrose E. J. 1968: Size changes in isolated nuclei of *Amoeba proteus* on treatment with polyionic substances. Nature, 217, 615–617.

Mast S. O. and Prosser C. L. 1932: Effects of temperature, salts and hydrogen-ion concentration on rupture of the plasmagel sheet, rate of locomotion and sol/gel ratio in *Amoeba proteus*. J. cell comp. physiol., 1, 333–354.

Pitts R. F. and Mast S. O. 1933: The relation between inorganic salt concentration, hydrogen ion concentration and physiological processes in *Amoeba proteus*. I. Rate of locomotion gel/sol ratio and hydrogen ion concentration in balanced salt solution. J. cell. comp. Physiol., 3, 449–462.

Pitts R. F. and Mast S. O. 1934 a: The relation between inorganic salt concentration, hydrogen ion concentration and physiological processes in *Amoeba proteus*. II. Rate of locomotion, gel-sol ratio and hydrogen ion concentration in solution of single salts. J. cell. comp. Physiol., 4, 237–256.

Pitts R. F. and Mast S. O. 1934 b: The relation between inorganic salt concentration and physiological processes in *Amoeba proteus*. III. The interaction between salts (antagonism) in relation to hydrogen ion concentration and salt concentration. J. cell. comp. Physiol., 4, 435–455.

Prusch R. D. and Dunham P. B. 1972: Ionic distribution in *Amoeba proteus*. J. exp. Biol., 56, 551–563.

Stockem W., Braatz-Schade K. and Haberey M. 1973: Electrophysiological investigations on the correlation between membrane potential and motile behaviour under normal and experimental conditions in *Amoeba proteus*. In: Progress in Protozoology, abstr. 4th Int. Congr. on Protozool. Clermont-Ferrand 1973, 403.

Wierciński F. J. 1955: The pH of animal cells. Protoplasmatologia, II B, 2 c, 1–56.

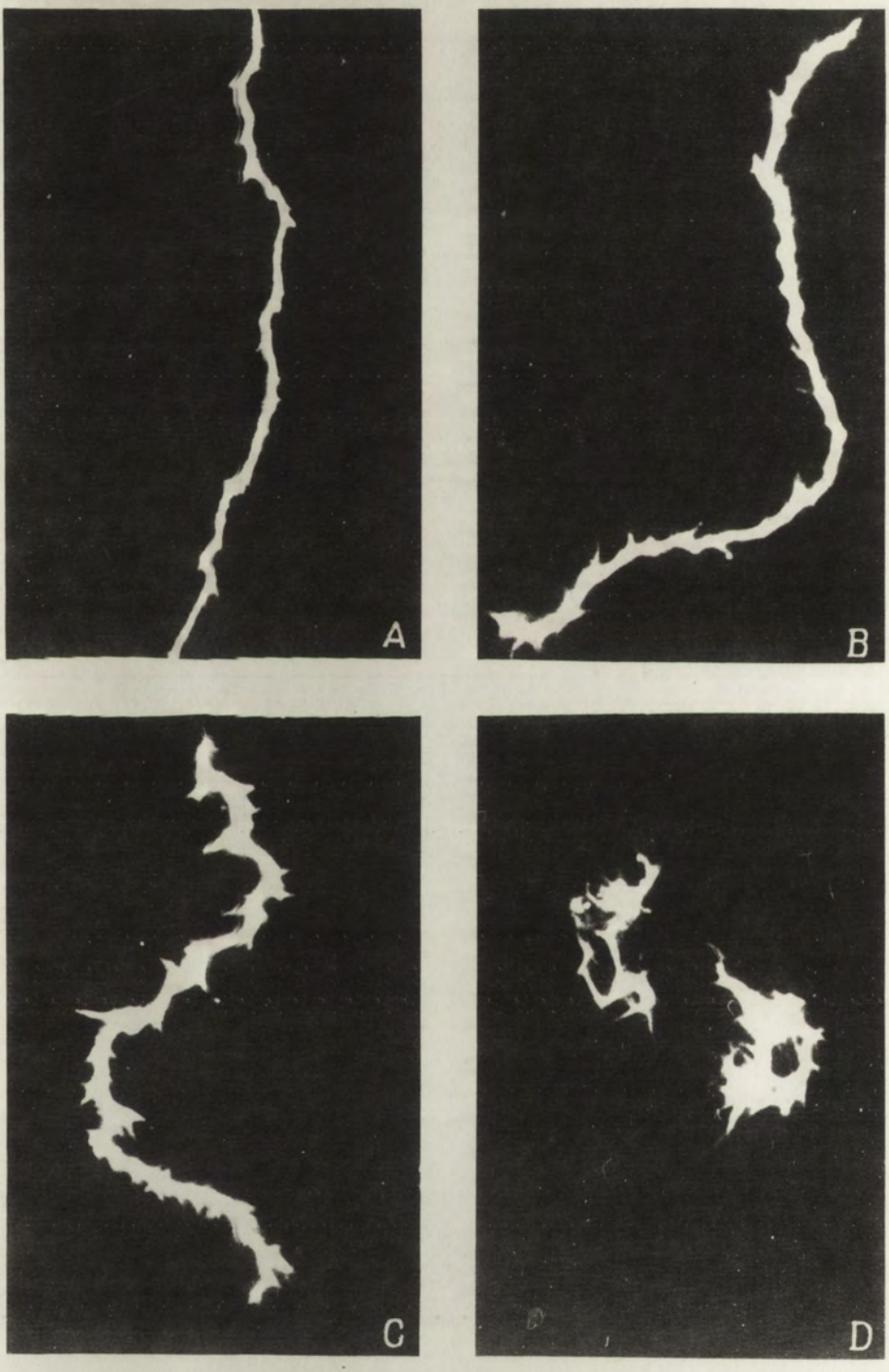
Wolpert L. and Gingell D. 1968: Cell surface membrane and amoeboid movement. In: Aspects of Cell Motility. University Press, Cambridge, 22, 169–198.

Received 12 September 1973

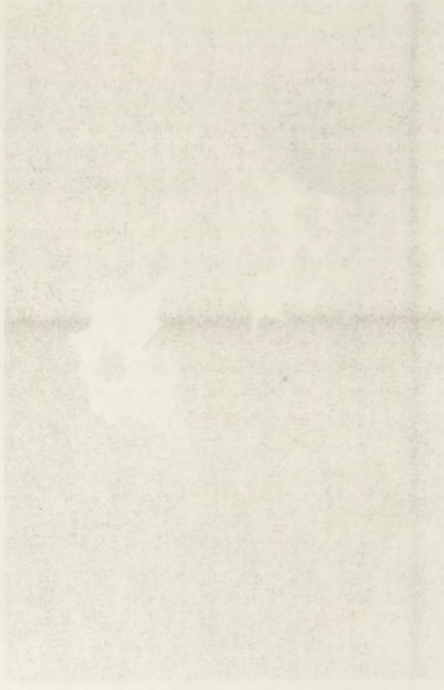
EXPLANATION OF PLATE I

Patterns of traces characterized by pseudopodial activities of *Amoeba proteus* from 1 to 4. Magn 14×

A: Pseudopodial activity equal 1
B: Pseudopodial activity equal 2
C: Pseudopodial activity equal 3
D: Pseudopodial activity equal 4



M. Opas auctor phot.



ACTA PROTOZOOLOGICA

Vol. XIII WARSZAWA, 15. X. 1975 FASC. 27

Department of Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 00-973 Warszawa, Pasteura 3, Poland

Zbigniew BARANOWSKI

Movements of the Slime Mould Strand. I. Cinematographic Analysis of some Kinetic Properties

Ruchy kanału plazmodium śluzowca. I. Filmowa analiza niektórych właściwości kinetycznych

Spiralling of the slime mould strand was first mentioned by Seifriz in 1946. The phenomenon was observed in a suspended strand of *Physarum polycephalum*. Kamiya and Seifriz (1954) provided experiments in order to establish the quality of the torsion, and stated that: (1) At room temperature the free end of the strand rotated to the right and left atlernately with a period of about 2 min. (2) The value of the deviations varied strikingly according to the specimen. (3) The angle of rotation of the free end in one direction was greater than in the opposite direction, hence the resultant torsion in one of the two possible directions. (4) Experiments showed the predominance of a counter-clockwise rotation.

To explain the polarization of the torsion Kamiya and Seifriz (1954) and Kamiya (1959), assumed the concept of sliding of the structural components, i.e., of a systematic reconversion of position of junctions linking those components.

A strand of the slime mould *Physarum polycephalum*, when suspended in a moist chamber, can not only rotate but also contract along its longitudinal axis and relax periodically with a period of 90 to 180 sec. Differences in the strand length between the contraction and relaxation phases amount to ca. 10% of its length. The hanging strand contracts longitudinally with the same period as it rotates. At the same time Kamiya and Seifriz (1954), did not find any direct connection between rotation and contraction. Neither did they establish any relationship between the time and direction of the protoplasmatic flow and the twisting motion.

To support this conclusion Kamiya and Seifriz (1954), carried out some experiments in a chamber resembling the "double chamber". By this method the flow could be accelerated, stopped or its direction inverted at any moment. The measurements of the torsion and the contraction did not show any effect of the artificial changes of the flow inside the strand on the movement of its outer layer,

The dynamic character of an isolated strand was presented by Kamiya (1970).

Kamiya et al. (1972), they measured the tension during isometric contraction. The tension values changed periodically and reached 50 g/cm^2.

The method used in tension measurements allowed to measure Young's modulus which describes mechanical properties. Its value equalled 10^5 dyne/cm^2. The Young's modulus changed its value according to the phase of the contraction cycle. It was greater by 50% during the contraction than during the relaxation.

The task of the present work is to study the movements of the surface of the slime mould strand in order to find whether there exists any relationship between the torsion and contraction and how it is manifested.

Material and Methods

Isolated fragments of a slime mould strand of *Physarum polycephalum* were the subject of investigations. *Physarum* was cultured by the method of Camp (1936) on filter paper. Segments of plasmodium used in the experiments were 10 mm long and 200–400 μm wide; they were isolated from the rest of plasmodium and placed directly in the chamber. In non-isolated fragments there sometimes occur changes of the strand diameter caused by the flow of plasma from two opposite directions to a definite area of the plasmodium. The use of relatively small fragments in the present study excluded secondary effects connected with the elasticity of the strand walls.

An inverted polarizing microscope was adapted for observation and microcinematgraphic technique was applied for recording the movement of surface of the strand hanging vertically or placed horizontally. To eliminate the mechanical effect of the substratum, the examined strand was, in both cases, suspended in the moist air. Before the experiment, grains of potato starch or small crystals of calcium oxalate were placed on the strand. The former markers proved to be better and were used most of the time. The movements of the grains or crystal reflected the movements of the strand surface.

The recording method consisted either on photographing the isolated fragment exposed for a long time or in filming the strand at slower rate (1 fps).

The surface movements of the isolated strand were filmed or photographed during 5 to 20 min since its separation from the plasmodium. The strand was placed on a Plexiglas ring, along its diameter. The inner diameter of the ring was of the same length (2.5 mm) as the middle fragment of the strand, suspended in the air. Both ends of the strand rested on the ring. The central part of the suspended strand, 1.5 mm long, was filmed.

The advantage of this method is the possibility of observation and recording displacements of the strand areas situated relatively near each other. An inverted microscope was used. The polarization and interference system was adapted to the microscope. The apparatus has been presented in Fig. 1.

The conditions of investigation demanded the construction of a special chamber. A diagram of this chamber has been presented in Fig. 2. The chamber was to keep the examined fragment of strand in moist air. The object glasses (1 and 5) allowed the object to observed while it rested on a ring (3) of a small inner diameter — 2.5 mm. A ring (4) fixed the object glass (5) at a certain distance off the strand surface. All the parts were pressed by an outer ring (6) which was screwed to the base (2). The inner apertures of rings (3 and 4) formed the space where the plasmodium strand with a few drops of water was enclosed.

The chamber was placed in the plane of the microscope table which was perpendicular to the

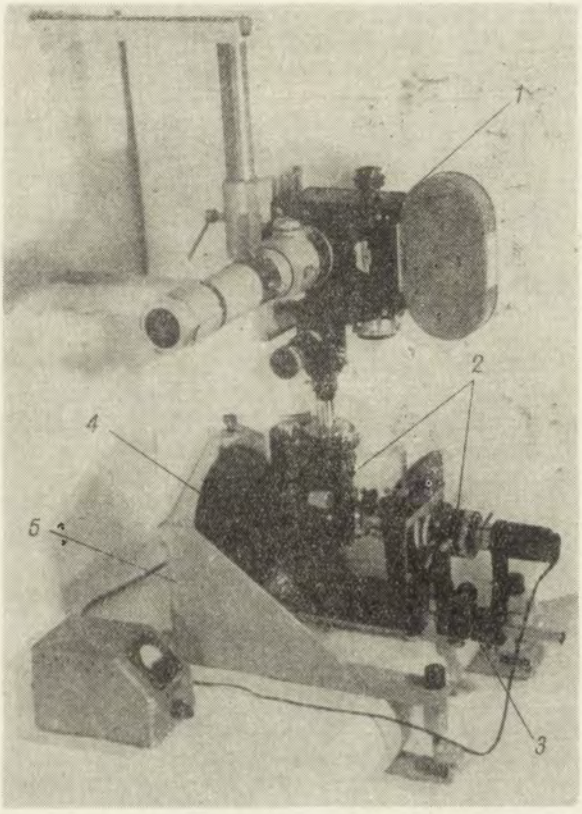


Fig. 1. Device for investigation of an isolated strand of *Physarum polycephalum*. 1 — Cinecamera, 2 — Polarization system, 3 — Chamber containing the examined strand, 4 — Inverted microscope, 5 — Base keeping the microscope in horizontal position

microscope axis. Owing to this the vertical position of the hanging strand could be changed into a horizontal position by turning the chamber by $1/2\ \pi$ in the plane of the table which rendered possible due to the cylindrical surface of the chamber base.

The analysis of the shots consisted on projecting the frames succsessive on a screen and record ing the position of the markers.

(1) A shift of the markers along the longitudinal axis of the strand showed the value of the contraction of the strand.

(2) A shift of the markers within the plane perpendicular to one of the edges of the strand considered as reference system, showed the value of the twist.

(3) Changes in diameter of the strand in respect to one of its edges, considered as reference system, showed the value of pulsation.

All those changes were analysed every 10 frames which corresponds to 10 sec intervals. It was assumed that the error of this method was the error in determination of the marker's position on the screen. The error in measurement of the torsion, resulting from the cylindrical shape of the strand, may be ignored as the markers selected for measurement were placed in the central part of the strand and the amplitude of their shifts was small.

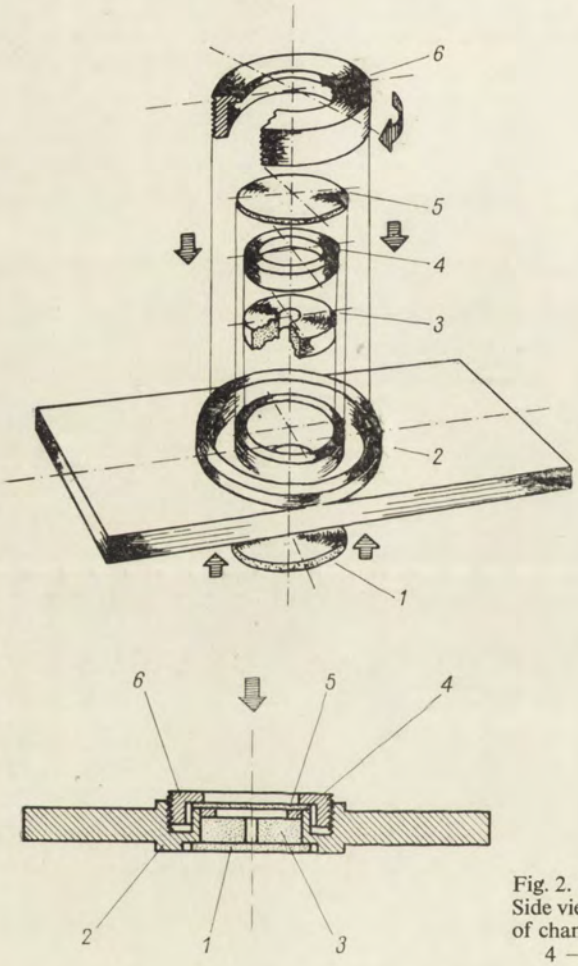


Fig. 2. Investigation chamber: a — Parts, b — Side view, 1,5 — Covering glasses, 2 — Base of chamber, 3 — Ring supporting the strand, 4 — Plexiglas ring, 6 — Pressing ring

Results

The technique of photographic with prolongated exposure has allowed to state that the surface of most of the examined fragments of the strand moved (Pl. I 1, 2, 3, 4). The results obtained indicated that the surface contract along the longitudinal axis of the strand, and twist. Traces left on the negative by markers moving together with the surface of the strand have shown that the torsion of the axis is always accompanied by the contraction of the strand (Pl. I 2).

This recording system does not allow to examine simultaneously the surface movements and the shape of the strand. The length of traces left by the moving markers was not an objective measure of their way, because of possibility of oscillatory movements occurring along the same way. The degree of blackening of the

negative cannot be a measure of time during which a grain or crystal stayed at a definite point of space because of changes in glare intensity during the experiment. In this situation the technique of recording the movements of the strand by photographing has been replaced by the filming technique.

A part of the investigated strands displayed no motion during 15 min of filming. The remaining greater part showed movement.

The movement could be explained by means of two kinetic models of the strand: a model of a standing wave on the strand and a model of the torsion. The advantage is that the both proposed models are not in contradiction with data published elsewhere.

Four markers placed on a strand hanging vertically were used in the analysis of the model of a standing wave. The disposition and denotation of the markers is shown in Fig. 3. The measurement of the twist has been neglected as the changes

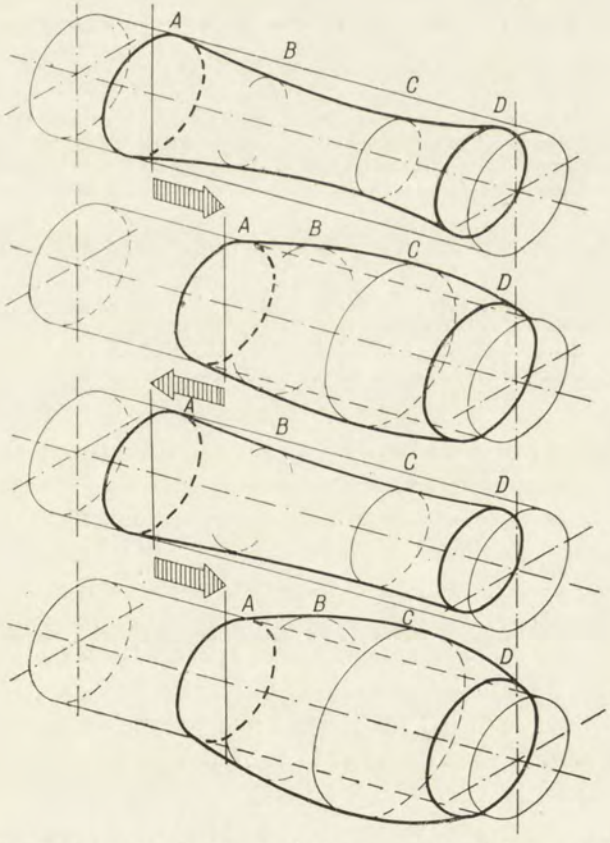


Fig. 3. Model of standing wave on the strand of *Physarum polycephalum* — changes of the strand shape during one full period of oscillation. A, B, C, D — cross-sections of the strand where the analyzed markers were placed. Arrows show the simultaneous motion of markers along longitudinal axis of the strand

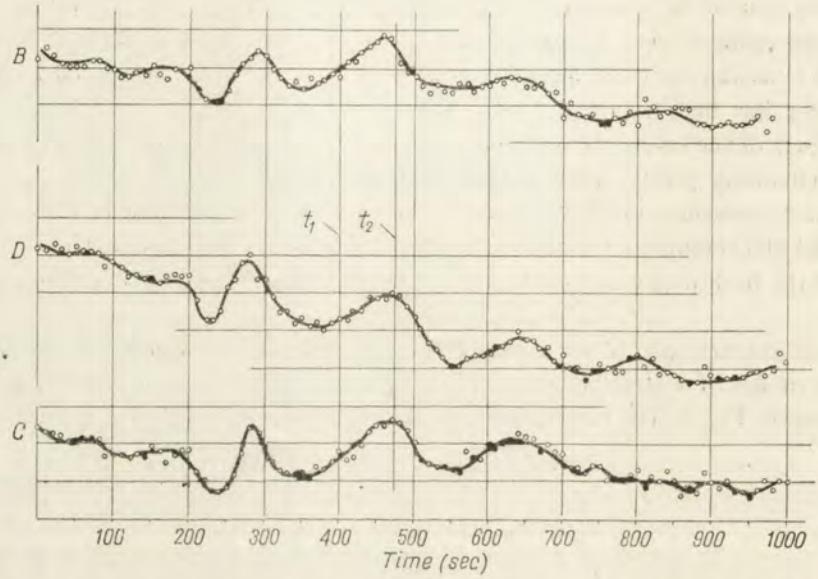


Fig. 4. Pulsation of *Physarum polycephalum* strand in a frame of reference connected with the strand. Changes of strand diameter at points B, C and D in relation to the observer placed in point A (see Fig. 3). The abscissae mean measured in seconds. The elementary unit on the axis of ordinates is 25 μm. Arrows on the model (see Fig. 3) correspond to moments t_1 and t_2 marked in the diagram

of position corresponding the twist remained within the method error limits. Analysis as described above concerned changes of position along the longitudinal axis of the strand and the value of the diameter.

A direct measurement of changes in the diameter of the strand in points A, B, C and D corresponding to the position of the four markers has shown that those changes had an undulatory character. This led to the supposition that the wave along the strand are standing or propagating one. In order to find the length of the standing wave, the diameters of strand, were analysed. Two neighbouring sites of the same diameter would indicate the nodes of standing wave. Figure 4 is a diagram of changes of the diameter changes. The period of these changes is approximately 180 sec. The phase of these changes is concordant. For better illustration of the present considerations the author has chosen two moments of time t_1 and t_2 which are marked on the diagram. At the moment t_1 all the points were in the minimum of their oscillations with respect to point A and at t_2 — in their maximum. The behaviour of the strand during 3 min had the character of shock caused by the excision from the plasmodium. In the following 10 min the strand behaved in a normal way.

In Fig. 5 the reference system transferred to point C is presented. Consequently the effect of undulation is smaller for points B and D but it is still distinct. All the three curves within the error limits have concordant phases. At moment t_1 all the three points examined have reached the maximum of their oscillation, and at t_2 — their minimum. The model (Fig. 3) illustrates the changes of shape of the strand

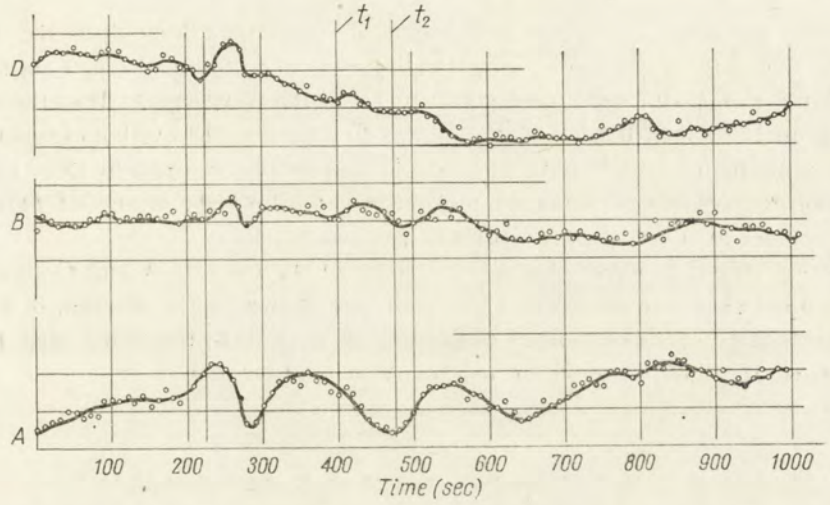


Fig. 5. Pulsation of *Physarum polycephalum* strand in a frame of reference connected with the strand. Changes of strand diameter at points A, B and D in relation to observer at point C. The abscissae mean time measured in seconds. The elementary unit on the axis of ordinates is 25 μm. Arrows on the model (see Fig. 3) correspond to moments t_1 and t_2 marked in the diagram

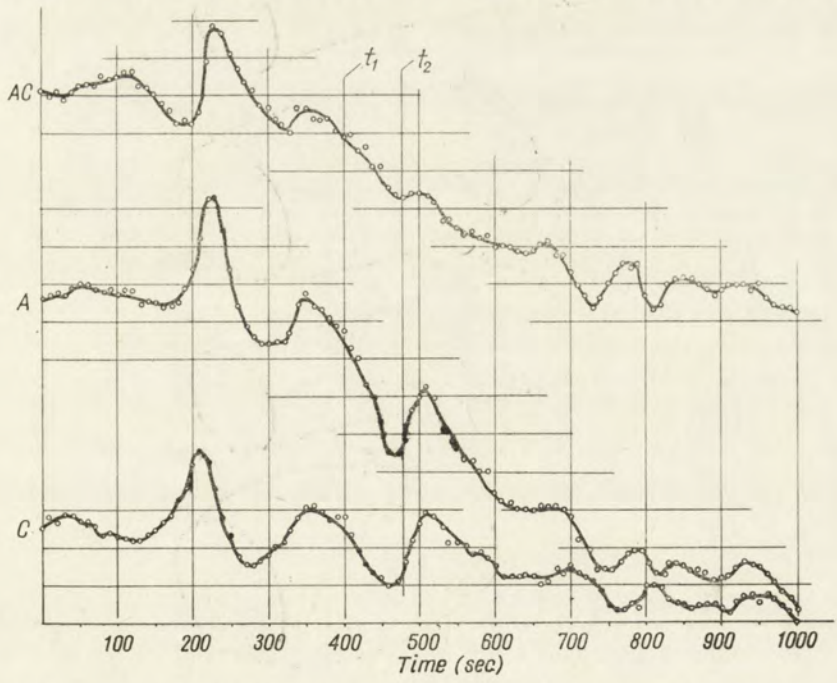


Fig. 6. Surface movement of *Physarum polycephalum* strand along longitudinal axis. Curve A and C — changes of position of markers A and C in relation to edge of the frame. Curve AC — movement of point A in relation to point C. Points A and C on the strand were 700 μm apart. The elementary unit on the axis of ordinates is 25 μm. Arrows on the model (Fig. 3) correspond to moments t_1 and t_2

(simplified diagram) during one full period of oscillation. Point A of the model may be considered as the node of the standing wave and point C — as its antinodes. The distance A–C (0.7 mm) corresponds to 1/4 of the wave-length. Thus the wave-length equals 2.8 mm. If we consider the standing wave as the overlap of two waves of the same period length and amplitude — propagating in opposite directions — we came to conclusion that wave may propagate along the strand of *Physarum polycephalum* at a speed of ca. 15 μm per second.

During strand diameter changes, the shifts of the markers A and C along the longitudinal axis were recorded. These shifts are shown by the diagram in Fig. 6. Curves A and C present changes of position in relation to the frame edge. Curve AC shows the movement of the marker A in relation to C.

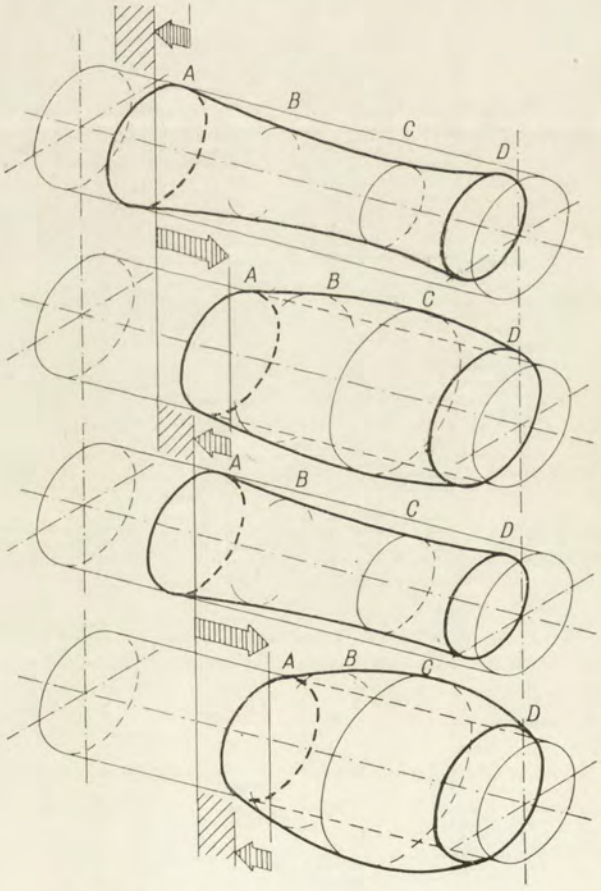


Fig. 7. Model of standing wave on *Physarum polycephalum* strand with regard to the constant rate of approach of node A to antinodes C. A, B, C, D — cross-section of strand where the analyzed markers were placed. Arrows show the movement of markers along the longitudinal axis of the strand. Hachured parts are the measure of the constant rate of approach of node A to antinodes C

Nature of the phenomenon which are responsible in approaching the points A and C remain unknown, however, more complicated model (Fig. 7) could be proposed. Diagram AC shows that the maximum diameter at point C corresponds to an approach of point A and vice versa. The changes of position of node A in relation to antinodes C have been marked on the model in Fig. 3. The model of a twist, has been presented in Fig. 8. The investigations were preceded by an analysis of changes of the diameter of the strand with a method analogous to that of the previous case. The diagram in Fig. 9 presents changes of diameter in points

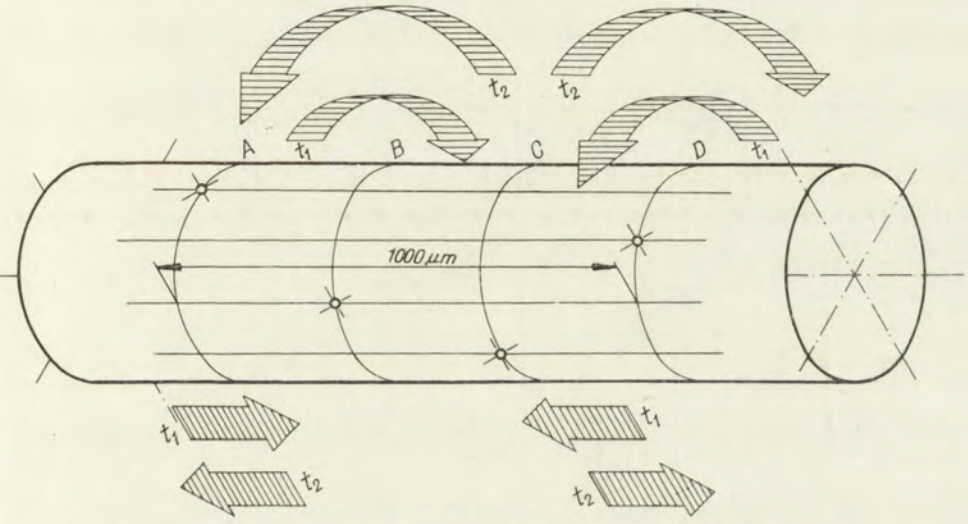


Fig. 8. Kinetic model of torsion of *Physarum polycephalum* strand. The position of markers on the strand corresponds with points A, B, C and D. Upper arrows show the torsion at moments t_1 and t_2. Lower arrows show the movement of markers along the longitudinal axis of the strand at moments t_1 and t_2 respectively

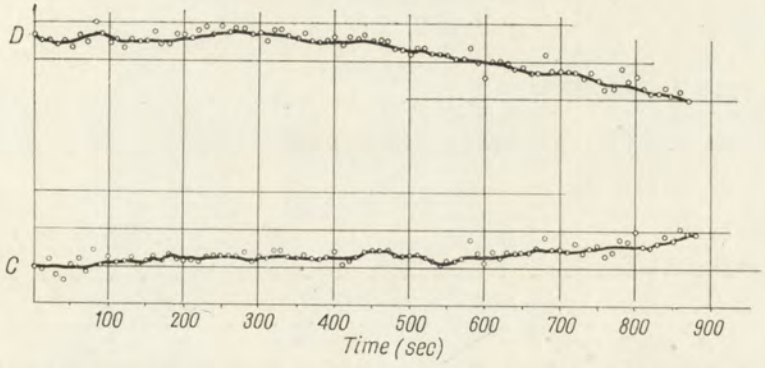


Fig. 9. Pulsation of the *Physarum polycephalum* strand in frame of reference connected with the strand. Changes of strand diameter in points C and D in relation to observer placed in point (see Fig. 8)

C and D in relation to the observer placed in point B. It can be clearly seen that the standing wave has not occurred on this strand. According to the method of analysis discussed in "Material and Methods", curves corresponding to the torsion of the strand have been plotted. These curves are characteristic of periodic changes.

The diagram in Fig. 10, shows the changes of position of the markers in a plane which is perpendicular to the longitutinal axis of the strand in the relation to point C. The movement of points B and D has the character of oscillation with opposite

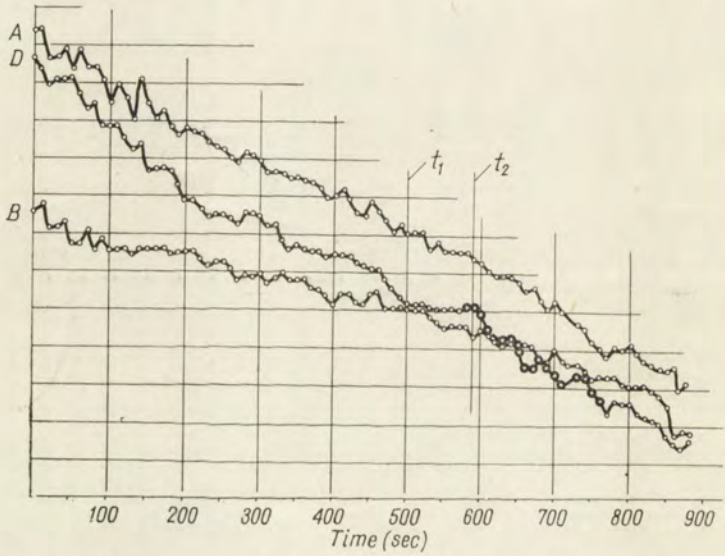


Fig. 10. Torsion of the *Physarum polycephalum* strand in frame of reference connected with the strand. Changes in position of markers A, B and D in a plane perpendicular to the longitudinal axis of the strand (see Fig. 8). At moment t_1 point B moved away from and point D approached to point C. At moment t_2 – point B approached, point D moved away

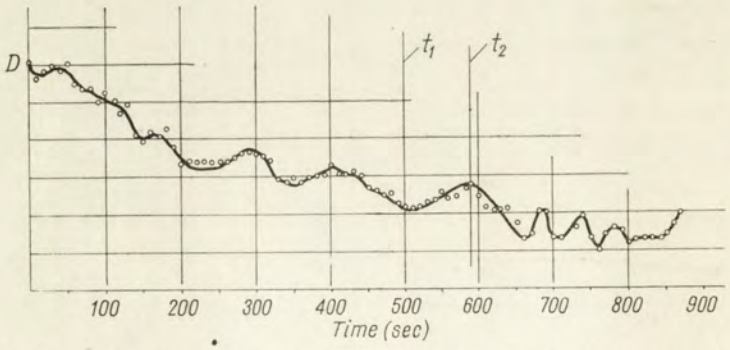


Fig. 11. Torsion of the *Physarum polycephalum* strand in frame of reference connected with the strand. Changes of position of marker D in a plane perpendicular to the main axis of the strand (see Fig. 8). The elementary unit on the axis of ordinates is 25 μm. At moment t_1 approach of points B and D, at moment t_2 – their moving apart

phases. At moments t_1 and t_2 marked on the diagram, points B and D approached and moved away from point C. At moment t_1 point B moved away and point D approached. At moment t_2 point B approached while point D moved away. As those changes were hardly visible the reference system was transferred to point B which has been shown on the diagram in Fig. 11.

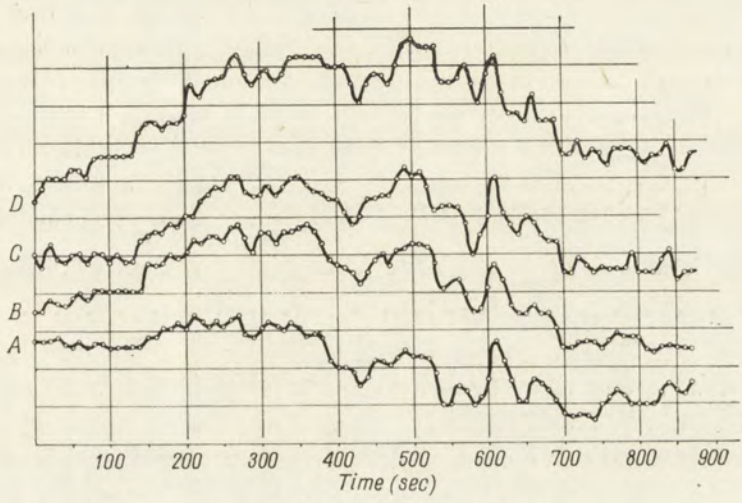


Fig. 12. Movement of strand surface of *Physarum polycephalum* along the longitudinal axis of the strand in relation to frame edge. Curves A, B, C, D show changes of position of markes A, B, C and D (see Fig. 8). The elementary unit on the axis of ordinates is 25 μm

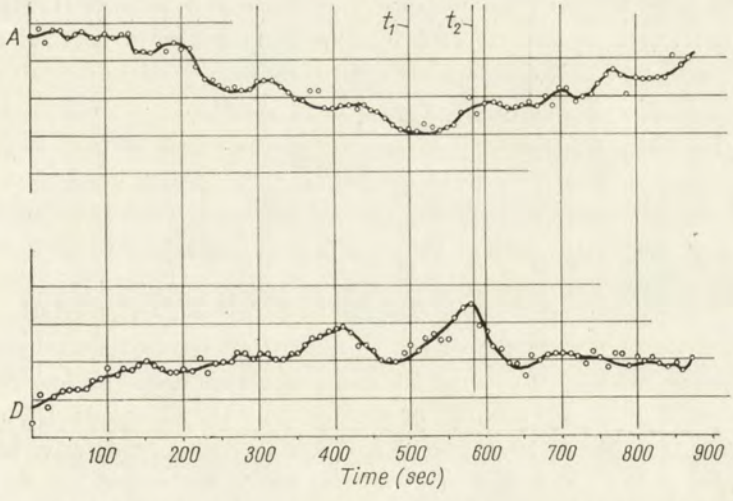


Fig. 13. Movement of strand surface of *Physarum polycephalum* along the longitudinal axis of the strand in frame of reference connected with the strand. Movement of markers A and D along the main axis of strand in relation to point C. The elementary unit on the axis of ordinates is 25 μm. At moment t_1 — approach of points A and D to point C. At moment t_2 — their moving away

The oscillatory character of the shifts of point D in relation to point B is very distinct here. The period of these changes is 150 sec. It can be seen that the approach of points B and D corresponds with moment t_1 and their moving apart — with moment t_2. In the model of the twist (Fig. 8) the upper arrows show the torsion of the area B and D in relation to C at moment t_1 and t_2. Simultaneously with the analysis of the torsion the author studied the movement of four points, A, B, C and D, along the longitudinal axis of the strand. The results have been presented in a diagram (Fig. 12). In this system the curves have a rather complicated course and it became possible to establish that points A and D oscillated in relation to each other when were analysed in relation to point C (Fig. 13).

Simultaneous analysis of twist and elongation of strand has shown that in the time t_1 the points A and D approach the point C, and in the time t_2 had moved away (Fig. 8). The lower arrows on the model (Fig. 8) illustrate this situation.

Conclusions

Until now no one has elaborated kinetic models of a *Physarum polycephalum* strand. That is why the discussion in the present paper is necessarily limited. It has not been noticed that a change of position of the strand from horizontal into vertical and vice versa should affect the behaviour of the strand. It is certain that there occur conformable periods of contraction and torsion when these two phenomena occur simultaneously. Considering the model of a twist described in the present paper it is also possible to explain why no visible connections between the twist and the contraction were observed in Kamiya and Seifriz's (1954) investigations. In the experiments performed by those authors both the torsion and the elongation were determined by the movement of the strand in its part above the free end. Thus the measured parameters were resultant parameters. Nothing is known of the character of the reciprocal displacements of parts of ectoplasma in a segment of few centimeters, while the model of torsion in a segment of 1 mm, eleborated in the present work, shows that the strand is not a tube twisting in the same way through the full length. The occurrence of pulsatory waves in the strands of *Physarum polycephalum* was established by Kamiya (1959, 1965, 1968) and by Stewart (1964). Until now it was thought that these waves propagate along the strand. Analysis of movements of the surface of an isolated strand reveals only a standing wave with length of ca. 2.8 mm and a period of 180 sec. Hence there exists a possibility of propagation of the wave by the plasmodium of *Physarum polycephalum* at a rate of 15 μm sec. The question whether this wave has a direct connection with the movement of cytoplasm inside the strand can be answered only in further investigations in which it is the author's purpose to measure simultaneously the pulsation and the torsion of the strand as well as the streaming of cytoplasm.

Summary

Fragments of the strand of *Physarum polycephalum* 0.5–1.0 cm of length and 0.2–0.4 mm of diameter, were isolated from the plasmodium and suspended by both ends in a moist chamber. Grains of potato starch or crystals of calcium oxalate were placed on the surface of the strand. During a space of time between 5 and 20 min after its separation from the plasmodium a 1.5 mm long fragment of the hanging strand was filmed or photographed in polarized light. The analysis of the films showed that on the isolated strand a standing wave of 2.8 mm of length and 180 sec period occurs. There exists a possibility of propagation of the wave along the strand of *Physarum polycephalum* at a rate of 15 μm sec. The connection of pulsation and of the simultaneous movement of the surface along the long axis of the strand has been illustrated on a model of a 1 mm long fragment of the strand. It has been demonstrated in the paper that there exists relation between the contraction of the strand and its torsion.

STRESZCZENIE

Izolowane z plazmodium fragmenty kanału *Physarum polycephalum* o długości 0,5-1,0 cm i średnicy 0,2-0,4 mm były zawieszone za dwa końce w komorze wilgotnej. Na powierzchni kanału znajdowały się naniesione ziarna skrobi ziemniaczanej względnie kryształy szczawianu wapnia. W okresie między 5 min i 20 min od czasu oddzielenia od plazmodium, 1,5 mm fragment wiszącego kanału był w świetle spolaryzowanym filmowany bądź fotografowany. W wyniku analizy filmów stwierdzono występowanie na izolowanym kanale fali stojącej o długości 2,8 mm i okresie 180 sek. Istnieje zatem możliwość propagacji fali po kanale plazmodium *Physarum polycephalum* z prędkością 15 μm/sek. Zależność pomiędzy pulsacją i jednoczesnym ruchem powierzchni wzdłuż osi podłużnej kanału przedstawiono w postaci modelowej dla odcinka kanału o długości 1 mm. W pracy wykazano istnienie związku pomiędzy skurczem kanału i jego skrętem.

REFERENCES

Camp W. 1936: A method of cultivating myxomycete plasmodium. Bull. Torrey Bot. Club, 63, 205–210.

Kamiya N. 1959: Protoplasmic streaming. In: Protoplasmatologia, (eds. Heibrunn L. V. and Weber F.), VIII/3a, Springer Verlag, Wienna, 1–199.

Kamiya N. 1965: Rheology of cytoplasmic streaming. Proc. Fourth int. Congr. on Rheology, 1, John Wiley and Sons, New York, 105–121.

Kamiya N. 1968: The mechanism of cytoplasmic movement in the myxomycete plasmodium. In: Aspects of Cell Motility, The 22nd Symp. Soc. exp. Biol., University Press, Cambridge, 199–214.

Kamiya N. 1970: Contractile properties of slime mold strand. Proc. Jap. Acad., 46, 1026–1031.

Kamiya N., Allen R. D. and Zeh R. 1972: Contractile properties of the slime mold strand. Acta Protozool., 11, 113–124.

Kamiya N. and Seifriz W. 1954: Torsion in a protoplasmic thread. Expl Cell Res., 6, 1–16.

Seifriz W. 1946: Torsion in protoplasm. J. Cell Sci., 1, 27–32.

Stewart P. A. 1964: The organization of movement in slime mold plasmodium. In: Primitive Motile Systems in Cell Biology (eds. R. D. Allen and N. Kamiya), Academic Press, New York, 69–78.

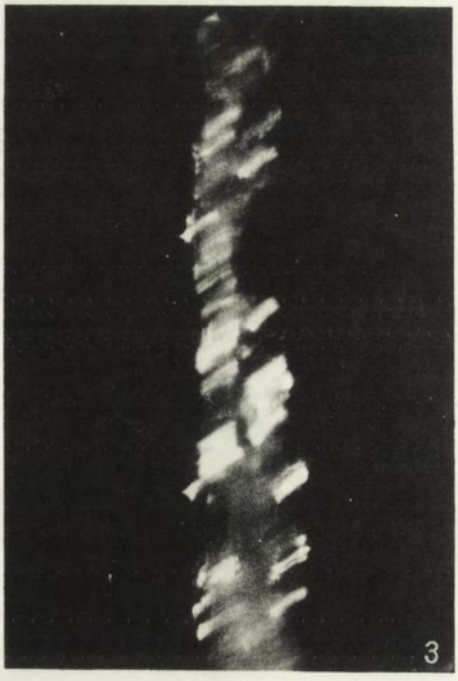
Received 15 October 1973

EXPLANATION OF PLATES I–II

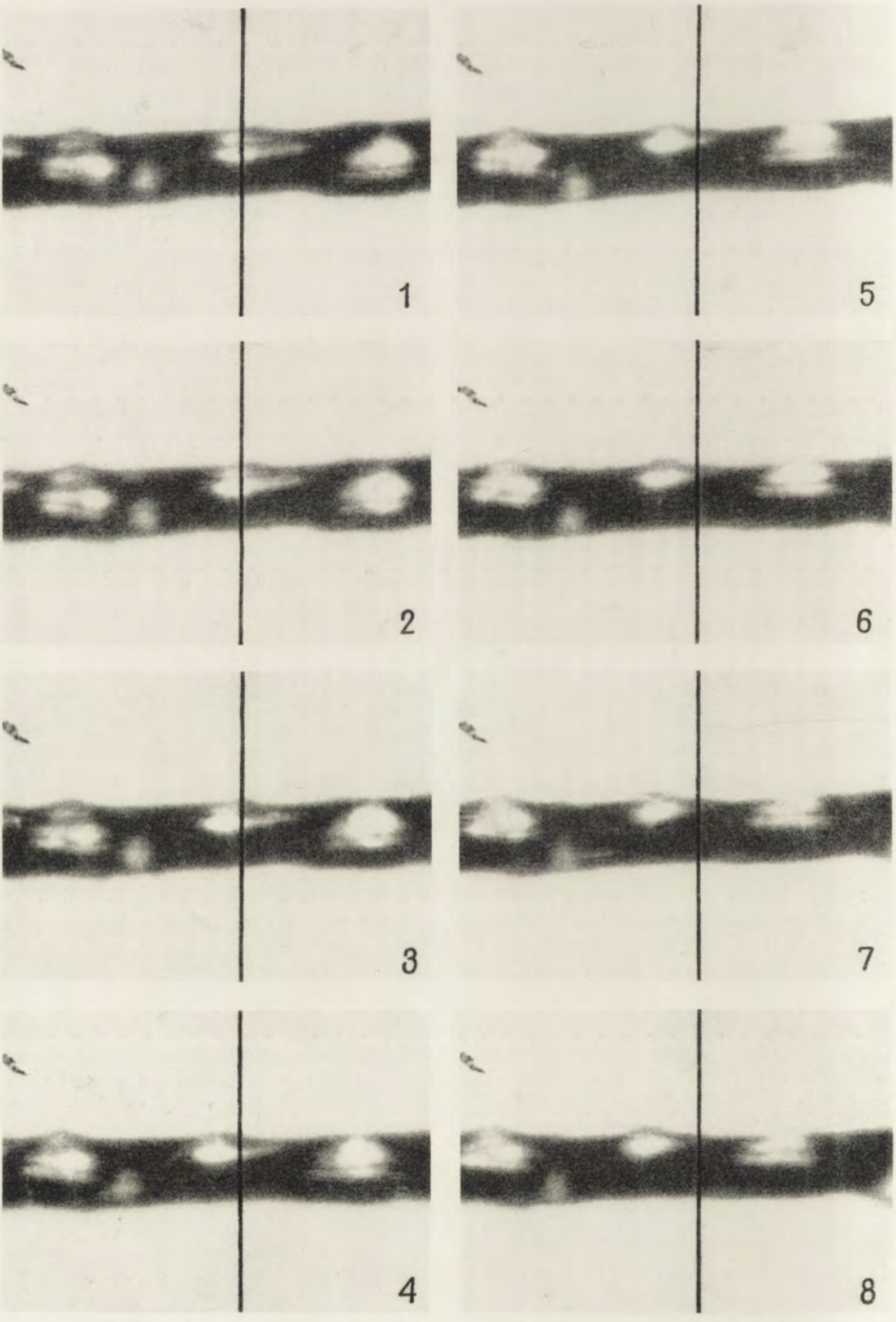
Plate I. The strand of *Physarum polycephalum* with crystals of calcium oxalate placed on surface. Pictures show successive phases of surface movement, taken in polarized light, exposure time 30 sec. Time intervals between shots: 1.2 — 32 sec, 2.3 — 1 sec, 3.4 — 32 sec

1: The surface of the strand does not move
2: Torsion with simultaneous contraction along the longitudinal axis of the strand
3: Surface movement causing the return of markers to
4: The surface of the strand does not move

Plate II. The strand of *Physarum polycephalum* with the grains of potato starch placed on surface. Time intervals between successive shots was 7 sec. The vertical line has been drawn with the purpose to show the moving markers along the longitudinal axis of the strand. Simultaneous twist of the markers is visible



Z. Baranowski auctor phot.



Z. Baranowski auctor phot.

Fasciculi praeparati:

M. Jerka-Dziadosz and I. Janus: Discontinuity of Cortical Pattern During Total Conjugation of a Hypotrich Ciliate *Paraurostyla weissei* — N. Ricci, R. Banchetti, R. Nobili and F. Esposito: Conjugation in *Oxytricha* sp. (*Hypotrichida, Ciliata*). I. Morphocytological Aspects — J. Kaczanowska: Shape and Pattern Regulation in Regenerants of *Chilodonella cucullulus* (O. F. M.) — A. Czapik: Les observations sur *Trimyema compressum* Lackey (*Ciliata, Trichostomata*) — I. G. Berezina: An Electron Microscope Study of Endosymbionts in *Blepharisma japonicum* — T. A. Шибалова, Т. И. Морозова и В. А. Кравцов: О соотношении роптрии и микронем у *Eimeria tenella* (*Sporozoa, Coccidia* [*Eimeria tenella* Microneme – Rhoptry Relationship] — G. B. Wilson, T. L. Jahn and J. R. Fonseca: Helical Nature of the Ciliary Beat of *Colpidium striatum* — T. V. Beyer, T. A. Shibalova and L. P. Ovchinnikova: A Comparative Cytophotometrical Investigation of the Dynamics of Cytoplasmic RNA in the Endogenous Development of *Eimeria tenella* Grown *in vivo* and in Tissue Culture. II. Macrogametogenesis — S. D. Amoji and S. S. Rodgi: On the Nature of Polysaccharide Reserve in the Gregarine, *Stylocephalus mesomorphi*. A Cytochemical Study — G. Csaba and T. Lantos: Effect of Amino Acid and Polypeptide Hormones on the Phagocytosis of *Tetrahymena pyriformis* — O. O. Dipeolu: The Infectivity to Tsetse Flies of Different Antigenic Variants of *Trypanosoma brucei* — J. Sikora: A New Method of Locomotion Arresting in Some Cilliates without Ciliary Immobilization

Państwowe Wydawnictwo Naukowe
(PWN — Polish Scientific Publishers)
Warszawa

Cena zł 22.—

Fasciculi: