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# On the variability in the norm of reaction of *Paramecium caudatum* to various cultivation temperatures

## Об изменчивости нормы реакции *Paramecium caudatum* на разные температуры культивирования

The vast experimental evidence obtained so far suggests that the thermoresistance of *Paramecium caudatum*, like that of other Ciliates, determined by the survival time at high lethal temperatures, changes significantly depending on the previous cultivation temperature (Poljansky and Orlova 1948, Poljansky 1957 a, b, 1971, Irlina 1960, Vogel 1966, Sukhanova 1968). The phenomenon of "heat hardening" (Aleksandrov 1956) is observed which means that thermostability much depends on the previous temperature regime: at relatively high heating thermostability increases, whereas at low temperatures it decreases. No exception has been found to this rule so far. Differences in the thermostabilities of "warm" and "cold" lines, expressed by the survival time at lethal temperatures (39 or 40°C) appear very remarkable. The survival time of the "warm" lines (cultured at 22–28°) exceeds that of the "cold" ones (cultured at 4–10°) by several times. This considerable variation lies within the limits of the norm of reaction and may be regarded as a modification being, in some cases, of adaptive value. The temperature induced changes in thermostability are completely reversible. However, prolonged treatment with temperature brings about prolonged modifications (Poljansky and Orlova 1948, Poljansky 1957, Genérmont 1966, 1970, Crippa-Franceschi 1958, 1970) whose nature still remains obscure.

Along with the above modificational changes, hereditary differences in thermostability are also observed. Ossipov (1966), using the conventional technique, examined thermostability of 59 clones of *P. caudatum* under standard cultivation conditions (25°). In the extremes, the thermostability of different clones differed more than by 7 times. Thus, in regard to the thermostability criterion, the occurrence of a pronounced intraspecies hereditary variation has been stated for *P. caudatum*. The latter co-existing with a broad modificational variation with regard to the same criterion.

The question of the species nature and structure in *Paramecium* and in other Ciliates, is rather complicated (Poljansky 1957 b, Sonneborn 1957, 1970). The

species, which is defined by morphologists and taxonomists as *Paramecium caudatum*, is known to involve a row of syngens (at least 16 have been described), each representing a closed genetic system unable to cross with other syngens and separating into two (in other species sometimes even more) mating-types. Thus, each syngen can be regarded as a genetically independent species ("biological species"), if its reproductive isolation is thought to be the main species criterion.

However, we are not fully aware if there are syngens in other Ciliates. The positive data available concern only few *Holotricha* species. In the majority of examined *Hypotricha* and *Heterotricha* (Demar-Gervais 1971) no syngens seem to occur. The whole reproductive isolation of syngens has not been proven even for *P. caudatum*. This enables us to consider *P. caudatum* not only as a "nomenclature unit", but also as a species with a complex interspecies pattern. It is in this sence that we use the notion "intraspecies variation" in *P. caudatum*. The literature data, yet unsufficient, suggest a great variety in species structure in ciliates.

The character of intraspecies variety in thermoresistance can be analysed by methods other than the above procedures. How extensive may be the variations of thermoresistance of different clones within the same species? Is there any "variation of variation" of this character and which is the extent of this variation? The present study is an attempt to solve these questions using *P. caudatum* as a material.

The questions raised may be regarded at least from two main points. From the cytophysiological aspect, the comparative study of variability in thermoresistance can provide evidence relevant for analysing the nature of changes underlying the temperature adaptation. From the genetical and evolutionary aspects, the study of intraspecies variation in respect to the physiological character in *Protozoa* is essential for characteristics of natural populations.

## Material and methods

Thirty six clones of *Paramecium caudatum* of different origin were examined. Most part of these were collected in the ponds of Leningrad district, others originating from the clones cultivated in the laboratory for several years. Some clones have originated from natural ponds of the Soviet Union — in the vicinities of Yakutsk, Sakhalin, fresh-water ponds of the Crimea, the vicinities of Frunze, Kaunas. Our clones have not been identified with the syngens established for *P. caudatum* by the American and Japan authors (Sonneborn 1957).

The cultivation was performed using the Sonneborn technique with lettuce infusion in addition to feeding on *Aerobacter aerogenes*. For thermostability-testing the cliates were examined on one and the same developmental stage — at the early stationary phase. The survival time of ciliates subjected to 39° was determined using conventional technique accepted at our Laboratory (Poljansky 1957). Each experiment involved not less than 100 animals. All the clones were cultivated at 28, 22, 17, 10 and 4°, resp., with rare missings in some temperature regimes. To provide the most strict identity of experimental conditions, the same ultrathermostate and even the same glace chamber were employed throughout the whole investigation.

The data obtained are treated statistically.

## Results

The results obtained are summarized in Table 1. Data of particular experiments are arranged in a definite order. Horizontal lines show data on the thermoresistance of each particular clone. The numerical material is oriented from the higher thermostability at 28° to the lower one. All the 36 clones tested display a picture which could be often observed by earlier authors: within each clone, lines previously cultivated at higher temperatures show higher thermostability. However, the extent of variation in respect to the thermostability trait appears to be different in different clones. A comparison of the extreme cases (No. 1 and No. 36) shows, for example, that the ratio of thermostability values, resp., at 28 and 4°, is 22.5 and 2.6, accordingly for clones Yak-5 and DVP-1. Again, the thermostability of clone Yak-5, if compared at equal temperatures of cultivation, appears to be much higher than that of clone DVP-1.

Obvious cases of variation in thermostability extent are seen in Fig. 1a, where thermostability curves for four clones are given: No. 1 (Yak-5), No. 3 (Sh-5), No. 11 (Li-7) and No. 34 (Gt-11-3).

A comparison of various clones (Table 1, Fig. 1) as to the extent of thermostability variation at various cultivation temperatures reveals a wide heterogenic within the limits of a species. Of particular interest seems thermostability of lines kept at 4°. When challenged with 39°, thermostability of these "cold" lines can be measured by several minutes in some clones (Table 1, clone 1 — 5.3 min, clone 7 — 5,4 min, clone 12 — 3.5 min, clone 28 — 3.8 min), whereas in other groups of clones the animals are seen to die within the first minute of the challenge. Accordingly, it seems appropiate to remind of a strong cold-resistance of some clones of *P. caudatum*. Poljansky and Posnanskaja (1964) reported that some lines of this species could be successively cultivated at 0° over several years, without any visible depressive events.

Significant interclone differences are seen in the shape of the thermostability curve. Fig. 1b illustrates some cases of sharply different profiles of the curves. In clones No. 6 (Mg-1a[3]) and 9 (DV-1) the increase in thermoresistance at elevated cultivation temperatures slips down rather smoothly and gradually. In other clones the curve shape appears quite different. The same Fig. 1b shows clones No. 13 (Li-9) and 24 (S-7) whose thermostability curves are moving up progressively and insignificantly from 4° to 22, whereas within the 22–28° interval thermostability rises up abruptly. Of special character is the curve shape in clone 36 (DVP-1) with a smooth, gradual and insignificant increase within the whole range of cultivation temperatures (4–28°C). In addition to these illustrations, other similar or intermediate positions of the curves can be easily figured out from the numerical data given in Table 1. Thus, within a species, the termostability curve configuration also involves a wide variability which is especially obvious when different clones are compared.

Table 1

The rate of survival of *Paramecium caudatum* (the average time in minutes) at 39° after preliminary cultivation at various temperatures

| No. | Clone | Locality | Preliminary temperatures of cultivation | | | | |
|---|---|---|---|---|---|---|---|
| | | | 28° | 22° | 17° | 10° | 4° |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | Yak-5 | Yakutsk | 119.3±0.4 | 60.9±1.1 | 25.9±0.5 | 11.1±0.2 | 5.3±0.1 |
| 2 | Yak-3 | Yakutsk | 71.0±0.3 | 17.25±0.2 | 7.2±0.5 | — | 2.2±0.1 |
| 3 | Sh-5 | Shapki, Leningrad district | 56.8±1.9 | 20.6±0.5 | 10.6±0.1 | 6.9±0.2 | 1.5±0.1 |
| 4 | Sh-4 | Shapki | 44.2±0.6 | 26.1±0.1 | 14.8±0.5 | 5.4±0.2 | 2.8±0.1 |
| 5 | Yas-2 | Yakutsk | 40.5±0.6 | 15.9±0.7 | 14.9±0.5 | — | 4.2±0.1 |
| 6 | MGI-a[3] | Moscow | 39.1±0.6 | 20.4±0.6 | 18.5±0.6 | 6.1±0.1 | 5.4±0.1 |
| 7 | DV-3 | Far East | 37.1±0.6 | 26.1±0.4 | 11.3±0.2 | 4.1±0.1 | 4.0±0.1 |
| 8 | Yas-4 | Yakutsk | 36.9±0.5 | 15.3±0.6 | 10.4±0.3 | 5.1±0.1 | 3.0±0.1 |
| 9 | DV-1 | Far East | 31.9±0.2 | 25.0±0.1 | 17.3±0.3 | 3.2±0.1 | 2.0±0.1 |
| 10 | Sakh-7 | Sakhalin | 30.5±0.2 | 8.1±0.1 | 9.0±0.2 | 5.5±0.1 | 3.0±0.1 |
| 11 | Li-7 | Kaunas | 30.3±0.2 | 21,6±0.4 | 13.6±0.3 | 5.8±0.1 | 1.2±0.1 |
| 12 | A-1 | Crimea | 28.1±0.2 | 13.4±0.1 | 11.2±0.2 | 4.8±0.1 | 3.5±0.1 |
| 13 | Li-9 | Kaunas | 28.1±0.2 | 5.8±0.1 | 2.5±0.1 | 1.8±0.1 | 0.5 |
| 14 | Bi-4 | B. Izhora, Leningrad district | 27.5±0.6 | 9.7±0.1 | 8.2±0.1 | 3.9±0.1 | — |
| 15 | Sh-3 | Shapki, Leningrad district | 27.2±0.5 | 11.2±0.4 | 5.8±0.4 | 1.8±0.1 | 1.0±0.1 |
| 16 | Li-6 | Kaunas | 26.7±0.3 | 12.5±0.2 | 11.7±0.2 | 5.0±0.1 | 2.0±0.1 |
| 17 | F11-9 | Frunze | 25.6±0.1 | 11.8±0.1 | 7.2±0.1 | 1.7±0.1 | 1.1±0.1 |
| 18 | F11-13 | Frunze | 25.0±0.2 | 13.7±0.2 | 8.9±0.1 | — | 2.3±0.1 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 19 | Bi-10 | B. Izhora, Leningrad district | 24.7±0.5 | 7.8±0.1 | 8.6±0.1 | 4.4±0.2 | 1.5±0.1 |
| 20 | Sakh-10 | Sakhalin | 24.4±0.1 | 8.6±0.2 | 5.2±0.1 | — | 1.0 |
| 21 | F11-10 | Frunze | 24.1±0.3 | 13.1±0.2 | 7.4±0.2 | 1.7±0.1 | 1.1±0.1 |
| 22 | D11-11 | Duderhoff, Leningrad district | 24.0±0.5 | 14.9±0.1 | 8.9±0.1 | 5.5±0.1 | 3.9±0.1 |
| 23 | Gt111-6 | Gatchima, Leningrad district | 23.7±0.3 | 7.1±0.2 | 5.5±0.1 | 3.4±0.1 | 1.2±0.1 |
| 24 | S-7 | Strel'na, Leningrad district | 22.6±0.3 | 7.7±0.1 | 6.6±0.4 | 4.1±0.1 | 1.8±0.1 |
| 25 | S-16 | Strel'na | 21.5±0.2 | 9.0±0.1 | 8.6±0.1 | 4.0±0.1 | 2.7±0.1 |
| 26 | Gt111-2 | Gatchina, Leningrad district | 20.2±0.2 | 8.7±0.2 | 2.3±0.1 | 2.6±0.1 | 2.2±0.1 |
| 27 | F11-15 | Frunze | 19.6±0.3 | 8.5±0.2 | 5.0±0.2 | 2.5±0.1 | 1.2±0.1 |
| 28 | P-4-1 | Pushkin, Leningrad district | 19.6±0.1 | 10.3±0.1 | 7.3±0.1 | 4.1±0.1 | 3.8±0.1 |
| 29 | ChR-5 | Chernaya rechka, (Black river), Leningrad | 19.0±0.3 | 11.0±0.1 | 6.1±0.2 | 3.6±0.1 | 1.3±0.1 |
| 30 | Li-2 | Kaunas | 18.5±0.4 | 7.6±0.2 | 2.5±0.1 | 1.0 | 1.0 |
| 31 | Bi-5 | B. Izhora, Leningrad district | 17.2±0.1 | 13.6±0.1 | 8.8±0.1 | — | — |
| 32 | Chr-3 | Chernaya rechka | 16.9±0.2 | 11.5±0.2 | 8.7±0.1 | 1.5±0.1 | 1.7±0.1 |
| 33 | F-8 | Petershoff, Leningrad district | 14.7±0.4 | 7.0±0.3 | 5.0±0.1 | 2.5±0.1 | 2.2±0.1 |
| 34 | Gt11-3 | Gatchina | 12.0±0.2 | 5.1±0.2 | 4.7±0.8 | 3.7±0.1 | 2.4±0.1 |
| 35 | P-7 | Pavlovsk, Leningrad district | 11.4±0.6 | 6.2±0.3 | 2.4±0.2 | 2.4±0.1 | — |
| 36 | DVP-1 | Far East | 7.0±0.1 | 5.1±0.1 | — | 4.3±0.1 | 2.7±0.1 |

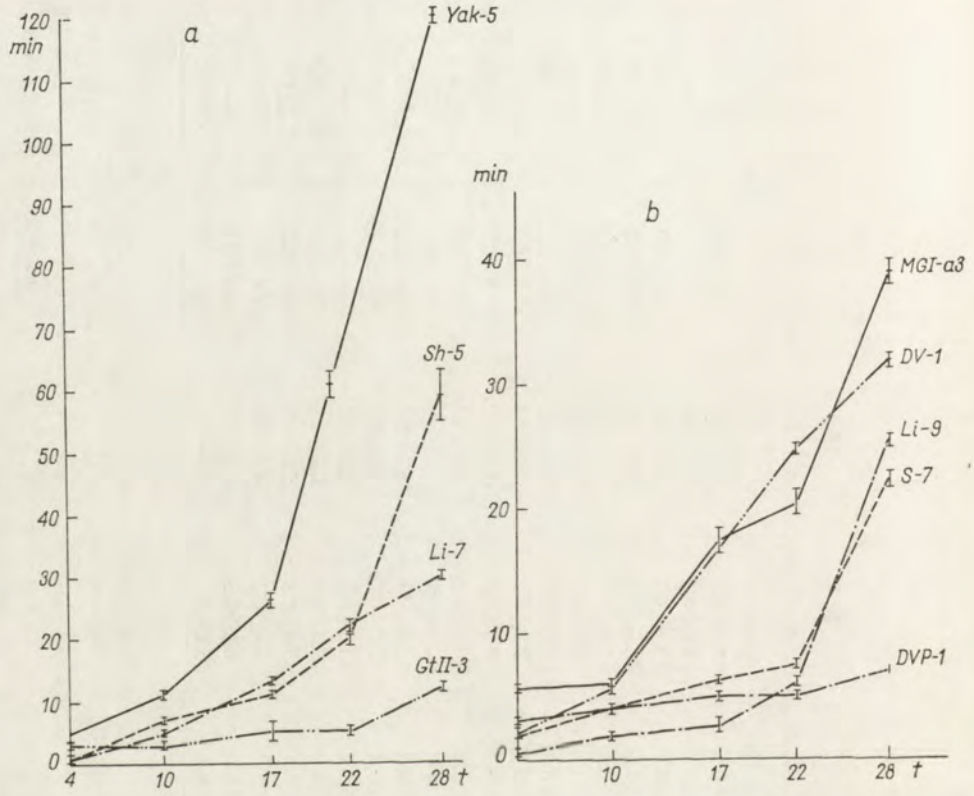


Fig. 1. Variations of thermostability among various clones of *Paramecium caudatum*, adapted to different temperatures of cultivation. a — clones which differ mainly with respect to the range of thermostability changes, b — clones which differ mainly with respect to the mode of temperature dependence of thermostability.
Abscissa — temperature of cultivation (°C)
Ordinate — mean survival time of cells at 39°C (min).

To what extent a certain level of thermostability, at a given temperature of cultivation, appears to be constant for one and the same clone? Experiments that involved repeated estimation of thermostability of some individual clones have suggested that within a clone, thermostability is conserved at a close level if the environmental conditions are similar (temperature of cultivation, density and development phase of the culture etc.).

Some data of experiments carried out in 1968 are given below. Lines of clone 1 (Yak-5) kept at 28° demonstrated the following values of thermostability while challenged at 39°: 70.0±1.0 min (16.II), 78.8±1.1 min (27.II), 119.3±0.4 min (9.IV). Lines of the same clone but kept at 22°: 69.5±1.3 min (27.II), 66.2±0.7 min (5.III), 67.7±0.9 min (12.III), 60.9±1.1 min (9.IV). The same at 17°: 24.0±0.5 (12.III), 25.8±1.0 (6.V). Lines of clone 8 (Yak-4) kept at 28°: 36.9±0.5 min (14.V), 24.3±0.6 min (18.VI), 30.4±0.8 min (28.VI). Lines of the same clone but kept

at 22°: 15.3±0.6 min (14.V), 13.2±0.3 min (18.VI), 15.6±0.5 min (28.VI). Lines of clone 6 (MG-1a[3]) kept at 28°: 38.1±0.3 min (28.II), 39.0±0.6 min (16.IV), the same clone but kept at 22°: 23.0±0.6 min (28.II), 20.0±0.6 min (5.III), 20.4± ±0.6 min (16.IV), 22.3±0.7 min (24.IV). Similar results, here omitted, were obtained for some other clones. Thus, the level of thermoresistance and the norm of reaction in respect to the character of thermoresistance represent clone properties to be inherited throughout asexual reproduction.

Among all the 36 clones examined, two exceptions to the general rule that thermostability changed with the environmental temperature have been observed. The thermoresistance of the two clones (No. 10, Sakh-7, and No. 19, Bi-10, Table 1) at 17° exceeded somewhat that at 22°. Since these discrepancies did not exceed one minute, this may be presumably due to some technical reasons (the number of animals that die at 39° is counted once a minute). An assumption may be drawn that in the above two clones no significant difference in thermoresistance exists between the lines kept at 17° and 22°, resp. More detailed examination of numerical data presented in Table 1 shows close values of thermostability at 17° and 22° for some other clones as well. It does not seem unlikely that the two temperature regimes, close to the optimal one, exert little, if any, effect on thermostability.

It has been reported elsewhere that changes in thermoresistance, arising due to cultivation at different temperatures, are accompanied with parallel non-specifically alterated resistance to lethal action of ethanol (Poljansky 1957 a, Irlina 1963, Sopina 1963, Sukhanova 1968). Simultaneously with the studies into thermoresistance of the above clones, their resistance (survival time) to 8 per cent alcohol was also examined. The clones of choice were those with contrast norm of reaction as to their thermostability. In all, six clones were subjected to the parallel examination. Table 2 summarizes data on thermoresistance of these clones at 10° and 28°, and on alcohol resistance of the corresponding material. The numerical material is oriented from clones with the wide norm of reaction to those with the poor one. For the sake of obviousness, the extent of variation is calculated in per cent.

Table 2

Comparison between levels of the rise of thermoresistance (TR) and of resistance to 8% ethanol (AR) (the average time in minutes) in various clones of *Paramecium caudatum* cultivated at 10 and 28° (clone designation as in Table 1)

| No. | Clone | TR | | $TR_{28} : TR_{10}$ | AR | | $AR_{28} : AR_{10}$ |
|---|---|---|---|---|---|---|---|
| | | at 10° | at 28° | in per cent | at 10° | at 28° | in per cent |
| 1 | Yak-5 | 6.1 | 78.8 | 1313 | 11.8 | 15.0 | 128 |
| 9 | DV-1 | 3.2 | 31.9 | 966 | 6.4 | 11.0 | 172 |
| 18 | Bi-10 | 4.4 | 24.7 | 630 | 5.3 | 9.4 | 177 |
| 11 | Li-7 | 5.8 | 30.3 | 522 | 7.3 | 9.3 | 130 |
| 34 | Gt11-3 | 3.7 | 12.0 | 325 | 3.4 | 18.8 | 553 |
| 36 | DVP-1 | 4.3 | 7.0 | 163 | 6.7 | 9.3 | 139 |

Similar data for alcohol-resistance are given in the right half of the Table 2. In all the six cases examined, the alcohol resistance of ciliates kept at 28° appeared to be higher than that at 10°. This has acknowledged once more the earlier traced paralel changes in resistance to temperature and to alcohol. The extent of variation (norm of reaction) in respect to the thermal and alcohol resistance is not the same. The most extended norm of reaction thermostability is observed in clone No. 1 (Yak-5) as has been pointed out above. This clone has displayed the most reduced norm of reaction in respect to its alcohol resistance. In contrast a strong variability in alcohol resistance (553%) was shown by clone No. 34 (Gt 11-3) which displayed a rather poor extent of the norm of reaction of thermostability.

## Discussion

Since the clones under examination have not been identified with the known syngens of *P. caudatum*, the studied variation is nothing but variability of random sample of clones with diverse origin. The most essential seem the sharp interclone differences traced in regard to the norm of reaction, rather than the occurrence of heritable intraspecific differences in regards to the thermostability character. From the point of this new approach, studies into intraspecific variation of *Protozoa* still remain insufficient, and to our knowledge hardly a few similar evidence has been reported so far. Poljansky and Strelkov (1938) who studied intraspecific variations of morphological characters with Ciliates of the *Ophryoscolecidae* family, provided evidence on significant differences in the extent of variation between forms A and B of *Entodinium caudatum*.

However, one question still remains unsolved: is different norm of reaction with regard to thermostability character of any adaptive value for the species existence under particular ecological conditions? It does not seem improbable that a widely extended variation may be of selective importance for the inhabitants of water pools with sharp temperature fluctuations.

Similar studies into the norm of reaction within a species are to be extended to include a variety of physiological characters with other species.

The clones under study are of diverse geographical origin (Table 1). A question arises if there is any relation between the geographical origin of the species, the level of its thermoresistance and the norm of reaction with regard to thermostability character. No such relation can be established from the analyses of Table 1. However, this statement is not to be regarded as conclusive, because the amount of material taken from each particular geographical point seems to be insufficient. Smaragdova (1941) reported the occurrence of geographical variation with regard to the temperature optimum of division for three species of *Paramecium* (*P. caudatum*, *P. aurelia* and *P. bursaria*), as well as to the body size (for *P. aurelia*). However, here material including only a few clones does not seem conclusive.

Different syngens of *P. caudatum* are far from being found everywhere (Sonneborn 1957) and seem to be confined to definite areas. Thus, from the total of 16 syngens, numbers 1 and 3 are met with in Japan and the USA, 12 and 13 — only in Japan, 2 — in North and South Americas, 15 and 16 — only in Europe etc. For solving the problem of intraspecies geographical variation of ciliates further studies involving greater comparative material are needed, taking into account both morphological and physiological characters.

How to explain the drastic differences in the shape of curves reflecting changes in thermostability between different clones (Fig. 1b)? Speculating on temperature depending changes in the definite physiological trait — thermostability, one could expect a priori, that the curve shape should be uniform, if not identical, in all the cases examined. This assumption is not, however, substantiated with facts available.

In recent years Aleksandrov (1965) advanced a hypothesis accordingly to which the cell thermostability level may depend on the protein molecule conformation. The level of the conformational flexibility of a protein molecule is thought to underlie the level of protein thermoresistance and, accordingly, the primary thermoresistance of the cell. If this hypothesis is true, it may appear that the conformational flexibility of protein molecules of Ciliates cultivated at various temperatures cannot be identical due to their different thermostability. This flexibility must vary in the course of the "hardening" to a definite temperature. Variations in the shape of thermostability curves may suggest that thermoresistance character is specified by more than one cause, i.e., by various protein groups. One may assume that thermoresistance of some particular clones could be determined by one kind of protein complexes, whereas another kind of these, with different run of temperature depending conformational changes in protein molecules, may be involved in other clones. The facts observed in our study are in agreement with the idea that thermoresistance is an "resultant" of many biochemical properties of the cell.

The above evidence of the present authors along with Ossipov's data (1966) show that a certain level of thermoresistance as well as the shape of thermoresistance curve are heritable traits by which individual clones of *P. caudatum* can be distinguished from each other. Which is the nature of these heritable differences? Are the latter genotypical differences or phenomena of another kind like those known as epigenetic variability (Nanney 1958, Ephrussi 1958, Olenov 1967)? The epigenetic changes involve no mutational transformation of the genotype, the gene system remaining unchanged, however, the activity of individual genes becomes different to be conserved throughout some cell generations. There is every reason to believe that the epigenetic changes may serve as one of the main machanisms of cell differentiation in the metazoan ontogenesis (Olenov 1967).

Ossipov (1966) who studied hereditary mechanisms underlying the thermoresistance of *P. caudatum*, reported the appearance of various inherited levels of thermoresistance in different caryonids originated from the same conjugating couple. It is bound to stress that all such caryonids have the same genotype. The intra-

caryonid differences in thermostability arise within a short period of differentiation of the macronucleus to persist throughout the whole period of asexual reproduction up to the next conjugation. This form of variability, operating with identical genomes, is presumably associated with changes in the genome machinery and must be determined as epigenetic variability.

Since the norm of reaction with regard to thermoresistance has not been analysed through the ex-conjugant progeny, we have no sufficient reason to speculate on the nature of the variability observed.

Further studies of intraspecific variation in Ciliates with regard to physiological characters seems to be essential for our knowledge of the species structure and of the nature of physiological adaptations to the environment.

## Summary

Using 36 clones of *Paramecium caudatum* from various geographical localities over the Soviet Union, a wide intra-specific inherited variability in the norm of reaction towards the thermoresistance character was followed.

The shapes of thermostability curves obtained at different temperatures of ciliate cultivation display an appreciable diversity, thus suggesting a complicated biochemical and functional conditionality of the thermoresistance level.

At similar temperature conditions of cultivation there is a certain parallelism between the patterns of changes in thermostability and in alcohol-resistance of the ciliates. However, no such parallelism was followed with respect to the extent of variation (norm of reaction).

## РЕЗЮМЕ

На клонах *Paramecium caudatum* показана широкая внутривидовая изменчивость нормы реакции по признаку теплоустойчивости.

Ход кривых изменения теплоустойчивости при культивировании инфузорий в разных температурах обнаруживает значительное многообразие, что указывает на сложную биохимическую и функциональную обусловленность уровня теплоустойчивости.

Имеет место параллелизм между направлением изменений теплоустойчивости и спиртоустойчивости при культивировании инфузорий в разных температурах. Однако по признаку размаха изменчивости (нормы реакции) такого параллелизма не существует.

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# An electron microscopic study of *Balantidium caviae*

## L'étude au microscope électronique de *Balantidium caviae*

The fine structure of several species of *Balantidium* has been described: *Balantidium xenopi* (de Puytorac and Grain 1965); *B. elongatum*, *B. coli*, (Grain 1966); *B. caviae* (Nelson 1969); and *B. testudinis* (Grain 1969 a). These studies have revealed the complex microtubular system associated with the vestibular ciliature and have aided in elucidating the taxonomic status of the genus *Balantidium* (Grain 1966). With the exception of Nelson's study (1969) little is known about the fine structure of *B. caviae*, particularly the macronuclear envelope, cortical fibrillar elements and the ecto-endoplasmic interface which according to Grain (1969 a) is variable.

This study examines the ultrastructural anatomy of *B. caviae* and compares it with other species of balantidia. Curious club-shaped and other abnormal cilia revealed in this investigation are described.

## Material and methods

*Balantidium caviae* was obtained from the cecum of the domestic guinea pig, *Cavia porcella*, and fixed for one hour in 2% osmium tetroxide (pH 7.4, veronal-acetate buffer). The cells were dehydrated in a graded series of ethanols and embedded in Mollenhaur's mixture of Epon and Araldite (Mollenhaur 1964). Thin sections were cut on a Porter-Blum $MT_2$ microtome; stained with saturated uranyl acetate (50% ethanol) and/or lead citrate before viewing in a Jem 6C or Siemens 101 electron microscope.

## Observations

It is our intent in this paper to report new pertinent observations on some aspects of the fine structure of *Balantidium caviae* Neiva, de Cunha et Travassas. Therefore, a detailed light microscopical description is not included.

*Balantidium caviae* is egg-shaped and uniformally ciliated, approximately 80 rows of cilia adorn the organism along the anterior posterior axis. A small slit-like depression (ca. $15 \times 5$ μm), the vestibulum, is found at the tapering anterior apex.

The vestibulum is not completely lined with cilia only the posterior ventral portion is ciliated, approximately 1/4 of the total vestibular surface. The remainder of the vestibular surface is barren. This small patch of vestibular cilia appears to be a continuation of the somatic ciliary meridians converging into the depression rather than specialized oral cilia.

A large sausage-shaped or bean shaped macronucleus and a small ovoid micronucleus are found in the vesicle filled cytoplasm.

Transverse (Pl. I 1) and longitudinal (Pl. I 2) sections through the somatic and vestibular ciliary meridians reveal four fibrillar structures associated with each kinetosome. These, according to the terminology of Grain (1969) and Pitelka (1969), are designated as the kinetodesmal fibrils, postciliary microtubules, left tangential microtubules (transverse) and the nemadesma.

The kinetodesmal fibrils (KD, Pl. I 1–2) originate near the anterior right margin of each kinetosome, extend upward toward the cell surface, turning anteriorly below the pellicular membrane. Each fibril appears to extend past its anterior neighbor, whether it extends beyond is difficult to ascertain. The compact stacking of kinetodesmal fibrils evident in *Paramecium* is not apparent in *Balantidium* (Pitelka 1965). In longitudinal sections a prominent 28 nm banding pattern is evident along the fibril (Pl. I 2), cross section reveals no such pattern (Pl. I 1).

The postciliary microtubules originate at the posterior right margin of each kinetosome (Pl. I 1–2). These microtubules (ca. 4–5 counted) course upward and posteriorly away from the kinetosome. In figure two it can be seen that the postciliarys (PC) diverge from the kinetosome and the kinetodesmal fibril; the kinetosome is out of the plane of section in this micrograph.

The left tangential microtubules originate near the anterior left margin of the kinetosome (Pl. I 1). This group of microtubules appears to be composed of a single row of four or five microtubules. However, in some sections a second row may be encountered (arrow, Pl. I 1). These microtubules like the postciliarys extend upward toward the cell surface, turning medially below the cell surface not posteriorly like the postciliarys. Their terminus is unknown, although they may extend to adjacent kineties as, in *Tetrahymena* (Allen 1967).

The nemadesma of the vestibular and circum-vestibular kinetosomes possess parallel rows of microtubules which extend into the endoplasm. Each nemadesmos consists of between 10–12 microtubules (Nelson 1970, reports 17) arranged in 2 parallel rows, arising from an electron dense amorphous mass below each kinetosome (Pl. II 3–4). An electron translucent region is present between the electron dense material and the microtubules (arrow, Pl. II 3). Collectively, the nemadesmal microtubules appear to form a large funnel or basket about the vestibulum and cytopharyngeal complex which is found at the base of the vestibular depression. Figure four is a section through the vestibular wall in which the barren ectoplasmic region (viewers left) and the ciliated portions can be compared. Note in particular the dense amorphous material and microtubules on the right. Figure three is a section

near the rim of the vestibulum the close apposition of the cilia, separated by low cytoplasmic ridges is characteristic of this region.

Somatic kinetosomes possess root-like microtubules similar to the nemadesma of the restricted oral region. The number of microtubules per kinetosome in the posterior extermities of the organism is reduced (i.e., 4–5 microtubules per kinetosome). Therefore, all somatic kinetosomes and vestibular kinetosomes bear microtubules which extend into the endoplasmic region.

The macronucleus when viewed in cross section is surrounded by a corrugated appearing double membrane (Pl. III 5–7). Within the electron translucient karyoplasmic matrix are found nucleolar-like bodies (NC) and electron dense chromatin clumps (arrows, Pl. III 5). Close inspection of the nuclear envelope (Pl. III 6) reveals that the two membrane-like structures are (arrows, Pl. III 6) separated by a space of approximately 23 nm. Although in our material two unit membranes can not be resolved, it does appear that they are present. Chromatin masses of varying size are frequently found attached to the inner "membrane" (Pl. III 6). Grain (1969 b) also observed similar clumping of the chromatin to the inner membrane of *B. testudinis*.

The micronucleus consists of clumped chromatin, two regions of karyoplasm; one dense almost nucleolar appearing in its consistency and a less dense translucent region (Pl. III 7). The micronucleus also appears to be surrounded by a double nuclear membrane (not shown).

A moderately electron dense region between 150–200 nm thick separates the ectoplasmic from the endoplasmic region (Pl. I, II 2–3, Pl. IV 8–9). The ecto-endoplasmic region is made up of minute filaments which form an anastomosing network; this is particularily evident in sections cut obliquely through the ectoplasmic region (Pl. I 1). The dense filamentous material is less distinct around the kinetosomes and is only found around the proximal ends of the kinetosomes (Pl. I 2, 3).

Abnormal cilia have been observed on the surface of *B. caviae*, particularly in the circum-vestibular region. These abnormal cilia are of three types: (1) those with ciliary axonemes which appear in the cytoplasm of the ectoplasmic ridges, often the axonemes are disorganized and do not show the typical 9+2 pattern (arrows, Pl. IV 8); (2) short clavate or balloon shaped cilia (Pl. IV 9); (3) two axonemes contained within a single limiting membrane and projecting from the cell surface (Pl. IV 10). The club shaped cilia (Pl. IV 9) are curious. The ground plasm surrounding the axoneme is more electron dense than the ground plasm of normal cilia (e.g. compare Pl. IV 8 with Pl. IV 9). The axoneme other than being short often has the typical 9+2 pattern when viewed in cross section (not shown).

## Discussion

Within the kinetosomal territory of each somatic kinetosome are found the kinetodesmal fibril, postciliary microtubules and transverse microtubules. These

somatic fibrillar and microtubular elements are topographically arranged about the kinetosome in a pattern strikingly similar to other holotrich ciliates (Grain 1969 a, b, Pitelka 1969) and some heterotrich ciliates (Paulin 1967); the kinetodesmal fibril not well defined in the latter.

Club-shaped or clavate cilia have been reported in *Didinium nasutum* (Dippell and Grimes 1966; Small et al. 1971). Their function is unknown, although in *Didinium* (Dippell and Grimes 1966) they appear near the primordial sites of the developing ciliary girdles in division. In *Balantidium* these abberrent cilia have not been previously reported. We have observed them in the vestibulum and on the somatic surface near the vestibular orifice. They are sparse and do not appear concentrated in a particular area which might suggest they may be possible primodial site(s) for new somatic or vestibular cilia. "Soie sensorielle" or sensory bristles have been observed on the dorsal side of the proboscis of *Dileptus cygnus* (Grain and Golińska 1969); Grain (1970) has also described similar clavate cilia on the dorsal surface of *Lagynophrya fusidens*. Their function in these two ciliates is unknown. Grain (1969 a, b) suggests they may have some osmotic sensing function. It is of interest that in our material and in *Dileptus* and *Lagynophrya* they are found in the vicinity of the oral area. Perhaps, in some unknown way they may be associated with the feeding process.

Intact axonemes as well as disorganized axonemal microtubules have been observed in the ectoplasmic region between the somatic and vestibular cilia. The possibility that these may be resorbed or cilia in various stages of neoformation needs to be investigated. Resorption of cilia has been reported in *Cyathodinium piriforme* (Paulin 1968) and *Diplodinium ecaudatum* (Roth and Shigenaka 1964). In *Diplodinium* the cilia are resorbed and contained in a vesicle and degraded by an autolytic process. In *Cyathodinium* no vesicles are formed to contain the resorbed cilia, although lysosomal-like bodies are seen in the vicinity of the resorbed cilia (Paulin unpublished observations). In balantidia examined to date no vesicles or lysosome-like bodies have been observed encapsulating the axonemes or in the vicinity of the axonemes. We can not find any evidence to suggest that these subpellicular axonemes are in stages of assembly, possibly leading to the formation of new cilia. Until further studies are made it is difficult to determine with any certainty if these intracellular axonemes are being resorbed or in stages of assembly. Whether there is any relationship between the club-shaped cilia which infrequently have irregular axonemes and these subpellicular axonemes can not be ascertained. The significance of both structure remains a problem warranting further investigation.

The electron dense region separating the ectoplasm from the endoplasm appears to be similar to the region described by Grain (1969a) in *Balantidium testudinis* and by Nelson (1969) in *B. caviae*. This region is lacking in *B. coli, B. elongatum* and *B. xenopi* (Grain 1969a). Although Krascheninnikow and Scholtyseck (unpublished research observations) have observed an electron dense region in their studies of *B. coli*. In *B. caviae* this interface is composed of an anasto-

mosing network of microfilaments, enclosing the endoplasm like a girdle. The proximal ends of the kinetosomes are embedded in this matrix. Nelson (1969) found this filamentous layer to consist of two parts, an outer layer of filaments arranged perpendicular to an inner layer. However, this double layering seemed to vary with the plane of sectioning. Its function is obscure but it may offer some resilience to deformations of the cell encountered while moving through the viscous particulate gut contents.

The macronucleus is enveloped by two membranes. No evidence for the bright halo surrounding the macronucleus of *B. coli* (Kretschmar 1963, Kraschenin-nikow 1968) could be found in *B. caviae*. The inner nuclear membrane is studded with dense bodies "chromatin bodies", similar to those described in *B. testudinis* by Grain (1969 a).

Kraschenin nikow (1968) in his light microscopic description of the macronucleus of *B. coli* found it to be of the heteromerous type (i.e., karyomeres easily distinguished into zones). We found, however, in *B. caviae* the macronucleus is homeomerous (i.e., karyomeres not distinguished into zones). Although, we are comparing light microscopial observations on *B. coli* with electron micrographs of *B. caviae*, it is possible that a similar study on *B. coli* may reveal similarities hereotfore unresoluable at the light microscopical level. The possibility that differences in nuclear structure may be a reflection of the physiological or state of activity of the macronucleus at the time of fixation must also be taken into consideration and must be looked at in greater detail.

## Summary

An electron microscopic study of the fibrillar elements and the dimorphic nuclei of *Balantidium caviae* has been described. It has been observed that each kinetosome of the cortex has a kinetodesmal fibril, postciliary microtubules, left tangential microtubules and a nemadesmos. A dense fibrous layer which consists of very dense filaments surrounds the base of each kinetosome and separates the endoplasm from the ectoplasm. Clavate or balloon-shaped cilia, cilia with multiple axonemes and intracellular axonemes have been observed.

The macronucleus is surrounded by two membranes. The internal membrane is often studded with blocks of chromatin. The cytoplasm of the micronucleus consists of two electron densities: the densest region is found around the central mass of chromatin.

## RÉSUMÉ

L'étude au microscope électronique des éléments fibreux et du nucléus dimorphique du *Balanditium caviae* ont été décrits. Il a été découvert que chaque cinétosome du cortex a une fibrille cinétodesmale; des microtubules postciliaires, des microtubules tangentielles de gauche et un némadesme. Une couche fibreuse dense qui consiste de filaments très fins entoure la base de cha que

cinétosome et sépare l'ectoplasme de l'endoplasme. Des cilia de forme clavée bizarre ou en forme de balon, des cilia avec axonemes multiples et des axonemes intracellulaires ont été observés.

Le macronucléus est entouré de deux membranes. Le membrane interne est souvent parsemée de blocs de chromatine. Le plasma de base du micronucléus comprend deux densités d'électrons; le plus dense se trouvant autour des masses de chromatine.

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## EXPLANATION OF PLATES I–IV

1: Oblique section through ectoplasmic region, the dense kinetodesmal fibrils (KD) postciliarys (PC) and left tangentials (LT) are indicated. What appear to be additional rows of microtubules associated with the left tangentials are indicated (arrow). The fine filamentous material between the ecto and endoplasm is evident (EE). 50 000×

2: The kinetosomes are out of the plane of section. The kinetodesmal fibril (KD) 28 nm banding is indicated at arrows, the postciliary microtubules (PC) diverge from their point of origin and extend posteriorly, the filamentous material is also evident (EE). 62 000×

3: Oblique section of somatic kineties near the anterior apex of the cell, the nemadesmal microtubules (N) extend into the endoplasm from an electron dense plaque at the base of the kinetosome an electron translucent space (arrow) is evident between the dense plaque and the microtubules. Kinetodesmal fibrils (KD) and postciliary microtubules (PC) can be seen in the cytoplasmic extensions between adjacent cilia. 41 800×

4: Cross section through vestibular kineties. In the upper right (viewers right) portions of the nemadesmal microtubules can be seen extending inward from the electron dense plaques at the base of each kinetosome. In the lower left a portion of the barren wall of the vestibulum is evident, note the dense filamentous band prevelant between the two regions. 37 000×

5: Macronucleus cut in cross section, the dense chromatin clumps (arrows within the nucleus) and nucleoli (NC) are evident, clumps of chromatin are appressed to the corrugated nuclear membrane (arrows). 6000×

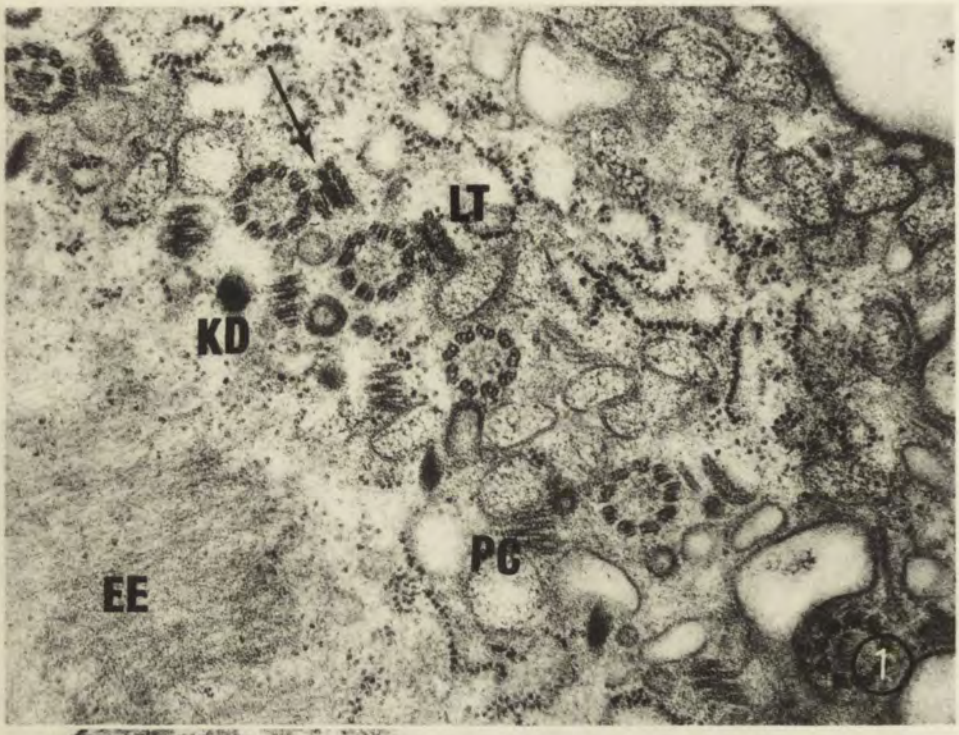
6: Macronuclear envelope, what appear to be two membranes (arrows) are separated by a 23 nm clear space. Chromatin masses appear appressed to the inner membrane, a nucleolar mass (NC) of a more granular texture is evident. 73 000×

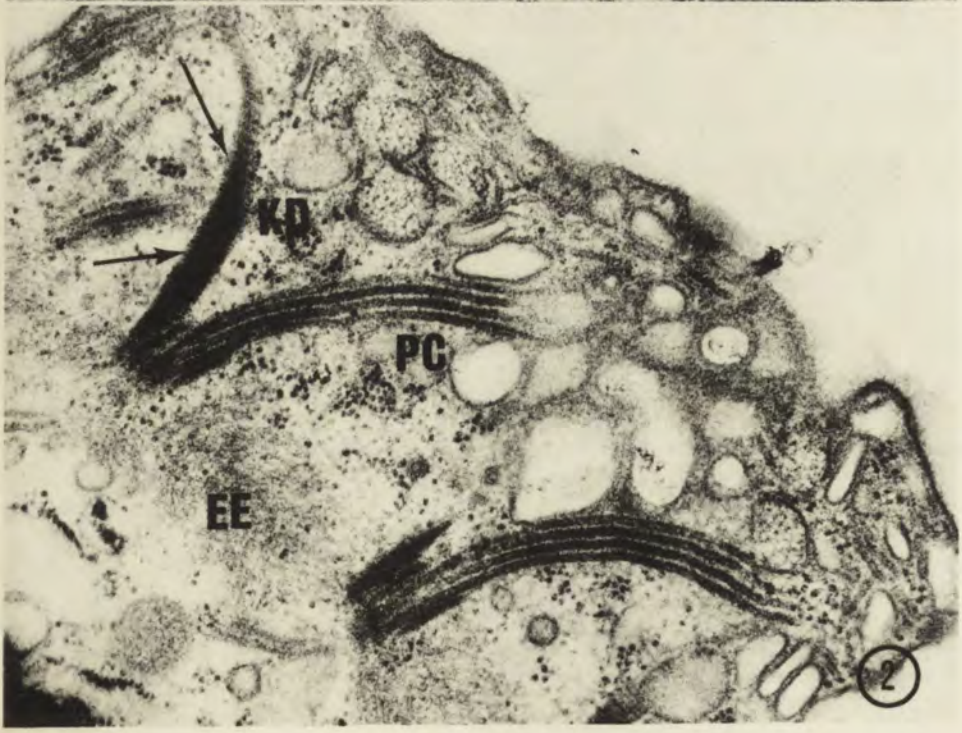
7: Micronucleus, the dense chromatin material is surrounded by a less dense granular material, an electron translucent area is found between the micronuclear envelope and the denser regions. The corrugated appearing macronuclear membrane is also evident. 21 000×

8: The axoneme of a cilium can be seen in the ectoplasmic ridge (arrows), doublet microtubules appear to be disorganized, normal cilia are observed on either side. 35 000×

9: Balloon-shaped cilium, the kinetosome is not in the plane of section. 90 000×

10: Two ciliary axonemes enveloped by the same ciliary membrane. 30 000×

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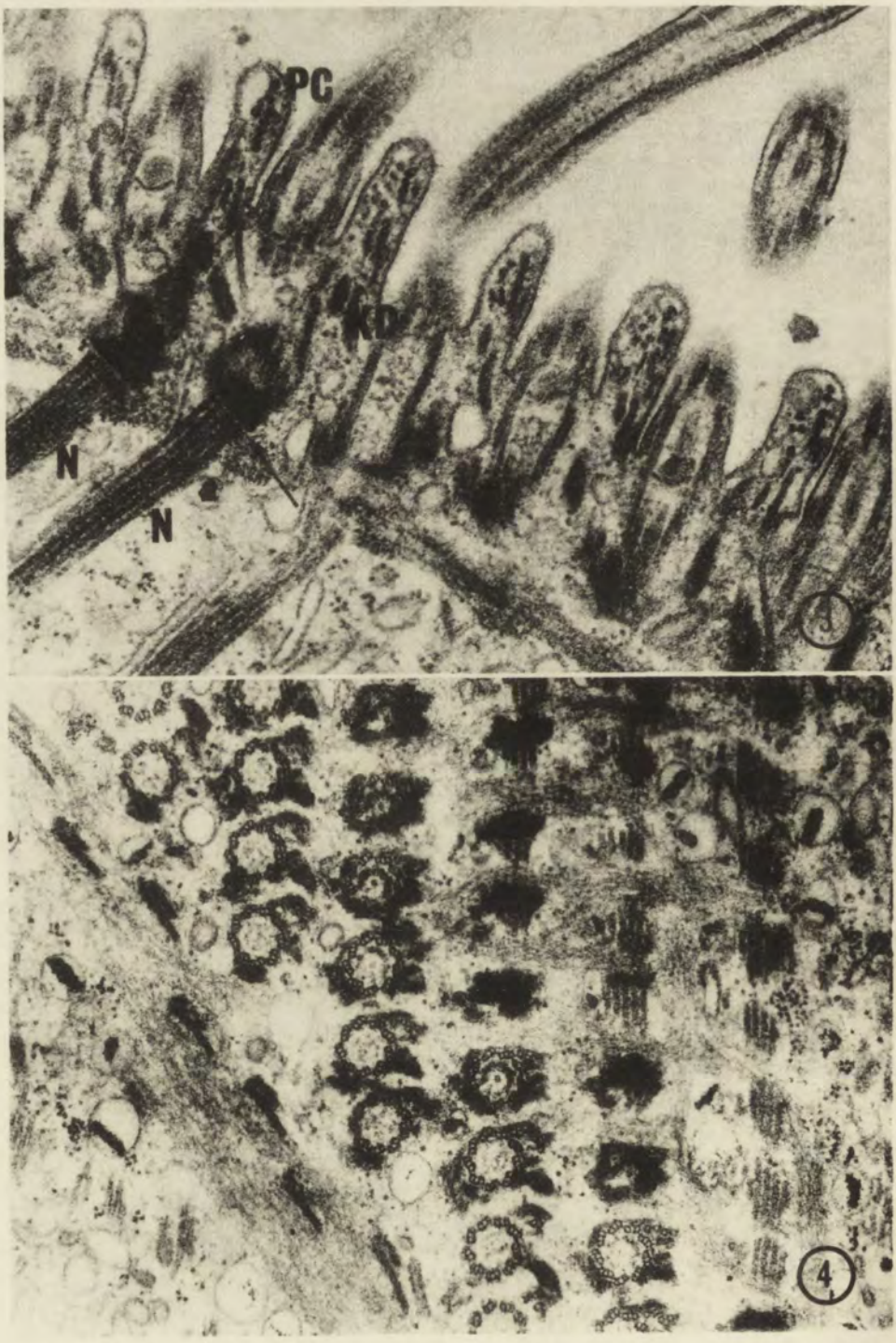


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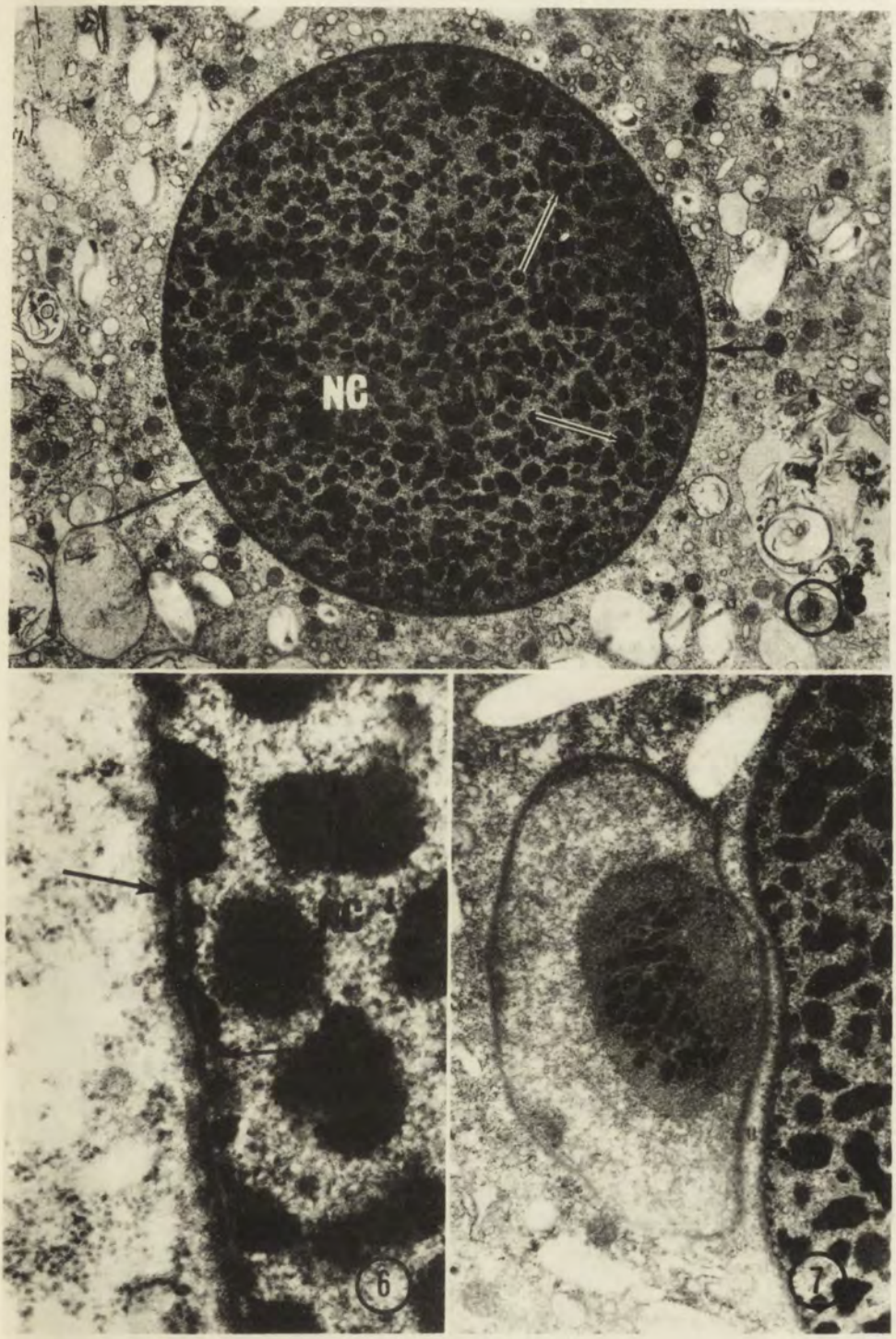


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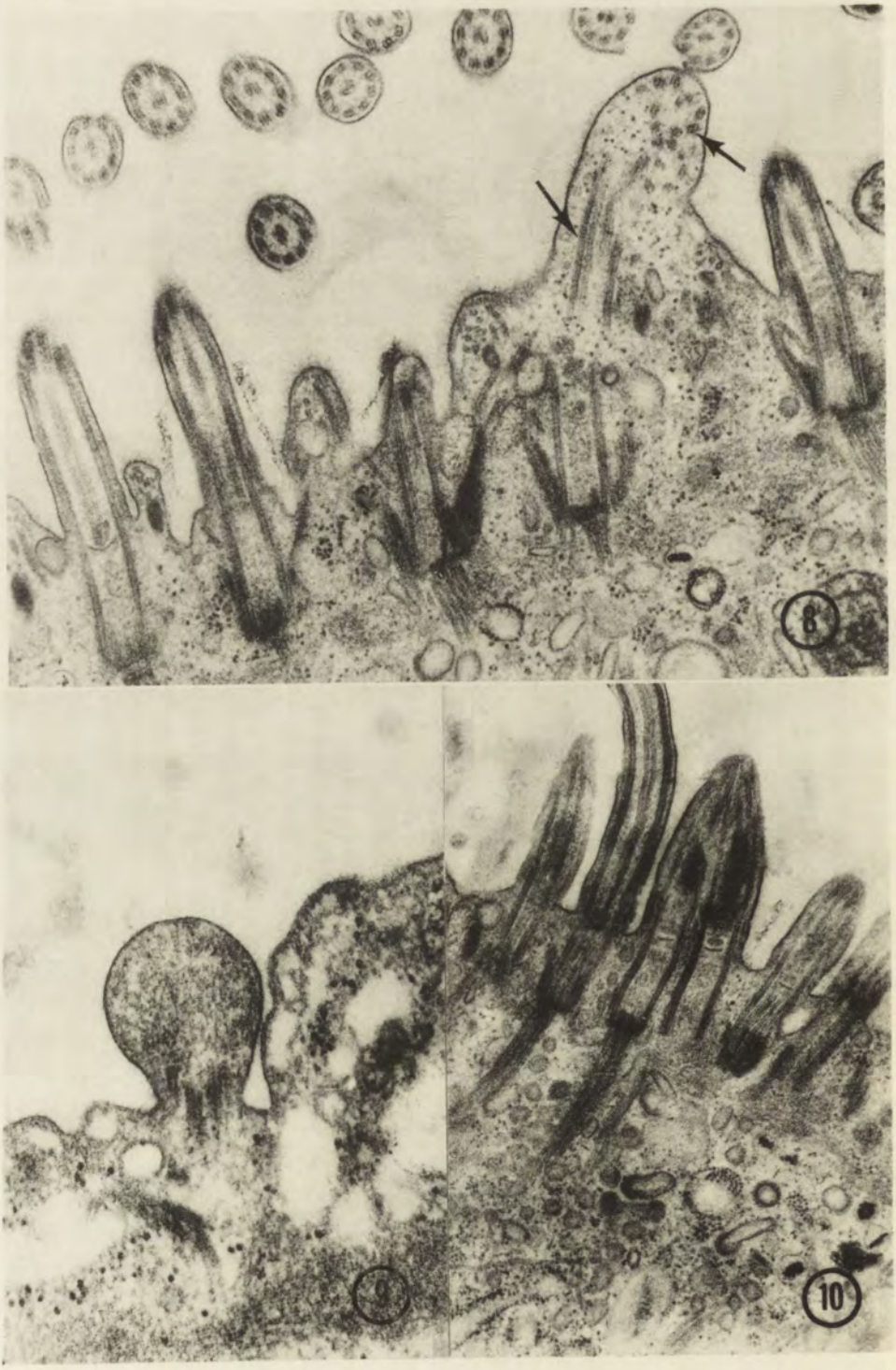


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# Elimination of chromatin from the micronucleus of *Chilodonella cucullulus* (O. F. M.) during the 1st meiotic division

Eliminacja chromatyny podczas pierwszego podziału mejotycznego z mikronukleusa *Chilodonella cucullulus* (O. F. M.)

The clones of *Chilodonella cucullulus* (O. F. M.), in which the mating type reaction as well as interclonal conjugation had been observed, were used for the research. The protozoans of different mating types were mixed together; pairing of cells was observed within 15 to 30 min. In the first phase of mating the ciliates join together with various parts of their body, then they come in a stable contact by their buccal cavities. Attainment of the permanent contact between both cells is the moment inducing the conjugation.

Within about 30 min after the mating of both partners the nuclear changes begin to undergo, the micronucleus (Mi) moves away from the macronucleus (Ma) (Pl. I 1) and, within 1 h, it assumes the shape of so called crescent stage, recorded in many ciliates (Pl. I 2–4). After 2 h more Mi elongates and transforms into a club-shaped structure; one end of this structure seems to be open (Pl. III 10–12). Rigid envelope of this structure is probably formed of the elongated nuclear membrane of Mi, strengthened by microtubular structures. The narrow end of this structure, which seems to be opened, is not rigid (Pl. III 12). The chromatin is prolonged into this narrow part and forms an agglomeration at the end, but the main chromatin mass is situated in the enlarged part of the "club". This stage corresponds with the "parachute" stage of pachytene.

At the end of prophase the broad part of the "club", with the main chromatin mass inside, shifts to the opposite end of the cell (Pl. II 6, 7) while the narrow end, containing a part of chromatin is eliminated to cytoplasm and resorbed (Pl. II 8, III 9).

Similar process of elimination of chromatin during the 1st meiotic division was observed in *Chilodonella ucinata* by Mc Dougall (1935).

The character of eliminated chromatin is not known till now. In all probability this phenomenon consists in elimination of genes of amplificated segments of heterochromatic chromosomes. These parts are not eliminated during vegetative divisions of cells.

The phenomenon of chromatin elimination was observed in oogonies and ootypes of *Tipula* by Lima de Faria (1960, 1962) and in *Acheta* by Bayreuther (1952, 1956). According to Lima de Faria et al. (1968) DNA accumulated by synthesis desintegrates in late diplotene and migrates to the nuclear sap. The phenomenon of synthesis of additional copies of DNA was also recorded in *Dytiscus* Gall et al. (1969). Brown and David (1968) observed the amplification of genes in amphibian oocytes.

On account of the material described above the possibility of amplification of some parts of chromosomes in *Chilodonella cucullulus* cannot be excluded. Consequently, it is the most probable that the Mi of this species contains some excessing chromatin and cannot be regarded as a genuine diploid.

The definitive elucidation of this problem is very difficult. It is not possible at present to determine whether the eliminated chromatine is responsible for r-RNA synthesis because morphologically distinquishable nucleoli are not present in the micronucleus.

## Summary

It is ascertained that during the 1st meiotic division in *Chilodonella cucullulus* (O. F. M.) occurs the elimination of a part of chromatine probably originating from the amplificated segments of chromosomes.

## STRESZCZENIE

Stwierdzono, że *Chilodonella cucullulus* (O. F. M.) z Mi podczas profazy podziału mejotycznego następuje eliminacja pewnej części chromatyny, która prawdopodobnie pochodzi z amplikowanych odcinków chromosomów.

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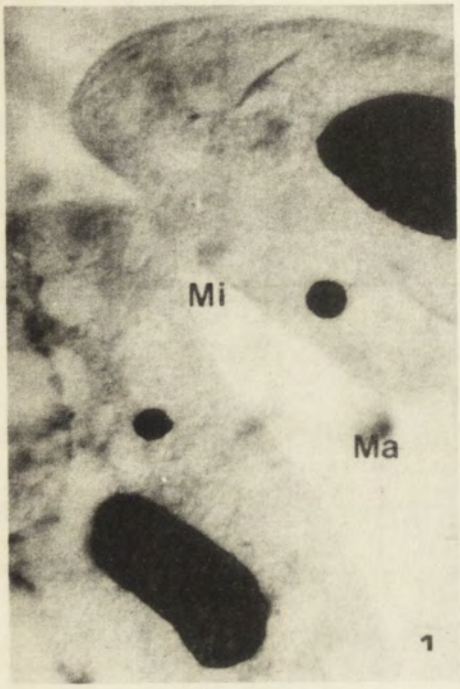
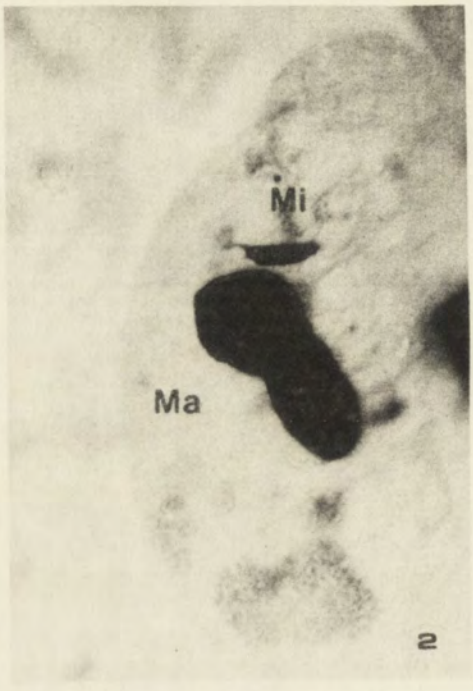
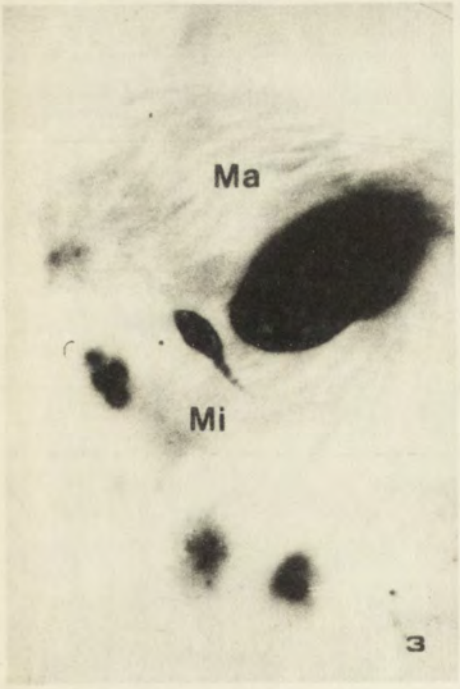
## EXPLANATION OF PLATES I–III

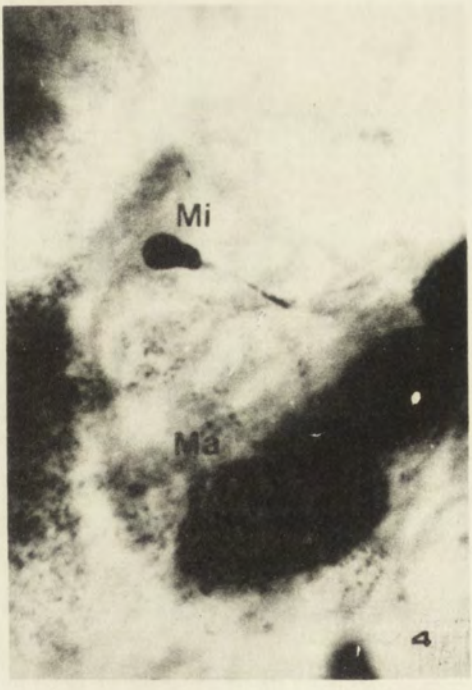
1–9: Succeeding stages of elongation of Mi of *Chilodonella cucullulus* (O. F. M.) during prophase of the 1st meiotic division with elimination of a part of chromatine

10–12: Structure of the "club", elongated to a maximum, with the elongated chromatine visible inside (phase contrast, the part of chromatine eliminated to cytoplasm is marked with arrows)

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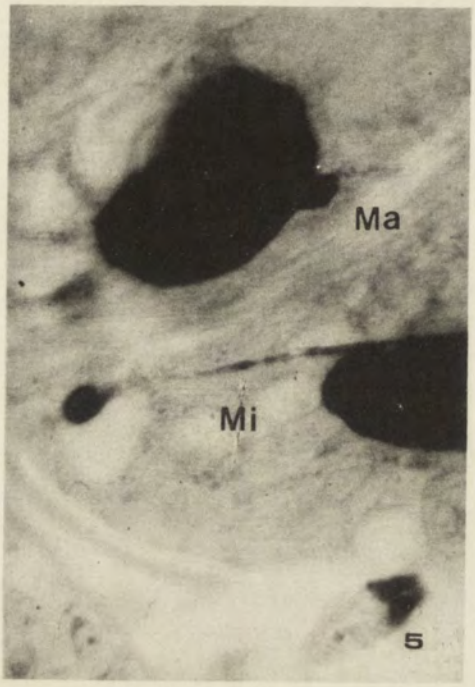


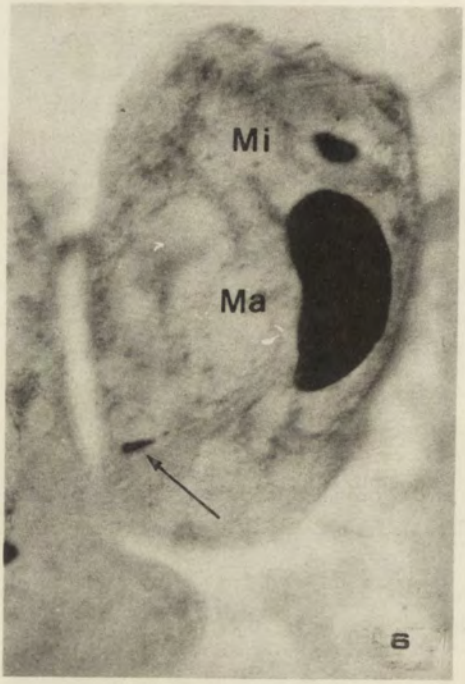


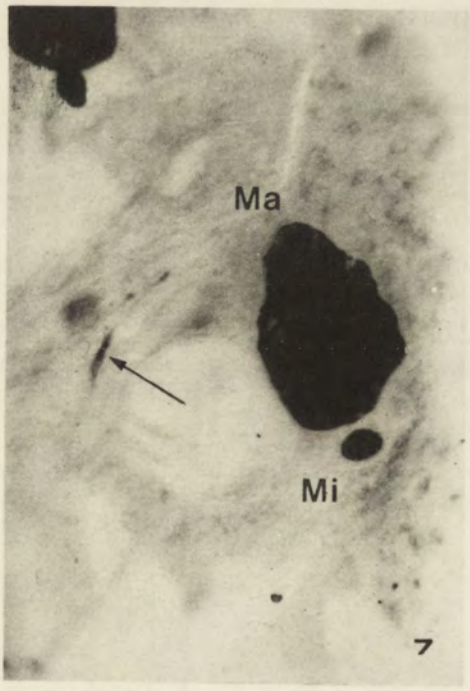


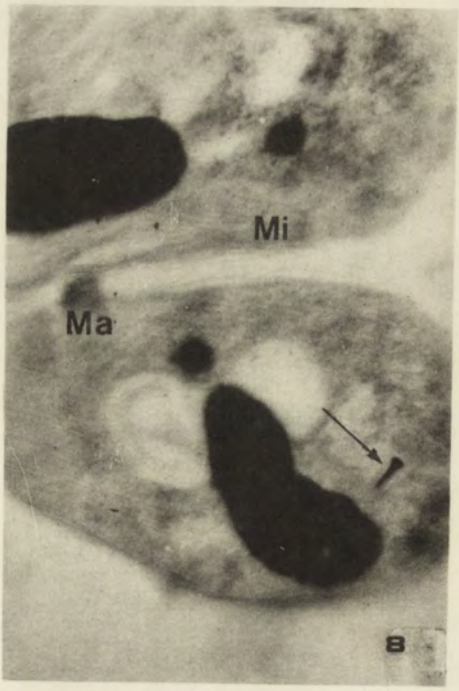


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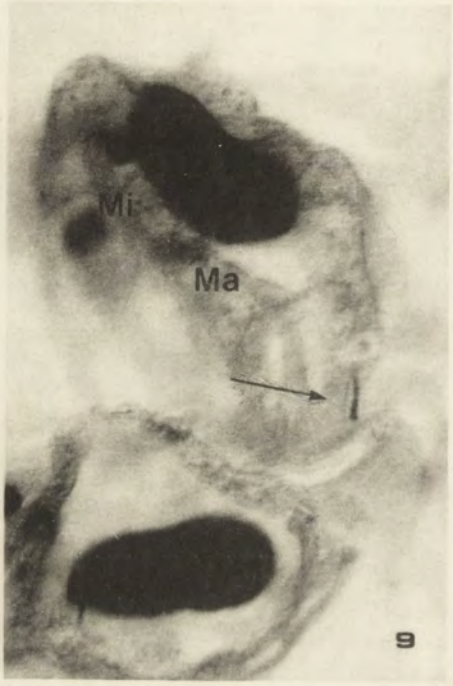


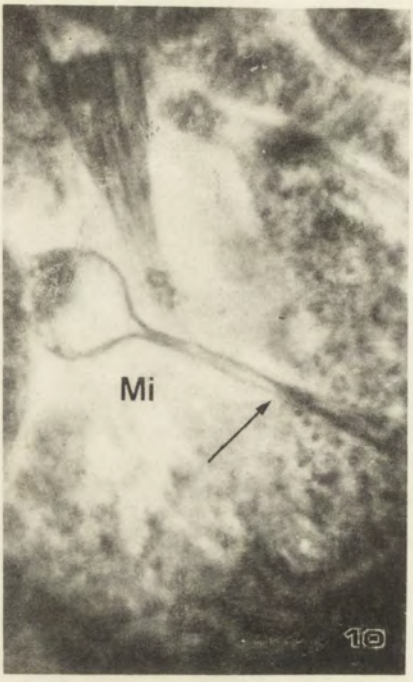


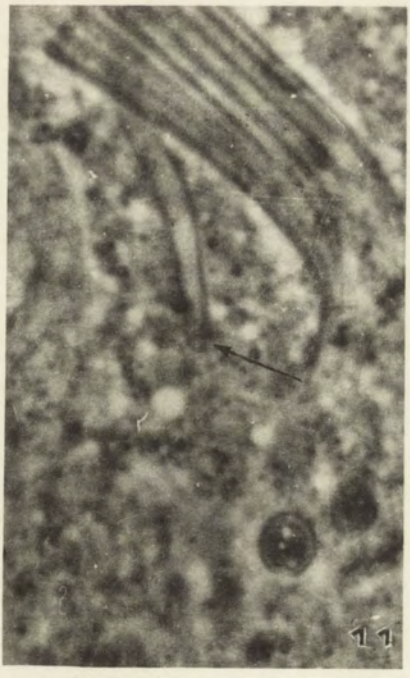


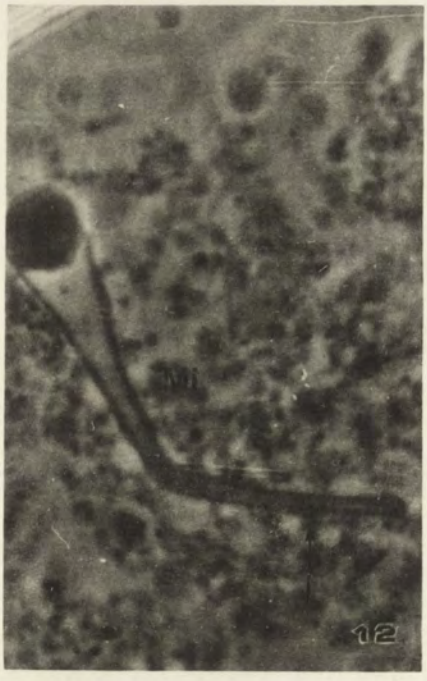


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# On *Babesiosoma*: *Haemosporidia* of cold-blooded vertebrates

Über *Babesiosoma*; *Haemosporidia* kaltblütig wirbeltiere

*Dactylosomidae* Jakowska et Nigrelli, 1955 includes *Dactylosoma* Labbé, 1894 and *Babesiosoma* Jakowska et Nigrelli, 1956. *Babesiosoma* was reduced to a junior synonym of *Haemohormidium* Henry, 1910 by Laird and Bullock 1969. This revision was accepted unequivocally by Levine 1971, who emended the family as *Dactylosomatidae* to include *Dactylosoma*, *Haemohormidium* and *Sauroplasma* du Toit, 1938. We do not agree with these additions and changes for the reasons discussed below.

## Discussion

The distinguishing features of *Dactylosoma* and *Babesiosoma* are clear cut. *Dactylosoma* has a granular cytoplasm, nucleus with an endosome, and merogony results in the formation of four to six merozoites arranged as in a "hand" or fan-shape; *Babesiosoma* has a less granular and more vacuolated cytoplasm, no endosome in the nucleus, and merogony results in the formation of not more than four merozoites arranged as a rosette or cross-shape. These characteristics were accepted by Kudo 1966, who does not refer to either *Haemohormidium* or to *Sauroplasma*, both of which are partially characterized by the absence of schizogony. However, the name *Haemohormidium cotti*, was proposed by Henry 1910 for a red blood cell parasite of the marine fishes *Cottus bubalis* and *Cottus scorpius* (family: *Cottidae*), which he figured but did not describe. Later 1913, he retracted this interpretation because he believed that the structures that he had seen in the red cells were "the peripheral chromatin phase of *Haemogregarina cotti*".

Nevertheless, Henry's 1913 description for *Haemohormidium cotti* is as follows:

> (1) "It is an oval or rounded globular body which exhibits great plasticity, and hence assumes many different shapes in the corpuscle cytoplasm.
>
> (2) Only the periphery of the parasite is readily stainable, and of this periphery one-third to two-thirds is taken up with a thin band of chromatin, which is often broken up into irregular beads and frag-

ments". Also, "no forms suggestive of either schizogony or of sporont formation... have been seen".

More recently, Laird and Bullock reported *Haemohormidium* sp. in the red cells of *Hemitripterus americanus* (family: *Cottidae*) and *Hippoglossoides platessoides* (family: *Pleuronectidae*) from St. Andrews, Canada, which was strikingly similar to the form described by Henry 1910. They characterized the organism as follows:

> The red cells "hold from one to four amoeboid bodies, the central zone of which assumes a paler stain than the surrounding host cell cytoplasm. The embayed periphery stains faintly blue except where the threadlike cytoplasm of the organism is obscured by granules and masses of chromatin. This stains deep red, and occupies from about one- to two-thirds of the peripheral zone". "No other stages were present, and haemogregarines are not known from either host".

It should be noted that the single figure that Laird and Bullock presented (Fig. 45, p. 1093) does not show these characteristics.

On the basis of this meager observation and from the description given (1913) or implied (1910) by Henry, Laird and Bullock invoked article 25 of the International Rules of Zoological Nomenclature, since they considered *Babesiosoma* and *Haemohormidium* to be generically similar, thus, making *Babesiosoma* Jakowska et Nigrelli, 1956 a synonym of *Haemohormidium* Henry, 1910. They further support their view for priority by accepting the conclusion made by Mackerras and Mackerras 1961 that the name *Haemohormidium* was validated by Wenyon 1926. We state categorically that Wenyon did not validate the name since he did not study the organism, hence he had no firsthand information; Wenyon only repeated the name and some of the features reported by Henry 1913. Thus, in discussing *Haemohormidium* (p. 1065), he wrote:

> "This parasite was described by Henry (1910 and 1913) as occurring in the red cell of two marine fish, *Cottus bubalis* and *Cottus scorpius*. It is an irregularly round or oval body, and varies in length from 2 to 4.5 microns. The chromatin is arranged along the margin, about two-thirds of which it occupies. As many as three of the organisms were found in a single cell. They bear some resemblance to ring forms of malarial parasites. Henry came to the remarkable conclusion that they represented the intracorpuscular stages of 'infective granules' extruded by haemogregarines".

Thus, insufficient written (or implied) features have been presented by Henry 1910, 1913 and by Laird and Bullock 1969 to definitely characterize *Haemohormidium*. It is exactly for these reasons that Wenyon 1926 included this genus among "Parasites of Doubtful Nature".

However, if it is assumed that *Haemohormidium* is a valid genus, its validity must rest solely on the description given by Henry 1913. Yet, Laird and Bullock concluded that *Haemohormidium* has the following characteristics:

"babesioids having rather large, oval, ellipsoidal or irregularly amoeboid trophozoites in which the well-stained periphery contrasts with a refractive central zone, and dividing (into no more than four merozoites, schizonts commonly being rosette-shaped or cruciform) by schizogony, binary fission, or budding".

It is apparent that Laird and Bullock, and accepted by Levine 1971, who had not referred to Henry's 1913 paper, advocate the acceptance of the genus *Haemohormidium* with characters not observed by them or by Henry. Further, we do not agree that there is sufficient evidence that *Babesiosoma* is a synonym of *Haemohormidium* (see Haldar, Misra and Chakravarty 1971). A close examination of the descriptive and pictorial features of *Babesiosoma* and *Haemohormidium* will support this view.

## Conclusion

The above discussion satisfies the separation of the genus *Babesiosoma*, *Haemohormidium* and *Sauroplasma*. Therefore, *Babesiosoma* and *Haemohormidium* should no longer be considered as synonyms. The re-establishment of *Babesiosoma* as a valid genus satisfies Article 59 (c) of the International Rules of Zoological Nomenclature. We reiterate that generic separation is possible by applying the following characters: (1) presence of schizogony; (2) production of definite number of merozoites in erythrocyte; (3) presence of endosome in the nucleus of the trophozoite.

At present the family *Dactylosomidae* Jakowska and Nigrelli, 1955 comprises only two genera: *Dactylosoma* Labbé, 1894 and *Babesiosoma* Jakowska et Nigrelli, 1956. However, the genus *Haemohormidium* Henry, 1910 may provisionally be accepted as a member of the family, but a more detailed description and definition is needed to establish its true systematic position. The genus *Sauroplasma* du Toit, 1938, which has no schizogonic cycle, should not be included in the family *Dactylosomidae*.

Finally, should future studies prove that schizogony is absent in *Haemohormidium* and *Sauroplasma*, then a separate taxon should be created for these genera, and other organisms of red blood cells of vertebrates in which this process does not occur.

Acknowledgement

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## Summary

The paper deals with the systematic position of the genera *Dactylosoma* Labbé, *Babesiosoma* Jakowska et Nigrelli, *Haemohormidium* Henry and *Sauroplasma* du Toit. The genus *Babesiosoma* has been revived and the family *Dactylosomidae*

Jakowska and Nigrelli, 1955 thus includes two genera *Dactylosoma* and *Babesiosoma*. *Haemohormidium* Henry may provisionally be accepted in the family *Dactylosomidae*, but not the genus *Sauroplasma* du Toit, which has no schizogony cycle in the vertebrate erythrocyte.

ZUSAMMENFASSUNG

Die systematische Stellung der gattungen *Dactylosoma* Labbé, *Babesiosoma* Jakowska et Nigrelli, *Haemohormidium* Henry und *Sauroplasma* du Toit wurde besprochen.

Die Revision der gattung *Babesiosoma* und der Familie *Dactylosomidae* Jakowska et Nigrelli, 1955 welche zwei Arten umfasst, wurde durchgeführt. *Haemohormidium* Henry kann provisorisch zur Familie *Dactylosomidae* eingegliedert werden, doch betrifft dass nicht der gattung *Sauroplasma* du Toit, welche den schizogenischen Zyklus in den Erythrozyten der Wirbeltiere entbehrt.

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# Relation between radiosensitivity and DNA content in nuclei of various *Amoeba proteus* strains

## Соотношение между радиочувствительностью и содержанием ДНК в ядре у разных штаммов *Amoeba proteus*

Amoebae proved to be a most convenient model for studying general regularities of radiation effects on the cell. The resistance of these organisms to ionizing radiation is immensely high. The nature of this phenomenon is usually attributed to high ploidy of their nuclei (Astaurov 1963). As has been recently shown, however, the radioresistance of various *Sarcodina* specimen is not correlated with the number of chromosomes in their nuclei and DNA content of the cell (Korogodin et al. 1971). At present there is no exact evidence concerning the organization of nuclear apparatus in amoebae and amounts of DNA in their nuclei. This can be explained by the fact that biochemical studies on the DNA content of *Amoeba proteus* nuclei are difficult to accomplish due to the presence in their cytoplasm of some unknown kind of DNA and lack of axenic cultures. Moreover, the sensitivity of biochemical methods is a severe problem for determining the DNA content in nuclei of individual specimens. Methods of absorption cytophotometry do not permit the estimation of DNA content in the nucleus since amoebae show weak Feulgen reaction.

In the present paper we determined DNA amounts in the nuclei of various *Amoeba proteus* strains differing in radiosensitivity using the cytofluorometric method (Rosanov and Kudryavtsev 1967).

## Materials and methods

The X- and γ-rays sensitivity of *Amoeba proteus* strains (L, B, C and Berkley) obtained from different natural populations but similar in their morphology has been investigated. The amoebae were cultivated according to the technique described by Yudin (1961) and Olenov et al. (1961). The animals were kept at 24°C and fed every other day (*Tetrahymena pyriformis*). Under such cultivation regime the life-span of on generation lasted about 40 h in all the strains tested.

The amoebae were irradiated in open standard glass vessels, X-rays were produced by the Roentgen apparatus RUM-7 60kV, 20mA, without filter, dose-rate 30 krad/min; γ-rays were used (the source of radioactive Cs LMB-γ-1, dose-rate 3 krad/min).

Simultaneously, 50–100 amoebae were irradiated on a layer of Prescott medium 1.6 mm thick. After irradiation amoebae were put one by one on plates of organic glass and and then the efficiency of cloning, i.e., the capacity of individual cells to yield clones (8–16 cells) was tested.

According to Nilova (1965), the length of cellular cycle stages in L strain cultivated in conditions similar to ours were as follows: presynthetic period — 7–12 h, synthetic period — 13 h and postsynthetic period — 4–6 h. But at the same time Ron and Prescott (1969) obtained different results for *Amoeba proteus* Berkley: $G_1$ lasted not longer than 15 min (practically, $G_1$ was lacking), S — 5–7 h, while the bulk of generation time is occupied by the period $G_2$ (30–36 h).

We measured DNA content in amoeba nuclei 28 h after division, i.e., probably in $G_2$ period. The nuclei from amoeba cells were isolated manually under binocular microscope in a solution of triton with spermidine (1 ml triton X-100 and 1 mg spermidine per 200 ml of the Prescott medium). The isolated nuclei were put on mount slips and fixed in methanol. For cytofluorometry the preparations were stained with auramine-$SO_2$, the reagent of Shiff type (0.3% auramine-00 solution) Reanal, Hungary and 0.2 ml thionil chloride ($SOCl_2$) per 100 ml dye at pH 1.25; T — 2–5°C). The cytochemical reaction was carried out as follows: 8 min hydrolysis in 6 N HCl at 20° (Fig. 1)

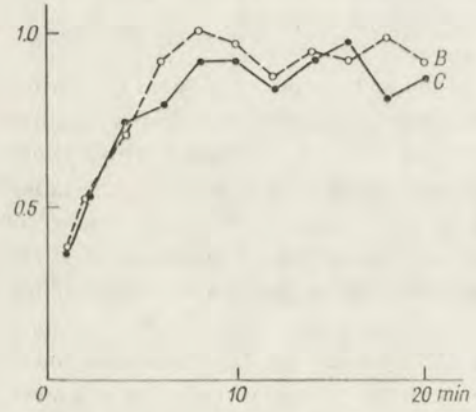


Fig. 1. Dependence of fluorescence of the isolated nuclei of *Amoeba proteus* (B and C) on the time of hydrolysis. Abscissa, time of hydrolysis (min). Ordinate, intensity of fluorescence (relative units)

was followed by staining for 1.5 h. For a more detailed description of optimal conditions for cytochemical reactions with auramine-$SO_2$ see the publications by Kudryavtsev et al. (1971 a, b, c). The photographs of *Amoeba proteus* nuclei treated with auramine-$SO_2$ are given in Plate I 1–4.

Simultaneously with the nuclei of *Amoeba proteus* we stained cells of rat liver taken as a control subject. Only tetraploid nuclei of liver cells were used for measurements. The fluorescence intensity of auramine-$SO_2$ treated nuclei was determined on a cytofluorimeter designed at the Institute of Cytology of the Academy of Sciences of USSR (Rosanov and Kudryavtsev 1967). The following photometric conditions were used: Exciting light was isolated by ФС-1.8 mm filter, 3 mm glass (жзс — 19 + жс — 18) was used as barrier filter.

## Results and discussion

Dose-effect curves showing the cloning efficiency of *Amoeba proteus* (L, B, C and Berkley strains) after X-ray and γ-irradiation are presented in Fig. 2. Radiosensitivity of these strains can be divided in two groups: The first group includes L and B strains ($LD_{50}$ — 25–30 krad) and the second one containing radioresistant strains C ($LD_{50}$ — 140–180 krad) and Berkley ($LD_{50}$ — 115 krad). When comparing

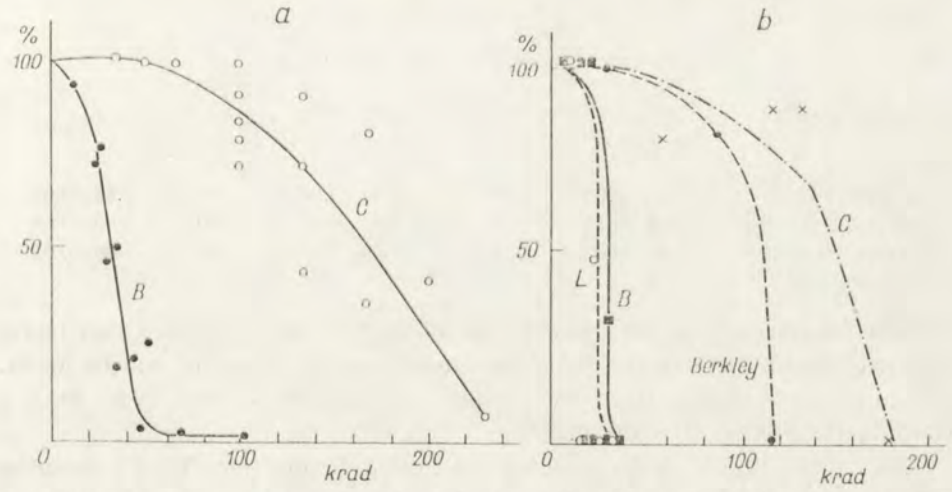


Fig. 2. Dose-effect curves of *Amoeba proteus* after X-ray (a) and γ-irradiation (b). Abscissa, dosage in krad. Ordinate, the cloning efficiency (%)

dose-effect curves after X-ray and γ-irradiation one must pay attention to a steeper slope in the case of γ-irradiation. It must be pointed out, however, that equal doses of radiation of different dose-rate produce the same effect on the cloning efficiency of amoebae. The dose-rate of X-rays more than 10-fold exceeds that of γ-irradiation source. But as has been shown earlier by one of the authors (Kalney 1967; Paribok et. al. 1968) the dose-rate of X-irradiation affects the degree of final response of the cell to radiation damage (strain B). With increase of dose-rate from 4.3 to 30 krad/ /min doses of the same intensity reduced two times. In case of γ-irradiation the effect of dose-rate, however, was not investigated.

Radiosensitivity tests of a few *Amoeba proteus* strains carried out for 5 years yielded stable results. Two groups of strains were found to differ by $LD_{50}$ about 5–6 times. It is known that the lethal effect of radiation energy is caused by the injury of unique cellular structures. The primary injurability of cells depends on the peculiarities of their reproduction apparatus among which such factors as DNA amounts in the nucleus play rather an important role. Therefore we compared the DNA content in the nuclei of two strains differing in sensitivity (B and C).

To compare DNA amounts in the nuclei of radiosensitive (B) and radioresistant (C) strains we conducted two series of experiments. The results are presented in the Table 1. They show that amoeba nuclei sharply differing in radiosensitivity contain the same amounts of DNA. Thus such factor as the DNA content per nucleus does not play the decisive role in these test-objects.

In order to estimate the DNA content per nucleus in relative units we used liver cells as a sample. On the average diploid rat liver cells contain approximately $6.6 \times 10^{-12}$ g DNA (Vaughan and Locy 1969; Rasch 1971). Assuming that in our experiments nuclei were fixed in postsynthetic period of the mitotic cycle and

Table 1

DNA content in *Amoeba proteus* nucleus

| Test-object | DNA content in relative units | | | | Mean |
|---|---|---|---|---|---|
| | experiment I | n | experiment II | n | |
| *A. proteus* B | 2.86±0.08 | 62 | 2.90±0.08 | 57 | 2.88±0.08 |
| *A. proteus* C | 3.06±0.12 | 21 | 2.72±0.04 | 90 | 2.90±0.08 |
| Liver cells (4c) | 1.00±0.02 | 90 | 1.00±0.02 | 46 | 1.00±0.02 |

that the fluorescence intensity of amoeba nuclei 2.89 times exceeded that of rat liver tetraploid nuclei (see the Table) we can measure DNA in the amoeba nuclei. Our calculations show that on the average amoeba nuclei contain $19.0 \times 10^{-12}$ g DNA in the presynthetic period.

Since there are numerous evidence concerning cytoplasmic DNA in amoeba it would be interesting to know how DNA is distributed in the amoeba cell and which portion of DNA falls on the nucleus. According to Korogodin et al. (1971), B and C strains of *Amoeba proteus* contain $100 \times 10^{-12}$ g DNA per cell. If these results are compared with the evidence we obtained about DNA amounts per nucleus of the amoeba cell we may see that the amoeba nucleus contains about one fifth of the whole cellular DNA, while the bulk of DNA is distributed along the cytoplasm.

At present the functional meaning of cytoplasmic DNA is not clear. It is not unlikely that it originates from symbionts and not from amoeba cells. It is not excluded also that variations in radiosensitivity of various *Amoeba proteus* strains can be associated with differences in intracellular distribution of DNA in different strains (Andreeva et al. 1972) and the important role played by cytoplasmic DNA in amoeba radiosensitivity. The presence of symbionts in the amoeba cytoplasm may also affect the radiosensitivity. According to Jagger et al. (1969), the presence of symbionts in the amoeba cytoplasm influences the length of the cell cycle. Thus *Amoeba amosinensis* a species recently discovered by Prescott, contained no symbionts and divided at two times higher rate than *A. proteus*. It is well known that delay in the first division after irradiation assists a more successful development of repair processes.

Scrutinizing our experimental results, we must point out another peculiarity of the amoeba nuclei. The volume of the latter makes up $6.2 \times 10^4$ $\mu^3$ and the volume of tetraploid liver cell nucleus about $3 \times 10^2$ $\mu^3$, i.e., amoeba nuclei exceed 200-fold in size those of liver cells and only 2.9-fold in their DNA content. Thus the DNA concentration in the nuclei of *A. proteus* is by two orders higher than in the nuclei of liver cells. This accounts for a weak staining of amoeba during cytochemical reactions. Such supposition may be supported by the morphological analysis of auramine-$SO_2$ stained nuclei (Pl. I 1–4). The distribution of DNA in amoeba nuclei shows the character of an open-worked network with large gaps between chromatin threads.

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## Summary

Among various natural populations there occur strains of *Amoeba proteus* greatly differing in their radiosensitivity: $LD_{50}$ for L and B strains makes up 25–30 krad, for Berkley strain 115 krad and for C strain 140–180 krad.

With the aid of the fluorometric technique for determining DNA amounts in auramine-$SO_2$ stained amoeba nuclei it has been shown that in B and C *Amoeba proteus* strains greatly differing in radiosensitivity the DNA content of the nuclei $G_1$ is the same: $19.0 \times 10^{-12}$ g. The calculations show that weak cytochemical reactions for DNA in amoebae are probably due to DNA low concentration in the nuclei.

## РЕЗЮМЕ

Среди различных природных популяций *Amoeba proteus* имеются штаммы, резко различающиеся по радиочувствительности: $ЛД_{50}$ для штаммов Л и В составляет 25–30 крад, для штамма Berkley — 115 крад, для штамма С — 140–180 крад. При использовании метода флуорометрического определения количества ДНК в ядрах амеб, окрашенных аурамином-$SO_2$, было показано, что у штамов *Amoeba proteus* В и С, резко различающихся по радиочувствительности, количество ДНК в ядрах одинаково и в пресинтетический период составляет $19.0 \times 10^{-12}$г. Проведенные расчеты показали, что слабые цитохимические реакции на ДНК у амеб связаны с низкой консентрацией ДНК в их ядрах.

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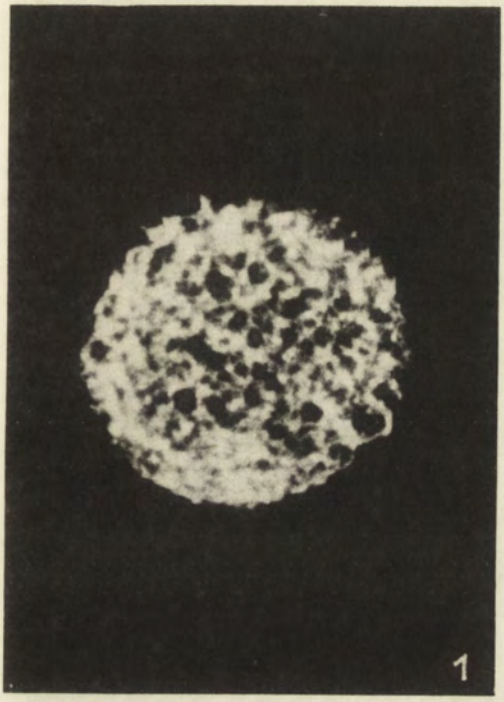
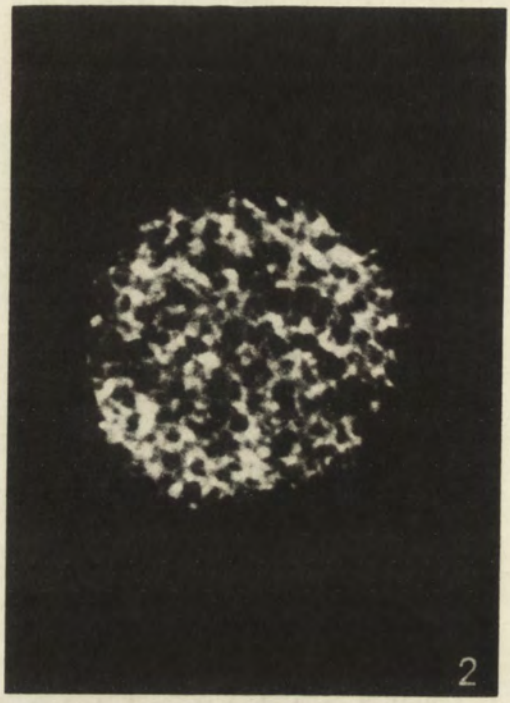
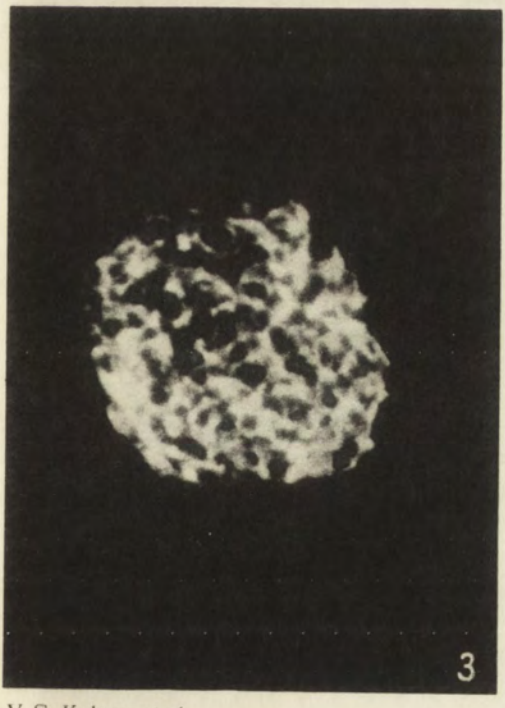
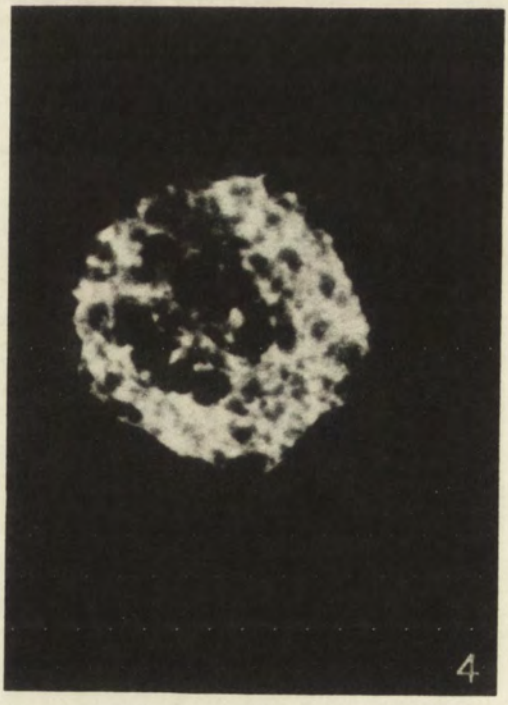
## EXPLANATION OF PLATE I

1–4: Photographs of isolated nuclei of *Amoeba proteus* B (1–2) and C (3–4) treated with auramine-$SO_2$




V. S. Kalney et al.

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# Effect of pH on *Blepharisma intermedium*. 1. Changes in fission rate and oxygen consumption

Der Einfluss das pH auf *Blepharisma intermedium*.
1. Die Änderungen im Teilungstempo und der Suerstoffverbrauch

Among the many factors that conjointly operate on life processes of protozoans temperature and hydrogen ion concentration of waters play an important role. Alteration of these factors influence the morphology, cell size, behaviour and reproductive rate in microorganisms. Information on the effects of temperature and pH of some ciliates is available. However, effect of pH on *Blepharisma intermedium* has not been studied so far. The influence of temperature on the fission rate of this ciliate has been reported (Kasturi et al. 1969). Changes in fission rate and oxygen consumption of *B. intermedium* with changes in pH are dealt with in this paper.

The effects of pH on ciliates grown in buffered media has not been systematically studied. Studies by Elliot (1933) on *Colpidium striatum*, Wingo and Anderson (1951) on the growth of *Tetrahymena geleii* E and of Loefer (1935, 1938, 1942) and others (Darby 1929, Jenkin 1927, Hetherington 1934, Slater 1953) on several protozoan species have shown that pH affects the growth rate of ciliates. In the present studies, media of different pH values with varying molarity have been used to determine the viability and reproduction of *B. intermedium*. Different buffering systems have been used to obtain the required pH. Changes in respiration rate with changes in external pH have also been described.

## Material and methods

Stocks of *B. intermedium* were grown as previously described (Kasturi et al. 1969) in a mixture of hay infusion and Erd Schreiber fortified with Horlicks malted milk. The buffer components were separately dissolved in this medium and mixed in requisite proportions to get the required pH and diluted with the original medium when necessary to the required molarity. The following buffer systems were used:

(1) Sodium dihydrogen phosphate — disodium hydrogen phosphate,
(2) Tris-maleate — sodium hydroxide,
(3) Potassium hydrogen phthalate — sodium hydroxide,
(4) Veronal — hydrochloric acid,

(5) Citric acid — disodium hydrogen phosphate,
(6) Citric acid — sodium citrate,
(7) Succinic acid — sodium hydroxide,
(8) Acetic acid — sodium acetate (A pH of 6.0 in the text with this buffer should be considered as 5.6).

The fission rate was determined by inoculating four ciliates from the stock culture into 10 ml of buffered medium in petri dishes. At the end of 24 h four organisms from this culture were reinoculated into 10 ml of fresh buffered medium and this process was repeated daily in all cases, generally for a period of seven days and in some cases for longer periods. The fission rate was determined by counting the number of doublings at 24 h intervals. All experiments were run in triplicate and average values were taken. In all cases temperature was maintained at 28°C.

Oxygen consumption studies were made on organisms grown at higher population density of 50 organisms per ml, as at this density the fission rate was same as in studies with four organisms per 10 ml. Organisms grown for 24 h were centrifuged and transferred to fresh medium maintaining the same population density. At the end of 48 h, organisms were centrifuged and suspended in buffered media such that 2 ml contained 4000 organisms and respiration studies were carried out for 1 h with Precision Warburg's apparatus. Oxygen consumption is expressed as $\mu$ $1/10^6$ cells/min.

## Results

### Fission rate

The rate of fission in buffers of different pH values and molarities is given in Table 1. It is observed that as long as the buffer molarity is not lethal to the organism, with all buffer systems tried, a pH of 6.0 is most favourable from the point of view of fission, the rate being 2 per day. But in buffer systems whose components are acids of TCA cycle, the rate of fission is 2 even at pH 5.0. The fission rate is reduced from 2 to 1 at pH values on either side of the pH at which there is maximum fission, provided the ciliate survives in the molarity of the buffer used.

The molarity of the buffer has no effect on the fission rate with the first four buffers of Table 1; the rate of fission is the same at a particular pH value with all the three molarities viz. 10 mM, 5 mM and 1 mM. With other buffers, however, the molarity proves lethal to the ciliate at 10 mM concentration at any of the pH values used. At 5 mM concentration, the organisms survive and divide only in succinate buffer at pH 5.0 and 6.0 whereas in acetate buffer they do so only at pH 6.0. The organisms survive in all buffers of 1 mM concentration.

As citrate and citrate-phosphate buffers at 10 mM and 5 mM concentrations proved lethal and ciliates survived at 1 mM concentration, the effect of these buffers at 2 mM and 3 mM concentrations was tried. The results given in Table 2 show that there was growth at these molarities as long as the pH was suitable.

### Oxygen consumption

The rate of oxygen consumption by the organisms grown in some buffers is given in Table 3. As could be seen from Fig. 1, the rate of oxygen consumption is more in acetate buffer than in Tris and citrate-phosphate buffers at pH 5.0, while

Table 1

Fission rate of *Blepharisma intermedium* in buffers of varying pH and molarities

| No. | Buffer | pH | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 mM | | | | | 5 mM | | | | | 1 mM | | | | | |
| | | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 |
| 1 | Phosphate | — | 2 | 1 | 1 | — | — | 2 | 1 | 1 | — | — | — | 2 | 1 | 1 | — |
| 2 | Tris-maleate-NaOH | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | — | 1 | 2 | 1 | 1 | 1 |
| 3 | Phthalate-NaOH | 1 | 2 | — | — | — | 1 | 2 | — | — | — | 1 | 1 | 2 | — | — | — |
| 4 | Veronal-HCl | — | — | 1 | 1 | 1 | — | — | 1 | 1 | 1 | — | — | — | 1 | 1 | 1 |
| 5 | Citrate-phosphate | L | L | L | — | — | — | L | L | — | — | L | 2 | 2 | 1 | — | — |
| 6 | Citrate | L | L | — | — | — | L | L | — | — | — | L | 2 | 2 | — | — | — |
| 7 | Succinic acid-NaOH | L | L | — | — | — | 2 | 2 | — | — | — | 1 | 2 | 2 | — | — | — |
| 8 | Acetate | L | L | — | — | — | L | 2 | — | — | — | 1 | 2 | 2 | — | — | — |

L – Lethal.

Table 2

Rate of fission of *Blepharisma intermedium* in Citrate and Citrate-phosphate buffers

| Buffer | pH | | | | | |
|---|---|---|---|---|---|---|
| | 3 mM | | 2 mM | | 1 mM | |
| | 5.0 | 6.0 | 5.0 | 6.0 | 5.0 | 6.0 |
| Citrate | 2 | L | 2 | + | 2 | 2 |
| Citrate-phosphate | 2 | 1 | 2 | 1 | 2 | 2 |

L – Lethal.
\+ – Survives and fission is blocked.

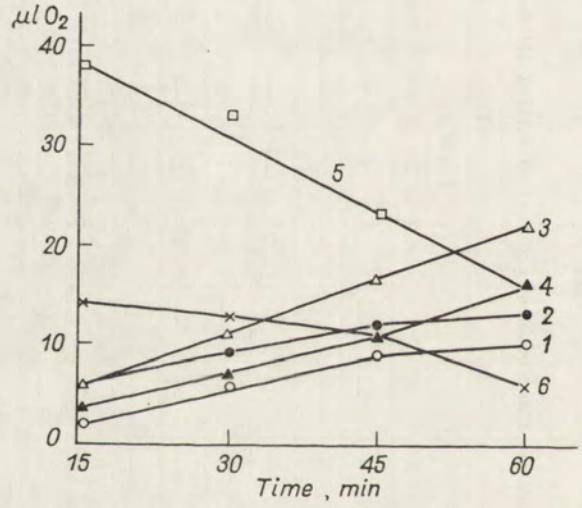


Fig. 1. Oxygen uptake of *Blepharisma intermedium* in different buffers
1 — citrate–phosphate buffer, 1 mM, pH 5.0; 2 — citrate–phosphate buffer, 1 mM, pH 6.0; 3 — acetate buffer, 1 mM, pH 5.0; 4 — acetate buffer, 1 mM, pH 6.0; 5 — Tris-maleate-sodium hydroxide buffer, 1 mM, pH 6.0; 6 — Tris-maleate-sodium hydroxide buffer, 5 mM, pH 5.0

Table 3

Effect of pH and buffer on oxygen consumption of *Blepharisma intermedium*

| Buffer | pH | Molarity in mM | $O_2$ consumption µ 1/$10^6$ cells/min |
|---|---|---|---|
| Tris-maleate-NaOH | 5.0 | 5 | 273 |
| | 6.0 | 1 | 684 |
| Citrate-$Na_2HPO_4$ | 5.0 | 1 | 186 |
| | 6.0 | 1 | 297 |
| Acetate | 5.0 | 1 | 366 |
| | 6.0 | 1 | 255 |

in Tris buffer at pH 6.0 it is more than double of what it is in the other two buffers. With Tris buffer at this pH the oxygen consumption is the same in 10 mM, 5 mM and 1 mM concentrations.

The oxygen consumption reaches a maximum in 15 min and then decreases in Tris buffer at all pH values and molarities. In citrate-phosphate buffer, the oxygen uptake almost reaches a constant value in 60 min while in acetate buffer it has still an upward trend.

## Discussion

Relatively little is known about multiplication rate of ciliates in relation to pH. While many ciliates survive over a wide range of pH, there is an optimum pH for maximum growth and fission. Darby's (1929) studies on *Paramecium* have shown that when all conditions except pH are kept constant *Paramecium caudatum* and *Paramecium aurelia* have optimum pH of 7.0 and 6.7 respectively for fission. Jenkin (1927) observed that a pH of 7.4 was optimal for growth of *Spirostomum ambiguum*. Similar studies of Loefer (1938) have shown that *Paramecium bursaria* could be cultured between pH 4.9 and 8.0 but the maximum growth is at pH 6.8. Loefer (1935) found this true of flagellates also. He observed that with *Chlorogonium euchlorum* the optimum pH for growth was 7.4. He also found that *Chilomonas paramecium* survives between the range of pH 4.1 to 8.4 but has two optima for growth at pH 4.9 and 7.0 *Stentor coeruleus* grew best in cultures adjusted to pH 7.7 to 8.0 as observed by Hetherington (1934). In his studies on the effect of pH of medium on the growth rate, Wingo and Anderson (1951) observed that *T. geleii* TP has two pH's of maximal growth rate; one maxima is slightly above pH 7.0 and the other extends from pH 5.0 to 6.8 Prescott (1958), however, observed that *T. geleii* has only one optimum pH at 7.25.

The growth and fission of *B. intermedium* extends over a wide range of pH 4.0–9.0 but there is an optimum pH for maximum growth rate and this is in conformity with the growth behaviour of other protozoans. In all the eight buffers used, at suitable molarity, the fission rate was maximum at pH 6.0. With the first four buffers of Table 1 the fission rate was 1 at pH 5.0 or 7.0, wherever these pH values were obtainable with the buffer.

The division rate of 2 is, however, not restricted to pH 6.0 with the buffers 5–8 (Table 1). The ciliates divide at the rate of 2 even at pH 5.0, if the molarity is favourable for growth. The above division rate at pH 6.0 also depends upon the molarity of the buffers used. While the ciliates divide at this rate in 10 mM, 5 mM and 1 mM of buffers 1–4, they do not survive in 10 mM concentration of buffers 5–8. But they divide at the rate of 2 in 5 mM succinate buffer at both pH values of 5.0 and 6.0, while they do so only at pH 6.0 in acetate buffer of the same molarity.

Table 2 shows the fission rate of *B. intermedium* in citrate and citrate-phosphate buffers at pH 5.0 and 6.0. In 3 mM citrate buffer of pH 6.0 the ciliate does not survive,

while in 2 mM buffer it survives but does not divide. At pH 5.0 in both molarities of the buffer the fission rate is, however, 2. In citrate-phosphate buffer the fission rate is 2 at pH 5.0 and 1 at pH 6.0 in both molarities. Thus the present investigations prove that the nature and molarity of the buffer play an important part in determining the optimum pH for growth and fission rate in *B. intermedium*.

The change of optimum pH of growth with variations in components of the medium has been reported for other protozoans. Earlier studies by Cramer and Myers (1952), Gross and Jahn (1958) and Hutner and Provasoli (1951, 1955), show that the growth of *Euglena* varies with the nature of the medium. Wilson et al. (1959) have shown that the pH of growth of *Euglena gracilis* var. *bacillaris* differs when succinate, fumarate, malate, acetate and citrate act as sole carbon sources. In our experiments the organic acids used for buffering action are not the only carbon sources for the organism. But the fact that we have noticed similar effects shows their influence in ciliate growth even when other carbon sources are present. The observations of Prescott (1958) have shown that at optimal pH of growth, generation time of *T. geleii* varies with change of media. This confirms the influence of buffering constituents on the optimum pH of growth of the ciliate.

The multiplication rate of a cell is closely dependent upon enzymatic processes of the cell. The external pH of the medium might influence the cell enzymatic activities in two ways: the environmental pH might effect enzymatic processes localized at the cell surface and thus interfere with the rate of nutrient uptake; the internal pH of the cell may change due to the penetration of the external constituents of the medium across the cell membrane, thus altering the normal cellular activities.

Investigations of Bates and Hurlbert (1970) on *Euglena gracilis* have shown that acetate causes death to the organism at pH values below 5.0 because the undissociated form of the molecule easily penetrates the cell membrane. The dissociation of the acid after entering the cell results in the lowering of the internal pH thus disturbing the cell enzymic processes. Their work with media containing other TCA cycle acids has also shown that all undissociated acids are not freely penetrable. The extent of dissociation and permeability of different acids depends upon the pH of the medium. Thus our observations of the differences in growth rate of *B. intermedium* with nature and molarity of the buffer is conceivably due to the difference in the dissociation and extent of permeability of the acid components. This also accounts for the difference noticed in the viability of *B. intermedium* in different buffers of varying molarity.

Differences have also been noticed in the oxygen uptake of *B. intermedium* with changes in pH and buffer. In acetate buffer, the oxygen uptake is more at pH 5.0 than at 6.0, but the division rate is the same at both the pH values. Studies on the oxidative metabolism of *Euglena* by Danforth (1953) have shown that oxygen uptake is pH dependent. Using different Kreb's cycle acids, he showed that this pH dependence was due to the difference in the ability of the organic acids to penetrate the biological membrane. The work of Bates and Hurlbert (1970) has shown

that not only growth but also respiration is pH dependent and varies with the nature of the carbon source.

In 1 mM Tris buffer at pH 6.0 the oxygen consumption is about twice that of the other two buffers at the same pH. This is true with 10 mM and 5 mM buffers also. It is observed that with this buffer the oxygen uptake is high initially and gradually declines. Similar observations have been made by Von Dach (1942) in his studies on the respiration of *Astasia klebskii* in inorganic medium.

It is reported by Seaman (1949) and Ryley (1952) that citrate did not have effect on the oxygen uptake of *Tetrahymena pyriformis* GL. With *B. intermedium*, however, the rate of oxygen uptake at pH 6.0 is the same as in standard culture (250 µl/$10^6$ cells/min of organisms grown in unbuffered hay infusion media) while at pH 5.0 it is less by about 30% showing that citrate also affects respiration rate depending on the pH of medium.

Thus the optimal growth of *B. intermedium* is pH and buffer dependent. Though the oxygen uptake differs with pH and buffer there is no correlation between growth and oxygen consumption. No such correlation has actually been found in studies with other organisms. The differences in both growth and oxygen consumption are said to be due to the differences in ionization and permeability of the substances across the cell membrane. Perhaps results on growth and respiration will be more meaningful if both the studies are made simultaneously.

Acknowledgement

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## Summary

The fission rate of *Blepharisma intermedium* between the pH range 4.0–9.0 with different buffer systems of varying molarity has been studied.

The optimum pH of fission is 6.0. This depends on the nature and molarity of the buffer. With some acids of the TCA cycle as buffering components the optimum pH is 5.0.

Though there are differences in the oxygen uptake of the organism with pH and buffer components there is no correlation between growth and oxygen consumption.

## ZUSAMMENFASSUNG

Es wurde untersucht: das Teilungstempo des *Blepharisma intermedium* zwischen pH 4.0 und 6.0 bei verschiedenen Puffersystemen und variirender Molarität.

Das Optimum des pH beträgt bei der Teilung 6.0. Es hängt aber von der Natur und der Molarität des Puffers ab. Mit einigen TCA Säuren, als Pufferkomponenten, beträgt das Optimum des pH 5.0.

Es gibt unterschiedlichen Verbrauch des Sauerstoffes aber keine Korrelation zwischen Sauerstoffverbrauch und dem Wachstum.

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# The effect of high intensity magnetic field on the entrance of various molecules into *Paramecium caudatum*

Der Einfluss eines magnetischen Feldes hoher Intensität auf den Eintritt verschiedener Moleküle in das *Paramecium caudatum*

Within the last 10 years there have been numerous well substantiated reports of magnetic fields influencing biological systems. These magnetic effects range from altered growth and orientation in both plants (Pittman 1965, 1967) and animals (Barnothy 1964, 1969), to alternations of enzyme activity (Hedrick 1964, Cook and Smith 1964). Interactions between magnetic fields and unicellular forms have also been studied; these studies indicate that *Chlorella* (Halpern and Konikoff 1964) and *Blepharisma* (Gibson and Isquith 1967, Isquith and Gibson 1969) have altered growth rates in both increased and diminished fields. *Volvox* (Palmer 1963) and *Paramecium* (Brown 1962; Kogan and Tikhonova 1965) move with respect to the direction of the field, and *Spirostomum's* susceptibility to various toxic compounds is altered (Rostkowska and Moskwa 1968). Magnetic fields have further been involved with protozoa in that there seems to be a strong correlation between reversals of the earth's magnetic field and extinction of various antarctic radiolarians (Hayes and Opdyke 1967).

It does not seem likely that each of these diverse effects, as well as numerous others reported in the literature, has its own unique mechanism. Rather it seems more probable that these effects are reflections of one or perhaps a few mechanisms. Some of the observed effects, especially those reported by Rostkowska and Moskwa (1968) on *Spirostomum*, indicated that cell permeability might be altered due to the field. Since permeability alterations could explain some of the observed magnetic effects, it was decided to perform a study of the effects of a high intensity magnetic field on the entrance of various substances into a cellular system. Due to the abundance of literature on osmoregulation (contractile vacuole activity) in *Paramecium* (e.g. Czarska 1964, Organ et al. 1968, 1969), *P. caudatum* was chosen for these studies.

The following premise was made: If the cell is to maintain itself, the total amount of water within the cell must remain relatively constant. Metabolic water's contribution to the contractile vacuole (c.v.) is negligible; water taken in with food vacuoles is voided with egested materials, and pinocytotic water is also negligible (Kitching

1967). Therefore, the only measurable source of water with which the c.v. is concerned, to any extent, is entering through the cell membrane.

Since vacuolar output equals vacuolar rate X vacuolar volume, if vacuolar volume is constant, any change in the rate of water penetrance into the cell should be compensated for by a change in vacuolar rate. Therefore, if a magnetic field caused a change in water entrance, this would be reflected in a change of vacuolar rate. This statement should be correct provided the size of the organism and contractile vacuole remains constant.

## Methods

*Paramecium caudatum*, being an extremely mobile form, required immobilization to allow the obtainment of data. The immobilization was accomplished with a one hour treatment of the organisms in a solution of $2.5 \times 10^{-3}$% nickel sulfate in 0.1% cerophyl. A random sampling of organisms was taken from a culture and transferred sequentially through three washes of the nickel solution in a three spot depression slide; the third depression being used as the experimental chamber. Organisms subjected to a magnetic field of 1 750 gauss (approximately 3 500 × the earth's magnetic field) were measured for body length, width, maximum diameter of the posterior contractile vacuole, and duration of the contractile vacuole cycle.

## Results

Table 1 contains data of morphological characteristics of *P. caudatum*. Analysis of variance was performed on the data. This study did not show any

Table 1

Morphological characteristics of *P. caudatum* in a magnetic field of 1 750 gauss at 24–25°C. Experimental organisms were exposed to magnetic field during the 30–59 min time interval. Each value represents the average of 6 experiments. For each experiment, 15–20 pieces of data were obtained starting at five minutes before the end of each time interval. These values represent the maximum diameter of the posterior c.v. All measurements are in micrometres

| Time after immobilization | Body length | | Body width | | Maximum posterior c.v. Diameter | |
|---|---|---|---|---|---|---|
| | exptl. | control | exptl. | control | exptl. | control |
| 0–29 min | 214.55 ±11.15 | 214.40 ±8.45 | 49.25 ±3.80 | 50.65 ±2.45 | 13.80 ±0.70 | 14.15 ±0.70 |
| 30–59 min | 213.24 ±4.20 | 214.75 ±8.55 | 47.90 ±2.55 | 50.05 ±2.85 | 14.05 ±0.50 | 13.90 ±0.85 |
| 60–89 min | 212.40 ±4.05 | 212.90 ±7.80 | 49.15 ±2.70 | 50.55 ±3.70 | 14.50 ±0.00 | 14.40 ±0.70 |
| 90–119 min | 210.75 ±4.55 | 211.50 ±7.80 | 48.80 ±3.15 | 51.50 ±2.80 | 14.55 ±0.50 | 14.30 ±1.00 |
| 120–149 min | 208.05 ±3.70 | 211.30 ±7.85 | 49.90 ±3.80 | 51.50 ±2.05 | 14.55 ±0.50 | 14.50 ±0.85 |

statistically significant change in either body length, body width, or vacuole diameter in either the controls or the magnetically treated organisms; therefore, we must assume any change in water entrance due to the magnetic field would be reflected by a change in vacuole cycle duration.

Analysis of variance was performed on the contractile vacuole duration data. The standard deviation for each time interval for both control and experimental groups was computed. In addition, in order to obtain a clearer picture of experimental differences, the variability introduced into the data due to different physio-

Table 2

Duration of contractile vacuole cycle. Data were gathered during intervals of five minutes each. Each experimental value represents the average of the six replicates; each control value represents the value of five replicates. Each replicate is the average of twelve pieces of data. The experimental organisms were exposed to the magnetic field for the time intervals 6–11. Time interval 1 began immediately after immobilization. The temperature range for the entire series was 25±1.5°C; there was less than 1°C for any one replicate. All measurements are in seconds

| Time interval | Experimental average | % Increase compared to interval No. 1 | Control average | % Increase compared to interval No. 1 |
|---|---|---|---|---|
| 1 | 12.13 | 0.0 | 10.82 | 0.0 |
| 2 | 12.25 | 1.0 | 11.33 | 0.5 |
| 3 | 12.39 | 2.1 | 10.98 | 0.1 |
| 4 | 12.34 | 1.7 | 10.99 | 0.1 |
| 5 | 12.37 | 2.0 | 10.81 | 0.0 |
| 6 | 12.39 | 2.2 | 10.48 | —0.3 |
| 7 | 12.21 | 0.7 | 10.72 | —0.9 |
| 8 | 12.06 | —0.5 | 10.80 | 0.0 |
| 9 | 12.25 | 1.0 | 11.04 | 2.0 |
| 10 | 12.30 | 1.4 | 10.75 | —0.6 |
| 11 | 12.49 | 3.0 | 11.14 | 3.0 |
| 12 | 12.53 | 3.3 | 11.12 | 2.8 |
| 13 | 13.88 | 14.4 | 11.30 | 4.4 |
| 14 | 14.40 | 18.4 | 11.57 | 6.9 |
| 15 | 15.03 | 23.9 | 11.64 | 7.6 |
| 16 | 15.39 | 26.9 | 11.91 | 10.1 |
| 17 | 15.89 | 31.0 | 12.02 | 11.1 |
| 18 | 15.51 | 27.9 | 11.87 | 9.7 |
| 19 | 16.19 | 33.5 | 12.58 | 16.3 |
| 20 | 16.91 | 39.4 | 12.70 | 17.4 |
| 21 | 16.97 | 39.9 | 12.98 | 20.0 |
| 22 | 17.98 | 48.2 | 13.53 | 25.1 |
| 23 | 18.09 | 49.1 | 13.92 | 28.6 |
| 24 | 18.59 | 53.3 | 14.51 | 34.1 |
| 25 | 18.94 | 56.1 | 15.10 | 39.6 |
| 26 | 20.19 | 66.5 | 14.93 | 38.0 |

logical conditions was eliminated. This was accomplished by obtaining the percent increase during each time interval compared to the time 1 interval. See Fig. 1.

It was found that the c.v. cycle duration for the control and experimental groups in the premagnetic and magnetic periods (i.e., the first 12 time intervals) was quite constant. No statistically significant difference was detected in the experimentals and all possible sources of variance were insignificant.

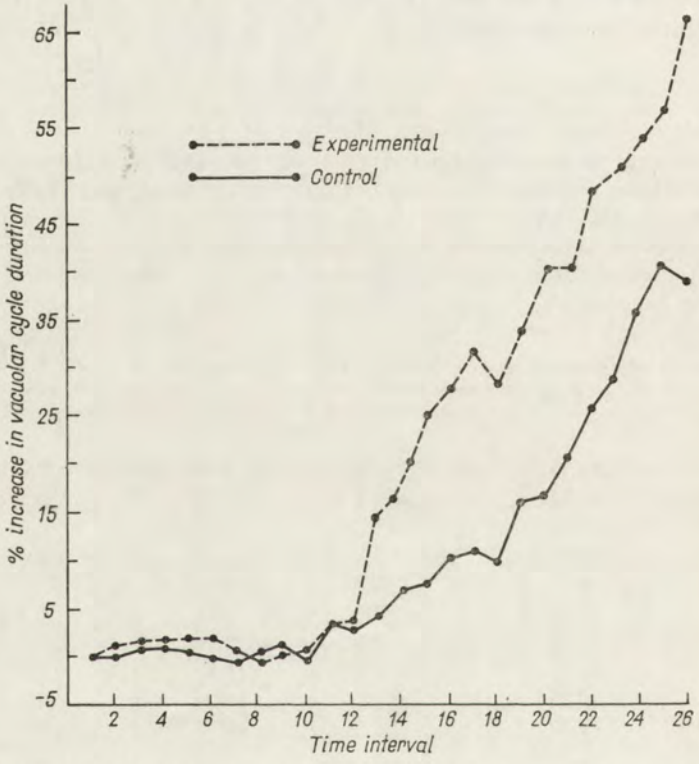


Fig. 1. Contractile vacuole activity. Five minute time intervals 1–6 are premagnetic period; 6–11 are period of magnetic treatment; and 12–26 are postmagnetic

The duration for the control organisms in the postmagnetic period increased compared to the first period. This change, however, was shown to be statistically insignificant. In contrast, the organisms subjected to the high intensity field were found to have a highly significant increase in duration when compared to the first period. All possible sources of variance were found to be statistically insignificant in the case of the controls. In the experimentals, however, one source of variance, that of magnetic treatment, was shown to be significant. In the premagnetic and magnetic periods, where the cycle of both control and treated organisms remained the same, we must assume that water entrance was constant. However, in the post-magnetic period, the cycle of the magnetically treated groups was found to be signi-

ficantly longer than the controls. Taking into account the previous data, this change in cycle duration must be assumed to be a reflection of a decrease in water entrance into the organism as caused by the magnetic field.

Since the organisms had been subjected to nickel ions for immobilization, the increased cycle duration observed in the control as well as part of the increase observed in the experimentals in the post magnetic period was possibly brought about to some extent by the role of the nickel ion in metabolism. Such an effect has been reported in the literature (Andrivon 1968). To show that the observed magnetic effect was not merely a reflection of altered nickel activity in the magnetic field, a nickel immobilization study was conducted.

This study was composed of 10 experiments, each control and experimental group consisting of 30 organisms. The experimental group was exposed to the field for the duration of the experiment. The study showed that organismic immobilization by $2.5 \times 10^{-3}$% nickel was accomplished in the same time for both controls and treated groups. Therefore nickel entrance into the cell and nickel's effect on metabolism were not being altered to a great extent by the magnetic field. If either one of these factors had been affected, the time required for immobilization would have changed. Also if nickel was having an accelerated deleterious effect on the c.v. activity in the magnetic field, and water entrance remained constant, the cell would be expected to swell. No statistically significant swelling was observed; therefore, the observed change in vacuolar rate was not due to the magnetic field's influence on nickel. None the less, this did not preclude the possibility that the observed change in vacuolar rate (the magnetic effect) was in some way being mediated by the nickel ion. To gather relevant to this possibility, a permeability study in which no nickel ions were involved was undertaken. This study involved alcohol immobilization of *Paramecium caudatum*.

The alcohols used were methanol, n-propanol and n-butanol. This group of alcohols was chosen because they form an homologous series different for each other in polarity and length of carbon chain.

The same concentrations of each alcohol could not be used. If the range of concentrations for all the alcohols had been lowered to 1–2%, immobilization would have taken too long a time for methanol and n-propanol; if it had been raised, it would have resulted in lethality for n-butanol and n-propanol.

Fifty percent immobilization of the organisms was the parameter assumed to reflect the rate of alcohol entrance into the cell. It was found, that the higher the concentration of alcohol, the less significant is the difference between the experimentals and the controls. Thus the effect was most pronounced in 7% methanol and 3.5% n-propanol where the magnetically treated organisms did not become immobilized after one hour. The difference in immobilization in the controls and treated organisms for each alcohol reflected a difference in the rate at which the alcohols entered the cells. See Fig. 2. Since this system contained no nickel ions, it seems that the magnetic effect does not depend upon the presence of nickel.

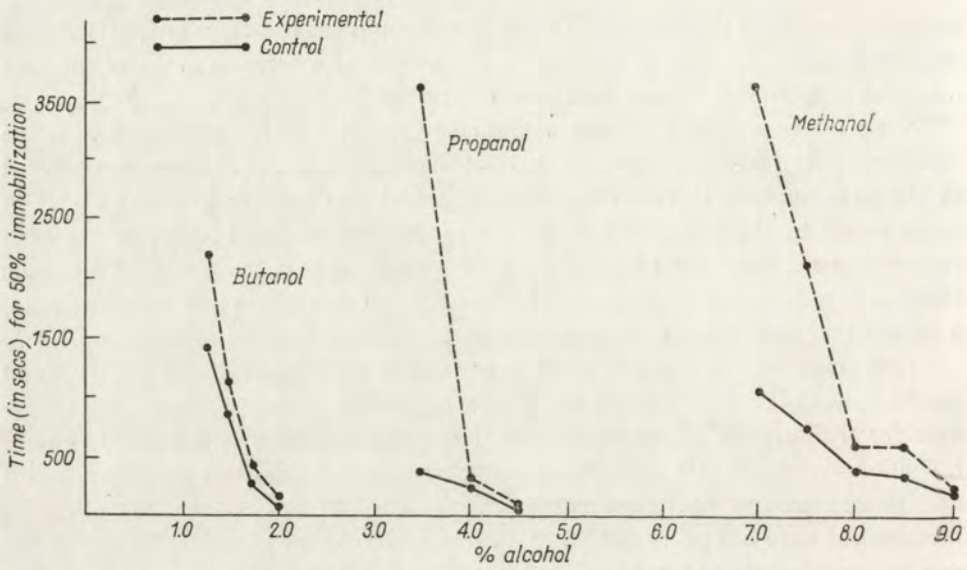


Fig. 2. Time required for immobilization of 50% of the population

## Discussion

From this group of studies the following conclusions appear at this time to be valid. First, and perhaps most significant is that the rate of water entrance into *Paramecium* is decreased by a high intensity magnetic field. In addition, the penetrance of the three alkane alcohols studies also show a decrease in penetrance into the cell. In contrast the rate of entrance of nickel ions into the cell seems to be relatively unaffected by a magnetic field. Although no precise mechanism is apparent at this time, it seems that these affects may be brought about by an alteration in membrane permeability especially to polar compounds.

If indeed alterations of membrane properties due to magnetic fields prove to be universal, it is quite likely that such effects could be the basis for many highly diverse biomagnetic effects reported in the literature.

## Summary

The entrance of various chemicals into *Paramecium caudatum* under the influence of a magnetic field of 1750 gauss has been investigated. The organism's contractile vacuolar activity was used as a criterion for alterations in the rate of water penetrance into the cell under the influence of the magnetic field. Magnetic fields were also shown to influence the rate of alkane alcohol penetrance into the cell, using the time required for immobilization of the cell as a criterion. Both the alkane alcohol and water penetrance decreased under the magnetic field's influence.

## ZUSAMMENFASSUNG

Der Eintritt verschiedener Chemikalen in das *Paramecium caudatum* unter dem Einfluss eines magnetischen Feldes von 1750 Gauss wurde untersucht. Die Beobachtung der Aktivität des Kontraktvakuolen führte zur Teststellung einer verminderten wasseraufnahme unter dem Einfluss des magnetischen feldes. Die Bestimmung des Zeitfaktors bezüglich der Unbeweglichkeit des *P. caudatum* ergab ebenfalls eine verminderte Aufnahme von Alkan alkohol.

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# Effect of some chemical factors on the euglenoid movement in *Euglena gracilis*

## Wpływ niektórych czynników chemicznych na ruchy euglenoidalne u *Euglena gracilis*

The mechanism of euglenoid movement as well as its localization in the cell have not been exactly determined and are still the open problem. Two following hypotheses regarding the localization and the mechanism of the body contraction are put forward: the first assumes that the mechanism responsible for changes of the body shape is present in the cortex (pellicular complex) of the cell (Dangeard 1902, Günther 1927, Dasgupta 1964, Diskus 1956, Mignot 1965, 1966, Schwelitz et al. 1970); the second one points to the fact that this mechanism is present in the interior part of the cell. According to the last hypothesis, the pellicular complex has a passive role in this movement, defining only the degree of the elasticity of a cell. Structure and thickness of the periplast determine changes of the shape only in certain patterns, characteristic for particular species (Conrad 1940, Pringsheim 1948, 1956, Łozina-Łozinski and Zaar 1963, Byrne and Marsland 1965, Leedale 1966, Leedale et al. 1965).

In the present report investigations on the influence of some chemical factors on the euglenoid movement were carried out in order to establish the ionic background of the excitation and localization of the structures responsible for changes of a body shape.

In many papers concerning the euglenoid movement few observations have been done which deal with the influence of chemical agents on this movement. Most of investigations were done on the action of pH on euglena cell (Le Févre 1931 Alexander 1931, Hall 1933, Kamiya 1939). Consequently very little is known about excitability and participation of $Ca^{2+}$ ions in the euglenoid movement. We could examine their role only by analogy with other *Protozoa*, since it is known that calcium ions play the most important role in the excitability and contraction of the cell (Kamada 1940, Kamada and Kinosita 1940, Jahn 1962, 1967, Grębecki 1964, 1965, Kinosita, Dryl and Naitoh 1964, Naitoh 1968, Kuźnicki and Mikołajczyk 1969, Sleigh 1970, Ettienne 1970). Jahn and Bovee 1968

suggested that excitation and contraction of euglena body should have a calcium mechanism. In this connection the first purpose of this work was to confirm experimentally the calcium hypothesis, to study the influence of agents removing the $Ca^{2+}$ ions from the surface on the body contraction and to attempt to clear up the role of calcium in the euglenoid movement.

The second purpose was to investigate the influence of 2,4-dinitrophenol on the euglenoid movement. The choice of this chemical agent was based on the previous experiments regarding influence on regeneration in *Blepharisma undulans* (Giese and McCaw 1963). Individuals which have been exposed to DNP regenerated at the same rate as the controls. However, it was observed that DNP acts on the rate of ciliary movement (Ryley 1952, Giese and McCaw 1963). Thus, all these date show that DNP is not penetrating the interior of the cell, but effects the cortical layer. Recently, Sleigh 1970 working on the excitability of *Spirostomum*, suggests that DNP acts at the surface of membrane or has influence on the Ca equilibrium in the superficial cytoplasm.

Some data relating to the effects of DNP and other chemical agents on the euglenoid movement in *Euglena gracilis* have been presented by the author elsewhere (Mikołajczyk 1969). The DNP appeared as an agent which causes the immediate inhibition of euglenoid movement. This phenomenon proved to be reversible since it was shown that after removing the poison from the medium, the ability to the body contraction is recovered. In the present paper these earlier observations were expanded and discussed.

## Material and methods

The material for experiments was *Euglena gracilis* strain Z. Cultures have been grown on Hutner's (Hutner et al. 1956) liquid medium, and have been kept at 25°C in artificial, constant illumination.

Speciments for experiments were condensed by centrifugation (max 30×G) no longer than 2 min. Densified cells were rinsed 3 times in phosphate–citrate buffer (Dryl 1959) or 10 mM Tris HCl buffer of suitable pH. The same buffer in experimental medium was applied as diluent.

Experimental procedure began not earlier that 2–3 h after centrifugation. Observations were carried out on a single cell as well as on numerous specimens (200–300 flagellates) at the same time. Each type of experiment was repeated no less than 100 times.

Experimental procedure: 0.002 ml of solution containing specimens was immersed in the 1 ml of the test solution. The control tests of the behaviour of specimens in pure buffer solution and at the same pH were carried out.

The influence of solutions EGTA, sodium citrate, KCl, NaCl, $CaCl_2$ and DNP on euglenoid movement was investigated.

The EGTA solution in concentrations 5–75 mM and sodium citrate in concentrations 5–100 mM were prepared on 10 mM Tris HCl buffer at pH 7.2–7.6.

The KCl in concentration 16–160 mM at pH 5.3 and 6.9, NaCl in contraction 16–100 mM at pH 5.5 and 7.2 were prepared on 10 mM Tris HCl and phosphate–citrate buffer.

DNP solutions in concentrations 0.25–2.50 mM at range of pH 4.4–8.1 were prepared on phosphate–citrate buffer.

For the purpose of examining the behaviour of individuals during recovery of euglenoid movement after removing of DNP from the medium, the following procedure was performed: the washing was carried out after 2 min action of 1 mM DNP at pH 5.0. The specimens were rinsed 3 times at 1 min intervals in pure buffers (phosphate–citrate at pH 5.1 and 10 mM Tris HCl at pH 8.1) or in buffer with 10 mM $MgCl_2$ at pH 7.5 and 2 mM $MgCl_2$+2 mM $CaCl_2$+2 mM ATP at pH 7.6.

After rinsing all the individuals regained the ability to contract, but this phenomenon was not synchronized.

Some experiments (e.g., recovery of euglenoid movement after action of DNP) were recorded by 16 mm Pentaflex cinematographic camera on colour UK 16 ORWO film. The motion picture were taken at 0.8 frames per sec. Changes of euglena shape were analyzed with a film analysing projector.

## Results

### The influence of cations on euglenoid movement

The investigation of the influence of KCl, NaCl and $CaCl_2$ on the euglenoid movement were carried out.

The potassium ions are most often used to evoked the ciliary reversal. In case of *Euglena gracilis* the concentration of potassium over 100 mM stimulates the body movements, but the reaction does not appear in 100% of individuals. It was found that the optimum of potassium activity as well as the other ions depends on the pH of the medium. Optimum of effectiveness was observed at pH 5.5. At pH 6.9 the stimulatory action of 100–160 mM KCl was not noticed. The potassium ions in the range of concentrations 100–160 mM independently on the pH of the medium had an inhibitory action on the flagellar movement. It was observed as the slow down or total inhibition of the flagellar beat.

It was found that the euglenoid movement appeared in about 90% of individuals in the sample at pH 5.0 when the concentration of sodium ions reached 100 mM. After 10–11 min the body movements disappeared. The same concentration of NaCl or higher — up to 160 mM but at pH 7.6 had no stimulatory action.

It was found that not only NaCl but also $CaCl_2$ stimulates the body contraction in *Euglena gracilis*. The differentiation was observed in the range of pH in which the reaction appeared. The optimum of $CaCl_2$ action was stated at pH 7.2. In this pH the 75–100 mM solutions of calcium evoked the contractile reaction of the euglena body, intensity of which was identical to the potassium action. At pH 5.5 the calcium ions in concentration 75–100 mM did not show the stimulatory action. It should be emphasized that calcium ions in this range of concentrations, as in the case of KCl, had a disturbing action on the flagellar movement.

Contractions under influence of KCl or $CaCl_2$ appear as frequently repeated responses one after another while the contractions in the sodium solution appear at lower frequency with longer intervals between each contraction.

In all cases the character of the euglenoid movements is practically the same. The first slight contractions cause little shortening of the body while the stronger

ones lead to the formation of a sphere or disc-like shape of the cell (Fig. 1). With the lapse of time kind of euglenoid movement changes to the peristaltic waves (Plate I). Detailed analysis of the euglenoid movement patterns was presented in the previous paper (Mikołajczyk 1972).

Effect of EGTA and sodium citrate

EGTA and sodium citrate proved to be very effective agents stimulating the euglenoid movements. At some range of concentrations they caused the body contr-

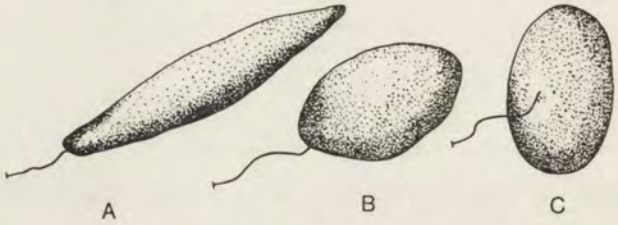


Fig. 1. Drawing the euglenoid movements leading to the formation, from elongated form (A), a sphere (B) and disc-like shape (C)

actions in 100% of individuals. The character of euglenoid movements was the same in both cases. The immersion of individuals in the test solution caused, at the beginning, contractions to a sphere or "disc" shape and next transformed into peristaltic waves.

Effect of EGTA: at pH 7.2–7.6 the lowest concentration which stimulates the euglenoid movements in 100% of individuals was 5 mM. The contractions persisted 3–6 min. The graduate increase of the concentration to 50 mM neither changes the character of contractions nor the time of their persistence.

In all cases of the examined concentrations of EGTA, individuals were kept 24 h. After this time the capacity of changing the body shape was maintained. It should be emphasized that the contractions appear in swimming individuals as well as in the substrate.

In the low concentrations (5–10 mM) no disturbing influence of EGTA on the flagellar movement was observed. After 24 h all specimens in the sample swam freely. However, in 50 mM solution all individuals were found laying on the substrate.

Effect of sodium citrate: the influence of the sodium citrate in the range of concentrations 5–100 mM on the euglenoid movement was investigated. The contractions in 100% of individuals appeared from 10 mM and persisted 2–5 min. The rise of concentrations, as in case of EGTA, did not change either the character of the body movements or the time of their persistence.

It was found that calcium ions in the concentration even up to 50 mM did not stop immediately the euglenoid movement caused previously by 10–20 mM EGTA or 25–50 mM sodium citrate.

The effect of DNP

The preliminary experiments on the influence of DNP on the euglenoid movements demonstrated that this agent at appropriate concentration causes their immediate immobilization (Mikołajczyk 1969) in the meaning that 100% of individuals are immobilized at the moment of contact with the applied agent. The specimens treated with DNP became non-sensitive to the action of intensive light, mechanical factors and EGTA. Next, it was found that the effect of DNP action depends on the pH of the medium (see Table 1). The inhibition of the body contractions was obtained at pH below 6.5. At pH 4.4 the concentration of 0.25 DNP

Table 1

Immobilizing effect of DNP in relation to the pH of external medium

| Concentration of 2,4-DNP in mM | pH of the medium | | | | | |
|---|---|---|---|---|---|---|
| | 4.4 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 |
| 0.25 | + | — | — | — | — | — |
| 0.30 | + | + | — | — | — | — |
| 0.50 | + | + | + | — | — | — |
| 1.50 | + | + | + | + | — | — |
| 2.50 | + | + | + | + | + | — |

+ — 100% of immobilized individuals.
— — absence of immobilization.

was sufficient for immobilization of the body movements. Immobilizing effects of DNP decreased parallel to increase of external pH value. At pH 5.5 the effect of immobilization was obtained in 1.5 mM DNP, while at pH 6.0 in 2.0 mM DNP solution. When pH reached 6.5 or higher 2.5 mM DNP had no an inhibitory action. Even though specimens were kept in 2.5 mM DNP at pH 6.5 during 24 h, neither the body movements were inhibited nor any changes in the work of the flagellum were noticed.

It should be emphasized, that together with the immobilization of the body contractions, the autotomy of the locomotory flagellum takes place. The point at which the flagellum breaks has not been exactly established.

It has been observed that immobilized individuals have lost their normal, elongate shape becoming shorter than control ones. Probably at the first moment of the contact with DNP, the shock contraction appears and the cell looks like "frozen" in this stage of contraction. The body immobilized by DNP is not elastic. This fact was examined by putting the specimens on the agar surface. The shape of control euglena is flattened on the agar surface by the gradual increase of the surface tension, while the immobilized one maintains its shape.

Recovery of euglenoid movements

It was observed that individuals immobilized by 2,4-dinitrophenol show visible morphological changes inside of cytoplasm after 2–3 h. These changes are visible as disappearance of chlorophyll. Places previously occupied by chlorophyll become white and transparent. In spite of such distinguished deformation of the cell structures, after DNP is removed from the medium, the synthesis of chlorophyll, as well as the ability to contract and regenerate the flagellum are recovered. It appears that at the beginning 2,4-DNP affects the structures of the pellicular complex and then very slowly penetrates inside of the cell. Therefore, the study of recovery of euglenoid movements was carried out after 2 min of exposure of euglena to the action of DNP.

It appeared that only pH value had distinct influence on the rate of the recovery of euglenoid movements. Neither 10 mM $MgCl_2$ at pH 7.5 nor $CaCl_2+MgCl_2+ATP$ had effect (Mikołajczyk 1969). It was noticed that external stimulating factors could only intensify the frequency of contractions.

Up to the moment the first contractions in buffers reappeared (at pH 5.1 after about 13 min, pH 8.1 — 6 min) individuals are still insensible to light and chemical agents (EGTA).

The character of these contractions was cinematographically recorded and then analysed. At the beginning euglenoid movements appear as slight contractions

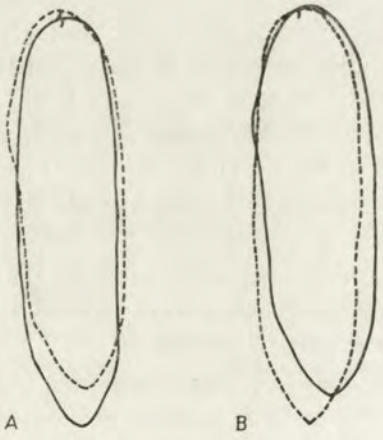


Fig. 2. One of the first contractions after 2.4-dinitrophenol is removed from the medium (analysis of the motion pictures). A — contraction, B — relaxation of the cell. Initial shape drown by continuous line, changed shape — discontinuous one

during which the posterior end of euglena moves towards the anterior one, while the anterior end practically does not change its position (Fig. 2). With the lapse of time the range of body movements gradually extends.

## Discussion

The results reported above do not give a very clear picture of the possible role of external calcium ions in the euglenoid movement. One important observation is that euglenoid movements could be induced by $CaCl_2$ and that calcium ions, added even in excess, do not stop contractions evoked by EGTA what may suggest that calcium ions do not play a specific role in the excitability of the euglena body.

However, the evidence of the existence of calcium — dependent mechanism is presented by experiments dealing with the decalcification of the surface membrane of the euglena. It was observed that EGTA as well as the sodium citrate cause the euglenoid movements in all specimens in the sample and this has been never observed in case of the action of cations. The fact that euglena kept even during 24 h in 50 mM EGTA solutions maintained the ability to change its shape, indicates that this chelating factor does not penetrate inside of the cell.

The appearance of the euglenoid movement under the influence of $CaCl_2$ may be explained as the result of the disturbing action on the flagellum. As it is known, the agents which cause the autotomy of the flagellum or its non-effective vibration stimulate the euglenoid movement (Dangeard 1902, Le Févre 1931, Hall 1931, Hilmbauer 1954, Fritch 1961, Mackinnon and Hawes 1961).

To summarize, it can be suggested that calcium ions play an important role in the excitability of euglenoid movement but this phenomenon needs more detailed investigations. Further experiments regarding this problem are planned. Results which have been obtained indicate, however, that in euglena, as well as in *Paramecium* (Jahn 1962, 1967, Kuźnicki 1970) the calcium ions, removed from the outer surface of the cell, cannot be the factor stimulating the ATP-ase of the contractile system.

Data regarding the inhibiting activity of the DNP action appeared to indicate a close correlation between the effectiveness of this agent and pH of the medium (Simon and Blackman 1949, Simon 1953). The activity of DNP increases parallel with decreases of pH. Such relation was also observed in pigment changes of *Euglena gracilis* by Greenblatt and Sharpless 1959. Zurzycki 1965 dealing with the chloroplast movements in *Lemna trisulca* found, that the effect of DNP at pH 5.8 was 10 to 20 times stronger than at pH 7.1. As it is known in the low pH DNP is in the undissociated molecules which are lipophilic and very well permeates across the membrane (De Deken 1955). In this connection the activity of DNP is determined not by the total concentration in the medium but by the concentration of its lipid — soluble form (Hemker 1962). This is probably the reason why a very low (0.25 mM) concentration of DNP at pH 4.4 is sufficient for causing the immediate inhibition in 100% of individuals. When pH is rising, the undissociated molecules of DNP dissociate to the anionic form, which possess lower lipid solubility and permeability.

Schwelitz et al. 1970 found the striated layer interior to the plasma membrane.

The 50 Å striations are separated from one another by 35 Å spaces and are grouped in 450 Å bands. Authors suggested that this structure might be involved in cell movements. The fact that euglenoid movement can be immediately but in a reversible way inhibited by action of DNP, seems to support the hypothesis derived from

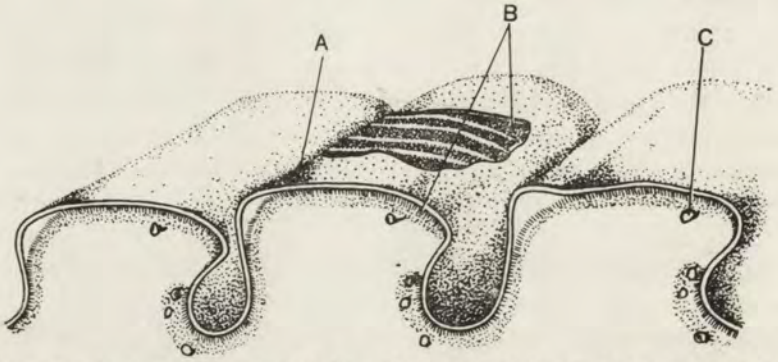


Fig. 3. Generalized scheme of the localization of the striated layer within the pellicular comples of the cell, drawing from the electron microscope pictures (Schwelitz et al). A — plasmalemma, B — striated layer, C — microtubule

the electron microscope studies by Schwelitz et al. 1970 which indicate that structures responsible for body movements are located within the pellicular complex, just below the plasmalemma (Fig. 3).

## Summary

The influence of some cations ($Na^+$, $K^+$, $Ca^{2+}$) sodium citrate and EGTA, as well as 2,4-dinitrophenol, at various range of pH of external medium, on euglenoid movements in *Euglena gracilis* was investigated.

Potassium, sodium as well as calcium ions induced the euglenoid movement maximum in 90% of individuals in *E. gracilis*, while the removing of the calcium ions from the outer surface of the cell by sodium citrate and EGTA caused the body contractions in 100% of individuals in the sample. This results indicate that calcium ions play important role in the excitability of the euglenoid movements, although it should be put in doubt whether calcium ions released from the outer surface of the cell could be the factor stimulating the ATP-ase of the contractile system.

It was found that at pH below 6.0, 2,4-dinitrophenol in concentration $\leqslant$2.5 mM caused the immediate, but reversible, immobilization of euglenoid movements. At pH 6.5 or above DNP, in concentration 2.5 mM, did not show inhibitory action. The inhibition of euglenoid movements is reversible. After DNP is removed from the medium, individuals regained the ability to change the body shape. The effect of DNP on the euglenoid movement points out to its action within the pellicular complex of the cell, and support the hypothesis suggesting the localization of the structures responsible for body movement in this part of the cell, just below the plasmalemma.

## STRESZCZENIE

Przeprowadzono badania wpływu niektórych kationów ($Na^+$, $K^+$, $Ca^{2+}$), cytrynianu sodu i EGTA oraz 2,4-dwunitrofenolu na ruchy euglenoidalne u *Euglena gracilis* w zależności od pH środowiska.

Stwierdzono, że zarówno jony potasu, sodu jak i wapnia stymulowały pojawianie się ruchów euglenoidalnych. Skurcze nie obejmowały jednak 100% pierwotniaków w próbce. Stwierdzono natomiast, że usuwanie jonów wapnia z powierzchni błony cytrynianem sodu lub EGTA powoduje pojawienie się ruchów euglenoidalnych u wszystkich wiciowców. Wyniki te pozwalają przypuszczać, że u *Euglena gracilis* jony wapnia pełnią istotną rolę w zjawiskach pobudzenia. Należy natomiast poddać w wątpliwość możliwość stymulowania ATP-azy systemu kurczliwego komórki przez jony wapnia zwolnione z zewnętrznej powierzchni błony.

Stwierdzono, że 2,4-dwunitrofenol w pH poniżej 6,0, w stężeniu $\leqslant 2.5$ mM wywołuje natychmiastowe zahamowanie ruchów euglenoidalnych, natomiast w pH 6,5 i powyżej związek ten w stężeniu 2,5 mM nie powoduje zahamowania skurczów ciała. Po przepłukaniu pierwotniaki odzyskują zdolność do zmieniania swego kształtu. Zarówno szybkość z jaką ruchy euglenoidalne ulegają zahamowaniu, jak i ich powrót po usunięciu DNP ze środowiska wskazują na jego działanie w obrębie kompleksu pellikularnego komórki, co potwierdza przypuszczenie, że struktury odpowiedzialne za zmiany kształtu ciała zlokalizowane są bezpośrednio pod plazmalemmą.


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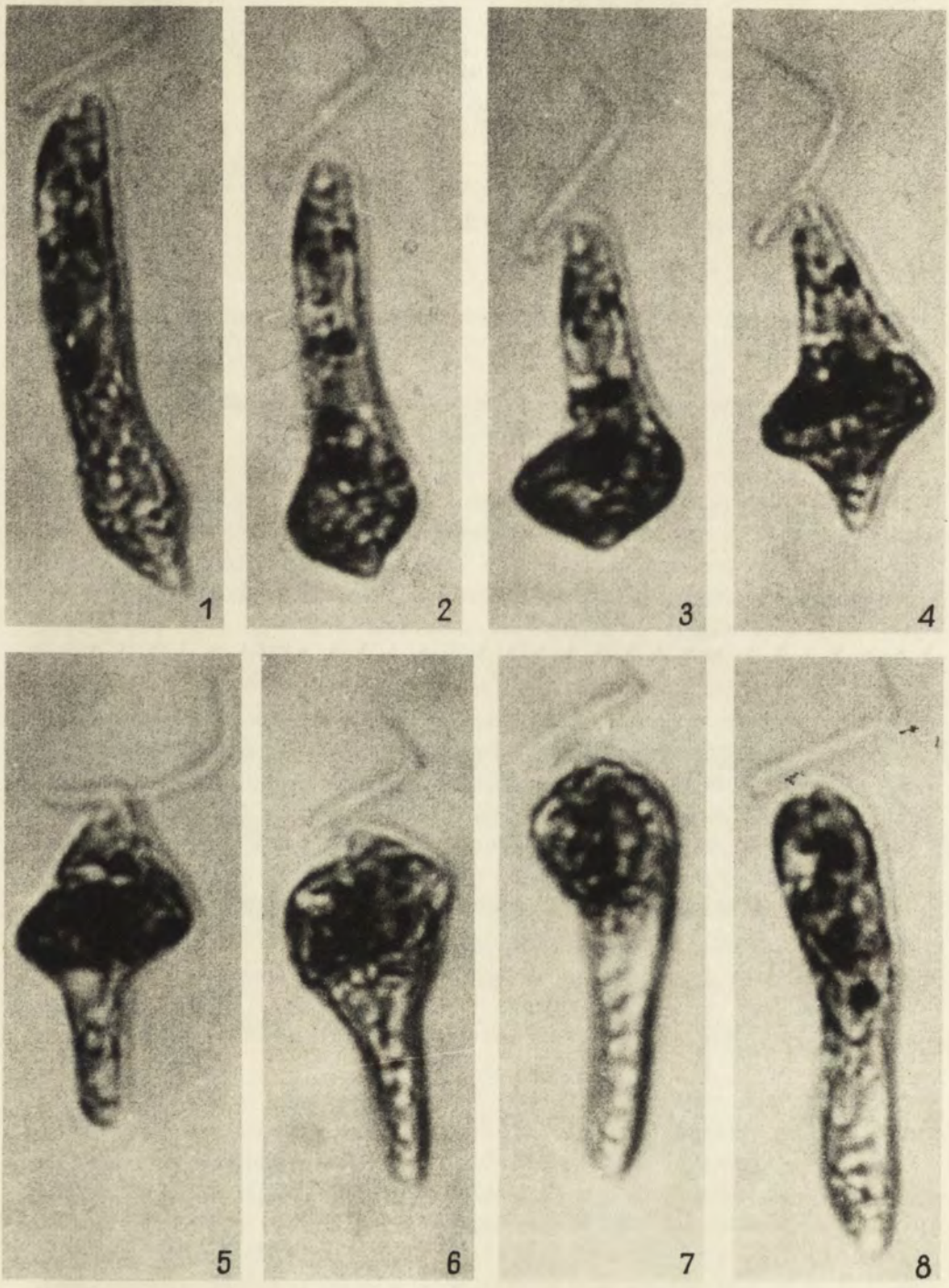
## EXPLANATION OF PLATE I

1–8: Consecutive stages of the peristaltic wave induced by EGTA




E. Mikołajczyk

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# Cytoplasmic streaming within *Paramecium aurelia*. III. The effect of temperature on flow velocity

Prąd cytoplazmatyczny u *Paramecium aurelia*. III. Wpływ temperatury na szybkość przepływu

There is number of studies on the effects of physical and chemical agents on the cytoplasmic streaming in various cells (Jahn and Bovee 1969). The physiological nature and mechanism involved within, remain unsolved yet. To find out the rules according to which this mechanism works, the influence of omnipresent and having most important significance, the temperature of external surrounding, were extensively studied (Bělehrádek 1957, Kamiya 1959, Troshin 1967). In sharp contrast with many quantitative data concerning plant cells and myxomycetes on the effect of temperature on cytoplasmic streaming, very little is known about the temperature — velocity relation in protozoa, especially in Ciliates (Jahn and Bovee 1967, 1969). The only available data origin from Koenuma 1954. He stated that the velocity of cytoplasmic streaming increases linearly with the change of external temperature and follows equation: $v=at+b$, where $v$ indicates velocity of particles suspended in moving cytoplasm in μm/sec, $a=0.1$ and $b=0.7$. The estimation was performed in temperature range from 5° to 30°C.

There are some qualitative data about the effect of long-lasting exposure of *Paramecium caudatum* to the low temperature. In *Paramecium caudatum* adapted to 0°C the cyclosis occurs, however the rate of movement is very slow, so the same vacuole remains within cell for more than two days (Poljansky and Posnanskaja 1964).

Our aim was to investigate the effect of external temperature on the velocity of cytoplasmic streaming within antiserum immobilized *Paramecium aurelia*. There are also several questions associated with this relation, particulary: (1) Is there any difference in velocity of cytoplasmic streaming at given temperature if it was obtained by heating or cooling. (2) At what range of temperatures does cytoplasmic streaming appear. (3) What are the conditions of recovery from critical temperature. (4) Dependence of cytoplasmic streaming rate on the rate of heating or cooling.

In the present paper, the estimation of cytoplasmic streaming velocity in the antiserum immobilized ciliates were performed because it is likely that, antigen-antibody complex covering pellicle and cilia of the surface of *Paramecium* (Mott, 1965) is less injurious factor than isopropyl alcohol (Hosoi 1937, Koenuma 1954, 1963), and more efficient in quieting of animals than methyl cellulose solution (Yamada 1969, 1970).

## Material and methods

Cultures of *Paramecium aurelia* stock 51, syngen 4 were maintained at 27° in lettuce infusion (Sikora 1966, Kuźnicki and Sikora 1971). Before observations, the animals were in each case, washed by centrifugation (up to 350×G for 2 min) in Dryl's 1959 citrate-phosphate buffer at pH 6.3, and kept in 27°C water bath for 1–4 h before use. The procedure of observations of the cytoplasmic streaming was as follows. The paramecia in experimental medium consisting of Dryl's 1959 citrate-phosphate buffer of pH 6.3 and appriopriate concentration of homologous antiserum (to immobilize paramecia of serotype 51A within 30 min) were placed on thin (0.6–0.7 mm) micro-

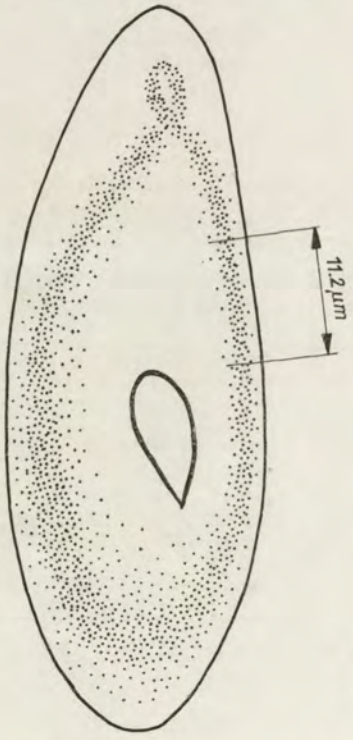


Fig. 1. Drawing of *Paramecium aurelia* cytoplasmic streaming with indication of 11.6 µm distance where velocity of crystals flow was measured

scopic slide and sealed under coverslip by vaseline to protect evaporation. Afterwards the slide was put on thermostatic device (designed and manufactured by M. Nencki Institute of Experimental Biology workshop) on microscope stage.

The thermostatic device unfortunately does not allow to apply the photographic method of Kuźnicki and Sikora 1971, 1972a, by use of polarizing light microscope. The bright field mi-

croscope technique was applied to follow the cytoplasmic streaming. The velocity of the streaming was obtained by measuring the time required for the given crystal (up to 3 μm in the length) to flow for 11.4 μm distance at the oral side of cyclosis routh (Fig. 1) of single *Paramecium* at the time of whole experiment

The crystals of 3 μm or less in length were the markers of cytoplasmic activity. Yamada 1969 stated that the granules of 1–3 μm in diameter flow with a constant velocity at a given time, so they were chosen. All measurements were done under 480 × magnification. As it was stated before (Kuźnicki and Sikora 1972 a) the most intensive cytoplasmic streaming within *P. aurelia* starts nearly simultaneously with complete immobilization by means of homologous antiserum. In few minutes cyclosis reaches the highest rate and afterwards, slowly decreases. Measurements of streaming velocity at different temperatures were performed usually after 30 min since the beginning

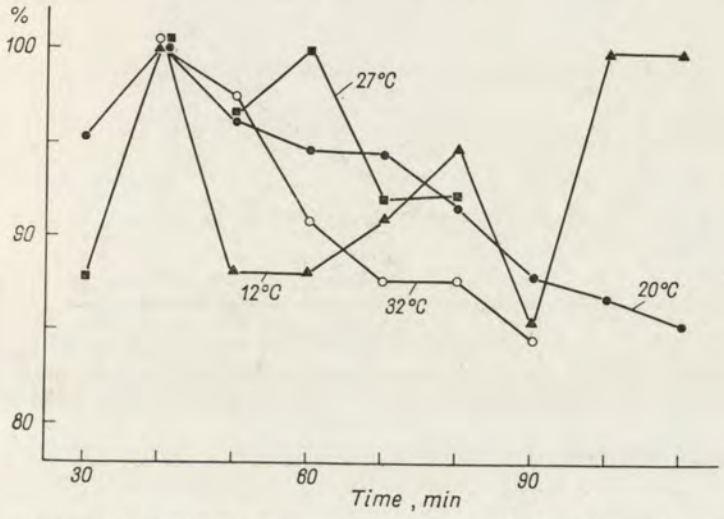


Fig. 2. Changes of initial velocity of cytoplasmic streaming in course of time. Each point is the value of at least of the mean of 10 measurements expressed in therms of percent of initial velocity reached by immobilized *P. aurelia* at forthieth minutes after begining of experiment

of immobilization and lasted up to 90–110 min. It should be pointed out that in course of the time, the change of cytoplasmic velocity at constant temperature does not exceed 15% of initial velocity (Fig. 2). There changes seem to have no essential influence on cytoplasmic streaming velocity relation to temperature, so were further neglected in description of cytoplasmic streaming values.

## Results

Paramecia treated by homologous antiserum, exhibiting cyclosis were used for quantitative analysis of the influence of external temperature on the rate of cyclosis. This phenomenon could be demonstrated by *P. aurelia* in range of temperatures between 3° to 36–37°C if change of temperature did not exceed about 1°C per minute.

The pattern of cyclosis (Kuźnicki and Sikora 1971, 1972 a) within above mentioned range of temperature remains constant and differs only in velocities.

Number of measurements performed at different temperatures in range of 3–27°C shows nearly linear character of dependence of cytoplasmic streaming velocity (Fig. 3). The Arrhenius plot for the temperature — velocity relation (Fig. 4) demonstrated breaks at 14° and 34°C. Calculation of temperature coefficient demonstrates value of approximately $\mu m = 11.000$ calories between 14–27°C and $\mu m = 9.500$ calories between 27–34°C. The $Q_{10}$ value was 2.35 between 14–34°C.

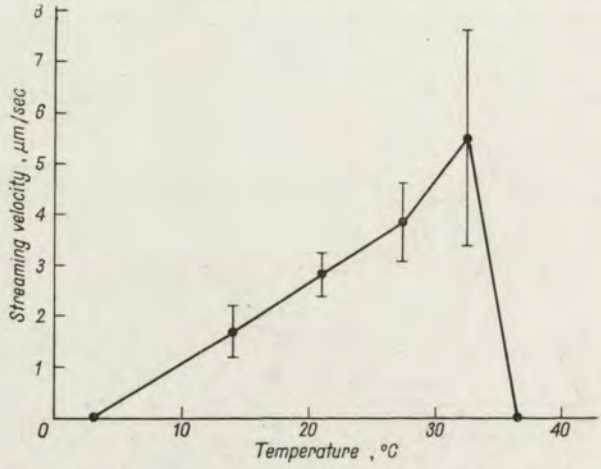


Fig. 3. Temperature — velocity of cytoplasmic streaming relation within *P. aurelia* expressed in µm/sec of the crystals velocity at given temperature in C°. Single point is the mean of at least 100 measurements

If the change of external temperature was gradual in rate 0.3–1°C per min, velocity of cytoplasmic streaming changes its value in range 3° to 27°C nearly simultanously and it does not matter whether it was reached by increase or decrease of temperature. In range from 27° to 36°C velocity of streaming increases up to 34°C and drops rapidly at 36–38°C, and in consequence cyclosis stops. Recovery of streaming is possible by decreasing temperature to lower value than 35°C, but velocity of streaming is about 30% slower than the velocity observed just before reaching the temperature which causes arresting of cytoplasmic streaming.

It should be pointed out that cessation of cytoplasmic streaming is reversible either at high or low temperatures, but patterns of cytoplasmic movements are different in both cases. At low temperature, movements of cytoplasm became slower and slower and eventually cytoplasmic streaming and any visible crystal movements stops. Recovery of cytoplasmic streaming, stopped at 3°C, takes place after increasing the temperature to 4–5°C. The recovery of cytoplasmic streaming arrested by high temperature (36–37°C) takes place if temperature falls down to 34–35°, but it should be claimed that arresting time at high temperature did not exceed 10–15 min, otherwise the cessation of streaming becomes irreversible. It has to be mentioned that in contrary to cessation effect at low temperatures, the high temperature although

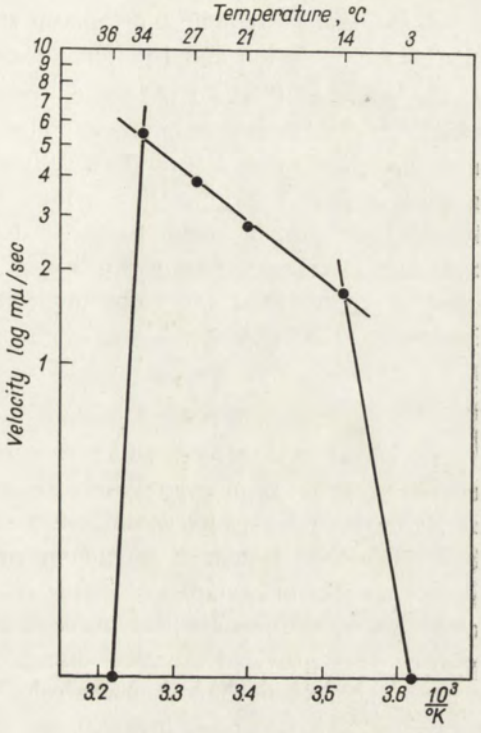


Fig. 4. Arrhenius plot of temperature — velocity relation of cytoplasmic streaming of antiserum immobilized *P. aurelia*

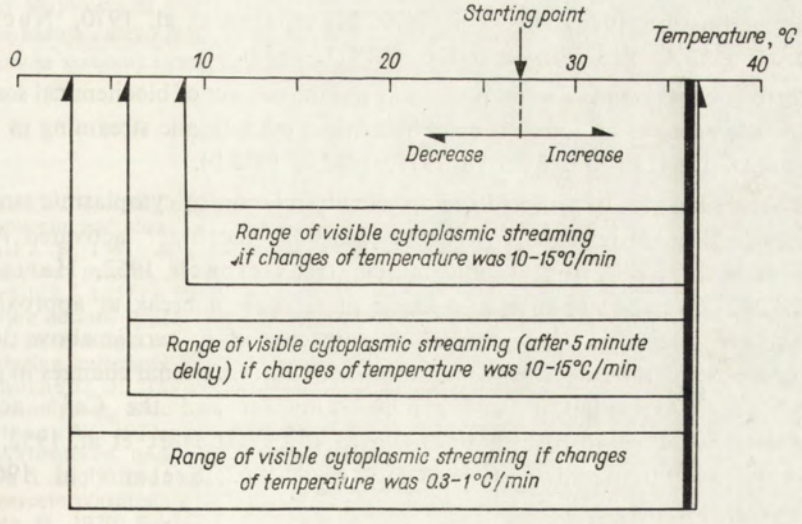


Fig. 5. Diagram of temperature relation of cytoplasmic streaming appearance within *P. aurelia* treated by homologous antiserum

stops cytoplasmic streaming, cytoplasm still possess power to agitate crystals, so they exhibit vibration and rotation movements.

The influence of rapid change of temperature, starting always from 27°C, on velocity of cytoplasm streaming was also studied, although there are some difficulties because *Paramecium aurelia* usually becomes swollen by pathological vacuoles. In spite of does difficulties it was found out that rapid change of temperature (10–15°C per min) in range from 10° to 36°C does not stop the cytoplasmic streaming. Decrease of temperature by 7° to 9°C stops cytoplasmic streaming for dozen or so minutes, and stops immediately in temperatures above 6°C or at higher than 37°C (Fig. 5).

## Discussion

It is known that the contraction of muscle is the consequence of the interaction between proteins: actin any myosin. Recently, mechanisms involved in propelling the cytoplasm movements within cell are also proposed to be closely related to above mentioned system of interacting proteins. Jahn and Bovee 1969 provide a list of number of examples of motile cells which contain actinoid and myosinoid proteins. Most of them are likely to be involved in contractility or related processes. Pollard 1971 provided a critical discussion of certain statements about presence of actin and myosin in non-muscle cells. The problem is whether mechanism of contraction and structures involved are based on unique system of actin-myosin interaction.

Besides, muscle actin and myosin-like system were identified in *Physarum polycephalum* and in *Acanthamoeba castellani* (Adelman and Taylor 1969, Hatano and Ohnuma 1970, Hinssen 1970, Nachmias et al. 1970, Nachmias and Ingram 1970, Weihing and Korn 1971, 1972).

Unfortunately *Paramecium* cytoplasm was not the subject of biochemical searching of contractile proteins although it exhibits distinct cytoplasmic streaming in certain conditions (Kuźnicki and Sikora 1971, 1972 a, 1972 b).

The Arrhenius plot of temperature — velocity relation of cytoplasmic streaming of *P. aurelia* (Fig. 4) resembles Arrhenius plots for the $Mg^{2+}$ activated ATPase activity of actomyosin from rabbit muscle (Hasselbach 1952, Hartshorne et al. 1972). The most common non-linear plots show a break at approximately 16°C. The non-linearity at high temperature (in case of *P. aurelia* above 34°C) is caused probably by the reversible denaturation or conformational changes in protein molecules. The Arrhenius plots for muscle myosin and the $Ca^{2+}$ activated ATPase activity of actomyosin is linear up to 40°C (Ouellet et al. 1952, Levy et al. 1959 a, 1959 b, Bendall 1961, Levy et al. 1962, Sreter et al. 1966 and Onishi et al. 1968).

The presented supposition seems to be the one of undirect proofs of presence of the actomyosin system propelling cytoplasmic streaming within *Paramecium aurelia.*

## Summary

The temperature — velocity relation of cytoplasmic streaming of antiserum immobilized *Paramecium aurelia* was studied. Cytoplasmic streaming occur within range 3–37°C provided that the rate of change of temperature does not exceed 0.3–1°C per minute. The higher rate of temperature change causes cessation of cytoplasmic streaming at 6°C, while cessation point at 36–37°C does not depend on the rate of temperature change. Either at low or high temperature, the cessation is reversible in certain conditions.

Arrhenius plot of temperature — velocity relation of cytoplasmic streaming of *Paramecium* resembles the Arrhenius plots for activity of rabbit muscle $Mg^{2+}$ activated ATP-ase actiomyosin system. This may suggest that temperature — velocity relation is the undirect proof for existence of actiomyosin system propelling cytoplasmic streaming in *Paramecium aurelia*.

## STRESZCZENIE

W pracy przedstawiono wyniki badań dotyczące zależności między temperaturą a szybkością ruchu strumienia cytoplazmy u *Paramecium aurelia*, immobilizowanych surowicą odpornościową. Ruch strumienia cytoplazmy obserwuje się w zakresie 3–37°C, jeżeli zmiany temperatury utrzymują się w granicach 0,3–1°C na minutę. Przy wyższym tempie zmian temperatury zatrzymanie ruchu strumienia cytoplazmy ma miejsce przy 6°C, podczas gdy górna granica wynosi zawsze 36–37°C. Zatrzymanie strumienia cytoplazmy w niskich i wysokich temperaturach jest odwracalne przy zachowaniu określonych warunków.

Zmiany szybkości ruchu strumienia cytoplazmy *Paramecium*, jak i aktywność ATP-azy aktomiozyny aktywowanej jonami $Mg^{2+}$ z mięśni królika, przedstawione w formie równania Arrheniusa wykazują daleko idącą zbieżność. Fakt ten uznano za pośredni dowód istnienia u *Paramecium aurelia* systemu aktomizoynowego warunkującego ruch strumienia cytoplazmy.

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## REFERENCES TO THE USE OF CULTURES OF ALGAE AND PROTOZOA

A sample survey of the literature on algae and protozoa has confirmed the impression that authors do not always give adequate reference to the strains used in work involving cultures.

The sample used was the 1971 issues of 14 journals taken at this establishment which publish original work on algae and protozoa. Papers dealing with fossils, larger seaweeds and organisms not so far cultured, were excluded.

Minimum adequate reference is considered to be the designation of the culture together with, where appropriate, indication of the source collection e.g., CCAP 211 /8d or Göttingen 11/6. A more complete reference would give also the name of the isolator and the date of isolation, but this information is usually available in the list published by the collection.

The survey shows that over three-quarters (153 out of 204) of the authors used cultures when this was possible. It also shows that well over half of the users (89 out of 153) gave inadequate or no reference to the cultures used. This is most unasatisfactory, especially when one considers the rigid insistence by authors and editors on proper bibliographic references.

References to specific names and a collection e.g. *"Chlamydomonas globosa* from CCAP" are not satisfactory as there may be now, or in the future, more than one strain fitting that description. Also, taxonomic names are liable to revision while strain designation should be immutable. References such as *"Tetrahymena pyriformis* "W" Strain" are inadequate without mention of the source. It has recently been shown by isozymal tests that strains of this species from different sources but with the same designation, may differ, while differently designated strains may be identical. The cause of this confusion presumably lies in mislabelling and failure to record the origin of stocks used. In one paper there was a serious orthographic error in a strain designation.

Among the advantages of using properly documented strains are:

a) that the work can be repeated or compared with other work on the same strain, and

b) the comparison of different strains of a species or of different species of a genus enables the significance of the particular characters to be assessed. Too often one sees a general statement about a species made from evidence derived from only one strain.

Wherever possible, cultures of new taxa or new strains used in important research should be deposited in at least one major collection. It is also of great value to a culture collection to receive reprints of work done with its cultures.

1st December, 1972

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The editors of ACTA PROTOZOOLOGICA appreciate very much the ideas expressed by Dr. E. A. George in his reasonable appeal concerning the necessity of proper references to the use and designation of protozoan cultures. We decided to publish the whole note with a strong recommendation to contributors to our Journal to follow in full extent the rules and suggestions of the author.

Editors of ACTA PROTOZOOLOGICA

ACTA PROTOZOOLOGICA 12, 151—152

## BOOKS RECEIVED

### Transport Processes in Living Organisms

*By:* Z. BOSZORMENYI, E. CSEH, G. GÁRDOS, P. KERTAI, Akademiai Kiado, Budapest 1972, 349 pages, 17 figs. *Translated by* I. BUTTYKAI.

### Environmental Physiology of Marine Animals

VERNBERG W. B., VERNBERG F. J. University of South Carolina, S. C., USA 346 pages, 116 figs. X, 1972. Cloth DM 62.40, US $ 19.80 Berlin - Heidelberg - New York: Springer Verlag ISBN 3-540-50721-8.

Results and Problems in Cell Differentiation

A Series of Topical Volumes in Development Biology

Editors: BEERMANN W., REINERT J., URSPRUNG H. Vol. 4.

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Editor: BEERMANN W., Max-Planck-Institut für Biologie. Tubingen, 227 pages, 110 figs. XV, 1972. Cloth DM 59, $ 18.70 Berlin-Heidelberg-New York: Springer Verlag, ISBN 3-540-05748-X. With contributions by Ashburner M., Beermann W., Berendes H. D., Lezzi M., Panitz R., Pelling C., Ribbert D., Robert M., Dudkin G. T.

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