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On the role of nucleus and cytoplasm in the inheritance of some characters in *Amoebae* (nuclear transfer experiments)

Роль ядра и цитоплазмы в наследовании некоторых признаков у амеб (опыты по трансплантации ядер)

The problem of the respective roles of the nucleus and cytoplasm in heredity, is a part of a more general and broader problem dealing with nucleocytoplasmic relationships and the role of the nucleus and cytoplasm in cell activity. As such it holds a central position in cytology and genetics since long. In the last few years interest to the problem was revived appreciably.

One of the reasons for this increasing interest is the progress in somatic cell genetics and studies on cell differentiation (Gurdon 1964). Detection of deoxyribonucleic acid in mitochondria, kinetoplast, plastids, and kinetosomes also stimulated interest to the problem of cytoplasmic inheritance. Despite the thorough investigation of the cytoplasmic DNA and demonstration of its biochemical activity no evidence has so far been obtained proving its participation in the transmission of any character in the line of descent (Sonneborn 1966, Olenov 1967). Finally, it must be stressed out that expectation for discovery of fundamentally new mechanisms of genetical continuity differing from classical nuclear mechanisms of storage, reproduction, and transmission of genetic information also inspired searching of examples of cytoplasmic inheritance (Olenov 1961, Nanney 1963).

To evaluate respective roles of the nucleus and cytoplasm in heredity various experimental procedures are used. Among them, nuclear transfer technique receives more and more attention in the last few years (Yudin 1965 a). The number of organisms which tolerate a microsurgical transplantation of nucleus from one cell into another increases continuously. Nuclear transfer alone or in combination with various special techniques can serve as an independent way of checking facts established by some different method. In other cases its application is the only source for obtaining singular and sometime very significant information (Gurdon 1964).

The technique of nuclear transplantation may vary according to the aims of investigation and the organism chosen, but in the final analysis it reduces to the microsurgical combination of the nucleus and cytoplasm from two different cells. Thus a viable cell is obtained which combines the nucleus and

cytoplasm of different origin. Needless to say, microsurgery is not the only way to do this. Certain variants of hybridization, for instance, a series of backcrosses, merogony or androgenesis (especially complete diploid androgenesis), etc.—produce similar results. These classical methods are known to be used successfully during a long time for studying the respective roles of the nucleus and cytoplasm in heredity. However, they are not free from some inherent defects (see, e.g. Astaurov 1948, Sokolov 1959).

Nuclear transplantation offers certain advantages over all these experimental procedures. In particular, it may be applied to the organisms which cannot be crossed. Nuclear transfer technique seems to be especially suitable for searching characters cytoplasmically inherited. This aspect of the matter must be particularily emphasized because of possible inadequacy or even absolute unsuitability of classical genetic procedures for cell heredity analysis which are from time to time pointed out by some authors (Caspari 1948, Sonneborn 1951, Ephrussi 1953, Nanney 1958, Danielli 1958, 1960 a, b). However, most if not all of the criticism usually made in this connection does not seem to be applicable to the method of nuclear transplantation (Danielli 1963).

The combinations of nuclei and cytoplasms produced by nuclear transfer technique are usually looked upon as nucleo-cytoplasmic chimerae or nucleo-cytoplasmic "hybrids". In unicellular organisms it is customary to evaluate the respective roles of the nucleus and cytoplasm in inheritance of the characters by which "parental" strains differ, according to the phenotype of a more or less remote progeny of the original "hybrid" cell obtained in result of the operation. The phenotype of this cell itself is of little value for such estimation, and may be taken into consideration only with reserve because, strictly speaking, it is not related to heredity—i.e. to the transmission of some peculiarities in the line of descent [1]. In principle, at least three basic types of results of a nuclear transfer experiment can be conceived. First, the progeny of a "hybrid" cell can be indistinguishable from that "parental" from which contributed the nucleus. In this case inheritance of differences between the original "parental" forms may be controlled exclusively by the nucleus. Such a result especially cannot be accounted for by a contamination of the transplanted nucleus with the donor cytoplasm when it is obtained in both reciprocal "hybrid" combinations. Second, the progeny of a "hybrid" cell may be similar to the "cytoplasmic parent" with respect to some characters. Such results lead us to the conclusion that the inheritance of characters in question are controlled exclusively by the cytoplasm. And finally, the "hybrid" progeny may differ with regard to some traits from either of the original, "parental" strains towards an intermediate or, on the contrary, more extreme expression of the character, or even some new peculiarities can appear. In these cases we are compelled to admit the dual, nucleo-cytoplasmic control of inheritance of the characters studied with additive or some other kind of interaction between nuclear and cytoplasmic determinants. It must be pointed out that the results of the two last mentioned types are less convincing and indisputable: a theoretical possibility of imitation of cytoplasmic inheritance

[1] It is just the case with the well-known data on the role of the nucleus and cytoplasm in cell morphogenesis which were obtained from interspecific nuclear transplantation experiments with *Acetabularia* (see reviews by Haemmerling 1953, 1963) and *Stentor* (see review by Tartar 1961).

phenomena exists even in nuclear transfer experiments (Gurdon 1964, see also Discussion).

The data available on the respective roles of the nucleus and cytoplasm in heredity which were obtained by nuclear transplantation technique (for review of literature up to the middle of 1965 see Yudin 1965 a) suggest an exclusively nuclear determination of inheritance of a great variety of characters in various taxonomically remote organisms. A large series of experiments performed on fresh-water uninuclear amoebae (from genus *Amoeba*) by Danielli and his co-workers (see reviews by Danielli 1958 a, 1959, 1960 a, b; of the recent papers see: Hawkins 1963, 1964, Hawkins and Cole 1965, Hawkins and Wolstenholme 1966, Jeon et al. 1967, Ord and Bell 1968, Hawkins and Willis 1969 a, b, c) is the only exception. As judged by the works of this group, the cytoplasmic share in heredity of amoebae appears to be enormously large, the cytoplasm, to some extent, participating in inheritance of most characters studied. Danielli 1963 considers the results to amend significantly our notion about material basis of heredity, which is founded to a large measure upon the application of classical genetic methodology.

Indeed, the importance of such kind of evidence for general biology can hardly be overstated. That is why a description of the experiments can be found in many modern monographs on general biology, genetics, cytology, etc. Since Danielli's data stand by themselves among the other evidence on the role of the nucleus and cytoplasm in heredity obtained by the nuclear transfer technique, further accumulation of as many data of the kind as possible with the same method and on the same object, seemed quite indispensable. Taking into account the situation we also undertook the investigation by the nuclear transplantation technique, respective roles of the nucleus and cytoplasm in inheritance of some characters in amoebae.

The present paper is for the most part a review of the data obtained and published by us at different times over the last eight years.

Materials and methods

Strains of amoebae

The following strains of amoebae (*Amoeba proteus*) of different origin were used. L, K, and Sh strains are clones originating from amoebae which were found in samples of protozoa from various lacalities of the Leningrad region [2]. L strain has been cultured under laboratory conditions since 1951 (in our laboratory since 1957), K strain, since 1962, and Sh strain, since 1967. The large uninuclear Sh amoebae markedly differ from other amoebae in their morphology and behaviour. Though not classified specially Sh amoebae are provisorily regarded as *Amoeba proteus* strain.

B strain was twice (in 1959 and 1960) sent to our laboratory from Medical University in Budapest by Dr M. Müller as being one of the strains cultured in Prof. J. F. Danielli's laboratory, London). P strain was brought by Dr L. N. Seravin from Poznań in 1962. C strain was obtained from the Leningrad Univer-

[2] In russian papers of Yudin and his co-workers the same strains of *Amoeba proteus* are used. Indication of strains is following: strain L is denoted as Л, K — К, Sh — Ш, B — В, P — П, C — С.

sity in 1964. Most of the strains were recloned more than once during their cultivation in our laboratory.

Cultivation technique

All the amoeba strains were cultivated under identical conditions by slightly modified Prescott and James' method (Prescott and James 1955, Prescott 1956, Olenov et al. 1961, Prescott and Carrier 1964). As a rule the culture medium was changed daily. *Tetrahymena pyriformis*, strain GL, serving as food, were grown on the yeast extract and given to amoebae every other day.

To obtain clones amoebae were isolated in microaquaria ("salt-cellars") with 0.50–0.75 ml of the Prescott medium or on plates of organic glass with depressions ca. 0.5 ml in volume (Zaar and Lozina-Lozinsky 1964). The plates were kept further in moist chambers. Conditions of feeding and medium changing in such individual cultures were the same as those in mass cultures. Under such conditions amoebae divided on the average once per 2–3 days. For the corresponding data see Yudin 1963. The clones were raised in the microaquaria until the number of amoebae in them reached several dozens,—i.e. for the first 1.5–2 months after their isolation, and then converted if necessary, into mass cultures in culture dishes or Petri dishes.

At different stages of the investigation all the strains were kept either at room temperature (18–22°C) or at 17 or 25°C in a thermostat.

Under established culture technique we, like the majority of investigators, never noticed any phenomena indicative of the sexual process in our strains. It should be emphasized, however, that there were almost no observations of mixed cultures combining amoebae from different clones.

Nuclear transplantation technique

Transplantation of nuclei in amoebae was carried out according to the Comandon and de Fonbrune technique. In our laboratory this technique was reproduced according to descriptions available in literature (Comandon et de Fonbrune 1939, Lorch and Danielli 1953) there is no need to expound it here, the more so, since some modifications of the method we use are discussed comprehensively in a special paper (Yudin 1968) [3].

It must be noted only that we applied a Soviet-make micromanipulator, MM-1 of Gleitmikromanipulator type though de Fonbrune's pneumatic micromanipulator is usually recommended for this purpose (Danielli 1960 a, Goldstein 1964). As a rule, the operation itself was carried out in 0.05 M NaCl, not in culture medium. This modification of the conventional procedure causes a sharp increase in the percentage of successful operations otherwise not very high under conditions of our laboratory (Yudin 1962). In every other respect it makes no alterations in the results usually obtained from nuclear transfer experiments with amoebae.

It must be also pointed out that recently some additional evidence became available showing that no significant amount of cytoplasm is carried over with the nucleus being transplanted by means of this technique (Craig 1967).

As a rule several "hybrid" clones of each type were produced and exam-

[3] Recently Dr. E. E. Makhlin who works in our group has tested the modification of nuclear transfer technique which was suggested by Jeon and Lorch 1968, and quite satisfactory results were obtained.

ined. In some cases clones from the intrastrain transplants were grown as controls, though such clones were known (Lorch and Danielli 1953 a, b) to be indistinguishable from the corresponding initial strains. The latter strains tested simultaneously always served as basic controls. "Hybrid" clones were tested for the first time several months (i.e. several dozens of cell generations) after the production of original "hybrid" cells.

Results

Hereditary differences between the amoeba strains which were used as genetic markers and methods of their testing

The presence of at least two hereditary different forms of the organism under investigation is the necessary precondition for analysis of heredity.

For a number of problems, the problem of the respective roles of the nucleus and cytoplasm in heredity inclusive, the physiological or biochemical nature of the differences (characters) is of little importance. It is much more essential that these characters can be easily tested and precisely estimated preferably in quantitative terms.

Hereditary variants of such kind might be looked for after mutagenic treatment of the original material. However, no one tried to induce mutations in amoebae so far (with the exception of Commandon et de Fonbrune 1942, and Ord 1968), and quite unexpected obstacles may be encountered on this way (see Kalinina 1967). Another no less common method is the utilization of the object's natural variation, i.e. the search of hereditary differences between individuals from different popuulations.

Comparative studies on a number of amoebae strains have revealed a series of differences between them which persist for a long time under identical conditions of cultivation. The description of these differences is characteristic for a certain part of natural variations in the proteus group of amoebae. As such, it can be of some importance for those interested in this protozoan group [4].

Initially only two strains of amoebae, L and B, (the first strains which appeared in our laboratory) were compared (Yudin 1961). No appreciable differences in appearance, mode of movement or in other external characters were observed under superficial examination of both cultures. No difference in the mean diameter of nuclei was revealed as well — the character which had been extensively used by Danielli and his colleagues.

Efficiency of cloning

One of the differences was found under usual conditions of cultivation and feeding when individual cultures had been started. More individual cultures of B strain died without giving rise to viable clones than those of L strain (18 from 100, or 18 per cent, and 8 from 100, or 8 per cent respectively). In other set of experiments 16 individual cultures from 75 died in B strain where-

[4] Specifically, detailed studies on variation, carried out with a number of amoeba strains can be of interest for analysis of the species problem in *Protozoa*. It is just the way Sonneborn 1957 suggests for revealing "biological species" in asexual *Protozoa*. Some studies on genetics of *Entamoeba* (Entner 1961, Entner et al. 1962, Siddiqui 1963, Entner and Most 1965, Goldman and Davis 1965) can serve as an example of such approach.

as in L strain only one culture was lost. This character was designated as "efficiency of cloning".

We fully realized that efficiency of cloning as well as the rate of reproduction, for example (Danielli 1959, Hawkins and Danielli 1963) must be strongly dependent on various environmental factors, and therefore it can hardly be a reliable genetical marker. However, the difference in question was regularly observed at different periods of time and what is more significant was reproduced in corresponding reciprocal nucleo-cytoplasmic "hybrids" (see below). That is why this marker was not rejected.

It should be noted that similar differences in the efficiency of cloning have been described by other authors. Thus Daniels (1962) observed different cloning efficiency (80–100%) in various strains of *Pelomyxa carolinensis* cultivated in identical conditions. Under the same conditions the strain of *Amoeba proteus* showed only a 70 per cent efficiency. Lorch and Danielli (1953) obtained clones from about 80 per cent of amoebae randomly selected from the mass culture of *A. proteus*. Working with B strain Kalny (1967) also observed the efficiency of cloning of about 80%.

Sensitivity to the toxic effects of high methionine concentrations

Olenov and his co-workers (1961) have shown on L amoebae that high methionine concentrations are rather toxic for this protozoan. By the end of incubation in methionine solution a considerable part of amoebae die. The survived individuals experience durable methionine post-effect, and a great majority die on the average 5–7 days after they have been transferred to the normal medium.

B strain proved to be more resistant to methionine than L strain. In 8 preliminary experiments in 0.05–0.10 M methionine solutions out of 447 L amoebae only 222—i.e. 46.5 per cent survived, whereas under the same experimental conditions out of 489 B amoebae 431—i.e. 88 per cent remained alive (Yudin 1961). In successive experiments the survival rate of initial strains (and "hybrid" clones) was checked in 0.15 M methionine solution after 18–20 hr of incubation, the counting of survived amoebae being made 2–4 hr after their transfer to the normal medium. In such condition the character of differences between the L and B strains remained the same (Yudin 1961, Kalinina and Yudin 1964). For example in a series of experiments ($n=105$) performed by Kalinina and Yudin the average survival rate of B strain was 32.6 ± 0.7 individuals and that of L strain, 0 ± 0.8 individuals from 50 used for the experiment.

The tests of strain K amoebae have shown that by their survival rate they are similar to strain B and, like the latter, are relatively stable to the toxic effects of methionine (Yudin 1964). On the contrary, P amoebae did not differ in that character form strain L (Sopina and Yudin 1965). Strain C amoebae were found to be highly resistant to methionine (Yudin et al. 1966). To illustrate a scope of differences between L and C strain amoebae histograms are presented in Fig. 1 which sum up the results of 176 tests of methionine resistance of this protozoan in various experimental series. The mean survival per cent of strain L calculated on the basis of these data was about 4 per cent (2.37 ± 0.26 individuals from 50 used in the experiment when *s.d.* = 3.5 individual) and that of C strain of about 78 per cent (38.8 individuals from 50 when *s.d.* = 6.9 individuals).

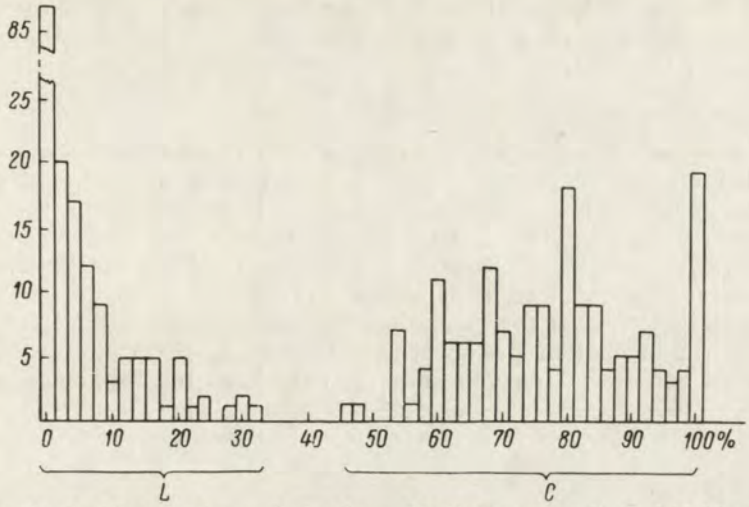

Fig. 1. Survival of L and C amoebae after a 18–20-hr exposure to 0.15 M methionine (Yudin, unpubl.). The abscissa is the percentage of amoebae which survived; the ordinate, the number of tests which showed a certain survival rate

Sh amoebae showed a relatively high resistance to methionine (89.8 per cent—449 individuals from 500 in 10 experiments [5].

We have been working with this character for rather a long time. Its phenotypic expression proved to be relatively stable under constant conditions of cultivation. A series of observations show, however, that it depends significantly on a great number of factors, and primarily on the cultivation temperature. Of some importance is the stage of cellular interdivision cycle passed by the animals tested, as well as the lapse of time since the last feeding.

Post-effect of methionine

A part of the strain L and strain B amoebae survived in the experiments with 0.05–0.10 M methionine solutions were converted into individual cultures. Our purpose was to see how many specimens being irreversibly damaged would die some time after the removal of the agent. In methionine-treated strain L amoebae the per cent of lethality of individual cultures was 44 (35 from 79 cultures). It was several times higher than that of individual cultures of that strain (see above) upon usual cloning (8%). In B strain amoebae the lethality per cent was 26—little differing from that obtained upon normal cloning (18%). Thus in this respect also B strain was found to be more resistant to methionine than L strain.

In successive experiments the post-effect of methionine was registered after a 68–72 hr incubation of amoebae in 0.05 M methionine solution. During that time the amoebae were fasting. The protozoans were daily transferred to a fresh solution. As a rule less than 10 per cent of the initial number of amoebae died by the end of such incubation. The survived amoebae (and corresponding controllers which had also been kept fasting for 3 days) were placed into solid watch glasses, and since then cultivated normally, the number of

[5] These data have been obtained by Dr. G. V. Nikolayeva.

protozoans in these individual cultures being counted daily. In 6 experiments of this type 20 died out of 75 individual cultures of L strain, and only 6, from 74 cultures of B strain.

Resistance to the toxic effects of ethyl alcohol

The resistance of amoebae to the toxic effects of ethanol was estimated by the number of individuals which survived after a 5-min treatment with 7% ethyl alcohol (Sopina and Yudin 1965). 15–20 min after the transfer of amoebae from alcohol to a fresh culture medium it was found out that dead protozoans cytolized while survived individuals stuck to the bottom of the microaquarium and resumed their normal movement.

The simplicity of tests for ethanol resistance in amoebae, and particularly, the short-term incubation period render this character most convenient for the work. It enables us, on one hand, to determine the resistance of clones

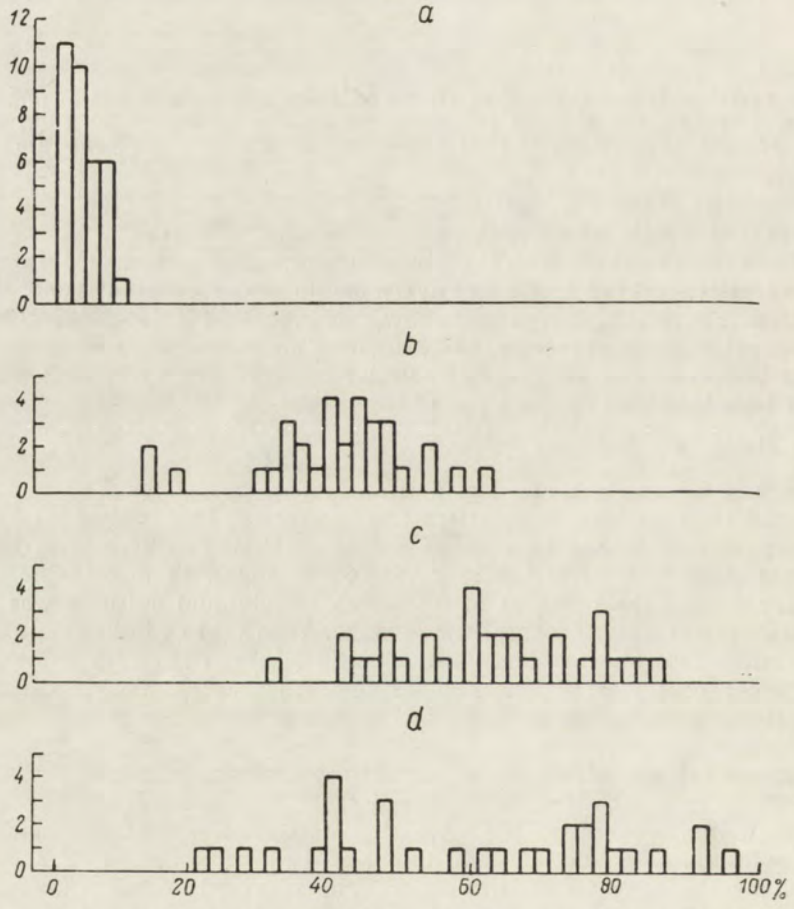

Fig. 2. Survival of different amoeba strains after a 5-min exposure to 7% ethyl alcohol (After Sopina and Yudin 1965). The abscissa is the percentage of amoebae which survived; the ordinate, the number of tests which showed a certain survival rate. (a) L strain; (b) P strain; (c) K strain; (d) B strain

rapidly at any given moment, and, on the other, to test a great number of various clones for a short length of time.

Repeated tests for the ethanol resistance of amoebae taken from one culture on the same day yielded slightly varying results. Additionally our experience shows that a general state of the culture may vary at different periods of cultivation by adopted technique. This affects the resistance of amoebae to toxic agents (Sopina 1966, Poljansky et al. 1967). Taking this factor into account, we preferred to test the resistance of each strain no more than once a day for several days, in the same way we did it when determining the resistance of amoebae to the toxic effect of methionine.

Figure 2 presents histograms showing the survival rates of amoebae of some investigated strains. The main parameters of these distributions—the arithmetic mean, standard error and standard deviation are summarized in Table 1.

Table 1
Survival rate of four amoeba strains after a 5 min incubation in 7% ethyl alcohol (After Sopina and Yudin 1965)

Strain	Average survival rate (mean number of survivors from 50 amoebae used for each test)	t	$s.d.$
K	32.5 ± 1.1		6.46
		0.95	
B	30.6 ± 1.6		9.22
		5.02	
P	21.2 ± 0.9		5.19
		21.10	
L	1.3 ± 0.2		1.18

The K and B strains are most resistant to the toxic effects of ethyl alcohol. These two strains do not differ in the average survival per cent. Strain L amoebae are most sensitive to ethanol. The survival level of P strain is intermediate between that of L strain, on one hand, and those of B and K strains, in the other (The difference of P strain from all those strains is statistically significant).

The ethanol resistance of L and B strains was also tested by Kalinina (1965 a). She found that the absolute value of differences in resistance between the above strains remained the same ($\bar{x}_L = 5.3$ individuals and $\bar{x}_B = 36.8$ individuals in Kalinina's experiments, while $\bar{x}_L = 1.3$ individuals and $\bar{x}_B = 30.6$ individuals in our own experiments).

Later on the ethanol resistance of C and Sh strains was determined. Strain C amoebae were compared with strain L amoebae and tested 101 times in the first experimental series, and 75 times in the second one. The results of testing are represented in Fig. 3. The average survival per cent of C strain in the first series was 91.8 (from 38 to 49 individuals from 50 used for the experiment), and in the second, 92.4 (from 32 to 50 individuals from 50). At

the same time the average survival per cent of L strain was 8.2 (from 0 to 8 individuals) in the first series, and 7.4 (from 0 to 11 individuals) in the second. In the following period the survival rate of L and C strains varied within the same range: 0–11 individuals from 50 for L strain and 35–50 specimens from 50 for C strain (Yudin and Nikolayeva 1968).

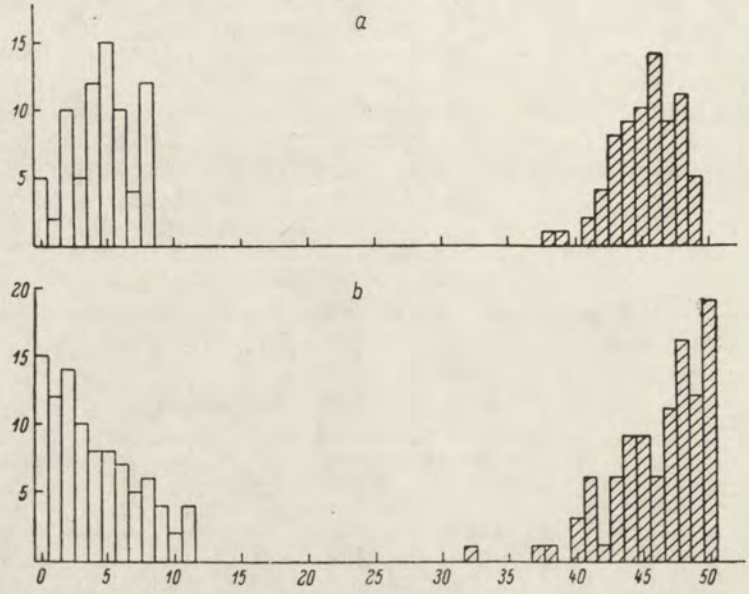

Fig. 3. Survival of L and C amoebae after a 5 min exposure to 7% ethyl alcohol. Two parallel series of tests (*a* and *b*) (After Yudin et al. 1966). The abscissa is the number of amoebae which survived (the initial number is 50 in each test), the ordinate, the number of tests which showed a certain survival rate. L strain is shown by clear columns; C strain, by cross-hatched columns

The survival rate of Sh strain (Nikolayeva, unpublished data) proved to be as high as that of C strain—99.9% (999 individuals from 1000 used in 20 experiments).

Hence, the testing of the survival rate of amoebae from 6 different strains (L, P, K, B, C and Sh) after a 5-min incubation in 7% ethyl alcohol showed that strain L amoebae were the most sensitive to this agent (about 3% of survival). P strain was found to be more resistant (about 40% of survival), but still less than K and B strains (about 60% of survival). C and Sh strains showed the highest resistance (about 100%).

Thermoresistance

The survival time at the action of the lethal temperature of 41°C was used as an index of thermostability for amoebae. During the experiment this time was registered with the accuracy of 1 min. Random samples (no more than 50–60 individuals each) of cells from mass cultures were tested. The death of protozoans was judged about by the rupture of their cell membranes and flow out of the cytoplasm. Values characterizing the mean survival time

of amoebae at lethal temperatures obtained from different experiments were summarized into total population and complex mean, as well as its standard error and standard deviation were calculated. This complex mean served to estimate thermoresistance of each clone.

After joining up the means from individual tests carried out during long periods of cultivation of C, B, K and L amoebae, into large populations with increased number of variants the following results were obtained (S o p i n a 1968 a). Most resistant to the effect of lethal temperatures are the amoebae from C (29.2±0.2 min) and B (28.8±0.2 min) strains which did not differ in their thermostability. K strain (25.6±0.6 min) occupies an intermediate position between C and B strains on one hand, and L strain (23.5±0.2 min) on the other, differing reliably by its thermoresistance from the both (Fig. 4).

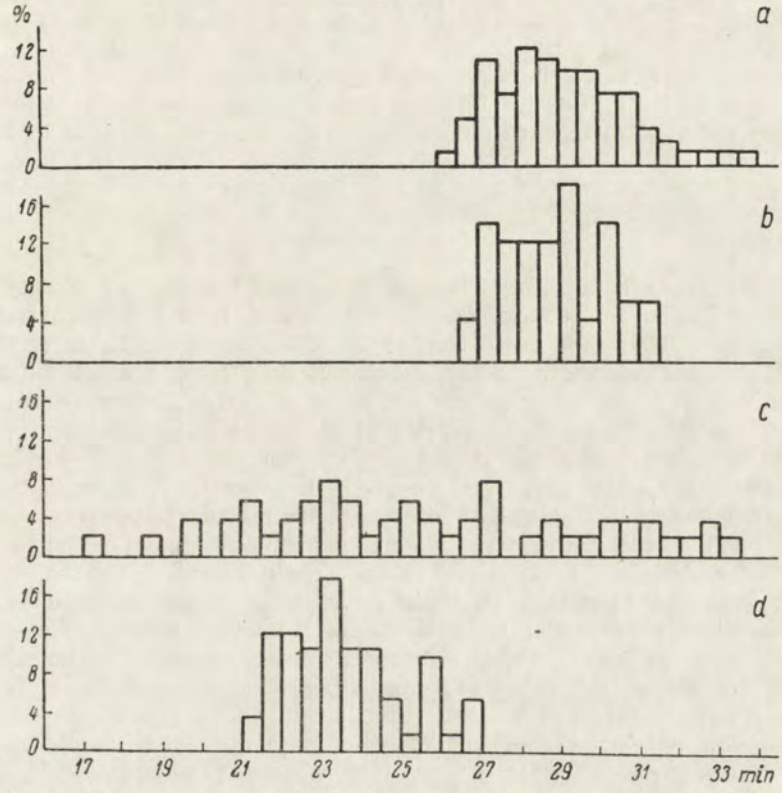

Fig. 4. Resistance of different amoeba strains to the lethal temperature 41°C (After S o p i n a 1968 a). The abscissa is the mean time of survival; the ordinate, the number of tests which showed a certain survival rate. (a) C strain; (b) B strain; (c) K strain; (d) L strain

Amoebae of strain K differ from those of C, B, and L strains not only in their mean survival time at 41°C, but also in dispersion of this character: unlike the clones above mentioned, they exhibit greater variability which is probably one of the components of the hereditary thermoresistance characteristic of this clone.

The tests for thermostability of amoebae of strain Sh and two clones Sh-1 and Sh-2 (Sopina, unpublished data) have shown that the mean survival time of strain Sh amoebae at the action of the lethal temperature was 1.1±0.1 min, that of clone Sh-1 amoebae, 1.2±0.1 min, and of clone Sh-2, 1.3±0.5 min (all numbers are complex means from 10 replications).

Hence, out of 5 tested clones of different origin clones C and B proved to be most resistant; clone K was intermediate, whereas clone L was sensitive to the effect of lethal temperature. The highest sensitivity was revealed in Sh strain amoebae.

The resistance of C, B, L and K clones to the effect of lethal temperature during a long time of cultivation under the same temperature remained constant. At different periods of testing during 2 years the thermostability of strain C amoebae was 28.4±0.4 min, 29.7±0.4 min, 28.8±0.4 min, 29.3±0.8 min, 29.0±0.6 min, 29.3±0.7 min, 29.3±0.4 min, 30.1±0.8 min (the means from different experimental variants, each being repeated no less than 6–10 times). The thermostability of strain K amoebae accordingly was 26.1±1.2 min, 25.3±±0.9 min, 25.0±0.8 min, 25.9±1.5 min. Similar data were obtained for strain L amoebae tested at different time during a year: 24.2±0.6 min, 23.6±0.4 min, 23.6±0.5 min, 23.1±0.6 min, 22.9±0.3 min, 23.5±0.4 min, and also for B strain: 28.6±0.5 min, 28.3±0.2 min, 28.6±0.3 min, 29.4±0.3 min, 29.7±0.9 min.

Morphology

The cultivation of strain K amoebae revealed one very characteristic difference between this strain, on one hand, and L and B strains, on the other (Yudin 1964). Not quite replete amoebae of strain L, as well as of strain B, are fastened tight to the substrate; they have a small number of large and not very long pseudopodia and move actively. Their appearance and behaviour are typical for the majority of *A. proteus* strains. Upon transfer to a fresh medium, mechanical stimulation or other treatment strain L and B amoebae, as a rule, pass quickly the cycle of nonspecific changes in the form described by Mast (1928), and then assume their typical appearance. Unlike these strains, the bulk of the strain K amebae are not fastened to the substrate, in the culture being in suspended state. Their pseudopodia are strongly elogated, thin and branched. In their appearance strain K amoebae differ from unfastened amoebae of L or B strain. However, after feeding or any kind of stimulation amoebae of K strain get quickly attached to the substrate, for a while assuming the shape quite indistinguishable from that of L and B strains (replete amoebae of all the three strains have the typical shape of "rosettes" attached to the glass, and in this state are not discernible). These differences are so obvious that in a great number of cases single cells as well as clones, even small ones, in all the cases may be unmistakably identified as belonging to a certain strain. The above morphological differences are presented in Fig. 5. Random samples of strain L and K amoebae of the same age have been drawn with the aid of the camera lucida.

This peculiarity of the shape is in compliance with a difference which can be quantitatively estimated in a very exact manner. It was established that the perimeter, i.e. the length of the external outline of a resting undisturbed cell in K strain on the average is significantly greater than that in L and B strains.

The smallest values for the perimeter are identical for both strains. It is

Fig. 5. Random samples of L and K amoebae of the same age

quite understandable if taking into account that some of the K amoebae are attached at given moment, and by losing their typical branched shape they become indistinguishable from L strain. But one third of the K amoeba cells have very large perimeters not observable in L amoebae. The fact is responsible for a greater variance of the character typical for K strain. The mean value of the perimeter (×148) in strain L amoebae is 24.9±0.4 cm, *s.d.* = 4.4 cm, in strain K amoebae, 35.1±1.0 cm, *s.d.* = 10.1 cm.

A similar difference was noted between amoebae of L and C strains, but no detailed study has been made on this pair of strains.

Control (intrastrain) nuclear transplantation

Control clones were obtained and tested to make sure that this transplantation procedure induced no genetic changes in the operated cell.

At that time the nuclear transplantation technique in 0.05 M NaCl had already been in use and the cultivation and testing of amoebae were carried out at constant temperatures. The results of comparison of the methionine resistance of control clones obtained from intrastrain transplants and simultaneously tested original strains are presented in Table 2 summarizing the data of different experimental series.

Similar data on the resistance of amoebae to ethyl alcohol appear in Table 3 and on the cell perimeter, in Table 4.

This evidence show that clones obtained as the result of intrastrain transplantation of nuclei do not differ in these characters from corresponding original strains. It means that this transplantation procedure by itself does not induce any change in the genetical constitution of transplant clones with respect to the properties tested.

As to thermostability the situation appeared to be more complicated. Taking into account the fact that this character depends more strongly on the environmental factors (see review by S u k h a n o v a 1968) we made attempts

Table 2

Survival rate of L, B, K and C amoeba strains and of clones grown from intrastrain transplants, after a 18–20 hr incubation in 0.15 M methionine

Strain	No. of tests	Total number of amoebae	Number of survivors	Percentage of survival	Reference
L	5	250	7	2.8	Yudin, unpublished
L_nL_c-2	5	250	10	4.0	
L_nL_c-4	5	250	4	1.6	
L_nL_c-5	5	250	8	3.2	
L	5	250	2	0.8	Yudin 1964
L_nL_c-4	5	250	0	0	
L	5	250	9	3.6	Yudin, unpublished
L_nL_c-6	5	250	0	0	
L	8	400	46	11.5	Yudin, et al. 1966
L_nL_c-2	8	400	2	0.5	
B	6	300	182	60.7	Yudin, unpublished
B_nB_c-3	6	300	184	61.4	
B_nB_c-4	6	300	191	63.7	
B_nB_c-5	6	300	173	57.7	
K	5	250	122	48.8	Yudin 1964
K_nK_c	5	250	141	56.4	
C	5	250	215	86.0	Yudin et al. 1966
C_nC_c-3	5	250	227	90.9	

Table 3

Survival rate of L and C amoeba strains and of clones grown from intrastrain transplants, after a 5 min incubation in 7% ethyl alcohol (After Yudin et al. 1966)

Clone	Number of tests	Total number of amoebae	Number of survivors	Percentage of survival
L	8	400	18	4.5
C	8	400	376	94.0
C_nC_c-3	8	400	373	93.5
L	10	500	35	7.0
C	10	500	462	92.4
L_nL_c-2	10	500	7	1.4

Table 4
Cell perimeter (cm × 148) in L and K amoeba strains and in clones grown from intrastrain transplants (After Yudin 1964)

Clone	Number of cells measured	$\bar{x} \pm s_{\bar{x}}$	*s.d.*
L	100	24.9±0.4	4.4
L_nL_c-4	50	25.7±0.7	5.0
K	100	35.1±1.0	10.1
K_nK_c-1	50	39.5±2.0	14.2

to investigate possible effects the nuclear transplantation may exert on this property (Sopina 1968 a). For this purpose intraclonal transplantations of three types were performed: $C_n \longrightarrow C_c$, $L_n \longrightarrow L_c$ and $K_n \longrightarrow K_c$. Clones grown from intraclonal transplants K_nK_c and L_nL_c were cultivated at 25°C, whereas clones obtained from intraclonal transplants C_nC_c, at 17°C. Original clones were kept under the same experimental conditions. We began to test clones L_nL_c 2 months after transplantation, clones K_nK_c, a year after obtaining intraclonal transplants, and C_nC_c clones, within a year after amoebae had been transferred to mass culture conditions. The thermostability of intraclonal transplants was determined simultaneously with that of original clones.

In all the clones grown from intrastrain transplants the thermostability was slightly decreased as compared with that of original C, K and L strains

Table 5
Thermoresistance of amoeba clones grown from intraclone transplants as compared to thermostability of the original amoeba clones (After Sopina 1968 a)

Temperature of cultivation	Clone	Mean survival time (min) at 41°C ($\bar{x} \pm s_{\bar{x}}$)	*t*
25	L	30.7±0.6	0.62
	L_nL_c-3	30.1±0.8	1.20
	L_nL_c-4	29.4±0.9	2.39
	L_nL_c-6	28.6±04	
	K	32.2±1.0	1.77
	K_nK_c-2	29.5±1.1	2.14
	K_nK_c-3	29.0±1.1	
17	C	29.7±0.4	2.50
	C_nC_c-1	28.0±0.5	2.49
	C_nC_c-2	27.9±0.6	2.57
	C_nC_c-3	27.6±0.7	3.51
	C_nC_c-4	27.1±0.6	

(Table 5). This difference was not statistically significant as judged by Student's *t*-test at 0.1% level of significance (except of C_nC_c-4 clone).

It was found out however, that a similar descrease in the resistance to the effect of lethal temperatures was observed upon simple subcloning. The decrease was only temporal and subclones when transferred to mass culture conditions (1.5–2 months later) did not differ in their thermostability from the original strains. Since all the clones obtained from transplants were nothing but "subclones", it can be suggested that a slight decrease in their thermostability should be entirely due to the procedure of cloning; as to the transplantation itself it does not cause any hereditary changes in the thermostability.

Hence, this evidence confirms the conclusion (Lorch and Danielli 1953 a, b; Danielli 1958 a) that the procedure of nuclear transplantation in amoebae does not induce by itself any hereditary changes in operated cells.

The role of nucleus and cytoplasm in the inheritance of discovered characters, as referred to the results of nuclear transplantation experiments

Sensitivity to the toxic effects of high methionine concentrations

At the first stage of investigation (no 0.05 M NaCl was used during the operation) at different periods of time we obtained 4 "hybrid" clones of the B_nL_c type and 4 clones of the L_nB_c type which enabled us to carry out a series of experiments on comparison of "hybrid" clones between each other and with original strains with respect to their methionine resistance (Yudin 1961). One clone of each type was studied in detail (clones B_nL_c-1 and L_nB_c-2). The first experiments with both "hybrid" clones were made 2–4 months after the obtaining of clones B_nL_c-1 and L_nB_c-2. During the last experiments the "age" of these clones was 5–8 months, i.e. approximately 40–60 cell generations respectively. The amoebae were cultivated at room temperature.

Before the "hybrid" clone L_nB_c-2 was obtained, we had compared the survival rate of clone B_nL_c-1 kept in 0.1 M methionine for 24 hr with that of the original strains. In 10 experiments out of 450 strain L amoebae 265 survived (59%), out of 450 strain B amoebae, 400 individuals remained alive (89%), and out of 450 amoebae of the "hybrid" clone B_nL_c-1, 407 (90%). These preliminary data seemed to show that it was the nucleus that evidently played a decisive role in the inheritance of the character.

The final confirmation of this hypothesis was obtained, however, only in the main experimental series when the percentage of survival in 0.15 M methionine was compared between the both reciprocal "hybrids" and original strains. The results of these experiments are presented in Table 6. The comparison by the chi-square test made in each experiment shows that in the majority of tests "hybrid" clones do not differ from the original strain which contributed the nucleus. The only exception is significant differences between L strain and L_nB_c-2 clone in the experiments 5 and 8 and between B strain and B_nL_c-1 clone in the experiments 2 and 5. These exceptional cases cannot be nevertheless, regarded as an evidence in favour of the participation of the cytoplasm in the inheritance of this character, since in the case of L strain and L_nB_c-2 clone the significance of the difference was rather due to the extraordinarily low resistance of strain L amoebae in the experiments in question than to increased survival rate of L_nB_c-2 clone. The survival per cent of clone B_nL_c-1 amoebae was even higher than that of B strain. These

Table 6

Survival rate of "hybrids" between L and B amoeba strains (the ratio of survivors to the initial number of amoebae) after a 18–20 hr incubation in 0.15 M methionine (After Y u d i n 1961)

Strain	Tests									Total	Percentage of survival
	1	2	3	4	5	6	7	8	9		
L	3 : 50	2 : 50	2 : 50	10 : 40	0 : 40	2 : 40	10 : 40	0 : 40	5 : 40	34 : 390	8.7
L_nB_c-2	6 : 50	6 : 50	3 : 50	5 : 40	9 : 40	7 : 40	9 : 40	11 : 40	11 : 40	60 : 383	15.6
B_nL_c-1	27 : 50	23 : 50	29 : 50	24 : 40	19 : 40	39 : 40	20 : 40	25 : 40	30 : 40	236 : 390	60.6
B	22 : 50	11 : 50	50 : 100	35 : 80	22 : 80	38 : 40* 26 : 40*	43 : 80	27 : 40	35 : 40	309 : 600	51.5

* Two replications.

Table 7

Survival rate of "hybrid" clones L_nB_c-2 and B_n L_c-1 (the ratio of survivors to the initial number of amoebae) after a 18–20 hr incubation in 0.15 M methionine, 3 years after obtaining the clones (Y u d i n, unpublished)

Strain	Tests								Total	Percentage of survival
	1	2	3	4	5	6	7	8		
L	4 : 50	0 : 50	0 : 50	0 : 50	0 : 50	2 : 50	5 : 50	0 : 50	11 : 400	2.8
L_nB_c-2	2 : 50	0 : 50	1 : 50	3 : 50	4 : 50	0 : 50	0 : 50	3 : 50	13 : 400	3.2
B_nL_c-1	30 : 50	40 : 50	28 : 50	26 : 50	26 : 50	30 : 50	29 : 50	31 : 50	240 : 400	60.0
B	31 : 50	40 : 50	34 : 50	26 : 50	32 : 50	33 : 50	29 : 50	40 : 50	265 : 400	66.3

differences perhaps might be put down to some uncontrollable variation in conditions of the experiment.

The characteristic of the clones B_nL_c-1 and L_nB_c-2 by their survival rate in 0.15 methionine underwent no changes even after a very durable cultivation of these clones, three years after their obtaining (Table 7). At that time both the cultivation and testing of amoebae were carried out at 17–18°C. Inspite such a long time, the survival level of "hybrid" clones showed no tendency to changing toward their cytoplasmic "parent" (Yudin 1965 b).

Analogous results were obtained for another pair of strains L and K (Yudin 1964). 5 "hybrids" of L_nK_c type and 3 "hybrids" of K_nL_c type were obtained and reproduced. As usually, the clones were cultivated under the same conditions as the original strains L and K. 2–3 months (approximately 20–30 cell generations) after the operation, the resistance of these clones to the effect of 0.15 M methionine was determined. The results given in Table 8 fully confirm the idea of exceptional nuclear determination of this character in heredity earlier discovered for the strains L and B. Chi-square test applied to the data presented in Table 8 shows that in each of the 5 experiments all

Table 8

Survival rate of L and K amoeba strains and of "hybrids" between them (the ratio of survivors to the initial number of amoebae) after a 18–20 hr incubation in 0 15 M methionine (After Yudin 1964)

Clone	Tests						Percentage of survival
	1	2	3	4	5	Total	
L	2 : 50	0 : 50	0 : 50	0 : 50	0 : 50	2 : 250	0.8
L_nK_c-1	0 : 50	0 : 50	1 : 50	0 : 50	2 : 50	3 : 250	1.2
L_nK_c-2	0 : 50	0 : 50	2 : 50	0 : 50	0 : 50	2 : 250	0.8
L_nK_c-3	0 : 50	0 : 50	4 : 50	0 : 50	0 : 50	4 : 250	1.6
L_nK_c-4	0 : 50	0 : 50	2 : 50	0 : 50	2 : 50	4 : 250	1.6
L_nK_c-5	0 : 50	0 : 50	0 : 50	0 : 50	3 : 50	3 : 250	1.2
K_nL_c-1	25 : 50	23 : 50	18 : 50	32 : 50	25 : 50	123 : 250	49.2
K_nL_c-4	16 : 50	27 : 50	25 : 50	32 : 50	27 : 50	129 : 250	51.7
K_nL_c-5	12 : 50	27 : 50	23 : 50	13 : 50	29 : 50	104 : 250	41.6
K	20 : 50	24 : 50	21 : 50	29 : 50	28 : 50	122 : 250	48.8

clones with L nuclei exhibit a significantly lower percentage of survival in methionine solution than clones with K nuclei. Differences observed within a group of clones with L nuclei are statistically insignificant. In a group of clones with K nuclei differences between some clones sometimes (see experiments 1, 3 and 4) are significant. A great variability of this character was already noted above for L and B strains.

Finally, still other pair of strains tested for survival in methionine was L and C amoebae (Yudin et al. 1966). Table 9 shows that in this "hybrid" combination differences are also determined exclusively by the nucleus.

Thus the same result was obtained from experiments with three "hybrid" combinations: inheritance of interstrain differences in the resistance of amoebae to the toxic effects of methionine in all the cases studied is determined exclusively by the nucleus.

Table 9

Survival rate of L and C amoeba strains and of "hybrids" between them after a 18 hr incubation in 0.15 M methionine (After Yudin et al. 1966)

Clone	Number of tests	Total number of amoebae	Number of survivors	Percentage of survival
L	5	250	49	19.6
C	5	250	242	96.8
C_nL_c-3	5	250	243	97.2
L	5	250	14	5.6
C	5	250	215	86.0
L_nC_c-5	5	250	14	5.6
L	8	400	36	9.0
C	8	400	293	73.5
L_nC_c-3	8	400	55	13.5

Efficiency of cloning and post-effect of methionine

The results of testing original strains L and B and corresponding "hybrids" B_nL_c-1 and L_nB_c-2 for the efficiency of cloning and survival rate after the incubation of amoebae in 0.05 M methionine solutions are represented in Table 10. The comparison of original strains with each other and with "hybrid" clones in the control series shows that these strains differ by the efficiency of cloning, while hybrid clones do not vary from original strains which contributed the nucleus.

The comparison of original strains with each other and with "hybrid" clones in the experimental series characterizing the survival rate of amoebae after long-lasting incubation in methionine solution demonstrates on one hand, significant differences between L and B strains, and, on the other, similarity between "hybrid" clones and original strains contributing nuclei.

Table 10

Efficiency of cloning (controls) and post-effect of the 68–72 hr incubation of amoebae in 0.05 M methionine (the ratio of number of individual cultures which died, to their initial number (After Yudin 1962)

Strain	Tests								Total	Percentage of individual cultures which died
	1	2	3	4	5	6	7	8		
L Control	0 : 10	0 : 10	0 : 10	0 : 10	0 : 10	0 : 10	0 : 10	0 : 10	0 : 80	0
Experimental	3 : 10	4 : 10	4 : 10	6 : 10	4 : 10	4 : 10	5 : 10	5 : 10	35 : 80	43.8±5.5
L_nB_c-2 Control	—	—	—	—	0 : 10	0 : 10	0 : 10	0 : 10	0 : 40	0
Experimental	—	—	—	—	3 : 10	3 : 10	6 : 10	5 : 10	17 : 40	42.5±7.8
B_nL_c-1 Control	3 : 10	2 : 10	3 : 10	3 : 10	3 : 10	2 : 10	2 : 10	2 : 10	20 : 80	25.0±4.8
Experimental	2 : 10	1 : 10	0 : 10	1 : 10	1 : 10	1 : 10	1 : 10	1 : 10	8 : 80	10.0±3.4
B Control	2 : 10	1 : 10	3 : 10	3 : 10	3 : 10	2 : 10	2 : 10	2 : 10	18 : 80	22.5±4.7
Experimental	3 : 10	1 : 10	1 : 10	1 : 10	1 : 10	0 : 10	1 : 10	1 : 10	10 : 80	12.5±3.7

The comparison of these data with corresponding control variants shows that differences by this character between L and B strains are rather qualitative than quantitative ones. While strongly pronounced post-effect of methionine is characteristic for strain L amoebae (and the "hybrid" L_nB_c-2), for B strain and the "hybrid" clone B_nL_c-1 under given conditions no negative methionine post-effect was revealed. We even observed a certain increase in the cloning efficiency of the latter clones of amoebae as compared with the corresponding controls, though it was statistically insignificant. It should be pointed out, however, that after the incubation in methionine in strain B amoebae and in clone B_nL_c-1 we noted the same morphological divergence from the normal as in strain L amoebae (see Olenov et al. 1961).

As was above mentioned, the efficiency of cloning and post-effect of methionine, owing to their sensitivity to the influence of various factors, can hardly be considered a priori as good genetic markers. Nevertheless all the results obtained give enough reason to believe that these characters are controlled solely by the nucleus.

Resistance to ethyl alcohol

The analysis of this character was carried out on nucleo-cytoplasmic hybrids of K_nL_c (2 clones) and L_nK_c (2 clones) types (Sopina and Yudin 1965). Since the obtaining of these "hybrids" till the onset of testing their resistance to ethanol about 30 cell generations had passed. In addition, we tested a "hybrid" clone B_nL_c-1 (the same we had tested for its resistance to methionine) which was at the age of more than three years by the time of experimentation. Unfortunately we had no reciprocal "hybrid" L_nB_c at our disposal at that time. As always, the ethanol resistance of original strains and "hybrid" clones was determined simultaneously. The clone K_nL_c-9 was checked 20 times; other clones and original strains by 32 times each.

The results of the tests of "hybrid" clones for their resistance to ethyl alcohol are presented in Table 11. One can see that the resistance of "hybrids"

Table 11

Survival rate of L, K, and B amoeba strains, and of "hybrids" between them, after a 5 min incubation in 7% ethyl alcohol (After Sopina and Yudin 1965)

Strain	Average survival rate (mean number of survivors from 50 amoebae used for each experiment)	t_d	*s. d.*
L_nK_c-1	1.4±0.3	0.18	1.49
L	1.3±0.2	0.64	1.18
L_cK_c-2	1.1±0.2		1.18
K_nL_c-6	29.1±1.3	2.00	7.30
K	32.5±1.1	0.43	6.46
K_nL_c-9	31.8±1.3		5.97
B_nL_c-1	30.3±1.6	0.14	8.53
B	30.6±1.6		9.22

does not differ from the resistance of those initial strains from which they got the nuclei. No statistically significant deviation toward the "cytoplasmic parent" was observed. Hence the inheritance of this character in successive cell generations is controlled exclusively by the nucleus.

The same results were obtained for another pair of strains, L and C (Yudin et al. 1966). The corresponding data can be found in Table 12.

Table 12

Survival rate of L and C amoeba strains, and of "hybrids" between them after a 5 min incubation in 7% ethyl alcohol (After Yudin et al. 1966)

Clone	Number of tests	Total number of amoebae	Number of survivors	Percentage of survival
L	5	250	21	8.4
C	5	250	247	98.8
C_nL_c-3	5	250	238	95.2
L	5	250	14	5.6
L	5	250	243	97.2
L_nC_c-5	5	250	27	10.8
L	12	600	61	10.1
C	17	850	609	71.6
L_nC_c-3	17	850	64	7.5

Note. All these "hybrid" clones were also tested for their methionine resistance (see Table 9).

Thermostability

The nuclear-cytoplasmic "hybrids" were obtained between C and L strains (combination I, 2 clones of C_nL_c type and 2 clones of L_nC_c type) and B and L strains (combination II, 1 clone of B_nL_c type and 3 clones of L_nB_c type), most strongly differing in their thermostability. Moreover, we investigated "hybrids" of less differing K and C strains (combination III, 2 clones of C_nK_c type and 4 clones of K_nC_c type). Sh strain most sensitive to heating has been found only recently and is still to be tested for the character in question.

Since the obtaining of "hybrids" C_nL_c and L_nC_c up to the analysis of their thermostability 16 months passed, i.e. about 140 cell generations. The "hybrid" clone B_nL_c-1 was obtained more than 6 years ago. Since the obtaining of nuclear-cytoplasmic "hybrids" L_nB_c till the onset of tests for their resistance to the effect of lethal temperatures 3 months, i.e. about 30 cell generations had passed. The analysis of thermostability of "hybrid" clones C_nK_c and K_nC_c was carried out 5 months after they had been obtained. The resistance of "hybrids" to the lethal temperature was determined simultaneously with that of original clones. Each experiment was repeated no less than 10 times. When the thermostability of nucleo-cytoplasmic "hybrids" B_nL_c and L_nB_c was tested, not only original clones L and B, but also the clones grown from intraclonal transplants L_nL_c-4 and B_nB_c-4 were used as controls (see part II).

The tests for thermostability of nucleo-cytoplasmic "hybrids" are represented in Table 13. One can see that the thermostability of "hybrid" clones C_nL_c and L_nC_c is lower than that of the corresponding original strains C and L, but this difference at 0.1% level of significance cannot be regarded as reliable.

The resistance of "hybrids" B_nL_c and L_nB_c to the effect of lethal temperature does not differ from the thermostability of corresponding clones grown from intraclonal transplants and of original clones B and L (S o p i n a 1968 b).

Table 13

Thermoresistance of L, C, B, and K amoeba clones, of "hybrids" between them, and of clones grown from intraclonal transplants (After S o p i n a 1968 d)

Clone	Mean survival time (min) at 41°C ($\bar{x}\pm s_{\bar{x}}$)	t
C	28.8±0.4	2.81
C_nL_c-1	26.7±0.6	1.29
C_nL_c-3	27.9±0.5	
L	24.2±0.6	2.62
L_nC_c-2	22.5±0.3	1.94
L_nC_c-3	22.9±0.3	
B	28.3±0.2	1.37
B_nB_c-4	27.9±0.2	1.63
B_nL_c-1	27.7±0.2	
L	23.6±0.4	1.25
L_nL_c-4	22.9±0.4	0.31
L_nB_c-1	23.3±0.7	1.00
L_nB_c-2	23.1±0.3	0.77
L_nB_c-4	23.1±0.5	
C	29.4±0.4	0.61
C_nK_c-5	29.0±0.5	0.87
C_nK_c-3	28.9±0.4	
K	25.9±1.0	0.47
K_nC_c-5	25.4±0.6	0.96
K_nC_c-4	24.7±0.7	1.57
K_nC_c-1	24.1±0.6	2.43
K_nC_c-3	22.9±0.6	

The case is the same with the "hybrid" combination III (S o p i n a 1968 d), although in this experimental series a difference in thermostability between original strains C and K, due to a small number of tests, was statistically significant only at 1% level of significance.

Thus the cytoplasm does not take part in the inheritance of thermostability. According to our data it is controlled exclusively by the nucleus.

Morphological differences

In order to evaluate the comparative roles of the nucleus and cytoplasm in inheritance of morphological differences between L and K strains 2–3 months (i.e. approximately 20–30 cell generations) after the obtaining of "hybrid" clones K_nL_c and L_nK_c, the perimeter was measured in amoebae of these clones just in the same way as in amoebae of original strains (the perimeter of the latter was measured at about the same time).

The results of the above experiments (Yudin 1964) are presented in Table 14, and also in Fig. 6 showing the histograms characterizing the variability of this character in original strains and in "hybrid" clones L_nK_c-1 and

Table 14

Cell perimeter (cm × 148) in L and K amoeba strains and in "hybrids" between them (After Yudin 1964)

Clone	Number of cells measured	$\bar{x}\pm s_{\bar{x}}$	*s. d.*
L	100	24.9±0.4	4.4
L_nK_c-1	100	24.1±0.4	3.7
L_nK_c-2	50	22.5±0.5	3.6
L_nK_c-3	50	21.9±0.4	2.7
L_nK_c-4	50	21.6±0.4	2.5
K	100	35.1±1.0	10.1
K_nL_c-1	80	33.6±0.9	8.4
K_nL_c-4	100	34.6±1.0	10.0
K_nL_c-5	58	31.9±1.5	11.3

Note: All these "hybrid" clones were also tested for their methionine resistance (see Table 8).

K_nL_c-4. It is clearly visible that all "hybrid" clones are similar in the mean perimeter and its standard deviation to that "parental" clone which yielded the nucleus. The comparison of original strains and "hybrids" does not permit to establish any statistically reliable participation of the cytoplasm in inheritance of this character.

No doubt that a small divergence between clones of the same genotype, whatever statistically significant it might be in some cases, occurs on another scale than much greater variation between clones with different nuclei. The smallest perimeter in a group of clones with K nucleus (in K_nL_c-5) is significantly larger than the largest perimeter in a group of clones with L nucleus (in L_nL_c-4): $t_{diff}=3.31$.

As was indicated, K amoebae are morphologically similar to C amoebae, but no detailed study was conducted on "hybrids" between L and C strains. The mere observation has shown, however, that reciprocal "hybrid" clones always differed in the shape of their cells depending on the origin of their nuclei.

Thus having used the method of nuclear transplantation for determining

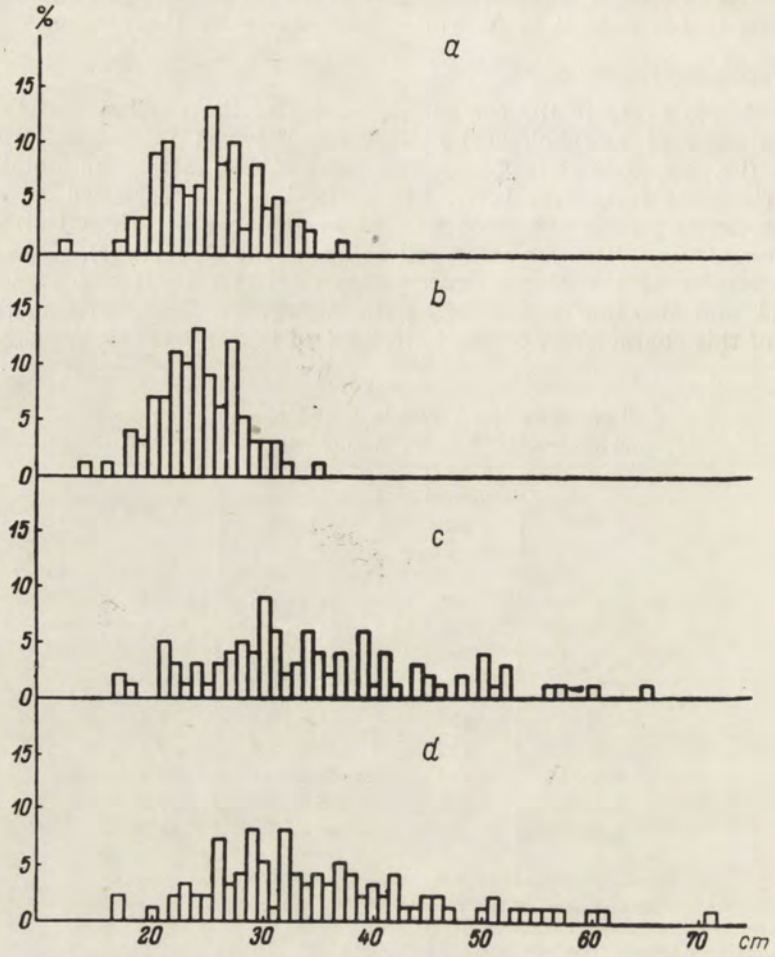

Fig. 6. Cell perimeter in L and K amoeba strains and nucleo-cytoplasmic "hybrids" between them (After Y u d i n 1964). The abscissa is the length of perimeter (×148); the ordinate, the percentage of cells with a certain perimeter. (a) L strain; (b) L_nK_c clone; (c) K_nL_c clone; (d) K strain

a respective role of the nucleus and cytoplasm in heredity of amoebae we have obtained definite results. The nucleo-cytoplasmic "hybrids" between strains of *Amoeba proteus* differing in a number of characters in all the cases were not discernible from those "parental" strains from which they got their nuclei. No deviation toward the "cytoplasmic parent" were registered. Since remote progeny of "hybrid" cells have been studied we may draw the conclusion that the inheritance of all these characters is controlled only by the nucleus. This conclusion concerns 6 randomly selected hereditary characteristics which enables us to say with certainty that the nucleus plays the leading role in heredity of amoebae. If there exist characters inherited via cytoplasm they are comparatively rare, and we have not so far observed any in our experiments.

It is evident that our data and conclusions differ significantly from the results obtained by Danielli and his collaborators, in experiments analogous with ours. This fact induces us to discuss, even if briefly, some general aspects of the evidence obtained and a possible cause of their divergence from the results of Danielli's group.

Discussion

On hereditary nature of interclonal differences

We have studied a respective role of the nucleus and cytoplasm of amoebae in the inheritance of various characters. That is why we need to be fully confident that interclonal differences we revealed are really hereditary. The following arguments can be adduced for this suggestion. All the above interstrain differences exhibit great stability for a long time under similar conditions of cultivation (it should be pointed out that some of these strains have been under observation for about 8 years). These differences are regularly reproduced upon cloning: all the clones show similarity and reproduce all the properties of the original strain. With the same regularity (rare exceptions are discussed below) these differences are reproduced by reciprocal "hybrid" clones. Finally, under certain conditions (changes of cultivation temperature, action of some chemical agents, bad state of the culture, durable fasting etc.) a strain may temporarily lose its characteristic property. The latter however is always restored as soon as amoebae are returned to normal conditions [6]. Thus we have every reason to believe that differences revealed between amoebae strains are hereditary.

The number of different genetic characters used in this investigation

A conclusion to be made about a comparative role of the nucleus and cytoplasm in heredity depends evidently on the number of characters studied from this point of view. However, the problem of differentiation of genetic characters appears to be rather complex when the variation is analyzed in amoebae (as well as in any object having no sexual process). By the work of Danielli and his co-workers one may see that a study of nucleo-cytoplasmic "hybrids" by itself does not, strictly speaking, allow to say what number of different genetic characters underlie our evaluation of a respective role of the nucleus and cytoplasm in the heredity of amoebae.

In every case a theoretical possibility of rather a broad pleiotropic effect make us doubt whether in reality we deal with different markers genetically conditioned in different way or only with different manifestations of the same hereditary determinant. Doubts increase when the question concerns hereditary characters similar in expression or in the mode of testing (see e.g. Danielli et al. 1955).

In our instance the resistance of amoebae to methionine, ethyl alcohol and high temperature may be different kinds of manifestation of some general

[6] In this connection it should be emphasized that the above description of genetic markers used for our investigation is fully valid on one hand, only for our strains, and, on the other, solely for our experimental technique of testing characters and for those conditions of incubation and feeding under which amoebae were cultivated. This condition usual in any genetic practice should be taken into account when attempts are made to find similar markers for some other material.

non-specific resistance of the cell to various deleterious effects tested in different way. The suggestion seems all the more likely since in many of the organisms studied (bacteria, infusoria, amphibians, insects and plants) during their adaptation to different temperatures a correlation was observed between the resistance to lethal temperatures and ethyl alcohol. These data allowed the conclusion that alcohol and relatively high temperatures are non-specific agents the resistance to which is determined by the stability of cell proteins to denaturating effects (Alexandrov 1948, 1963, 1965).

These considerations induce us to seek in genetic experiments on nuclear transplantation in amoebae an evidence that each newly tested character is different from those previously investigated—i.e. it is otherwise genetically conditioned. The absence of the sexual process and insufficient elaboration of techniques for induction and quantitive estimation of mutations in amoebae render inapplicable classical criteria of discontinuity of genetic material—i.e. the ability for recombination and independent mutation. We suggest however, that for this purpose some of analogs, a kind of partial substitute of such criteria can be used.

Different combinations of characters in strains of different origin, in our opinion, testify in favour of different genetic control of these characters. Thus the amoebae of C and B strains are most resistant and the amoebae of L strain are most sensitive to the effects of not only lethal temperatures, but also of ethyl alcohol and methionine. At the same time in strain P amoebae a relatively low resistance to methionine (no higher than in L strain) goes together with rather high (intermediate as compared with other strains) resistance to alcohol. Amoebae of K strain resistant to ethanol and methionine show moderate thermostability, whereas strain Sh amoebae which are also tolerant to the first two agents have very low thermostability. Similarly, ethanol and methionine-resistant amoebae may have a normal (B strain) as well as increased (K and C strains) cell perimeter and vice versa: amoebae with the normal cell perimeter may be both sensitive (L strain) and resistant (strain B) to these agents. All these facts are easily explicable assuming the given characters are capable of changing independently, and, hence, they are genetically conditioned in a different way.

Another evidence of genetic difference of the characters tested is probably the ability of nuclei to become destabilized in heterokaryons with respect to a given character independently of all the others. For example, L nuclei after interaction in heterokaryons with C nuclei at certain exposure appear to be destabilized with respect to methionine resistance without altering stability to alcohol (Nikolayeva 1966; Yudin et al. 1966; Yudin et al. 1967). A study of the ability of amoebae for independent hereditary destabilization is also regarded as a peculiar analog of testing the capability of the character to undergo independent mutational changes (Yudin and Nikolayeva 1968).

All these considerations give a good reason to assert that the conclusion about the exclusive nuclear control of inheritance was derived with respect to at least five different characters.

On the nature of characters used in our investigation

It is no coincidence that we have started this section of the paper with a discussion of evidence showing the hereditary nature of the characters studied.

When studying cell heredity, by nuclear transplantation technique inclusive, it is not always to discriminate such phenomena as long-lasting modifications and so-called "pseudo-heredity" (Hagemann 1964) connected with a passive transfer of intracellular symbionts, parasites, some biologically active substances etc. from one cell generation to another. In this connection, one can understand why Danielli and his collaborators attached such an importance to durable observations over the same "hybrid" clones (see, e.g. Danielli et al. 1955, Danielli 1960 b).

Specifically, with the aid of different techniques a great number of constantly living organisms were detected in the cytoplasm of amoebae of certain strains (Roth and Daniels 1961, Rabinovitch and Plaut 1962 a, b; Wolstenholme and Plaut 1964, Wolstenholme 1966). Although the nature of these organisms is still unclarified, the majority of the authors tend to regard these organisms as symbionts of amoebae — bacteria or rickettsia-like organisms. According to Prescott and Stone (1967), they occur in all the examined strains of *Amoeba* and *Pelomyxa* (cf., however, Wolstenholme 1966).

Clearly, these facts have no direct reference to our case since the inheritance of all our markers is determined by the nucleus. It is conceivable anyhow that all our characters, or many of them are due to the presence of intranuclear symbionts in the nuclei of some strains under comparison. It should be readily apparent that the nuclear transplantation technique alone would not have enabled us to tell this variant of "pseudo-heredity" from the true nuclear control. However, if our characters are conditioned by the existence of intranuclear symbionts one) could expect to reveal any sings of their infectious nature.

In fact, Kalinina and Yudin (1964) have found that if diheterokaryotic cells of amoebae obtained by nuclear transplantation technique are cut into halves which give rise to new clones, such clones as a rule differ from the both original forms. When tested by a number of markers the heredity of which is controlled by the nucleus (as judged by "true" nucleo-cytoplasmic "hybrids"), each such clone exhibits a phenotypical instability with respect to these characters. In some of the experiments it was found to be similar to one of the original strains, and in other experimental series, to another. And sometimes it exhibited intermediate phenotype. Such instability was found to be hereditary and could be transferred by transplantation of nuclei of the unstable clone into the cytoplasms of any of the original stable strains (Kalinina 1965 b). This phenomenon was interpreted by Kalinina and Yudin (1964) as a result of peculiar genetic interaction between interphase nuclei in heterokaryons brought about via cytoplasm.

Naturally, in case of "pseudo-heredity" differences between two amoebae strains induced by the presence of symbionts in the nuclei of one of these strains would be of "presence-absence" type. However, this is at variance with the fact that in the course of genetic nuclear-nuclear interaction in diheterokaryons both nuclei usually undergo hereditary changes (Kalinina and Yudin 1964, Kalinina 1965 a, Yudin et al. 1966, Yudin and Nikolayeva 1968). The data of Kalinina (1964) on the resistance of methionine of di- and triheterokaryons with different ratio of L and B nuclei are also inconsistent with the suggestion that differences in methionine resistance are of "presence-absence" type.

It is even less likely (though not excluded as yet) that each of our charac-

ters is determined by the presence of two different (perhaps mutant) forms of symbionts in the nuclei of both strains under comparison. In that instance these characters might not be the differences of "presence-absence" type, and, hence, in the fact that genetic interaction of nuclei is two-way there would be nothing inconsistent with the hypothesis of infectious nature of characters. As was indicated, however (Yudin 1967), this suggestion permissible for any one character is absurd when several different characters are concerned. It would make us to admit the presence of a great number of different symbionts in nuclei of the strains compared which affect the cell phenotype and are capable for independent transfer.

Hence with a great deal of assurance one may assert that hereditary characters tested connot be considered as examples of "pseudo-heredity". Nevertheless, strictly speaking, we have no reason to regard them as genetic in the narrow sence of the word—i.e. related to differences of mutational origin. The hereditary differences can be genetic as well as epigenetic (Ephrussi 1958; Nanney 1958) in nature. As we know, the above mechanisms of hereditary variation may be responsible for similar results, and their differentiation, for practical puroposes, presents many difficulties, particularly while working with organisms inaccessible for cross breeding analysis (Nanney 1963, Olenov 1967).

A good deal of important evidence pertaining to the problem under discussion was obtained from a study of hereditary destabilization in amoebae (Yudin 1967). At present we are aware of five methods for obtaining hereditarily unstable clones in *A. proteus:* 1. Joint stay of genetically different nuclei in heterokaryons (Kalinina and Yudin 1964); 2. Injection of RNA extracted from one strain to the amoebae of another (Kalinina et al. 1967); 3. X-irradiation of original strains (Kalinina 1967), 4. Treatment of original strains with actinomycin D or 5. RNAase (Kalinina 1968, 1969). Here it is most important to stress out the following characteristic traits of destabilization process. The bulk of clones obtained by any of the techniques used would prove to be unstable (practically 100%), many of them being unstable with respect to two different markers simultaneously. Arising changes are usually similar, no matter by which of the five methods they were obtained (at the same time only two first non-stability induction techniques involve with the introduction to a cell of one strain of material taken from another). It is also essential that in literature there is no indication of the mutagenic activity of RNAase or actinomycin.

These peculiarities prevent from considering changes leading to the hereditary non-stability to be mutational. Proceeding from these results Olenov (1965, 1967, 1968) made the suggestion that arising changes are epigenetic (epigenomic by Olenov's terminology). It can be assumed so far (see also Kalinina 1967) that not only induced non-stability of a given character, but also the initial hereditary differences between strains with respect to destabilizing characters are epigenetic. Although it is difficult to adduce forcible arguments in favour of this assumption it offers certain advantages as a working hypothesis. It permits, in particular, to compare the phenomenon of instability in amoebae with the behaviour of so-called selfing clones in paramecia exhibiting cyclic changes of mating type (Sonneborn 1966).

In fact, the above considerations are concerned only with those of our characters which displayed their ability for hereditary destabilization: re-

sistance to methionine (K a l i n i n a and Y u d i n 1964), resistance to ethyl alcohol (K a l i n i n a 1965 a) and thermostability (S o p i n a 1968 c). As for the nature of other markers, it is still hard to say something definite.

Causes due to which our evidence are inconsistent with the results obtained by Danielli's group

D a n i e l l i (1955) advanced the hypothesis that the cell nucleus, at least in amoebae, "controls the n a t u r e of the macromolecules which are synthesized, but that in the cytoplasm self-reproducing o r g a n i z a t i o n s of molecules are responsible for many cytoplasmic activities" [7].

He upholds and develops his idea in a series of his later works. A most detailed formulation of his hypothesis is given in his publications of 1958 (D a n i e l l i 1958 a, 1958 b) where he lays emphasis to the fact that impetus to the hypothesis has been given by the results obtained from the experiments on nuclear transplantation carried out by himself and his co-workers. Indeed, issuing from their experimental results it was easy to believe that the inheritance of such characters as antigenic specificity, peculiarities of free amino acid pool and ability to adapt to antiserum was controlled exclusively by the nucleus. On the other hand, in the inheritance of such morphological and physiological characters as the nucleus size and peculiarities of its distribution within the clone, the shape of moving amoebae and the form they assume in antiserum specific for the clone, the reproduction rate and resistance to streptomycin, the cytoplasm plays a very important and sometimes even dominating role (D a n i e l l i 1960 b) [8].

Perhaps, the incompatibility of our data with the results obtained by D a n i e l l i's group is due to the fact that in one instance markers were chosen for investigation which are controlled exclusively by the nucleus, and in another, mainly by the cytoplasm? This supposition, however, seems most unlikely, for, to begin with, the conclusions drawn by both groups are based on a substantial number of characters but in either case the latter were chosen randomly. In addition, the division of hereditary characters into chemical, physiological, and morphological ones seems to be rather relative. But even if we accept this temporal classification there will be every reason to doubt the correctness of D a n i e l l i's hypothesis. In our instance the inheritance, for example, of ethanol resistance and thermostability (i.e. characters which can be classified with certainty as physiological) is controlled exclusively by the nucleus. The same holds true for the morphological character tested.

It is also unlikely to attribute the discordance of the obtained results to the fact that the work was carried out with different amoeba strains, since we have tested various "hybrid" combinations and obtained identical results. Finally, we cannot suggest that regularities revealed by Danielli and his coworkers are characteristic for interspecific transplantations, whereas ours, for intraspecific ones. Except "hybrids" between *Amoeba proteus* and *A. discoides*, D a n i e l l i described "hybrids" between different strains of *A. proteus*. Furthermore, at the very beginning of the experiment status of *A. discoides* gave rise to doubts (L o r c h and D a n i e l l i 1953 a), some addi-

[7] As such this hypothesis touches on a subject whether any additional genetic information, besides that determining the primary structure of macromolecules and dynamic of their synthesis, is required to build supramolecular structures.

[8] As to the latter marker, cf. K a l i n i n a 1969.

tional evidence (K a t e s and G o l d s t e i n 1964) made it even more unlikely. Causes for the divergence in question are to be found elsewhere.

Let us return to the phenomenon of genetic nuclear interaction in heterokaryons of amoebae. In this connection of primary importance is the fact that an averaged (for all the tests) phenotype of the majority of unstable clones resulting from this interaction was intermediate between the phenotypes of original strains more or less approximating one of them (see e.g. Figs. 4 and 5 in review by Y u d i n 1967). On such estimation, the non-stability of the clones imitated a dual nucleo-cytoplasmic control of corresponding characters. For some of the latter this imitation was aggravated by the mode of distribution of characters. Thus, thermostability values of unstable clones show identical one-peak distributions as for original strains, though with increased standard deviation (S o p i n a 1968 c).

Very seldom such unstable clones arise when conventional nucleo-cytoplasmic "hybrids" are obtained by ordinary technique, although the overwhelming majority of these hybrids inherit, according to our data, characters and properties of that parental strain which has contributed the nucleus, and show the same stability of phenotype. A very odd behaviour of one of numerous L_nB_c "hybrids" provides an excellent example in this respect (Table 15). In some

Table 15

Survival rate of the exceptional "hybrid" clone L_nB_n-1 (the ratio of survivors to the initial number of amoebae) after a 18–20 hr incubation in 0.15 M methionine (Y u d i n, unpublished)

Strain	Tests								Total	Percentage of survival
	1	2	3	4	5	6	7	8		
L	0:50	7:50	7:50	—	5:50	0:50	0:40	15:40	34:330	10.3
L_nB_c-1	0:50	30:50	30:50	27:50	7:50	0:50	0:40	25:40	119:370	32.2
B	30:50	20:50	31:50	—	20:50	19:50	10:40	15:30	145:320	45.3

of the experiments this particular clone was as methionine resistant as the "parental" B strain, and in others, as much methionine sensitive as the "parental" L strain. On the average, for all the tests, the stability level of this clone was intermediate between the resistance level of L and B. Thus based only on these total results one could conclude that the inheritance of methionine resistance in a given clone is more or less equally controlled by the nucleus and cytoplasm.

The appearance of such exceptional unstable nucleo-cytoplasmic "hybrids" in result of conventional transplantation procedure was also explained by the genetic interaction of nuclei, in this instance, by the interaction of the donor nucleus with the products of just removed nucleus of the recipient cell, these products being temporarily preserved in enucleated cytoplasm (Y u d i n 1965 b). Rare occurrence of the unstable clones under such conditions was attributed to the rapid loss of activity of the nuclear products in the enucleated cytoplasm (K a l i n i n a and Y u d i n 1964, Y u d i n 1967, Y u d i n and N i k o l a y e v a 1968). Later on this suggestion was proved experimentally.

The progeny of nucleo-cytoplasmic "hybrids" appeared to be indistiguishable from its nuclear "parent" only when no less than 2–5 min had passed since the moment of enucleation of the recipient cell till the moment of its renucleation by the nucleus of another strain. If this time is reduced to 10–25 sec, many "hybrid" clones exhibit the inheritable instability with respect to the same characters or some of then (Y u d i n 1968 a). Thus obtained "hybrid" clones of C_nL_c type proved to be typically unstable with respect to alcohol resistance; 10 of them were also unstable in methionine resistance. Out of 10 clones of L_nC_c type 3 were unstable with regard to methionine resistance; the remaining clones did not differ in this character from the original L strain. None of 10 "hybrids" of this group showed instability of their ethanol resistance.

The summary evaluation of the average survival rate of these "hybrid" clones yields striking results (Table 16). When arranged by their increasing

Table 16

The averaged survival rate of the original clones L and C and of "hybrids" between them (mean number of survivors from 50 amoebae used for each test), the latter having been obtained with 10–50 sec intervals between de- and renucleation of a recipient cell (Y u d i n, and N i k o l a y e v a unpublished)

0.15 M methionine resistance			7% ethyl alcohol resistance		
clone	number of tests	$\bar{x}\pm s_{\bar{x}}$	clone	number of tests	$\bar{x}\pm s_{\bar{x}}$
C_nL_c-1	20	0.2±0.2	L	45	2.9±0.4
C_nL_c-2	22	0.7±0.3	C_nL_c-1	10	4.5±1.6
L	36	2.3±0.5	C_nL_c-2	10	6.5±1.9
C_nL_c-3	20	10.6±2.6	C_nL_c-6	13	20.0±4.5
C_nL_c-4	20	19.6±3.0	C_nL_c-11	12	21.1±4.8
C_nL_c-6	16	26.1±3.1	C_nL_c-3	13	21.7±5.0
C_nL_c-10	16	26.6±2.7	C_nL_c-5	13	25.6±5.0
C_nL_c-8	13	28.7±3.3	C_nL_c-10	13	26.7±5.5
C_nL_c-5	16	30.2±2.1.2	C_nL_c-7	12	27.7±5.0
C_nL_c-9	16	32.4±2.4	C_nL_c-9	13	30.4±4.8
C_nL_c-11	12	32.4±3.7	C_nL_c-8	12	31.8±4.4
C_nL_c-7	11	32.9±2.2	C_nL_c-4	13	32.5±5.5
C	36	38.1±1.1	C	24	46.3±0.6

mean (averaged through all the test) resistance, the clones C_nL_c constitute almost a continuous series of survival values beginning from the level of the original strain L to that of the original strain C. Some of these clones (C_nL_c-1 and C_nL_c-2) seem to be even more sensitive to methionine than L. Had the respective roles of the nucleus and cytoplasm in heredity been judged about by the behaviour of one of these clones we could have made the conclusion about any degree of participation of the cytoplasm in the inheritance of these characters depending on a clone chosen. It must be recalled once again that with respect to these characters "true" nucleo-cytoplasmic "hybrids" of this

type are similar to each other and to the original strain which has contributed the nucleus.

The finding of the phenomenon of hereditary destabilization of characters in amoebae allowed the supposition (Yudin 1965 b) that the the evidence obtained by Danielli and his co-workers which seemed to be demonstrating a dual nucleo-cytoplasmic determination of the bulk of the characters studied may offer another explanation (see also Gurdon 1964, Lorch and Jeon 1969). We suggested that, instead of "true" nucleo-cytoplasmic "hybrids" these authors had obtained and tested "hybrid" clones destabilized (in the above sense of the word) with repect to many characters controlled in reality by the nucleus [9].

True, the evidence we have at our disposal (Yudin 1968 a) seem to show that for obtaining unstable "hybrid" clones in our conditions it is required, for example, to reduce sharply an interval between the denucleation of the recipient cell and introduction to the latter of nucleus of the donor-cell, or to set up during the operation even if temporal state of heterokaryosis. This is accounted for by the fact that in our experimental conditions nuclear products of the recipient cell capable of inducing instability in the donor-cell nucleus lose their activity very quickly after they get into the cytoplasm. However, it can be easily admitted that under other experimental conditions their activity in the cytoplasm is retained for a longer time. In this instance not "true" nucleo-cytoplasmic "hybrids", but unstable "hybrid" clones would be the typical result of the nuclear transplantation procedure. It can be easily imagined that under such conditions any correct interpretation of the data obtained by this technique would present extraordinary difficulties.

All the above show that very important requirements should be placed upon experiments conducted to obtain nucleo-cytoplasmic "hybrids". The nucleus of a donor-cell is to be introduced only some time after the removal of the host nucleus of a recipient cell. It would be desirable to obtain and test a few clones of each "hybrid" combination, all the clones of one type (provided they are "true" nucleo-cytoplasmic "hybrids") must have the same phenotype. By contrast, there is every reason to suggest that Danielli and his co-workers have studied only one clone of each "hybrid" type (see Cole and Danielli 1963). Finally, in instances when the obtained "hybrid" clones are of intermediate phenotype, it seems reasonable to make transplantations of nuclei from such a "hybrid" clone back to the cytoplasm of its "cytoplasmic parent". If the intermediate phenotype is retained in all thus obtained subclones, no one would be able to explain this phenomenon by the participation of cytoplasm in inheritance of the character tested (cf. Kalinina 1965 b).

The evidence accumulated over the past few years on the phenomenon of destabilization of hereditary characters in amoebae still further supports the above supposition. These new evidence enable us to explain in detail many peculiarities of the results obtained by Danielli's group and consider them as a more or less complete imitation of phenomena of cytoplasmic heredity.

First it seems that a great many of hereditary characters of amoebae are capable for destabilization. This was established for at least three randomly chosen characters tested in this respect: methionine resistance, ethanol resis-

[9] The material obtained by Danielli's group contains a direct reference to the appearance of heritable changes of nuclei under the influence of "alien" cytoplasm (Danielli 1959, 1960 b et al.).

tance and thermostability. The same clones were simultaneously destabilized by these three characters. This fact enables one easily to explain, on the basis of our suggestion, why characters with a double nucleo-cytoplasmic control are prevalent among those studied by Daniellis group.

However, for some reason or other, identical clones may be destabilized with respect to some of the characters remaining stable to the other (Yudin et al., 1966, Yudin and Nikolayeva 1968). From this point of view it becomes clear why, concurrent with the characters exhibiting a dual genetic control, Daniellis group has also obtained traits conditioned exclusively by the nucleus.

Even the above evidence (Table 16) show that the degree of approximation of unstable clones to their "cytoplasmic" parent is unbounded. In restricted series of tests a given unstable clone may even not exhibit values of a character typical for its "nuclear" parent. However, in the majority of cases, providing the number of tests was sufficiently great, the averaged estimation of unstable clones yielded characteristic values intermediate between those typical for original "parental" forms with a greater or lesser degree of approximation to the "cytoplasmic" parent. This explains, within the limits of the suggested hypothesis, why Daniellis and his co-workers have found not a single character inherited only via cytoplasm (Daniellis 1963).

The resulting instability appears to be a stable hereditary property. No instance has so far been known when previously unstable clones get stabilized. On the contrary, there is evidence (Sopina 1968 c) that this property can persist for years. Hence, in this respect also the instability may well imitate genetic functions of the cytoplasm.

Tests with injection of cytoplasm from one strain to another (Hawkins et al. 1962, Hawkins 1964, Hawkins and Wolestenholme 1966, Hawkins and Cole 1965) intended to support results of nuclear transplantation are not inconsistent with our hypothesis. As was indicated, the experiments on genetic interaction of nuclei show that a certain amount of agent capable of inducing nuclear instability constantly enters the cytoplasm of amoebae and persists there for some time. This agent may be evidently transferred from one cell to another upon injection. Therefore, it can be assumed that in the case of intracellular injection of cytoplasm taken from another strain the collaborators of Daniellis have obtained destabilized clones.

In this regard of particular interest is one of the recent works of Daniellis group (Jeon et al. 1967). It shows that the behaviour of *Amoeba proteus* clones injected with the cytoplasm of *A. discoides* with respect to the character with previously detected dual nucleo-cytoplasmic control closely resembles that of our unstable clones in respect to characters the heredity of which was found to be determined exclusively by the nucleus. In this connection it is appropriate to mention the work of Kalinina et al. (1967). The authors showed that amoeba strains may be destabilized by injections of total RNA isolated from an other strain (see also Hawkins 1969).

No doubt, the offered explanation of discrepancies between our data and results of Daniellis group is not the only one possible. But however that may be, the fact is that at present, using the same technique for identical amoeba strains with respect to the same characters and varying a few seemingly insignificant details of this procedure (Yudin 1968 a, b), we are able to obtain

either evidence demonstrating an exclusively nuclear determination of inheritance of these characters or some data imitating the dual nucleo-cytoplasmic control. Thus fact enables us to maintain that at present the experimental material obtained by D a n i e l l i ' s group, as well as their conclusions are far from being indisputable.

From our point of view the picture on the whole resembles that we repeatedly observed in genetics of protozoa (N a n n e y 1963) and in other fields of genetics. It happened time and again that phenomena first taken for cases of cytoplasmic heredity, when analysed in detail, turned to be more or less complete imitation of the latter. A case of the inheritance of mating types and serotypes in B group of syngenes of *Paramecium aurelia* seems to be very similar to our instance. It was found out that in *Paramecium* seeming cytoplasmic heredity disguises regulating influences of the "old" prezygotic macronucleus on the direction of epigenetic differentiation of "new" macronucleus: i.e. influences exerted via cytoplasm and possibly analogous to nuclear-nuclear interinfluence in amoebae.

Summary

The comparison study of 6 *Amoeba proteus* strains of different origin—L, K, B, P, C, and Sh—revealed hereditary differences in 6 characters: efficiency of cloning, resistance to the toxic effects of methionine, post-effect of methionine, resistance to the toxic effects of alcohol; thermostability and average cell perimeter.

Nucleo-cytoplasmic "hybrids" obtained by nuclear transplantation technique were compared between each other and with original strains. In neither of the 6 characters did the "hybrid" clones differ from the original strain which had contributed the nucleus. For some characters this result was obtained on several different "hybrid" combinations. On the basis of the above it is concluded that in amoebae the inheritance of all these characters in successive cell generations is controlled by the nucleus. The data obtained furnish no indication of the participation of cytoplasm in this process.

Under certain conditions among nucleo-cytoplasmic "hybrids" there occur "hybrid" clones destabilized with respect to characters the inheritance of which is conditioned by the nucleus. Such clones are similar to clones grown from diheterokaryotic amoebae. Their hereditary instability is the result of genetic nuclear-nuclear interaction and imitates dual nucleo-cytoplasmic control of the inheritance of corresponding characters. It is suggested that instead of true nucleo-cytoplasmic "hybrids" D a n i e l l i and his co-workers obtained and tested "hybrid" clones destabilized with respect to many nuclear characters. If this was the case the results could lead D a n i e l l i's group to the conclusion about an essential role of the cytoplasm in heredity of amoebae.

РЕЗЮМЕ

При сравнительном изучении шести штаммов амеб *Amoeba proteus* различного происхождения — Л, К, В, П, С и Ш — были выявлены наследственные различия между ними по шести разным признакам: эффективности клонирования, устойчивости к токсическому действию метионина, последействию метио-

нина, устойчивости к этиловому спирту, теплоустойчивости и среднему периметру клетки.

Ядерно-цитоплазматические „гибриды", полученные методом трансплантации ядер, сравнивались по этим признакам друг с другом и с исходными штаммами. По всем шести признакам „гибридные" клоны не отличались от того исходного штамма, от которого они получили ядро. Этот результат для некоторых признаков был получен на нескольких разных „гибридных" комбинациях. На основании полученных данных делается вывод, что наследование всех этих признаков в ряду клеточных поколений амеб обусловлено ядром. Полученный материал не дает никаких указаний на участие цитоплазмы в этом процессе.

Показано, что при получении ядерно-цитоплазматических „гибридов" в некоторых условиях возникают „гибридные" клоны, дестабилизированные по ряду признаков, наследование которых контролируется ядром. Такие клоны сходны с клонами-потомками амеб-дигетерокарионов. Их наследственная нестабильность является результатом генетического взаимодействия ядер и имитирует двойственный ядерно-цитоплазматический контроль наследования соответствующих признаков, наследование которых контролируется ядром. Такие клоны сходны „гибридов" Даниэлли и его сотрудники получали и исследовали „гибридные" клоны, дестабилизированные в отношении многих ядерных признаков. Это и привело группу Даниэлли к выводу о существенной роли цитоплазмы в наследственности у амеб.

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Thécamoebiens *(Rhizopoda, Testacea)* nouveaux des eaux souterraines littorales de la Mer Noire

Нови тестацеи (*Rhizopoda, Testacea*) от крайбрежните подпочвени води на Черно море

Les quatre nouvelles espèces de thécamoebiens décrites dans le présent travail ont été rencontrées par nous dans les eaux souterraines littorales des plages de la Mer Noire. Elles se répartissent en trois genres dont l'un est aussi nouveau pour la Science.

Dans quelques publications précédentes (Golemansky 1968, 1969 a, b) nous avons eu déjà la possibilité de montrer que les eaux souterraines littorales des plages sableuses des mers sont habitées par une cénose thécamoebienne peu connue pour le moment, mais bien caractéristique et spécifique pour ce milieu écologique. Les quatres thécamoebiens nouveaux, dont la déscription est faite ci-déssou, nous considérons aussi comme des intégrants spécifiques de cette cénose, n'habitant que le psammal littoral des mers et des océans.

Alepiella gen. n.

Typus generis: *Alepiella tricornuta* sp. n.

Diagnose: Thèque allongée, comprimée dorso-ventralement et ornée de trois cornes: une terminale caudale et deux latéraux. Revêtement en général assez léger et consititué de petites écailles et plaquettes de forme différente, noyées dans un ciment chitinoïde clair. Le pseudostome, placé obliquement par rapport de l'axe longitudinal, est muni d'un évasement de la thèque ayant en vue ventrale l'aspect d'un disque large et clair.

Alepiella tricornuta gen. n., sp. n. Figs. 1, 2, Pl. I 1, 2

Déscription: Thèque allongée, comprimée dorso-ventralement, incolore et translucide. Sa partie postérieure se prolonge en une corne creuse et tubulaire. La thèque est ornée aussi de deux cornes latéraux aussi creuses et tubulaires, mais dirigées en avant—ce que donne à l'espèce un aspect assez bizare (Fig. 1).

Revêtement chitinoïde et constitué de petites écailles et de plaquettes de forme différente, soudées dans un ciment chitinoïde clair. Souvent on observe des éléments exogènes plus gros dans la région du pseudostome.

Pseudostome circulaire et placé obliquement par rapport de l'axe longitu-

dinal. Dans la région du pseudostome la thèque s'évase en une collerette large et claire, entourant l'orifice buccal. En vue latérale cet évasement a l'aspect d'un entonnoir large et court, tandis qu'en vue ventrale il sé présente en forme d'un disque entourant le pseudostome.

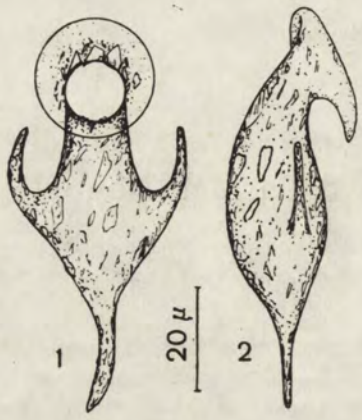

Fig. 1—2: *Alepiella tricornuta* gen. n., sp. n., 1—vue ventrale, 2—profil

Dimensions observées:
longueur totale: 85—90 μ,
largeur totale: 40—45 μ,
largeur sans cornes: 20—25 μ,
épaisseur: 16—21 μ,
longueur des cornes: 15—22 μ,
pseudostome: 10—13 μ,
évasement du pseudostome: 27—32 μ.

Animal vivant non observé.

Ecologie: *Alepiella tricornuta* gen. n. sp. n. a été observée jusqu'à présent d'une seule localité au bord de la Mer Noire: plage Alepou, située à 20 km environs au sud de v. Sosopol. L'espèce a été trouvée à 5–7 m de la mer à une profondeur dans le sable égale à 90 cm (25.IV.1968 et 9.X.1968). Il semble que c'est une espèce assez rare dans le psammal littoral de la Mer Noire.

Une préparation type de l'espèce est déposée dans la collection de l'auteur à l'Institut Zoologique et Musée de Sofia.

Psammonobiotus linearis sp. n. Figs. 3, 4

Description: Thèque de petite taille, allongée, plagiostome et semblable à celle d'une *Trinema*. Sa partie postérieure est effilée en une corne non aiguë et relativement courte. En vue ventrale la thèque est plus au moins rétrécie dans sa partie antérieure. En vue latérale elle est légérement comprimée avec une face dorsale bombée et face ventrale peu bombée ou plate. L'ensemble est incolore et parfaitement transparente.

Le pseudostome, tronqué obliquement, est circulaire et pourvu de même collerette chitineuse que les autres espèces connues du genre *Psammonobiotus*.

En vue ventrale cette collerette a l'aspect d'un disque large et hyalin, entourant le pseudostome.

Le revêtement est chitinoïde. Rarement sur la thèque on observe et des plaquettes polygonales de différente grandeur disposées sans ordre et noyées dans la substance chitinoïde.

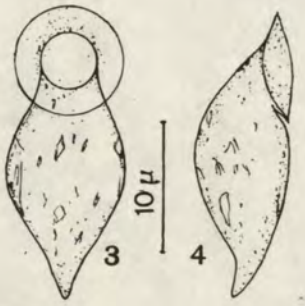

Fig. 3—4: *Psammonobiotus linearis* sp. n., 3—vue ventrale, 4—profil

Dimensions observées:
longueur: 21—24 µ,
largeur: 10—12 µ,
épaisseur: 7—9 µ,
pseudostome: 3—4 µ,
évasement du pseudostome. 8—11 µ.

Animal vivant non observé.

Ecologie: *Psammonobiotus linearis* sp. n. a été observée une seule fois dans le psammal littoral de la plage près de l'embouchure du fleuve Ropotamo: 10 m de la mer; profondeur dans le sable: 50—60 cm (1.IX.1966). C'est probablement une espèce assez rare dans les eaux souterraines littorales de la Mer Noire.

Discussion: *Psammonobiotus linearis* sp. n. est la troisième espèce du genre *Psammonobiotus*, connu jusqu'à maintenant seulement du psammal littoral des mers et des grands lacs (Golemansky 1969 c). La nouvelle espèce diffère de *Ps. communis* et *Ps. minutus* par sa taille plus réduite, sa forme allongée (largeur/longueur = 0.4—0.5) et la présence du corne caractéristique au fond de la thèque.

Amphorellopsis maximus sp. n. Figs. 5, 6; Pl. I 3

Description: Thèque ovoïde-globuleuse, rétrécie et étirée dans la région du pseudostome en un col relativement court, mais bien developpé. En vue frontale le fond de la thèque est largement arrondi. En vue latérale elle est comprimée et l'aplatissement se manifeste plus en approchant le col et la région du pseudostome. Le fond, en vue latérale, est prèsque ogival.

Le pseudostome est terminal, oval ou élliptique et entouré d'une large collerette chitineuse, formée de l'évasement de la thèque dans la région de l'orifice buccal. Cette collerette parfois se renverse en arrière comme le cas de *Nadinella tenella*, décrite par Penard (1899) du lac de Génève.

La membrane est de nature chitinoïde, incolore et translucide. Sur sa surface et surtout dans la partie antérieure sont empâtées des particules silicieuses amorphes, anguleuses ou arrondies et souvent parsemées de pla-

ques plus grosses. Chose caractéristique pour *Amphorellopsis maximus* sp. n. c'est la présence des éléments quartzeux plus gros au fond de la thèque qui sont souvent disposés en une seule rangée (Fig. 5, 6 Pl. I, Fig. 3).

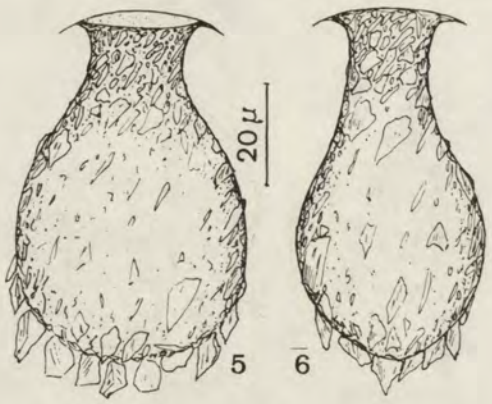

Fig. 5—6: *Amphorellopsis maximus* sp. n., 5—vue frontale 6—vue latérale

Dimensions observées:
longueur: 63—77 μ,
largeur: 33—54 μ,
épaisseur: 25—40 μ,
col: 19—24 μ,
collerette du pseudostome: 32—35 μ.

Cytoplasme hyalin et clair presque remplissant la thèque de l'animal. Epipodes et vacuoles pulsatives non observés. Pseudopodes peu nombreux, filopodes. Ils dépassent souvent 2—3 fois la longueur totale de l'animal. Noyau unique, assez gros (20—23 μ) et globuleux.

Ecologie: *Amphorellopsis maximus* sp. n. a été trouvé dans le psammal littoral de la plage de v. Pomorie: 4 m de la mer; profondeur dans le sable: 15—20 cm t°=16.5°C; Cl′—8.5 g/l; 10.X.1968. Le sable dans cette localité était beaucoup plus gros que celui des autres plages voisines et mêlé de coquilles et de débris de coquilles des Mollusques marins.

Trois préparations (1 Holotype et 2 Paratypes) de la nouvelle espèce sont déposées dans la collection de l'auteur à l'Institut Zoologique et Musée de Sofia.

Amphorellopsis lucida sp. n. Figs. 7—9

Description. Thèque pyriforme, hyaline et transparente, plus au moins allongée, ogivale ou lancéolée en arrière et étirée en avant en un col, qui s'évase dans la région du pseudostome. Cette évasement forme une collerette, entourant le pseudostome, qui a l'aspect d'un disque hyalin en vue apicale. Au fond de la thèque on observe une pointe courte de même matière que l'enveloppe (Figs. 7, 9). Il est intéressant de noter que dans une même population de l'espèce on rencontre parfois des formes dont les thèques sont dépourvues de pointe et sa panse est arrondie (Fig. 8). La séction transversale de la thèque et le pseudostome sont circulaires.

Le revêtement est chitinoïde avec des écailles amorphes de différente forme et grosseur disposées par-ci par-là sur l'enveloppe complètement transparente.

Dimensions observées:
longueur: 17—26 μ, (m) 22 μ,
diamètre: 8—12 μ, (m) 10.5 μ,
col: 4—6 μ,
évasement du pseudostome: 7.5—11 μ.
Animal vivant non observé.

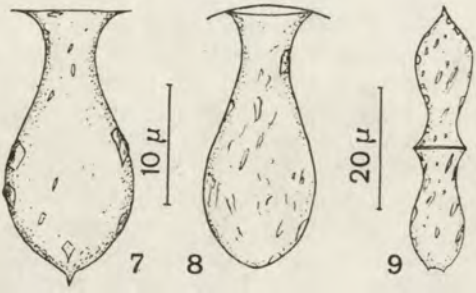

Fig. 7—9: *Amphorellopsis lucida* sp. n., 7—Individu typique avec pointe caractéristique au fond de la thèque, 8—forme aberrante de l'espèce, dépourvue de pointe au fond de la thèque, 9—formes en réproduction

Dans un prélevement du psammon littoral nous avons observé des formes en réproduction que nous illustrons au Fig. 9. Les deux individus étaient prèsque de même dimensions et forme.

Ecologie: *Amphorellopsis lucida* sp. n. est une espèce commune dans les eaux souterraines littorales de la Mer Noire. Au cours de nos investigations l'espèce a été trouvée dans les localités suivantes:

1. Plage près de l'embouchure du fleuve Ropotamo: 2 m de la mer; profondeur dans le sable: 20 cm; 8.III.1967.

2. Plage de Slantchev brjag: 7 m de la mer; profondeur dans le sable: 60 cm; t°=20.8°C; Cl′—8.75 g/l.; pH=8.7; 26.VIII.1967.

3. Plage près de l'embouchure du fleuve Potamjata:

a. 7 m de la mer; profondeur dans le sable: 103 cm; t°=9.6°C; Cl′—9.6 g/l.; pH=8.6; 24.IV.1968.

b. 5 m de la mer; profondeur dans le sable: 40 cm; t°=16°C; 8.X.1968.

4. Plage de v. Sinemorec:

a. 6 m de la mer; profondeur dans le sable: 90 cm; t°=9.8°C; Cl′—9.6 g/l.; pH=8.6; 24.IV.1968.

b. 6 m de la mer; profondeur dans le sable: 75 cm; t°=15.8°C; 8.X.1968.

5. Plage de v. Kiten: 12 m de la mer; profondeur dans le sable: 90 cm; t°=17.2°C; 8.X.1968.

6. Plage de v. Kranevo: 3 m de la mer; profondeur dans le sable: 65 cm; t°=15.9°C; 12.X.1968.

Discussion: *Amphorellopsis lucida* sp. n. est la quatrième espèce du genre *Amphorellopsis*, connue jusqu'à maintenant. Par ses caractères morphologiques notre nouvelle espèce est la plus proche d'*Amphorellopsis taschevi*, décrite par nous, des eaux souterraines littorales de quelques plages de Cuba (Golemansky 1969 b). Mais entre les deux espèces citées existent des différences morphologiques assez nettes qui nous donnent la raison de les considérer comme deux taxons à part bien délimités. *Amph. lucida* sp. n. est une forme monaxone, bien symétrique et n'est jamais courbée dans la région du col comme le cas d'*Amph. taschevi*. L'ensemble est plus clair et transparent chez *Amph. lucida* sp. n. que chez d'*Amph. taschevi*. La taille de notre nouvelle espèce est aussi plus réduite que chez l'espèce de la Mer

Caraïbienne. Enfin, le caractère taxonomique le plus important, d'après nous, qui sépare nettement les deux espèces, c'est la présence de pointe caractéristique au fond de la thèque d'*Amph. lucida* sp. n., qui n'a été jamais observée ches l'espèce du psammon littoral de Cuba.

Résumé

Nos investigations sur la faune rhizopodique des eaux souterraines littorales des mers faites pendant les dernières quelques années nous ont montré, que ce milieu écologique est habité par une cénose thécamoebienne spécifique, dont les intégrants, avec peu d'exceptions, n'habitent pas les eaux douces, le sol et les mousses. Dans la présente publication est faite la déscription d'un genre nouveau pour la Science, ainsi que de quatre espèces aussi inconnues jusqu'à présent, notamment: *Alepiella tricornuta* gen. n., sp. n., *Psammonobiotus linearis* sp. n., *Amphorellopsis maximus* sp. n. et *Amphorellopsis lucida* sp. n.

Cette nouvelle contribution complète nos connaissances actuelles sur les espèces, la répartition et l'écologie de la cénose thécamoebienne du psammal littoral des mers, restée prèsque entièrement ignorée jusqu'à nos jours.

РЕЗЮМЕ

Направените от нас изучвания през последните години върху ризоподната фауна на крайбрежните подпочвени води на моретата показа, че тази екологическа среда се обитава от една специфична тестацейна ценоза, чиито компоненти с малки изключения не се срещат в сладките води, почвата и мъховете. В предлаганата работа е направено описание на един нов за науката род и четири нови вида тестацеи от крайбрежните подпочвени води на Черно море, а именно: *Alepiella tricornuta* gen. n. sp. n., *Psammonobiotus linearis* sp. n., *Amphorellopsis maximus* sp. n. u *Amphorellopsis* lucida sp. n., с което се допълват нашите досегашни познания за видовия състав, разпространението и екологията на тази малко позната ризоподна ценоза на крайбрежния морски псамал.

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Golemansky V. 1969 c: *Psammonobiotus communis* Gol., 1968 (*Rhizopoda, Testacea*)—une rélicte marine du lac d'Ohrid. Izv. zool. Inst., Sof., (in press).

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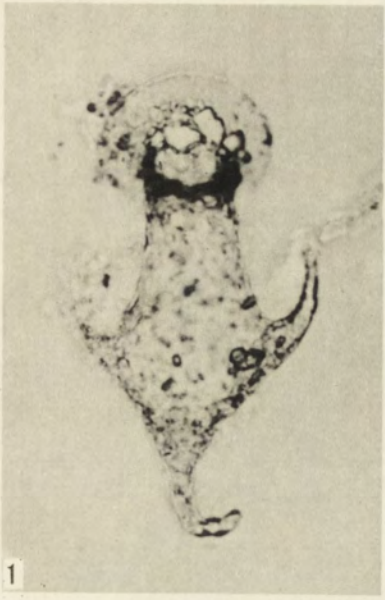
EXPLICATION DE PLANCHE I

1: *Alepiella tricornuta* gen. n., sp. n., vue ventrale. 850×
2: Ibid., profil. 850×
3: *Amphorellopsis maximus* sp. n., vue frontale. 1300×



V. Golemansky auctor phot.

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Инфузории мезопсаммона Кандалакшского залива (Белое море). II

The ciliates of the mesopsammon of the Kandalaksha Gulf (White Sea). II

В настоящей работе продолжается описание некоторых новых и ранее неизвестных для Белого моря видов. Описание даётся на основании прижизненных наблюдений и изучения фиксированного материала, импрегнированного серебром (Chatton et Lwoff 1930) или окрашенного гемалауном. Материал собран в районе биологической станции Московского университета в июне-сентябре 1967—1968 гг.

Prorodon moebiusi Kahl, 1930 (Рис. 1)

Тело продолговато-овоидное, слабо сократимое. Размеры 200—300 × 100—150 μ. Отношение длины к ширине 2 : 1 = 3 : 1. Рот терминальный, щелевидный, ведёт в протяжённую глотку, снабжённую 36—40 извитыми трихитами. Ресничный покров густой, равномерный, около 120—160 меридиональных рядов. Растояние между кинетосомами, находящимися в одном ряду 1.3—1.5 μ. Около ротового отверстия реснички расположены в 7—8 колец.

Эктоплазма прозрачная, хорошо выраженная, толщиной 2—3 μ. Эндоплазма мелкозернистая, светлая, прозрачная, содержит отдельные включения. Ядерный аппарат центральный, состоит из овального или продолговатого макронуклеуса (Ма) размером 30—50 × 25—30 μ и 3—5 микронуклеоусов (Ми) размером около 3 μ. Сократительная вакуоль (СВ) единственная, терминальная.

Широко распространённый в Кандалакшском заливе вид. Встречается преимущественно в крупнозернистом и среднезернистом песках, в придонной воде и реже в скоплениях нитчатых водорослей и в детрите.

Беломорская форма отличается от формы, описанной Калем (1930), отсутствием характерной для этого рода борозды, отходящей дорсально от ротового отверстия, а также более вариабильным числом Ми.

Placus dogieli sp. nov. (Рис. 2)

Тело овоидное или эллипсоидное, слегка уплощенное. Размеры 80—130 × × 40—80 μ. Отношение длины к ширине 3 : 2—2 : 1. Наиболее часто встречаются формы размером 100—110 × 50—70 μ. Ротовое отверстие помещается на переднем полюсе тела, окружено обычными ресничками. Глотка снабжена тонкими три-

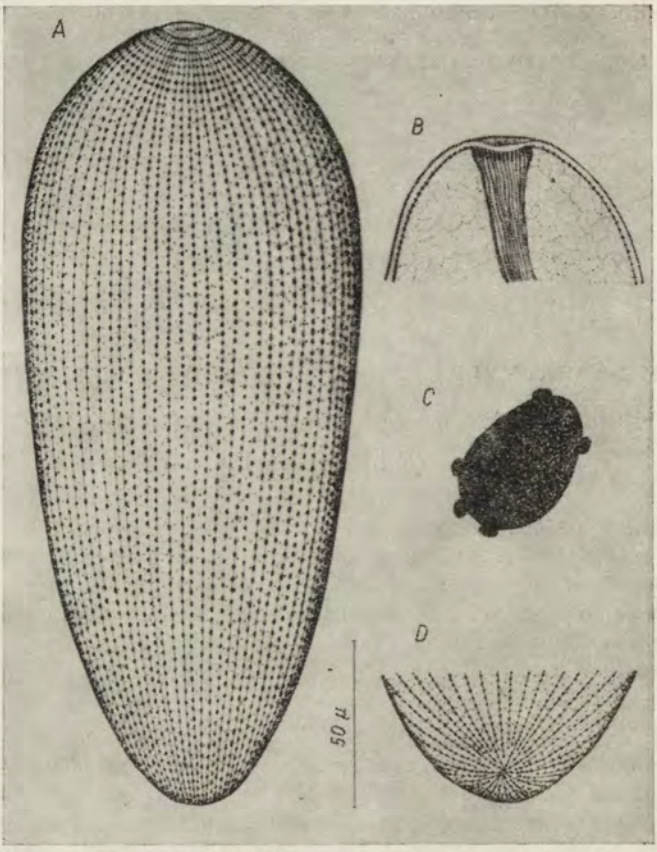

Рис. 1. *Prorodon moebiusi* Kahl, 1930. A — общий вид (тотальный препарат, серебрение), B — передний конец тела (оптический разрез), C — ядерный аппарат (гемалаун), D — задний конец тела (серебрение)

Fig. 1. *Prorodon moebiusi* Kahl, 1930, A—general view (whole preparation, silver impregnation), B—front end of body (frontal section), C—nuclear apparatus (haemalum), D—back end of body (silver impregnation)

хитами. Пелликула плотная, панцировидная, блестящая, несёт 22—24 спиральные борозды, вдоль которых тянутся ряды ресничек. 16—18 кинет подходит к ротовому отверстию, в то время как остальные 6 кинет, не доходя до переднего полюса, упираются в один особый ряд. Вдоль этого ряда тянется короткая псевдомембрана (не более 1/3 длины тела), образованная двумя тесно сближенными рядами плотно прилегающих друг к другу ресничек. На заднем полюсе имеется узкое, продолговатое поле, лишенное ресничек. Степень спирализации борозд находится в связи с формой тела. У продолговатых форм спираль выражена сильнее, у округлих — слабее. Несколько экземпляров, обнаруженных нами, характеризуются 32 спиральными кинетами, из которых 26 кинет подходят к ротовому отверстию. Размеры этих экземпляров 130 × 80 μ.

Эндоплазма мелкозернистая, прозрачная, бесцветная. Ма продолговатый, сильно вытянутый, или червеобразный (40—60 × 15—18 μ). Ми не обнаружен. СВ терминальная.

Широко распространённый в Кандалакшском заливе вид. Встречается преимущественно в крупнозернистом и среднезернистом песках, в придонной воде, а также в детрите.

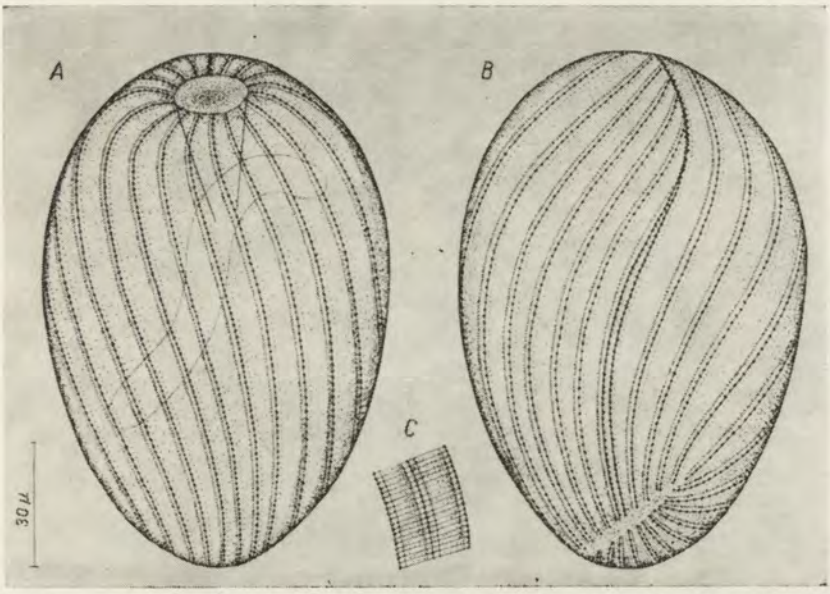

Рис. 2. *Placus dogieli* sp. nov. A, B — общий вид (тотальный препарат, серебрение), C — фрагмент аргирома

Fig. 2. *Placus dogieli* sp. nov. A, B—general view (whole preparation, silver impregation), C—fragment of argyrome

Эта форма занимает промежуточное положение между двумя группами видов *Placus*. От форм, имеющих мембрану (*P. luciae* Kahl, *P. socialis* Fabre-Domerque, *P. buddenbrocki* Sauerbrey, *P. levida* Kahl) настоящий вид отличается отсутствием характерного для этих видов углубления в основании мембраны. От форм, не имеющих мембраны, (*P. ovum* Kahl, *P. striatus* Cohn) настоящий вид отличается присутствием такой мембраны и значительно более крупными размерами тела. От тех и других форм новый вид отличается своеобразным расположением и числом кинет и борозд.

Lagynophrya maxima sp. nov. (Рис. 3)

Тело продолговатое, асимметричное, несократимое. Вентральная сторона прямая или вогнутая, дорсальная — всегда выпуклая. Размеры 140—180 × 30—50 μ. Отношение длины к ширине 3 : 1—4 : 1. Рот слегка смещён на вентральную сторону. „Головка" полусферическая, крупная, хорошо выраженная (как у *L. mutans* Kahl), с длинными трихоцистами (около 20 μ). Реснички собраны в 36—40 продолных рядов, кинетосомы мелкие, тесно прилегающие друг к другу.

Эндоплазма сильно вакуолизированная, бесцветная, но непрозрачная. Ма продолговатый или червеобразный (50—60 × 8—10 μ). Ми не обнаружен. СВ терминальная.

Встречается в мелкозернистом песке литорали и в детрите.

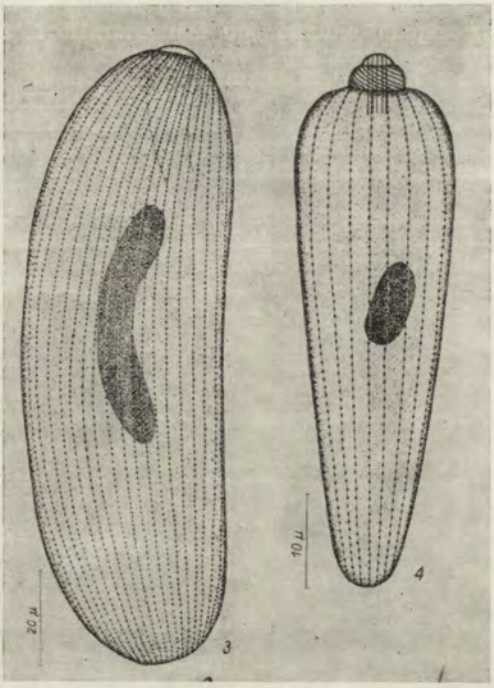

Рис. 3. *Lagynophrya maxima* sp. nov. Общий вид (тотальный препарат, серебрение)
Рис. 4. *Lacrymaria conifera* sp. nov. Общий вид (тотальный препарат, серебрение)
Fig. 3. *Lagynophrya maxima* sp. nov. General view (whole preparation, silver impregnation)
Fig. 4. *Lacrymaria conifera* sp. nov. General view (whole preparation, silver impregnation)

Этот вид отличается от всех ранее описанных видов этого рода очень большими размерами, продолговатой формой тела и червеобразным Ма. Ближайший вид — *L. armata* Kahl.

Lacrymaria conifera sp. nov. (Рис. 4)

Тело продолговатое, почти коническое, сзади сужено и закруглено, слабо сократимое. Размеры 50—70 × 15—20 μ. Отношение длины к ширине 3 : 1—4 : 1. „Головка" двучленная, высокая, со спиральными ресничными рядами. Глотка с трихоцистами. Всё тело покрыто мелкими ресничками, собранными в 18—20 меридиональных рядов.

Эндоплазма непрозрачная, содержит разнообразные включения, от чего инфузория кажется чёрной. Иногда задний конец свободен от включений. Ма продолговатый с одним Ми. СВ терминальная.

Широко распространённый в Кандалакшском заливе вид. Встречается преимущественно в мелкозернистом песке литорали, а также в детрите.

От ближайшего вида *L. delamarei* Dragesco отличается чёткой конической формой тела, вдвое меньшими размерами тела, иным строением ядерного аппарата, отсутствием трихоцист.

Plagiopogon loricatus Kahl, 1931 (Рис. 5)

Тело продолговато-овальное, несократимое. В сечении овальное или эллипсоидное. Размеры 50—70 × 20—25 μ. Отношение длины к ширине 2 : 1—3 : 1. Всё тело покрыто панцирем, состоящим из 12—16 продолных или слегка косых рядов прямоугольных пластин. Каждый ряд включает 20—22 пластины. Крупные реснички по две вместе собраны также в 12—16 рядов. Рот терминальный, трихоцисты длинные.

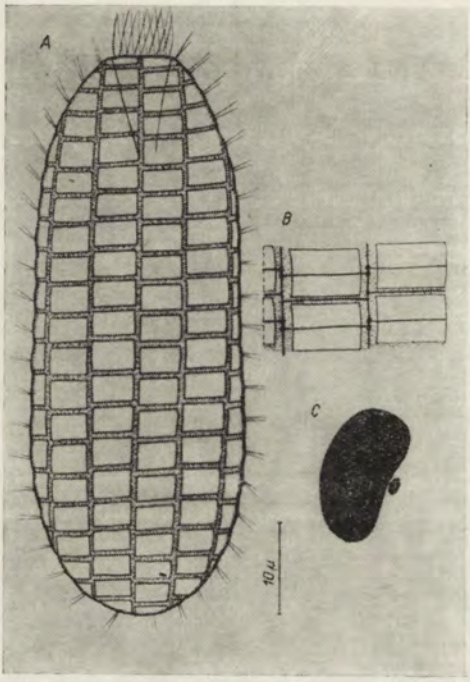

Рис. 5. *Plagiopogon loricatus* Kahl, 1931. A — общий вид (тотальный препарат, серебрение), B — фрагмент аргирома, C — ядерный аппарат (гемалаун)
Fig. 5. *Plagiopogon loricatus* Kahl, 1931. A—general view (whole preparation, silver impregnation), B—fragment of argyrome, C—nuclear apparatus (haemalum)

Эндоплазма мутная, непрозрачная, сильно вакуолизированная. Ядерный аппарат представлен продолговатым или овальным Ма и лежащим рядом Ми. СВ терминальная.

Широкораспространённый в Кандалакшском заливе вид. Встречается в песках разнообразной зернистости, в детрите и в придонной воде.

Беломорская форма отличается от формы, описанной Калем (1933) для Балтийского моря, некоторыми деталями строения инфрацилиатуры и отсутствием каудальной реснички.

Hemiophrys salmica sp. nov. (Рис. 6)

Тело продолговатое, сплющенное с боков, сужено и вытянуто спереди, но не образует „шеи"; сзади также сужено, с обоих концов закруглено, несократимое. В поперечном сечении овальное. Размеры 70—90 × 25—35 μ. Отношение длины

к ширине 2 : 1—3 : 1. Щелевидный рот занимает 1/4 длины брюшного ребра, окружен длинными ресничками. Правая сторона покрыта мелкими ресничками, расположенными в 20—22 ряда, левая сторона лишена ресничек и фибриллярных структур.

Эндоплазма мелкозернистая, оветлая, непрозрачная. Трихоцисты отсутствуют. Ядерный аппарат состоит из 2 частей. 8—10 сократительных вакуолей лежат вдоль дорсальной стороны тела.

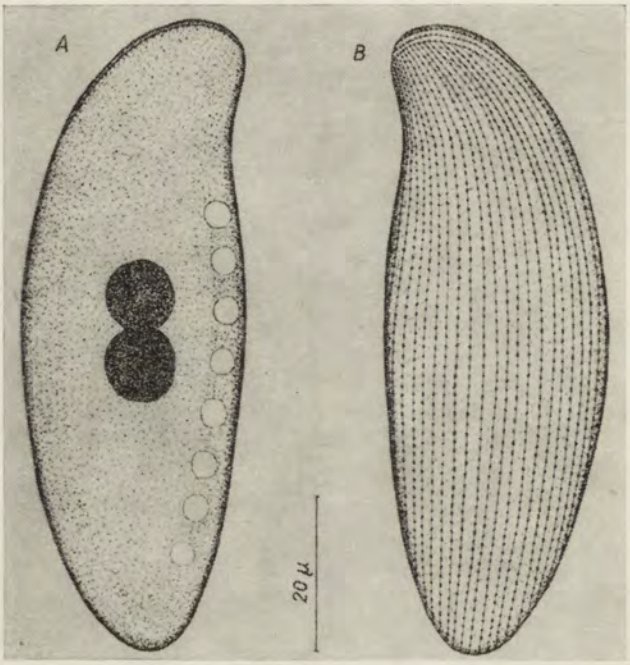

Рис. 6. *Hemiophrys salmica* sp. nov. A, B — общий вид, левая и правая стороны (тотальный препарат, серебрение)

Fig. 6. *Hemiophrys salmica* sp. nov. A, B—general view, left and right sides (whole preparation, silver impregnation)

Встречается в крупнозернистом и среднезернистом песке литорали. Высокой численности достигает в августе-сентябре.

От ближайшего вида *H. marina* Kahl отличается главным образом иной формой тела.

Loxophyllum variabilis (?) Dragesco, 1960 (Рис. 7)

Тело продолговато-овоидное, сильно уплощенное, несколько вытянуто спереди, сзади широко закруглено. Размеры 90—180 × 40—70 μ. Отношение длины к ширине 2 : 1—3 : 1. Щелевидный рот занимает от 1/4 до 1/3 длины тела, окружен длинными ресничками. Правая сторона несёт 30—60 продолных ресничных рядов, левая — 20—30 тонких фибрилл без кинетосом или с редкими кинетосомами.

Эндоплазма мелкозернистая, прозрачная, гетерогенная. Эктоплазма прозрачная, бесцветная, гомогенная. Брюшная и спинная краевые полоски несут параллельно расположенные тонкие трихоцисты. Ядерный аппарат состоит из

4—9 частей. Чаще встречаются формы с 4 ядрами. СВ в середине тела, вблизи спинной стороны.

Широко распространённый в Кандалакшском заливе вид. Встречается преимущественно в крупнозернистом и среднезернистом песках, реже в скоплениях нитчатых водорослей или в детрите.

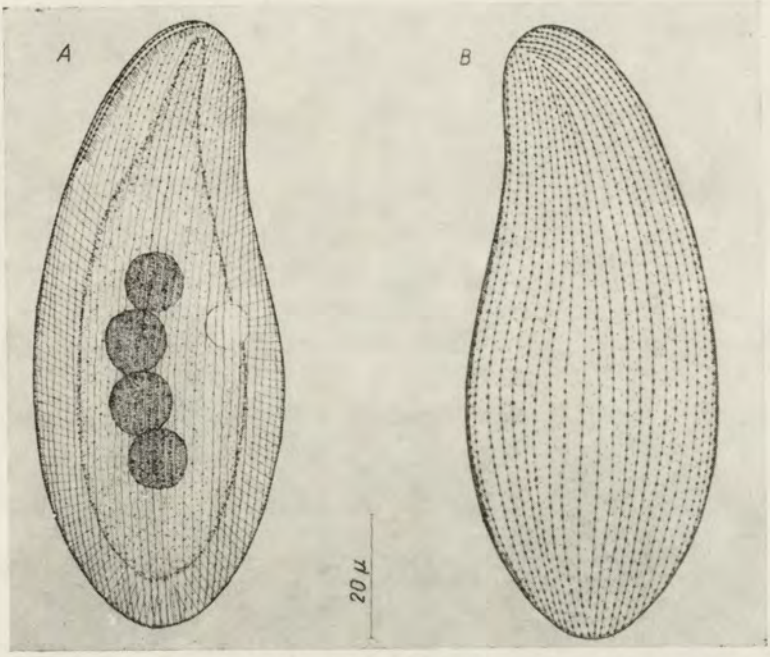

Рис. 7. *Loxophyllum variabilis* (?) Dragesco. A, B — общий вид, левая и правая стороны (тотальный препарат, серебрение)

Fig. 7. *Loxophyllum variabilis* (?) Dragesco. A, B—general view, left and right sides (whole preparation, silver impregnation)

Беломорская форма отличается от формы, описанной Dragesco, главным образом иным расположением и большим числом фибрилл на левой стороне, более вариабильным числом ядер и несколько иной формой тела.

Loxophyllum schewiakoffi sp. nov. (Рис. 8)

Тело листовидное, сильно уплощенное, почти несократимо, спереди сужено и заострено в виде клюва, сзади расширено и широко округлено. Форма тела постоянная. Вентральное ребро гладкое, дорсальное — волнистое, но не несет папилл. Размеры 80—100 × 50—60 μ. Отношение длины к ширине 1.5 : 1. Правая сторона несёт 25—30 характерных рядов ресничек, левая — своеобразно расположенные прерывистые фибриллы.

Эктоплазма плотная, прозрачная, бесцетная, панцировидная. Эндоплазма также бесцветная и прозрачная. Дорсальная и вентрльная краевые полоски несут параллельно расположенные трихоцисты. Ядро только одно. СВ не обнаружена. Движение очень медленное, по субстрату. Инфузория сильно тигмотактична.

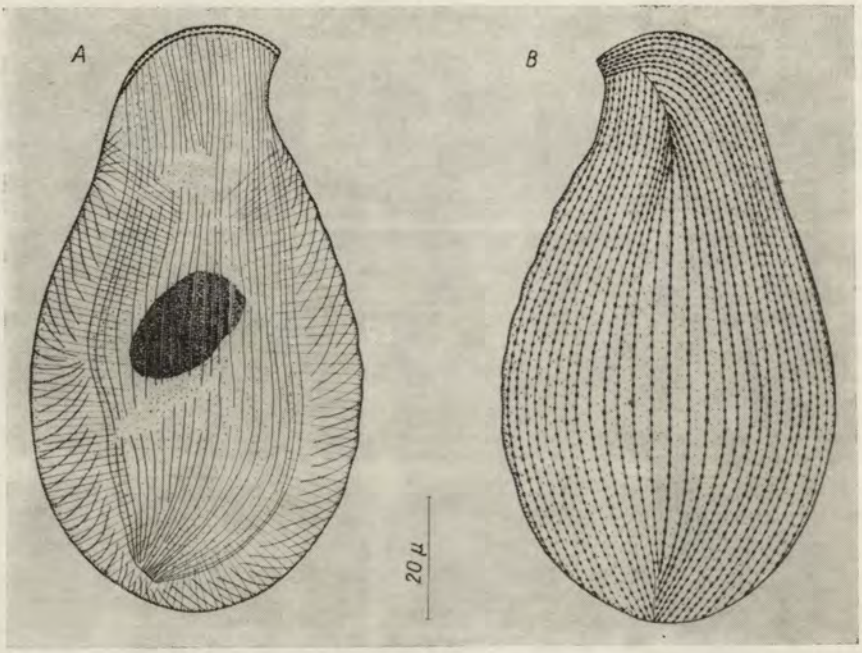

Рис. 8. *Loxophyllum schewiakoffi* sp. nov A, B — общий вид, левая и правая стороны (тотальный препарат, серебрение)

Fig. 8. *Loxophyllum schewiakofi* sp. nov. A, B—general view, left and right sides (whole preparation, silver impregnation)

Очень редкий вид. Обнаружен в среднезернистом песке литорали.

Формой тела настоящий вид напоминает *L. raikovi* Dragesco, но существенно отличается от него размерами тела, строением ядерного аппарата, плотной, почти панцировидной эктоплазмой и рядом других признаков.

Chilodontopsis vorax (?) Stokes, 1887 (Рис. 9)

Тело продолговато-овоидное, асимметричное, слегка уплощенное в дорсовентральном направлении. Левая сторона прямая, правая — выпуклая. Передний конец широкий, задний постепенно сужается и закруглен. Размер 130—160 × × 50—65 μ. Отношение длины тела к ширине 2 : 1—2.5 : 1. Глоточное отверстие в первой пятой тела, овальное или круглое. Глотка коническая (около 30 μ) с 16—18 опорными элементами. Всё тело покрыто ресничками, образующими 50—80 рядов, некоторые из которых сходятся на вентральной стороне. На вентральной стороне, на уровне глоточного отверстия, начинается послеротовой ряд и пересекает всю вентральную плоскость, заканчиваясь справа, несколько ниже середины тела (реже на середине или чуть выше).

Эндоплазма прозрачная, мелкозернистая, бесцветная. Ядерный аппарат состоит из овального Ma (30 × 18 μ) и эллипсоидного Ми (6 × 4 μ).

Встречается в мелкозернистом песке и в детрите.

Настоящая форма существенно отличается от солоноватоводной формы *Ch. vorax* Stokes, переописанной Kahl 1930—1935 и Dragesco 1960 формой и пропорциями тела, а также протяжённостью посторального ряда. Однако, A g a-

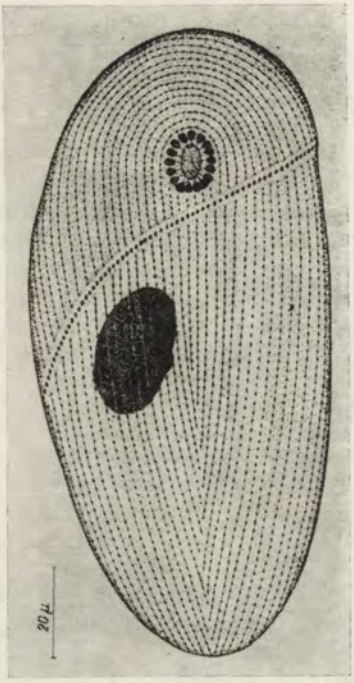

Рис. 9. *Chilodontopsis vorax* (?) Stokes, 1887. Общий вид, вентральная сторона (тотальный препарат, серебрение)

Fig. 9. *Chilodontopsis vorax* (?) stokes, 1887. General view, ventral side (whole preparations, silver impregnation)

maliew 1967 для Каспийского моря приводит в качестве примера форму *Ch. vorax* с длинным посторальным рядом, пересекающим всю вентральную сторону. Беломорская форма близка к каспийской.

Coelosomides vermiformis sp. nov. (Рис. 10)

Тело вытянутое, слегка асимметричное, в сечении круглое, с обоих концов закруглено, червеобразное, слабо сократимое. Размер 350—400 × 50—60 μ. Отношение длины к ширине 7 : 1—8 : 1. Всё тело покрыто ресничками, собранными в 30—36 продольных рядов. Кинетосомы крупные. Рот на переднем полюсе тела. Буккальная полость коротко цилиндрическая, пократа мелкими ресничками, собранными в косые ряды, глотка узкая, изогнутая, трихоцисты и трихиты отсутствуют.

Эндоплазма непрозрачная, содержит огромное количество разнообразных преломляющих свет включений, отчего инфузория выглядит чёрной. Задний конец тела свободен от включений. Ядерный аппарат состоит из продолговатого Ма (70—80 × 18—20 μ) и 3—5 крупных Ми (4 × 6 μ). Множество СВ.

Встречается в крупнозернистом и среднезернистом песках литорали, а также в придонном слое воды.

От ближайшего вида *C. marina* Anigstein (переописан Dragesco 1960) отличается значительно более вытянутой, червеобразной формой тела, продолговатым Ма и присутствием нескольких Ми.

Plagiopyla ovata Kahl, 1931 (Рис. 11)

Тело овальное или овоидное, слегка сплющенное, асимметричное. Размеры 110—130 × 70—90 μ. Отношение длины к ширине 1 : 1.5—1 : 2. Щелевилный рот

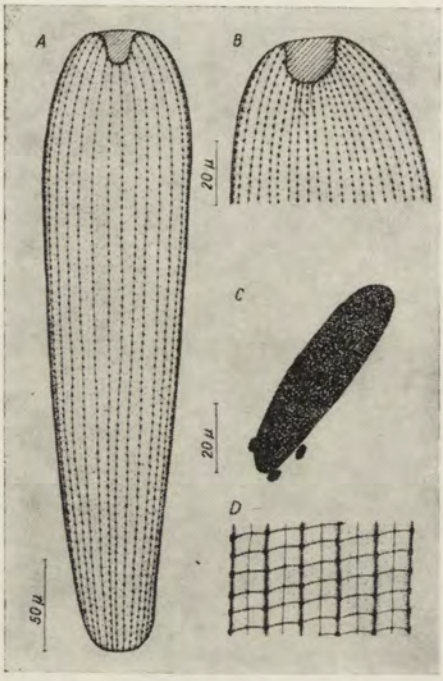

Рис. 10. *Coelosomides vermiformis* sp. nov. A — общий вид (тотальный препарат, серебрение), B — передний конец тела, C — ядерный аппарат (гемалаун), D — фрагмент аргирома

Fig. 10. *Coelosomides vermiformis* sp. nov. A—general view (whole preparation, silver impregnation), B—front end of body, C—nuclear apparatus (haemalum), D—fragment of argyrome

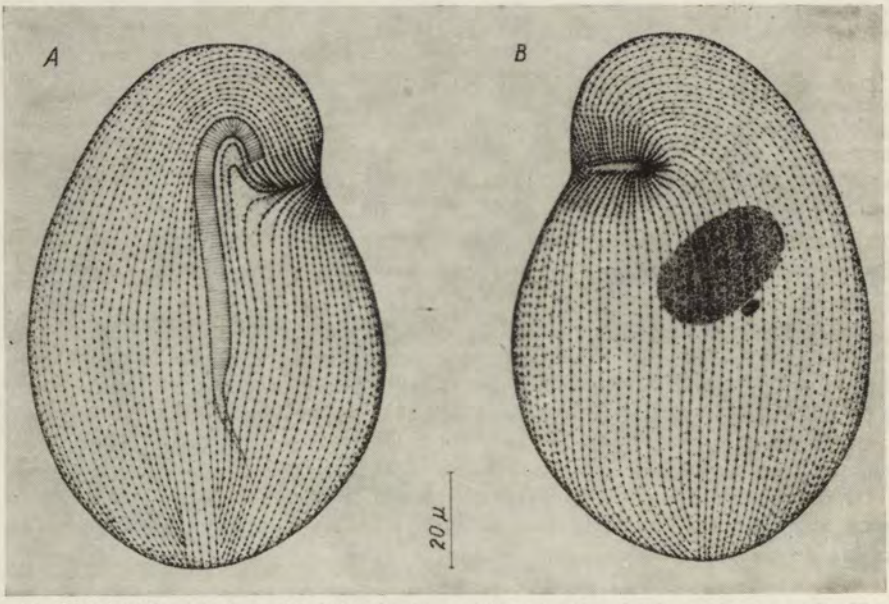

Рис. 11. *Plagiopyla ovata* Kahl, 1931. A, B — общий вид, дорсальная и вентральная стороны (тотальный препарат, серебрение)

Fig. 11. *Plagiopyla ovata* Kahl, 1931. A, B—general view, dorsal and ventral sides (whole preparation, silver impregnation)

в первой четверти тела, окружен многочисленными тесно сближенными ресничками. Глотка короткая, коническая. Большое околоперистомальное поле усеяно многочисленными мелкими ресничками, собранными в тесно прилегающие друг к другу сходящиеся ряды. В глубине перистома, в первой четверти тела, на вентральной стороне — щелевидное ротовое отверстие, ведущее в короткую коническую глотку. На дорсальной стороне к перистомальному полю подходит широкая изогнутая поперечноисчерченная „лента". Всё тело покрыто ресничками, расположенными в 75—85 характерно расположенных рядов. Ход рядов на брюшной стороне и спинной различен.

Эндоплазма мелкозернистая, прозрачная, бесцветная. Ядерный аппарат состоит из овального Ма и сферического или эллипсоидного Ми. СВ терминальная с двумя порами.

Встречается в среднезернистом и крупнозернистом песках литорали, в детрите и в придонной воде, а также в наскальных заплесках.

Беломорская форма идентична форме, описанной Kahl 1931 для Балтийского моря.

Frontonia elongata sp. nov. (Рис. 12)

Тело продолговатое, асимметричное, слегка сократимое. Спереди несколько расширено и округлено, сзади постепенно сужается и также округлено. Вентральная сторона вогнутая, дорсальная выпуклая. Размер 200—250 × 40—50 (сократившаяся 180—200 × 50—60 μ). Отношение длины к ширине 4 : 1—5 : 1. Буккальная полость (15 μ) лежит на расстоянии 30—40 μ от переднего полюса тела. Буккальная цилиатура состоит из 4 вестибулярных кинет, приоральной кинеты и 3 пенникулусов. Фронтальная кинета отсутствует. 2—3 посторальные кинеты. Всё тело покрыто ресничками, собранными в 45—50 рядов, имеющих характерное для рода расположение.

Эндоплазма прозрачная, бесцветная. Ядерный аппарат состоит из одного продолговатого, червеобразного Ма, часто распадающегося на несколько узелков (3—6) и 2—3 Ми. СВ на вентральной стороне, в середине тела.

Встречается в мелкозернистом песке литорали.

Настоящий вид отличается от ближайшего вида *F. microstoma* Kahl, переописанного Roque 1961, Borror 1963 главным образом иной формой тела и присутствием нескольких Ми.

Ophryoglena marina sp. nov. (Рис. 13)

Тело продолговатое, асимметричное, несократимое. Спереди и сзади оно сужено и закруглено. Правая сторона прямая или слегка выпуклая, левая всегда сильно выпуклая. Размеры 180—300 × 50—75 μ. Отношение длины к ширине 1 : 3—1 : 4. Ротовое отверстие помещается в первой четверти тела. „Стекловидное тело" с чёрным пигментным пятном. Буккальная цилиатура имеет типичный для рода состав. Всё тело покрыто мелкими ресничками, расположенными в 160—200 продолных рядов. Кинетосомы мелкие, тесно прилегающие друг к другу.

Эндоплазма сильно вакуолизированная и пигментированная, коричневая, непрозрачная. Сверху над буккальной полостью имеется „стекловидное тело" с чёрным пигментным пятном. Ядерный аппарат представлен продолговатым Ма (около 60 μ) и лежащим рядом крупным Ми (около 6 μ). СВ не обнаружена.

Широко распространённый в Кандалакшском заливе вид. Встречается в песке, в детрите и в придонном слое воды.

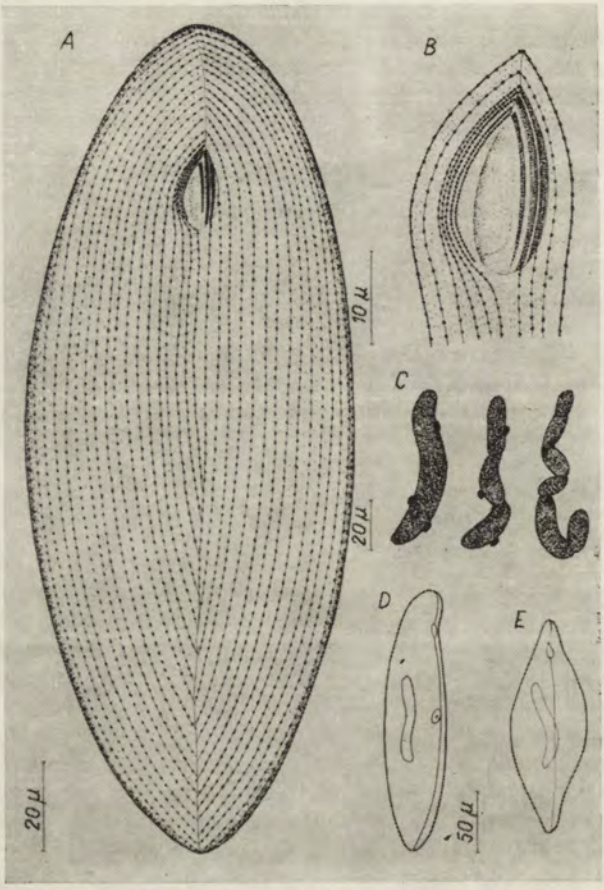

Рис. 12. *Frontonia elongata* sp. nov. A — общий вид, вентральная сторона (тотальный препарат, серебрение), B — цилиатура буккальной полости, C — типи ядерного аппарата (гемалаун), D — типичная форма тела и относительное расположение органелл, E — форма тела фиксации

Fig. 12. *Frontonia elongata* sp. nov. A—general view, ventral side (whole preparation, silver impregnation), B—ciliature of buccal cavity, C—types of nuclear apparatus (haemalum), D—typical shape of body and relative situation of organelles, E—shape of body after fixation

От ближайшего солоноватоводного вида *O. macrostoma* Kahl отличается формой тела и строением ядерного аппарата. Настоящий вид, вероятно, идентичен форме, встреченной К а л е м 1930—1935: стр. 361, рис. на стр. 352 (23), но не выделенной им в самостоятельный вид.

Condylostoma curva sp. nov. (Рис. 14)

Тело продолговатое, асимметричное, спереди расширено и косо срезано, сзади сужено и также срезано. На заднем конце часто имеется выступ. Правая сторона выпуклая, левая вогнутая. Размер 120—200 × 30—40 μ. Перистом большой, составляет не менее 1/3 длины тела. Адоральная зона мембранелл (АЗМ) хорошо развита. Справа от перистома лежит ундулирующая мембрана (УМ). Всё пери-

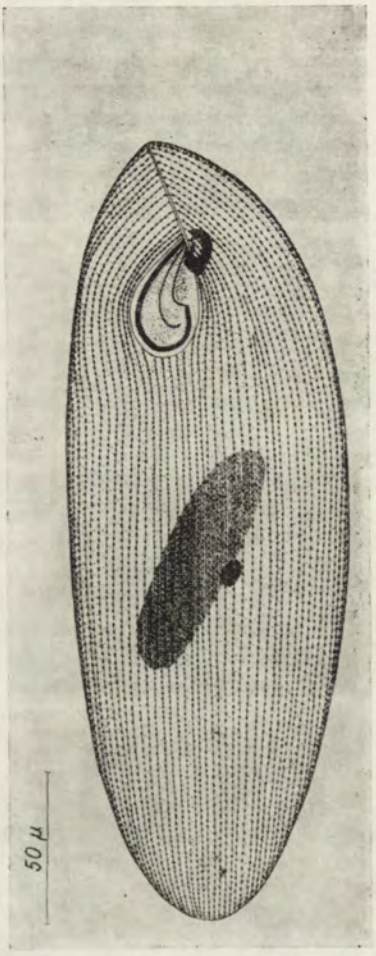

Рис. 13. *Ophryoglena marina* sp. nov. Общий вид, вентральная сторона (тотальный препарат, серебрение)
Fig. 13. *Ophryoglena marina* sp. nov. General view, ventral side (whole preparation, silver impregnation)

стомальное поле покрыто многочисленными ресничками, образующими 20—25 поперечных рядов. Справа от УМ, на вентральной стороне тела, вблизи переднего конца, имеются 5 цирр. Всё тело покрыто ресничками, расположенными в 20—25 рядов.

Эндоплазма мелкозернистая, прозрачная. Ядерный аппарат состоит из 7—9 узелков, образующих вместе длинную цепочку. СВ терминальная.

Этот вид широко распространён в Кандалакшском заливе. Встречается, главным образом, в среднезернистом песке.

Настоящий вид отличается от ближайшего вида *C. arenaria* forma proturostyla Kahl совершенно иной формой тела. От другого близкого вида *C. minima* Dragesco настоящий вид отличается формой тела, присутствием цирр справа от УМ, формой перистома и нередуцированными кинетами на дорсальной стороне.

Trichotaxis multinucleatus sp. nov. (Рис. 15)

Тело продолговатое, закруглено на концах. Передний конец тела вытянут в виде „головы". Максимальная ширина приходится на последнюю треть тела.

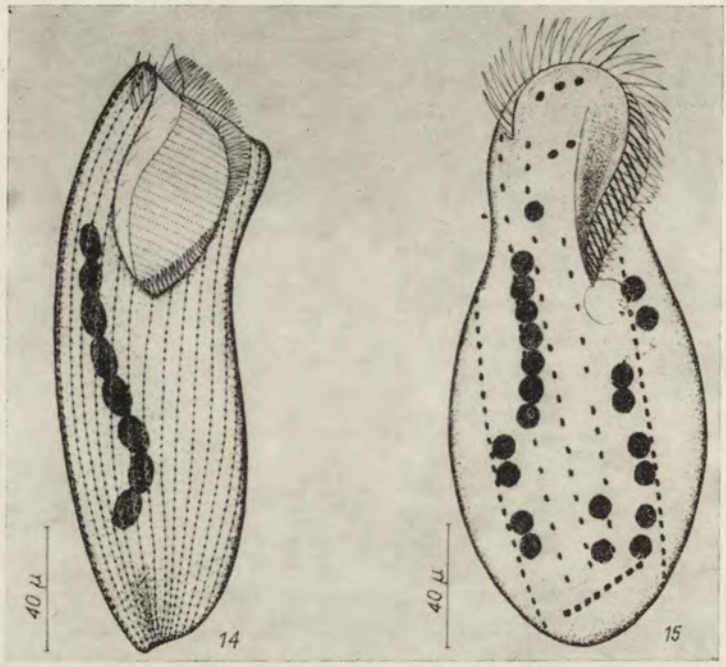

Рис. 14. *Condylostoma curva* sp. nov. Общий вид, вентральная сторона (тотальный препарат, серебрение)
Рис. 15. *Trichotaxis multinucleatum* sp. nov. Общий вид, вентральная сторона (тотальный препарат, серебрение)
Fig. 14. *Condylostoma curva* sp. nov. General view, ventral side (whole preparation, silver impregnation)
Fig. 15. *Trichotaxis multinucleatum* sp. nov. General view, ventral side (whole preparation, silver impregnation)

Размер 120—200 × 40—50 μ. АЗМ хорошо развита, начинается справа на вентральной стороне, огибает передний конец тела и возвращается слева на вентральную сторону, где тянется до конца первой трети тела. Брюшная сторона несёт 2 маргинальных, 3 вентральных и один поперечный ряд цирр. Последний включает 8 цирр. Вентральные цирры крупнее маргинальных, но мельче поперечных. Из 5 фронтальных цирр 3 передние особенно мощные.

Эндоплазма мутная, непрозрачная, мелкозернистая. Ядерный аппарат состоит из большого числа округлых образований (до 30). СВ лежит в середине тела.

Встречается в среднезернистом и крупнозернистом песках литорали.

От ближайшего вида *T. fossicola* Kahl отличается формой тела и составом ядерного аппарата.

Gastrostyla pulchra Perejaslawzewa, 1885 (Рис. 16)

Тело продолговатое, сужено и закруглено на концах. Максимальная ширина приходится на переднюю треть тела. Размеры сильно варьируют, так что можно выделить две различные формы этого вида. Форма А имеет размеры 240—300 × × 50—60 μ. Отношение длины к ширине 4 : 1—5 : 1. Форма В имеет размеры 160—200 × 50—60 μ. Отношение длины к ширине 2.5 : 1—3 : 1. Обе формы встречаются вместе. АЗМ у обеих форм хорошо развита и состоит из 42—50 мем-

бранелл, занимая от 1/3 до 1/4 длины тела. Брюшная сторона несёт 2 маргинальных ряда и один редуцированный вентральный ряд цирр. 5 поперечных цирр имеют характерное расположение, рядом с ними обнаруживаются ещё две цирры. У формы А семь фронтальных цирр, у формы В — шесть фронтальных цирр. На дорсальной стороне 5—6 продольных рядов щетинок.

Эндоплазма зернистая, непрозрачная, содержит разнообразные включения. Ядерный аппарат состоит из 4 Ма и 4 Ми у форм А и из 2 Ма и 4 Ми у форм В. Под эктоплазмой располагаются многочисленные трихоцисты.

Широко распространённый в Кандалакшском заливе вид. Встречается в песках различной зернистости, в детрите и в скоплениях нитчатых водорослей. Неоднократно отмечался также на поверхности непористых субстратов.

Наиболее обстоятельное переописание этого вида дано Borror 1963 a.

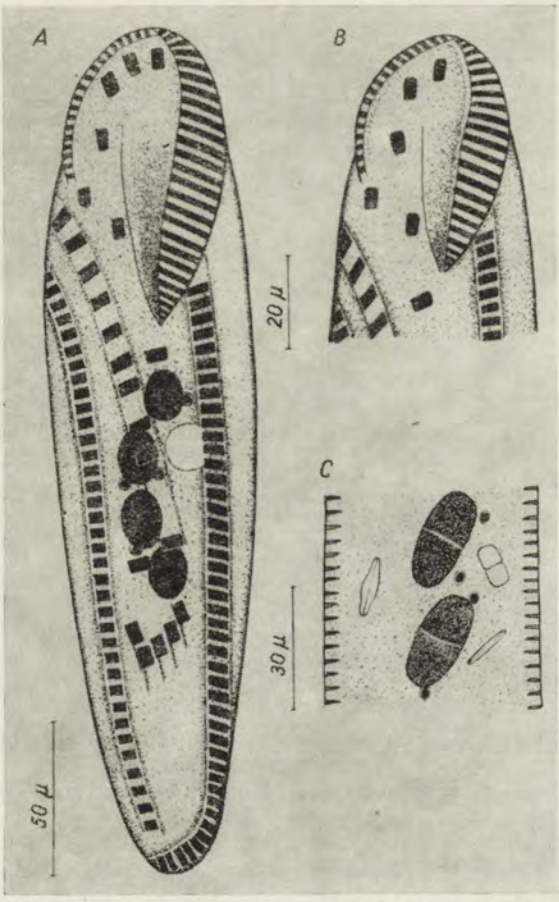

Рис. 16. *Gastrostyla pulchra* Perejaslawzewa, 1885. А — общий вид, вентральная сторона, форма А (тотальный препарат, серебрение), В — передний конец тела, форма В, С — фрагмент тела с ядерным аппаратом (гемалаун), форма В

Fig. 16. *Gastrostyla pulchra* Perejaslawzewa, 1885. A—general view, ventral side, form A (whole preparation, silver impregnation), B—front end of body, form B, C—fragment of body with nuclear apparatus, form B (haemalum)

Описанная им форма идентична беломорской форме B. Сходство распространяется на форму тела, число Ма и фронтальных цирр. (Ми Borror не обнаружил). Различия касаются расположения фронтальных цирр и числа рядов дорсальных щетинок. Беломорская форма A существенно отличается от формы, описанной Borror для атлантического побережья США.

Aspidisca fusca Kahl, 1928 (Рис. 17)

Тело овоидное, сильно уплощенное, асимметричное, несократимое. На концах тело закруглено. Размер 40—60 × 25—30 μ. Слева на уровне перистома имеется большой острый зубец. В передней половине тела 7 одинаковых мембранелл, в задней — 5 поперечных цирр.

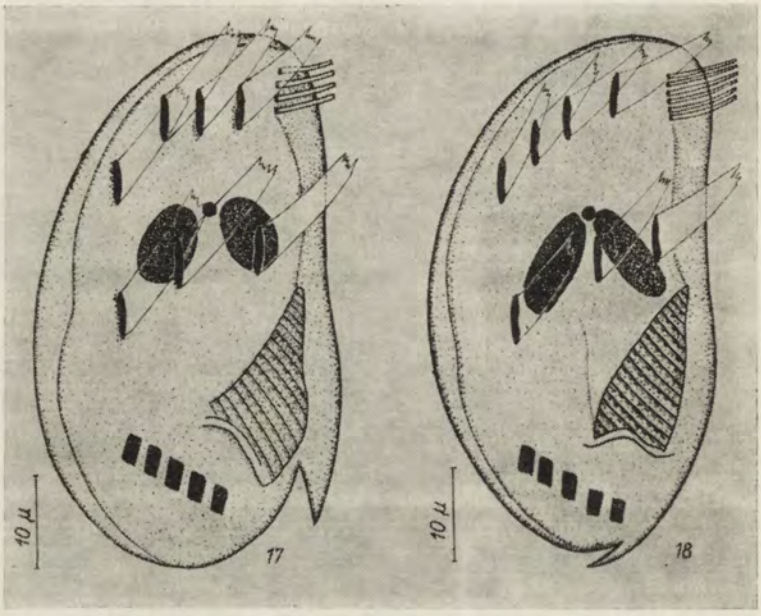

Рис. 17. *Aspidisca fusca* Kahl, 1928. Общий вид, вентральная сторона (тотальный препарат, серебрение)

Рис. 18. *Aspidisca irinae* sp. nov. Общий вид, вентральная сторона (тотальный препарат, серебрение)

Fig. 17. *Aspidisca fusca* Kahl, 1928. General view, ventral side (whole preparation, silver impregnation)

Fig. 18. *Aspidisca irinae* sp. nov. General view, ventral side (whole preparation)

Цитоплазма прозрачная, бесцветная, мелкозернистая, Ядерный аппарат состоит из двух овальных Ма и одного Ми. СВ в задней половине тела.

Широкораспространённый в Кандалакшском заливе вид. Встречается в песках разной зернистости, в детрите и в скоплениях нитчатых водорослей.

Беломорская форма существенно не отличается от форм этого вида, описанных Dragesco 1965, Agamaliev 1967, Kahl 1930—1935.

Aspidisca irinae sp. nov. (Рис. 18)

Тело овальное, сильно уплощенное, асимметричное, несократимое, на концах закруглено. На заднем конце тела, слева, небольшой острый шип. Размер 45—75 × 30—40 μ. АЗМ состоит из двух частей. В передней половине тела 7 одинаковых мембранелл, в задней — 5 поперечных цирр.

Цитоплазма прозрачная, бесцвтная, мелкозернистая. Ядерный аппарат состоит из двух продолговатых Ма и одного Ми. СВ в задней половине тела.

Встречается в мелкозернистом и среднезернистом песках литорали, а также в детрите и в скоплениях нитчатых водорослей.

Настоящий вид несомненно очень близок к *A. fusca* Kahl, но отличается от него формой тела и расположением зубца. Промежуточных форм нами обнаружено не было.

РЕЗЮМЕ

Описано 18 видов инфузорий: *Prorodon moebiusi* Kahl, 1930, *Placus dogieli* sp. n., *Lagynophrya maxima* sp. n., *Lacrymaria conifera* sp. n., *Plagiopogon loricatus* Kahl, 1931, *Hemiophrys salmica* sp. n., *Loxophyllum variabilis* (?) Dragesco, 1960, *L. schewiakoffi* sp. n., *Chilodontopsis vorax* (?) Stokes, 1887, *Coelosomides vermiformis* sp. n., *Plagiopyla ovata* Kahl, 1931, *Frontonia elongata* sp. n., *Ophryoglena marina* sp. n., *Condylostoma curva* sp. n., *Trichotaxis multinucleatus* sp. n., *Gastrostyla pulchra* Perejaslawzewa, 1885, *Aspidisca fusca* Kahl, 1928, *A. irinae* sp. n. найденых в песчаном дне Белого моря. Одиннадцать видов являются новыми.

SUMMARY

Description of eighteen infusorian species discovered in the sandy bottom of the White Sea, founded on studies of living ciliates and fixed material impregnated with silver by the Chatton and Lwoff 1930 method. Eleven species are new.

1. *Prorodon moebiusi* Kahl, 1930 (Fig. 1). Body egg-shaped, 200–300×100–150 μ. 120–160 somatic kineties, circumoral rings of cilia. Macronucleus (Ma), 3–5 micronuclei (Mi). Contractile vacuole (CV) posterior.

2. *Placus dogieli* sp. nov. (Fig. 2). Body oval or ellipsoid, 80–130×40–80 μ, 22–24 (seldom 32) spiralled somatic kineties. Ma worm-shaped, single Mi (?). CV posterior.

3. *Lagynophrya maxima* sp. nov. (Fig. 3). Body elongated, ventral side concave, dorsal side convex. 140–180×30–50 μ. 36–40 somatic kineties. Ma elongated or worm-shaped. CV posterior.

4. *Lacrymaria conifera* sp. nov. (Fig. 4). Body conic, "neck" absent, 50–70×15–20 μ. 18–20 somatic kineties. Endoplasm with many regular granules packed in the two-thirds of the body. Ma elongated, single Mi. CV posterior.

5. *Plagiopogon loricatus* Kahl, 1931 (Fig. 5). Body ellipsoid, 50–70×20–25 μ. 12–16 slightly spiralled somatic kineties. Ma oval, single Mi. CV posterior.

6. *Hemiophrys salmica* sp. nov. (Fig. 6). Body lancet-like, semicircular in cross-section, ends rounded. 70–90×25–35 μ. 20–22 somatic kineties on the right body side. *Trichocysts* (Trc) absent. Nuclear apparatus (Ma ?) consists of 2 oval parts. 8 CV along dorsal side.

7. *Loxophyllum variabilis* (?) Dragesco, 1960 (Fig. 7). Body lancet-like, 90–180×40–70 μ. 30–60 powerful somatic kineties on the right side, 20–30 reduced kineties on the left side. Trc along ventral and dorsal sides. Nuclear apparatus (Ma ?) consists of 4–9 oval parts. CV dorsal, central.

8. *Loxophyllum schewiakoffi* sp. nov. (Fig. 8). Body leaf-shaped, incontractile, 80–100×50–65 μ. 25–30 powerful somatic kineties on the right side fibrils on the left side. Trc along ventral and dorsal sides. Nuclear apparatus (Ma ?) constitutes a single part.

9. *Chilodontopsis vorax* (?) Stokes, 1887 (Fig. 9). Body elongated, 130–160×50–65 μ. Gullet with 16–17 elements. 50–80 somatic kineties. Postoral line crosses the ventral side. Ma oval, single Mi.

10. *Coelosomides vermiformis* sp. nov. (Fig. 10). Body worm-shaped, 350–400× ×50–60 μ. 30–35 somatic kineties. Buccal cavity with fine cilia. Ma elongate, 4–5 Mi.

11. *Plagiopyla ovata* Kahl, 1931 (Fig. 11). Body oval, 110–130×70–90 μ. 75–85 somatic kineties. Ma oval, single Mi. CV posterior.

12. *Frontonia elongata* sp. nov. (Fig. 12). Body elongated, 200–250×40–50 μ. 45–50 somatic kineties. Buccal cavity 15 μ long. Buccal ciliature includes 4 vestibular kineties, a single paroral kinety (undulating membrane) and 3 peniculi. Frontal kinety absent. 2–3 postoral kineties. Ma worm-shaped, 2–3 Mi. CV ventral, central.

13. *Ophryoglena marina* sp. nov. (Fig. 13). Body elongated, 80–300×50–75 μ. "Glass-shaped body" with black pigment spot. 160–200 somatic kineties. Buccal ciliature of typical composition. Ma elongated, with a single Mi.

14. *Condylostoma curva* sp. nov. (Fig. 14). Body elongated, incontractile, left side concave, right side convex. 120–200×30–40 μ. 20–25 somatic kineties, 5 cirri. Buccal cavity with fine cilia. Nuclear apparatus (Ma ?) consists of 7–9 oval parts. CV posterior.

15. *Trichotaxis multinucleatus* sp. nov. (Fig. 15). Body elongated, 120–200×40–50 μ. 2 marginal, 3 ventral rows of cirri, 5 frontal and 8 anal cirri. Nuclear apparatus (Ma ?) consists of 25–30 Oval parts. CV ventral, central.

16. *Gastrostyla pulchra* Perejaslawzewa, 1885 (Fig. 16). Body elongated, 160–300×50–60 μ. AZM with 50–55 membranelles. 2 marginal, a single reduced ventral rows of cirri, 6–7 frontal and 5 anal cirri. Bristles in 5–6 dorsal rows. 2–4 Ma, 4 Mi.

17. *Aspidisca fusca* Kahl, 1928 (Fig. 17). Body oval, left margin with a single tooth. 40–60×20–25 μ. AZM two-parted. 7 frontal and ventral, 5 anal cirri. 2 oval Ma, one Mi.

18. *Aspidisca irinae* sp. nov. (Fig. 18). Body oval, posterior margin with a single tooth. 40–65×20–25 μ. AZM two-parted. 7 frontal and ventral, 5 anal cirri. 2 ellipsoid Ma, one Mi.

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Electron microscopic studies of *Thigmophrya macomae* Ch. et Lw., an arhynchodine thigmotrichid ciliate

L'étude ultrastructurale de *Thigmophrya macomae* Ch. et Lw., un Cilié Arhynchodine Thigmotrichide

During the past decade electron microscope studies covered most of the main groups of *Protozoa*, but the order *Thigmotrichida* was neglected, (Pitelka 1968). Recently Lom and Kozloff 1966 and Lom, Corliss and Noirot-Timothée 1968 have studied three species of the family *Hemispeiridae* and one representative of the family *Ancistrocomidae*. In an attempt to fill the gap, three species have been studied at Svancea, representing the families *Thigmophryidae, Hemispeiridae* and *Ancistrocomidae*. Studies on *Ancistrumina nucellae* Khan, and *Ancistrocoma pelseneeri* Chatton et Lwoff which belong to the two latter families, have already been completed (Khan 1970 a, 1970 c). The present study is on *Thigmophrya macomae*, representing those specialized thigmotrichs which possess a posteriorly situated cytostome and a dense ciliation of the body. Chatton and Lwoff 1926, Raabe 1936 and others who studied this ciliate found no adoral ciliature in their studies. This led them to believe it to be amongst the most primitive members of the order *Thigmotrichida*, as members of the *Hemispeiridae*, the other family in the *Arhynchodina*, possess a well developed adoral ciliature and a reduced general ciliature of the body. Fenchel 1964, found that *Thigmophrya saxicavae* has an adoral zone of membranellae and an "undulating membrane" but these were not well developed. In the present studies of silver impregnated *Thigmophrya macomae* the condition which was described by Fenchel has not been naticed at all, but the fine structural studies agree fairly well with his observations.

This ciliate is found browsing on the gills of *Macoma balthica*, holding to the gill surface by its densely ciliated thigmotactic field. It is elongated, measuring 94–110 μ in length and 40 μ in width. The cytostome is situated postero-ventrally and is surrounded by a funnel-shaped infundibulum, which is very similar to the one in *Paramaecium*. The walls of this carry a continuation of the ciliature of the body. The silver line system is composed of 29–33 kineties on each side of the body, of which six kineties from the left side of the cytostome enter the infundibulum and continue posteriorly on the right

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side. The granules of each kinety are connected by thick argentophilic fibres, thus giving the kinety the appearance of a continuous line from the anterior to the posterior tip of the body. There is also a network of fine argentophilic fibres joining the kineties transversely, (Figs. 1–5).

Materials and methods

Macoma balthica were collected from the sands of Swansea Bay, at about mid-tide level, and were examined for thigmotrichids. Moderate infestations of *Thigmophrya macomae* were found in about 20% of the bivalves examined. The ciliates were isolated and dropped into 3% glutaraldehyde in 0.1 M sodium cacodylate and 0.25 M sucrose solution, buffered to PH 7.2 at 0–5°C, and fixed for one hour. They were then washed in three changes of 0.1 M cacodylate and 0.25 M sucrose solution, for 15 minutes in each grade, and then washed for 24 hours in 0.1 M cacodylate solution alone. The material was then post-fixed in 2% osmium tetroxide solution, buffered to pH 7.2 with veronal acetate at 0–5°C for one hour, after which it was washed briefly in distilled water and then rapidly dehydrated in a series of cold ethanols. It was left for 1 hour each in absolute ethanol and propylene oxide, and embedded in Epon 812. The polymerization was carried out first overnight at room temperature and then at 45°C (in partial vacuum) and 60°C, for 24 hours at each temperature. Ultra thin sections were cut on a Huxley ultramicrotome with glass knives and the sections were stained in lead citrate for 2.5 minutes. Some were double stained, in 2% uranyl acetate for 7 minutes and then in lead citrate for 2.5 minutes. The sections were viewed either on an Akashi Tronoscope TR50 at 50 kv or an A.E.I. EM. 6G, electron microscope at 75 kv. Photographs were taken at original magnifications of ×3 500 to × 50 000.

Electron microscopy

The pellicle is thrown into longitudinal folds, which appear in transverse sections as large finger-like projections. Each kinety lies in a deep trough between adjacent folds (Figs. 6, 8.). The pellicle comprises three distinct membranes, the inner membrane being separated from the two outer membranes by a clear area, This inner membrane is more electron dense than the outer two (Fig. 8). Immediately under it, there are various cytoplasmic inclusions, such as mucocysts, mitochondria and layers of post ciliary sub-pellicular microtubules. The clear material between the membranes extends deeply into the cytoplasm of the thigmotactic region, in the form of long narrow channels. These lie under the kinetosomes and in cross section appear to be double-membraned (Fig. 7). In some of the electron micrographs they have been found to join parasomal sacs, suggesting that they are possibly ducts connected with these (Fig. 7).

In most parts of the body the kineties show a typical tetrahymenid arrangement of the kinetosomes, which lie in pairs forming a zig-zag pattern in each kinety (Figs. 6, 13). Every kinetosome gives off a long cilium, thus making the kinety a "Haplokinety". Associated with the kinetosomes there are microtubular and fibre systems similar to those found in *Tetrahymena*. The

cilia are 12 μ long and show the well known 9+9/2 pattern of fibres. The central fibres pass into a dense circular disc at the level of the body pellicle and end immediately at that point. There is one transverse partition under the base of the central fibres, which may be regarded as demarcating the cilium proximally (Fig. 9). The peripheral fibres under this point become triplets and form the wall of the kinetosome. In the distal portion of each kinetosome these triplets are joined to each other by dense strands from inside and have a typical cart-wheel appearance (Figs. 10, 12).

The kinetosomes of each kinety conform with the classical description of desmodexy, given by Chatton and Lwoff 1935. From the right side of each kinetosome a conspicuous, densely stained, striated fibrous bundle arises and runs anteriorly to join a similar fibrous bundle from the kinetosome immediately in front of it (Figs. 11, 12, 13, 14). These are the kinetodesmata, which thus form a longitudinal line on the right side of and parallel to each kinety (Fig. 14). These fibres seem to be very similar in fine structure to the KF fibres described earlier by Pitelka and various other authors in many other ciliates. They bear cross striations throughout their lengths and gradually taper as they run anteriorly (Fig. 14). Each kinetodesmos starts as a bifurcated fibrous bundle from two anteriorly situated triplet fibres of a kinetosome. The area between the bifurcated end is filled with dense substance, which obscures the fibrous part of the kinetodesmos at the point of its emergence (Figs. 12, 14). Two sets of microtubules arise from each kinetosome. Immediately under the kinetodesmos, a set of 8–12 microtubules arises anteriorly and on the right, then runs longitudinally just under the pellicle, as subpellicular microtubules (Figs. 8, 9) which form a regular layer. From the opposite side, slightly above the base of the kinetosome, another set of 8–10 microtubules ascends curving on the left side of the kinetosome and then runs transversely to form bands which lie beneath the subpellicular microtubules. (Figs. 10, 16), just mentioned. Both the longitudinal and transverse subpellicular microtubules ("post-ciliary" microtubules of Pitelka 1969) are of similar size.

Also associated with the kinetosomes are single microtubules, one of which runs under the kinetosomes along each kinety (Fig. 15). This basal microtubule is not as straight as the other two sets of microtubules connected with the kinetosomes, but is a sinuous structure not showing any obvious connection with the kinetosomes. A careful study shows that there are long narrow channels, possibly extensions of parasomal sacs, aggregated in groups below the pellicular ridges (Figs. 7 and 11).

Large pear-shaped, mucus-ejecting "trichocysts" are seen under the pellicle (Figs. 17, 18) and may be termed 'mucocysts' as they resemble those of *Ancistrocoma pelseneeri* (Khan 1970 a). Their position, however, is similar to that of the mucigenic bodies in *Ancistrocoma* and *Ancistrumina* and contain two types of granular contents. One type is very dense and finely granular, the other coarsely granular, less dense and containing a few large microtubules filled with a fuzzy substance. These tubules run lengthwise within the mucocyst. This latter type seems to be similar to an early stage in the development of trichocysts of *Paramaecium* and *Frontonia* (Yusa 1963, 1965) except that it possesses large microtubules. Both types of mucocysts always lie just under the pellicle and not deeper in the cytoplasm. They are situated, as in other ciliates, on the secondary meridians and their distal ends come into

contact with the two outer pellicular layers. At these points of contact, the two layers seem to fuse into one layer. Usually no clear openings were seen, but a few mucocysts ejecting their mucoid contents were noticed in some electron micrographs. The ejected mucoid substance appears to be less electron-dense and coarsely granular (Figs. 17, 18, 19).

The cytoplasm is vesicular, with some spaces apparently empty, and others fluid filled (Figs. 6, 16, 17). The latter are seen as electron-opaque vesicles, scattered in the cytoplasm among dense areas of free ribosomal and glycogen granules. The endoplasmic reticulum, which is often smooth, forms the boundaries of many of the spaces. The peripheral areas of the body contain some darkly stained membranes in the form of channels which are connected with the parasomal sacs and appear as double membraned vesicles when cut transversely (Figs. 11, 15). Large droplets of phospholipids, with dense material inside, occur in the cytoplasm, all over the body (Figs. 6, 17). Some of them can be seen surrounded by membranes, suggesting that they are inside food vacuoles, parts of which appear as empty spaces (Fig. 6).

Though the food vacuoles are not seen as prominently as in other arhynchodine ciliates, a few large vacuoles containing algal cells have been noticed in some sections (Fig. 26). One of them had formed a large dense residual body (Fig. 26). A few smaller vacuoles containing bacteria were also seen in the posterior region of the body, around the cystostome. Many small osmiophilic vesicles were observed, but no typical golgi vesicles. The mitochondria form the most abundant inclusions of the cytoplasm, as they are seen in large numbers under the pellicle, arranged in rows parallel to the kineties, and in the cytoplasm surrounding the nuclei, the food vacuoles and the contractile vacuole (Figs. 11, 13, 30). They seem to be highly active, as they are often branched or extended. In some of them, a dense, finely granular mass was noticed (Figs. 23, 26). Degenerating mitochondria were also seen frequently.

As is characteristic of *Thigmophrya*, the mouth is situated in a vestibulum, the vestibulum itself occurring about 4/5 of the distance posteriorly on the ventral aspect. In a superficial section through the vestibulum, its shape is oval and in its walls, the somatic kineties continue to run posteriorly (Fig. 21). Anteriorly, the vestibulum becomes narrower and triangular in shape. The mouth is situated in the left corner of the anterior side of the triangle and that area seems to be devoid of general ciliature, except for two kineties on either side of the mouth (Fig. 23).

These kineties described below seem to be like those which Fenchel 1964 termed adoral kineties in *Thigmophrya saxicavae*. The left side of the triangle, which leads to the cytostome, is also devoid of ciliature but shows well developed oral ribs, supported underneath by fine dense fibrous structures, which run posteriorly along the margin of the triangle (Figs. 23, 24) and extend about half way along the posterior part of the vestibule. The right wall of the triangle is ciliated all along its length (Fig. 24).

What may be termed the "adoral kinety" appears in the bare area of the cytostomal wall and runs a very short distance beside the mouth. It is accompanied by a still shorter kinety, like that which was called an undulating membrane by Fenchel 1964. Apart from their shortness, however these kineties are similar to the ordinary somatic kineties (kineties of the general ciliature) some of which continue to run inside the vestibulum. They have similar types of fibrillar and microtubular systems associated with their

kinetosomes (Figs. 22, 23, 24). Each of these kineties is made up of a zig-zag row of paired cilia-bearing kinetosomes and, further more, each kinetosome gives off microtubules from its sides to join the longitudinal and transverse layers under the pellicle.

Close to the mouth, two openings of the contractile vacuole may be seen clearly (Figs. 20, 22). They seem to be the openings of a sac similar to the discharge sac of *Ancistrocoma* and *Ancistrumina*, with walls supported by fine fibrils. The contractile vacuole itself possesses fibrous structures in its wall, (Fig. 26). Around the triangular space of the cytostome, large numbers of elongated canaliculi are seen. These possess thick, electron-dense walls and are similar to some of the channels connected with the parasomal sacs, which lie in the cytoplasm of the thigmotactic region. In one electron micrograph a cluster of such canaliculi is seen around a fluid-filled space (Fig. 27).

The macronucleus is spherical, but may have two deep invaginations of the nuclear membrane on one side (Figs. 26, 28). Close to the two nuclear membranes, there is always an endoplasmic reticulum which forms layers of smooth membranes around it, producing a lamellar appearance (Fig. 25). Nuclear pores penetrate the membranes conspicuously at various places (Fig. 32). The nucleoplasm contains small, spherical, evenly distributed DNA bodies (Figs. 25, 29). The nucleoli are few and very large compared with DNA bodies, between which they are scattered (Figs. 29). They are finely granular, irregular masses of electron-dense material, including spaces which are less densely stained.

The micronucleus is oval and lies close to the macronucleus, but somewhat more posteriorly (Figs. 26, 30). The smooth endoplasmic reticulum surrounds this closely too, giving rise to a multi-membraned structure round the nuclear membranes. At one point a similar lamellar structure extends inside the nuclear membranes. The two nuclear membranes are set more closely together than those of the macronucleus and nuclear pores are not clearly visible (Fig. 30). There are always aggregations of ribosomes around the outer nuclear membrane which are sometimes attached to the nearby endoplasmic reticulum. The nuclear contents consist of small bodies of DNA, often joined loosely to each other and appearing as small intertwined chromosomes, with small gaps among and around them. These are filled with a less dense, coarsely granular matrix, within which a few fibrous structures are also seen occasionally. In the middle of the nucleus, a larger space with scanty granular matrix is seen. What would appear to be spindle microtubules seem to be embedded in the denser chromosomes (Fig. 30).

Discussion

The over all impression derived from studying the ultrastructure of this ciliate is that it is definitely a more primitive thigmotrichid than those chosen to represent the *Hemispeiridae* and *Ancistrocomidae*. The folding of the pellicle is slighter than in these families and the adoral ciliature is simpler than in the *Hemispeiridae*. On the other hand the basal body cilium complex is more elaborate in *Thigmophrya*, resembling the subpellicular lattice-work of tetrahymenid ciliates. These characters seem to be important in this case as they show on one side the affinity of this group of ciliates with the hymeno-

stomes. On the other side the very simple adoral ciliature provides more evidence supporting Raabe's and Fenchel's conclusions (1967, 1964) that the members of the *Thigmophryidae* are more primitive than other forms in the arhynchodine group of thigmotrichid ciliates.

The most prominent fibrillar system in the pellicle of this ciliate is formed by the kinetodesmata, which are also found in *Ancistrum* and *Boveria* (Lom et al. 1968), but lacking in *Ancistrumina* and *Ancistrocoma*, (Khan 1970 a, 1970 b). These were first described from electron-micrographs of *Tetrahymena* by Metz and Westfall 1954, consisting of short, tapering striated fibrils arising from kinetosomes in bundles, each of which extends to the right and anteriorly to join other bundles obeying the rule of desmodexy. They were subsequently found in *Colpidium, Glaucoma* and *Paramaecium* by Pitelka 1961. The kinetodesmata of *Thigmophrya* greatly resemble those of *Tetrahymena, Colpidium, Glaucoma* and *Paramaecium* in origin and extent, except that the two sets of microtubules which arise from each kinetosome, form just two subpellicular layers of microtubules, whereas in the tetrahymenid ciliates there are two further layers making four layers in all (Allen 1967). In *Ancistrumina* and *Ancistrocoma* transverse microtubules are not present, however, in *Ancistrocoma* both the microtubular sets given off by each kinetosome are incorporated into a subpellicular layer, of which all the microtubules run longitudinally (Khan 1970 a). The origin from each kinetosome, of one bunch of fibres and two sets of microtubules going off in three different directions, probably provides each kinetosome and its cilium with a very firm anchorage. The well extended and regularly arranged subpellicular layers of microtubules also provide structural support to the pellicle.

A single sinuous microtubule running under the bases of the kineties is another link with *Tetrahymena*, for that has a similar basal microtubule (Allen 1967). According to Allen, this microtubule may not be merely structural, but may rather function as a communication pathway along each kinety. Various arguments have been put forward for and against such function in ciliates (Pitelka 1969, Roth 1958, Pitelka and Child 1964).

The mucocysts observed here appear similar to the mature mucocysts described in *Tetrahymena* by Tokoyasu and Scherbaum 1965 and Zebrun, Corliss and Lom 1967 and in *Frontonia* by Rouiller and Fauré-Fremiet 1957. At the same time they also resemble the primordia of trichocysts of *Frontonia* and *Paramaecium* (Yusa 1961, 1965), but differ from all above in having large microtubules inside. Allen 1967, was not able to decide whether these bodies are surrounded by two membranes in *Tetrahymena pyriformis*, but could clearly demonstrate one membrane surrounding the vacuole in which these bodies lie. In *Thigmophrya* the two membranes fit closely around the mucocysts and the outer has a wavy structure. This was also noticed in mucocysts of *Tetrahymena rostrata* by Zebrun, Corliss and Lom. The two types of contents in mucocysts of *Thigmophrya* possibly relate to stages in the development of individual mucocysts. The mucigenic bodies of *Tetrahymena* (Allen 1967) and *Ancistrocoma* (Khan 1970 a) are filled by a crystalline substance, which seems to be more electron dense than in this ciliate. In *Thigmophrya*, coarse granular contents probably denote maturing mucocysts within each of which 3–4 large microtubules are arranged longitudinally. These could not be seen clearly amongst the fine granular dense contents which are believed to characterize mature mucocysts.

The canaliculi surrounded by dense thick membranous walls are another characteristic feature of the cytoplasm, though similar tubular structures were noticed in the cytostomal walls of *Blepharisma* (K e n n e d y Jr. 1965). They have been noticed in *Thigmophrya*, immediately under the pellicle and their bounding membranes look very similar to the pellicular membranes. They may perhaps be deep invaginations of the pellicular membranes acting as simple passages for exchange of fluid and other materials with the external medium. Parasomal sacs of the thigmotactic region seem to be connected with another but somewhat similar tubular system, the bounding membranes of which are much thinner.

Although F e n c h e l 1964, was probably justified in regarding this form as having an adoral ciliature, the adoral kineties are in fact no different from those of the general ciliature and certainly not so specialized as the adoral kineties of *Conchophthiridae* and *Hemispeiridae*. The fact that adoral kineties could not be seen in silver impregnated specimens of *Thigmophrya macomae*, whereas they were seen by F e n c h e l in *Thigmophrya saxicavae*, suggests that these kineties may be shorter in the former than in the latter species.

The *Thigmophryidae* would indeed appear to be the most primitive group among the arhynchodine thigmotrichids, as in all other families except *Conchophthiridae* the adoral kineties tend to curve around the cytostome posteriorly (R a a b e 1967), whereas in *Thigmophrya* they are very short and do not curve around the cytostomal groove. Further evidence that these kineties are primitive comes from the ultra-structural details of the associated fibre system and the fact that they are not differentiated to form polykineties and diplokineties (K h a n 1969 b). The shortness of the adoral kinety in *Thigmophrya macomae* is not associated, however, with any specialized trend, as suggested by F e n c h e l 1964. The electron microscopic studies clearly show that these kineties are just as complex as the kineties of the general ciliature.

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Summary

The kineties are formed by a double zig-zag row of kinetosomes, each of which gives off a transverse and a longitudinal set of subpellicular microtubules and also from the side a cross-striated tapering bundle of fibres, the kinetodesmos. Mucocysts are abundant under the pellicle, some with a dense, finely granular contents and others with less dense, coarsely granular contents and a few large microtubules. They are surrounded by two clear membranes.

Large numbers of mitochondria are seen in the thigmotactic region and scattered in the cytoplasm. Some of them contain a large dense body. The vestibulum does not show any well marked fine structural differentiation. The entrance to the cytostome is a wide triangular space with two adoral kineties, but the latter are structurally similar to the kineties of the general ciliature. Oral ribs are well developed in the cytostome. This receives two openings from a contractile vacuole, the walls of which are supported by bundles of fibres. The macronucleus has two deep invaginations of its nuclear membrane and large aggregations of ribosomes were noted in this area. Smooth ER forms a lamellar structure close to the nuclear membrane. The micronucleus contains beaded chromosomes and scanty matrix.

RÉSUMÉ

Les kineties se forment par un double rang en zigzag, des kinetosomes, chacun desquels donne un complexe transversal et longitudinal des microtubules subpellicullaires et aussi du coté une botte de fibres transversalement striées diminuant de largeur, les kinetodesmos. Les mucocystes avec un contenu dense et finement granulaire sont abbondantes sous la pellicule et aussi on trouve des autres avec un contenu moins dense avec des granules plus rudimentaires et quelques larges microtubules. Elles sont entourées par deux membranes clairement visibles. Un grand nombre des mitochondries peut être observé dans la region thigmotactique et eparpillés dans la cytoplasme. Quelques uns d'eux contiennent un corps large et opaque. Dans le vestibulum on ne peut pas discerner une fine differentiation structurelle bien developée. L'entrée au cytosome est une large espace triangulaire avec deux kineties adorales mais les dernières sont structurellement semblables aux kineties de la ciliature génèrale. Les côtes orales sont bien developées dans le cytostome. Cela reçoit deux ouvertures d'une vacuole contractile les parois desquelles sont supportées par des bottes de fibres. Le macronucleus a deux invaginations profondes de sa membrane nucléaire et on a observé des aggregations des ribosomes dans cette région. Le RE lisse forme une structure lamellaire près de la membrane nucleaire. Le macronucleus contient des chromosomes en chapelet et peu de matrix.

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EXPLANATION OF PLATES I–IX

Fig. 1–5. Silver linie system (Chatton and Lwoff's technique) of *Thigmophrya macomae*, consisting of 31–33 kineties on each side of which 6 or 7 of the ventral

side enter the walls of the posteriorly situated vestibulum. The adoral kineties are not visible in these preparations.

Fig. 6. A longitudinal section of the anterior region of the body showing the pellicular folds of thigmotactic field (Pf). The kineties (K) run longitudinally and each consists of a double zig-zag row of cilia in a pellicular groove formed between the adjoining two pellicular folds. There are many mitochondria (M) scattered in the cytoplasm, which also contain large number of small vesicles (V) and of highly electron dense bodies (Pl), which seem to be droplets of phospholipids. Mucocysts (Mc) are also seen in between the kinetosomes. 8000×

Fig. 7. An oblique section through the anterior part of the body showing bunches of double-membraned channels (Psc) just under the pellicle in the cytoplasm in between the kineties. 32 000×

Fig. 8. Almost transverse section of the thigmotactic region showing long pellicular folds (Pf), wavy outer pellicular membranes (large arrow) and a thick dense inner membrane (arrow head) inside these folds. The microtubular systems (small arrows and Smt) associated with the kinetosomes (K) come to lie under the pellicle. The kinetodesmata (Kf) are seen originating from the right side of each kinetosome nearest the observer and adjoining the Smt tubules). 32 000×

Fig. 9. A cilium with its kinetosome seen in L.S. shows a dense granule (small arrow) at the base of the cilium through which the central fibrils pass immediately before they end. Below it there is another transverse partition (arrow head), which marks the beginning of the kinetosome. The central space of the kinetosome is almost empty except for its base (double arrow) where dense patches fill up the space. The kinetodesmal fibre (Kf) and the subpellicular microtubules (large arrow) arise from the same side of the kinetosome. 64 000×

Fig. 10. An oblique section cutting the kinety at different levels, shows transverse sections of the kinetosomes empty inside except at the base (arrow), where dense material from each triplet converges in the centre to form a cart-wheel structure. The kinety is sandwiched between two mitochondria (M) and here its kinetosomes form a single row, not a double zig-zag one. 64 000×

Fig. 11. Three kineties sectioned obliquely, show the origins of the kinetodesmal fibres (Kf) and the transverse subpellicular microtubules (arrow heads). Due to different orientations of the kinetosomes the origin of Kf fibres from the top kinetosome is seen clearly but the corresponding kinetosomes of the middle and lower kinety show part of it. The subpellicular microtubules (small arrow) are seen arising from the anterior side of each kinetosome. 32 000×

Fig. 12. Another oblique but superficial section shows how broad the Kf fibre bundles are, where they arise from kinetosomes. Large number of thin-walled channels are seen between the kineties and a thick membraned canaliculus (arrow head) with a dense lumen is seen close to the pellicular membrane. The cart-wheel structure of the base of a kinetosome (arrow) is also shown. 32 000×

Fig. 13. Kinetosomes of four kineties (K) show (from left to right) the bundles of kinetodesmal fibres (Kf) being cut at different levels in each kinety so that only the left two show the fibre bundles complete. The cross striations on these fibres may be distinguished with difficulty. The arrow heads show the origin of the transverse microtubules. A parasomal sac (Psc) is also seen adjecent to the tip of the kinetosome. 32 000×

Fig. 14. The Kf fibre bundle of Fig. 13 is magnified to show the cross striations (arrows and arrow heads). 72 000×

Fig. 15. A section passing through the area just below the kinetosomes showing a single sinuous basal microtubule (Bmt) under each kinety. A thick membraned canaliculus (arrow head) lies close to the basal microtubule. 40 000×

Fig. 16–17. Near the pellicle the transverse subpellicular microtubules are seen (arrows and arrow heads) close to an elongated mitochondrion (M). The longitudinal subpellicular microtubules (small arrows) are seen forming a dense zone between the inner pellicular membrane and the transverse microtubules. Fig. 17 also shows a mucocyst (Mc) with dense finely granular contents surrounded by clear double membrane (arrow head) and large droplets of phospholipids (Pl) above which the smooth ER makes a stack. 32 000× and 40 000×

Fig. 18–19. Two types of mucocysts (Mc) are seen lying immediately under the pellicle and in the adjacent cytoplasm, some with coarsely granular less dense contents and 4–5 large microtubules (arrow) and others with finely granular dense

contents. Both the types are surrounded by a double membrane (arrow head). 32 000× and 80 000×

Fig. 20–22. At the anterior margin of the cytostome (Cyt) two structures like discharge sacs (Ds1, Ds2) open into the cytostome (large arrows) presumably to drain the large contractile vacuole (Cv). The walls of the contractile vacuole and the discharge sacs contain bundles of fibres (small arrows). 32 000 and 32 000×

Fig. 21. A longitudinal section passing superficially through the vestibulum (Vtm) shows the kineties of the general ciliature in its walls. 3500×

Fig. 23–24. The triangular vestibulum which leads into a broader cytostome (Cyt) possesses a bare anterior and left marginal wall where the two adoral kineties (Adk) and the oral ribs (Or) are present. The oral ribs are supported from inside by a bundle of fibres (small arrows). Anterior to the base of the triangle large numbers of mitochondria are present in the cytoplasm, with rough ER (large arrow head) between them. Figure 23 also shows a mitochondrion (large arrow) containing a large dense body. 10 000× and 25 000×

Fig. 25. Part of the nuclear membrane of macronucleus (Mac) closely invested by smooth ER (arrows) giving it a lamellar appearance. 40 000×

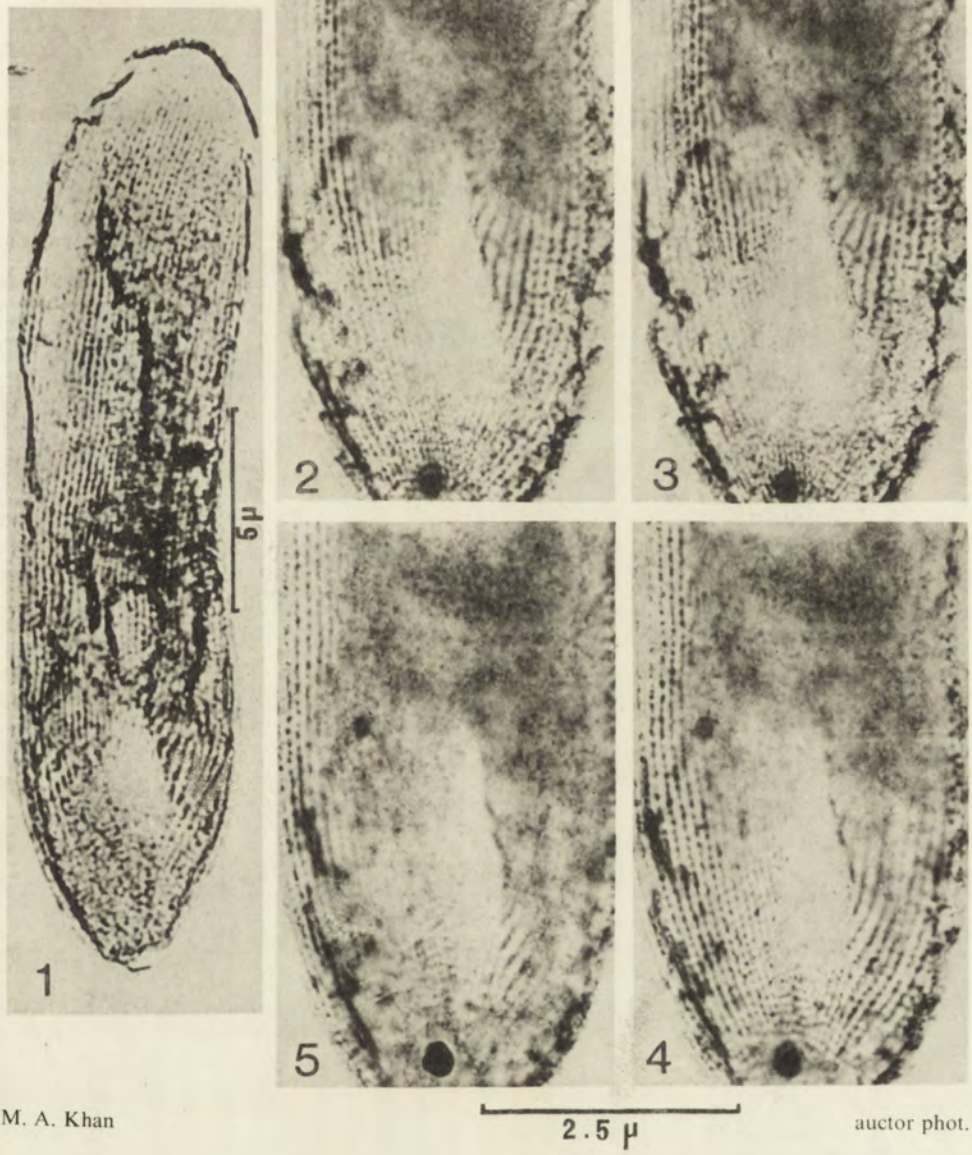
Fig. 26. A low power electron micrograph showing the macronucleus (Mac) with two deep invaginations on one side (arrows) and an oval micronucleus (Mic), an algal cell (A) and also a residual body (Rb) close to the macronucleus. The arrow heads show the dense inclusions in mitochondria. 3500×

Fig. 27. Close to the walls of the cytostome, dense canalicules (Cl) form a cluster around a fluid-filled space. 40 000×

Fig. 28. The nuclear membrane of the invaginated area between the two parts of the macronucleus contains large aggregations of free ribosomes and rough ER. 20 000×

Fig. 29. The macronucleus (Mac) contains a few less dense nucleoli (N), which are larger than dense DNA bodies (DNA) and coarsely granular. 32 000×

Fig. 30. The micronucleus shows beaded dense chromosomes (Chr) spread in a granular matrix (Mx). Close to the nuclear membranes, one or two more membranes give a lamellar appearance (arrow head). The spindle microtubules are seen embedded in the beaded chromosomes (small arrows). 24 000×



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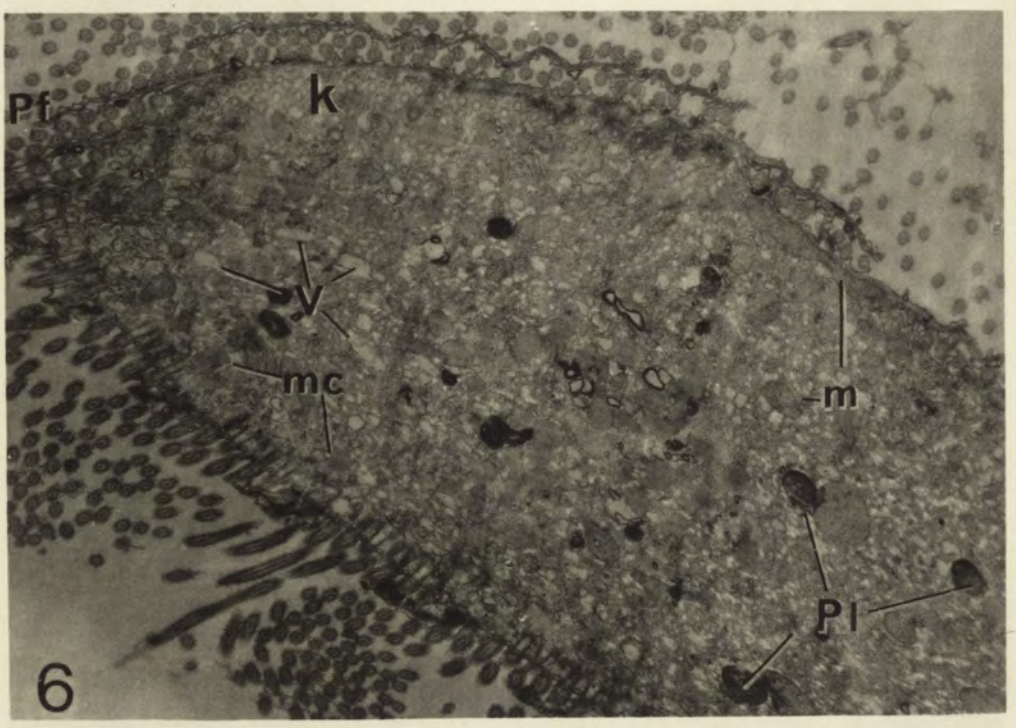

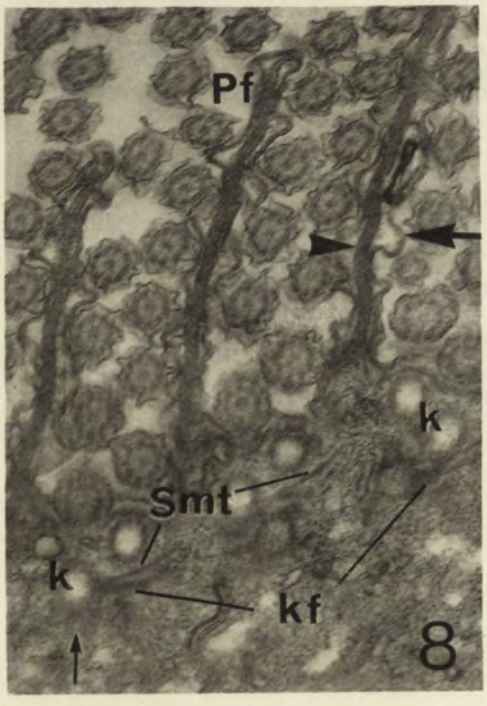

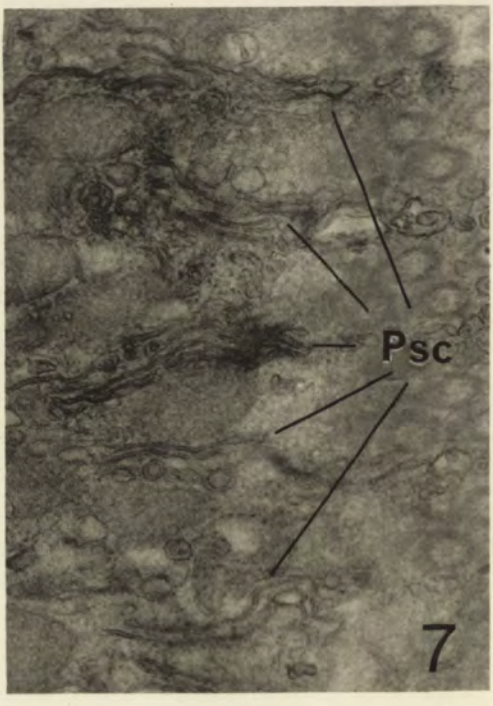

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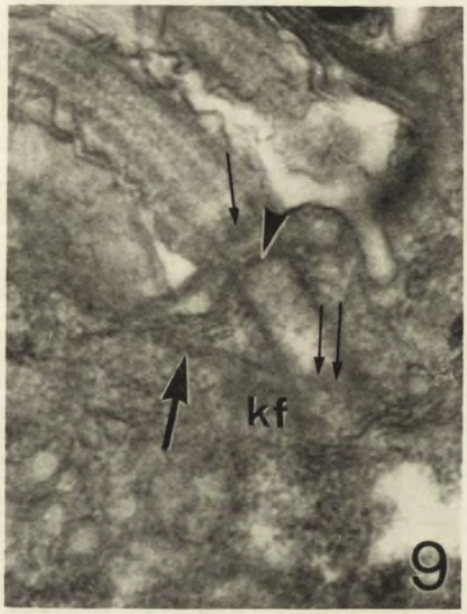

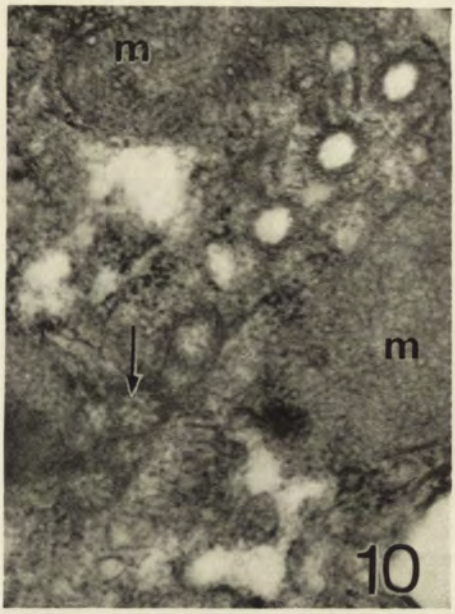

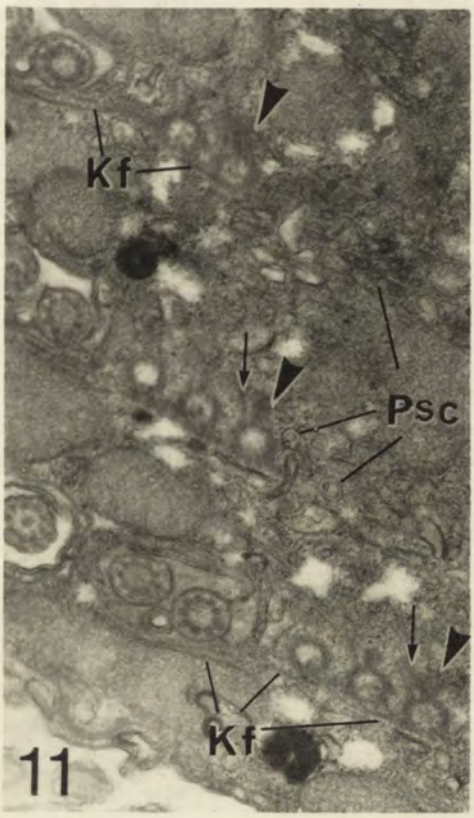

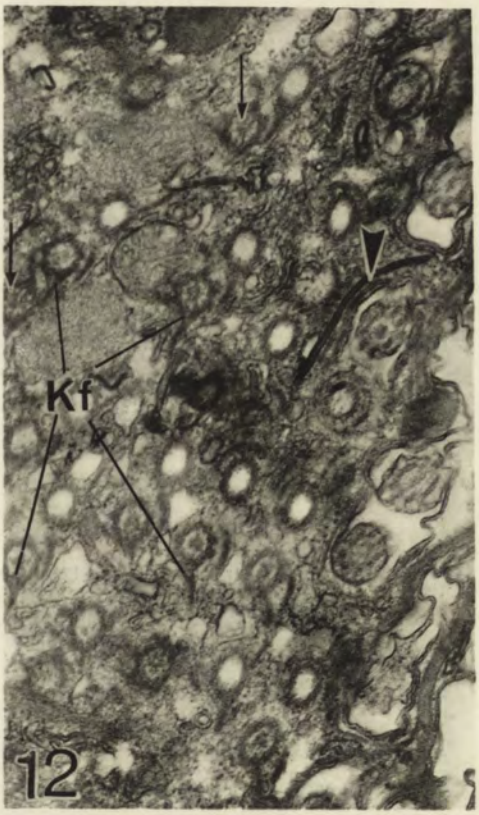

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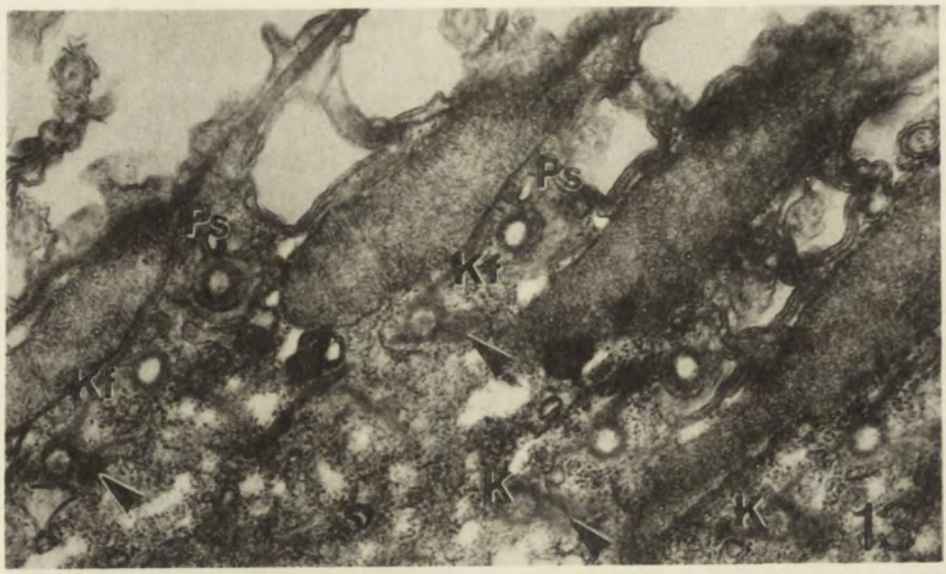

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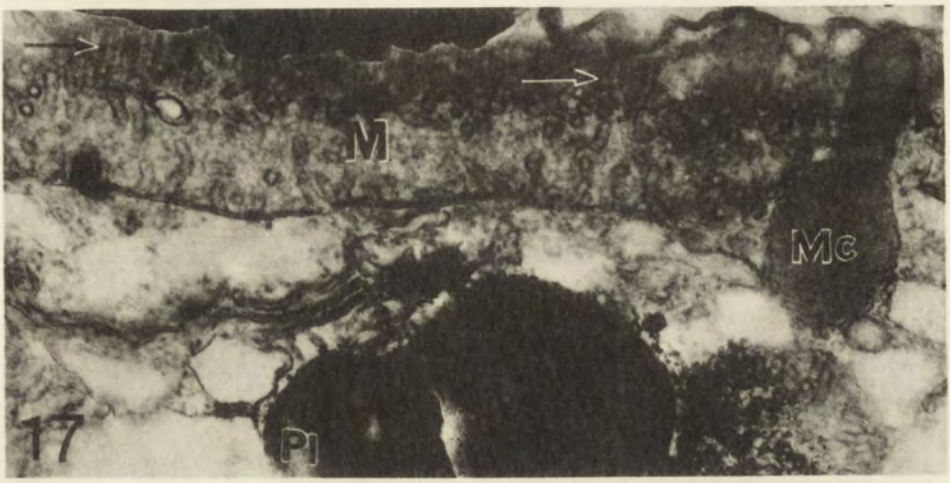

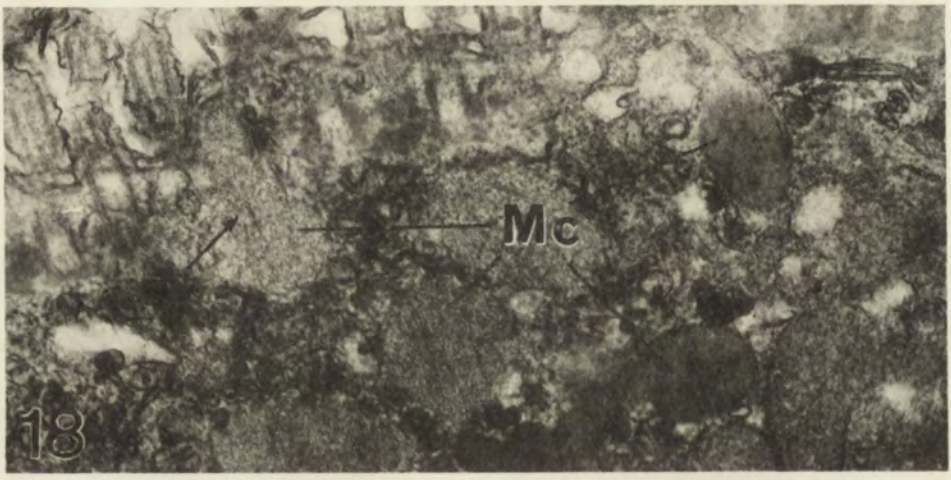

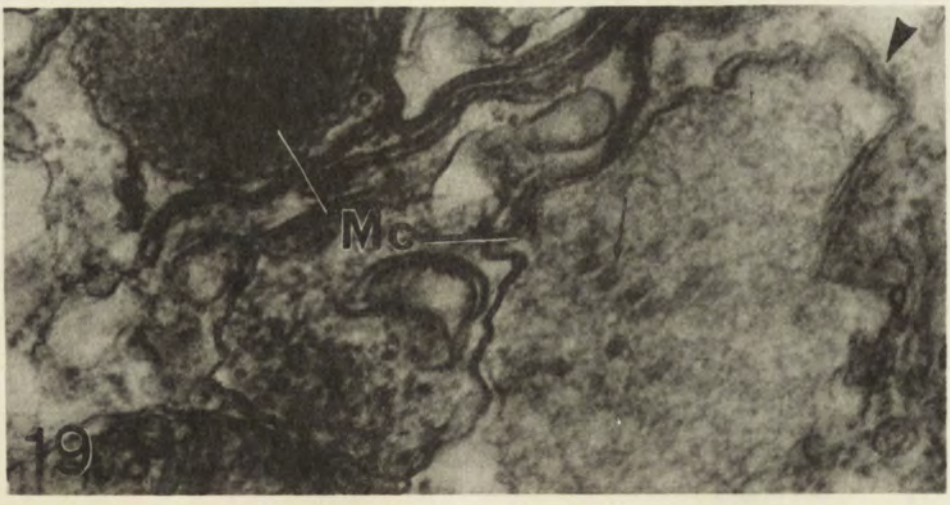

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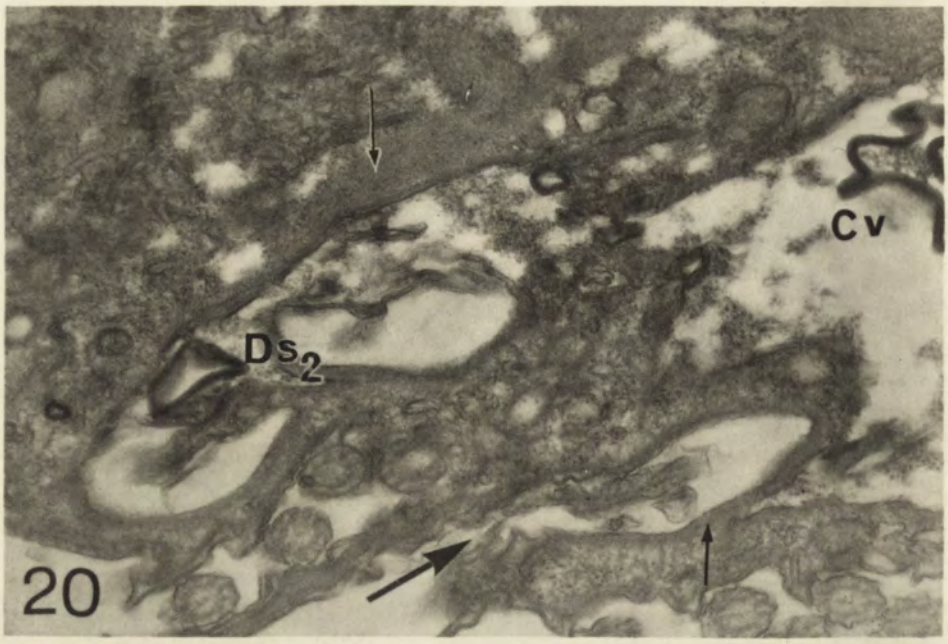

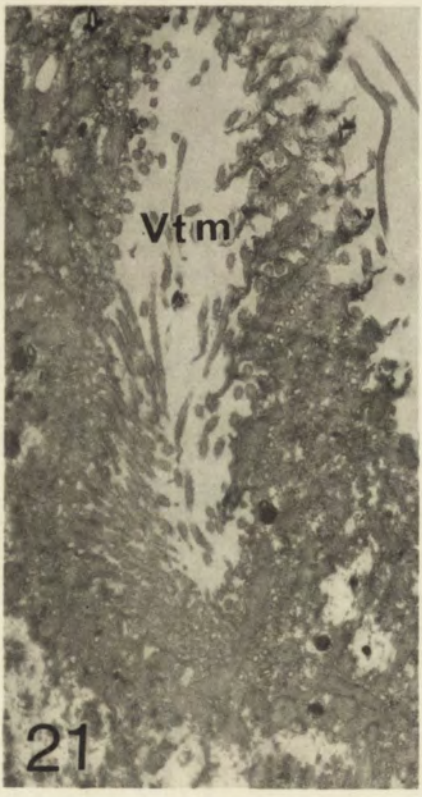

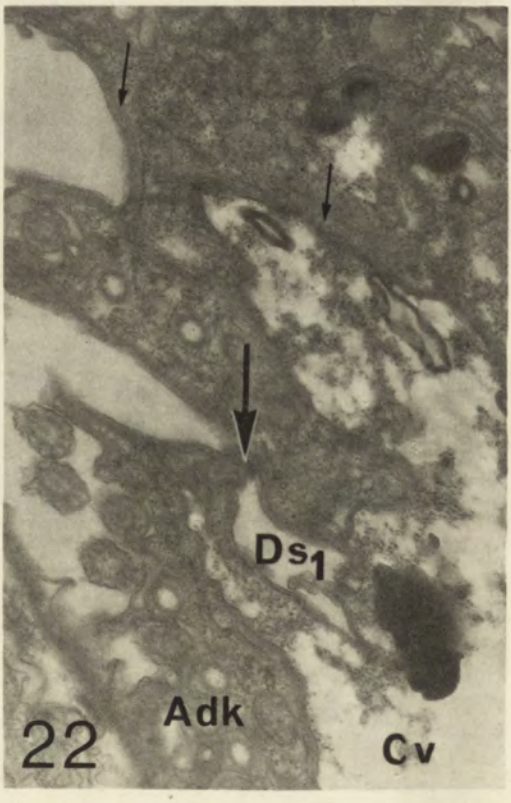

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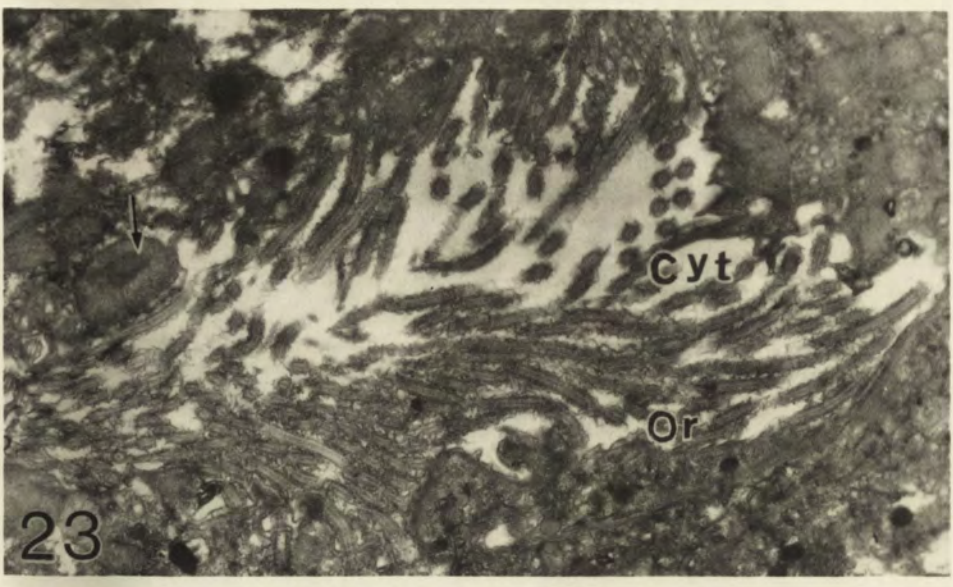

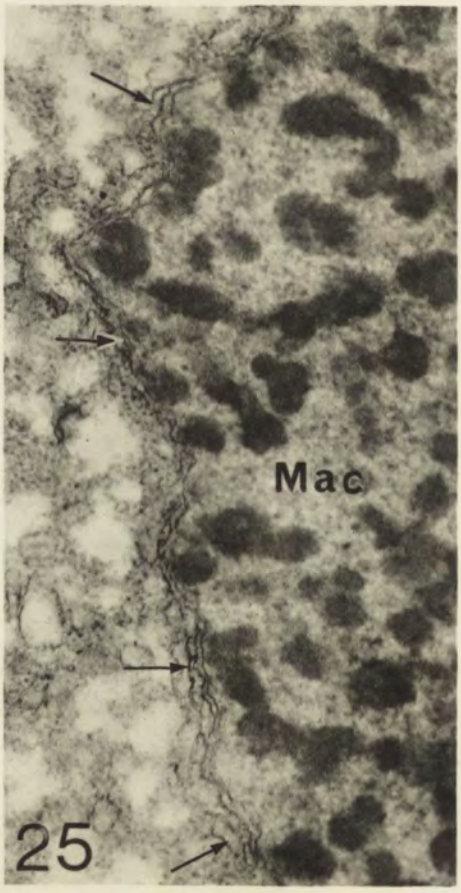

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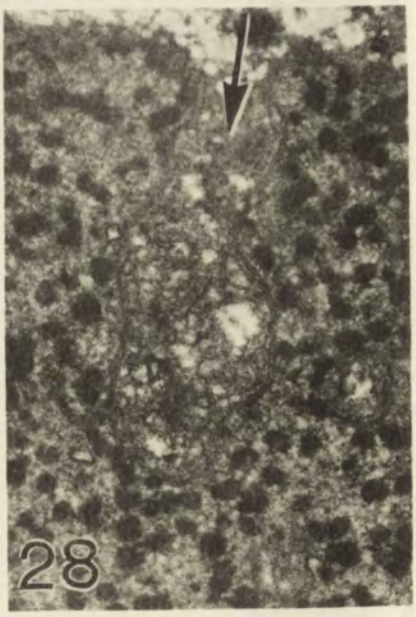

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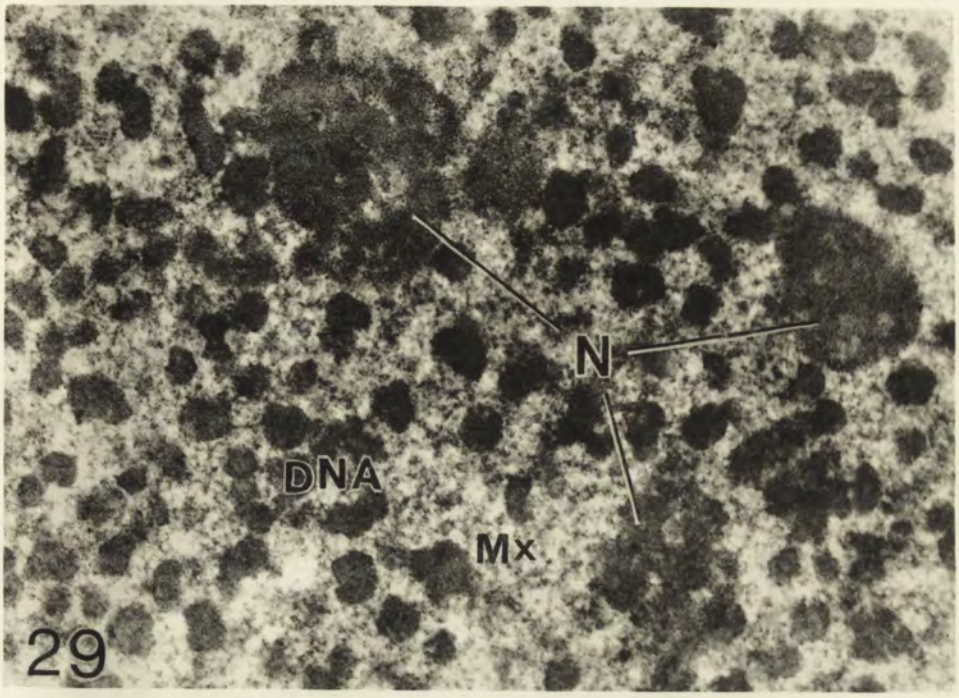

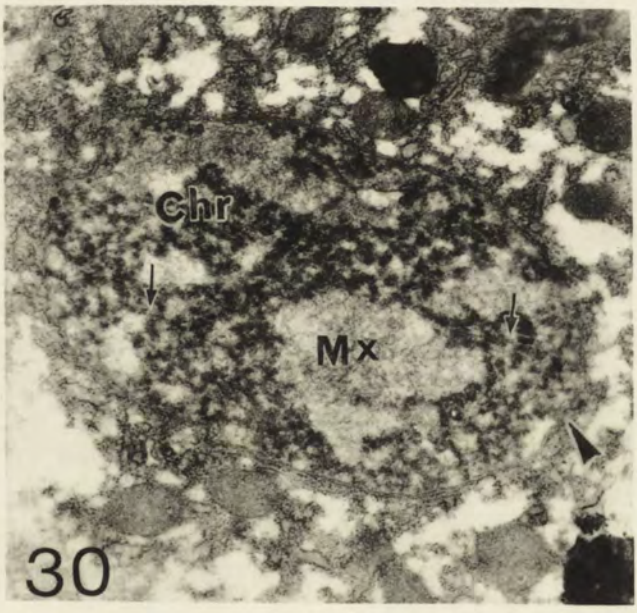

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Иммуногенность ереванского штамма *Trypanosoma lewisi*

Immunological properties of armenian stock *Trypanosoma lewisi*

Широко распространенный кровопаразит *T. lewisi* привлекает внимание исследователей как хорошая модель для изучения иммунитета при трипаносомозах (Эпштейн 1931, Sanders and Wallace 1966). Наши предыдущие эксперименты показали, что данный трипаносомоз у молодых белых крыс продолжается около трех месяцев, после чего в их крови паразиты исчезают и они становятся не восприимчивыми к новым заражениям (Хачоян 1967).

В настоящей работе изучались иммуногенные свойства штамма *T. lewisi*, выделенного в городе Ереване. Штамм длительное время сохранялся в среде NNP (Packchanian 1959), вследствие чего он потерял одно из основных свойств паразита — способность развиваться в организме животного. Для получения трипаносомного антигена штамм выращивали в мясопептонном бульоне с добавлением 10% дефибринированной, инактивированной кроличьей крови. После 5-6 дней выращивания в посевах проверялось наличие роста и чистота культуры. При отсутствии посторонней микрофлоры, паразиты отделялись от среды и концентрировались методом флотации (Хачоян и Налбандян 1967). Полученный концентрат отмывался трехкратно физиологическим раствором и число паразитов доводилось до 500 тыс. особей в 1 мм^3 (Petana 1963). Полученная взвесь использовалась в качестве антигена. При определении биуретовым способом взвесь содержала в 1 мл 3.85 мг. белка. Значительное число паразитов в взвеси были подвижными, живыми. Для иммунизации использовались 3 беспородных кролика весом 3—3.5 кг. Во время иммунизации кролики получали разнообразную, богатую витаминами пищу. Антиген вводился кроликам внутривенно пятикратно. Первый раз 0.5 мл, а остальные по 1 мл через день. После последней инъекции на 8-ые сутки из сердца кроликов получали кровь и отделяли сыворотку обычным способом. Для реакции агглютинации полученную сыворотку разводили физиологическим раствором до 1 : 3200, а трипаносомный антиген 1 : 20. В качестве контроля брали сыворотку нормальных кроликов.

Опыты показали, что реакции агглютинации с сывороткой иммунных животных положительна и резко положительна в разведениях I/1600, тогда как у контрольных кроликов она отрицательна (Таблица 1).

При микроскопии агглютината хорошо видно, как трипаносомы в разведенных иммунных сыворотках собираются группами или склеиваются жгутиками, но остаются живыми, подвижными, то есть наблюдается феномен агглюмерации. Контрольная реакция агглютинации с антигеном *T. cruzi* была или отрицательна или положительная в малых разведениях (до 1 : 25—1 : 50). Небезынтересно отметить, что добавление этих имунных сывороток к питательным средам не препятствовало бурному размножению *T. lewisi*. В дальнейшем полу-

Таблица 1

Table 1

Реакция агглютинации сыворотки кролика No 46 с трипаносомными антигенами
Agglutination reaction of serum from rabbit No. 46 against *Trypanosoma* antigen

Антигены (1:20) Antigens (1:20)	Степень разведения сыворотки Dilution of serum								Контроль антигена Control of antigen	Контроль сыворотки Control of serum
	1:25	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200		
T. lewisi	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++	—	—
T. cruzi	++ +	++	—	—	—	—	—	—	—	—

ченную сыворотку инактивировали при температуре +56° в течение 30 мин. и испытывали в реакции связывания комплемента. Трипаносомный антиген применяли в рабочей дозе 1 : 80 в виду выраженной антикомплементарности. Комплементом служила свежая сыворотка морских свинок, гемолизин использовали в рабочей дозе (1 : 400). Эритроциты получали от барана обычным способом и в реакции применяли 3%-ую взвесь.

Реакция связывания комплемента, которая ставилась в общем объеме 2.5 мл со специфическим трипаносомным антигеном, дала положительные и резко положительные результаты в разведениях до 1 : 800 при отрицательных контролях. (Таблица 2).

Эти же иммунные сыворотки с другими неспецифическими трипаносомными антигенами (*T. cruzi*, *T. mesnili*) дали положительные результаты только в разведениях до 1 : 100. На основании этого мы пришли к выводу, что местный штамм *T. lewisi*, несмотря на потерю способности развиваться в организме животного, обладает такими же хорошо выраженными антигенными свойствами как и до помещения в культуру, и попадание этого антигена парэнтерально в организм кролика, вызывает выработку высокоспецифичных антител, которые проявляют себя очень активно как в реакции агглютинации, так и в реакции связывания комплемента. Добавление этих иммунных сывороток к питательной среде даже в количестве 10% не препятствует бурному размножению трипаносом, но последние по сравнению с трипаносомами, выращенными в нормальных условиях, малоподвижны, расположены группами, склеены

Таблица 2
Table 2

Сыворотка кролика No 46 с трипаносомными антигенами в РСК
Complement fixation test with serum from rabbit No. 46 and trypanosoma antigens in CFT

Антигены (1:80) Antigens (1:80)	Степень разведения сыворотки Dilution of serum						Контроль антигена Control of antigen	Контроль сыворотки Control of serum
	1:50	1:100	1:200	1:400	1:800	1:1000		
T. lewisi	++ ++	++ ++	++ ++	++ ++	++ ++	++	–	–
T. cruzi	++ ++	++	–	–	–	–	–	–
T. mesnili	++ ++	++ +	–	–	–	–	–	–

жгутиками. При пересевах на привычные среды они снова становятся подвижными и не отличаются от обычных культур.

РЕЗЮМЕ

Изучались иммуногенные свойства штамма *Trypanosoma lewisi*, выделенного в городе Ереване. Штамм вследствие длительного культивирования потерял одно из основных свойств паразита — способность развиваться в организме животного. Однако в условиях культуры он сохранил свои прежние антигенные свойства и при иммунизации кроликов вызывал образование высокоспецифичных антител, которые проявляли себя очень активно как в реакции агглютинации, так и в реакции связывания комплемента. Добавление этих иммунных сывороток к питательной среде, даже в количестве 10%, не препятствовало бурному размножению трипаносом, но последние были малоподвижны и склеены жгутиками.

SUMMARY

The immunogenic properties of *Trypanosoma lewisi* isolated in Yerevan (Armenian SSR) were studied. After a long time of cultivation, this strain lost one of its main properties i.e. the ability to develop in the host organisms but retained the immunogenecity and former antigenic properties.

Sera obtained from rabbits immunized with *Trypanosoma lewisi* possess antibodies of high specificity as it was shown by means of agglutination test and complement fixation test.

When 10% of immune sera was added to the nutritional medium, the multiplication of *Trypanosoma* was not inhibited and the organisms were immobilized because their flagella were sticking together.

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Mechanisms of the motor responses of *Paramecium*

Mechanizmy reakcji ruchowych *Paramecium*

The changes of movement character are the fundamental—and in many cases the only one—protozoa response to stimuli which may be analysed qualitatively and quantitatively (Mitchison 1961, Jahn and Bovee 1967). Since the time of the pioneer works of Jennings 1897, 1899 ciliates of *Paramecium* species of the *aurelia* group belong to the objects which have been most intensely investigated from this point of view. This preference is explainable by a number of reasons. Paramecia are organisms suitable for the study of many problems of a general biological significance. As to their structure and function ciliates are single cells which give ground to analyses comparative with the metazoa cells (Pitelka 1963, Jahn and Bovee 1967). The study on protozoa material evokes no objection which are put often forward to the studies on metazoa cells that they concern only a part of organism isolated from the whole by the experimental procedure. *Paramecium* remains independent organism keeping simultaneously the structure and function of a single cell.

In culture and in many experimental media, paramecia of the "aurelia" group swim forwards spiralling left i.e. anticlockwisely (Bullington 1925, 1930, Kuźnicki 1966 a, Grębecki et al. 1967 a). This type of movement (Fig. 1), arbitrarily called "normal", has been proposed to be marked FLS (forward left spiralling) in contrast to FRS (forward right spiralling), (Dryl and Grębecki 1966). Forward movement with right rotation (Fig. 1) appears as a mass phenomenon, in the first place in the conditions of hindered work of the ciliary apparatus evoked by different factors: rise of medium viscosity (Alverdes 1922, Ludwig and Schlicksupp 1951, Grębecki et al. 1967 a), homologous antiserum (Kuźnicki and Sikora 1966) nickel ions (Párducz 1961, Grębecki et al. 1967 b).

When paramecium swimming forwards meets a mechanical obstacle, chemical or mechanical stimuli or others, it responses by avoiding reaction in most cases. This phenomenon has been described by Jennings 1897, 1899, 1906 and analyzed by Párducz 1956, 1959, 1967 as consisting of: 1. rapid withdrawal followed by a shortlasting swimming with the posterior body end forwards, 2. a stop accompanied by rotation movement of the anterior body part, 3. a return to the movement with the anterior body part forwards under a certain angel to the course of the covered paths (Fig. 2).

Withdrawal of the ciliate is involved by a synchronic (Kinosita et al.

1965, Párducz 1967, Kuźnicki et al. 1969 a) change of the cilia position on the whole body surface except cytopharynx. If cilia start their work again in the now position *Paramecium* continues swimming spiralling in the same direction as at FLS. This movement of the ciliate with its posterior body end forwards is traditionally determined in literature as "ciliary reversal". This seems not to be sufficiently precise as for the present state of knowledge.

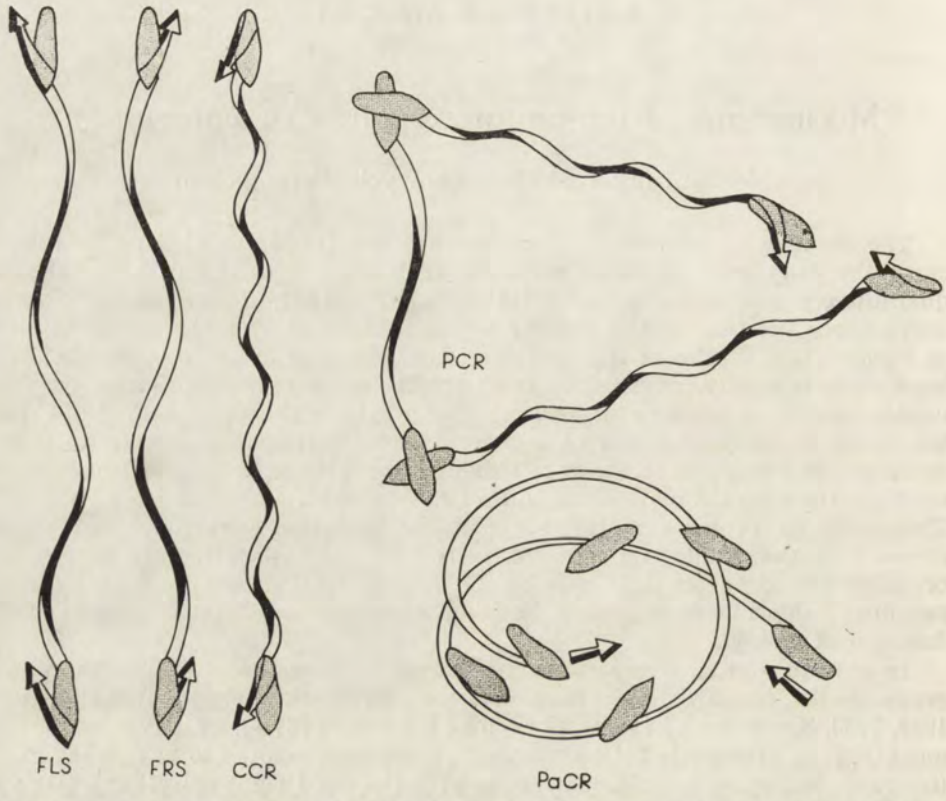

Fig. 1. Main types of motor responses in *Paramecium caudatum*. FLS—forward movement with left spiralling. FRS—forward movement with right spiralling. CCR—continuous ciliary reversal. PCR—periodic ciliary reversal. PaCR—partial ciliary reversal

A number of factors lowering the level of calcium ions adsorbed on the *Paramecium* membrane as a result of desorption, chelation or precipitation, evoke a movement with the posterior body end forwards which may last up to several hundreds of seconds (Kamada 1940, Kamada and Kinosita 1940, Jahn 1962, Seravin 1963, Grębecki 1964a, 1965, Kuźnicki 1966a, b, Naitoh 1968, Kuźnicki and Mikołajczyk 1969). So the ciliary reversal may be only one of the motor response phases—as in the case of avoiding reaction—or a distinctly independent phenomenon. In the last case for sake of precise distinction, it should be called a continuous ciliary reversal (CCR) (Fig. 1), applying the term "ciliary reversal" exclusively to

the change of the cilia position which may occur as well in beating as in non-beating undulipodies (Naitoh 1966, Grębecki and Mikołajczyk 1968, Kuźnicki et al. 1969 a).

Fig. 2. Typic avoiding reaction in *Paramecium caudatum* (after Párducz 1959). A—source of stimulation, 1–9—successive positions of protozoan during avoiding reaction

FLS, FRS, avoiding reaction and CCR do not embrace all the types of motor responses of *Paramecium*. Besides them, some others were distinguished: periodic ciliary reversal—PCR (Dryl 1961) and partial ciliary reversal—PaCR (Párducz 1959, Grębecki 1965 and Kuźnicki 1966 a). In PCR (Fig. 1) the forward backward movements appear alternatively, approximately every 0.5–1 sec. In contrast to CCR this reaction may be very long-lasting. In a suitably selected mixture of Ba^{2+} and Ca^{2+} ions it may last over ten hours. During PaCR (Fig. 1) paramecia describe a tight spiral. This type of movement is involved by an asymmetric pattern of cilia position, a part of them working in reversal position whereas the others work in the normal one.

Appearing of ciliary reversal is accompanied by depolarization of the cell membrane (Yamaguchi 1960, Kinosita, et al. 1964, Kinosita, et al. 1965, Naitoh 1966). In PCR evoked by Ba^{2+} ions, a start directed to the reversal of the membrane potential precedes the ciliary reversal by 22–36 msec. (Kinosita, et al. 1965). The changes in the level of calcium ions adsorbed on the membrane have an essential influence on appearing of ciliary reversal, independently of the factor evoking it (Kamada and Kinosita 1940,

Grębecki 1965, Kuźnicki 1966a, Naitoh 1968), whereas this phenomenon seems to be independent of the cellular metabolism. Those facts indicate that change of cilia position from the normal to reversal is an indicator that in *Paramecium* processes occurred, analogical to the state of excitation of nerve and muscle cells in metazoa.

If the motor response of ciliate is orientated in relation to the stimulus, the phenomenon is described in the term of taxis (Jennings 1904, 1905, 1906, Mast 1941, Wichterman 1953, Jahn and Bovee 1967). The mechanism of some taxes of *Paramecium* e.g. geotaxis remains still an unsolved problem (Kuźnicki 1967, 1968). In the other cases e.g. galvanotaxis, our knowledge is much more advanced owing to a significant degree of demonstration of analogy between the behaviour of the protozoan cell and neuron (Kamada 1931, Kinosita 1936, 1938, 1939, Jahn 1961, Grębecki 1962, 1963).

In the direct current field, at its defined intensity, *Paramecium* turns its anterior body end to cathode and swims in this direction (cathodic galvanotaxis). Turn and directional movement occur as consequence of reversal of ciliary beat from the side of cathode accompanied by intensification of its activeness in the normal direction from the side of anode (Ludloff phenomenon). The area of the ciliary field working in the reversal position augments with the density of current which involves a fall of swimming velocity. In this case ciliary reversal is also correlated with the depolarizing action of cathode (cathelectronus) and activation of cilia on the anodal side with hyperpolarization (anelectronus). The assumption that the protozoon cell posesses the properties of a core-conductor (Jahn 1961) explains the Ludloff phenomenon.

The fundamental problems concerning the phenomena of stimulation and reactions may be analysed on the protozoa material as well as on the excitable cells of metazoa. In comparison with the latter, protozoa show a number of convenient properties. The study of excitability processes on the cells of metazoa is a rather complex technical operation whereas in *Paramecium* it appears in an easily observable form as the ciliary reversal. In metazoa, excitation on the cellular level and response or any form of behaviour can scarcely be observed and studied simultaneously. In the case of *Paramecium*, the change of cilia position—total or partial—provides directly definite behavioural manifestations which are described either in the terms of typical motor responses or of taxes. In consequence, the phenomena of excitation, of motor responses and behaviour of the ciliate are accessible to analysis in their association.

Progress in studies on ciliary movement

The behaviour of *Protozoa* is manifested in motor responses. The organs of movement in *Paramecium* are numerous, the whole cell surface is covered by undulipodia called cilia. The problematics of ciliary structure and function as well as, of the motoric reactions are closely connected with one another and in a number of questions—difficult to isolate. In both field, a remarkable progress has been achieved in the course of the last 15 years and found an extensive elucidation in review articles and in monographies (Kitching

1961, Sleigh 1962, 1966, Pitelka 1963, Pitelka and Child 1964, Jahn and Bovee 1964, 1967, Dryl and Grębecki 1966, Kinosita and Murakami 1967, Párducz 1967, Seravin 1967, Andrivon 1969).

A cilium of *Paramecium* has a conventional structure (Pitelka 1965), i.e. the same as the prevailing majority of undulipodia occurring in nature (cilia of the other protozoa, flagella, tails of spermatozoa, cilia of epithelial cells of metazoa). The surface of cilium is covered by plasma membrane which is a continuation of the membrane covering the whole cell. Inside the cilium, parallel to its long axis, run 11 fibrils (filaments) arranged also in a typical manner (Fig. 3). The central filaments disappear on the level of cell surface,

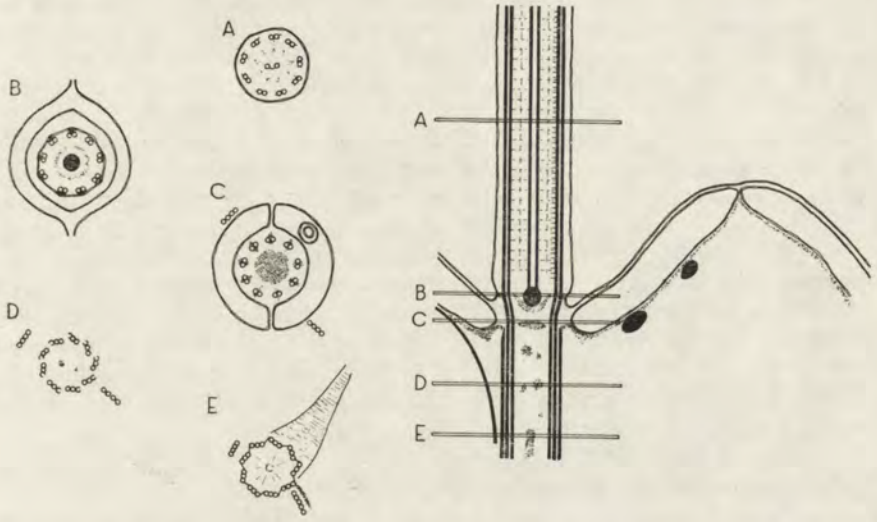

Fig. 3. Structure of cilium and adhering cortical structures in *Paramecium* (after Pitelka 1965). Right—longitudinal section, left—cross sections at different levels, A—free cilium, scalloped profile of cilium, B—densities between the peripheral fibrils and membrane, C—terminal plate including the attenuated tip of the tapered third subfibril of each of the peripheral triplet fibrils. D—kinetosome in its upper half showing the gradually tapering third subfibril and adjacent transverse and posterior tubular fibrils, E—base of kinetosome showing the cartwheel structure and origin of transverse and postciliary tubular fibrils and kinetosomal fibril

the peripheral ones penetrate into kinetosome. The behaviour of filaments during the beat of cilium as well as the biochemical processes occurring on it, are intensely investigated however on other protozoa not on *Paramecium*. Considering the generally accepted uniformity of structure and function in all the undulipodia, it may be accepted that the mechanism of bending of the *Paramecium* cilium lies in the nine peripheral which are built of actinomyosinolike proteins supplied with energy by ATP. An open problem remains still the question whether an alternate contraction and relaxation of filaments takes place during movement (Bradfield 1955, Roth and Shigenaka 1964), or sliding of filaments occurs, similarly as in the model of muscular

contraction according to Hanson and Huxley 1955. The latter hypothesis gains gradually more convincing arguments (Horridge 1965, Satir 1965, Sleigh 1968).

Independently of the further results of investigations on the behaviour of filaments, the automatism and relative functional autonomy of the undulipodia is undisputable. This theory, put forward by Verworn 1889, 1890 and Gray 1928 found a convincing support. After isolation from the cell, undulipodia—including the cilia of *Paramecium* (Kuźnicki 1963 a, Seravin 1967) preserve their activity for some time. This phenomenon takes place also in the case when cilium is deprived of kinetosome. Stop of the movement of an isolated undulipodium is evoked by exhaustion of the energetic reserves. Addition of ATP + Mg^{2+} to the medium restores their activeness to the isolated cilia of *Tetrahymena pyriformis* (Gibbons 1965). The same factors reactivate the cilia movement in saponine models of *Spirostomum ambiguum, Euplotes patella* (Seravin 1961, Seravin, Skoblo and Bagnjuck 1965), of *Paramecium* (the experiments of Aleksandrov and Arronet cited by Seravin 1961). Within each cilium, there exist an autoregulating mechanism securing its rythmic bending which may take place when the inflow of energy either from the medium secured.

Cilium is as well an effector as an excitable structure reacting to different external stimuli which may modify its movement. If cilium is a part of organism, its activity remains simultaneously under the cellular control. In *Paramecium*, the most evident manifestation of this fact is the ciliary reversal especially when it occurs as a total response to a local stimulus.

Basing on the above as well as other facts, Kinosita and Murakami 1967 proposed to analyse the ciliary activity and the mechanisms of its control on 3 levels of organization of the excitable structures:

1. Filaments and other ultrastructures within cilium,
2. Cilium together with kinetosome,
3. Ciliated cell as an independent excitable structure.

Morphological differentiations and the relative functional autonomy give grounds to this distinction. It is however clear that in *Paramecium*, each of the consecutive level of the excitable structures should exist in some physiological organizing relations of the subsequent level. The problem of mechanism of motor response resolves itself to establishing those relations. It should be established precisely in the first place, which aspects of the ciliary activity follow from the properties of undulipodia themselves and which are under the control of the cell.

The analysis of cell function is impossible without description of the manner of its activity as the form of its movement. It was believed till recently that in this field the fundamental problems have been solved except for some details. This view was based on illusory grounds, especially concerning the ciliature of ciliates.

Till 1968 a conviction was generally accepted of a technical impossibility of direct observation of cilia on the surface of swimming *Paramecium*. This view was explicitly stated by Párducz 1967 and resulted from the following data:

1. Small dimensions of cilia—length 10–12 μ, diameter—0.27 μ (Sedar and Porter 1955).
2. Relatively rapid frequency of beats—28/sec, (Sleigh 1962).

3. Similar optic properties of cilium and cell, as effect—an insignificant contrast between them.

4. Microscopic difficulties in operation. It was found impossible to keep a sharp picture under a high magnification of undulipodia on the surface of a ciliate swimming with velocity reaching up to 1300 μ/sec.

In consequence all the microscopic studies on living protozoa were limited exclusively to the observation of the profile of cilia on the edge of the cell. In these conditions, nobody succeeded in executing microphotograms of undulipodia as well of individuals of *Paramecium* as of any other ciliated protozoa cells (Pitelka and Child 1964). Since the photographic documentation failed to provide any informations about the behaviour of ciliary apparatus in *Paramecium* or in other ciliates during movement, the only source of data in this problem have been the preparations gained after instantaneous fixation with $OsO_4 + HgCl_2$ and hematoxylin staining (Párducz 1952, 1967, Grębecki 1964 b). According to Párducz 1954, (Pl. I 1, I 4) the pictures gained by him with his method—modified by him—of instantaneous fixation reflect objectively not only the character of the course of the metachronal waves—but also the successive phases of the work of a single cilium. The view of Párducz found no controversion, however some authors anticipated (Brokow 1966) that the problem of ciliary movement might be fully elucidated only after gaining the photographic documentation.

Kuźnicki et al. (1968 a, 1968 b, 1969 b) executed motion pictures presenting the behaviour of ciliary apparatus of *Paramecium multimicronucleatum* in the conditions of culture, in phosphate-citric buffer, in the methylcellulose solutions. They succeeded—among others—to register precisely on film the beat of single cilium. Its feature proved to be essentially different from its generally accepted description.

The recent theories of ciliary movement and of its coordination

Till the present time, the coordinated movement of cilium on the surface of *Paramecium* as well as on all the ciliated protozoan and metozoan cells has been described as a pendular i.e. consisting of an effective stroke and of recovery stroke. In the case of *Paramecium*, the differences in interpretation concerned only details. The majority of earlier authors believed that the effective stroke and the recovery stroke occur in the same plane. Cilium assumes the form of a straight line in the moment of effective stroke and bends in the phase of the recovery stroke. Párducz 1954, 1958, 1967 put forward another interpretation. The cilium of *Paramecium* fails to assume a fully right feature at any phase of coordinated movement and its work runs in a three-dimensional space (Pl. I 3). At the forward movement with left spiralling (FLS), the effective stroke of cilium occurs from left to right, backwards to the swimming direction. After having accomplished the effective stroke, cilium bends rotating still right i.e. counterclockwise, till the moment of attaining the exit for the next effective stroke. According to Párducz 1954, 1967, the backward movement is nothing else as a short phase of a so-called autonomous rotation of cilium. Rotation becomes interrupted by the metachronal impulse which evokes an effective stroke. After the metachronal

excitation, the period of refraction follows, and cilium enters the phase of autonomous rotation again till it is reached by another metachronal impulse. So the coordinated ciliary movement would be an effect of cooperation of two spontaneous processes. One of them being the autonomous rotation with an exciting mechanism within the cilium itself, the second one—excitation waves of an endogenic character, running at regular intervals over the cortical ectoplasm from one cell end to the other (in the normal FLS movement, forwards from behind, and during CCR—inversely). The metachronal impulse would be consequently a control mechanism of the cell ensuring a coordinated work of the whole ciliary apparatus (Párducz 1967).

The idea of "autonomous" "non-coordinated" ciliary movement arose as contrast to the interpretation of "coordinated" movement, as result of direct observation and not of analysis of preparations.

Since a long time, the fact was known that under the action of impairing factors, separation of pellicle fragments from ectoplasm may be evoked, followed by formation of the so-called hyaline blebs. Owing to their transparency, cilia become visible on the bleb surface and their behaviour may be observed. Besides, their analysis has been facilitated by the fact that appearing of a hyaline blebs is a pre-mortal symptom accompanied by arrest or a considerable slowing of the ciliate movement. Párducz 1954 after having treated ciliates with ammonium vapours, supported the former observations that cilia in hyaline blebs—more distinctly seen from above than in profile—exhibit a regular rather slow rotatory movement (Fig. 4) of an counterclock-

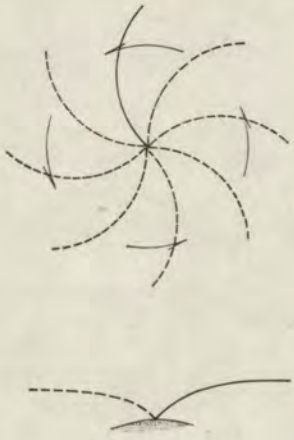

Fig. 4. The "autonomous rotation of uncoordinated cilia" from above and in side view (after Párducz 1967). Evidently the drawing illustrates conicoidal beat of cilia in stationary *Paramecium*. Compare with Pl. II and Pl. III

wise direction (constant polar rotation). Later on, it has been stated (Pitelka and Child 1964, Párducz 1967) that the conical rotatory movement of cilia appears as well without the presence of hyaline blebs in *Paramecium, Colpidium, Didinium, Opalina* under the action of different factors (narcotics, inorganic salts) which paralyse the progressive movement of *Protozoa*. In the immobilized individuals, all the cilia rotate with the same rate. The phenomenon is accompanied by vanishing of the metachronal waves. In the interpretation of Párducz 1967, this is an evident effect of exclusion of cilia from the control of cellular mechanism coordinating their work. The base of the Párducz's theory of ciliary movement following from its own investiga-

tions mostly on *Paramecium*, was the analysis of direction of the course of metachronal waves and its correlation with the effective stroke of cilium. In this field he came to statements fully controversive to the formerly accepted views. Till 1954 all the authors—including P á r d u c z—agreed that in *Paramecium* during the FLS movement, the metachronal waves initiated on the anterior body part and propagated backwards, whereas at CCR their course is inverse. The effective stroke of cilium, independently of the swimming direction of *Paramecium*, is perpendicular to the front of metachronal wave. In its publications 1954 and 1956, P á r d u c z revoked his former views stating that at FLS, the ciliary waves are initiated on the posterior body end and run forwards, and at CCR—from forward backwards. The effective stroke of cilium occurs at any type of movement, almost parallel to the wave front. This is the behaviour of body ciliature of *Paramecium*. The ciliary effective stroke of the oral groove cilia is perpendicular to the wave front. Direction of the metachronal wave propagation is the same as on the other areas of cell surface.

K n i g h t - J o n e s 1954 distinguished four main types of metachronal coordination: 1. symplectic metachronism, 2. antiplectic metachronism, 3. dexioplectic metachronism, 4. leoplectic metachronism. In the first case the direction of effective stroke of cilia is in harmony with the direction of propagation of the metachronal wave, in the second case it is opposed, in the third—rightward, in the fourth leftward. After P á r d u c z 1967, the somatic ciliature of *Paramecium*, when working in a coordinated manner, shows a dexioantiplectic metachronism, whereas the cilia of the oral groove—a purely antiplectic one.

In preparations, after the method of instantaneous fixation (Pl. I 1, and 4) the dark areas fail to correspond to the crests of metachronal waves neither to the light ones to their valleys. The dark stripes arose as result of mutual overlapping of cilia during their backward movement i.e. when they are in a position nearest the body surface whereas the light zones are the places of effective stroke of cilia.

The ideas of P á r d u c z developed by him in a number of experimental works published in the years 1954–1964 and collected in a summarizing article from 1967 have became generally accepted in majority. This concerned in the first place all the features of cilium movement, propagation direction of the metachronal waves and their relation to the effective stroke and the values of pictures gained after the method of instantaneous fixation. As a controversive problem remained only the mechanism of coordination in the metachronal movement.

P á r d u c z himself since 1954 till his last work 1967 consequently declared himself against describing inductive functions to any fibrillar systems of *Paramecium*. In his opinion, even kinetodesma which forms a real junction between the cilia, fails to posess a character of a primitive nervous system, conducting the metachronal impulses in a neuroidal way. In this problem, the opinions of majority of the present authors are in agreement with the Párducz theories, although D o r o s z e w s k i 1958 does not exclude the possibility of conduction of metachronal movement stimuli by the silvering system.

P á r d u c z 1954, 1958, 1962, 1967 decidedly rejected the hypothesis about any functional associations between metachronism and the subpellicular fiber systems, keeping simultaneously the notion of endogenic metachronal

impulse which is conducted by non-specialized cortical ectoplasm (Fig. 5). Till the present time, the electrophysiological methods failed to demonstrate any changes of the membrane potentials in *Paramecium* connected with propagation of metachronic ciliary waves on the surface. According to Párducz 1967 this is a result of not sufficiently precise techniques which does not permit to the registration of relatively small cyclic changes in the electric properties of membrane.

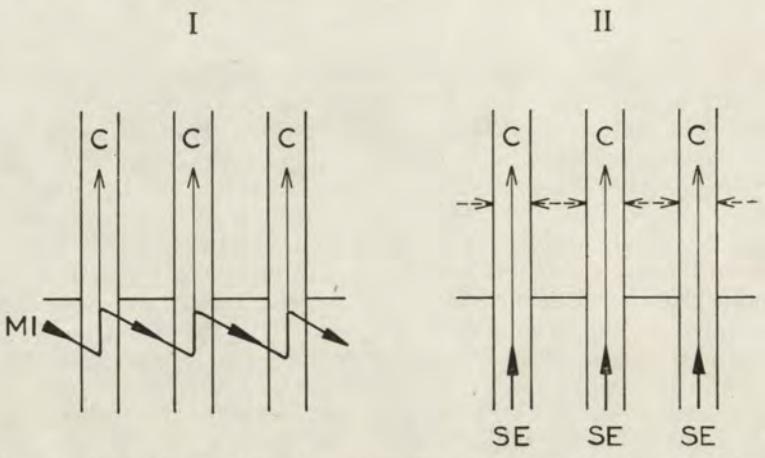

Fig. 5. Illustration of two theories of metachronal coordination in *Paramecium:* I—metachronism by metachronal impulse, II metachronism by mechanical interaction. (based on the drawings of Sleigh 1969). C—contraction, MJ—metachronal impulse, SE—spontaneous excitation

The concept of endogenous metachronal impulses is supported and developed before all by Seravin 1967. The other authors have either accepted an ambivalent position (Kuźnicki 1963a, Dryl and Grębecki 1966) or express the view (Jahn and Bovee 1964, 1967, Pitelka and Child 1964, Sleigh 1965, 1966, 1969), that the interpretation of the metachronic movement of *Paramecium* cilia may be most adequately elucidated by the hydrodynamic regulation i.e. the "mechanical interaction" of cilia. Each cilium beats on the basis of its own spontaneous excitation. The adjacent cilia, however are so close together during some beat cycle, that mutual interaction involved a smaller phase difference between neighbouring undulipodia (Fig. 5). In this situation, their "mechanical interference" (Sleigh 1969) may be conceived in a feature of metachronal wave without any endegenous impulses. Depending on the rate of cilium beat (Sleigh 1966, 1969), the metachronal wave on the *Paramecium* surface would propagate forwards (high rates) or backwards (low rates) as it takes place in *Paramecium* in media of increased viscosity.

Ciliary activity in stationary *Paramecium*

Toward the end of 1967 Kuźnicki et al. made an attempt of executing motion pictures of cilia of *Paramecium multimicronucleatum* during their normal activity. It was initially assumed that application of Hycam Redlake

Laboratories Camera may bring about a partly overcoming of the hitherto existing difficulties in observation of undulipodia. Among the different variations of microscopic technique (bright and dark field, phase contrast, Namarski system, interference-polarization microscopy) for taking photographs of cilia with the speed of 300–4000 frames/sec (fps), only the phase contrast lenses proved to be useful.

Paramecia taken directly from the cultures or rinsed previously in phosphate-citric buffer (D r y l 1959) at pH 5.2–5.6 were placed in chambers in which the distance between the slide and cover glass amounted approx. 720 μ. In the first stage of work—keeping in view the general opinion that a direct observation of cilia on the surface of swimming protozoon was impossible—attention was concentrated exclusively on the undulipodia on the edge of the body of stationary individuals (not swimming, "thigmotactic").

The "thigmotactic" response of *Paramecium* on the walls of the vessel or on detritus occurs in masses in the cultures rich in the bacterial flora. It has all the symptoms of feeding reaction, however even in the conditions of a full lack of food in medium, the protozoon coming into a direct contact with the surface of any body—relatively solid—may react by a more or less prolonged arrest.

J e n n i n g s 1906 gave a general description of the behaviour of ciliary apparatus in *Paramecium* at its transition from swimming to the stationary phase and in its duration. Meeting a fragment of filter paper, bacterial detritus, cotton fibres etc., protozoon responses by a slightly marked avoiding reaction. Subsequently a group of cilia, most often on its anterior body part on the aboral side, coming into contact with the object again, adheres to its surface and straightens simultaneously. In such an "anchored" *Paramecium*, the oral groove cilia work intensely whereas the beats of the remaining ones are much slowed and in some areas of the cell, may even be stopped.

In the first phase of experiments, K u ź n i c k i, et al. applying low magnifications (128–300 ×) fixed on film the moment of "anchoring" of *Paramecium* as well as the general picture of ciliary activity in a stationary individual ("thigmotactic").

Analysis of the motion pictures supported the correctness of the Jennings' description which required only some supplementation. In stationary *Paramecium*, the activity of cilia of the oral groove is in full agreement with the P á r d u c z's definition of the coordinated movement. Metachronal waves running out of vestibule, propagate towards the anterior body part. On the remaining areas of cell surface, the metachronal waves are not so regular or fully invisible.

Metachronal beating of the oral groove cilia of *Paramecium* attracted attention of many authors. However quantitative data concerning it have been exceptionally scarce. This gap could be to some degree supplemented on the base of our films. In the individuals studied, the distance between two metachronal waves (wave length) was a value of a low variability and persisted within the limits 17.2–20.5 μ of a mean 18.6 μ. In contrast to this, the rate of metachronal wave propagation fluctuated in large limits from 455 μ/sec to 1280 μ/sec. Even in the same individual, within approx. 2 sec this rate might have increased from 482 μ/sec to 788 μ/sec. In the case of a relatively constant rate of propagation of wave, of a mean value 932 μ/sec, the number of waves propagated in the oral groove amounted on the average 53.8 waves/sec. On

the base of films executed under low magnification, only the metachronal movement could be analysed. Therefore attempts were made of photographing cilia using immersion phase contrast lenses. The films taken at high magnification (up to 1000×) gave amazing results. It has been stated (Kuźnicki et al. 1968 b, 1970) that in the stationary *Paramecium* the form of cilia beat Pl. II is very similar to that described by Párducz as the autonomous rotation of uncoordinated cilia (Fig. 4).

The results obtained inclined us to try an experiment essentially different from the way of observing living protozoa as it was done up to the present time, i.e. photographing cilia directly on the surface of *Paramecium*. Those attempts concluded only in a partial success. In the majority of films the contrast between cilium and cytoplasm was very slight. In all the cases however when cilia were visible, their spiral gyration was beyond discussion. Gyration which has been most successfuly photographed in the peristomal cilia, occurs in the anticlockwise direction. Considering the fact that during this movement, cilium fails to acquire a straight line in any plane, it was determined as a conicoidal beat (Kuźnicki et al. 1970). This feature of cilium beat, determined as conicoidal, has really a character of helix with an increasing diameter from the base to the tip of undulipodium.

It has been generally accepted until now that the form of a cilium beat is the same in the swimming and non-swimming individuals. At a high magnification, only a rather small segment of the protozoon body is observable. The view of the whole *Paramecium* can never be gained. In all the stationary individuals however, independently of the fact which cell fragment has been photographed, all the beating cilia showed a conicoidal movement.

The conicoidal movement, described until now in the terms of "rotational", should not be a pathological symptom but is a normal phenomenon in stationary *Paramecium*. This was the next among the number of conclusions contradictory to the presently accepted views.

Párducz 1954, 1958, 1967, as well as the other authors, has stressed that in the moment of appearing of the autonomous rotation of cilia, a full vanishing of the metachronal waves follows and that this movement is not effective. Really the conicoidal movement of the oral groove cilia is always accompanied by a very well visible and regular metachronism (Pl. III), while the body cilia behave variably in this respect. On one body area metachronism may be visible, on the others it may fully disappear. This distinct difference in the behaviour of the oral groove cilia and the body cilia finds a clear explanation in the differences of their movement rate. Frequency of work of the *Paramecium* cilium when analysed on films taken at a high speed, proved to be much higher than it has been reported until now. The cilia of oral groove performed a conicoidal movement of a rate from 25.4 cycles/sec to 78.2 cycles/sec and in these cases were always accompanied by metachronism. In the stationary *Paramecium*, the rate of beat of body cilia is varying from 0 to 35.8 cycles/sec. No doubt, there exists a zone of definite velocity at which transition occurs from the metachronal to ametachronal movement. A precise definition of this value is however actually impossible because this process has in the transition zone a rather indistinct character. Together with the fall of gyration rate of cilia, gradually disappear the metachronal waves becoming less and less visible. It may be however roughly stated that the gyration of cilia at the rate of 20 cycles/sec in any zone of the cell, would

always be accompanied by appearing of metachronal waves. This accounts for the various behaviour of body cilia in the stationary *Paramecium*. All the present observations on conicoidal movement, determined until now as "rotational" or "funnel-shaped" have been carried out on the ciliates immobilized by the action of impairing factors, involving a highly slowed movement of cilia. Consequently the unanimous view of all the authors that the appearing of "rotational" movement is always accompanied by vanishing of ciliary waves—is explicable.

The idea of the non-effective autonomous rotation was not precise in its formulation. Really any ciliary beat—independently of its form—evokes a movement of the particles in the surrounding medium. Effectiveness of functioning of cilia in *Paramecium* may be considered in two aspects:

1. as a swimming organelles,
2. as organelles putting food to cytopharynx.

In the stationary *Paramecium*, the first aspect is negligible. For the second one a direct, positive answer has been gained. Plate III illustrates the moment of promoting the bacterial cells towards the cytostome by gyrating oral groove cilia as recorded on successive film frames. Simultaneously, on the same sequence, the movement of the metachronal wave is seen in the opposite direction i.e. towards the body anterior.

Technique of direct observation of cilia on the cell surface

An essential methodical conclusion follows from the study of ciliary activity in stationary *Paramecium*. The record of cilia directly on the cell surface is possible although the pictures gained in this way are fairly contrastfull which is involved as well by similar optic properties of cilium and cytoplasm as by large-grain films which may be applied in the high speed cameras. This caused a serious restriction, nevertheless the manner of photographing presented the only way of gaining objective ground for evaluation of the pattern of beat in the swimming individuals.

Spiralling is a component of the progressive movement of the type FLS, FRS, CCR or PCR in *Paramecium*. Photographing undulipodia in profile on the edge of cell of a swimming individual is not sufficient for the characteristic of the feature of the cilium beat, being effective for stationary *Paramecium*. Moreover they may be the source of optical illusions involved by escaping of some cilia from the focussed plain and appearing of some others instead. Gaining photograms of cilia on the surface of swimming *Paramecium* proved to be an exceptionally difficult task because of the reasons mentioned above but—despite the opinion of P á r d u c z—possible to be performed.

Plate IV contains a series of cinemicrographs presenting the behaviour of cilia in the course of FLS ciliary reversal and CCR. Despite the low contrast, cinephotographs unmisticably indicate that in the swimming *Paramecium*, cilia do not show effective and return stroke but an undulatory movement which has been stated until now exclusively for flagella. Merely the change of cilia position from the normal to the reversive, reminds in its form the phase of return stroke. In the FLS or CCR movement, cilia always perform an undulatory beat not changing in a perceptible way the angle of inclination to the cell surface.

Because of the technical difficulties, the films gained by means of the high speed camera was quantitatively not sufficient and too little contrastfull for being the only base for drawing conclusions essentially different from the theories accepted till now. There arose a problem of finding a technique giving much better quantitative results.

It was postulated that the difficulties in observations of cilia at the surface of swimming ciliates may be overcome if simultaneously: 1. the contrast between cilium and cell is increased, 2. the speed of swimming paramecia is reduced without evoking impairing effects on the course of the other vital processes. Both postulates have been achieved (Kuźnicki et al. 1968 a) by applying the following procedure.

P. multimicronucleatum taken from the culture were rinsed in one of the series of phosphate-citrate buffers (Dryl 1959) at pH 5.2–5.6 and remained in them at least for 1 hour. Then ciliates were placed in methyl cellulose solutions prepared on buffers of the same pH. The sample of paramecia was added to the solution of methyl cellulose so that its concentration amounted 0.8–1.2%. After a 3-hr or longer incubation in this medium—cilia observed on the cell surface under immersion phase contrast lenses, became distinctly visible which made possible the recording of their beat on the motion pictures (Pl. V) or on photomicrographs as well (Pl. VI).

The high viscosity of methyl cellulose involved a considerable fall of speed of swimming in *Paramecia*. Speed falls to over ten to several tens of microns/sec depending on the concentration applied but in the 1.2% solutions, the movement of paramecia is not arrested—even for a while—for at least 24 hr. The methyl cellulose solutions applied provide simultaneously an exceptionally favourable experimental situation. In these conditions, the ciliary activity of *Paramecium* is manifested as (FRS ⟶ CCR ⟶ FRS ⟶ CCR .....*n*) (Grębecki et al. 1967 a). Transitions from the movement of the type FRS to a continuous ciliary reversal (CCR) alternate at intervals of several seconds. Owing to this, in the methyl cellulose solutions, the form of cilium beat may be analysed during different types of motor responses (Pl. V, Pl. VI 59, 60) as well as the phenomenon of ciliary reversal itself (Pl. VI 61–63), as the transition from the continuous ciliary reversal to swimming with the anterior body end forwards (FRS), not applying in any case additional stimulation.

Methyl cellulose solutions seem to be a medium practically neutral for *P. multimicronucleatum* as far as the possibilities of the course of fundamental life functions are concerned. Even after having remained 5 days in the 1.2% methyl cellulose, 100% of individuals live.

The optimal conditions for a uniform study of all the manifestations of ciliary activity—as described above—coincide with the period between 3–24 hr after the introduction of specimens into the methyl cellulose solutions. Prolongation of the exposure time permits however to collect observations inaccessible in another way. E.g. keeping paramecia over 24 hr in 1.2% or higher methyl cellulose solutions may involve in some, exceptionally rare cases, the loss of some body cilia while the others preserve their normal activness securing the possibility of motor responses of *Paramecium*. Rarefying of the ciliary apparatus is advantageous for the analysis of the single cilium beat making it much more precise that in the case when undulipodia have covered densely the whole cell.

The method of direct observation of cilia on the cell surface (Kuźnicki et al. 1968 a) involves a number of other essential faciliations. In the first place the high speed cameras become unnecessary. The fall of speed of swimming, and of the cilia movement permit to apply the standard cameras of a velocity up to 60 fps. This—in turn—makes possible the use fine grain films. The best results have been obtained with Kodak-Ektachrome commercial film. At last, this technique may be successfully applied for the study of activity of undulipodia in the other species of ciliates and flagellates e.g. *Trichonymphida.*

Form of cilia beat in swimming *Paramecium*

The technique of direct observation of cilia on the *Paramecium* surface made possible the collection of an extensive film documentation. It has been stated on this base and owing to the photograms executed with high speed cameras (Kuźnicki, et al. 1968 b, 1969 b, 1970), that in swimming *P. multimicronucleatum* cilia beat with a travelling wave. Plate VII presents a typical picture of the beat of the same body cilium, observed from above on the cell surface and recorded on 20 successive film frames. The undulatory waves are moving upward from base to tip of cilium. The same character of the undulating movement is seen in cilia observed in lateral view on the body edge (Pl. VIII). This indicates univocally that we have to do with a three dimensional travelling wave (Fig. 6). Such a wave oriented distally produces a locomotory

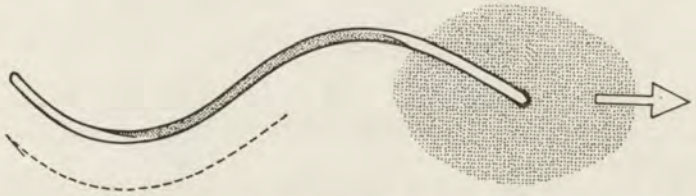

Fig. 6. Diagram showing the travelling helical wave in an individual body cilium of swimming *Paramecium*. Dotted arrow indicates the wave movement, arrow at right—direction of swimming of *Paramecium*. Based on the drawing of Jahn and Bovee 1968, made for another purpose

force directed from the cilium base to its tip and—in effect—pushes *Paramecium* in an opposite direction. During this movement, cilium does not rotate around its axis, although an illusion of rotation may in direct observation. Really this movement is undulatory occurring simultaneously in two perpendicular planes with phases shifted for 90° in relation to each other. If waves are sinusoidal, of the same amplitudes and of the same wave length, their combination gives in effect a perfect helix. The character of such wave has been described by Jahn and Bovee 1968 for trophozoites of *Plasmodium relictum* anchored to their parent cell.

In swimming *Paramecium*, ciliary wave observed as well on the surface as on the cell edge, is not an ideal sine wave but has symmetrical character. The curved and straight regions which appear during the cilium movement, are fully comparable to the undulatory movement of protozoan flagella and

to the movement of some spermatozoa tails. The travelling helical wave directed from the base of undulipodium to its tip has been described—among others—in spermatozoa of bull (Gray 1958, Rikmenspoel 1965) and in the transverse flagellum of *Ceratium* (Jahn et al. 1963). Possibly in the same way move the free-swimming spermatozoa of man (Zorgniotti et al. 1958).

In *Paramecium* placed in the methyl cellulose solution, all cilia—as well somatic as oral groove ones—beat in a way of travelling helical wave. The form of beat is fully independent of the fact whether the protozoon swims forwards or during its continuous ciliary reversal (CCR). Only the cytopharyngeal cilia which form systems of membranelles perform the three dimensional pendular beat. The effective stroke of those cilia is orientated to the posterior of cytopharynx, at the end of which a food vacuole is formed. The direction of the effective stroke of cytopharyngeal cilia is the same, independently of the motor responses: FLS, FRS, CCR or in stationary *Paramecium*.

The direct observations and in the first place the extensive film documentation (Kuźnicki et al. 1969 b) solve all doubts that in *P. multimicronucleatum* swimming in methyl cellulose solutions, body and oral groove cilia beat always in the pattern of travelling helical wave. This only fact involves the necessity of revision of the present views concerning the ciliary movement and the functional distinctness of cilia from the other undulipodia (protozoan flagella, tails of spermatozoa etc.).

The pictures gained owing to the technique of direct observation of cilia on the cell surface permitted simultaneously to a more extensive—than it was possible until now—analysis of the films executed with high speed cameras. It may be stated on the same ground that in protozoa either swimming in the culture medium or in buffors, cilia beat in the same manner as in solutions of methyl cellulose. It is a helical wave travelling from base to tip. The curve of the wave bending and the angle of inclination between cilium and the cell surface increase only insignificantly in the media of normal viscosity when compared with these parameters in methyl cellulose solutions.

Films which have been gained by application of high speed cameras were quantitatively much more scarce and show a less sharp contrast than the motion pictures revealing the ciliary activity of *P. multimicronucleatum* in methyl cellulose solutions. On this ground, the objection may be put forward that the wavy character of the ciliary beat in *Paramecium* swimming in the media of a normal viscosity has not been documented in a sufficiently convincing manner. The consequence of this objection would be the hypothesis that the increase of medium viscosity evokes the transition from the back and forth movement—considered until now as the only form in which cilia may beat—to the travelling helical wave. The importance of this objection requires a more extensive discussion, the more so as the answer to it would simultaneously indicate the reasons for which our results are so essentially different from all the former descriptions and theories concerning the ciliary movement.

Increase of the medium viscosity is one of the fundamental methods applied for years in analysis of movement of different undulipodia. Among the factors experimented, most recognition found recently methyl cellulose as a compound acting almost exclusively on the increase of viscosity.

The study of the influence of increased viscosity upon the form of cilia

beat was not possible as yet because of the lack of methods of their observation and recording. Flagella of flagellates or spermatozoa tails were studied from this point of view frequently and extensively being rather convenient for filming or photographing.

On this ground, Holwill 1966 in his review of the physical aspects of flagellar movement, stated explicitly that increase of medium viscosity fails to influence the form of undulipodia movement but only changes such parameters of their function as frequency, amplitude and length of their wave. Exceptions of this rule are rare and appear as changes of a small scale. E.g. the tails of bull spermatozoa which beat normally in the form of a helical wave, perform in the media of increased viscosity a sinusoidal movement (Rothschild 1961). The absence of influence of the increase of medium viscosity upon the form of cilium movement in *Paramecium* is consequently in agreement with the behaviour of the other undulipodia and its eventual change would be an exceptional phenomenon.

The cilia of cytopharyngeal membranelles in *Paramecium* work in the same pattern in methyl cellulose solutions as in the media of a normal viscosity. Independently of conditions, their movement is composed of the effective stroke and return stroke. This is an indirect argument speaking in favour of the helical form of beat of body and oral groove cilia in a medium of normal viscosity. The difference of the beat pattern of membranelles from this of the remaining ciliature seems to be a rather common phenomenon in ciliates. This has been proved by the studies of Sleigh (1957) on the movement of membranelles in *Stentor* occurring in the edge of peristome. Lack of observation methods of the body cilia on the ciliates surface is the simplest answer to the question why the presented results deviate from the former descriptions.

In all the former theories on the ciliary movement of *Paramecium*, an unverified postulation remained that undulipodia of stationary or immobilized individuals, beat in the same pattern as in the swimming ones. However the latter have been fully out of reach of direct observation. As consequence, any studies on the forms of cilium movement and attempts to define its rate by stroboscopic methods were carried out on stationary or immobilized paramecia. In those conditions the conicoidal beating cilia when observed in profile on the body edge, continuously disappear from the view of a focus plane and appear again after a moment. In consequence, an illusion arises that cilium performs a "backward and forward" movement.

Párducz, the most prominent investigator of ciliary movement in ciliates, was fully aware of all drawbacks and limitations of the direct observation of cilia in protozoa. Just for this reason he ascribed such a great value to the instantaneous fixation technique corrected by him. According to his postulations when protozoa have been treated with the solution of OsO_4+HgCl_2, "true-to-life snapshots" of cilia on swimming individuals may be gained in the course of 1/200 sec. Unfortunately this postulation was not based on verifiable data. Nevertheless if we have accepted the postulations of Párducz as axiom, as it has been previously done by the author (Kuźnicki 1963 a, Grębecki et al. 1967 b), cilium is even then capable to perform movement. Pictures of cilia disposed in regular "metachronal waves" (Pl. I 1–4) correspond in fact to the first phase of movement of cilia entering ciliary reversal (Pl. IX 97, 98, 99). The full cycle of this process may last 15 msec

only (Kuźnicki et al. 1969 a), and manifestation of the initial phases of this process appears after a few msec already. The "typical" pictures gained after the technique of instantaneous fixation would be rather a reflection of protozoon reaction to the first contact with the fixative and not the real picture of behaviour of cilia in movement. Such an interpretation fails however to account for the reasons of appearing and of regularity of "metachronal waves" in preparations. This problem remains still an open question but not so essential as it seems to be.

Párducz 1967 ascertained that if the individuals in sample manifest all the same motor responses, so after the introduction of fixative they have the same metachronal pattern in nearly 100%. The author of the present article, applying many times the technique instantaneous fixation, failed to state this regularity in any case. In every mass preparation, the disposition of metachronal waves in separate individuals was variable and far from being regular. A number of paramecia had no metachronal waves at all, producing pictures which are characteristic for a simultaneous beat at the reversal of cilia (Pl. I 5) or at disturbances evoked by the action of chloroform (Pl. I 6).

Similar observations have been done previously by Doroszewski 1958, however this author did not object the value of the method itself.

The last problem which should be put forward, is the lack of harmony between even the most regular metachronal coordination in preparations (Pl. I 1, 4) of Párducz and the scheme of single beating cycle (Pl. I 3). When comparing the drawing with photograms, the homologues of position a, b, c, d may be found with no difficulty whereas the positions e, 0, 1 cannot be detected in any case. In preparations of instantaneous fixation technique, the phase of effective stroke of cilia cannot be stated.

Until now, the only means affording the possibility of an exact analysis of movement of single body cilium was to isolate it from the cell. Such observations have been carried out recently by Gibbons 1965 on *Tetrahymena* and by Seravin 1967 on *Paramecium*. Their results agreed with that stated previously. After having been isolated from the cell, cilia of *Paramecium* perform an undulating movement exclusively. Similar is the behaviour of reactivated cilia of *Tetrahymena*. The form of their movement has a character of a helical wave (Gibbons 1965).

According to Seravin 1967, those facts indicate that cilium returns to the primitive feature of flagellar movement after having been excluded from the coordinating (metachronal impulses) cellular mechanisms. Párducz 1967 interpreted the appearing of autonomous rotation of cilia in a similar way. Our results (Kuźnicki et al. 1968 b, 1969 a, 1970) differ only from the former theories of the ciliary movement in ciliates but are simultaneously fully in agreement with the observations carried out on cilia when their movement may be precisely analysed.

Uniformity of cilia and flagella

The pioneers of the contemporary research on ciliary movement had been Verworn 1890, 1894 and Gray 1922, 1928, 1930 Verworn's descriptions and diagrams of the movement of cilia in *Paramecium* were based on direct

visual observations. However the ciliary beat of swimming *Paramecium* is too fast for the eye to be followed. The observations of Gray concern mainly cilia (correctly abfrontal cirri) of *Mytilus* gills. From the point of view of the observation possibilities the object was excellently selected but the manner of movement of abfrontal cilia in *Mytilus* proved to be unique (Kinosita and Kamada 1939, Yoneda 1960, Sleigh 1962). The contemporary authors (Rivera 1962, Sleigh 1962, 1968) have stated that many forms of ciliary movement exist, however this movement is not continuous and consists in every case of effective stroke and of return stroke.

The fundamental achievement of the last twenty years in the field of cilia and flagella biology has been the revealing of common features of their ultrastructure and biochemical processes securing their activity. One of the ways of differentiation of cilia from flagella became the form of their movement. Criteria of this division are illustrated of Fig. 7 cited by Sleigh

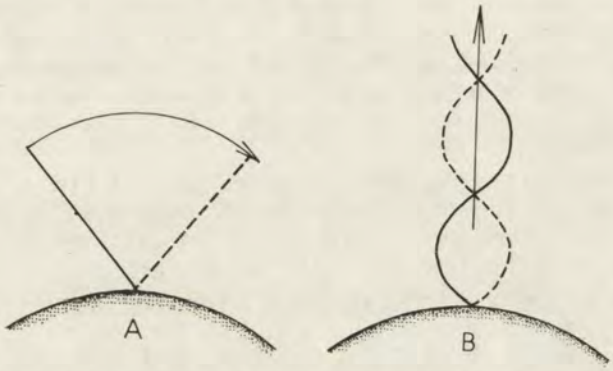

Fig. 7. Diagram illustrating after Sleigh 1962 "typical" form of beat: A—cilium, B—flagellum. Arrows indicate the movement of water. Compare with Pl. VII

1962. Postponing the unavoidable simplification in the attempt of a uniform presentation of this problem, the form of flagellar movement is only one of the many possible forms.

Ulehla 1911 proposed a distinction of three main forms of flagellar movements: 1. three-dimensional (helical) waves, 2. planar undulatory waves and 3. oar-like movement. The essential criteria of this division are fully actual (Holwill 1966), and the number of the investigated cases has much increased. It has been demonstrated among others, that number of flagella beat in a manner typical for the "ciliary movement". This type of movement occurs in *Mastgamoeba setosa, Entosiphon sulculatum* of a gliding *Peranema trichophorum* or *Petolomonas* as well as in the third anterior flagellum in the course of swimming (Jahn and Bovee 1967). The latero-posterior beat of flagellum camposed of the effective stroke and return stroke, proved also to be equally "typical" for flagella as the helical or sinusoidal waves. According to Jahn and Bovee 1967 these facts remove any critically functional distinction between flagella and cilia. The rule of differentiating cilia from flagella has remained always valid since it was generally assumed that the form of undulating movement never occurs in cilia.

This last limitation has been removed by demonstration (Kuźnicki et al. 1968 b, 1969 b, 1970) that the body and oral groove cilia of *P. multimicronucleatum*—and possibly in many other ciliates beat in the same manner as "typical" flagella and spermatozoa tails. This conclusion should be proved for the other *Paramecium* species of the "aurelia" group.

It has been known since a long time that the phenomenon of metachronism may accompany any movement of undulipodia if they occur side by side in a great number. The flagellate species—symbionts of xylophague insects similarly as the representatives of the class *Opalinata*, included till recently into ciliates, possess numerous undulipodia which cover either the whole cell or its extensive areas.

Distinct metachronal waves running from forward backwards occur in all the *Trychonymphida*. They may be especially distinctly observed after placing protozoa in methyl cellulose solutions. In these conditions, the behaviour of undulipodia of *Trichonymphida* sampled from termites, indicates a similitude of its character and of propagation direction of metachronal waves with those phenomena in *Paramecium* in the media of increased viscosity. In turn, the *Opalina* species are the best objects among protozoa for direct observations as well of metachronism as of reversal of undulipodia. Consequently, all the manifestations of undulipodia activity in ciliates may occur also in flagellates.

Sleigh 1962, Pitelka 1963, Pitelka and Child 1964, Seravin 1967, determined cilia and their function as one of the modifications of flagella. Presently a much more progressing thesis may be formulated. Cilia and flagella are not differing from each other in any aspect.

The terminological problems are of a secondary importance. According to the tradition, undulipodia of ciliates and of somatic cell of metazoa may be called "cilia" and undulipodia of flagellates and spermatozoa—"flagella". Postponing this discrimination would however be more in agreement with real state of things. In this case, the most general term embracing all the structures in question would be "undulipodium". Nevertheless a rigorous rejection of the terms "cilium" and "flagellum" would possibly introduce more chaos than profit.

Ciliary movements and its role in the motor responses of *Paramecium*

Recently the most developed and experimentally documented theory of ciliary movement of *Paramecium* has been presented by Párducz (1967 and other works from 1954–1964). Its essential theses may be summarized as follows:

1. Autonomous rotation is the only type of cilia movements stimulated by mechanism within a cilium. Cilia rotate in a non-coordinated manner and—in consequence—*Paramecium* remains motionless.

2. Metachronal (coordinating) impulse of endogenous origin are passing constantly over the cortical ectoplasm of a normally functioning *Paramecium*. They reach single cilia and evoke effective stroke of cilia. Endogenous impulses enable protozoon to perform any progressive movement and gaining food. In

swimming forwards (FLS, FRS), the metachronal impulses are produced in the caudal part of protozoon and proceed forwards. In the course of continuous ciliary reversal (CCR), direction of their way becomes inverse.

3. To every coordinated type of motor responses e.g. FLS, FRS, CCR corresponds a definite pattern of disposition of metachronal waves on the cell surface (Pl. I 1, 4). The structure of ciliary waves, their profile and direction of propagation are basic source of information for the analysis of motor responses in *Paramecium.*

A number of essential arguments may be put forward against every of all the theses cited above. Those arguments permit simultaneously to formulate an interpretation of ciliary activity in *Paramecium* different from the concept of P á r d u c z.

1. It has been ascertained previously that the same undulatory form of beat appears as well when cilium is a part organism as after its isolation from the cell. This indicates that each cilium is an independent oscillator with its own excitating mechanism. The classical postulations of V e r w o r n and G r a y remain fully valid for *Paramecium.* Autonomy of cilium in propulsive functions is of course not full. The cell, as provider of energy, acts by means of metabolic rate on the beating frequency of cilium. In the motor responses, this involves only the changes in the swimming rate of protozoon, or in effectiveness of food intake. The cellular control mechanisms of propulsive functions of cilium are limited to the above facts.

2. The conicoidal movement (autonomous rotation after P á r d u c z)—which appears in all the stationary *Paramecia*—is effective and may be accompanied by metachronism. On the other hand, M i l i c e r 1935 already ascertained that in paramecia remaining in an unchanged medium, the pattern of metachronal waves undergoes constant changes without exerting influence upon their motor responses. In the specimens remaining in the culture liquid, the percentage of individuals manifesting metachronism fluctuates from 4–90%. Basing on those observations, M i l i c e r drew the conclusion: "Metachronal waves are presumably only an expression of regulation between cilia, however their role in the progressive movement of the ciliate seems doubtfull".

The publication of M i l i c e r has been overlooked by majority of protozoologists however the recent studies supported her views. According to the observations of P á r d u c z 1954 the postero-anterior direction of propagation of metachronal waves is characteristic for the movement of the type FLS or FRS. However in fact, in the media of increased viscosity, the direction of metachronal waves propagation undergoes inversion (S l e i g h 1966). The direction of spiralling in CCR is the same independently of the medium viscosity (G r ę b e c k i et al. 1967 a).

In methyl cellulose solutions, the continuous ciliary reversal (CCR) is accompanied by metachronal waves passing from the caudal cell part forwards, whereas in salt solutions—e.g. KCl—the direction of their movement is inverse. Consequently the different directions of metachronal wave propagation correspond to the same types of motor responses.

It has been stated on the base of films (K u ź n i c k i et al. 1969 b) that as well in the case of stationary as in swimming *Paramecia*, appearing and vanishing of metachronal waves depend exclusively of the movement rate of cilia and of the medium conditions. In 1.2% solutions of methyl cellulose, metachronal waves may fully disappear without any influence on the motor

responses of *Paramecium*. The absence of associations between the motor responses of *Paramecium* and metachronism, together with the simultaneous dependence of appearing of metachronal waves and directions of their propagation of the beating rate of cilia and of the medium viscosity—present strong arguments that coordination is purely mechanical. This would be the same mechanism which conditioned metachronism of flagella in *Trichonymphida* or metachronism of symbiotic spirochetes on the surface of the flagellate *Myxotricha paradoxa* (Cleveland and Grimstone 1964). The only controversive phenomenon of *Paramecium* is the postero-anterial direction of metachronal wave course during FLS and the antero-posterial one in CCR. This problem remains unsolved and its solution—independently of results—would clarify only the types of mechanical interaction of undulipodia. The absence of connection between metachronism and the motor responses seems however to be sufficiently documented, similarly as the fact that propagation of metachronal waves or the ciliary beat itself are not accompanied by any changes of the membrane potential. The only manifestation of ciliary activity correlated with membrane depolarization is the ciliary reversal (Yamaguchi 1960, Kinosita et al. 1964, Kinosita et al. 1965, Naitoh 1966). The conception of metachronal impulse has consequently no ground.

3. The leading idea of the presented concept is the demonstration that the body and oral groove cilia in *Paramecium* perform two functions: a. as propulsing organelles, b. as organelles steering the cell movement. The first function manifests either as a travelling helical or spiral wave, the second one—as the changes in cilium position in relation to the long body axis. The most frequent and most extensively studied form of change in the cilia position is the ciliary reversal (Pl. VI 61–63, Pl. IX). If cilia initiate their undulating movement in a new position, the protozoon swims with its posterior body end forwards (CCR). Ciliary reversal is a manifestation of undulipodia activity which is—in the first place—under the cellular control—as a result of changes in the state of excitation of the cell. Both function of cilium of propulsing and of steering—show a distinct diversity which is manifested in: a. possibility of excluding one of them when the other is fully preserved, b. both functions are not performed simultaneously, c. the geometry of cilia reversal and the forms of cilia beats are different, d. they have different ionic activation mechanisms. The facts supporting those theses will be successively presented.

Worley 1934 noticed after having acted on paramecia with novocaine, that under the action of this narcotic the capability of reversal disappears at first, then metachronism, and at last the movement of cilia ceases. On this base he put forward a hypothesis of existence of three different mechanisms responsible for the above manifestations of ciliary activity. The recent investigations have demonstrated that inhibition of ciliary beat may also precede the inhibition of capability of reversal.

Kuźnicki 1963 b studying the influence of Ni^{2+} on *P. caudatum* stated that during the physiological immobilization, the motionless ciliates perform, at several intervals, withdrawal for a distance not exceeding their body length, and a return to their exit position. Those shiftings are involved by a synchronic transit to the reversal position of cilia, and then—to their exit position on the whole body surface. This result suggested that in paramecia immobilized by Ni^{2+}, the capability of ciliary reversal is preserved.

This postulation has been documented experimentally by Naitoh 1966

and Grębecki and Mikołajczyk 1968. *Paramecia* remained in solutions of $NiCl_2$ to the stage of traumatic immobilization, when not only the beat of cilia but also the spontanic reversal are fully annihilated. Cilia of those immobilized individuals subjected to the action of direct current or of chemical stimulation (KCl, $BaCl_2$) behaved in the same manner as in the free-swimming organisms. In the immobilized paramecia, the transit of cilia to the reversal position was associated with the simultaneous depolarization of membrane (Naitoh 1966).

Owing to the method of direct observation of cilia on the cell surface, not only the behaviour of undulipodia in swimming could be registered on film but also the transit from FRS to CCR and to FRS again (Kuźnicki et al. 1969 a, 1970). For gaining a material for comparison—applying the high speed cameras—behaviour of *Paramecia* in PCR induced by Ba^{2+} ions was also photographed. As well in methyl cellulose solutions as in $BaCl_2$+ +$CaCl_2$ solutions, ciliary reversal is expressed in a simultaneous change of position by all the cilia, on the whole cell surface. Keeping paramecia in 1.2% methyl cellulose solutions for over 24 hr may evoke a short-lasting stop of ciliary beating. However the non-beating cilia, still preserve their capability of reversal and of their return to the "forward" position which occurs similarly as in swimming individuals (Pl. IX).

Most essential conclusion following from the above results has been the ascertaining that the undulating cilium movement and its reversal are not only independent but mutually excluding phenomena. In a situation when any of mechanisms is not hampered, cilium is not capable of beating and changing its position simultaneously. During the transition from the "forward" position to reversal, the travelling helical waves of cilia fully disappear and reappear after the transit to a new position. The geometric feature of movement is so different in both cases (compare Pl. V and VII with IX) that it suggests the existence of two separate kinds of filaments interaction within cilium for every type of movement. As far as this conclusion is only a postulation, so the existence of two different ionic mechanisms of activation seems to be certain. On the saponine models of protozoa (Seravin 1961, Seravin et al. 1965) or after separation of cilia from the cell (Gibbons 1965), ATP+Mg^{2+} are the factors activating the movement of cilia. The ciliary reversal on the glycerol models of *Paramecium* may be evoked only by Ca^{2+}+ATP, or Zn^{2+}+ATP (Naitoh 1968).

Ciliary reversal is a universal reaction of *Paramecium* to chemical, electric, thermic and mechanical stimuli. It may be total as during the avoiding reaction (which in turn presents a mechanism of negative or positive chemotaxis or thermotaxis) or may be partial as during galvanotaxis or PaCR.

In the reversal position, cilia may perform propulsive movements for a rather long time period (CCR) or return rapidly to their exit position (avoiding reaction). A constant alternation may also occur of reversal position and return to the forward position (PCR).

A problem of essential importance for elucidation of bases of the excitation processes of cell, of control of the ciliary activity and of the ciliate behaviour—is the knowledge of reversal mechanism and of the factors which involve in some cases the beat of cilia in reversal position for a long time (CCR), and their rapid return to the normal position in the others.

The phenomenon of ciliary reversal—exactly of CCR—has recently been

investigated best by the study of movement reactions following the chemical stimulation. This field of research will constitute the essence of the further course of the present discussion.

The role of calcium ions in excitability of *Paramecium*

The first studies on CCR stimulated chemically were carried out by Jennings (1897, 1899). When paramecia have been placed in KCl solutions (of concentrations from over ten to several tens mM), a continuous ciliary reversal appeared immediately embracing always 100% of individuals. The subsequent studies (Mast and Nadler 1926, Kamada 1938, 1940, Oliphant 1938, 1942, Kamada and Kinosita 1940) revealed a various capacity of inorganic ions of stimulating CCR and a distinctly different action of Ca^{2+}. It was ascertained that the majority of monovalent cations evoke CCR prolonged for several tens of seconds whereas among the bi- and trivalent cations, this capacity is manifested by Ba^{2+} and Mn^{2+} ions. All the anions proved to be fully uneffective unless they simultaneously involved changes of pH. In contrast to the latter ones, Ca ions were not neutral. This ion proved to be the only antagonist against all the factors evoking CCR. It raised the threshold of excitability in paramecium in the field of direct current and inhibited the cathodal reversal. The continuous ciliary reversal evoked by K^{1+} ions may be at any moment interrupted by introduction of Ca^{2+} into the medium. However a full lack of calcium ions in medium suppressed any excitability. Even after application of high K^{1+} concentrations, CCR could not be evoked.

It was evident in the light of those facts that Ca^{2+} ions should play a role in the motor responses of *Paramecium* which was supported by the microinjection experiments (Kamada 1938). Injection of KCl into cytoplasm was fully ineffective but introduction of oxalates into the cell evoked inversion. On the base of this, Kamada 1940 put forward the hypothesis that in cytoplasm, ion Ca^{2+} is bound with a rather unprecised anion X. The external action of K^{1+} (in result of diffusion) or internal action of oxalates (in consequence of binding Ca^{2+}) would liberate anion X which initiates CCR.

For a more intrinsic precision of the ionic competition potassium—calcium (Kamada and Kinosita 1940), exact observation were carried out on the time of duration of CCR in *P. caudatum*, in dependence of the difference of ratio K^{1+}/Ca^{2+}. The concentration ratio of both cations in the media evoking reversal (stimulating medium) as well as in the exit ones (adaptation medium) were taken into account. The results failed to reveal any general regularities. They have been demonstrated later by Jahn 1962. He calculated the data of Kamada and Kinosita 1940 not from the point of view of a direct dependence $\frac{[K^{1+}]}{[Ca^{2+}]}$ but in the terms of Gibbs–Donnan equilibrium $\frac{[K^{1+}]}{\sqrt{[Ca^{2+}]}}$.

Then it was ascertained that for the individuals originating from a definite adaptation medium, the maximal time of CCR duration is always the same independently of the absolute ion concentration if

$$\frac{[K^{1+}]}{\sqrt{[Ca^{2+}]}} = \text{const.}$$

On this ground, considering simultaneously the fact that Ca^{2+} is antagonistic against all the factors evoking reversal Jahn 1962 put forward a hypothesis that the membrane of *Paramecium* has properties of a physical cation exchanger, and that the ciliary reversal is involved regardless of the cause of the loss by the loss of divalently bound cation, usually Ca^{2+}, associated with the membrane.

On the surface of *Paramecium*, there are fixed negative charges (e.g. carboxyl, phosphate groups). At a physiological ion concentration, each of those sites has an associated cation H^{1+}, Na^{1+}, K^{1+}, Ca^{2+}, Mg^{2+} which are the main components of media in which protozoa live. The associated cations are subjected to thermic movement and may be exchanged for cations of the same kind or others. According to the concept of Jahn 1962, 1967 during the maximal time of CCR evoked by K^{1+} ions, the state of membrane is characterized by constant ratio of calcium and potassium ions adsorbed on it, according to

$$\frac{[K^{1+}]_s}{[Ca^{2+}]_s} \propto \frac{[K^{1+}]_m}{\sqrt{[Ca^{2+}]_m}}$$

where: s—surface, m—medium.

This is of course correct if we assume that Ca^{2+} is "bound" by both valences. The idea of applying the Gibbs–Donnan ratio to explanation of mechanism of ciliary reversal (Jahn 1962) initiated a now course of investigation.

It was revealed among others (Grębecki 1965, Kuźnicki 1966 a) that not only FRS and CCR but also other types of motor responses (PCR, PaCR) depend of the level of calcium ions associated on the membrane. The gradual decalcification of the cellular membrane complex in *Paramecium* may be evoked by the agents: precipitating (oxalates), binding (citrates) or chelating (EDTA), and involves the following sequence of reactions: FLS → PCR → CCR → PaCR → FLS (Grębecki 1965). This sequence is reversible. A new gradual increase of calcium ions level in medium evokes appearing of all the above types of motor reactions in an inverse sequence. Similar regularities were stated (Kuźnicki 1966 a) in the case if action of uni- and bivalent inorganic cations (K^{1+}, Rb^{1+}, Cs^{1+}, Tl^{1+}, Ba^{2+}, Sr^{2+}, Mn^{2+}).

The motor responses of *P. caudatum*, manifested directly after addition to the medium of any of the inorganic cations cited above may be brought to four fundamental types FLS, PCR, CCR, PaCR. Those reaction types appear in a definite sequence in proportion as the concentration of cation increases. In dependence of the kind of cation and of calcium level in medium, motor responses may produce a sequence FLS → PCR → CCR → PaCR → FLS (identical with the sequence produced by chelating and calcium precipitating agents), or a reduced (e.g. FLS → PCR → CCR) or a modified one (Fig. 8). Those modifications concern in the first place the change of sequence of appearing of PaCR. Each series of motor reactions independently of the ion, may be inversed by a gradual increase of Ca^{2+} in medium.

As conclusion of those results, Kuźnicki 1966 a put forward a hypothesis that all the motor responses of *Paramecium* evoked by chemical stimulation are involved by desorption of calcium ions from the outside membrane of ciliate.

This theory has been supported by Naitoh and Yasumasu 1967. Those

authors, applying the calcium isotope ^{45}Ca described exactly a cation exchange phenomenon in the binding of K^{1+}, Na^{1+}, Rb^{1+}, Mg^{2+}, Ba^{2+} taking place on the membrane of living paramecia.

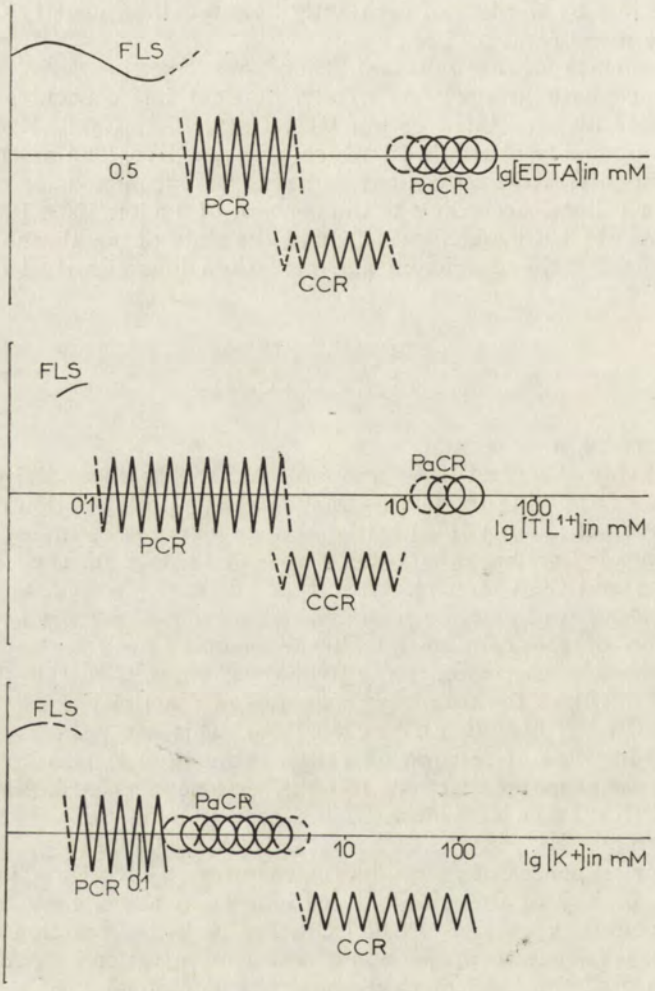

Fig. 8. Sequence of motor responses of *Paramecium caudatum* varying according to: EDTA, Tl_2SO_4 and KCl concentration. Diagrams represents the behaviour of ciliates in the first minute following the chemical stimulation. Levels of Ca^{2+} in medium for EDTA—0.1 mM, for Tl^{1+} and K^{1+}—0.5 mM. Result of EDTA after Grębecki 1965, of Tl_2SO_4 and KCl after Kuźnicki 1966a

The maximal saturation of all the anionic sites on *Paramecium* occurs at concentration 0.6 mM of Ca in medium (Naitoh 1968). This author, basing on his own empiric data, established the equation determining the quantity of adsorbed Ca^{2+} by the whole *Paramecium* membrane in the case when other cations besides calcium occur also in medium calcium:

$$[PCa\tfrac{1}{2}] = \frac{P_t}{kJ_a+1}$$

in which $PCa\frac{1}{2}$ represents the amount of calcium bound by the cation exchange system,
P_t—the total binding capacity of the system,
k—the equilibrium constant

J_a—the ratio $\frac{\text{Monovalent cation}}{\sqrt{[Ca^{2+}]}}$ or $\frac{\sqrt{[\text{Bivalent cation}]}}{\sqrt{[Ca^{2+}]}}$ in the medium

It follows from the above equation that the amount of calcium ions bound on the surface of *Paramecium* is correlated with the J_a value. An increase in J_a leads to a fall of calcium bound by the *Paramecium* surface.

The key-role of calcium ions in excitability of protozoan cell my be considered as documented. This does not mean however that the mechanism of motor responses of *Paramecium* were elucidated. The diversity of views in this field is very significant. Jahn 1962, 1967, after having ascertained that loss of Ca^{2+} from the outer surface of *Paramecium* membrane evokes CCR, obviously avoided the answer which is the physiological association between those two phenomena. He stressed only in his considerations that the hypothesis proposed by him—in contrast to the concepts of the recent cytophysiology—does not posulate the necessity of ions penetration across cell membranes as an indispensable condition of occurrence of excitation phenomena (ciliary reversal) in the protozoan cell. Grębecki 1965, and Kuźnicki 1966 a raised objections as well against the former theory of Kamada 1938, 1940, as against the recent one (Jahn 1962) concerning the loss of calcium ions. *Paramecia* in which the superficial cellular complex has been highly decalcified, become fully insensible to any chemical stimulation except Ca^{2+}. A gradual calcification of medium involves appearing of CCR and of the other motor response types in a sequence inverse to that which took place during decalcification. According to Grębecki 1965, not the process of calcium loss itself but its actual level remaining in the anionic sites on the membrane surface, defines the degree of stimulation i.e.—the type of motor response. In this way, according to Grębecki 1965 the theory postulating analogy between ciliary reversal and muscle contraction, has no foundation.

A fully opposite view has been expressed by Naitoh 1968 who criticized the experiments of Grębecki 1965 with EDTA. EDTA as well as other factors chelating or binding the external calcium fail to evoke—in his opinion—CCR in *Paramecium*. Ciliary reversal takes place as result of contraction of a contractile system which has not been identified by the author. This contraction is expressed in the change of ciliary position. Calcium ions liberated from the external surface of *Paramecium*, penetrate across the membrane and activate ATP-ase of the ciliary contraction system. Ca^{2+} released from the cation exchange system, would be the factor controlling ciliary reversal in a manner analogous to its role in the muscle contraction. The duration of CCR is related to the time of release of bound Ca^{2+}.

Kuźnicki and Mikołajczyk 1969 proved in experiments the hypothesis of Naitoh. It was ascertained that EGTA the specific chelator of calcium evokes CCR of *Paramecium* in the limits of concentration 0.05–0.15 mM. In solution of EGTA, CCR of *Spirostomum ambiguum* may be pro-

longed up to 21 min. A conclusion follows, that Ca^{2+} ions released from the sites of cation exchange system of ciliates cannot perform any other direct function in the ciliary reversal.

Mechanism of ciliary reversal

The present theories of ciliary reversal and of the motor responses associated with it in *Paramecium*, seem to be either not full or not convincing. A demonstration that the propulsive functions of cilium and its function of steering possess some different and mutually excluding mechanisms, placed this problem in another light. In the first place, it became evident that ciliary reversal and CCR are not uniform phenomena. The first one is a reaction of undulipodia to the state of excitement (if the stimulus is local and weak) or of the whole ciliature, the second one—is a consequence of persisting of this state with a simultaneous initiation of propulsing functions by cilium.

The theory of Naitoh 1968 that ciliary reversal (in the meaning of the change of cilium position) is analogous to muscle contraction seems to be a postulation of solid grounds.

The contemporary views (Szent-Gyorgyi 1968, Perry 1968) on muscle contraction may be summarized as follows:

1. Contraction is a result of cooperation of actine, myosin and ATP,
2. Contraction takes place in result of sliding mechanism which—in this way—fails to secure a permanent change of length or of configuration of protein molecules,
3. Calcium ions are an activating agent of the whole system i.e. appearing of Ca^{2+} ions near the myofilaments is the indispensable condition for contraction.

Analogy between ciliary reversal and muscle contraction cannot evoke objection. The difference between the "reversal" contraction and the undulating cilium movement (which evidently is also involved by the mechanism of filaments contraction) consists in the differences in the ionic sources of their activation. Reversal is activated by Ca^{2+} and, consequently, the essential problem of this process is appearing of calcium ions activating the contraction of myofilaments.

Out of two diametrically different theories (Jahn 1962, 1967, Naitoh 1968) the first one seems to be justified.

For appearing of reversal, penetration of ions across the membrane into the cell is not indispensable. Depolarization of membrane is of course accompanied by increase of permeability but penetration is a secondary phenomenon and has no influence upon the filament contraction of undulipodia.

The continuous ciliary reversal (CCR) may be interrupted at any moment by the increase of external concentration of Ca^{2+}. Considering this fact for support of his hypothesis, Naitoh 1968 postulated that only the ions released of the *Paramecium* surface are able to penetrate to the cell inside. For the free Ca^{2+} ions, membrane is a unpermeable barrier.

Another interpretation of reversal mechanism is more compatible with the present state of knowledge concerning the relations between excitation and contraction. Cilia, as well as the whole cell, are covered by a continuous plasma membrane. In the immediate vicinity of the cilium shaft and kineto-

some embedded in cytoplasma, occur the intracellular plasmic membranes producing the so-called alveoles. Those membranes are presumably the zones of direct contact with the external membrane (Fig. 3). Liberation of calcium ions adsorbed on the outer surface of membrane is a trigger mechanism promoting the release of calcium ions occurring on the inside surface of plasma membrane, as well as on the intracellular membrane system. The liberated calcium ions would penetrate directly to the zone of cilia filaments and evoke their contraction expressed in reversal. Reversal mechanism would be the same as the mechanism of muscle contraction (P o d o l s k y 1968).

For the actinomyosin systems of smooth and striated muscles, a change of Ca^{2+} ion concentration in the limits 10^{-5}–10^{-7} M, corresponds to the state of maximal contraction and full relaxation (P e r r y 1968, R ü e g g 1968). No reasons exist for assuming that for the ciliary filaments this scale is not similar or identical. The quantity of calcium on the intracellular membranes of the cortical zone of protozoon is fully sufficient for evoking a contraction reaction manifested in the change of cilia position. Consequently from this point of view as well, the postulation of compulsory penetration of calcium ions into the cell—becomes not necessary.

G r ę b e c k i 1965, and D r y l and G r ę b e c k i 1966 not only contradicted the analogy between muscle contraction and ciliary reversal, but stressed simultaneously the ionic distinctness of excitation processes in fresh-water protozoa and in the metazoa cells. In *Paramecium*, the membrane depolarization evoked by chemical or electric stimulation occurs as well in conditions of a full absence of Na^{1+} ions as of any monovalent cations in the medium.

Until recently, a general conviction existed that this phenomenon cannot occur in the cells living in conditions of "high salinity". T a s a k i 1968, T a s a k i et al. 1969 demonstrated on the classical object which are the giant axons of *Loligo* (exactly their models) and that this thesis has no foundation. The only condition for appearing of the excitation processes is the presence of monovalent cations inside the cell, and of bivalent cations (usually calcium) in medium. This means that not only the phenomena of contraction but also the excitation processes on the cellular level occur according to the same pattern.

The time of duration of continuous ciliary reversal in *Paramecium* depends of the amount of calcium ions released from the anionic sites on the external membrane surface. CCR is however a type of motor response which disappears in any case after several tens or several hundreds of seconds although the factor which has evoked it is still present in medium. In contrast to CCR, the reactions of the PCR nature which undergo permanent alternation: "forward--swimming–backward-swimming" at intervals of several seconds, may persist with no change for tens of hours (much longer in methyl cellulose solutions).

This is the more striking that keeping cilia in the reversal position does not presumably require any energy (similarly as the state of prolonged contraction in smooth muscles of many molluscs—R ü e g g 1968), inversely to the permanent changes of the swimming direction.

K u ź n i c k i 1966 a remarked that this difference in the behaviour of *Paramecium* is the principal problem and without the study of its nature, the problem of mechanism of motor responses in ciliates cannot be solved. For the phenomenon of spontaneous disappearing of reversal the term accommodation was proposed (J a h n 1961). This would include however this pheno-

menon to the same type of processes as accommodation in nerve (sensoric adaptation)—which was strongly stressed by J a h n 1967. This process remains not elucidated in both cases.

The spontaneous disappearing of CCR may also be determined as relaxation. Reversal is correctly interpreted as contraction. This fails to solve the problem itself before we know why contraction and relaxation alternating at short intervals of time (PCR) do not change the threshold of cell excitability and show no signs of adaptation (accommodation, habituation) whereas after a spontaneous disappearing of CCR ciliates behave in a different manner. Any phenomena of accommodation are—no doubt—manifestations of homeostatic phenomenon. Their knowledge is one of the most important problems of the present cytophysiology.

Summary

The most complete theory of ciliary movement and coordination in *Paramecium*, has been presented by P á r d u c z in the series of his works from 1954–1967. The author of the present study objects the fundamental theses of this theory after having ascertained the following facts:

1. In swimming *Paramecium*, the body and oral groove cilia beat with a travelling helical wave from the base of undulipodium to its tip. In stationary *Paramecium*, the beat of cilia is conicoidal. The criterion of distinguishing cilia from flagella, based on geometry of their movement, is deprived of real foundation.

2. The conicoidal beat of cilia (autonomous rotation according to the terminology of Párducz) may be accompanied by regular metachronism.

3. No distinct association exists of the metachronal waves pattern and direction of their propagation on one, and motor responses of *Paramecium*—on the other side.

4. The postulation of existence of endogenous origin of metachronal impulses passing at regular intervales over ectoplasm along the periphery of the cell finds no support in facts. Metachronism in *Paramecium* may be most convincingly explained by a mechanic interaction of cilia.

5. Pictures gained by the instantaneous fixation technique are artifacts, not being objective reflection of cilia behaviour in the course of motor responses of *Paramecium*.

The leading idea of the present article has been to demonstrate that the body and oral groove cilia of *Paramecium* perform two functions: 1. as propulsing organelles, 2. as organelles steering the cell movement. The first function has an activation mechanism within the cilium itself and manifests the form of cylindrical or conicoidal helical wave. Steering functions appear in the changes of cilia position related to the long body axis of *Paramecium*. Ciliary reversal is the most frequent and best investigated manifestation of cilia functions which is preceded by the change of state of cell excitability. Consequently, ciliary reversal is a type of cilia activity which is under the control of the cell.

Propulsing and steering aspects of the cilium function are also expressed in: a. possibility of exclusion of one of them while the other one is fully preserved, b. the fact that both functions are not performed simultaneously, c. and have different ionic activation mechanisms.

Ciliary reversal is in *Paramecium* a general reaction to the stimuli which desorbe calcium ions from the plasma membrane of cell. Liberation of calcium ions is a trigger mechanism promoting the desorption of calcium ions from the internal surface of plasmic membranes and from the intracellular system of membranes. Liberated calcium ions penetrate to the zone of myofibrils and evoke their contraction which is expressed in ciliary reversal. Consequently, the mechanism of reversal would be fully analogous to muscle contraction.

STRESZCZENIE

Teoria ruchu rzęskowego i mechanizmów jego koordynacji u *Paramecium* została najbardziej wszechstronnie opracowana przez Párducza (seria prac z lat 1954–1967). Autor kwestionuje jej zasadnicze tezy wykazując, że:

1. U pływającego *Paramecium* rzęski somatyczne i peristomalne biją w postaci fali śrubowej, wędrującej od podstawy undulipodium do jego wierzchołka. W stanie stacjonarnym forma ruchu rzęsek ma charakter stożkowaty. Zasada odróżniania rzęsek od wici na podstawie geometrii ich ruchu pozbawiona jest obiektywnych podstaw.

2. Rzęskom wykonującym ruch stożkowaty (autonomiczną rotację wg terminologii Párducza) może towarzyszyć regularny metachronizm.

3. Nie istnieje żaden jednoznaczny związek między wzorcami fal metachronicznych i kierunkiem ich rozchodzenia się a reakcjami ruchowymi *Paramecium*.

4. Założenie o istnieniu stale przebiegających po ektoplazmie endogennych metachronalnych impulsów nie znajduje potwierdzenia w faktach. Metachronizm u *Paramecium* najbardziej przekonywająco daje się wyjaśnić mechanicznym współdziałaniem między rzęskami.

5. Obrazy uzyskiwane metodą szybkiego utrwalania są artefaktami, a nie obiektywnymi odwzorowaniami zachowania się rzęsek podczas reakcji ruchowych.

Ideą przewodnią pracy jest wykazanie, że somatyczne i peristomalne undulipodia *Paramecium* spełniają dwie funkcje: 1. organelli napędowych, 2. organelli sterujących ruchem pierwotniaka. Pierwsza posiada mechanizm aktywacji w obrębie samej rzęski i przejawia się w formie cylindrycznej bądź stożkowatej fali śrubowej. Sterujące funkcje przejawiają się w zmianach położenia rzęsek w stosunku do podłużnej osi ciała. Najczęściej występującą i najlepiej poznaną funkcją sterującą undulipodiów jest rewersja rzęskowa. Rewersję rzęskową poprzedza zmiana stanu pobudzenia komórki—jest to forma aktywności ruchowej, znajdująca się pod kontrolą komórkową.

Napędowa i sterująca odrębność funkcji rzęski wyraża się również w: a. możliwości wyłączenia jednej z nich podczas gdy druga jest w pełni zachowana, b. obie funkcje nie są wykonywane jednocześnie, c. mają one inne jonowe mechanizmy aktywacji.

Rewersja rzęsek jest uniwersalną reakcją *Paramecium* na bodźce, które wypierają jony wapnia zaadsorbowane na błonie plazmatycznej komórki. Zwolnienie jonów wapnia jest mechanizmem spustowym, wyzwalającym zwolnienie jonów wapnia występujących na wewnętrznej powierzchni błon plazmatycznych oraz z układu błon wewnątrzkomórkowych. Zwolnione jony wapnia dostają się do strefy fibrylli rzęskowych i wywołują ich skurcz wyrażający się rewersją rzęskową. Mechanizm rewersji byłby więc pełnym analogiem skurczu mięśniowego.

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EXPLANATION OF PLATES I–IX

Plate I: Instantaneous fixtion technique. 1—*Paramecium multimicronucleatum* fixed during normal movement (FLS), 2—scheme of disposition of metachronal waves in FLS, 3—pattern of single beating cycle of cilium: 0, 1, a—effective stroke, b, c, d, e, 0—recovery stroke (autonomous rotation), 4—pattern of metachronal waves typical for CCR, 5—cilia performing reversal beat, 6—*Paramecium* treated previously with vapours of chloroform.
Plate II: Conicoidal beat of oral cilia in *P. multimicronucleatum* (portions of successive frames of motion picture). Stationary individual, cilia observes in profile on the edge of body. Speed of camera—720 fps.
Plate III: Effectiveness of conicoidal beat of cilia. 19–38—a series of successive frames of cinemicrograph. Speed of camera—780 fps. Metachronal waves proceed forwards while the bacterial cell is propelled towards cytopharynx.
Plate IV: Picture of cilia on the surface of *P. multimicronucleatum* swimming in a medium of normal viscosity. 39–50—every tenth frame of motion pictures taken at, 960 fps. 43, 44, 45—transition from the normal to reversal position.
Plate V: The body cilia on the surface of swimming *P. multimicronucleatum* in 1.2% methyl cellulose solution. 51–58—series of successive cinemicrographs taken at 50 fps.
Plate VI: Photomicrographs (Polaroid) illustrating different manifestation of ciliar activity of *P. multimicronucleatum*. 59—oral groove cilia, 60—transition of body cilia to reversal position on the anterior body end, 61—normal position of cilia, 62—middle phase of change of cilia positon from the normal one to reversal, 63—cilia in reversal position.
Plate VII: From of cilium beat in swimming *P. multimicronucleatum*. 64–83—successive frames of the same cilium. Transverse white line—portion of a cross hair in the ocular permitting to determine the direction of specimen movement. Speed of camera—50 fps., magnification—1250×, medium—1.2% methyl cellulose solution.
Plate VIII: The form of cilium beat in swimming *P. miltimicronucleatum*, seem in profile on the edge of the body, 84–95—successive frames, speed—50 fps., medium—1.2% methyl cellulose solution.
Plate IX: Ciliary reversal occurring in non-beating cilia (85–104), *P. multimicronucleatum* after having remained over 24 hr in 1.5% methyl cellulose solution, 1000×, speed 50 fps.

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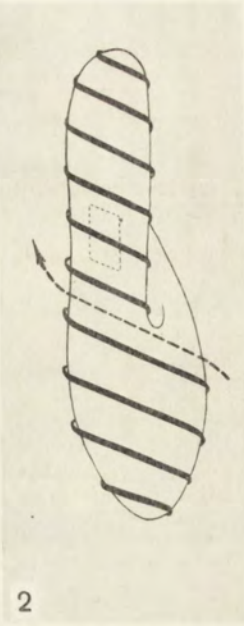

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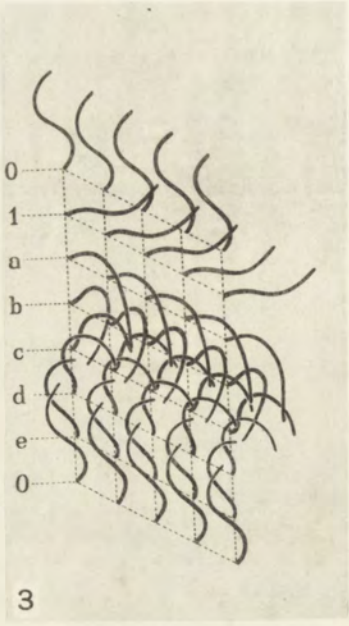

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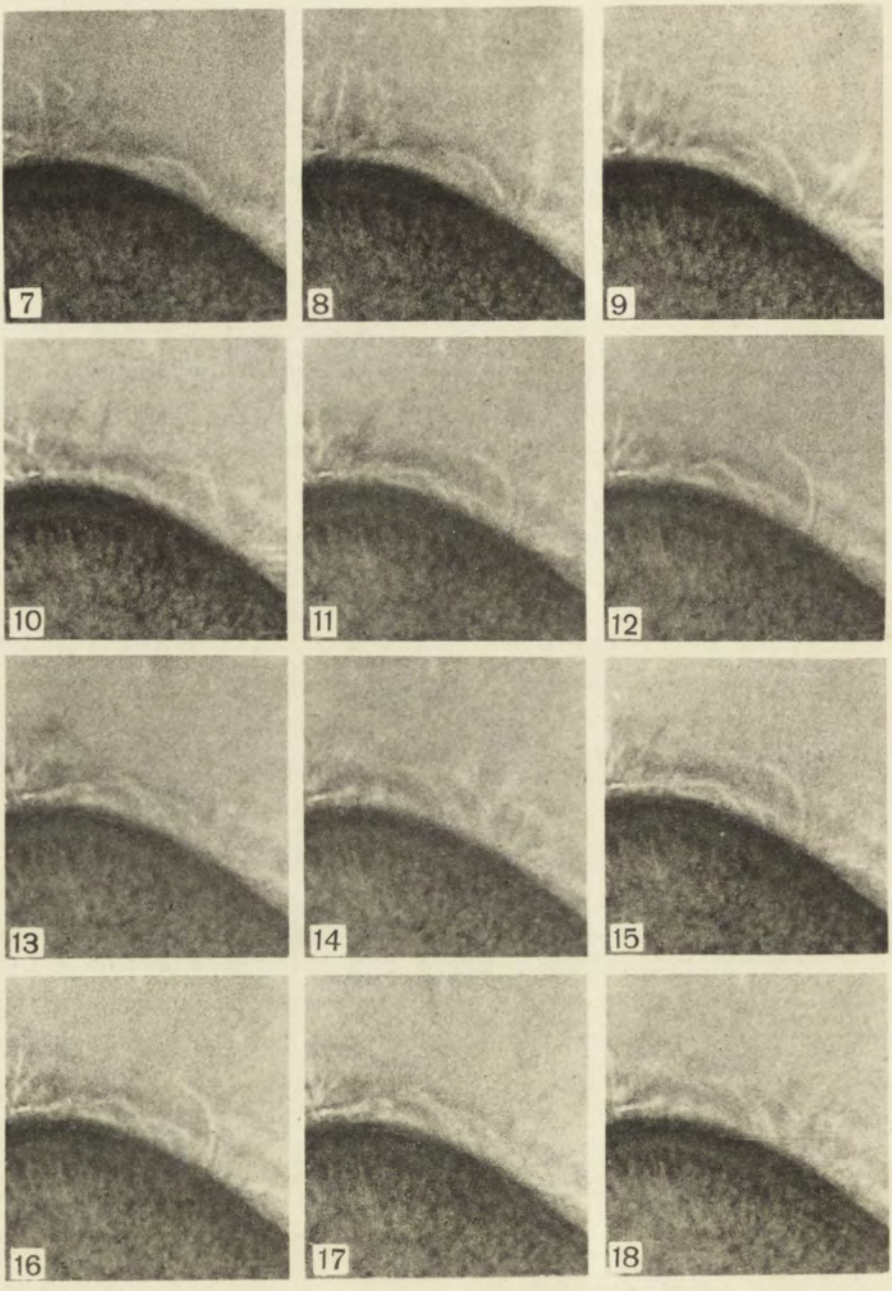

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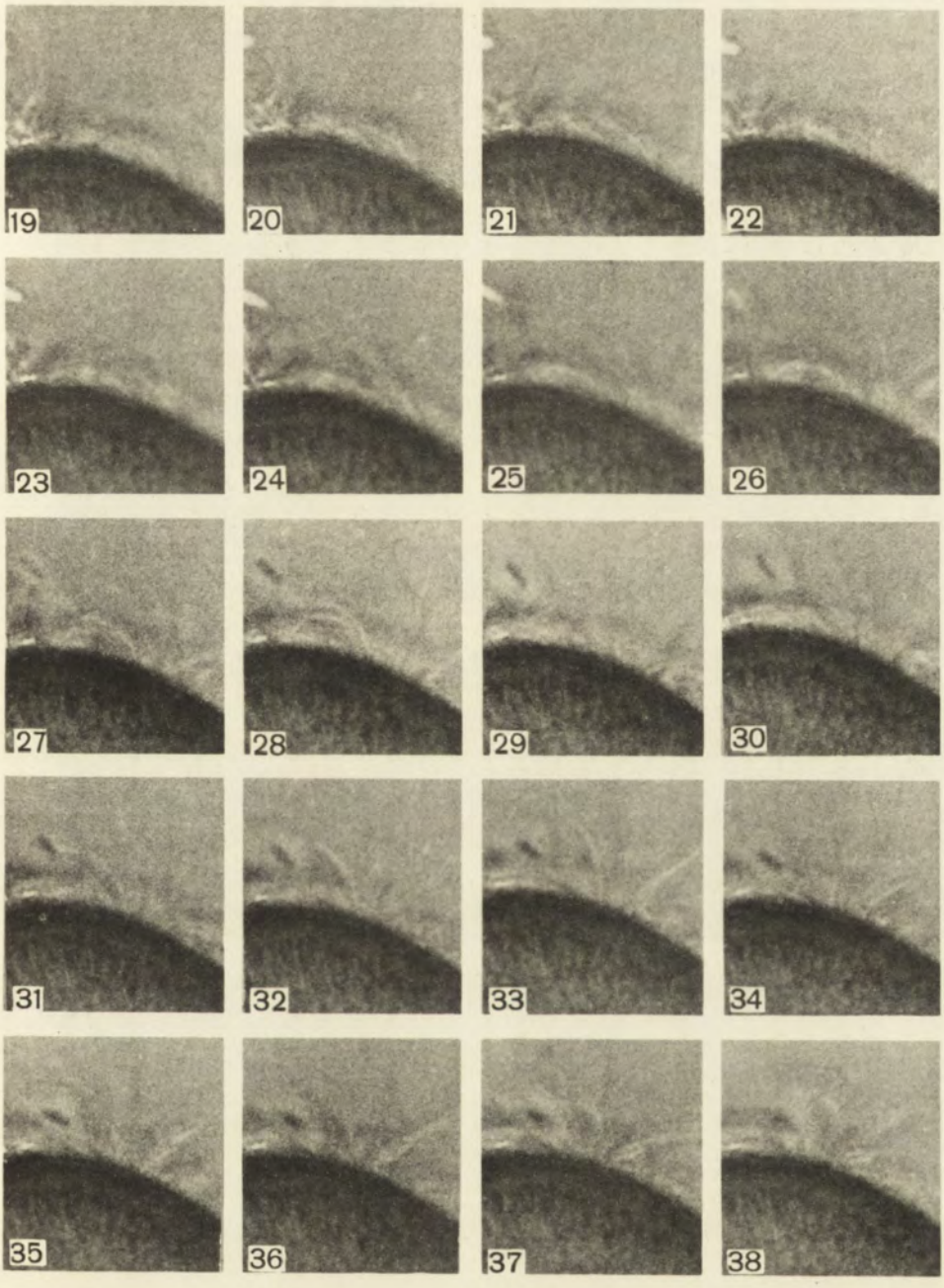

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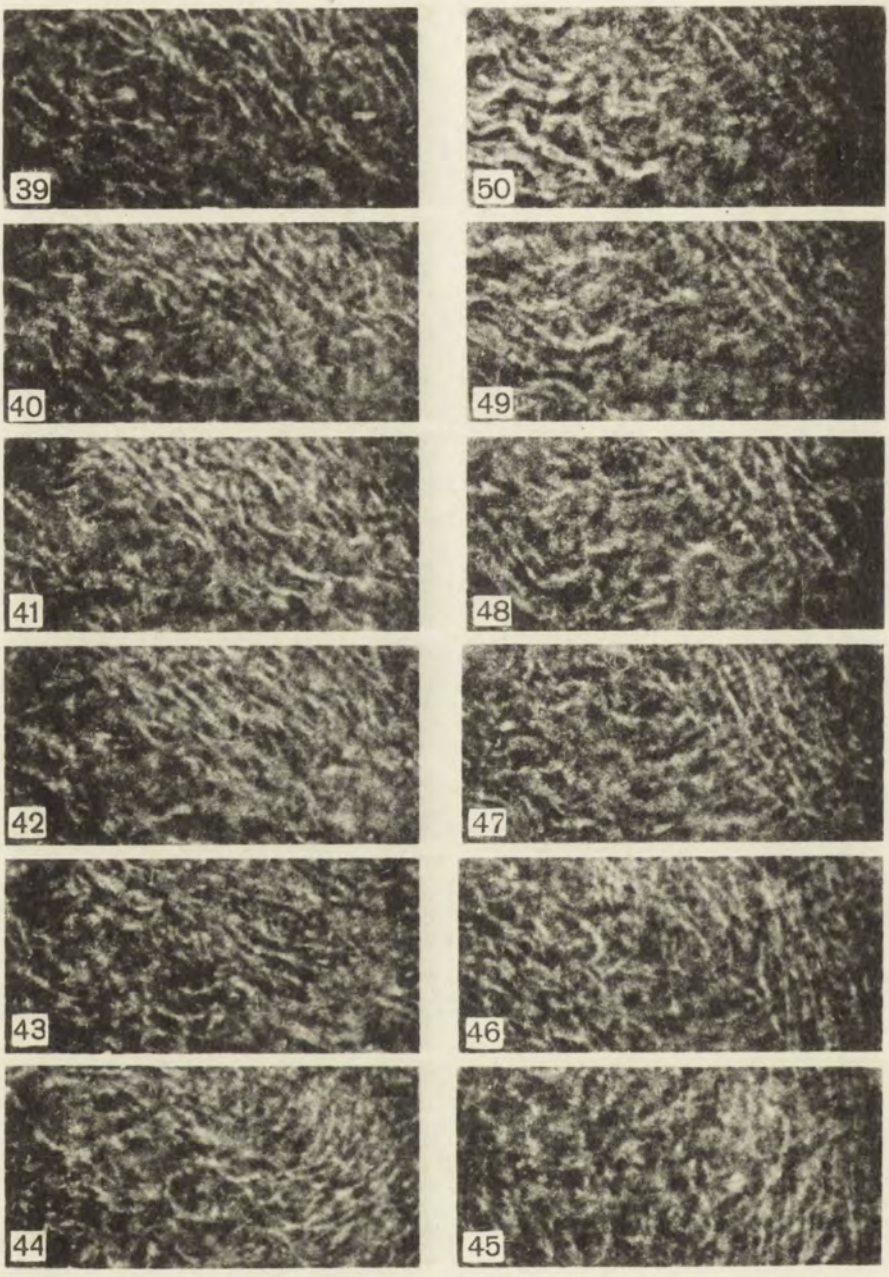

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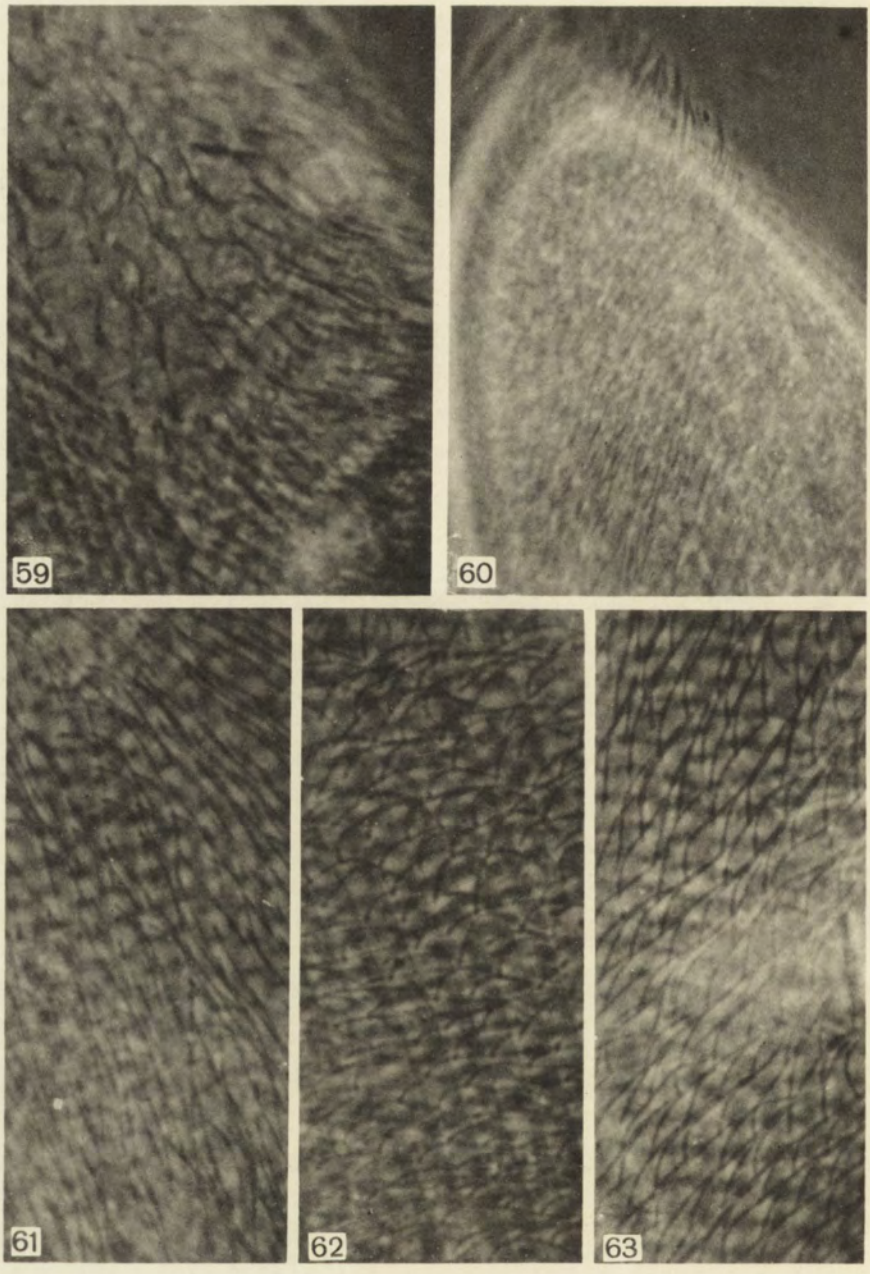

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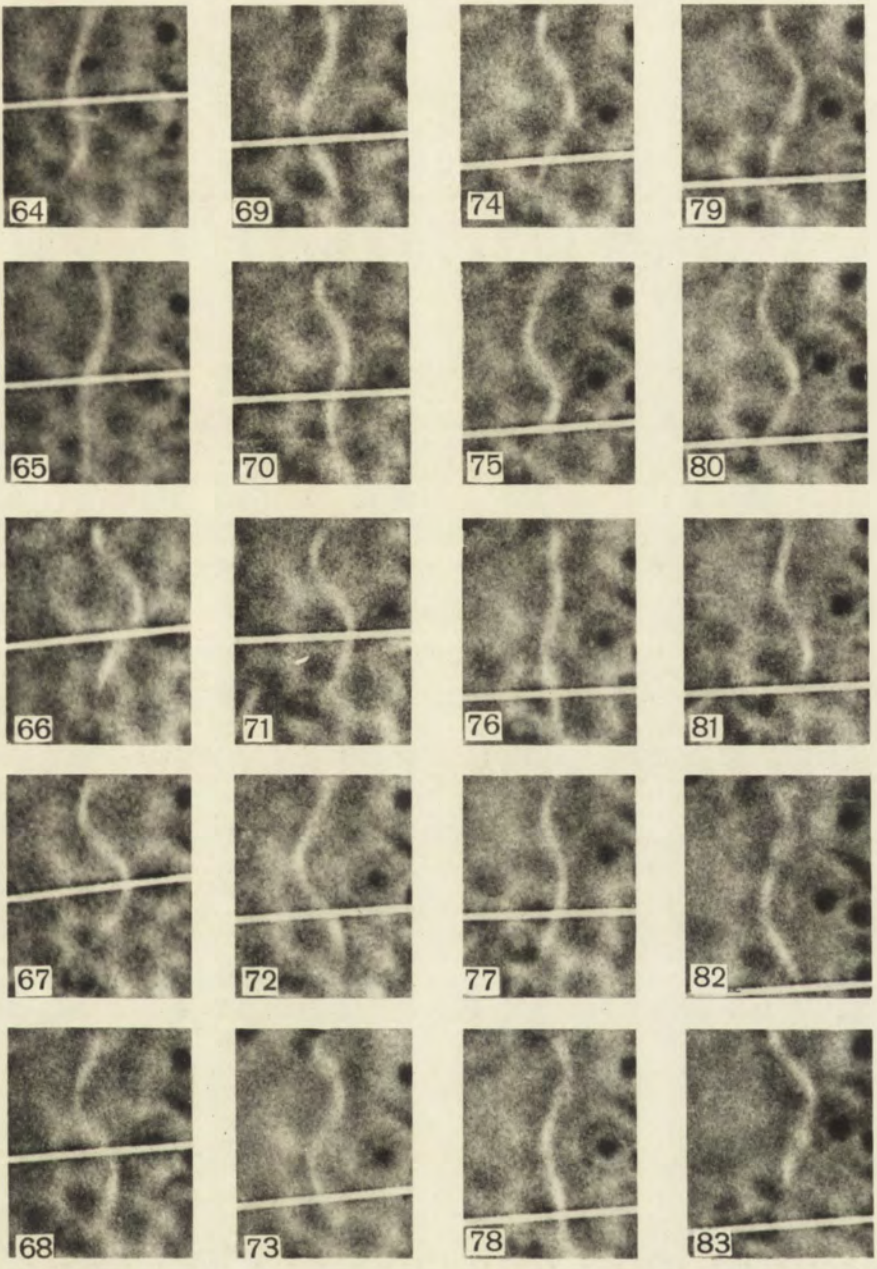

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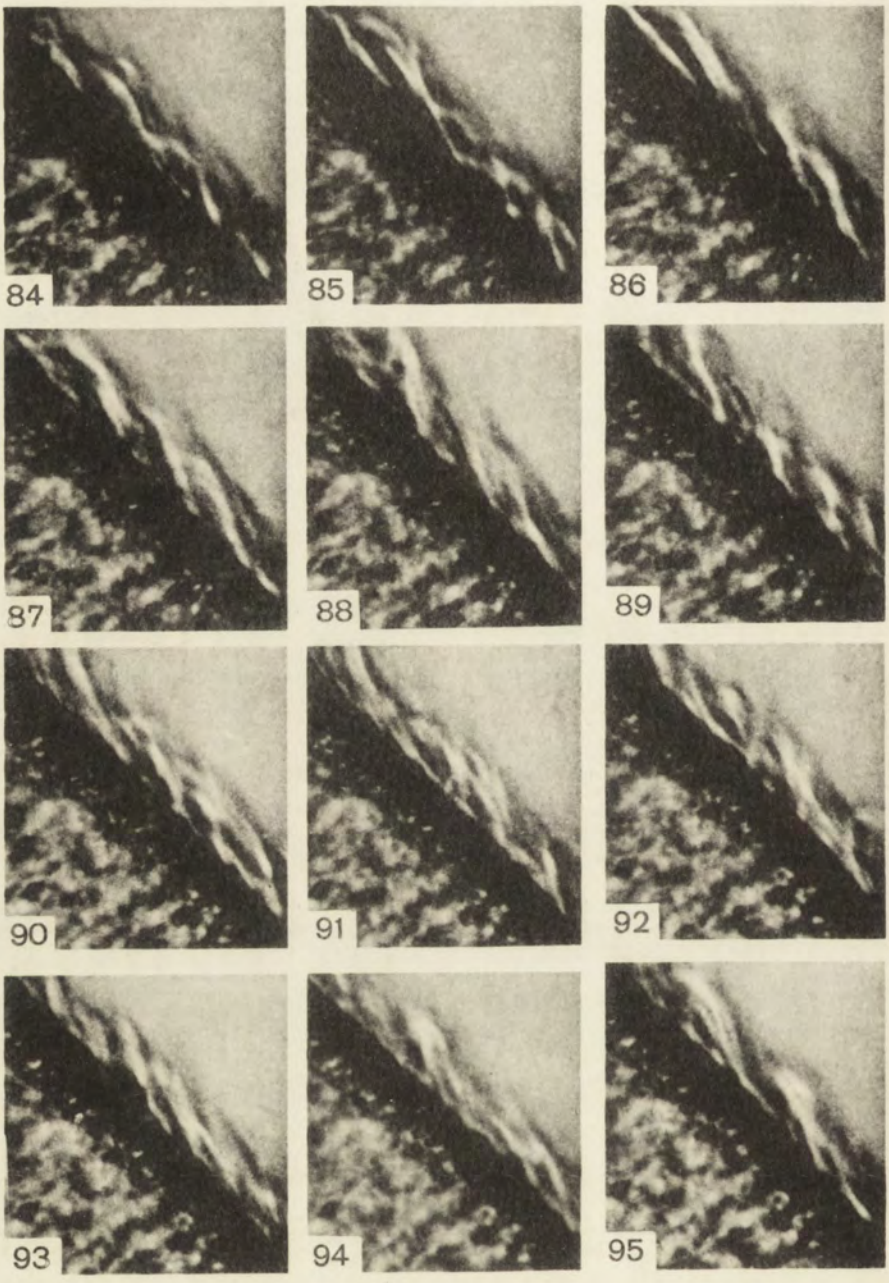

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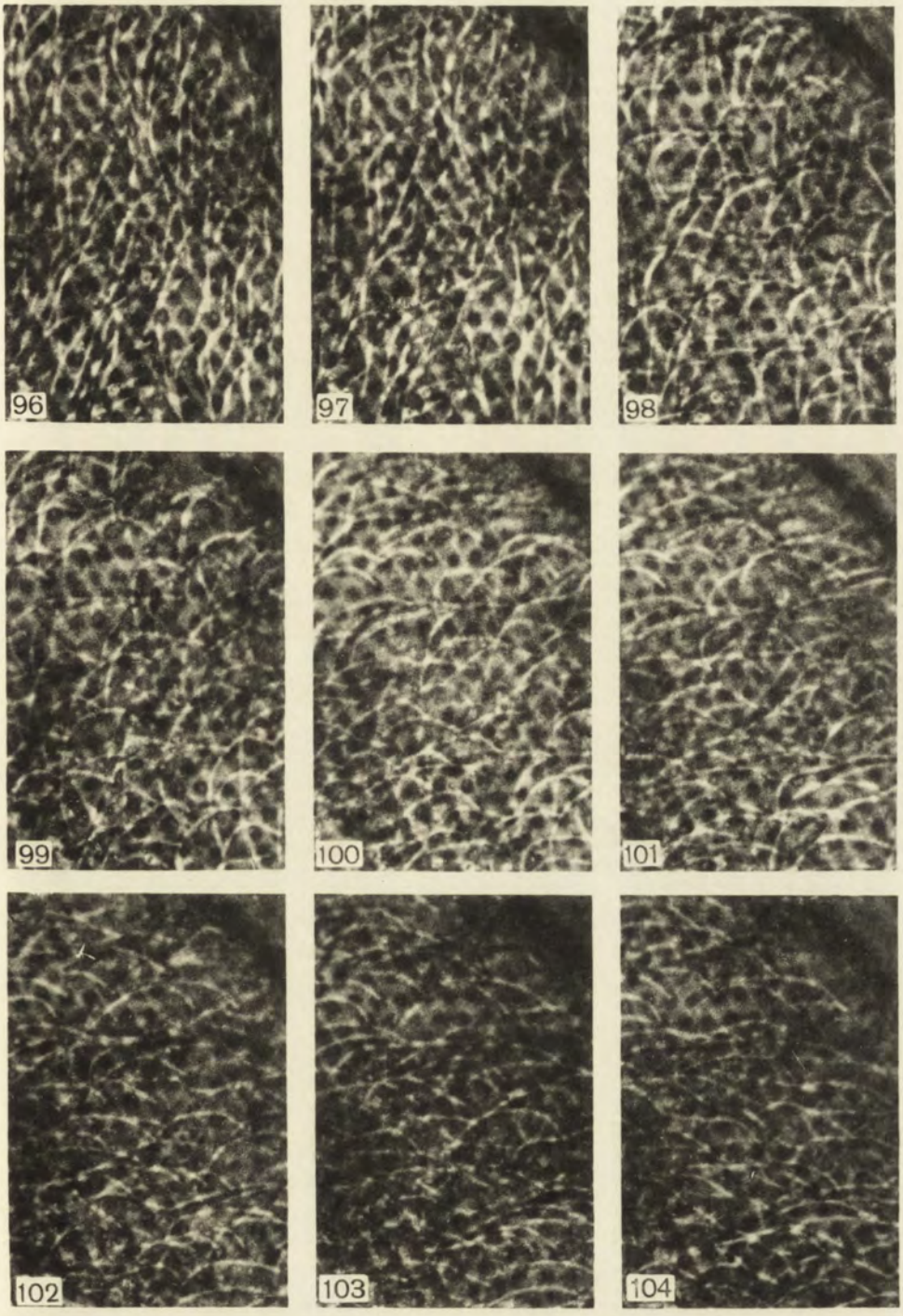

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The distributed solation hypothesis: an explanation of protoplasmic movement in slime mold plasmodia

La hypothèse de la solation distribuée: une explication du mouvement de la protoplasma dans les plasmodia de Mycétozoaire

The protoplasm of a cell has the ability to set up patterns of movement within itself. In both plant and animal cells, the occurrence of circular streaming patterns within protoplasm is termed cyclosis. In some organism, the protoplasm may stream in one direction and result in a translocation of the cell over a substratum, in which case it is referred to as amoeboid movement. In other organisms the protoplasm may flow first in one direction and then in another, in which case it is termed shuttle flow, and if net flow occurs in one direction during shuttling, translocation of the organism over a substratum results. It has been argued that there are different mechanisms for these three types of protoplasmic movement, but during the course of the life cycle of the slime molds, they proceed from cyclosis to amoeboid movement to a shuttle type of protoplasmic flow and at one stage in their life cycle they are even capable of shifting between the latter two types of protoplasmic movement. From this alone, we adduce that a common basis underlies all three types of movement in this organism.

Thus fair, no hypothesis for the various movement phenomena has successfully related the three types of movement. The hypothesis presented herein is general in form and provides a common basis for all three types of movement, thereby necessarily relating them in a coherent manner.

Biologists have thought in terms of "protoplasmic contraction" for a century, although their models usually were not as specific as the actomyosin-like system (Bary 1864, Voulk 1910, Hyman 1917, Mast 1926, Lewis 1942, Seifriz 1943). The secondary concern, but one which is intimately related to the identification of the motive force, is the localization within the cell of the place where the force is applied. Traditionally, there have been four schools of thought regarding the location of the motive force: 1. at the front, where it pulls the organism forward (Hofmeister 1865, Allen 1961), 2. at the rear, where it pushes the organism forward (Bary 1864, Rinaldi and Jahn 1963, 1964), 3. in the sol (Schaeffer 1920, Buy 1940, Stewart and Stewart 1959, Kavanau 1963), distributed throughout both the gel and sol (Hofmeister 1865, Kamiya 1959) or 4. localized at the

interface between the two (Loewy 1949). These alternate sets of arguments are not, of course, mutually exclusive.

Closely related to both development and localization of the motive force is the internal control of protoplasmic movement or streaming. It is significant that different amoebae have different protoplasmic flow patterns (Bovee 1964). In addition, in myxomycete plasmodia and some other organisms, protoplasmic streaming can occur within the organism without any external translocation (Kamiya 1959). Even more significant is the ability of slime mold microplasmodia to alternate between amoeboid streaming and shuttle flow streaming. The method of control of protoplasmic movement has neither been thoroughly investigated nor have many hypotheses been developed, although there have been some noteworthy attempts (Goldacre 1964, Rinaldi and Jahn 1964).

All of the hypotheses for the development of motive force, including the two predominating concepts have, in addition to their strong points, some very serious shortcomings. On the one hand, the "fountain flow" hypothesis (Allen 1961) does not adequately explain how contractile fibers at the anterior end of a pseudopod effectively exert tensile strength on sol material nor exert a force sufficient to effect movement and why multipodial amoebae do not pull themselves apart (Goldacre 1964). While the "Contraction-hydraulic hypothesis" (Jahn 1964) does not provide for an independent control of pseudopodia, on the other hand it does require a positive internal pressure throughout the organism. The retraction of a pseudopod or the formation of pinocytotic vacuoles against a high positive internal pressure would seem to preclude general validity for this argument, especially since plasmodia and myxamoeba pinocytose and take up substrate materials during their most rapid migration.

Many recent observations have pointed to the need for an hypothesis which could explain how isolated plugs of endoplasm in an organism can undergo independent motion within the cell. One case in particular is that in which it has been found that the contractile vacuole in many amoeboid forms is actually pushed shut by plugs of endoplasm (Jahn et al. 1967). In this case the force is clearly exerted upon the vacuolar membrane as a push. A similar situation exists in the elimination of diatoms from amoebae (Abé 1964).

In any area of research a working model or hypothesis is necessary; that is, there must be a framework within which meaningful questions can be asked and to which probing questions can be directed. It seems to us that currently the area of protoplasmic movement has no such unifying theory and that, moreover, there have been no attempts to draw upon the attractive ideas of the current theories while somehow avoiding the incorporation of their weaknesses.

There are certain minimal requirements that should be satisfied by, or at least be compatible with, a general hypothesis of protoplasmic movement. It is clear for example that it would be desirable to avoid incorporation of any mechanisms leading to an excessive, overall, positive internal pressure against which pinocytosis would have to expend an inordinate amount of energy. In order for there to be local occurrence and control, motive force development and protoplasmic movement must be capable of occurring in localized regions (Miller and Anderson 1966). From this last, one infers that cytoplasm must be able to exist in different states in different local

regions and that these localized states must be capable of variations, temporally as well as spatially.

In view of these considerations, an hypothesis has been developed to provide a general explanation of protoplasmic movement, to provide some suggestions for alternate physicochemical processes which may underlie protoplasmic movement, to provide an example of a biochemically coupled motile system, and to serve as a tool in the design of experiments on protoplasmic movement. It should be explicitly stated that the present work did not begin as an attempt to integrate the noteworthy features of models currently extant, but it nevertheless provides, in its ramifications, explication of the attractive features of those models.

The hypothesis

The four distinct features of the distributed solation hypothesis not in order of importance are: 1. spontaneous contraction of sol to form gel (and ultimately slime), 2. the enzymatic conversion of gel to sol, 3. the development of motive force for movement, and 4. the development of rhythmic activity by the solation centers.

Spontaneous contraction of sol to form gel

Plasmodial protoplasm in the sol contains large numbers of negatively charged groups which have positive ions (mostly potassium) and water associated with them (the term "associated" is used in the same sense as Ling 1957). A transitional, gelation-like process takes place spontaneously and involves a cross linking (possibly by electrostatic interaction) between adjacent macromolecules to form a very weak, gel-like state. With further elapse of time, the molecules of the cytoplasm form still more cross-linkages, with the result that the cytoplasm of the organism loses its potassium selectivity, loses associated water, decreases its volume, and as a result of these processes, may be thought of as passing toward the more elastic end of the visco-elastic spectrum. For concreteness, the foregoing process may be thought of as being like that of gelation and syneresis of gels.

Alternatively, the slightly gelated cytoplasm in the more sol-like state may be thought of as being capable of a fluid-like response at lower stresses because of structure that is relatively easy to disrupt and break down. If there are no other biochemical or mechanical processes influencing the gelation-like process, then it continues and the material becomes more gel-like and capable of sustaining tensile stresses; greater local stress differences are then necessary to break down the material structure and produce flow. As this gelation process continues, the cytoplasm would contract still further, and it would lose associated potassium and water and become, eventually, a thick slime to be rejected by the organism. This formation of slime is a final, irreversible step, and the slime would thus be considered as a waste (or by-) product of the gelation process.

The conversion of gel to sol

It is proposed that the more gel-like protoplasm of the plasmodium is converted to sol by an enzymatic process which is thought to be distributed more or less continuously throughout the protoplasm of the organ-

ism. At times, however, it may seem to be localized only in certain regions of the protoplasm. For convenience, the term "solation element" is used to connote this instantaneous locus of solation, but the use of the term "solation element" should not be construed as meaning that these are discrete structures or permanently located regions within the organism. A "solation element" is, generally speaking, a heterogeneous group of cytoplasmic organelles located within a particular spatial region at a particular time and associated with one another by their proximity and the mutual availability of biochemical substrate that permits them to act in concert. Under differing physiological conditions, solation elements would be expected to vary in size, number, and activity.

Development of the motive force for movement

Because the cytoplasm is normally undergoing "contraction" and a process much like syneresis, there is a shift of water and ions throughout the more gelatinous, ectoplasmic structure. In the process of solating, and perhaps to some extent solvating the cytoplasm, the solation elements incorporate water and ions (primarily potassium) into the structure of the cytoplasmic sol, and this results in a local increase in volume. This volume increase when the gel is enzymatically converted to sol is postulated to be similar to that described in amoeba by Y a g i (1961). This important volume increase in the area of a solation element stresses the tighter, gel-like structure within the immediate neighborhood if there is a more fluid and less elastic region nearby, then under increased stress, it would tend to flow. This would, for example, provide the basis for movement of isolated plugs of endoplasm within the protoplasm.

Development of rhythmic activity by the solation centers

The inherent periodicity of many biochemical reactions and/or organelles must be compatible with a detailed explanation of any cellular activity. This rhythmic activity forms an integral part of the control of solation in the distributed solation hypothesis. In its several ramifications this subterranean periodic behavior provides not only a basis for the rhythmic movement of protoplasm which might seem plausible enough, even without a detailed explanation of the mechanism—but strikingly it does not conflict with the more or less steady movement of protoplasm that occurs in amoebae, nor with the varieties of patterns of protoplasmic movement that occur in the several amoebae. This underlying rhythmic biochemical activity is central to the control of the various types of protoplasmic movement, according to the distributed solation hypothesis.

We make the following suppositions: 1. the key organelles of a particular solation element each have an intrinsic rhythm of biochemical activity (P y e and C h a n c e 1966), 2. if there are several types of key organelles, their periods are taken to be roughly the same under the same circumstances, 3. this biochemical activity is a part of the metabolism of the protoplasm, 4. there is a given key compound (or compounds—the argument may be generalized), and 5. the rate of consumption of that compound in one of the organelles is greater than in the others. (Here and in the sequel we are speaking, for simplicity in this initial description, of the biological reactions as if they were equilibrium chemical reactions for which catalysts—that is, enzymes—were

always available; for the simple arguments that follow to hold, we also must exclude zero order reactions.) Finally, we introduce the following terminology: 1. the availability of a given compound in the immediate neighborhood of an organelle at a given time for a reaction taking place at (either in or on) that organelle—that is, the local instantaneous biochemical substrate—is referred to as contemporaneous microsubstrate availability, and 2. the instantaneous biochemical substrate in the region of a solation element is termed contemporaneous macrosubstrate availiability.

Under the above 5 suppositions, regardless of the initial phases of the different organelles utilizing the key compound, a decrease in the contemporaneous microsubstrate availability brought about primarily by one of them or possibly due more or less equally to all of them, would limit all of the reactions. If the microsubstrate availability later increased, the near equality of the periods of the organelles would cause synchrony. Moreover, if there were other kinds of organelles providing this compound by one or more equilibrium reactions, these second kinds of organelles would be more active at lower concentrations of the compound and less active at higher concentrations of the compound due to the shift in equilibrium; then all other things being equal (that is, no other microsubstrate appreciably influencing the reaction equilibrium inhibiting the enzymes), this would accelerate the tendency to synchrony of the first kind(s) of organelles within the solation element.

This argument, of course, attributes a dominant role to the first kind of organelles and was used only for simplicity and ease in presentation: any one or combination of the organelles either utilizing or producing a key compound (the argument, as indicated above, is not limited to one compound) could conceivably dominate. Thus, suppose for example that certain of the organelles produce a certain, key compound. As more of the compound is produced, equilibrium reactions producing it would be retarded because of the shifts in equilibria. Regardless of the initial phases of the organelles producing it, an increase in concentration of the compound produced by one or several of them would inhibit them all. When the concentration decreased, they would begin to produce the compound again, and if their periods were approximately the same, the synchrony would result. Moreover, if there were other kinds of organelles utilizing this compound, they would be more active at higher concentrations of the compound, all other things being equal, and regardless of their periods, they could therefore only accelerate the tendency to synchronous behavior of the first kind of organelles.

Synchronization between solution elements

Although the argument for synchrony of the two dominant kinds of organelles within a given solation element would appear to hold for the interaction between two solation elements, it generally does not, for the synchronization of the dominant organelles is primarily dependent upon microsubstrate availability (that is, locally produced substrate), whereas any synchronization between solation elements would depend primarily upon the presence or absence of cytoplasmic substrate or macrosubstrate availability (the counterpart of the term microsubstrate availability for organelles is macrosubstrate availability in the case of solution elements), and to some extent the movement of other solation elements. Any synchronization of solation elements that occurred would be a result of a peculiar relationship between the macrosub-

strate availability, and the transfer of the key compounds by diffusion or convection or both. Because of the critical relations among these factors, solation elements of similar periods can have any phase difference ranging from synchrony to asynchrony. It does not seem unreasonable, however, to single out macrosubstrate availability as perhaps the most important of all these factors in the overall rhythmic pattern of several solation elements.

In addition, the movement of the solation elements would affect synchrony. A solation element is "created" by an aggregation of organelles, so it may be "destroyed" by a separation of the organelles constituting the solation element, say simply by their drifting apart. The organelles could also "drift" in the same direction because of, and leading to the interpretation that, the solation element itself is moving.

This could result from inhomogeneities and perhaps anisotropies in the surrounding cytoplasm—that is different structure in different locations and weaker structure in one direction—and an appropriate anisotropy and inhomogeneity in the stress field could contribute to drive the solation element in that direction. Solation elements may thus vary in activity and spatial location with the passage of time and variation of the physiological state, and they may even disintegrate.

Implication of the hypothesis and discussion

In this hypothesis the gelation and "contraction" of the cytoplasm is a spontaneous process; the organism must expend energy through an enzymatic step to solate the cytoplasm. When solation does occur, it is limited to small areas distributed throughout the endoplasm called solation centers. In order for the solation element to function, the organism must have appropriate biochemical substrates.

When solation of the endoplasm in a gel tube takes place, the outer ectoplasm is stressed. When the solation element moves, an accompanying pulse or wave of stretching (and, subsequently, contraction) of the outer ectoplasm would be expected to occur as described by Seifriz 1937 and Stewart 1964. Another outward manifestation of the movement of solation elements would be a local loss of water and ions to the environment. That is, externally, there would be a release of water and ions which could be correlated with the previously described waves of contraction in the ectoplasm. This release of ions would produce a diffusion potential and would explain the correlation between the dynamoplasmogram and electroplasmogram which Kamiya obtained (Kamiya 1959). There is another effect of solution elements that would be outwarly apparent i.e. if the ectoplasm were punctured, then the endoplasm would be expected to flow out to an extent dependent among other things, upon the amount of endoplasm that had been solated, as has been described by Bélár 1930, Anderson and Pollock 1952, Jahn et al. 1964.

The ability of the slime mold to develop shuttle flow from typical amoeboid movement is explained as an increase in the number of solation centers and the synchronization of the activities between the solation centers. The motive force is due to the anisotropic stress created when a solation element incorporates water and potassium to solate protoplasm. The force for protoplasmic movement is therefore distributed throughout the endoplasm of the plasmo-

dium. Inasmuch as the anisotropic stress of the solation element only lasts for a short time in a limited area and would be attenuated within a relatively short distance, there is no overall (turgor) pressure inside the plasmodium to inhibit pinocytotic activity during movement. The control of movement is perhaps due to two prime factors: the availability of free water to solate the protoplasm and, the synchrony which develops between solation elements.

As a consequence of the hypothesis, water and potassium ions would be expected to be more abundant in the less structured regions of the cytoplasm (that is, the sol and, to a lesser extent, the newer, gel-like cytoplasm) than in the regions of older, more highly structured gel and slime (Anderson 1962, 1964).

It has been explained that a loss of water and ions would be accentuated in the neighborhood of the solution elements and therefore diffusion potentials would be detectable from the external surface of the organism. In addition, a base level of these potentials would be expected to vary with the age of the gel because the newer gel is higher in both water and ions. Therefore, a higher base level of diffusion potential would be measurable from areas of newer gel. Superimposed upon the base level diffusion potential would be periodic increases due to the presence of solation elements, (Miller et al. 1968).

Many investigators have attributed a dominant role to the membrane or outer covering in the process of protoplasmic movement. Other investigators fell that it has no role at all. It is clear that the hypothesis presented herein does not provide a dominant role for the external covering of a plasmodium. This, however, is not to say that the presence of an outer covering, the amount of slime formed, its thickness, the degree to which it is folded, and its relative permeability to certain materials will not have a secondary effect on protoplasmic movement.

In this hypothesis, it is implied that coupled enzymatic processes are instrumental in the conversion of gel to sol (for other indications of the enzymatic conversion of gel to sol see Rudzinska 1967). It is important to point out that biochemically the detectable difference in sol and gel would be in the functional group which the solating enzyme(s) or agent(s) attacks in order to change the gel to the sol condition. There are several possibilities along these lines. For instance, the interconversion may involve action on polysaccharide complexes. Alternatively, it is also possible that polysaccharide complexes are not involved or only indirectly involved. Thus the solating agent could conceivably operate on protein structure or on protein associated with polysaccharides. Differences in the staining of sol and gel have already been found in slime mold (unpublished work by Sheen, Gailey, Miller, Anderson, Bargman and Carter 1969).

The solation process is thus conceived of as a system of coupled enzymatic reactions which supply the energy necessary to keep the cytoplasm in the sol condition. The coupling may differ under anabolic as opposed to catabolic conditions, and therefore a difference in solation activity would be expected under conditions of starvation and feeding.

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a revision of a hypothesis contained in a doctoral thesis (Miller 1965). Professors John D. Anderson, Tommy T. Dunagan and Mr. Charles A. Petty made timely criticisms. The senior author is grateful for partial support by a USPH service Predoctoral fellowship number NIH-IFL-6N-23-041-01 Division of General Medicine. Portions of this work were supported by NSF grant NSF-GK-3676 awarded to X. B. Reed.

Summary

A hypothesis proposed to elucidate the phenomenon of protoplasmic movement in slime molds incorporates the concept of functional solation elements. Central to the hypothesis are the notions that (1) primarily due to gelation, the cytoplasmic matrix spontaneously contracts, (2) gelation continues and increases with elapse of time unless opposed by the action of solation elements, (3) solation elements convert gel to sol, (4) associated ions are released during gelation, but, during solation, water is absorbed and local volume increases occur in the neighborhood of the solation elements. Surrounding gel regions are stretched, (5) protoplasmic flow results from the local stress variations and structural anisotropies and inhomogeneities developed by the solation process.

RÉSUMÉ

Une hypothèse proposée pour l'élucidation du phenomène du mouvement protoplasmatique chez les Mycétozoaires contient l'idée des élèments de solation fonctionelle. Les notiens centrales de cette hypothèse sont les suivantes: (1) le matrix cytoplasmatique se contracte d'une façon spontanée grâce à la gelation, (2) la gelation continue et accroît au cours du temps si elle n'est opposée par l'action des éléments de soltion, (3) les éléments de solation changent le gel en sol, (4) des ions associés sont relachés pendant la gelation mais pendant la solation l'eau est absorbée et le volume local accroît en proximité des éléments de solation. Les régions de gel entourants ces éléments sont allongés, (5) le mouvement de la protoplasme est un résultat de variations de tension locale et aussi les anisotropies structurelles et inhomogèneites produits par le procès de solation.

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