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Stefan RADZIKOWSKI *

Study on morphology, division and postconjugation morphogenesis in *Chilodonella cucullulus* (O. F. Müller)

Badania nad morfologią, morfogenezą podziałową i postkonjugacyjną *Chilodonella cucullulus* (O. F. Müller)

Chilodonella cucullulus (O. F. Müller, 1786) is a species of fresh and brackish waters. The classical studies of its morphology and morphogenesis carried out by F a u r é - F r e m i e t (1950) failed to give a full picture of morphogenesis in division and conjugation. A more detailed analysis of those processes has been the subject of the present study.

Material and method

Material for study was collected in the sewage plant at Otwock near Warszawa. Protozoa were grown on a nutrient of a following composition: NaCl — 2 g, KHPO$_4$ — 0.5 g, arabic gum — 5 g, water — 1000 ml. Nutrition was supplemented by yeast. Observation of living material was carried out using the negative phase contrast optics. Permanent preparations were impregnated following the dry silver impregnation method of K l e i n.

Results

Morphology

The protozoon is flattened dorso-ventrally and measuers 100—150 μ in length and 40—50 μ in width. Ciliature occurs on the ventral side and is arranged in two systems: the left and the right one, in a joint number of 18—21 kineties. In the anterior body part, on its ventral side is a cytostome which is supported by 10—14 trichits producing a basket. The anterior part of cytostome is surrounded by 2—3 short buccal kineties. Kineties of the right side — 8—11 in number — of different size, in the anterior body part, reach anteriorly as far as the preoral kinety which runs from the cytostome obliquely to the left side. On the right side there is the kinety x which arises de novo a short time prior to the division (C h a t t o n et L w o f f 1931) in *Chilodonella uncinata*. During the division, it shifts to the dorsal side, producing the "transverse kinety" in the anterior body part. The number of the left side kineties fluctuates from 10 to 13 Those kineties are variable in length. Since the number of kineties is variable and there is no possibility of their numbering — there will be marked by numbers only these the segments of which shift in morphogenesis.

Morphogenesis

The first signs of the approaching division are: the increase of body size, the increase of number of basal corpuscles in kineties (D o b r z a ń s k a - K a c z a n o w s k a 1963) and formation of the kinety x (Fig. 1 A) Division begins with bending of kineties B, C in the middle region of protozoon (Fig. 1 B, Pl. I 1) and with differentiation of the area between the kineties A and B. At that time the whole zone becomes concave. K a n e d a (1960) called this zone the cytostomatogenic field, and F a u r é - F r e m i e t (1948) — the embryonic area. An increase in number of meshes of the argyronymal network between the kineties A and B may also be observed. This fact suggests the increase of surface at this zone as well as increase of number of basal corpuscles in kineties B, C, D (Pl. I 1) Subsequently the kineties A, B, C break off — the kinety A in one — and kineties B and C in two places (Fig. 1 C). In these segments occurs an alteration of the ciliary movement: from the metachronal to the membranellar one. As a result, two segments B_1 and C_1 arise. Sometimes a third segment D_1 arises of the kinety D. It subsequently joins the buccal ciliature.

About that time the basket is also detached and shifts inside the cytoplasm. After this period, formation of new basket rudiments in prother and in opisthe (Pl. I 4) may be observed in form of rosettes composed of very small trichits. On the apical part of each trichit two ellipsoid corpuscles are present, connected with the trichit by means of two thin fibers. Those corpuscles (d e P u y t o r a c 1964) originate from the basal corpuscles. During morphogenesis trichits undergo contortion to the left.

The next stage is the breaking of kinety A in several points. As result, several segments arise: segment A_1 which gets folded, segment A_2, as well as segment A_3 and A_4. The segment A_3 may arise in the course of division however this is not a rule (Fig. 1 D). The segments B_1 and C_1 and sometimes D_1, shift by a rotatory movement round the newly formed trichits and become located in front of them (P. I 4, 5) D o b r z a ń s k a- K a c z a n o w s k a 1963), (Fig. 1 E However the segment A_1 is shifted from between the kineties A_2 and B_2 of the opisthe to the ends of the segments B_1 and C_1 (Pl. I 5). At this phase the division furrow appears on the dorsal side and the breaking of kineties of the left side may be observed. The segment A_1 shifts to the left side and straightens producing the preoral kinety of the opisthe. This is in agreement with the findings of F a u - r é - F r e m i e t 1961, (Pl. I 6, II 7, 8, Fig. 1 F). The segment A_3 is not involved in the rotatory movement but shifts to the segment C and joins the buccal ciliature (Fig. 1 E, F). At this time the basal corpuscles in the middle part of the right system kineties increase in number and allomatric growth of kineties is observed. Finally the opisthe is orientated at an angle towards the prother)Fig. 1 I).

As the division furrow deepens, the segments x shifts to the dorsal side and produces the transverse kinety (Fig. 1 H). This period is characterized by a decline of the movement synchronization. In result the protozoa detache from each other. The superior segments of the kineties A, B, C, which have been transmitted to the prother, grow up and produce the kineties A, B, C, of the prother. In contrast to this, the kinety A arises of the segment A_4 which shifts to the right side near the segment A_2 and grows up to its normal size (Fig. 1 D—J). The kinety B of the opisthe is formed from the segment A_2, kinety C from the segment B_2 etc. The above process is a result of the fact that the
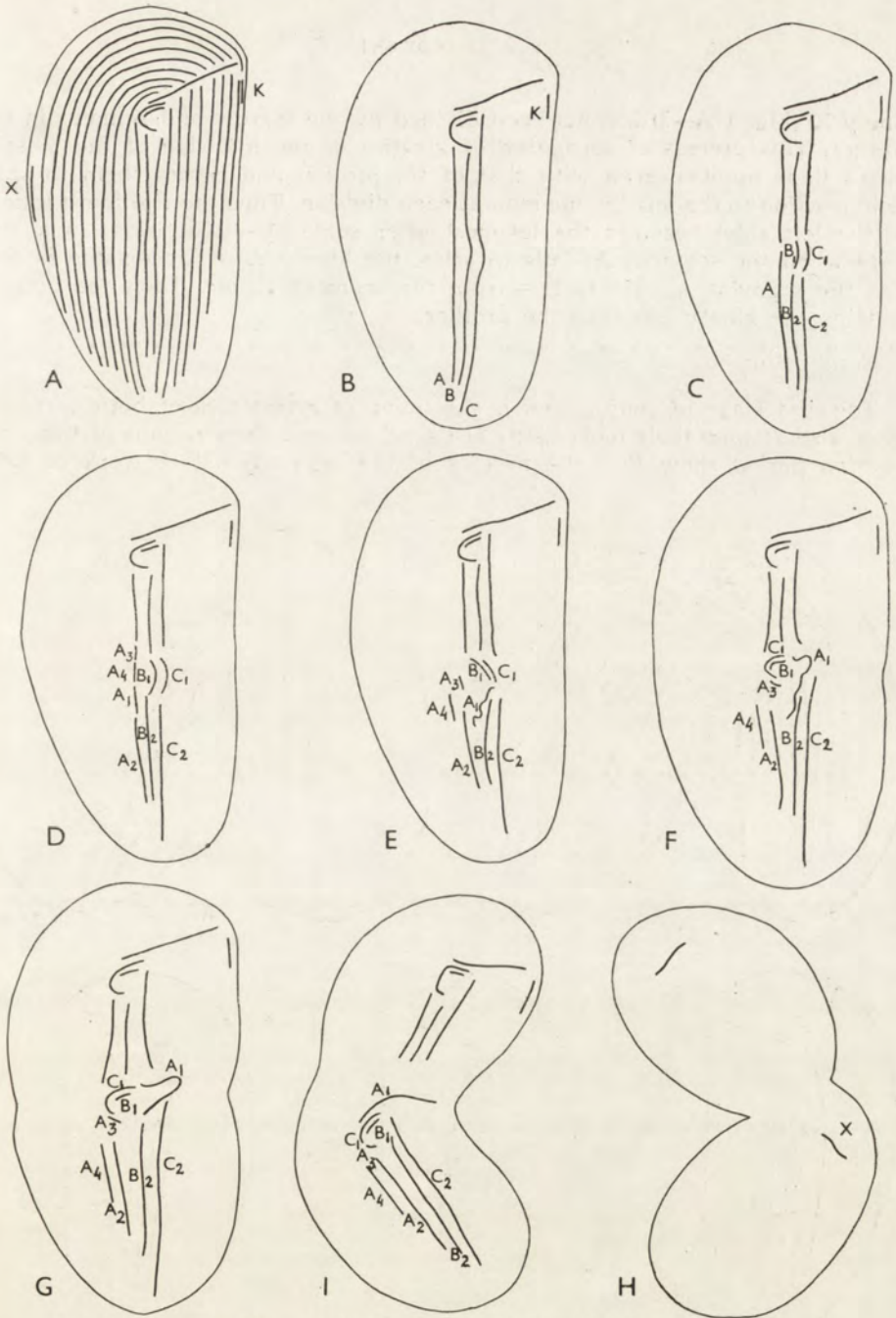
Fig. 1. *Chilodonella cucullulus* (O.F. Müller), division morphogenesis
A. Situation of kineties on the ventral side, B. Bending of kineties B, C. C. Forma-
tion of segments B_1, C_1 and B_2, C_2. D. Differentiation of segments A_1, A_2, A_3. E. Shif-
ting of segments B_1, C_1, folding of segment A_1. F. Shifting of segment A_1 from seg-
ments B_1, C_1 and B_2, C_2. G. Formation of the new buccal ciliature. Segment A_3 takes
position on the side of segment C_1. Further shifting of the segment A_1. H. Formation
of the preoral kinety of the segment A_1. Position of protozoa at an angle. I. Position
of the kinety x (dorsal side)

kinety K (Fig. 1 A—I) has not been divided by the furrow and remains in the prother. This process of formation of kineties of the left side of the opisthe makes their number even with that of the prother and causes them to shiff their position to the left by one step at each division. Thus the rightomst kinety (of the left side) becomes the leftmost after some 11—12 division. In a few cases when the segment A_4 fails to arise, the kinety A of the opisthe is formed the segment A_2, kinety B — from the segment B_2 etc. Then the opisthe contains one kinety less than the prother.

Conjugation

The first stage of conjugation is the union of cytostomes of both partners. After a short time their movements are synchronized. Preparations of this conjugation period show that the meshes of the argyronymal network of both
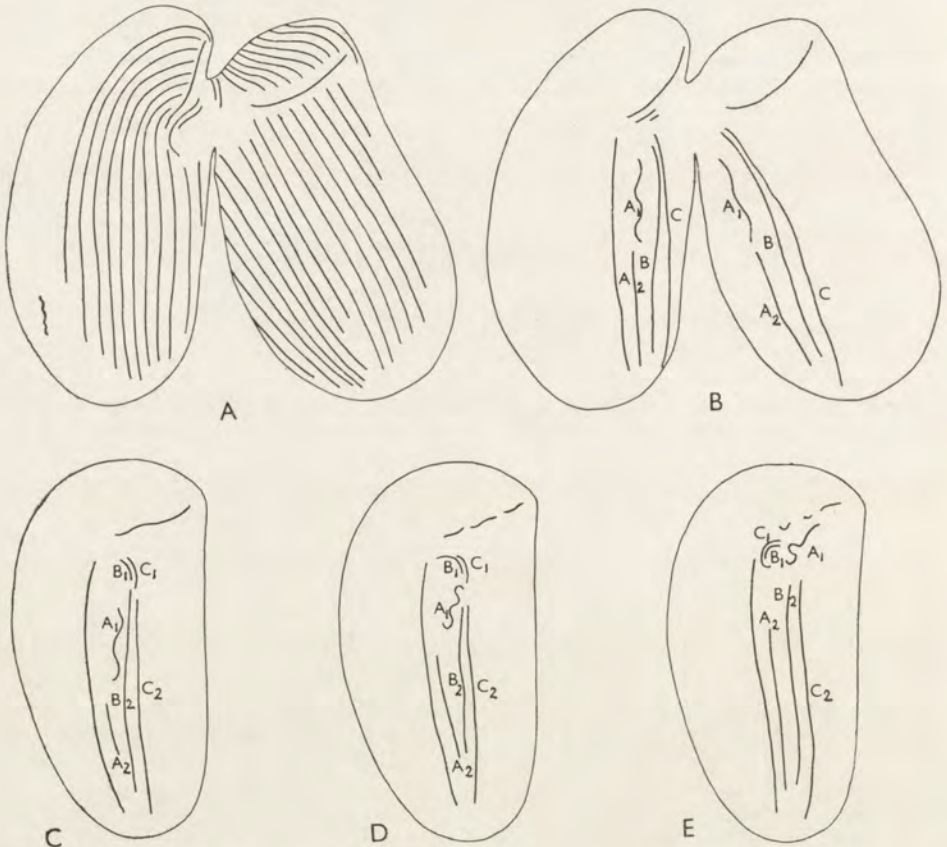

Fig. 2. *Chilodonella cucullulus* (O.F. Müller), postconjugation morphogenesis A. Position of protozoa during conjugation, B. Differentiation of the segments A_1, A_2 and approaching of the superior parts of kineties B, C, to one another. C. Folding of the segment A_1. Differentiation of segments B_1, C_1 and B_2, C_2. D. Shifting of the segment A_1 towards the ends of segments B_1, C_1. Fragmentation of the old preoral kinety. E. Formation of the new preoral kinety from the segment A_1

partners are connected (Pl. II 9). Similar pictures were found by K l e i n 1928 in *Chilodonella uncinata.*

The protozoa remain at this stage for about 24 hrs. After 18 hrs. of union, the old baskets detach and become shifted inside the cytoplasm. Then the two buccal kineties disappear. On the free space arise the primordia of trichits of the new basket in the same manner as it occurs in division. The kinety marked as A breaks in its middle part and its superior segment A_1 is folding (Fig. 2 B, Pl. III 10). The segment A_1 may also arise from the inferior part of the kinety A and the segment A_2 from its superior part. The kineties B and C approach each other and break in their superior part, cutting off the segments B_1 and C_1 (Fig 2 C, Pl. III 11) which subsequently shift by a rotatory movement upon the trichits of the newly formed basket (Fig. 2 D, Pl. III 12). The position of the segments B_2 and C_2 remains unalterd. At this thime, in the majority of cases, separation of both partners occurs. In each of them the segment A_1 becomes displaced from the segments A_2 and B_2 to the left side similarly as in division, producing the preoral kinety (Pl. III 13). It takes place on the side of the old one (Fig. 2 D, E) which disappears in the course of the first postconjugation division. The segments A_2, B_2, C_2 grow up by increase of the basal corpuscles in the upper part, producing the kineties A, B, C. The kinety A may break into four segments i.e. A_1, A_2, A_3 and A_4 similarly as it occurs in division of the opisthe. Consequently the post-conjugant has one kinety more from the segment A_4 than in the former case. In result the cilliate has one kinety more than before conjugation.

Besides the normal conjugation in *Chilodonella cucullulus* occurs also another type of conjugation in which major part of one partner is absorbed by the other. Two individuals join by their buccal apertures — like in the normal conjugation — then the area of their junction enlarges. Subsequently a flow of cytoplasm into one of the partners occurs which results in a regular shortening of kineties and diminution of the surface of conjugant (Pl. IV 14—17) the other. Duration of this type of conjugation form the moment of junction up till the complete absorption amounts about 8 hrs. A similar course of conjugation was also observed by N o l a n d 1927 in *Metopus sigmoides* and by H e c k m a n n 1965 in *Urostyla hologamma.* This is the so-called total conjugation.

Discussion

The above observations seem to indicate that the initial moment of division as well as of formation of the new buccal ciliature is the isolation of the area between the kineties A and B. During the division this moment can be stated by the number of meshes of the argyronymal network since their number increases at this period. The number of basal corpuscles in the segments B_1 and C_1 increases also which is in conformity with the observations of D o b r z a ń- s k a - K a c z a n o w s k a 1963. However the isolation of a similar area in conjugation occurs in a slightly different manner: it is preceded by disappearing of two buccal kineties and displacing of the basket inside the cytoplasm.

At those areas, in division as well as in conjugation, trichits of the new baskets — a more stiff element — are formed. According to d e P u y t o r a c 1964 in *Prorodon viridis* the rudiments of trichits initiate from the basal corpuscles arising in this zone. K a n e d a 1960 in *Chlamydodon pedarius* determi-

ned all this area as the cytostomatogenic field whereas F a u r é - F r e m i e t 1948 suggested that these are the embryonic areas performing a role of organizer.

It may be presumed that this area exerts also an influence upon the neighbouring kineties e.g. in the case of shifting of the segments B_1 and C_1 which sometimes concerns also the segment D_1. I suppose also that this influence may account for formation of the segment A_1 of various length in different individuals. Possibly the movements which take place in this area during conjugation, are morphogenetic movements with a certain degree of independence.

In division however, the displacing of segments exerts influence upon incising of the division furrow. It would be interesting to follow this process in regeneration. Incising of the division furrow from the right side and smoothening of kineties over the preoral kineties are evoked by the allometric growth of the right side kineties, i. e. the most external kineties show the highest increase in size. The increase of kineties diminishes towards the left side of protozoon. The existence of zones of different intensity of increase was ascertained also by D o b r z a ń s k a - K a c z a n o w s k a 1965 in *Chilodonella cucullulus.*

Shifting of the segment x to the dorsal side depends also on the division furrow in which it is displaced to the anterior part of the body.

S u m m a r y

In *Chilodonella cucullulus* (O.F.M.) the differantiation of the area at which the adoral and preoral kineties are to arise is the initial moment of division as well as of formation of the new buccal ciliature in post-conjugants. It is formed in a different manner in both cases. In division it occurs by an increase of surface and in conjugation by disappearing of the old buccal kineties. It is suggested that this area exerts influence upon the surrounding kineties. Movements occurring in this area are morphogenetic movements. The total conjugation was also observed in *Chilodonella cucullulus.*

STRESZCZENIE

U *Chilodonella cucullulus* (O. F. M.) momentem początkowym podziału, jak również powstawania nowego urzęsienia gębowego u postkonjugantów, jest wyodrębnienie się pola na którym tworzyć się będą kinety adoralne i preoralna. W obu przypadkach pole to tworzy się w odmienny sposób. W czasie podziału — przez przyrost powierzchni, a w czasie konjugacji — przez zanikanie starych kinet gębowych. Autor sugeruje, że pole to wywiera wpływ na otaczające kinety. Ruchy zachodzące na tym polu są ruchami morfogenetycznymi.

Autor obserwował u *Chilodonella cucullulus* występowanie konjugacji totalnej.

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EXPLANATION OF PLATES I—IV

Chilodonella cucullulus (O. F. Müller)

Division

1: Bending of kineties B and C, formation of stomatogenic area
2: Further stage of bending of kineties
3: Breaking of kineties A, B, C
4: Formation of the rudiment of the new basket
5: Shifting of the segments B_1, C_1 and of the segment A_1
6: Straightening of segment A_1
7: Further stages of straightening of segment A_1 and breaking of the right side kineties

Conjugation

9: Place of junction of the conjugants
10: Formation of the segment A_1 and its folding
11: Differentiation of segments B_1, C_1
12: Shifting of segments B_1, C_1
13: Formation of the preoral kinety from segment A_1
14, 15, 16, 17: Consecutive stages of total conjugation.

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PLATE III

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PLATE IV
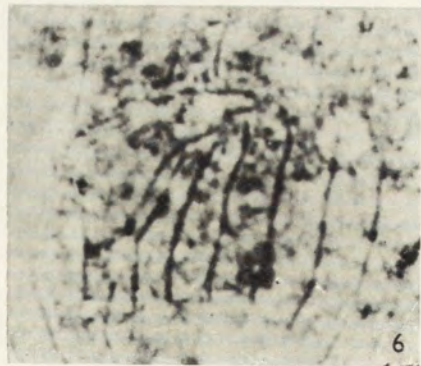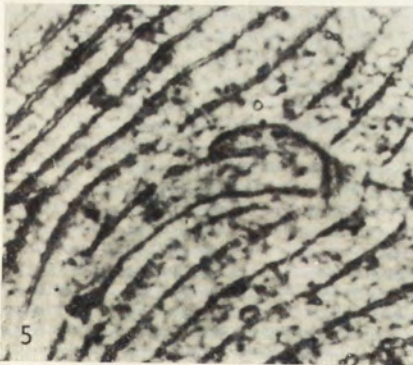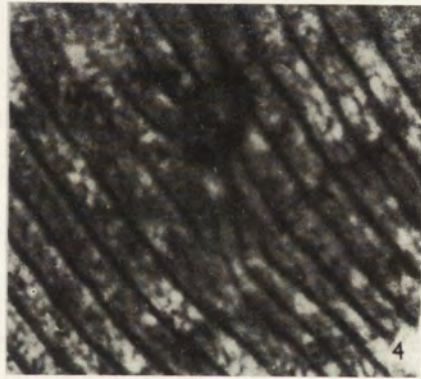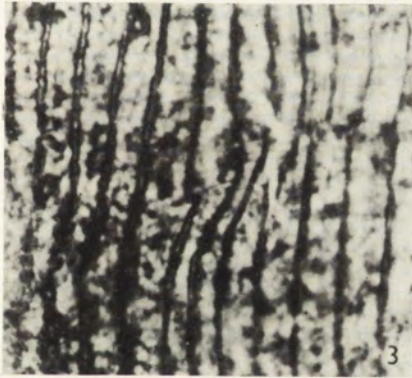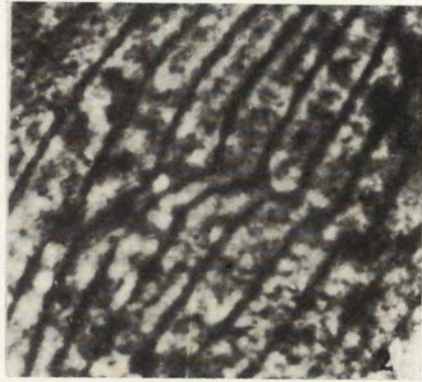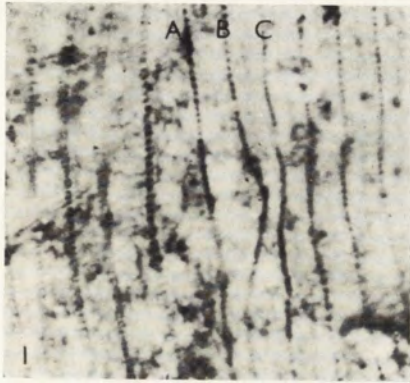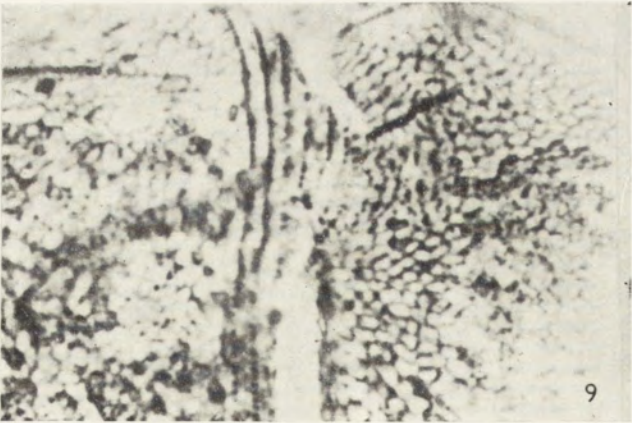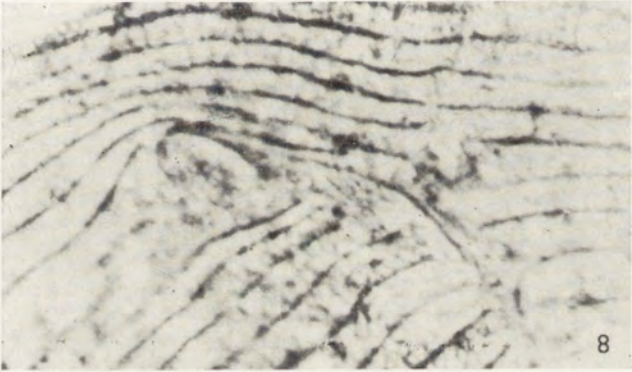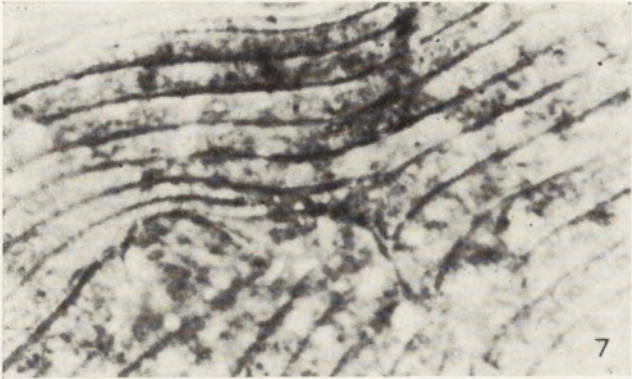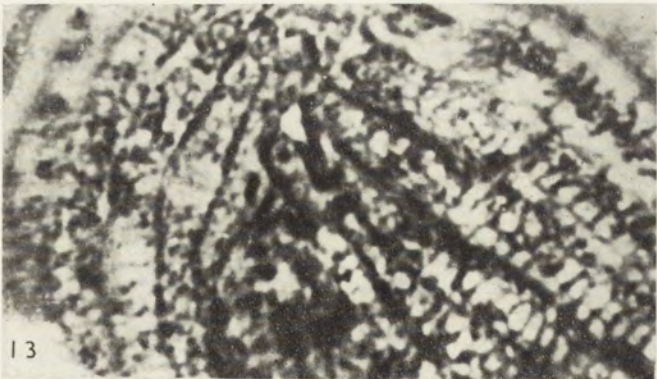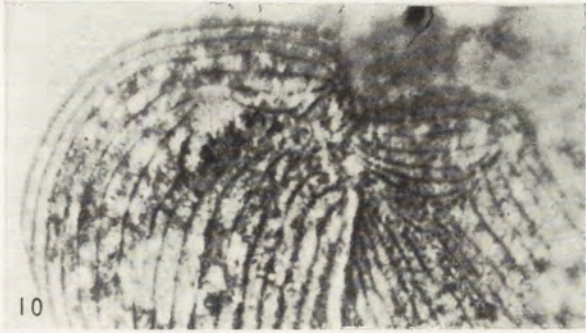
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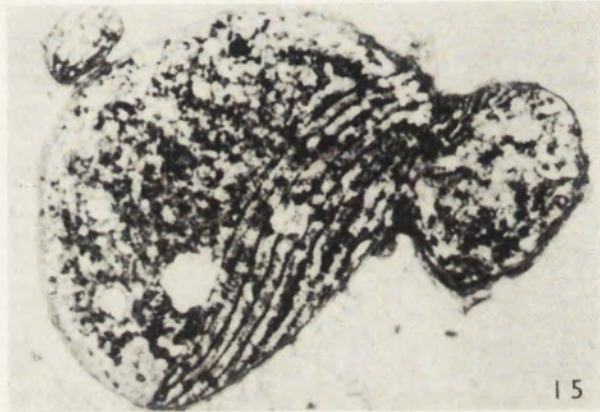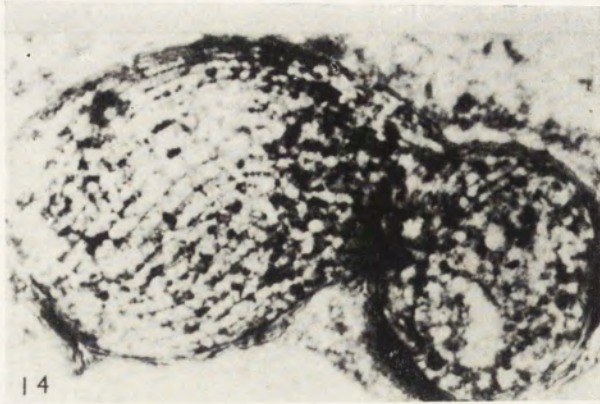
Maria WOLSKA

Study on the family *Blepharocorythidae* Hsiung
I. Preliminary remarks

Badania nad rodziną *Blepharocorythidae* Hsiung I. Wiadomości wstępne

The study on the representatives of the genus *Blepharocorys* was initiated by F i o r e n t i n i 1890, who described two new species of ciliates from the horse intestine: *Diplodinium uncinatum* and *Entodinium valvatum*.

B u n d l e 1895 described *Blepharocorys jubata* from horse coecum and transferred the two above species of F i o r e n t i n i to the genus *Blepharocorys* which was established by himself.

Subsequently other species were included to the genus *Blepharocorys*: *B. equi* Schumacher, 1915 (synonym of *B. uncinata* according to H s i u n g 1930 b, and S t r e l k o v 1939), *B. curvigula* Gassovsky, 1918, *B. micorocorys* Gassovsky, 1918, (considered by H s i u n g as a synonym of *B. valvata,* and raised to the range species again by S t r e l k o v 1939), *B. angusta* Gassovsky, 1918.

D o g i e l 1926 descibed *B. bovis* from the cattle rumen. The some species was reported previously by J a m e s o n 1925 as *Charon ventriculi*. Strelkov 1939 transferred the species of J a m e s o n to the genus *Blepharocorys,* and in this way the name *B. bovis* became a synonym of *B. ventriculi* (Jameson).

H s i u n g 1930 b described *B. cardionucleatum* from the horse colon. S t r e l k o v 1939 transferred the species *Ochoterenaia appendiculata* described by C h a v a r i a 1933, to the genus *Blepharocorys*.

The genus *Blepharocorys* Bundle was included to the family *Chiliferidae* Buetschli, 1887 by H i c k s o n 1903, whereas P o c h e 1913 included this genus to the family *Colpodidae*. D a C u n h a 1917 established the family *Paraisotrichidae* and included the genus *Blepharocorys* into it. H s i u n g 1929 established a new family *Blepharocoridae* for the genus *Blepharocoris* Bundle nad *Charon,* Jameson. S t r e l k o v 1939 gave a new name *Charonnautes* instead of the *Charon* because the type-species of this genus — *Charon ventriculi* Jameson — showed all the characters of the genus *Blepharocorys* and was included to it, whereas *Charon equi* described by H s i u n g 1930 a differed distinctly from the representatives of the genus *Blepharocorys*.

Ciliates of the genus *Blepharocorys* occur in the coecum and colon of *Equidae* (except *B. ventriculi* which occurs in cattle rumen). As to the genus *Charonnautes* — *Ch. equi* Hsiung, 1930 it occurs in horse coecum and *Ch. nuda* Hsiung, 1932 — in rumen of cattle.

The family *Blepharocorythidae* has been included to the order *Trichosto-mata* but — as many families of this order — without sufficient documentation. F a u r é - F r e m i e t 1963 stressed the necessity of a detailed revision of seve-ral families included into the order *Trichostomata*. The same concerns the fa-mily *Blepharocorythidae*. The silver impregnation method has not been applied as yet to any species of this family.

In the present study, an attempt was made to examine the infraciliature of representatives of the family *Blepharocorythidae* by means of silver impregna-tion method, and to follow the division morphogenesis, in order to obtain some data which might be useful for a more detailed characteristic of this family and its adequate classification.

Material and methods

Material was provided from the content of horse coecum (of the Łódź dis-trict) and from that of horse colon (of the Warszawa district), two samples of the latter were kindly offered to me by prof. dr. Marian Rybicki from the Zo-ological Institute, University of Warszawa.

Ciliates were fixed in 10% formalin and coated by a layer of gelatine on the slide following C h a t t o n, and impregnated subsequently with ammoniacal silver solution of R i o H o r t e g a or of B i e l s z o w s k i.

Following species had been examined in this study: *Blepharocorys uncinata* (Fior.), *B. valvata* (Fior.), *B. jubata* Bundle, *B. curvigula* Gass., *B. angusta* Gass. Other species of the same genus are very rare in the horse intestine. Detailed description of these species based on silver impregnation method, will be repor-ted in the course of subsequent study. In the present communication, only some data have been discussed which complement the characteristic of the genus and are of essential importance.

Results

The characteristic of the genus *Blepharocorys* is as follows. The assymetri-cal, non-contractile, elongated body is flattend laterally. On the anterior end of the body, the so called frontal appendix (helmet according to G a s s o v s k y) is present which limits the buccal overture on the dorsal side. The ciliature is reduced to two zones on the anterior body part (one on the frontal appendix, another one around the buccal overture on the so called ventral lip), and to a group of aboral cilia on the posterior pole. Besides those ciliary groups, the buccal depression (called by H s i u n g and S t r e l k o v — vestibulum) is coa-ted by ciliature as well as the „pharynx" — a long tubule connected with the buccal depression. S t r e l k o v applies the term „glotka" (pharynx) to it and H s i u n g calls it „oesophagus". The anal tubule is well marked on the poste-rior body end. The single contractile vacuole is near the anal tubule.

It should be stressed however that the term „vestibulum" has in this case not the same meaning in which it is presently used for determination of the concavity occurring in *Trichostomata*. The term „oesophagus" used in a meaning not precisely determined should be postponed in the description of ciliates. For characterization of the buccal apparatus of the ciliate, 5 terms of C o r l i s s 1955 are the most convenient: cytostome, cytopharynx, vestibulum, buccal cavity (eventually its synonym — peristome) and buccal overture.

The silver impregnation method allows to examine more in detail the cilia‑ ture of *Blepharocorys*. The schematic drawing in Fig. 1 concerns the species *B. jubata* Bundle which was the most abundant in my material. In all the species of the genus *Blepharocorys,* the ciliary zones and the buccal apparatus are constructed according the same pattern. The division process is also essentially the same.
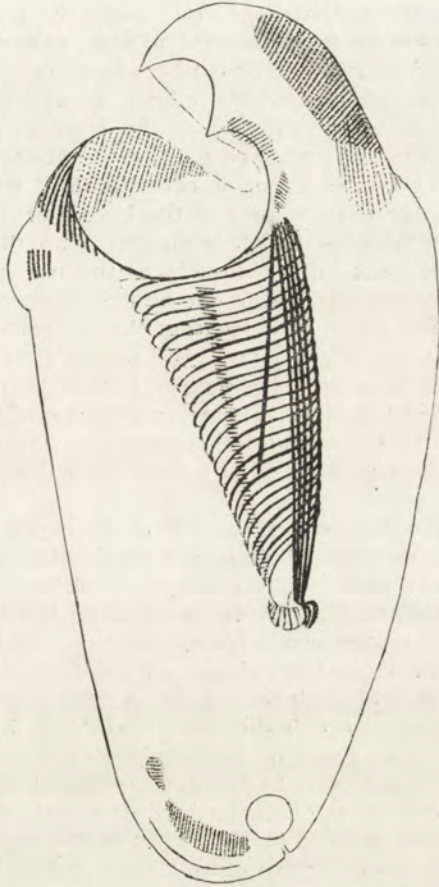
Fig. 1. *Blepharocorys jubata* Bundle, scheme

It should be pointed out in the first place that the somatic cilia form compact, sharply delimited groups like in the order *Entodiniomorpha*. They form 3 bands composed of short, oblique, densely located kineties. Those characteristic zones are not connected with one another. No infraciliature was stated on the non-ciliated body part.

The zone lying at the base of the frontal appendix has a form of irregular band, broader on the left side of the appendix. It breaks along oblique line on the dorsal margin of the frontal appendix and passes with its narrow part upon the right side and dives there into a depression.

The zone encircling the ventral margin and the lateral margins of the buccal overture, has its anterior margin incised corresponding to the shape of this part of the ciliate body.

The small aboral zone lies in a depression. It seems to be covered by a valve and may pass upon its interior surface (Pl. I 1). In most species this zone is situated ventrally in relation to cytopyge, on the left side of body.

The buccal cavity is characterized by a complex specialized ciliature which is not connected with the somatic one. It appears in form of a deep funnel shaped structure directed towards the ventral side, endowed with two groups of cilia and with numerous fibers. Those fibers have not been described as yet in the genus *Blepharocorys*. One of the ciliary groups forms a narrow band with rows of oblique kineties similarly as the somatic zones just described. This group lies in the anterior enlarged part of the buccal funnel on the right dorsal side (Pl. I 2). The second group is constituted of several long and thick kineties running along the narrow part of the buccal cavity on the dorsal side (Pl. I 2—3). One of the kineties which is shorter than the others, turns aside from the remaining ones and runs obliquely across the right wall of the funnel. Long kineties which run parallel to one another, approach abruptly in their lower part near the ends which are looking like connected by fibers. Beyond the „bond", kineties diverge slightly and bend towards the ventral side (Pl. I 3). In those kineties single separate kinetosomes cannot be distinguished because they closely adhere to one another. Therefore any one of those kineties should be recognized as base of the undulating membrane. The ciliature of the dorsal side of the funnel is visible in Pl. I 4 (longitudinal section in more or less saggital plane).

The left ventral and right sides of the funnel are supported by fibers (Fig. 1). One of their groups is constituted of long semicircular fibers. They are connected with kinetosomes of one of the longitudinal kineties. They run across the left and ventral side and scarcely encroach upon the right one — especially the anterior fibers. The second group consists of short fibers which are slightly visible. They lie on the right ventral side of the funnel producing a ladder-shaped structure which is broadened on its posterior and anterior ends. On its posterior part this structure is distinctly visible, it bends and approaches very much the posterior segments of the longitudinal kineties (Pl. II 5), joining somehow the semicircular fibers. In the species *B. jubata* the terminal semicircular fiber seems to bend strongly backwards and to encircle the „ladder". Nevertheless it cannot be defined exactly what is the mutual relation of those two groups of fibers or even the structure of the „ladder". Possibly some longitudinal fibers exist which belong to this system. One short fiber always visible, connects the posterior part of the ladder-shaped structure with the longitudinal kineties (Fig. 1). This system of fibers and kineties described in the case of the species *B. jubata*, becomes more complex in the other species in which the longitudinal kineties — and consequently the fibers — are twisted. However not any new elements seem to appear in the buccal apparatus of other species (Pl. II 7—8).

The two groups of buccal cilia described above are not associated with the somatic ciliature, and — as follows from the observation of dividing ciliates — fail to originate from the somatic ciliature. Consequently the genus *Blepharocorys* does not correspond to the characters of the order *Trichostomata*. Moreover, its division morphogenesis indicates that it can hardly be included to the

Holotricha. Its division occurs in a similar way as in *Entodiniomorpha.* The ciliature of opisthe arises inside the vacuole with no connection (at least without any visible one) with the ciliature existing already i.e. it is formed de novo in the meaning of C o r l i s s.

In the present study morphogenesis has not been followed in all the stages. Most frequently the division stage represented in Pl. III 9—11 was found. This stage or a similar one was observed in all the species occurring in presented material. The presence of the vacuole in the division zone may be regarded as certain. Possibly 3 separate vacuoles arise: one on the dorsal side, the second one on the ventral and the third on the left side. In the first vacuole develops the ciliature of the frontal appendix of the opisthe. In the second one — the ciliature of the ventral lip, the ciliature of the anterior zone of the buccal cavity of the opisthe and the aboral zone of the prother. In the third vacuole develop the longitudinal kineties of the buccal cavity and fibers. The number of the vacuoles is not quite certain. In the vacuole or vacuoles, occurs the growth and formation of the zones, the processes of invagination, protrusion and shifting of zones, till at an anvanced stage of division all the elements assume a feature and position which corresponds to final one (Pl. III 12).

Discussion

D o g i e l 1926 stated for *B. ventriculi (B. bovis)* that the division initiates not by a circular constriction but by formation of the subpellicular peripheral canal as in *Ophryoscolecidae.* At a later stage, this canal communicates with the external medium. The observation of author contribute nothing essentially new. They introduce some more details and concern several species but are not a full. Nevertheless even such fragmentary information about the character of morphogenesis seem to be of a certain importance. D o g i e l limited himself to the above statment, without drawing conclusions suggested by his observations which would concern systematics and phylogenesis of the genus *Blepharocorys.* It seems that the observations of D o g i e l as well as my own, introducing some new data about morphology allow in conclusion to exclude *Blepharocorythidae* from the order *Trichostomata* if the information about one genus may be extended to the whole family.

At present time an adequate ranging of this family in the ciliate system would be difficult. At any rate a position should be found for it close to *Entodiniomorpha* or even within the order *Entodiniomorpha.* Equally difficult would be to establish the origin of *Blepharocorythidae* since this family is endowed with characters of an order standing on one of the peaks of the phylogenetic tree of ciliates and shows simultaneously the features of *Holotricha.*

In the structure of their buccal apparatus which is endowed with longitudinal kineties and fibers, *Blepharocorythidae* resemble to *Paraisotrichidae* — *Holotricha* of uncertain position.

Another structural detail which has been stated in the present study seems to approach *Blepharocorythidae* to the family *Buetschliidae (Holotricha, Gymnostomata).* According to the observations of the author in all the species of the genus *Blepharocorys* the presence of a vacuole similar the the "Konkrementenvacuole" of *Buetschliidae* has been stated on the ventral side of the anterior part of body (Pl. I 4). S c h u m a c h e r 1915 in his mo-

nograph on *Blepharocorys equi* pointed out that — so far as it could be stated — the vacuole with concretions did not occur. This statement proves that S c h u-m a c h e r was not certain as to the presence or absence of the vacuole. I failed to ascertain the occurrence of the concretions in the vacuole in *Blepharocorys* but for some other reasons it seems to be the counterpart of the "Konkremen-tenvacuole" or perhaps this structure is in state of atrophy. A group of big non-ciliated kinetosomes (Pl. II 6) is namely present on this vacuole as it was described by the author previously (W o l s k a 1964) for *Didesmis ovalis* and *Blepharozoum trizonum* and was also seen in other species of *Buetschliidae* from the horse intestine (W o l s k a, unpublished). Presence of special kind of kinetosomes — or rather short kineties — on the "Konkrementenvacuole" seems to be consequently a general feature in the family *Buetschliidae* and evidently such special kinetosomes occur in *Blepharocorythidae*.

If the "Konkrementenvacuole" would occur in *Blepharocorythidae* in its typical form, it could be considered as a sign of convergence evoked by the same medium conditions. However if we accept that it is a remnant organelle — which seems probable — its presence would prove not convergence but rather the affinity of the families in question; in one of those families this organelle becomes atrophied in course of evolution. Another possibility would be to consider it as the rudiment of the "Konkrementenvacuole" which appears here somewhat "in statu nascendi". The latter concept seems less probable because the development of *Blepharocorythidae* follows the line similar to that of *Entodiniomorpha* in which "Konkrementenvacuole" never occurs.

The evidences mentioned above not very convincing, but may be, they show a relation between *Buetschliidae* and *Blepharocorythidae*. The con-siderations on evolution of highly specialized parasitic forms are always based on rather elusive foundations. It should be added that in *Buetschliidae* a ten-dency to reduction of the ciliature is marked distinctly and in some species, cilia form only narrow zones on the surface of the body.

At present, many indepedent evolutionary branches are traced from *Gymno-stomata*. *Blepharocorythidae* are perhaps one of the branches which is derived from *Gymnostomata* and the similitude of their evolution to the evolution of *Entodiniomorpha* provides a reason for considering the possibility of tracing the order *Entodiniomorpha* directly from *Gymnostomata*.

S u m m a r y

The somatic and buccal ciliature of genus *Blepharocorys* Bundle has been characterized as a result of observations of several species. The general outline of the division morphogenesis is presented.

The systematic position of *Blepharocorythidae* and the relation of this family to other groups of ciliates is discussed.

STRESZCZENIE

Autorka charakteryzuje orzęsienie somatyczne i gębowe u rodzaju *Blepharocorys* Bundle, na podstawie obserwacji kilku gatunków i przedstawia w ogólnym zarysie morfogenezę podziałową.

Autorka zastanawia się nad stanowiskiem systematycznym *Blepharocorythidae* i nad związkami jakie zachodzą pomiędzy *Blepharocorythidae* i innymi grupami orzęsków.

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EXPLANATION OF PLATES I—III

1: *B. jubata*, left side view. 2000 ×
2: *Blepharocorys* sp. right side view, 2000 ×
3: *B. jubata*, left side view (optical section). 2000 ×
4: *B. jubata*, longitudinal section, 2600 ×
5: *B. jubata*, view from the dorsal side, optical secton, 2000 ×
6: *B. curvigula*, on the right side of microgram a group of kinetosomes on the vacuole are seen, 1300 ×
7: *B. uncinata*, view from the left side, optical section 2300 ×
8: *B. uncinata*, view from the right side, optical section, 1700 ×
9: *B. jubata*, division 2000 ×
10—11: *B. jubata*, division, optical section in two different plains, 2000 ×
12: *B. jubata*, division, advanced stage, 2000 ×

PLATE III

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auctor phot.

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Application of the ammonium-silver impregnation method to the investigation of ciliates from the rumen of herbivorous mammals

O wartości impregnacji amoniakalnym roztworem srebra orzęsków ze żwacza trawożernych ssaków

The silver impregnation method has been applied by G r a i n 1962, 1963 a, 1963 b, 1964, 1965 to the representatives of the family *Isotrichidae*. As results, the infraciliature and morphogenesis of three species: *Isotricha intestinalis* Stein, *I. prostoma* Stein and *Dasytricha ruminantium* Schuberg which are common in the rumen of cattle — has been described in details. The ciliature of the vestibulum was discussed and compared with the vestibulum ciliature of other representatives of the order *Trichostomata*.

The aim of the present study is only to point out some morphological features which were revealed specially distinctly in those species by means of the ammonium-silver impregnation method. Precisely, I wish to stress the applicability of this solution to impregnation of the representatives of the family *Isotrichidae* and to show the slight differences in the impregnation results of G r a i n and those of the present study.

The material was collected from the rumen content of catle of the Łódź district. Ciliates were fixed in 10⁰/o formalin, rinsed in water, placed on a slide and coated with a layer of gelatine after C h a t t o n, impregnated with the ammoniacal silver solution of B i e l s z o w s k i and — at last — treated with 10⁰/o formalin.

Results

Dasytricha ruminantium Schuberg (Pl. I 1)

It may be stated in the impregnated ciliates that the somatic kineties near the posterior pole loose their regular array. Only at the very margin of the vestibulum, in a reduced number they form a closely textured zone which is highly spiralized (Pl. I 1, 2). According to G r a i n on the ventral side some of the short kineties are prolonged as far as the vestibulum. It is a new finding of the author that several marginal kineties penetrate entirely into the vestibulum and loose the connection with the body surface (Pl. I 3).

The distinct impregnation of the transverse fibers on the dorsal wall of the vestibulum (Pl. I 4) should be also stressed. Presence of those fibers has been ascertained by G r a i n 1965 b in electron microscopy.

Isotricha prostoma Stein (Pl. II 5, 6)

G r a i n 1963 a described the vestibulum in *I. prostoma* as a cornet with its apex curved ventrally, coated entirely with spiral kineties. The photographs of my preparations (Pl. II 8, III 9—12) confirm the desciption of G r a i n except that lining of the vestibulum with kineties is not full. The gap between the kineties which are lining the vestibulum begins near its ventral margin and spreads broadly upon the dorsal side in accordance with the spiralization of kineties of different length. The Pl. II 7 illustrates the distinction of the vestibular kineties from the somatic ciliature which is marked in the drawings of G r a i n. In Pl. II 8 fibers which originate at the final kinetosomes are seen.

Isotricha intestinalis Stein (Pl IV 13)

The vestibulum of *I. intestinalis*, coincides with the description of G r a i n 1962, being bordered by the right lip — nearly parallel to the body axis — and the left lip which is oblique to it (Pl. IV 14). The ciliature of the vestibulum looks in my preparations as follows. In full accordance with description by G r a i n the somatic kineties which bend on the right lip, run on the wall of the vestibulum obliquely to its axis. They spread on a part of the ventral and anterior wall of the vestibulum and break off on the anterior wall along an arcuate line. Similarly, the kineties which bend on the left lip, are lining a part of the dorsal and anterior wall of the vestibulum — as reported by G r a i n, they break off on the anterior wall at a certain distance from the kineties of the oposite side. Kineties of the left lip are vertical to the neighbouring ones, as described by G r a i n (Pl. IV 14). The posterior wall of vestibulum as well as a part of the ventral and dorsal ones are lined with kineties which penetrate from behind into the vestibulum. This configuration is illustrated in Pl. IV 15—17. Consequently the vestibulum is not completely lined with cilia. On the anterior wall there remains a zone free of kineties. This part of vestibulum which is lined with kineties, forms a sort of a curved gutter, open at its anterior part with its margins being slightly tucked up.

The characteristic of the vestibular ciliature in the genus *Isotricha* should be supplemented by the statment that this ciliature is not complete and is more contorted in the species *I. prostoma* than in *I. intestinalis*. The distiction of the vestibular kineties from the somatic ones in the species studied — most strongly expressed in *Dasytricha ruminantium* — should also be stressed.

The ammoniacal silver solution seems to produce a very contrastfull impregnation of kinetosomes in *Isotrichidae*.

In my publication W o l s k a 1965 I have described the infraciliature of a ciliary group situated near the ventral margin of the ciliophore in *Ophryoscolecidae*. The term "free cilia" was applied to this ciliary group. I failed to know that this structure had already been described in details by B r e t s c h n e i d e r 1962 as "Paralabialorgan", and subsequently discussed by R o t h et S h i g e n a k a 1964. My publication from 1965 — its part concerning the "free cilia" — is in some degree a repetition of their findings. Nevertheless it retains its value as a proof of applicability of the ammoniacal silver solution (when applied according a definite procedure) for revealing the infraciliature of the rumen ciliates

Summary

Results of impregnation with ammoniacal silver solution are reported in the case of three species of the family *Isotrichidae: Dasytricha ruminantium* Sch., *Isotricha intestinalis* St., and *I. prostoma* St. Some deviations of those results from the former findings are indicated.

It has been ascertained that the "free cilia" described in the author's publication 1965, are identical with "Paralabialorgan" reported previously by B r e t s c h n e i d e r 1962.

STRESZCZENIE

Podane są wyniki srebrzenia amoniakalnym roztworem srebra trzech gatunków z rodziny *Isotrichidae: Dasytricha ruminantium* Sch., *Isotricha intestinalis* St. i *I. prostoma* St. i wykazana pewna rozbieżność tych wyników z dotychczasowymi danymi.

Stwierdzone jest, że opisane w publikacji autorki 1965 „wolne rzęski" Ophryoscolecidae są identyczne z opisanymi poprzednio przez B r e t s c h n e d e r a 1962 jako „Paralabialorgan".

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Addendum

In paper: W o l s k a M. (1965): Remarks on the adoral ciliature in the order *Entodiniomorpha*. Acta Protozool 3, 321—324 the following changes should be made: Page 322, line 31, instead of: (Pl. I 2) should be: (Pl. I 2, 6). Page 322, line 50 instead of: (Pl. I 5, 6) should be: (Pl. I 5). Page 323, line 19—23 instead of erroneously printed text should be: Its presence — besides some other characters — places according F e r n a n d e z - G a l i a n o the family *Cycliposthiidae* at a lower level of the phylogenetic tree then the family *Ophryoscolecidae*. Nevertheless similarity of adoral ciliature in two families is more conspicuous than it could be assumed as yet. Page 325, line 10 instead of: 5, 6 should be: 5.
Page 325, it should be added: 6. *Cycliposthium* sp., rows of free cilia.

EXPLANATION OF PLATES I—IV

Dasytricha ruminantium Schuberg

1: General view of cilate. 1000 × approx
2: Posterior body part, the zone of spiral kineties around the vestibulum is seen. 2500 × approx
3: Ventral wall of vestibulum, optical section. 2500 × approx
4: Dorsal wall of vestibulum, optical section. 2500 × approx

Isotricha prostoma Stein

5: General view of ciliate. 500 × approx
6: Anterior body part. 1000 × approx
7: Posterior body part, a part of vestibulum is seen. 1000 × approx
8: The same individual as in 7 in optical section, kineties of vestibulum and fibers are seen
9: Ventral wall of vestibulum, optical section. 2500 × approx
10: The same individual as in 9 in optical section, dorsal wall of vestibulum is seen
11: Incomplete ciliature of vestibulum is seen, optical section. 2500 × approx
12: Vestibulum is seen in another position than in 11, dorsal and ventral vestibular kineties are seen simultaneously, optical section. 2500 × approx

Isotricha intestinalis Stein

13: Posterior body part and vestibulum; surface view, a part of vestibulum in optical section. 1000 ×
14: Surface view near the vestibulum. 2500 ×
15: Vestibulum in optical section. 2500 ×
16: Vestibulum in optical section, in a slightly different position than in 15. 2500 × approx
17: Kineties penetrating into the posterior wall of vestibulum. 2500 × approx

M. Wolska   auctor phot.

PLATE II

M. Wolska

auctor phot.

PLATE III
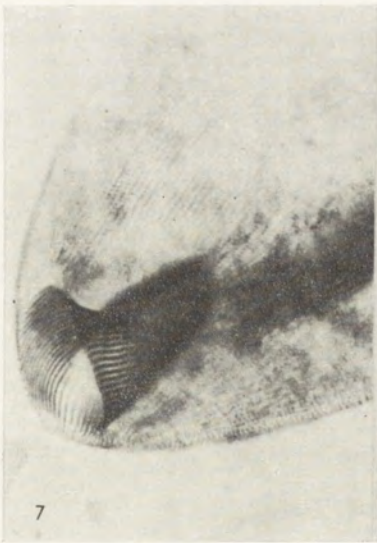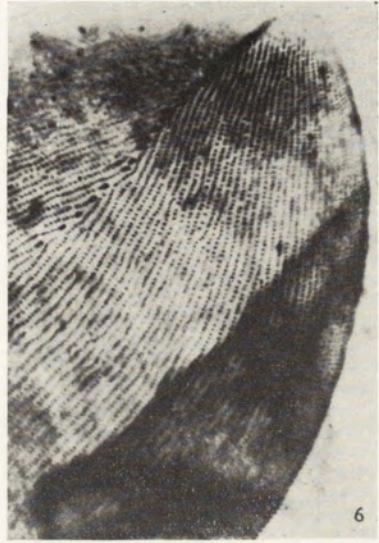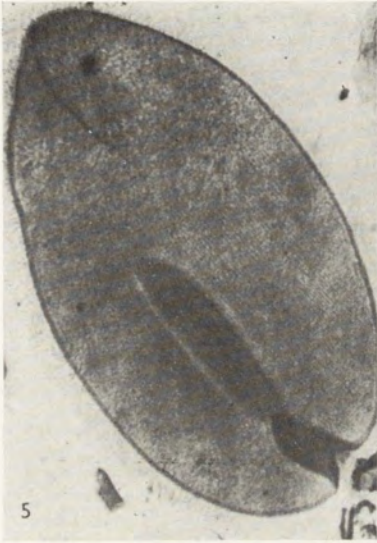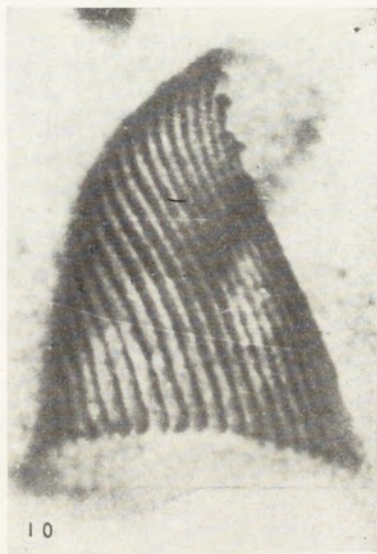
M. Wolska

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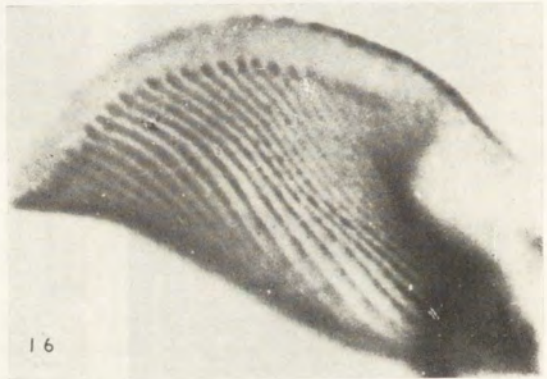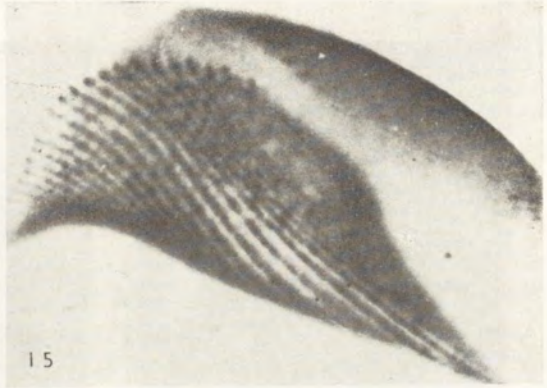
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Irena DWORAKOWSKA*

The structures of the nuclear apparatus in *Plagiotoma lumbrici* Dujardin and their transformations in division cycle

Struktury aparatu jądrowego *Plagiotoma lumbrici* Dujardin i ich przemiany w cyklu podziałowym

Plagiotoma lumbrici Dujardin has been chosen as the subject of the present study on account of the specific structure of its nuclear apparatus and the possibly specific course of transformations in division.

Plagiotoma lumbrici Duj. has been included to the family *Plagiotomidae* together with *Nyctotherus, Paranyctotherus* and *Nyctotheroides*. Informations concerning the biology of this species have been reported by P e r t z e w a 1929. This author, similarly as H e i d e n r e i c h 1935, sygnalized its occurrence in the intestine of *Lumbricus terrestris* and *L. rubellus*. The material for the present study derived also from those species and from *L. castaneus*.

The body of *P. lumbrici* is flattened, with the outline of an elongated oval. The AZM is very long and runs along the left margin of the body as far as cytostome which lies at the distance of 2/3 from the anterior body end. In the posterior part of the ciliate, behind the cytostome, spreads the territory of the digestion cytoplasm. This territory is precisely limited and no food vacuoles occur beyond its boundary. The nuclear apparatus of *Plagiotoma lumbrici* comprises most frequently 2 Mi and Ma which is fragmented into a variable number of parts.

Material and methods

Earthworms used in this study: *Lumbricus terrestris* and *L. rubellus* were collected mostly in the park of the Białowieża Mansion, a part of them — in Warszawa. *Lumbricus castaneus* originated exclusively from Warszawa [1].

Earthworms were captured from April till August 1964. The material collected in August was kept in the laboratory over winter till April 1965.

The density of ciliates in earthworms fluctuated considerably with the season of the year, locality, age of the host individual and the species. In winter the density of population increased considerably in all the individuals.

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[1] Earthworms were determined by dr J. Plisko from Institute of Zoology, Polish Academy of Sciences.

The content of the posterior intestine of several earthworms was submerged with water at room temperature. After 30 min. the liquid containing the free-swimming protozoa was carefully decanted and centrifuged. Protozoa remained unimpaired after this procedure.

After fixation in the Schaudinn's fluid, protozoa were stained with the iron haematoxylin of Heidenhain. The Feulgen reaction was carried out after fixation in the Carnoy fluid. A certain number of preparations were executed after the following procedure: living protozoa were placed after centrifugation in 1⁰/₀ aqueous solution of sodium citrate for 20—45 min. or even more. Subsequently they were centrifuged and fixed in the Carnoy fluid for 5—10 min. and dried completely. The Feulgen reaction was carried out immediately after desiccation. Sodium citrate was applied for a group of preparations in order to loosen the structures.

The method of Pappenheim-Unna controlled by preparations digested with saliva ribonuclease was also used.

Results

In the division cycle of *Plagiotoma lumbrici*, 4 phases may be distinguished (in relation to Ma) similarly as it was done in the case of *Blepharisma undulans* (H e l s o n et al. 1959), namely: interphase, precondensation, condensation and postcondensation.

The division of Ma and of Mi will be described separately, with distinction of those periods. Conjugation was not observed.

Macronucleus

The interphase

The macronucleus occupies a triangular area which lies at a distance of approx. 1/3 of the body length from the anterior end of the protozoon. A part of Ma is located however on the territory adjacent to the digestion cytoplasm, always near its left margin.

The main mass of Ma as well as its "tail" which lies in the posterior body part, are in fragments. The number, size and shape of those fragments are very variable. They may be completely detached from one another or linked by bridges. Their number may fluctuate from several to thirty.

The chromatin material in the fragments fails to form distinct regular structures but forms rather agglomerations with neither definite shape nor regular distribution (Pl. I 1). In the nucleus, treated in this phase with sodium citrate, fragments are enlarged, chromatin forms no agglomerations but is dispersed uniformely. Staining with pyronine at this stage presents some more clear pictures: the nucleus is highly stainable but the accumulations appear more regularly than the chromatin structures (Pl. I 2). In many places appear big spheroid pyronine-positive agglomerations. Sometimes they occur simultaneously in all the fragments (Pl. I 3). In preparations stained after the Unna-Pappenheim method, those places are pyronine-positive and fail to stain with methyl green. In the control treated with ribonuclease, they are negative as well to pyronine as to methyl green. Those structures are unstained after the Feulgen's method being then visible as vacuoles (Pl. I 4).

Precondensation

The vacuoles in the fragments disappear gradually and the structure of fragments becomes more uniform. Zones of a rather faint stainability appear in them. They have usually a shape of stripes which are sharply delimited at least on one side. Most frequently more than one fragment in an individual contains such zones, however individuals were found in which those structures were present in all the fragments (Pl. II 11). Usually in one fragment one zone occurs but fragments were found with a higher number of them (Pl. II 9). Faintly stainable zones may be located in different places of the fragment (Pl. I 6, Pl. II 11); they always are oriented transversally to its axis.

In the period when the zones appear, the structure of the fragments is different than it was before. Chromatin becomes arranged in the form of agglomerations that lie along the fragments vertically to the zones (Pl. I 5—7). This pattern reminds somewhat the chromatin elements in division. They are often cords of grains being sometimes double (Pl. I 6).

In many cases, a stripe of highly stainable chromatin, parallel to the zone, lies closely to the faintly stained zone (Pl. II 10). In this period, fragments are of the same size as in interphase but the gradual reduction of the digestion cytoplasm causes their proximity to the middle region of the body.

Condensation

In fragments of Ma, the faintly stainable zones disappear whereas the chromatine structures become manifested more and more distinctly: chromatin bands running across the fragments (Pl. II 12), as well as distinct rows of grains touching one another and arranged along the fragments (Pl. II 13—14).

Fragments show an inclination to fuse together into one mass. Simultaneously the chromatin structures differentiate more distinctly (Pl. II 14). They are grains linked by filaments of a Feulgen-positive material. A longitudinal arrey — along the thread — as well as a transverse one (of similar size) of grains may also be observed. A still more distinct regular pattern of grains appears after application of sodium citrate (Pl. II 15). In the subsequent condensation of the fused Ma, grains begin to disappear and highly developing filaments arise instead. Towards the conclusion of this process, no grains are present in Ma but only filaments of variable thickness which somatimes are hardly visible in microscope (Pl. III 16—17).

In the Ma of this stage, pyronine stains intensely the structure of the thick threads (Pl. III 19). Subsequently the chromatin filaments become thick again, the structure is no longer so distinct as it was before and Ma assumes gradually a fusiform shape as if the lateral fragments were drawn inside (Pl. III 20—22). Chromatin structures are much less distinct than before. The grain rows however running along the whole Ma, are still distinguishable. It is impossible to discern details in this compact mass of chromatin. The nucleus in condition of maximal condensation is shown in Pl. III 22.

Postcondensation

Ma remains for a short period of time in condition of maximal condensation. It undergoes distension to the offspring cells very quickly. Simultaneously with the onset of distension, the high stainability with pyronine, disappears. The nucleus at this stage is nearly stainless (Pl. IV 23).

Before separation of Ma to the offspring individuals, its synchronic fragmentation in the future prother and opisthe takes place (Pl. IV 24). Chromatin is in form of grains and filaments which seem to constitute an uniform structure.

In some cases between the offspring individuals, an undivided part of chromatin remains (Pl. IV 25) which is possibly resorbed in cytoplasm, similarly as it occurs with the fragment not included during the condensation of Ma. As a result of subsequent fragmentation, several parts arise, lying linearly sharply delimited from one another. The chromatin structures in the shape of grains on filaments are still preserved, similar to those which occur in the beginning of fragmentation.

In this period, Ma continues initially to stain faintly with pyronine but some swollen cords of a higher stainability are seen (Pl. IV 26). Position of those structures corresponds to that of the rows of chromatin grains. Later on the affinity to pyronine abruptly increases up to the level observed in interphase but the structures observed previously, still remain visible (Pl. IV 27).

In this phase, the offspring individuals are in most cases separated from one another. The fragments of Ma divide gradually into smaller particles, loose their regular array of chromatine and change their position in respect to one another.

In Pl. IV 28, the posterior part of Ma is pushed sidewards by the digestion cytoplasm being formed, and as a result the distribution of Ma chromatine arises which is typical for the interphase stage.

Micronucleus

Interphase and precondensation

Mi of *Plagiotoma* measures about $8\,\mu$ in diameter. The protozoon contains usually two Mi. They are situated near the left margin on side of the main Ma mass. Sometimes individuals with one Mi occur, more rarely with three of them and those with no Mi are observed also. After all the staining methods Mi is homogenous and the Feulgen's reaction reveals a high content of DNA. In all the phases of the cycle, Mi is much more Feulgen-positive than Ma.

If the ciliates have been treated with sodium citrate prior to the staining, the diameter of Mi increases two times and then it is possible to discern agglomerations of chromatine in it which are dispersed regularly over its whole volume. Then the intensity of its staining is similar to that of Ma fragments. The effect of staining with pyronine is homogenous and is much more faint in Mi than in Ma (Pl. I 2—3, II 9).

Condensation

In the late phase of Ma condensation, Mi undergoes division. Initially Mi elongates, remaining still homogenous. Then its volume considerably increases and highly stainable chromatin filaments appear in it. They are disposed regularly and run along its whole length. They fill the whole Mi lying closely one on the side of the other being very numerous. The exact count of the filaments is impossible (Pl. III 17). This picture may be assumed as the pattern of pro-metaphase.

Later on, in proportion as the nucleus elongates, its picture becomes clearer and the long parallel chromatin filaments are seen (Pl. III 18). In some places, the disposition of filaments in pairs, one on the side of the other is distinct

which is not visible in the photogram. At this stage Mi is pyronine-negative (Pl. III 19).

Subsequently the chromatine filaments are grouped over towards the direction of two poles of the elongated Mi and the boundary between the two complexes of chromatin mass is manifested. This stage may correspond to anaphase. In a later period, chromatin maintains its filamentous structure in its middle part, and becomes homogenous at the poles. This marks the beginning of telophase (Pl. III 20—22). The polar parts of dividing Mi may assume various shape and size. Gradually the remnants of chromatine filaments disappear, and Mi with a structure of chromatine characteristic for interphase are formed.

Postcondensation

In the postcondensation phase of Ma, the chromatine pattern of Mi does not differ from that of interphase but the nucleus is initially fully pyronin-negative (Pl. IV 23). The affinity of Mi to pyronine increases with the advancing fragmentation of Ma. Towards the end of postcondensation stage of Ma, it reaches the degree characteristic for interphase (compare Pl. IV 26 with 27).

Transformations of Ma in the division cycle last longer than those of Mi. The correlation decribed above is the most frequent but exceptions occur, e.g. the case represented in Pl. IV 25 where the beginning of anaphase in Mi coincides with the postdivision fragmentation of Ma. Usually both Mi undergo the same division stages simultaneously but here exceptions occur also (e.g. the picture in Pl. III 22).

It seems that the amount of chromatin in Ma as well as in Mi may differ considerably in single individuals (Pl. III 21—22, IV 24). Pl. IV 25 shows an uneven division of one of Mi which may possibly present a manner of formation of an individual without Mi. As a result of fission of the offspring individuals, one protozoon would arise without Mi and another one with three Mi and with a resorbed particle of chromatin.

Discussion

Chromatin of macronucleus.

Three ways of arising of polyploidy in Ma in course of its formation from the products of the synkarion division have been known till the present time:
1. fusion of diploidal nuclei, as in *Trachelocerca phoenicopterus* (R a i k o v 1958),
2. the endomitotic polyploidization as in *Ephelota gemmipara* (G r e l l 1953 a),
3. polyteny, as in *Nyctotherus cordiformis* (G o l i k o v a 1964) or in *Stylonychia* (A m m e r m a n n 1964, A l o n z o and P è r e z - S i l v a 1965). This variability of way in which polyploidy is effected, may be partly explained by the variability of chromatin structures existing in the vegetative nucleus which are assumed by a number of authors as chromosome aggregates.

In the resting Ma of many ciliates, rows of grains or rods were observed J u r a n d et al. 1962, G r e l l 1950 b, G r e l l 1953 b, M ü g g e 1957, G o l i k o-v a 1964). Grains which failed to show regularity in the preceding period, become organized into regular bead strings during division (C a l k i n s 1919, 1930 a, W e y e r 1930, T i t t l e r 1935, H. R a a b e 1947, K o r m o s and K o r-

m o s 1957), or chromatin filaments appear in this period (D i l l e r 1928, G r e l l 1950 a, K a n e d a 1960, R u t h m a n n and H e c k m a n n 1961, R u t h- m a n n 1963).

Very important seems to be the finding of S e s h a c h a r 1960 that the chro- matin grains of the interdivision Ma may be experimentally transformed into bundles of fibrils. S e s h a c h a r achieved this by intense centrifugation of the *Spirostomum* and *Blepharisma* nuclei. A similar effect was obtained by application of KCN or NaCN solution (S e s h a c h a r 1963).

The electron microscopic study revealed that the chromatin elements in Ma are small bodies (R u d z i ń s k a and P o r t e r 1955, J u r a n d and al. 1962) however fibrillar elements have been described as well (R u t h m a n n and H e c k m a n n 1961, D i p p e l and S i n t o n 1963, K l u s s 1962). S e s h a c h a r 1964, 1965 stated that in *Blepharisma intermedia* and in *Spirostomum ambi- guum,* the small bodies are cross sections of the fibrils which are seen in the light microscope. S e s h a c h a r assumes that they are chromosomal structures.

The hypothesis of the subnuclear organization of Ma chromatin (S o n n e- b o r n 1947) assumes that Ma contains diploidal elements able to autoreplica- tion and to segregation in successive divisions. The hypothesis of "Sammel- chromosomen" (G r e l l 1950 b) postulates that chromosomes belonging to the same genoms join one another by ends and form an aggregation which repli- cates and segregates in division as an entity. The fundamental difference bet- ween the subunits suggested by both hypotheses is the number of genoms in their composition. The subnucleus would be constituted of two "Sammelchro- mosomen".

"Sammelchromosomen" are known in several protozoa (G r e l l 1953 c, 1953 d, R u t h m a n n 1963). R a i k o v 1964 proved that the Ma of *Nassulopsis elegans* contains "Sammelchromosomen". The calculation based on the degree of polyploidy and on the number of structures observed, indicated that they are diploidal, and consequently correspond to subnuclei.

Chromatin in the Ma of *Plagiotoma* undergoes transformations in the course of division cycle. It appears in the interphase as irregular agglomerations, as grains connected by fibrils in precondensation and in early condensation, as filamentous structures, grains of fibrils again in the period of postocondensa- tion and at last returns to the interphase structure. In the other ciliates similar transformations are explained as the result of changes in the spiralization of chromatin fibrils (S e s h a c h a r 1960, 1963, 1964).

In the interdivision period of *Plagiotoma lumbrici,* the existence of condensed chromatin is proved by the effect of sodium citrate action when the loose, indistinct nearly homogenous chromatin appears. Results obtained after this method resemble to the interphase nuclei of higher plants and animals.

Existence of chromatin structures in the resting stage of Ma inclined (G r e l l 1964) to include this nucleus into the category of chromosomal nuclei. The nucleus of *Dinoflagellata* has been included to the same category, because its structure in interphase is similar to that of Ma and is equally rich in DNA.

In the case of *Plagiotoma lumbrici,* the chromatin structures of its resting nucleus are not distinct enough for determination of their pattern. In the con- clusive phase of precondensation and in the beginning of condensation, chro- matin cords — swollen in some parts — become visible (Pl. I. 5), as well as grains on the fibrils (Pl. I 7). R u t h m a n n and H e c k m a n n 1961 interpreted the nature of similar grains in *Bursaria truncatella* as a result of fibrils spira-

lization. The structures in Pl. I7 resemble to that seen in endomitoses in the rudiments of Ma in *Ephelota gemmipara* (G r e l l 1953 a, tab. 12, fig. 1, 2). It may be observed in the period of condensation in *Plagiotoma,* that the rows of grains are also parallel (Pl. II 14, 15). The similitude with the former case suggests that this array may be assumed as result of endomitoses. This interpretation is put forward by R u t h m a n n 1963 for analogical structures in Ma of *Loxophyllum meleagris.*

In the late period of condensation, grains disappear in Ma of *Plagiotoma* and filaments appear instead. Filaments are of various thickeness and are disposed more or less along the axis of the elongated nucleus. It can hardly be ascertained whether they run along the whole length of Ma or only along a part of it (Pl. III 17). They resemble somewhat to fibrils obtained from Ma of *Blepharisma* by S e s h a c h a r. Possibly they are similar elements as those described in *Bursaria truncatella* by R u t h m a n n and H e c k m a n n.

Those elements might have arisen by despiralization of grain rows which were observed previously, and this moment seems to be a critical point in the division process of Ma. At this time, the most essential rearrangments of the chromatin material should occur in connection with the division. Such rearrangments have been ascertained by means of the autoradiographic method in the condensing Ma of *Euplotes eurystomus* (K i m b a l l and P r e s c o t t 1962). In *Loxophyllum meleagris* division takes place in course of formation of the fibrillar structure which arose as result of respiralization of grains (R u t h m a n n 1963). In *Plagiotoma lumbrici,* Ma does not divide at this phase: a spiralization occurs again as well as shrinkage of the whole nucleus. Grains appear once more and distension of the nucleus follows.

Together with the fission of Ma to the offspring individuals its fragmentation in each of the two parts occurs. During this process, chromatin forms cords of beads. The nucleus is however so highly condensed that the details of their partition cannot be discerned. It may be clearly observed that fragmentation is not accompanied by constriction of the nuclei as it takes place in the other ciliates in which numerous Ma fuse together into one nucleus in division (C a l k i n s 1919, 1930 a, W e y e r 1930, H. R a a b e 1947). Fragmentation is here the disruption of Ma which produces fragments with sharply delimited margins. Such a disruption may occur simultaneously in two or in a higher number of spots within one Ma.

P e r t z e w a 1929 reported that in the division of *Plagiotoma lumbrici,* a smaller amount of chromatin is transmitted to the prother. In the present study this regularity has not been ascertained. It was proved however that the division may begin when variable amounts of chromatin are present in the Ma of individuals.

In majority of higher ciliates, the segregation apparatus in Ma — analogous to the mitotic one — has not been revealed, although in *Nassula ornata,* distinct fibrillar structures associated with division occur (R a i k o v 1962). In *Plagiotoma lumbrici* no traces of similar structures were found.

The interesting problem of the nuclear membrane of the macronucleus during the transformations in chromatin has not been solved either. The character of Ma of this ciliate is rarely found in protozoa; it occurs in some parasitic ciliates of the order *Apostomata* (P e r t z e w a 1929). The division cycle of Ma in the cyst of *Foettingeria actinarum* resembles to some structures in the Ma division in *Plagiotoma lumbrici* (D e M o r g a n 1924).

Chromatin of micronucleus

In the majority of ciliates Mi is a small nucleus measuring 1—4 μ. In *Plagiotoma lumbrici* Mi is exceptionally big; its diameter amounts approx. 8 μ. Mi of this size are known also in *Pleurotricha lanceolata* (R e g i n a l d and M a n w e l l 1928) and in *Uroleptus halseyi* (Calkins 1930 b). In the majority of cases, Mi is a homogenous body, with a high affinity to nuclear dye-stuffs (W e y e r 1930, C a l k i n s 1930 b, H. R a a b e 1946). In *Plagiotoma lumbrici* the nucleus is also highly condensed, in interphase no structures are discernable in it, and the Feulgen's reaction is much more intense than in Ma. The density of the chromatin material is posssibly much higher in it than in Ma although perhaps not so considerable as it has been reported for *Bursaria truncatella* (R u t h m a n n 1964). It is difficult to evaluate the degree of chromatin condensation in Mi compared with that of Ma, basing only on observation. After application of sodium citrate, preparations were obtained in which chromatin of both the nuclei acquired approximately the same degree of looseness in both nuclei and its approximate amount could be compared. Those comparisons seem to indicate that Ma contains only 5—15 times more chromatin in relation to one Mi.

During the division of Mi in *Plagiotoma,* numerous long filaments become visible. They are more or less parallel to one another and to the division axis and run along the whole length of Mi (Pl. III 17). The array and homogenity of those chromatin elements remind the division of other ciliates. These chromatin elements — usually called chromosomes — assume always a position parallel to one another and to the division axis when they lie in the equatorial plan of the division figure. Such a chromatin pattern was described as metaphase by R e g i n a l d and M a n w e l l 1928, C a l k i n s 1930 b, K i d d e r 1933, H. R a a b e 1946, D i l l e r 1954. In those cases, the length of the chromatin elements equaled no more than half of the nucleus length and they were accompanied by fibrillar achromatin structures of the division spindle apparatus.

The division spindle was described in the majority of Mi. It was found in *Plagiotoma lumbrici* by P e r t z e w a 1929 and was accepted as metaphase stage together with the chromatin agglomerations which occur simultaneously. P e r t z e w a found this stage only twice (pictures 27 and 28 of P e r t z e w a) and was not sure as to its interpretation. She considered it as a simplified form of mitosis which is common among Protozoa.

Structures similar to that described above or even still more irregular, were known in many ciliates but were rather considered as forms of degeneration (C a l k i n s 1930 b). Divisions illustrated by P e r t z e w a were presumably of the same character.

The only stage corresponding to the metaphase would be in *Plagiotoma lumbrici* that of the filaments running along the whole length of Mi (Pl. III 17). A case of division of Mi similar to that which has been found in *Plagiotoma lumbrici* was described by W e n r i c h 1926 in division of *Paramecium trichium*. Mi is filled with long parallel chromatin filaments. W e n r i c h failed to ascertain a typical metaphase or distinct subsequent division stages in the case studied by him. The Mi filaments gradually separate from one another and condense at the poles.

In *Plagiotoma lumbrici* the division of Mi is similar. It was observed in some dividing nuclei that the filaments are double but it was hard to establish

whether they separate or pass as a whole to one pole. In most cases, chromatin of Mi exhibits some deviations from the mitotic scheme. G r e l l 1950 b — considering the above facts — claims that the Mi chromosomes of ciliates occur as "Sammelchromosomen" which divide however owing to the spindle.

Chromosomes linked by their ends often occur in Mi during meiosis in the maturation divisions (N o l a n d 1927, D i l l e r 1948, R a y 1956, W i c h t e r-m a n 1937) or in pronuclei (C a l k i n s 1930 b, W i c h t e r m a n 1937). In the divisions of vegetative Mi, chromatin appears in the form of bead strings e.g. in *Gastrostyla steini* (W e y e r 1930) and undergoes the stages of different spiralization which reminds the behaviour of "Sammelchromosomen" in division of Ma in *Loxophyllum meleagris* (R u t h m a n n 1963). In the division of Mi in *Uroleptus halseyi,* a band constituted of chromatin grains occurs (C a l k i n s 1930 b) and in *Urostyla grandis* the grains in this band are disposed in pairs (H. R a a b e 1946). During division of Mi the position of the chromatin elements, which is parallel to the division axis, is similar to the positon of "Sammelchromosomen" in division of *Aulacantha scolymantha* (G r e l l 1953 a), as well as in *Dinoflagellata* (G r e l l 1964).

In those protozoa chromosomes form aggregations and in division the sister chromatides shift to one of the poles. Such a division would possibly occur in the Mi of ciliates. In a number of ciliates complex segregation of the chromatin material takes place during meiosis. It is often associated with peculiar mitotic figures, the so-called "parachute stages" as in *Oxytricha fallax* (G r e g o r y 1923). N a n n e y and R u d z i ń s k a 1960 postulate that the complex processes are associated with the transformation of the chromatin aggregations into chromosomes. This would be in agreement with the view of D e v i d e and G e i t l e r (after A l f e r t and B a l a m u t h 1957) that in ciliates, the individual chromosomes in Mi are clearly discernable only during meiosis or immediately after its completion.

The chromatin filaments in Mi of *Plagiotoma lumbrici* might be the aggregations of chromosomes. In this case the occurrence of Mi with various amounts of chromatin would be accounted for (Pl. III, 20—22). In *Hyalospora caridinae* the difference in the Mi size is a regular phenomenon. In the vegetative individual its diameter amounts 7μ and only 2μ in the foront (M i y a s h i t a 1933). This phenomenon may be connected with the reduction of the number of aggregations comprised in the nucleus. Division of such a "Sammelchromosome" Mi might possibly be analogous to the segregation of genoms in Ma. In division of Mi in *Plagiotoma lumbrici* no division centres occur.

It is difficult to follow the fata of the nuclear membrane of Mi during division. It seems that the membrane remains unchanged in the polar parts whereas it is disrupted in the central region during the separation of chromatin mass.

The division period of Mi in *Plagiotoma lumbrici* as related to the division changes in Ma is essentially the same as in most other ciliates (S a i t o and S a t o 1961 a, C a l k i n s 1930 b, W i c h t e r m a n 1937).

Nucleoli and RNA

The Feulgen-negative vacuoles visible in Pl. I 4, giving a positive reaction after pyronine (Pl. I 3) are nucleoli. Nucleoli in the ciliate Ma are considered as analogous structures to nucleoli of other animal and plant cells on account of their cyclic appearance and disappearance during division, as well as of

their content of RNA. Disappearing of nucleoli is considered to be associated with the passage of RNA to cytoplasm, which may occur — according to the view of some authors — more or less explosively and synchronously. Nucleoli may be emitted to cytoplasm as e.g. in the rudiment of Ma in *Paramecium bursaria* (E h r e t and P o w e r s 1955), or may pass to cytoplasm prior to the division of the cell (K a n e d a 1960, W e n r i c h 1926, S a t o 1963), or are emitted to cytoplasm in the interphase period (G r e l l 1964).

Most probably the granules x eliminated prior to the division as described in some ciliates (C a l k i n s 1930 b, S u m m e r s 1935, T i t t l e r 1935) correspond to nucleoli.

In the Ma of *Plagiotoma lumbrici* nucleoli are visible for a long time since the beginning of interphase. Towards the end of this phase their number diminishes till they disappear completely. Passage of this elements trough the nuclear membrane was not observed. Presumably they disappear gradually within the nucleus similarly as in the other ciliates (S a t o and S a i t o 1959, R u t h m a n n 1963, S c h w a r z 1956).

In protozoa the nucleoli-organizing chromosomes are known in *Zeleriella* (*Opalina*) (C h e n 1936). In the course of development of the Ma primordium, the nucleolar structures similarly as chromatin, show a more expressed regularity in ciliates. The association of nucleoli with the site of chromatin formation in the chromosomal structure becomes more distinct. In *Bursaria truncatella* e.g. (P o l i a n s k y 1934) and in *Paramecium caudatum* (S a i t o and S a t o 1961 b), the heterochromatic segments of chromosomes are connected with nucleoli. E g e l h a a f 1955 ascertained the occurrence of pairs of nucleoli or even groups of pairs in the development of Ma in *Paramecium bursaria*. He even postulated its association with the homologous structures of chromatin. In all those cases, any regularity in the nucleolar system fails to be observable after formation of the resting Ma. Possibly the connection of nucleoli with the nucleoli-organizing sites in chromosomes is preserved, however different secondary links arise as well with chromatine as with the other nucleoli (G r e l l 1953 a, R u t h m a n n 1963).

Nucleoli in the Ma fragments in *Plagiotoma lumbrici* are distributed more or less evenly but without a perceptible regularity and with no connection with distinct chromatin structures. This is possibly involved by the complexicity of the chromosomal structure in the interphase Ma.

Ma of *Plagiotoma lumbrici* contains a considerable amount of RNA which is not located in nucleoli. This RNA persists in the nucleus for a long period of the life cycle, and disappears only in the phase of the early postcondensation. In respect to the period of its disappearing from the nucleus, it reminds the chromosomal RNA of *Metazoa* (J a c o b s o n and W e b b after K a u f m a n n and al. 1960).

The non-nucleolar RNA structures in Ma of *Plagiotoma lumbrici* resemble to the DNA structures in the division cycle, being however bigger, seemingly embracing the chromatin elements. It may be postulated that this is the chromosomal RNA like that which was reported in Ma of other ciliates (S c h w a r z 1958, R u t h m a n n and H e c k m a n n 1961, R u t h m a n n 1963).

R u t h m a n n 1963 reported that the chromosomal RNA may be associated with numerous loci and in the given time only some chromosomes contain RNA. Such details could not be observed in the picture of chromosomal RNA in *Plagiotoma lumbrici*.

Mi of *Plagiotoma lumbrici* is comparatively slightly pyronine-positive and — similarly as in the case of the Feulgen's test — its staining is homogenous. The DNA content in Mi exceeds many times that of RNA, what is still more clear when comparing the quantity of those two substances in Mi with their quantity in Ma. If the production of RNA-protein is accepted as indicator of metabolic activness of DNA, this example would reflect properly the dominating importance of Ma in the life processes of the ciliate and in the expression of phenotype (S o n n e b o r n 1947). This follows from the fact that the Ma chromatin has other capability of RNA production than the Mi chromatin.

M o s e s 1950 studying the content of DNA, RNA and protein in Ma and Mi of *Paramecium aurelia*, came to conclusion that the difference between those nuclei consists only in the different quantity of those compounds, but their quantity ratio is in both nuclei the same. The functional difference — according to M o s e s — would consist in the quantitative domination of the Ma chromatin.

It is clear that in the case of *Plagiotoma lumbrici,* the quantitative ration of the DNA and RNA content is inverse in both nuclei studied. Since the differences in the structural organization of chromatin in those nuclei are insignificant a suggestion arises that those differences are only qualitative. The different function of both nuclei in ciliates has been put forward long time ago and the substance evoking this differentiation was named idiochromatin (Mi) and trophochromatin (Ma).

This was applied by D i l l e r 1928 for explaining the role of elimination of chromatin from the Ma rudiments in *Trichodina pediculus.* According to his view, after elimination of some amount of chromatin material which interfered with the trophical functions, only the genuine Ma trophochromatin remains. In the light of this view, Mi would differ from Ma in posessing a certain amount of chromatin non present in Ma and — possibly blocking its trophic functions. It would also indirectly explain the ground of difference in the DNA and RNA content in both nuclei in *Plagiotoma lumbrici.*

At present it is difficult to ascertain whether this proportion of nucleic acids content in the Ma and Mi of ciliates is a rule.

According to conception of the ciliates Mi is nearly free of RNA whereas Ma is very rich in this compound. Results obtained by M o s e s 1950 in *Paramecium aurelia* would be — in this respect either erroneous or this ciliate is an exception. The differentiation of Ma chromatin from Mi chromatin by elimination of a part of this substance — as suggested by D i l l e r 1928 for *Trichodina pediculus* — may be a more common phenomenon in ciliates than it is supposed. The cases of chromatin elimination during the development of the Ma rudiments are known for a long time in many ciliates (M u s l o w 1913, C a l k i n s 1919, K i d d e r 1933, E l l i s 1937, D i l l e r 1954, G o l i-k o v a 1964).

In the development of Ma primordium, in most of cases described, a coalesced mass of Feulgen positive material appears. It is also mentioned by D o g i e l et al. 1962 who called it a kariosom-like body. In some cases this mass is the eliminated chromatin described above. If the elimination of chromatin from the Ma rudiments proved to be a common phenomenon, a real ground for the functional differentiation of both nuclei would be found. In this case such

a differentiation would remind the differentiation of somatic and generative tract cells known in many *Metazoa* e.g. *Ascaris megalocephala, Cyclops* or *Cycidomyidae (Diptera).*

Faintly stainable zones

In the Ma interphase in *Plagiotoma lumbrici,* stripes of a low stainability, distinctly delimited from the remaining chromatin, become distinctly visible. Their aspect is similar to that of the reorganization areas occurring in *Hypotricha.* In ciliates endowed with a complex Ma, those stripes appear prior the condensation but the time of their persistence may embrace even the whole interphase. The reorganization stripes occur synchronously in all the nuclei of an individual; the nucleoli disappear earlier than reorganization stripes.

On the boundary of the stripe and the non-reorganized territory, lies a band of a highly stainable chromatin. Outside this band staining of the territory is faint and closely to the reorganized part the territory fails to stain at all (C a l k i n s 1930 a, W e y e r 1930, T i t t l e r 1935, H. R a a b e 1947, S u m m e r s 1935).

The morphological description of the stripe has been supported by the subsequent autoradiographic investigations, which proved that incorporation of thymidin occurs only in the last narrow, completely unstainable region (G a l l 1959).

The zones of a low stainability in *Plagiotoma lumbrici* resemble to the reorganization areas of *Hypotricha* in their general aspect and in their stainability. Nevertheless the differences in the intensity of stainability of different regions cannot be observed. In many cases only the band of a highly stainable chromatin is visible (Pl. II 10—11).

In Pl. II 8 the faintly stainable zone is swollen. Similar pictures are found rather often and it may be supposed that they appear as a result of a strong despiralization of DNA fibrils in this region, similarly as it was ascertained in the reorganization area (K l u s s 1962). This region is only faintly and homogenously stained with pyronin; it is however not clear whether this character of staining is due to despiralization or to loss of a part of RNA. At any rate the above observations fail to supply evidences in favour of the possibility that RNA synthesis is inhibited in this territory.

In *Plagiotoma lumbrici,* the slightly stainable zones are accompanied by chromatin elements parallel to them, lying along the fragments of Ma (Pl. I 5—7). Such structures were not found in reorganization areas of *Hypotricha,* however in *Chlamydodon pedarius* a chromatin network was seen at this place (K a n e d a 1960). The chromatin strings near the faintly stainable zones in *Plagiotoma lumbrici* distinctly precede the grains on fibrils which are found in the later periods of the cycle. Such structures are known in the division cycle of *Hypotricha* in the stage of the Ma division (C a l k i n s 1930 a), W e y e r 1930, H. R a a b e 1947).

S o n n e b o r n 1947 explained the transition of the reorganization strands in the Ma of *Euplotes* as a wave of endomitoses progressing across the consecutive subnuclei. This theory fails to explain why the reorganization strands occur only in a certain group of ciliates although the subnuclei are supposed to be common for all the ciliates.

In ciliates possesing no stripe structure, incorporation of thymidin occurs in the whole nucleus uniformly (G u t t e s and G u t t e s 1960, R u t h m a n n

1964). Besides in *Hypotricha,* the reorganization stripes were found in *Chlamydodon pedarius* (K a n e d a 1960) and in *Heliochona scheuteni* (D o b r z a ń-s k a - K a c z a n o w s k a 1963). K i m b a l l and P r e s c o t t 1962 suggest the term "replication stripes" for the reorganization stripes as more suitable for processes associated with this structure. In connection with the functional determination of this structure, there is lack of evidence as to the nature of the zones of a faint stainability in *Plagiotoma lumbrici.* Only the autoradiographic study could solve this problem.

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Summary

The transformations of structures occurring in Ma and Mi of *Plagiotoma lumbrici* in the course of their division cycle have been described. In the division cycle, following periods were distinguished: interphase, precondensation, condensation and postcondensation according to similar phases of this process in the other ciliates. Ma in *Plagiotoma lumbrici* becomes fragmented into a variable number of irregular parts which fuse together and form one mass during division. Filamentous and granular structures are observed in the course of this process. They are transformed one into another during division. They are assumed to be aggregations of chromosomes. Mi is a big structure and its diameter amounts $8\,\mu$ approximately. A great number of uniform filaments appear in Mi during division. It may be postulated that they are aggregations of chromosomes of a structure similar to those occurring in Ma. Mi shows a very low content of RNA and a very high content of DNA when compared to that in Ma. This fact may be considered as expression of difference between the Mi and Ma chromatin. Nucleoli are present in Ma and disappear in the period of precondensation. At a certain time before the condensation. zones of a low stainability appear in Ma. The possibilities of considering them as replication bands known in *Hypotricha* is discussed.

STRESZCZENIE

Praca zawiera opis przemian struktur występujących w Ma i Mi *Plagiotoma lumbrici* podczas cyklu podziałowego. Cykl podziałowy został podzielony na okresy: interfaza, prekondensacja, kondensacja i postkondensacja, w oparciu o podobnie przebiegające fazy tego procesu u innych orzęsków. Ma u *Plagiotoma lumbrici* jest rozczłonkowany na zmienną liczbę nieregularnych części, które przed podziałem zlewają się w jedną masę. Podczas tych procesów widoczne są przechodzące w siebie wzajemnie nitkowate i ziarniste struktury, interpretowane jako agregaty chromosomów. Mi posiada stosunkowo duże rozmiary; jego średnica wynosi około $8\,\mu$. Podczas podziału jądro to wykazuje obecność dużej liczby jednakowych nitek. Można wnioskować, że są to agregaty chromosomów zbliżone morfologią do występujących w Ma.

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Mi wykazuje w porównaniu z Ma bardzo niską zawartość RNA przy bardzo dużej zawartości DNA. Można to uważać za wyraz różnicy jakościowej między chromatyną Mi a chromatyną Ma. W Ma występują nukleole, które znikają w okresie prekondensacji. Przez pewien okres przed kondensacją występują we fragmentach Ma strefy słabo barwliwe. Przeprowadzono dyskusję o możliwości uznania ich za pasma replikacyjne znane u *Hypotricha*.

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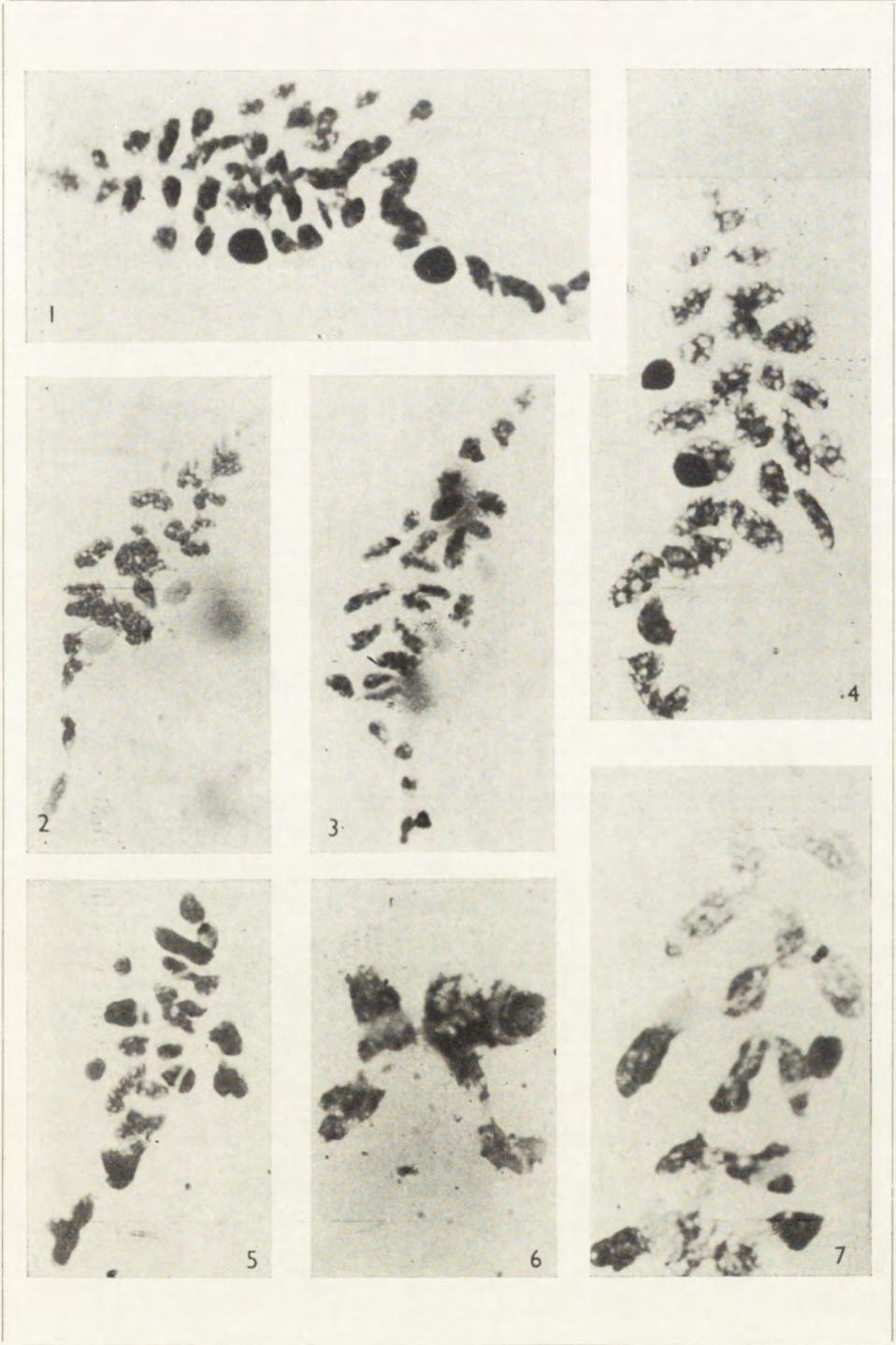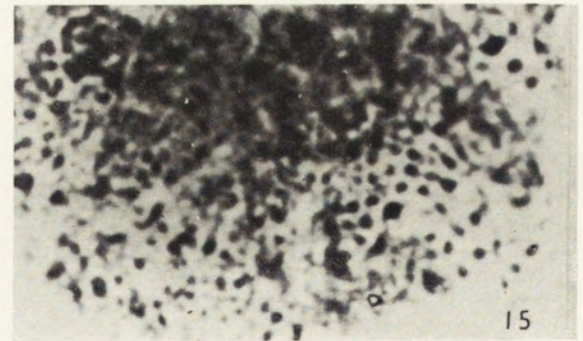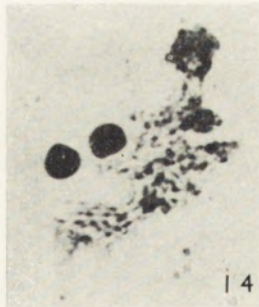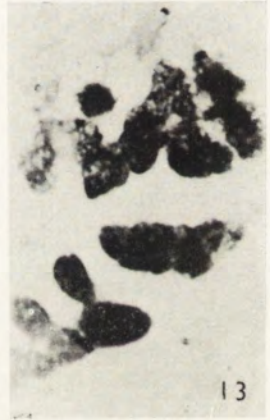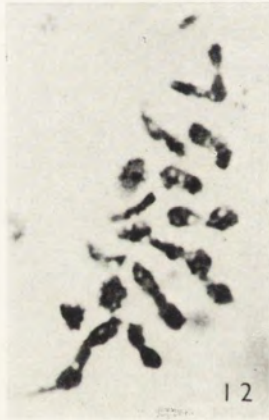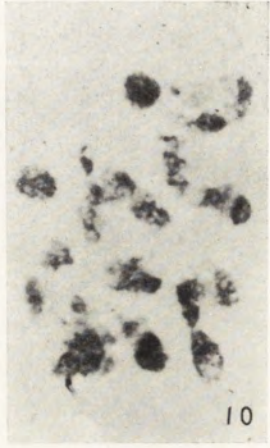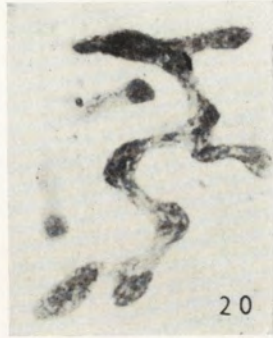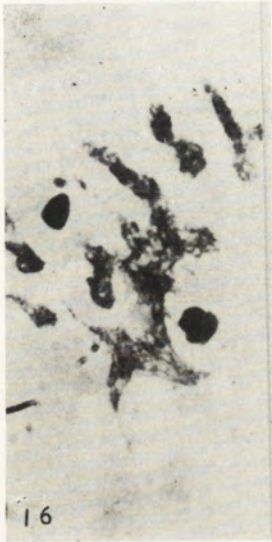
PLATE I

I. Dworakowska

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I. Dworakowska

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PLATE IV

EXPLANATION OF PLATES I—IV

Nuclear apparatus of *Plagiotoma lumbrici* Dujardin

Interphase

1: Numerous Ma fragments with aggregations of chromatin and nucleoli, two highly stainable Mi. Feulgen. 750 ×
2: Nuclear apparatus Pyronine. 760 ×
3: Nucleoli in Ma. Pyronine. 650 ×
4: Nucleoli in Ma. Feulgen. 760 ×

Precondensation

5—7: Chromatin structures vertical to the faintly stainable zones. 5 — Feulgen. 760 ×, 6—7 sodium citrate. Feulgen 760 × and 1000 ×
8: Faintly stainable zone distended, ballonlike. Feulgen. 3000 ×
9: Faintly stainable zone. Pyronine. 800 ×
10: Chromatin band adjacent to the faintly stainable zone. Sodium citrate. Feulgen 740 ×
11: Faintly stainable zones in all the Ma fragments (one Mi above) Sodium citrate, Feulgen. 760 ×

Condensation

12—13: Chromatin in Ma with swellings; "striation" produced by swelling is seen. Sodium citrate. Feulgen. 12 — 740 ×, 13 — 780 ×
14: Chromatin grains joined by fibrils and forming beads cords. Regular pattern of grains in rows is seen. Two highly stainable Mi. Feulgen. 760 ×
15: Loose structure similar to that in phot. 14. Parts of Ma much enlarged. Sodium citrate. Feulgen. 2000 ×
16: Chromatin filaments in Ma. One Mi on the left. Feulgen. 760 ×
17: Chromatin filaments in Ma. Prometaphase in Mi. Filaments in Mi much similar to that in Ma at this phase. Feulgen. 800 ×
18: Beginning of anaphase in Mi. Sodium citrate. Feulgen. 1000 ×
19: Beginning of anaphase in Mi. Pattern corresponding to the structure of chromatin filaments in Ma. Mi stainless. Pyronine. 2300 ×
20: Chromatin filaments in Ma. Telophase in Mi. Feulgen. 760 ×
21—22: Early telophase in Mi. Rows of chromatin grains on filaments. Feulgen. 760 ×

Postocondensation

23: Ma distended before fission. Mi almost stainless on the left. Pyronin. 760 ×
24: Secondary fragmentation of Ma before fission to offspring individuals. Chromatin grains bound with the filaments and producing bead cords are seen. A resorbed chromatin fragment on the right. Feulgen. 760 ×
25: Fragmentation of Ma. Undivided chromatin part remains between the individuals. Prother failed to obtain Mi, opisthe contains one Mi at the beginning of the anaphase and another one after its second uneven division. Feulgen. 760 ×
26: Fragmentation of Ma. One Mi is more stainable (?) Pyronin. 900 ×
27: Fragmentation of Ma. Two Mi on the left. Pyronin. 760 ×
28: Subsequent fragmentation of Ma. Feulgen. 760 ×

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Изменения количества ДНК и РНК в течение жизненного цикла *Ichthyophthirius multifiliis*

Quantative changes of DNA and RNA during the life cycle of *Ichthyophthirius multifiliis*

Цитохимические исследования разных стадий развития *Ichthyophthirius multifiliis* показали, что в течение его жизненного цикла происходят значительные колебания в количестве питательных веществ (Mc Lennan 1935, Успенская 1964). Получить представление о динамике нуклеиновых кислот с помощью только цитохимических методов не удается. Для того, чтобы проследить изменения количества РНК на протяжении жизненного цикла ихтиофтириуса, выяснить происходит ли во время палинтомического деления, не сопровождающегося промежуточными стадиями питания и роста, синтез ДНК мы использовали более точную количественную методику. В настоящей работе сообщаются результаты изучения количественных изменений РНК и ДНК на различных стадиях жизненного цикла ихтиофтириуса, полученные с помощью цитофотометрии.

Материал и методика

Материалом для исследования послужили разведенные в лабораторных условиях на меченосцах (*Xiphophorus helleri*) популяции ихтиофтириуса. Подопытные рыбы и ихтиофтириусы на всех стадиях содержались при температуре 20°С. Трофонты, томонты, томиты на разных этапах палинтомического деления и бродяжки приклеивались к предметному стеклу сулемовым фиксатором Ниссенбаума (Nissenbaum 1953).

Для получения количественных данных по РНК использовалась окраска аллоцианином в течении 48 часов (Овчинникова и Селиванова 1964), для измерения количества ДНК — нуклеальная реакция Фельгена время гидролиза 6 минут). Окрашенные тотальные препараты фотометрировались на спектрофотометре МУФ-4 методом сканирования. Для определения количества ДНК фотометрирование производилось в зеленой линии спектра (длина волны 546 mμ), а для определения количества РНК — в желтой линии спектра (длина волны 579 mμ).

Методика фотометрирования и последующей обработки результатов описана в предыдущих работах (Овчинникова и др. 1963, Cheissin and oth. 1963, Raikov and oth. 1963). Площади исследуемого объекта (S), выраженные в квадратных сантиметрах, определялись по микрофотографиям, полученным на МУФ-4. Количество ДНК и РНК (Q) дается в относительных единицах.

Для того, чтобы выяснить, сколько делений претерпевает трофонт при 20°C производились подсчеты числа бродяжек, полученных из одной особи, а кроме того вычислялся объем восьмидневного трофонта (объем шара) и объем томитов, полученных в результате последнего деления (готовые к выходу бродяжки), и по отношению объема трофонта к объему бродяжки судили о количестве вышедших из трофонта бродяжек, а следовательно и о числе проделанных делений. Как показали подсчеты, при температуре 20°C ихтиофтириусы проделывают обычно десять делений, в результате чего, в случае нормального равномерного деления томитов, получается 1024 бродяжки.

При определении среднего количества РНК ($Q_{РНК}$) у различных стадий ихтиофтириуса расчет велся на весь объем клетки без вычета объема макронуклеуса так, как будто бы клетка не имеет ядра, и РНК равномерно распределена во всем ее объеме. Дело в том, что на стадиях деления макронуклеус ихтиофтириуса имеет очень неправильную форму и вычислить точно его объем трудно. Однако, чтобы оценить значение этой ошибки, мы вычислили величину Q цитоплазмы, равную $Q_{РНК}$ минус Q макронуклеуса, у томонта и у бродяжек.

Макронуклеус на этих стадиях имеет правильную форму эллипсоида вращения и, таким образом, его Q может быть легко вычислено, исходя из формулы объема эллипсоида вращения (V). Было вычислено, что у бродяжки V ядра составляет в среднем 9/100 от объема целой клетки, а у томонта V ядра составляет в среднем 8/100 от V целой клетки. Соответственно $Q_{РНК}$ ядра бродяжки будет равно 9/100 от $Q_{РНК}$ бродяжки, а $Q_{РНК}$ ядра томонта 8/100 от $Q_{РНК}$ томонта. Внося потравку в среднее значение $Q_{РНК}$ на этих стадиях, мы убедились, что допущенная ошибка не влияет существенно на получающуюся картину и порядок величин остается прежним. Так у томонта $Q_{РНК}$ без поправки равно 512.96×10^{-6}, а с поправкой — 471.92×10^{-6}; у бродяжки $Q_{РНК}$ без поправки равно 1.81×10^{-6}, а с поправкой — 1.65×10^{-6}. Поэтому, хотя приводимые нами в таблице (Таблица 5) значения $Q_{РНК}$ оказываются несколько завышенными, ошибкой этой можно пренебречь.

Измерения количества РНК производились нами у стадии трофонта в первые сутки после заражения рыбы; у трофонтов в возрасте 8 суток; у только что осевших для инцистирования томонтов; у стадии 2, 8, 32, 512 томитов и у бродяжек в первые сутки их свободного плавания (1024 томита).

Ядерный аппарат ихтиофтириуса состоит из одного крупного макронуклеуса и одного микронуклеуса. В стадии трофонта микронуклеус плотно примыкает к макроиуклеусу (Mc Lennan 1935, Nie Dashu and Lee Lien-Siang 1960), или даже находится в особом углублении в макронуклеусе (Haas 1933), и на препаратах, окрашенных по Фельгену, совершенно неразличим. После выхода трофонта из рыбы, в период его свободного плавания и образования цисты, микронуклеус мигрирует к периферии клетки и приступает к митотическому делению. Во время митоза он обнаруживает очень слабое окрашивание по Фельгену. Кроме того, на первых этапах палинтомического деления цитоплазма ихтиофтириуса буквально забита заглоченными клетками тканей хозяина, ядра которых интенсивно красятся по Фельгену, что затрудняет измерение количества

ДНК в слабоокрашивающихся в этот период микронуклеусах. На более поздних этапах деления (8—16 томитов) иногда можно найти микронуклеусы в интерфазном периоде, но интерфаза, видимо, очень коротка и собрать достаточное количество материала с такими микронуклеусами трудно. На этих же стадиях происходит эпиминация хроматина из макронуклеуса, и появлется опасность спутать микронуклеус с выделенными глыбками хроматина, тем более, что они имеют часто совершенно округлую форму и близки по размерам к микронуклеусу (Haas 1933, Buschkiel 1936, Успенская 1964 и др.).

Наиболее надежным материалом для количественных измерений ДНК в микронуклеусе являются последнии стадии деления, бродяжки и молодые трофонты. Поскольку при 20°С ихтиофтириусы проделывают десять делений и дают 1024 бродяжки, то, следовательно, образованию бродяжек предшествует стадия 512 томитов. Измерения количества ДНК в микронуклеусе производились на стадии 512 томитов; у бродяжек на первые сутки после выхода из цисты (1024 томита) и у молодых трофонтов в первые двое суток после заражения рыбы.

Измерение количества ДНК в макронуклеусе производилось у трофонтов на первые сутки после заражения рыбы, у трофонтов в возрасте восьми суток, способных уже покинуть рыбу и перейти к размножению, у стадии 2 томитов, 8 томитов, 16 томитов, 64 томитов, 512 томитов и у бродяжек в первые сутки после выхода их из цисты.

Результаты исследования

Изменение количества ДНК в микронуклеусе на разных стадиях жизненного цикла ихтиофтириуса.

Приведенные в Таблице 1 данные показывают, что на стадии 512 томитов среднее количество ДНК в микронуклеусе каждого томита в два раза больше, чем у бродяжки в первые сутки ее свободного плавания. Можно думать, что у томитов перед последним делением микронуклеусы находятся в постсинтетическом периоде (G_2), затем происходит еще одно деление, и образовавшиеся бродяжки покидают цисту. У бродяжек микронуклеус находится в пресинтетическом состоянии (G_1). Период свободного плавания бродяжек очень непродолжителен и при 20°С не превышает обычно двух суток. Достигнув хозяина и внедрившись в него бродяжка превращается в молодого трофонта. Только в хозяине вновь начинается синтез ДНК в микронуклеусе. В первые сутки паразитирования трофонтов их микронуклеусы, находясь на промежуточных стадиях синтеза (S), обладают большим, чем у бродяжек, но не двойным количеством ДНК. На вторые сутки паразитирования количество ДНК в микронуклеусе удваивается по сравнению с таковым у бродяжки, т. е. микронуклеус достигает постсинтетического периода. На этом этапе микронуклеус уже приближен к поверхности макронуклеуса и вскоре примыкает к нему и становится неразличимым. Таким образом, пресинтетический микронуклеус ихтиофтириуса имеет в среднем $0.0174 \pm 0.0010 \times 10^{-6}$ единицы, а постсинтетический — $0.0370 \pm 0.0020 \times 10^{-6}$ единиц ДНК.

Изменения количества ДНК в макронуклеусе в течение жизненного цикла ихтиофтириуса.

Большим количеством работ (Sonneborn 1947, Grell 1950, 1953, Moses 1950, Walker and Mitchison 1957, Gall 1959, Woodard, Gelber and Swift 1961, Ruthmann and Heckmann 1961, Raikov and oth.

Таблица 1

Изменение количества ДНК (Q днк) в микронуклеусе ихтиофтириуса

Quantitative changes of DNA (Q$_{DNA}$) in micronucleus of *Ichthyophthirius*

Стадия Stage	Количество микронуклеусов Number of Mi	Среднее количество ДНК (Qднк) в Ми в усл. един. Mean content of DNA (Q$_{DNA}$) in Mi in arbitr. units	Средняя площадь Ми (s) в см². Mean area of Mi in cm²
512 томитов 512 tomits	32	0.0370 ± 0.0020	0.00136 ± 0.00005
Бродяжка (1024 томита) Free-swimming individual (1024 tomits)	30	0.0174 ± 0.0010	0.00100 ± 0.00005
Трофонт в 1-ые сутки после заражения Trophont 1 day after penetration into the fish	8	0.0260 ± 0.0007	0.00175 ± 0.00015
Трофонт на 2-ые сутки после заражения Trophont 2 days after penetration into the fish	35	0.0360 ± 0.0020	0.00310 ± 0.00016

В Таблицах 1—5 в значениях количества ДНК и РНК множитель $\times 10^{-6}$ а в значениях площади Ми и Ма — $\times 10^{-6}$ опускается.

In Tables 1—5 in determinations of DNA and RNA content the coefficient $\times 10^{-6}$ and in determinations of Mi and Ma area the coefficient of $\times 10^{-4}$ are omitted.

1963, Cheissin and oth. 1964 и др.), доказано, что макронуклеус многих инфузорий является высокополиплоидным ядром. То же можно сказать и о макронуклеусе ихтиофтириуса. Макронуклеус зрелого трофонта имеет во много раз большее количество ДНК, чем микронуклеус.

Как было показано в предыдущем параграфе, бродяжка, после выхода из цисты, обладает пресинтетическим микронуклеусом. Количество ДНК в макронуклеусе бродяжки в это время в два раза меньше количества ДНК в макронуклеусе томитов перед последним делением (512 томитов) (Таблица 2), т. е. синтез ДНК в нем еще не начался. После же попадания бродяжки в рыбу, уже на вторые сутки, количество ДНК в макронуклеусе молодого трофонта увеличивается вдвое по сравнению с бродяжкой, а на восьмые сутки паразитирования среднее количество ДНК в макронуклеусе трофонта достигает уже $130.56 \pm 7.23 \times 10^{-6}$ единиц (Таблица 2).

Отношение пресинтетического макронуклеуса к пресинтетическому микронуклеусу бродяжки составляет 24. Если считать микронуклеус диплоидным, то плоидность макронуклеуса бродяжки будет равна 48 n. Что касается плоидности макронуклеуса восьмидневного трофонта, то ее довольно трудно определить точно, по той причине, что число $130.56 \pm 7.23 \times 10^{-6}$, характеризующее среднее количество ДНК в макронуклеусе этой стадии является средним, полученным

Таблица 2

Среднее количество ДНК (Qднк) в Ma разных стадий ихтиофтириуса

Mean DNA content (Q) in Ma of different stages of *Ichthyophthirius*

Стадия Stage	Количество Ma Number of Ma	Среднее количество ДНК (Qднк) в усл. един. Mean content of DNA (Q_{DNA}) in arbitr. units	Достоверность различия между Q Significance of differences between Q	Средняя площадь Ma (S) см² Mean area of Ma (S) in cm²	Достоверность различия между S Significance of differences between S	Средняя оптическая плотность Ma (D) Mean optical density of Ma (D)	Достоверность различия между D Significance of differences between D
Трофонт 8 суток Trophont 8 days old	29	130.56 ± 7.23	$t = 2.7$	1.62 ± 0.09	$t = 0.31$	0.8 ± 0.01	$t = 5$
2 томита 2 tomits	34	101.47 ± 8.16	$t = 3.05$	1.56 ± 0.17	$t = 6.2$	0.69 ± 0.02	$t = 7.3$
8 томитов 8 tomits	34	66.17 ± 8.20	$t = 2.2$	0.69 ± 0.03	$t = 3.1$	0.85 ± 0.01	$t = 0.9$
16 томитов 16 tomits	33	47.10 ± 2.88	$t = 12.2$	0.56 ± 0.03	$t = 10.8$	0.83 ± 0.02	$t = 4.1$
64 томита 64 tomits	29	11.16 ± 0.66	$t = 15.51$	0.17 ± 0.01	$t = 15.3$	0.74 ± 0.01	$t = 9.1$
512 томитов 512 tomits	32	0.88 ± 0.06	$t = 7.46$	0.017 ± 0.002	$t = 2$	0.54 ± 0.02	$t = 6.4$
1024 томита (бродяжка) 1024 tomits (free-swimming individual)	30	0.41 ± 0.02	$t = 7.40$	0.013 ± 0.001	$t = 8.6$	0.36 ± 0.02	$t = 5$
Трофонт на вторые сутки после внедрения в рыбу Trophont 2 days after penetration	35	0.81 ± 0.05		0.025 ± 0.001		0.23 ± 0.02	

от количества ДНК в ядрах, находящихся на разных стадиях синтеза после последнего эндомитоза.

Однако, поскольку у бродяжек, на первые сутки их свободного плавания, макронуклеус определенно находится в пресинтетическом периоде ($Q = 0.41 \times 10^{-6}$), то можно вычислить, сколько эндомитозов должен проделать макронуклеус бродяжки после внедрения ее в рыбу, чтобы количество ДНК в нем достигло величины, характерной для макронуклеуса зрелого трофонта.

Оказывается, что 8 эндомитозов привели бы к образованию ядра со средним количеством ДНК равным 104.96×10^{-6} единиц, а 9 эндомитозов дали бы 209.92×10^{-6} единиц ДНК. Таким образом, число 104.96×10^{-6} должно было бы характеризовать среднее количество ДНК в пресинтетическом макронуклеусе после восьмого эндомитоза (период G_1) а число 209.92×10^{-6} в постсинтетическом макронуклеусе (период G_2) промежуточные же значения Q принадлежали бы ядрам, находящимся на различных этапах синтеза ДНК, т. е. в периоде S. Эти цифры укладываются в ряд значений QДНК, полученных с помощью цитофотометрии для восьмидневных трофонтов (величина Q колеблётся от 70.56 до 286.55×10^{-6}).

После достижения ядрами периода G_2 происходит их разделение. Среднее количество ДНК в макронуклеусе каждого из двух дочерних томитов, получившихся в результате первого деления, по цитофотометрическим данным равно 101.47×10^{-6}, т. е. довольно близко к цифре 104.96×10^{-6} полученной при расчете, исходя из ядра бродяжки. Должно быть среди измеренных нами на этой стадии жизненного цикла макронуклеусов большинство находилось в периоде G_1.

Действительно, если разбить все промеренные у восьмидневных трофонтов и на стадии двух томитов макронуклеусы на группы, одна из которых объединит ядра, находящиеся в периоде G_1 (до 105×10^{-6} ед.), другая — ядра в периоде
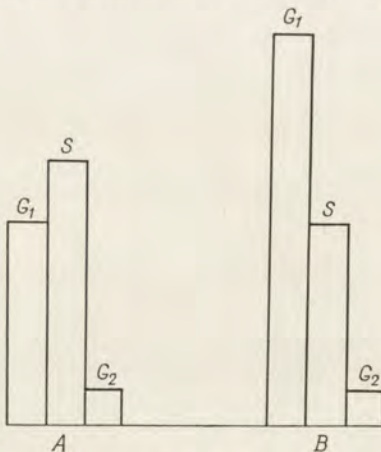

Рис. 1. Сравнение количества макронуклеусов на различных уровнях синтеза ДНК на стадии трофонта (А) и стадии 2-х томитов (В)

Fig. 1. The comparison of amount of Ma at different levels of DNA synthesis at the stage of trophont (A) and the stage of 2 tomits (B)

S (до 210×10^{-6} ед.) и третья в периоде G_2 (210×10^{-6} ед. и выше), то получится следующая картина, отраженная в диаграмме (Рис. 1 А, В). Так как мы и ожидали, среди восьмидневных трофонтов у большинства уже начался синтез ДНК (Рис. 1 А) и потому среднее значение Q их макронуклеуса занимает промежуточное положение между Q пресинтетического и Q постсинтетического макронуклеуса. Среди же макронуклеусов особей, закончивших первое деление, боль-

шая часть еще не приступила к синтезу ДНК (Рис. 1 B) и находится в периоде G_1.

Приведенные данные говорят о том, что полученная нами при расчете из макронуклеуса бродяжки величина Q пресинтетического ядра трофонта равная 104.92×10^{-6} единиц близка к истинной, и число эндомитозов, которое проделывает Ма трофонта за 8 суток паразитирования, действительно равно 8. Тогда плоидность макронуклеуса трофонта, проделавшего с момента внедрения в рыбу 8 эндомитозов, возрастет с 48 n (плоидность макронуклеуса бродяжки) до 12608 n.

На восьмые сутки паразитирования при 20°C трофонт достигает зрелости, достаточной для того, чтобы приступить к размножению, и будучи снят с рыбы, начинает делиться, давая в результате полноценных инвазионных бродяжек. Но, как уже говорилось, при 20°C совершается обычно 10 делений и, таким образом, оказывается, что при равномерном делении макронуклеуса последовательно на два, имеющегося в нем количества ДНК не хватило бы для того, чтобы в результате получился 48 плоидный макронуклеус бродяжки, содержащий 0.41×10^{-6} единиц ДНК.

В Таблице 3, в графе 3 приведены истинные количества ДНК в макронуклеусе каждой из изученных стадий, в графах же с 4-ой по 9-ую показано, как должно было бы меняться количество ДНК в макронуклеусе по мере деления, в том случае, если бы синтеза вообще не было (4-я графа), если бы он прекратился на стадии двух томитов (5-я графа), восьми томитов (6-я графа), 16 томитов (7-я графа), 64 томитов (8-я графа), 512 томитов (9-я графа).

При рассмотрении приведенных в таблице столбцов чисел, можно видеть, что уже начиная со стадии восьми томитов имеющегося в их макронуклеусе количества ДНК достаточно, чтобы в результате простого последовательного деления на два, не сопровождающегося синтезом ДНК, из них получились бы макронуклеусы 1024 томитов с нормальным для этой стадии количеством ДНК (0.41×10^{-6}). Если мы сравним цифры граф 7, 8, 9 и 10 с цифрами из графы 3, то увидим, что предполагаемые и истинные значения Q довольно близки. Например, приведенные в графе 7-й предполагаемое значение Q для 64 томитов — 11.78×10^{-6}, тогда как истинное значение Q для этой стадии — 11.16×10^{-6} Начиная с графы 7 предполагаемые значения Q для бродяжек (1024 томита) завышены по сравнению с истинными, но именно со стадии 16 томитов начинается элиминация части хроматина (H a a s 1933, M c L e n n a n 1935, Успенская 1964 и др.). Как показали измерения, количество ДНК в выброшенных кусках довольно велико, и на стадии 16 томитов составляет 0.1 часть, а на стадии 64 томитов от 0.03 до 0.1 части от Q макронуклеуса. Благодаря выбросу хроматина должна сгладиться разница между предполагаемым и истинным значением Q на последних этапах деления. Подобная элиминация хроматина из макронуклеуса отмечена и у многих других инфузорий (C a l k i n s 1930, K i d d e r 1934, K i d d e r and C l a f f 1938, Burt, K i d d e r and C l a f f 1941, T a y l o r and G a r n j o b s t 1941, P a i n t e r 1945, S e s h a c h a r 1960, 1963, B e e r s 1963 и др.). Одни авторы считают, что таким образом происходит „очищение" макронуклеуса от продуктов обмена (C a l k i n s 1930, K i d d e r 1934), другие отмечают, что выброс части хроматина способствует обогащению цитоплазмы нуклеиновыми кислотами (P a i n t e r 1945, S e s h a c h a r 1947, D a s s 1950, D y s a r t 1963 и др.).

Существует также предположение, что при этом происходит регуляция плоидности макронуклеуса и тем самым предотвращается гиперплоидия

Faure-Fremiet 1953). Таким образом можно думать, что при 20°С восьмиднев-
ный трофонт, покинувший рыбу и инцистировавшийся, во время первых 3-х —
4-х делений продолжает синтезировать ДНК за счет каких-то имеющихся
в клетке рессурсов, начиная же со стадии 8—16 томитов синтез прекращается
и происходит простое, приблизительно равномерное распределение геномов
между дочерними особями.

Изменение количества РНК в течение жизненного цикла ихтиофтириуса.

Как показали измерения, проведенные на галлоциановых препаратах
среднее количество цитоплазменной РНК у восьмидневного трофонта составляет
687.00 $\pm$ 56.50 $\times$ 10^{-6} единиц (Таблица 4).

Макронуклеус его в это время содержит многочисленные нуклеолы. После
выхода ихтиофтириуса из рыбы, во время свободного плавания и инцистиро-
вания происходит, видимо, какой-то расход цитоплазменной РНК и среднее ее
количество у томонта снижается до 512.96 $\pm$ 35.14 $\times$ 10^{-6}. Сразу же после инци-
стирования, в связи с подготовкой макронуклеуса к делению наблюдается исчез-
новение нуклеол (Успенская 1964), количество же РНК цитоплазмы в пер-
иод первого деления сильно возрастает, в связи с чем у каждого из двух
дочерних томитов среднее количество РНК превышает таковое в материнской
особи (Таблица 4). Существует взгляд, что РНК нуклеол, после их разрушения
может переходить в цитоплазму и этот взгляд подкрепляется эксперименталь-
ными данными (Браше 1960, Цанев и Марков 1964). Имеются также
данные о выбросе нуклеол в цитоплазму некоторых инфузорий (Calkins 1919,
1930, Ehret and Powers 1955, Суханова 1960, Райков 1959, 1963).
Возможно, что это справедливо и для ихтиофтириуса, и тогда обогащение цито-
плазмы рибонуклеиновой кислотой, в начале деления, можно отнести за счет
выхода нуклеол в цитоплазму.

В результате 10 последовательных делений образуются бродяжки со средним
количеством РНК равным 1.81 $\pm$ 0.14 $\times$ 10^{-6} единиц. Для того, чтобы выяснить,
происходит ли во время деления расход цитоплазменной РНК, в Таблице 5
сравнивается истинное количество РНК на разных стадиях жизненного цикла
(графа 3) с количеством ее, которое должно было бы получиться, если бы име-
ющаяся в цитоплазме томонта РНК распределялась во время деления поровну
между дочерними особиями (графа 4). Сравнение проводится начиная со второго
деления, т. к. перед первым делением предполагается обогащение цитоплазмы
РНК за счет выхода нуклеол. Рассматривая приведенные в таблице цифры,
мы видим, что везде истинные количество РНК выше ожидаемых на той же
стадии и, таким образом, суммарное количество РНК в цисте не снижается по
сравнению с ее количеством у томонта. Напротив, создается даже впечатление,
что для получения бродяжек со средним количеством РНК равным 1.81 $\times$ 10^{-6}
единиц нужно было бы предположить некоторое увеличение РНК во время де-
ления, т. к. эта величина превышает ожидаемую. Как уже упоминалось раньше,
начиная со стадии 16 томитов происходит частичная элиминация хроматина
из макронуклеуса и если правилен взгляд, что выбрасывание хроматина в ци-
топлазму способствует обогащению последней нуклеиновым материалом, то мо-
жет быть этот выброс, составляющий у ихтиофтириуса от 0.03 до 0.1 части от
ДНК макронуклеуса как-то влияет на колебания количества РНК цитоплазмы
во время деления. Однако правильность такого предположения не имеет в на-
стоящее время достаточных доказательств.

Сравнение истинного и ожидаемого количества ДНК в Ма ихтиофтириуса в случае прекращения ее синтеза на разных этапах

The comparison of the real and expected DNA content in Ma of *Ichthyophthirius* in the case of the cessation of its synthesis at different stages

1	2	3	Ожидаемое значение Q при прекращении синтеза ДНК на стадиах: Expected DNA content in the case of the cessation of DNA synthesis at the stages:						
Стадия Stage	Количество особей Number of specimens	Истинное значение $Q_{днк}$ в Ма каждой стадии Real DNA content at each stages of division	4 трофонта trophont	5 2 томитов 2 tomits	6 8 томитов 8 tomits	7 16 томитов 16 tomits	8 64 томита 64 tomits	9 512 томитов 512 tomits	10 1024 томита 1024 tomits
Трофонт 8 суток Trophont 8 days old	29	130.56 ± 7.23	130.56						
2 томита 2 tomits	34	101.47 ± 8.66	65.28	101.47					
4 томита 4 tomits	—	—	32.64	50.74					
8 томитов 8 tomits	34	66.17 ± 8.20	16.32	25.37	66.17				
16 томитов 16 tomits	33	47.10 ± 2.88	8.16	12.69	33.09	47.10			
32 томита 32 tomits	—	—	4.08	6.35	16.55	23.55			
64 томита 64 tomits	29	11.16 ± 0.66	2.04	3.18	8.28	11.78	11.16		
128 томитов 128 tomits	—	—	1.02	1.59	4.14	5.89	5.58		
256 томитов 256 tomits	—	—	0.51	0.80	2.07	2.95	2.79		
512 томитов 512 tomits	32	0.88 ± 0.06	0.26	0.40	1.04	1.48	1.40	0.88	
1024 томита (бродяжки) free swimming individuals — 1024 tomits	30	0.41 ± 0.02	0.13	0.20	0.52	0.71	0.70	0.44	0.41

Таблица 4

Среднее количество РНК (QРНК) у разных стадий жизненного цикла ихтиофтириуса

Mean RNA content at different stages of life cycle of *Ichthyophthirius*

Стадия Stage	Количество особей Number of specimens	Среднее количество РНК (QРНК) в усл. ед н. Mean content of RNA (QRNA) in arbitr. units	Достоверность различия между QРНК Significance of differences between QRNA	Средняя площадь (S) в см² Mean area (S) in cm²	Достоверность различия между S Significance of differences between S	Средняя оптическая плотность D Mean optical density D	Достоверность различия между D Significance of differences between D
Трофонт 8 суток Trophont 8 days	27	687 ± 56.50		9.46 ± 0.58		0.72 ± 0.013	
			t = 3.9		t = 1.1		t = 9.7
Томонт осевший и инцистировавшийся Tomont just after encysting	27	512.96 ± 35.14		8.75 ± 0.34		0.65 ± 0.015	
			t = 1.6		t = 2.1		t = 5.6
2 томита 2 tomits	42	550 ± 41.88		7.48 ± 0.51		0.75 ± 0.01	
			t = 10.1		t = 15.7		t = 22.5
4 томита 4 tomits	—	—		—		—	

8 томитов / 8 tomits	18	153.55 ± 14.89		2.83 ± 0.45		0.59 ± 0.02	
16 томитов / 16 tomits	—	—	$t = 6.6$	—	$t = 4$	—	$t = 5$
32 томита / 32 tomits	32	53.06 ± 2.64		1.066 ± 0.023		0.490 ± 0.017	
64 томита / 64 tomits	—	—	$t = 18.6$	—	$t = 3.2$	—	$t = 7.5$
128 томитов / 128 tomits	—	—		—		—	
256 томитов / 256 tomits	—	—		—		—	
512 томитов / 512 tomits	55	4 ± 0.21	$t = 14.1$	0.124 ± 0.006	$t = 5$	0.35 ± 0.01	$t = 5.9$
1024 томита (бродяжка) / free-swiming individuals (1024 tomits)	32	1.81 ± 0.14	$t = 9.9$	0.089 ± 0.004	$t = 5.1$	0.220 ± 0.016	$t = 6.2$
Трофонт 1 сутки после заражения / Trophont 1 day after penetration	31	5.42 ± 0.39		0.15 ± 0.011		0.390 ± 0.018	

Таблица 5

Сравнение истинного количества РНК (Q_{PHK}) у каждой стадии деления ихтио-
фтириуса с количеством РНК ожидаемым в случае отсутствия расхода и синтеза
при равномерном ее распределении между дочерними особями

The comparison of the real RNA (Q_{RNA}) content at each stages of division of
Ichthyophthirius with the expected RNA content in the case of its equal distribu-
tion among daughter individuals

Стадия Stage	Количество особей Number of specimens	Истинное количество РНК (Q_{PHK}) у каждой стадии в усл. един. Real RNA content (Q_{RNA}) in each stages. in arbitr. units	Ожидаемое при равномерном распределении количество РНК (Q_{PHK}) в усл. един. Expected RNA content (Q_{RNA}) in the case of equal distribution, in arbitr. units
Трофон 8 суток Trophont 8 days old	27	687.00 ± 56.50	687.00
Только что осевший и инцистировшийся томонт Tomont immediately after encysting	27	512.96 ± 35.14	512.96
2 томита 2 tomits	42	550.00 ± 41.88	550.00
4 томита 4 tomits	—	—	275.00
8 томитов 8 tomits	18	153.55 ± 14.89	137.50
16 томитов 16 tomits	—	—	68.75
32 томита 32 tomits	32	53.06 ± 2.64	34.38
64 томита 64 tomits	—	—	17.16
128 томтов 128 tomits	—	—	8.58
256 томитов 256 tomits	—	—	4.29
512 томитов 512 tomits	55	4.00 ± 0.21	2.15
Бродяжки Free-swimming individuals	32	1.81 ± 0.14	1.08

На стадии 512 томитов в макронуклеусе каждого из них вновь появляются крупные нуклеолы, которыми обладает и бродяжка. После попадания бродяжки в нового хозяина она превращается в трофонта, и уже в первые сутки паразитирования количество РНК становится в нем в 3 раза больше, чем у бродяжек (Таблица 4). На протяжении паразитической стадии идет синтез РНК.

Данные о колебаниях количества РНК носят еще очень предварительный характер и не могут расцениваться как точные, хотя бы потому, что галлоцианин дает суммарное окрашивание различных типов РНК, доля которых в разные моменты жизненного цикла ихтиофтириуса пока еще совершенно не ясна. Кроме РНК галлоцианин может также неспецифически окрашивать некоторые другие компоненты цитоплазмы (П и р с 1963). Приведенные нами количественные данные могут считаться лишь ориентировочными, могущими помочь при дальнейшем изучении обмена у ихтиофтириуса.

Р е з ю м е

У *Ichthyophthirius multifiliis* макронуклеус (Ма) высокополиплоиден: у бродяжек, полученных при 20°C, плоидность равна 48 n, плоидность Ма зрелого трофонта определена приблизительно как 12000 n.

В цисте в процессе палинтомии, при 20°C, ихтиофтириус проделывает 10 делений. Во время палинтомического деления на первых его этапах (3-4 деления) происходит синтез ДНК в Ма, а начиная со стадии 8-10 томитов синтез ее прекращается и совершается простое разделение геномов, приблизительно поровну, между дочерними особями.

У бродяжек в течение их свободного плавания микронуклеус (Ми) и макронуклеус находятся на пресинтетическом уровне. После внедрения бродяжек в хозяина начинается вновь синтез ДНК в ядрах. На вторые сутки паразитирования, при 20°C, Ми достигает постсинтетического состояния. К этому времени Ми приближается к Ма и становится неразличимым. В таком состоянии он, видимо, сохраняется до первого деления. Ма за 8 суток паразитирования, при 20°C, проделывает 8 эндомитозов, после чего ихтиофтириус становится способным к размножению.

Количество РНК цитоплазмы увеличивается у трофонта с каждым днем его паразитирования. После того, во время короткого периода плавания и инцистирования томонта, количество РНК сильно уменьшается, а во время подготовки к первому делению вновь сильно возрастает, возможно за счет выхода в цитоплазму нуклеол из Ма. Во время палинтомического деления не происходит снижения общего количества РНК, напротив, создается впечатление, что суммарное ее количество в цисте несколько возрастает. Объяснение этому пока еще не найдено и данные по динамике РНК нужно рассматривать как предварительные и ориентировочные.

SUMMARY

Macronucleus of *Ichthyophthirius multifiliis* is a highly polyploid nucleus. Poploidy degree of presynthetic macronucleus of free-swimming stages of *Ichthyophthirius* appeares to be 48 n. Polyploidy degree of macronucleus of the mature trophont may be approximately estimated as 12000 n. 8 endomitosis take place in macronucleus for 8 days of parasitic life.

During the process of palintomy *Ichthyophthirius* undergoes 10 divisions inside the cyst at 20°C. At the first stages of palintomic division (3 or 4 divisions) the synthesis of DNA takes place. At the stage of 8-16 tomits (at 20°C) synthesis of DNA stops and simple, equal distribution of genoms among daughter idividuals occurs.

At the free-swimming stage macronucleus and micronucleus are at the presynthetic level. Only after the penetration of free-swimming individual into a host skin the synthesis of DNA in micronucleus and macronucleus begins again. In 48 hours the amount of DNA of micronucleus of a young trophont reaches the postsynthetic level. At this moment micronucleus approaches macronucleus and soon becomes invisible. At this level it is obviously preserved till the first division. Macronucleus of trophont undergoes 8 endomitoses for 8 days of parasitic life at 20°C. Afterwards *Ichthyophthirius* is able to multiply.

The RNA content in cytoplasm during the parasitic stage of ciliates increases. After leaving the fish, during the short period of free-swimming and encysting of tomont a certain decrease of RNA content takes place. Then, before the first division considerable increase of RNA amount occurs apparently due to RNA of nuceoli wich are thrown out from macronucleus. There is some evidence that during palintomic division the decrease of RNA does not occur and the RNA is distributed among the daughter individuals. The amount of RNA at some stages of palintomic division seemes to increase somewhat but we have not sufficient data to explain this phenomenon at present. The data on RNA dinamics must be considered as preliminary.

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Immobilization by homologous antiserum and antigenic transformation in *Paramecium aurelia* in relation to the ionic composition of medium

Immobilizacja pod wpływem homologicznej surowicy i transformacja antygenowa *Paramecium aurelia* w zależności od jonowego składu środowiska

The extensive studies of antigens in *Paramecium aurelia* (S o n n e b o r n 1943 a, b, 1948, 1950 a, b, 1957, S o n n e b o r n and L e S u e r 1948, A u s t i n 1951, 1957, 1959, 1963 a, b, A u s t i n, W i d m a y e r and W a l k e r 1956, B e a-l e 1948, 1952, 1954, 1957) gave foundation to the knowledge of the role of immobilizing antigens in cytoplasmic inheritance in those protozoa.

The immobilization in *P. aurelia* by the homologous antiserum is evoked by the immunological reaction of the antigen proteins of the pellicle and cilia with the antibodies (B e a l e and K a č s e r 1957, B e a l e and M o t t 1962). This reaction has possibly a character and a course which are slightly different from those of the reaction "in vitro" of the pure fraction of antigen protein and a pure fraction of antibodies, as it occurs in the gel diffusion method (P r e e r 1959 a, b, B a l b i n d e r and P r e e r 1959, F i n g e r 1956, 1957, F i n g e r and H e l l e r 1963, 1964, F i n g e r, H e l l e r and G r e e n 1962, F i n g e r, H e l l e r and S m i t h 1963, S e e d, S h a f e r, F i n g e r and H e l-l e r 1964).

The analysis of physiological consequences of immobilization in paramecium, requires a determination of conditions in which the immobilization reaction occurs.

The appropriate high concentration of the antiserum (antibodies) is the necessary condition of occurrence of the immobilization reaction. B e a l e 1948, 1954 demonstrated that the "immobilization time" is proportional to the degree of dilution of the serum used i.e. the higher the dilution of serum the longer the time till all the paramecia are immobilized. This rule holds true in the range of immobilization time above 10 min. Observations of B i s h o p 1963 proved that the values of immobilization time below 10 min. assume a character of a non-rectilinear interdependence, i.e. the fall of the serum dilution (or rise of antibodies concentration) is not accompanied by a proportional acceleration of the immobilization rate.

A u s t i n 1959 observed the influence of pH of the medium upon the antigen transformation. B e a l e 1953, 1954 provided a number of data about the

possibility of induction of the antigen transformation by means of high (sublethal) concentrations of various salts as: NaCl, $CaCl_2$ and others.

Other factors of external medium which might influence the immobilization phenomenon, have not been investigated in a regular way as yet. An attempt of the present study was to analyse the action of pH and some cations on the immobilization reaction and transformation of serotype in *Paramecium aurelia* induced by treatment with homologous antiserum.

The author wishes to express his thanks to Dr. S. Dryl, Department of Biology, M. Nencki Institute of Experimental Biology in Warsaw, for his patient guidance throughout the work and for critical reading of the manuscript.

Material and methods

The object of study were serotypes 51 A and 51 B of *Paramecium aurelia*, sygen 4 (free of "kappa")[1].

The monobacterial culture was carried out according to the slightly modified method of S o n n e b o r n 1950 b: lettuce was dried in a continuous current of air, at a temperature of about 60—70°C for 48 hours. For preparing the aquous extract (water bidestilled in glass) 1,5 g. of lettuce powder per 1 l was used and sterilized in autoclave at 1,5 Atm. for 1 hour in glass containers of a capacity not exceeding 2 l. The aquous lettuce extract was inoculated with *Aerobacter aerogenes* (strain K — 7, from the collection N. C. T. C. in London).

Antisera were produced according to the method of S o n n e b o r n 1950 b. A dense suspension of paramecia (10^5 specimens/ml) was prepared from *P. aurelia* culture 99—100% pure in relation to the represented antigen. Suspension was kept frozen at $-20°C$ in refrigerator, melted before the use and injected intravenously to the rabbits twice a week, consecutively in increasing doses: 0.1, 0.2, 0.4, 0.5, 0.6, 0.7 ml.

Serum obtained from the immunized rabbits (10 days after the last injection) was heated at 56°C for 30 min. Samples of antisera were kept in refrigerator in sealed-up ampoules.

Identification of defined serotypes was performed at the temperature of 27°C. Antiserum was diluted in M i c h a e l i s phosphate buffer at pH 7.1 in which K_2HPO_4 was substituded by Na_2HPO_4.

As a measure of intensity of immobilization process in paramecia by the homologous antiserum, the t i m e o f i m m o b i l i z a t i o n was accepted i.e. the time from the moment of placing the ciliates in the homologous antiserum (in a defined dilution), till the moment of complete immobilization of 90% of individuals. The rate of immobilization may be expressed as inverse of immobilization time.

The study of the influence of the ion composition of medium upon the immobilization time of serotype 51 A was carried out in the following manner:

a. The homologous anti-51 A serum (experimental series) was diluted in the buffer solution of a known composition and concentration of cations. As control, the non-homologous anti-51 D serum in analogous buffer solutions was applied.

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[1] The strain of *P. aurelia* as well as the standard antisera were provided by the Zoological Institute of Indiana University owing to the kindness of prof. T. M. Sonneborn.

b. pH of the prepared solutions was determined potentiometrically by means of he pH-meter of the type "Cambridge".

c. Over 100 individuals of paramecia in the volume of 0.1 ml. of experimental fluid were introduced into 2 ml of diluted antiserum, and mixed subsequently. Time of exposure was measured with a stop-watch.

The same procedure (as in b and c) was applied in the control series, except that the ciliates were exposed to the solution of non-homologeus antiserum.

In this way the influence of pH as well as that of chlorides of Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+} was examined. Observations were carried out during two houres and in some cases even longer. The titre of the antisera used amounted about 1:400 at pH 7 according the criterion of titre evaluation applied by B e a l e 1948.

The influence of the ion composition in medium upon the antigenic transformation 51 A to 51 B induced by the homologous antiserum was stutied by the following method:

a. Experimental solutions of a defined pH and of a suitable concentration of cations (chlorides) were prepared.

b. Paramecia were exposed to solution containing the homologous anti-51 A serum (experimental series), or the non-homologous anti-51 D serum (control series) for 2 hours at 18°C.

c. Paramecia were rinsed with the nutrient inoculated with bacteria.

d. Individuals which were previously isolated were incubated for 72 hours at 18°C and the serotypes of the obtained clones were ascertained.

Procedure in the control series was the same as in b, c, d, except paramecia were exposed to the non-homologous anti-51 D serum.

By this procedure the influence of external pH and of different concentrations of NaCl, KCl, $CaCl_2$ and $MgCl_2$ was examined. The titre of the sera applied amounted 1 : 400 at pH 7.

Another series of experiments concerned the influence of the experimental medium of a known ion composition upon transformation of 51 A to 51 B or other serotypes without exposure of animals to homologous antiserum. Paramecia were exposed to a long-lasting (2—72 hrs.) action of different salt concentrations: NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$, $SrCl_2$, $BaCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$, NaH_2PO_4, Na_2HPO_4, sodium citrate and EDTA. Following buffer solutions were applied: phosphate-citrate buffer according to Dryl 1959, 1961, phosphate buffer after M i c h a e l i s, tris with HCl, tris maleic acid, acetate buffer and phthalate buffer.

The intensity of transformation was expressed as t r a n s f o r m a t i o n l e v e l i.e. by percentage of individuals or clones acquiring a serotype different from the initial one as a result of the antigenic transformation.

R e s u l t s

The immobilization time at different pH of medium

Paramecia of the serotype 51 A were subjected to the action of the anti-51 A serum in tris buffer with maleic acid (I) at the range of pH 5.7—8.2 and in modified Michaelis phosphate buffer (II) at pH 5.6—8.3. As follows from the immobilization time is the shortest i.e. immobilization occurs most rapidly at pH 5.8. With the gradual alkalinization of medium, the time of immobilization

becomes prolonged and at pH 7.0 for tris with maleic acid and at pH 7.4 for Michaelis phosphate buffer, it reaches 120 minutes. More prolonged observations proved that between pH 7 and 8 only a partial immobilization occurs after 120 min. i.e. a decrease of the movement rate of a variable intensity. A complete immobilization of more than 50% individuals takes place only after 4—5 hours. Above pH 8 no immobilization was observed and only in some individuals a slight reduction of the movement rate was noticed.
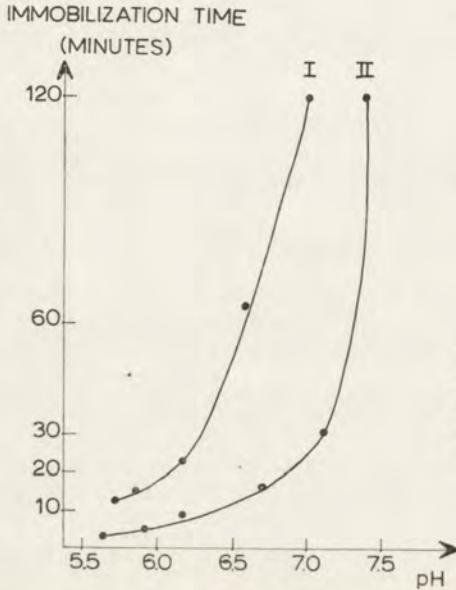
Fig. 1. Immobilization time of serotype 51 A *P. aurelia* evoked by anti-51 A serum, in relation to the pH of medium. Observations carried out in buffers: "I" tris with maleic acid and "II" phosphate buffer of Michaelis (modified)

In the medium in which immobilization occurs most rapidly, it concerns not only the somatic cilia but the peristomal ones as well.

The curves which illustrate the immobilization time at various pH values in phosphate buffer of Michaelis and tris buffer with maleic acid have a similar and nearly parallel course. Possibly the difference of values after separate measurments of both curves is involved by different concentration of electrolytes in both buffers.

The observations described above have been supported by the study of immobilization time in serotype 51 B after the action of anti-51 B serum at different pH values.

The influence of cations on the immobilization time

The influence of ions upon binding the antigen with antibody cannot be separated from the influence of external pH. Some of the chlorides hydrolize when dissolved in water and their solutions show therefore different pH values. Besides, the slightly alkaline reaction of the serum involves a necessity of studying the effects of cations in modified phosphate buffer of Michaelis. Its modification consisted in replacing K_2HPO_4 by Na_2HPO_4. As a result, in the experimental buffer solution sodium cations were present only (except for some

insignificant concentration of electrolytes contained in the diluted serum), which made possible the analysis of action of single cation only.

The scheme of experiments on the effects of different cations was similar to the experiments on the influence of pH upon the immobilization time.
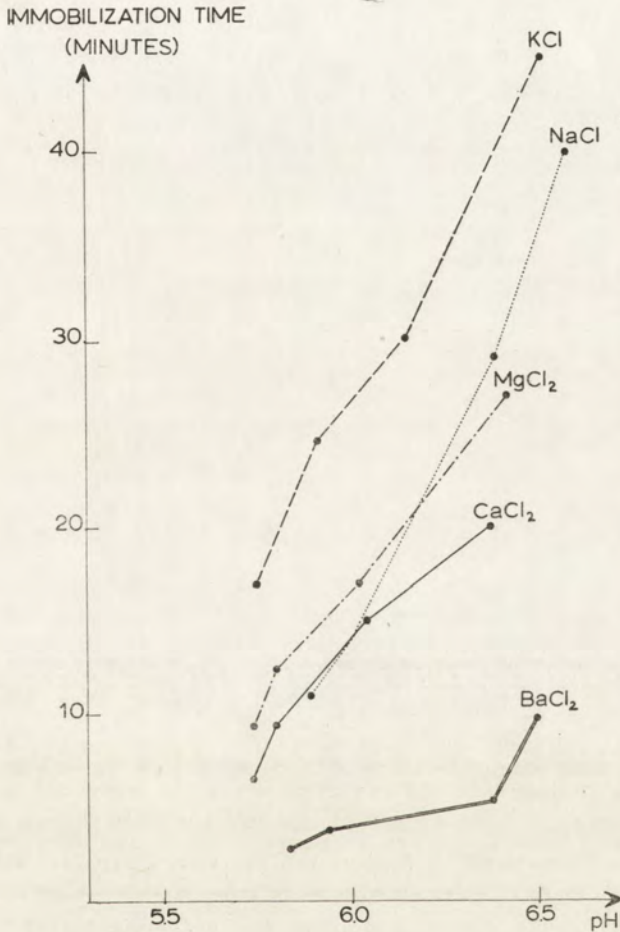
Fig. 2. Immobilization time of serotype 51 A. *P. aurelia* evoked by anti-51 A serum, in relation to pH and different cationic composition of the medium. Observations carried out in phosphate buffer of Michaelis (modified). Salt concentration 4 mM.

The results are presented in Fig. 2. They show the dependence of immobilization time on the presence of cations: Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+} in the phosphate buffer medium in concentration 2 mM.

Curves of different values but of similar course were obtained (Fig. 3 and 4). In all the cases the alkalinization of medium evoked an elongation of immobilization time.

IMMOBILIZATION TIME
(MINUTES)
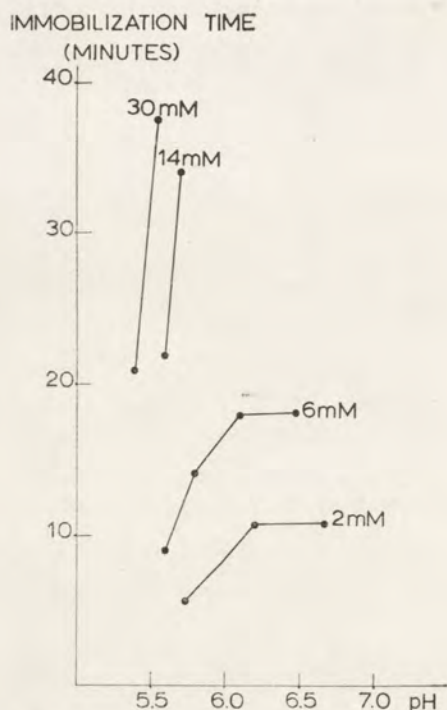
Fig. 3. Immobilization time of sero-
type 51 A. *P. aurelia* evoked by
anti-51 A serum in various concen-
trations of CaCl₂ and at various val-
ues of pH obtained by means of
phosphate buffer of Michaelis (mo-
dified)

IMMOBILIZATION TIME
(MINUTES)
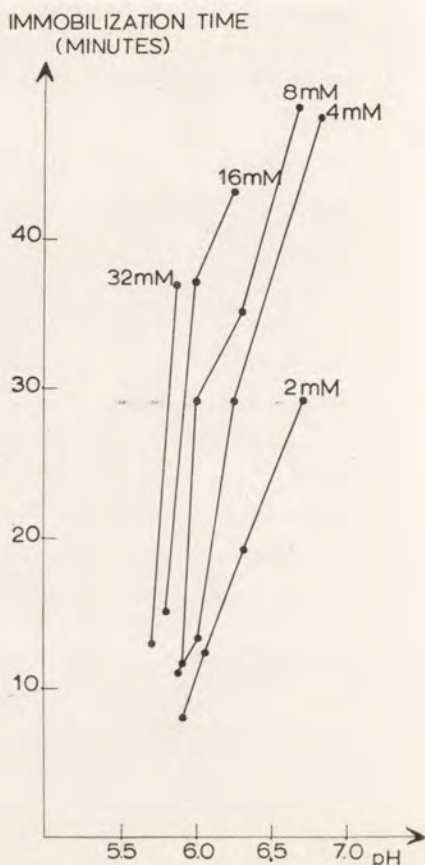
Fig. 4. Immobilization time of serotype 51 A *P. aurelia* evoked by anti-51 A serum
in different concentrations of NaCl and at different pH valeus obtained by means
of phosphate buffer of Michaelis (modified)

The influence of external pH upon the antigenic transformation of sero-
type 51 A to 51 B

The influence of pH of medium upon the antigenic transformation was
studied after a short-lasting exposure of paramecia to the experimental me-
dium. It seemed that since external pH is a decisive factor for demonstrating
the products of binding the antigen with the antibodies in form of a visible
reaction i.e. immobilization — consequently the antigenic transformation, which
is caused by the same factor, would also depend on this factor in a decisive
degree. The mean results obtained after application of the modified phosphate
buffer of Michaelis and the phosphate-citrate buffer of Dryl are illustrated
in the diagram (Fig. 5). It may be concluded from the above data that in the
acidic range, the value near pH 4.8 is the boundary marking the inhibition
of antigenic transformation. The first maximum of frequency of transformation
was found at pH 5.6 followed by a slight decrease with the lowest point at
pH 7.1, and then by an increase with a second maximum at pH 8.3. The sub-

sequent alkalinization involves decrease of transformation frequency. A distinct interdependence between external pH values and the level of transformation was not observed. At pH 5.8 even a very short-lasting exposure (10 min.) of paramecia to homologous antiserum induced almost 100% transformation of serotype 51 A to 51 B.

In all the control experiments negative results were obtained i.e. transformation failed to occur without homologous antiserum treatment of after treatment with non-homologous antiserum at all pH values examined.
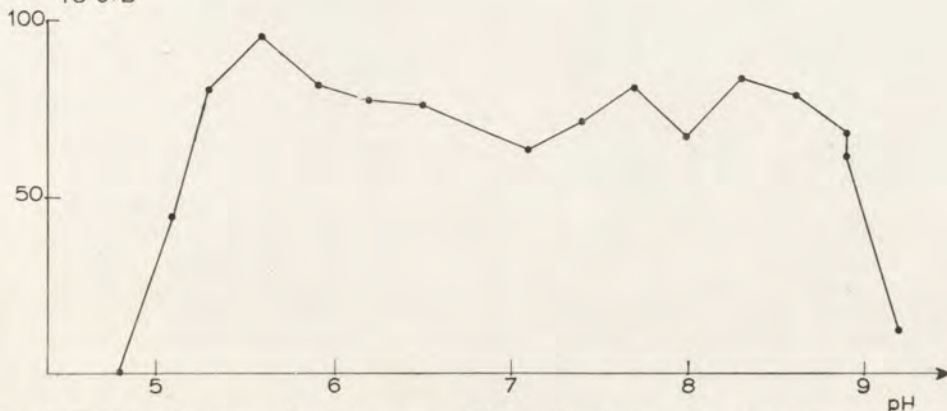
Fig. 5. Relation between the frequency of the antigenic transformation 51 A to 51 B *P. aurelia* induced by the homologous antiserum treatment at temp. 18°C and the pH of the exposure medium

The effects of cations on the antigenic transformation 51 A to 51 B

The influence of cations upon the frequency of antigenic transformation induced by homologous antiserum treatment depends both on their concentration and on the kind of applied buffer. As a rule the frequency of antigenic transformation decreases as the concentration of cation increases and this is more distinct in the phosphate buffer at pH 7.0 (Fig. 6) than in tris buffer with HCl (Fig. 7). In the phosphate buffer the level of transformation is nearly twice as high as at the same concentrations of salts dissolved in tris buffer with HCl. However essential differences in the action of actions were not found.

No transformation of antigenic type could be stated when paramecia were exposed to different concentrations of NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$, $BaCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$, NaH_2PO_4, Na_2HPO_4, sodium citrate and EDTA, provided that paramecia were not treated simultaneously with homologous antiserum. It should be pointed out in this connection that no antigenic transformation was noticed in controls, i.e. in animals exposed to non–homologous antiserum.

Discussion

Immobilization of *P. aurelia* after homologous antiserum treatment is the result of an immunological reaction between the antigen proteins of pellicle and cilia and the homologous antibodies as it was suggested by B e a l e and K a č s e r 1957, B e a l e and M o t t 1962, M o t t 1965.

The results of the present study concerning the influence of the ion composition of medium upon the course of immobilization reaction seem to support the above hypothesis since there are many points similar to the data achieved in studies on the dynamics of binding of antibodies with the antigens in other organisms. This seems to indicate the common background of those phenomena.

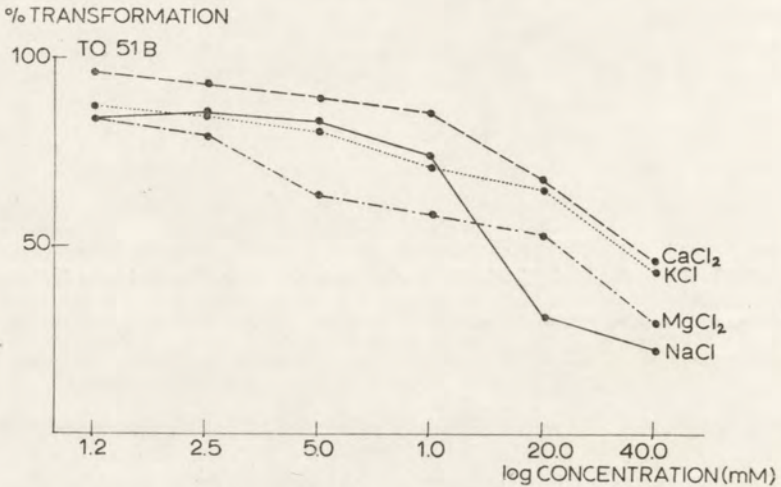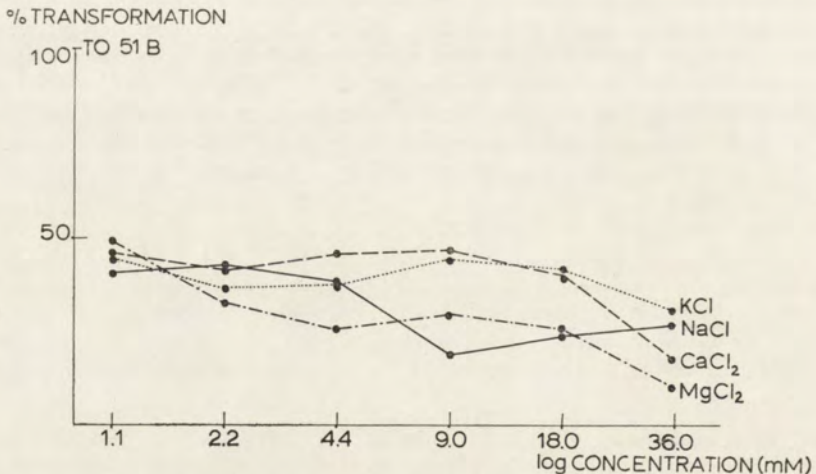Cann and Clark 1956 analyzed the kinetics of the reaction antigen —

Fig. 6. Relation between the frequency of the antigenic transformation 51 A to 51 B
P. aurelia induced by homologous antiserum treatment and the concentration of salts in the Michaelis phosphate buffer (modified) at pH 7.0 and 18°C

Fig. 7. Relation between the frequency of the antigenic transformation 51 A to 51 B
P. aurelia induced by homologous antiserum treatment and the concentration of salts in the tris — HCl buffer at pH 7.0 and temp. 18°C

antibody using the antigen of bacteriophage T2r$^+$ and a purified fraction of specific antiserum. It follows from their study that the reaction consists in binding the antigen with antibody by means of electrostatic forces. Changes in external pH and salt concentration may modify — according to C a n n and C l a r k 1956 — the range and optimal points of the highest activity by altering the number of electrostatic charges in the proteins of bacteriophage and antibodies. At low concentrations of NaCl, (3 mM) the optimum of neutralization of bacteriophage by pure fractions of homologous antiserum (of rabbit) takes place at pH 5.7 with a simultaneous abrupt fall of activity both in the acidous and the alkaline range of pH. The rise of concentration of NaCl up to 150 mM evokes a general decrease of activity level of the reaction elements.

Similar results have been reported by T s u j i, D a v i s and S o w i n s k i 1960 in their work on inhibition of oxidation of luciferine by luciferase (enzyme of the marine crustacean *Cipridina hilgendorfii*) caused by antibodies specific for luciferase. Those findings indicate that pH 5.4 presents the optimal condition for the rate of binding the antigen with the antibody. Further analysis of the pH influence as well as that of NaCl concentration (T s u j i, D a v i s and G i n d l e r 1962) is essentially consistent with the findings of C a n n and C l a r k 1956. T s u j i, D a v i s and G i n d l e r 1962 indicate that the rate of inactivation of luciferase rises with the acidification of medium from pH 7.0 to pH 5.0 and this process is the more intense the lower is the initial concentration of NaCl. The activating influence of the decreasing NaCl concentration has the character of an asymptote.

Considering the above results it may be assumed that the data of the present study on the action of ion composition of medium upon the immobilization of *Paramecium* by the specifie antiserum — seem to support the concept of reaction mechanism: antigen — antibody, as put forward by T s u j i, D a v i s and G i n d l e r 1962. This hypothesis suggests that electrostatic forces of proteins with opposite charges are responsible for binding antibody with antigen. The degree of differentiation of charges depends on the pH of isoelectric points. Therefore in the case of bacteriophage T2r$^+$, the isoelectric point of its proteins is at pH 4 and the highest point of reactivity is pH 5.7. The highest intensity of inactivation of luciferase (isoelectric point at pH 3.3) was at pH 5.4. It has been reported by S t e e r s 1961, 1962, that the isoelectric point of antigen protein of *Paramecium aurelia* 51 A is at pH 4.0 whereas the isoelectric point of rabbit gamma globuline is at pH 5.8 (T i s e l i u s and K a b a t 1939). Consequently the immobilization optimum in *Paramecium aurelia* reported in the present study might be in agreement with the above hypothesis.

The role of various electrolytes in the course of immobilization is essentially similar. Nevertheless a range of cations was determined according to their capability of diminishing the rate of immobilization by homologous antiserum as follows: $Ba^{2+} > Ca^{2+} \geqslant Mg^{2+} > Na^+ > K^+$. This range resembles to that reported by G r o s s b e r g, C h e n, R e d i n a and P r e s s m a n 1962 ($Ba^{2+} > Ca^{2+} > Mg^{2+} > Cs^+ > Rb^+ > NH_4^+ \geqslant K^+ > Na^+ \geqslant Li^+$) concerning the influence of ions upon acceleration of hapten binding by antibodies. In contrast to pH, the role of various ions is less distinct. The role of electrolytes concentration is not elucidated either. T s u j i, D a v i d and G i n d l e r 1962 suggest that the role of of higher concentrations of NaCl consists in diminishing the potential ζ both in antigen and in the antibody. This promotes its saluting out by dehydration and entrance of their molecules in the action field of the

Van der Waals forces. In this case the rise of the NaCl concentration would inhibit the activeness of the substrate in reaction antigen — antibody by diminishing the effective concentration of its components. The probability of their collision diminishes and the rate of reaction decreases as well.

The author failed to evoke the antigen transformation of the serotype 51 A in *Paramecium aurelia* by means of various (even sublethal) concentrations of electrolytes. This was an attempt of repeating the experiments of B e a l e 1953, 1954. However, the antigen transformation reaction evoked by the homologous antiserum may be modified by salts and by different values of external pH. These facts seem to indicate that antigenic transformation may be considered as a phenomenon accompanying the process of binding antigen with antibody although this process is not always revealed in a visible form owing the unstable physicochemical conditions of medium. In the light of this concept the results of pH influence upon antigenic transformation at the range above 8 (Fig. 1 and 5) become comprehensible. Despite the lack of immobilization, the transformation occurs at a rather high level.

A suggestion arises that the phenomena under study: immobilization and the process which evokes directly the antigenic transformation by homologous antiserum, are two phases of binding antigen and antibody (Z a b ł o c k i 1959). The first phase of this process — which fails to appear in a visible form — would correspond to the process which initiates the antigenic transformation. The next phase would correspond to immobilization as to the stage when reaction products become visible. This phase is evidently connected with a number of factors in external medium as pH and kind and concentration of salts.

Summary

Results of the study indicate that in *Paramecium aurelia*, the intensity of immobilization after treatment with homologous antiserum, measured by the time of immobilization, depends on the pH of medium and on concentration of salts. The maximal immobilization rate occurs at pH 5.8. It was ascertained, that in the experimented range of chloriedes the intensity of immobilization reaction is inversely proportional to concentration of electrolyte. The bivalent cations ($Ba^{2+} > Ca^{2+} \geqslant Mg^{2+}$) act stronger than the univalent ($Na^+ > K^+$).

The antigenic transformation of the serotype 51 A to 51 B evoked by the homologous antiserum, may be modified by the ionic composition and by pH of the medium to which paramecia are exposed in the serum solution. It is suggested that transformation may be induced by the real binding of the immobilization antigen of cilia and of the pellicle with homologous antibodies without revealing this process in the form of immobilization since this second phenomenon depends on the appropriate physico — chemical conditions in external medium.

STRESZCZENIE

Otrzymane wyniki wskazują, że intensywność immobilizacji serotypów *Paramecium aurelia* pod wpływem homologicznej surowicy odpornościowej, mierzona czasem immobilizacji, jest uzależniona od pH środowiska i od stężenia soli. Maksimum szybkości immobilizacji występuje przy pH 5.8. Stwierdzono, że w bada-

nym zakresie stężeń soli chlorowych intensywność przebiegu immobilizacji jest odwrotnie proporcjonalna do stężenia. Dwu-wartościowe kationy $Ba^{2+} > Ca^{2+} \geqslant \geqslant Mg^{2+}$ działają intensywniej niż jednowartościowe $Na^+ > K^+$.

Transformacja antygenowa serotypu 51 A do 51 B wzbudzona homologiczną surowicą może być modyfikowana składem jonowym i pH środowiska w którym następuje ekspozycja pantofelków do roztworu surowicy. Sugeruje się, że transformacja może być indukowana przez rzeczywiste połączenie się antygenu immobilizacyjnego rzęsek i pellikuli z homologicznymi przeciwciałami, bez uwidocznienia tego faktu w postaci immobilizacji, wymagającej odpowiednich warunków fizykochemicznych środowiska.

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Acriflavin-induced akinetoplasty in *Crithidia fasciculata*

L'état akinetoplastique induit par l'acriflavine chez *C. fasciculata*

The kinetoplast or kinetonucleus may be considered a characteristic organelle of members of the family *Trypanosomatidae* although it has been reported to be absent from a certain percentage of the individuals of various strains of *Trypanosoma evansi* and from all individuals of *T. equinum*. It now seems agreed that this organelle is distinct from both the blepharoplast and the parabasal body. Recently, it has become an object of renewed interest following the demonstration by two different techniques (C o s g r o v e and A n d e r s o n 1954, S t e i n e r t, F i r k e t and S t e i n e r t 1958) that it contains deoxyribonucleic acid. However, its stainability with Janus green and other mitochondrial stains and the interpretation of its appearance in electron micrographs led C l a r k and W a l l a c e 1960 to identify it as a mitochondrion, an interpretation which scarcely suffices to account for the presence of a relatively large amount of deoxyribonucleic acid in it. S t e i n e r t and S t e i n e r t 1962 have advanced the idea "d'un kinétonucléus de structure et de function typiquement nucléaires, qui transmettrait les informations génétiques nécessaires à la synthèse des enzymes respiratoires généralement associés aux mitochondries."

W e r b i t z k i 1910 showed that akinetoplastic strains of *Trypanosoma* sp. could be produced by drugs; H o a r e reported the spontaneous appearance of akinetoplastic strains of *T. evansi* and *T. equiperdum* and of akinetoplastic individuals of various other species (reviewed by H o a r e 1954). T r a g e r and R u d z i n s k a 1964 have shown that acriflavin, added to a defined medium for *Leishmania tarentolae,* either inhibited growth without producing akinetoplastic forms (at limiting levels of riboflavin) or, at higher concentrations of riboflavin, produced up to 70% akinetoplastic individuals. C o s g r o v e and M c S w a i n 1960 reported the spontaneous occurrence, in cultures, of from 0.1 to 0.5% akinetoplastic individuals in seven species (representing the genera *Leptomonas, Crithidia* and *Blastocrithidia*) of trypanosomids from insects and the production of 25% to 30% akinetoplastic individuals in cultures of *Crithidia fasciculata* to which acriflavin had been added. This paper presents the results of further studies of the production of akinetoplastic individuals of *C. fasciculata*.

Materials and methods

Crithidia fasciculata (ATCC 11745) was grown axenically at 25°C in the yeast extract-hemin medium used in other investigations (Cosgrove 1959). A stock solution of 1% acriflavin, neutral USP in glass-distilled water was autoclaved separately and added aseptically to the desired concentrations. For microscopic study, an aliquot of culture was removed and the organisms in it concentrated by centrifugation, then resuspended in a few drops of diluted duck plasma (organisms suspended in plasma stain much more satisfactorily with Giemsa than those suspended in salt solutions). Smears were air-dried and stained with Giemsa or wet-fixed (Carnoy) and stained with Lillie's Feulgen. In determining the percentage of akinetoplastic individuals, all organisms in 100 randomly selected fields were counted and classified. Organisms in division were counted as two individuals only if two separate nuclei were present. Population counts were made with a hemocytometer on aliquots removed from a well-mixed culture and fixed with an equal volume of Bouin's fixative.

Results

Populations of this flagellate growing on both the routine yeast extract-hemin medium and on the much richer diphasic medium of Phillips 1951 used for maintaining stock cultures contain about 0.1% akinetoplastic individuals. As can be seen from Fig. 1, this value can be raised to approximately 48% by growth in a medium containing 30 µg acriflavin per ml. When the medium contained greater concentrations of acriflavin, the percent of akinetoplastic individuals decreased sharply. In all concentrations of acriflavin which resulted in increased percentages of akinetoplastic individuals, some increase in numbers of flagellates occurred, but in the higher concentrations of acriflavin (above 80 µg per ml.) no increase in total population or in percent of akinetoplastic individuals occurred. These relationships are to be expected if the production of akinetoplastic individuals requires cell division. Stained preparations of control and experimental cultures showed many stages of cell-division like those of Plate I 7 with two complete kinetoplasts formed before nuclear division begins. Division figures like those in Plate I (9, 10) were found only in experimental acriflavin-containing cultures. Note that completion of cytokinesis in the individual of Plate I (10) would have produced one akinetoplastic (marked with the arrow), and one normal daughter; the single kinetoplast of this individual appears to be the same size as the kinetoplasts in Plate I (1). Careful examination of these slides failed to yield any evidence for dissolution of the kinetoplast; the organelle is either absent or present in at least the same mean size and with the same Giemsa and Feulgen stainability as in dividing individuals from control cultures.

Growth of cultures was noticeably slower in the presence of 30 µg acriflavin per ml. The curve of Fig. 2 shows that growth is linear, as opposed to the characteristic sigmoid curve shown by control cultures. The linear growth agrees well with the microscopical evidence that an akinetoplastic individual results from failure of the kinetoplast to duplicate before cytokinesis is complete, if we assume that akinetoplastic individuals are either incapable of division or divide much less frequently than normal individuals.

A systematic search for dividing akinetoplastic individuals resulted in find-
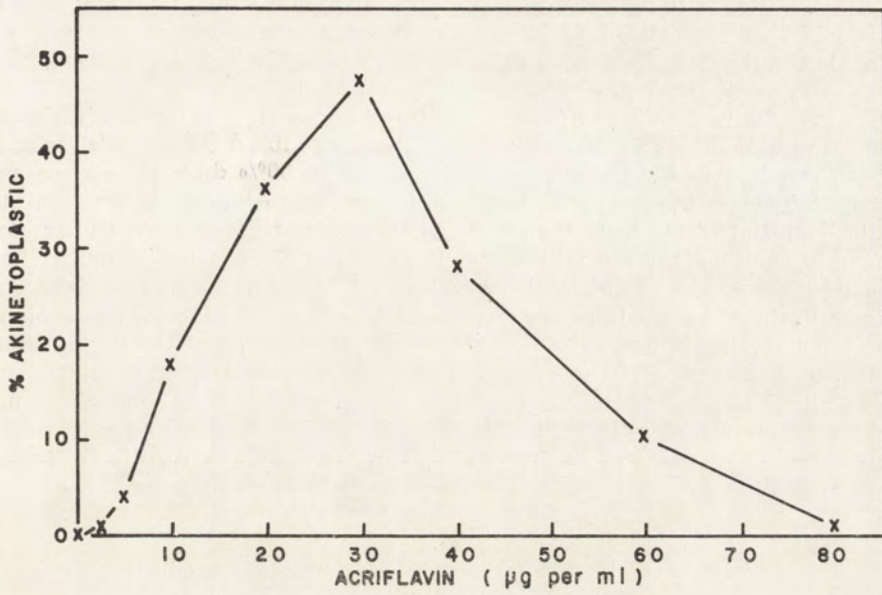
Fig. 1. Maximum percentage of akinetoplastic individuals in cultures containing various concentrations of acriflavin
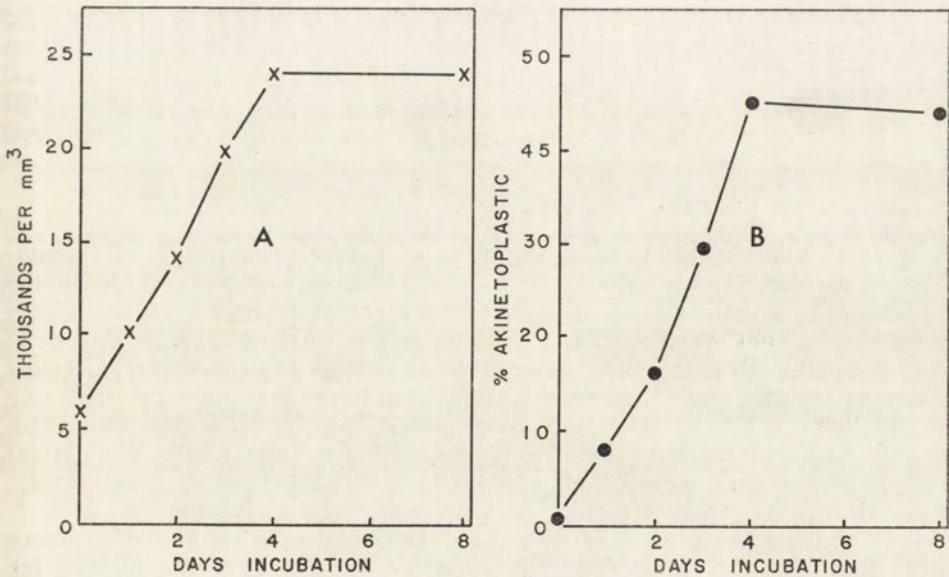

Fig. 2. A. Growth curve in yeast extract-hemin medium with 30 μg acriflavin/ml. B. Percentage of akinetoplastic individuals in the populations of curve A

ing (Pl. I 11) three binucleate, biflagellate individuals without kinetoplasts out of 10 345 akinetoplastic individuals examined (a percentage occurrence of 0.029%). On the same slides, 0.60% of the kinetoplastic individuals were in division by these criteria.

To test further this assumption, cultures containing about 45% akinetoplastic individuals were diluted with sterile amphibian Ringer with glucose, or with sterile Phillips' overlay or with sterile 50% duck plasma, so as to contain between 500 and 1000 individuals per ml. Aliquots (0.1 ml) of this diluted material were pipetted on the surface of blood agar plates, and smeared uniformly over the surface with a sterile glass rod. Incubation in moist chambers for three weeks at 25°C, with examination at intervals of five days, gave the results in Table 1. It is apparent that the number of colonies is always approximately the same as the number of normal individuals in the inoculum, as would be expected if the akinetoplastic individuals are incapable of producing viable daughter cells through several successive generations. Since most of the colonies were easily visible after five days incubation and remained as discrete pin-point colonies for the entire three weeks, the results in Table 1 are not distorted by overgrowth of akinetoplastic colonies by normal ones.

Table 1

Results of plating experiments to determine reproductive ability of akinetoplastic individuals

% akinetoplastic individuals in inoculum	mean number of organisms per 0.1 ml. inoculum	colonies per plate	
		predicted**	observed
45	62 (8)*	34	25 (5)*
43	75 (6)*	32	34 (7)*
0.5 (control)	99 (11)*	99	101 (12)*

* standard errors
** assuming that akinetoplastic individuals cannot reproduce
Medium: Yeast extract-hemin plus 5% agar. Inoculum grown in medium without acriflavin (control) or with 30 μg/ml acriflavin.

Discussion

From the literature on the spontaneous or induced occurrence of akinetoplastic individuals and strains, three questions become apparent: (1) how is the akinetoplastic condition produced? (2) can a kinetoplast, once lost, be regenerated? (3) in what ways are akinetoplastic and normal individuals different? The mammalian trypanosome material which has been used so far for most studies of akinetoplastic phenomena is not particularly favorable for answering any of these questions since the species used can be cultivated only with difficulty and, in most species, the kinetoplasts are quite small. While final answers to these three questions cannot be given as a result of this study, probable answers can be offered.

Loss of the kinetoplast might be due either to dissolution of the structure under the influence of the drug, or to failure of it to duplicate in time for a daughter kinetoplast to be distributed to each daughter flagellate. The present study shows that an akinetoplastic individual resuls from failure of

the kinetoplast to duplicate prior to cell division; such kinetoplasts are of normal size and careful examination of stained preparations fails to reveal any diminution in size, stainability or other aspect which might be interpreted as part of a process of dissolution. This interpretation is strengthened by the finding that the drug does not produce akinetoplastic individuals at concentrations which prevent all multiplication, nor does it affect kinetoplast appearance in surviving individuals at such concentrations. It is also consistent with the spontaneous occurrence of akinetoplastic individuals in *C. fasciculáta* and in various species of *Trypanosoma*. On the other hand, the report of T r a g e r and R u d z i n s k a 1964 indicates that dissolution of the kinetoplast may well occur in *Leishmania tarentolae*. Their electron micrographs show extensive disorganization of the DNA-containing portion, as well as other serious structural defects. An attractive hypothesis for all these effects is that acriflavin interferes with the reading of the DNA for replication, which is sufficiently delayed so that replication of the entire kinetoplast is either prevented or imperfect at the following division as a result of insufficient DNA. Depending upon the time relations of kinetoplast duplication and cell division, there would be produced either the effect described in this paper or the degenerative effect described by T r a g e r and R u d z i n s k a. Using the technic of S t e i n e r t and S t e i n e r t 1962 it has been shown that division of the kinetoplast in *C. fasciculata* precedes cell division by only 5—7 minutes; even a minor interference with DNA duplication would seem inevitably to produce akinetoplastic offspring.

The mechanism for such interference seems most probably to lie in the known ability of DNA to bind acriflavin (and related molecules) in so intimate a fashion as to produce steric interference with duplication of the nucleic acid molecule. The greater effect of acriflavin on kinetoplast DNA than on nuclear DNA would seem to have its basis in the binding at adenine-thymine (A-T) base pairs (C h a m b r o n et al. 1964) and the probable higher adenine-thymine content of kinetoplast as opposed to nuclear DNA (M a r m u r et al. 1963; D u B u y et. al. 1965). Differences in A-T content of nuclear and kinetoplastic DNA, as indicated by the report of M a r m u r et al, would explain differences in the effectiveness of acriflavin in producing akinetoplasty in different species.

S u m m a r y

Cultivation of *Crithidia fasciculata* in the presence of acriflavin (30 µg/ml) results in about 48% of akinetoplastic individuals compared with about 0.1% in cultures without acriflavin. Akinetoplastic individuals result from cytokineses without preceding divisions of the kinetoplast rather that by dissolution of the kinetoplast in the presence of acriflavin. These findings are discussed with regard to information on drug-induced and spontaneous occurrence of the akinetoplastic condition in trypanosomes. An hypothesis for the mode of action of acriflavin in producing akinetoplasty is proposed, based on interference with DNA reading or replication as a result of bonding of acriflavin at adenine-thymine base pairs.

RÉSUMÉ

Quand on cultive *Crithidia fasciculata* dans un milieu contenant de l'acriflavine (30 µg/ml.), on obtient jusqu'à 48% des flagellés sans kinétoplaste, comparé à 0.1% dans les cultures sans l'acriflavine. Ces flagellés akinétoplastiques sont produit par

des cytokinèses qui se complètent devant la duplication du kinétoplaste, et non par la dissolution du kinétoplaste en présence de l'acriflavine. On discute ces découvertes concernant l'occurrence, spontanée ou causée par les drogues, de la condition akinétoplastique parmi les Trypanosomes. On propose que le méchanisme par lequel l'acriflavine produit l'état akinétoplastique est l'interférence avec la transcription ou la réplique de l'acide desoxyribonucléique par l'acriflavine liée aux plateaux A-T.

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EXPLANATION OF PLATE I

Crithidia fasciculata

1: Individuals from culture with no acriflavin
2: Individuals from culture with 80 µg acriflavin/ml
3 and 4: Individuals from culture with 30 µg acriflavin/ml and 46⁰/₀ akinetoplastic individuals
5, 6, 7, 8: Stages in normal division, from same culture as 1
9 and 10: Stages in abnormal division, from same culture as 3
11: Apparent division of akinetoplastic individual, from same culture as 3

Photomicrographs of dried smears, fixed with methanol and stained with Giemsa.

10 μ
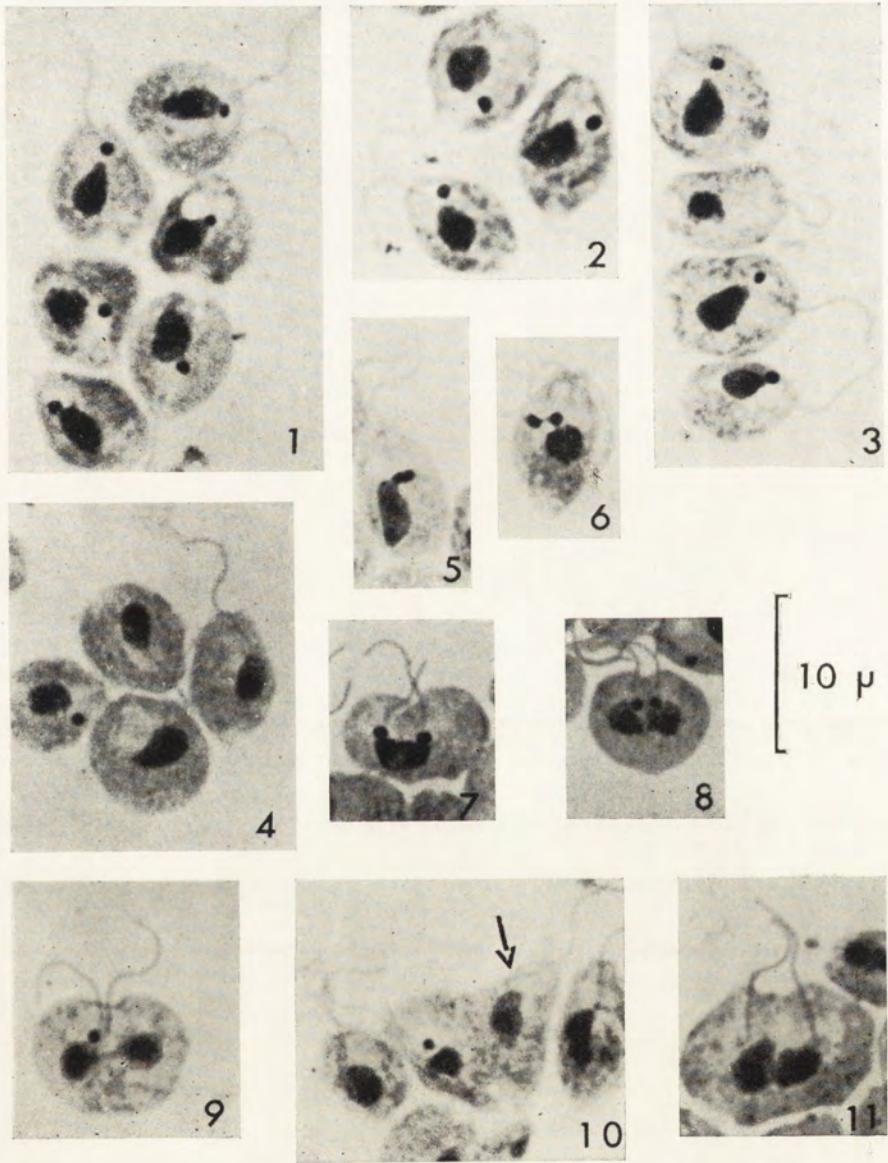
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L. M. GORDEEVA

О культивировании *Entamoeba invadens* Rodhain, 1934, при температуре 37°C

On cultivation of *Entamoeba invadens* Rodhain, 1934 at 37°C

Температурные границы для роста в культуре являются одной из особенностей, характеризующих виды и штаммы амеб рода *Entamoeba*.

Оптимальной для роста *E. histolytica* в культуре является температура 37°C, но недавно появилось сообщение о выделении штамма „Laredo", который способен расти в очень широком диапазоне температур 10—35°C (Dreyer 1961).

Entamoeba invadens Rodhain, 1934 — патогенная для рептилий — морфологически сходна с *E. histolytica* (Geiman and Ratcliffe 1936, Ratcliffe and Geiman 1938, Steck 1963 и др.). Однако, в культуре этот вид амеб размножается при 16—35°C с оптимумом температур 20—30°C.

Литературные данные свидетельствуют о неспособности *E. invadens* расти при температуре 37°C (оптимальной для *E. histolytica*). В Таблице 1 представлены температурные границы для размножения *E. invadens* и максимальное время наблюдения переживания амеб при различных температурах (по литературным данным).

Установлено (Siddiqui 1963), что способность *E. invadens* адаптироваться к высоким температурам меняется в зависимости от вида организма, сопутствующего ей в культуре. В культуре с *Clostridium acidi-urici* *E. invadens* размножалась при температуре не выше 33°C, в ассоциации с *Trypanosoma cruzi* — при 34°C (не выше), и в ассоциации с *Clostridium perfringens*, после культивирования в течение 17 недель при 34.5°C, оказалась способной размножаться при 35°C (но не выше). В смешанной полибактериальной культуре *E. invadens* размножалась на протяжении 70 дней при 35.5°C (Lachance 1963). При 36°C наблюдался слабый рост амеб, культура просуществовала только 29 дней. При 36,5°C размножения амеб в культуре не наблюдалось, но амебы переживали до 12 дней, а при 37°C — только 7 дней.

При работе с культурами энтамеб мы обнаружили, что имеющийся в нашей лаборатории штамм *E. invadens*, культивируемый при комнатной температуре, способен адаптироваться к температуре 37°C. Настоящее сообщение посвящено изучению некоторых особенностей амеб адаптированного штамма.

Материал и методика

Исходный штамм *E. invadens*, обозначенный нами как штамм „И", был получен от профессора Jirovec (Прага) в октябре 1960 года.

Культура амеб со смешанной неидентифицированной бактериальной флорой поддерживается на среде Павловой с рисовым крахмалом. Пробирки 150×16 мм закрываются ватными пробками. Культивирование ведется в темноте, за исключением коротких интервалов во время пересевов (через 2—3 недели), при комнатной температуре (20—25°С).

Культура амеб, размножающихся при 37°С, условное обозначение — штамм „ИТ", содержится в термостате. Температура в термостате поддерживается на уровне 36.5—37°С при колебаниях в 0.5—1°С во время открывания термостата. Во время пересевов температура снижается на короткий срок (5—10 минут) до 33°С. Перед пересевом пробирки со свежей средой подогреваются до 37°С. В связи с более бурным ростом бактерий при 37°С среда истощается быстрее, и требуются более частые пересевы (через 2—3 дня) по сравнению с культурами штамма „И".

Развитие культуры отмечается путем наблюдения за относительным количеством амеб на стенках пробирки. Пробирки просматриваются под микроскопом в проходящем свете при увеличении $100 \times$. Относительное количество амеб обозначается от $\pm$ до $+ + + +$, что соответствует (при просмотре капли из осадка культуры) единичным амебам в поле зрения, более 5 амеб, более 25 амеб, 125 амебам в поле зрения и более 125 амеб. В последнем случае, при просмотре пробирки в проходящем свете, отмечается сплошной рост амеб.

Результаты и обсуждение

В культурах штамма „И" почти постоянно отмечается рост амеб на $+ + + +$. Образование цист не наблюдается. Амебы в культурах подвижны, наполнены зернами крахмала. В культурах месячной давности лишь единичные амебы содержат небольшое количество зерен крахмала.

В мае 1963 г. мы обнаружили небольшое количество очень крупных подвижных амеб, с зернами крахмала, в пробирке с культурой *E. invadens*, помещенной пять дней назад в термостат при 37°С. Был сделан пересев на свежую среду и отмечен незначительный рост амеб в культуре при 37°С. Всего было сделано 5 пересевов в течение 3-х недель. Этот срок существования *E. invadens* в культуре при 37°С превышал все ранее известные данные (Таблица 1). Поскольку в литературе имелись указания о невозможности культивирования *E. invadens* при 37°С было интересно пронаблюдать как долго сможет существовать при 37°С культура, имеющегося в лаборатории штамма амеб.

В связи с этим 13 сентября 1963 года в термостат (37°С) была поставлена пробирка с 72-часовой (рост на $+ + + +$) культурой *E. invadens*, растущей при комнатной температуре. Стабильный рост амеб (на $+ +$, а затем и на $+ + +$) в культуре при 37°С установился через 1.5 месяца. Эта культура, условно обозначенная как штамм „ИТ", поддерживается и в настоящее время, уже сделано свыше 190 пересевов.

Амебы в пробирках располагаются на стенках, поднимаясь довольно высоко (20 мм и более) над крахмалом, но у границы с крахмалом их плотность наибольшая.

Таблица I

Температурные границы размножения *Entamoeba invadens* и максимальное
время наблюдения ее переживания при различных температурах

Temperature ranges of proliferation of *Entamoeba invadens* and observed
survival time (maximum) of *E. invadens* exposed to various temperatures

Размножение Proliferation		Переживание Survival time		Автор Author
максимальное maximum	замедленное r duced	вегетативные формы trophozoites	цисты cycts	
23—25°		37°		Rodhain 1934
20—30°		10°, 33°		Geiman and Ratcliffe 1936
20—30°	30°, 35° (10 месяцев—months)		5° (10 дней —days)	Ratcliffe and Geiman 1938
24—30°	16—20°	37° (7 дней —days)	8° (14 дней —days)	McConnachie 1955, 1958.
	33.5—35°	8° (35 дней —days)	24° (7 дней —days)	
			37° (7 дней —days)	
20—25°	30° (в эмбрионе ципленка—in chicken embro)		4—5° (3 дней —days)	Meerovitch 1956, 1958, 1960, 1961
	35° (без инцистирования—without incystation)			
20—30°		37° (24 часа —hours)		Steck 1963
30°	33°, 34.5 (17 недель—weeks), 35°.			Siddiqui 1963
25°	12.5° (295 дней —days)	4° (23 дня —days)		Lachance 1963
	35° (541 день —days)	36.5° (12 дней —days)		
	35.5° (70 дней —days)	37° (7 дней —days)		
	36° (29 дней —days)			

В первые 2-3 суток после пересева амебы содержат огромное количество зерен крахмала, и поэтому кажутся темными в проходящем свете. В отдельных пробирках можно обнаружить единичных подвижных амеб без крахмала и на 15—20-й день после посева, но, как правило, на 5—7-й день после посева в культуре появляется довольно много округлившихся неподвижных амеб. Образования цист у штамма „ИТ" не отмечено.

На кривой развития амебной популяции в культуре при 37°C (Рис. 1), видно, что пик популяции отмечается через 48—72 часа после посева, затем количество амеб начинает уменьшаться.
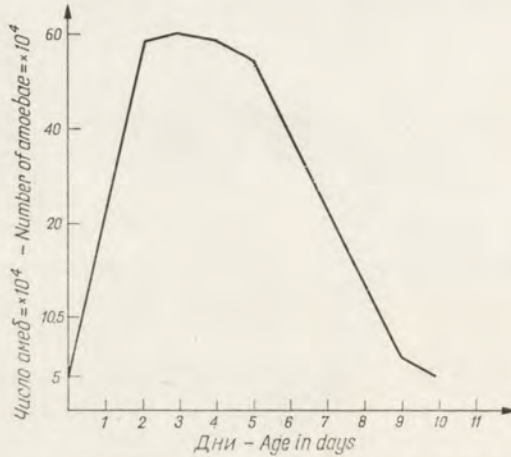
Рис. 1. Кривая развития популяции *E. invadens* в культуре при 37°C.
Fig. 1. The course of growth of population of *E. invadens* in culture at 37°C

В отличие от культур штамма „И", в культурах штамма „ИТ" гораздо реже отмечается рост амеб на $++++$, обычно это совпадает с понижением температуры в термостате до 36.5—36.7°C.

Морфология амеб из культуры штамма „ИТ"

Морфология амеб из культуры штамма „ИТ" изучалась на постоянных препаратах (фиксирующая жидкость Шаудина и Карнуа, окраска — железным гематоксилином по Гейденгайну, метиловым зеленым — пиронином по Браше на РНК и для выявления ДНК ставилась реакция Фельгена). Постоянные препараты амеб из культуры получались с помощью плексигласовой камеры по методике описанной ранее (Гордеева 1964).

Морфологических различий у амеб, культивируемых при 37°C (штамм „ИТ") и при комнатной температуре (штамм „И"), при изучении постоянных препаратов не выявлено.

В 24-часовой культуре амебы наполнены зернами крахмала. Ядро пузырьковидное, под тонкой оболочкой обнаруживается пристенный слой хроматина в виде гранул почти одинакового размера, расположенных в один или несколько слоев. Иногда можно отметить скопление гранул на одной стороне ядра. В не-

которых ядрах гранулы располагаются более плотно, и тогда пристенный слой хроматина выявляется в виде темноокрашенного кольца. Гранулы пристенного слоя хроматина окрашиваются пиронином в малиновый цвет, что говорит о содержании в них РНК. Кариосома в виде точечного образования расположена в центре ядра, реже субцентрально. После реакции Фельгена в кариосоме выявляется слабая розовая окраска, что свидетельствует о присутствии в ней ДНК. Между кариосомой и пристенным слоем хроматина обнаруживаются нежные мелкие менее плотные гранулки (Табл. I A). Часть этих гранул содержат ДНК, так как дают положительную реакцию Фельгена, а некоторые окрашиваются пиронином и, следовательно, содержат РНК.

В 48-часовых культурах амебы содержат зерна крахмала на разных стадиях переваривания и некоторое количество бактерий. Картина строения ядра такая же, как у амеб из 24-часовой культуры. Цитоплазма умеренно вакуолизирования (Табл. I B).

В 72—96-часовых культурах попадаются амебы, в цитоплазме которых имеются хроматоидные тела (Табл. I C, D).

Наши данные о наличии ДНК в кариосоме и гранулах между кариосомой и пристенным слоем хроматина в ядре *E. invadens* согласуется с результатами, полученными N a r a s i m h a m u r t i 1964, который отметил также, что гранулы под оболочкой ядра были всегда Фельген-отрицательными. Последнее замечание согласуется с нашими данными о присутствии РНК в гранулах пристенного слоя хроматина.

Известно, что при повышении температуры размеры микроорганизмов уменьшаются. Тенденцию к уменьшению размеров *E. invadens* в культуре по мере повышения температуры отмечал S i d d i q u i 1963. Однако, при измерении амеб на постоянных препаратах нами не было обнаружено существенной разницы в размерах вегетативных форм штаммов „И” и „ИТ”. Амебы из 48-часовой культуры штамма „ИТ” были почти одинакового размера с амебами из 7—10-дневной культуры штамма „И”. Результаты измерений представлены в Таблице 2.

Таблица 2

Размер вегетативных форм *Entamoeba invadens* — исходного штамма „И” и адаптированного штамма „ИТ” (в микронах)

Size of trophozoites of *Entamoeba invadens* of initial strain "I" and temperature-adapted strain "IT" (in microns)

	Штамм „И“ Strain „I“	Штамм „ИТ“ Strain „IT“
Длина Length	$33.75 - 53.75^{x}$ 42.95 ± 6.43^{xx}	$36.75 - 53.75^{x}$ 42.56 ± 5.1^{xx}
Ширина Width	$7.5 - 21.25^{x}$ 12.42 ± 4.7^{xx}	$8.5 - 21.25^{x}$ $12.85 \pm 3.8xx$

x — диапазон — range
xx — среднее и среднее квадратичное отклонение — mean and mean square deviation

Наши данные в какой-то степени согласуются с результатами наблюдений Lachance 1963, который отметил, что при температуре 35.5°C размеры амеб в полибактериальных культурах *E. invadens* сначала увеличивались, а затем возвращались к норме, но не были меньше по сравнению с размерами амеб, культивируемых при комнатной температуре.

Дополнительно нами был поставлен ряд опытов с целью выяснить: а. возможно ли штамм „ИТ" реадаптировать к комнатной температуре, б. какова температурная устойчивость этого штамма, и в. может ли штамм „ИТ" заражать теплокровных животных.

а. Реадаптация штамма „ИТ" к комнатной температуре. 17. II. 1964 г. — 4 пробирки с 48-часовой культурой штамма „ИТ" (рост на $+++$) из термостата (37°C) были поставлены в шкаф при комнатной температуре. Через 24 часа количество амеб уменьшилось до $++$, а еще через 2 дня до $+$. В пересеве (21. II. 1964) на свежую среду был отмечен рост амеб на $++$, и уже во втором пересеве (1. III. 1964) рост амеб был такой же обильный, как и в культурах штамма „И", постоянно растущих при комнатной температуре. Таким образом, реадаптация к комнатной температуре произошла быстрее, чем адаптация к 37°C.

б. Температурная устойчивость штамма „ИТ". Было отмечено, что даже незначительное повышение температуры (над 37°C) действует неблагоприятно на культуру амеб штамма „ИТ". При повышении температуры до 37.3°C в 48- и 72-часовых культурах обнаруживаются только единичные округлившиеся амебы, которые при пересеве на свежую среду дают очень слабый рост ($\pm$ и $+$) в двух последовательных пересевах.

Опыты по культивированию амеб штамма „ИТ" при 38°C после длительного (в течение 5—6 месяцев) культивирования при 37°C дали отрицательные результаты. Были поставлены 3 опыта, в которых 48-часовая культура амеб (рост $+++$) помещалась в термостат при 38°C. Уже через 24—48 часов в пробирках оставались лишь единичные неподвижные амебы. В пересевах, сделанных через 24 часа, обнаруживались единичные малоподвижные очень крупные амебы, но размножения амеб не наблюдалось. Культура погибала на 3—4-й день. Амебы не росли и в пересевах не свежую среду при 37°C, это свидетельствует о том, что при температуре 38°C уже через 24 часа амебы теряют способность к размножению.

Гибель амеб может быть вызвана либо непосредственным действием температуры на амеб, либо отсутствием благоприятных условий для развития амеб, которые обеспечиваются сопутствующей бактериальной флорой (косвенное влияние температуры).

Отрицательный окислительно-восстановительный потенциал среды является одним из основных физико-химических факторов, обеспечиваемых сопутствующими бактериями и являющихся необходимым условием развития амеб в культуре. В связи с этим в одном из описанных опытов в пробирки со средой перед посевом добавлялось по капле 0,1%-ного водного раствора метиленового голубого. Контрольные пробирки с культурой помещались при 37°C, опытные — при 38°C. Как в контрольных, так и в опытных пробирках через 24 часа наступило обесцвечивание, что указывало на установление отрицательного окислительно-восстановительного потенциала. Таким образом, причиной отсутствия размножения и гибели амеб при 38°C не могло быть несоответствие окислительно-восстановительного потенциала среды.

в. Способность к заражению теплокровных животных: Было поставлено 3 опыта, заражено 60 крысят культурой штамма „ИТ", растущей в течение 3, 7 и 8 месяцев при 37°С, и 30 крысят контрольных — культурой штамма „И", растущей постоянно при комнатной температуре.

В опыт для заражения брались молодые крысята весом 20—25 гр., свободные от инфекции *E. muris*. 48-часовая культура штамма „ИТ" (125 амеб в поле зрения при увеличении 100 ×) в количестве 0.2 мл вводилась интрацекально. Контрольные крысята заражались 7-дневной культурой штамма „И". В одном из опытов крысятам вместе с культурой амеб было введено по 20 единиц лиофилизированной гиалуронидазы. Крысят вскрывали на 7-е сутки. Ни одно животное не заразилось. У животных, забитых и вскрытых на следующие сутки после заражения, амеб в слепой кишке обнаружить также не удалось.

Резюме

Из культуры штамма „И" *Entamoeba invadens*, длительно культивируемого при комнатной температуре, выделен штамм „ИТ", адаптированный к росту при 37°С. Штамм „ИТ" культивируется непрерывно в течение более 18 месяцев, на протяжении которых сделано свыше 190 пересевов. На постоянных препаратах амебы штамма „ИТ" морфологически не отличаются от амеб исходного штамма „И".

При повышении температуры в термостате до 37.3°С амебы округляются и теряют подвижность, но сохраняют жизнеспособность и при пересеве дают рост при температуре 37°С. При 38°С амебы переживают в культуре 3—4 дня, но уже через 24 часа теряют способность размножаться.

Штамм „ИТ", растущий при 37°С, не заражает молодых крысят при введении им культуры интрацекально.

SUMMARY

A strain ("IT") adapted to growth at 37°C was obtained from the culture of the "I" strain of *Entamoeba invadens* grown at room temperature for a long time. The "IT" strain was cultured at 37°C for above 18 months (190 subcutures). In stained preparations the amoebae of adapted strain did not differ morphologically from the initial ones.

When the temperature was raised up to 37.3°C the amoebae became motionless and rounded up but remained viable and recovered when inoculated to fresh medium and maintained at 37°C. At 38°C the amoebae survived in the culture for 3—4 days but had lost totaly the capacity of multiplying in 24 hours.

The strain of *E. invadens* adapted to 37°C was not infective for young rats when the amoebae were injected in the coecum.

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ПОДПИС К ТАБЛИЦЕ I

Морфология амеб из культуры адоптированного штамма *E. invatens:* A. 24-часовая культура, B. 48-часовая культура, C. 72-часовая культура, D. 96-часовая культура

EXPLANATION OF PLATE I

The morphology of amoebae from the culture of temperature-adopted strain of *E. invadens*: A. 24 hrs. culture, B. 48 hrs. culture, C. 72 hrs. culture, D. 96 hrs. culture

PLATE I

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Fasciculi: