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Size Dependent Regulation of Serially Repeated Structures of a Protozoan *Paraurostyla weissei*

Received on 28 May 1980

Synopsis. Size dependent regulation was shown for left marginal cirri (LMC) of *Paraurostyla weissei.* Normal and size reduced cells were morphometrically analysed. The number of kinetosomal rows in LMC (KsR) decreases posteriorward in normal cells from four to two and in small cells from 3 to 2. The ratio KsR/LMC is size invariant. Reduction of cell size is connected with disappearance, first of cirri having four then three KsR. Reduction of the number of adoral membranelles (AM) is greater than LMC. The ratio of AM/LMC and AM/KsR as well as the internal architecture of cirri is characteristic for a given cell size.

Generation of serially repeated structures is one of the basic features of morphogenesis of different organisms. The cell surface pattern of various one-celled organisms is reformed after disturbance. This can be achieved either by regrowth of lost parts or by reorganization of the fragment into a new whole. Regrowth of the lost parts e.g., in *Paramecium* (Tartar 1954) or in *Chilodonella* (Kaczanowska 1975) leads to the formation of qualitatively and quantitatively species-specific cortical pattern. Adjustment of the investigated parts of ciliature to the cell size has been found in *Stentor* (Tartar 1941, 1961, 1967) and *Stylonychia* (Dembowska 1938). The pattern of cell structures that responds in a size dependent manner to the alternation of the organism's dimensions is exemplified by the set of ciliary organelles of *Paraurostyla weissei.* The surface of this ciliate can be divided into sets of zones that have species-typical fate of differentiation. In a fully differentiated cell each of these zones is characterized by the unique arrangement of ciliary elements — membranelles and cirri. The previous investigations (Jerka-Dziadosz 1976, 1977) showed that the number of elements of ciliary pattern was correlated with the cell

length. Further morphometric studies (Bąkowska and Jerka-Dziadosz 1980) of regulation of the adoral zone of membranelles revealed that the size of membranelles (the total number of kinetosomes) adjusted regulatively to their total number. Instead, the number of transverse kinetosomal rows (kineties) was preserved in each membranelle regardless the overall cell dimensions. This rises the question of whether the capacity for such regulation exists in a group of other also serially repeated structures — the cirri. The analysis of regulation of left marginal cirri has shown that their number is closely but not absolutely controlled at species and size typical value. The number of kinetosomal rows in individual structures undergoes regulation according to the total number of cirri. The smaller the cell is the less marginal cirri is formed and the fewer transverse and longitudinal rows of kinetosomes they contain. The ratio between the number of adoral membranelles and left marginal cirri changes with cell size. Smaller cells possess relatively more LMC than AM. Thus, this ratio is also species-typical for a given organism's size.

The results are discussed in relation to the influence of morphogenesis of structures on the mode of their regulation and the ideas of generation of serially repeated structures.

Material and Methods

Cells used in these investigations were a clone of *Paraurostyla weissei*, line Z-6 isolated after total conjugation (Jerka-Dziadosz and Janus 1975). The cells were kept in sterile Pringsheim solution and fed on green algae *Chlorogonium* grown on earth medium after Heckmann (1963).

Observations were performed on cells of normal and reduced size. Reduction of cell size was achieved by: (1) starvation in sterile Pringsheim solution where cells passed through repetitive physiological reorganizations (Dembowska 1938), (2) repeated cutting of normal cells into nucleated pieces which regenerated into small complete organisms.

The counts of adoral membranelles, left marginal cirri and kinetosomal rows in cirri were performed on preparations stained with protargol after Jerka-Dziadosz and Frankel (1969) under light microscope (Ortholux Leitz-Wetzlar). Kinetosomes in left marginal cirri were scored on ultrathin sections examined under an electron microscope JEM 100B. For electron microscopy the cell were prepared as described previously (Bąkowska and Jerka-Dziadosz 1978). For better contrast of microtubular structures in some experiments the cells before dehydration were stained in 0.1% solution of tannic acid for 15–30 min.

The results were statistically analysed using regression analysis. Additionally, two tests for proportionality were used: (1) 95% confidence interval of Y-intercept, (2) confidence interval of coefficient b calculated for the regression Y/X on X. These routine techniques are described by Sokal and Rohlf (1969). Prepara-

tion of the statistic programs and computations were performed by the Computing Center of the Polish Academy of Sciences.

Results

The ciliature of *P. weissei* cells has been described in the earlier paper (Jerka-Dziadosz and Frankel 1969). The ciliary organelles of the ventral surface (membranelles and cirri) are disposed in highly asymmetrical species-specific manner (Fig. 1). The ventral pattern is composed exclusively of multikinetosomal elements. Regulation of oral ciliature i.e., adoral and preoral membranelles has been analysed previously (Bąkowska and Jerka-Dziadosz 1980). Presently investigated left marginal cirri (LMC) are aligned in longitudinal row situated along the left margin of the ventral surface. Cells of normal size possess about fifty left marginal cirri. In cells twice reduced the number of LMC decreases to thirty two (Jerka-Dziadosz 1976).

Ultrastructure of Cirri. The kinetosomes composing each cirrus are surrounded by fibrous basket (fb). Kinetosomes within the basket are disposed in longitudinal and transverse rows (Fig. 2). According to Puytorac et al. (1976) the row formed by kinetosomes possessing prominent posticiliary microtubules can be considered as kinety. The adjacent kinetosomal rows in cirri and mebranelles are subsequent kineties or the multiplication of the first one. In cirri of *P. weissei* the longitudinal rows of kinetosomes are kineties while in adoral membranelles the transverse rows could be so called. Kinetosomes of consecutive kineties form transverse rows in marginal and ventral cirri. The first, anterior row is often shorter by one kinetosome in respect to the subsequent rows. The length of cilia in each cirrus decreases from right to left and from posterior to anterior.

Kinetosomes within each cirrus are interconnected by fibrous material which integrates marginal kinetosomes with the cirrial basket (Fig. 2). Kinetosomes of the same kinety are connected by the two connectives: right (RC) and left (LC). The left one is usually thicker both in transverse and perpendicular plane. Kinetosomes forming transverse rows are interconnected by two connectives — anterior (AC) and posterior (PC). The latter is thicker similarly as the right connective mentioned above. The last, oblique connective joins two kinetosomes of adjacent kineties. The left kinetosome of this pair is located in the posterior row in respect to the right kinetosome.

The accessory filamentous and microtubular structures are connected with kinetosomes forming each cirrus. The type of kinetosomal

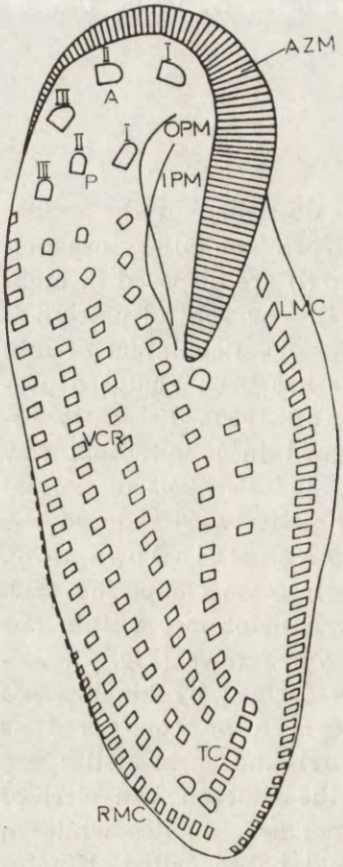

Fig. 1. Schematic representation of the ciliary pattern of the ventral surface of *Paraurostyla weissei* cell. A — anterior row of frontal cirri, AZM — adoral zone of membranelles, IPM — inner preoral mebranelle, LMC — left marginal cirri, OPM — outer preoral membranelle, P — posterior row of frontal cirri, RMC — right marginal cirri, TC — transverse cirri, VCR — rows of ventral cirri, I–III — enumerate of frontal cirri

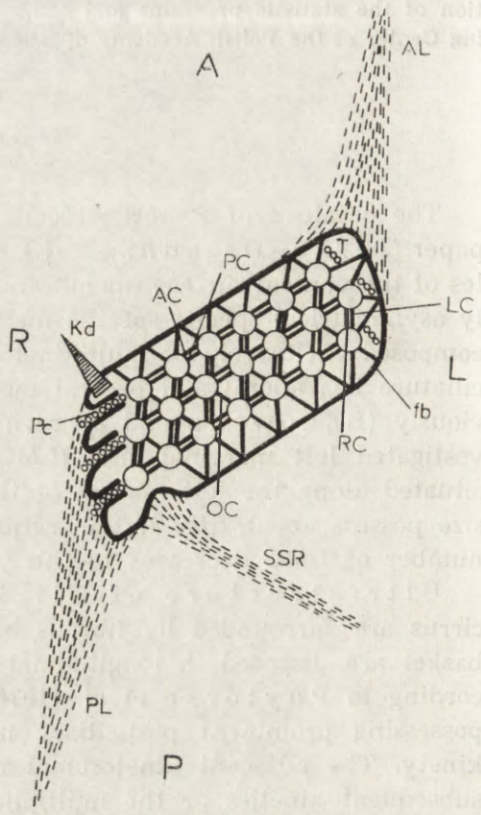

Fig. 2. Schematic representation of ultrastructure of left marginal cirrus. A — anterior margin of the cell, AL — anterior longitudinal fiber, AC — anterior connective, fb — fibrilar basket, L — left side of the cell, LC — left connective, kd — kinetodesmal fiber, OC — oblique connective, P — posterior margin of the cell, Pc — postciliary microtubules, PC — posterior connective, PL — posterior longitudinal fiber, R — right side of the cell, RC — right connective, SSR — small subectoplasmic rootlet, T — transverse microtubules

derivatives depends on the position of a kinetosome within the cirrus. Kinetosomes of the first, right kinety possess postciliary microtubules and kinetodesmal fibers. In fully developed marginal cirrus all kinetodesmas disappear except the one associated with the anterior kinetosomes of the right kinety. Kinetosomes forming the remaining kineties

possess only one short postciliary microtubule. Kinetosomes of the last, left kinety are accompanied by transverse microtubules.

Three microtubular fibers are associated with each marginal cirrus. These are: anterior longitudinal (AL) and posterior longitudinal (PL) fiber and small subectoplasmic rootlet (SSR). The fibers are formed of microtubules originating in the cirrial basket. Additionally, transverse microtubules of kinetosomes of left kinety contribute to AL formation, while postciliary microtubules associated with kinetosomes of right kinety join the PL. The cirrial fibers of subsequent cirri overlap providing the stability of cirrial row and probably play some role in cell shape formation.

The ultrastructure of left marginal cirri i.e., the system of connectives between kinetosomes, the accessory fibers connected with kinetosomes and microtubular fibers associated with cirri are similar in normal and size reduced cells.

The basic feature of architecture of marginal cirri are common for all kinds of cirri in *P. weissei*. They are also similar to the previously described for *Gastrostyla* (Grim 1972), *Oxytricha* (Grimes and L'Hernault 1978), *Stylonychia*, *Onychodromus* and *Euplotes* (Tuffrau et al. 1968).

Disposition of Kinetosomes within Left Marginal Cirri. Marginal cirri of a given cell differ in the number and disposition of kinetosomes. In the anterior part of normal cells there are cirri composed of three transverse rows of kinetosomes (Pl. I 1). Among them cirri having four kinetosomal rows can be found. The localization of these cirri seems to be random similarly as in *P. hymenophora* (Grimes and L'Hernault 1978). In the posterior portion of the cell there are cirri consisting of two rows of kinetosomes (Pl. I 2). The number of kineties (longitudinal rows of kinetosomes) in subsequent cirri decreases posteriorly from seven to five. In the anterior portion of size reduced cells there are cirri composed of three rows of kinetosomes (Pl. I 3). Cirri located in the posterior part of cell are built up by two transverse kinetosomal rows (Pl. I 4). The number of kinetosomes in transverse rows decreases posteriorly from five to three. In tiny cells the posterior cirri can be composed of two transverse rows with only two kinetosomes in each of them.

Analysis of the Regulation of Left Marginal Cirri. The number of AM, LMC and the total number of transverse kinetosomal rows composing the left marginal cirri were scored in 48 cells of different size. The results are listed in Table 1. The cells are grouped according to the number of AM they possess. The number of adoral membranelles is highly correlated with the cell length. The AM

Table 1

The number of adoral membranelles, left marginal cirri and the total number of transverse rows of kinetosomes in left marginal cirri and the disposition of transverse kinetosomal rows among the left marginal cirri in cells of different size

Group of cells	Number of AM	Number of LMC	Disposition of kinetosomal rows in LMC			Total number of KsR	Number of cells
			4-rows	3-rows	2-rows		
Normal	50—61 (56)	36—49 (41)	0—3 (1)	2—37 (24)	1—47 (17)	94—121 (112)	11
Medium	30—49 (37)	28—42 (33)	0—2 (0.1)	1—27 (13)	2—35 (19)	64—99 (80)	24
Small	19—23 (26)	23—34 (29)	— —	0—5 (1.3)	19—34 (28)	50—68 (60)	13

The mean numbers are given in parentheses.

number being independent of physiological state of cells and conditions of fixation reflects well enough the degree of cell size reduction. The results presented in Table 1 indicate that decreasing of cell dimensions, leading to the formation of smaller number of adoral membranelles is connected with the reduction of the number of LMC as well as the total number of transverse kinetosomal rows in LMC. Cells of normal size possess less marginal cirri than AM. Cells of medium size have nearly equal number of both ciliary organelles. In tiny cells the number of LMC is larger than AM. The ratio between the number of AM and the total number of transverse kinetosomal rows in LMC is about 1 : 2 for all cells regardless their size. There are only few cirri composed of four kinetosomal rows even in cells of normal size. The cells of reduced size do not possess cirri having four transverse rows of kinetosomes. In normal cells the number of cirri composed of three transverse rows of kinetosomes changes in a wide range. This range becomes the narrower, the smaller cells are analysed. The minimal number of cirri having two transverse kinetosomal rows increases together with cell size reduction. The range of changes of the number of two-row cirri narrows when the size of cell decreases.

Three aspects of LMC regulation were analysed:

(1) Influence of the cell size alterations on the quantitative relation between the two groups of serially repeated structures — adoral membranelles and left marginal cirri,

(2) The relation between the total number of kinetosomal rows in left marginal cirri and the number of these cirri,

(3) Regarding the constant number of transverse kinetosomal rows

in each AM and the changes of this number in left marginal cirri, the relation between the number of AM and the total number of kinetosomal rows in LMC were analysed.

ad 1. The relation between the number of AM and LMC. Reduction of cell size causes decrease of the number of adoral membranelles and left marginal cirri. The statistical analysis showed that the number of LMC is highly correlated with the number of AM ($r = 0.84$). The linear character of this relation is illustrated in the Fig. 3. The value of the regression coefficient b indicates that the reduction of the number of AM by one is connected with disappearance of 0.4 cirrus. The tests for proportionality showed that the ratio between the number of AM and LMC depends on the cell size. The number of adoral membranelles is reduced faster that the number of marginal cirri. Thus, small cells possess relatively more LMC than AM. It means, that not only the number of different serially repeated structures is characteristic for a given cell size, but also the ratio between them.

ad 2. The relation between the total number of transverse kinetosomal rows in left marginal cirri and the number of LMC. The number of LMC as well as the total number of kinetosomal rows composing them decreases together with the cell size (Table 1). The statistic analysis showed that the number of AM is highly correlated with the number of kinetosomal rows in left marginal cirri ($r = 0.84$). The value of correlation coefficient is similar to the one calculated for the relation between the number of adoral membranelles and LMC. The Fig. 4 illustrates the linear relationship between the numbers of analysed structures. Both tests for proportionality revealed that the ratio between the number of LMC and the total number of kinetosomal rows forming LMC is size invariant. The results of statistical analysis indicate that the number of kinetosomal rows in LMC is reduced more readily than LMC. The number of kinetosomal rows in a given cirrus depends both on the total number of transverse kinetosomal rows in LMC and the position of that cirrus within the cell.

ad. 3. The relation between the total number of transverse kinetosomal rows in left marginal cirri and the number of adoral membranelles. The total number of kinetosomal rows in LMC is strongly related to the number of AM ($r = 0.99$). The linear character of this relation is illustrated on Fig. 5. Both tests for proportionality showed that the ratio between analysed pair of data depends on the cell size. The value of regression coefficient b indicates that the reduction of the number of AM by one is equal to disappearance of 1.6 transvers kinetosomal row in LMC. Thus, the number of kinetosomal rows in LMC decreases faster than the number of AM. The number of adoral mem-

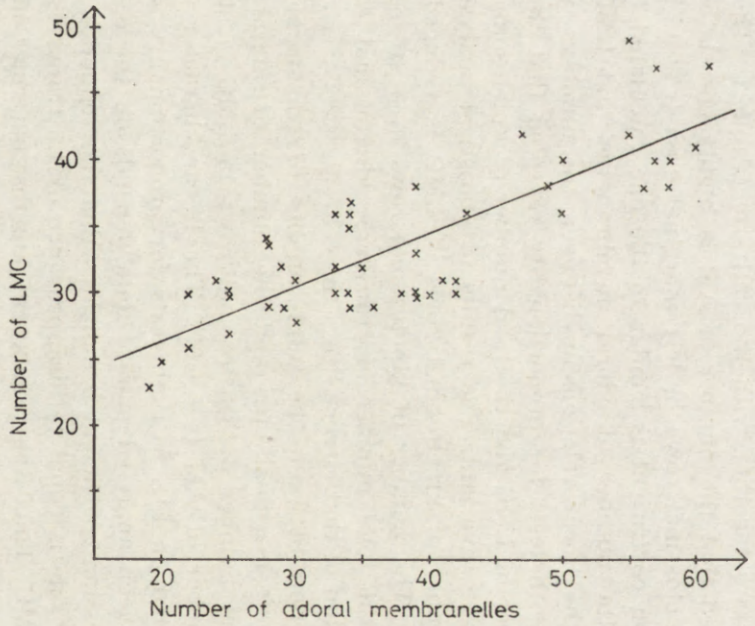

Fig. 3. The relation between the number of LMC and AM. The regression line is described by the equation: LMC = 18.332+0.40352AM

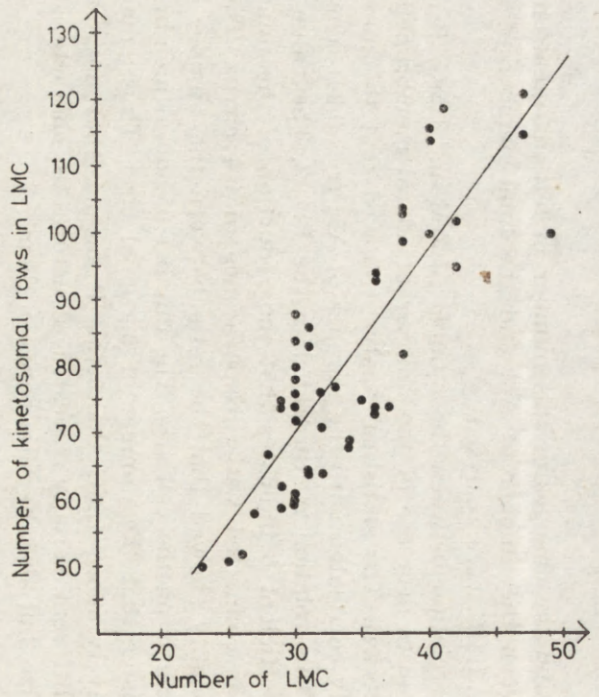

Fig. 4. The relation between the total number of kinetosomal rows in LMC and the number of LMC. The line fitted by regression analysis is described by the equation: KsR = −12.928+2.7661LMC

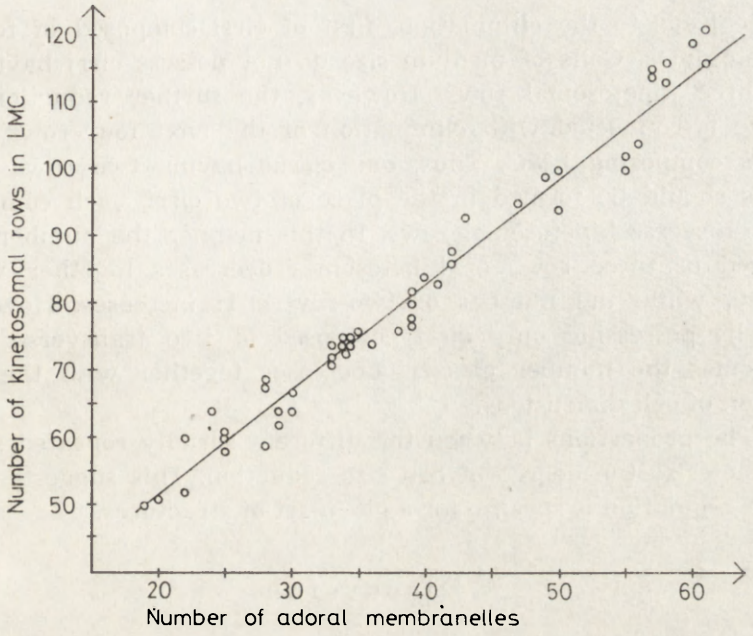

Fig. 5. The relation between the total number of kinetosomal rows in LMC and the number of AM. The regression line is described by the equation: KsR = = 19.354+1.597AM

branelles can be taken as a measure of the length of AM primordium, while the total number of transverse rows of kinetosomes in LMC is equal to the length of LMC primordium. Therefore, it can be concluded that the length of primordia is regulated according to the cell size. Regulation of different primordia do not preserve the constant proportions between them. Thus, the ratio between the length of two different primordia is characteristic for a given cell size.

Summarizing the results presented above the following conclusion can be drawn: (1) The relation between the total number of transverse kinetosomal rows in LMC and the number of AM is stronger than in the case of the relation connecting the total number of kinetosomal rows in LMC with the number of LMC. This suggests the stronger control of the length of LMC primordium than the number of LMC eventually differentiating from this primordium. (2) Regulation of the number of left marginal cirri leads to the changes of pattern of kinetosomal rows distribution among cirri which depend on the degree of cell size reduction. On the basis of results of statistical analysis it can be said that the reduction of the AM number by 2.5 membranelle is connected with disappearance of one cirrus, composed of four transverse kinetosomal rows. That means, the reduction of the

cell size leads to the elimination, first of cirri composed of four rows of kinetosomes. Cells of medium size do not possess cirri having more than three kinetosomal rows. However, the further reduction of the cell size is connected with elimination of the next four rows of kinetosomes composing LMC. Thus, one cirrus having two rows of kinetosomes should be formed in the place of two cirri, each composed of three transverse kinetosomal rows. In this manner the number of cirri composed of three rows of kinetosomes decreases together with the cell size, while the number of two-row cirri increases. However, in tiny cells possessing only cirri composed of two transverse rows of kinetosomes the number of cirri decreases together with the further reduction of cell dimensions.

(3) The proportions between the different serially repeated structures characterize the degree of cell size reduction. This suggests that the mode of regulation is specific for a given set of structures.

Discussion

Regulative development in a classical embryological meaning is a process leading to the formation of normally ordered proportional body pattern, regardless the disturbances in early stages of morphogenesis. Regulative embryos despite of reduced size develop species-specific number of serially repeated structures. As a consequence of this phenomenon structures of a reduced size are formed. The regulative development can be exemplified by segmentation of arthropod embryo (Hearth and Sander 1973, Sander 1975), or by mesoderm segmentation occurring in vertebrate somitogenesis (Cooke 1975 a, b, 1979 a, b, Flint et al., 1978, Pearson and Elsdale 1979, Elsdale and Pearson 1979). Among one-celled organisms, ciliates possessing high regeneration ability (Jerka-Dziadosz and Golińska 1977) reform the pattern of cortical structures after alteration of the cell size. The regulation of ciliary pattern of *P. weissei* can be achieved only by formation of the new set of structures adjusted to the changed cell dimensions and the resorption of the old set of organelle (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz 1974, 1976, 1977). Thus the possibility of regulation of cortical pattern is restricted in time and can take place only during morphogenesis.

The study on the regulation of ciliary pattern of *P. weissei* (Jerka-Dziadosz 1976, 1977) showed that the number of serially repeated structures was correlated with the cell length. The morphometric analysis of regulation of oral ciliature (Bąkowska and Jer-

ka-Dziadosz 1980) revealed that the reduction of cell size did not bring about elimination of pattern elements existing as a single structure, e.g., outer and inner preoral membranelles. The number of serially repeated structures (frontal and ventral membranelles) were proportionally reduced. The size of each analysed ciliary structures decreased together with the cell dimensions. However, the four transverse kinetosomal rows in each adoral membranelle were preserved regardless the degree of cell size reduction.

The results of the present analysis showed that the number of left marginal cirri similarly as the number of frontal and ventral membranelles decreased together with the cell size. The number of transverse kinetosomal rows composing individual cirri depended also on the cell dimensions. The preservation of four transverse rows of kinetosomes in adoral membranelles and possibility of changing the number of these rows in left marginal cirri explains why the number of cirri is reduced in lower rate than the adoral membranelles.

Generation of ciliary pattern occurs as a series of morphogenetic events such as proliferation of new kinetosomes, their ordering and distribution among differentiating structures (Tartar 1967, Grain and Kaczanowska 1977). In *P. weissei* during the first visible stage of morphogenesis, new kinetosomes arises and quickly form pairs. This process of proliferation ended by kinetosomal pair formation has been called the first round of proliferation. The further differentiation of the kinetosomal pairs depends on the developmental fate of primordium. Kinetosomes develop various primordium-specific accessory structures as microfilamentous and microtubular fibers. The formation of kinetosomal derivatives determines the possibility of ordering of kinetosomal pairs. The final orientation of kinetosomes is gradually established. In close vicinity of already ordered kinetosomal pairs, new kinetosomes are formed. New kinetosome addition to the preexisting paired kinetosomes is called — the second round of proliferation. The orientation of pairs delimits the direction of new kinetosomes addition. Except one (the primordium of preoral membranelles) all ciliary primordia of *P. weissei* undergo a process of segmentation. The stage of morphogenesis in which the segmentation occurs is primordium specific. It can occur before the second round of proliferation is initiated (AM primordium) or during the second round of proliferation (LMC primordium) (Jerka-Dziadosz 1980 a, b).

The results presented here as well as described in previous paper (Bakowska and Jerka-Dziadosz 1980) indicate the existance of a strong correlation between the intensity of both rounds of proliferation. The extent of the first round of proliferation is directly related

to the number of repeated structures. Both the number of adoral membranelles and left marginal cirri decreases with the cell size. The intensity of the first proliferation influences also the length of structures. The intensity of the second round of proliferation determines the number of kineties in each structure i.e., the structure width. The correlation of both rounds of proliferation conditions the formation of proportional structures with characteristic architectural regularities.

The question arises which of the morphogenetic events of ciliary pattern formation are of regulative character. No quantitative differences in ultrastructure of oral ciliature between normal and size reduced cells were found (Bąkowska and Jerka-Dziadosz 1980). The architecture of left marginal cirri is also similar in both groups of analysed cells. This indicates that the information required for formation, ordering and orientation of kinetosomes (Tucker 1977, Kaczanowska and Grain 1977, Sonneborn 1975, Jerka-Dziadosz 1980 a, b) is independent on the size of differentiating ciliary pattern. Thus, the mechanism controling formation of basic elements of pattern is of nonregulative character and can be explained by propagation of conformational changes (Roth et al. 1970, Roth and Pihlaja 1977). The information of the kind of "nearest neighbour interactions" can be transmitted from the one to the next cortical zone. In this way the information can spreed over considerable intracellular areas (Sonneborn 1975). However, the mechanisms of regulative control should be superimposed upon local, nonregulative processes to generate the pattern quantitatively adjusted to the size of the whole.

The number of serially repeated structures of *P. weissei* is regulated according to the cell size. The stage of morphogenesis in which the segmentation occurs is constant and specific for a given primordium. The AM primordium segmentates immediately after the first round of proliferation. The reflection of this is the ordering of kinetosomal pairs into rows perpendicular to the antero-posterior cell axis (Jerka-Dziadosz 1980 b). The direction of new kinetosome addition (the second round of proliferation) is determined by the differentiation of kinetosomes in pairs. The second round of proliferation is restricted to the anterior margin of each double row. This leads to the separation of successive paired rows. Therefore, after the final ordering of kinetosomal pairs each adoral membranelle differentiates as a separate structure. The number of kinetosomal rows added to the first two is constant in the case of adoral membranelles. Thus, the spatial repetition of this morphogenetical event does not change together with the cell size. The reduction of the AM number concomitant with the reduction of the cell size indicates that only the length of primordium

through which the process of segmentation passes, is regulated. Therefore, the segmentation of AM primordium is of non-regulative character ensuring the constant size of "cut off" units regardless the cell size. The changes of the length of the two posterior kinetosomal rows of adoral membranelles depending on the cell dimensions indicate that the primordium width also undergoes regulation.

Kinetosomal pairs in LMC primordium are set in order, in a different manner than it is in the case of AM primordium. Paired rows of kinetosomes are formed paralell to the antero-posterior axis of a cell. New kinetosomes are added along the left margin of the paired row. Thus, the second round of proliferation does not lead to the separation of kinetosomal rows. The primordium segmentation begins during the second round of proliferation and not before its initiation as it is in the case of AM primordium. The number of "cut off" segments is proportionally related to the primordium length, adjusted to the cell size and the number of kinetosomes in each unit decreases together with their number. This indicates the regulative character of segmentation of LMC primordium.

The data listed in Table 1 indicate that the similar number of rows of kinetosomes can be disposed among left marginal cirri in a quite variable manner. However, the high values of correlation coefficients calculated for analysed pairs of data suggest that there exists a strong tendency for disposition of the similar number of kinetosomal rows according to one pattern. This indicates that the extreme patterns arise sporadically and they are consequence of "errors" in primordium segmentation, due to unknown factors. They lead to the formation of too numerous three-rows cirri or two-rows cirri as well as to "cutting off", too large segments i.e., cirri composed of four transverse kinetosomal rows. In tiny cells the cirri formed by four kinetosomal rows do not appear. Additionally, the range of variations of the number of cirri composed of three rows, as well of two rows of kinetosomes changes in narrower range. This suggests that the reduction of cell size is connected with the limitation of "errors" in segmentation of primordium. Thus, the pattern of distribution of kinetosomal rows among cirri becomes more stable.

The data concerning the relation between the number of adoral membranelles and the total number of transverse kinetosomal rows in LMC indicate that cell counts with great accuracy the number of kinetosomes disposed paralelly to its antero-posterior axis. That means, cell monitors the length of its primordia in a precise way, while the

number of structures formed from cirrial primordium seems to be not so important and is less strictly controlled.

The question may be raised concerning the source of observed differences in regulation of primordia in *P. weissei*. It can be supposed that separate mechanisms are responsible for the different regulation of primordia. However, in such a case the additional assumption of superior mechanism coordinating the development of all primordia of a cell would be needed to explain the precise relation between the number of different serially repeated structures. The alternative possibility is that the same mechanism controls the size of developing primordia and structures differentiated from them. However, the same information can be "read off" differently by various cell zones leading to generation of local differences in development of structures and their regulation.

The spatial aspect of pattern development associates with a factor gradually distributed over cell surface. On the other hand, the temporal aspect of the pattern formation suggests the passing of a wave-front of some factors through the developing system. However, the nature of regulating mechanism(s) operating in cortical layer of *P. weissei* responsible for adjustement of ciliary pattern to the cell size remains obscure.

Summarizing the above considerations it can be said that the dimensions of all primordia being the zones of one morphogenetic field (Grimes and Adler 1978) are regulated according to the cell dimensions. In this way the extent of both rounds of proliferation can be determined. The occurrence or the lack of segmentation as well as the character of this process is primordium specific. It seems that it is the factor responsible for kinety orientation, which defines the character of segmentation but certainly influences neither the occurrence nor the lack of this process and its antero-posterior direction. The observed differences in regulation of adoral membranelles and preoral membranelles as well as left marginal cirri could result from separate regulative mechanisms. However, it seems more probable that the local differences modify the answer of a given cortical zone to the same common regulative factor.

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RÉSUMÉ

L'analyse morphométrique des individus normaux de *Paraurostyla weissei* et ceux à volume réduit montre que la régulation du developpement des cirres marginaux de gauche (LMC) dépend des dimensions de la céllule. Le nombre de rangs des cinétosomes dans le LMC (KsR) décroît dans la direction postérieure: il tombe de 4 à 2 chez les cellules normales, et de 3 à 2 chez les céllules réduites. Le quotient KsR/LMC ne change pas avec les dimensions. La diminution de la céllule est liée à la disparition des cirres à 4 KsR d'abord, et ensuite de ceux à 3 KsR. La réduction du nombre de membranelles adorales (AM) est plus forte que celle du LMC. Les quotients AM/LMC et AM/KsR ainsi que l'architecture interne des cirres sont caractéristiquement liés aux dimensions actuelles de la céllule.

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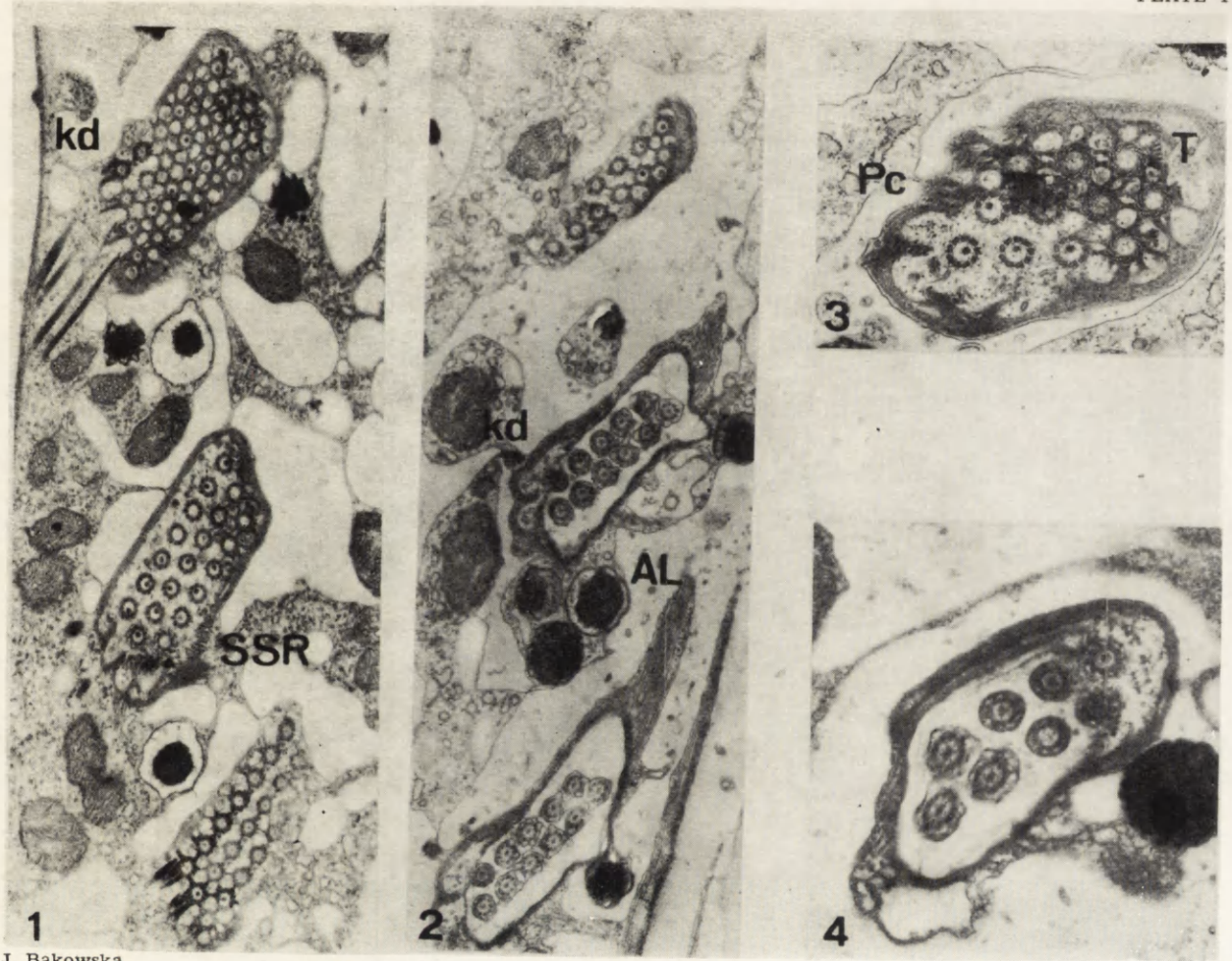
EXPLANATION OF PLATE I

The left marginal cirri of normal and size reduced cells. AL — anterior longitudinal fiber, kd — kinetodesmal fiber, Pc — postciliary microtubules, SSR — small subectoplasmic rootlet

1: The left marginal cirri of the anterior part of a cell of normal size, fixed 15 min after division. 10560 ×
2: The left marginal cirri of the posterior part of a cell of normal size fixed 4–5 h after division. 11040 ×
3: The left marginal cirrus of the anterior part of size-reduced cell fixed 5 h after operation. 19200 ×
4: The left marginal cirrus of the posterior part of size-reduced cell fixed after several reorganization. 19296 ×



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Особенности организации ядерного аппарата инфузорий *Paramecium bursaria*, зараженных симбиотическими бактериями *Holospora acuminata*

Some Pecularities in Organization of the Nuclear Apparatus in a Ciliate, *Paramecium bursaria*, Infected with Symbiotic Bacteria, *Holospora acuminata*

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Синопсис. Проведено электронномикроскопическое изучение ядер „чистых" и инфицированных клеток инфузорий *P. bursaria*. Симбиотические бактерии *Holospora acuminata* локализуются только в микронуклеусе (МИ). Присутствие нескольких сот бактерий в МИ не сказывается на ультраструктуре макронуклеуса (МА). Морфология и структура зараженных МИ характеризуется целым рядом существенных изменений. В десятки раз увеличивается объем ядра. Хроматиновые тяжи агрегируют в крупные компактные тела, однако в кариоплазме выявляется тонкофибриллярная сеть, представляющая собой, вероятно, деконденсированные районы хромосом. В кариоплазме интерфазных МИ сохраняются элементы ахроматинового аппарата, в норме присутствующие только в период митоза. В интерфазном МИ обнаружено белковое тело — центр образования микротрубочек; в кариоплазме под ядерной оболочкой выявляются мультимембранные структуры. Ядерная оболочка зараженных МИ образует систему выростов и инвагинаций, в том числе и развитый комплекс внутриядерных каналов; на внешней поверхности наружной мембраны располагаются рибосомы; частота пор такая же, как и в оболочке МА. Отмеченные особенности организации зараженных МИ указывают на наличие их высокой функциональной активности в отличие от „чистых" МИ. Учитывая ранее полученные нами данные по *Holospora undulata* из МИ *Paramecium caudatum* (Ossipov et al. 1976, Podlipaev and Ossipov 1979), проведен сравнительный анализ специфических преобразований ядерных структур в симбиотической системе.

Возможность использования „симбиотического" метода при анализе структуры и функций ядерного аппарата инфузорий была нами ранее обоснована (Ossipov et al. 1976, Ossipov 1978). Перспективность предложенного метода

определяется его следующими биологическими особенностями. Во-первых, клетки, содержащие в своих ядрах бактерий, не теряют способности к репродукции, в результате чего можно получать клеточные линии, в которых симбионты поддерживаются стабильно. Во-вторых, для всех изученных внутриядерных симбионтов инфузорий характерна строгая специфичность их локализации в определенных компартментах гетероморфного ядерного аппарата (Ossipov et al. 1976, 1980, Harrison et al. 1976 a, b, Gromov 1978, Estève 1978). В-третьих, присутствие симбиотических бактерий так или иначе сказывается на структуре и функции зараженных ядер. Логично предположить, что симбионты являются своего рода регуляторами генной активности и тем самым могут служить своеобразными инструментами для анализа функций ядер. Совершенно очевидно, что эффективность предложенного метода во многом определяется разнообразием симбионтов, используемых в такого рода исследованиях.

Настоящая работа посвящена сравнительному изучению морфологии и ультраструктуры ядерного аппарата „чистых" клеток *Paramecium bursaria* и парамеций, зараженных симбиотическими бактериями *Holospora acuminata* (Ossipov et al. 1980). Ранее нами было установлено, что *H. acuminata* поддерживается только в микронуклеусах (МИ) парамеций. Результаты светооптических и электронномикроскопических наблюдений самих симбионтов приведены в нашем предыдущем сообщении (Ossipov et al. 1980).

Материал и методика

Работа выполнена на зараженном клоне AC61-10 и на трех „чистых" клонах: AC56-1 AM21-1 и ДВ-25 *P. bursaria* (Ossipov et al. 1980). Приемы работы с культурами инфузорий, светооптические и электронномикроскопические методы описаны в предыдущих публикациях, и аналогичны ранее использованным для анализа внутриядерных симбионтов. Ультраструктура ядерного аппарата исследована на срезах инфузорий, фиксированных OsO_4 на какодилатном буфере (pH 7.4). Срезы изготавливались на ультрамикротоме LKB III, дополнительно их контрастировали уранилацетатом и цитратом свинца. Образцы просматривались на электронном микроскопе Hitachi HU-11 E при ускоряющем напряжении 75 kV.

Результаты и обсуждение

Проводилось сравнительное электронномикроскопическое изучение ядер „чистых" и инфицированных клеток (Pl. I 1–6). Морфология и ультраструктура ядерного аппарата „чистых" клонов *P. bursaria*, изученных нами, вполне соответствует имеющимся в литературе описаниям (Schwartz: 1976, 1978, Lewis et al. 1976). Контур ядерной оболочки макронуклеуса (МА) относительно ровный, хроматиновые элементы МА образуют крупносетчатую

структуру, состоящую из тяжей диаметром 140–150 nm (Pl. II 7, Pl. III 8). Нуклеолярный аппарат МА — агрегаты нуклеол 0.7–1.0 μm. Их размер, как полагают отмеченные авторы, зависит от стадии клеточного цикла и функционального состояния клетки. В кариоплазме МА наблюдается сеть тонкофибриллярных элементов, представляющих собой, вероятно, фибриллы деконденсированного хроматина и различные формы внутриядерных РНП. В цитоплазме в непосредственной близости от оболочки МА со стороны глотки лежат параллельные ряды микротрубочек. По-видимому, за счет этих структур обеспечивается фиксация положения МА относительно ротового аппарата клетки.

Микронуклеусы (МИ) „чистых" клеток на срезах имеют округлые очертания. Ядерная оболочка состоит из двух мембран, разделенных перинуклеарным пространством 25–30 nm (Pl. III 8, Pl.IV 9–11). В ядерной оболочке видны многочисленные поры диаметром около 60 nm. Конденсированный хроматин представлен тяжами диаметром около 60 nm, соединенными тонкой сетью фибриллярного материала в плотные агрегаты. В кариоплазме МИ видна тонкая сеть фибрилл диаметром около 11 nm (Pl. III 8). Как и в МИ других изученных видов, в МИ *P. bursaria* не обнаруживаются никакие элементы нуклеолярного материала. На одном из полюсов МИ выявляется „полярная шапочка", не содержащая хроматиновых структур, но заполненная короткими фрагментами микрофиламентов и деполимеризованных, хаотично расположенных микротрубочек. По-видимому, „полярная шапочка" МИ содержит скопления элементов остаточного веретена (Schwartz 1976, Raikov 1978).

Морфология ядерного аппарата клона AC61-10, инфицированного симбиотическими бактериями *H. acuminata*, характеризуется целым рядом отличительных особенностей. Обращает на себя внимание значительное увеличение размеров инфицированных МИ (Pl. I 2–6), они могут достигать 1/2–2/3 длины клетки, превышая, таким образом, МА по объему. Наиболее крупные МИ занимают большую часть цитоплазмы клетки, они имеют вытянутую, S-образную или подковообразную форму со слегка заостренными полюсами (Pl. I 3, 4). Небольшая часть клеток культуры содержит по два зараженных МИ. Инфицированные МИ обычно тесно прилегают к МА. В зараженном МИ бактерии лежат свободно в кариоплазме; на некоторых срезах их число достигает 200 (Pl. V 12).

Хроматиновые элементы МИ, которые представлены тяжами диаметром 60 nm, обычно образуют довольно плотные агрегаты (Pl. VI 13, Pl. VIII 14). Однако на многих срезах видно, что значительная часть кариоплазмы МИ лишена хроматиновых тяжей, на других — они вообще отсутствуют (Pl. V 12). В том случае, когда в клетке имеется два МИ, степень конденсации хроматиновых элементов может быть несколько различной (Pl. VII 14, Pl. VIII 16, Pl. IX 17). В кариоплазме выявляется сеть фибрилл (диаметром около 10–13 nm), которые, по-видимому, представляют собой деконденсированные участки хромосом. В зараженных МИ отсутствуют элементы нуклеолярного материала.

Характерной особенностью ультраструктуры зараженных МИ *P. bursaria* является то, что в интерфазе здесь сохраняются многочисленные ахроматиновые элементы. Одиночные или, реже, сдвоенные микротрубочки и микрофиламенты пронизывают во всех направлениях плотные агрегаты хроматиновых тяжей (Pl. VIII 16, Pl. IX 17, Pl. X 18). В ахроматиновых участках кариоплазмы и особенно в зоне, прилегающей к ядерной оболочке, располагаются пучки микротрубочек диаметром 24 nm (Pl. X 18, Pl. XI 19). Некоторые из них довольно тесно прилегают к оболочкам бактерий.

В нескольких зараженных интерфазных МИ были обнаружены структуры размером до 0.9 μm, характеризующиеся умеренной электронной плотностью и имеющие в некоторых плоскостях сечения форму усеченного округлого тела (Pl. VIII 15). На срезе ядра выявляется не более одного такого образования. Со стороны плоского края структуры отчетливо видны перпендикулярно отходящие короткие фрагменты микротрубочек длиной до 140 nm, диаметром около 19 nm. В дальнейшем они, вероятно, превращаются в микротрубочки диаметром 24 nm, разбросанные в большом числе в кариоплазме МИ. Полимеризующиеся микротрубочки лежат параллельными рядами. Остальная часть тела заполнена относительно аморфным веществом. Во всех случаях это образование находилось в кариоплазме под ядерной оболочкой МИ, но непосредственно не было с ней связано. Все сказанное позволяет считать, что описанная структура соответствует внутриядерному центру образования микротрубочек (ЦОМТ). Для некоторых видов инфузорий доказано, что подобное белковое тело в профазе митотического цикла МИ служит центром образования внутриядерных микротрубочек веретена (Hauser 1968, 1970, 1972, Karadzhan 1976, Raikov 1978). Ни наши, ни отмеченные выше литературные данные не отвечают на вопрос о существовании ЦОМТ в „чистых" МИ *P. bursaria*. Однако, учитывая относительно крупные размеры этой структуры в зараженном МИ *P. bursaria*, можно предположить, что присутствие симбиотических бактерий в ядре приводит к изменению типа биогенеза микротрубочек веретена.

Одна из наиболее интересных особенностей организации зараженных МИ заключается в том, что у них и в интерфазе выявляются кинетические элементы, описанные ранее для „чистых" МИ *P. bursaria* только в митозе на стадии профазы — анафазы (Lewis et al. 1976, Schwartz 1976) и отмеченные нами у чистых клонов (Pl. IV 9–11). На основании анализа ультраструктуры делящихся МИ этими авторами было высказано предположение о том, что у митотических хромосом *P. bursaria* отсутствуют типичные кинетохоры, но их функцию при анафазном движении хромосом выполняют совершенно необычные, сложно организованные комплексы, названные Шварцем киносомами (Fig.1). В незараженных МИ полярные микротрубочки, согласно данным Lewis et al. (1976) прямо не контактируют с конденсированными хромосомами, а присоединяются к микрофиламентам, которые, в свою очередь, прикрепляются

к воронкообразной структуре. Вся эта сложная система киносомы возникает в профазе; размер и пространственное отношение ее отдельных элементов закономерно изменяются на последовательных стадиях митоза. К телофазе киносомы исчезают, сохраняются только рассеянные по ядру одиночные микротрубочки и тонкая сеть микрофиламентов.

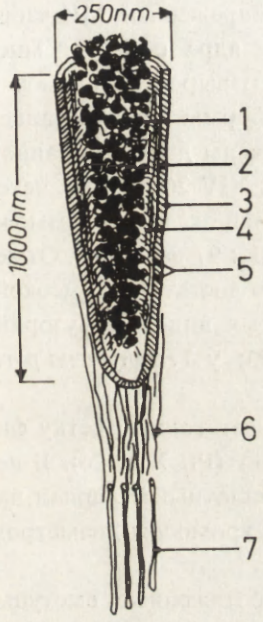

Рис. 1 Организация кинетических элементов „чистых" микронуклеусов *Paramecium bursaria* на стадии мета-анафазы (по данным Lewis et al., 1978). 1 — конденсированная хромосома, 2 — микроламелла, 3 — внешняя субламелла, 4 — внутренняя субламелла, 5 — микросепты (период около 15 nm), 6 — микрофиламенты, вероятно, содержащие актиноподобные белки, 7 — микротрубочки, направленные к полюсу ядра

Fig. 1. Schematic organization of kinetic elements in noninfected macronuclei of *Paramecium bursaria* during metaphase-anaphase (after Lewis et al. 1978). 1 — condensed chromosome, 2 — microlamella, 3 — outer sublamella, 4 — inner sublamella, 5 — microsepta (periodicity about 15 nm), 6 — microfilaments probably comprising aktine-like proteins, 7 — microtubules directed towards the micronuclear pole

Практически во всех просмотренных нами интерфазных зараженных МИ клона AC61-10 *P. bursaria* выявляются структуры, которые, несомненно, можно считать производными киносом (Pl. VI 13, Pl. VIII 16, Pl. IX 17, Pl. XII 20–22, Pl. XIII 23–25). Так, обнаруживаются воронковидные образования диаметром 230–300 nm, состоящие из двух слоев: ламеллярного и филаментозного (Pl. XII 20–22). Во внутреннем пространстве некоторых воронок выявляются хроматиновые тяжи. Вдоль, а иногда и внутри этих структур проходят одиночные микротрубочки диаметром 24 nm. На поперечных срезах видно, что некоторые воронки снаружи плотно окружены одним рядом продольно ориентированных микротрубочек (до 20–25 по периметру). На Pl. XIII 23 представлен продольный срез через несколько тесно прилегающих друг к другу воронковидных образований, организация которых в общем напоминает мета-анафазные киносомы (Fig. 1). В воронке четко выражены внутренняя субламелла, состоящая из сети тонко фибриллярного материала, имеющего период ячейки около 14 nm, и наружная — из одного слоя микрофиламентов диаметром около 14 nm (Pl. XIII 25). Вершины воронок ориентированы в направлении полюса ядра. Вдоль воронок лежат одиночные микротрубочки, параллельно им, между отмеченными структурами, располагаются палочковидные бактерии.

Ядерная оболочка зараженных МИ также имеет ряд весьма характерных особенностей организации, что может указывать на усиление метаболической активности этих ядер по сравнению с „чистыми" МИ. Следует отметить, что, несмотря на значительное увеличение объема зараженных МИ по сравнению с „чистыми" МИ, их ядерная оболочка на всем протяжении интактна (Pl. V 12, Pl. VI 13, Pl. VII 14). Она состоит из наружной и внутренней мембран и пронизана порами диаметром около 65 nm. Зараженные МИ часто неправильной формы вследствие того, что оболочка ядра образует многочисленные выросты (Pl. VII 14). Очертания и размер выростов оболочки различны: пальцевидные, округлые, почковидные и формы протуберанцев (Pl. XIV 26, 27, Pl. XV 28, Pl. XVI 29). Выросты окружены двухмембранной оболочкой и также содержат многочисленные поры (Pl. XIV 26, 27). На некоторых тангенциальных срезах выростов ядерной оболочки оказалось возможным подсчитать число пор на единицу поверхности МИ: 97 на 1 μm^2. Отмеченную частоту пор в зараженном МИ следует признать достаточно высокой, если сравнивать по этому показателю с МА некоторых видов инфузорий: у *Paramecium aurelia* — 60–80; у *Didinium nasutum* — 100; у *Tetrahymena pyriformis* — до 190 (Raikov 1978).

Кариоплазма в выростах ядерной оболочки МИ имеет тонкую сетку фибриллярного материала с диаметром фибрилл 11–13 nm (Pl. XIV 26). В некоторых выростах кроме того обнаруживается электронноплотный материал, напоминающий фибриллы несколько деконденсированных хромосом диаметром около 20 nm (Pl. XVI 29).

Наружная оболочка зараженного МИ образует протяженные выступы, которые внешне ничем не отличаются от цистерн ретикулума (Pl. XIV 27, Pl. XV 28). На наружной поверхности внешней мембраны оболочки МИ и ее многочисленных выростов располагаются рибосомы (диаметр около 20 nm); на тангенциальных срезах оболочки МИ видны полисомы (Pl. XVII 33). В околоядерной цитоплазме параллельно ядерной оболочке МИ лежат и в отдельных местах непосредственно контактируют с ядерной оболочкой цистерны шероховатого ретикулума. Таким образом, есть основание считать, что хотя бы часть эндоплазматического ретикулума, локализованного около зараженного МИ, происходит за счет выростов наружной мембраны его ядерной оболочки. Следует напомнить, что для „чистых" МИ *P. bursaria* и всех других исследованных к настоящему времени инфузорий не было получено никаких доказательств участия внешней мембраны ядра в биогенезе эндоплазматического ретикулума (Raikov 1978). Несомненно, что отмеченные выросты ядерной оболочки значительно увеличивают поверхность МИ, что может являться отражением интенсификации метаболических процессов в зараженном ядре (Raikov 1968, 1978).

Интересной особенностью ядерной оболочки зараженных МИ являются выросты внутренней ядерной оболочки, которые уходят вглубь ядра в виде

извитых каналов диаметром около 60 nm, окруженных одиночной мембраной (Pl. XVI 31, 32). Таким образом, кариоплазма зараженного МИ пронизана внутриядерными каналами.

Еще одной характерной чертой организации зараженного МИ является присутствие в кариоплазме многослойных мембранных образований, состоящих из закрученных уплощенных цистерн и извитых трубчатых отростков диаметром 30 nm (Pl. XVI 30). Эти необычные для ядер мембранные структуры располагаются непосредственно под оболочкой МИ. Следует отметить, что параллельно лежащие сдвоенные мембраны не содержат элементов, напоминающих ядерные поры и рибосомы. Другая особенность этих многослойных образований состоит в том, что их мембраны не имеют разрывов и лишены дополнительного, легко идентифицируемого электроннoплотного слоя. Этим они совершенно определенно отличаются от ранее отмеченных нами (Ossipov et al. 1980) спирально закрученных фрагментов мембранного материала, лежащего в кариоплазме зараженных МИ и имеющих, несомненно, бактериальное происхождение. Такие фрагменты наружной клеточной мембраны возникают в результате развития спор бактерий. Можно предположить, что возникновение многослойных мембранных структур связано либо с внутриядерным депонированием мембран, по-видимому, производных ядерной оболочки, либо с особым характером активности хромосом зараженного МИ. Первое предположение основано на том, что у инфицированных парамеций регуляция количества ядерных мембран должна иметь место в связи с резкими изменениями объема МИ в зависимости от количества симбиотических бактерий, присутствующих в ядре. Второе соображение основано на литературных данных по гаметогенезу у некоторых беспозвоночных, где на строго определенной стадии мейоза (образование синаптенимального комплекса) возникают необычные впячивания внутренней ядерной мембраны и из них образуются своеобразные мембранные завитки (Rasmussen 1976, Danilova and Shileiko 1978). Авторы связывают проявление такого рода активности ядерной мембраны с осуществлением одной из важных функций генетического аппарата — закономерным передвижением и сближением мейотических хромосом перед их гомологичным спариванием.

Ультраструктура МА зараженных клеток (Pl. XVII 34) не отличается от описанных выше структур соматического ядра „чистых" клонов. Следует отметить, что количество пор на единицу площади оболочки ядра по нашим измерениям составляет 88 на 1 μm^2.

Итак, представленный материал позволяет сделать вывод о том, что выявленные нами особенности структуры ядерного аппарата инфузорий *P. bursaria*, зараженных *H. acuminata*, проявляют много общих черт с изменениями характерными для ядер *P. caudatum*, инфицированных *H. undulata* (омега-частицами) — также симбиотическими бактериями МИ (Gromov et al. 1975, 1976, Ossipov et al. 1973, 1976, Podlipaev and Ossipov 1979). Во-первых, не отмечено

никаких существенных изменений в ультраструктуре МА зараженных клеток двух видов парамеций, по сравнению с МА тех же клонов „чистых" инфузорий. По-видимому, пребывание симбионтов в МИ не вызывает резкого изменения активности другого компонента ядерного аппарата — МА у инфицированных парамеций.

Во-вторых, у обоих видов парамеций имеет место увеличение объема зараженного МИ в десятки раз, однако при этом ядро не теряет способности к делению. Репродуктивная активность зараженных ядер тем более удивительна, если учесть, что в кариоплазме присутствуют сравнительно крупные инородные тела — симбиотические бактерии (0.5×6.0 μm), число которых достигает нескольких сотен на ядро. Способность инфицированных МИ к многократным делениям связана, по-видимому, с наблюдающимися компенсаторными изменениями их ахроматинового аппарата. По нашим данным, общими для кинетических элементов зараженных МИ у обоих видов парамеций можно считать следующие черты. В интерфазных МИ не происходит полной деполимеризации микротрубочек, и в кариоплазме сохраняются многочисленные их пучки, за счет чего происходит ориентировка симбиотических бактерий вдоль продольной оси ядра. Наличие относительной упорядоченности расположения бактерий в кариоплазме МИ можно рассматривать как фактор, способствующий осуществлению нормального цикла спирализации хромосом и распределению дочерних хромосом при митозе. У *P. bursaria* это, по-видимому, обеспечивается и сохранением элементов киносом в течение всего интерфазного цикла.

В-третьих, степень компактизации хроматиновых элементов в зараженных МИ изменяется по сравнению с нормой, хотя направления этого процесса у *P. bursaria* и *P. caudatum* противоположны. Напомним, что у *P. caudatum* было отмечено отсутствие гетерохроматизированных хромосом в интерфазных зараженных МИ — весь хроматин ядра находится в деспирализованном состоянии. Для *P. bursaria*, напротив, характерна гетерохроматизация и агрегация хроматиновых тяжей в плотные компактные образования, тогда как деспирализация затрагивает, по-видимому, только незначительную часть хромосомного материала.

В-четвертых, основные типы изменения компонентов ядерной оболочки зараженных МИ у обоих видов парамеций сходны и указывают на наличие относительно высокой функциональной активности этих ядер, что отличает их от неинфицированных МИ инфузорий (Borchsenius 1975, Borchsenius and Ossipov 1978, Fokin 1978, Raikov 1978). Общее увеличение поверхности зараженного МИ достигается не только за счет значительного увеличения объема ядра, но и благодаря разного рода выростам оболочки и ее внешней мембраны, в том числе и развитой системой внутриядерных каналов, свойственной как *P. caudatum* при заражении *H. undulata* (Podlipaev and Ossipov 1979), так и *P. bursaria* при заражении *H. acuminata*. Установлено участие внешней

мембраны зараженного МИ в процессе формирования шероховатого ретикулума. У *P. bursaria* на внешней поверхности ядерной оболочки МИ выявлено наличие рибосом и полисом, что никогда раньше не отмечалось для „чистых" МИ. Количество пор в ядерной оболочке на единицу поверхности МА и инфицированных МИ составляет, по нашим подсчетам, у *P. bursaria* соответственно 88 и 97 на 1 μm^2. Такое близкое значение частот пор в ядерной оболочке соматических ядер и инфицированных МИ, по-видимому, отражает существование высокой активности двустороннего транспорта макромолекулярных компонентов между зараженным МИ и цитоплазмой.

Таким образом, изучение ультраструктуры ядер, инфицированных симбиотическими бактериями, позволяет получить дополнительную информацию о структуре и функциях ядерного аппарата инфузорий. Проведенный сравнительный анализ специфических преобразований ядерных структур в результате инфекции у близких видов парамеций позволяет выявить общие черты процесса морфо-функциональной адаптации клеточных органелл и ультраструктур в симбиотической системе.

SUMMARY

Electron microscope investigation has been carried on the nuclei of noninfected cells of *Paramecium bursaria*. Symbiotic bacteria, *Holospora acuminata*, are localized exclusively in the micronuclei (MI). Presence of some hundreds of bacteria in MI does not affect the ultrastructure of MA. In the structure of infected MI a series of morphological changes has been observed. The capacity of MI enlarges ten times, chromatin threads aggregate forming compact bodies and a mesh-work of thin fibrilles, being probably a remnant of decondensed chromosomes, appears in the caryoplasma. In the caryoplasma of MI during interphase occur the elements of achromatic apparatus which in normal cells may be seen only during mitosis. An albuminous body — a centre of microtubules formation, has been found in MI during interphase as well as multimembranaceous structure, situated in caryoplasma under the nuclear envelope. The envelope of infected MI forms a system of processes and invaginations including a developed complex of inner canals. On the surface of outer membrane ribosomes are situated. Density of pores in the envelope of MI is the same as in MA. The above pecularities of organization of infected MI indicate their high functional activity in comparison with noninfected ones. On the base of present observations as well as of earlier data (Ossipov et al. 1976, Podlipaev and Ossipov 1979) a comparative analysis of specific transformation of nuclear structures in a symbiotic system is made.

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ПОДПИСИ К ТАБЛИЦАМ I-XVII

1-6. Светооптические микрофотографии "чистой" и зараженных клеток *Paramecium bursaria*
1: "Чистая" клетка клона AC56-1. Окраска по Фельгену. × 1100
2: Зараженная обесцвеченная клетка клона AC61-10. Реакция Фельгена. × 1100
3: Зараженная клетка клона AC61-10. Окраска азуром. × 1100
4: Зараженная обесцвеченная клетка клона AC61-10. Окраска азуром. × 1100
5: Выделенный из клетки зараженный микронуклеус. В кариоплазме видны селективно окрашенные симбиотические бактерии. Окраска азуром. × 2000
6: Участок клетки клона AC61-10 с зараженным микронуклеусом. Видны хроматиновые тяжи. Реакция Фельгена. × 1500

7–11. Электронограммы ядерного аппарата "чистых" клеток *Paramecium bursaria*
7: Участок клетки клона AM21-1. × 13 500
8: Микронуклеус и участок макронуклеуса клетки клона AM21-1. × 31 000
9: Участок микронуклеуса, находящегося на стадии метафазы митоза, клетки клона ДВ-25. × 53 000
10–11: Участки микронуклеусов, находящихся на стадии профазы митоза, клеток клона AC56-1. × 30 000

12–34. Электронограммы зараженных клеток клона AC61-10 *Paramecium bursaria*
12: Участок клетки. × 9500
13: Микронуклеус. × 20 000
14: Участок клетки, содержащей два микронуклеуса. × 6600
15: Центр образования микротрубочек в кариоплазме зараженного микронуклеуса. × 111 000
16, 17: Участки микронуклеусов. Фрагменты. 14, 16 — × 65 000. 17 — × 83 000
18, 19: Участки микронуклеусов. В кариоплазме видны многочисленные пучки микротрубочек. 18 — × 55 000. 19 — × 36 000
20–25: Элементы ахроматинового аппарата (киносомы, микротрубочки и микрофибриллы) в интерфазных микронуклеусах. 20 — × 88 000. 21 — × 58 000. 22 — × 88 000. 23–25 — × 69 000
26–29: Разные типы выростов ядерной оболочки микронуклеусов. 26 — × 42 000. 27 — × 37 000. 28 — × 71 000. 29 — × 55 000
30: Многослойное мембранное образование в кариоплазме микронуклеуса. × 55 000
31, 32: Каналы, образованные протяженными выростами внутренней мембраны оболочки микронуклеуса. 31 — × 28 000. 32 — × 53 000
33: Тангенциальный срез ядерной оболочки микронуклеуса. Стрелкой обозначены полисомы. × 55 000
34: Участок макронуклеуса зараженной клетки. × 17 000

Обозначения: cb — хроматиновые тела, cp — цитоплазма парамеций, fv — пищеварительная вакуоль, ks — киносома, ma — макронуклеус, mf — микрофибрилла, mi — микронуклеус, ml — микроламелла, nc — внутриядерный канал, ne — ядерная оболочка, nl — нуклеола макронуклеуса, np — ядерная пора, sb — симбиотическая бактерия *Holospora acuminata*.

EXPLANATIONS OF PLATES I–XVII

1–6. Light microscope photographs of noninfected and infected cells of *Paramecium bursaria*

1: Noninfected cell of AS56 - 1 clone. Feulgen reaction × 1100
2: Infected colourless cell of AS61 - 10 clone. Feulgen reaction. × 1100
3: Infected cell of AS61 - 10 clone. Azur stain. × 1100
4: Infected colourless cell of AS61 - 10 clone. Azur stain. × 1100
5: Isolated infected micronucleus. Selectively stained symbiotic bacteria are visible in caryoplasma. Azur stain. × 2000
6: Fragment of a cell of the clone AS61 - 10 with infected micronucleus. Chromatin threads are visible. Feulgen reaction. × 1500

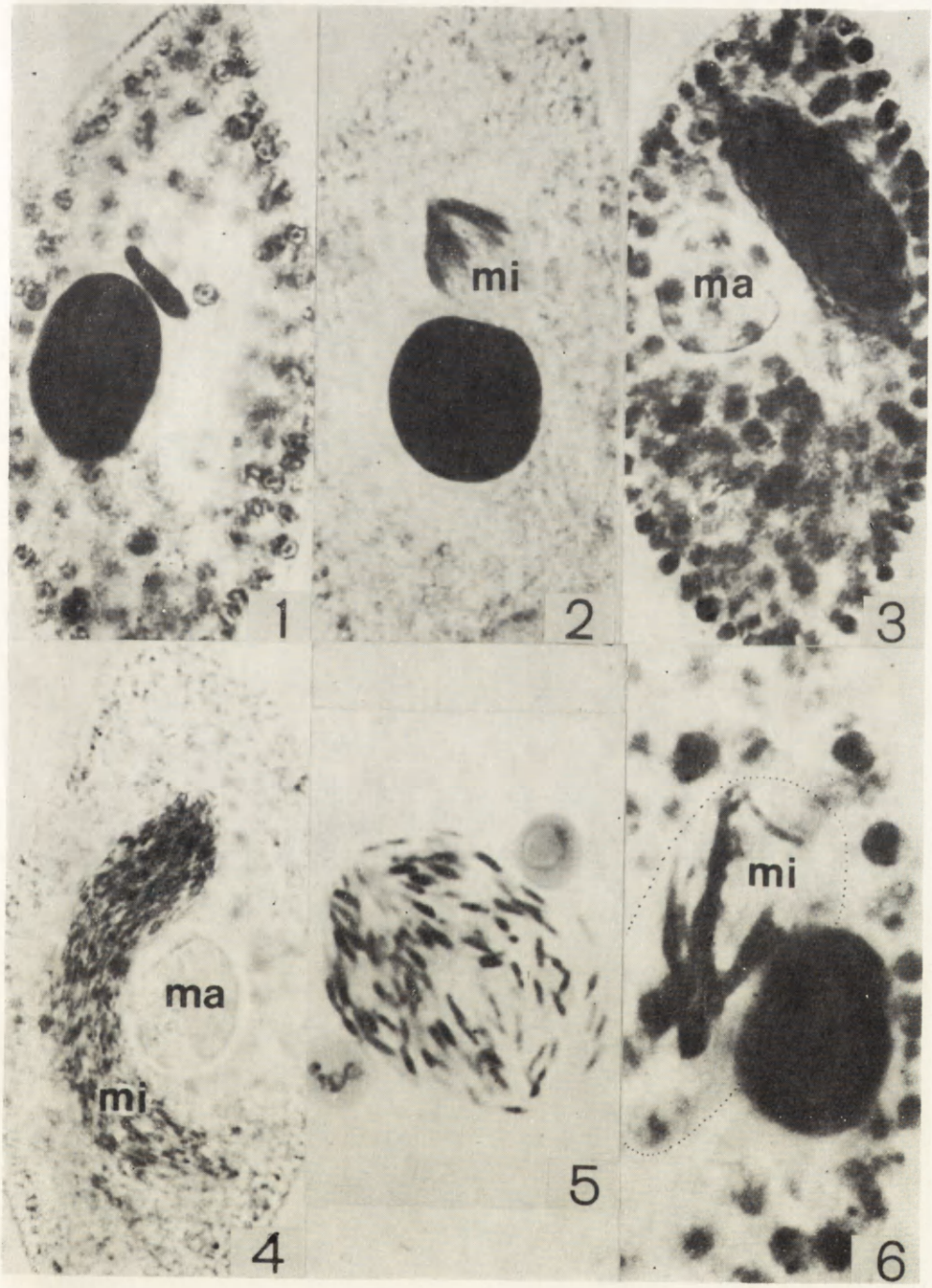
7–11. Electronograms of nuclear apparatus in noninfected cells of *Paramecium bursaria*

7: Fragment of a cell, AM21 - 1 clone. × 13 500
8: Micronucleus and a fragment of macronucleus in a cell of the clone AM21 - 1. × 31 000
9: Fragment of micronucleus during mitotic metaphase, DV = 25 clone. × 53 000
10, 11: Fragments of micronuclei during mitotic prophase, AS56 - 1 clone. × 30 000

12–34 Electronograms of infected cells of *Paramecium bursaria*, clone AS61 - 10

12: Fragment of a cell. × 9500
13: Micronucleus. × 20 000
14: Fragment of a cell containing 2 micronuclei. × 6600
15: Centre of microtubules formation in caryoplasma of infected micronucleus. × 111 000
16, 17: Fragments of micronuclei seen in Fig. 14 at greater magnification. 16 — × 65 000, 17 — × 83 000
18, 19: Fragments of micronuclei. Numerous bundles of microtubules are visible in caryoplasma. 18 — × 55 000, 19 — × 36 000
20–25: Elements of achromatic apparatus (kinetosomes, microtubules, and micronuclei during interphase. 20 — × 88 000, 21 — × 58 000, 22 — × 88 000, 23–25 — × 69 000
26–29: Various types of processes of the nuclear envelope of micronuclei. 26 — × 42 000, 27 — × 37 000, 28 — × 71 000, 29 × 55 000
30: Multilayered membranaceous structure in caryoplasma of a micronucleus. × 55 000
31, 32: Canals formed by elongate processes of the inner membrane of nuclear envelope of a micronucleus. 31 — × 28 000, 32 — × 53 000
33: Tangent section through the nuclear envelope of a micronucleus. Polisomes marked by an arrow. × 55 000
34: Fragment of macronucleus of infected cell. × 17 000

cb — chromatin bodies, cp — cytoplasma, fv — food vacuoles, ks — kinosome, ma — macronucleus, mf — microfibrilla, mi — micronucleus, ml — microlamella, nc — inner canal, ne — nuclear envelope, nl — nucleole of macronucleus, np — nuclear pore, sb — symbiotic bacteria.



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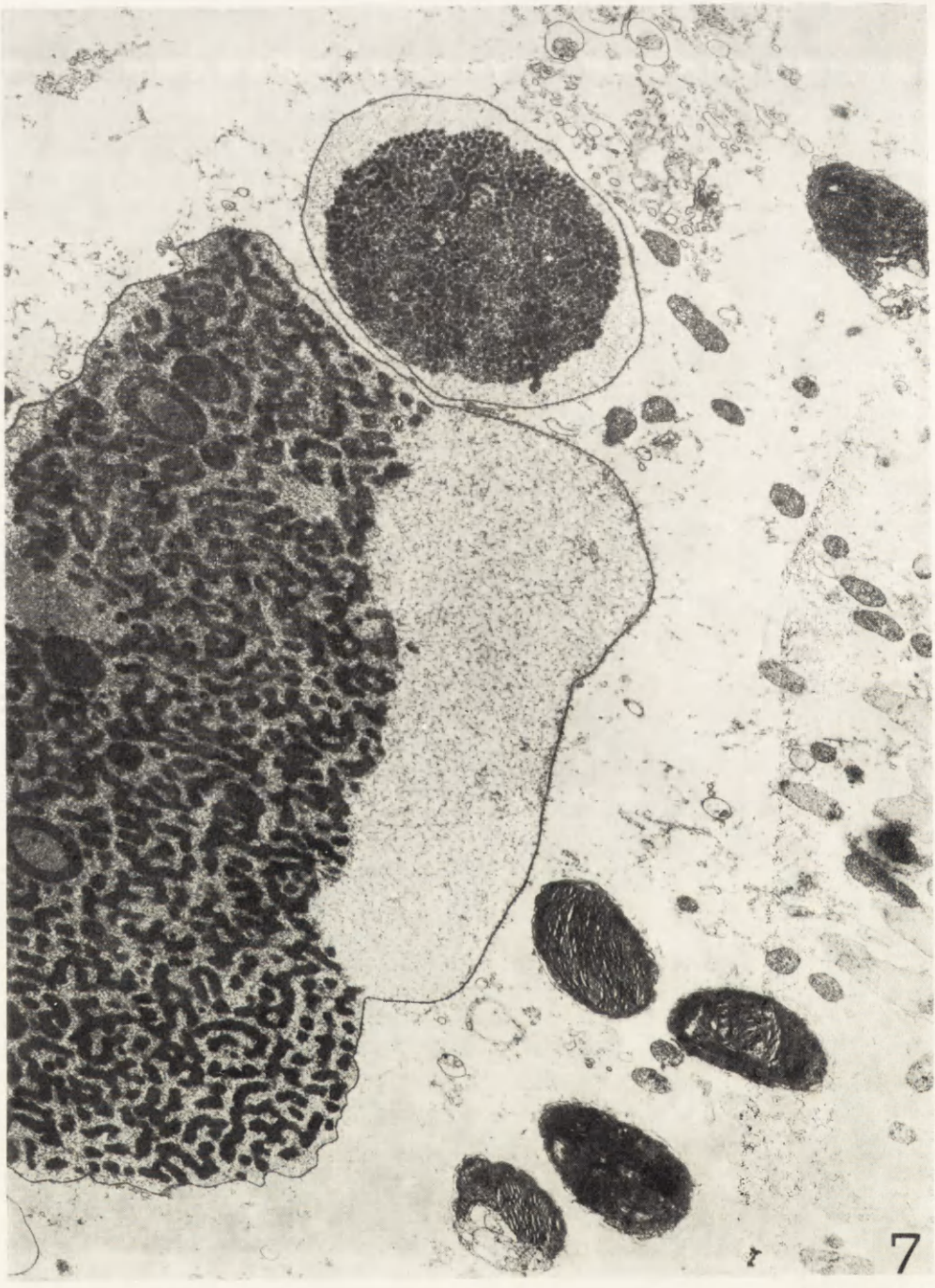

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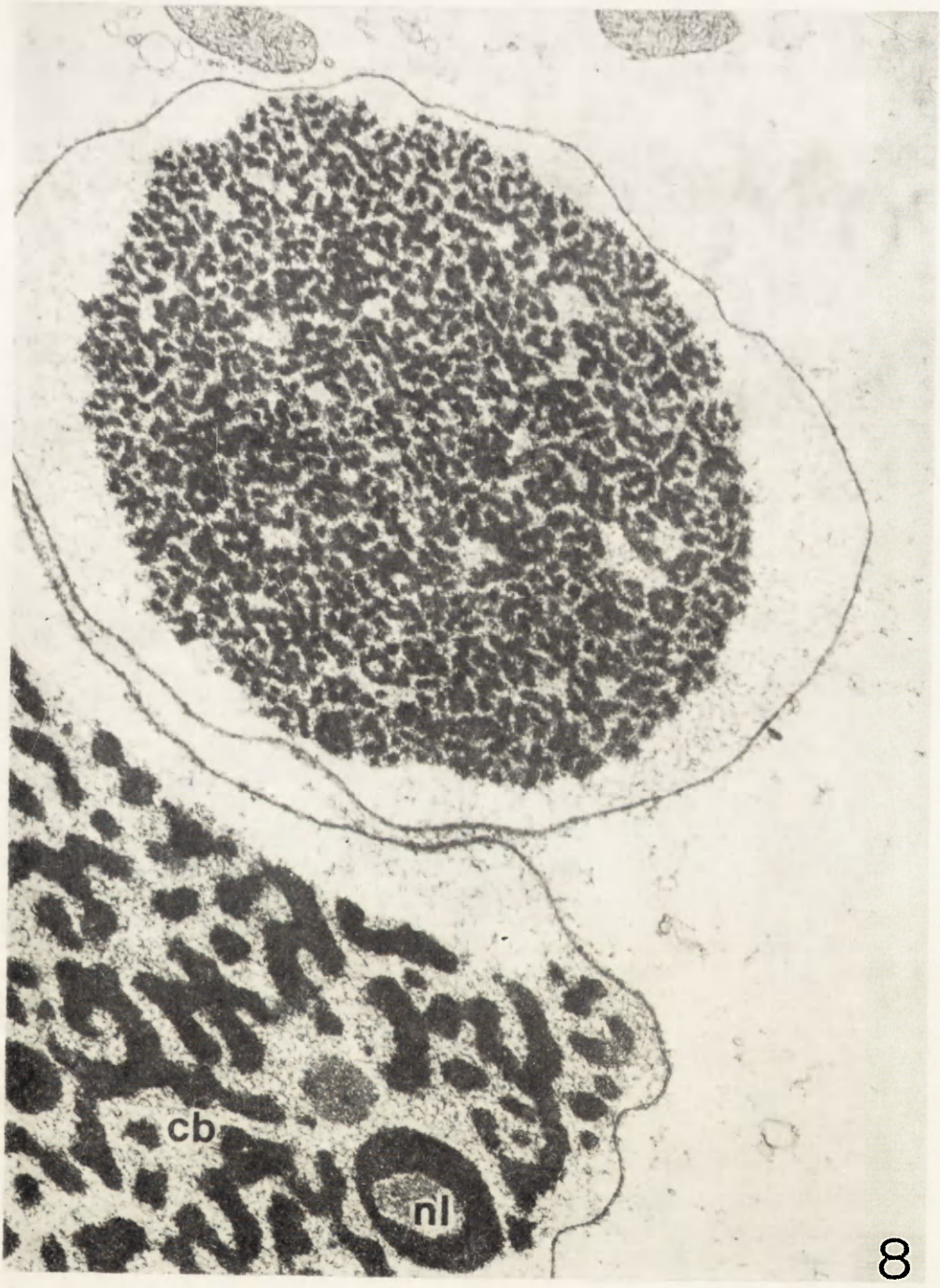

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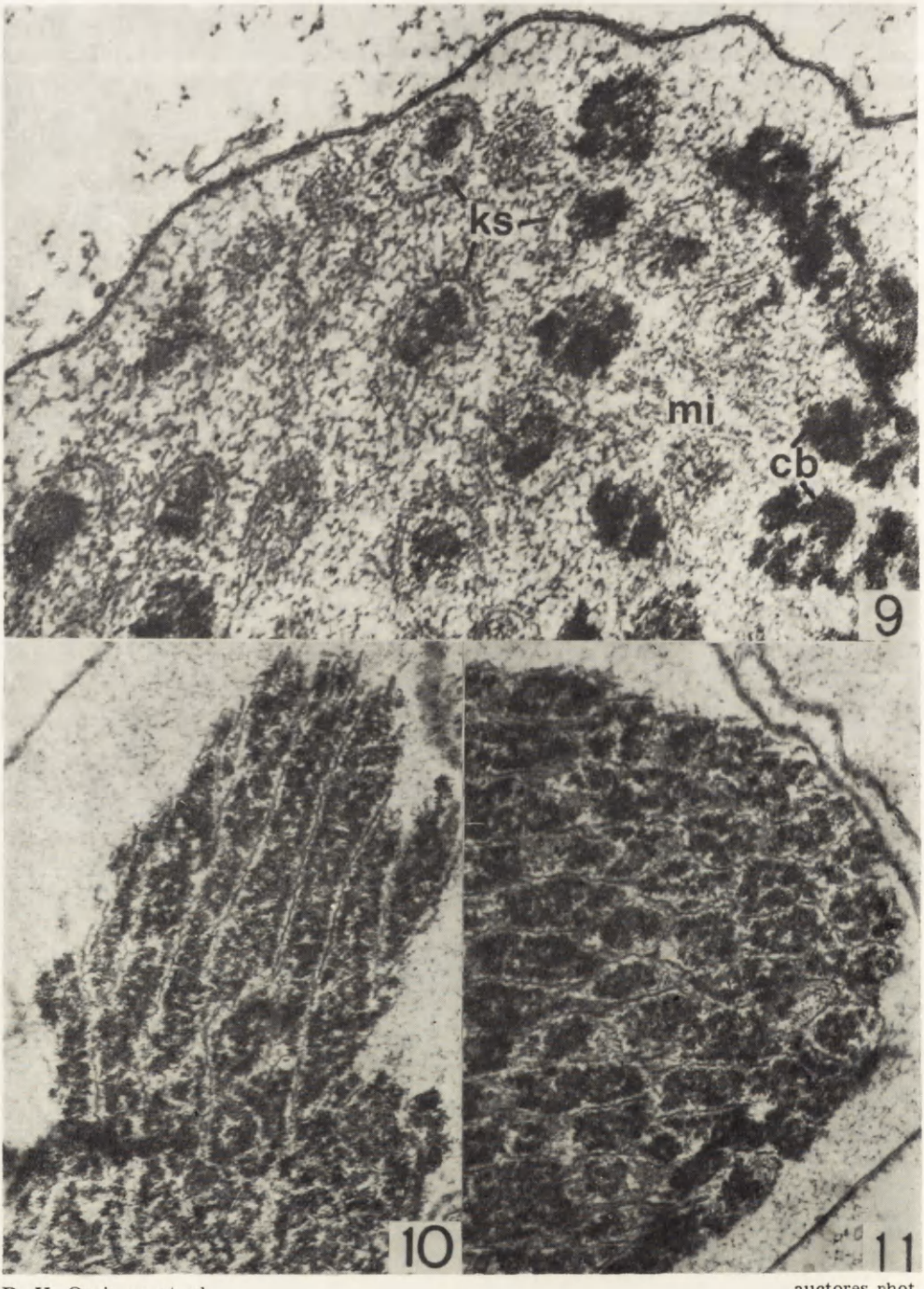

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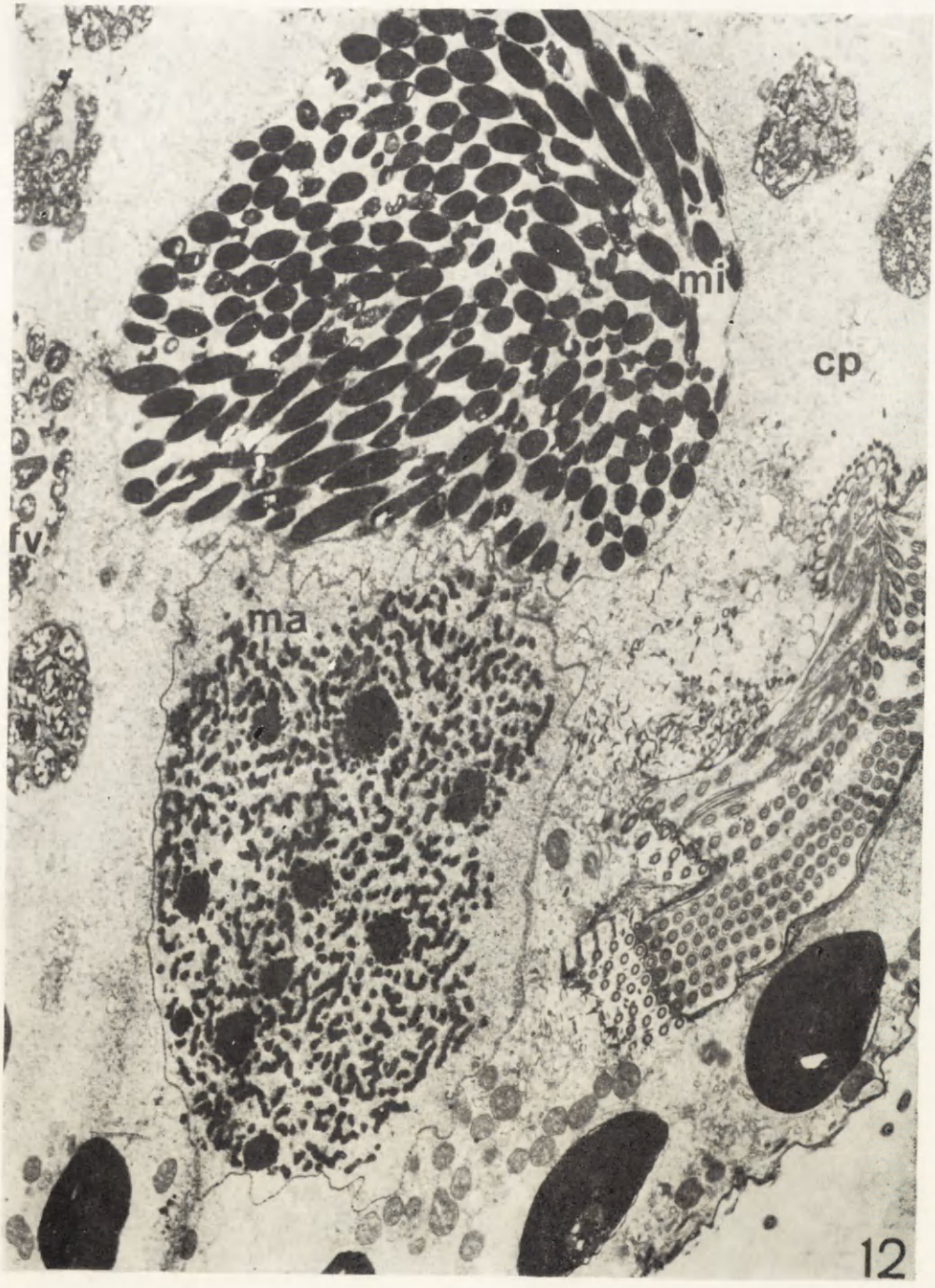

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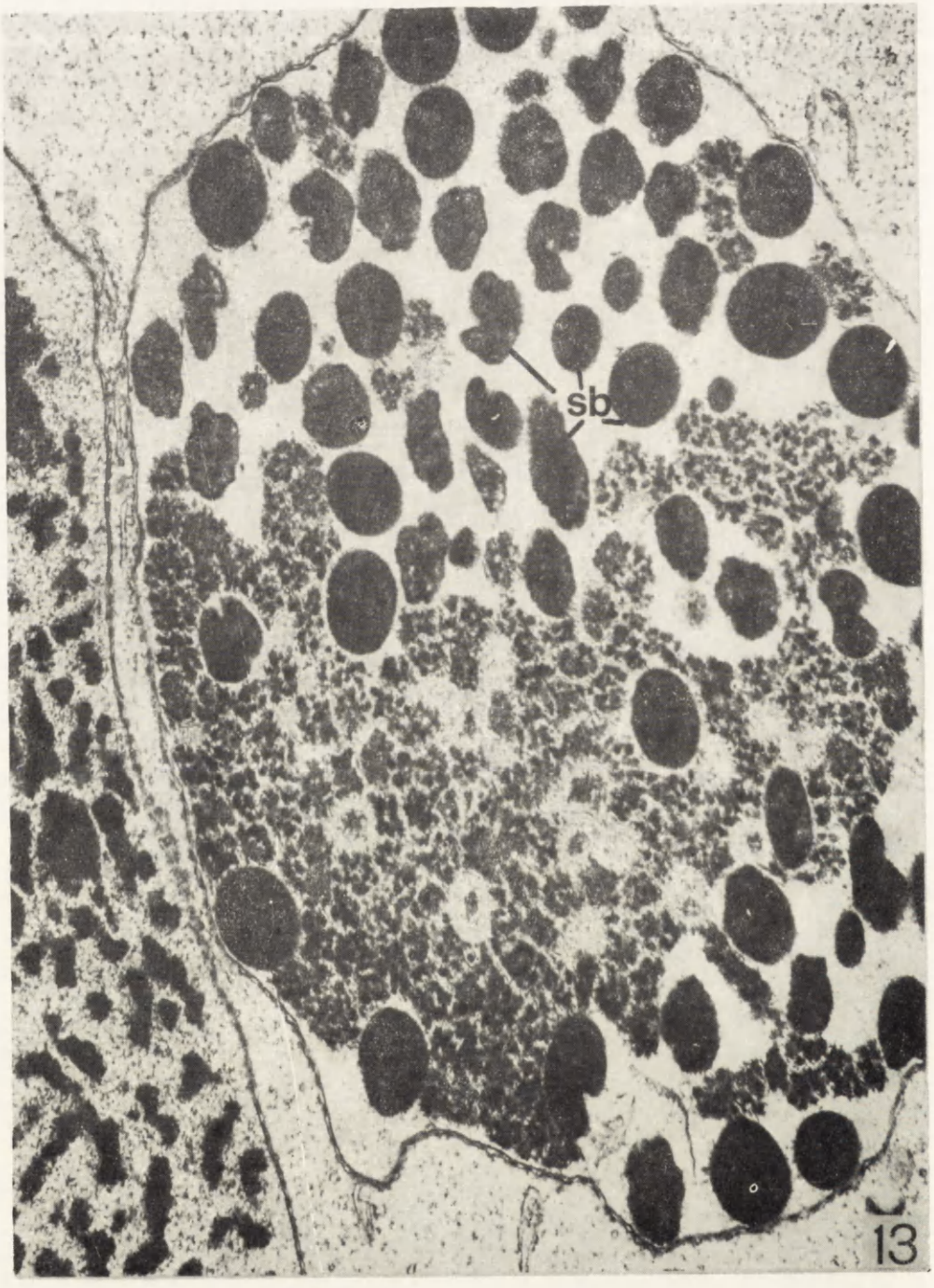

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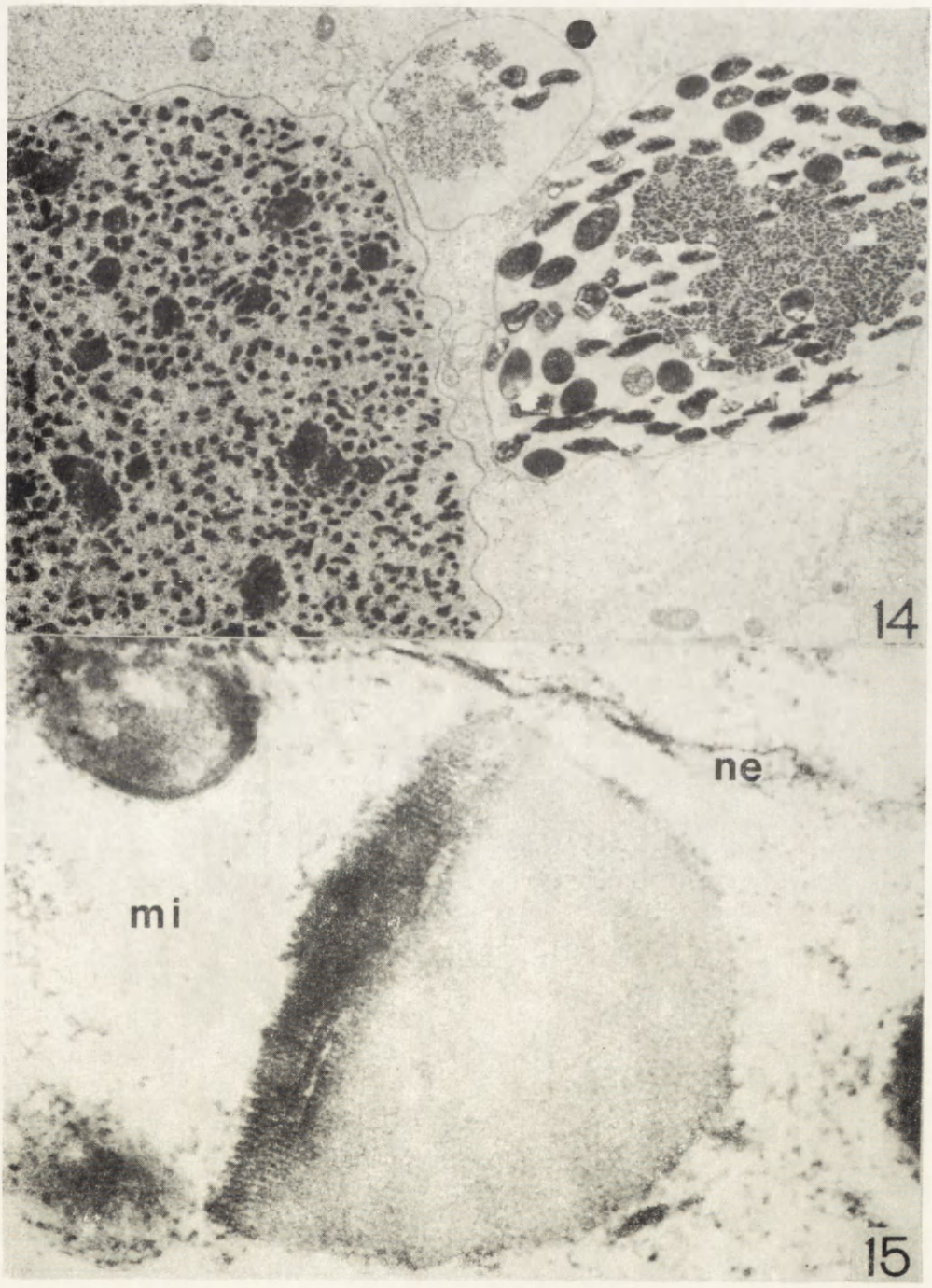

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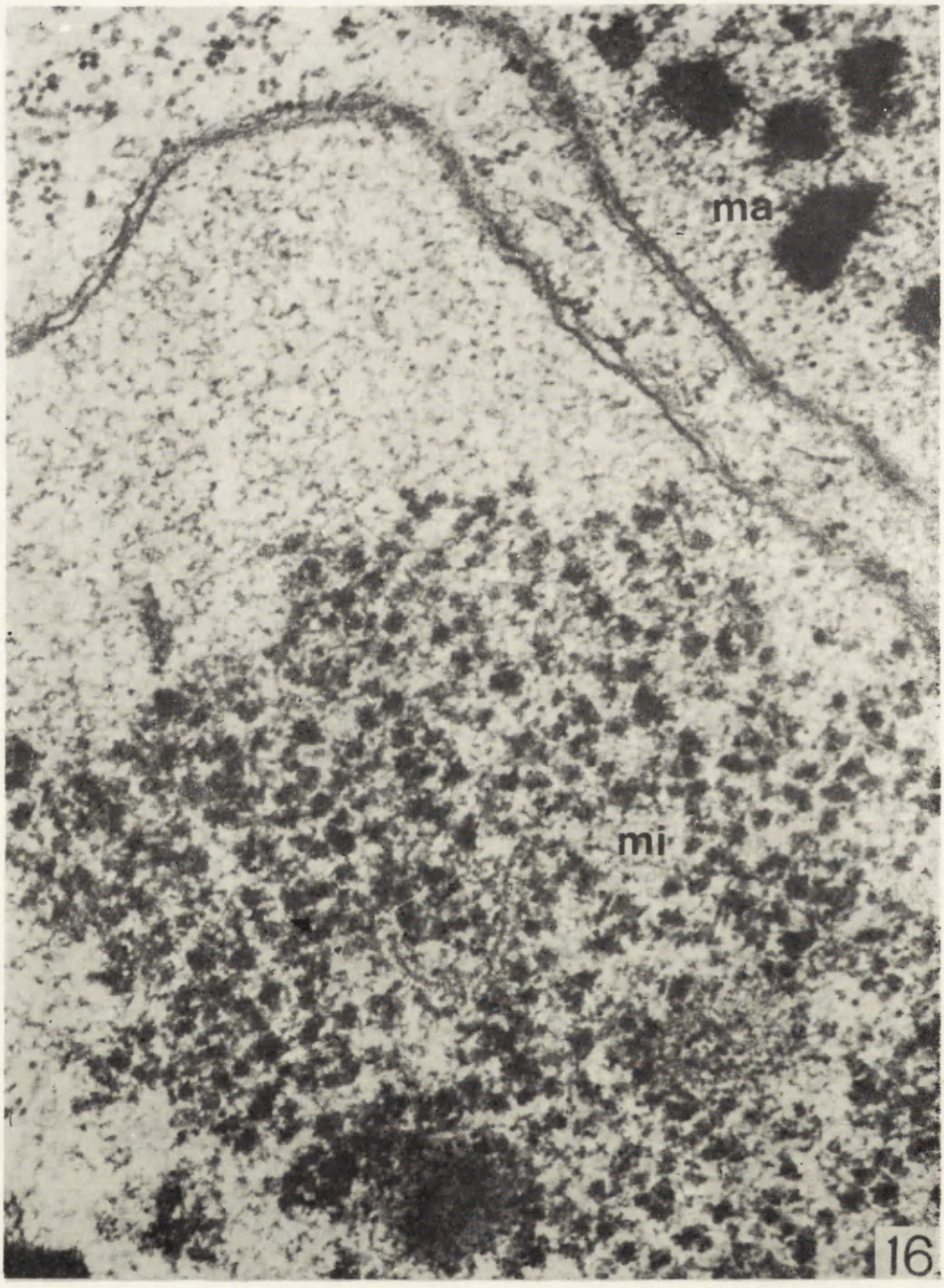

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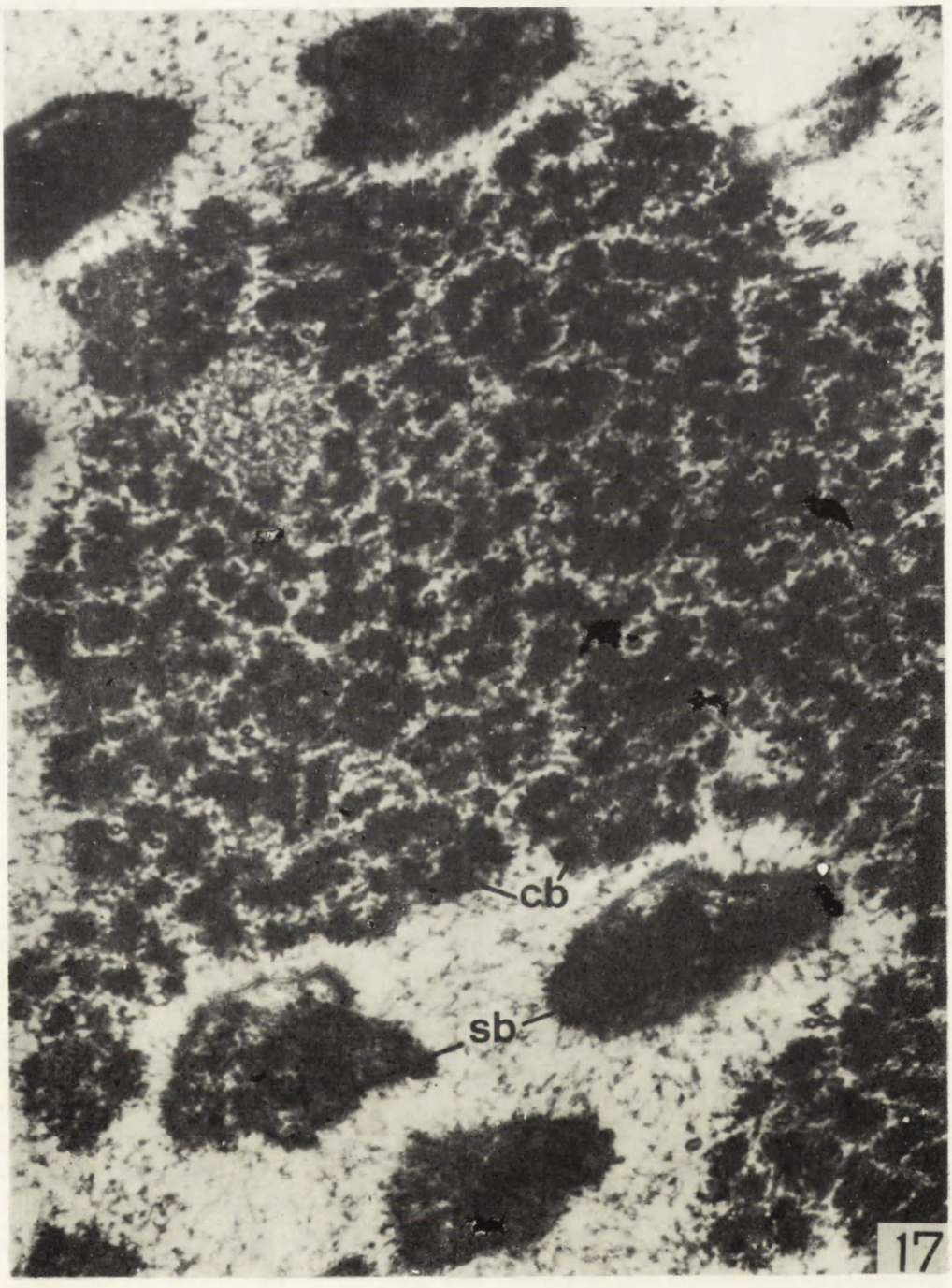

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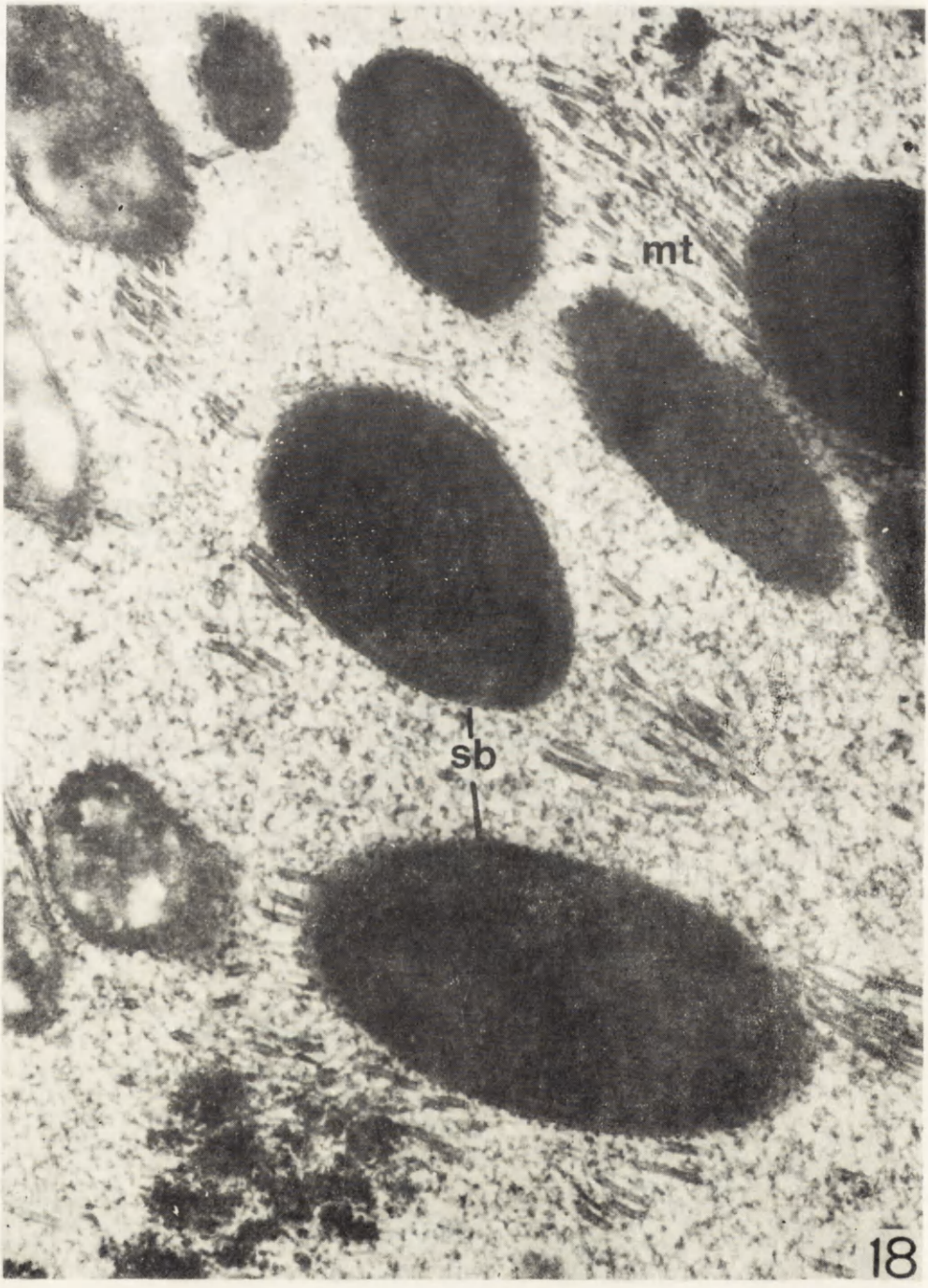

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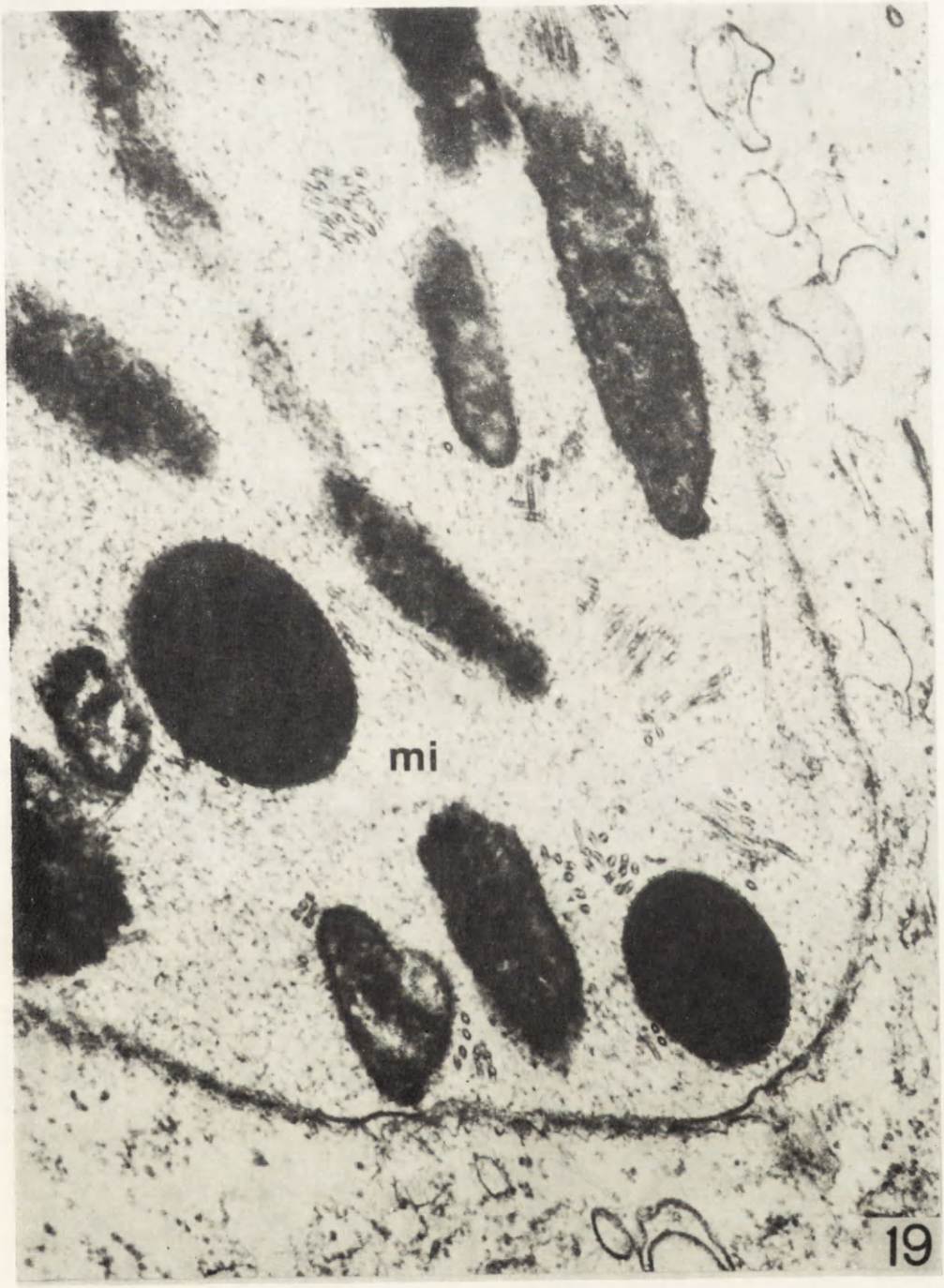

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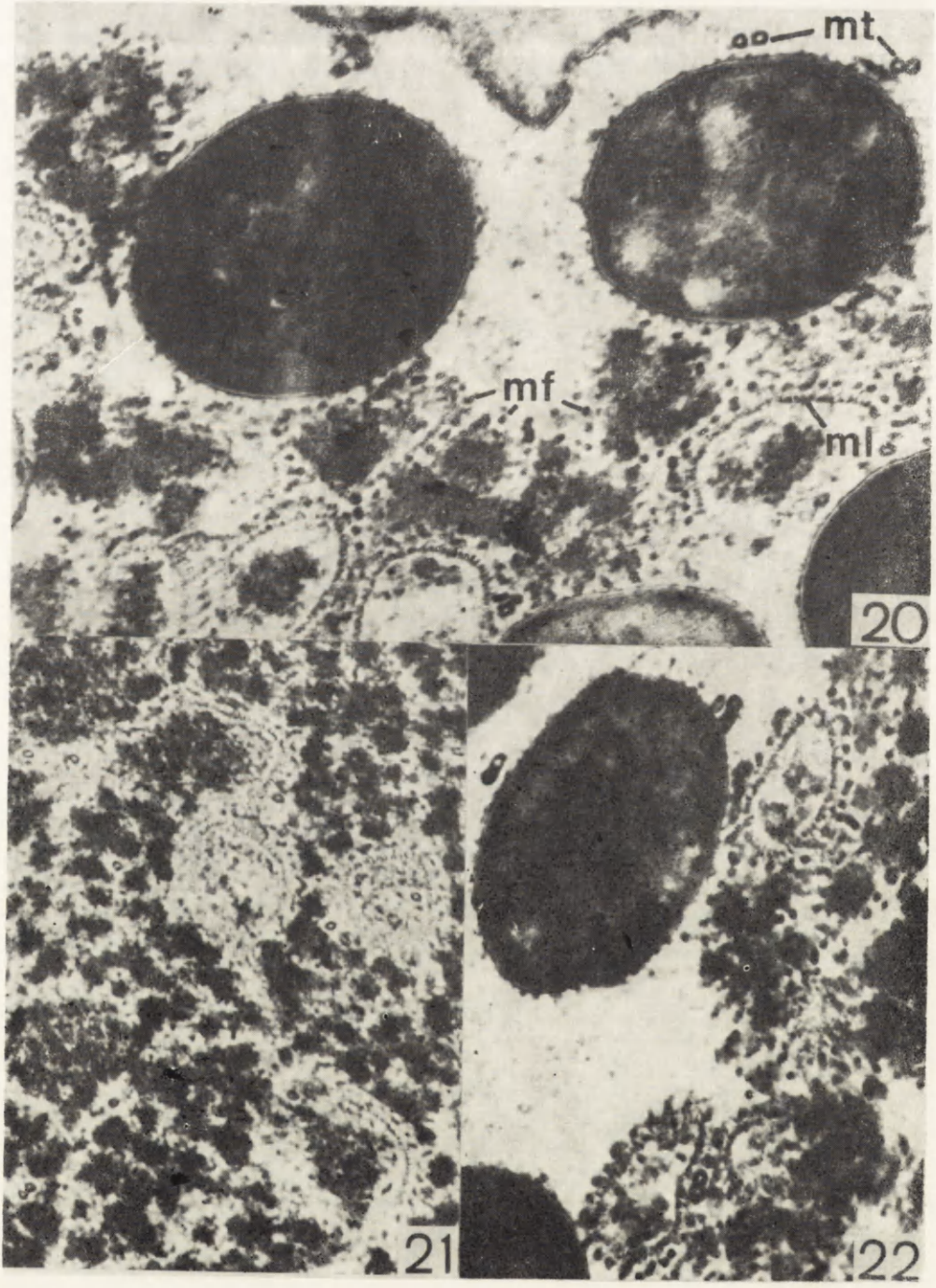

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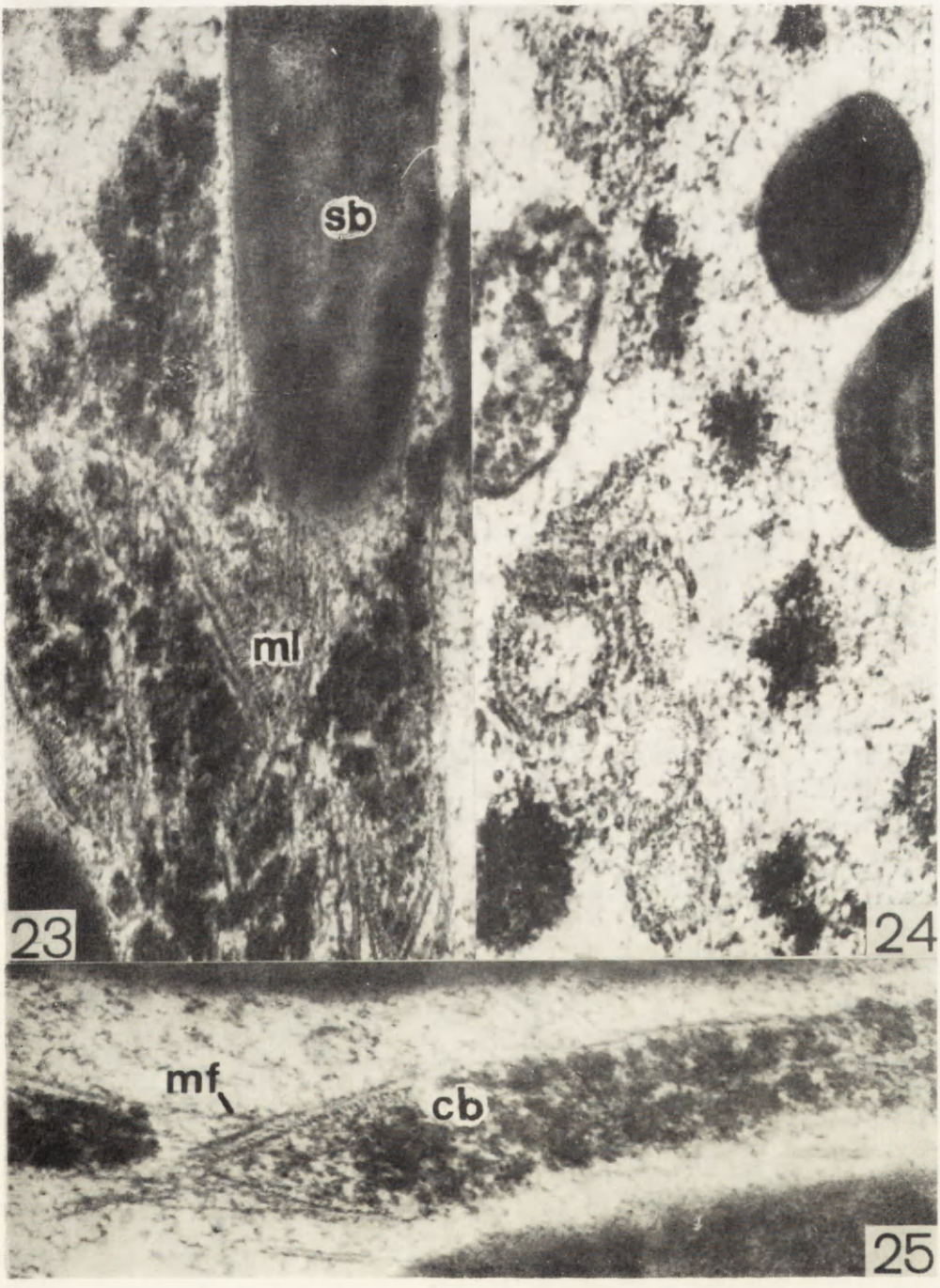

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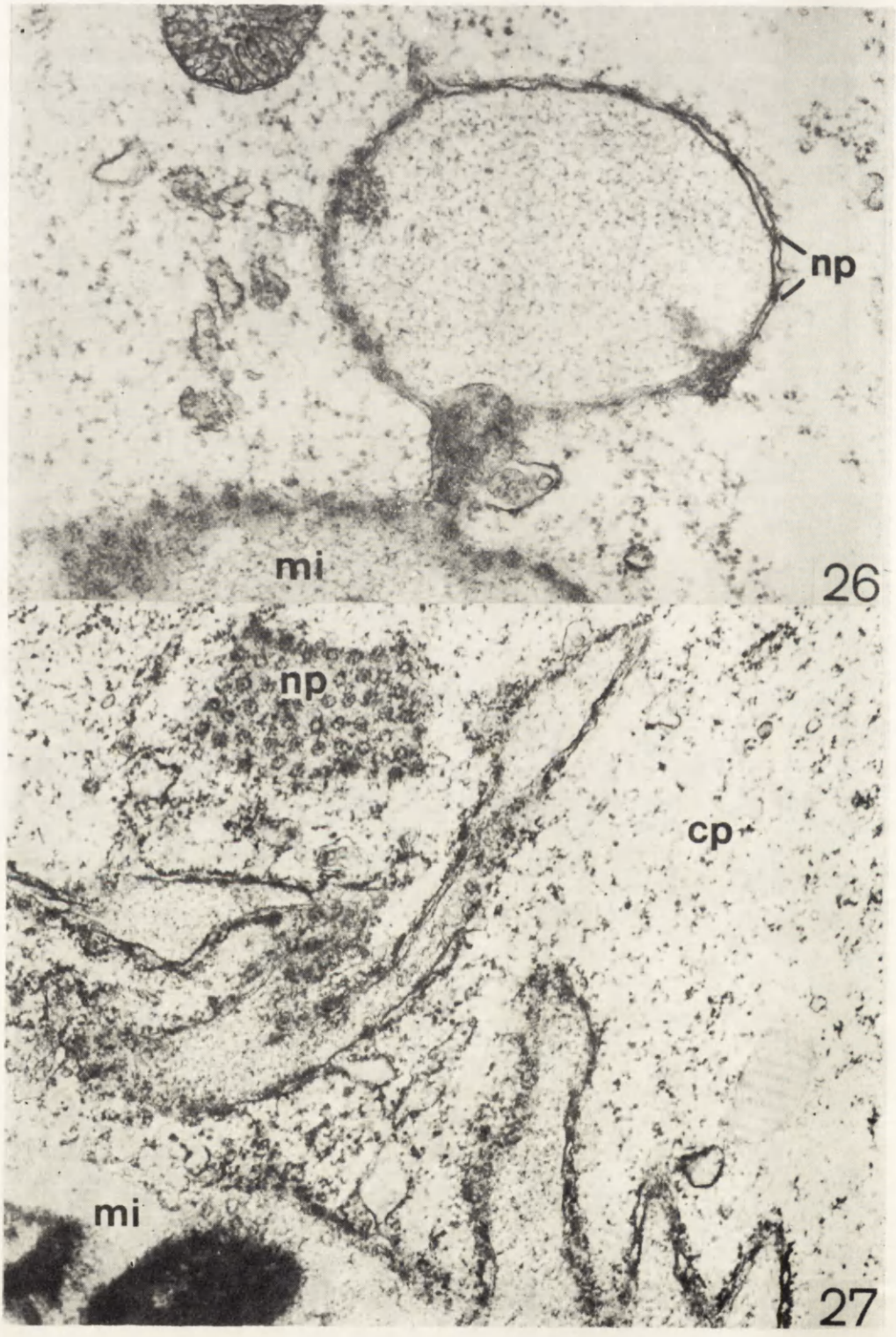

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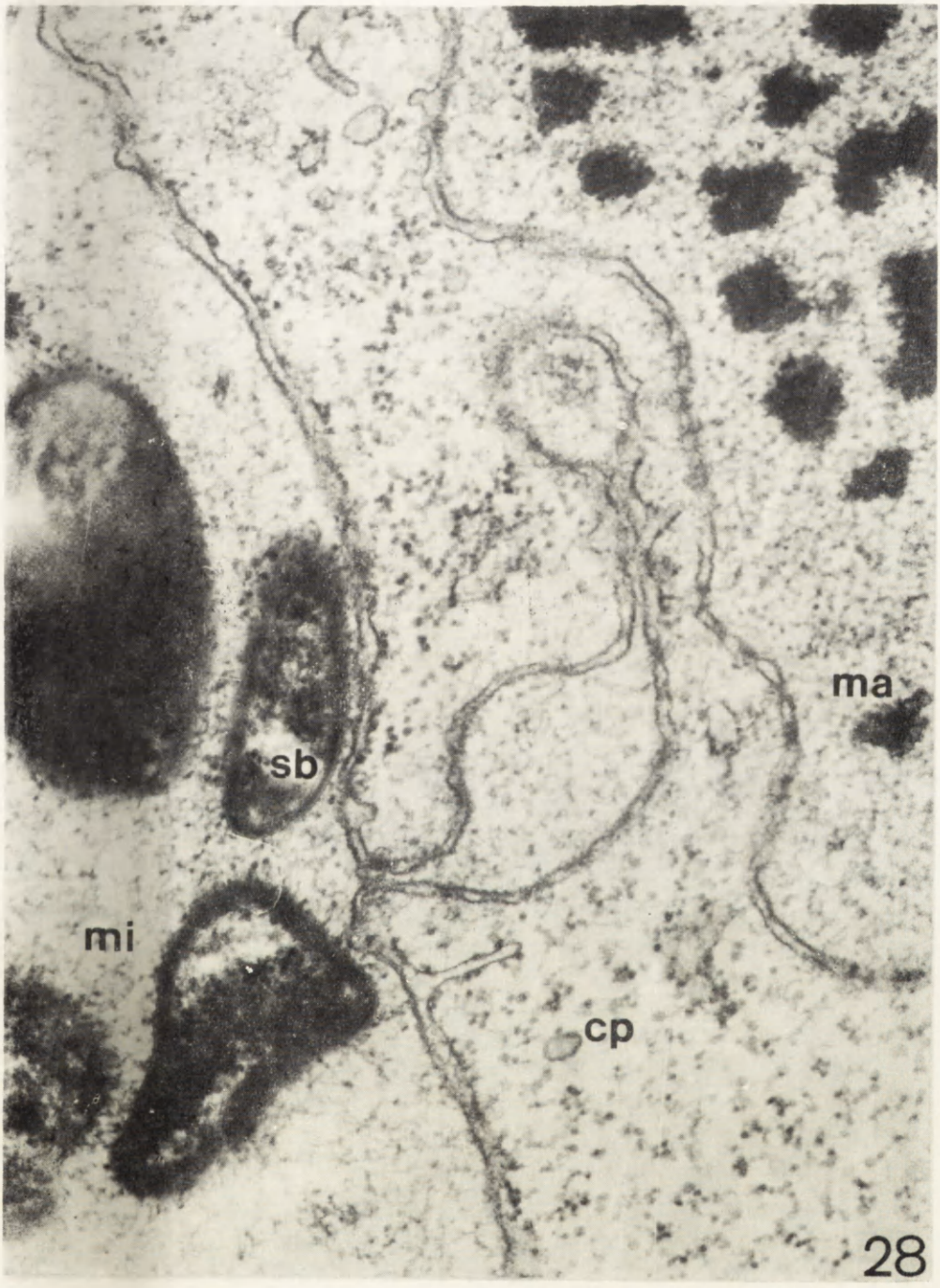

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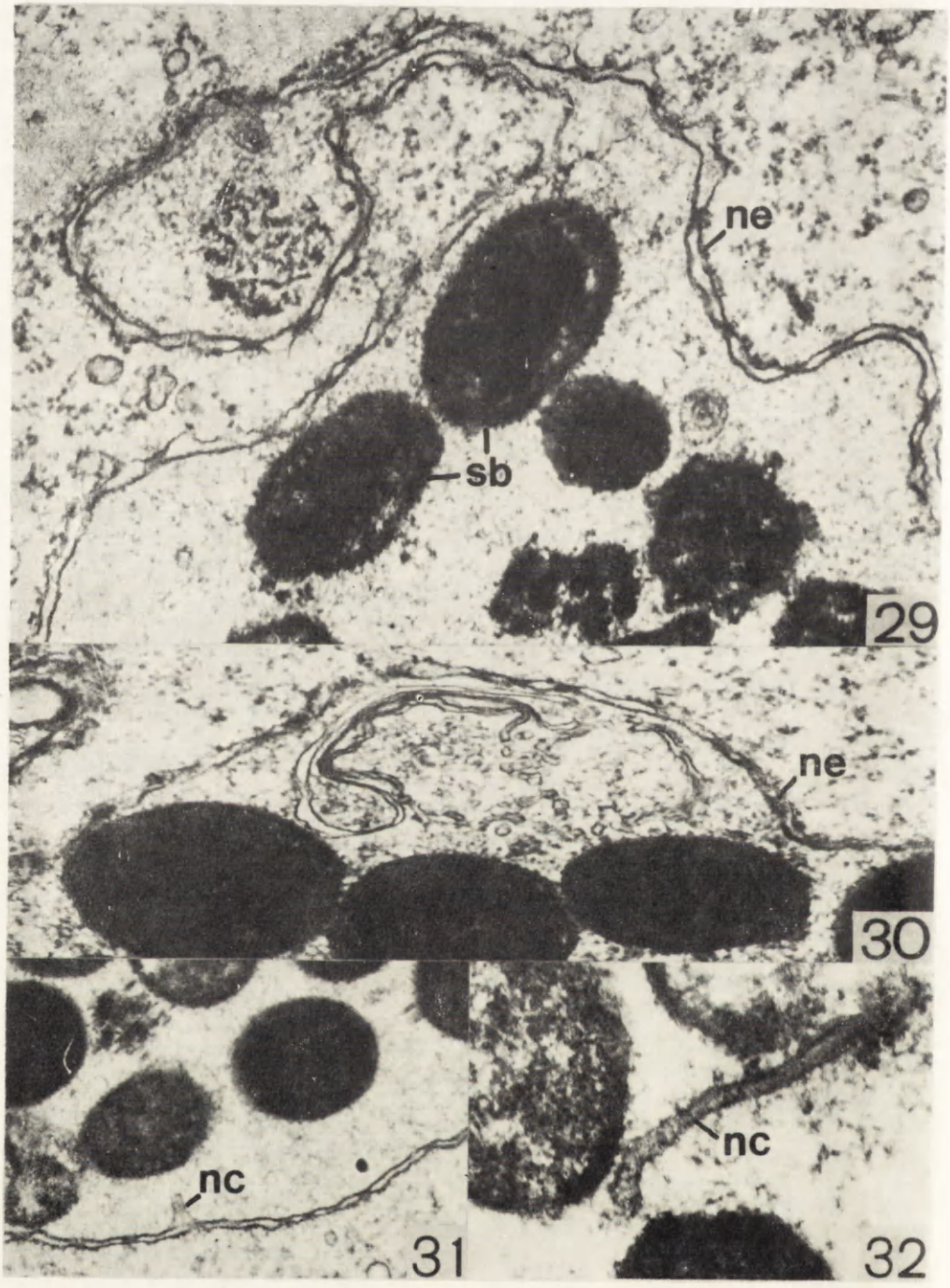

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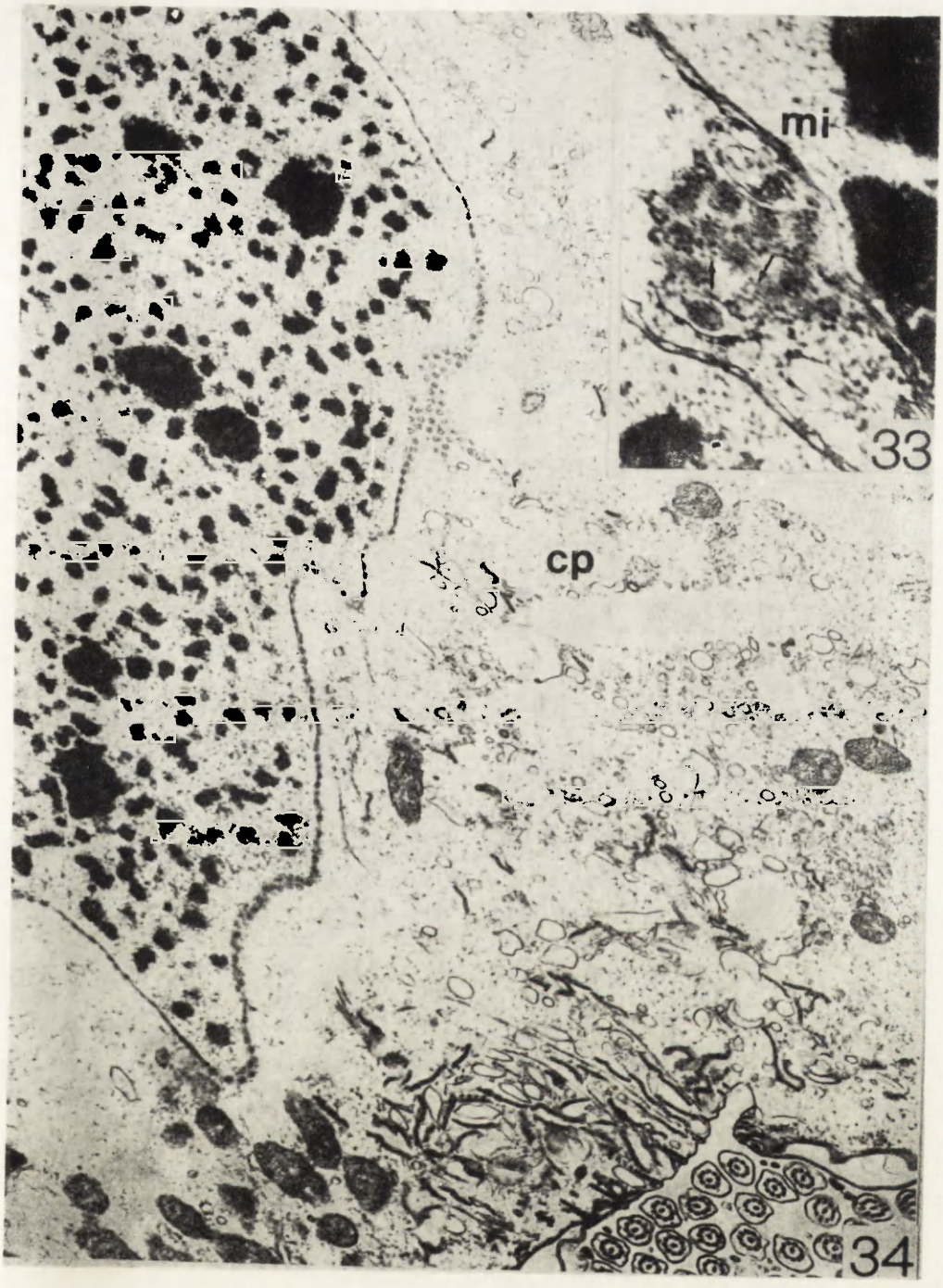

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Transparent *Amoeba proteus* Originated from the Strain C

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Synopsis. The viable transparent "mutant" of *Amoeba proteus* obtained by means of hydroxylamine treatment and the original strain C were compared. The main feature of the "mutant" designated C_t is the lack of isotropic bipyramidal and platelike crystals which causes transparency of the cells. Only few birefringent clusters remain in the cytoplasm. Changes in the crystal content are accompanied by changes in the shape and size of the cells, fission rate, number of large "spherical" multinuclear cells and a distinct change in actinomycin D resistance.

Amoeba proteus "mutants" partially devoid of crystals were obtained by Ord and Bell (1968) and Ord (1970, 1973) by means of N-methyl-N-nitrose-urethane treatment of single amoebae at a selected stage of the cell cycle. Instead of single cells, Kalney and Nikolajeva (1977) have treated mass cultures of random cell cycle stage using hydroxylamine. The "mutant" they obtained lacked diffused crystals and was easily recognizable. Nikolajeva and Selivanova (1979) had showed that both the diffused crystals of strain C and the birefringent clusters in the strain C_t might consist of the same material, i.e., triuret, according to Grunbaum et al. (1959), Griffin (1960) and Carlström and Møller (1961). In the C_t strain of *A. proteus*, not only were the crystals changed but also several other features. The aim of the present paper was to review some characters of the original strains C and of the transparent "mutant" C_t.

Material and Methods

Strain C of *Amoeba proteus* and strain C_t were cultured according to Prescott and Carrier (1964) at 20 ± 2°C. *Tetrahymena pyriformis* GL was used as a food organism. Amoebae before examination were starved for 24–48 h. Dimens-

ions of cell were measured by estimation of the area covered by the outline of cells and their nuclei using preparations fixed in methyl alcohol and stained by the Giemsa-Romanovsky method. Fixed and living preparations of amoebae were examined under bright field and polarized light microscopy. The mean generation (G) times was estimated after 10 days of cloning and calculated using the formulae applied by Hawkins (1973):

$$N = \frac{\log b - \log a}{\log 2} \quad \text{and} \quad G = \frac{T}{N},$$

where: N — number of generations, a — initial number of cells, b — final number of cells, T — time (in hours) during which growth occurred.

Alcohol resistance was estimated by 15 min exposure of 50 specimens in 7% ethyl alcohol (Gorjunova and Kalinina, 1978).

Samples of 200 cells of both strains under investigation were treated with actinomycin D at a concentration of 1 mg/ml in Prescott and Carrier's medium (1964) for 15, 30, 60 and 120 min. After rinsing cells were isolated in order to establish their capacity to produce viable clones.

Results

The transparency of the strain designated C_t was caused by the absence of bipyramidal and plate-like crystals. Within some of the C_t cells a few plate-like crystals were sometimes present. Usually one or a few clusters are the only structures in the cells visible under a bright field (Pl. I 1), while under the polarized microscope these clusters appear highly birefringent (Pl. I 2) in contrast to the bipyramidal and plate-like crystal of strain C (Pl. II 3, 4). The main feature of the "mutant" C_t, the transparency, is accompanied by other changes. The morphology of the C_t cells is different in comparison with the original strain C. The C_t cells are larger (Table 1) and their pseudopodia tend to be shorter, wider and less numerous (Pl. I 1 and Pl. III 5). The size of the nucleus is little changed, while the frequency of multinuclear specimens increases up to 6% in comparison with the original strain where they appear less frequently (1%). The number and sizes of vacuoles and of refractile spherical bodies are increased in C_t (Pl. III 5). The length of the cell cycle becomes approximately twice as long, and the subsequent fissions of C_t cells take place at intervals of about 105 h resulting in 78% viable offspring which is lower than in the normal strain C (Table 2).

Resistance to ethyl alcohol remains at the level of the normal strain C, while sensitivity to actinomycin D is increased (Table 2). In contrast to the original strain, the number of specimens C_t with two or more nuclei is far greater. Most of them are changed into "sphere shape"

Table 1

Comparison of *Amoeba proteus* strains: C and transparent C_t. In brakets the number of cells measured. For details see text

Designation of strains	Mean generation time in hours	Rough estimation of area covered by outline in μm^2	
		Cell	Nucleus
C	50 (210)	27 000 (60)	850 (60)
C_t	105 (189)	39 000 (60)	940 (60)

Table 2

Cloning efficiency expressed in percent of viable off spring after exposure of *Amoeba proteus* strains C and C_t to actinomycin D at a concentration 1 mg/ml

Designation of strains	Cloning efficiency in percent				
	Control	Duration of actinomycin D treatment in min			
		15	30	60	120
C	98	92	84	70	6
C_t	78	54	27	3	0

individuals (Pl. III 6) with 2, 3 or 4 nuclei. "Spherical" cells as a rule do not divide and eventually die during the following month.

The *Amoeba proteus* strain C_t described above has retained its features since February 1976. It is grown in the Institute of Cytology of the Academy of Sciences U.S.S.R., Leningrad, collection.

Discussion

Lack of the sexual process in *Amoeba proteus* makes it impossible to define genetically any heritable changes in experimentally-obtained viable clones as real mutations. Therefore the strain C_t is described as a "mutant" in inverted commas.

O r d (1970) has produced "mutants" from *Amoeba proteus* by exposure of single amoebae to the N-methyl-N-nitrosourethane. "Mutations" expressed as the change of crystal content have been divided into four types (O r d 1973). The type designated PM — pale "mutant", with birefringent platelets replacing the isotropic bipyramidal crystals of control amoebae (O r d and B e l l 1968) to some extent resembles the transparent C_t strain described above. It is characterized by approximately half the normal division rate and by an increase of the nuclear size. It is noteworthy that the crystals of the C_t strain in con-

trast to the PM "mutant" are gathered into indistinct birefringent clusters (Pl. I 1, 2). It seems that the C_t strain is the one most changed among the several *Amoeba proteus* "mutants" partially devoid of crystals as described by Ord (1973). According to Ord (1968, 1973) the PM and other crystals "mutants" obtained in her laboratory might revert to normal type under favourable experimental conditions such as change of temperature or culture medium. The C_t strain does not change its character even if cultured under different temperature conditions (4–25°C) and in different media for month (unpublished).

The multinuclear "spherical" cells in the C_t clones probably result from nuclear divisions which are not followed by cytokinesis (Gorjunova and Kalinina 1977). Untill now it has not been established what mechanism is responsible for promoting the transformation into "sphere shape" specimens.

It is remarkable that the highly birefringent clusters which remain in the cytoplasm of C_t specimens resemble very much in their form and birefringence the recrystalized native triuret (Grunbaum et al. 1959, Griffin 1960, Carlström and Møller 1961). It is probable that the mutagen changed the metabolic pathway of nitrogen turnover, so that the original strain C after "mutation" lost the ability to produce bipyramidal or plate-like crystals.

Although the two strains used in this study show a comparable sensitivity to ethyl alcohol, the effect of actinomycin D is much stronger on the "mutant" C_t. This difference suggests a certain distinction of physiological organization between the transparent strain C_t and the original C of *Amoeba proteus* which is also reflected in their morphology.

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RÉSUMÉ

Les caractéristiques d'un transparent viable "mutant" de l'*Amoeba proteus* produit par le traitement à l'hydroxylamine ont été comparées à celles de la souche C originelle. Le trait principal de ce "mutant" nommé C_t est l'absence des cristaux isotropiques plats ou bipyramidales, ce qui explique la transparence des céllules. Il ne reste dans leur cytoplasme qua très peu d'agglomérations des réfractions doubles. Ces changements sont accompagnées per des certaines modi-

fications de la forme et des volume des céllules, du taux des divisions, du nombre de grandes sphériques céllules multinucléaires et par une importante différence de résistance à l'actinomycine D.

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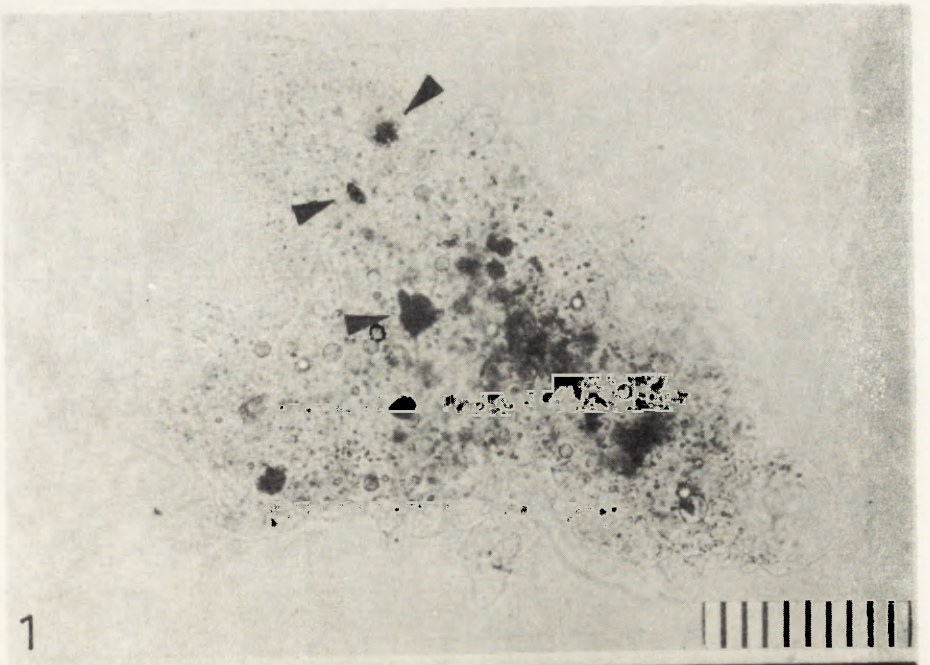
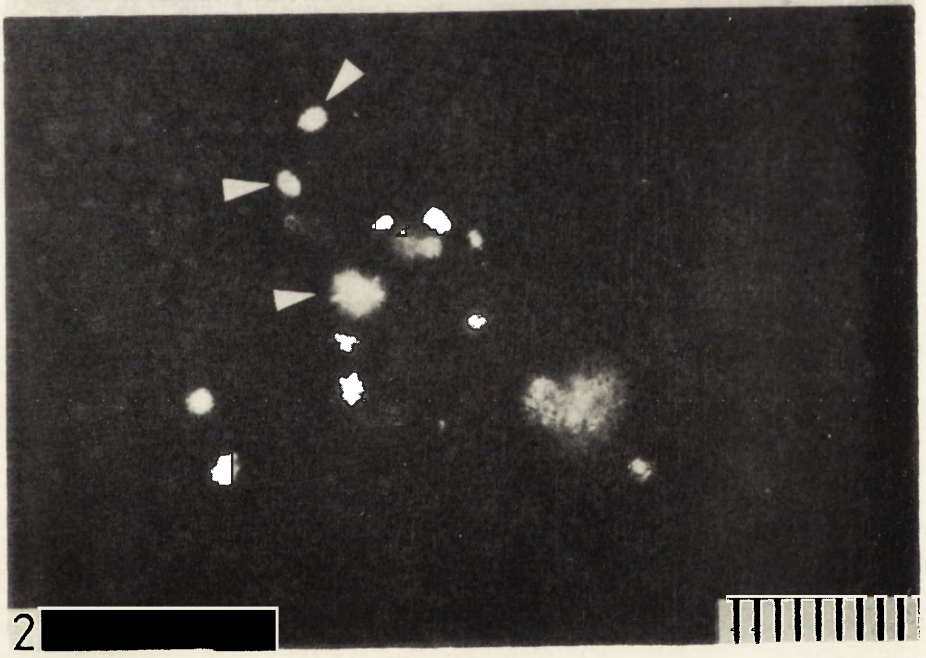
EXPLANATION OF PLATES I–III

1: Transparent *Amoeba proteus* designated C_t originated from strain C (for comparison, see Phot. 3). No bipyramidal or plate-like crystals only few clusters are seen (arrowheads). Scale div. = 10 μm
2: The same specimen as described in Phot. 1 seen with polarization optics. Clusters are highly birefringent (arrowheads). Scale div. = 10 μm
3: *Amoeba proteus* strain C containing large number of bipyramidal and plate-like crystals randomly distributed within the cell. Scale div. = 10 μm
4: *Amoeba proteus* strain C in the "spherical" state, usually multinuclear. Scale div. = 10 μm
5: *Amoeba proteus* C_t photographed in differential interference contrast. Some of vacuoles are indicated by arrowheads, while arrows indicate nucleus (n) and refractile bodies (r). Scale div. = 10 μm
6. *Amoeba proteus* C_t in the "spherical" state, usually multinuclear. Scale div. = = 10 μm





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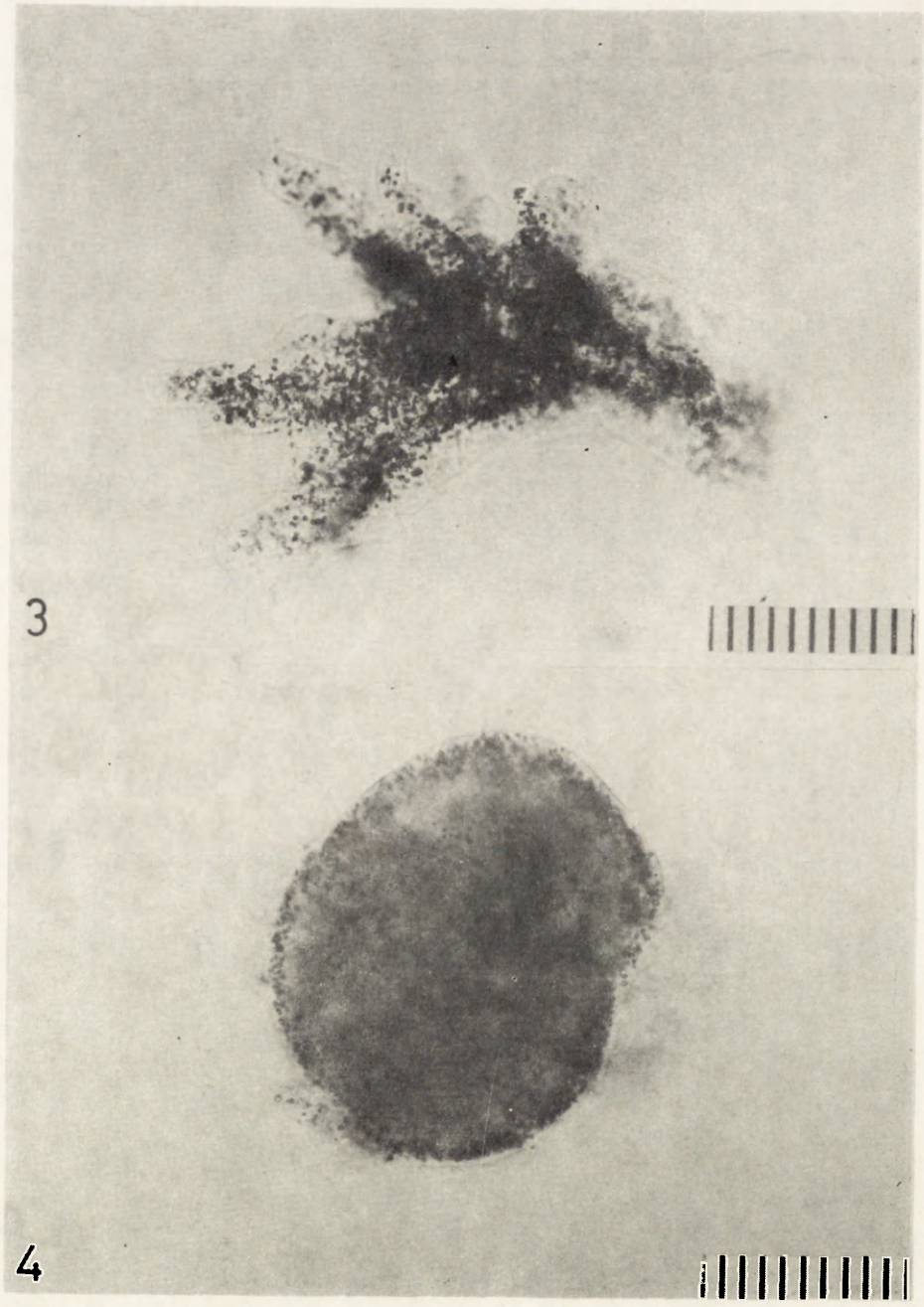

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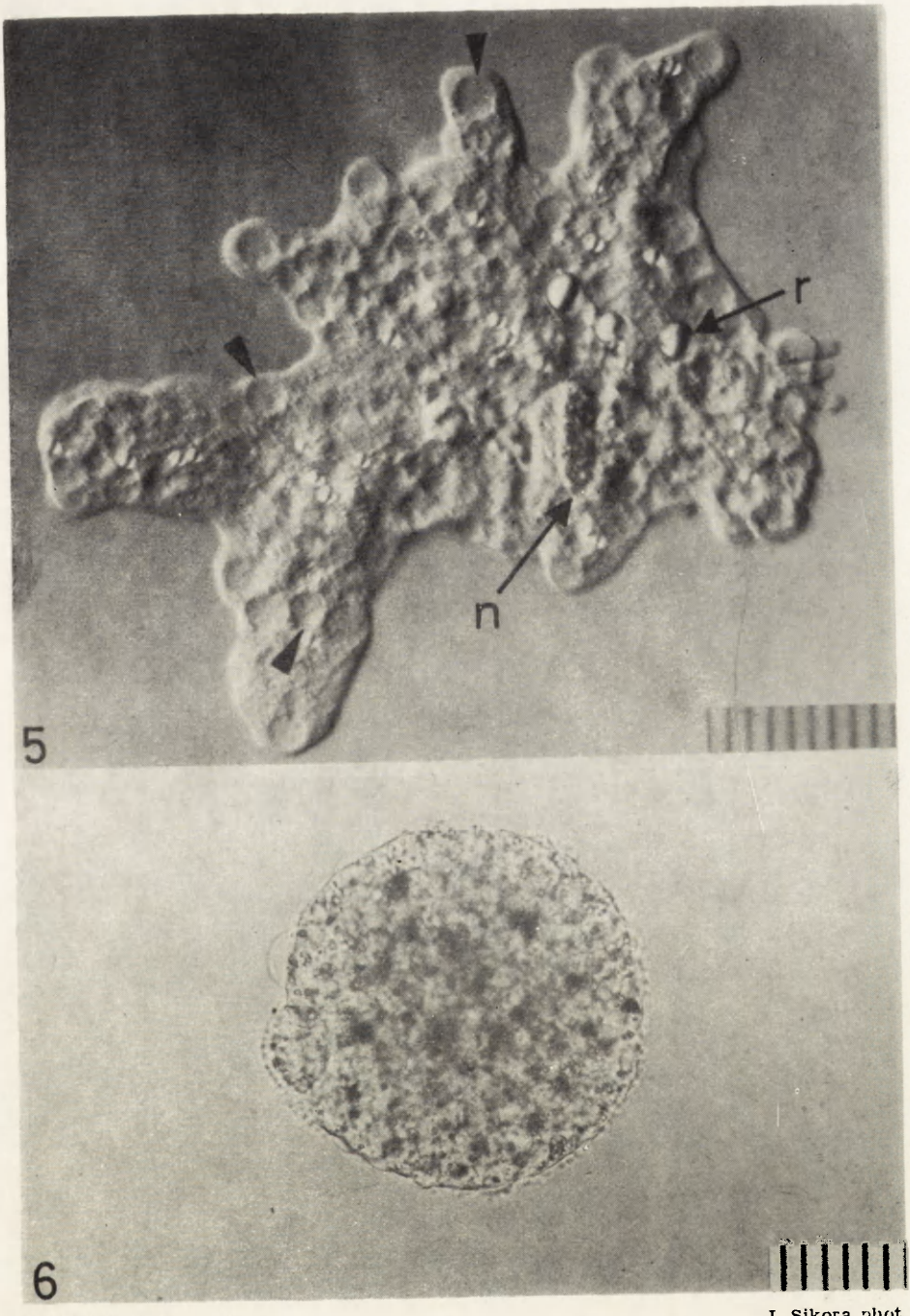

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Antigenic Specificity of *Amoeba proteus* Nuclear Transplants

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Synopsis. Some strains of *Amoeba proteus* and of morphologically proteus-like amoebae can be distinguished by use of a substrate detachment test based on the treatment of amoeba by a dilute solution of antiserum. These strains vary in sensitivity to this test. It was proved by the use of nuclear transplantation that antigenic specificity is of nuclear origin.

Antigenic diversity of large free-living amoebae has been investigated by several authors (Heathman 1932, Danielli et al. 1955, Wilson 1957, 1958, Danielli 1959, 1960, 1963, Wolpert and O'Neill 1962, Kates and Goldstein 1964, O'Neill 1964, Jeon et al. 1967, Lorch and Jeon 1969, Jeon and Lorch 1973, Friz 1974, Sikora and Kalinina 1975, Kalinina et al. 1976). However, this diversity is not as specific as was shown to be the case for *Paramecium* species (for Ref. see: Sonneborn 1950, 1970, Beale 1954, Finger 1974). The substrate detachment test proposed by Sikora and Kalinina (1975) allows some strains of *Amoeba proteus* and morphologically proteus-like amoebae to be distinguished. The question concerning the role of the nucleus and the cytoplasm in the control of antigenic expression of *Amoeba proteus* has been studied in Danielli's laboratory (Danielli et al. 1955, Wilson 1957, 1958, Jeon et al. 1967) although attempts were mainly done by use of non-compatible *Amoeba proteus* and *Amoeba discoides* (Jeon and Lorch 1969). Therefore it was considered worthwhile to investigate the antigenic properties of the "hybrids" obtained by a nuclear transfer operation between compatible strains Bk and Da of *Amoeba proteus*.

Material and Methods

Amoeba proteus strains used were: Da = DP = X65 obtained from M. J. Ord (University of Southampton, Southampton, Great Britain) and Bk obtained from D. M. Prescott (University of Colorado, Boulder, U.S.A.) and two types of clone originated from nuclear-cytoplasmic "hybrids" $Da_{nucleus}$ $Bk_{cytoplasm}$ and $Bk_{nucleus}$ $Da_{cytoplasm}$ obtained from A. Yudin (Institute of Cytology, Academy of Sciences USSR, Leningrad, USSR). All strains have been cultured at 20±2°C in Prescott and Carrier's (1964) medium. *Tetrahymena pyriformis* GL was used as a food organism.

Before treatment amoebae were starved for 24–48 h in Prescott and Carrier's medium. The substrate detachment method described previously (Sikora and Kalinina 1975) has been applied to estimate the detachment frequency. Samples of 100–120 cells taken from mass culture were pipetted gently into hollows of triple depression slides in fresh Prescott and Carrier's solution. After 30 min when amoebae had settled and were adhering to the glass, the medium was sucked out and replaced by the experimental solution consisting of the antiserum against strain C, diluted by Prescott and Carrier's medium. In control experiments the normal serum and culture medium were added into appropriate depression slides. After 2 h of incubation the number of amoebae detached from the substrate was counted under a dissecting microscope.

Results

In order to estimate the substrate detachment of *Amoeba proteus* strains Da and Bk and their nucleo-cytoplasmic "hybrids" Da_nBk_c (3 clones) and Bk_nDa_c (3 clones), samples have been treated by means of diluted antiserum against strain C in concentration 1 : 160. As is shown in Table 1, the strain Da is far more sensitive and detaches readily from the substrate (almost 90% of individuals) while strain Bk does not respond to this concentration (only 9% of individuals). "Hybrids" consisting of Bk cytoplasm and Da nucleus respond to antiserum like the original strain Da, while "hybrids" consisting of Bk nucleus and Da cytoplasm remain almost unaffected by antiserum, resembling its nuclear "parent strain" Bk. In Prescott and Carrier's (1964) medium and in the corresponding concentration of control serum, not more than 5% of specimens of strains Da, Bk and their "hybrids" detach from the substrate.

Table 1

Substrate detachment expressed in percent (±SE) of Da and Bk strains of *Amoeba proteus* and their nuclear transplants — "hybrids", after 2 h treatment with antiserum against strain C in concentration 1:160 (diluted with Prescott and Carrier's 1964 medium)

Strains of *A. proteus* and their "hybrids" (designation)	Number of tests	Percent of specimens detached (±SE)
Da	23	88.8±1.4
Da_nBk_c No. 2	13	84.6±1.8
Da_nBk_c No. 8	17	88.7±2.1
Da_nBk_c No. 11	17	86.2±2.7
Bk	25	9.0±1.5
Bk_nDa_c No. 5	13	7.5±1.6
Bk_nDa_c No. 12	18	8.8±1.9
Bk_nDa_c No. 13	18	6.7±1.3

Discussion

The substrate detachment test (Sikora and Kalinina 1975) has enabled us to investigate to what extent the nucleus controls antigenic expression by comparison of the two strains of *Amoeba proteus* and their nucleo-cytoplasmic "hybrids". The original strains Da and Bk differ in their sensitivity to the antiserum. Yudin (1973) had found that these strains are compatible, forming viable "hybrids" by cross-nuclear transplantation and giving clones which were suitable to investigate antigenic expression. "Hybrids" consisting of Bk cytoplasm and Da nuclei detached from the substrate as readily as the original strain Da, while the strain Bk and its "hybrids" containing nuclei of strain Bk remain almost insensitive to the action of the antiserum. It appears that the results obtained have shown clearly that antigenic character in the examined cases depends on the origin of nucleus, therefore it might be suggested that the antigenic specificity in *Amoeba proteus* is under nuclear control.

First attempts to study the role of the nucleus and cytoplasm in antigen inheritance in free-living large amoebae have been done by Danielli et al. (1955). As immunological tests, cytolytic effect, change in body shape and change in division rate after homological antiserum treatment were used. It was suggested (Danielli et al. 1955, Jeon et al. 1967) that cytolytic sensitivity is under the control of the cytoplasm, while other effects like change in body shape and change if division rate depends on the nucleus. These conclusions have not been confirmed in the present paper. The contradiction may be due to the fact that a different test was used here. Furthermore, so far there is no evidence of antigenic transformation as for *Paramecium* serotypes (for Ref. see: Sonneborn 1950, 1970, Beale 1954, Finger 1974) but it could not be excluded. The tests presented in this paper were made during the period of one year, but standard culture conditions might induce a uniform expression of the surface antigen; so far changes of antigens have not been observed.

In spite of its limitations, the substrate detachment test appears to be suitable for the examination of the antigenic character of *Amoeba proteus* strains.

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RÉSUMÉ

Certaines souches de l'*Amoeba proteus* et des amibes qui morphologiquement ressemblent à cette espèce se diffèrent entre elles par leur susceptibilité à l'action d'une solution diluée de l'antiserum qui les détache de substratum. Les transplantations des noyaux démontrent que cette spécificité antigénique est d'origine nucléaire.

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Comparison of Locomotion and Adhesion in Four Strains of *Amoeba proteus*

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Synopsis. Four strains of *Amoeba proteus* were examined with respect to their rate of locomotion, strength of adhesion and area of adhesion. The rate of locomotion was found to be inversely correlated with the strength of adhesion. The area of adhesion was not correlated with any of these parameters, however. An assumption is put forward that a nature of adhesion in *Amoeba proteus* might be strain specific.

Among several strains of *Amoeba proteus* there is a substantial morphological and physiological diversity (Jeon and Lorch 1973, Sikora and Kalinina 1975, Yudin and Sopina 1970). Besides morphological characters, nucleo-cytoplasmic compatibility, resistance to chemical and physical factors, the rate of attachment (detachment) to a substratum has been investigated (Jeon and Lorch 1973, Sikora and Kalinina 1975, Ord 1973, Yudin and Sopina 1970). It was therefore of interest, whether the obvious morphological differences between several strains of *Amoeba proteus* are accompanied by differences in their locomotory and adhesive behaviour.

Material and Methods

Four strains of *Amoeba proteus* (C, Ct, Sh, As) from the collection of the Institute of Cytology, USSR Academy of Sciences, Leningrad were used in the experiments. Amoebae were cultured in Pringsheim's medium and fed on *Tetrahymena*. For the experiments 24 h starved amoebae were chosen exclusively.

Measurements of Rate of Locomotion.

Paths covered by amoebae during 15 min were recorded by means of dark

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field long-exposure photomacrography (Opas 1975). Length of individual pathways was averaged and the rate of locomotion was counted in micrometers per minute.

Measurements of Strength of Adhesion

The method of estimating the strength of adhesion of amoebae consisted in application of known centrifugal acceleration to the attached cells. Amoebae (500–700 cells per test tube) were allowed to settle on the side of a test tube placed horizontally. After 20–30 min amoebae were counted and then centrifuged at 3000 rpm for 10 min in Janetzki K-70 centrifuge. The distance from the center of a horizontal rotor to the bottom of a test tube was 19.5 cm. The percentage of cells which remained attached after centrifugation gave an indication of the strength of adhesion of amoebae.

Microscopy

A NU 2 (Zeiss-Jena) equipped with interference reflection device (Patzelt 1976) and OPTON Standard equipped with differential interference contrast device microscopes were used. For interference reflection microscopy amoebae were fixed in ice-cold 2.5% glutaraldehyde.

Results and Discussion

Plates I and II present the main morphological features of the four *A. proteus* strains used.

C strain fits very well general descriptions of *Amoeba proteus* (Andresen 1973, Page 1976). For sake of comparison it may be characterized as an amoeba of a cell volume c.a. 2.5×10^{-6} cm^3, moderate number of crystals (Pl I a), moderate speed of locomotion (Table 1). The number of pseudopodia formed during migration varies, but usually is reduced to one or two big ones as the speed of locomotion increases.

Sh amoebae are usually larger than C (app. 1.5 x) and are easily distinguishable by being almost black, due to very high number of fine crystals (Pl I b). They form less and shorter pseudopodia during locomotion than C amoebae. They are also slower in migration (Table 1).

High number of fine crystals (and blackish colour) is also typical for As amoebae. These small amoebae (0.2–0.5×C) are usually polypodial (Pl II a), with the strong tendency to monopodiality during fast migration. They are approximately twice as fast in locomotion as C amoebae (Table 1).

Ct amoebae are nearly transparent due to lack of crystals (Pl II b). Their cytoplasm is highly vacuolized and abundant in refractile bodies (Nikolajeva et al. 1980). The cell size in Ct amoebae is approximately twice normal (C amoebae) and the speed of locomotion is similar to that of Sh amoebae (Table 1).

Table 1

Rate of locomotion and strength of adhesion in four strains of *Amoeba proteus*

Strain	Ct	Sh	C	As
Rate of locomotion (μm/min)	86±32	86±54	118±32	246±54
Strength of adhesion[1]	—[2]	58±6	35±11	29±9
Detaching force (dynes)	—	4×10^{-1}	1×10^{-1}	$0{,}4\times10^{-1}$

[1] Measured as the percentage of cells which remained attached after centrifugation.
[2] Not measured.

The present results show that the strains which are less adhesive have higher rate of locomotion (Table 1). This relationship is graphically shown in Fig. 1. The amoebae of different strains were centrifuged with the same centrifugal acceleration. Since there are obvious morphological differences between these strains (cell volume and density), the actual detaching force was different for each strain. Rough estimation of detaching force (Table 1) gives basis to an assumption that the differences in position of areas of strength of adhesion (SA) / rate of locomotion (RL) relationship should be greater than those shown in Fig. 1. The area of SA/RL relationship for Sh amoebae should be shifted toward higher percentages, whereas the SA/RL relationship area for As amoebae should be shifted toward lower percentages. Direction's in which SA/RL areas for Sh and As amoebae should be shifted in the case of centrifugation of all the strains with uniform detaching force acting are shown in Fig. 1 by arrows.

Although strength of adhesion is inversely correlated with the rate of locomotion in investigated strains of amoebae, the area of adhesion does not follow this correlation. From comparison of interference reflection images shown in Plates III–VI it is clear that the two slower strains (Ct and Sh) are characterized by broad adhesions, whereas the faster strain (C) adheres to the glass by very limited area of the amoeba underside. The fastest strain (As), however, is adhering to the glass with a greater part of the cell underside than slower strain C. Therefore, the percentage of an amoeba underside being in contact with a substratum is not correlated with a mean speed of locomotion characteristic for a given strain and with strength of adhesion either.

Low numerical aperture of the optical system used does not permit for analysis of distance of separation between surfaces of amoebae and glass (O p a s 1979). It seems probable, however, that the physical features of adhesions of fast locomoting amoebae and of slow ones are not uniform. A force necessary to detach a square unit of adhesion area in Ct or Sh amoebae (slow strains) would be much higher than

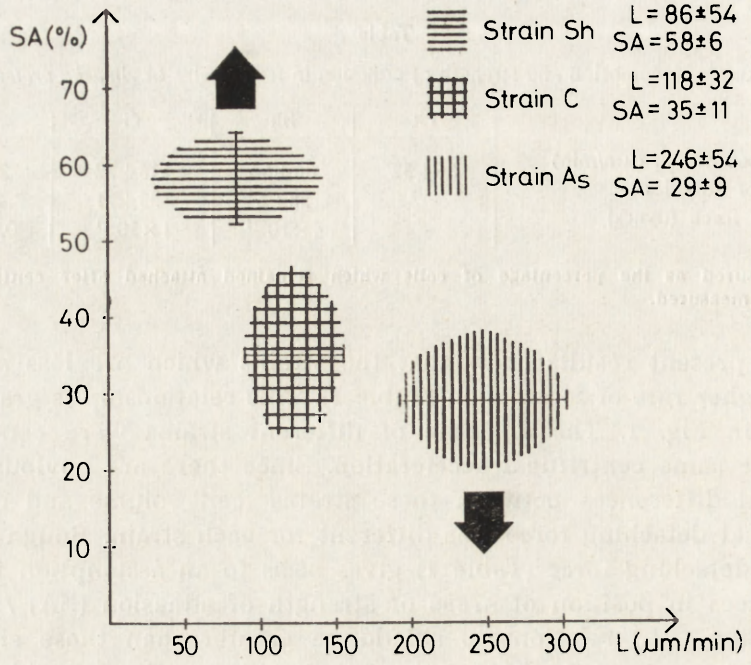

Fig. 1. Rate of locomotion (L) and strength of adhesion (SA) in three strains of *Amoeba proteus*. SA is shown as the percentage of cells which remained attached after centrifugation. Further explanation in the text

a force needed to detach the same square unit of adhesion area in As amoebae (fast strain). Since differences in physicochemical character of surfaces between these strains of *A. proteus* have not been investigated, the question of a nature of the observed diversity remains open.

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We are grateful to Ms Krystyna Tabeńska for excellent technical assistance.

RÉSUMÉ

La vitesse de locomotion, ainsi que l'intensité d'adhesion et l'étendu des surfaces adhésives ont été étudies chez quatre souches de l'*Amoeba proteus*. La vitesse de migration se trouve inversemment corélée avec l'intensite d'adhesion. Cépendant l'étendu de la surface adhésive n'est pas corélé à ces deux parametres. On peut supposer que l'adhésivité chez *Amoebâ proteus* présente un caractère spécifique dépendant de la souche.

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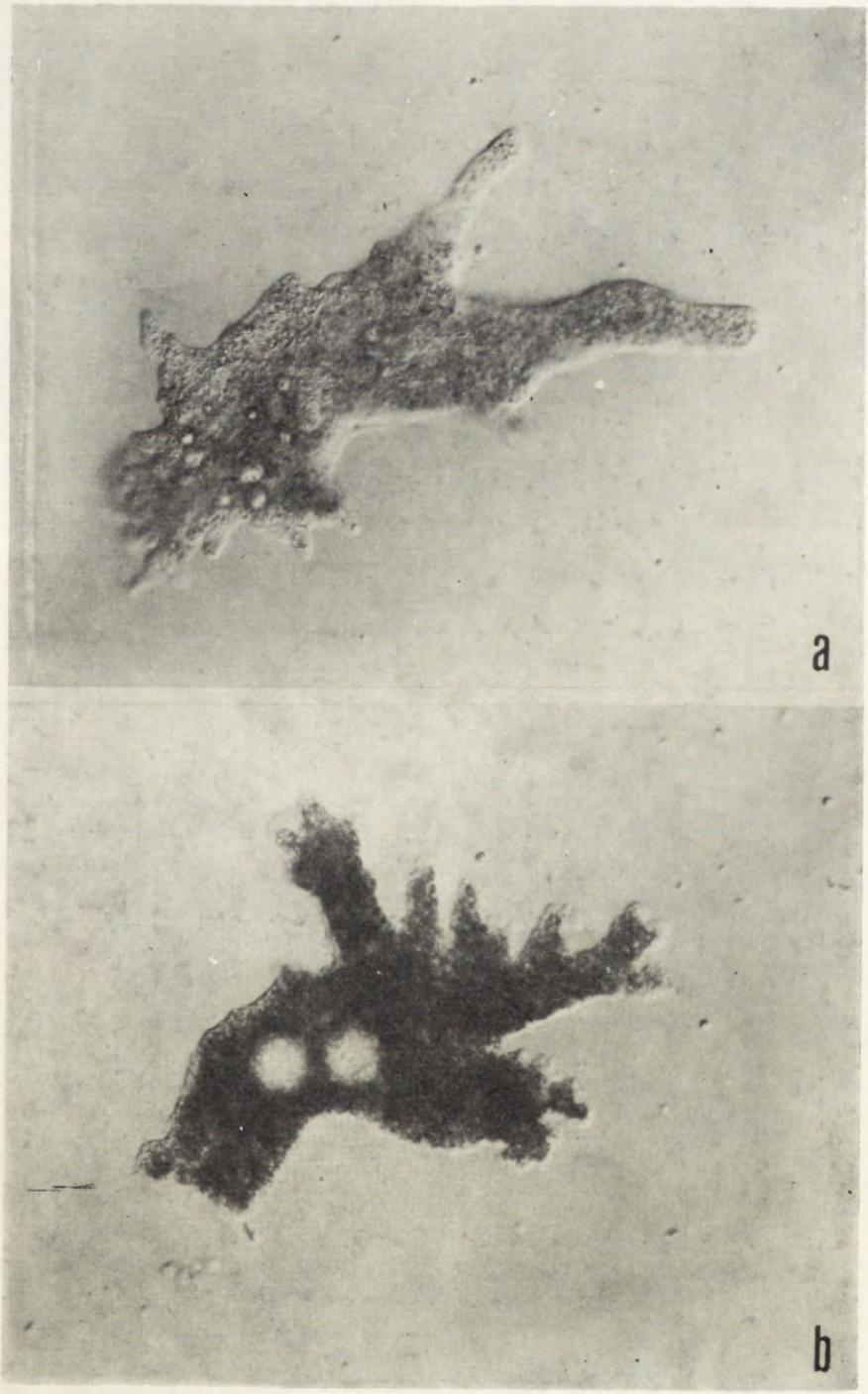
EXPLANATION OF PLATES I–VI

Plate Ia: *Amoeba proteus* strain C. Differential interference contrast, × 220
Plate Ib: *Amoeba proteus* strain Sh. Differential interference contrast, × 220
Plate IIa: *Amoeba proteus* strain As. Differential interference contrast, × 220
Plate IIb: *Amoeba proteus* strain Ct. Differential interference contrast, × 220
Plate III: Bright field and interference reflection image of *Amoeba proteus* strain C. × 190
Plate IV: Bright field and interference reflection image of *Amoeba proteus* strain Sh. × 190
Plate V: Bright field and interference reflection image of *Amoeba proteus* strain As. × 190
Plate VI: Bright field and interference reflection image of *Amoeba proteus* strain Ct. × 190



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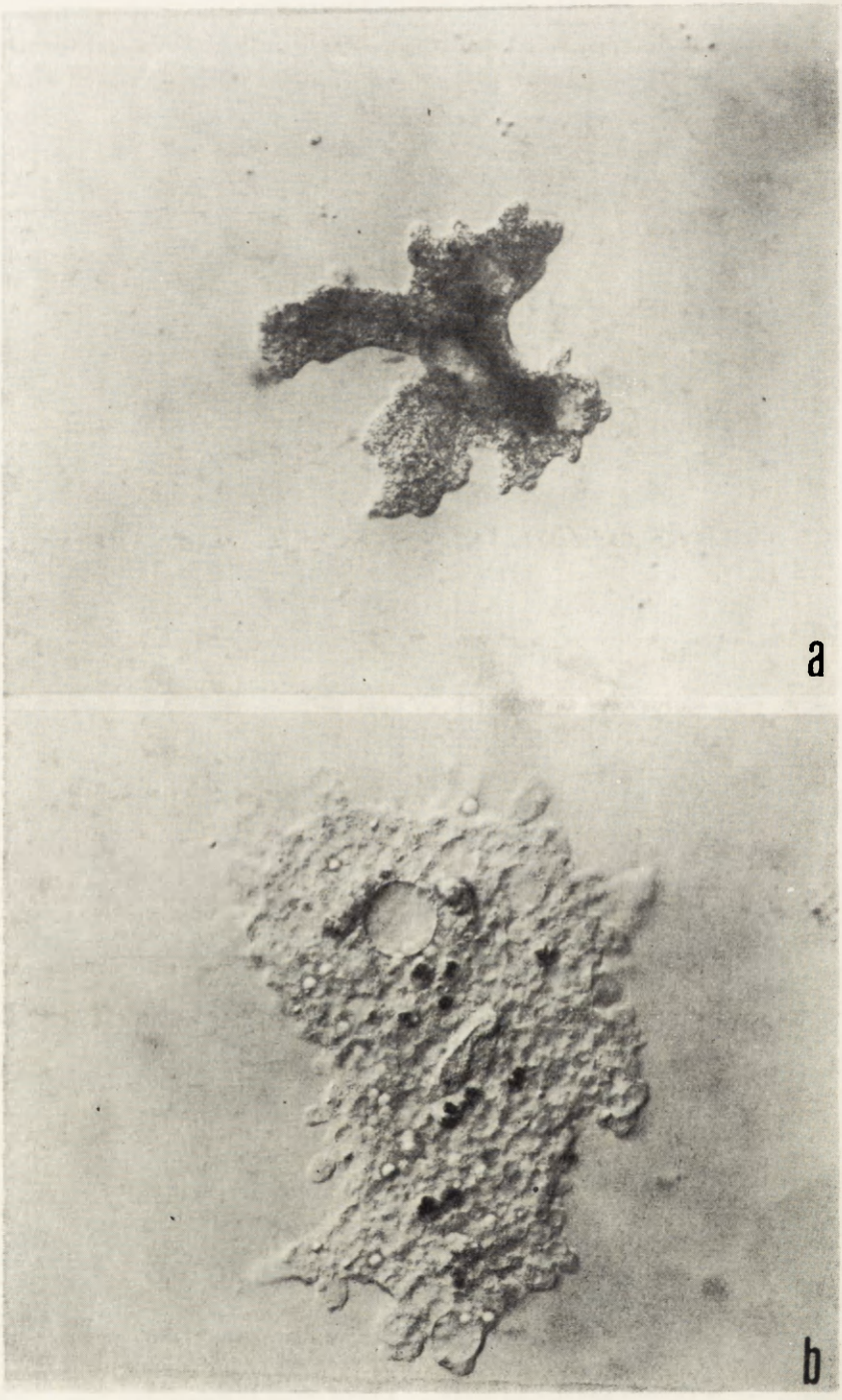

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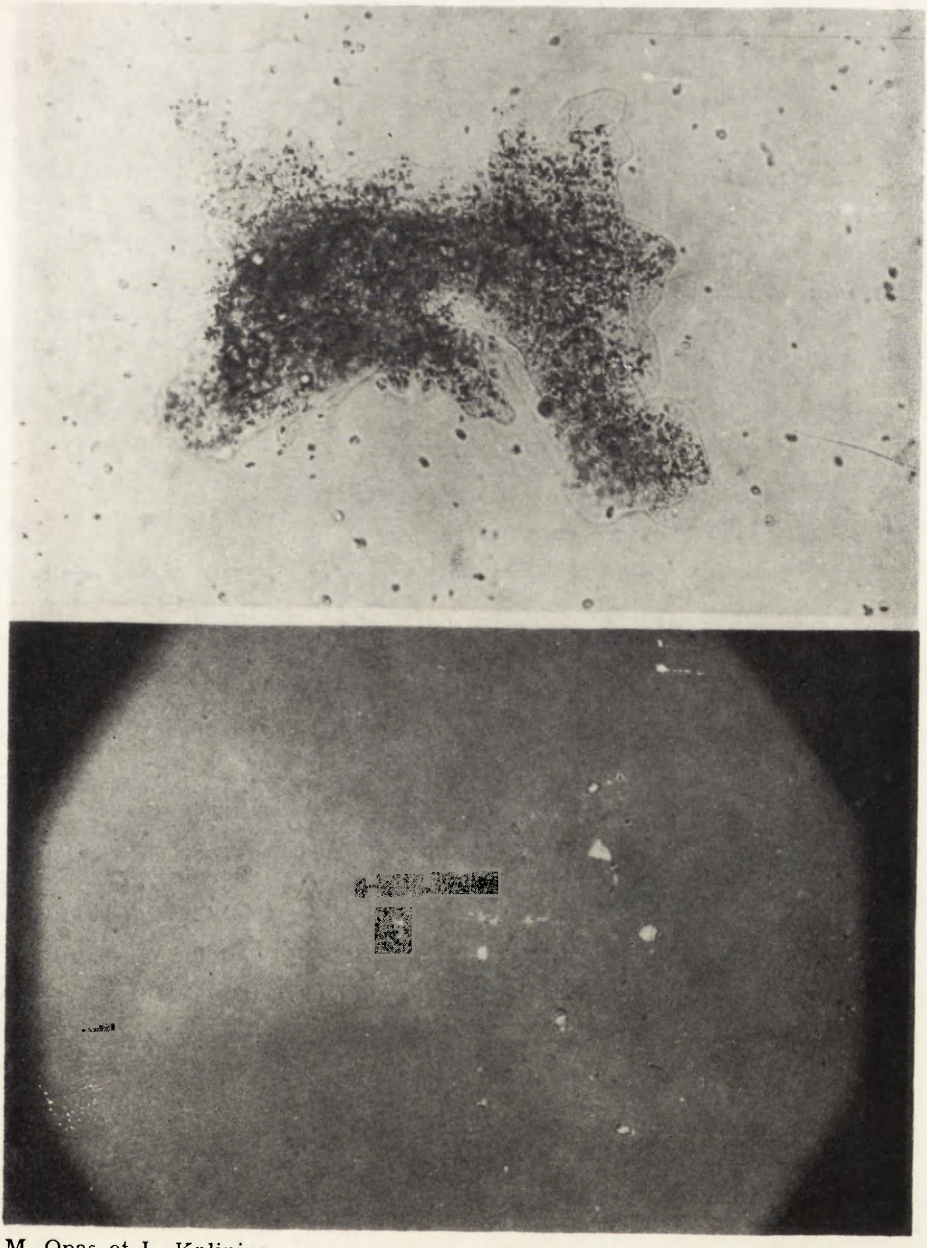

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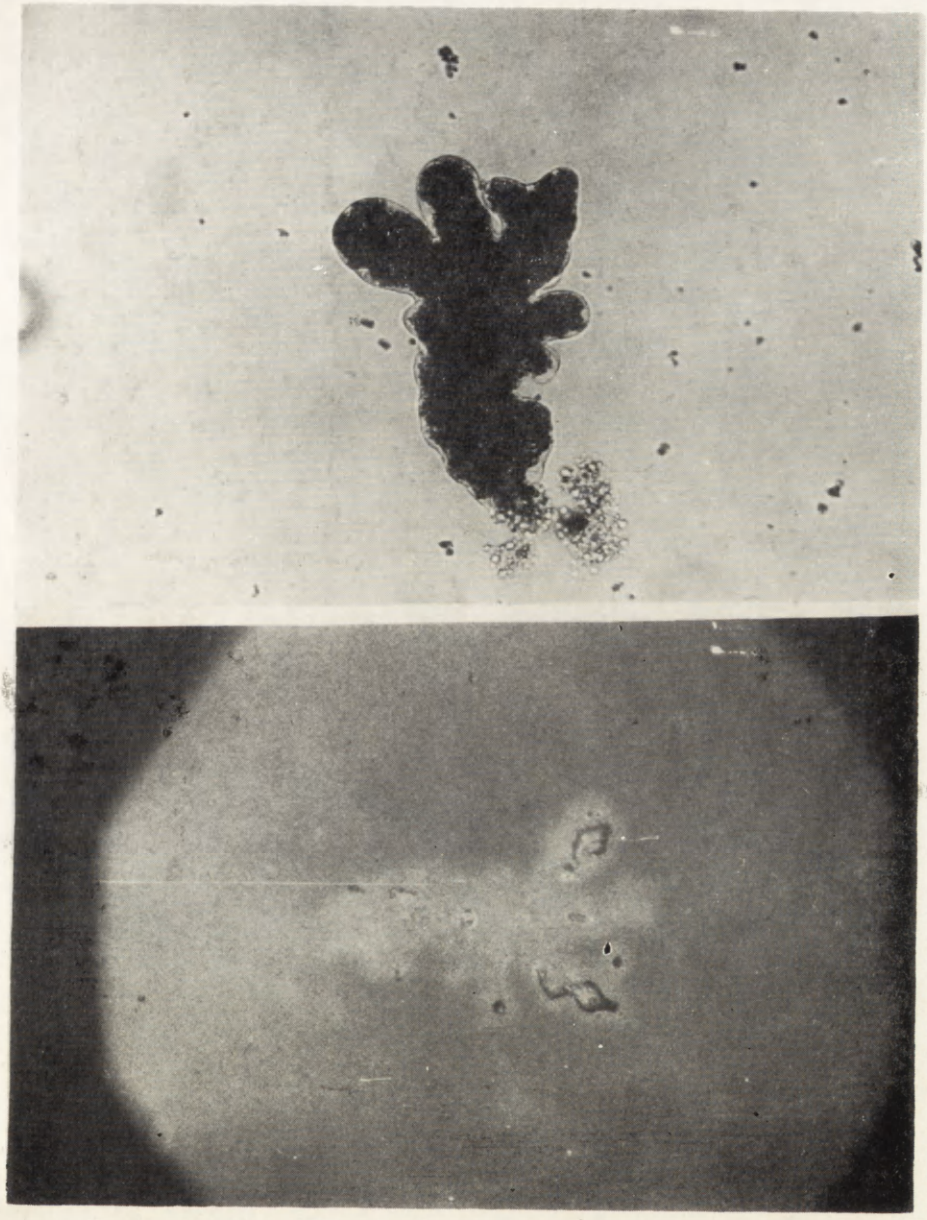

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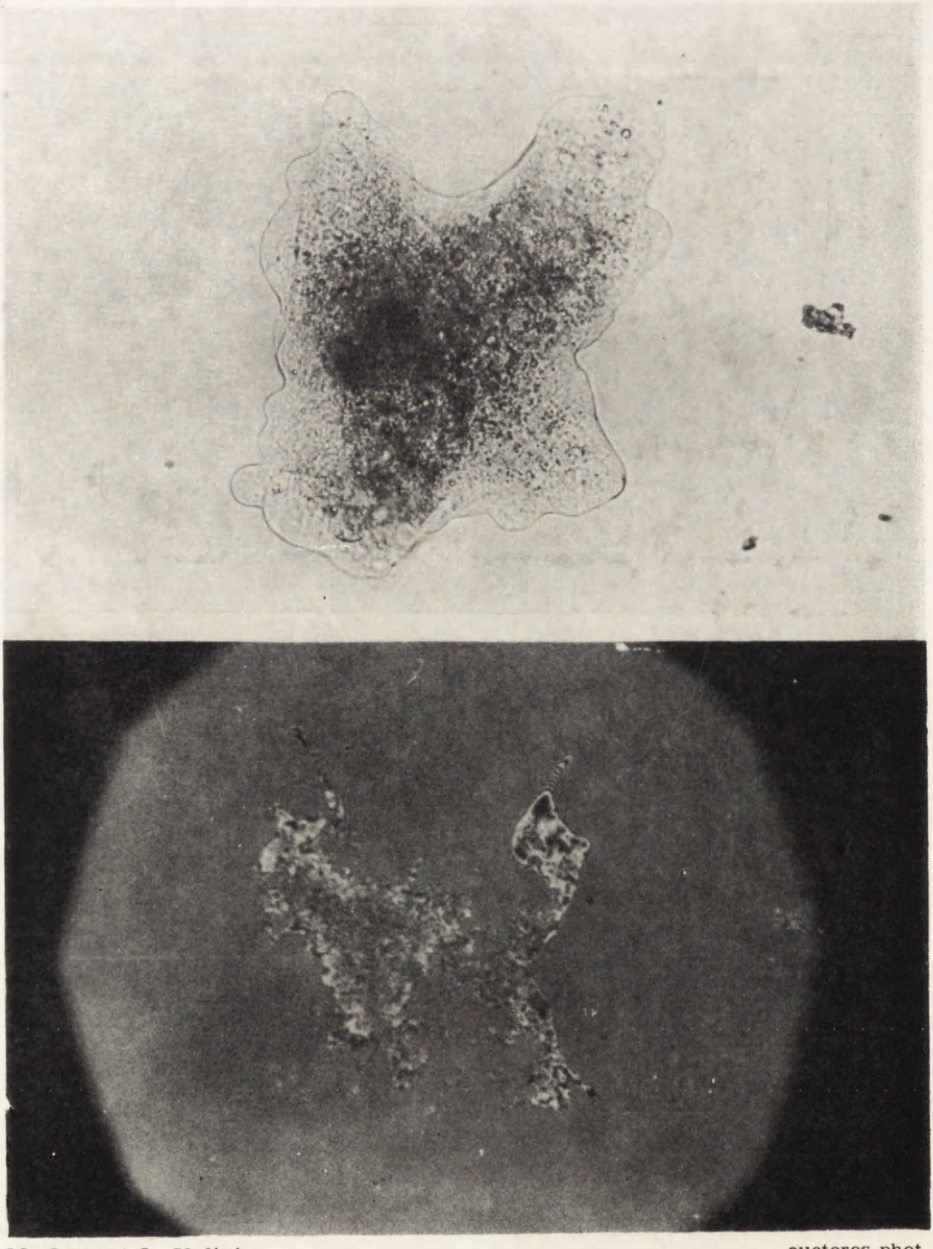

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Cephaline Gregarine, *Hoplorhynchus ramidigitus* sp. n. (*Protozoa: Sparozoa*), Parasite of an Odonate *Agrioenemis pygmaea* (Rambur) from India

Received on 19 May 1980

Synopsis. The cephaline gregarine (*Protozoa*: *Sporozoa*) *Hoplorhynchus ramidigitus* sp. n. is described from the midgut of an odonate insect *Agrioenemis pygmaea* (Rambur). The gregarine has the ratio of LP:TL = 1:4.9 and WP:WD = 1:0.97. The genus is reported for the first time from India.

Carus in 1863 established the genus *Hoplorhynchus*. Later, Kamm in 1922 summarized the generic characters of *Hoplorhynchus* as follows: (1) trophozoite with a long neck, (2) epimerite is a flat disc

Table 1

A chronological list of the different species under the genus *Hoplorhynchus* Carus, 1863 described so far is given below:

Parasite	Host	Reference
Hoplorhynchus oligocanthus (Siebold) Schneider, 1875	*Gomphus vulgatissmus* L. *Onychogomphus uncatus* Charp.	Schneider (1875)
H. actinotus (Leidy)	*Scolopocryptops sexspinosus* *Scolopocryptops* sp.	Crawley (1903)
H. scolopendras Crawley, 1903	*Scolopendra woodi*	Crawley (1903)
H. ozakii Hukui, 1952	*Otocryptops rubiginosus* Koch.	Hukui (1952 a)
H. bouruiensis Hukui, 1952	*Otocryptops rubiginosus* Koch.	Hukui (1952 b)
H. orthetri Hoside, 1953	*Orthetrum albistylum speciosum* Uhler	Hoside (1953)
H. hexacanthus Obata, 1953	*Coenagrion quodrigerum* Selys	Obata (1953)
H. ozaki Hukui, ssp. corcica Theodoridès, Désportes and Jolivet, 1976	*Otocryptops rubiginosus* Koch.	Théodoridès et al. (1976)

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with 8 to 10 digitiform processes at apex, and (3) spores biconical.

The literature available reveals that Schneider (1875), Crawley (1903), Hukui (1952 a, b), Hoside (1953), Obata (1953), Desportes (1963), Baudoin (1967) and Théodoridès et al. (1976) made significant contributions on the genus *Hoplorhynchus* Carus, 1863 and described several new species. A chronological list of the species under the genus *Hoplorhynchus* is given in Table 1.

In course of our investigations on the cephaline gregarines of insects, we have obtained a species of *Hoplorhynchus* from the midgut of the odonate, *Agrioenemis pygmaea* (Rambur). The organism is described hereunder as *Hoplorhynchus ramidigitus* sp. n. for some of its diagnostic characters.

Material and Methods

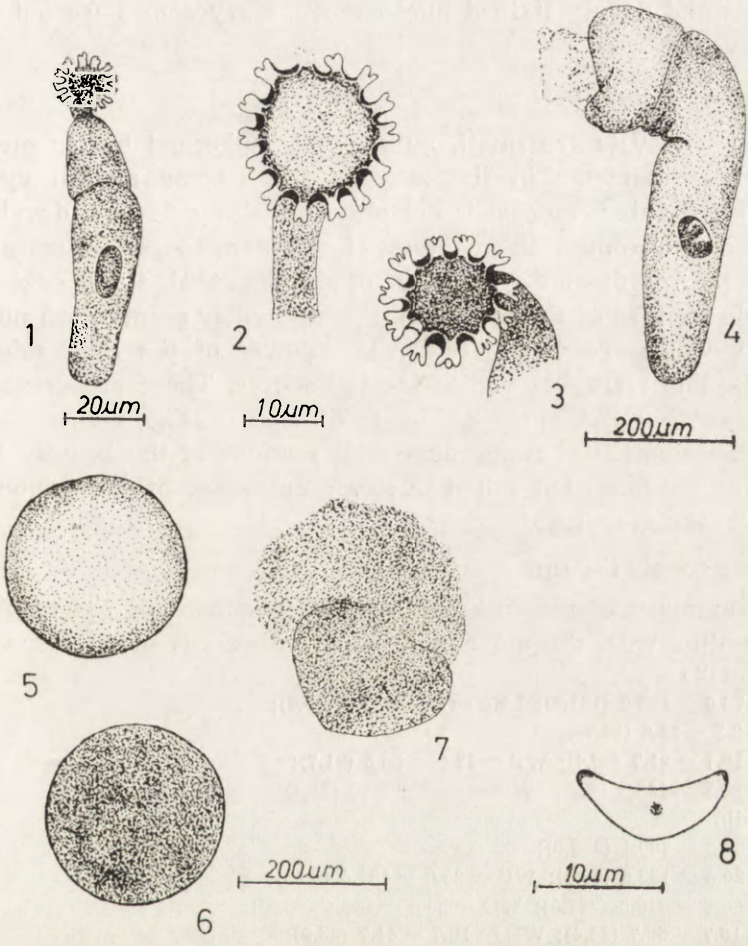
The odonate insects *Agrioenemis pygmaea* (Rambur) were collected from the campus of Chinsurah hospital (about 40 km north of Calcutta) in different dates of the year 1977. These were brought alive to the laboratory, and examined for their protozoan parasites on the same day. The methods followed in this study were the same as described in our previous paper (Haldar and Sarkar 1979). The ratios used here are those of length of protomerite to total length and the width of protomerite to width of deutomerite. The figures have been drawn with the aid of a Camera lucida.

Observations

Structure of the Trophozoite and Sporadin

The earliest stage obtained from the smears of the midgut contents of the odonate is a trophozoite having a disc-like epimerite, a conical protomerite and a cylindrical deutomerite with an ellipsoidal nucleus at its centre (Fig. 1 *1*). On an average, tropozoites measure 131.0 µm in length and 21.7 µm in width. The epimerite is placed on a neck which may reach a length of up to 33.3 µm. From the disc of the epimerite 11 to 14 short, stumpy, digitiform processes are radiated peripherally which may be bifid or trifid (Fig. 1 *2* and *3*). The epimerite measures 16.7 µm in diameter in the average. The septum between the neck and the protomerite is not very distinct but that between the protomerite and deutomerite is distinct and lenticular. The deutomerite is almost cylindrical with a truncated end.

The sporadins (Fig. 1 *4*) are solitary cylindrical bodies measuring



Camera lucida drawings of *Hoplorhynchus ramidigitus* sp. n. 1 — A trophozoite — having bifid processes of its epimerite. 2 — The epimerite — enlarged, 3 — The epimerite — enlarged to show its trifid nature, 4 — A fully grown sporadin, 5 — Gametocyst — early stage, 6 — Gametocyst — advance stage, 7 — Gametocyst — bursts by simple rupture, 8 — A spore — crescentic

302.0 µm in length and 83.6 µm in width in the average. The protomerite is somewhat rectangular and the deutomerite is cylindrical with an ovoidal nucleus in the later.

In trophozoites, the cytoplasm is differentiated into an outer hyaline epicyte and inner granular endocyte in protomerite and deutomerite; in sporadins, however, this differentiation is not clearly discernable.

The nucleus is ellipsoidal to ovoidal in shape, measuring 34.4 µm × 23.9 µm in dimensions in the average and situated more or less in the middle of the deutomerite. It consists of a distinct nuclear

membrane and deeply stained nucleoplasm. Karyosomes are not distinctly observed.

Gametocyst and Spore

The gametocysts are small, milky white spherical bodies measuring 240.0 μm in diameter in the average and having a thin cyst wall (Fig. 1 5). An outer ectocyst is not observed. After 24 h of development in the moist chamber, the content of the gametocyst becomes highly granular (Fig. 1 6) and after 96 h of development, these ooze out by the simple rupture of the cyst wall at a particular point when numerous refractile bodies are visible under low power of the light microscope (Fig. 1 7). The refractile bodies are the spores. These are crescentic in shape measuring 12.0 μm × 3.5 μm in dimension (Fig. 1 8).

The examination of numerous serial sections of the heavily infected midguts of the hosts has failed to locate any stage of endogenous development of the gregarine.

Measurements (in μm)

The summary of measurements of 20 specimens of the trophozoites and sporadins with the mean within parenthesis is given below:

Trophozoite
TL = 75.0 – 175.0 (131.7); LNo = 8.3 – 33.3 (16.6);
DE = 8.3 – 25.0 (16.7);
LP = 16.7 – 41.7 (28.4); WP = 12.5 – 33.3 (21.7);
LD = 41.7 – 108.3 (76.7); WD = 16.7 – 25.0 (21.7);
Sporadin
TL = 87.5 – 700.1 (302.0);
LP = 20.8 – 133.6 (60.5); WP = 25.0 – 183.4 (85.9);
LD = 66.7 – 616.8 (241.5); WD = 25.0 – 183.4 (83.6);
LN = 16.7 – 66.7 (34.4); WN = 16.7 – 41.7 (23.9);
LP:TL = 1:4.9; WP:WD = 1:0.97

Details of measurements of 20 specimens are given in Table 2.

Seasonal Intensity

The host insects were collected during the months of March to November, 1977. During other months these were not available at all. The parasites were mostly found during March-April and September-October. On an average, 31.6% of the insects were found to be infested in their midguts.

Material

Holotype — on slide No. Od_6 H-5, prepared from the midgut contents of *Agrioenemis pygmaea* (Rambur) collected at Chinsurah, West Bengal, India on 3 September, 1977; Paratype — many, on the above

Table 2

Showing details of measurements of the different parts of 20 specimens of *Hoplorhynchus ramidigitus* sp. n. (in μm)

Serial No.	TL	DE	LP	LD	LNc	LN	WP	WD	WN
1	150.0	25.0	41.7	66.7	8.3	16.7	25.0	25.0	—
2	175.0	16.7	41.7	83.3	33.3	—	33.3	33.3	—
3	125.0	16.7	25.0	66.7	16.7	—	12.5	16.7	—
4	75.0	8.3	16.7	41.7	8.3	—	16.7	20.9	—
5	133.5	16.7	16.7	83.5	16.6	—	20.8	20.8	—
6	668.8	—	133.5	535.3	—	66.7	183.3	166.7	41.7
7	166.71	—	50.0	116.7	—	—	33.3	50.0	—
8	433.4	—	100.0	333.4	—	—	183.3	150.0	—
9	133.6	—	33.3	100.3	—	20.8	41.9	41.7	—
10	233.4	—	83.4	150.0	—	33.3	58.3	58.3	16.7
11	87.5	—	20.8	66.7	—	—	25.0	25.0	—
12	300.0	—	66.7	233.3	—	—	75.0	75.0	—
13	116.7	—	29.2	87.5	—	—	37.5	41.6	—
14	250.0	—	50.0	200.0	—	25.0	66.7	58.3	16.7
15	208.4	—	41.6	166.8	—	—	58.3	50.0	—
16	116.7	—	41.7	125.0	—	—	50.0	41.6	—
17	233.4	—	50.0	183.4	—	—	54.2	66.6	—
18	216.7	—	41.7	175.0	—	33.3	66.6	58.3	28.8
19	616.8	—	83.3	533.5	—	—	183.4	183.4	—
20	700.1	—	83.3	616.8	—	58.3	150.0	166.7	33.3

LP:TL = 1:4.9; WP:WD = 1:0.97

numbered slide as well as on other slides; other particulars are same as for holotype material.

Affinities

In having a disc-like digitate epimerite and smooth crescentic spores, the gregarine undoubtedly belongs to genus *Hoplorhynchus* Carus, 1863. Although its epimerite exhibits ramification, its spores are smooth and crescentic and not biconical with spines and as such differs from the genus *Ramicephalus* Obata. It resembles *H. oligocanthus* (Siebold) Schneider in the nature of spores, but differs in possessing characteristic epimerite having 12 to 14 peripheral bifid or trifid digitiform processes. It is, therefore, considered as a new taxon and is named *Hoplorhynchus ramidigitus* sp. n. The specific trivial name has been given to stress the very characteristic feature of the epimerite of the gregarine.

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RÉSUMÉ

Le travail contient les déscriptions de la morphologie et du cycle de development d'une nouvelle espèce des grégarines (*Protozoa*: *Sporozoa*), *Hoplorhynchus ramidigitus* (LP:TL = 1:4.9; WP:WD = 1:0.97) de l'intestin de l'odonate, *Agrioenemis pygmaea* (Rambur) aux Indes.

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