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ACTA PROTOZOOLOGICA

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ACTA PROTOZOOLOGICA
VOL. VII WARSZAWA, 30.IX.1969 FASC. 1

Institute of Zoology, Warsaw University, Warszawa 64, Poland

J. KACZANOWSKA and D. KOWALSKA

Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O.F.M.) I. The cortical organelles and intraclonal dimorphism

Studia nad topografią organelli kortykalnych *Chilodonella cucullulus* (O.F.M.) I. Organelle kortykalne i dymorfizm wewnątrzklonalny

The aim of the present series of studies is a tentative description of cortical organelles disposition on the flat ventral surface of *Chilodonella cucullulus* (O.F.M.) The studies include both the interdivisional period, as well, as the stages of the development of offspring structures in the parental pattern.

The present paper contains the result of the first section of studies. The papers which will follow, would deal with the relation of the total number of kineties to the CVPs (contractile vacuole pores) disposition (Kowalska and Kaczanowska 1969 in press) and the geometry with topography of the cortical organelles.

We would like to thank Prof. Dr. Z. Raabe for valuable comments and critisism of the manuscript.

Materials and methods

Studies were carried out on stocks marked B, C, S and clone B_1 of *Chilodonella cucullulus* (O.F.M.) isolated from mass cultures from the sewage treatment centre at Józefów near Warsaw. Stock were isolated in March 1967.

Stock C died out in May of the same year, while stock S died out in April 1968. Stock B underwent conjugation in the fall of 1967 and from one exconjugant from one caryonide clone B_1 was isolated. In this clone mass intraclonal conjugation is periodically observed (experimentally tested by S. Radzikowski M. Sc. unpublished). After the mass conjugation of B_1 exconjugants were not re-isolated and the conventional mark B_1 is applied for each subclone or substocks isolated from the periodically conjugating pool of initial clone B_1. This pool B_1 is cultivated till the present time.

Protozoans are cultivated in room temperature in Petri dishes in a small amount of tap water to which a drop of fresh baker yeast suspension is added. The protozoans are fed every second day and inoculated every 4—6-th

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day. The sporadic infection of cultures with *Chilodonella uncinata*, or fungi was only removed by means of re-isolation of protozoans.

The cultures used in experiments were started from a single ciliate isolated from the stock cultures.

The infravital observations were conducted by contrast phase microscopy. Observations were also made of stained ciliates with nigrozin and silvered after Klein and most of them after Chatton-Lwoff in Corliss (1953) modification.

Three series of experiments with protozoans of the same age were conducted on stock S and clone B_1 in a period when the ciliates were in good physiological condition. Individual samples contained about 10 dividers were isolated during five minutes in stage of advanced cytokinesis. The process of cytokinesis was completed in isolated specimens within 5 to 8 min. Next, samples were fed with fresh dose of yeast and placed in a moist chamber.

The individual samples were successively fixed hour by hour from the moment of isolation up to the moment when the next cytokinesis was observed in at least one specimen. This means that the last samples was fixed in a period of less than an hour. The remaining samples were further observed to test the degree of the cell division synchrony. It appeared that the remaining control protozoans in general divided quite synchronously within half an hour or so. However, in few controls single (one or two) ciliates were recorded that were few hours late in comparison with the remaining ones. This results points to the possibility of similar cases also occuring in the examined samples. It should be added that isolated and fixed ciliates can considerably differ from each other, as to degree of number of yeasts ingested even if they were in the same sample. This difference occurs always in well fed stocks and results in the tendency of specimens to form clusters on the bottom.

All the above data taken together prove that methods of cultivation have not been sufficiently elaborated and only supply limited data.

Finally, after the silvering of the samples had been made only 7—13 well-stained specimens were analised from each sample. Additional experiments are described in the main section of paper.

Results

The cortical pattern of *Chilodonella cucullulus*

The description of the morphology of ciliate corresponds to the redescription of this species by Fauré-Fremiet 1950, Dobrzańska-Kaczanowska 1965 and Radzikowski 1966.

The ventral surface is characterized by kineties arranged in the furrows interspaced by ridges. Somatic ciliature consists of kineties of the right system curved to the left in the apical region, in front of the oral complex. Next, usually, there are three postoral kineties. Kineties of the left system of ventral ciliature are regularly interspaced with the rest of kineties. Three marginal left kineties are successively shorter and shorter counting from right to left. So the extreme left is very short and appears only in anterior part of the cell body.

The left kineties reach anteriorly the preoral kinety (one of the oral kineties) which separates the left and right systems of kineties.

Oral ciliature consists of two or tree circumoral kineties and the preoral kinety. These kineties are inverted in relation to the main body axis and to the rest of the ventral ciliature (according to Deroux 1968 statement for the other cyrtophorous ciliate) and have membranellar-like character (Radzikowski 1966) and a different fine structure (Sołtyńska personal communication).

The arched dorsal surface is devoid of cilia except for one oblique kinety X (after Radzikowski 1966) originated from the right, kineties system during division.

The infravital observations (Pl. I, 1) and stains (Dobrzańska-Kaczanowska 1965) point to the existence of alveolar system with a liquid content, able to mutual deformation under the pression and to the fusion.

The silvered dorsal cortex (dry method after Klein) consists of argyrome and conspicous disseminated dark granules. In Chatton-Lwoff slides these granules can be seen clearly. (Pl. I 2, IV 17). The role of these structures is unknown. In some young nondividers (opisthes) they are absent in the apical region. It is suggested that they multiply in the interdivisional period.

The oral apparatus of this ciliate consists of oral kineties and oral complex. Cytostome is elongated and covered with membrane bearing a slit. The margin of membrane around the slit is more strongly silvered that the remaining part of the cortex and it is possible to distinguish its external part and the dark edge of the oral slit (Pl. I 3).

The oral basket is made up of closely arranged rods of nemadesmes with the two apical processes of triangular small nemadesmes on each rod, and of the cytopharyngeal tube. The disposition of the oral slit towards the apical-antiapical axis may vary. It can be situated almost transversally in relation to the meridional kineties, or obliquely or even almost paralelly to them.

The most frequently occuring configuration of the slit is oblique in relation to the apical-antiapical axis and perpendicular to the preoral kinety (Pl. I 2).

Few separate silvered dots can occur posteriorly to the oral basket. They are not kinetosomes and their function is unknown (Pl. I 3).

The cytoproct is seen as more or less longitudinal depression on the dorsal surface. In Chatton-Lwoff preparations it is seen as an undulating slit placed in the posterior part of the cell body along its right margin (Pl. I 5). This is most probably a permanent structure because it can be seen in any well-silvered specimen. This structure was observed without any comment and drawn in the exconjugants of *Ch. cucullulus* by Radzikowski 1966. Infravital observations were also made on the defecation.

Many authors have observed contractile vacuoles (CVs) of this species. Fauré-Fremiet 1950 drew the contractile vacuole pores (CVPs) between kineties on the ventral surface giving no comments. The observations in vivo and on nigrozin preparations (Pl. I 4) and finally on silvered preparations prove that these are permanent structures. In nigrozin preparations the stain accumulates in the apertures; in the silvered specimens the pore ring is darkened. The diameter of pores is usually 1—1.2 μ and does not change depending on the size of the specimen. One contractile vacuole can be discharged by one, or rarely, two pores. In the latter case the two pores are near to each other, more or less closely in the adjacent interkinetal spaces, or one above the other in the same interkinetal space.

Few cases were observed of two pores of a single vacuole being at a cosiderable distance from each other, up to two interkinetal spaces. In silvered preparations such specimens were classified into class of anomalies bacause there was no clear evidence whether the pores really represent the same vacuole.

The infravital observation has proved that the nondividers have various number of kineties, of CVPs and show different topography of the latter, as well as different frequency of doubling of CVP.

Observations of silvered slides confirmed these statements, but certain regularities of topography of CVPs can be seen. Two main patterns of CVPs topography can be observed in specimens in all stocks and clone B_1. The topography of CVs is described as the intraclonal dimorphism on the base of disposition of CVPs. As it was said above, the cases of doubling of pores of one vacuole (except the class of anomalies) were classified as the one place.

Intraclonal dimorphism

The variability of kineties and vacuoles (each represented by a single or double pores) were examined on the ventral surface in one sample of an initial stock B. The same results were obtained in the clone B_1 too. Table 1 shows the results of analysis of 200 well-silvered specimens of stock B. The drawing made up with Camera lucida on which the topography of CVPs on the cortex were marked allowed in all examined cases to distinguish two patterns of CVPs topography.

Table 1

The number of CVPs and the number of kineties (stock B)

Number of CVPs \ Number of kineties		18	19	20	21	Total
3		3	38	30	4	75
4		—	34	37	1	72
5		1	4	1	—	6
Double CVPs	3	2	10	10	—	22
	4	—	3	8	—	11
	5	—	—	2	—	2
Underdeveloped or anomaly		2	4	6	—	12
Total		8	93	94	5	200

The first pattern represents the tri-vacuoles ciliates. The two anterior vacuoles are situated behind the mouth and are represented by CVP-1 (pore, or two pores) for the right and respectively CVP-2 for the left vacuole. Then the right upper vacuole is usually represented by the pore (or at least one of two pores) in the interkinetal space between the kinety of right system and the first postoral kinety. At a distance of at least five kineties to the left there is at least one pore of the left upper vacuole CV-2.

Cases of smaller or larger distance measured in terms of the number of kineties between the pores of upper vacuoles (CVP-1 to CVP-2) are examples of an increase of total number of kineties, variants of doubling of CVP or some special cases.

The posterior vacuole, represented on the cortex by the pore (or at least one of the pores) CVP-3 is situated in the posterior part of the body, at a distance of four kineties to the left in the relation to CVP-1, and respectively at a distance of one kinety in relation to CVP-2.

The second pattern of CVs disposition is represented by specimens possessing four or five vacuoles. As a rule, the CVPs topography was as follows.

CVP-1 was situated in the same interkinetal space as in the former case i.e. between the right kinety and the first postoral kinety. CVP-2 occurs usually at a distance of six kineties to the left from CVP-1. In some specimen, particularly those with increased body width, the distance between CVP-1 and CVP-2 reaches even eight kineties. But the six kineties distance is most prevailing. CVP-3 occurs in the posterior part, at a distance of four kineties to the left in relation to CVP-1 and respectively of two kineties in relation to CVP-2.

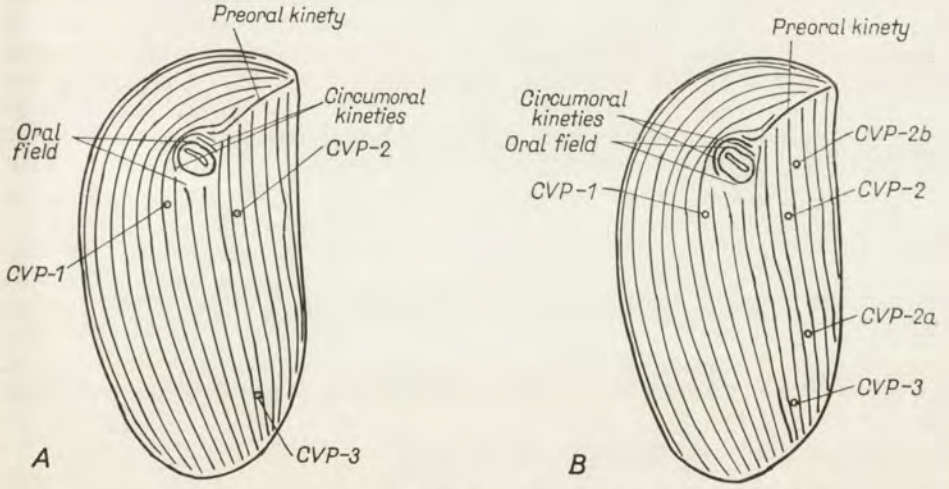

Fig. 1. Pattern of topography of CVPs on the ventral surface of *Chilodonella cucullulus*. A — Typical tri-vacuoles specimen — proter; B — Typical five-vacuoles specimen — opisthe. The four-vacuoles specimen has same pattern, but without CVP-2b

The fourth vacuole represented by CVP-2a occurs in the middle part of the body in the same interkinetal space as CVP-2. In certain cases of ciliates growing very strongly in length, additional pore of CVP-2c was seen posteriorly to CVP-2a in the same interkinetal space. In these rare specimens three vacuoles can function simultanously in a single interkinetal space.

In all stocks, the specimens were found in which the four basic vacuoles namely: CVP-1, CVP-2, CVP-2a, CVP-3 were accompanied by the fifth additional CV-2b represented by single pore CVP-2b. The CVP-2b does not modify

the topography of the other ones. It is situated at a distance of one kinetal space to the left and a little ahaed of CVP-2. This vacuole is situated behind the preoral kinety and to the left of the mouth.

No case was found of simultanous existence of CVP-2b and CVP-2c in the same specimen.

The Figures 1 A and B and Pl. II 6,7 illustrate two patterns of topography of CVPs on the ventral surface.

This pattern of topography of CVPs was seen in the ciliates with different total number of kineties, being of various size and obtained from various stocks.

Observations on the ciliates of the same age

Observations carried out on ciliates of the same age from stock S and a stocks obtained from a progeny of a single cell during four days from clone B_1 allow to state that the number of CVs does not increase in the interdivisional period. Two patterns of CVPs topography are also preserved in every samples.

During about two thirds of the whole generation time gradual elongation of the ciliates has been observed. About two hours before expected division an allometric intensive growth of the region of the right margin of the flattened cell body was observed. The ciliates acquire a kidney-like shape and all kineties are curved as a bow in the equatorial zone. In that period all kinetosomes are equally intensely silvered and are rarely distributed along kineties.

About 90—60 minutes prior division characteristic changes of shape were seen. The entire ciliature is straightened in the equatorial zone and the external kineties of the left system which were very short are now being lengthened.

Yet the scope of variability of total number of kineties does not change in these experiments.

There is no folding of the cortex and no visible changes of stiffness. This suggests that these are allometric and gradient phases of surface growth. The straightening of the whole ciliature in the equatorial zone most probably takes place behind the oral region and spreads out to the left margin. This interpretation is supported by the facts, that the oral slit is strongly curved downwards and so it becomes almost parallel oriented to the main body axis, the CVP-2 is displaced posteriorly and the kineties of the left system are lengthened except the extreme one.

The growth of the left part of the ventral surface does not however balance the strong allometry of the right margin of the flattened body which continues to be strongly curved. This characteristic body shape caused by the allometry of cortex growth is shown in Pl. II 8.

This shape corresponds to the first signs of divisional morphogenesis. On the whole equatorial zone (with some exception, see below) kineties are crowded with kinetosomes. These kinetosomes are silvered more strongly and they are a little bigger than the less silvered kinetosomes of apical and antiapical region of the same kineties. Some counting of the first postoral kinety kinetosomes supports the suggestion that it is really a period of intensive synthesis of kinetosomes. It should be added that during the repair of transversal cutting of kineties also more intensive and extensive darkening of kinetosomes was observed in the region of healing. The nature of this phenomenon is unknown.

However in the equatorial zone the segments of somatic kineties in area of presumptive future oral segments do not silver strongly.

In some specimens the stage of total resorption of the segments of somatic ciliature was observed exactly in the region of the future differentiation of the oral segments (Pl. II 9) and was followed by the stage of new synthesis of kinetosomes in this region. Such resorption can also take place simultaneously with the differentiation of the oral ciliature and this process can be detected only at two ends of synthesized oral kineties of the future opisthe.

In this period the other symptoms of approaching division could be observed, as the differentiation of kinety X″ for the future opisthe and the formation of local cavity (a little ring-like deepening for the oral anlagen) for opisthe.

The data based on the comparison of the samples of the same age are not quite consistent as to the size, number of kineties and kinetosomes in the first postoral kinety and permit us only to make general remarks on change in shape body as it is visualized in Table 2.

Table 2
Comparison of the length of time of the succesive stages of growth of ciliates of the same age

Stock	Minimal generation time in hours	Period of growth of right kineties in hours	Period of early division in hours
S	9.5	7—8	8—9
Clone B_1	9.2	7—8	8
Clone B_2	10.6	9—10	10

Morphogenesis in division and the development of cortical structures

The characteristic shape observed in the predivisional stage allowed to observe a number of processes in isolated specimens in the earliest stages of division. The whole process is arbitrary divided into some stages. This proposition may change however if comparative nuclear and cortical phases will be tested.

1. Early induction stage. This stage lasts about 30 min. It begins about 90 min before the separation of the offsprings. The first symptom is the characteristic change of shape of the cell as described in previous section. The order and sequence of these symptoms may vary in a considerable limits. This different level of advancement of the cortical structures in each particular specimen is very characteristic feature of early stages of morphogenesis of *Chilodonella*. So one of the first differentation is arising of kinety X″. This kinety arises as follows. Above the equatorial zone on the right margin of the ventral ciliature a slight local cavity appears. The first kinetosomes were noticed in this cavity. Therefore the stages of delineation of kinety X″ were not observed as it was described for *Ch. uncinata* (Chatton et al. 1931). It is possible that we omitted such stages however the youngest kinety X″ observed in our slides had only four kinetosomes. In any case further development proceeds by multiplication of kinetosomes along the cavity. Cilia

and interkinetosomal fibrillar systems are formed in a very early phase of the differentiation of this region.

In the same period, or a little earlier the resorption of somatic ciliature in the region of future oral opisthe ciliature may occur. Resorption included both: kinetosomes and fibrills among them and the surface in this region is smoothened. Simultanously intensive synthesis of oral ciliature can be observed. These kinetosomes are quickly equipped with cilia and form membranellar structures. Their differentiation was observed in vivo and in the slides in the cases when single oral segment were composed of no more than a few kinetosomes.

On the "in vivo" individuals the oral ring as the oral anlagen could be seen in the middle part of ventral surface. May be that it is represented as a dot in silvered specimens in the interkinetal space between the second and the third postoral kinety.

The fourth phenomenon (which can be the next after differentiation of kinety X″ however) in this period is the appearance of the primary sites of future CVPs. The primary sites are formed, as a little spots adjacent to the proper kinety on their left side. The new CVPs are formed in various distance from parental structures and they are not their products. The spots change into transversal slits across the half of interkinetal distance. As a rule a little more sites of induction of CVPs are formed in the posterior part of the ciliate in its left system (Pl. III 10) and only few of them develop, while the remaining ones are resorbed.

The arising of new CVPs does not cause any visible changes in the structure and function of the old CVs. The new pores are formed in a characteristic order in following sequence: the new upper CVPs for proter, opisthe and posterior CVP for opisthe and a little later CVP-2a″ and CVP-3′ (i.e. fourth vacuole for opisthe and posterior vacuole for proter). Moment of appearing of CVP-2b″ was not observed, but it probably takes place early. The cases of the formation of CVP-2c″ are probably connected with the supporting of the development of the one site more of primary differentiation.

2. The stage of complete synthesis of the oral segments and separation of somatic segments A_4 involved in morphogenesis. This stage lasts about 20 min and may overlap with the former one (Pl. III 11, 12). Kinety X″ is crowded with kinetosomes on full length.

The rapid synthesis of kinetosomes of oral ciliature causes the seperation of the distinct, subequatorial segments: In the second postoral kinety in the subequatorial zone short segment of somatic ciliature (which was not resorbed and replaced) A_4 precedes the proper oral segment A_1 placed more posteriad than the others. The segment A_1 will become preoral kinety, and segment A_4 is a somatic primordium of kinety that will later supplement the ciliature of the future opisthe. These abbrewiations used here are following to Radzikowski 1966 description.

The next two or sometimes three oral segments are situated in the equatorial zone. In this stage the oral primordium for opisthe in the form of rosette appears beneath the oral ring. The development of the proter's rosette was not observed in this stage in silvered slides. There was not any change in the oral area of parental structure too.

In this stage stability of the activated sites is attained. Some of the sites of primary differentiation disappear. The growing sites transform into an oval

transversal openings and lose their contact with kineties. Beneath them the new vacuoles are formed and their function is initiated. The structure and function of parental CVs is still preserved.

3. The stage of morphogenetic movement of the oral ciliature of future opisthe. It lasts about 20 minutes or even less.

The course of this puzzling process is consistent with Radzikowski 1966 description. The morphogenetic movement involves the rotation of the following segments: somatic segment A_4 is pushed to the posterior part of the body, the second, third and eventually also fourth oral kineties being moved to the right, beginning with their anterior ends and the first oral kinety A_1 is drawn upwards and placed in front of the curved oral kineties that have already been put into rotation. The local displacement of these all segments and the oral primordium is accompanied by an allometric growth of the right kineties particularly in the zone just posterior to the future fission line.

The anterior part of the cell body which will form future proter grows too. This growth is connected mostly with the isometric growth of equatorial zone. In that stage all vacuoles can function, old and new as well. The new CVPs are already completely round but somewhat smaller than the old ones. It is possible that the ring of CVPs is not still thickened. Pl. III 13, 14. Fig. 2. The oral field of the future proter does not change.

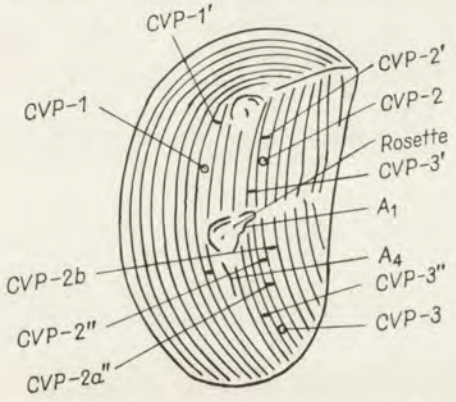

Fig. 2. Pattern of topography of old and new CVPs. It is tri-vacuoles parental specimen (CVPs) that will divide into tri-vacuoles proter (CVPs') and five-vacuoles opisthe (CVPs"). Somatic segment A_4 is formed from the second postoral kinety of parental specimen. CVPs numbers marked with figures

Nevertheless cases have been recorded of the resorption of the old basket in that stage and the appearance of the rosette of the future proter.

In the same stage considerable lengthening of cytoproct has been seen and the appearance of a line in the respective part of proter. Plate IV 16 showing the dorsal side of a ciliate in that stage supplies a picture of a short segment (?) before the kinety X" of the opisthe. We think that this picture is an example of the first stage of appearence of the future proter cytoproct. However, the possibility that the anterior segment represents abnormal, isolated anterior segment of kinety X" can not be excluded, though it seems rather unlikely.

The outline of elongated cytoproct in the part of future opisthe is clearly visible. This cytoproct is silvered more strongly in anterior than in the posterior part.

Usually kinety X″ is already displaced to the dorsal surface of ciliate. The arched dorsal surface is clearly swollen in its equatorial zone.

4. Early stage of cytokinesis. It lasts for only 10 min. or so. It is characterized by the fact that in majority cases the oral field of the parental specimen looks as in the interdivisional period, through the rosette of the future proter is clearly seen in the stage of basket formation. The furrowing of the cell body in the equatorial zone is marked, and it is accompanied by the disruption of kineties on the left side of the oral basket. The extreme kinety of the left system does not reach the equatorial zone of furrowing. This kinety belongs to the proter only. So it is a case of „evolution par decalage" after Chatton et al. 1931 model. The ciliature of the opisthe is supplemented with the segment A_4 ingrowing between the preexisting kineties as the second postoral kinety.

Particularly rapid allometric growth of the right kineties and cortex in the equatorial zone causes that the arising proter and opisthe are not in the same axis. However in the proter part isometric growth predominates and the allometry prevails in the opisthe. In this stage the whole surface of the future proter is equal with the opisthe surface.

The opisthe cytoproct is now shorter than before, and the growth of the proter cytoproct can be seen Pl. IV 17.

In that stage also the function of the parental CVs dissappears. The new CVPs have already attained their normal size and form. On the dorsal side the furrowing of the cell is clearly seen (Pl. IV 14, 15).

5. Advanced cytokinesis. This stage lasts from 5 to 7 minutes and in normal condition does not exceed 12 min.

The external structure of the parental oral complex is completely resorbed (Pl. IV 14). The new oral aperture of the proter has the same shape and form as it is found in the opisthe. The details of this process are obscure and the further study on this subject is needed. On the silvered specimens the round oral aperture of proter or opisthe is bordered by thin strongly darkening ring.

The old CVPs are gradually resorbed. In the final stage of cytokinesis, or immediately after the separation of cells they are represented as little dots on the cortex. These dots also disappear and there is no trace of the old CVPs on the cortex of the offsprings.

The final stage of cytokinesis is very complex phenomenon. It includes the displacement of the opisthe kinety X″ to its proper, final position, the putting up of the right opisthe kineties ahaed of the oral complex, and stretching preoral kinety to the full length which separates the right and the left system of kineties in the opisthe apical region.

Relation of topography of CVPs of the parental cells with the patterns of topography of CVPs of the offspring

As many as 150 dividers from all stocks and clone B_1 was examined in the various stage of division. The results of the analysis of the parental and daughter patterns in the same specimens agree together and are consistent to the case of stock B visualized in Table 3.

It was found that two patterns of disposition of CVPs (marked as CVPs′ for proter and CVPs″ for opisthe) correspond to proters and opisthes regardless of their parental pattern (Pl. IV 15).

Table 3
Comparison of the number of CVs of parental specimens and the number of CVs of their offsprings (stock B)

Number of CVs in parental specimen	Proter				Opisthe			
	3	4	5	anomaly	3	4	5	anomaly
3	16	—	—	—	—	12	3	1
4	22	—	—	—	—	14	8	—
?	6	—	—	1	—	4	1	2
Total	44	—	—	1	—	30	12	3

So the tri-vacuoles ciliate with one-kinetal space between CVP-2′ and CVP-3′ represents proter, while four or five-vacuoles ciliate with the two--kinetal space between CVP-2″ and CVP-3″ and with additional vacuole CVP-2a is characteristic for opisthe pattern.

Cases of tri-vacuoles specimens with two-kinetal space between CVP-2 and CVP-3 usually belong to the group of under-developed opisthes, but it may happen that they are defective proters, as well. These are rare cases of the CVPs pattern nonhomogenous as one regards their origin.

Stability of the patterns of CVPs in the interdivisional period

Studies on the ciliates of the same age, and the analysis of dividers points that the differentiation of two patterns takes place during division. The above conclusion has been supplemented with the analysis of pattern of isolated proters and opisthes during division and fixed 2—6 hours later.

Table 4
Number of CVs and total number of kineties in isolated proters and opisthes (stock B)

Number of CVs \ Number of kineties		18	19	20	21	?	Total
Proter	3	3	4	2	3	2	14
Opisthe	3*	2	1	—	—	—	3
	4	4	2	3	2	—	11

* Ciliate with underdeveloped four-vacuoles pattern.

The results (Table 4) have confirmed the supposition. Three specimens tri-vacuolated, which were opisthes, represent the underdeveloped opisthe pattern (only lack of CVP-2a″).

Discussion

Observation on changes of the cell body in the predivisional stages point to the allometric growth of cell surface in various periods. This problem calls for studies on genetically uniform material kept in defined set of conditions.

During the cell division, resorption of many cortical organelles and their production de novo in the daughter cells was observed. The resorption includes oral complex (oral basket and the membrane bearing slit), old CVs and their CVPs and probably a part of somatic ciliature.

Observation of the elongation and shortening of the original cytoproct observed during division can be interpreted as the resorption of the old and formation of the new cytoproct for opisthe. Nevertheless data are not sufficient to draw the final conclusion from these observations.

The new structures: kinety X″, oral ciliature for opisthe, new CVPs and CVs, and probably the cytoprocts are formed in a new topography in the offsprings regardless the parental patterns of these structures. There are at least two exceptions i.e. the same localization of the parental and proter oral complex and the pattern of right somatic kineties.

As it could be based on such limited data these structures are the base for cytotactic influence on the topography of the daughter cells (Sonneborn 1964).

There are no data proving whether the oral kineties of the proter are replaced in situ and about the earliest stages of morphogenesis of the oral complex. All above data suggest that the morphogenesis encompasses the entire ventral surface.

The phenomenon of the reconstruction of CVs and CVPs in every division is similar to phenomena described in *Euplotes* (Diller and Maloney 1968).

The sites of primary differenciation of the new CVPs during division are always adjacent to the left side of kinety. So it seems that the kineties could be activated and become the local centers for differentiation of CVPs. The activation only some of them would be concordant with studies of Beisson and Sonneborn 1965 and Nanney 1967.

In the divisional morphogenesis two kinds of resorption take place: that of fully mature CVPs of the parental specimen, and that of some sites of primary induction of CVPs. The second case is similar to that of the formation of the two oral anlagen for opisthe in certain specimens of *Tetrahymena* in the phenomenon of cortical slippage (Nanney 1967). In the strain U.M. 981 natural resorption of one of them was noticed.

The observed intraclonal and in the stocks dimorphism arises during morphogenesis in division. Similar phenomenon was described in *Euplotes vanus* by Hufnagel and Torch 1967, concerning the different number of the right caudal cirri in the proter and in the opisthe. The same authors pointed to the epigenetic and cortical character of this process.

Tartar 1961, Sonneborn 1963, 1964, Nanney 1966 a, b gave the evidence of the existence of some pattern of cortical factors regulating the topography and number of arising structures during morphogenesis.

On the other hand de Haller 1964 and Heckmann and Frankel 1968 tested the genic control of morphogenesis and cortical pattern.

Within the limited scope of this study the intraclonal dimorphism seems

cortically determined. It is suggested by us that the different number of CVs in proter and opisthe may be related with the smaller part of parental surface organized in to the future proter in comparison with the future opisthe in the stage of arising of CVPs. The another supposition is that the earlier stomatogenesis in opisthe is related with more intensive primary induction of CVPs in comparison with the proter, which may be is more inhibited by the old parental pattern. However all these assumptions are very unsufficient for description of the phenomenon. Further studies on the topography of CVPs should be undertaken and more experiments are desired.

The second problem is beyond the scope of this study. This is the problem of the possibility of genic control of intraclonal dimorphism. May be, that the contradiction between the cortical or genic control of this phenomenon can be overtaken, if we asume that the divisional morphogenesis as a whole is one process under the genic control. The individuality of daughter units is attained gradually in the course of parental further development (i.e. divisional morphogenesis itself). If so, any event no homothetigenic in sensu Corliss 1961 will give two different morphological forms of offsprings.

Summary

The paper contains a description of a number of cortical organelles of *Chilodonella cucullulus* (O.F.M.) e.g.: the oral complex, contractile vacuole pores (CVPs) and cytoproct.

The study of morphogenesis in division has proved or suggested that old structures are resorbed and the new ones are formed in the proter and opisthe.

Interclonal dimorphism has been found, based with two patterns of topography and number of CVPs on the ventral surface.

The study of morphogenesis in division has revealed that the dimorphism results in the formation of a different pattern of CVPs disposition in the proter and in the opisthe. It has been found that specimens representing both morphological types have the same pattern and type of morphogenesis.

STRESZCZENIE

W pracy tej opisano, lub uzupełniono opis szeregu struktur kortykalnych *Chilodonella cucullulus* (O.F.M.) takich jak: aparat gębowy, otworki wodniczek tętniących (CVPs) i cytopyge.

W badaniach nad morfogenezą w podziale stwierdzono, lub sugerowano resorpcję starych struktur i wytworzenie ich de novo w proterze i opistorze.

Stwierdzono dwupostaciowość wewnątrzklonalną związaną z dwoma typami ułożenia i liczby CPVs na orzęsionej powierzchni. Badania podziału orzęska wyjaśniły, że źródłem dymorfizmu jest wytwarzanie innego wzoru rozmieszczenia CPVs dla protera, a innego dla opistora. Stwierdzono, że przystępujące do podziału osobniki obu typów morfologicznych mają ten sam wzór i typ morfogenezy.

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EXPLANATIONS TO PLATES I—IV

1. Ventral surface of *Chilodonella cucullulus*. Phase contrast.
2. Argyrome and dark dots on the dorsal surface. Impregnation after Klein.
3. The oral region. Kineties of the oral ciliature, oral slit surrounded with internal and external margin of the mebrane. Behind the oral complex dots of unknown function and postoral kineties are shown. Chatton-Lwoff method.
4. Ventral surface. CVPs are shown. Negative staining with nigrozin.
5. Dorsal surface. The undulating line of cytoproct is clearly visible. Chatton-Lwoff method.
6. Ventral surface of tri-vacuoles specimen. Micrograph made from the dorsal side, so that the pattern is reversed. CVPs are indicated by arrows. Chatton-Lwoff method.
7. Ventral surface of four-vacuoles specimen. Arrows indicate the disposition of CVPs. Chatton-Lwoff method.
8. Ventral surface of tri-vacuoles specimen in predivisional stage. Arrows indicate the CVPs disposition. Chatton-Lwoff method.
9. Ventral surface of specimen in the stage of total resorption of somatic ciliature in the region of future synthesis of oral ciliature for opisthe. Micrograph made from the dorsal side, so the pattern is reversed. Chatton-Lwoff method.
10. Induction of new CVPs. Chatton-Lwoff method.
11. Differentiation of the oral segments. Segment A_4 is not still separated. This is a four-vacuoles ciliate in division. Chatton-Lwoff method.
12. The stage of differentiation of the oral kineties. The arrow points to the presumable position of the rosette of the future opisthe which will be attained after morphogenetic movement. A tri-vacuoles specimen in division. Chatton-Lwooff method.
13. Morphogenetic movement of segments of ciliature of future opisthe. Segment A_4 (arrow) is displaced to the rear between somatic kineties. Segment A_1 of the future preoral kinety is drawn upwards. Tri-vacuoles specimen in the division. The new CVPs have form of the oval pores. No changes are seen in the parental oral region or of the old CVPs. Chatton-Lwoff method.
14. The cytokinesis. Disruption of the left somatic ciliature. The morphogenetic movements of the oral kineties is nearly completed. Strong allometry of the right part of the ventral surface. Resorption of the parental mouth (arrow). Chatton-Lwoff method.
15. Advanced cytokinesis. The drawing of preoral kinety is completed. Apical region of the opisthe is formed. The total resorption of old CVPs. Proter represents tri-vacuoles pattern, opisthe four-vacuoles pattern. The oral field of the proter and opisthe in the same stage. Circular margin surrounds the round oral aperture. Micrograph made from the dorsal side and the pattern is reversed. Chatton-Lwoff method.
16. Stage of morphogenetic movement. Dorsal surface. The primordium of anterior cytoproct (?), kinety X" and elongated cytoproct of opisthe is seen. Chatton-Lwoff method.
17. Early cytokinesis. Dorsal side. Fully developed cytoproct of proter is clearly visible. Opisthe cytoproct shortened and situated in the posterior part of specimen. This micrograph is a montage of two focal plane of one specimen. Chatton-Lwoff method.

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Eine neue Art aus der Gattung *Colpidium* (Stein, 1860): *Colpidium kleini* sp. n. *(Hymenostomatida, Tetrahymenidae)*

A new species of the genus *Colpidium* (Stein, 1860): *Colpidium kleini* sp. n. (*Hymenostomatida, Tetrahymenidae*)

Dem Andenken des kürzlich verstorbenen Entdeckers des Silberliniensystems bei Infusorien, Dr. B. M. Klein gewidmet

Die Unterscheidung der verschiedenen Arten der Gattung *Colpidium* bereitete schon den älteren Forschern große Schwierigkeiten (Stockes, zitiert aus Kahl 1930—1935). Bresslau 1922 konnte schließlich mit Hilfe der verschiedenen Zahl der Cilienreihen zwei sichere Arten unterscheiden: *Colpidium colpoda* Ehr. und *Colpidium campylum* Stockes. Genannter Autor stellt fest, daß bei *Colpidium colpoda* 40—50, bei *Colpidium campylum* 20—25 Cilienreihen charakteristisch sind. Schneider 1930 führt als sicheres Unterscheidungsmerkmal die Tektinhülle an. Nach Reizung sondert *C. colpoda* eine dünne aus sehr kleinen, schwer unterscheidbaren Stäbchen bestehende Hülle, *C. campylum* dagegen eine sehr voluminöse aus deutlich unterscheidbaren Stäbchen, ab. Die Größenverhältnisse der beiden Arten sind zur Bestimmung fast unbrauchbar, da sie je nach Fundort und Ernährungszustand starken Schwankungen unterlegen sind. Dies findet auch Ausdruck in der Literatur. Kahl 1930—35 führt als Länge für *C. colpoda* 100—150 µ, für *C. campylum* 70—120 µ an. Matthes und Wenzel 1966 fanden dagegen für *C. colpoda* nur 90—120 µ, für *C. campylum* nur 50—70 µ.

Später konnte Klein 1928 mit Hilfe des Silberliniensystems *C. colpoda* und *C. campylum* genau differenzieren. Corliss 1953 (zitiert aus Czapik 1968) unterscheidet fünf Arten: *C. colpoda* (Ehrenberg, 1831), *C. striatum* Stockes, 1886, *C. colpidium* (Schewiakoff, 1889), *C. campylum* Stockes, 1886 und *C. truncatum* Stockes, 1885.

Kahl 1930—35 gibt für die Gattung *Colpidium* Stein, 1860 folgende Diagnose:

„Diese Gattung stimmt in der Organisation ihres Mundes durchaus mit *Glaucoma* überein, hat also nur den Wert einer Untergattung (Fig. S. 331, *24, 26*). Der Md. ist an die r. Schmalseite des mäßig abgeflachten Körpers verlagert, während er bei den *Glaucomen* auf der Breitseite liegt. Ein weiterer geringer Unterschied liegt darin, daß die Reihen der dors. Breitseite auf dem präoralen Abschnitt nach r. geknickt sind. Die Caudalborsten dors. am Hinterende sind deutlicher als bei *Glaucoma*. Bisher sind zwei sichere Arten bekannt (*C. colpoda* und *campylum*), deren tatsächliche

Verwandtschaft in interessanter Weise dadurch bestätigt wurde, daß beide gleichmäßig auf einen bestimmten Bazillus mit pathologischer Kettenbildung reagieren, während andere *Glaucomen* diese Reaktion nicht aufweisen (Untersuchungen von E. u. Ch. Chatton über Dystomie bei Infusorien).

Zwei andere, bisher nicht bestätigte Arten, die Stockes aufgestellt hat, werden im Anschluß kurz erwähnt; beide sind *campylum* sehr ähnlich und wohl identisch.

Colpidium (*Paramaecium*) *colpoda* (Ehrb. 1831) Stein, 1860 (*Tillina helia* Stockes, 1885) (Fig. S. 331, *21, 22*). Gr. 100—150 µ. Plump bis schlank ovid, vorne nach r. vorgebogen. Eng gestreift. Die Torsion welche von machen Autoren erwähnt wird, ist nur eine scheinbare; der Eindruck wird durch das Übergreifen der vorderen Dorsalreihen auf die Ventralseite erzeugt, wodurch die präorale Naht stark nach l. gekrümmt wird. Ma. rund oder ellipsoid, mit einem Mi. Sehr verbreitet, zahlreich in fauligen Gewässern, aber nicht im Sapropel. Wohl nur im Süßwasser. Die bisher aus dem Salzwasser gemeldeten Funde bedürfen der Nachprüfung. Eine interessante sehr kleine und ganz konstante Degenerations?-form fand ich einmal recht zahlreich in verjauchtem Wasser (Fig. S. 331, *20*; 40 µ) der Kern war auffallend groß; die präorale Naht nicht nach l. gebogen.

Colpidium (*Tillina*) campylum (Stockes, 1886) Bresslau 1922 (*Glaucoma colpidium* Schew., 1896, *Cryptochilum griseolum* var. *marinum* Gourr.u.R.1886) (Fig. S. 331, *17—19*). Gr. 50—120 µ. Gestalt sehr wechselnd, ja nach Fundort und Ernährung lang fingerförmig bis kurz ovid. Wimperreihen viel weitläufiger als bei *C. colpoda*. Dors. zeigt sich die Knickung der Reihen ± deutlich, aber nie die adorale Depression. Die vtr. präorale Reihennaht ist nicht gekrümmt.

Sehr häufig, zahlreich in fauligen Gewässern, nicht sapropel. Kommt in Oldesloe auch in schwachen Salzwässern nicht vor; es ist daher zweifelhaft, ob das sehr ähnliche *Cryptochilum gris.* var. *mar.* Gourr u.R. ein *Colpidium* ist.

Zwei kleine von Stockes aufgestellte unsichere Arten:

a. *Colpidium truncatum* Stockes, 1885 (Fig. S. 348, *9*). Gr. 50 µ. Als Kennzeichen dienen die vordere schräge Abstützung die etwas engere Streifung und die Lage der c.V., dors. auf dem letzten Fünftel, links der Meridiane.

b. *Colpidium striatum* Stockes, 1886 (Fig. S. 348, *10*) Gr. 50 µ. Kaum von *campylum* zu unterscheiden. Die c.V. liegt weiter hinten. Man darf diese beiden Arten wohl in Zukunft fallen lassen".

Kurze Charakteristik des Silberliniensystems von *Colpidium colpoda* Ehr.

Das System ist ein typisches Streifensystem. Unterschieden werden leistungsmäßig zwei Arten von Fibrillen: 1. die Basalfibrillen (Meridiane 1. Ordnung), die die Basalkörner tragen, und 2. die Protrichozystenfibrillen (Meridiane 2. Ordnung), denen die Protrichozysten angeschlossen sind (Taf. V 17, 19, 20). Charakteristisch ist die dreieckige Cytostombildung mit den drei adoralen Membranellen (AZM) (Taf. V 18). Rechts im Cytostom eine undulierende Membran. Durch die starke Linkskrümmung der präoralen Naht bildet sich eine ausgeprägte Polspirale (Taf. V 18). Cytopyge ventral, kontraktile Vakoule mit dorsal liegenden Exkretionsporus ungefähr in der Mitte des Tieres (Foissner 1967). Bei den anläßlich der Protrichozystenregeneration ablaufenden formativ-plastischen Veränderungen der Meridiane 2. Ordnung teilen sich diese in 4—5 parallel laufende Fibrillen auf, die durch zahlreiche Anastomosen verbunden werden. Dadurch entsteht ein Engmaschengitter (Taf. V 19, 20), welches nach erfolgtem Anschluß der Protrichozysten soweit rückgebildet wird, bis der normale Formzustand wieder erreicht ist (Foissner 1968).

Kurze Charakteristik des Silberliniensystems von *Colpidium campylum* St.

Das Silberliniensystem ist ein typisches Streifensystem. Genau wie bei *C. colpoda* können Silberlinien, denen Basalkörner und solche, denen Protrichosystenkörner angeschlossen sind, unterschieden werden (Taf. VI 21, 22, 24). Charakteristisch ist auch hier die dreieckige Cytostombildung mit den drei adoralen Membranellen und einer undulierenden Membran (Taf. VI 22, 24). Der apikale Polbereich wird durch das einfache Konvergieren der Silberlinien gebildet. Die Silberlinien-Meridiane 1. Ordnung biegen sich um das Cytostom, wodurch das charakteristische Dreieck entsteht. Zur Regeneration der Protrichozysten teilen sich die Meridiane 2. Ordnung nur in zwei parallel laufende Fibrillen auf, die ganz sporadisch durch Anastomosen verbunden werden (Taf. VI 23). Cytopyge ventral, Exkretionsporus dorsal (Taf. VI 21).

Lebenddiagnose der neuen Art, die zu Ehren des Entdeckers des Silberliniensystems der Ciliaten Herrn Dr. B. M. Klein benannt sein soll.

Colpidium kleini sp. n., Größe ca. 100 µ. Form und Aussehen ähnlich *Colpidium colpoda*. Größe je nach Ernährungszustand schwankend. Bei meist gleicher Länge wie *C. colpada* fällt die weitaus schlankere Form, die ungefähr der von *C. campylum* gleicht, auf. Makronuleus ellipsoid mit einem Mikronukleus. Kontraktile Vakoule mit dorsal liegenden Exkretionsporus ungefähr in der Mitte des Tieres. Massenhaftes Vorkommen in Pflanzenaufgüssen, jedoch nicht sapropel. Das Tier ist lebend oft schwer von den andern Arten zu unterscheiden. Als grobes Unterscheidungsmerkmal kann auch bei *C. kleini* sp. n. die Zahl der Cilienreihen angesehen werden: nämlich 35—40.

Charakteristik des Silberliniensystems von *Colpidium kleini* sp. n.

Das verläßlichste Unterscheidungsmerkmal der neuen Art ist das Silberliniensystem. Hier zeigen sich klare Unterschiede in der Protrichozystenregeneration und im Bereich des apikalen Pols. Bei den anläßlich der Protrichozystenregeneration ablaufenden formativen Veränderungen der Meridiane 2. Ordnung teilen sich diese in 2—3 parallel verlaufende Fibrillen auf (Taf. III 9, 10). Nach erfolgtem Anschluß der Protrichozysten wird der ursprüngliche Zustand — ein Meridian 1. Ordnung und ein Meridian 2. Ordnung durch zusammenrücken der aufgeteilten Protrichozystenfibrillen wiederhergestellt (Taf. III 11). An der Regeneration der Schleuderorganellen beteiligen sich im auffalenden Maße auch die Basalfibrillen, wodurch oft der Eindruck einer dreifach aufgeteilten Protrichozystenfibrille hervorgerufen wird (Taf. III 9, s. Pfeil). In fortgeschrittenen Stadien der Regeneration liegen in diesen Ausläufern der Basalfibrillen immer Protrichozystenkörner (Taf. III 9. 10). Dieselben werden gegen Ende der Regeneration ebenfalls den Meridianen 2. Ordnung eingegliedert. Die Ausläufer der Basalfibrillen werden rückgebildet (Taf. III 10, 11). Eine Anastomosenbildung zwischen den aufgeteilten Fibrillen ist wenig ausgeprägt, es wird nie das typische Engmaschengitter wie bei *C. colpoda* gebildet.

Der *C.colpoda* kennzeichnende spiralige Polbereich ist bei der neuen Art nur andeutungsweise vorhanden (vgl. Taf. V 18 mit Taf. IV 13). Es ist nur eine leichte Linkskrümmung der präoralen Naht erkennbar (Taf. I 1, IV 13). In

der Form und Ausbildung des Oralapparates lassen sich keine Unterschiede zu den anderen Arten nachweisen. Der Oralapparat ist typisch tetrahymenid, mit drei adoralen Membranellen und einer undulierenden Membran ausgestattet (Taf. I 4, IV 12—15).

Diskussion

Die bei der Protrichozystenregeneration auftretende Aufteilung der Meridiane 2. Ordnung in mehrere parallel verlaufende Fibrillen, kann für die Gattung *Colpidium* als sicheres Artunterscheidungsmerkmal gewertet werden. Die Meridiane 2. Ordnung teilen sich während der Protrichozystenregeneration bei *C. colpoda* maximal in 4—6 (Taf. V 19), bei *C. kleini* sp. n. in 2—3 (Taf. II 8, III 9) und bei *C.campylum* in 2 parallel verlaufende Fibrillen auf. Die bei *C. colpoda* vorhandene starke Anastomosenbildung zwischen den aufgeteilten Fibrillen der Meridiane 2. Ordnung, zeigt sich in diesem Ausmaß weder bei *C. campylum* noch bei *C. kleini* sp. n. Die bei *C. kleini* sp. n. festgestellte Beteiligung der Meridiane 1. Ordnung an der Protrichozystenregeneration (Taf. III 9) konnte bei *C.campylum* nicht und bei *C.colpoda* nur im geringen Ausmaß beobachtet werden (vgl. Taf. III 9 mit Taf. VI 23, V 19).

Der kennzeichnenste Unterschied der drei Arten untereinander ist jedoch der apikale Polbereich (Taf. IV 13, V 18, VI 22). Bei *C.campylum* wird der apikale Pol durch das einfache Konvergieren der Silberlinien gebildet (Taf. VI 22). Durch die starke Linkskrümmung der präoralen Naht zeigt sich bei *C.colpoda* eine ausgeprägte Polspirale (Taf. V 18). Bei *C.kleini* sp. n. ist nun ebenfalls eine leichte Linkskrümmung der präoralen Naht vorhanden, diese ist jedoch nie so ausgeprägt wie bei *C.colpoda*. Vor allen ist die Naht, welche durch das Zusammenstoßen der ventralen und dorsalen Silberlinien gebildet wird, nicht so kompliziert wie bei *C. colpoda* (vgl. Taf. IV 13 mit Taf. V 18).

Eine Verwechslung von *C.kleini* sp. n. mit C. *campylum* ist durch die Linkskrümmung der präoralen Naht ausgeschlossen. Eine Verwechslung mit *C.colpoda* ist durch die weit einfachere Protrichozystenregeneration nicht möglich.

Diese Unterschiede im Silberliniensystem dürften die Einordnung als neue Art rechtfertigen. Das Tier wurde bis jetzt an drei verschiedenen Orten im Raume von Ober-Österreich gefunden. Zur Kultur eigneten sich Pflanzenaufgüsse am besten. In denen konnten sie massenhaft gezüchtet werden. Oft kamen die drei Arten *C.colpoda*, *C.campylum* und *C. kleini* sp. n. gemeinsam in der Kultur vor. Reinkulturen wurden noch nicht versucht. Einmal trat *C. kleini* sp. n auch in der Umgebung von im Wasser faulenden Plankton auf.

Ob diese neue Art mit den von Stockes (zitiert aus Czapik 1968) beschriebenen Arten *C.truncatum* oder *C.striatum* indentisch ist, konnte nicht sicher entschieden werden, da die von Stockes gegebenen Bestimmungsmerkmale zu wenig differenziert sind (vgl. auch Kahl 1930—35). Die neuere Beschreibung von *C.striatum* durch Corliss 1953 (zitiert aus Czapik 1968) nähert sich *C.kleini* sp. n. am meisten. Allerdings ist nach den angegebenen Merkmalen eine sichere Bestimmung des Tieres kaum möglich, da ja Größe und Zahl der Cilienreihen je nach Fundort und Ernährungszustand starken Schwankungen unterworfen sind. Die Bestimmung der Art mit Hilfe

des Silberliniensystems wurde von diesem Autor nur im geringeren Maße durchgeführt. Gegen eine Identität spricht vor allem die Größ. Kaum identisch mit der neuen Art dürfte das von Gelei 1932 beschriebene *C.pannonicum* sein, obwohl Größe und Zahl der Cilienreihen annähernd den bei *C.kleini* sp. n. festgestellten gleichen. Die Ausprägung des Cytostoms sondert *C.pannonicum* aber von den anderen Colpidiumarten ab. Kahl 1930—35 schlug vor, diese Art in einer neuen Gattung einzuordnen. Eine Identität mit der von Kahl 1930—35 beschriebenen Degenerationsform von *C.colpoda* ist nach den angegebenen Bestimmungsmerkmalen ausgeschlossen.

Das Silberliniensystem erscheint bei *C.kleini* sp. n. als Bindeglied zwischen *C.colpoda* und *C.campylum*. Die Zahl der Cilienreihen, die mit 35—40 ebenfalls in der Mitte steht, bestärken diese Auffassung.

Die angeführten Daten erlauben daher die Vermutung, die neue Art als phylogentisch zwischen *C. colpoda* und *C. campylum* stehend einzuordnen.

Zusammenfassung

Mit Hilfe des Silberliniensystems konnte eine neue Art in der Gattung *Colpidium* Stein, 1860, gefunden werden. Die Unterschiede in der Protrichozystenregeneration (Aufteilung der Meridiane 2. Ordnung in 2—3 parallel verlaufende Fibrillen) und im apikalen Polbereich (leichte Linkskrümmung der einfachen präoralen Naht, ohne spiralige Polzone) rechtfertigen die Einordnung von *C. kleini* sp. n. als neue Art. Phylogenetisch dürfte es sich um ein Bindeglied zwischen *C.colpoda* und *C.campylum* handeln. Darauf deutet die Zahl der Cilienreihen (35—40) und die eigenartige Ausprägung des Silberliniensystems hin.

SUMMARY

A new species of *Colpidium* Stein, 1860, has been described with a help of the silverline-system. The description of *C.kleini* sp. in., as a new species, is justified by the differences in the regeneration of the protrichocysts (dividing of the second meridians into 2—3 parallel fibrils) and the different apical polar zone (slight left turn of the simple präoral suture). The new species seems to be a connecting link between the *C.colpoda* and *C.campylum*. This is indicated by the number of the ciliary rows (35—40) and the specific formation of the silverline-system.

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LEGENDE ZU DEN TAFELN I—VI

Colpidium kleini sp. n.

1: Ventralansicht (1 500 ×)
2: Dorsalansicht mit Exkretionsporus (Ex) (1 500 ×)
3: Teilansicht der Dorsalseite mit Exkretionsporus (Ex) (2 500 ×)
4: Teilansicht der Ventralseite mit Cytostom. Deutlich sind die drei adoralen Membranellen AZM und die leichte Linkskrümmung der präoralen Naht sichtbar (2 300 ×)
5: Teilansicht der Dorsalseite. Deutlich erkennbar die Meridiane 1. Ordnung (M.1.) mit den ihn ihnen liegenden Basalkörnern. Die Meridiane 2. Ordnung (M.2.) sind ohne Relationskörner, da die Protrichozysten ausgestoßen wurden (4 500×)
6: Teilansicht der Ventralseite. Beginnende Regeneration der Protrichozysten (4 500×)
7: Teilansicht der Dorsalseite. Frühes Stadium der Protrichozystenregeneration. Beginnende Aufteilung der Meridiane 2. Ordnung (4 500×)
8: Teilansicht der Dorsalseite. Mittlere Phase der Protrichozystenregeneration. Die Meridiane 2. Ordnung haben sich in zwei deutlich unterscheidbare Fibrillen aufgeteilt. Die Basalfibrillen senden viele Ausläufer zu den aufgeteilten Meridianen 2. Ordnung. Vereinzelntes Vorkommen von Relationskörnern der neugebildeten Protrichozysten (P) (4 500×)
9. 10: Teilansicht der Dorsalseite. Maximale Aufteilung der Meridiane 2. Ordnung (s. Pfeil) zum Zwecke der Protrichozystenregeneration. Vermehrtes Auftreten von Protrichozysten (5 000×)
11: Teilansicht der Ventralseite. Dem System sind wieder Protrichozysten in normaler Anzahl angeschlossen. Die Regeneration der Protrichozysten ist abgeschlossen. In den Meridianen 2. Ordung sind die Relationskörner derselben sichtbar. Meridiane 1. Ordnung (M.1.) mit Basalkörnern, Meridiane 2. Ordung (M.2.) mit Protrichozystenkörnern
12: Ventralseite mit Oralapparat (2 300×)
13: Blick auf den apikalen Pol (2 300×)
14: Blick auf das Cytostom (3 000×)
15: Blick auf das Cytostom. Deutlich erkennbar die drei adoralen Membranellen und die Basalkörner der undulierenden Membran (UM) (3 000×)
16: Blick auf den distalen Pol mit Cytopyge (Pfeil) (2 600×)

Colpidium colpoda Ehrb.

17: Ventralansicht. Meridiane 1. Ordnung mit den sehr regelmäßig ihn ihnen liegenden Basalkörnern. Meridiane 2. Ordnung zum Zwecke der Protrichozystenregeneration aufgeteilt (1 000×)
18: Blick auf den apikalen Polbereich (1 800×)
19: Teilansicht der Ventralseite. Maximale Aufteilung der Meridiane 2. Ordnung (M.2.) zum Zwecke des Neuanschlusses der Protrichozysten. Ausgeprägte Engmaschengitterbildung der Meridiane 2. Ordnung (5 400×)
20: Teilansicht der Ventralseite. In den aufgeteilten Meridianen 2. Ordnung sind bereits viele Protrichozystenkörner erkennbar (P). Die Gitterbildung beginnt rückläufig zu werden (5 400×)

Colpidium campylum Stockes

21: Dorsalansicht mit Exkretionsporus (Ex) (1 500×)
22: Ventralansicht mit Cytostom (1 500×)
23: Teilansicht aus der Dorsalseite. Maximale Aufteilung der Meridiane 2. Ordnung zum Zwecke des Neuanschlusses der Protrichozysten (4 500×)
24: Blick auf das Cytostom. (UM) Basalkörner der undulierenden Membran (5 400×)

Die den Mikrophotographien vorgestellten Präparate wurden ausnahmslos mit der vom Autor (Foissner 1967) veröffentlichten „trockenen" Silbermethode angefertigt.

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A propos de *Deltopylum rhabdoides*

Remarks on *Deltopylum rhabdoides*

Avec H. Mugard, j'ai décrit en 1946 sous le nom de *Deltopylum rhabdoides* n. gen., n. sp., un Cilé tetrahyménien histophage dont l'aspect évoque à première vue *Glaucoma sagitta* Kahl mais qui se caractérise à la fois par son cycle physiologique et par une ouverture buccale triangulaire, d'où la dénomination générique proposée. A. Czapik 1968 a retrouvé cette espèce près de Cracovie.

La forme caractéristique de cette espèce est celle des individus chasseurs, ou thérontes, nageant activement à la recherche d'une proie; lorsque le théronte a trouvé un fragment d'*Enchytraeus* ou quelques petit organisme blessé, il dilacère les tissus et en ingère avidement les débris; c'est le stade trophonte au cours duquel la taille du Cilié s'accroît notablement. Puis le trophonte commence à digérer les ingêsta dont il est rempli, et effectue la synthèse de ses propres constituants, tandis que son appareil buccal se résorbe et disparaît presque totalement. Ce nouveau stade prélude à la multiplication et l'Infusoire, devenu tomonte, subit deux divisions successives. Au cours de la première bipartition une nouvelle ciliature buccale se reconstitue chez le proter comme chez l'opisthe, à partir des cinéties somatiques 1 et 2; l'ouverture buccale est alors de forme elliptique allongée, et l'ébauche ciliaire se différencie en donnant une membrane parorale et trois membranelles adorales M_1 M_2 et M_3, étendues parallèlement les unes aux autres. Au début de la 2ème bipartition les structures buccales régressent à nouveau, puis se reconstituent chez le proter et chez l'opisthe, comme au cours de la première bipartition; mais cette fois les trois membranelles adorales se développent davantage, et s'incurvent dans la cavité buccale qui devient fonctionnelle avec l'ouverture triangulaire caractéristique du théronte, le retour à ce stade fermant ainsi le cycle des transformations. Ces données, déjà indiquées dans la note de 1946, ont été confirmées et précisées en 1956 par Mugard et Lorsignol.

A. Czapik retrouve chez ses exemplaires de *Deltopylum* les caractères généraux décrits par les auteurs précités, mais écrit: „Cependant en ce qui concerne la structure de la bouche, je veux y ajouter quelques détails. Le péristome a une forme plutôt ovale que triangulaire (Pl. I 1). Chez un théronte mesurant 120 µ de long la bouche a 27×6 µ. La membrane vibratile (UM) contourne le côté droit de la bouche formant un faible arc. Les trois membranelles situées parallèlement à la bouche inclinent légèrement leurs extrémités antérieures vers le côté droit; M_1 et M_2 sont de la même longueur, M_3 est plus courte. Chaque membranelle consiste en deux rangées de cinétosomes".

Cette description, parfaitement exacte, comme la microphotographie qui l'accompagne, ne justifierait plus la dénomination générique *Deltopylum*; mais elle se rapporte, de toute évidence, d'après les indications qui viennent d'être rappelées, aux deux tomites isssus de la première bipartition et non pas aux thérontes proprement dits issus de la deuxième bipartition.

J'insiste sur ce point car le cas des *Deltopylum* présente un certain intérêt en ce qui concerne les processus généraux de la stomatogenèse par le fait que ceux-ci s'effectuent, chez ce Cilié, en deux étapes distinctes entre lesquelles s'intercalent l'autolyse des deux premieres structures buccales et une seconde bipartition.

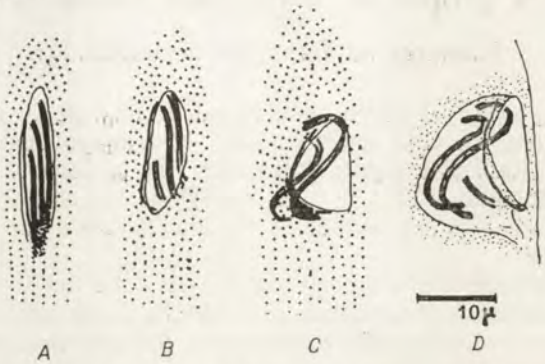

Fig. 1. *Deltopylum rhabdoides* A. Ciliature buccale en voie d'organisation avec les trois membranelles adorales et la membrane parorale à peu près parallèles dans une très légère dépression ovalaire de la surface du corps. B. Ciliature buccale des deux tomites issus de la 1ère bipartition; les membranelles s'enfoncent dans la dépression de contour ovale. C. Ciliature buccale des quatre tomites issus de la 2ème bipartition; la nouvelle ciliature s'est constituée suivant les deux stades précédents, puis les membranelles M_1 et M_2 se sont allongées et contournées en distendant la cavité péristomienne dont l'ouverture devient deltoïde. D. Cavité péristomienne du théronte vue de profil pour montrer sa profondeur. Les quatre figures ont été dessinées à la même échelle, avec la chambre claire, sur des préparations imprégnées à l'argent. Le trait représente 10 µ

La première étape réalise, chez les deux premiers tomites, une ciliature buccale tétrahyménienne avec les trois membranelles adorales parallèles comme chez diverses espèces du groupe *Glaucoma-Colpidium*. Après régression et disparition de cette ciliature, la seconde étape réalise, chez les quatre tomites issus de la seconde bipartition, une structure plus complexe qui n'est pas sans évoquer celle plus complexe encore qui caractérise le cytostome des Ophryoglènes (Mugard 1949, Canella 1964, Canella e Rocchi-Canella 1964, Roque et al. 1965, Roque et de Puytorac 1967). Dans ce dernier cas, cependant, c'est au cours d'un processus continu que se réalisent la structure initiale à membranelles adorales parallèles, puis leur double courbure dans la profondeur de la fossette buccale, stade final qui caractérise le théronte ophryoglenien.

Chez *Deltopylum* la structure buccale caractéristique du théronte est probablement liée à l'accroissement légèrement hypertélique des membranelles adorales de la deuxième génération qui, à l'étroit dans la cavité buccale, se contournent en S et déforment son ouverture en lui donnant un contour deltoïde.

Résumé

Le cycle normal de *Deltopylum rhabdoides*, Cilié Tetrahymenien histophage, comporte entre les stades trophonte et théronte, deux bipartition successives. La forme triangulaire de l'ouverture péristomienne qui caractérise le genre est réalisée, à la seconde bipartition, chez les quatre tomites frères; les deux tomites issus de la première bipartition possèdent une ouverture buccale transitoire dont la forme ovale peut prêter à confusion.

SUMMARY

The normal cycle of *Deltopylum rhabdoides*, a histophagous Tetrahymenial ciliate, comportes two successive bipartitions between the trophont and the theront stages. The triangular form of the peristomial aperture which characterizes the genus is realized in the four sister tomites at the time of the second bipartition; the two tomites formed from the first bipartition possess a transitory buccal opening of which the oval shape can cause confusion.

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Fine structure of *Ancistrocoma pelseneeri* (Chatton et Lwoff), a rhynchodine thigmotrichid ciliate

Ultrastructure de *Ancistrocoma pelseneeri* (Chatton et Lwoff)
Cilié thigmotriche rhynchodine

This commensal ciliate is commonly found attached to the surface of the mantle and gills of marine bivalve Molluscs. Chatton et Lwoff 1926 first described it from *Macoma balthica* and Kozloff 1946 from many other species of clams and mussels. Chatton et Lwoff 1950 gave a short but vivid description of this ciliate again in their monographic study of thigmotrichid ciliates.

The lack of an ultrastructural study of the whole group of *Thigmotrichida* was pointed out first by Pitelka 1963 in her book "Electron microscopic studies of Protozoa". The only work published since then, on the ultrastructure of this group, has been an abstract of the joint work of Lom and Kozloff 1966 on the rhynchodine *Ignotocoma sabellarum*. The present study is an attempt to describe in detail the fine structure of *Ancistrocoma pelseneeri* of the family *Ancistrocomidae*.

Materials and methods

Five clams, *Mya truncata* were examined for thigmotrichid ciliates in March, 1967 and found to harbour numerous *Ancistrocoma pelseneeri*. The ciliates were examined alive under the binocular microscope and by phase-contrast microscopy.

Schaudin's, da Fano's, Hollande's, Bouin's and Nissenbaum's fixatives were used to fix the ciliates for light microscope studies and were followed by staining with Heidenhain's haematoxylin. The silver line system was revealed by Chatton and Lwoff's technique and Bodian's protargol method, whilst Feulgen's method was used to stain the nuclei and their details.

Small pieces of gills with attached ciliates were fixed in Bouin's fluid, embedded in polyster wax and sectioned at 6 μ. Various cytochemical methods were used at the light microscope level, Pearse 1960, to demonstrate the carbohydrates, proteins, nucleic acids and lipid contents of the ciliate cytoplasm.

For electron microscopy, ciliates were fixed in 3% glutaraldehyde in 0.25 M

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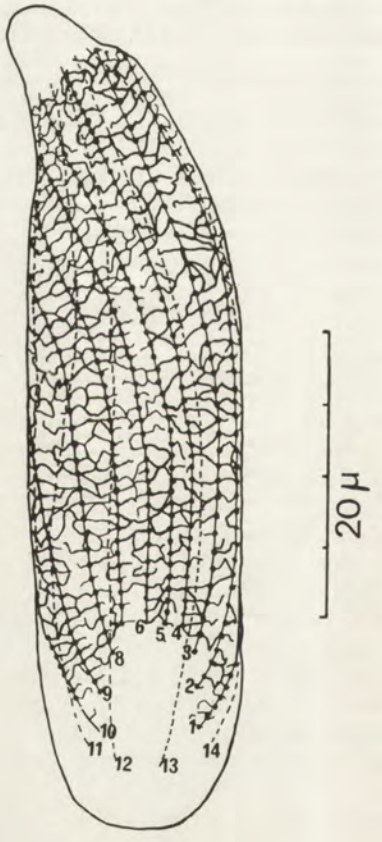
sucrose solution buffered with 0.2 M sodium cacodylate solution at a pH of 7.2 for two hours at room temperature, postfixed by 2% osmium tetroxide for two hours and embedded in epon 812. Ultra-thin sections were cut on a Huxley ultramicrotome using glass knives. Silver or grey sections were selected and picked up on 400 or 200 mesh grids and then double stained by 2% uranyl acetate for 7 minutes and lead citrate for 2—3 minutes. The electron micrographs were taken on an Akashi Tronscope TR50 and an AEI EM6 G at original magnifications of ×5 000 to ×50 000.

Observations

From the light microscopy it may be useful to give a brief general description to relate with the fine structure. These ciliates (Fig. 1 and Text fig. 1) do not possess a cytostome and in this respect differ from those numerous thigmotrichs which are associated in the sub-order *Arhynchodina*. Instead, like the remaining thigmotrichs, the *Rhynchodina*, they posses a retractile tentacle at the anterior tip of the body (Text fig. 2), by which they attach themselves



Text fig. 1. *Ancistrocoma pelseneeri* (Chatton et Lwoff), diagram showing the kineties

to the host surfaces (Fig. 2) and have been said to feed on the host cell contents. Staining for the argentophilic system shows that there are fourteen kineties of which five are ventrally placed, closely associated and run from the anterior tip to two-thirds the length of the body (Text fig. 1 and Fig. 5). The remaining kineties are broadly spaced, reach almost to the posterior end and cover the dorso-lateral surfaces of the body. The mid-dorsal surface is thus devoid of kineties. A net-work of fine fibres is also revealed in the pellicle joining the kineties to each other (Figs 3, 4). The cilia are long, beat metachronally slowly when the ciliate is attached but faster when it is freed. The tentacle, when extended, emerges from the dorsal aspect of the anterior tip of the body and about 22° to the right (Fig. 36).

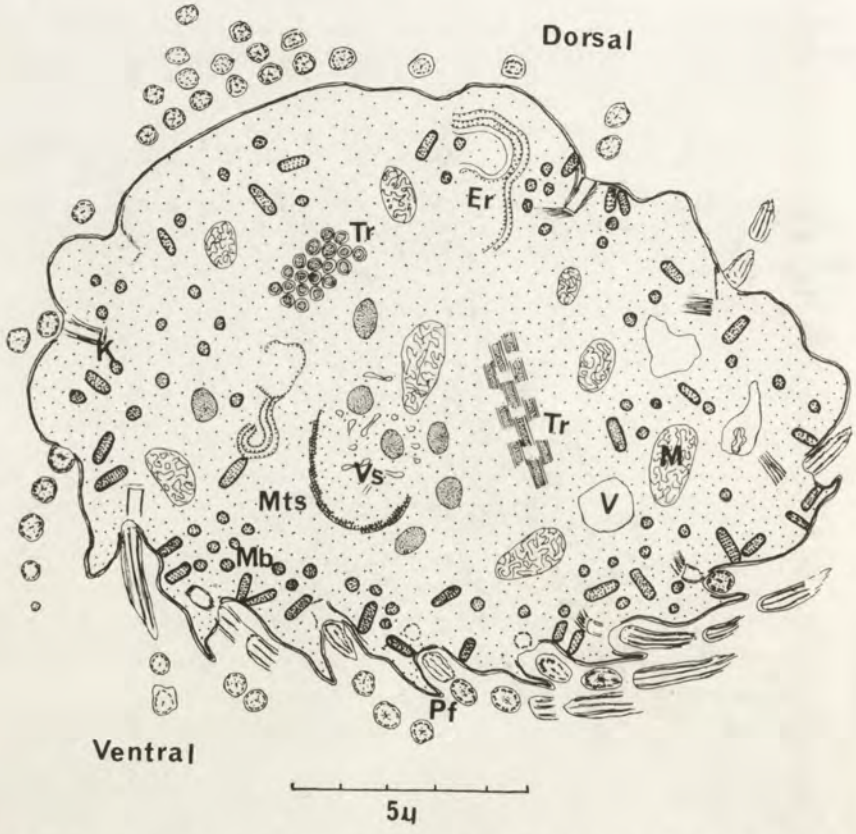
Electron microscopy

The pellicle is two layered, the inner layer being thicker and more dense (Figs 8, 17). Only at certain sites can gaps between the cytoplasm and the

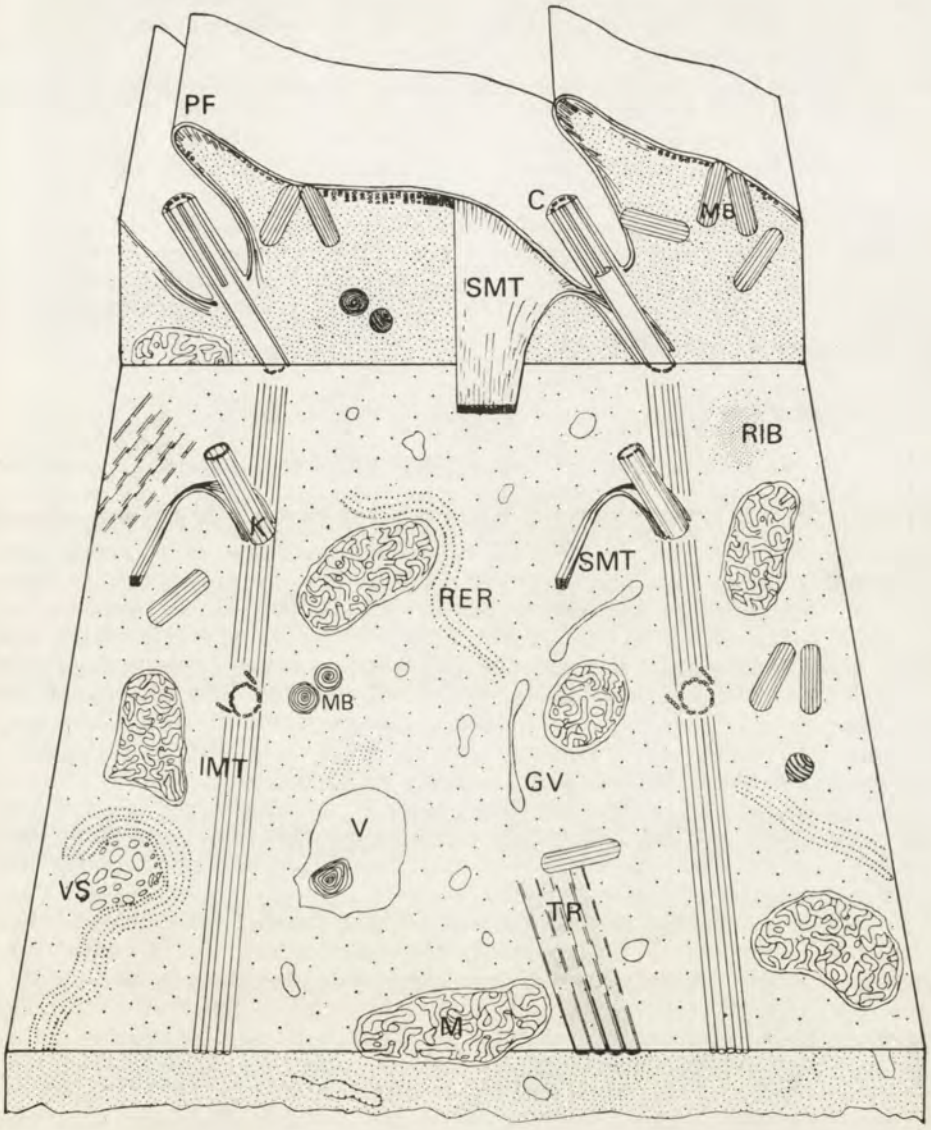
Text fig. 2. A longitudinal section of *Ancistrocoma pelseneeri* attached to the inner mantle epithelium of the host *Mya truncata*. The tentacle (T) is embedded in one of the host's mucus secreting cells (Muc). Mucigenic bodies (Mb); Vacuoles (V); Trichites (Tr); Rough endoplasmic reticulum (Rer); Macronucleus (Mac); Micronucleus (Mic)

pellicular layers be seen. Otherwise the pellicle is well stretched and closely apposed to the endoplasm (Figs 9, 19). It is thrown into sharp longitudinal folds ventrally (Text fig. 3) and the closely set thigmotactic cilia arise from the deep troughs of these folds (Figs, 7, 34, 38).

The cilia have the familiar 9/9+2 fibrillar system (Figs 8, 9). Each kinetosome is approximately 420 mμ long and consists of 9 triplets of peripheral fibrils only (Fig. 8). The kinetosome thus shows a space in it with occasional granular inclusions (Fig. 9). A septum similar to the axial plate (axosome) of hypotrichid cilia, G l i d d o n 1966, demarcates the distal end of the kinetosome from the proximal end of the cilium. This appears as an electron--dense disc in a transverse section of the cilium (Figs 8, 9). The parasomal sacs lie on the left of each kinetosome. Granular rings have often been noticed near the parasomal sacs (Fig. 10, 11).



Text fig. 3. Transverse section through the middle of the anterior region of the body to show the pellicular folds (Pf) of the ventral thigmotactic region. The cilia are close together ventrally, and set deep into the pellicular folds. The cytoplasm shows the kinetosomes (k) sectioned at various angles, a large number of mucigenic bodies (Mb) arranged under the pellicle and scattered in the cytoplasm, and the basal root of the tentacle cut across about three-quarters of the way back along its length. At that level it consists of ventral microtubular sheets only, with mucocysts and a few empty vesicles within and around it



Text fig. 4. A schematic representation of the sub-pellicular structures of *Ancistrocoma pelseneeri*. The origin of the two sets of microtubules from each kinetosome (K) to form a continuous layer of sub-pellicular microtubules (SMT) is shown. The infra-kinetosomal bundle of microtubules (IMT) are also shown to form a continuous band under the kinety. Various cytoplasmic inclusions include rough endoplasmic reticulum (RER), mitochondria (M), golgi-like vesicles (GV), food vacuoles (V), small vesicles surrounded by stacks of rough ER (VS) and long straps of trichites (TR). The mucigenic bodies are seen arranged under the pellicle (MB) and also scattered in the cytoplasm. The pellicle on the ventral side is thrown into folds (PF)

There seem to be two sets of microtubules each measuring approximately 21 mμ in diameter, arising from the kinetosome. Each set comprises 2—4 microtubules arising slightly above the base of the kinetosome, one anteriorly and the other posteriorly. These run up towards the pellicle, the latter set curving round the kinetosome, and then both run longitudinally just under the pellicle to form the thick layer of sub-pellicular microtubules (Figs 6, 7, 9, 10). It seems possible that each of these microtubules may run the whole length of the body. Arising from the base of each kinetosome there is a third set of 4—6 microtubules, which run longitudinally for about the distance marked by 4—5 kinetosomes of the same kinety and end successively one after the other, forming a continuous band of microtubules under each kinety. This infra-kinetosomal band of microtubules is structurally quite different from the kinetodesmata of other ciliates. There are no kinetodesmata found in this ciliate (Figs 6, 7, 10 and Text fig. 4).

Trichocysts

Three types of trichocysts are seen scattered in the cytoplasm. They can be easily distinguished by their structure, size and topography. There seems to be an aggregation of all three types in the anterior region and relatively much fewer posteriorly (Figs 6, 8, 12—14). The rudimentary stages of all three types look similar. They lie deep in the endoplasm but, as they grow to their maximum size, they move to various parts of the body to be arranged as described below. They can be shown cytochemically, by mucicarmine and mucihaematin, to contain mucoid substance which appears to be mucoprotein rather than mucopolysaccharide. In electronmicrographs they display grades of electron density ranging from a highly dense crystalline material to a grey and finely granular one. The three types of trichocysts will be referred to here as (1) mucigenic bodies, (2) mucocysts and (3) trichites.

Mucigenic bodies are the most common type of "trichocysts" seen in this ciliate (Figs 6, 8, 36, 37). They have a capsule-shaped body with both ends rounded and are occasionally pear-shaped. They are arranged radially just under the pellicle with one end attached to its inner surface (Fig. 8). They lie in groups just behind each kineosome forming parallel rows, the so-called secondary meridians (Figs 6, 8). Each body is a solid capsule full of an electron-dense crystalline material. In transverse sections through these bodies the tiny component crystals seem to be arranged in closely packed concentric rings. The bodies are not surrounded by limiting membranes as are similar bodies in other ciliates (Figs 8, 16). Various stages of developing mucigenic bodies, which may be called protrichocysts, can be recognised in the endoplasm. They first appear as single membrane-bound vesicles containing an electronopaque substance (Fig. 16). In the next stage crystals start appearing in concentric whorls, which start near the centre of each vesicle and circle anti-clockwise until they reach the periphery. The number of whorls may be one, two or three. In the last stage, the protrichocysts are fully packed with 7—9 whorls of crystals and the outer membrane may perhaps become part of the outer-most whorl. Each of the tiny crystals which form the whorls first appears as an empty hexagonal structure, which later fills with a dense substance. These early stages in the development of trichocysts are seen scattered in the cytoplasm. Fully grown mucigenic bodies then seem to move towards the pellicle and finally become

arranged in rows just under it. Though these bodies have not been noticed emptying their contents, a dense substance always seems to form a layer outside the pellicle and this is similar to the substance in the bodies (Figs 6, 8).

Mucocysts are large, oval or spherical bodies packed with a fine granular substance which is less dense than the contents of the mucigenic bodies (Figs 14–16). Large numbers of mucocysts are found in the tentacle base and anterior cytoplasm, but they may occur in smaller numbers throughout the cytoplasm. In the tentacle they are arranged fairly regularly in longitudinal rows. Each mucocyst is covered by a membrane. The empty mucocysts can be seen in electron-micrographs giving the cytoplasm of the tentacle a vacuolated appearance (Figs. 14, 15). In these structures, the outer membrane probably ruptures and the contents are squirted out. The membranes of the emptied mucocysts are seen forming a loose network in this area. The extruded mucus forms a fibrous layer extending from the surface of the pellicle in the embedded region of the tentacle (Figs 36, 46). This fibrous layer is similar to that which is produced by the contents of certain trichostomatid ciliates, de Puytorac 1956. These mucocysts appear to be derived from vesicles in the endoplasm similar to those which form the other types. Indeed it seems that similar protrichocysts probably give rise to all the three kinds of "trichocysts". Among the developing protrichocysts the whorled arrangement shown by the crystalline contents of the first type and the homogenously distributed granular contents of the second type can be easily distinguished, however, (Fig. 16).

Trichites are bundles of long fibro-crystalline straps which are seen criss-crossing the cytoplasm (Figs 12, 13). Some are longitudinally placed and are found running from the anterior to the posterior tip of the body. Each bundle contains 4—10 trichites each arranged at an angle to the other, so that in an oblique section they seem to have a matted arrangement. In structure, these trichites differ from the other two types in having an electrondense, fibrous medulla with a less dense material forming the cortex (Fig 13). Some of them have a crystalline structure similar to that in the mucigenic bodies. Perhaps these represent early stages in the development of trichites, when they may resemble the protrichocysts of the former two types. Cytochemical tests for proteins have revealed the substance in the medullary region of the trichites to be a fibrous protein, giving an intense reaction with Millon's reagent. Mercury bromophenol blue also stains these structures.

Mitochondria and Golgi vesicles

The tubular mitochondria show the familiar double limiting membrane (Figs 6, 17), the tubules being continuous with the inner membrane. The average diameter of the tubule is 75 mμ. In sections of glutaraldehydefixed material the outer membrane always appears wavy. A number of membrane-bound inclusions can also be seen inside the mitochondria. Otherwise the tubules contain a material with a uniformly fuzzy appearance whilst some appear to be empty (Fig. 20). Mitochondria are occasionally surrounded by endoplasmic reticulum. They may also be associated with large granular bodies and enclosed with them by a common membrane (Fig. 18). Their shapes are elongated, looped or swollen in different parts of the cytoplasm (Fig. 19). They occur close to the kinetosomes, particularly in the ventral

thigmotactic region, around the vacuoles and sometimes close to the nuclear membrane (Figs 6, 29, 55).

A large number and variety of vesicles lie in the endoplasm, the most peculiar and common being elongated sacs (Fig. 21). These greatly resemble the elongated golgi vesicles of flagellate and metazoan cells. It was only on one or two occasions that they have been seen in typical golgi stacks and otherwise they lie singly in the cytoplasm. They seem to be aggregated in the cytoplasm in and around the base of the tentacle (Fig. 22). Each sac possesses a thin limiting membrane and has a coarse granular content. These granules are less dense than the ribosomes of the cytoplasm, so it is assumed that they may be secretion products or glycogen granules. It was impossible to localise them cytochemically under the light microscope so as to be positive for glycogen staining, but enzymic digestion by a-amylase for one hour partly digests them.

Endoplasmic reticulum and vacuoles

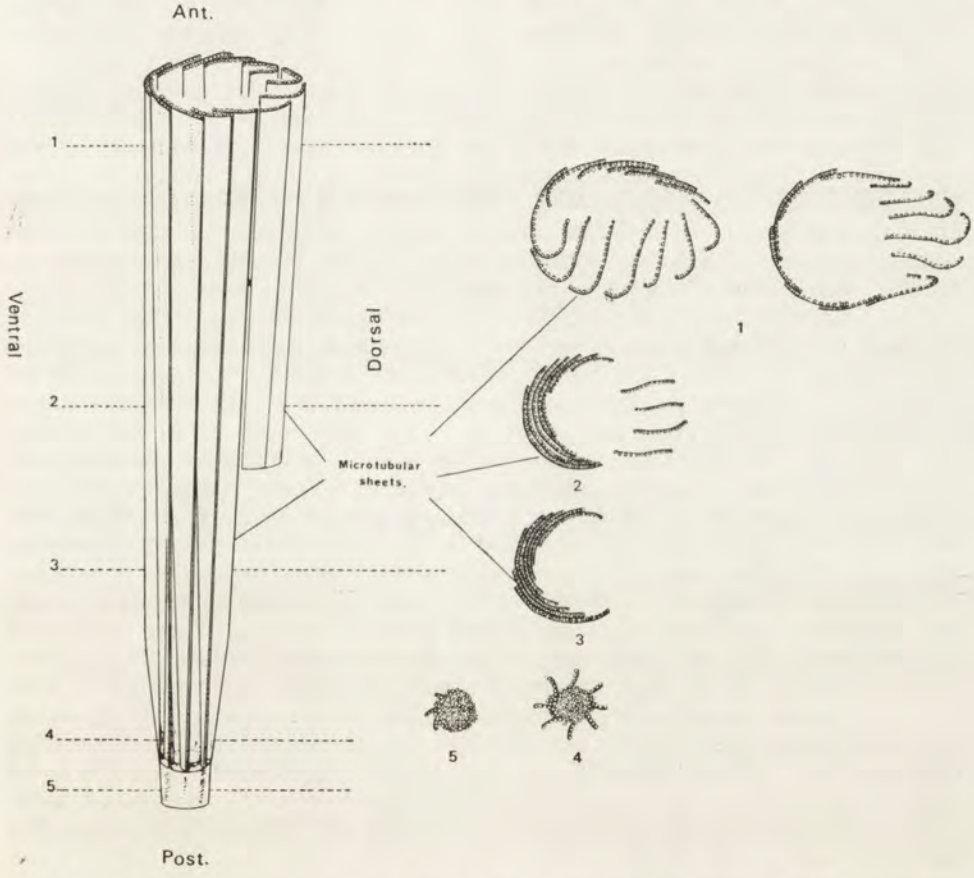
The cytoplasm is very rich in free ribosomes and glycogen, the two types of granules being easily distinguished by their different densities of staining, the former being more electron-dense (Fig. 23). Ribosomes are found in association with the outer nuclear membrane of both the macro- and micronucleus. The cytoplasm also contains a large number of small rings of ribosomes. These can be seen free in the cytoplasm and also inside the large food vacuoles. The Feulgen reaction and methyl green/pyronin stain show some reaction in these food vacuoles, indicating that they contain DNA and RNA.

The endoplasmic reticulum (ER) is very distinctive and the rough ER in particular forms characteristic configurations, including large stacks which are often whorled and are found in association with food vacuoles, the nuclear membranes and dense amorphous bodies in the cytoplasm (Fig. 24). They are also seen surrounding groups of small vesicles (Figs 25, 26) and larger, round dense bodies. The smooth ER is seen around the contractile vacuole forming a network of tubules (Fig. 33) or in the cytoplasm forming cisternae (Figs 25, 26).

The contractile vacuole and the surrounding tubular network are also characteristic of this ciliate. The vacuole is always seen on the ventral side, in the middle region of the body close to the pellicle, the tubular network of smooth membranes forming a typical nephridial structure as in *Paramecium*. These tubules are long coiled structures opening by one end into the vacuole and joining with the ER at the opposite end (Figs 33—35). This nephridial network is always surrounded by a dense fuzzy substance which may also occur within the tubules, but the vacuole itself has never been seen to contain this substance. The vacuole does contain particles perhaps of undigested food and frequently lamellar structures, a typical residue after lipid digestion. The outer parts of the tubular network have a compact arrangement, but closer to the vacuole the arrangement becomes less compact, whilst the membranous walls become more distinct (Fig. 34). A small discharge sac with a fibrous zone around its walls is found in between the vacuole and the pellicle (Figs 33—35). In some instances the nephridial tubules directly join this sac (Fig. 35). It is always situated close to a kinetosome and seems to open by a minute pore through the pellicle (Fig. 35).

A large number of food vacuoles are seen displaying various stages in the

digestive process (Figs 29—31). Newly formed vacuoles can be easily identified, being full of food particles (Figs 29, 30). Some other vacuoles, perhaps half way through digestion, contain a variety of inclusions such as small thick membranous vesicles which show a double membrane also, large dark bodies, stacks of lamellar structures and amorphous substances (Figs 30, 31). These vesicles always have a thick membrane around them, which sometimes appears to be double. There are also a large number of empty vacuoles in which the process of digestion has probably been completed. These are covered by thin single membranes and sometimes contain one or two concentric lamellar inclusions (Fig. 31). Small vesicles occasionally with less dense material in them are seen joining the food vacuoles at various stages (Fig. 29), suggesting the possibility that these food vacuoles may in fact be autophagic.



Text fig. 5. Diagrammatic reconstruction of the microtubular sheets which surround the basal root of the tentacle, running from the anterior tip almost to the middle of the body. The diagrams of the transverse sections of the tentacle base at various levels (1, 2, 3, 4 & 5) are based on electronmicrographs such as Figs. 37—40. The microtubular sheets have a whorled arrangement, especially ventrally. The ventral sheets converge to form a very compact bundle of microtubules at the posterior end. The dorsally placed microtubular sheets seem to be confined to the anterior half of the basal root

Tentacle

The tentacle has a basal root (Text fig. 5) which extends almost into the middle of the body, curving slightly at its posterior end. This is surrounded by a special system of microtubules associated together to form 14 to 16 longitudinally running sheets (Figs 36, 37). These microtubular sheets can be seen in a transverse section to have a whorled arrangement (Figs 37, 38). Towards the ventral side they are closely pressed together but on the dorsal side they have spaces between them (Figs 37, 38, 41, 42). As the basal root runs posteriorly the dorsal sheets of microtubules have increased gaps between them and ultimately they disappear somewhere in the middle of the tentacle (Fig. 42). The ventral counterparts maintain their arrangement further posteriorly, then, just before they end, they form a close bundle of microtubules (Figs 39, 40). A number of free kinetosomes have been noticed surrounding this microtubular bundle, suggesting that these microtubules may also originate from kinetosomes as the other microtubules seem to do (Text fig. 5).

A number of larger microtubules have also been noticed running longitudinally inside the basal root of the tentacle. They contain a finely granular less electron-dense substance which appears similar to the contents of the mucocysts. These tubules measure approximately 70 mμ in diameter and seem to be the largest of all the microtubules in this ciliate. They are wavy and are flexed at a number of points in their length, which suggests that they are not as rigid as the other microtubules (Figs. 14, 21). Sometimes the ends are swollen and seem to be empty (Fig. 47).

The microtubules of the sheets which surround the basal root measure approximately 31 mμ in diameter and those forming each sheet are arranged regularly on a common basement membrane (Fig. 43). The cytoplasm of the tentacle root seems to be continuous at its posterior end with the surrounding cytoplasm, but is distinguished by an apparent absence of mitochondria (Fig. 42). Towards the anterior region of the root a number of membranous vesicles, which are probably empty mucocysts, give the cytoplasm a vacuolar appearance (Figs 14, 15). Where the tentacle proper emerges from the cell, the lumen is divided into an outer and inner part separated by a membrane (Figs. 44, 45). The inner part contains a spongy material which is marked by longitudinal bands in a section of the extended tentacle. Adjacent bands are joined to each other by fine fibres (Figs 36, 44, 45). These structures are not seen in a retracted tentacle of the unattached ciliate. The pellicle at the anterior tip of the retracted tentacle is slightly invaginated to give rise to a folded margin with a sunken portion in the middle (Fig. 6). In an attached ciliate, the tentacle extends out and develops a terminal swelling embedded in a host cell (Figs 36, 46). The pellicle of the extended part is much thinner, and its surface in the embedded region bacomes dense and more prominent (Figs 36, 46). Outside this is found the fibrous layer described earlier (p. 34).

The nuclei

The macro- and micronuclei differ in their staining properties with the Feulgen reaction. The macronucleus shows a larger number of darkly stained bodies, between which the nucleoplasm remains negative or slightly stained. The micronucleus on the other hand shows a compact granular structure with a uniform reaction.

The macronucleus is a very conspicuous elongated structure, measuring 11—16 μ×4—7 μ and lying in the middle of the body. Under the electron microscope, it is bounded by a double membrane which is wavy (Fig. 48). In almost all cases the outer membrane, which is continuous with the ER at some places, has been found studded with ribosomes. Minute openings in the nuclear membrane can be seen clearly (Fig. 49). Occasionally narrow prolongations of the nuclear membrane have been observed, arising from one side of the macronucleus, and one of these seems to bring the nuclear contents directly in contact with cytoplasm (Fig. 48). Staining with uranyl acetate gives a clear picture of the contents. Three different categories of contents can be seen: (1) large, finely granular, compact electron-dense bodies of DNA, (2) spherical or ovoid, coarsely granular, less dense nucleoli and (3) a fibrous and granular matrix filling up the gaps of the nuclear space (Figs 48—51). Many of the electronmicrographs show what is probably a condensation phase of the macronucleus, where the large DNA bodies are gathered in the middle, leaving the peripheral space with just a scanty matrix (Figs 49, 50). The matrix contains fibrous structures which sometimes extend from the central granular mass and join the inner nuclear membrane. In the central region the matrix shows a more granular pattern and fills the gaps between the large DNA bodies and the nucleoli. Towards each end, the nucleus usually contains a loose fibro-granular matrix only (Fig. 51).

The micronucles is oval, sometimes with a narrow end, measures 2 μ×3 μ and lies immediately beside the macronucleus (Figs 48, 52, 56). The double nuclear membrane is quite distinct and the outer bears ribosomes (Figs 53, 57). In one of the stages, which may perhaps be an interphase, the micronucleus has uniformly granular contents, which fill it completely (Fig. 48). Close to the inner nuclear membrane, longitudinally arranged microtubules are present, but they do not seem to run the whole length of the nucleus. In some instances the small space between the macro- and mirconuclei is filled up with ribosomes (Fig. 48). In another electron-micrograph, probably in early prophase, the granular contents of the micronucleus aggregate in certain areas, producing denser patches. Rounded areas with less dense granular contents can be seen clearly among the dense areas. The peripheral space seems to be more open now and the spindle microtubules can be seen clearly in this zone. Mitochondria can also be seen close to the outer nuclear membrane (Figs 52, 55).

At another stage the micronucleus contains highly condensed, very electron-dense material, within a somewhat extended nuclear membrane. Small areas of lesser density, which form pockets in the condensed mass, are probably the RNA rich nucleoli. At the poles, a loose granular matrix can be distincly seen, with spindle microtubules and some fine fibres running longitudinally. A small lamellar body is also seen in this area (Fig. 54).

A late prophase stage shows the micronucleus in a highly vesicular state with a network of fine fibrous structures filing up the entire space (Figs 56, 57). The granular contents of the chromosomes are seen in the process of condensation. Spindle mircotubules can be distinguished in the peripheral zone. A number of annular openings in the nuclear membrane can be seen and at some places the outer nuclear membrane joins the rough ER.

Micronuclei seen in a metaphase stage, reveal a more familiar structure. Chromosomes of varied size are seen arranged on the equatorial plate of the

spindle. They consist of a highly condensed material with some fibrous structures in them. Spindle microtubules, surrounded by a grainy material here and there, can also be seen clearly extending from one pole to the other. The scanty matrix appears to be more fibrous at this stage leaving large empty spaces towards the poles. Lamellar inclusions are again distincly visible in the empty spaces. The nuclear membranes seem to be still intact (Figs 58, 59).

Discussion

Thigmotaxis

All *Thigmotrichida* are commensal with or parasitic on various molluscs. They walk about on the surfaces of these, particularly inside the mantle, and whilst doing so they seem to adhere quite strongly to the host epithelium. This locomotion has been called thigmotaxis and has been thought of as involving specialised cilia in the ventral or thigmotatic region, Chatton et Lwoff 1926. It now appears, however, that there is nothing morphologically unusual about the ventral cilia and that the only ventral specialisations for thigmotaxis are the closeness of the kineties, the large number of mucigenic bodies associated with them and the microtubular anchoring of the kinetosomes to the remarkably deep pellicular folds. Mitochondria are numerous ventrally and the cytoplasm there has a rich ribosomal content, with highly active ER and many golgi-like sacs. The organisation is therefore very suitable for supporting ciliary activity and mucoprotein secretion.

The deep pellicular folds are well designed for strengthening the ventral pellicle against forces which would tend to buckle it transversely. They recall the folds in roofing sheets of iron or asbestos, except ahat they taper sharply and thus interfere as little as possible with the free movement of the cilia. The associated microtubular system would appear to have structural and supporting functions, as in *Actinosphaerium* and *Tetrahymena*, Tilney and Porter 1965, Tilney, Hiramoto and Marsland 1966. The kinetosomes are thus anchored firmly to the pellicle, whilst the infrakinetosomal bundles, which are so different from kinetodesmata, are in a good position to fix the kinetosomes rigidly by anchoring them deeply to one another, whilst the cilia beat metachronally fore and aft. It also seems possible that the infrakinetosomal bundles may act as communication lines along the kineties. In *Tetrahymena* a large single microtubule has been noticed linking the cilia in a similar fashion, Allen 1967. In *Ancistrocoma*, however, the arrangement is different, in that each microtubule runs for a distance equivalent to only 4 or 5 kinetosomes, whilst contributing to a bundle which continues for the whole length of the kinety. It is this neuronlike arrangement which suggests a function in communication.

Contractile vacuole

These have been studied in detail in many Protozoa, but mostly in fresh water species, Kitching 1967. Many marine Protozoa seem to lack contractile vacuoles altogether, but *Ancistrocoma* possesses this organelle with its elaborate structures. The nephridial tubules form a network like that of *Paramecium*, but they open directly into the vacuole without forming radial

canals or ampullae, and seem to remain permanently in communication with it as in *Tetrahymena*, Elliot and Bak 1964. The densely stained area around these tubules is strikingly like the "spongiome" (nephridioplasm) of peritrichid ciliates described by Fauré-Frémiet and his colleagues. The wall of the vacuole is simple in structure and lacks the fibrillar supports observed in the suctorian *Tokophrya*, Rudzinska 1958, the peritrichs *Campanella* and *Ophrydium*, Fauré-Frémiet et Rouiller 1959 and in *Paramecium*, Schneider 1960. The small, adjoining discharge vacuole, however, does have some fibrous structures in its wall. Close association of this vacuole with its nearby kinetosome is similar to what has been described in *Tokophrya* and *Stentor*, Weisz 1954, Rudzinska 1958. None of the electron-micrographs which show the contractile vacuole seem to show any stages in pulsation, such as systole. Perhaps the vacuole of *Ancistrocoma* does not pulsate, because it may be concerned only with gradual processes of ionic regulation.

Trichocysts

Many ciliates and flagellates possess small bodies just under the pellicle, which may be caused to discharge their contents by various stimuli. They have been referred to as trichocysts, trichites, Kudo 1954, protrichocysts and mucoid trichocysts, Pitelka 1961, mucigenic bodies and mucocysts, Pitelka 1963 and secretory ampoules, Cheissin and Mosevitch 1962. All these are said to discharge a mucoid material, which may be either fibrous, filamentous or amorphous. The coexistence of three different kinds of such structures in *Ancistrocoma* necessitates the adoption of the terms mucigenic bodies, mucocysts and trichites. The term "trichocysts" has been used here as a general term for all such bodies, the mucoid contents of which may be either filamentous or amorphous.

Earlier ultrastructural studies of various kinds of trichocysts have dealt with their structure and possible functions in *Paramaecium*, *Tetrahymena*, Pitelka 1961, Tokuyasu and Scherbaum 1965, Allen 1967, Zebrun, Corliss and Lom 1967, *Frontonia*, Rouiller et Fauré-Frémiet 1957, Yusa 1963, *Colpidium*, Pitelka 1961, Cheissin and Mosevitch 1962, *Holophrya*, de Puytorac 1964 and *Ophryoglena*, Roque, de Puytorac et Savoie 1965. The mucigenic bodies of *Ancistrocoma* resemble the "trichocysts" in most of these ciliates, in being situated just under the pellicle and in the secondary meridians. They differ markedly, however, in having an elongated capsular shape, without a limiting membrane, and in possessing a uniformly electron-dense crystalline content. The absence of a surrounding limiting membrane is a characteristic feature of these bodies, whereas those of all other ciliates referred above are surrounded by a clear membrane. Cheissin and Mosevitch have observed the mucigenic bodies in large numbers under and around the cytostome of *Colpidium colpoda*. Those of *Ancistrocoma* are aggregated around the basal root of the tentacle and in the anterior region of the body.

The mucocysts of *Ancistrocoma* lie within the basal root of the tentacle, into which they appear to discharge their contents. This discharged substance seems to be granular and electron-lucid inside the tentacle stalk, but immediately outside the tip of the embedded tentacle it forms a fibrous dense zone within the host cell. It seems that the secretion, shown by the cytoche-

mical tests to be a mucoprotein, may polymerise or coagulate when it is discharged into the host cell.

Trichites are seen in a number of gymnostomatid and trichostomatid ciliates, and electron microscopic studies on these tubular, rod-like structures have dealt with their structure, nature and function in *Didinium*, Yagiu and Shigenaka 1965, *Coleps*, *Nassula*, and *Frontonia*, Rouiller, Fauré-Frémiet and Gauchery 1956. Lom and Kozloff 1966 have described trichite-like structures associated with the tentacle of *Ignotocoma* and called them toxicysts. The trichites of *Ancistrocoma* are characterised by their tubular, somewhat crystalline protein fibres arranged in concentric whorls. These long rods are found in bundles which run lengthwise at different angles, appearing as inter-woven bands in oblique sections. They probably serve a skeletal function in this ciliate. They strikingly resemble those associated with the pharynx in certain gymnostomatids, and differ markedly from the toxicysts of *Ignotocoma*, for they are not associated with the tentacle nor seen to eject any toxic substance.

The development of all the three types of "trichocysts" can be traced from small vesicles connected with the ER, through a common protrichocyst stage to well formed capsular mucigenic bodies, spherical mucocysts or elongated trichites. Cheissin and Mosevitch described mucosysts in *Colpidium colpoda* as permanent vesicles connected with the ER. According to them, the mucoid material is synthetized in the cytoplasm and transported through the cisternae of the ER to the mucocyst vesicles, where it is accumulated, condensed and discharged upon external stimulation. Tokuyasu and Scherbaum have observed vacuoles originating from the ER in *Tetrahymena* and then invaginating to form double-membraned blebs. They increased in size by accumulation of material, which turned crystalline, and they then moved towards the pellicle and attached themselves under it. Those authors could not apply the sequence described in *Colpidium* to their study of *Tetrahymena*. The electron-micrographs of *Ancistrocoma* show a series of events, the initial stages of which are strikingly similar to the description given by Cheissin and Mosevitch in *Colpidium*. The important difference in *Ancistrocoma* is that three different types of "trichocysts" subsequently develop from one similar precursor stage.

The tentacle

This is the most specialised organelle of *Ancistrocoma*, functioning for attachment and possibly for feeding. The basal root does not possess a complete boundary wall, but the investment of microtubular sheets must play an important role in anchoring and probably in the extension and retraction of the tentacle. Sliding of the microtubules relative to one another may cause changes in pressure within the basal root that could result in the tentacle being extended or sucked back again. From the brief description (in abstract) published on the rhynchodine thigmotrichid *Ignotocoma sabellarum*, Lom and Kozloff 1966, it is noticed that a similar tentacle is present in that ciliate also, but insufficient details are given to allow a comparison of the fine structure. The tentacle of *Ancistrocoma*, however, presents a number of structures different from any in *Ignotocoma*.

Feeding

The light microscope studies of Kozloff, 1946 suggested that *Ancistro-*

coma pelseneeri are branchial parasites which feed by means of the suctorial tentacle. He also observed typical food vacuoles containing ingested fragments of epithelial cells and globular masses usually of a dense, homogenous character in the posterior part of the body. The electron-micrographs show a variety of vacuoles which are similar to those described by Kozloff and contain solid particles of a considerable size. The absence of any large opening in the attached part of the tentacle discounts the possibility of any ingestion of such food particles there. It is possible that absorption of food material is carried out by the pellicular surface of the attached tentacle as well as by the general body surface. Electron-cytochemical work on *Ancistrocoma* and the host cells will be nececcary in future studies, if a parasitic relationship is to be confirmed.

Acknowledgment

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Summary

The ventral thigmotactic region is characterised by deep pellicular folds. Three sets of microtubules originate from each kinetosome, two joining a sub-pellicular layer of microtubules and the third forming a continuous band of infra-kinetosomal microtubules under each kinety. There are two mucus-secreting organelles, which may be termed mucigenic bodies and mucocysts, the latter being associated with the tentacle. There are also long trichites criss-crossing the cytoplasm, possibly skeletal in function. The cytoplasm has rich and active ER. The tentacle is a highly specialised organ, used for attaching the ciliate to the host cell and also possibly for feeding. It has a large basal root surrounded by a number of microtubular sheets. These may slide relative to one another to bring about extension and retraction of the tentacle. The contractile vacuole may not in fact pulsate, but it is surrounded by a typical nephridial network of tubules. Vacuoles containing food materials are large and conspicuous, but the method of ingestion is problematical.

RÉSUMÉ

Les profonds plis pelliculaires sont caractéristiques de la region thigmotactique ventrale. Trois groupes des microtubules commencent à chaque kinetosome, desquelles deux joignent la couche sous-pelliculaire des microtubules et la troisième forme une ceinture continue des microtubules infra-kinetosomales en dessous de chaque cinétie. Il y a deux organelles muqueuses qu'on peut déterminer comme les corps mucinogeniques ou les mucocystes associées avec le suçoir (le tentacule). II y a aussi des trichites longues croisant le cytoplasme, probablement d'une fonction squelettique. Le cytoplasme contient un réticulum endoplasmique abondant et actif. Le suçoir, un organ bien specialisé, serve à l'attachement de l'infusoire à la cellule-hôte et — peut être — à l'alimentation. Sa large partie basale est entourée par

des nombreuses lamelles microtubulaires. Elles peuvent glisser, changeant leur position mutuelle, effectuant l'extension et rétraction du suçoir. La vacuole contractile n'est pas probablement pulsative. Elle est entourée par un typique système nephritique des tubules. Les vacuoles contenant de nourriture sont volumineuses et remarquables, mais la façon d'ingestion est problématique.

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EXPLANATION OF PLATES I—XIII

Fig. 1. Phase-contrast micrograph showing the general features and cilia of a free-swimming *Ancistrocoma*

Fig. 2. Section of the gill of the host *Mya truncata*, showing an attached ciliate (arrow head). Haematoxylin and eosin stain

Figs. 3. and 4. Silver nitrate impregnated ciliate (Chatton and Lwoff technique) to show the sub-pellicular net-work of fibres (arrow head)

Fig. 5. (a—d). Light micrographs of ciliates treated with protein silver (Bodian's protargol method) to show the kineties

Fig. 6. A longitudinal section of the anterior half of the body showing a kinety (K) in section at various levels, the arrangement of mucigenic bodies (Mb) under the pellicle and a special system of microtubules (T) which surrounds the basal root of the tentacle. The section goes near the edge of that system and does not show the tentacle itself. The origin of sub-pellicular microtubules (arrow heads) and the infra-kinetosomal microtubules (double arrow heads) are shown. The latter form a longitudinal track running basally along the kinety (×12 000)

Fig. 7. A superficial longitudinal section as above showing subpellicular microtubules (arrow heads) and the infra-kinetosomal microtubules (Imt) forming a track under the kinety (double arrow heads). The arrow shows large microtubules with a dense material inside them (×12 000)

Fig. 8. A longitudinal section of a cilium and its kinetosome showing the peripheral and central fibres. The arrow indicates the circular disc (axial plate or axosome) where the central fibres end (×40 000)

Fig. 9. Part of the regular layer of sub-pellicular microtubules (Smt) is shown in a subtangential section. An axial plate is seen in transverse section (arrow). (×18 000)

Fig. 10. Higher magnification electronmicrograph showing Smt., Imt. and the parasomal sacs (Ps). A granular ring is also seen (arrow head). It would adjoin a parasomal sac of the next kinety (×40 000)

Fig. 11. The triplet peripheral fibres of the kinetosomes are shown. A parasomal sac (Ps) lies on the left of each kinetosome (×42 000)

Fig. 12. Two groups of trichites (Tr) (×16 000)

Fig. 13. A bundle of trichites in transverse section showing clearly a dense crystalline medullary region surrounded by a less dense cortex. The smaller trichites show gradual development of the crystalline contents (×32 000)

Fig. 14. A longitudinal section through the base of the tentacle showing mucocysts (Mc), arranged in longitudinal rows between the microtubular sheets of the tentacle. The arrow shows large microtubules filled with a homogenous substance. They are seen inside the tentacle base (×12 000)

Fig. 15. Highly magnified area of the same showing empty spaces left by the mucocysts, with remains of their limiting membranes (arrow head) (×35 000)

Fig. 16. Vesicles (Vs) growing to become protrichocysts, some containing crystalline material which forms whorled patterns (arrow). A large protrichocyst, which will presumably develop into a mucocyst, has finely granular instead of crystalline contents (arrow head) (×32 000)
Fig. 17. A mitochondrion (M) lying just under the pellicle shows its wavy outer membrane (arrow head) and tubules with fuzzy contents (arrow) (×50 000)
Fig. 18. A mitochondrion and a granular body of similar size, surrounded by empty food vacuoles (×24 000)
Fig. 19. Irregularly shaped mitochondria under the pellicle (×25 000)
Fig. 20. Stacks of rough endoplasmic reticulum (Rer) one of which surrounds a mitochondrium on one side (arrow). Some of the tubules seem empty (×36 000)
Fig. 21. Golgi-like stacks near to the basal root of the tentacle (arrow). End of a large microtubule (small arrow) which occurs inside the tentacle base shows a dense homogenous substance in it (×32 000)
Fig. 22. Elongated golgi-like vesicles (Gv) with granular contents (×36 000)
Fig. 23. Free ribosomes (Rib) and some attached to circular membranes (arrow head) (×36 000)
Fig. 24. Various configurations of the highly active Rer in the endoplasm (×16 000)
Fig. 25. A group of vesicles (Vs) surrounded first by one or two layers of smooth endoplasmic reticulum (arrow) and then by Rer (×32 000)
Fig. 26 An earlier stage, perhaps, in which the vesicles are surrounded by stack of Rer (×32 000)
Fig. 27. Another stage in which the Rer is shown loosing its ribosomes and surrounding a few vacuoles (arrows) (×32 000)
Fig. 28. A group of vesicles (Vs), probably after their release from the endoplasmic reticulum (×32 000)
Fig. 29. A large food vacuole (V) with a variety of inclusions, among which a lamellar structure (arrow) in the middle is very prominent. A double arrow shows the Rer, surrounding the vacuole on one side (×16 000)
Fig. 30. Another food vacuole with a large double-membraned structure in the middle (arrow head) and a number of small round vesicles (arrow head) around it (×16 000)
Fig. 31. Two empty food vacuoles — one with a lamellar residue inside it (arrow), the other surrounded by stacks of Rer (×24 000)
Fig. 32. Large, dense bodies in the food vacuole encircled by Rer (×28 000)
Fig. 33. The contractile vacuole with its surrounding nephridial tubules (Nt) and adjoining discharge sac (Ds), which lies close to the pellicle. The arrow indicates a tubule with denser contents (×24 000)
Fig. 34. A low power electron-micrograph of the contractile vacuole (Cv) and surrounding cytoplasm (×10 000)
Fig. 35. The nephridial tubules (Nt), some of which are seen joining the discharge sac (Ds) directly. The arrows indicate the denser contents of the tubules in Figs. 35 and 37. The discharge sac shows a small opening through the pellicle (arrow head) (×20 000)
Fig. 36. The inner epithelium of the mantle of *Mya truncata* with a ciliate attached by its extended tentacle (T) to one of the mucus producing cells (Muc). The tentacle when extended always makes an angle of about 22° with the long axis of the ciliate. Darkly staining mucigenic bodies adjoin the basal root of the tentacle (×7 000)
Fig. 37. Somewhat oblique transverse section through the basal root of the tentacle, showing the arrangement of microtubular sheets (Mts) and darkly staining mucigenic bodies (×20 000)
Fig. 38. Mts of the tentacle root in transverse section, with mucocysts (Mc) inside the tentacle root and some trichites outside (×20 000)
Fig. 39. The tentacle root sectioned through the posterior end shows a cart-wheel arrangement of the microtubular sheets (arrow) originating around a bundle of microtubules (Mt) (×14 000)
Fig. 40. A transverse section of the tentacle root slightly anterior to the previous one (×15 000)
Fig. 41. The basal root of the tentacle sectioned transversely, about halfway along it, where the dorsally placed microtubular sheets are sparse and arranged almost radially, whilst the ventral ones are closely packed and almost concentric (arrows) (×9 000)

Fig. 42. Only the ventrally placed microtubular sheets are seen in this transverse section, slightly behind the middle of the tentacle's basal root (×10 000)
Fig. 43. Electron-micrograph of the microtubular sheets, at a higher magnification than shown in Fig. 44. The microtubules are arranged on a thin basement membrane (arrow) (×60 000)
Fig. 44. Base of the extended tentacle seen in a longitudinal section. Bands of amorphous material are shown running towards the tip (arrow). The tentacle is lined inside by a membrane (arrow head). The microtubular sheets bounding the basal root are seen very close to the sub-pellicular microtubules (double arrow) (×40 000)
Fig. 45. In transverse section the above mentioned bands are clearly seen to be connected to each other by fine strands (arrow) (×14 000)
Fig. 46. The tip of the extended tentacle embedded in the host cell, showing an electron-dense fibrous zone, probably of ejected mucus, around its surface (×50 000)
Fig. 47. A section passing through the posterior end of the tentacle's basal root, showing the terminal bundle of microtubules (Mt) closely associated with a few kinetosomes (large arrow). The small arrow indicates one of the larger microtubules associated with the root of the tentacle (×20 000)
Fig. 48. Closely situated macro-(Mac) and micronuclei (Mic), in a low power electron-micrograph. Arrow shows an aggregation of ribosomes in between the nuclei. The nuclear membrane of the macronucleus is evaginated at one end, forming a process which seems to open into the cytoplasm terminally (double arrows) (×6 000)
Fig. 49. The nuclear membrane of part of the macronucles. The outer membrane is studded with ribosomes (arrow heads). The nuclear pores are very minute. Also seen are the dense DNA bodies (DNA), small coarsely granular nucleoli (Nu) and the matrix (Mx) (×21 000)
Fig. 50. Cross section of the end of the macronucleus showing large gap between dense DNA bodies and the nuclear membrane. The fibro-granular matrix (Mx) fills this gap loosely (×16 000)
Fig. 51. Extension of the nuclear membrane (double arrow) with a nucleolus at its base (×24 000)
Fig. 52. Micronucleus, probably at an early prophase stage. The finely granular material is seen condensed (arrow). The loose matrix shows a few spindle microtubules (arrow head). The rough endoplasmic reticulum is seen joining the outer nuclear membrane (double arrow) (×16 000)
Fig. 53. A part of the previous electron-micrograph magnified to show the ribosomal association of the nuclear membrane. The arrow head shows spindle microtubules (×32 000)
Fig. 54. Increased condensation of the contents in another stage of prophase with patches of less dense, granular nucleoli (arrow). A lamellar body is seen in the loose matrix (arrow head) (×24 000)
Fig. 55. A similar stage associated with mitochondria (M) (×24 000)
Fig. 56. A late prophase stage where the condensed material has become coarsely granular and forms long chromosomes (Chr). The matrix (Mx) is highly vesicular (×16 000)
Fig. 57. More magnified portion of the previous electron-micrograph, to show the gap between the two nuclear membranes and the nuclear pores more prominently (×32 000)
Fig. 58. The micronucleus in metaphase stage showing the chromosomes (Chr) arranged on the equatorial plane, the spindle microtubules running from one pole to the other (arrow heads) and scanty matrix (Mx) with lamellar inclusions (arrows) (×15 000)
Fig. 59. A similar stage showing spindle microtubules more prominently (arrow heads) (×20 000)

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Некоторые данные по ультратонкому строению макрогамет *Eimeria tenella* (*Sporozoa, Coccidia*)

Some data on the ultrastructure of macrogametes of *Eimeria tenella* (*Sporozoa, Coccidia*)

В последние годы тонкая структура эндогенных стадий развития кокцидий подотряда *Eimeriidea* (*Sporozoa, Coccidia*) интенсивно исследовалась с электронным микроскопом. В этом плане наиболее полно оказались изучены кокцидии кроликов и крупного рогатого скота: *Eimeria intestinalis, E. magna, E. perforans, E. stiedae, E. bovis* и *E. auburnensis* (Мосевич и Хейсин 1961, Scholtyseck 1962, 1963, 1965, Cheissin 1964, 1965, Scholtyseck und Voigt 1964, Cheissin and Snigirevskaya 1965, Scholtyseck und Piekarski 1965, Scheffield and Hammond 1965, Scholtyseck, Volkmann und Hammond 1966, Scholtyseck, Hammond and Ernst 1966, Hammond, Scholtyseck and Miner 1967).

Однако до сих пор не проводились исследования ультратонкого строения разных эндогенных стадий развития кокцидий из кишечника кур. Единственная известная нам работа (Scholtyseck und Weissenfels 1956) посвящена исследованию тонкого строения оболочки ооцист *E. tenella* (Raillet et Lucet, 1891). Изучение ультратонкой организации этого паразита существенно само по себе. В то же время оно интересно в сравнительном плане. Сопоставление данных по *E. tenella* с ранее полученными сведениями о тонком строении эндогенных стадий развития других кокцидий, таких как *E. intestinalis, E. perforans, E. bovis* и др. позволит получить более чёткое представление об общих закономерностях строения, роста и развития внутриклеточных паразитов. В данной статье приводятся некоторые данные об ультраструктуре макрогамет *E. tenella*.

Результаты электронно-микроскопических исследований других стадий цикла развития этого вида будут приведены в последующих публикациях.

Материал и методика

Для получения макрогамет *Eimeria tenella* 7—10 дневных цыплят заражали спорулированными ооцистами *E. tenella*. Вскрытия производили на 6—7 сутки

после заражения, когда в слепых отростках кишечника имеется множество ранних и поздних стадий развития макрогамет. Небольшие кусочки слизистой (1—2 мм³) иссекали из слепых отростков цыплят и немедленно помещали в капельку забуференного 2% раствора четырехокиси осмия, разведенной на изотоническом буфере при pH 7,4. Материал фиксировали в течение 1,5 часов при 0°С. Обезвоживание производилось в спиртах возрастающей концентрации от 40 до 100° с интервалом в 10°. Время экспозиции в каждой смене спиртов составляло 15—20 минут. Зафиксированный материал заключали в аралдит. Полимеризацию производили в термостате при температурах 37, 45 и 60°С. Ультратонкие срезы готовились на микротоме УМТ-2 с последующей комбинированной окраской уранил-ацетатом (Watson 1958) и гидратом окиси свинца (Karnovsky 1961). Исследование срезов проводилось с помощью электронного микроскопа JEM-5g при увеличении до 10 000—12 000 раз с последующим светооптическим увеличением.

Результаты исследований

При исследовании со световым микроскопом срезов слизистой оболочки слепых отростков цыплят на 6-е сутки после заражения обычно можно видеть большое количество ранних стадий развития макрогамет *E. tenella*, локализованных в эпителиальных клетках. Размеры таких макрогамет составляют 6—7 мк. На 7-й день встречаются в большом количестве более крупные макрогаметы, занимающие всю цитоплазму эпителиальной клетки хозяина. Такие макрогаметы имеют большое ядро с резко очерченной кариозомой (Gill and Ray 1957). Цитохимические исследования макрогамет выявляют в молодых формах небольшое количество гликогена, локализованного в виде мелких гранул вокруг ядра (Edgar et al. 1944, Tsunoda and Ichikava 1954 и др.). В крупных макрогаметах *E. tenella*, так же как и других видов *Eimeria*, вся цитоплазма заполнена гликогеном, и кроме того, видны большие округлые тельца, многие из которых занимают периферическое положение. Эти тельца демонстрируют сильную реакцию на белки и полисахариды и принимают участие в образовании оболочек ооцисты (Gill and Ray 1954, 1957).

При исследовании с электронным микроскопом ранних стадий развития макрогамет (на 6 день после заражения), имеющих около 6—7 мк в длину и 4—5 мк в ширину, были выявлены следующие особенности их строения.

Молодая макрогамета, находящаяся в клетке хозяина, ограничена трехслойной мембраной (Табл. I 1 Pm), толщиной 75—80 Å и окружена паразитофорной вакуолью, имеющей вид узкой щели, расположенной между паразитом и цитоплазмой клетки хозяина (Табл. I 1, II 4 PV). Паразитофорная вакуоль по мере роста макрогаметы увеличивается в размерах (Табл. I 2, II 4). Элементарная мембрана, покрывающая молодую макрогамету, может считаться первичной наружной мембраной, сохраняющейся от трофозоита. В цитоплазме молодых макрогамет очень мало запасных питательных веществ и пластического материала. На Табл. I 1 виден участок молодой макрогаметы с немногочисленными гранулами гликогена (gl), располагающимися как по периферии, так и в центре гаметы. Размеры таких гранул колеблются от 1 000 до 2 000 Å в длину и 300—400 Å в ширину. В цитоплазме молодых макрогамет, кроме гликогена, заметны тельца овальной формы (LB) диаметром от 3 000 до 4 000 Å, состоящие из тесно переплетенных плотных осмиофильных тяжей, которые образуют клубки раз-

ной плотности в виде сети лабиринта. Эти тельца, которые можно назвать „лабиринтными", не имеют собственной мембраны, а окружены гранулярной эндоплазматической сетью.

„Лабиринтные" тельца весьма сходны с аналогичными образованиями, найденными Шолтизеком с соавторами в макрогаметах *E. perforans* (Scholtyseck und Schäfer 1963, Scholtyseck, Hammond and Ernst 1966).

Цитоплазма макрогамет богата каналами эндоплазматической сети, которые расположены беспорядочно по всей клетке. На мембранах, их ограничивающих, всегда много рибосом (Табл. I 1 R). Последние разбросаны вне связи с мембранами эндоплазматической сети, как в центральной так и периферической части макрогамет. В цитоплазме можно видеть вакуоли различной структуры и размеров. Различаются три типа таких вакуолей. Первый тип — вакуоли с малоконтрастным содержимым, ограниченные гладкой элементарной мембраной (Табл. I 1,2 V_1), чаще всего расположенные одиночно. Эти вакуоли не имеют строго округлой формы, их диаметр колеблется от 2 000 до 3 000 Å. Некоторые из них располагаются ближе к периферии (например, Табл. I 2 V_1 снизу). Кроме того, в цитоплазме макрогаметы *E. tenella* находятся вакуоли другого строения, которые мы обозначаем как вакуоли второго типа (Табл. I 1,2, II 3, III 6, 7 V_2). Они частично окружены каналами эндоплазматической сети и располагаются в любой части макрогаметы (Табл. III 7 ER). Стенки вакуолей второго типа, в отличие от вакуолей первого типа, образованы прерывающимися мембранами толщиной до 60 Å.

Вакуоли третьего типа представляют собой неограниченные мембраной полости с прозрачным содержимым (Табл. II 3,4 V_3). Они чаще встречаются в центре макрогаметы. Митохондрии немногочисленны и представляют собой продолговатые тельца с короткими трубочками и зернистым матриксом (Табл. I 1 M).

Большое ядро (Табл. I 1 N) с тёмным плотным ядрышком (n) занимает значительную часть цитоплазмы макрогаметы. В кариоплазме ядра находятся равномерно распределенные относительно плотные гранулы, которые, по-видимому, соответствуют хроматину.

По мере роста макрогаметы (на 7 день после заражения) увеличивается количество и размеры „лабиринтных" телец (Табл. II 3,4, III 5 LB). Они достигают в диаметре около 0.2—0.5 мк и располагаются одиночно и группами, причем эти тельца заключены в большие вакуоли (Табл. II 2 VER). Подобно Шолтизеку и др. (Scholtyseck, Hammond and Ernst 1966), мы считаем, что эти вакуоли, по-видимому, являются производными эндоплазматической сети.

Помимо „лабиринтных" телец, в сравнительно крупных макрогаметах на более поздних стадиях их развития (начало 7 дня после заражения) появляются немногочисленные „тёмные" тельца (Табл. I 2, II 3, 4, III 6, IV 11 DB). Они имеют шарообразную форму с электронноплотным содержимым, в котором видны светлые зернистые включения. Тельца окружены элементарной мембраной (Табл. III 6, IV 11) и достигают в диаметре от 0.8 до 1 мк. В зрелых макрогаметах они располагаются по периферии правильными рядами (Табл. III 6). Эти тельца, наряду с „лабиринтными", которые также концентрируются по периферии макрогаметы, возможно принимают участие в образовании оболочки ооцисты. Вокруг таких „тёмных" телец распюлагаются многочисленные рибосомы (Табл. III 6 R).

В растущей макрогамете накапливается большое количество гликогена, гранулы которого становятся крупнее, чем в молодых макрогаметах (Табл. II 3,

4, III 6, IV 10—12, V 14 gl). Их размеры достигают 0.3—0.5 мк в длину и 0.1—0.2 мк в ширину. На поверхности первичной мембраны растущей макрогаметы (достигшей 8—9 мк) можно видеть многочисленные пузырьки диаметром 180—200 Å, расположенные в один ряд (Табл. III 8, 9 V). У макрогамет более раннего возраста, они не были найдены. На тангентальных срезах через такие макрогаметы (Табл. IV 10 V) заметно, что эти пузырьки представляют собой профили перерезанных поперек сравнительно коротких трубочек, длиной от 1 000 до 1 500 Å. У более крупных макрогамет (на 7 день) над первичной мембраной можно видеть вторую мембрану толщиной 80 Å (Табл. IV 11 Fm_2). Местами на поверхности макрогаметы заметны только её фрагменты, которые на поперечных разрезах имеют вид дугообразных складок. Нередко на срезах через макрогаметы видны переходы от пузырьков к дугообразным фрагментам и от них к сплошной мембране. Можно предположить, что эта вторая мембрана образуется за счет слияния мембран трубочек, расположенных на поверхности первичной мембраны на ранних стадиях развития макрогаметы (Табл. IV 12 m).

В мембранах макрогамет *E. tenella* нами были обнаружены микропоры такого же строения как и у *E. intestinalis* (Снигиревская 1967). На Табл. V 13 mp видны микропоры в поперечном и продольном разрезах. На поперечном разрезе (Табл. V 13) микропора имеет вид кольца, ограниченного двумя мембранами. Диаметр внутреннего отверстия микропоры составляет 600—700 Å. Наружный диаметр равен 1 200—1 500 Å. Расстояние между внутренней и наружной мембранами микропоры составляет 300—400 Å. На продольном разрезе (Табл. V 14) видно, что микропора образована инвагинацией поверхностных мембран в цитоплазму. Поэтому микропора на продольном разрезе имеет форму короткого цилиндра.

В крупных макрогаметах, также как и в мелких, встречаются вакуоли всех трех типов. По мере роста макрогаметы количество вакуолей третьего типа в ней увеличивается и вакуоли располагаются как в центре, так и по периферии макрогаметы (Табл. II 3, 4).

Каналы эндоплазматической сети в крупных макрогаметах менее развиты, чем у молодых макрогамет. В цитоплазме крупных макрогамет много свободных рибосом.

Обсуждение

Макрогаметы различных видов кокцидий имеют много общего. В цитоплазме накапливается большое количество питательных веществ (гликогена и липидов) и пластического материала в виде белковых и гликопротеиновых телец, которые впоследствии участвуют в построении оболочек ооцист. При элекронномикроскопическом исследовании *Eimeria perforans* и *E. stiedae* (Scholtyseck and Voigt, 1964; Scholtyseck et al., 1966) в макрогаметах были выявлены ультраструктурные особенности гранул гликогена и липидов, а также пластических телец, идущих на построение оболочек гамет. Шолтизек обнаружил округлые тельца разной величины, содержащие извитые нити. Эти тельца, похожие на клубок нитей или лабиринт, были первоначально названы „лабиринтными" (Scholtyseck 1962), а в последующих работах Шолтизек дал им название H_1 — тельца; по наблюдениям этого автора они принимают участие в образовании оболочек ооцисты *E. perforans*. Кроме того, Шолтизек обнаружил ещё и другие „лабиринтные" тельца, которые он обозначил как H_2 — тельца. В последних нити закручены плотнее и имеют кольцеобраз-

ные завитки (Scholtyseck 1964). Они тоже участвуют в образовании оболочек ооцисты. В макрогаметах *E. tenella* найдены тельца подобные „лабиринтным". Они появляются в цитоплазме вокруг ядра и по мере роста макрогаметы передвигаются к периферии и участвуют в построении оболочек. По данным Снигиревской (личное сообщение) макрогаметы *E. intestinalis* обладают пластическими тельцами „ячеистой" структуры, но не лабиринтной. „Ячеистые" тельца найдены также у *E. stiedae, E. bovis* и *E. auburnensis* (Scholtyseck et al. 1966). У *E. intestinalis* „ячеистые" тельца участвуют в построении внутренней мембраны оболочки ооцисты и содержат протеины.

В макрогаметах *E. tenella* наряду с „лабиринтными" тельцами встречается много плотных телец, аналогичных таковым макрогамет *E. intestinalis*. Шолтизек предполагает, что эти тельца могут участвовать в построении оболочки ооцисты. У *E. intestinalis* такие плотные тельца исчезают после образования оболочек и, по-видимому, тоже участвуют в их образовании, хотя непосредственно этот процесс не был нами обнаружен.

Различия, наблюдающиеся у некоторых видов кокцидий в ультраструктуре пластических телец, определяются, по-видимому, неодинаковым химическим составом этих телец. Можно предположить, что пластические тельца содержат разные протеины.

В цитоплазме макрогамет были найдены вакуоли разных типов, происхождение которых до сих пор ещё не вполне ясно. Вакуоли третьего типа, возможно, представляют собой липидные капли. Их содержимое растворяется в процессе обработки материала. По нашему мнению, вакуоли 1 и 2-го типов являются пиноцитозными вакуолями, ограниченными типичной элементарной мембраной. Если это так, то возникновение этих вакуолей должно быть связано с цитостомом. Однако функционирующего цитостома наблюдать на нашем материале не удалось, хотя микропора, выполняющая функцию цитостома, и была найдена. Между тем, у макрогамет *E. intestinalis* было обнаружено образование на конце канала — цитостома (микропоры) пищеварительной вакуоли, что свидетельствует о функционировании этой органеллы (Снигиревская и Хейсин 1968). Аналогичного функционирующего цитостома в макрогаметах *E. tenella* найти не удалось. Поэтому пока неясно, осуществляется ли питание у этих внутриклеточных простейших через цитостомы или оно происходит каким-либо иным путем.

Шолтизек и Шэфер (Scholtyseck und Schäfer 1963) предполагают, что питание макрогамет *E. perforans* может осуществляться не только через микропору, но и при помощи других приспособлений. Эти авторы обнаружили на поверхности макрогамет *E. perforans* трубочки диаметром 650 Å и длиной 1.3 мк, которые соединяют макрогаметы с клеткой хозяина. Их стенки имеют характерную периодическую структуру.

Такие трубочки не были обнаружены у *Eimeria tenella*. Однако на поверхности растущих макрогамет этого вида над мембраной были обнаружены более тонкие и короткие трубочки. Они намного тоньше, чем найденные Шолтизеком у *E. perforans* и не связывают макрогамету *E. tenella* с клеткой хозяина. Такие трубочки были найдены только у макрогамет, имеющих крупные гранулы гликогена и большие „лабиринтные" тельца. У молодых макрогамет они не были обнаружены. По нашему мнению, эти трубочки образуют путем слияния составляющих их стенки мембран вторую мембрану макрогаметы, которая появляется во время роста, когда в цитоплазме накапливаются крупные гранулы гликогена.

Резюме

В макрогаметах *Eimeria tenella* электронномикроскопически обнаруживаются два типа пластических телец в цитоплазме: тельца, напоминающие клубок нитей, или „лабиринтные тельца", и „тёмные" тельца. У молодых макрогамет первый тип включений локализован вокруг ядра, но на более поздних стадиях развития макрогаметы они перемещаются по направлению к её периферии.

В зрелых макрогаметах „тёмные" тельца расположены в периферической зоне цитоплазмы, образуя правильные ряды. „Лабиринтные" и „тёмные" тельца, по-видимому, образуют стенку ооцисты.

В цитоплазме макрогамет всегда можно различить, кроме гранул гликогена, ещё три типа вакуолей. Одни из них ограничены сплошной элементарной мембраной, вторые — прерывистой; третьи представляют собой полости с прозрачным содержимым и не ограничены мембраной. Третьи могут быть липидными тельцами, а вторые, вероятно, связаны с процессом пиноцитоза. На поверхности макрогаметы было обнаружено несколько нефункционирующих цитостомов (микропор), а также короткие тонкие микротрубочки. Последние не связывают макрогамету с цитоплазмой клетки хозяина. Заметно, что при росте макрогаметы эти трубочки образуют путём слияния составляющих их стенки мембран вторую мембрану макрогамет. В момент образования второй мембраны, гранулы гликогена, обычно присутствующие в изобилии в цитоплазме макрогамет, имеют уже значительные размеры.

SUMMARY

The fine structure of the *Eimeria tenella* macrogametes has been studied with electron microscope. Two types of plastic bodies were detected in the cytoplasm: the clew-like or labyrinth bodies and the dark ones. In young macrogametes, the former are seen around the nucleus spreading towards the periphery in larger macrogametes.

In mature macrogametes the dark bodies are located in the peripheral zone of the cytoplasm arranged in regular rows. The two types of bodies are likely to be associated with the oocyst wall formation.

In addition to the glycogen granules, three types of vacuoles can be distinguished in the cytoplasm of macrogametes. Some are underlined with a continuous unit membrane, the other — with a fragmented one, while the third ones are cavities with a transparent content lacking membrane. The latter vacuoles may be lipid bodies. Vacuoles of the 2nd type are likely to be associated with pinocytosis.

On the surface of the macrogamete, some non-functioning cytostomes and thin short microtubules are seen. These do not connect the parasites body with the host cell cytoplasm. It can be shown that during the growth of the macrogamete, membranes constituting the walls of the microtubules may contribute to the formation of the second membrane which occurs only in mature macrogametes. By the time of the second membrane formation, glycogen granules abundant in the cytoplasm reveal considerably large dimensions.

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ПОДПИСИ К ТАБЛИЦАМ I—V

Ультратонкое строение макрогамет *Eimeria tenella*

1: Формирование лабиринтных телец (LB) и гранул гликогена (gl) в молодой макрогамете, 15 000 ×
2: „Лабиринтные" тельца (LB) внутри большой вакуоли (VER) в цитоплазме растущей макрогаметы, 12 000 ×
3 и 4: В растущей макрогамете видны „лабиринтные" тельца (LB) и гранулы гликогена (gl) вокруг них, 12 000 ×
5: Участок растущей макрогаметы с деталями строения лабиринтных телец (LB). Видны фрагменты эндоплазматического ретикулума (ER), окружающего тельца, 30 000 ×
6: „Тёмные" тельца (DB) в периферической зоне растущей макрогаметы, 36 000 ×
7: Фрагмент зрелой макрогаметы; V_2 — вакуоли второго типа, 30 000 ×
8 и 9: Многочисленные пузырьки (V) на поверхности растущей макрогаметы, 50 000 × и 24 600 ×
10: То же самое на тангенциальном срезе, 30 000 ×
11 и 12: Образование второй мембраны $(m)_2$ над плазматической мембраной растущей макрогаметы (стрелка). Fm_2 — фрагменты второй мембраны, 50 000 ×
13: Зрелая макрогамета с двумя микропорами (mp) перерезанными поперек, 35 000 ×
14: Продольный срез через микропоры (mp) зрелой макрогаметы, 48 000 ×

Принятые сокращения:

Pm — плазматическая мембрана, PV — паразитофорная вакуоль, gl — гликоген, LB — „лабиринтные" тельца, DB — „тёмные" тельца, ER — эндоплазматический ретикулум, V_1 — вакуоли первого типа, V_2 — вакуоли второго типа, V_3 — вакуоли третьего типа, R — рибосомы, M — митохондрии, Fm_2 — фрагменты второй мембраны, mp — микропора, VER — большие вакуоли эндоплазматического ретикулума, V — пузырьки, локализующиеся над плазматической мембраной растущей макрогаметы, m_2 — сформированная вторая мембрана.

EXPLANATION OF PLATES I—V

Ultrastructure of macrogametes in *Eimeria tenella*

1: Formation of labyrinth bodies (LB) and glycogen granules (gl) in young macrogamete, 15 000×
2: Labyrinth bodies (LB) within large vacuole (VER) in the cytoplasm of growing macrogamete, 12 000×
3 and 4: Labyrinth bodies (LB) and glycogen granules (gl) surrounding them in growing macrogamete, 12 000×
5: Fragment of growing macrogamete with details of structure of labyrinth bodies (LB) Fragments of endoplasmic reticulum (ER) surrounding these bodies are visible, 30 000×
6: Dark bodies (DB) in the peripheral zone of growing macrogamete, 36 000×
7: Fragment of mature macrogamete; V_2 — second type vacuole, 30 000×
8 and 9: Numerous vesicles (V) on the surface of growing macrogamete, 50 000× and 24 000×
10: The same on tangential section, 30 000×
11 and 12: Formation of the second membrane (m_2) above the plasma membrane of growing macrogamete (arrow). Fm_2 — fragments of the second membrane, 50 000×
13: Mature macrogamete with two micropores (mp) in transverse section, 35 000 ×
14: Longitudinal section of the micropore in mature macrogamete, 48 000×

Abbreviations used:
Pm — plasma membrane, PV — host vacuole, gl — glycogen, LB — labyrinth bodies, DB — dark bodies, ER — endoplasmic reticulum, V_1 — firsth type vacuole, V_2 — second type vacuole, V_3 — third type vacuole, R — ribosomes, M — mitochondria, Fm_2 — fragments of second membrane, mp — micropore, VER — large vacuoles of endoplasmic reticulum, V — vesicles lying above plasm membrane of growing macrogamete, m_2 — formed second membrane

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Электронномикроскопическое изучение процесса шизогонии *Eimeria intestinalis*

Electron microscopic study of the schizogony process in *Eimeria intestinalis*

Для внутриклеточных паразитических простейших, относящихся к классу споровиков, характерно наличие в их жизненном цикле особого типа бесполого размножения — шизогонии, рассматриваемой как множественное деление клетки, или синтомия. Изучение шизогонии с помощью электронного микроскопа несколько изменило это представление. Имеются электронномикроскопические наблюдения шизогонии у некоторых видов *Eimeria* и *Plasmodium*, но данные разных авторов, а также их выводы относительно способа образования мерозоитов различны. Шолтизек (Scholtyseck 1965) называет этот процесс „отделение отщеплением"; Шеффилд и Хаммонд (Sheffield and Hammond 1967), Хеплер и др. (Hepler et al. 1966), Аикава и др. (Aikawa 1966, Aikawa et al. 1967) считают что образование мерозоитов у изученных ими видов происходит путем почкования.

В связи с тем, что электронномикроскопические картины шизогонии, обнаруженные у разных видов споровиков, отличаются друг от друга, нам представляется интересным изучение этого процесса еще у одного из видов кокцидий — *Eimeria intestinalis* Cheissin, 1948, паразита кишечника кролика.

Материал и методика

Исследование проводилось на эндогенных стадиях развития *Eimeria intestinalis*: мерозоитах и шизонтах. Кусочки ткани тонкого кишечника кролика, забитого на 7—8 день после заражения, фиксировались 2% р-ром осмиевой кислоты по Шьёстранду и заливались в аралдит.

Ультратонкие срезы приготавливались на ультрамикротоме УМТ2 и контрастировались уранил-ацетатом (Watson 1959) и гидроокисью свинца (Karnovsky 1961). Микрофотографии были получены на электронном микроскопе JEM-5g. Электронноптическое увеличение составляло 12—18 000 × с последующим фотоувеличением.

Результаты исследования

Внедрившись в клетку хозяина, спорозоит округляется и начинает расти, превращаясь в трофозит — будущий шизонт. Вокруг паразита в клетке хозяина образуется паразитофорная вакуоль, ограниченная одной мембраной. Вдоль этой мембраны в цитоплазме клетки хозяина собираются митохондрии и иногда вплотную к ней прилегает аппарат Гольджи (Табл. V 10). Между стенкой паразитофорной вакуоли и паразитом обнаруживается узкая щель с электронно-прозрачным содержимым. Иногда в ней содержатся короткие трубочки, диаметром 200 Å, которые не связаны ни с телом паразита, ни с мембраной вакуоли (Табл. I 1, 2, II 3). Т. к. трубочки видны, обычно, вблизи участков разрушенной мембраны паразитофорной вакуоли, можно предполагать, что они представляют собой её фрагменты.

Одноядерный трофозоит представляет собой клетку округлой формы, ограниченную одной плазматической мембраной (Табл. I 1, 2, II 3). В нем нами ни разу не обнаружены органоиды, характерные для мерозоита. Лишь в некоторых одноядерных трофозоитах видны фрагменты второй мембраны, являющейся, вероятно, остатками внутренней мембраны мерозоита (Табл. I 2, 3). Иногда в цитоплазме трофозоитов могут быть обнаружены пучки трубчатых фибрилл такого же диаметра (около 200 Å), как и субпелликулярные фибриллы мерозоитов. Однако, в округлившемся трофозоите они теряют свое упорядоченное расположение и встречаются не закономерно (Табл. I 2 f).

Ядро трофозоита (N) крупное, до 3 мк в диаметре, ограничено двумя мембранами с редко расположенными в них порами, диаметром 0.1 Å. Вблизи пор в нуклеоплазме и в околоядерной зоне цитоплазмы видны более светлые участки (Табл. III 5p). Наружная мембрана ядерной оболочки покрыта рибосомами. В ядре содержится относительно большое число осмиофильных глыбок, образованных мелкими гранулами, представляющих собой, по-видимому, скопления хроматина (chr). Кроме того, по всей нуклеоплазме рассеяны тонкие плохо различимые нити, входящие в разных местах в глыбки хроматина. Хроматин в ядре располагается неравномерно: его меньше в той части ядра, где находится ядрышко (Табл. I 1 n). Довольно крупное ядрышко располагается эксцентрично, вблизи от ядерной оболочки. Оно представляет собой плотное тело, состоящее из извитой, многократно свернутой ленты — неклеолонемы. Последняя выглядит зернистой, вероятно, в связи с тем, что содержит мелкие гранулы, а также тонкие нити (Табл. I 1).

Цитоплазма трофозоита, обладающая высокой базофилией в световом микроскопе, заполнена большим количеством свободных рибосом и довольно многочисленными канальцами шероховатой эндоплазматической сети (Табл. I 1, 2, II 3 ER). В цитоплазме некоторых трофозоитов встречаются скопления мелких гранул, имеющие диаметр 0.3—0.4 мк (Табл. I 1, II 3) не ограниченные мембраной, эти скопления напоминают парануклеарные тела спорозоитов *Eimeria nieschulzi* (Colley 1966).

Гладкие мембраны представлены в трофозоите пищеварительными вакуолями и элементами аппарата Гольджи (GC). Последний имеет вид типичной для простейших диктиосомы. Диктиосома трофозоита состоит из системы немногих уплощенных мешочков (Gc), переходящих на одной из сторон в небольшие вакуоли (Gv) а на другой — в мелкие пузырьки, диаметром 250 Å (Gves). Последние обычно прилегают к каналу шероховатой эндоплазматической сети (Табл. III 6). Мелкие пузырьки, располагающиеся по 5—6 в ряд, имеют элек-

тронноплотное содержимое, в то время, как просвет канала эндоплазматической сети намного прозрачнее каналов, не связанных с гладкими цистеранми. Довольно часто видна связь диктиосомы с оболочкой ядра с помощью мелких пузырьков; перинуклеарное пространство в месте контакта с диктиосомой несколько просветлено (Табл. III 4). На Табл. III 5 видна диктиосома, связанная со складкой, образованной наружной мембраной оболочки ядра, выпячивающейся в цитоплазму.

В одноядерном трофозоите нами обнаружена своеобразная структура, имеющая в своем составе некоторые элементы диктиосомы (Табл. III 3). К участку оболочки ядра, вогнутому в сторону нуклеоплазмы, примыкает канал шероховатой эндоплазматической сети. В пространстве, ограниченном мембранами ядра и канала эндоплазматической сети, в одну линию расположены прозрачные пузырьки, диаметром 250 Å. Пузырьки такого же диаметра, но с электронноплотным содержимым, прилегают к наружной мембране ядерной оболочки и к мембране канала эндоплазматической сети. Указанные признаки дают основание предполагать, что эту структуру можно рассматривать как одну из стадий формирования диктиосомы.

Митохондрии трофозоита — овальные или вытянутые, иногда неправильной формы, в срезе их может насчитываться по 5—10. Матрикс митохондрий гомогенный, светлый, трубочки располагаются в основном по периферии (Табл. II 3 M, IV 7).

В цитоплазме шизонтов обнаружены тельца, преимущественно овальной формы, приблизительно таких же размеров, как и митохондрии. В редких случаях их форма может быть неправильной. Как и митохондрии, они ограничены двумя элементарными мембранами (Табл. I 1, II 3 dM). Эти тельца содержат тонкогранулярный матрикс и не содержит крист. Вблизи ограничивающей оболочки, в их матриксе встречаются скопления электронноплотного вещества, благодаря чему создается впечатление, что внутренняя мембрана толще наружной. Однако, на некоторых фотографиях можно различить ее многослойный характер. Кроме того обнаружены подобного рода тельца, матрикс которых имеет более плотный и гранулярный характер, чем матрикс митохондрий. В них имеются неотчетливые мембранные структуры, соответствующие трубочкам митохондрий (Табл. IV 8). Недостаточный контраст внутренних мембран, по-видимому, связан с относительно высокой плотностью окружающего их матрикса.

Таким образом, прослеживаются переходные формы между митохондриями и плотными овальными тельцами. Эти образования мы интерпретируем как дегенерирующие формы митохондрий (dM).

В плазматической мембране одноядерного трофозоита имеется одна микропора, оставшаяся, по-видимому от мерозоита (mp). Подробно микропора эндогенных стадий развития уже описана нами (Snigirevskaya 1967, Снигиревская и Хейсин 1968).

На стадии трофозоита паразит усиленно растет, увеличивается масса цитоплазмы и ядра. Это свидетельствует об его активном питании, которое осуществляется, по-видимому, с помощью микропоры, являющейся ультрацитостомом. Наличие у конца канала микропоры в цитоплазме вакуолей подтверждает это предположение (Снигиревская и Хейсин 1968). Пищеварительные вакуоли, ограниченные одной мембраной, распределены в различных областях тела паразита (fv).

Содержимое этих вакуолей, так же, как и содержимое паразитофорной

вакуоли прозрачно (Табл. II 3, III 4, V 10). В цитоплазме паразита встречаются в небольшом количестве прозрачные или невысокой плотности включения, представляющие собой, вероятно, капли жира (Табл. III 4 l).

Трофозоит в процессе развития дифференцируется в шизонт или гаметоцит. От молодой макрогаметы одноядерный шизонт одних и тех же размеров отличается тем, что в макрогамете содержатся крупные осмиофильные включения, принимающие участие в образовании оболочки зиготы. Одноядерные же шизонты и микрогаметоциты различить по электронномикроскопическим фотографиям не представляется возможным. Деление ядер начинается тогда, когда рост паразита еще не закончился. Вместе с ростом паразита увеличивается и паразитофорная вакуоль так, что клетка хозяина превращается в узкую полоску цитоплазмы.

Ядро паразита делится сначала на два дочерних, затем каждое из них опять делится до тех пор, пока не образуется число ядер, характерное для шизонтов данной генерации. Деление происходит путем митоза, при этом ядерная оболочка сохраняется, ядрышко же исчезает. Вероятно, оно принимает участие в образовании нитей веретена, обнаруженного нами внутри ядра (Табл. V 9). Нити веретена прикрепляются к ядерной оболочке, с наружной стороны которой в цитоплазме находится центриоль (c).

Вслед за одноядерным шизонтом следует двуядерная форма и затем многоядерная. Ядра многоядерных шизонтов крупные, с большим ядрышком, расположенным эксцентрично. Хроматин распределен в ядре более или менее равномерно (Табл. VI 11). Это отличает их от ядер микрогаметоцитов, хроматин которых, в основном, локализируется по периферии. Параллельно с делением ядер происходят изменения и в цитоплазме шизонтов. Увеличивается число митохондрий, в ограничивающей оболочке шизонта можно видеть уже не одну микропору, а несколько (Snigirevskaya 1967). Однако, определение их общего количества возможно лишь при специальном исследовании.

В процессе развития в многоядерном шизонте появляется большое количество гладких мембран, представляющих собой инвагинации плазматической мембраны. Поверхность тела шизонта преобретает волнистый вид. В некоторых участках под плазматической мембраной появляется внутренняя мембрана (Табл. VI 12 im). Эти участки поверхности тела шизонта, ограниченные двумя мембранами, выпячиваются в виде конусов. Такие конусовидные выпячивания цитоплазмы образуются как на поверхности шизонта, обращенной в паразитофорную вакуоль, так и в щелях, образованных инвагинациями клеточной мембраны. На вершине выпячивания виден типичный коноид и в цитоплазме вблизи от него круглое осмиофильное включение, ограниченное мембраной шероховатой эндоплазматической сети (Табл. VII 13, 14). Структура ядра к этому времени несколько изменяется: ядрышко обнаружить не удается, хроматин заполняет все ядро, но образует более плотные участки по периферии (Табл. VII 14, VIII 15).

Конусовидные выпячивания с коноидом (C) и осмиофильным включением, являющимся, по-видимому, зачатком парной органеллы, представляет собой начальную стадию формирования дочерних клеток шизонта — развивающуюся почку мерозоита. На следующей стадии развития почка вытягивается в длину, появляется связь между коноидом и парной органеллой (Табл. VIII 15), появляются упорядоченно расположенные под внутренней мембраной субпеликулярные фибриллы (Табл. VIII 16 sf). Образуется полностью сформированный передний конец мерозоита со специфическими для него органоидами.

Довольно часто в почке мерозоита обнаруживается центриоль, которая имеет атипичное строение: в центре цилиндра, состоящего из девяти периферических фибрили проходит еще одна, центральная фибрилла (Табл. VII 13, VIII 15 c). Нахождение центриоли у *Eimeria intestinalis* является интересным фактом т. к. в цикле развития паразита имеется микрогамета — единственная стадия, у которой возникают жгутики. Можно предположить, что возникновение жгутиков происходит не de novo, (Хейсин 1965), а связано с этими центриолями (Хейсин 1967). Т. к. центриоли встречаются на срезах очень редко, можно предположить, что они есть не у всех мерозоитов, а только у мерозоитов с определенной половой потенцией, а именно, у тех мерозоитов, которые в дальнейшем разовьются в микрогаметоциты. На Табл. VIII 13 видно, что почти все почки мерозоитов одного шизонта имеют центриоли, т. е. мерозоиты, образовавшиесяз из одного шизонта, обладают одной и той же потенцией.

В процессе образования мерозоита почки вытягиваются еще больше и плазматическая мембрана врастает вглубь тела шизонта. На том уровне, где наружная мембрана почки мерозоита переходит в ограничивающую мембрану шизонта, оканчивается внутренняя мембрана почки. Около каждого ядра многоядерного шизонта формируется две почки. Ядро делится последний раз митотическим путем так, что каждая почка получает одно ядро. Так, на Табл. VIII 14 видны попавшие в срез две почки, в каждую из которых вдается вырост материнского ярда, представляющий собой будущее ядро мерозоита. В ограничивающей оболочке почки, уже содержащей ядро, обнаруживается микропора, образованная инвагинациями наружной и внутренней мембран (Табл. VIII 16 mp). Позади ядра в полностью сформированной почке появляются белковые тельца и зерна гликогена (Табл. IX 18 pr, gl). Такие готовые мерозоиты некоторое время еще остаются прикрепленными своими задними концами к остаточному телу шизонта. По-видимому, эта стадия довольно продолжительная, т. к. не распавшиеся шизонты встречаются на срезах в большом количестве (Табл. IX 18). Ограничивающая мембрана остаточного тела переходит в наружную мембрану оболочки мерозоита внутренняя мембрана и субпелликулярные фибриллы оканчиваются в месте связи заднего конца мерозоита с остаточным телом шизонта (Табл. IX 19).

Остаточное тело шизонта *E. intestinalis* сравнительно небольшое, ограничено одной мембраной (Табл. VIII 17, IX 18). Оно содержит незначительное количество рибосом и гладких мембран. Кроме того, в остаточных телах некоторых шизонтов встречаются крупные скопления мелких гранул, не ограниченные мембраной, такие же, как и в одноядерных трофозоитах.

Мерозоиты отделяется от остаточного тела, видимо, одновременно, после чего они выходят из зараженной клетки и проникают в новые.

Структура зрелого мерозоита была уже описана нами (Хейсин и Снигиревская 1965). Здесь мы можем дополнительно отметить обнаруженные нами в теле мерозоита диктиосомы. Единичная диктиосома всегда располагается перед ядром, причем мелкие пузырьки соединяют параллельно лежащие цистерны с оболочкой ядра (Табл. X 20). Мембраны диктиосомы мерозоита контрастируются слабее, чем у шизонта. В цитоплазме имеюстя митохондрии, более компактные и содержащие несколько большее число крист, чем митохондрии шизонта; рибосомы, гликогеновые включения в виде зерен и белковые в виде круглых телец ячеистой структуры; капли жиры не встречаются. Кроме того, изредка наблюдаются в незначительном количестве овальные тельца с двумя ограничивающими мембранами.

Обсуждение

Полученные нами данные показывают, что образование мерозоитов у *E. intestinalis* происходит путем синхронного почкования, аналогично обнаруженному у видов *Plasmodium* (Aikawa 1966, Hepler et al. 1966) и у *Eimeria bovis* (Sheffield and Hammond 1967).

Процесс почкования на примере видов *Plasmodium* Hepler et al. 1966 расчленили на три фазы: фаза дедифференциации, во время которой мерозоиты теряют многие свои структуры; фаза роста, включающая ядерные деления и увеличение числа цитоплазматических органоидов; и фаза редифференциации, при которой возникают специализированные органоиды новых мерозоитов. Ход шизогонии *Eimeria intestinalis* также укладывается в эту схему.

Внедрившись в клетку хозяина, спорозоит или мерозоит округляется и начинает расти, превращаясь в шизонта. В трофозоите происходит деление ядра и параллельно с этим изменения цитоплазматических структур. Для *Eimeria intestinalis* характерно наличие трех генераций шизонтов, из которых развивается различное число мерозоитов.

Существенная роль в проникновении мерозоита приписывается его специализированному переднему концу и, в частности, парной органелле, которая, по предположению ряда авторов, выделяет протеолитические ферменты (Garnham et al. 1960, Aikawa 1966).

Округлившийся в клетке хозяина трофозит уже не имеет специфических для переднего конца мерозоита органоидов. Можно продположить, что эти органоиды растворяются с помощью тех же протеолитических ферментов, которые способствуют проникновению паразита в эпителиальную клетку кишечника.

Вокруг паразита, проникшего внутрь клетки кишечника, образуется паразитофорная вакуоль, ограниченная одной мембраной. На многих микрофотографиях в клетке хозяина видна высокая концентрация вблизи этой мембраны клеточных органоидов: митохондрий, эндоплазматической сети, аппарата Гольджи, которые могут быть источником синтеза мембраны вакуоли. Поэтому мы присоединяемся к мнению Аикавы и др. (Aikawa et al. 1967) и Хеплера и др. (Hepler et al. 1968), считая мембрану, окружающую паразитофорную вакуоль, производным клетки хозяина.

Все деления ядра происходят путем митоза, подобно тому, что описано у *Plasmodium* (Hepler et al. 1966, Aikawa 1966, Aikawa et al. 1967). Ядерная оболочка при делении ядра сохраняется, ядрышко исчезает, вероятно, принимая участие в образовании нитей веретена, которые проходят через ядро, прикрепляясь к центриоли, находящейся в цитоплазме у оболочки ядра. Последнее деление ядер шизонтов происходит тогда, когда уже образовались почки мерозоитов и напоминает деление ядра при внутреннем почковании.

Отпочковывание мерозоитов происходит следующим образом: к концу деления ядер в цитоплазме шизонта сильно увеличивается количество гладких мембран благодаря тому, что плазматическая мембрана врастает в тело шизонта (Рис. 1). Она становится наружной мембраной мерозоита. Специфические органоиды переднего конца мерозоита и внутренняя мембрана образуются, по-видимому, de novo. В пользу этого предположения говорит тот факт, что никаких предшественников этих структур в развивающемся шизонте обнаружено нами не было. Они появляются лишь тогда, когда деление ядер почти закончилось и образовались почки мерозоитов. Образование этих структур

de novo возможно, т. к. в клетке происходит интенсивный синтез белка. На это указывает большое число рибосом и шероховатой эндоплазматической сети в цитоплазме шизонта, а также большое ядро с крупным ядрышком фибриллярногранулярной природы.

Зрелые мерозоиты некоторое время прикреплены своими задними концами к остаточному телу. Отпочковываются они, видимо, одновременно, после чего они выходят из больных клеток.

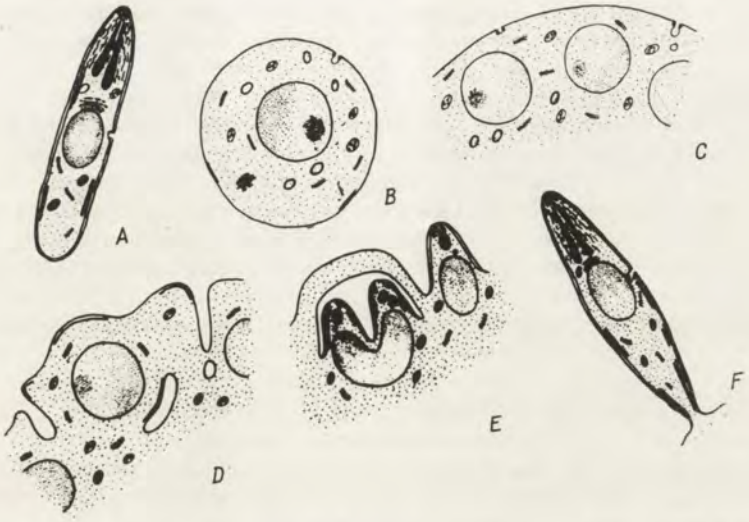

Рис. 1. Схема процесса шизогонии *Eimeria intestinalis*. A — зрелый мерозоит со специфическими органоидами, B — округлившийся трофозоит, лишенный специфических для мерозоита органоидов, C — стадия многоядерного шизонта, D — появление инвагинаций плазматической мембраны и образование внутренней мембаны будущих мерозоитов, E — формирование почек мерозоитов, обладающих характерными для переднего конца мерозоита органоидами, F — отпочковывающийся мерозоит

Fig. 1. Scheme of the schizogony process in *Eimeria intestinalis*. A — mature merozoite with characteristic organellae, B — rounded trophozoite without organellae characteristic of the merozoite, C — stages of multinuclear schizont, D — appearance of invagination on plasmatical membrane and formation of the inner membrane of future merozoites, E — formation of merozoite buds with organellae characteristic of the anterior end of merozoite, F — budding merozoite

На стадии трофозоита паразит усиленно растет, накапливая клеточную массу. Вещества, необходимые для своего развития, паразит получает из клетки хозяина, которые сначала поступают, по-видимому, в паразитофорную вакуоль, а затем уже из нее проникают частично через мембрану паразита, а частично (вероятно. высокополярные соединения) поглощаются ультрацитостомом, роль которого у *Eimeria intestinalis* выполняет микропора (Снигиревская и Хейсин 1968). На стадии трофозоита наблюдается одна микропора, оставшаяся от мерозоита. Количество микропор шизонта должно соответствовать количеству ядер, т.к. при распадении шизонта на мерозоиты, каждый мерозоит получает по одной микропоре, в то время как в остаточном теле шизонта микропоры не обнаружены.

О высокой метаболической активности одноядерного трофозоита говорят большие размеры его ядра, которое увеличивается с ростом паразита. Структура ядра на протяжении процесса шизогонии несколько изменяется. В одноядерном трофозоите ядро — крупное с более или менее равномерным распределением хроматина, с крупным ядрышком. Структура, обнаруженная в ядрышке, также свидетельствует о высокой метаболической активности клетки. Как показано Ченцовым 1966 в период синтетической активности ядрышко имеет фибриллярногранулярную природу, сходную с тем, что видно на наших снимках. Структура ядра изменяется перед последним делением: ядра становятся более компактными, хроматин, в основном, локализуется по периферии ядра, ядрышко исчезает, т.е. ядро преобретает тот вид, который оно имеет у мерозоита.

Имеющийся в клетке белок, по-видимому, не весь используется развивающейся клеткой. Часть его откладывается в цитоплазме в виде скоплений, состоящих из мелких гранул, не ограниченных мембраной. Эти частицы не входят в состав вновь образующихся мерозоитов, а остаются в остаточном теле шизонта вместе с небольшим количеством рибосом и гладких мембран. По всей вероятности этот белок представляет собой продукт экскреции. Доказательством этого является то, что эти скопления мелких частиц не включаются в дочерные клетки, а отбрасываются вместе с остаточным телом. Это происходит таким путем, вероятно, потому, что специальные экскреторные органоиды у этих паразитов отсутствуют. Включения, которые мы интерпретируем как экскреторные, очень напоминают описанные у спорозоитов *Eimeria nieschulzi* (Colley 1967) парануклеарные тела, которые автор связывает с секрецией.

Активное участие в общем обмене паразитической клетки принимают митохондрии, обнаруженные на всех стадиях развития. Количество митохондрий увеличивается по мере роста шизонта и деления его ядер.

В одно- и многоядерных шизонтах встречаются митохондрии неправильной формы, наличие которых, возможно, связано с образованием новых митохондрий. Косвенно это подтверждается тем, что в мерозоитах, образующихся из шизонтов, мы не наблюдали разветвленных митохондрий.

Как и у всех организмов, ведущих анаэробный или полуанаэробный образ жизни, митохондрии шизонтов *Eimeria intestinalis* имеют мало трубочек, в основном, расположенных по периферии митохондрии. Трубочки несколько более плотно упакованы в мерозоитах, которые некоторое время существуют, активно двигаясь в более богатой кислородом среде, в просвете кишечника.

Наряду с такими типичными, хотя и бедными кристами, митохондриями, в цитоплазме шизонтов обнаружены дегенерирующие митохондрии, полностью лишенные внутренних структур. Они также ограничены двумя мембранами, их содержимое имеет тонко-гранулярный вид. Митохондриальное происхождение этих структур подтверждается тем, что нами обнаружены переходные картины в виде митохондрий, содержащих плотный гранулярный матрикс и лишь остатки внутренних мембран. В матриксе наблюдаются скопления электронноплотного вещества по периферии, вблизи ограничивающей оболочки. Можно полагать, что эти скопления представляют собой оттесненные к периферии в процессе деградации мембранные системы крист, в некоторых участках слившиеся в одну толстую мембрану.

Подобная деградация внутренних структур митохондрий у организмов, ведущих анаэробное существование, не раз описывалась в литературе. Это явление обнаружено у грегарин (Vivier et Schrevel 1966), у паразитов печени

Fasciola (Bjorkmann 1962), у облигатных анаэробов — дрожжей (Birjusova 1964, McClary and Bowers 1967), при инактивации системы дегидрогеназы в мышцах (Moore et al. 1956).

По-видимому, аналогичные процессы, имеющие место у *Eimeria intestinalis*, также связаны с их анаэробным существованием. Как показали опыты Бейер на *E. intestinalis* с сукциндегидрогенезой, этот фермент почти полностью отсутствует во внутриклеточных стадиях развития — шизонтах и макрогаметах. Слабая сукциндегидрогеназная активность появляется у мерозоитов, которые некоторое время существуют в более кислородной среде, активно двигаясь в ней (Бейер 1962).

При сравнении стадий шизонта и мерозоита обнаружено, что большое количество митохондрий с меньшим количеством крист и полностью дегенерировавшими кристами встречается в шизонтах. Их становится меньше в почкующемся шизонте и в уже сформированных мерозоитах. Интересно отметить тот факт, что указанные изменения претерпевают не все митохондрии (в шизонтах имеются типичные митохондрии, хотя и с небольшим количеством крист). Вероятно, оставшиеся митохондрии дают возможность быстро перейти к обмену за счет дегидрогеназ, локализованных на митохондриальных мембранах, при выходе мерозоитов в просвет кишечника. Большое количество зерен гликогена в теле мерозоита обеспечивает выделение достаточных количеств энергии, необходимой для его активного движения. Этот факт свидетельствует о том, что перестройка структур происходит не под влиянием внешних условий жизни, а что она обусловлена генетически, т.е. организм, еще не перешедший к новому образу жизни, уже полностью подготовлен к нему.

Аппарат Гольджи представлен типичными диктиосомами, образованными стопкой гладких цистерн, которая с помощью мелких пузырьков соединяются с каналом шероховатой эндоплазматической сети. Такая же структура диктиосомы описана у других паразитических простейших *Selenidium* (Vivier et Schrevel 1966), у *Sacrocystis* и *Toxoplasma* (Senaud 1967). Довольно часто в одноядерных и многоядерных шизонтах *E. intestinalis* и всегда в мерозоитах диктиосомы связаны с ядром. Возможно, через них осуществляется ядерно-цитоплазматический обмен. На эту возможность указывали Zeigel and Dalton 1962. Они предполагали, что ядерное содержимое идет в цитоплазму через мелкие пузырьки аппарата Гольджи к ламеллярным структурам. Возможно, это относится и к диктиосомам *E. intestinalis*, мелкие пузырьки которых заполнены электронноплотным содержимым. То же может относиться и к диктиосомам, не связанным с ядром. В этом случае через мелкие пузырьки осуществляется перенос веществ из каналов шероховатой эндоплазматической сети.

Однако, в большинстве случаев диктиосомы шизонтов не связаны с ядром, а располагаются в цитоплазме в связи с каналом шероховатой эндоплазматической сети. Так что связь диктиосомы с ядром временная. Возможно, ядро индуцирует образование аппарата Гольджи. С формированием аппарата Гольджи мы связываем своеобразную структуру, найденную в одноядерном трофозоите, описанную выше. Прозрачные пузырьки, расположенные в одну линию между каналами ядерной оболочки и шероховатого ретикулюма, вероятно, являются цистернами, образующимися из мелких пузырьков, расположенных вдоль мембран каналов. Образование новых мешочков с гранулярными мембранами связывает Гримстоун (Grimstone 1961), который также обращает внимание на тесную связь диктиосомы *Trichonympha* с ядром. На то, что мелкие пузырьки или гранулы являются первичной основной структурой аппарата Гольджи

указывал и Куросуми (Kurosumi 1961). По-видимому, сформированный аппарат Гольджи, связанный еще с оболочкой ядра, является следующим этапом развития диктиосомы. Возможно, в этот период и происходит ядерно-цитоплазматический обмен. Затем диктиосома отделяется от ядра и мигрирует в цитоплазму. При отделении диктиосомы от ядерной оболочки отслаивается канал шероховатой эндоплазматической сети так, как это описано для образования шероховатой сети из наружной мембраны ядерной оболочки. На связь образования диктиосомы с ядром указывают и Moore and McAlear 1963, которые наблюдали образование диктиосомы около ядра клеток грибов из наружной мембраны ядерной оболочки. Содержимое цистерн и вакуолей диктиосомы всегда прозрачно, так же, как и содержимое канала шероховатой эндоплазматической сети, связанного с диктиосомой. Заполнены осмиофильным веществом лишь мелкие пузырьки, находящиеся между каналом ретикулюма и цистернами. Возможно, в них локализуются те белки, которые синтезируются в канале шероховатой эндоплазматической сети.

Резюме

Электронномикроскопическое изучение процесса шизогонии *Eimeria intestinalis* показало, что образование мерозоитов у этого вида кокцидий осуществляется путем синхронного почкования. Внедрившийся в клетку хозяина мерозоит превращается в шизонта, ядро которого делится повторно путем митоза. Параллельно ядерным делениям происходят изменения цитоплазматических органоидов паразитической клетки. Возникновение специфических для мерозоита органоидов (коноида, парной органеллы, токсонем), вероятно, происходит de novo, что возможно при наличии в клетке синтеза белков.

Обсуждается роль парной органеллы в проникновении паразита в клетку хозяина, роль микропоры в его питании, а также роль остаточного тела в экскреторных процессах. Описаны изменения митохондрий и аппарата Гольджи в ходе процесса шизогонии.

SUMMARY

Electron microscopy study of the schizogony process of *Eimeria intestinalis* has shown that the merozoites in this coccidian species are formed by synchronous budding. Appearance of specific organelles of merozoites occurs de novo most likely, what is possible in the case of intensive synthesis of protein in the cell. The role of paired organelles in the invasion of parasite into the host cell, the role of micropore in the nutrition, and that of the residual body in the excretory processes are discussed. Changes in mitochondria and Golgi complex during the schizogony process are also described.

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ПОДПИСИ К ТАБЛИЦАМ I—X

Процесс шизогонии у *Eimeria intestinalis*

1: Одноядерный шизонт, 25 000 ×
2: Участок одноядерного шизонта с фрагментами внутренней мембраны (стрелки) и пучком фибрилл, 50 000 ×
3: Одноядерный шизонт; скопления белка (стрелка), 34 000 ×
4: Связь аппарата Гольджи с оболочкой ядра, 45 000 ×
5: Диктиосома, связанная со складкой ядерной оболочки (стрелка), 40 000 ×
6: Диктиосома одноядерного шизонта, 60 000 ×
7: Разветвленная митохондрия одноядерного шизонта, 80 000 ×
8: Переходные формы между нормальными митоходриями и дегенерирующими. Стрелками указаны ещё не полностью дегенерировавшися внутренные мембраны, 28 000 ×
9: Ядро в состоянии митотическово деления; видны веретено и центриоль, 46 000 ×
10: Двуядерный шизонт, 27 000 ×
11: Многоядерный шизонт, 30 000 ×
12: Появление инвагинаций поверхностной мембраны и образование внутренней мембраны, 32 000 ×
13: Почкующийся шизонт. В почках шизонтов видны центриоли, 23 000 ×
14: Разделение ядра между двумя почками мерозоитов, 34 000 ×
15: Почка мерозоита. Видна связь парной органеллы и коноида (стрелка), 38 000 ×
16: Почкующий шизонт. Видны упорядоченные субпелликулярные фибриллы и микропора, 30 000 ×
17: Остаточное тело шизонта. Стрелкой указан неиспользованный белок, отбрасываемый вместе с остаточным телом, 50 000 ×
18: Нераспавшийся шизонт, 30 000 ×
19: Задние концы мерозоитов, связанные с остаточным телом, 43 000 ×

Принятые сокращения:

N — ядро, n — ядрышко, chr — хроматин, p — поры в оболочке ядра, M — митохондрии, dM — дегенерирующие митохондрии, ER — эндоплазматический ретикулум, f — фибриллы, sf — субпелликулярные фибриллы, fv — пищеварительные вакуоли, l — капля жира, GC — аппарат Гольджи, Gves — пузырьки аппарата Гольджи, Gc — цистерны аппарата Гольджи, Gv — вакуоли аппарата Гольджи, c — центриоль, C — коноид, PO — парная органелла, mp — микропора, pr — белковые тельца мерозоитов, gl — зёрна гликогена, om — наружная мембрана пелликулы мерозоита, im — внутренняя мембрана пелликулы мерозонта

EXPLANATION OF PLATES I—X

Process of schizogony in *Eimeria intestinalis*

1: Young, mononuclear schizont, 25 000×
2: Part of young schizont; fragments of the inner membrane (arrows) and bundle of fibrils are seen, 50 000×
3: Young, mononuclear schizont; accumulation of albumin (arrows), 34 000×
4: Communication between Golgi complex and membrane of the nucleus, 45 000×
5: Dictyosome connected with the fold of the membrane of the nucleus (arrows), 40 000×
6: Dictyosome of mononuclear schizont, 60 000×
7: Branched mitochondrion of mononuclear schizont, 80 000×
8: Intermediate forms of mitochondria between normal and degenerating ones. Arrows schow the inner membranes not yet fully degenerated, 28 000×
9: Nucleus in the period of mitotic division; the spindle and centriole are seen, 46 000×
10: Binuclear schizont, 27 000×
11: Multinuclear schizont, 30 000×

12: Appearance of invagination on the surface membrane and formation of the inner membrane, 32 000×
13: Budding schizont. Centrioles are seen in the buds, 23 000×
14: Parting of the nucleus between two buds of merozoites, 34 000×
15: Merozoite bud. Connection of the paired organellae with conoid is seen (arrows), 38 000×
16: Budding schizont. Arranged subpellicular fibrils and micropore are seen, 30 000×
17: Residual body of schizont. Arrows show nonutilized protein thrown away together with resting body, 50 000×
18: Nondisintegrated schizont, 30 000×
19: Posterior ends of merozoites connected with residual body, 43 000×

Abbreviations used:

N — nucleus, n — nucleolus, chr — chromatin, p — pores on the nucleus membrane, M — mitochondria, dM — degenerating mitochondria, ER — endoplasmic reticulum, f — fibrils, sf — subpellicular fibrils, fv — food vacuole, l — lipid, GC — Golgi complex, Gves — vesicle of Golgi complex, Gc — cistern of Golgi complex, Gv — vacuole of Golgi complex, c — centriole, C — conoid, PO — paired organellae, mp — micropore, pr — protein body of merozoite, gl — glycogen granules, om — outer membrane of the merozoite pellicle, im — inner membrane of the merozoite pellicle

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Ультратонкое строение некоторых стадий *Myxidium gasterostei* Noble, 1943

Ultrastructure of some stages of development of *Myxidium gasterostei* Noble, 1943

За последнее время в печати появилось довольно большое количество работ, касающихся ультратонкого строения различных микроспоридий (Grassé 1960, Хейсин, Шульман и Винниченко 1961, Lom and Vavra 1963, Lom and de Puytorac 1965a, b, Шульман 1966, Успенская 1966 и др.). Накопление такого материала нужно для того, чтобы разобраться в процессах дифференцировки и морфогенеза, которые у этих сложно организованных животных до сих пор остаются не вполне ясными.

В настоящей статье приводятся данные об ультратонком строении некоторых стадий *Myxidium gasterostei* Noble, 1943 паразита желчного пузыря колюшек *Gasterosteus aculeatus* и *Pungitius pungitius*.

Материл и методика

В Советском Союзе *Myxidium gasterostei* до сих пор был встречен лишь в колюшках водоемов Камчатки. Нами материал собирался из озера Дальнего на базе лаборатории ТИНРО (Камчатская обл. Елизовский район).

Различные стадии *M. gasterostei*, извлеченные из желчного пузыря или вместе с пузырем, фиксировались 2% раствором OsO_4 разведенным жидкостью Палада, и заливались в метакрилат и аралдит. Залитый материал резался на ультрамикротоме, и срезы исследовались под электронным микроскопом JEM-5g.

Самые молодые из обнаруженных нами стадий представляли собой 8-ядерных мелких трофозоитов, прикрепяющихся к стенке желчного пузыря, охватывая эпителиальную клетку в виде шапочки.

Из-за редкой встречаемости этих стадий в период сбора материала, нам не удалось получить данных по их ультратонкому строению, и здесь приводится реконструкция, сделанная под световым микроскопом с гистологических срезов, окрашенных железным гематоксилином (Рис. 1).

Более поздние стадии, уже описывавшиеся Ноблем (Noble 1943) в его статье о ядерном цикле *Myxidium gasterostei* имеют вид узких червеобразных плазмодиев, свободно плавающих в желчи, в полости пузыря. По мере роста

трофозоит принимает листовидную форму, а плазмодии, с уже сформированными спорами внутри, обычно имеют вид толстых округло-овальных лепешек до 8 мм в диаметре. Такие листовидные и лепешковидные плазмодии закручиваются, по несколько штук вместе, причем более мелкие оказываются внутри более крупных. Все эти последовательные стадии трофозоитов изучались нами под электронным микроскопом.

Рис. 1. Молодой трофозоит *Myxidium gasterostei*, прикрепленный к стенке желчного пузыря; реконструкция по срезам окрашенным железным гематоксилином

Fig. 1. Young trophozoite of *Myxidium gasterostei* attached to the wall of biliary bladder; reconstruction after sections stained with iron haematoxylin

Результаты исследования и их обсуждение

Строение трофозоитов

Изучение ультратонкого строения трофозоитов показало, что поверхность их, подобно таковой у *M. lieberkühni* (Lom et de Puytorac 1965 a, b, Успенская 1966) имеет очень тонкую мембрану, приблизительно 60 Å, и образует длинные и тонкие плазматические выросты — микроворсинки (1.7—1.4 μ длиной, 0.1 μ шириной), густой щеткой покрывающие все тело плазмодия (Табл. I 1). На поверхности, у основания ворсинок, обнаруживаются очень мелкие впячивания мембраны, от которых отшнуровываются мельчайшие пузырьки (0.07—0.1 μ в диаметре) — видимо, пиноцитозные вакуоли, транспортирующие вглубь цитоплазмы пищевые частицы (Табл. I 2, 3). Непосредственно под мембраной у трофозоитов разных возрастов располагается плотный слой цитоплазмы с мелкими вакуолями, мелкими осмиофильными гранулами и пронизанный каналами эндоплазматической сети. Этот слой под световым микроскопом кажется гомогенным и обозначается как эктоплазма. Далее вглубь следует слой, богатый митохондриями, с некоторым количеством некрупных вакуолей (0.4 μ в диаметре) (Табл. I 4). Митохоидрии у *M. gasterostei* длинные, лентовидные типичного строения с хорошо развитыми гребнями (Табл. II 5, 6), однако реакция с нитросиним-тетразолием показывает, что окисление красителя идет лишь до образования гранул монофармазана, что говорит о пониженной сукциндегидразной активности у *M. gasterostei*, живущего в полуанаэробных условиях желчного пузыря рыб.

В центре тела молодого червеобразного трофозоита располагаются вегетативные и генеративные ядра. Уже у таких плазмодиев ядра дифференцированы, и начинается обособление участков цитоплазмы с митохондриями и жировыми включениями вокруг генеративных ядер (Табл. I 4). В некоторых участках цитоплазмы, в средней части тела наблюдается очень сильное развитие шероховатой эндоплазматической сети, что обычно связывают с усиленным синтезом белка в этих участках. На фотографии 7 Таблица II видно, что двойная мембрана шероховатой эндоплазматической сети переходит непосредственно в двойную мембрану генеративной клетки (стрелка). Этот факт, с одной стороны, указывает на то, что многочисленные мембраны, возникающие в генеративной клетке в период спорогенеза, вероятно, развиваются из мембран эндоплазматической сети. С другой стороны, в этот период на построение спор расходуется большое количество белковых веществ, которые при наличии таких связей могут поступать в генеративную клетку по каналам эндоплазматической сети. На фото 8 Таблице II такой же „рулон" мембран шероховатой сети расположен вблизи вегетативного ядра и связан с его оболочкой, что наводит на мысль аб участии вегетативного ядра в процессах морфогенеза. На наличие связи эргастоплазма с оболочкой ядра капсулогенной клетки указывают в своей работе так же Лом и Пюторак (Lom et de Puytorac 1965 a, b).

У листовидных трофозоитов центральная часть цитоплазмы начинает вакуолизироваться. Вакуоли здесь уже достигают 1.5 μ в диаметре (Табл. III 9).

У наиболее крупных лепешковидных трофозоитов, приступивших к спорообразованию, вакуоли эти неимоверно разрастаются и вся центральная часть плазмодия превращается в альвеолярный слой (Табл. III 10). Между крупными вакуолями остаются лишь цитоплазматические мостики. Стенки вакуолей представлены универсальной мембраной и создается впечатление, что возникли они в результате сильного вздутия цистерн эндоплазматической сети. Иногда между двумя такими альвеолами не остается цитоплазматического мостика и ограничивающие их мембраны почти примыкают друг к другу (Табл. III 10, стрелка).

Под электронным микроскопом альвеолы либо кажутся пустыми, либо содержат хлопья каких-то свернувшихся при фиксации веществ (Табл. III 10). Иногда внутри них попадаются глыбки, дающие положительную реакцию PAS. Часть содержимого дает реакцию с нильским голубым на кислые жиры. Кое-где в утолщениях цитоплазматических мостиков альвеолярного слоя встречаются вегетативные ядра, генеративные клетки или жировые включения, окруженные митохондриями. В основном же ядра и генеративные клетки располагаются между эктоплазмой и альвеолярным слоем.

Вегетативные ядра имеют, видимо, уплощенную форму, так как на поперечных разрезах они вытянутые, а под световым микроскопом кажутся округлыми. Оболочка ядра двойная с порами (Табл. IV 11), крупная кариозома лежит эксцентрично, как у большинства видов миксоспоридий (Шульман 1966). Генеративное ядро округлое в сечении, часто с неровными краями и с эксцентричной кариозомой (Табл. IV 12).

Как уже говорилось, по мере развития плазмодия вокруг генеративных ядер обособляются участки цитоплазмы с митохондриями и жировыми включениями, возникают генеративные клетки, и плазмодий становится многоклеточным (Табл. V 13). Вначале цитоплазма генеративных клеток плотная и при малых увеличениях клетки кажутся темными. Генеративные клетки *Myxidium gasterostei* становятся панспоробластами.

По схеме Нобля (Noble 1943), ядро панспоробласта делится, в результате

чего образуется одно вегетативное и одно генеративное ядро. Вегетативное ядро делится еще рез. Образовавшиеся два ядра, остаточные ядра двуспорового панспоробласта (Табл. VII 17), которые впоследствии дегенерируют. Генеративное ядро делится четыре раза и дает восемь ядер. В сумме с двумя дегенерирующими вегетативными ядрами их десять в панспоробласте.

При выделении клеток споробласта на границе их раздела каналы эндоплазматической сети вздуваются, образуя большое количество вакуолей. Цитоплазматические мостики между вакуолями утончаются и клетки-споробласты отделяются друг от друга (Табл. V 14).

По мере формирования спор в панспоробласте наблюдается явная дегенерация всех органелл: дегенерируют остаточные ядра и митохондрии, исчезает эндоплазматическая сеть, и сформированшиеся споры оказываются в вакуоле подвешенными к ее стенке на тонких фибриллах (Табл. VI 16).

Внутри каждого споробласта обособляются две клетки створкообразовательницы или вальвогенные клетки, две капсулогенные клетки и двуядерный амебоидный зародыш (Табл. VI 15). Развитие двух споробластов у этого вида идет не вполне синхронно. Например, может быть так, что в одном из них уже развились стрекательные капсулы, а в другом только начинается их закладка.

Образование створок споры

Отделившиеся собственной мембраной вальвогенные клетки в споробласте распластываются по его периферии, охватывая со всех сторон спороплазм (амебоидный зародыш) и капсулогенные клетки как бы футляром. Благодаря неравномерному уплотнению цитоплазмы этих клеток (Шульман 1966) образуются продольные ребра оболочки споры, поэтому на поперечном разрезе споробласт выглядит свездчатым (Табл. VII 17, VIII 21). Два крайних ребра каждой вальвогенной клетки, примыкая друг к другу, в месте стыка клеток образуют шовный валик (Табл. VII 17). По линии стыка двух клеток-створкообразовательниц происходит раскрытие створок споры.

В начале формирования спор вальвогенные клетки имеют нормальные ядра и митохондрии, видна сеть эндоплазматических каналов (Табл. VI 15, VII 17). Постепенно же цитоплазма их становится плотной, гомогенной, митохондрии дегенерируют. Ядра тоже дегенерируют, хотя и продолжают давать положительную реакцию Фельгена и во вполне сформированных створках.

Формирование стрекательных капсул

У *Myxidium gasterostei* так же как это описано Ломом и Пютораком (Lom and de Puytorac 1965 b) для других видов миксоспоридий, образование стрекательной капсулы внутри капсулогенной клетки начинается с развития продолговато-овального зачатка, имеющего мелко-гранулированный осмиофильный наружный слой, более светлый внутренний слой и скопление мелких электронноплотных гранул в середине. Затем один конец зачатка вздувается, превращаясь в зачаток собственно капсулы, другой начинает расти, вытягиваясь в наружную трубку стрекательной нити. Наружный слой капсулы уплотняется, образуя оболочку, которая концентрируется в довольно плотный матрикс. Находящийся между наружной оболочкой и матриксом более светлый слой утол-

щается и становится электронно прозрачным (Табл. VIII 18, 19, 20). По мере вворачивания в полость капсулы стрекательной нити, гранул матрикса становится меньше, и в капсуле с полностью ввернутой нитью от него почти ничего не остается (Табл. VIII 21).

Наружная трубка имеет то же ультратонкое строение, что и капсулярный зачаток, правда электроннопрозрачный слой в ней развит гораздо слабее (Табл. IX 24, 25). На некоторых срезах хорошо видно место перехода собственно капсулы в наружную трубку стрекательной нити (Табл. VIII 22, 23). Согласно гипотетической схеме Лома и Пюторака (Lom and de Puytorac 1965 b), стрекательная нить появляется в наружной трубке у ее конца и растет в сторону стрекательной капсулы, врастая в нее. Однако, прямых подтверждений этому предположению до сих пор нет. Картины, которые наблюдали эти авторы, могут трактоваться и иначе. В их статье отмечается, что нужно еще набрать материал, иллюстрирующий рост внутренней стрекательной нити и дегенерацию наружной трубки.

Стрекательная нить *Myxidium gasterostei* очень длинная, соответственно и наружная трубка образует много витков в капсулогенной клетке (Табл. IX 25), проследить ее по всей длине, не имея полной серии большого количества срезов невозможно, поэтому наш материал не вносит в этот вопрос ясности.

На микрофотографии 26 (Табл. IX) видно, что матрикс наружной трубки концентрируется и образует как бы тяж с собственной тонкой оболочкой. На фотографии 27 (Табл. IX) виден конец такого тяжа. Если принять схему Лома и Пюторака то можно было бы думать, чго этот тяж — растущая по направлению к капсуле еще не сформированная инть. Однако тяж этот не скручен, как это характерно для стрекательной нити. Возможна и другая трактовка этой картины: если срезы наши прошли через каспсулогенную клетку, наружная трубка которой еще не достигла окончательной длины, то можно думать, что гранулярный матрикс постепенно подтягивается к концу трубки и за счет материала матрикса происходит ее достройка. Однако, для правильной трактовки полученных картин у нас еще недостаточно материала.

Ввернутая стрекальная нить довольно широка на тех срезах, где она перерезана вдоль. Ширина ее достигает 0.5 μ (Табл. VI 16). На поперечных разрезах видно, что она перекручена и образует характерную восьмерку, описанную для других видов (Хейсин и др. 1961, Lom and Vavra 1963, Шульман 1966).

В начале закладки капсулогенного зачатка в капсулогенной клетке имеется сильно развитая сеть эргастоплазматических каналов, митохондрии, ядро. По мере роста капсулы цитоплазма клетки сильно вакуолизируется, митохондрии и ядро оттесняются к периферии (Табл. X 29). Передний конец полярной капсулы у этого вида покрыт осмиофильной крышечкой с пробочкой (Табл. VIII 21).

Амебоидный зародыш, или спороплазм

Амебоидный зародыш у *Myxidium gasterostei* занимает центральное положение в споробласте между двумя капсулогенными клетками, так как стрекательные капсулы этого вида расположены на противоположных полюсах (Табл. X 28). Амебоид имеет два ядра и зернистую цитоплазму, содержащую митохондрии и эндоплазматическую сеть каналов (Табл. X 29). Нам не удалось наблюдать аппарата Гольджи. Но поскольку у других видов миксоспоридий у спороплазма обнаружен аппарат Гольджи, то должно быть он есть на этой стадии и у *Myxidium gasterostei*.

Из приведенного описания видно, что ультратонкое строение *M. gasterostei* существенно не отличается от такового у других видов, а лишь в деталях. Полученные нами данные по электронной микроскопии этого вида, хотя и сообщают ряд дополнительных сведений, все же не вносят окончательной ясности в такие важные вопросы как закладка стрекательной нити и дегенерация наружной трубки. Для решения этих вопросов нужны дальнейшие электронно-микроскопические исследования.

Резюме

Было проведено электронномикроскопическое исследование стадий развития *Myxidium gasterostei* Noble. В результате исследования выяснилось, что поверхность тела *M. gasterostei* покрыта тонкой мембраной приблизительно 60 Å толщиной, образующей длинные и тонкие микроворсинки. От поверхностной мембраны отпочковываются мелкие пиноцитозные вакуоли. Мембрану подстилает слой мелкозернистой эктоплазмы, затем следует слой, богатый митохондриями и жировыми включениями, далее располагается основная масса генеративных и вегетативных ядер. У крупных зрелых трофозоитов внутренняя часть тела занята альвеолярным слоем. Создается впечатление, что альвеолы образуются за счет раздутия цистерн эндоплазматической сети. В определенный период в цитоплазме наблюдается сильное развитие шероховатой эндоплазматической сети, „рулоны" которой связаны то с мембраной генеративных клеток, то с вегетативным ядром. Видимо, в период морфогенеза происходит усиленный синтез белка и транспортировка его по каналам шероховатой эндоплазматической сети в генеративную клетку. Вегетативное ядро должно быть принимает участие в синтезе белка в этот период.

У молодых червеобразных трофозоитов уже начинается обособление генеративных клеток. По мере роста и созревания трофозоита количество генеративных клеток увеличивается и плазмодий становится многоклеточным.

Было подробно прослежено развитие споробластов и дифференцировка внутри них вальвогенных и капсулогенных клеток и спороплазма (амебоидного зародыша). Была прослежена также закладка капсулярного зачатка и наружной трубки стрекательной нити.

Развитие споробластов у этого вида происходит не вполне синхронно. По мере формирования споры цитоплазма, митохондрии и ядра панспоробласта дегенерируют, и сформированная спора оказывается подвешенной к стенкам вакуоли на тонких фибриллах.

Благодаря тому, что стрекательная нить и соответственно наружная трубка стрекательной нити очень длинны, проследить их по всей длине не удается, а поэтому наш материал не вносит достаточной ясности в вопрос о дальнейшей судьбе наружной трубки и о способе закладки стрекательной нити. Для решения этих важных вопросов морфогенеза нужны дополнительные материалы.

SUMMARY

The investigation of the stages of development of *Myxidium gasterostei* Noble under electron microscope was carried out. It was found that the surface of trophozoit of *M. gasterostei* is limited by a thin membrane 60 Å thick with thin and long microvilli. The pinocytotic activity can be observed. Under the surface of the mem-

brane is a thick ectoplasmic layer, then follows the layer rich in mitochondria and fat inclusions.

Next is the layer with generative and vegetative nuclei. In the mature trophozoit the inner part of the body is occupied by the alveolar layer.

Alveoles seem to be formed due to swelling of the ergastoplasmic vesicles.

At the certain stage of morphogenesis the strong development of the granular ergastoplasmic reticulum is observed connected either with the membrane of generative cell or with the vegetative nucleus.

The intensive synthesis of protein and its transport along the canals of ergastoplasmic reticulum seems to take place in the process of morphogenesis.

The vegetative nucleus must have take part in the protein synthesis during this period. The separation of generative calls begins already inside the young wormlike trophozoites. As the trophozoit is growing mature the number of generative cells increases and plasmodium becomes multicellular. The development of sporoblasts and the separation of valvogenic, capsulogenic cells and sporoplasm inside them was thoroughly followed.

The development of sporoblasts of this species is not completely synchronous.

As the spores are being developed the cytoplasm, mitochondria and the nuclei of pansporoblast degenerate and the spores appear to be attached to the vocuol walls by means of thin fibrilles.

The problem of the further fate of the external tube and of the development of the internal filament could not be solved on the base of our material. The internal filament and the external tube of *Myxidium gasterostei* are too long to be followed from one end to another. Much more is to be done to solve some important problems of morphogeneses in *Myxosporidia*.

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ПОДПИСИ К ТАБЛИЦАМ I—X

Ультратонкое строение *Myxidium gasterostei*

1: Поверхность трофозоита *M. gasterostei* с микроворсинками и эктоплазма, 18 000 ×
2 и 3: Пиноситозные вакуоли у трофозоита *M. gasterostei* (стрелки), 75 000 × и 72 500 ×
4: Поперечный разрез через молодого червеобразного трофозоита, 18 900 ×
5 и 6: Лентовидные митохондрии (M) трофозоита, 40 000 × и 40 950 ×
7: Шероховатая эндоплазматическая сеть (ER), связанная с генеративной клеткой (стрелка), 43 920 ×
8: Шероховатая эндоплазматическая сеть (ER), связанная с вегетативным ядром (NV), 27 200 ×
9: Поперечный разрез через листвидного трофозоита, 15 000 ×
10: Поперечный разрез через зрелого лепешковидного трофозоита, 9 000 ×
11: Вегетативное ядро (NV), 17 000 ×
12: Генеративные клетки с генеративными ядрами (NG) у молодого трофозоита, 29 000 ×
13: Зрелый трофозоит с выделившимся генеративными клетками (gc), 9 300 ×
14: Разделение споробластов, 31 500 ×
15: Споробласты с обособившимися вальвогенными (vc), капсуогенными (cc) клетками и амебоидным зародышем (SP), 16 000 ×
16: Зрелая спора, 26 840 ×
17: Панспоробласт с остаточными (RN) ядрами и двумя споробластами (SB), 34 500 ×
18: Капсуогенный зачаток (ca), 24 400 ×
19: Дальнейшее развитие капсулярного зачатка (ca), 17 200 ×
20: Полярная капсула с еще не ввернутой стрекательной нитью, 24 800 ×
21: Споробласты с полярными капсулами с ввернутыми стрекательными нитями (cr — пробочка, cp — крышечка), 16 000 ×
22 и 23: Места перехода капсулярного зачатка (ca) в наружную трубку стрекательной нити (et), 24 400 × и 22 000 ×
24 и 25: Наружная трубка стрекательной нити (et), 24 400 × и 16 000 ×
26 и 27: Наружная трубка стрекательной нити (et), с концетрированным матриксом (mt), 30 000 × и 21 000 ×
28: Расположение стрекательных капсул в зрелой споре *M. gasterostei*, 19 800 ×
29: Двуядерный амебоидный зародыш в зрелой споре *M. gasterostei*, 18 133 ×

1—15, 18—21, 26—28 — заливка в метакрилат; 16, 17, 22—25, 29 — заливка в аралдит

EXPLANATION OF PLATES I—X

Ultrastructure of *Myxidium gasterostei*

1: Surface of trophozoite of *M. gasterostei* with microvilli and ectoplasm, 18 000×
2 and 3: Pinocitotic vacuoles in trophozoite of *M. gasterostei* (arrows), 75 000× and 72 500×
4: Transverse section of young trophozoite, 18 900×
5 and 6: Mitochondria (M) of trophozoite, 40 000× and 40 950×
7: Granular ergastoplasmic reticulum (ER) connected with generative cell (arrow), 43 920×
8: Granular ergastoplasmic reticulum (ER) connected with vegetative nucleus (NV), 27 200×
9: Transverse section of leaflike trophozoite, 15 000×
10: Transverse section of mature trophozoite, 9 000×
11: Vegetative nucleus (NV), 17 000×
12: Generative cells with generative nuclei (NG) in young trophozoite, 29 000×
13. Mature trophozoite with separated generative cells (gc), 9 300×

14: Fission of sporoblasts, 31 500×
15: Sporoblasts with separated valvogenic (vc) and capsulogenic (cc) cells and sporoplasm (SP), 16 000×
16: Mature spore, 26 840×
17: Pansporoblast with residual nuclei (RN) and two sporoblasts (SB), 34 000×
18: Capsular anlagen (ca), 24 400×
19: Further development of capsular anlagen (ca), 17 200×
20: Polar capsule without a filament, 24 800×
21: Sporoblasts with polar capsules and inverted filaments inside the capsules (cr — cork, cp — cap), 16 000×
22 and 23: Capsular anlagen (ca) and its connection with external tube (et), 24 000× and 22 000×
24 and 25: External tube of filament (et), 24 400× and 16 000×
26 and 27: External tube of filament (et) with concentrated matrix (mt), 30 000× and 21 000×
28: Mature spore of *M. gasterostei*, 19 800×
29: Sporoplasm in mature spore of *M. gasterostei*, 18 133×

1—15, 18—21, 26—28 — embedded in methacrylate; 16, 17, 22—25, 29 — embedded in araldit

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Enzyme systems in ciliates *Blepharisma intermedium* and *Spirostomum ambiguum:* phosphatases, urease and dehydrogenases

Die Enzymsysteme bei Ciliaten *Blepharisma intermedium* und *Spirostomum ambiguum*: Phosphatasen, Urease und Dehydrogenasen

An understanding of the enzyme systems is a basic prerequisite for the study of metabolism in ciliates. The information available regarding phosphatases, dehydrogenases and other enzymes of intermediary metabolism in ciliates is confined to only a few genera, e.g. *Tetrahymena*, *Paramecium*, *Frontonia*, *Stentor* and *Stylonychia* (see Seaman 1955, Holz 1964). In this paper some of the enzymes of intermediary metabolism of *Blepharisma intermedium* and *Spirostomum ambiguum* have been described.

Material and methods

Animals were grown at 25—27°C in hay infusion fortified with Horlicks malted milk. Ciliates at log phase of growth were used for the experiments.

Air dried animals fixed in cold acetone were used for the demonstration of alkaline and acid phosphatases (Gomori 1957). Only air dried animals were used for the detection of glucose-6-phosphatase (Wachstein and Meisel 1956 vide Pearse 1960), urease (Sen 1930), succinate dehydrogenase (Nachlas et al. 1957), malate dehydrogenase and lactate dehydrogenase (Hess et al. 1958). Adequate controls were maintained.

Since preformed calcification can be mistaken for the activity of alkaline phosphatase, some slides were preincubated with citrate-phosphate buffer, pH 4.5, for 10 minutes. Controls for phosphatases were run by omitting the substrate from the incubation mixture. Sodium fluoride (0.005 M) was also used as inhibitor of acid phosphatase. For phosphatases, time of incubation varied between 1—20 hrs.

For urease, incubation was done at various pH ranging from 5 to 8. As suggested by Seaman 1959, CO_2 free distilled water was used throughout the procedure.

In case of dehydrogenases, a few slides were incubated in a mixture containing 0.1 M sodium cyanide, since oxygen possibly through cytochrome system may compete with the tetrazolium salt as hydrogen acceptor. For controls, substrate was omitted from the incubation mixture for dehydrogenases.

Observations

Phosphatases

Alkaline phosphatase was localized in the periphery of the macronucleus, in mitochondria and basal granules. The cytoplasm also gave weak reaction (Pl. I 1). The intensity of reaction reached its maximum within 3 hrs. If the pretreatment in citrate phosphate buffer pH 4.5 was omitted, the whole posterior half of the animal showed dark reaction and no organelles could be identified in such preparations.

Acid phosphatase was chiefly localized in nucleoli, mitochondria and basal granules. Food contents, food vacuolar membrane and cytoplasm were also positive (Pl. I 2). Acid phosphatase activity could be detected around food vacuole within 15 minutes of its formation and reached its maximum within 2 hrs.

Glucose-6-phosphatase was chiefly found in mitochondria and cytoplasm, accompanied by a negative reaction in controls.

Urease

No urease activity was detected in *Blepharisma*. The reaction was positive in *Spirostomum ambiguum*. Mitochondria and cytoplasm were positive for the reaction. The highest activity was seen at alkaline pH. The reaction was erratic below pH 5.5.

Dehydrogenases

Both *Blepharisma* and *Spirostomum* gave strong reaction for dehydrogenases (Pl. I 3, 4), accompanied by a negative reaction in controls. The enzymes were chiefly localized in basal granules, mitochondria and cytoplasm. Absence of sodium cyanide from the incubation medium for succinate dehydrogenase did not appreciably affect the intensity of reaction. The activity for malate and lactate dehydrogenases was low as compared with of succinate dehydrogenase.

Discussion

Phosphatases

Alkaline phosphatase has been described in a few ciliates, e.g. *Colpidium campylum* (Sullivan 1950), *Tetrahymena* (Elliott and Hunter 1951), *Paramecium caudatum* (Honigberg 1955) and *Stylonychia pustulata* (Hunter 1959). Hunter 1959 demonstrated acid phosphatase in mitochondria and alkaline phosphatase in both mitochondria and macronucleus of *Stylonychia pustulata*, but basal granules were shown to be lacking these enzymes. In the present study, both *Blepharisma* and *Spirostomum* showed intense staining for alkaline and acid phosphatases in basal granules. The perinuclear distribution of alkaline phosphatase is probably related to metabolism of nucleotides and nucleic acids (Sullivan 1950).

The presence of acid phosphatase was first reported by Weisz 1949 in *Stentor coeruleus* around the macrouncleus, basal granules, body cilia and endoplasmic fat vacoules. Since then it has been reported in *Tetrahymena* (Elliott and Hunter 1957), *Opalina* (Hunter 1957), *Stylonychia* (Hunter 1959) and *Paramecium* (Müller and Törö 1962). The high

concentration of acid phosphatase in food vacuoles, found in the present study (Pl. I 2), is suggestive of its role in intracellular digestion, particularly in view of the work done by Müller and Törö 1962, Müller et al. 1963, 1965. Müller and Törö 1962 cytochemically demonstrated acid phosphatase activity in food vacuole of *Paramecium multimicronucleatum* and correlated the pH changes in the food vacuole with the corresponding changes seen in acid phosphatase activity. They suggested a probable role of acid phosphatase activity in intracellular digestion, especially in view of the findings of de Duve 1959 and Novikoff 1960 on the part played by lysosomes in intracellular digestion, wherever phagocytosis is taking place. In general, nonspecific acid phosphatases are associated with lysosomes (Seed et al. 1967). Müller et al. 1963 showed that food vacuoles meet the criteria for lysosomes. Later, Müller et al. 1965 showed that appearance of acid phosphatase activity in the food vacuole was dependent on vacuole formation and not on the contents of the vacuole. Further, they showed that the early appearance of acid phosphatase activity in food vacuole (such as 15 mts. in *Blepharisma* and *Spirostomum*) was not due to synthesis of enzymes or their activation in the food vacuole but was due to redistribution of the pre-existent activity i.e., due to migration of enzymatically active structures to the vacuoles. This observation has also been supported by ultrastructure study of ingestion and digestion in *Tetrahymena pyriformis* by Elliott and Clemmons 1966. They showed that hydrolases (including acid phosphatase) from lumina of endoplasmic reticulum are packaged in primary lysosomes. These primary lysosomes coalesce with the food vacuoles either directly or through pinocytic vacuoles and thus initiate the degradation of contained food.

Glucose-6-phosphatase is a relatively less known specific acid phosphatase enzyme from protozoa, which dephosphorylates glucose-6-phosphate. Recently it has been reported in *Trypanosoma gambiense* (Seed et al. 1967). It has been reported from mitochondrial and microsomal fractions of rodent liver and can, therefore, be used as a marker for the presence of microsomes (Pearse 1960). In *Blepharisma* and *Spirostomum* its activity was chiefly found in mitochondria and cytoplasm.

Urease

The urease activity has been a point of debate in ciliates. Seaman 1954, 1959 showed urease activity in *Tetrahymena pyriformis*. Hunter 1959 found urease activity in *Stylonychia pustulata* located in mitochondria. In *Spirostomum*, urease activity was chiefly localized in mitochondria and cytoplasm. The urease activity in *Spirostomum*, in *Tetrahymena* (Seaman 1959) and in *Stylonychia* (Hunter 1959) is interpreted to mean that Krebs-Hensleit cycle is functional in these ciliates. However, it would appear that this cycle is not operative in *Blepharisma intermedium* as in a few strains of *Tetrahymena pyriformis* (Dewey et al. 1957), *Paramecium putrinum* (Hunter 1959), and *Entodinium caudatum* (Abou Akkada and Howard 1962).

Dehydrogenases

Succinate dehydrogenase has been reported in a few ciliates e.g. *Paramecium caudatum*, *Tetrahymena pyriformis*, *Stylonychia pustulata* and *Colpoda cucullus* (see Holz 1964). Seaman 1951 and Eichel 1954 found that homogenates of *Tetrahymena* oxidize succinate aerobically. In *Blepharisma*

and *Spirostomum*, succinate dehydrogenase could be demonstrated both under aerobic and anaerobic conditions. Koehler and Fennell 1964 identified enzymes of Krebs' cycle in *Tetrahymena pyriformis* W by localizations of isocitrate, succinate and malate dehydrogenases. On the basis of their observations, they concluded that metabolic pathways of *Tetrahymena pyriformis* W show characteristics similar to those described for higher organisms. Succinate and malate dehydrogenase activity indicates the operation of Krebs' cycle in *Blepharisma* and *Spirostomum*.

The presence of glycolytic enzymes was first reported by Geddes and Humphrey 1951 (vide Seaman 1955). They showed the production of lactic acid upon anaerobic incubation of *Paramecium caudatum* with glycogen, glucose, fructose and phosphorylated sugars. Later, Seaman 1955, and Koehler and Fennell 1964 made a detailed study of glycolytic enzymes in *Tetrahymena pyriformis* as evidence for the operation of glycolysis. The presence of lactate dehydrogenase in *Blepharisma* and *Spirostomum* is of importance as both ciliates show glycogen as their reserve food and so the pyruvate formed as a part of glycolytic cycle can be reduced to lactic acid under anaerobic conditions. The reverse of this conversion can give rise to pyruvate from lactic acid which can undergo either reverse glycolytic changes or can follow the Krebs' cycle.

The presence of phosphatases and dehydrogenases in the basal granules of both *Blepharisma* and *Spirostomum* is of added importance and needs special mention here. It is now established that the energy for beating of cilia is derived from glycolysis and citric acid cycle and is carried to the sites of utilization by the high energy phosphate bonds of ATP (Sleigh 1962).

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Summary

The different cytochemical tests performed for the detection of various enzymes in *Blepharisma intermedium* and *Spirostomum ambiguum* are described. Alkaline phosphatase is present in basal granules, mitochondria, cytoplasm and periphery of the macronucleus. Acid phosphatase is detected in the nucleoli, mitochondria, basal granules, cytoplasm and food vacuolar membrane. Mitochondria and cytoplasm contain glucose-6-phosphatase also. *Spirostomum* shows high urease activity while *Blepharisma* lacks it. Succinate, malate and lactate dehydrogenases are detected in basal granules, mitochondria and cytoplasm. The significance of these enzymes is discussed.

ZUSAMMENFASSUNG

Es wurden verschiedene cytochemicale Proben beschrieben für die Entdeckung der mannigfaltigen Enzyme in *Blepharisma intermedium* und *Spirostomum ambiguum*. Alkalische Phosphatase befindet sich in den Basalkörnchen, Mitochondrien, in der Cytoplasma und in der Periferie vom Makronucleus. Saure Phosphatase

wurde entdeckt in den Kernkörperchen, Mitochondrien, Basalkörnchen, in der Cytoplasma und in der Membran der Nahrungsvakuole. Mitochondria und Cytoplasma enthalten auch Glukose-6-Phosphatase. *Spirostomum* zeigt eine starke Aktivität der Urease, die bei *Blepharisma* überhaupt nicht vorkommt. Succinat-, Eisenmalat- und Lactatdehydrase wurde entdeckt in Basalkörnchen, Mitochondrien und in der Cytoplasma. Es wurde die Bedeutung dieser Enzyme beschprochen.

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EXPLANATION OF PLATE I

1. A portion of whole mount of *Spirostomum* showing alkaline phosphatase reaction. Basal granules are clearly seen ×600.
2. A portion of whole mount of *Blepharisma* showing acid phosphatase activity. High concentration of acid phosphatase near food vacuole, nucleoli and mitochondria can be seen ×600.
3. Whole mount of Spirostomum showing succinate dehydrogenase activity ×150.
4. A portion of whole mount of *Blepharisma* showing lactate dehydrogenase activity. Basal granules and mitochondria are seen ×600.

b.g. — basal granules, Mi — mitochondria,
nl. — nucleoli, F.v. — food vacuole.

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Cultivation of *Eimeria tenella* in tissue cultures I. Further development of second generation merozoites in tissue cultures

Kultivace kokcidie *Eimeria tenella* v tkáňových kulturách
I. Další vývoj merozoitů druhé generace v tkáňové kultuře

The first reports on cultivation of coccidia in tissue cultures were published by the end of 1965. Patton 1965 observed development of 1st generation merozoites in various tissue cultures infected with sporozoites of *Eimeria tenella*. After inoculation of different tissue cultures with sporozoites of *E. acervulina* Strout et al. 1965 recorded multiple infection of one cell and rarely multinucleated schizonts of 1st generation. Doran and Vetterling 1967 a, b cultivated sporozoites of *E. meleagrimitis*, *E. necatrix*, *E. acervulina* and *E. gallopavonis* in tissue cultures; sporozoites of *E. meleagrimitis* and *E. necatrix* gave rise to the 1st generation of merozoites. Fayer and Hammond 1967 obtained the development of 1st generation merozoites in the species *E. bovis*, also in tissue cultures. The remaining papers published till now on this problem (Bedrník 1967 a, b.) report inoculation of tissue cultures with 2nd generation merozoites of *E. tenella*. The use of 2nd merozoites for the initial study of cultivation of coccidia in tissue cultures has certain advantages in comparison with the methods using sporozoites. In using sporozoites it is necessary to collect large amounts of sterile oocysts from which sporozoites have to be obtained. The method of excystation in vitro seems to be rather unprofitable. On the other hand by using considerably smaller doses of oocysts a quite unlimited number of merozoites can be obtained. Furthermore, the fundamental knowledge derived from experiments with merozoites, from their requirements on the type of cells, media, temperature etc, can be applied in experiments with sporozoites. A preliminary knowledge on the cultivation of merozoites can thus save much efforts and time in the first steps towards the cultivation of coccidia in tissue culture. The results of these two papers on the development of 3rd generation merozoites from the inoculated 2nd generation and especially the development of sexual stages and oocysts in tissue culture, which have not been described elsewhere, confirm the assumptions on the suitability of 2nd generation merozoites for cultivation studies.

Material and methods

The source of merozoites and preparation of inoculum

Merozoites were obtained from the caeca of chickens killed by decapitation on the 5th or 6th day after experimental infection. The chickens used, mostly Leghorn 2 up to 3 weeks old, were inoculated per os with 50 up to 150 thousand sporulated oocysts. Merozoites were released from the caeca by two ways:

1. The caeca were cut longitudinally and their contents consisting mostly of blood and different quantity of merozoites, were agitated in tissue culture medium used as inoculum. Besides merozoites and erythrocytes this inoculum, indicated as N inoculum, contained a small number of different cells originated from the inflamed mucosa of the caeca of infected chickens, only few bacteria and quite exceptionally some fungi. It contained sometimes also old schizonts of 2nd generation enclosing mature merozoites of 2nd generation. Several times oocysts were observed in the inoculum. As for other developmental stages only the schizonts containing several nuclei were observed on rare occasions inside the released caecal cells.

2. The second way of preparing the inoculum was the releasing of merozoites closed in the envelopes of old schizonts and in host cells by means of trypsin. The cut coeca were rinsed with PBS, put in 1.5% trypsin (for TC), agitated at intervals and kept at 39° C for 1 hour. Then trypsin was removed either by centrifugation or by filtration through membrane filters. Sediment containing merozoites and other elements was re-suspended in the tissue culture medium. This inoculum designated as N TRYPS inoculum contained fewer erythrocytes than the N inoculum and more caecal cells, either single or in groups, released by trypsinisation. It contained also more cells harbouring trophozoites or schizonts with several nuclei.

Used types of tissue cultures

He-La cells maintained in medium with LAH and 10% calf serum.
CHE-A cells derived from 10 up to 13 days old chicken embryos.
CHE-B cells derived from 10 up to 13 days old chicken embryos from which the digestive tract was removed before trypsinisation.
CHE-C cells derived by trypsinisation of the digestive tract and of the liver from 17 up to 19 days old chicken embryos.

The CHE-A and CHE-B cultures were formed mostly by fibroblasts growing on the slides often in 2 or more layers. Among the fibroblasts we observed epithelial cells forming islets of different sizes. In the CHE-C cultures a greater quantity of different epithelial cells was found. In all three types of chicken cultures macrophages were present in various numbers. Chicken cells were maintained on TC medium 199 + 10% calf serum + 0.5% Bactopeptone + 1% $NaHCO_3$ and 100 units penicillin and streptomycin per ml. The number of cells in the inoculation dose mounted on the slides was 700 up to 900 thousand per ml. The CHE-A and CHE-B cultures formed a continuous layer of cells on slides after 24 up to 48 hours, in CHE-C cultures the layer was formed later, sometimes not at all. At a higher concentration of cells in the inoculum a rapid fall off of the cell layer from the slides was recorded. The cells were maintained on cover slides in test-tubes or in Roux's bottles. With several exceptions the primary cultures were always used in our experiments. The cells that were not mounted on slides immediately after trypsinisation,

were kept in the tissue culture medium at 4°C and mounted on the slides during the next week if needed.

Method of inoculation of tissue cultures with merozoites

After trying different inoculation methods and determination of factors influencing a further development of merozoites in tissue culture, the following method of infection was used: the tissue culture in which the cells were maintained was removed. 1 tube was filled with 1 up to 2 ml of inoculum, the Roux's bottles according to their size with 30 or 50 ml of inoculum. The concentration of merozoites in the inoculum fluctuated between 0.3 up to 2 millions per ml. mostly making about 1 million per ml. Morozoites originated from various chickens showed very different developmental properties so that it was impossile to determine a commonly optimal concentration of merozoites in the inoculum. After four hours approximately, during which the cultures were kept at 39 up to 40°C, the inoculum was removed, the tissue washed with fresh medium and re-covered with the medium containing 2% $NaHCO_3$. Subsequently the tissues were incubated at 39 up to 40° C until conclusion of the experiment. In long-term experiments the medium was changed every 48 hour. Though the contents of caeca used were not sterile, the number of bacterial contaminations was minimal and fungal contaminations were very rare.

Methods of transferring coccidia

Roux's bottles with grown CHE-A cultures were inoculated as described above. After 24 hours the medium from bottles containing multiplied coccidia was removed and the tissues were rinsed with PBS added to the removed medium. Afterwards the tissue in the bottle was rinsed with a small amount of trypsin. After being kept at 39° C for several minutes, the removed tissue was agitated in a small amount of medium and distributed either in tubes containing cover slides with a continuous layer of CHE-A cells or into tubes with pure slides without cells. The removed old medium with PBS was centrifuged for 4 minutes at 700—800 G, the sediment containing merozoites was agitated in a small amount of fresh medium and divided into test-tubes with tissues on cover slides. Further procedure was the same as in normal infection. Only when cells were inoculated from bottles to pure slides the medium was not changed.

Methods of observation of coccidia in tissue culture

Living coccidia were observed in the light microscope either through the bottle glass or in samples of extirped tissue and on cover slides which had been taken out of the tubes. Tissues with coccidia were fixed with the aid of Schaudinn's fluid and stained with Harris's haematoxylin, Mayer's haematoxylin, Heidenhain's iron haematoxylin, Delafield's haematoxylin and Protargol (after fixation with Bouin-Holland). After fixation with formalin we used also the MacManus stain.

Results

Merozoites of 2nd generation not only entered the cells of all the used types of tissue culture, but underwent a further development.

Further development of 2nd generation merozoites in He-La cells

Merozoites entered He-La cells very quickly. The first intracellular merozoites were seen as early as 5 minutes after inoculation. Later on it was possible to see multiple infections of one host cell (Pl. I 1). In He-La cells only the development of 3rd generation merozoites was observed in sporadic cases. The development of sexual stages did not take place at all. Besides merozoites the inoculum contained also different other cells derived from the caecum of the infected chicken (as mentioned above). We ascertained that such "misintroduced" cells were sometimes attached among He-La cells and were invaded by merozoites which underwent further development. The number of coccidia developed inside these cells, in comparison with the number of coccidia developed in the tissue derived from chicken embryos, was however quite unsignificant.

Further development of 2nd generation merozoites in tissues derived from chicken embryos

Similarly as He-La cells, these cells were entered by merozoites very quickly (as early as seven minutes after inoculation). The distribution of merozoites in such cells was rather irregular, areas of uninvaded cells alternating with those consisting of cells invaded frequently. Often we observed one cell harbouring two or more merozoites while the neighbouring cells were not infected at all. It seems to be possible that merozoites were actively picking out their host cells. Once we observed an intranuclear merozoite. Two types of merozoite development were recorded in the cells derived from chicken embryos: development into the 3rd and 4th asexual generation on the one hand and into sexual stages (with subsequent fertilization and origin of oocysts) on the other.

1. The development of further asexual generations

The first developmental stages — uninucleate round trophozoites were observed as early as 1 or 2 hours after inoculation. First multinucleated schizonts developed in tissue culture were seen 2 hours after inoculation, and their number gradually increased. The size of schizonts was variable. Their shape was prevalently round, but we found also lobular schizonts. (Pl. I 2). The first rosettes of new merozoites of 3rd generation were observed 14 hours after inoculation. After 24 hours of cultivation mature schizonts of the 3rd generation, enclosing developed 3rd merozoites, began to prevail (Pl. I 3). In the beginning merozoites were arranged in regular rosettes. With their inner ends they attached the residual bodies. The number of merozoites within one rosette, the same as the number of nuclei in the schizonts, varied from several individuals up to several tens. Merozoites measured approx. from 7 up to 12 μ. The size of the rosettes was 10—50 μ, most frequently approx. 30 μ. Later on the regularly arranged rosettes changed into irregular clusters of moving merozoites.

The released extracellular merozoites of 3rd generation appeared in variable quantity in some cultures after 24 hours. Sometimes they invaded new cells. We may suppose that the small schizonts occurring in cultures after

24 or 48 hours of cultivation were grown from these merozoites developed in tissue culture. We can hardly presume that such small schizonts, frequently forming colonies concentrated in one place (Davies, Joyner and Kendall 1965) had arosed from 2nd merozoites the development of which started later or was stopped at this developmental stage.

The individuals which ceased to develop showed signs of degeneration. The non-developing intracellular merozoites were quickly resorbed by the host cell. After 24 hours they were found only in small numbers and after 48 hours practically not at all. This speaks for the fact that such small schizonts of normal appearance are a further asexual generation developed in the tissue culture. Our conclusions in this sense are supported also by the fact that the first merozoites of 3rd generation were mature as early as 14 hours after inoculation so that within 24 hours, when these small schizonts were observed, they had enough time to be released from the host cells and enter the new ones. The multinucleate schizonts and the rosettes of merozoites within the tissue were distributed similarly as the intracellular merozoites. They occurred either separately or formed "colonies".

Up from 24 to 48 hours the number of schizonts, especially of the large ones, decreased and the number of rosettes increased, but the whole quantity of developing coccidia, i.e. the total amount of schizonts and rosettes, was smaller after 48 hours than after 24 hours. The number of schizogonies disappearing in a natural way after releasing of merozoites was small in relation to the whole number of schizogonies and could not explain the decrease of developing coccidia between 24 and 48 hours. From the onset of the cultivation process different degenerated coccidial stages appeared in the cultures (Pl. II 4, 5). Their number increased with prolonged cultivation time. This could explain also the decrease of developing coccidia between 24 and 48 hours. In this time also the living conditions in the inoculated tissue culture deteriorated.

Breaches appeared in the cell layer, cells began to vacuolize and sometimes they lost their sharp delineation. It was also ascertained that in the inoculated cultures the cells fell off the slides sometimes after 24 hours and quite regularly after 48 hours of cultivation. On the contrary in noninfected cultures the cells were firmly attached to slides. This phenomenon was probably not due merely to exhaustion of nutritional substances or to accumulation of metabolic products, because it could not be stopped by a timely exchange of medium. It is a question whether it was due to coccidia or to the influence of other cells "misintroduced" into the tissue culture simultaneously with the N inoculum. The desintegration of the tissue in the surroundings of developing coccidia and the formation of microscopical breaches in their neighbourhood indicate a possible influence of coccidia. This conclusion is supported also by the results of several other authors (Burns 1958, 1959, Sharma and Foster 1964, Strout and Holman 1965). They observed the lethal influence of an extract from *E. tenella* oocysts and from caecal scrapings of chickens infected with *E. tenella*, on the rabbits and tissue cultures.

The origin of a great number of degenerated coccidial stages and the suppression of a further growth of the merozoites which developed in tissue culture and, after being relased from envelopes of schizonts, re-invaded suitable cells, may be due to the deterioration of living conditions within the tissue culture. We suppose furthermore that the suppression of further growth of coccidia may partly be due to a lack of mechanical influences supporting

the breaking of mature schizonts and releasing of 3rd merozoites (movements of chicken caecum, abbrasion of its walls by food etc.). On stained preparations we observed degenerated merozoites within the host cells. In fresh cultures we saw clusters of moving merozoites within the host cells unable to escape from them.

Table 1

Comparison of the development of coccidia in tissue cultures and in cells from N inoculum attached to pure slides 24 hours after inoculation

Experiment No.	Tissue cultures		Pure slides	
	asexual stages	sexual stages	asexual stages	sexual stages
38/39	+++	+	+++	0
73/2	++	0	++	0
76/1	+	0	0	0
76/2	0	0	0	0
77/1	0	0	0	0
77/2	0	0	0	0
77/3	0	0	0	0
77/4	0	0	0	0
81/1	+	0	0	0
81/2	++	++	0	0
82/2	+	+	+	+
82/3	+	+	+	+
83 A/1	+	0	+	0
83 B/1	+	+	+	+
83 B/2	++	+	+	+
83 B/3	0	0	0	0
85 B/1	++	+	0	0
87 B/1	++	++	+++	0
87/2	1 rosette	++	0	0
88/2	++	+	+	0
89 TRYPS	++	++++	0	0
			rarely attached noninfected cells	
96 TRYPS	++++	++++	++−+++	++−+++
97 TRYPS	+	++++	+	+

Explanations

\+ — 1 —25 asexual or sexual stages on one slide
++ — 26—50 „
+++ — 51—100 „
++++ — 101 and more „

The development of coccidia in cells from the N inoculum

This idea was induced by the presence of different cells in the inoculum and their ability of attachment in He-La cell cultures. Simultaneously with the inoculation of tissues also pure slides without cells were covered with the N

inoculum. The medium was not changed and slides were stained after 24 or 48 hours. In most instances the cells usually appearing as macrophages, rarely as fibroblasts, were attached to the slides. Some of these cells were infected with merozoites which underwent a further development within them.

Development of coccidia in cells from the inoculum which were attached to pure slides and absence of development in the inoculated tissue culture have never been noted. On the contrary, we recorded several times an intensive development of coccidia in tissue cultures and absence of development in cells attached to pure slides (Table 1). Even when in some instances the number of developing coccidia in cells on pure slides was the same as that in tissue cultures (once even greater), we must take into consideration that tissue cultures were washed twice with medium after 4 hours at the latest and plenty of the „misintroduced" cells were removed, whereas on pure slides the inoculum was left for 24 hours.

The experiments with He-La cells in which the "misintroduced" cells were easily discernible revealed the actually small number of such cells. Cells harbouring coccidia were "misintroduced" into the tissue culture from caeca of chickens with the N- as well as with the N TRYPS inoculum. These cells remained above the tissue and attached neither in tissue culture nor on pure slides. It was possible to distinguish them from proper tissue culture cells harbouring developing coccidia. The cells of small pieces of caecal tissue "misintroduced" into the culture were arranged more densely than the cells of the tissue culture; they were also more clearly demarcated. From all cell elements "misintroduced" into the tissue culture only macrophages were indistinguishable from the macrophages of tissue culture. The increased occurrence of degenerated stages of coccidia which developed in cells attached to pure slides was remarkable (Pl. II 4, 5). It seemed that the host cell macrophages defended themselves against the developing coccidia which were sometimes destroyed.

Attempted passage of coccidia

As mentioned above, the living conditions in the tissue culture deteriorate quickly in 24 up to 48 hours after inoculation. We tried to prepare better conditions for a further development of coccidia by transferring 3rd merozoites, developed in tissue culture, into fresh cultures. Using this method we succeeded in obtaining the development of a further, i.e. 4th generation merozoites as well as of sexual stages. Further development of coccidia was observed both on the slides with developed tissue and in the tissue established on pure slides after inoculation with trypsinized tissue and with merozoites from Roux's bottles. In cultures inoculated only with merozoites without the presence of cells from Roux's bottles only intracellular merozoites were observed which did not undergo a further development.

2. Development of sexual stages

The gametogony and sporogony occurred simultaneously with the development of further asexual generations in tissue derived from chicken embryos inoculated with 2nd merozoites.

Microgametocytes

The first microgametocytes developed within the first 24 hours after inoculation. They were also observed after 48 and 72 hours. Only very rarely they occurred in isolated form. In most instances they formed islets of different sizes containing from a few up to several tens of individuals (Pl. II 6). One islet enclosed individuals in different developmental stages. Microgametocytes were arranged closely one to the other and their basal oval shape was irregularly deformed. Their size ranged from 7.5 to 21 μ. One microgametocyte gave rise to numerous microgametes (Pl. II 7). After releasing of microgametes a large residual body was sometimes observed (Pl. IV 12). The morphology of microgametocytes grown in tissue cultures was similar to that of microgametocytes developed in vivo.

Microgametes

Approx. 4 μ long, slender, mostly more or less bent bodies. On preparations they were observed in clusters originated from microgametocytes after gamogony (Pl. II 7). Their vitality was proved by fertilization of macrogametes and the origin of zygotes and oocysts.

Macrogametocytes and macrogametes

Macrogametes appeared in tissue culture simultaneously with microgametocytes. Their number was conspicuously smaller than that of microgametocytes. (It is a characteristic feature of *E. tenella* also in the development in vivo). Macrogametocytes occurred not only in close islets together with microgametocytes, but were sometimes also dispersed in the surroundings of these islets. Their basal shape was oval, sometimes more or less protracted or lobular (Pl. III 8). They displayed dark stainable cytoplasm and large nuclei with a clear zone around the large nucleolus. Their size was approx. 15 μ.

Zygotes

The origin of zygotes was followed by rounding off of macrogametes and by thickening of their outer walls after fertilization. Zygotes displayed a more regular oval shape than macrogametes. Simultaneously they occurred in different developmental stages from individuals with slightly thickened outer walls to individuals with granules accumulated along the walls and other ones with thick outer walls (Pl. III 9, 10, IV 11).

Oocysts

After 48 or 72 hours of cultivation intracellular or free oocysts appeared in cultures. Their morphology was the same as in oocysts developed in vivo (Pl. IV 11). As already mentioned, the oocysts were sometimes „misintroduced" into tissue cultures. For oocysts developed in tissue culture we took only the oocysts from those experiments where they were not observed in the inoculum and where intracellular zygotes were found after 24 hours.

The distribution of sexual stages in tissue culture

While the developing asexual stages were distributed very irregularly in fibroblasts, epithelial cells and in macrophages, the developing sexual stages were limited mainly to islets of epithelial cells. The infection rate of epithelial cells in islets was very high (Pl. IV 12). Merozoites probably

concentrated in these iselts. Nevertheless islets of epithelial cells with occasional sexual stages were observed, too. The number of sexual stages in different experiments varied considerably and depended upon the properties of merozoites used and on the type of cells used. The largest number of sexual stages was ascertained in CHE-C cells. It is very interesting that development of sexual stages was observed also in CHE-B cells. This indicates that sexual stages do not need to develop in glandular epithelium of the caecum. Sexual stages developed also in macrophages from the inoculum attached to pure slides. The epithelial cells resisted better than fibroblasts to deterioration in tissue culture mentioned above. Sexual stages within these epithelial cells developed further even after degeneration of asexual stages grown out of epithelial cells.

Factors influencing a further development of 2nd merozoites in tissue culture

This subject being described in detail in our other reports we wish to mention shortly only two factors:

1. Merozoites obtained from chickens on the 6th day after their infection differ from merozoites obtained on the 5th day after infection by their ability of developing into sexual stages. When the former were developed within the tissue culture, sexual stages were always noted, whereas in using the latter ones, sexual stages developed only in some instances.
2. After inoculation of tissue culture with the N TRYPS inoculum a stormy development of asexual as well as sexual stages was regularly observed, being considerably more intensive than the development in the tissue culture infected with the N inoculum from the same chicken.

Discussion

The results obtained revealed that the type of cells used for cultivation may condition the outcome of the experiment. The type of cells used may be one of the factors responsible for only a limited success achieved till now in cultivation of coccidia in tissue culture. In most of the published reports the authors used the tissues very different from natural host organisms. E.g. Doran and Vetterling 1967 used the tissues derived from kidneys of different mammals for the cultivation of poultry coccidia. In He-La cells we observed the development of 3rd generation merozoites only on very rare occasions. In chicken cell cultures not only the 3rd generation of merozoites developed, but probably multinucleated schizonts of 4th generation as well. After the transfer of coccidia developed in tissue cultures into fresh cultures, even merozoites of 4th generation were obtained. The importance of the type of cells used was confirmed still more expressively by the development of sexual stages. In He-La cells they developed not at all whereas in cultures derived from chicken embryos they developed mostly in epithelial cells and scarcely also in macrophages.

We suppose that the results of passaging coccidia developed in tissue culture into fresh cultures are of great importance. It is possible that this is the correct way of cultivating coccidia not only from merozoites but also from sporozoites. The development of the 4th generation of merozoites in tissue

culture indicates that the number of asexual generations need not be strictly limited which may play an important role in the problem of chronic coccidiosis.

It remains an open question whether merozoites are giving rise to sexual stages because they enter the epithelial cells by chance or whether they invade them for the purpose to develop into sexual stages.

According to the opinion which has prevailed till present *E. tenella* develops only in the glandular epithelium of caeca (Challey and Burns 1954, Patillo 1959, Doran 1965). Only Scholtyseck 1953 reported the development of merozoites in fibroblasts and Gresham and Cruickshank 1959 observed multinucleated schizonts in macrophages. Our experiments have confirmed that both these observations overlooked till now were correct. In tissue cultures the development of asexual stages takes place in fibroblasts but from the distribution of developing coccidia in the cultures it is evident that every cell is not suitable as a host cell. Several authors (Doran 1966, Horton 1967, Patillo 1959, Van Dornick and Becker 1957) suppose that macrophages serve only as transporters of sporozoites from lamina propria to the glandular epithelium but we have observed that in macrophages from tissue culture asexual as well as sexual stages developed. This was observed in macrophages „misintroduced" into He-La cell cultures after incolation with N inoculum and mainly when macrophages from the N inoculum were maintained on pure slides. In macrophages we observed also many degenerated stages of coccidia. This confirms the idea that macrophages serve as a defence against infection.

Summary

Second merozoites obtained from chickens on the 5th or 6th day after infection underwent further development after inoculation into tissue culture. In He-La cells they rarely gave rise to 3rd asexual generation. In chicken cell cultures they completed the development of 3rd asexual generation and gave rise to beginning stages of the 4th asexual generation (multinucleate schizonts) as well as to sexual stages developing to zygotes and oocysts after fertilization. After being transferred into a fresh tissue culture, 3rd generation merozoites, developed in the tissue culture, gave rise to 4th generation merozoites and sexual stages. Asexual stages grew in fibroblasts, epithelial cells and macrophages. The developing sexual stages were limited essentially to islets of epithelial cells. They were observed also in macrophages „misintroduced" into cultures with the inoculum from the caeca of infected chickens. Merozoites obtained from caecal contents underwent further development in a substantially smaller number than those released from caecal cells.

Merozoites obtained on the 6th day after infection of chickens gave rise to sexual stages in all instances, those obtained on the 5th day following the infection developed to such stages only in some instances.

SOUHRN

Druhé merozoity získávané z kuřat 5. nebo 6. den po jejich infekci jsou schopné po inokulaci do tkáňových kultur pokračovat ve svém vývoji.

V He-La buňkách vzácně tvoří třetí asexuální generaci, v buňkách odvozených

od kuřecích embryí tvoří 3. asexuální generaci a začátek 4. generace (mnohojaderné schizonty) a sexuální stadia, z nichž se po oplození vyvíjejí oocysty. Kokcidie vyvinuté v tkáňové kultuře se po přenosu do další pasáže tkáňových kultur vyvíjely v 4. generaci merozoitů a sexuální stadia.

Vývoj asexuálních stadií probíhal ve fibroblastech, epitheliálních buňkách a makrofágách. Vývoj sexuálních stadií byl omezen v podstatě pouze na uzavřené ostrůvky epitheliálních buněk. Mimo tyto ostrůvky byla vyvíjející se sexuální stadia pozorována také v makrofágách, zavlečených do kultur z coek nakažených kuřat spolu s inokulem.

Merozoity získávané výplachem z obsahu coek nakažených kuřat měly podstatně menší vývojové schopnosti než merozoity uvolňované trypsinisací ze střevních buněk. Merozoity odebírané z kuřat 6. den po jejich infekci se vždy vyvíjely v sexuální stadia, merozoity odebírané 5. den po infekci kuřat dávaly vznik sexuálním stadiím pouze v některých případech.

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EXPLANATION OF PLATES I—IV

Cultivation of *Eimeria tenella* in tissue culture

1: Intracellular merozoites of 2nd generation in He-La cells 5 hours after inoculation. Harris's haematoxylin, ×2 500

2—3: Cultures derived from chicken embryos

2: Multinucleate schizonts of 3rd generation 24 hours after inoculation. Harris's haematoxylin, ×3 200
3: Rosettes of merozoites of 3rd generation 24 hours after inoculation. In the smaller rosette a residual body is clearly visible. Harris's haematoxylin, ×1 500

4—5: Degenerated coccidia developing in macrophages from the N inoculum attached to pure slides, in different stages of resorption, 24 hours after inoculation. Harris's haematoxylin, ×2 000

6—12: Cultures derived from chicken embryos

6: Microgametocyte 48 hours after inoculation. Harris's haematioxylin, ×3 500
7: In the upper part multinucleated microgametocyte, in the centre microgametocyte releasing microgametes with a clearly visible residual body, 48 hours after inoculation. Harris's haematoxylin, ×3 700
8: Macrogamete 48 hours after inoculation. Harris's haematoxylin, ×3 500
9: Formation of oocyst 24 hours after inoculation. Heidenhain's haematoxylin, ×2 700
10: Formation of oocyst with granules clearly visible in the cytoplasm, 24 hours after inoculation. Heidenhain's haematoxylin, ×3 000
11: Zygote and intracellular oocyst 48 hours after inoculation. Harris's haematoxylin, ×3 000
12: Islets of sexual stages with atypically large number of zygotes. In the lower part a large residual body after gametogony is visible. Mayer's haematoxylin, ×1 000

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Some aspects of the study of soil protozoa

Некоторые аспекты изучения почвенных простейших

According to many authors, soil is a complex body constituted of organic and inorganic components. A great number of microscopic organisms are concentrated in soil, living there actively. Their interconnections and interrelations are very complex and remain not elucidated in many aspects. However, no doubt, various microorganisms are the principal members of microbiocoenoses being fundamentally associated with one another. They influence the character and the course of the biogenic processes of soil.

In the course of development of our knowledge about the soil protozoa, their significance has appeared more important. The presence of definite interrelations between protozoa and bacteria becomes gradually elucidated. At the present stage of our knowledge it may be stated with certainty that the whole assembly of the soil population, its interconnections and interrelations in the defined conditions of environment should be investigated, for understanding the biodynamic processes occurring in soil and for gaining the possibility to rule over them.

In the Soviet Union, the most extensive pedo-protozoological investigations have been developed by Brodskij. After having demonstrated that soils are inhabited by a great number of protozoa, he concentrated his attention on establishing their importance for soil fertility and on the necessity to influence upon the soil biocoenoses (Brodskij 1935, 1943).

The aim of the present study has been to demonstrate the importance of protozoa in soil processes in order to detect the character of their interrelations with bacterial flora and with the vegetation. We tried to answer the question whether protozoa are useful or harmful agents in the biodynamic processes of soil and what are the factors of their action in the soil biocoenosis. The importance of the problem of soil protozoa is clearly demonstrated by data concerning the numerosity and distribution of protozoa at various places and consequently in various soils of the Soviet Union (Table 1).

The data assembled prove that the soils of Soviet Union are inhabited by a number of protozoa which belong to various species.

The problem whether protozoa in soil are active or remain as cysts for the major time was raised by the investigators since a long time. The solution of this problem is closely connected with the elucidation of their role in the biogenic soil processes i.e. with their role in fertility.

In the laboratory experimental conditions, this problem has been first solved with a positive result by Lozina-Lozinsky i Martynov

Table 1

Number of individuals and species of protozoa occurring at different places of USSR (in 1 g of soil)

Place and soil	Flagellates	Amoebae	Ciliates	Total number of protozoa	Author
RSFSR					
Turf-podsol loamy	190 750*	131 500	250	322 500	Gelcer
soils	28**	26	25	79	1967
Azerbaijan SSR					
Grey meadow soils	90	910	910	1 910	Amiraslanowa 1967
	22	13	16	51	
Lithuanian SSR					
Turf-podsol sabulo-	1 000 000	100	100	1 000 200	Lepinis
as-clayey soils	24	11	16	51	1963
Uzbek SSR					
Typical grey soils	1 000 000	1 000	10 000	1 011 000	Nikoljuk
of old culture	26	13	13	52	1965
Light grey soils of	100	1 000	100	1 200	Nikoljuk
first year culture	6	9	14	29	1965

* Total number of organisms
** Number of species ascertained

1930. Amoebae inoculated in sterile soil on Petri dishes spread actively. However a convincing solution could be gained only by direct observation in the natural field conditions. The experiments were carried out directly on the virgin grey soils and on those with cotton crops (Nikoljuk 1965). As a criterion of the vital activity of protozoa was considered their penetration from the surrounding soil into the regions sterlized previously. The experiment demonstrated an active penetration of protozoa into the sterilized regions since the first days of experiments. The numerosity of the penetrating organisms depended on the humidity of soil.

The experiments proved as well that the conditions of life in soil permit reproduction and active movement of protozoa. They are natural permanent members of the soil biocoenoses.

Our experiments were supported by the study of Gelcer 1964 for the Moscow region, of Lepinis 1967 for Lithuania and of Amiraslanowa 1967 for Azerbaijan.

Russel and Hutchinson 1909, 1913, carrying out experiments on partly sterilized soil, were first who payed attention to the fact that the dynamic of microflora is associated in a definite way with the intensity of the development of protozoa. The subsequent investigations stated the bacterial feeding of soil protozoa and their selection of food material (Sewertsowa 1916, Hill 1916, Oehler 1916, Cutler and Sandon 1921 and others).

Cutler 1923, 1927 concluded that the dynamic of bacteria and protozoa in soil depend on each other and that an abundant development of protozoa supresses bacteria and, after a certain time, their content becomes stabilized.

The development rate of amoeba depends on food supply and on the species of bacteria.

Hino 1926 stated that soil protozoa feed, as a rule, on bacteria and influence in this way the course of the soil processes.

The investigations on the bacteria-feeding protozoa (Fedorowa-Winogradowa 1927, Winogradowa und Gurfein 1928) are much convincing when amoebae are cultivated on azotobacter which proved to be a fully acceptable food for them.

The analysis of numerous investigations, concerning the character of interrelations of soil protozoa and bacteria, suggests a conclusion that all the authors found an interrelation of the development of protozoa and that of bacteria This is based on the selective feeding of the main protozoa species on bacteria. (Hino 1927, 1934, Singh 1941 a, 1941 b, Waksman 1932, Heal 1963 and others).

The data gained in the course of the present investigations on the dynamic of the protozoa and bacteria development in natural soil conditions of Uzbekistan (on cultivated and virgin grey soils) and on turf-podsol soils of RSFSR studied by Gelcer 1967 (Table 2) — permit to state that the development of bacteria and that of protozoa show a definite interdependence i.e. an intense development of bacteria corresponds to a raised number of protozoa.

Table 2

Number of bacteria and protozoa in different soils (in 1 g)

Month	Light grey soils		Typical grey soils		Turf-podsol soils*		Light grey soils, non-irrigated	
	Bacteria	Protozoa	Bacteria	Protozoa	Bacteria	Protozoa	Bacteria	Protozoa
March	75×10^6	6400	93×10^6	20 100	—	—	5×10^6	4200
June	6×10^6	4600	27×10^6	10 000	50×10^5	42 370	6×10^6	380
August	10×10^6	3010	9×10^6	3 800	6×10^5	7 870	2×10^6	100
October	19×10^6	3560	175×10^6	201 000	—	—	1×10^6	20

* After Gelcer 1967

The comparison of the species composition of the most frequently and widely occurring protozoa (Nikoljuk 1965 and others) with the data about their mode of feeding and about the character of their food (Sandon 1932, Brodskij 1935) has demonstrated that the most characteristic soil protozoa are mostly the bacteria-feeding and omnivorous forms with prevailing of the bacterial food (Table 3).

The investigations of our laboratory (Nikoljuk 1965) carried out in Uzbekistan concerning the food selectivity of protozoa, most widely occurring in soil, permitted to conclude that protozoa feed most intensely on the oligonitrophil bacteria (Table 4). They multiply most intensely on azotobacter.

A question arises how this would reflect on the activity of these bacteria which occupy the most fundamental place among the factors of soil fertility. As known, feeding on azotobacter by protozoa fails to reflect on the production of nitrogen. The opinion about the ground of this phenomenon is not

Table 3

Feeding mode of protozoa and their principal species characteristic for the soils of Uzbek SSR

Protozoa	Kind of food (after Sandon 1937)	Species of protozoa in soils of Uzbek SSR
Flagellates		
Bodo sp. sp.	as a rule bacteria	*Bodo globosus* Stein *Bodo variabilis* Lemm.
Cercobodo sp. sp.	bacteria	*Cercobodo ovatus* Lemm. *Cercobodo digitalis* Lemm.
Monas sp. sp.	bacteria prevailing or exclusively bacteria	*Monas minima* H. Meyer *Monas vulgaris* Senn
Oikomonas sp. sp.	partly bacteria, partly saprophytes	*Oikomonas steini* Kent *Oikomonas termo* Kent
Amoebae		
Amoeba sp. sp.	bacteria prevailing	*Amoeba limax* Duj. *Amoeba albida* Nägler *Amoeba annulata* Penard *Amoeba aquarum* Jollos
Ciliates		
Colpoda steini	bacteria	*Colpoda steini* Maup.
Colpoda cucullus	bacteria and flagellates	*Colpoda cucullus* Ehrbg. *Colpoda maupasi* Enriques

Table 4

Intensity of protozoa development at different bacterial nutrition
(% of oligonitrophils on 7, 14 and 30 days)

Physiological group	Amoebae			Ciliates			Flagellates		
	Days								
	7	14	30	7	14	30	7	14	30
Oligonitrophils bacteria	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Denitrifying bacteria	35.5	30.0	19.6	6.7	19.0	6.8	11.0	2.9	10.9
Nitryfying bacteria	12.5	18.5	4.6	3.8	7.3	4.1	7.3	2.0	7.5
Ammonificying bacteria	1.7	5.6	0.2	0	0	0	0	0	0
Ammonificying bacteria with spores	1.0	0	0	0	0	0	0	0	0
Cellulose destroying bacteria	1.5	0.7	2.8	5.4	2.0	4.1	13.4	4.8	11.6

uniform (Hino 1926, Hervey and Greaves 1941, Nikoljuk 1952, 1965).

Solution of this problem became possible after the study of the character of influence exerted by ubiquitous protozoa occurring in great number in soil, upon the development and intensity of nitrogen fixation by azotobacter. Determination of factors which involve a rise of nitrogen fixation in the mixed

cultures of bacteria and protozoa, permitted to solve the above problems (Nikoljuk 1965). In general, protozoa exert a positive influence upon the azoto-fixation activity of bacteria. Flagellates show a lower stimulation, and sometimes, absence of it. In all those cases, in the mixed cultures the number of bacteria falls and the quantity of accumulated nitrogen rises (Table 5).

Different views exist concerning the factors of the intensity rise in azoto--fixation by azotobacter in the mixed cultures with protozoa.

In the present study, a new method (Nikoljuk 1953) and that of Hervey and Greaves 1941 were applied giving uniform results (Table 6). They indicated that the stimulating action of protozoa upon azotobacter is based on organic substances secreted by the former ones. The accumulating action of the root system of plants upon protozoa and bacterial flora suggests an inverse problem concerning the influence of protozoa upon the development

Table 5

Amount of nitrogen fixed by azotobacter in common cultures with protozoa in liquid media

Combination of microorganisms	Cells in 1 ml of medium		Nitrogen fixation	
	azotobacter	protozoa	in mg/1 g of sugar	% control
1951				
Azotobacter — control	10 000 000	0	6.24	100.0
Azotobacter+flagellates	1 000 000	8 200	6.00	96.0
Azotobacter+amoebae	1 000 000	200 000	7.70	123.0
Azotobacter+ciliates	1 000 000	50 000	8.20	131.0
1952				
Azotobacter — control	100 000 000	0	7.92	100.0
Azotobacter+ciliates	10 000 000	100 000	9.59	121.0
1953				
Azotobacter — control	100 000 000	0	11.15	100.0
Azotobacter+amoebae	10 000 000	1 000	14.90	134.0
Azotobacter+ciliates	10 000 000	1 000	15.35	137.0

Table 6

Influence of substances secreted by ciliates upon the development of azotobacter and the process of azotofixation

Combination of microorganisms	Cells in 1 ml of medium		Fixation of nitrogen	
	azotobacter	ciliates	in mg/lg of sugar	% control
Azotobacter — control	100 000 000	0	7.92	100.0
Azotobacter+ciliates	10 000 000	100 000	9.59	121.0
Azotobacter+secretion of ciliates	10 000 000	0	10.60	134.0
Azotobacter+secretion (of viable ciliates, through pores of the plate)	100 000 000	0	10.35	130.8

of plants (Nikoljuk 1954, Gellert 1958). Protozoa emit substances which stimulate azotobacter. It became interesting to determine the action of those substances upon plants.

The aim of the study consisted in determining the action of protozoa upon the germination of seeds and the subsequent development of plants (Nikoljuk 1965).

The germination ability of cotton seeds provided with amoebae and ciliate cultures, as well as suspension of killed organisms, was followed in Petri dishes. In some cases it proved to be twice higher than normally. This result was also observed in the vegetation experiments. The plants germinating from seeds with protozoa are bigger (Table 7).

Table 7

Germination of cotton and development of roots after a previous treatment of seeds with living protozoa and with a suspension of the dead (%/control)

No. of experiment	Control	Treatment of seeds with:				
		azoto-bacter	living amoebae	ciliates	supension of dead: amoebae	supension of dead: ciliates
Germinated seeds (Petri dishes)						
1	100.0	100.0	174.0	200.0	189.4	186.6
2	100.0	100.0	111.2	113.7	108.7	103.7
Root length						
1	100.0	97.5	124.4	139.0	141.4	134.1
Germinated seeds (plant pots)						
1	100.0	118.2	136.5	123.6	136.5	141.0

Further investigations concerned the determination of the nature of substances which exert a positive action upon the vitality of bacteria and upon the development of plants and are emitted by protozoa (Nikoljuk i Tapilskaja 1968).

After a number of preliminary laboratory experiments, the authors concentrated their attention on experimenting the capability of protozoa to production of growth-stimulating substances. The experiments were executed on two strains of *Amoeba limax* isolated from the rhizosphere of lucerne. Amoebae were cultivated on azotobacter in the medium of Fiodorow. The presence of growth substances was determined in the culture liquid. As indicator of the growth activeness of the culture medium of amoebae, the coleoptile of oat was applied (the method of Bojarkin 1966).

As a control of every experiment served the solution of kalium salt and of 3-indolylacetic acid (IAA) in concentration 10^{-7}. In the evaluation of results, the mean accretion of five coleoptiles (one reiteration of the experiment) was calculated and subsequently the reiteration in per cent related to the exit size and to the control in water was established. The difference in size after immersion in distilled water and in the experimental solution was considered as accretion caused by the growth substance.

The experiments with the biotest (Table 8) indicated that the culture liquid of the soil amoebae exerts a growth-activating action upon the coleoptiles of oat, similar to that of 3-indolylacetic acid i.e. heteroauxine. The capability to produce the growth substances is not equal in different strains: the strain 1 evoked an accretion of coleoptiles of 13.5% i.e. more than in the control with synthetic IAA.

After having established with the biotest the presence of growth substances in the culture medium of amoebae by means of determining the character of their action which is similar to that of heteroauxine — their identification was carried out.

Table 8

Capability of production of growth-stimulating substances in the strains of amoebae, increment of coleoptiles in %

Strains	Reiteration	Increment in water		Increment/issue value		
		amoebae	IAA control	amoebae	IAA	water
A. limax 1	10	13.5	5.6	120.8	112.4	106.4
A. limax 2	10	11.4	9.3	114.8	122.4	112.0

Note: IAA — 3-indolylacetic acid

The primary identification of growth substances was performed by means of paper chromatography (K e f e l i i T u r e c k a j a 1966). After centrifugation, the culture liquid was evaporated under vacuum at temp. 40—60°, the residue was extracted with ether. This procedure was repeated 3 times. The dry sediment was diluted in 4 ml of ethanol and used for chromatogram. As control served a solution of 3-indolylacetic acid in ethanol.

The development was performed in an acid mixture: n-butanol: acetic acid: water (40 : 12 : 28) and in an alkaline mixture: n-butanol: ammonia: water (10 : 1 : 1). After application, chromatograms were examined in day light and in ultraviolet light, in ammonia vapours or omitting them. Chromatogram spots were detected with specific reagents: of Ehrlich, of Salkowsky and with $FeCl_3$. The development of the culture liquid of amoebae and azotobacter on the chromatogram paper demonstrated that the biologically active substances of the indol nature are also present in the culture medium of *Amoeba limax* (Table 9). As shown in the table, the spots gained after development of the medium of amoeba culture, have the same indicators (Rf), luminescence in UV, reactions to specific stains as 3-indolylacetic acid used for control.

In the extracts of azotobacter cultures which was the growth medium for amoebe, 3-indolylacetic acid has not been revealed. This proves that the strain of *Azotobacter chroococcum* applied as food for amoebae, fails to produce 3-indolylacetic acid and that for its presence in the mixed cultures with amoebae, only the latter ones are responsible.

It may be stated in conclusion:

In the process of vital activity, the soil protozoa secrete biologically active substances, which in the case of amoeba, are identified as 3-indolylacetic acid or heteroauxine.

The positive action of soil protozoa upon the bacterial flora, on the

Table 9

Identification of spots of the amoeba culture medium

Culture medium	Solvents											
	n-butanol — CH_3COOH — H_2O						n-butanol — NH_4OH — H_2O					
	Rf	Luminescence in UV		Stain reaction			Rf	Luminescence in UV		Stain reaction		
		without NH_3	in NH_3 vapours	Ehrlich	Salkov-sky	$FeCl_3$		without NH_3	in NH_3 vapours	Ehrlich	Salkov-sky	$FeCl_3$
Amoebae	0.94	light violet	light violet	blue	magenta pink	pink	0.17	light violet	light violet	blue	magenta pink	pink
Azotobacter	—	—	—	—	—	—	—	—	—	—	—	—
Control (IAA)	0.94	light violet	light violet	blue	magenta pink	pink	0.17	light violet	light violet	blue	magenta pink	pink

Note: IAA — 3-indolylacetic acid

development of plants and on the soil processes, may be accounted for, in a first place, by their capability of producing such a powerful activator as heteroauxine.

Summary

The soil protozoa are a microorganism group widely spread in different soil zones and conditions. They lead in soil an active mode of life. The main representatives of those organisms feed on soil bacteria and their relation to the representatives of different physiological groups is not uniform, with preference of oligonitrophils.

It has been stated that feeding on bacteria, partly on azotobacter, protozoa exert a positive influence on the activity of bacteria. In mixed cultures, a high quantity of nitrogen is being accumulated.

The application of protozoa prior to semination of seeds has a positive effect on germination and on the initial development of plants.

It has been stated in the above study:

In the process of life activity soil protozoa secrete biologically active substances which in the case of amoeba are defined as 3-indolylacetic acid i.e. heteroauxine.

The positive action of soil protozoa upon the bacterial flora, on the development of plants and on the soil processes may be accounted for by their capability to produce such a powerful activators as heteroauxine.

РЕЗЮМЕ

Почвенные простейшие — группа микроорганизмов широко распространенная в различных почвенных зонах и условиях. Простейшие ведут в почве активный образ жизни. Основные представители этих организмов питаются почвенными бактериями дифференцированно относясь к представителям тех или иных физиологических групп, отдавая предпочтение олигонитрофилам.

Установлено, питаясь бактериями, в частности азотобактером, простейшие на их деятельность влияют положительно, в совместных культурах азота обычно накапливается значительно больше. Предпосевная обработка семян простейшими положительно сказывается на их всхожести и начальном развитии растений.

Последующими исследованиями установлено:

1. В процессе жизнедеятельности почвенные простейшие выделяют биологически активные вещества, которые на примере амеб идентифицируются как индолил-3-уксусная кислота или гетероауксин.

2. Положительное влияние почвенных простейших на бактериальную флору, развитие растений и на почвенные процессы в целом можно объяснить, в первую очередь, их способносью продуцировать столь мощный биологический активатор как гетероауксин.

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PROTISTOLOGICA

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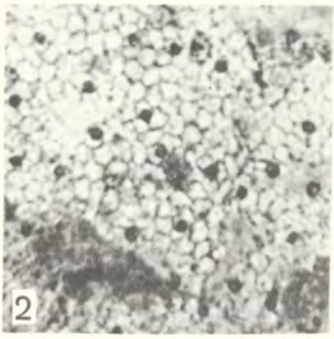

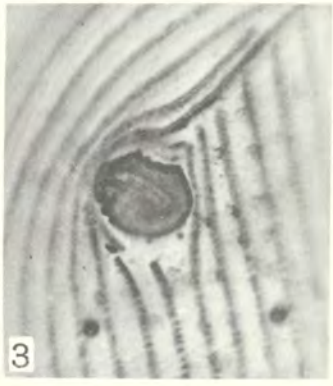

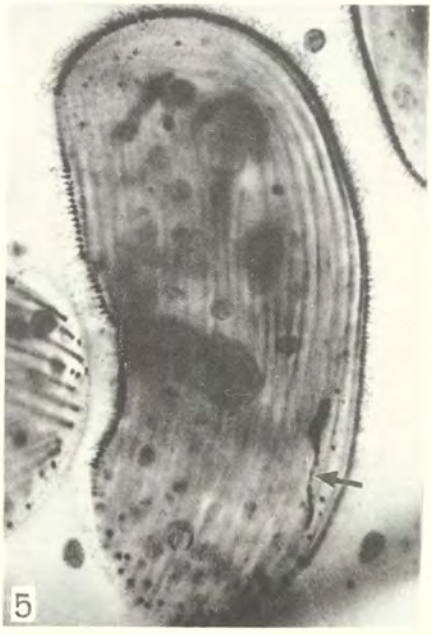

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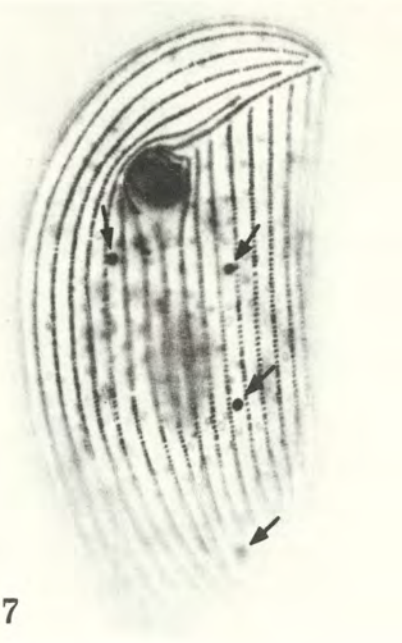

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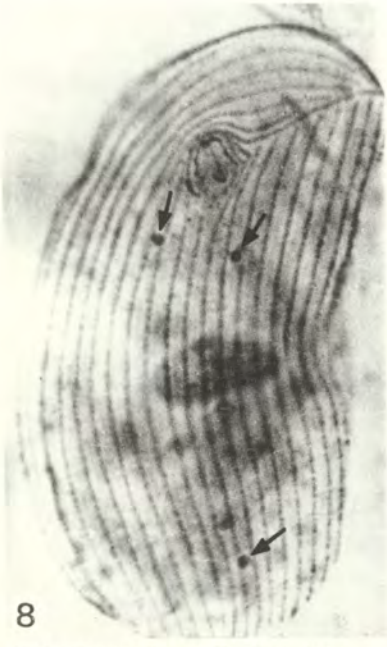

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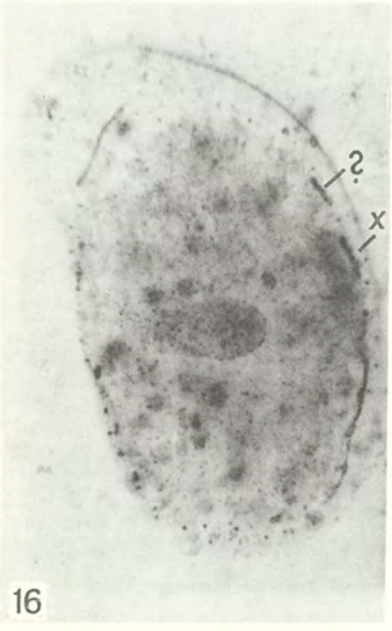

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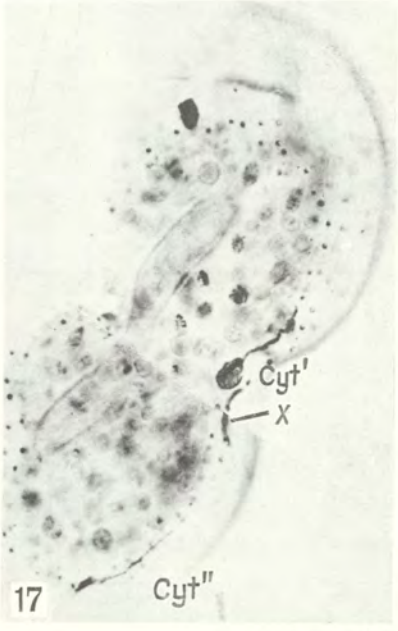

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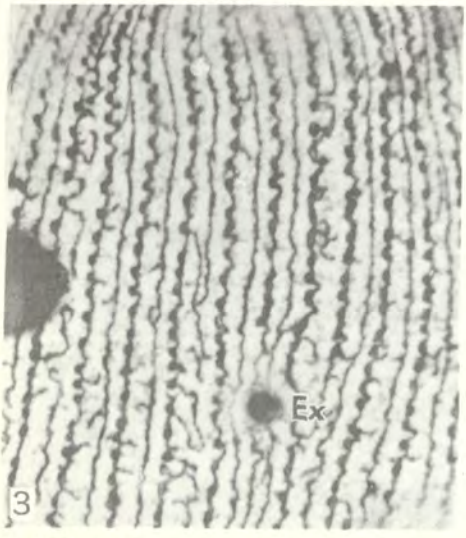

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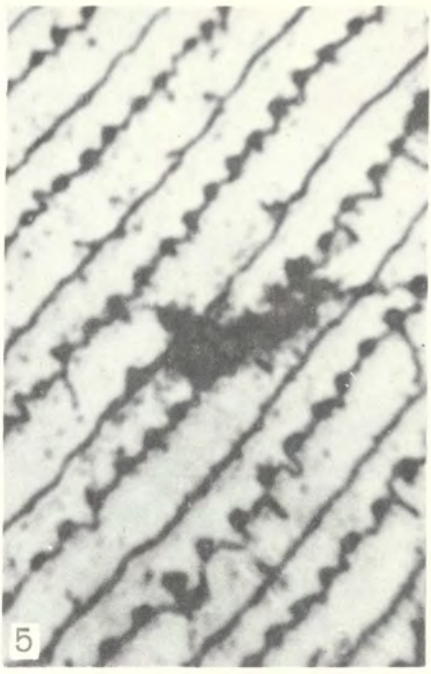

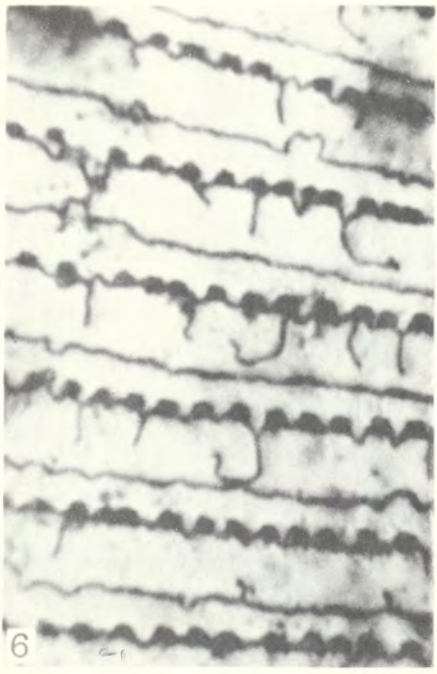

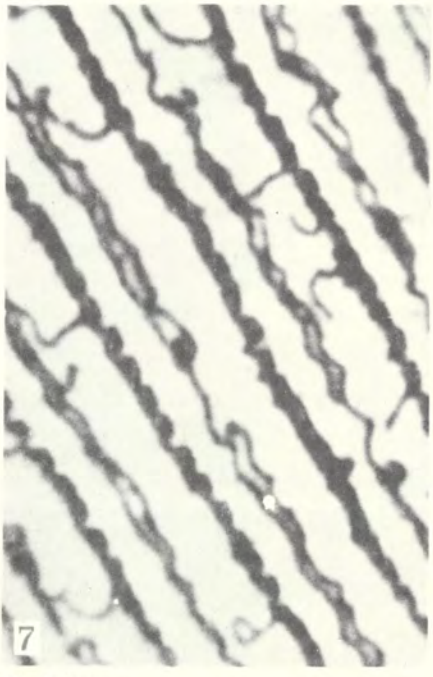

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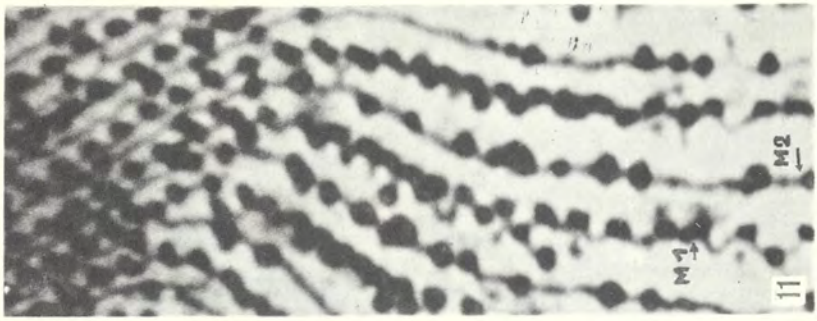

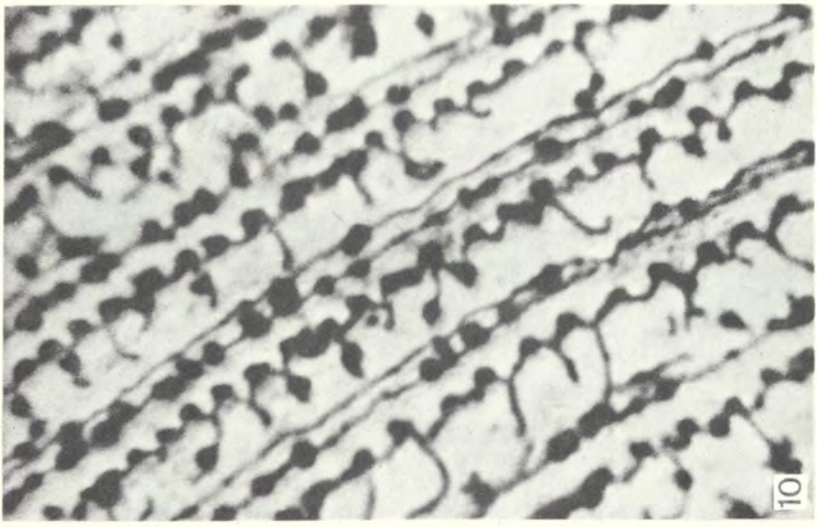

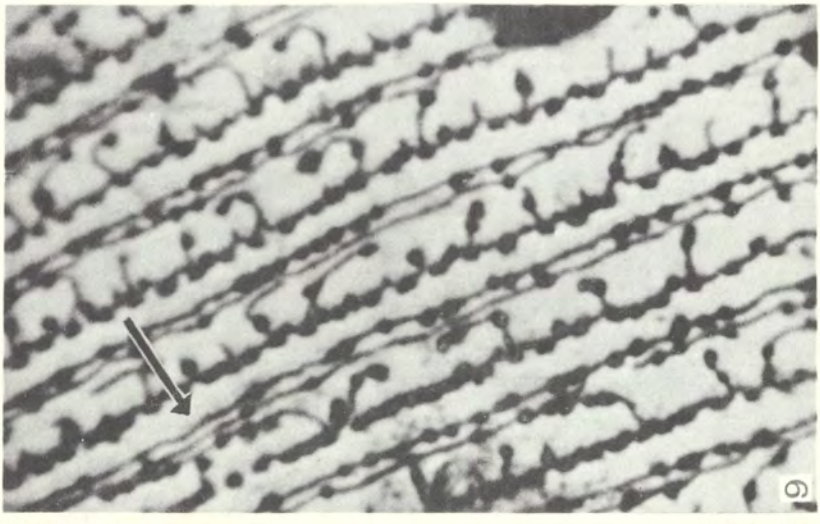

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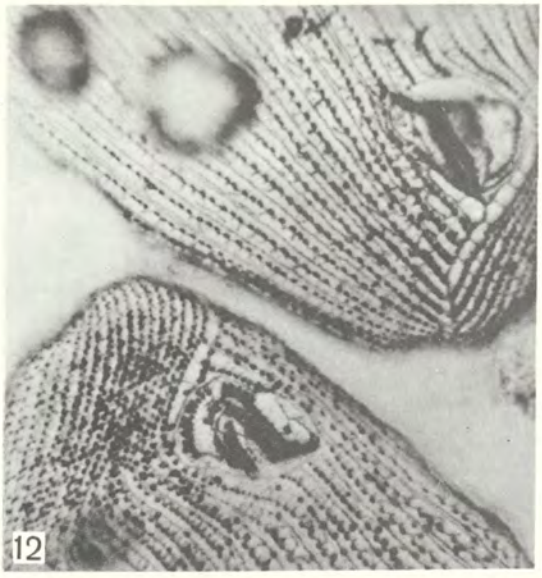

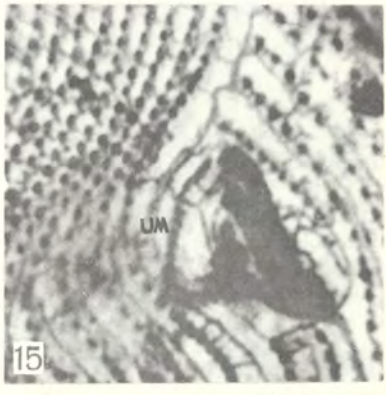

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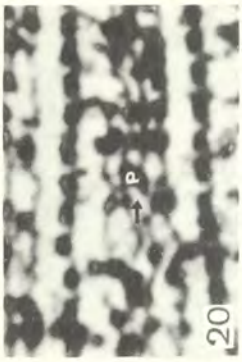

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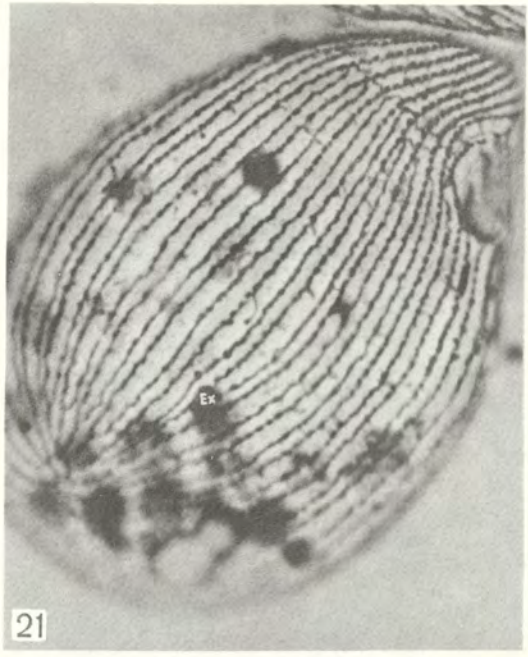

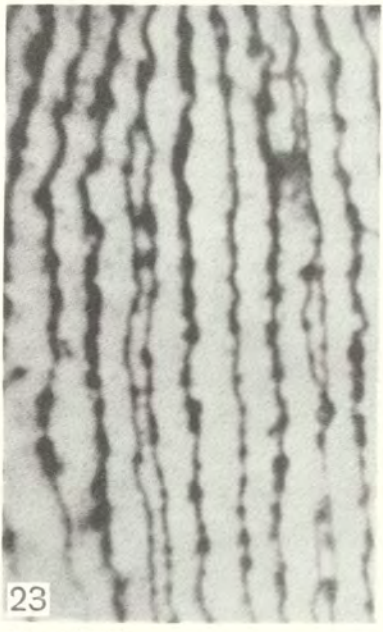

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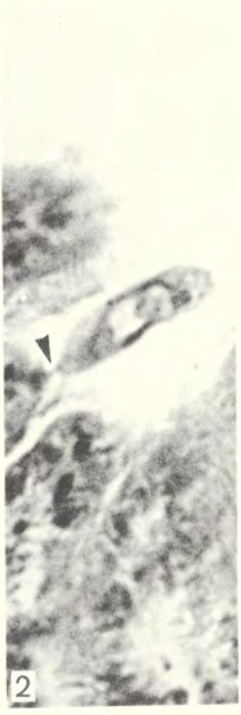

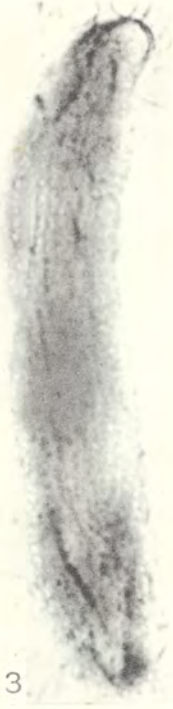

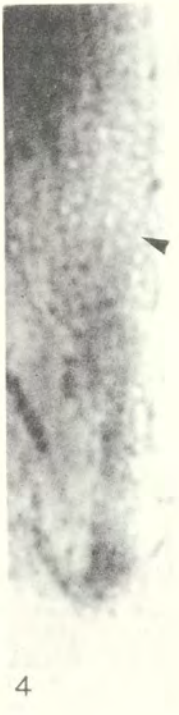

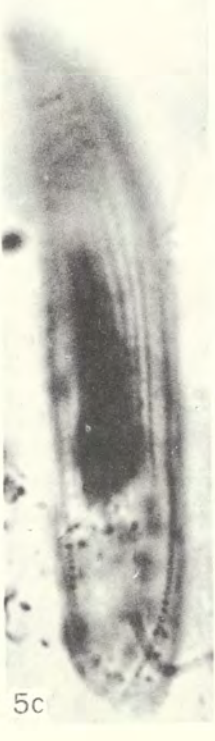

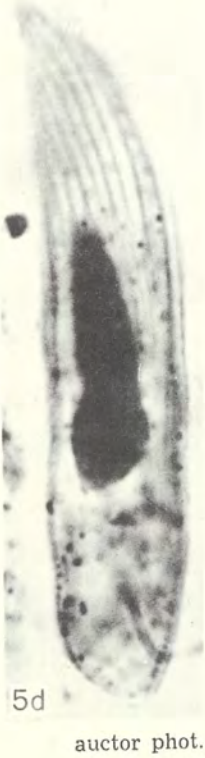

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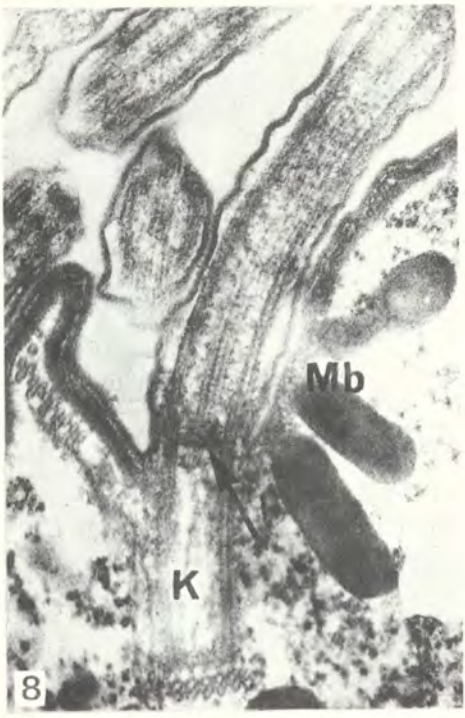

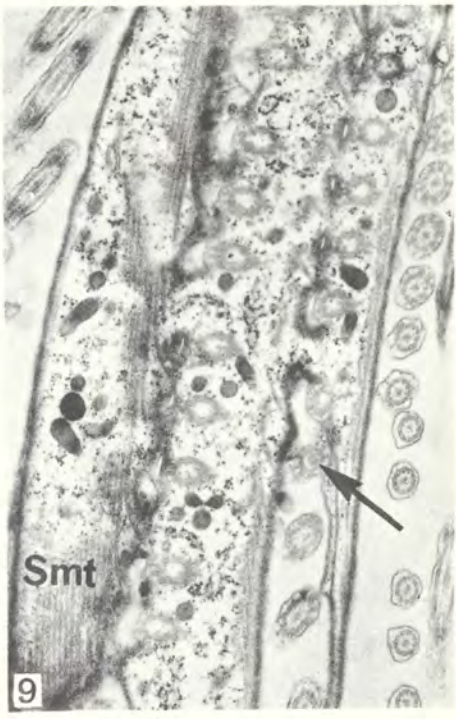

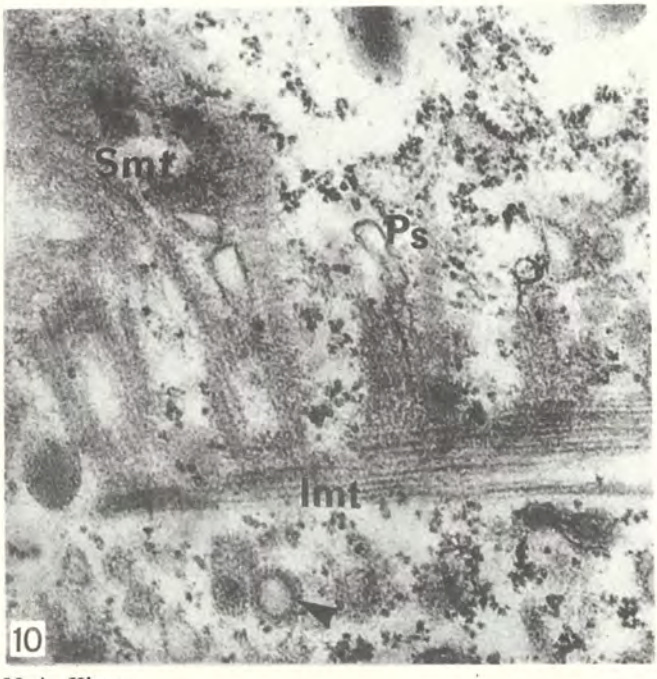

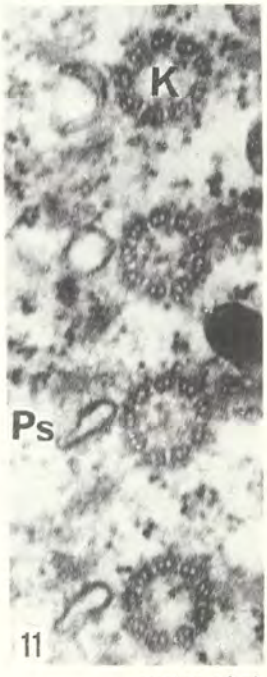

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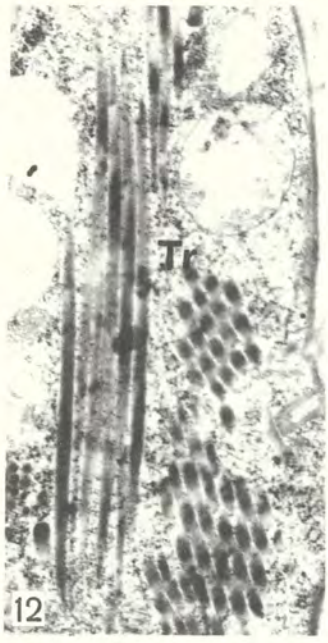

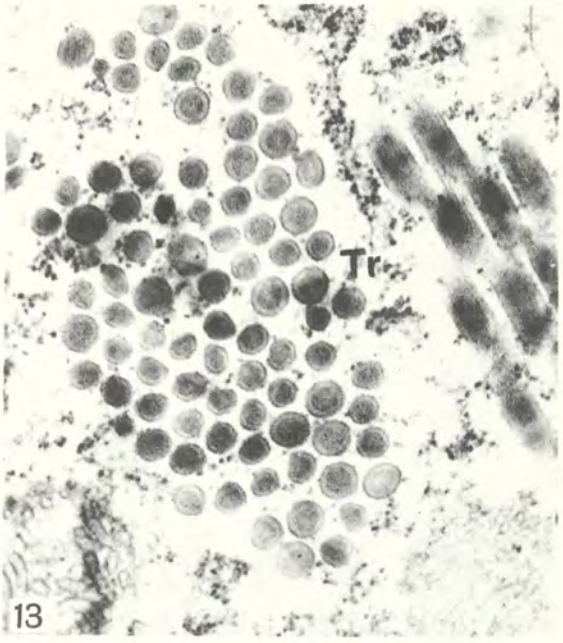

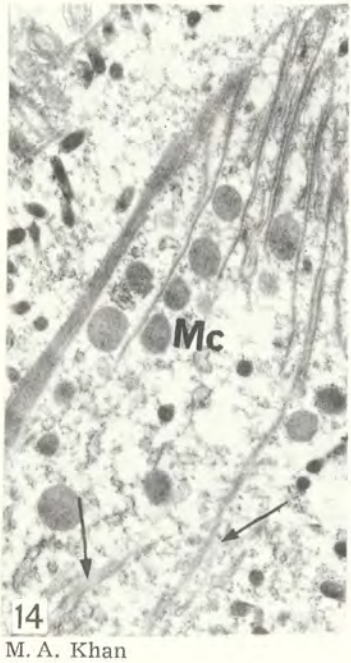

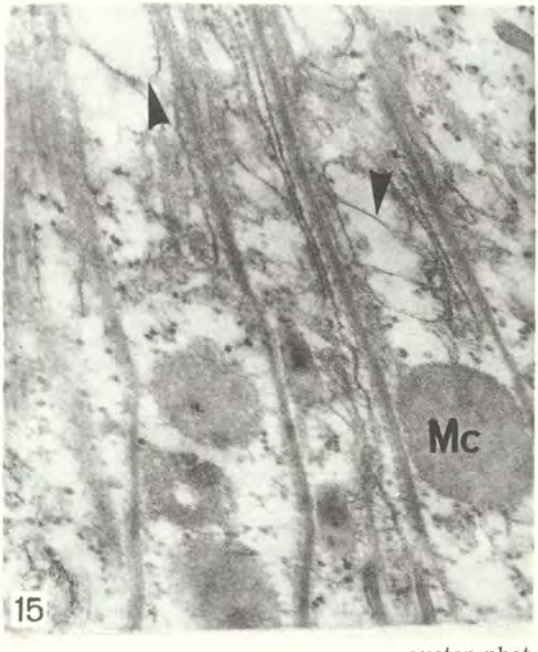

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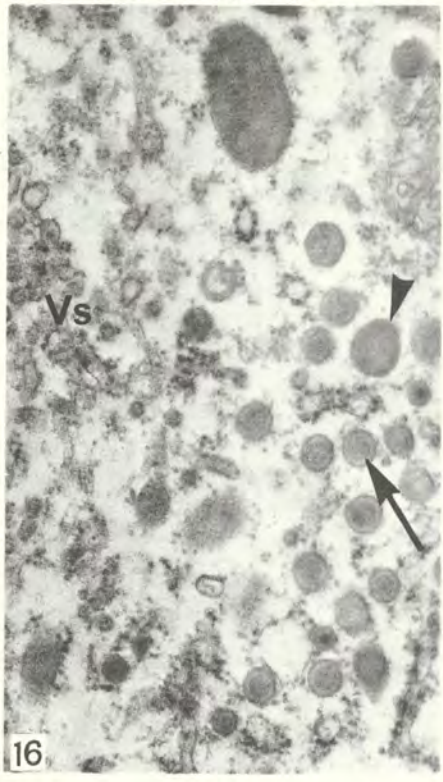

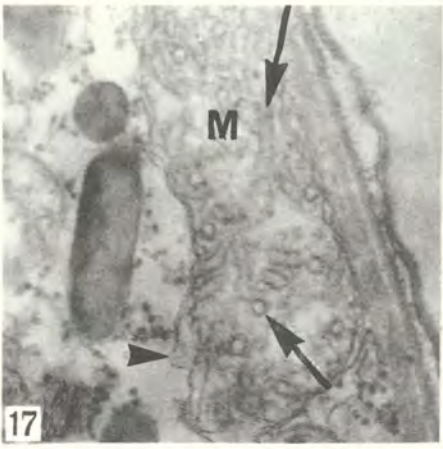

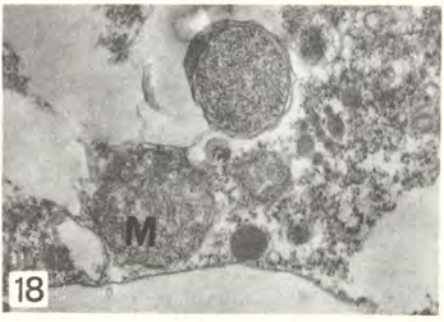

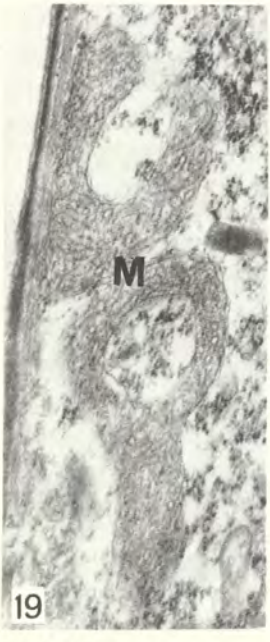

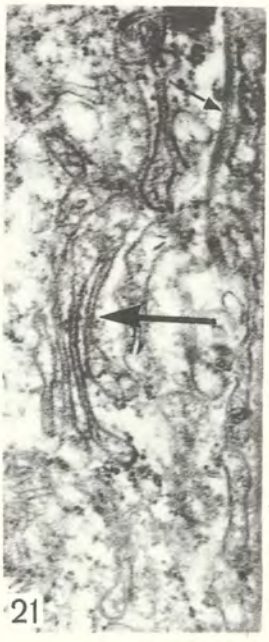

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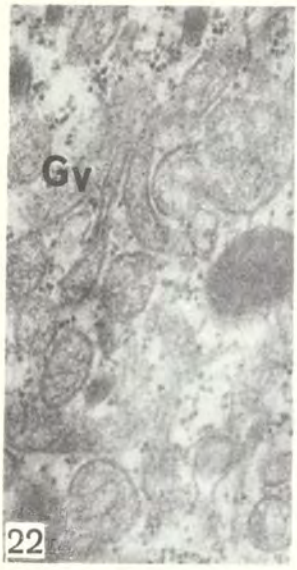

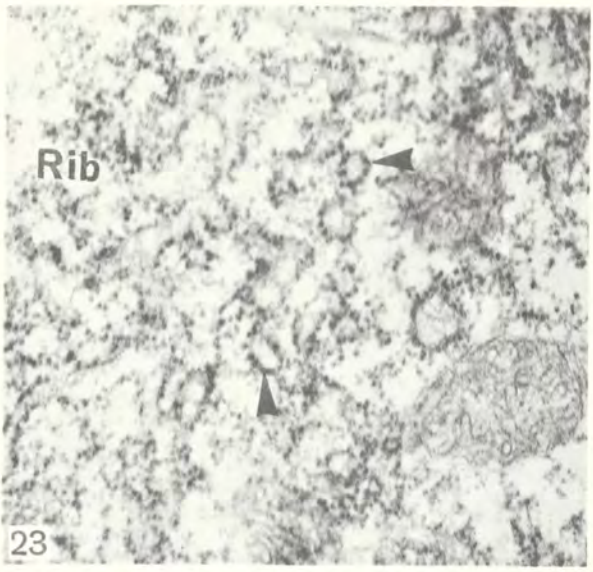

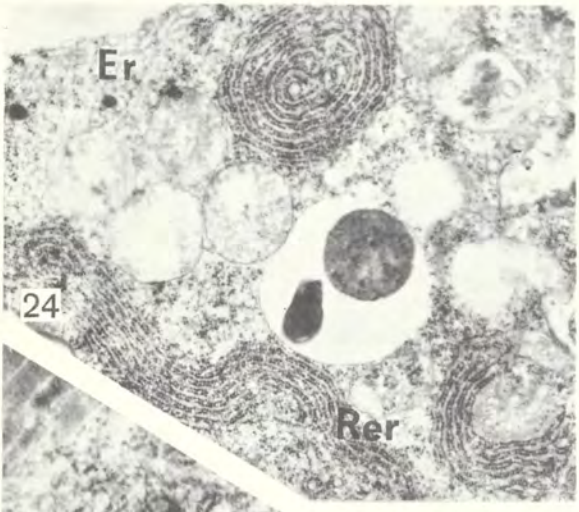

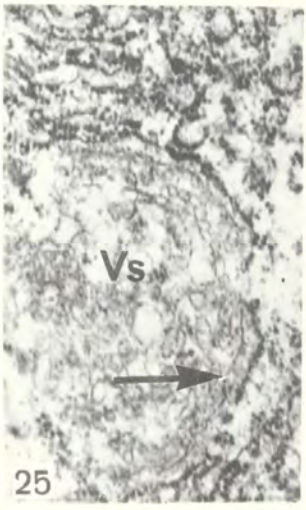

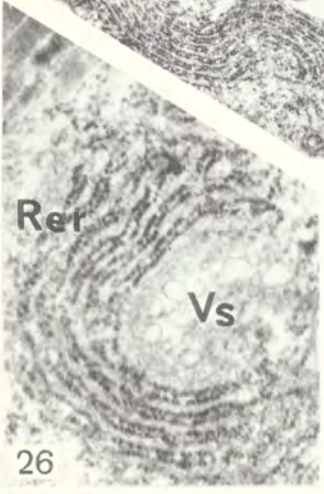

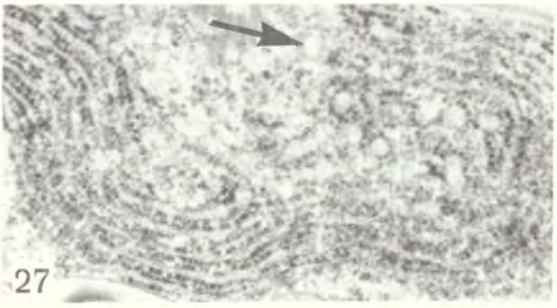

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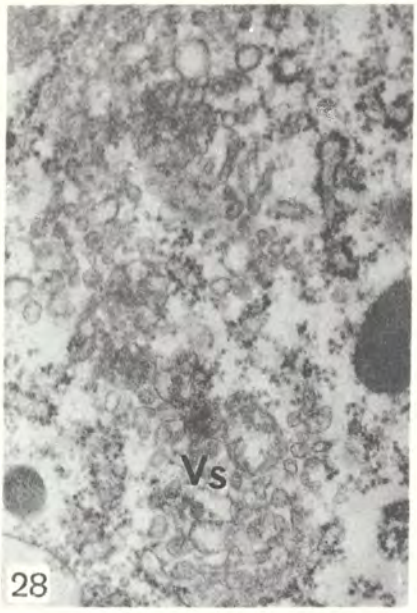

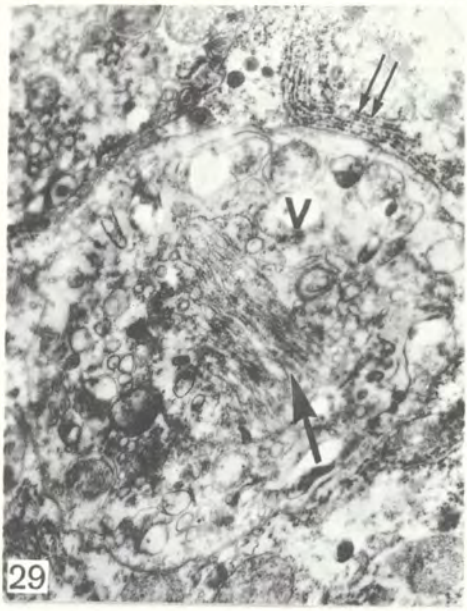

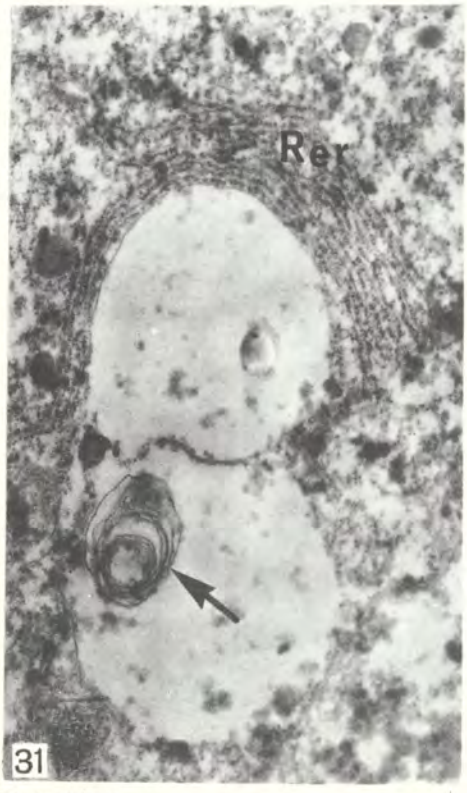

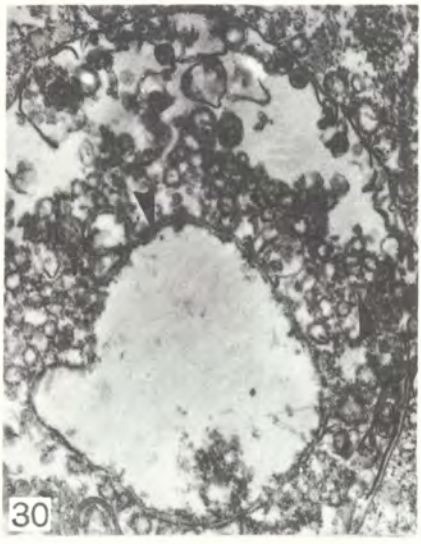

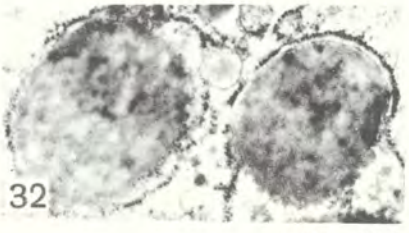

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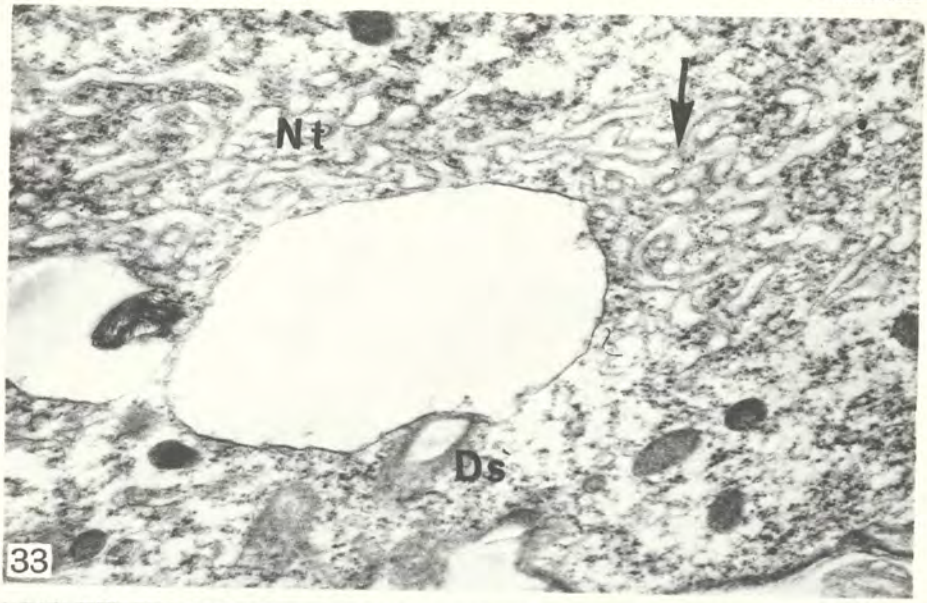

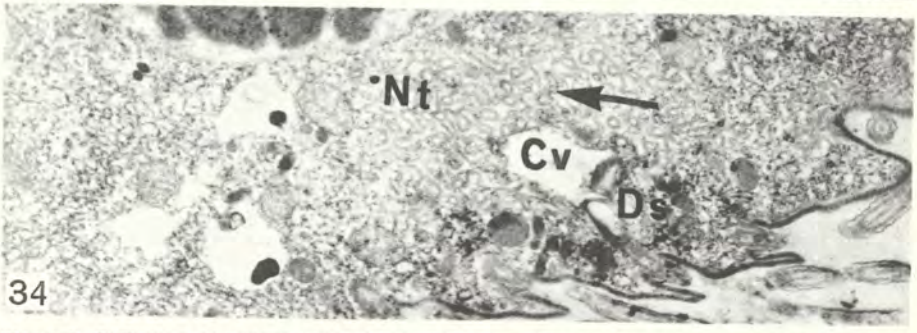

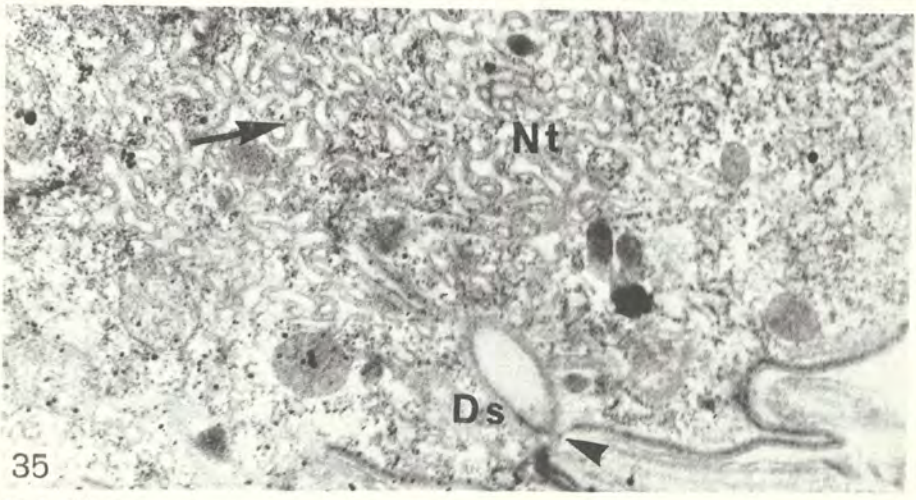

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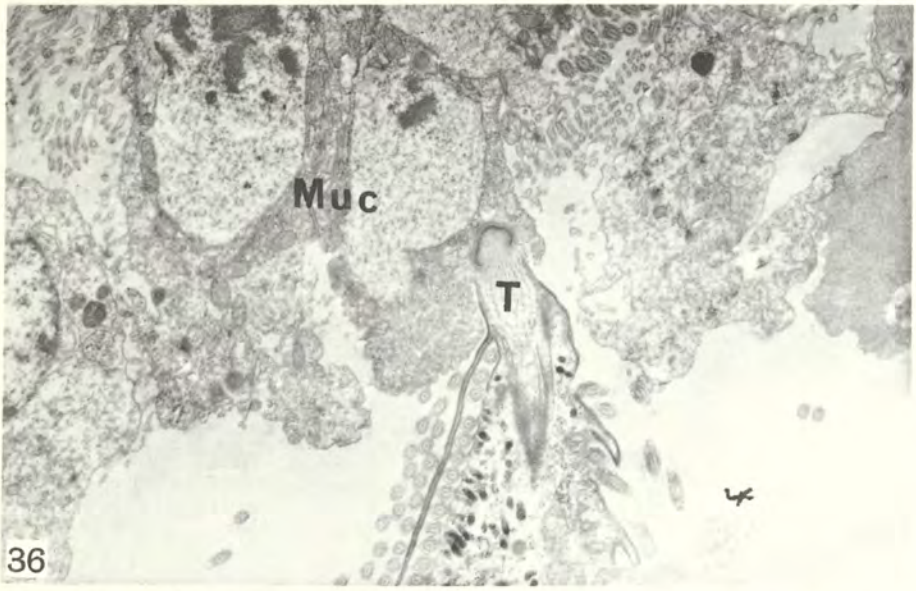

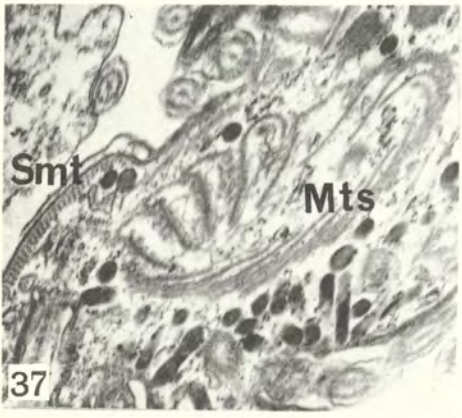

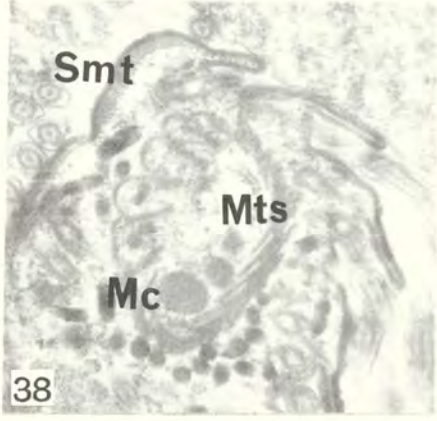

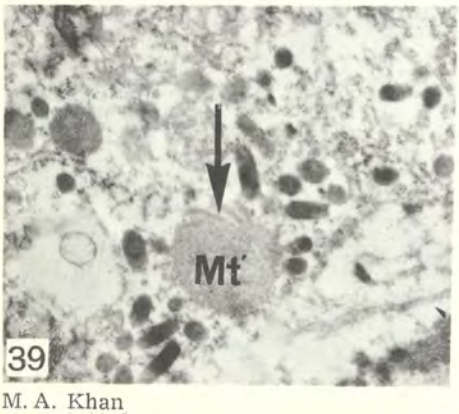

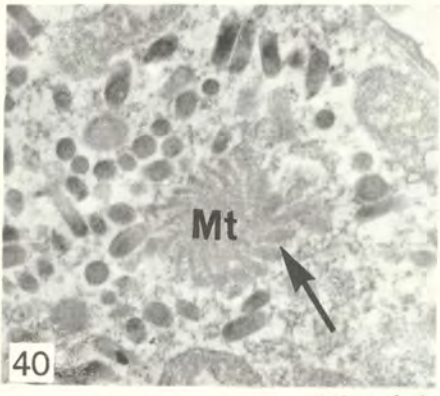

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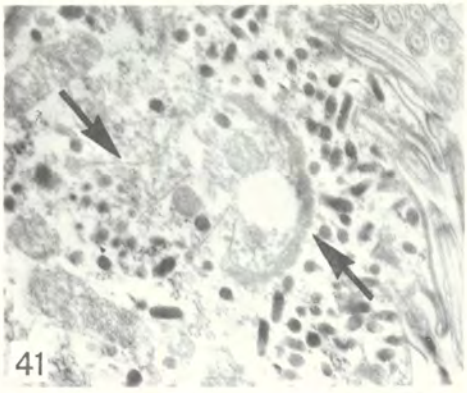

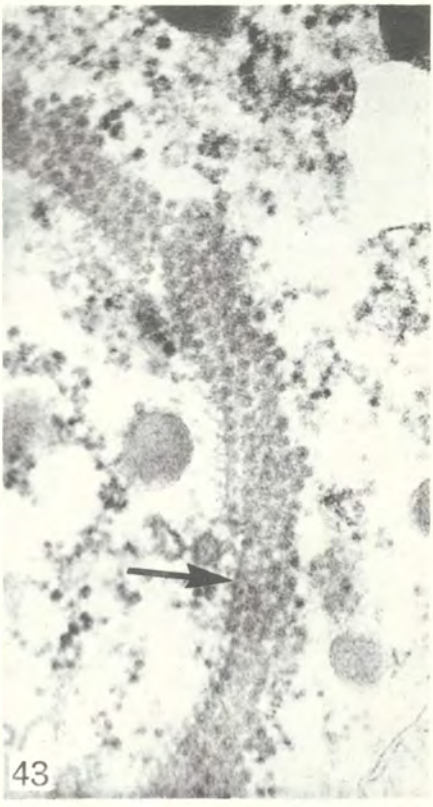

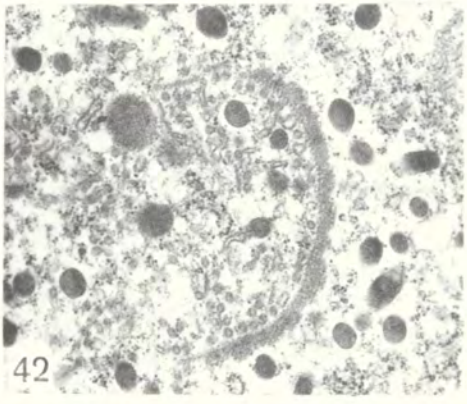

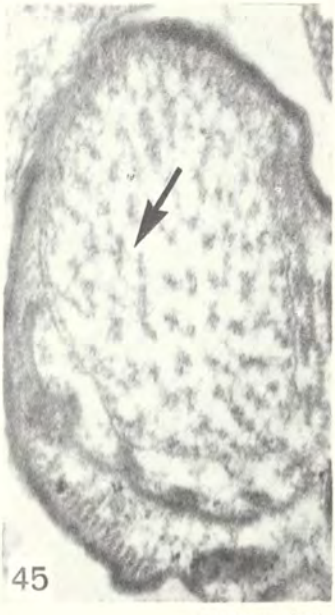

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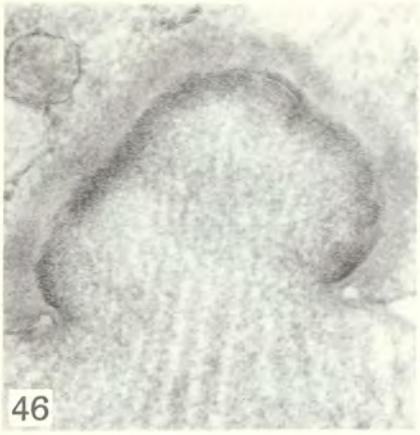

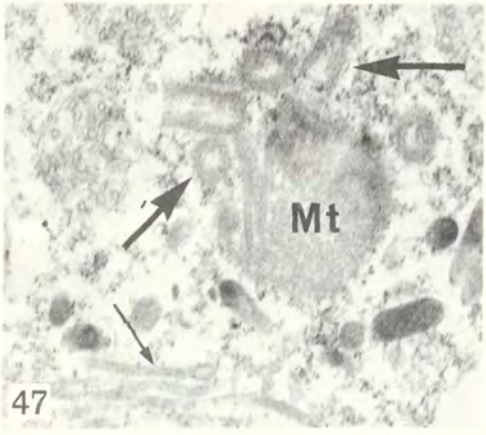

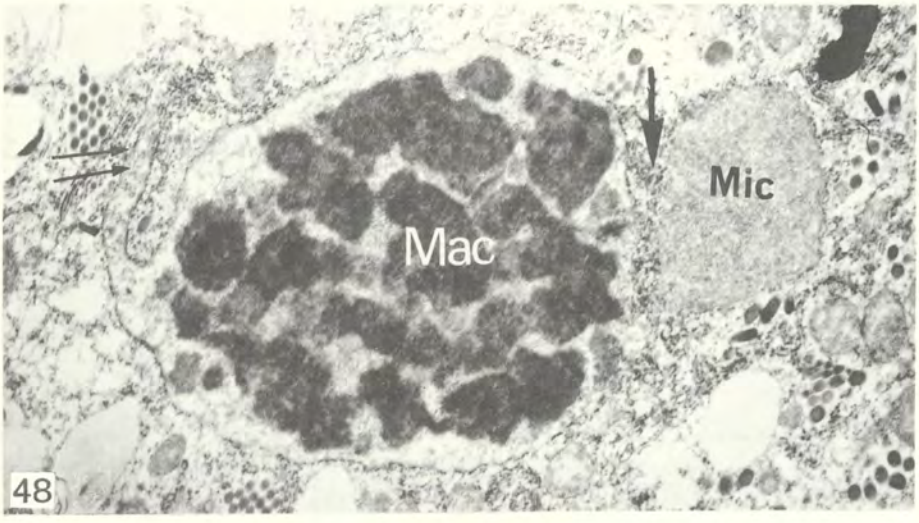

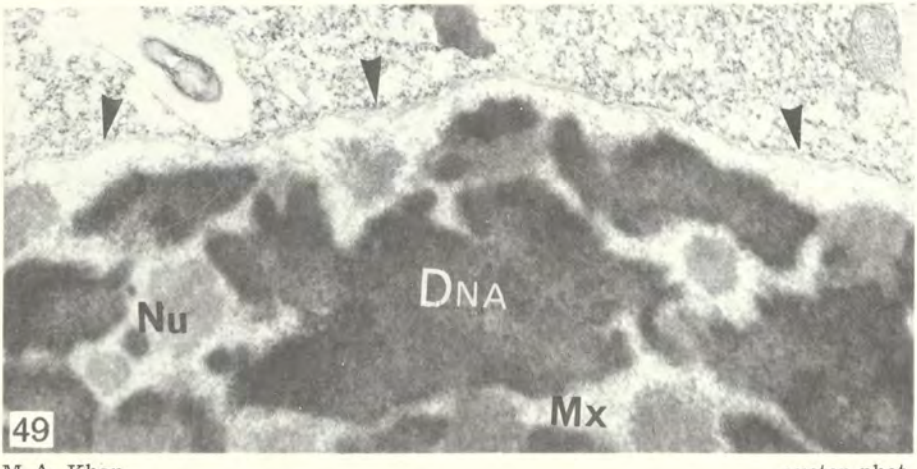

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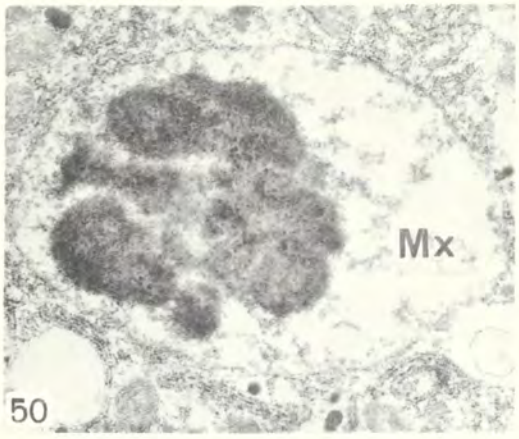

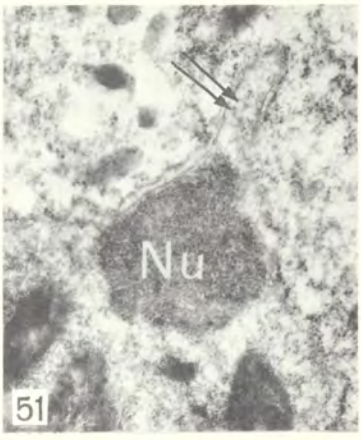

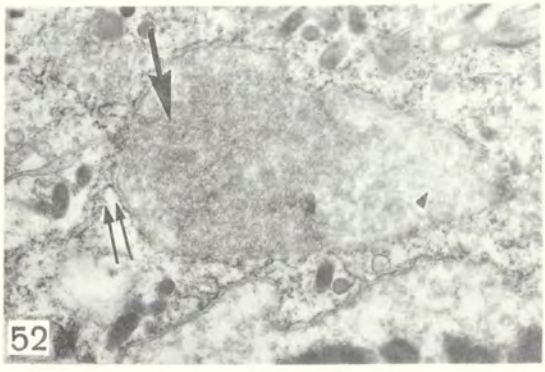

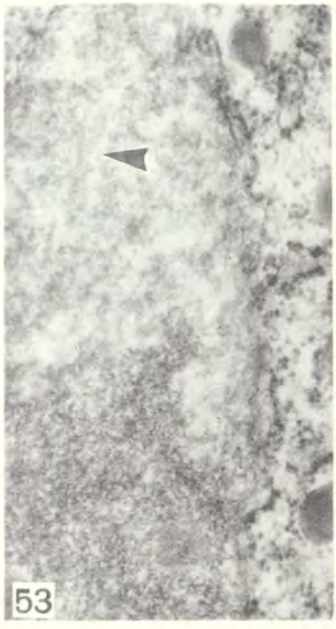

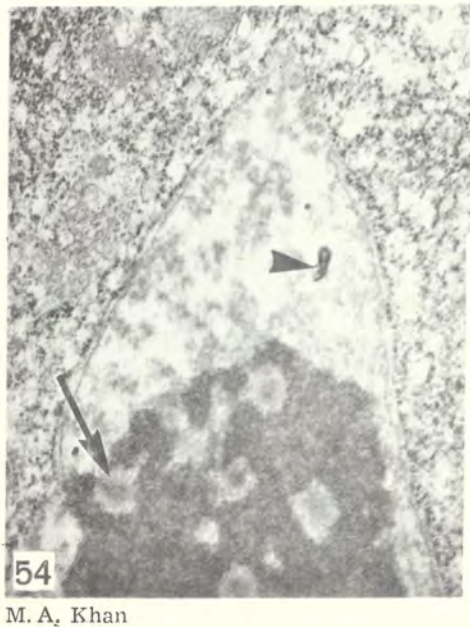

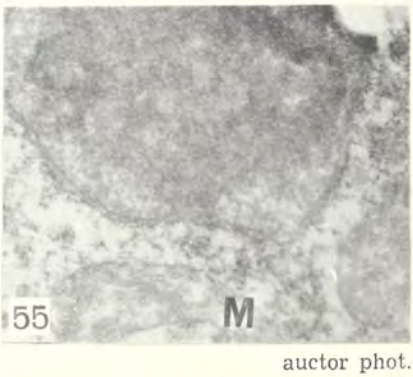

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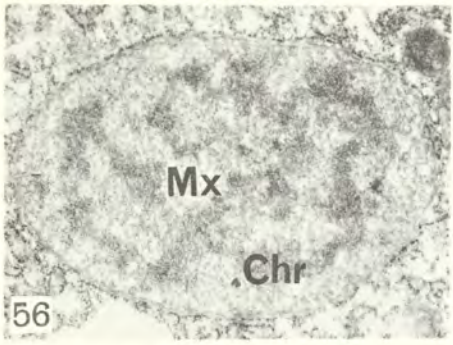

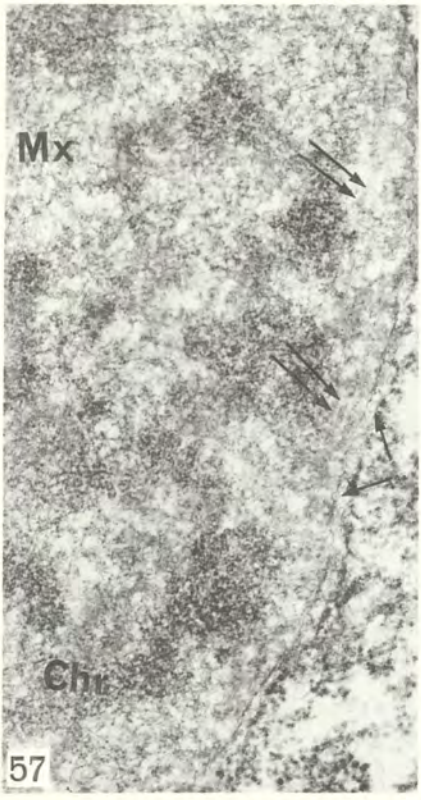

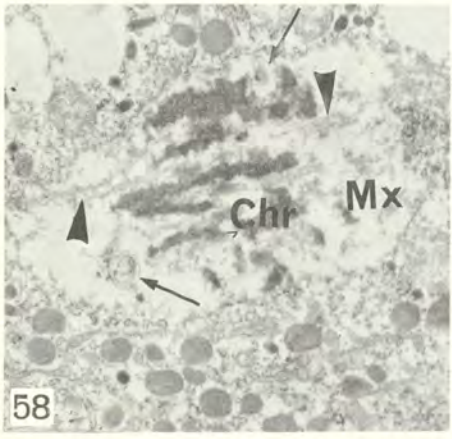

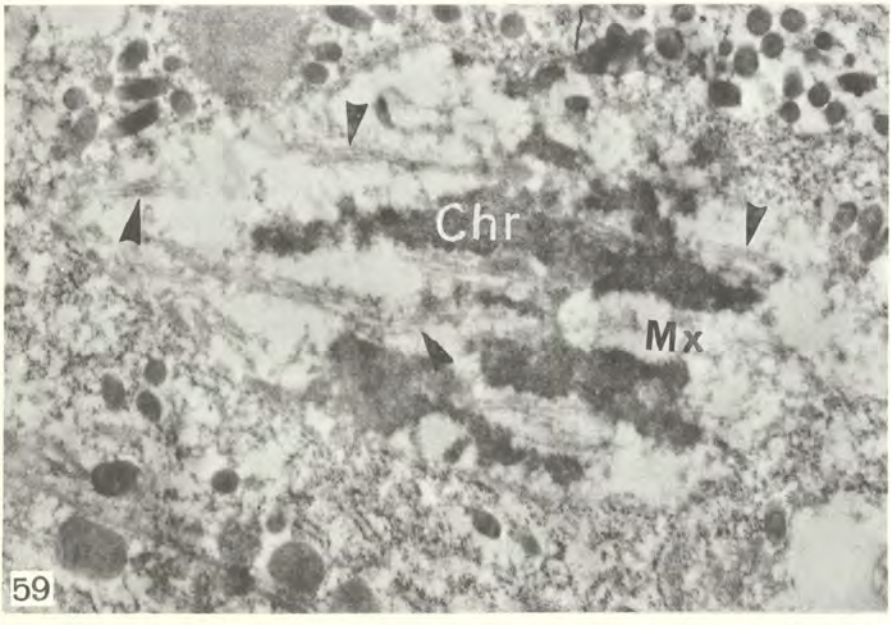

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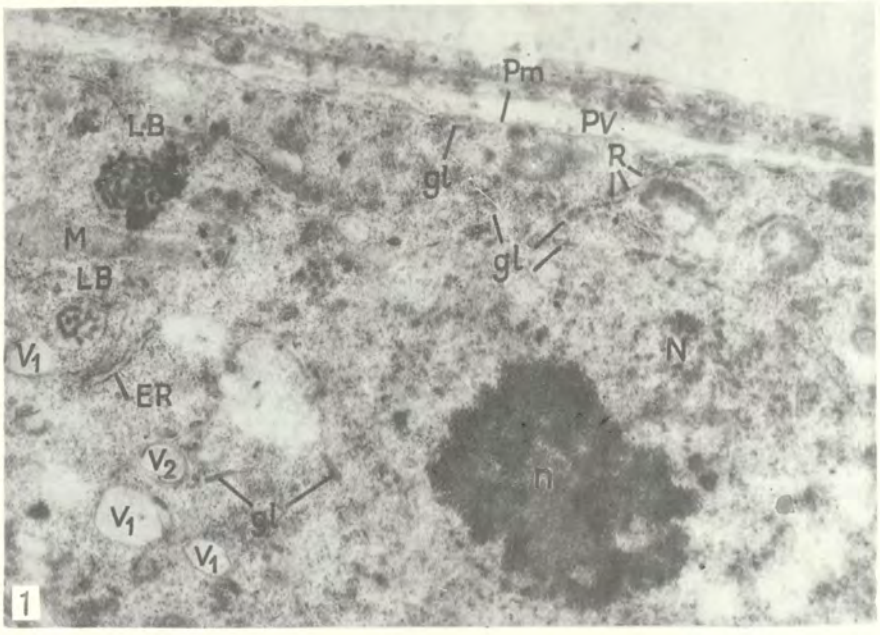

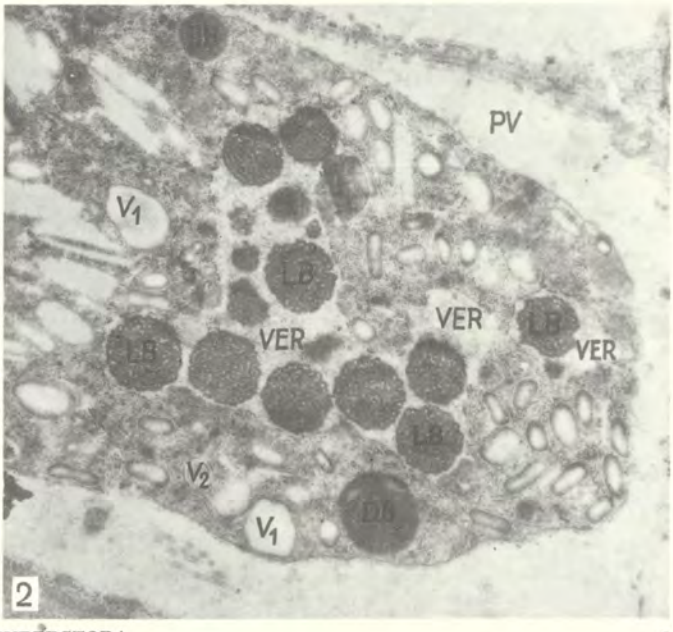

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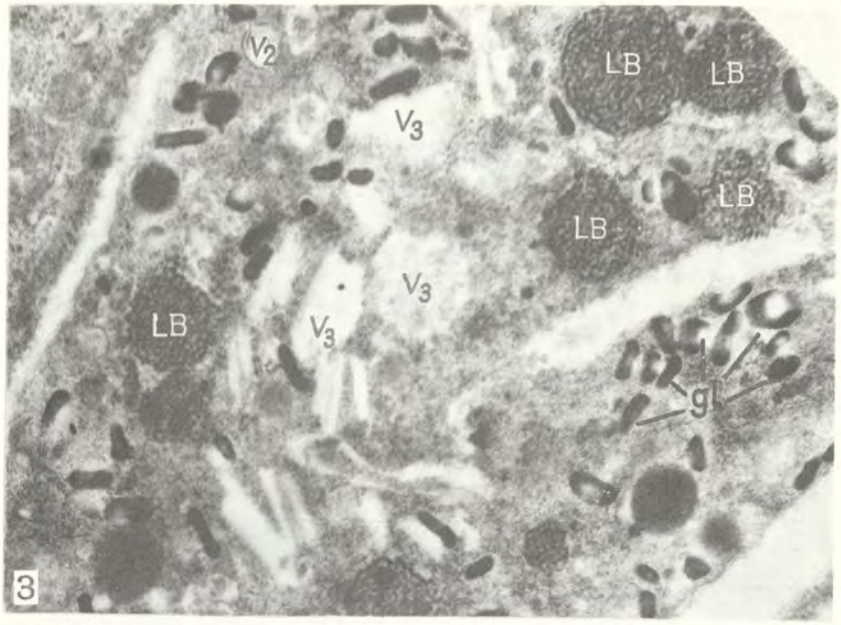

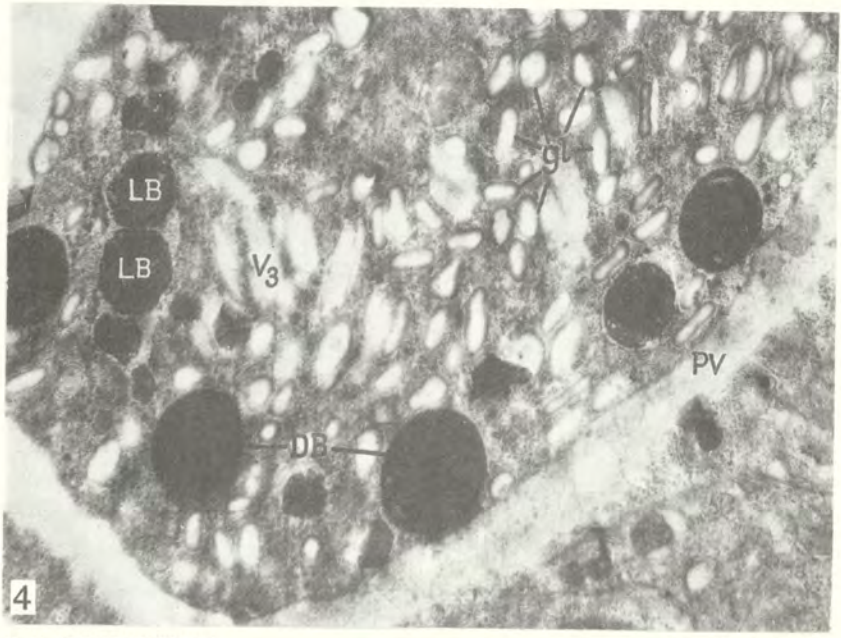

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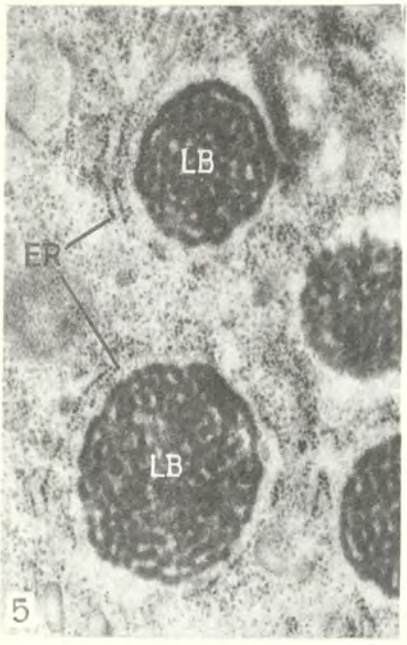

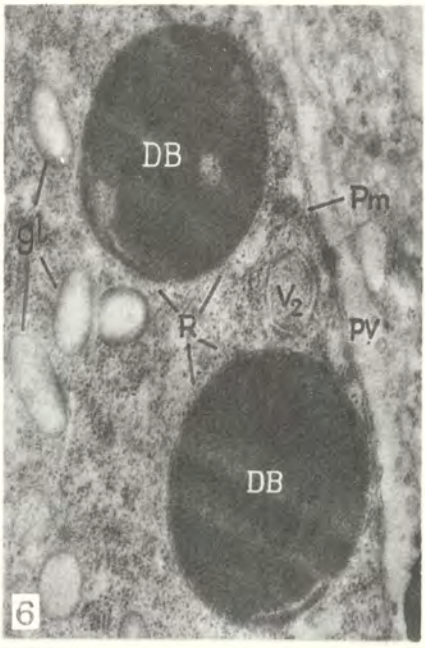

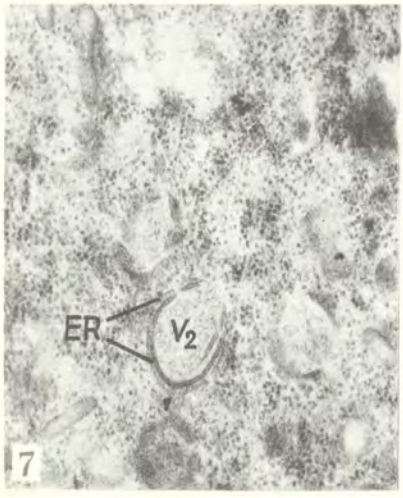

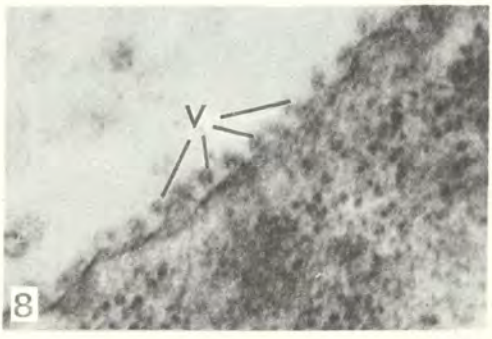

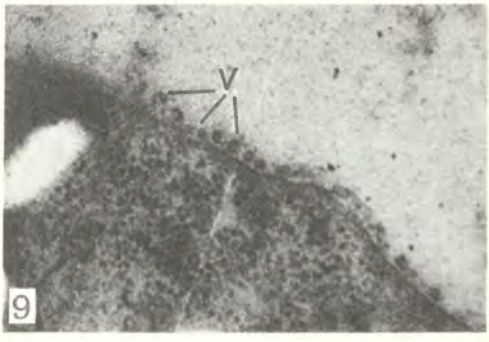

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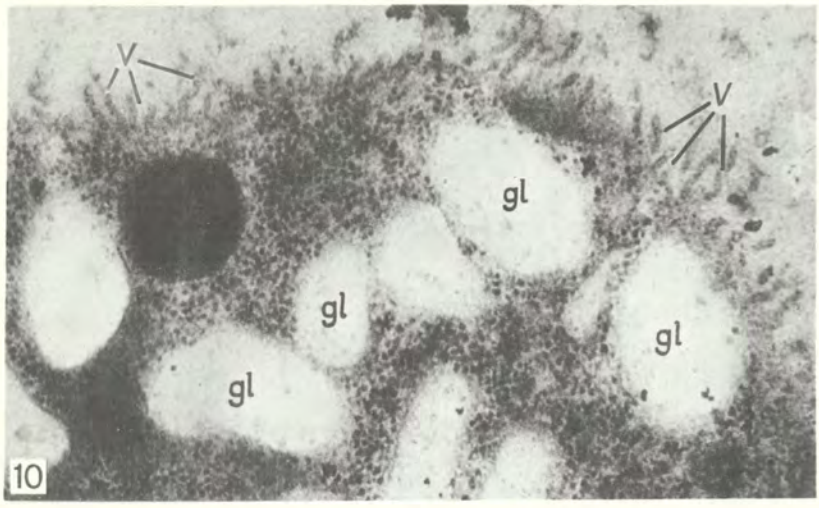

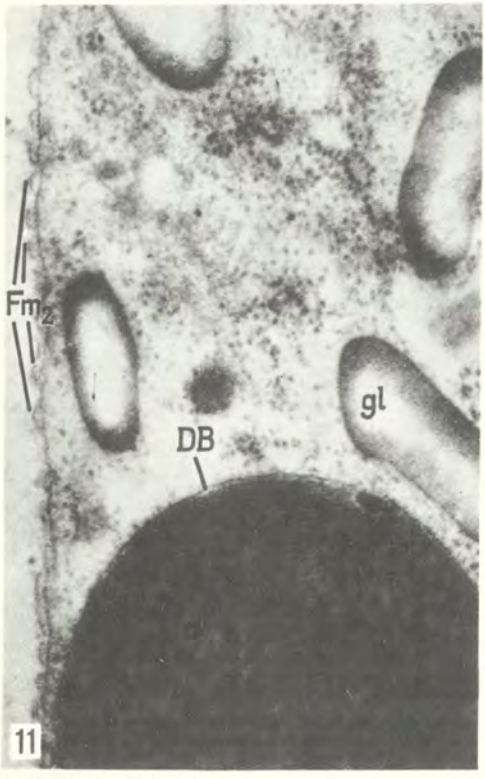

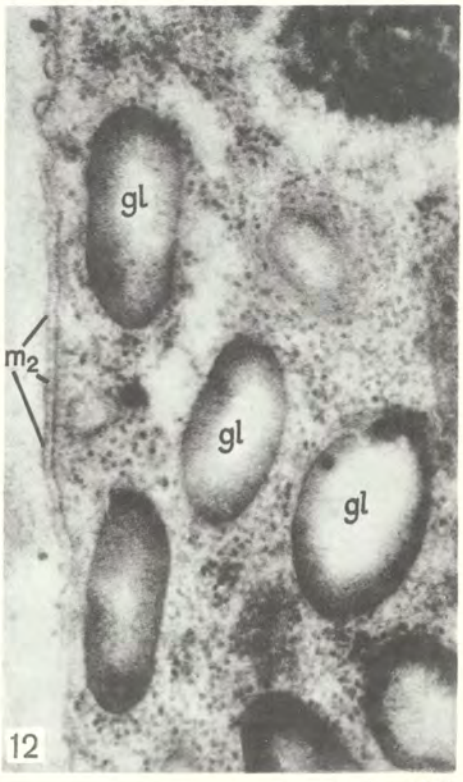

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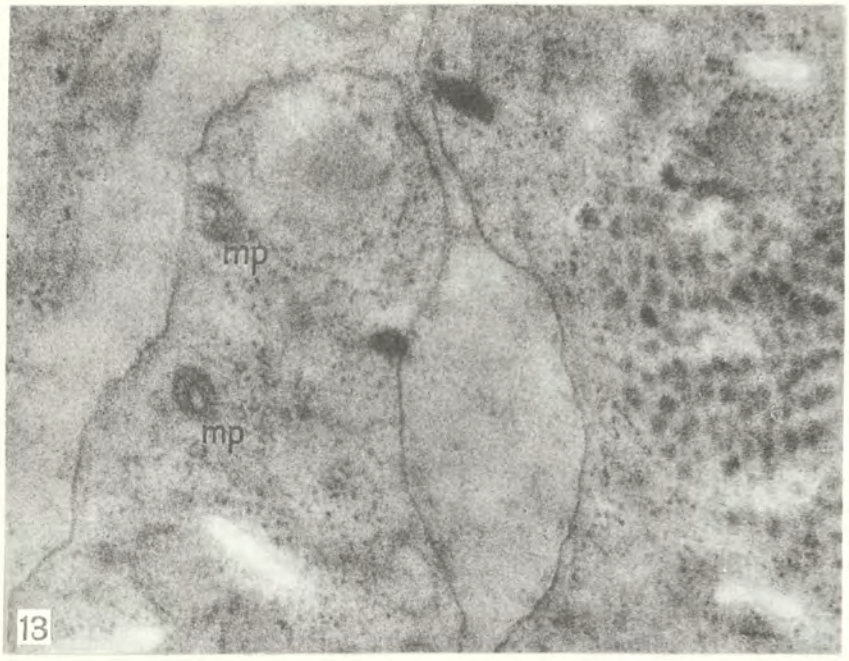

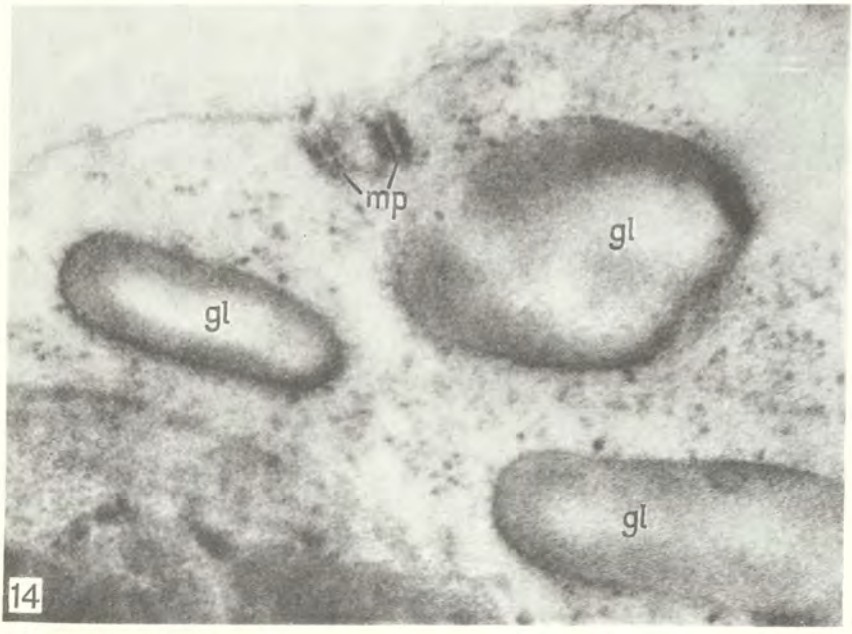

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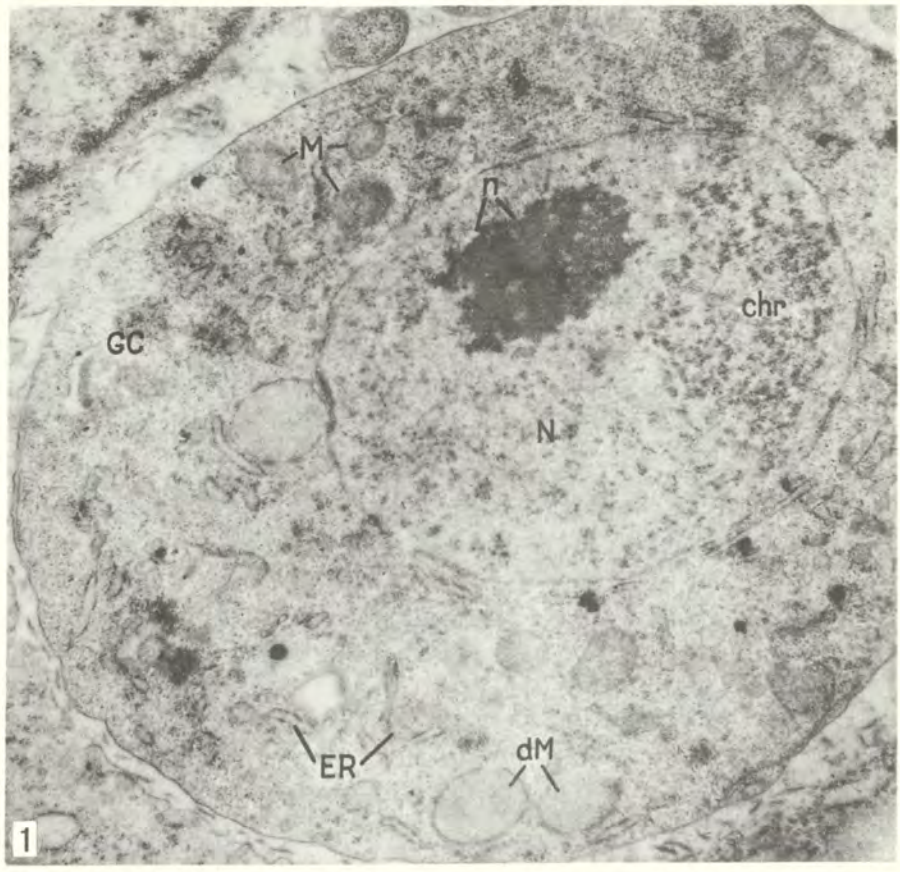

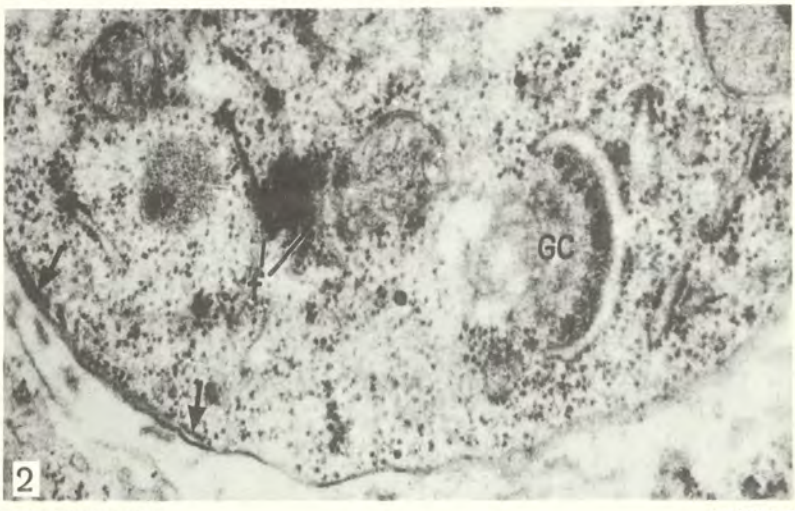

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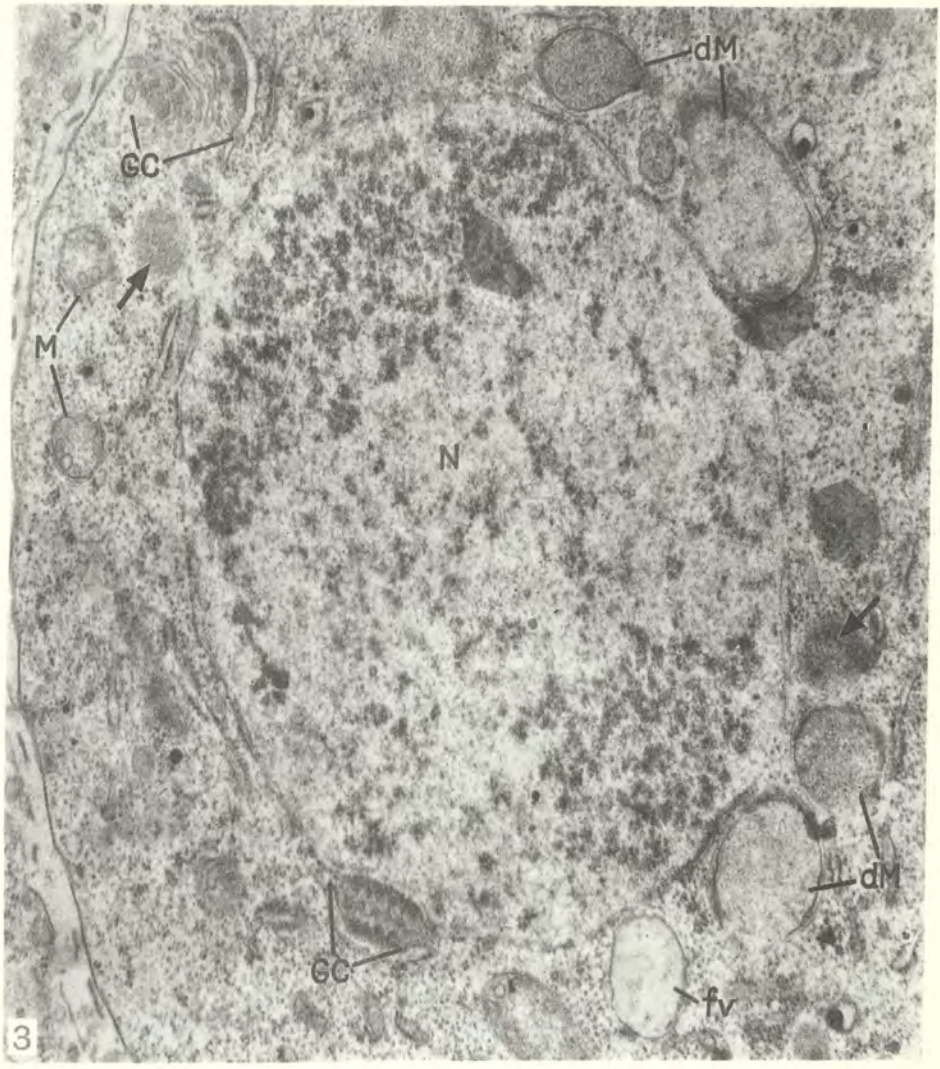

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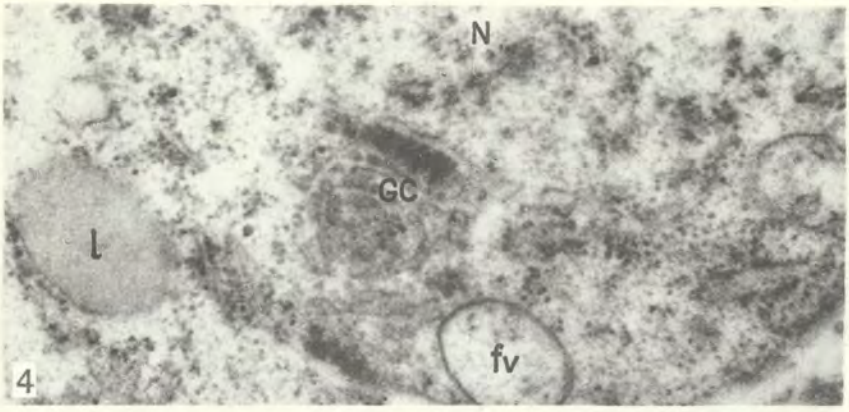

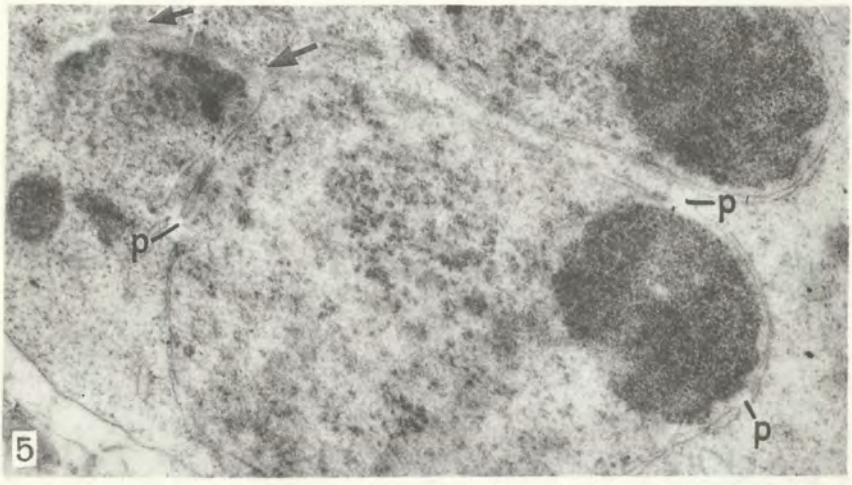

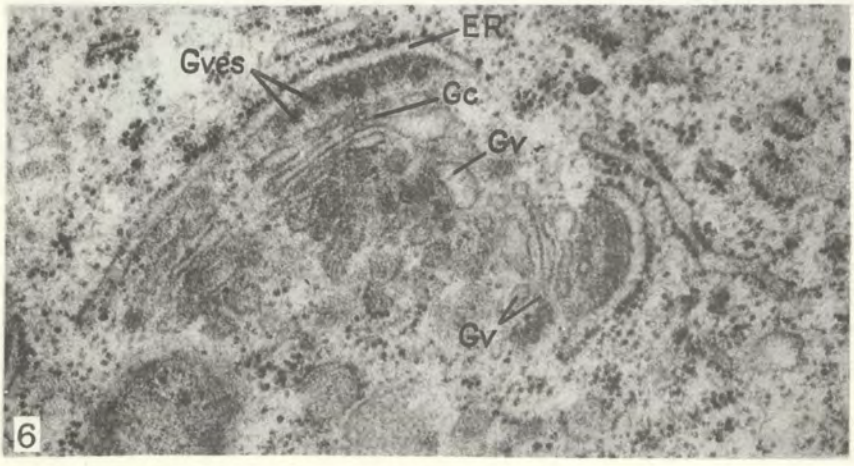

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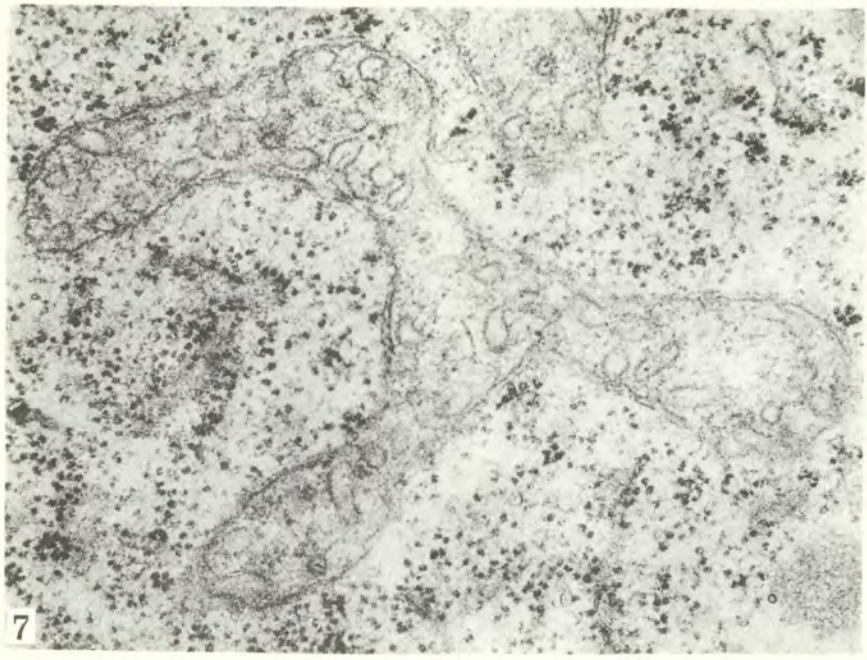

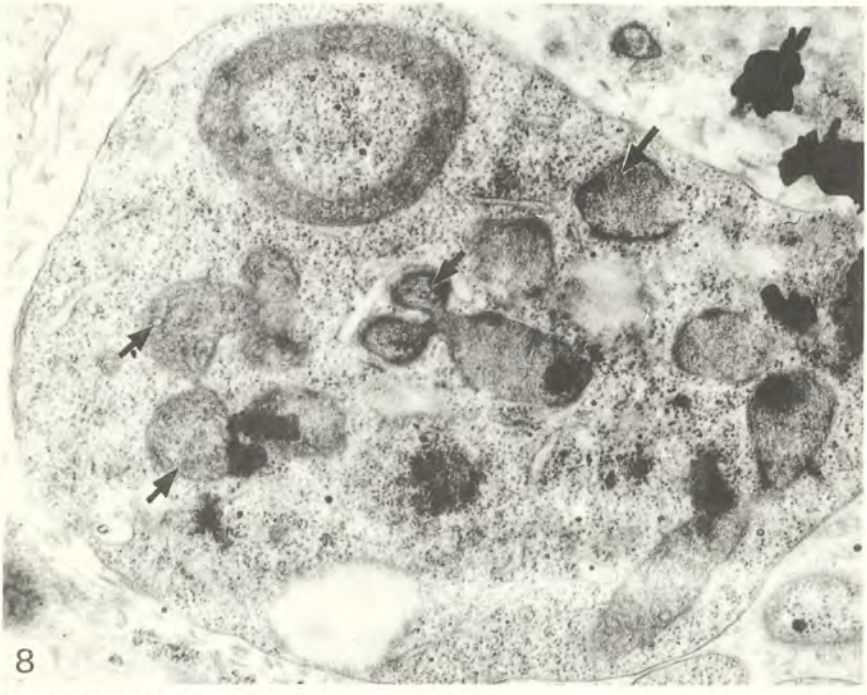

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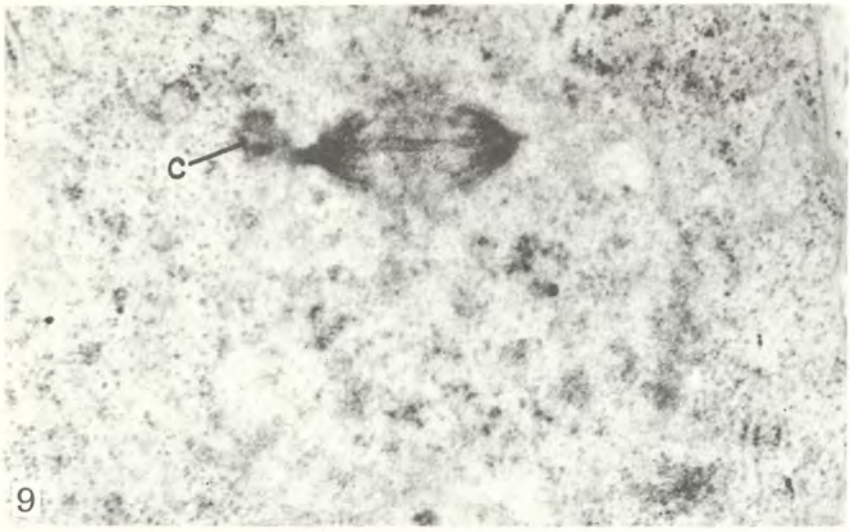

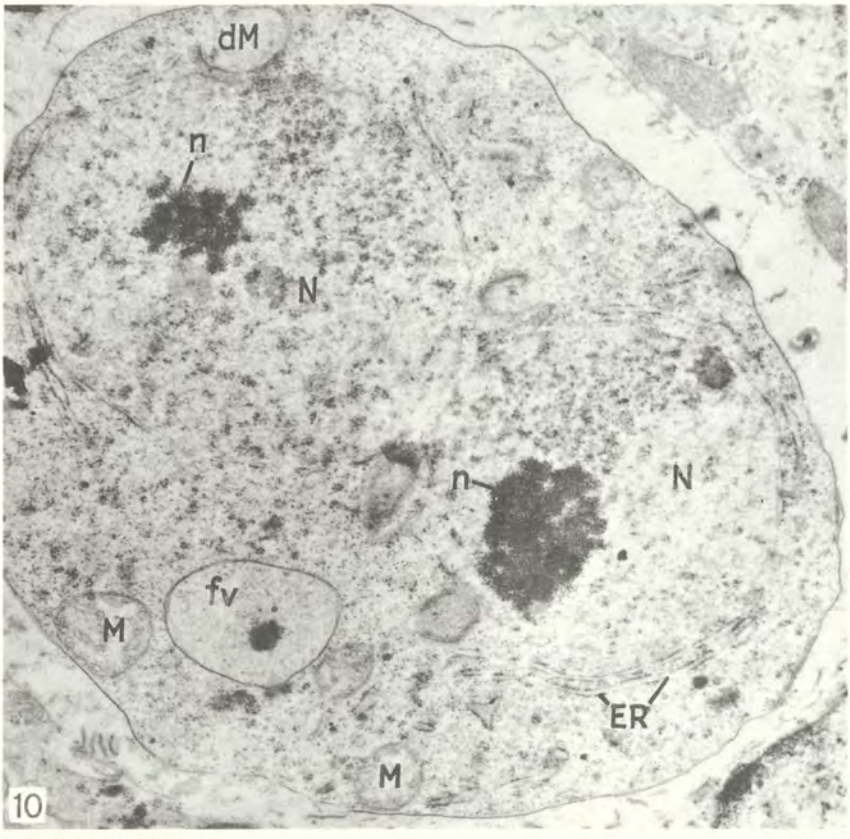

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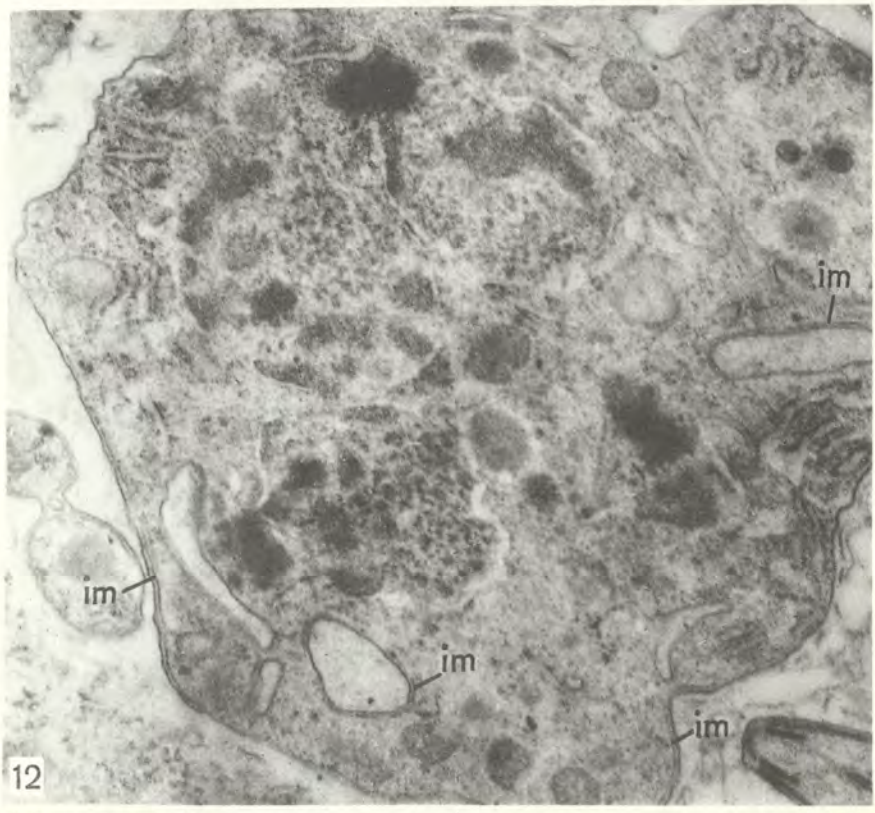

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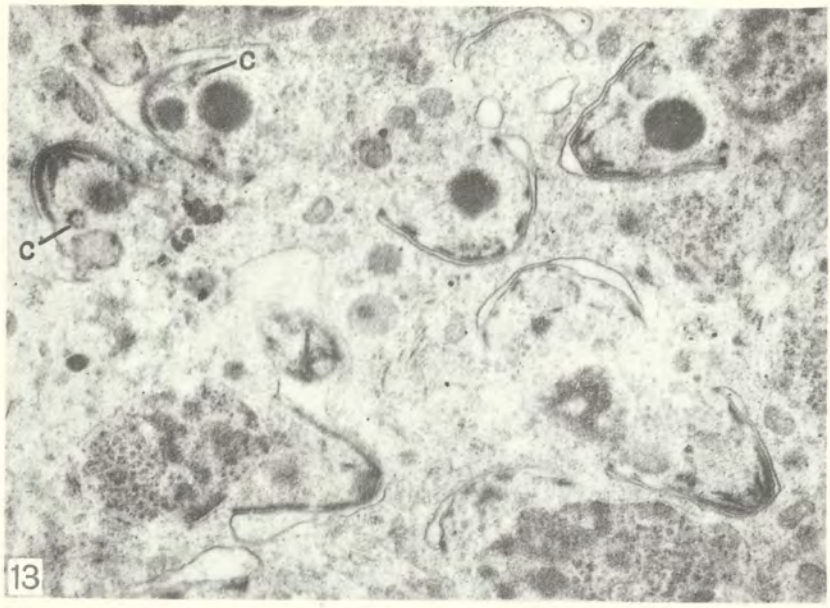

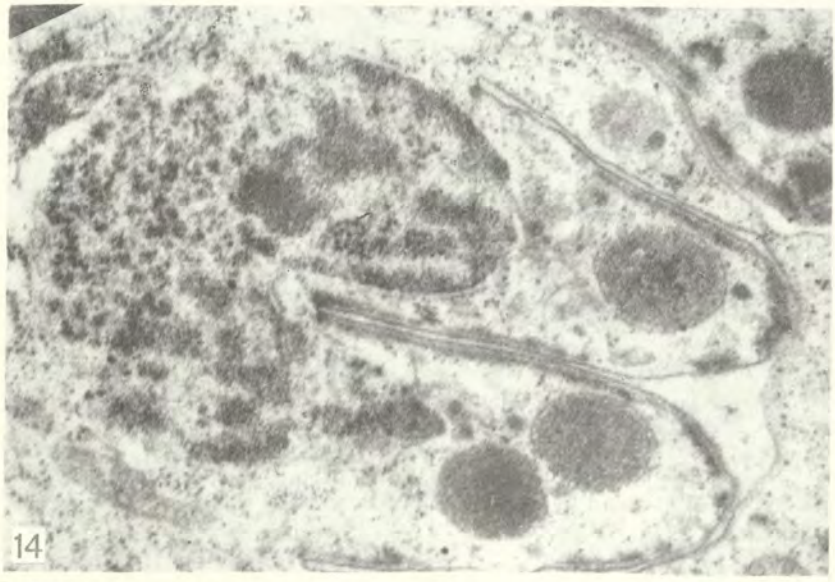

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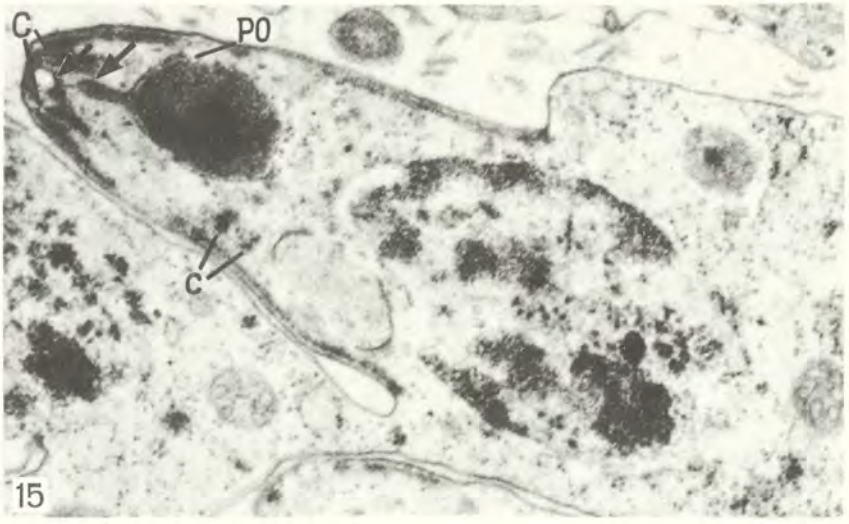

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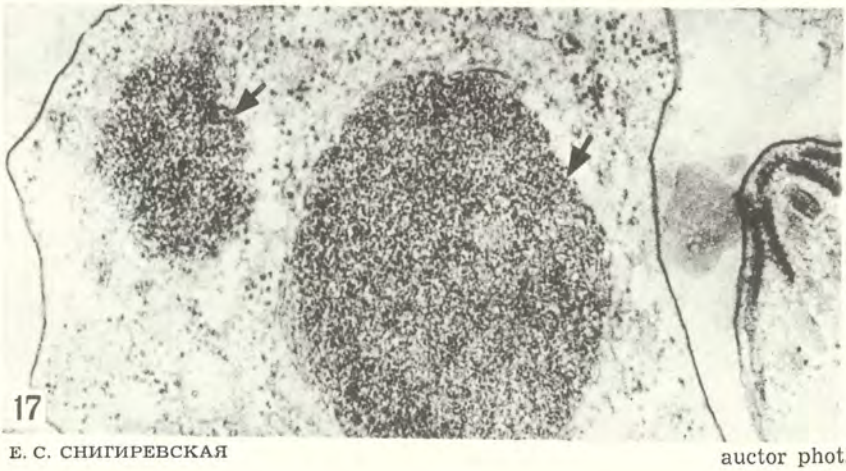
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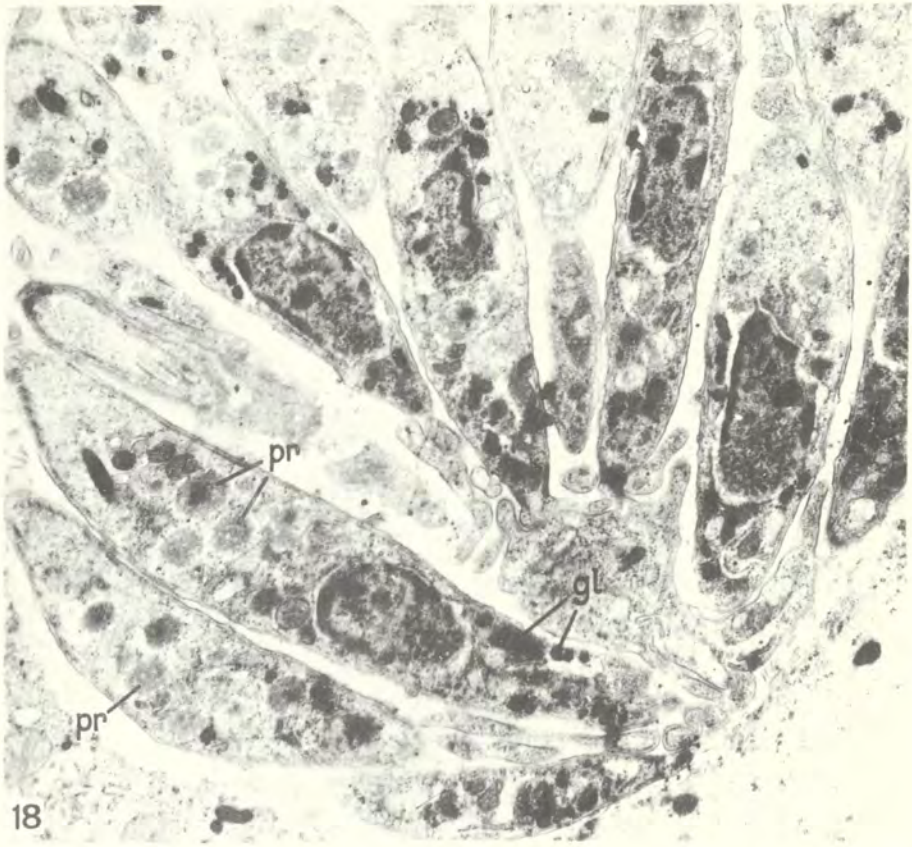

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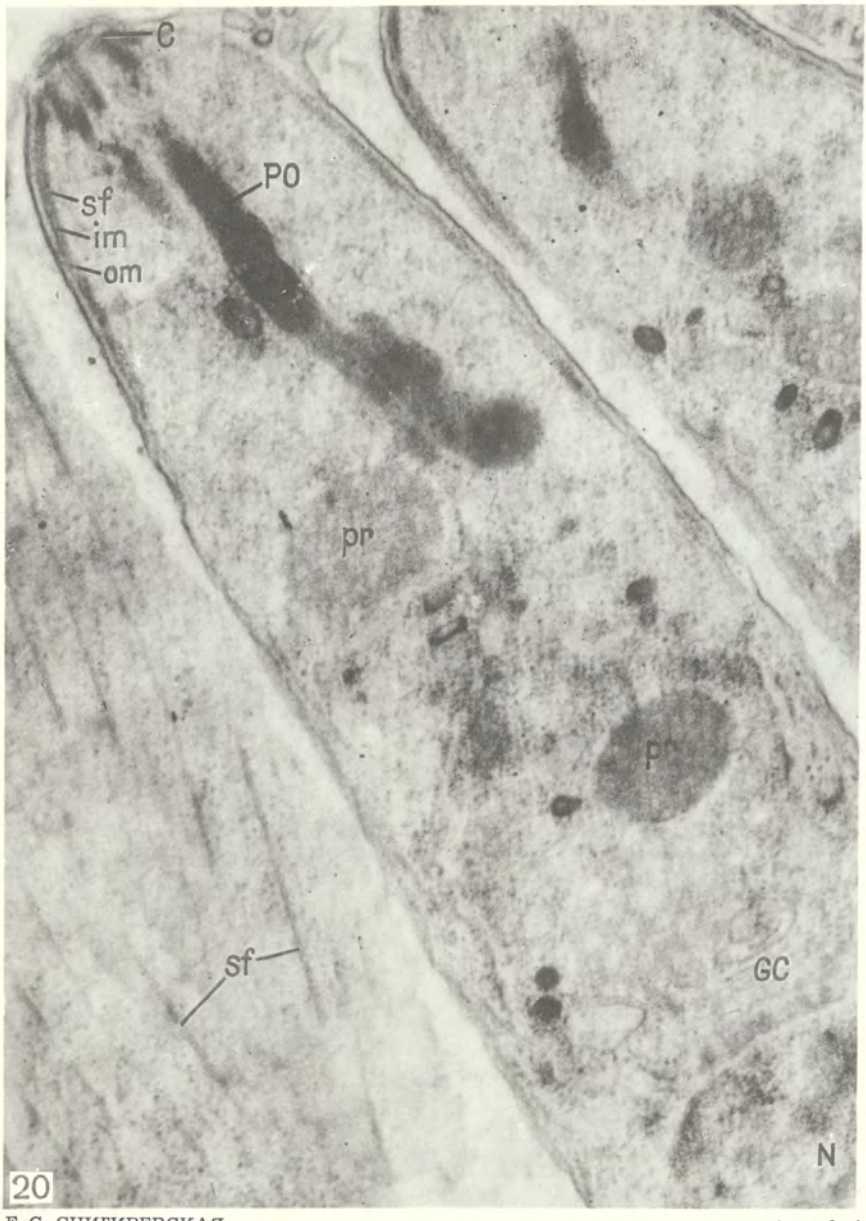

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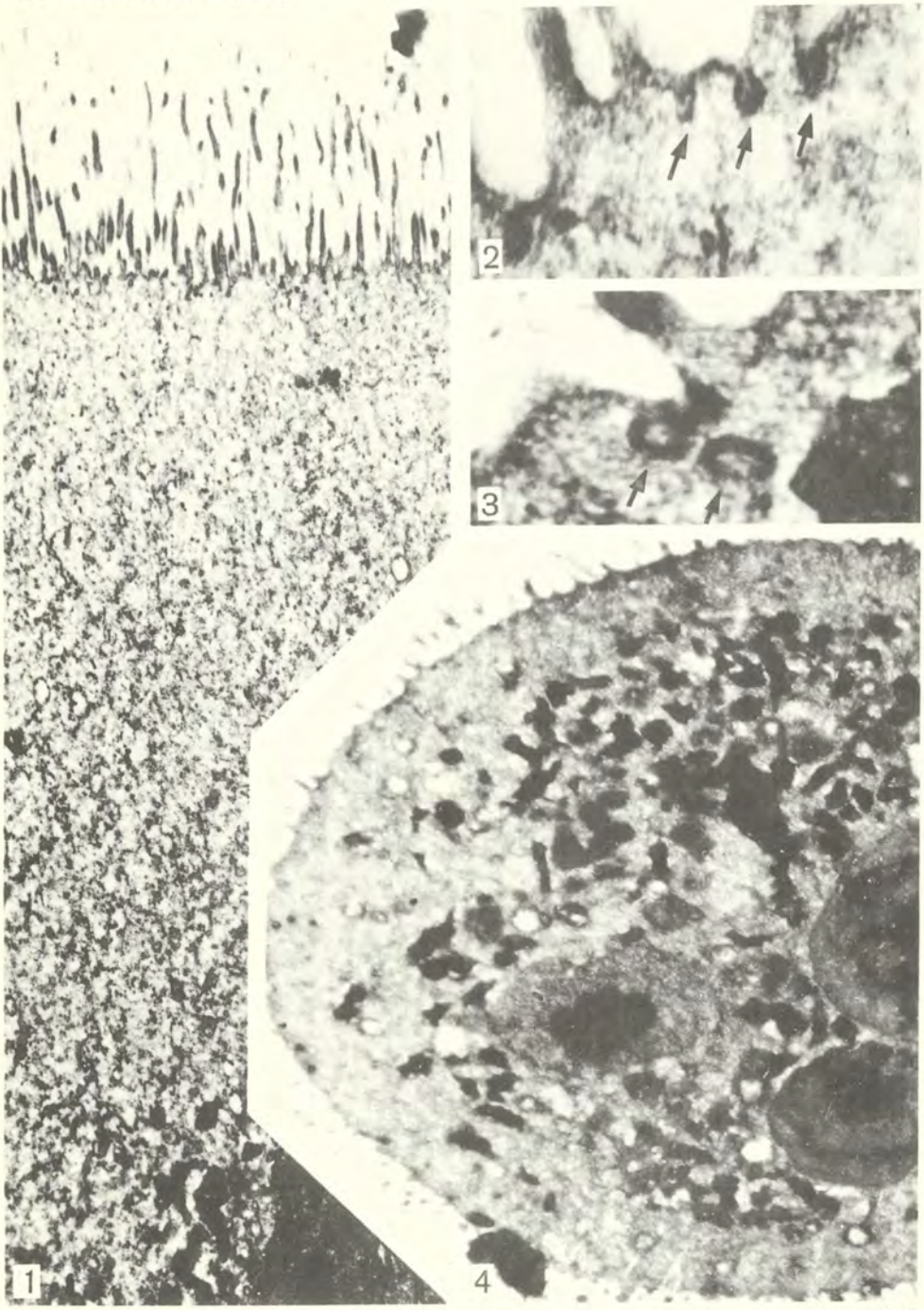

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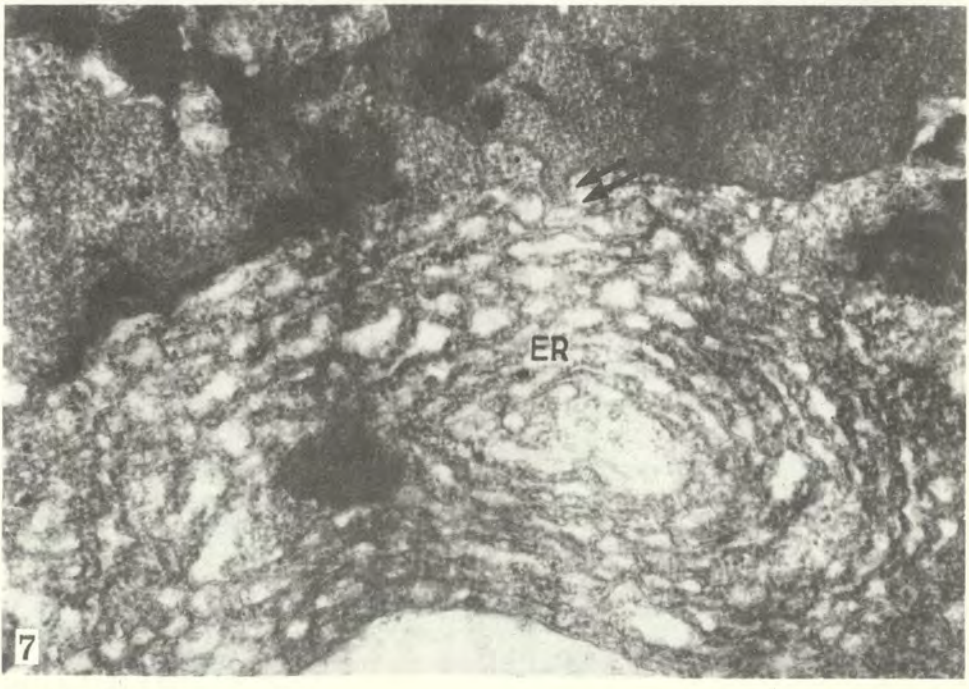

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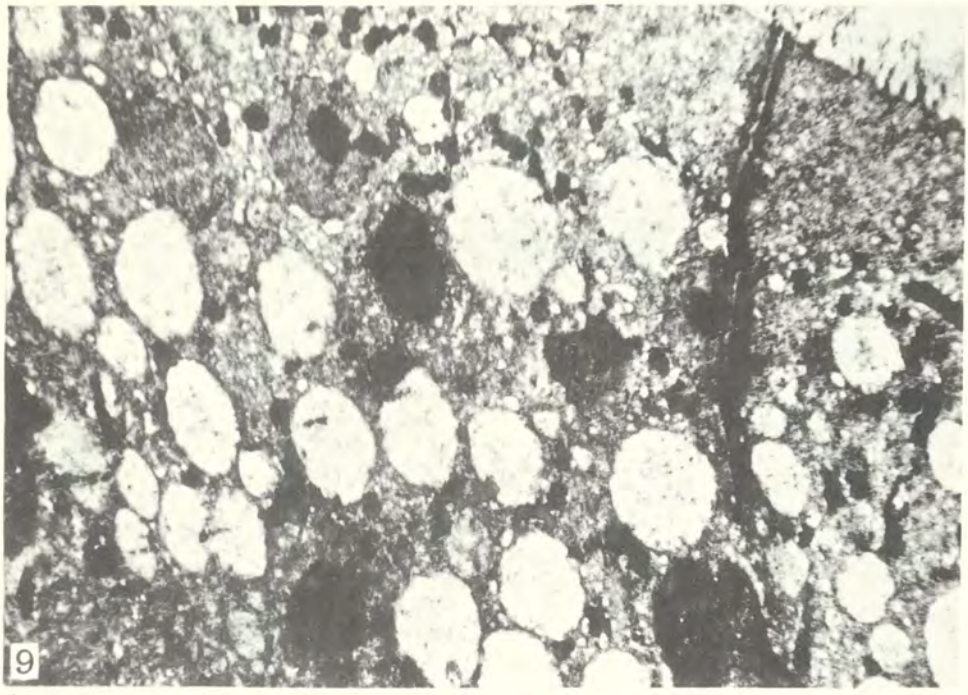

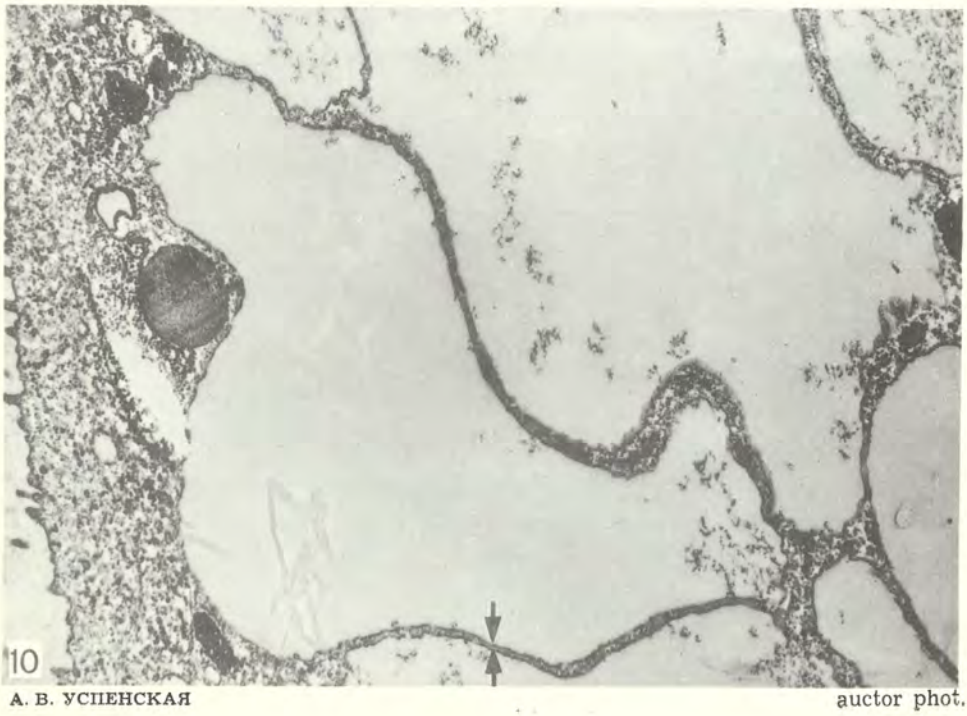

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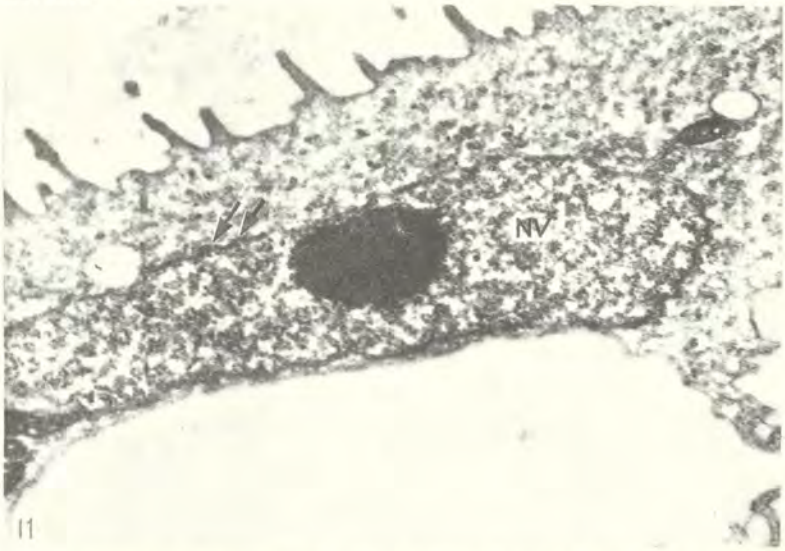

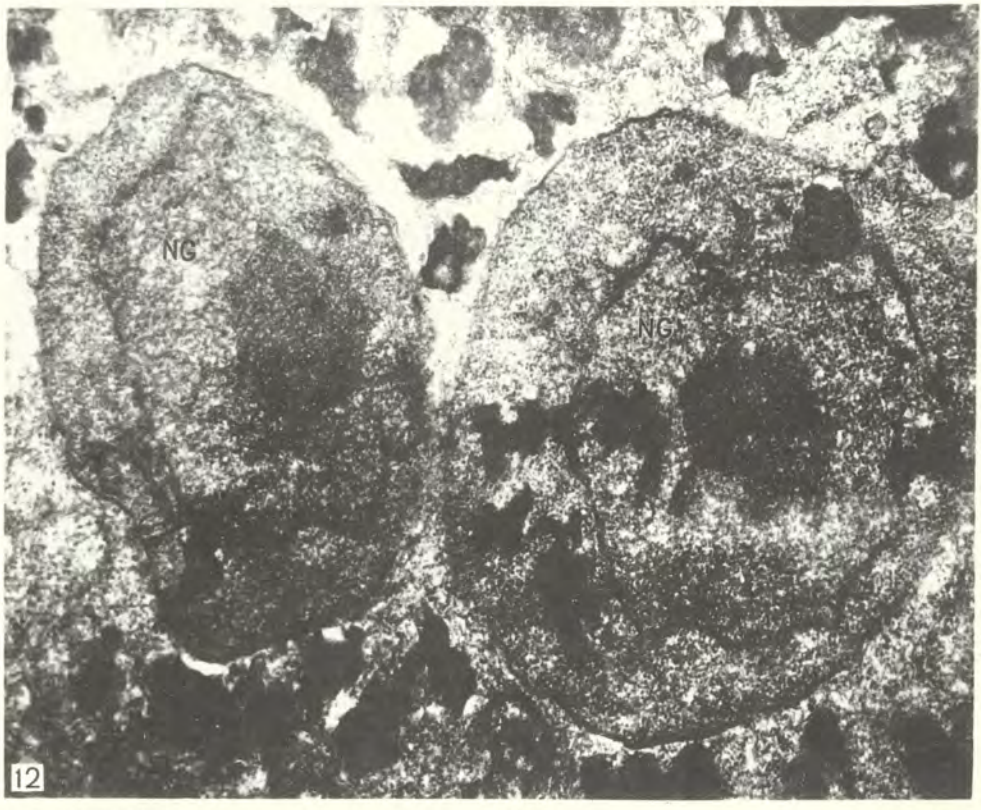

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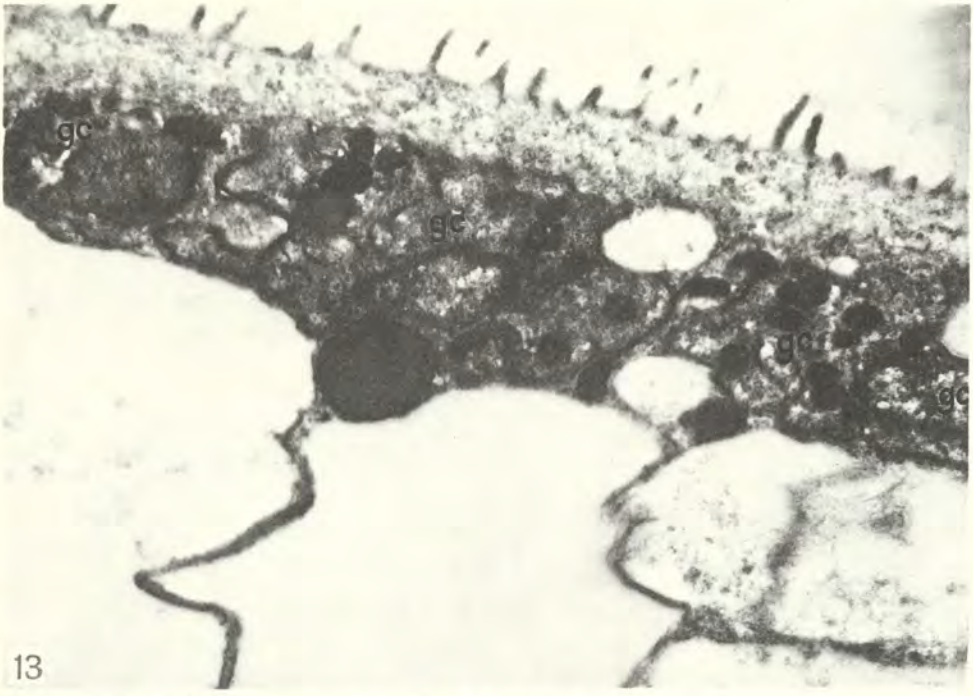

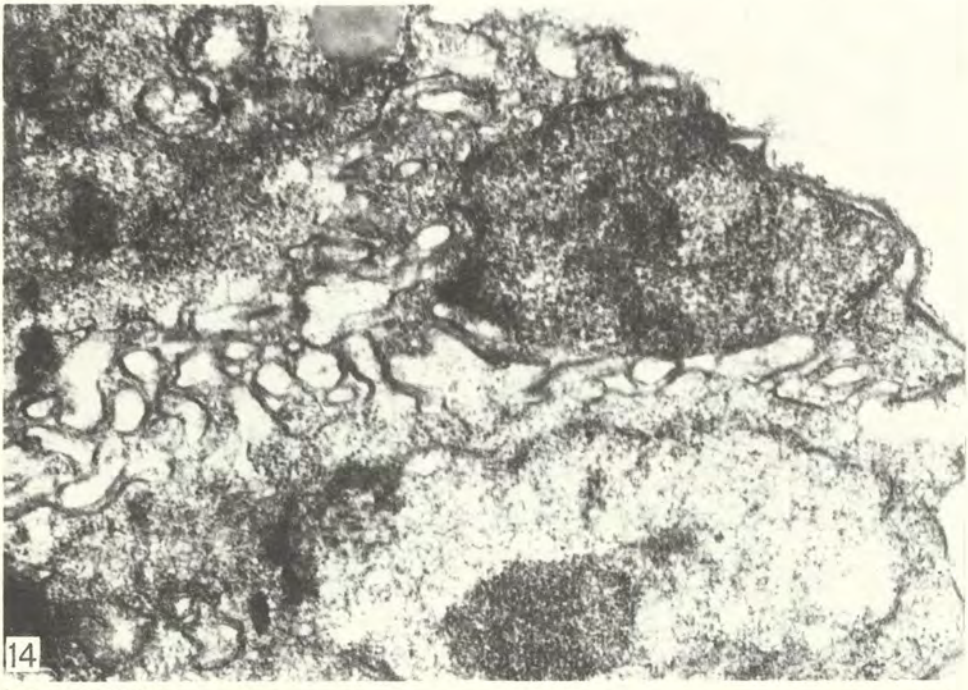

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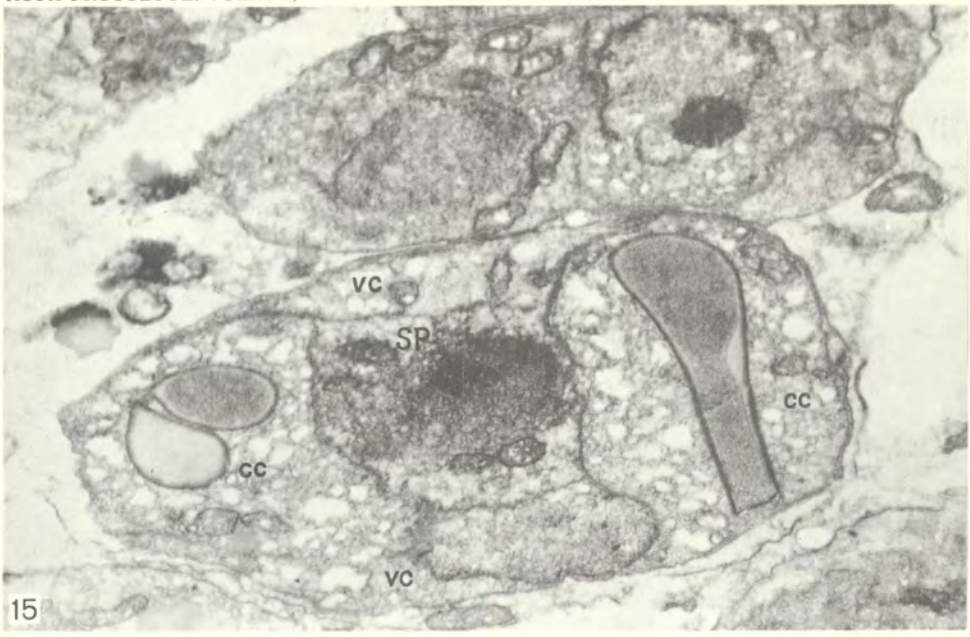

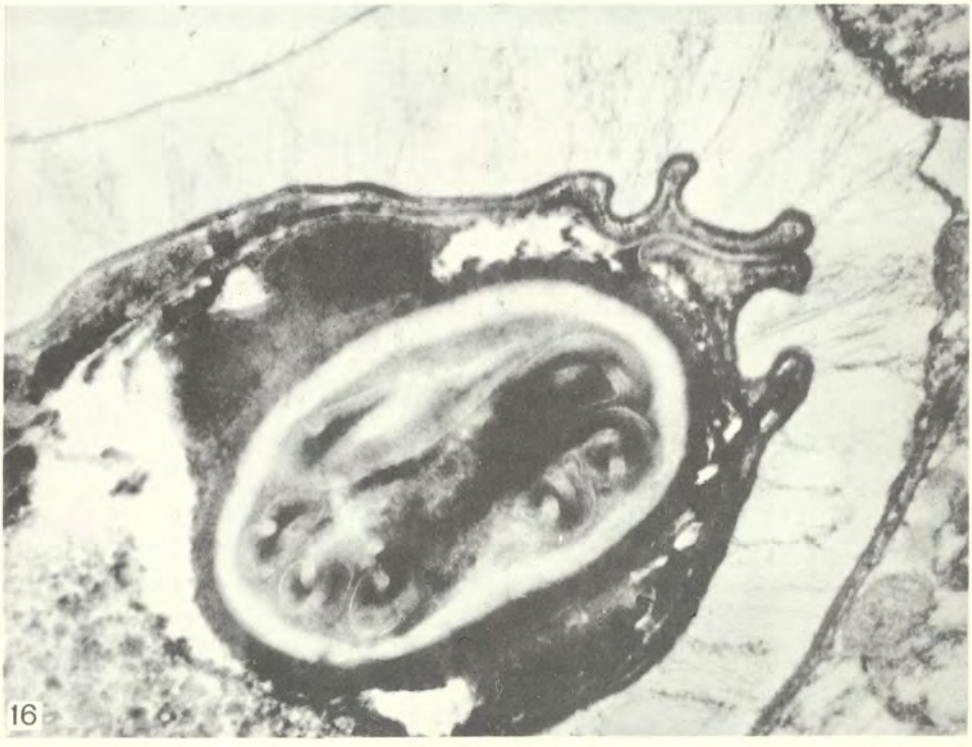

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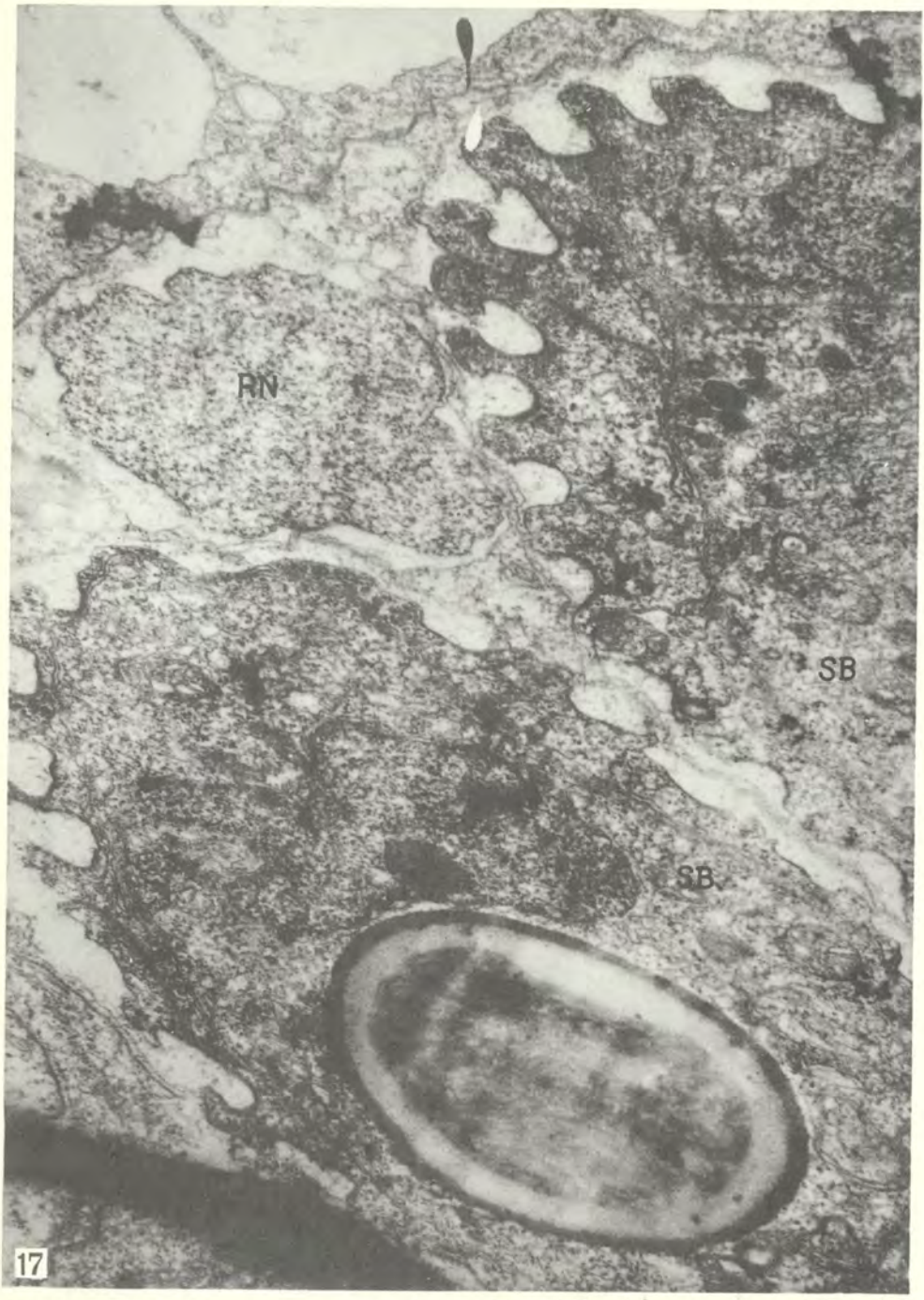

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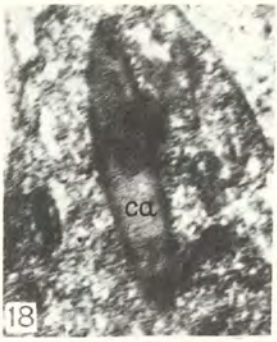

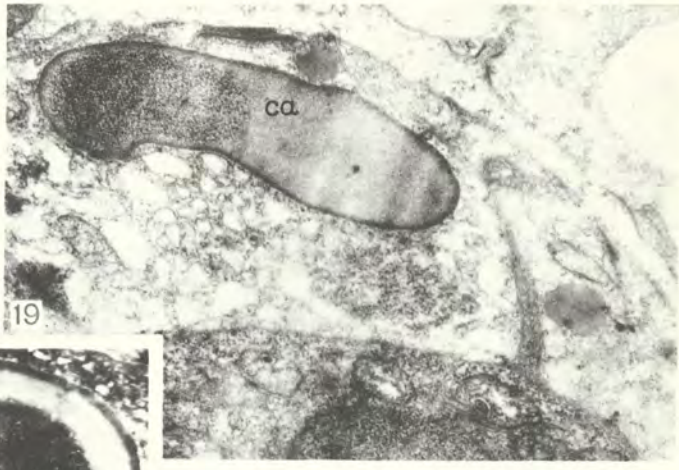

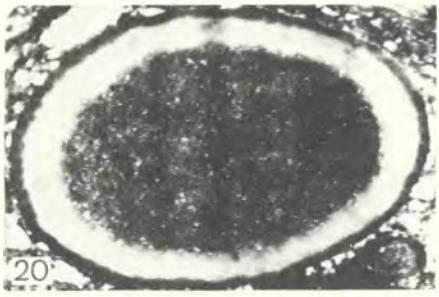

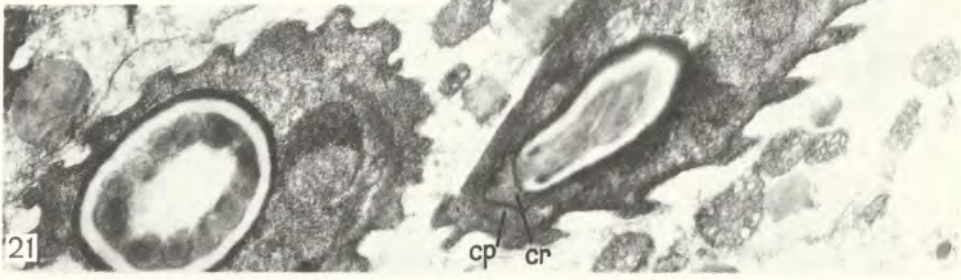

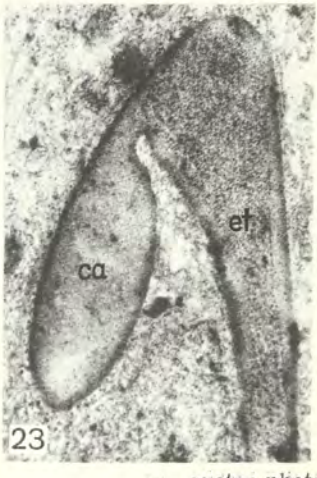

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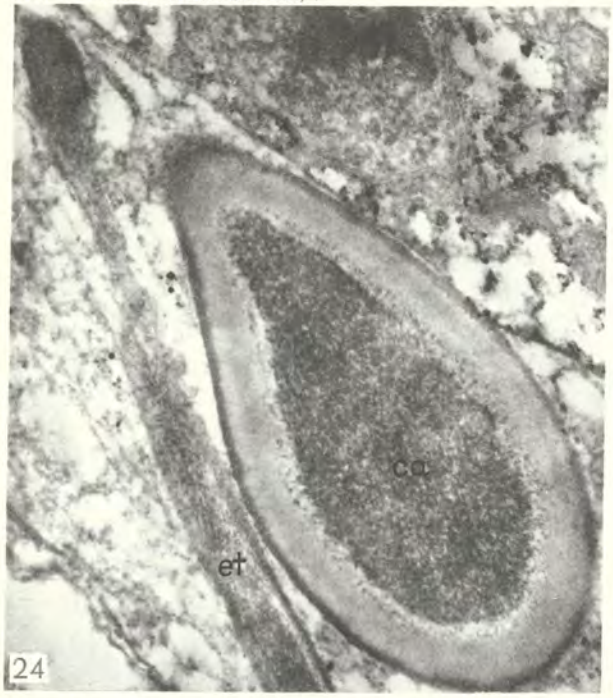

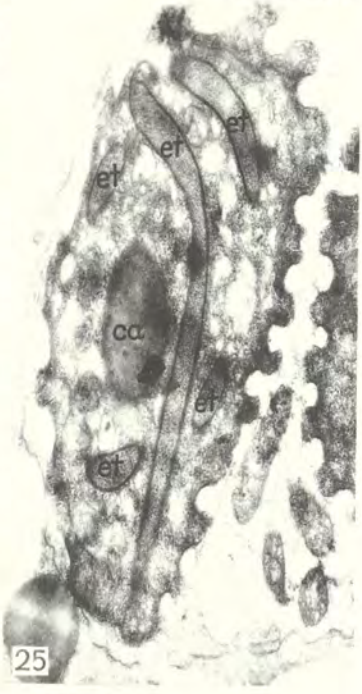

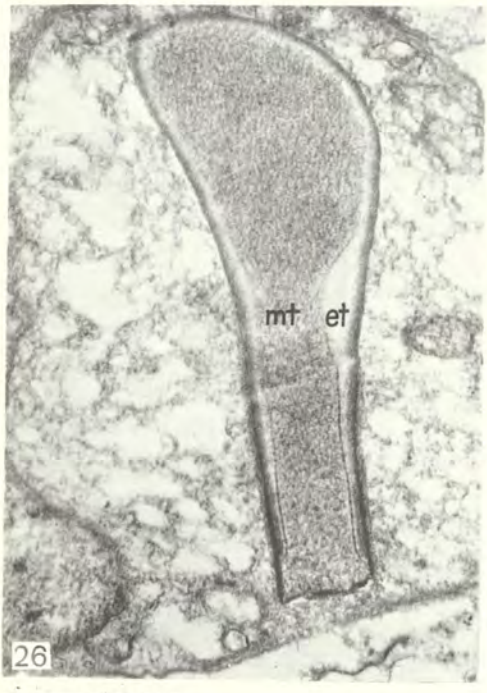

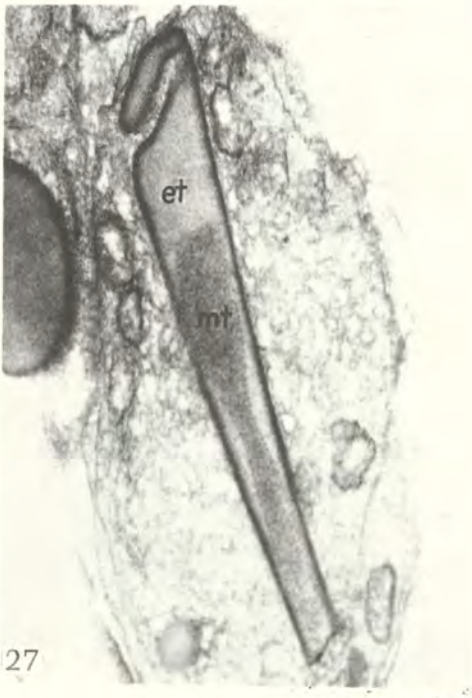

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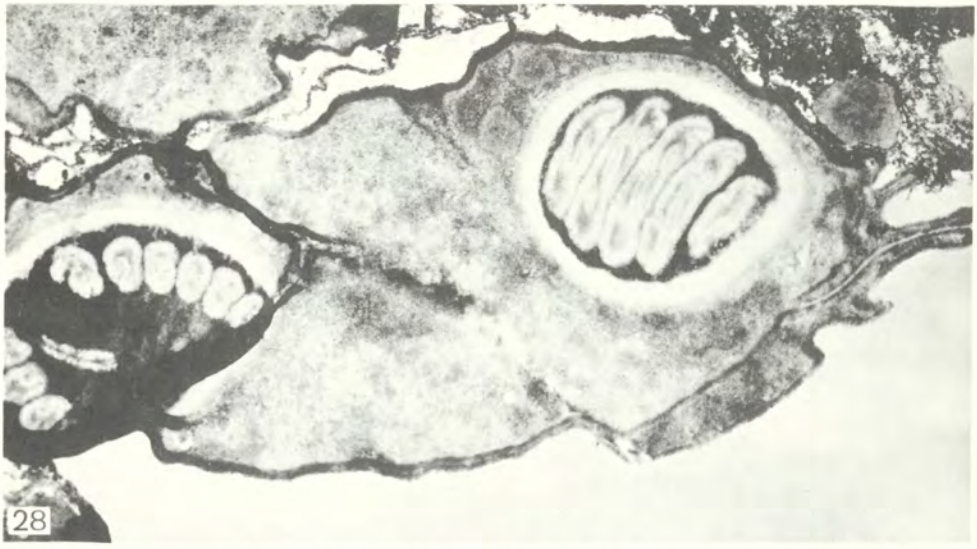

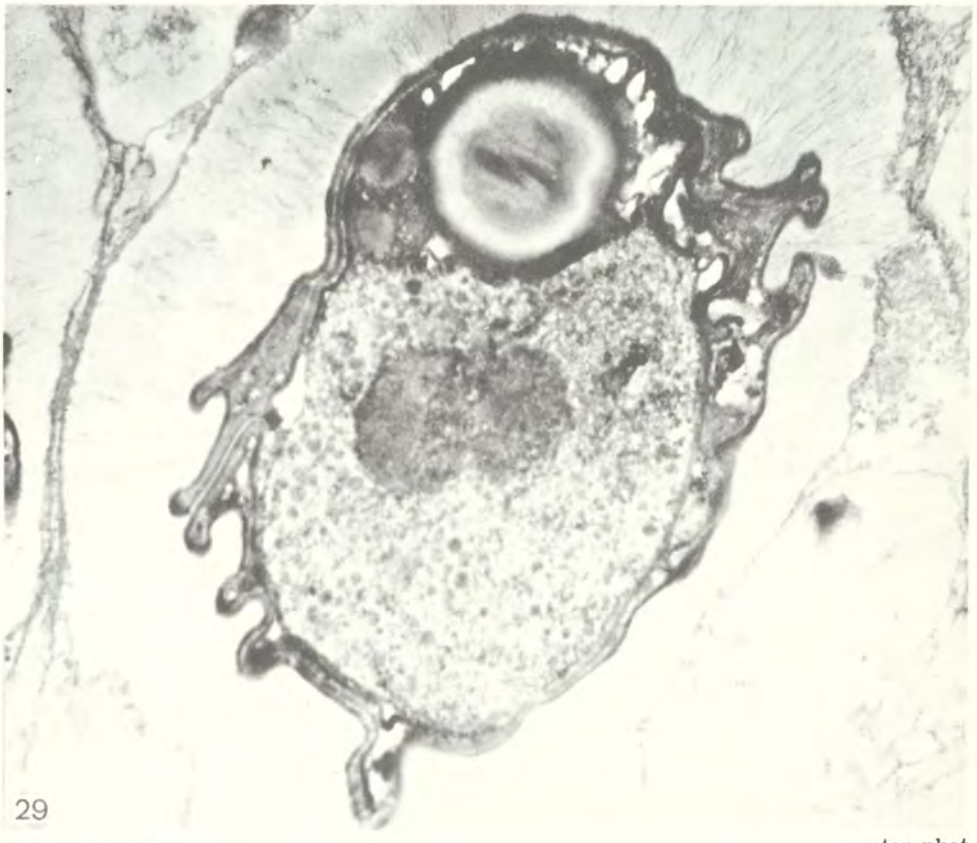

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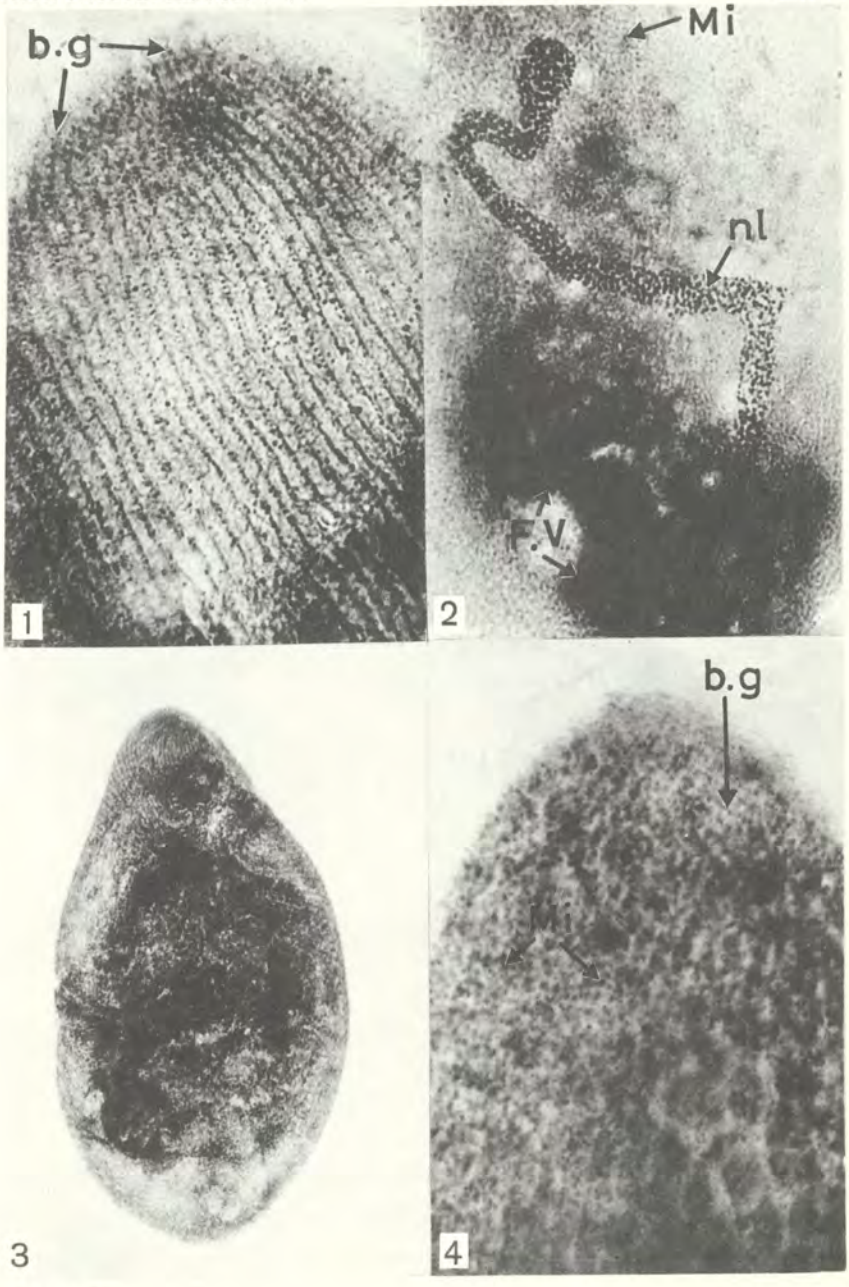

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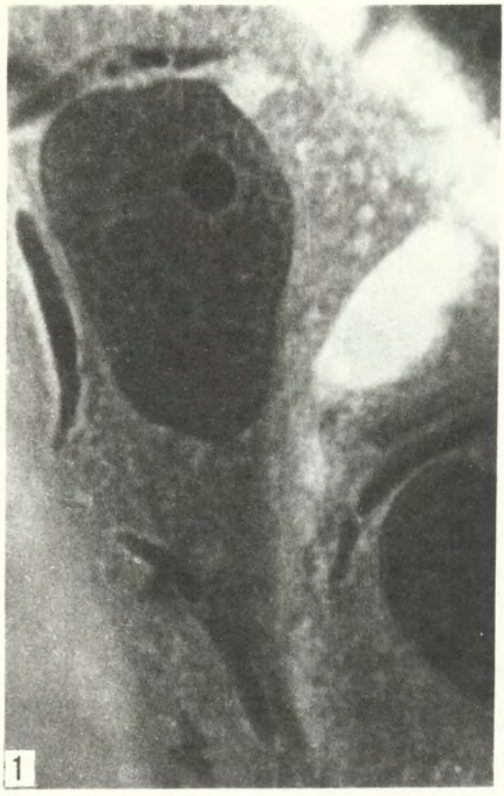
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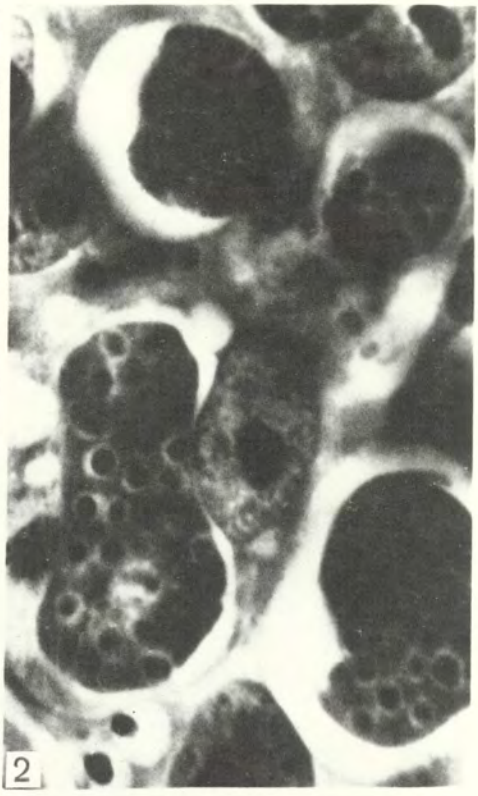
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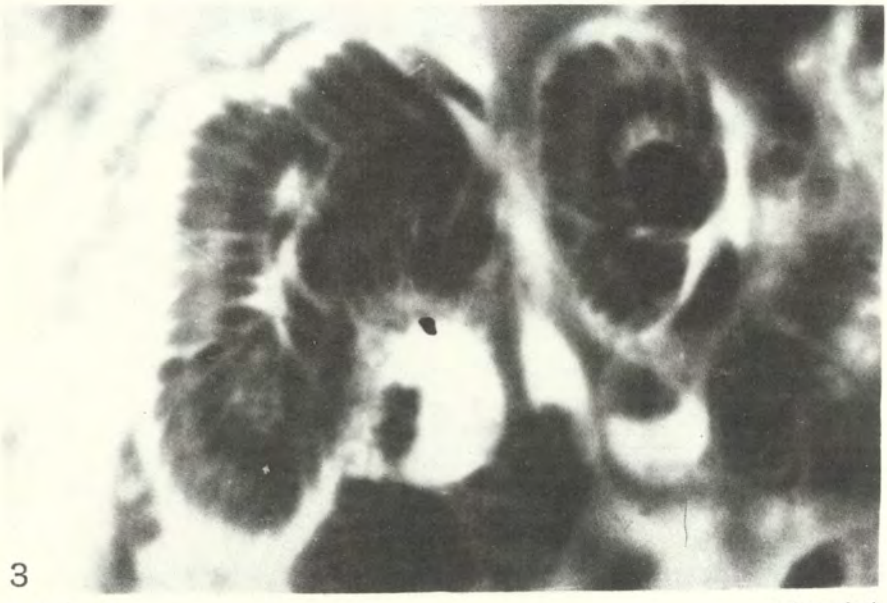
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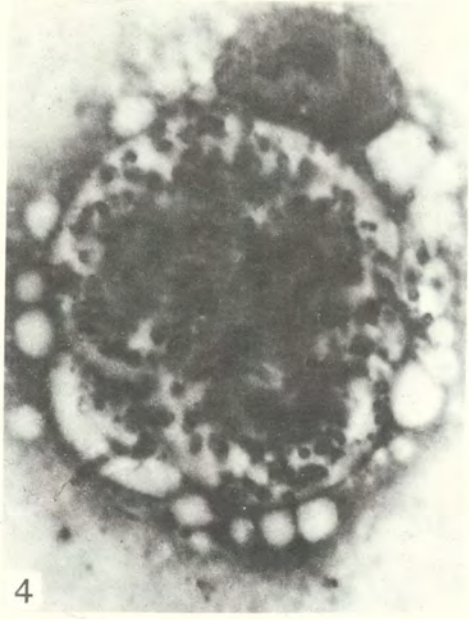

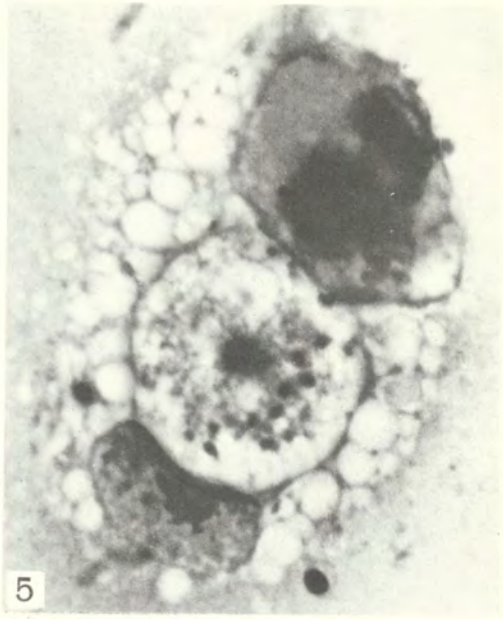

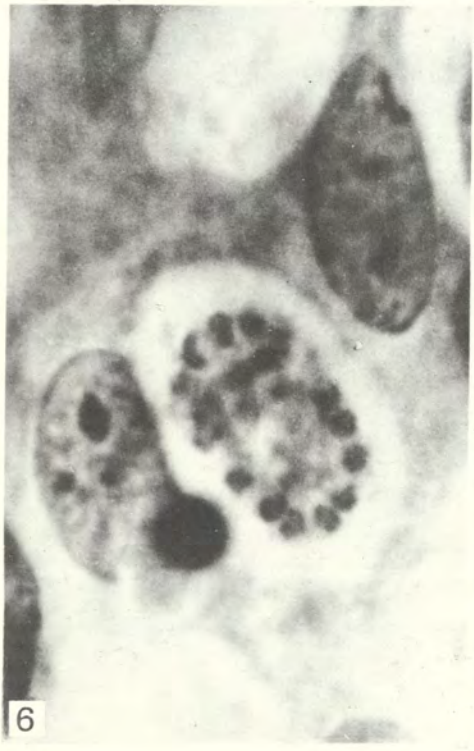

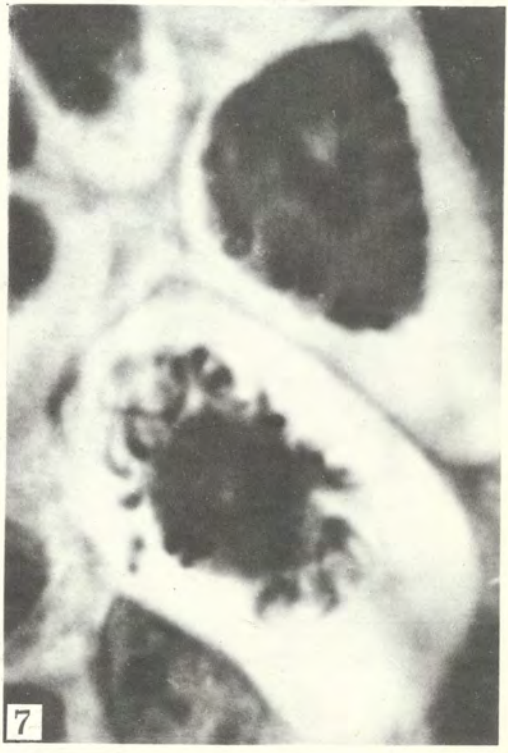

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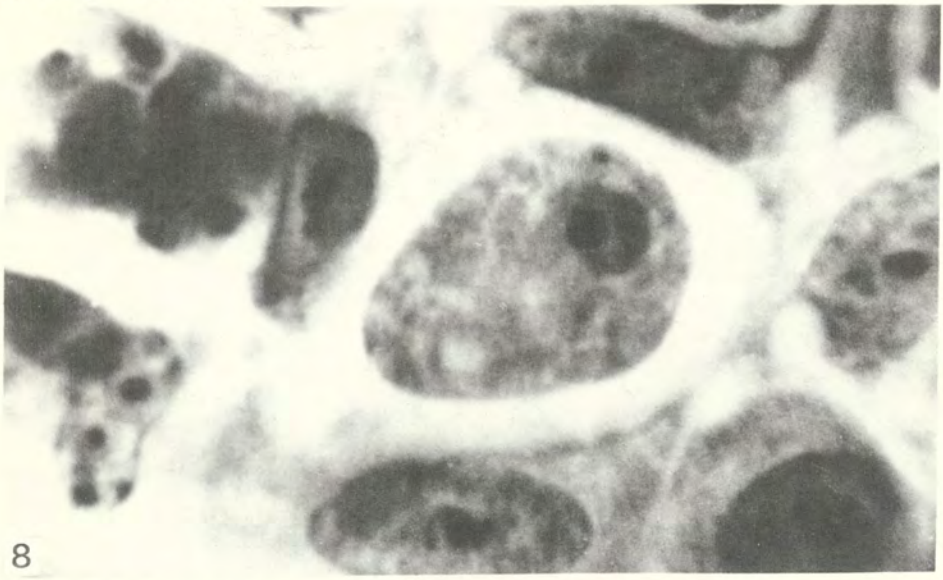

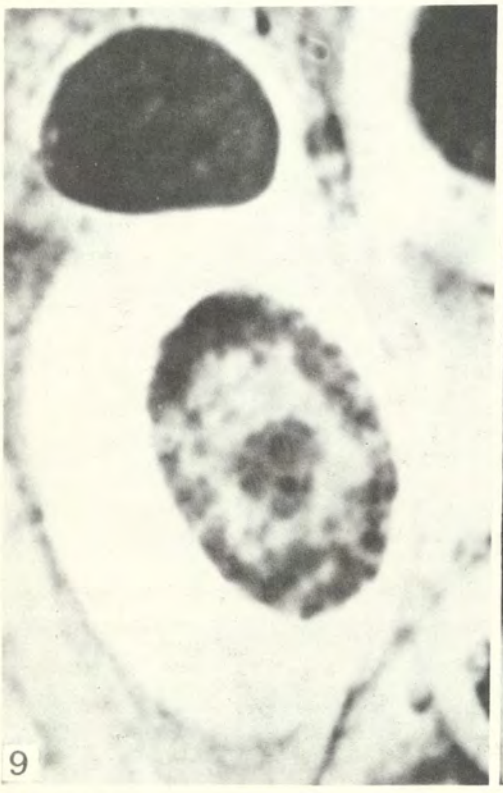

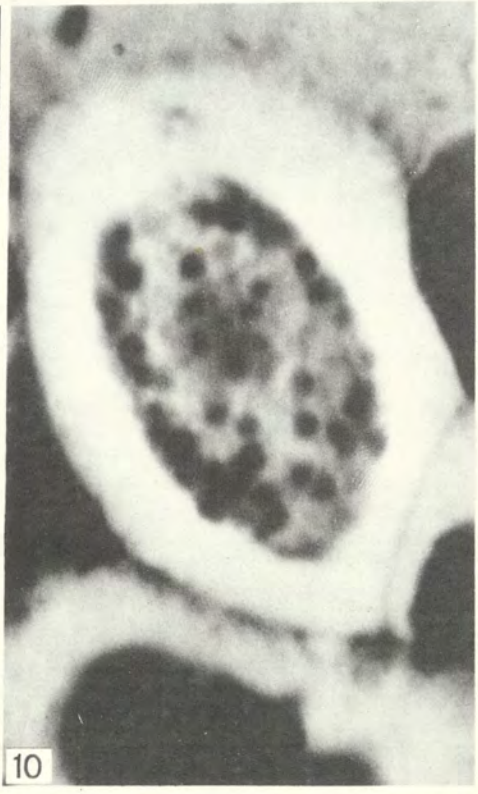

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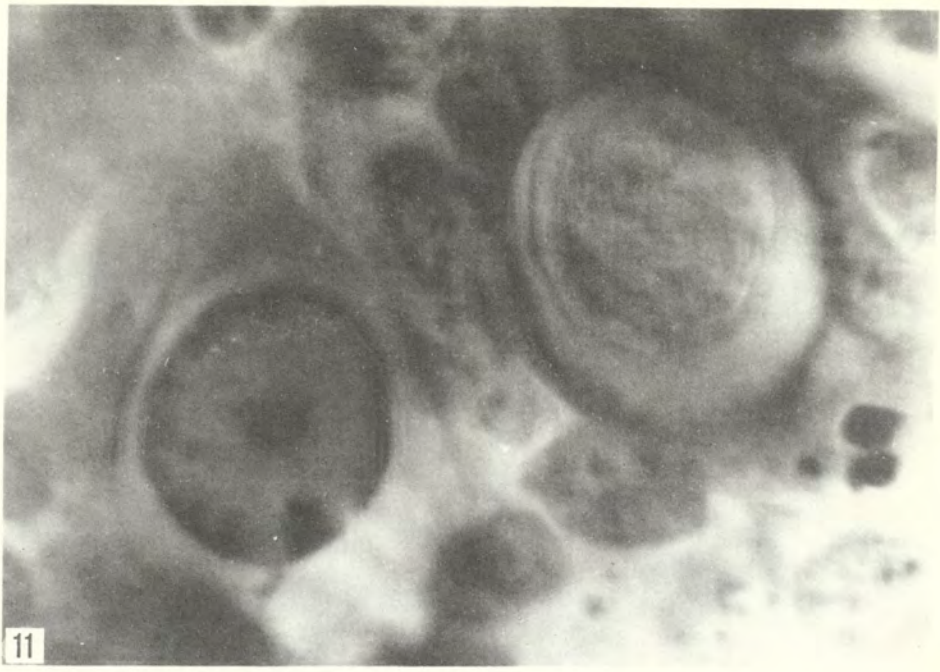

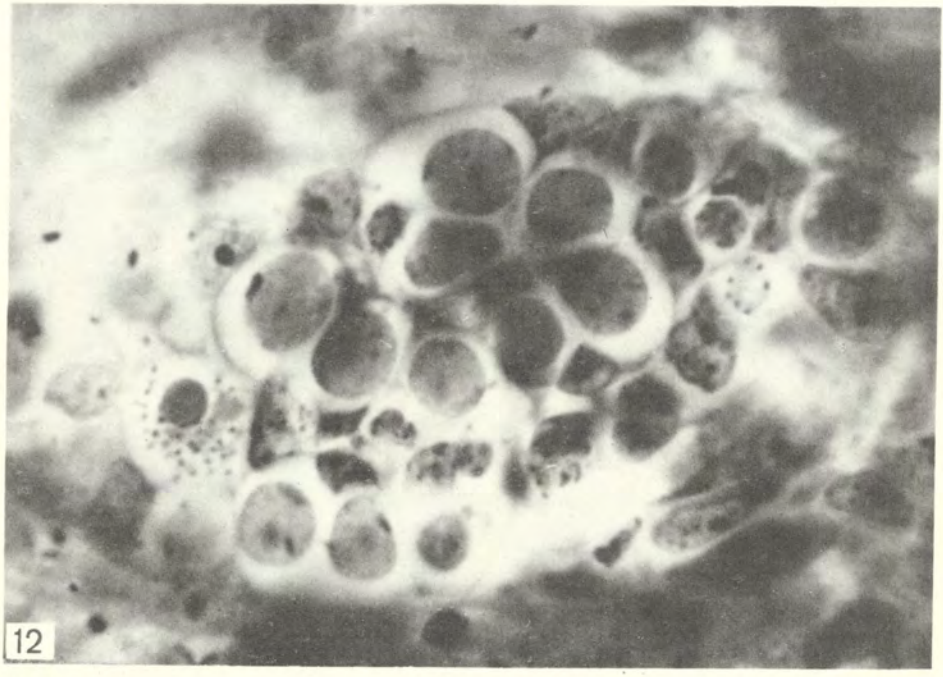

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