ELECTRON MICROSCOPE STUDY OF COLPODA FASTIGATA KAHL

By
F. Biczók
From the Zoological and Biological Institute of the University of Szeged (Hungary) and the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illions (U. S. A.).

The experiences referring to the submicroscopic structure and properties of the protoplasm have been completed by a number of data gained from the electron microscope studies of *Protozoa*. Some of the data point to the changes taking place in the protoplasm of certain *Protozoa* as a consequence of encystment (Barker & Deutsch, 1958, Deutsch & Zaman, 1959, Osada, 1959). Nevertheless only a restricted number of data refer to the far-reaching changes occuring within the plasm structure of *Protozoa* before, during and after encystment (Biczók, 1957, 1959). As the electron microscope research of the different phases of encystment leads to a more exact perception of the function and the dynamic changes of the plasm parts, I want to follow this method in laying morphological basis for my physiological studies. This paper wants to give some informations about the submicroscopic structure of *Colpoda fastigata* Kahl immediately after excystment.

Method

Colpoda fastigata Kahl cultivated in root extract was transferred into sterile garden soil and made encysted by the desiccation of the soil. In this form cysts have been available for years. The animals were activized from the cysts by means of root extract and made encysted again.

Before embedding the organisms being in culture were centrifuged. It did not aim only at the concentration of the substance, but the activization of the animals being weakly encysted as well. Fixation was achieved by means of 4 per cent formalin diluted with pH 7,4 acetatveronal buffer solution, and futher after a twofold washing 2 per cent osmiumtetroxid was used. The purpose of the prefixation was the getting rid of the great aggregation of the by-products and bacteria resulting of the encystment — which is unavoidable when fixed in osmium. The fixed subject after washing was dehydrated by means of 70-, 96- and 100 per cent alcohol in intervals of 30—30 minutes.

The embedding was made — after Roth — into 60 per cent n-butyl- and 40 per cent methyl-methacrylate. The substance was held in this solution, under 0° C, for 24 hours. Then the preparation treated with 0,5 per cent 2—4 dichlorobenzoil peroxid was polymerized in a thermostate, on 60° C.

Results and Discussion

As it is well-known, the metabolism of cysted animals leading a latent life is decreased to the minimum (Pigon, 1961). It may be supposed that this phenomenon is closely connected with the change of structure and inclusions

of the protoplasm. This supposition is based on several informations (BARKER & DEUTSCH, 1938, DEUTSCH & ZAMAN, 1959, OSADA, 1959). The question seems to have an interesting and new solution, with the examination of the electron microscope structure of the excysted *Protozoa.*

1. Pellicle: The excysted *Colpoda fastigata* is bordered by a densely compacted mass of osmiophil granules of 90—150 Å crowded together within the cytoplasm. The cytoplasmic part, which is cca 0,1 μ thick is sometimes divided into two layers. One may have the impression of a regeneration of the pellicle structure after excystment. It is supported by the fact that the excysted animal is in a strongly metabolic state for a certain time.

2. Endoplasmatic reticulum (Ergastoplasm): The tube-like pieces of the intraplasmic structure bordered by a twofold membrane (mentioned by PORTER, 1953, PALADE, 1956) may be well seen here and there. The lumens of 0,1—0,2 μ diameter of the tube refer to the fact that the reticulum is sometimes arranged in a cluster-formation (Fig. 1). (To a certain extent it remembers of the cross section of the thinly lengthened vacuolar elements of the GOLGI-apparatus). PALADE-granules are rearly to be seen on the ergastoplasmic membrane. Near the endoplasmatic reticulum there are some vesicles surrounded by membranes mentioned by certain authors (SEDAR and RUDZINSKA, 1956, ROTH, 1957, WOHLFARTH—BOTTERMANN, 1959) in connection with other *Protozoa.* It is striking that a number of vesicles are open towards the cytoplasm, especially those lying closely under the pellicle-like part Fig. 2). It cannot be decided as certain whether these are formations connected pynocytose on the one hand and whether they are related to the ergastoplasm on the other.

3. Mitochondria: They are oval bodies sometimes having a size of nerly 1 μ, which are bordered by a relatively thin (140 Å) twofold membrane. As in the case of most ciliates, also mitochondria are dissected by elements of tubular structure the relation of which to the inner membrane is obscure. Similarly to the tubular parts, there are a lot of scattered small granules of lower electron density in their environment (Fig. 3). Here I point to the interesting, problematic formations of the cytoplasm, viz. the inclusions, appearing in a very great number either in a strongly osmiofil, lengthened or in an almost rounded form. On the basis of their dye a number of them seems to be inhomogenious without having a special structure. Anyhow, in certain forms the tubuli appear thinner and lighter than the inner substance. It may be supposed that these inclusions are mitochondria in a state of development. Strikingly, between these inclusional forms of the size of 1—2 μ and the mitochondria certain transitional forms are to be seen. It is noteworthy that DEUTSCH and SWANN (1959) discovered a relation between mitochondria and the light, empty inclusions being similar to the former as to form and size.

4. Ground-substance: The submicroscopic point of the cytoplasm not yet discussed will be here summarized. As it is wellknown, the parts of the size of 500—3000 Å are called microsomes (after CLAUDE, 1943), the smaller ones are referred to as PALADE-granules (1955). Because of their high RNA content they are of great importance in the life of the plasm, especially in the protein-synthesis. Here I want to deal primarily with the latters because of their more frequent occurence. Their size is 70—120 μ. They are the most osmiofilical among the several parts of the cytoplasm. They seem to be analogous with the small granules appearing in the cytoplasm of birds and mammals (PALADE,

1955), but in the above mentioned part they are not to be found in inclusions being without a structure. They are scattered in the whole cytoplasm densely and almost proportionally. It is striking that they are sometimes arranged in the form of a circle, an ellipse or in an undulating line. Besides the granules mentioned above dense and empty bodies of a microsomal size („vesicular microsome") occur only in a very few number.

5. Cyst-wall: *Colpoda fastigata* is one of the best encysting cilial *Protozoa.* Sometimes in a short time, in 1—2 days a cystwall with a crumpled or smooth surface appears around the animals and may be as thick as 1—2 μ. This transparent wall is much more complex than that of the cysts *Entamoeba* (DEUTSCH and ZAMAN, 1959, OSADA, 1959). The cyst-wall of the *Colpoda fastigata* consists of three well distinguishable layers: of a dense outer one of 1600 A visible on the microphotograph, of a less dense middle one of 5200 Å and of an inner one of 1500 Å constructed of four membranes. The outer and middle layers are connected by a light part being cca 500 Å thick. The inner layer and a considerable part of the middle one is broken by a narrow breach. It may be supposed that the breaches occur in a greater number at the admission of certain materials especially of water being necessary for excystment, and may have an important role.

Summary

The characteristics of the submicroscopic structure of *Colpoda fastigata* KAHL examined immediately after excystment are as follows:

1. The cytoplasm is bordered outwards by a lot of small granules being of great electron density.

2. The parts which may be classified into the realm of the PALADE-granules occur in a great number and with an almost proportional density within the cytoplasm as well, where they are arranged side by side even in regular forms. It seems that these granules are of great importance in the reorganization of certain cytoplasmatic constituents, after excystment.

3. The coming about of mitochondrion within the cytoplasm seems to be observable through the inclusions showing a primitive structure or being without any structure.

4. The cyst-wall is transparent, consisting of three layers. The outer and middle layers are connected by a thin part without a structure. The two inner layers are broken by a narrow breach.

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Bibliography

(1) BICZÓK, F.: Physiology of the cystment examined on the Colpoda fastigata (Acta Univers. Szegediensis, Acta Biol. 5, 109, 1957).
(2) BICZÓK, F.: A contractilis fehérjék szerepe az osmoregulatióban (MTA. Biol. Csop. Közl. 3, 183, 1959).

(3) CHEISSIN, L. M.—MOSSEVITCH, T. V.: Electron microscope study of Colpidium colpoda (First Intern. Conf. of Protozool. Abstr. Praha, p. 18, 1961).
(4) CLAUDE, A.: The constitucion of protoplasm (Sci. 97, 451, 1943).
(5) DEUTSCH, K.—SWANN, M.: An electron microscope study of a small free living Amoeba Hartmanella astromyxis) (Quart. J. of Microsc. Sci. 100, 13, 1959).
(6) DEUTSCH, K.—ZAMAN, V.: An electron microscopic Study of Entamoeba invadens Rod (Exper. Cell. Res. 17, 310, 1959).
(7) OSADA, M.: Electron-Microscopic Studies on Protozoa. I. Fine structure of Entamoeba histolitica (Keio J. Med. 8, 99, 1959).
(8) PALADE, G. E.: A small particulate component of the cytoplasm (J. Biophys. Biochem. Cytol. 1, 59, 1955).
(9) PALADE, G. E.: The endoplasmic reticulum (J. Biophys. Biochem. Cytol. Suppl. 2, 83, 1956).
(10) PAPPAS, G. D.: Electron microscope studies on Amoeba (Ann. of the New-York Acad. of Sci. 78, 448, 1959).
(11) PIGON, A.: Observations on the physiology of encystment of a Ciliate (First Intern. Conf. of Protozool. Abstr. Praha, p. 166, 1961).
(12) PORTER, K. R.: The fine structure of a submicroscopic basophilic component of cytoplasm (J. Exper. Med. 97, 727, 1953).
(13) ROTH, L. E.: An electron-microscope study of the cytology of the protozoan Peranema Trichophorum (The J. of Protozool. 6, 107, 1959).
(14) ROTH, L. E.: An electron-microscope study of the protozoan Euplotes patella (J. Biophys. Biochem, Cytol. 25, 985, 1957).
(15) SEDAR, A. V.—RUDZINSKA, M. A.: Mitochondria of Protozoa (J. Biophys. Biochem. Cytol. Suppl. 4, 331, 1956).
(16) WOHLFARTH—BOTTERMANN, K. E.: Die elektronmikroskopische Untersuchung cytoplasmatischer Structuren (Verhandl. der Deutsch. Zool. Geselsch. Münster—Westf. 45, 303, 1959).

Plate I

Section of *Colpoda fastigata* showing mitochondria (mi), mitochondrion-like bodies (mib), larger (Gs) and numerous small dense granules (gs_1) scattered freely or ordered (gs_2) in the cytoplasm, elements of the endoplasmic reticulum (er), vesicles (v) and membranes (me). (Fig. 1. ×11,000, Fig. 2. ×34,000)

Plate II

A section demonstrating the tubular structure of mitochondria (mi), the endoplasmic reticulum (er) and membranes of cytoplasm (me). Fig. 3. ×54,000, Fig. 4. ×34,000, Fig. 5. The wall of the cyst. ×24,000)

Plate I.

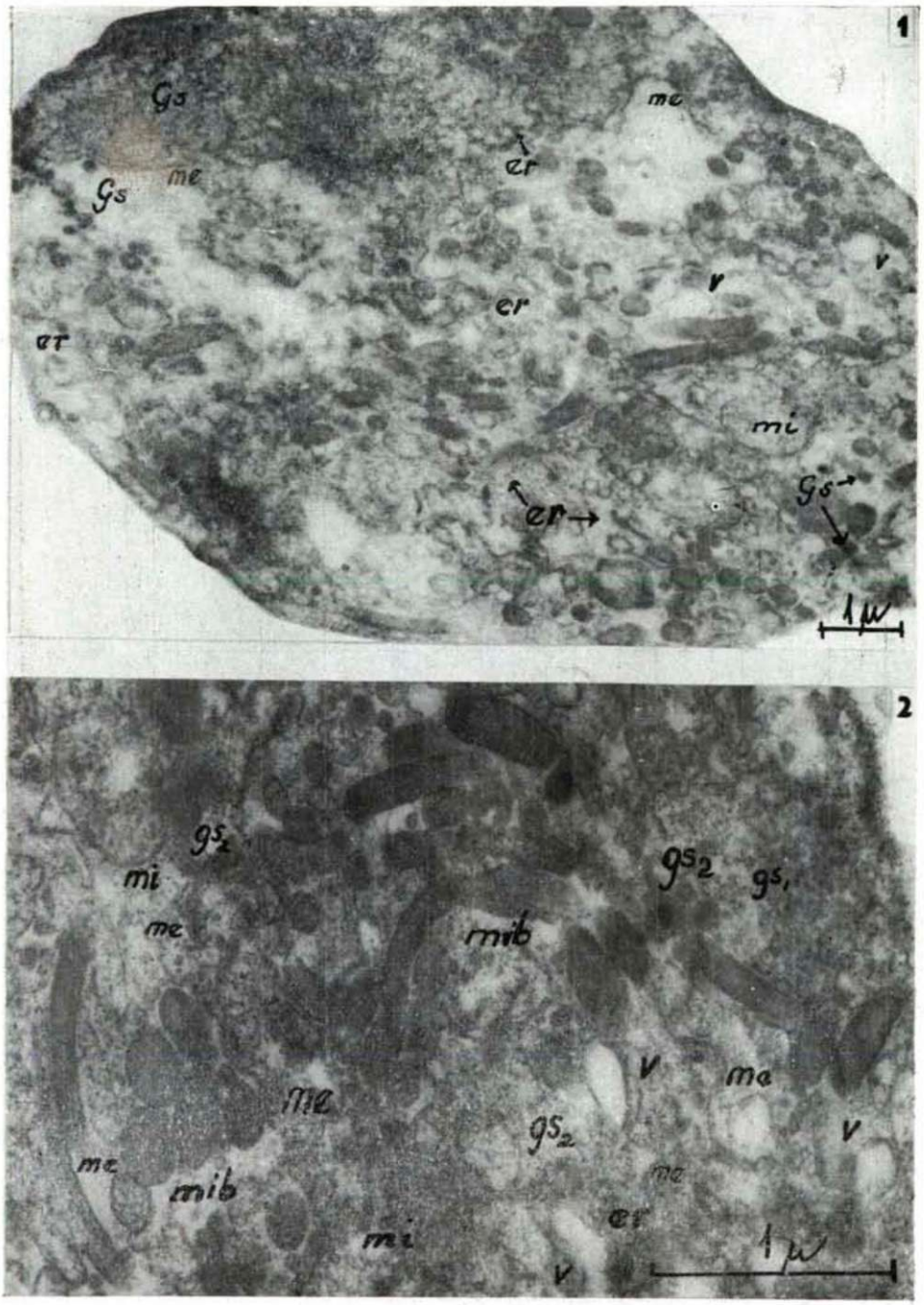
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Plate II.