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Ultrastructural Features of *Caudospora simulii* Weiser (Protozoa, Microsporidia)

J. VÁVRA

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and Laboratory of Parasitology, Faculty of Medicine, Rennes

Abstract The fine structure of some life cycle stages of the microsporidian *Caudospora simulii* is described. The plasmodium is limited by a single membrane which is bordered by a layer of small vesicles or flat cisternae. The cytoplasm of the plasmodium contains various vesicles, several cisternae of endoplasmic reticulum, zones of a primitive Golgi apparatus and nuclei of normal appearance. Neither plasmodia nor other life cycle stages of *Caudospora* contain mitochondria. Sporoblasts are covered with a double membrane and their cytoplasmic content is polarized by a couple of nuclei shifted to one pole of the cell. The single polar filament of the *Caudospora* consists of the same layers as the polar filaments of other microsporidia. The filament is anchored to the spore shell by a simple swelling of its anterior part which later changes into a complicated anchoring disc. The cauda develops as a hollow projection of the outer layer of the spore membrane. Of a similar origin are the two long filamentous projections situated at the anterior part of the spore.

The spore shell consists of two main layers of different appearance and chemical composition and originating at different times. For these layers, the names exo- and endospore are proposed. The microsporidian suborder Dicnidea should be abolished as both its representatives (*Caudospora* and *Telomyxa*) have only a single polar filament in their spores.

Recently the ultrastructure of Microsporidia has been discussed in a number of papers, which were mostly dealing with mature spores (e.g. WEISER 1959; KUDO and DANIELS 1963; HUGER 1960; LOM and VÁVRA 1963; VIVIER 1965; SCHOLTYSECK and DANEEL 1962; DE PUYTORAC 1961, 1963). Others again referred also to the fine structure of various life cycle stages (e.g. VÁVRA 1965; VIVIER 1966; DE PUYTORAC 1962; LOM and CORLISS 1967). Until the present about 18 microsporidians belonging to 8 genera have been studied in electron microscopy. In comparison with the number of microsporidia estimated at roughly 300 species of 17 genera, the necessity of further electron-microscopical investigation of these parasitic protozoa seems inevitable. Furthermore, VÁVRA 1965 showed that in spite of a general similarity in the ultrastructure of the whole class Microsporidia, important differences occur among the individual species. To add to the knowledge on microsporidian ultrastructure, the present paper deals with the rare and atypical genus *Caudospora*,

thus completing the brief note on the fine structure of *C. simulii* included in the paper of DOBY et al. (1965).

MATERIAL AND METHODS

Infected *Simulium latipes* Meig. larvae were collected in a brook running through a meadow near Guichen (Ille-et-Vilaine), France.

Minute fragments of infected fat body were fixed in 1% OsO_4 buffered with Veronal-acetate buffer, dehydrated in ethanol and embedded in Durcupan ACM-Fluka (Araldite) epoxy resin. Ultrathin sections were cut on Sorvall's Porter-Blum ultramicrotome with a glass knife. It is most difficult to cut blocks with the spores, because a) the embedding resin never penetrates some of the spores, b) the fully mature spores are extremely hard and are easily shifted in the block when contacting the edge of the knife. This disturbs the integrity of the section during further manipulation and during observation in the electron beam. The sections showing silver or grey interference colours were stained with an ethanol solution of phosphotungstic acid and were observed under the JEM 6 A electron-microscope operating at 80 or 100 kV.

OBSERVATIONS

In the examined material, we found four developmental stages of the sporogonial part of the life cycle of *Caudospora:* the plasmodia, sporoblasts, young spores and mature spores. The ultrastructure of these developmental stages considerably differs and will be separately dealt with in the following text.

Plasmodia

Plasmodia are represented by large (up to 10 μ) formations of a generally circular outline (Plate I. Fig. 1). Up to 4 nuclei were observed in a single ultrathin section of a plasmodium which is in relatively good accord with the number of nuclei observed under the light microscope, varying from 4 to 16 (WEISER 1961; DOBY et al. 1965). The plasmodium is limited by a thin single sheath membrane of about 100 A which is bordered by an almost continuous layer of vesicles clearly originating from the fusion of small, irregular protrusions of the plasmodium membrane (Plate II, Fig. 5). In the individual plasmodia, this vesicular layer is more or less distinctly developed, sometimes appearing as a flat cisternal sheath enveloping the whole plasmodium (Plate I, Fig. 1). An amorphous substance of moderate electron density occurs in the thin space between the vesicular layer and the proper surface of the plasmodium (Plate I, Fig. 2; Plate II, Fig. 3). Small vesicles are nipped off from the internal side of the plasmodium membrane, thus indicating the uptake of substances by pinocytosis. In some cases also the detachment of rather large vesicles from the plasmodium membrane was observed. These vesicles are drawn deep into the cytoplasm where they evidently become part of the endoplasmic reticulum (Plate II, Figs. 1, 2).

The cytoplasm of a plasmodium is rather thin in appearance having a few membranous structures either in the form of large, irregular vesicles loosely scattered throughout the volume of the cell (Plate I, Fig. 1; Plate II, Fig. 1) or in the form of a few cisternae which are characteristic for the endoplasmic reticulum (Plate I, Fig. 3). Despite the fact that the localization of individual ribosomes is not very well preserved in phosphotungstic acid stained sections, it seems that most of the vesicles and cisternae belong to the so-called "rough-surface type" of endoplasmic reticulum. Furthermore, several arbitrarily distributed areas with an accumulation of very small vesicles of considerable density can be found in the cytoplasm of a plasmodium (Plate I, Figs. 1, 2). Similar formations considered to be a primitive Golgi apparatus (VÁVRA 1965) were found in the developmental stages of other microsporidia.

Similar to other microsporidia studied so far, mitochondria are also completely absent in *Caudospora simulii* cells.

The nucleus of a plasmodium is of typical appearance being surrounded by a double membrane which sometimes forms large perinuclear cisternae (Plate II, Fig. 1). So far we have not been able to observe pores in the nuclear membrane of *Caudospora*. Very often, "diplocarya", i.e. two closely adjacent nuclei, can be found in plasmodium sections (Plate I, Fig. 1). Such nuclei are in very intimate morphological contact, their membranes adhering to each other over a large area. Therefore, some authors consider diplocarya to be geneticaly active elements, which exchange their nuclear substances or fuse by autogamy. However, we did not observe any definite morphological support of this assumption in the electron microscope, as each of the twin nuclei of the diplocaryon retains its double membrane over the whole nuclear surface.

Sporoblasts

There were very few sporoblasts present in our material suggesting that they represent only a short-lasting and transitional link in the life cycle.

The first feature by which the young sporoblast may be distinguished from the plasmodium, is the appearance of the cell membrane. Contrary to the plasmodium, the sporoblast is no longer covered by a single membrane, but its surface is wrapped in a double membrane 300 A thick with two dense lines of 100 A each and an interspace of 100 A (Plate II, Fig. 4). Its outer sheath is very finely undulated. Older sporoblasts can be easily recognized by their elongated shape (Plate III, Figs. 1, 2). The sporoblast membrane is not quite compact, being interrupted by small openings at irregular distances (Plate III, Fig. 1, arrows). It seems improbable that these "pores" are actually open for an exchange between the cell and the environment. Some of our photomicrographs indicate that the pore is covered by a very thin membrane, which is much thinner than the inner sheaths of the original sporoblast membrane. Furthermore, the "pores" are the sites of origin of the vesicular expans-

ions protruding from the sporoblast into the environment (Plate III, Fig. 1, v). These protrusions, although less numerous, resemble vesicular projections limiting the plasmodium surface. Unfortunately it is impossible to ascertain the length of these protrusions because the space between the cells of the parasite is filled with membranous and vesicular formations of unknown origin.

The internal fine structure of the sporoblast slightly differs from that of the plasmodium. While in the latter the cytoplasmic components are distributed at random throughout the volume of the cell, there is a marked polarization in the cell of the sporoblast. This is partly due to the elongated shape of the sporoblast and it is also the necessary step preceding the formation of the spore. The most conspicuous sign of this polarization is the shifting of the two nuclei in each sporoblast to the lower end of the cell. This end is the basal part of the future spore where the cauda starts to differentiate. Like in the plasmodium, each of the two nuclei retains its own double membrane. The endoplasmic reticulum is more plentiful in the sporoblast and forms about 15 parallel cisternae closely in front of the nuclei. In the anterior "nonnuclear" half of the sporoblast only irregular vesicles are present. The Golgi zones are of the same appearance as in the plasmodia and usually occur at two sites of the sporoblast cytoplasm: close to the nuclei and in the mid-cell between the nuclei and the anterior end of the sporoblast.

Young spores

For the relative scarcity of sporoblasts in our material, the start of the transformation of sporoblasts into spores could not be traced. All young spores found in our section had already developed several threads of polar filament.

In comparison with the sporoblast, the young spore is more oval in shape and its inner content is more condensed and more electron dense. At this stage, the enveloping membrane of the young spore is still composed of two sheaths as in the sporoblast, but they are no longer perforated by porelike openings (Plate I, Fig. 3, Plate V, Fig. 4).

The appearance, number and position of the nuclei remains unchanged, if compared with the sporoblast.

The rapidly increasing number of ribosomes in the young spore is responsible for a darkening of the spore content in the electron microscope and obscures the details of its fine structure.

The threads of the polar filament appear as a large electron transparent canal coiled just beneath the membrane in the posterior half of the spore. The *Caudospora* is evidently not suitable for studying the substructure of the developing filament, but it is possible to distinguish in its threads the same layers as those described in filaments of other microsporidia (VÁVRA et al., 1966), although their mutual proportions are different. The outmost layer is very well developed and occupies approximatively 2/3 of the diameter of the whole filament. The second electron dense layer occupies about 1/3 of the filament diameter, but its subdivision into two addi-

tional layers is indistinct and can only be viewed in some pictures (Plate V, Fig. 5). In the young spore, the filament is anchored to the spore pole by a simple swelling of its apical part (Plate IV, Fig. 1; Plate V, Fig. 1). Later, a fine granular substance impregnates the tip of the anchoring structure.

The internal electron dense part of the filament seems to penetrate the anchoring mass. On transverse or nearly transverse sections through the terminal part of the filament axis, deeply embedded in the above mentioned granular mass, often several concentric circles of small electron dense granules of an unknown significance may be observed. They probably represent the terminal portions of the presumptive filamental fibrilous bundles (Plate V, Fig. 1a).

The cauda, a unique structure of *Caudospora*, appears as a small protuberance on the posterior pole of young spore, growing exactly from the spot where the sporoblast had become separated from the mass of cell differentiated from the same plasmodium. The cauda is actually a projection of the spore membrane and, as in the latter, its wall also consists of two sheaths. In its younger stage, the cauda is not quite hollow, but filled with small electron dense vesicles originating from long tubules convoluted into a ball at the posterior end of the spore (Plate V, Figs. 6, 7, 8). Because the cauda grows considerably both in length and width during the ripening of the spore, these vesicles and tubuli may be considered to be the path along which its building material is transported.

Two protuberances resembling the cauda but much thinner occur on the anterior pole of the spore (Plate IV, Figs. 3, 4). In the light microscope, these expansions cannot be distinguished from the extruded polar filaments (Plate VI, Fig. 4). This fact was responsible for the erroneous listing of *Caudospora* to Dicnidea, which are microsporidia with two polar filaments (Weiser 1961). Viewed under the electron microscope, these structures represent simple filamentous protuberances of the spore membrane (Doby et al. 1965).

Mature spores

Contrary to the young spore with its relatively thin, double layered outer membrane, the mature spore is covered by a thick envelope consisting of several sheaths. During development, these layers are formed successively. First, the two layers of the original double membrane fuse together into one electron dense envelope of 300 A. Then a thick, electron transparent layer is deposited between this membrane and the thin membrane limiting the plasmatic spore content. In the fully maturated spore, this transparent layer is very distinct and attains up to 2,000 A. After the formation of this layer, the electron dense layer covering it rapidly thickens (up to 1,000 A). This is the sign of maturity. In fully developed spores the last mentioned layer is not of equal thickness on the spore surface, but it forms two opposite longitudinal ribs (4,000 A) extending along the entire length of the spore (Plate V, Fig. 9). These ribs are identical with the ornamentation of the spore, visible under the optical microscope (Plate VI, Fig. 5).

In the ripe spore, the polar filament is anchored to the spore pole by a flattened disc situated perpendicularly to the straight top part of the polar filament. This adhesive structure originated from the top part of the proper filament. The upper part of this disc is convex, exactly following the inner curve of the spore case. Its bottom part is almost straight or slightly undulated. The whole formation, sectioned longitudinally at the site of the entrance of the filament, gives the impression of a sectioned mushroom (Plate V, Figs. 2, 3). There are two layers present in this disc: its convex side is covered by thin layer exhibiting moderate electron density, which cements the adhesive structure to the innermost layer of the spore case. The rest of the disc consists of electron dense material into which the polar filament merges. In the absence of serial longitudinal sections through the adhesive disc, the exact interpretation of the filament termination in this structure is rather difficult, but it is evident that the filament or some of its layers deeply penetrate the adhesive disc (Plate V, Fig. 2). It still remains to be decided, whether the entire filament dips into the disc or whether its outer membrane is a continuation of the membrane of the disc. In the latter case, the disc could be interpreted as a swollen part of the anterior tip of the filament. Observations on the origin of this structure in *Plistophora debaissieuxi*, which will be reported elsewhere, seem to corroborate this assumption. On the other hand, it is difficult to interpret the layers seemingly bridging the tip of the polar filament (Plate V, Fig. 2). At the point of entrance of the filament into the disc, it is usually possible to observe a small swelling of its axis (Plate V, Figs. 2, 3). The significance of this structure, however, remains unknown.

Recently Lom and Corliss (1967) called the anchoring structure of the filament in *Plistophora hyphessobryconis* the "polar cap-polaroplast complex". They claim this structure to be identical with the so-called PAS positive polar cap known from light microscope observations (Vávra 1965). The basal part of the filament "is closely associated" with the polar cap-polaroplast complex and the substance of the polar cap "evidently passes for a short distance into the lumen of the filament". This statement seems in need of further confirmation by histochemical reactions on an ultrastructural level, but the observations made in the light microscope may indicate the PAS positive material to be located in the wall of the spore.

In mature spores of *Caudospora*, the whole filament winds in 12—13 turns arranged in one layer at the posterior half of the spore. From the number of turns and from their diameter, the total length of the filament can be estimated to be 60 μ.

In all examined spores the polaroplast became swollen during fixation, taking on a foamy appearance with large bubbles slightly angular in outline (Plate IV, Figs. 1, 2, 3, 4).

So far we have not been able to observe the so-called posterior vacuole in the spores of *Caudospora*. This structure, quite common in spores of other microsporidia, is in *Caudospora* replaced by a mass of irregularly convoluted tubuli (Plate IV, Fig. 2; Plate V, Figs. 6, 7, 8).

The plasma which surrounds the plasmodium and the sporoblast, is filled with numerous vesicles and mitochondria (Plate III, Fig. 1). It seems reasonable to interpret most of these vesicles to be an integral part of the host cell cytoplasm. On the other hand, it seems justified to consider at least some part of the above mentioned vesicles as expansions of the external membranes of the parasite. As the development of the parasite proceeds the vesicles, dispersed among its cells, gradually decrease in number. Finally the ripe spores seem to be located quite freely in the fat tissue (Plate VI, Fig. 1).

DISCUSSION

Our study, although made on limited material because of the extremely rare occurrence of *Caudospora*, shows that the general outline of the fine structure of *Caudospora simulii* is in accord with the ultrastructural features of other microsporidia. Similarities were found 1. in plasmodia and sporoblasts: in the character of the outer membrane; the appearance of endoplasmic reticulum and Golgi zones; the complete absence of mitochondria. 2. In the spore: in the number, sequence and time of formation of spore shell layers; the general location of organellae in the spore; the number and sequence of the polar filament layers.

The distinctly differentiating characters of *Caudospora* are: In the plasmodium, the presence of abundant vesicular protrusions sometimes forming an almost continuous layer bordering the plasmodial membrane. No formation of sporoblasts inside the plasmodium was observed in *Caudospora*, although this is a typical feature of polysporoblastic microsporidia (VÁVRA 1965) to which according to DOBY et al. (1965) *Caudospora* belongs. In this connection a fact well known from light microscope observations should be pointed out. In individual microsporidia, the durability and preservation of the pansporoblast membrane greatly varies. In some species, this membrane is retained until maturity of the spores, thus keeping the cluster of spores originating from the pansporoblast together. In other species, the sporoblasts lose their common membrane at an early developmental stage. *Caudospora* evidently represents one of the extreme members of this second group.

Another typical feature is the abnormally developed outer layer of the polar filament, while its electron dense axis seems to be very compressed. Although the polar filaments of all microsporidia examined under the electron microscope are, in fact, composed of the same number of layers in the same arrangement (VÁVRA, JOYON, DE PUYTORAC 1966), there are certain differences in the individual species.

Fixation and embedding may distort the picture characteristic for the individual species, such as the polaroplast structure observed in the electron microscope, due to swelling during fixation and dehydration. However, the picture of swollen polaroplasts is typical for the individual species: in this respect, the foamy

appearance of the polaroplast in *C. simulii* is unique, as the polaroplasts of other microsporidia are rather lamellated.

The present study corroborates the opinion previously expressed by VÁVRA (1966) that the two main layers of the spore shell should be named exo- and endospore respectively. These two layers differ not only in their appearance under the electron microscope, in the time of origin during spore maturation, but also in their chemical composition and function.

Caudospora, the external layer (exospore) of which is very conspicuous, demonstrates the developmental plasticity of this layer. It is exclusively the exospore which forms the external ornamentation of the spore as well as the cauda and the two filamentous projections situated at the anterior pole of the spore. There are no data available on the function of these structures, but it may be assumed that they somehow influence the floating of the spore in the water and its trapping by the host. A form resembling the *Caudospora* occurs in the spores of other parasitic protozoa, e.g. in the myxosporidian *Henneguya*, in the coccidian *Barrouxia caudata* and in the sporocyst of the gregarine *Gonospora*.

The solubility test (a 20 % solution of potassium hydroxyde) shows the difference in the chemical composition of the exo- and endospore. While this alkali causes a swelling and dissolution of the exospore, this has no influence on the endospore (Plate VI, Figs. 2, 3). Recent investigations which will be reported elsewhere have shown that the endospore is of a chitinous, whereas the exospore is of a protein nature.

The taxonomic result of this study indicates the necessity to abolish the suborder Dicnidea. Originally the suborder was created by LÉGER and HESSE (1922) for the microsporidian *Telomyxa*, the spores of which were believed to contain two polar filaments each. In 1961, *Caudospora* was claimed by Weiser to be the second representative of the suborder Dicnidea. The present study fully confirms the opinion of DOBY et al. (1965) that *Caudospora* should be withdrawn from Dicnidea. The further existence of the suborder Dicnidea is not justified, as CODREANU (1961) proved that the spore of *Telomyxa* is of a special type, representing the equivalent of two inseparably joined spores.

It is obvious that nearly all microsporidian spores are built according to a uniform scheme, their variability being due only to different proportions of the singular morphological components.

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J. V. Parasitologický ústav ČSAV, Flemingovo n. 2, Praha 6, ČSSR

EXPLANATION TO THE PLATES

Used abbreviations: A, anchoring of the filament; C, cauda; D, "endospore", the internal chitinous layer of the spore shell; E, endoplasmic reticulum; F, polar filament; G, Golgi zone; H, host mitochondria; M, single sheath membrane of the plasmodium; N, nucleus; P, polaroplast; T, tubules replacing the posterior vacuole; V, vesicular layer and vesicular protrusions bordering the plasmodium or sporoblast membrane; X, "exospore", the external electron-dense layer of the spore shell;
In all figures the line indicates 0.5 μ, except in Figs. 25—28 where the line corresponds to 5 μ.

Plate I

Fig. 1. Plasmodium of *C. simulii* with two adjacent nuclei (diplocaryon). The proper membrane of the plasmodium (M) is bordered by the vesicular layer (V).
Fig. 2. Enlarged part of Fig. 1 showing the relation between the membrane of the plasmodium and the vesicular layer. Both are separated by a layer exhibiting moderate electron density.
Fig. 3. Comparison of the ultrastructure of a plasmodium (on the right) and a young spore (left). Arrows point to double membrane of the spore.

Plate II

Fig. 1. Plasmodium with large vesicles in cytoplasm but very poor in Golgi zones. The nucleus exhibits large perinuclear cisternae.
Fig. 2. Enlarged part of Fig. 1 demonstrating the origin of a cytoplasmic vesicle (E) from the external membrane.
Fig. 3. Enlarged part of Fig. 1 in which the cisternal sheath bordering the plasmodium is well visible.
Fig. 4. A very young sporoblast having just developed its double membrane (arrows).
Fig. 5. Section through the membrane of a plasmodium showing the origin of a vesicular protrusion.

Plate III

Figs. 1, 2. Sporoblasts of *Caudospora* showing the polarization of the spore content. Note the appearance of the outer membrane the two sheaths of which fuse into one electron dense envelope with a few vesicular protrusions (V) and "pores" (arrows). The sporoblast is surrounded by vesicles of unknown origin and a few mitochondria belonging to the host cell (H).

Plate IV

Fig. 1. Very young spore of *Caudospora*. Note the increasing density of the content of the cell. The outer membrane is still double.
Fig. 2. Older spore with an even darker spore content. The membrane consists of one electron dense layer.
Figs. 3, 4. Sections through the anterior part of the spore showing the origin of one of the two filamentous spore projections (arrow).
The cross-sectioned spore on the right of Fig. 4 clearly demonstrates the presence of only one polar filament in *Caudospora*. Cross sections of cauda of neighbouring cells indicated by C.

Plate V

Figs. 1, 2, 3. Details of the disc of *Caudospora*. Fig. 1 indicates that this structure originates as part of the filament proper. The inset „a" shows the deposition of electron dense substance in the anchoring disc and the penetration of the filament axis into this structure. The dots may represent the cross sections through the fibriles of the filament. In further development the disc becomes more complicated and difficult to interpret (Figs. 2, 3). Note the swelling on the filament axis (arrows).
Figs. 4, 5. Details of the spore wall structure in a very young (Fig. 4) and in an older (Fig. 5) spore. The latter figure shows also that the polar filament differentiates in the cytoplasm as a whole and is later separated into individual coils.
Fig. 6. A very young spore with its cauda.
Fig. 7. Cauda growing in the form of a hollow tube into which tubuli protrude (arrow) from the posterior part of the spore (T).
Fig. 8. Detail of the basal part of the spore showing the continuity of the cauda membrane with the envelope of the young spore.
Fig. 9. Cross section of a mature spore of *Caudospora*. The two main layers of the spore shell and their form are visible.

Plate VI

Fig. 1. Longitudinal section through an advanced spore with very developed exosporal layer (X). The two insets show the separation of individual coils of the polar filament (arrow).
Fig. 2. Swelling of the exosporal layer and of the cauda caused by 20% KOH solution.
Fig. 3. Further dissolution of the exosporal layer in the lye. The endospore remains intact.
Fig. 4. Spore of *Caudospora* in phase contrast microscope. The two filamentous projections and the cauda well visible.
Fig. 5. The typical appearance of *Caudospora simulii* in the light microscope.