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ULTRASTRUCTURE OF SPOROGENESIS OF *THELOHANELLUS NIKOLSKII* AKHMEROV, 1955 (MYXOZOA: MYXOSPOREA) FROM THE COMMON CARP, *CYPRINUS CARPIO*

Sherwin S. Desser, Kalman Molnar,* and Iris Weller
Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1, Canada

ABSTRACT: Plasmodia of *Thelohanellus nikolskii* occur in epidermal cysts in the fins of carp (*Cyprinus carpio*) from Hungary. The plasmodium, believed to be a grossly hypertrophied host cell, was limited by a membrane with numerous pinocytotic channels and associated vesicles. The peripheral ectoplasmic zone of the plasmodium contained large vesicular nuclei, mitochondria, Golgi apparatus, smooth endoplasmic reticulum, and lipid inclusions. Among these organelles and extending into the central region of the plasmodium were various stages of sporogenesis. These consisted of single and associated generative cells and sporoblasts ranging from the two-cell stage to pansporoblasts containing mature spores. In young sporoblasts, the development of the second capsulogenic cell was abortive resulting in the formation of a single polar capsule in each of the two spores. The capsular primordium and external tubule developed simultaneously and the rudimentary polar filament appeared in both structures. The external tubule was withdrawn into the capsule by a process of invagination and the mouth of the mature polar capsule was plugged by a corklike stopper. Posterior to the capsulogenic cell was a typical binucleate sporoplasm. The two halves of the spore wall were formed by the flattened valvogenic cells that were joined around their circumference by a long, continuous junctional apparatus which was located in a flangelike projection. The outer edge of the flange contained up to 50 microtubules in each valvogenic cell. The junctional apparatus was reinforced by dense fibrillar structures. The components of the tripartite wall of mature spores were formed in the valvogenic cell.

Although ultrastructural studies on several species of myxosporean parasites have been conducted (e.g., Cheissin et al., 1961; Lom and de Puytorac, 1965; Schubert, 1968; Lom, 1969; Current and Janovy, 1976, 1977; Desser and Paterson, 1978; Grassé and Lavette, 1978; Current et al., 1979; Yamamoto and Sanders, 1979), nothing has been reported on *Thelohanellus* species, whose spores are characterized by a single polar capsule.

In the present study, ultrastructural features of *Thelohanellus nikolskii* from the fins of common carp from Hungary are described with emphasis on the nature and probable origin of the plasmodium, development of the polar capsule and tubule, and formation of the spore wall.

MATERIALS AND METHODS

Cysts containing plasmodia of *T. nikolskii* were dissected from the fins of common carp, 7 to 9 cm in length, from a nursery pond in Eastern Hungary. Fresh spores were removed from mature plasmodia and photographed. For electron microscopic study, cysts of different sizes were excised and fixed at 20 C in an aqueous solution containing 2.5% (v/v) glutaraldehyde and 2% (v/v) OsO_4, and buffered with sodium cacodylate to pH 7.2 to 7.4 according to the method of Franke et al. (1969). The tissues were subsequently processed as described previously (Desser and Paterson, 1978). For localization of glycogen, sections were collected on gold grids and stained by the periodic acid–thiosemicarbazide–silver proteinate (PA–TSC–Ag protein) method of Thiery (1967), using 30 min oxidation in 1% (w/v) periodic acid, 30 min in 1% (w/v) thiosemicarbazide, and 30 min in a 1% (w/v) solution of strong silver proteinate (Protargol, from Roboz Surgical Instrument Co., Washington, D.C.). A control sample was processed as above omitting the periodic acid oxidation. The sections were examined in a Zeiss E.M. 9A electron microscope.

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* Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary.

RESULTS

Cysts (1–2 mm in diameter) were localized in the connective tissue adjacent to the finrays, particularly in the caudal and dorsal fins (Fig. 1). The cysts contained spores whose morphological features and dimensions conformed with those of *Thelohanellus nikolskii,* which was described by Akhmerov (1955) from similar cysts in the fins of common carp from eastern territories of the Soviet Union. The spores had a single polar capsule with a loosely coiled filament (Fig. 2).

Cyst Wall and plasmodium

Plasmodia containing various stages of developing spores were surrounded by a prominent cyst wall. External to this wall were perichondrial cells and scattered chromatophores containing

dense pigment granules (Fig. 3). The cyst wall surrounding immature plasmodia ranged in thickness from 2 to 3.5 μm and contained many closely-spaced collagen fibrils in which periodic banding was apparent at higher magnification (Fig. 3, inset). The surface of young plasmodia was thrown into palisadelike folds and was limited by a membrane, overlain by a closely-applied membranelike layer which was coated by granular material (Fig. 4). Numerous pinocytotic channels extended from the inner membrane into the fine, granular ectoplasmic zone of the plasmodium (Figs. 3–6). The walls of cysts surrounding mature plasmodia were more homogeneous in appearance and individual collagen fibrils could not be discerned (Figs. 5, 6).

In the peripheral ectoplasmic zone, beneath the pinocytotic channels and vesicles, were numerous mitochondria with dense matrices and well-developed cristae (Figs. 5, 6). Also prevalent in this region were Golgi apparatus, smooth endoplasmic reticulum (Er), and lipid droplets (Figs. 5, 6). Many large vesicular nuclei were located in the ectoplasmic zone. Interspersed in the more central region of the plasmodium (the endoplasm) were stages of sporogenesis, ranging from single "generative cells" to mature spores, which lay among profiles of loose membrane (Fig. 5).

Early sporogenesis

The earliest recognizable stages of sporogenesis were morphologically similar, closely associated generative cells (Fig. 7). Both cells contained a large vesicular nucleus with marginally-arranged heterochromatin and (when sectioned in the appropriate plane) a prominent nucleolus. Their dense, homogeneous cytoplasm contained closely-spaced ribosomes and occasional short segments of membrane which were closely associated with large, oval mitochondria. The latter organelles contained sparse cristae in a relatively electron-lucent matrix (Fig. 7). The mitochondria of generative cells differed markedly from those in the plasmodial ectoplasm, which had more elaborate cristae and a denser matrix (see Figs. 5, 6, 9, 21). A small dense structure comprised of several closely packed microtubules appeared to form a bridge between many of such paired generative cells (Fig. 7 and inset).

In a subsequent stage of development, one of the generative cells was enveloped by the other, the "envelope" cell (Fig. 8). The cytological fea-

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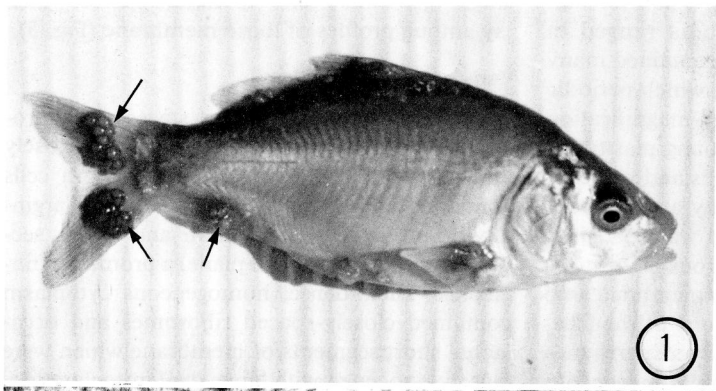
FIGURES 1–5. *T. nikolskii.* **1.** Young carp whose fins contain many cysts (arrows) of *T. nikolskii* (actual size.) **2.** Photomicrograph of spores containing a single polar capsule with loosely-wound filament. ×1,200. **3.** The cyst wall (Wc) surrounding a young plasmodium contains many closely-spaced collagen fibrils (Cf). At higher magnification periodic banding was apparent in these fibrils (inset ×64,300). Pigment granules can be seen in the cytoplasm of chromatophores (Ch) which lie external to the cyst wall. The outer region of the plasmodium (ectoplasm—Ep) contains many pinocytotic channels (Pc) and vesicles. Dense mitochondria (Mi) lie beneath the pinocytotic region. ×17,940. **4.** The interface between a young plasmodium and its cyst wall illustrating the membranelike boundary of the cyst (arrow) which overlies the limiting membrane (Lm) of the plasmodium. ×34,850. **5.** A more advanced plasmodium enclosed by a dense, homogeneous cyst wall. Vesicular nuclei (Nv), dense mitochondria (Mi), loose membrane profiles (arrow), single generative cell (Ge), a generative cell within an envelope cell (Ec), a young sporoblast (Sn), and a portion of a mature spore (Sp) lie in the hypertrophied cytoplasm of the plasmodium. ×3,600. Abbreviations: Br, intercellular bridge; Cc, capsulogenic cell; Cf, collagen fibrils; Ch, chromatophores; Cp, capsular primordium; Dp, dense plaque; Ec, envelope cell; Ep, ectoplasm; Er, endoplasmic reticulum; Et, external tubule; Gc, dense granular coat; Ge, generative cell; Gl, glycogen granules; Go, Golgi apparatus; Ip, peculiar inclusion; Ju, junctional apparatus; Li, lipid inclusion; Lm, limiting membrane; Mf, microfilamentous girdle; Mi, mitochondria; Nv, vesicular nucleus; Pc, pinocytotic channels; Sn, sporoblast; Sp, mature spore; St, corklike stopper; Vc, valvogenic cell; Wc, cyst wall.

FIGURES 6–9. **6.** Individual collagen fibrils cannot be discerned in the cyst wall surrounding a mature plasmodium. Pinocytotic channels (Pc) and vesicles occur in the ectoplasmic zone which also contains mitochondria with dense matrices (Mi), short segments of smooth ER (Er), a Golgi apparatus (Go), and a portion of a large, vesicular, plasmodial nucleus (Nv). ×28,800. **7.** Two generative cells joined by a dense intercellular "bridge" (Br) comprised of closely spaced microtubules. ×13,800. **Inset.** Intercellular bridge. ×24,800. **8.** Two-celled sporoblast. The mitochondria (Mi) in both cells contain sparse cristae in a light filamentous matrix and are associated with segments of smooth ER (Er). A peculiar inclusion (Ip), consisting of a dense core and smaller satellite bodies of similar appearance occurs in the cytoplasm of both cells. ×11,700. **9.** A recently-divided, generative cell with a persistent microtubular bridge (Br), between the adjoining daughter cells. The limiting membrane (arrows) of the lower cell is sectioned obliquely. The nucleus of the envelope cell (En) and part of its cytoplasm (asterisks) can be seen between the daughter cells. ×13,800. **Inset.** Intercellular bridge. ×28,500.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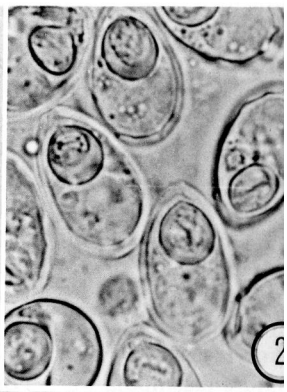
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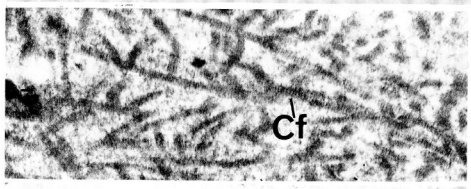
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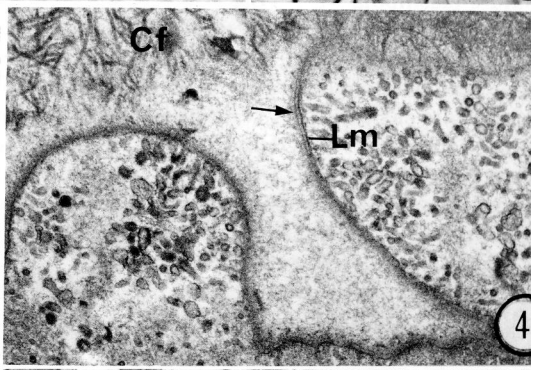
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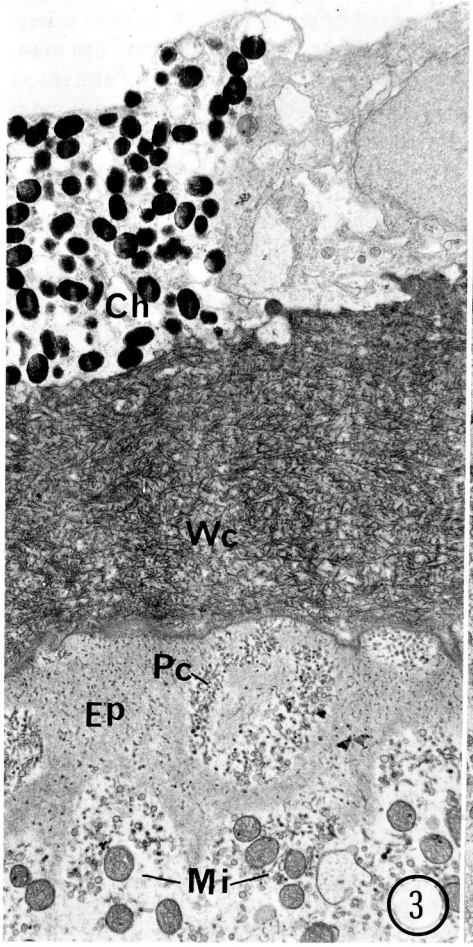
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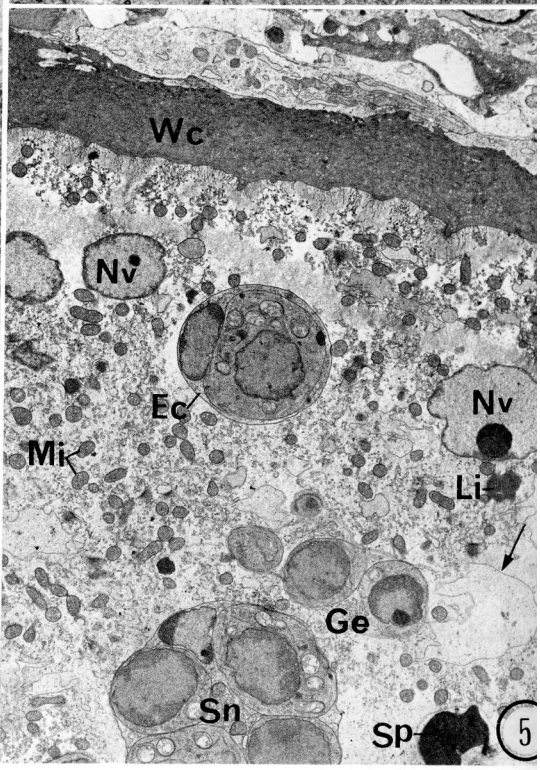
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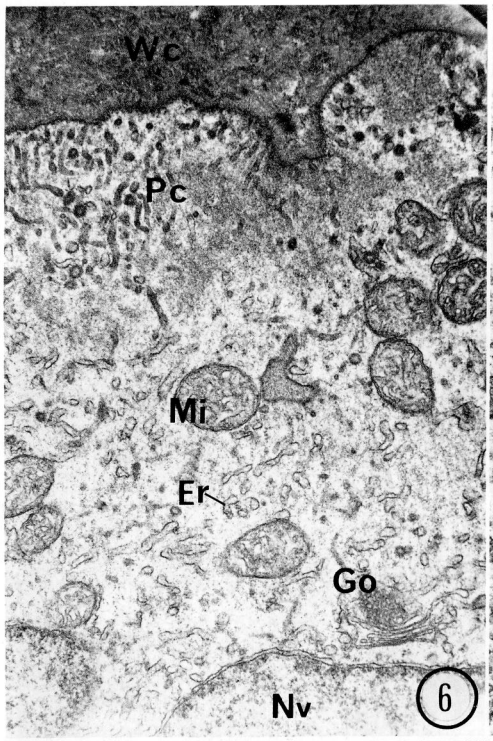
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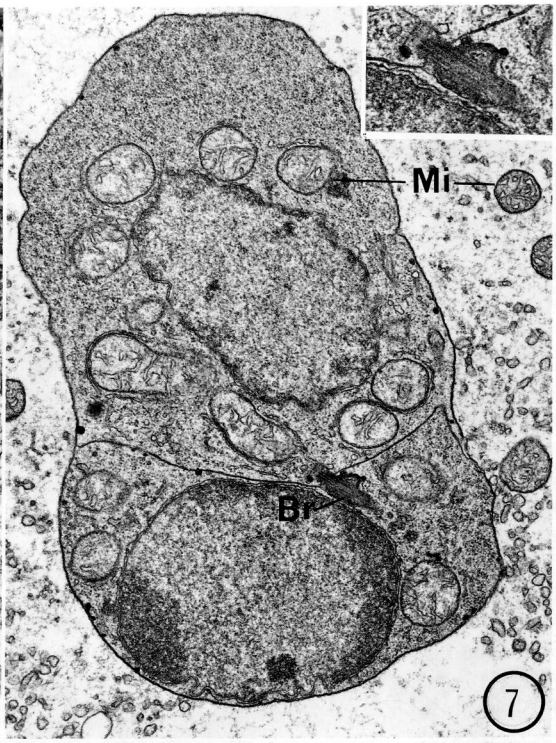
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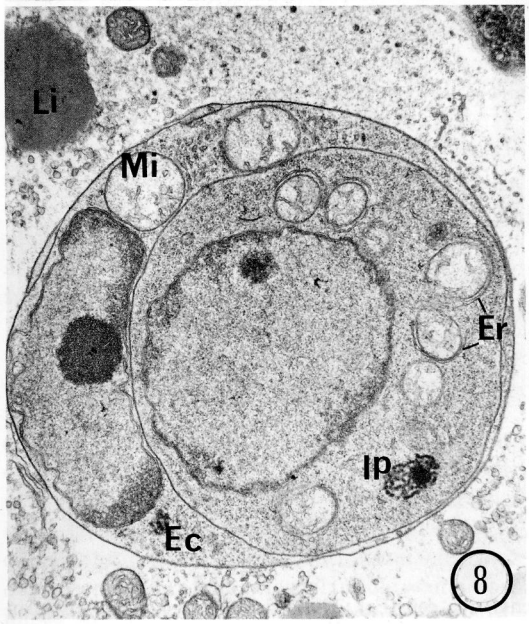
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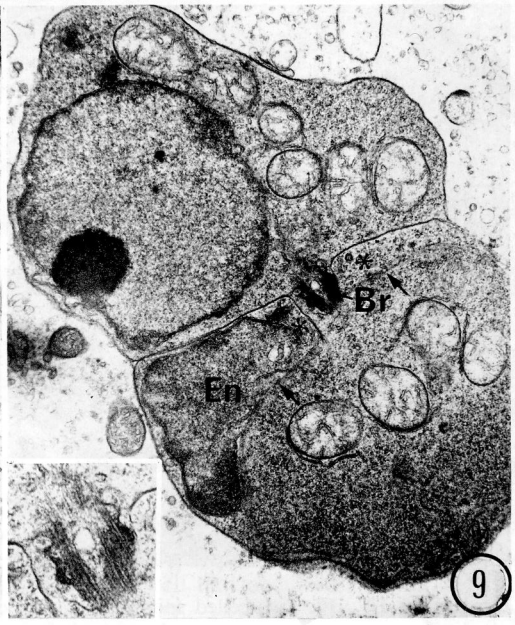
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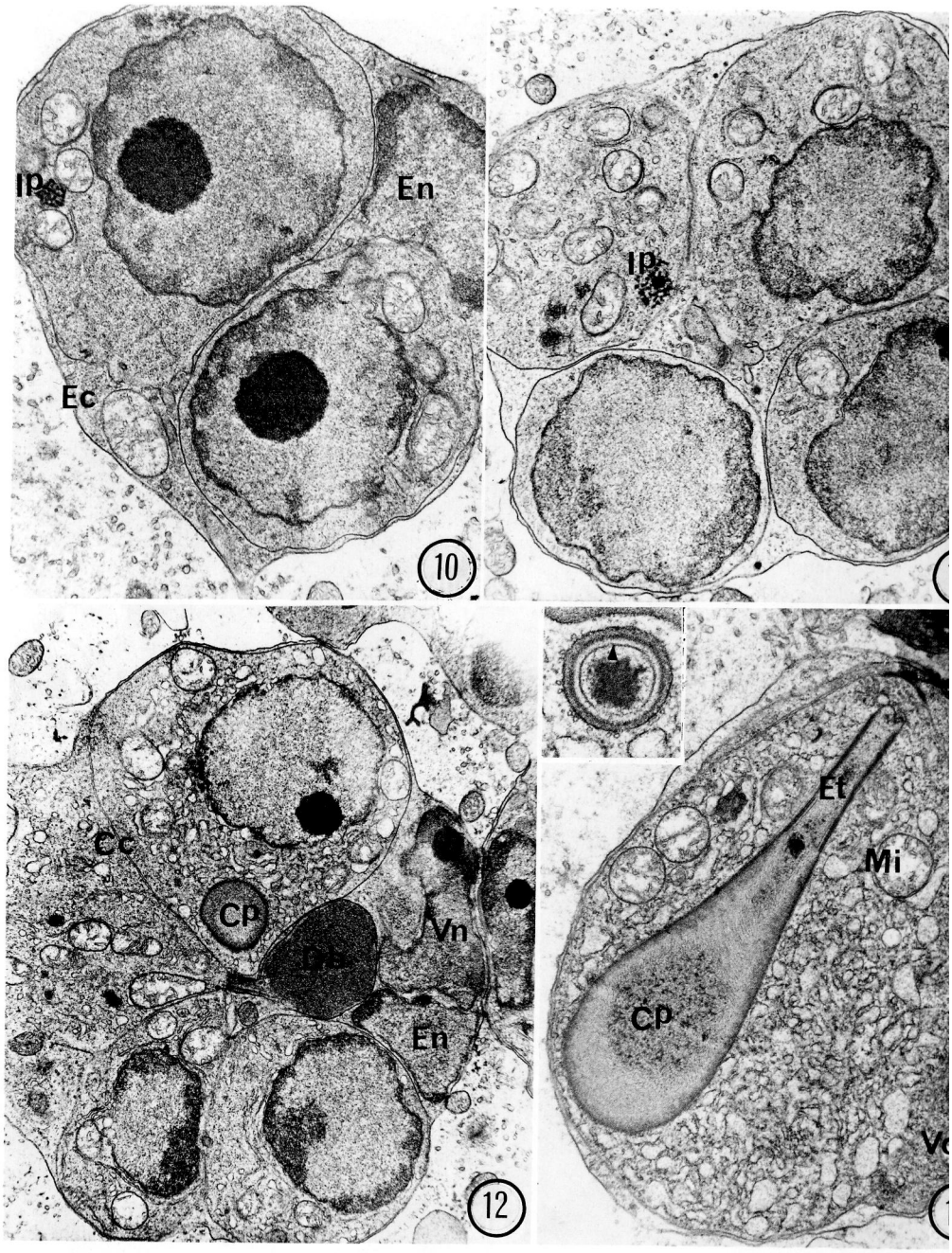


FIGURES 10–13. **10.** Two-celled sporoblast within envelope cell (Ec). The cells contain a large vesi nucleus with a prominent nucleolus. ×11,700. **11.** Four-celled sporoblast. The identity of individual cells i apparent at this stage. ×11,100. **12.** Sporoblast with two capsulogenic cells (Cc) one of which is connecte an intercellular bridge to a dense body (Db). The envelope cell nucleus (En) and valvogenic cell nucleus can be recognized. One of the capsulogenic cells contains a small capsular primordium (Cp). ×8,400. **13.** stage of polar capsule primordium and external tubule. The capsular primordium (Cp), a membrane-boun is attached to a short external tubule (Et), which is surrounded by microtubules. ×9,900. **Inset.** Transec through neck region between capsular primordium and external tubule illustrating inner membranelike c (arrowhead). ×36,100.

tures of the envelope and enclosed generative cell resembled that of their predecessors except for the presence of a peculiar inclusion which consisted of a central dense body, surrounded by several smaller satellite bodies of similar appearance (Figs. 8, 10, 11).

The enclosed generative cell subsequently divided. An intercellular bridge was seen in some bicellular sporoblasts (Fig. 9). Sporoblasts with two, four, or more cells were seen (Figs. 10–12) and in some sections different cell types could be recognized. For example, a small ovoid "capsular primordium" was seen in the cytoplasm of a young capsulogenic cell. An adjacent capsulogenic cell was connected by a microtubular bridge to a dense spherical body (Fig. 12). The valvogenic cells became flattened and surrounded the capsulogenic and sporoplasm cells (Figs. 13, 14).

Polar capsule formation

In early stages of polar capsule formation, the cytoplasm of capsulogenic cells contained many dilated cisternae of smooth ER, Golgi apparatus, and lipid inclusions. A dilated, membrane-bound sac connected to a short external tubule, which was surrounded by several randomly-arranged microtubules, was sometimes seen (Fig. 13 and inset). With further development, the volume of the dilated sac (the capsular primordium) and the length of the external tubule increased considerably (Fig. 14). The tubule was bound by two closely-applied membranes while the terminal portion, which was heavily reinforced, lay near an electron-dense plaque situated adjacent to the sutural plane of the valvogenic cells (Figs. 14, 16). The reinforced terminal region of the external tubule was surrounded by a girdle of helically-arranged microtubules (Figs. 15, inset a; 16) and was lined internally by similarly-arranged, dense fibrils (Figs. 15, inset b; 18, inset). Growth of the external tubule appeared to be restricted to the region between its terminus and the forming polar capsule. The limiting membrane of the capsular primordium was continuous with the outer membrane of the external tubule which was joined to the capsule. The inner membrane of the tubule appeared to be formed de novo at the capsule–tubule junction, and in the neck region, an inner, short, membranelike collar was seen (Figs. 13, inset; 15). The capsular primordium contained a central core of loosely arranged, dense material, some of which also occurred in the lumen of the external tubule (Figs. 14, 15). Peripheral to the dense material in the capsular primordium was a fine granular zone which was surrounded by a network of fine microfilaments (Figs. 15, 24).

In a more advanced stage of polar capsule formation, the electron-lucent band surrounding the periphery of the capsule was more distinct (Figs. 17–19). The microfilamentous girdle was no longer apparent and the outer, finely granular region of the capsular matrix surrounded an inner core of denser material with which were associated short segments of the forming polar filaments (Figs. 17, 18). The external tubule, rarely seen at this stage, contained a polar filament forming similarly to that seen in the capsular primordium, and occasionally a portion of the tubule was seen connected to the capsule (Fig. 18). When sections through such tubules were viewed at higher magnification, helical, dense fibrils characteristic of the terminal region of the external tubule, were seen (Fig. 18, inset).

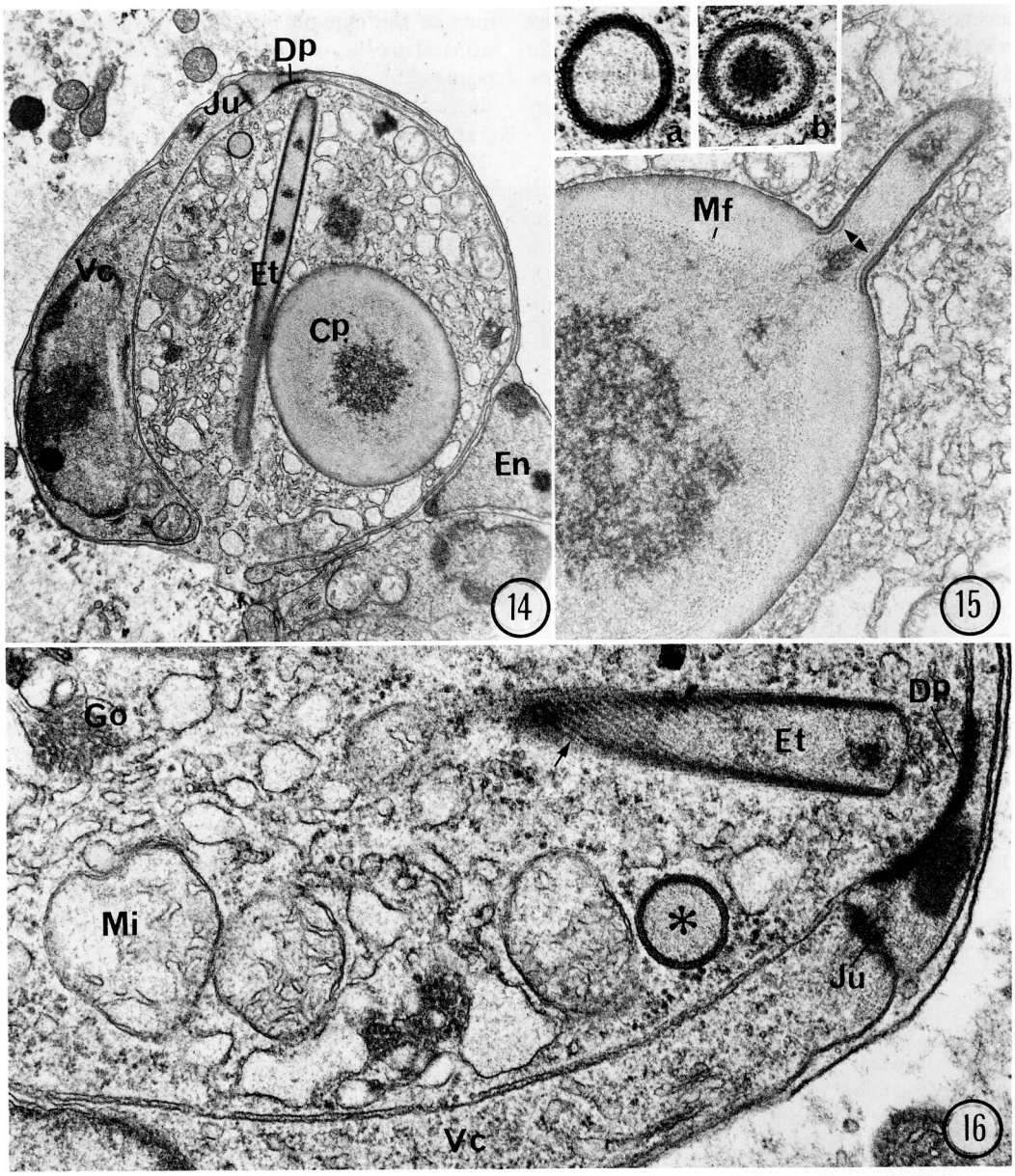
In a subsequent stage of development of the polar capsule, the diameter of the polar filament was considerably enlarged and the lumen appeared uniformly electron-dense. The dense core of the capsule, characteristic of earlier stages, was not apparent and the external tubule was no longer seen in the cytoplasm of the capsulogenic cells (Fig. 19).

Mature polar capsules were pyriform in shape and contained a dilated, spirally-arranged filament, which lay in a dense granular matrix. Beneath the limiting membrane of the polar capsule a narrow dense band surrounded the electron-lucent zone of the wall (Fig. 20). At the apex of the capsule the electron-lucent walls were reflexed inwards forming the walls of the polar filament. A corklike stopper plugged the opening of the capsule into the discharge channel (Fig. 20 and inset).

The sporoplasm cell

By the time the capsulogenic cell of each sporont was at the external tubule stage, the adjacent sporoplasm cell had acquired its characteristic appearance. The ribosome-studded cytoplasm contained cisternae of smooth ER, Golgi apparatus, and large mitochondria with sparse cristae in a lucent matrix. Small, spheroidal, electron-dense bodies were concentrated mainly in the peripheral cytoplasm (Fig. 21).

In sections stained by the PA–TSC–Ag protein



FIGURES 14–16. **14.** The flattened valvogenic cells (Vc) surround each maturing sporoblast and are joined on either side by a junctional apparatus (Ju). The enclosed capsulogenic cell contains a large, membrane-bound capsular primordium (Cp) with a dense core and light granular cortical region. Similar dense material lies in the lumen of the external tubule (Et), the terminal end of which lies beneath a dense plaque (Dp) near the junction conjoining the valvogenic cells. ×9,000. **15.** The capsular primordium and external tubule are bound by a common, limiting membrane. In the area of their junction an inner membrane appears in the tubule. Beneath the latter membrane is a short, dense ring (arrowheads). Some of the dense core material of the capsular primordium lies in the lumen of the external tubule. The light, granular cortical zone of the capsular primordium is surrounded by a microfilamentous girdle (Mf). ×28,600. **Inset A.** A transection through terminal region of external tubule with helically arranged microtubular girdle. ×37,200. **Inset B.** Transection through similar region with both inner fibrils and outer microtubles. ×37,200. **16.** Capsulogenic cell with terminal end of the external tubule (Et) with helically-wound microtubular girdle (arrow). A transection through the central region of the tubule (asterisk) illustrates the unreinforced double-membraned wall. ×30,380.

method, β-glycogen particles were seen scattered in the cytoplasm of immature capsulogenic cells (Fig. 23). They were particularly abundant in the sporoplasm cells. Electron-dense material was also concentrated beneath the limiting membrane of adjacent valvogenic cells (Fig. 22). The aforementioned dense spheroidal inclusions in the sporoplasm cell were unstained by this method.

The capsulogenic and sporoplasm cells were surrounded by two concave valvogenic cells joined on either side of the sporoblast by a continuous junctional apparatus. This apparatus was located in a flange formed by cytoplasmic extensions of each valvogenic cell, and extended around the circumference of the forming spore (Figs. 24, 25). In transverse sections through the outer edge of the flange, up to 50 microtubules were seen beneath the limiting membrane of each valvogenic cell (Fig. 24). Below the peripherally-arranged microtubules, the junctional apparatus adjoining the valvogenic cells was reinforced by two rows of dense fibrillar material. When viewed in transection each row was arranged in five dense bands on either side and parallel to the junction. The dense bands were connected to the junction and each other by microfilaments arranged at right angles (Fig. 24). A longitudinal section through the flange in this region revealed the extent of the junctional apparatus and the parallel rows of the fibrillar supporting structures (Fig. 25).

Examination of different stages of sporogenesis revealed the sequence of formation of the spore wall. Four distinct membranes were apparent along the outer margins of young sporonts. They were bound externally by the persistent membrane of the envelope cell. Internal to the latter were the two membranes of the flattened valvogenic cell and beneath these, the limiting membrane of either the sporoplasm or capsulogenic cells, depending on the level of the section (Fig. 26). With further maturation, electron-dense material began to accumulate beneath the limiting membrane of the valvogenic cells (Fig. 27). This dense material was more abundant and became confluent at a later stage (Fig. 28). Eventually, a narrow, homogeneous layer was formed in both the proximal and distal cytoplasm of the flattened, dense valvogenic cell (Fig. 29). The limiting membrane of the valvogenic cell became separated from the dense cytoplasm and short segments of dense material were laid down between the limiting membrane and the underlying, dense material.

The wall of mature spores consisted of an out-

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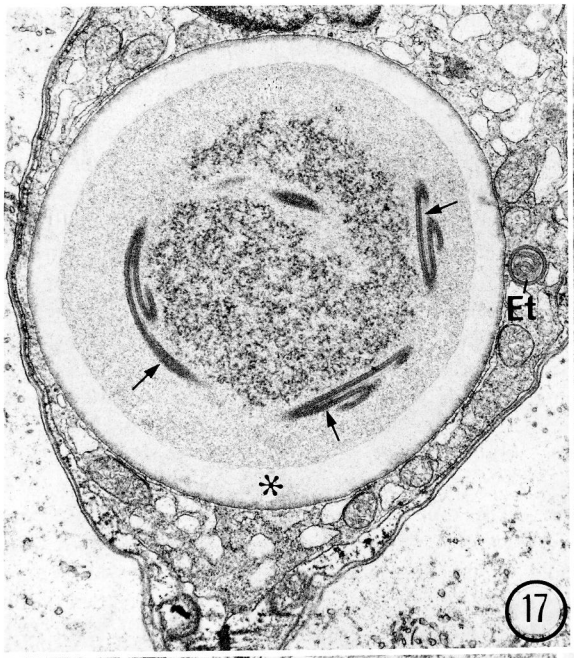
FIGURES 17–20. **17.** Maturing capsular primordium containing rudimentary filament (arrows) which lies in the light granular zone adjacent to the dense-core material. An electron-lucent zone (asterisk) underlies the limiting membrane of the capsular primordium. Note the transversely-sectioned, external tubule (Et) containing filaments similar to that seen in the capsule. ×19,450. **18.** Capsular primordium at similar stage as that in Figure 17, but sectioned so that rudimentary filaments appear "s"-shaped. A short portion of the terminal region of the external tubule containing filament material (arrow) is connected to the capsule and contains dense fibrils. ×18,700. **Inset.** Terminal portion of external tubule containing filament and dense fibrils. ×30,100. **19.** More advanced stage of polar filament development. The diameter of the filament has enlarged and the lumen contains homogeneous, dense material. The dense, core material of the capsule is no longer apparent. ×17,800. **20.** Maturing polar capsule containing an enlarged polar filament, coiled in a dense granular matrix. The apex of the pyriform capsule is plugged by a dense, corklike stopper (St) which faces the discharge channel (Dc). **Inset.** The lucent wall of the polar capsule is invaginated (arrowheads) in the apical region to form the wall of the polar filament. ×32,650.

FIGURES 21–23. **21.** The cytoplasm of binucleate sporoplasm cell in a maturing sporont, contains large mitochondria with few cristae in a lucent matrix, smooth ER (Er), Golgi apparatus (Go), and peripherally-concentrated, dense, spherical inclusions (Di). A portion of the adjacent capsulogenic cell (Cc) and the surrounding valvogenic cells (Vc) are also seen. The sporoblast mitochondria contrast strongly with those (arrows) in the plasmodial cytoplasm. ×14,850. **22.** Peripheral region of a maturing sporont stained by the PA–TSC–Ag protein method. Densely stained material (arrows) lies beneath the peripheral limiting membrane of the flattened, valvogenic cell (Vc). Several β-glycogen particles can be seen in the cytoplasm of the underlying sporoplasm cell. The dense, spherical inclusions (Di) in the latter cell are unstained. ×19,980. **23.** A maturing pansporoblast stained by the PA–TSC–Ag protein method. β-glycogen particles can be seen in the cytoplasm of the capsulogenic and sporoplasm cells (asterisk). The asynchronous development of the adjacent sporont is apparent. In the upper capsulogenic cell, a rudimentary filament can be seen in sections of the external tubule (Et), while in the lower cell, neither the capsular primordium nor the external tubule contains a filament. ×15,130.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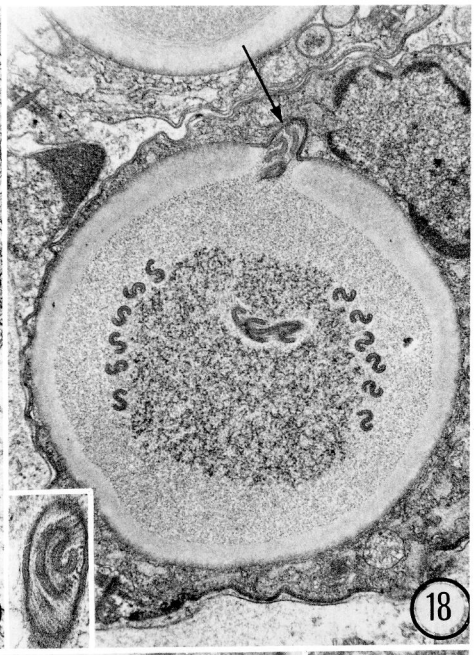
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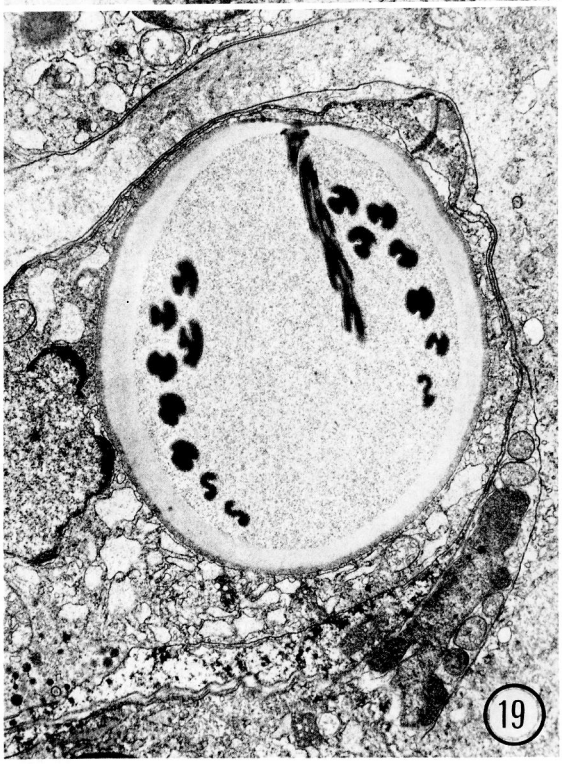
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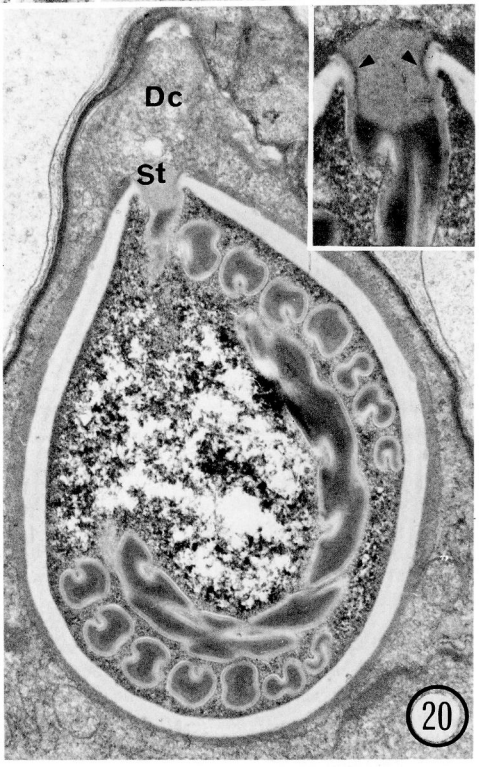
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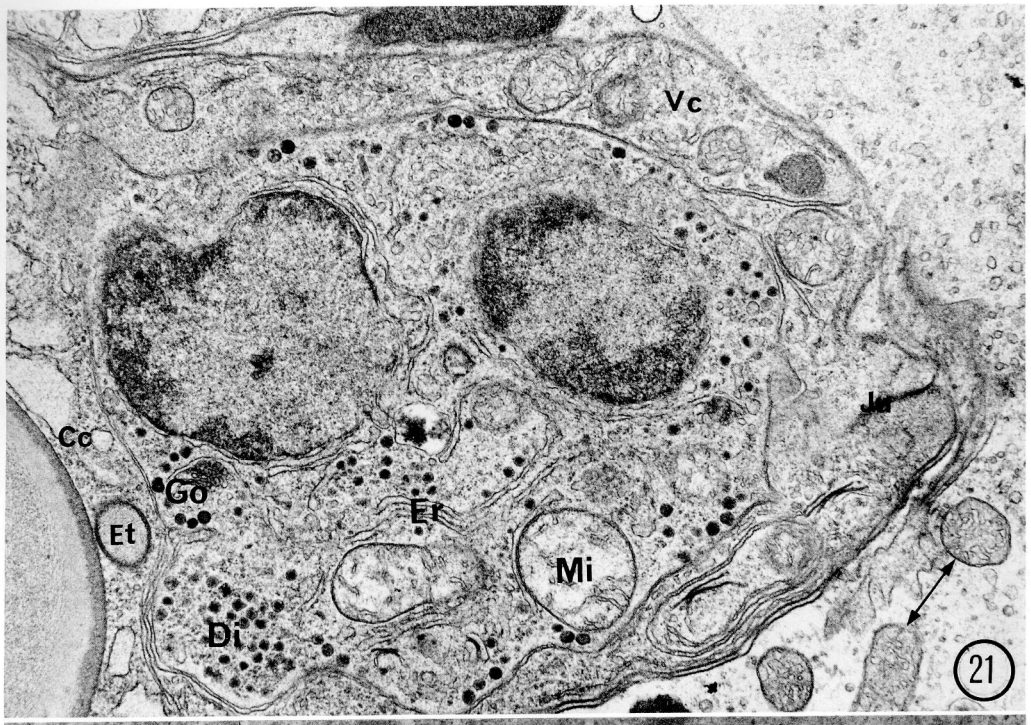
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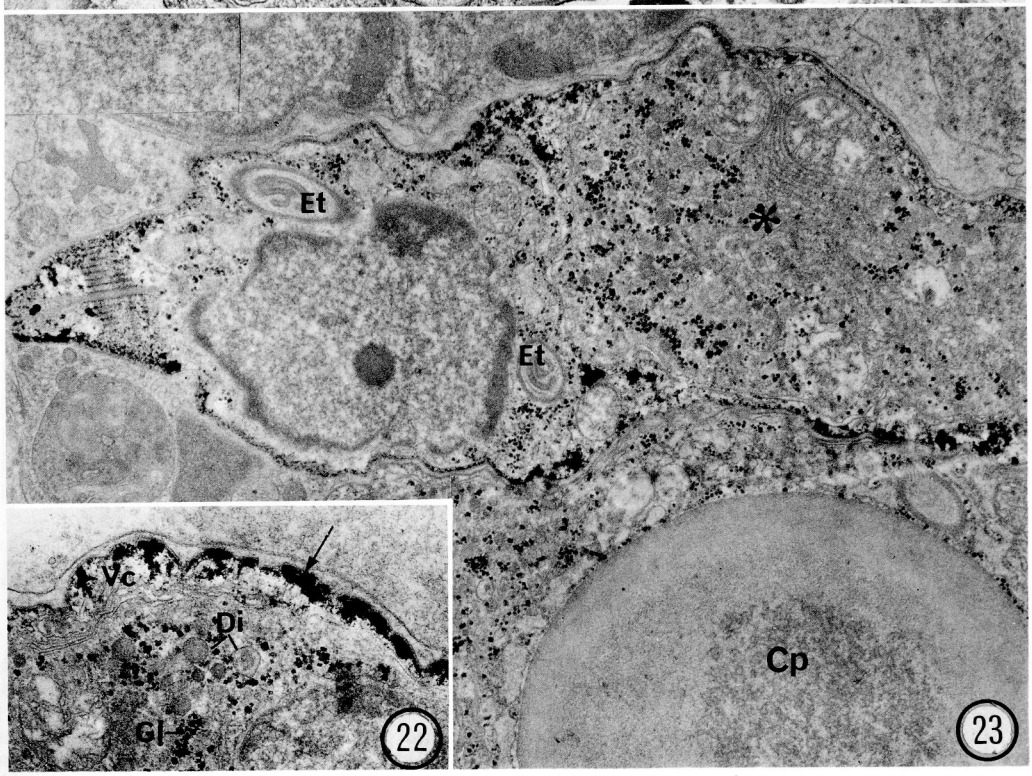
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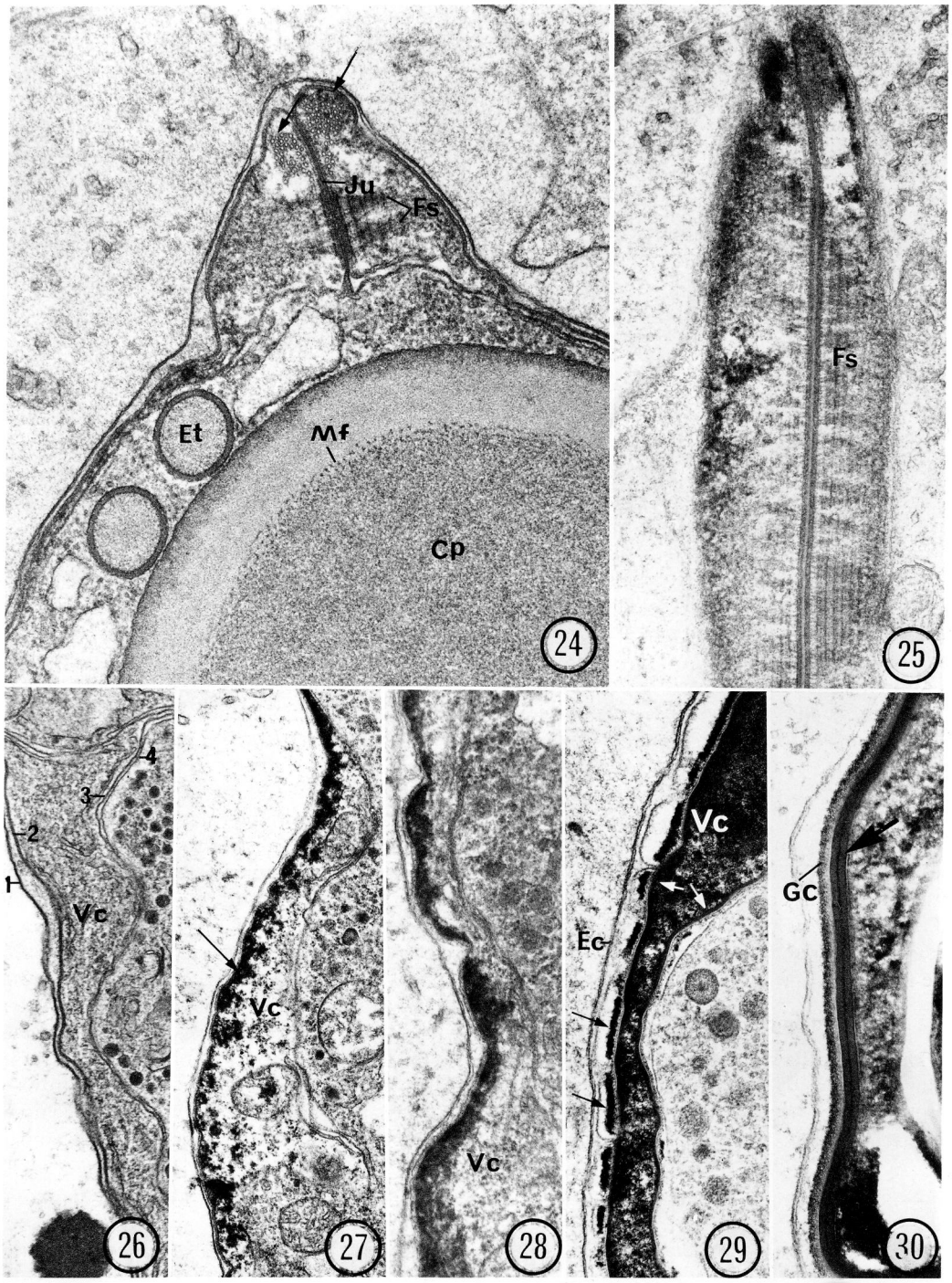

FIGURES 24–30. **24.** Transversely-sectioned, sutural flange formed by conjoined valvogenic cells. Numerous microtubules (arrows) are concentrated beneath the limiting membrane at the tip of the flange on either side of the junctional apparatus (Ju). Beneath the microtubular zone the junction is reinforced by two rows of banded fibrillar material (Fs). ×31,200. **25.** Longitudinal section through sutural flange illustrating the continuous junctional apparatus and adjacent, parallel, supporting, fibrous structure (Fs). ×32,400. **26–30.** Stages in the development of the spore wall. ×19,550. **26.** The membranes in an immature sporoblast: 1, the persistent envelope

er, dense, granular coat which overlay a well-defined, bilayered, electron-dense band (Fig. 30). The band consisted of the previously-described, closely-appressed upper and lower, dense layers formed in the cytoplasm of the valvogenic cells. The two layers of the band were separated in the sutural flange region where the dense cytoplasm of the valvogenic cells intervened. The plasmalemma of the underlying cells was closely applied to the dense band. The limiting membrane of the valvogenic cell often persisted around the outer granular layer of mature spores and remnants of the membrane of the envelope cell were also sometimes seen.

DISCUSSION

The cyst wall

Plasmodia of *T. nikolskii* were surrounded by a prominent cyst wall of host origin. Cyst walls around young plasmodia were comprised of closely-spaced, collagen fibrils. Individual fibrils were indistinct in more mature cysts that were relatively homogeneous in appearance, although the periodic banding which characterizes collagen was still apparent.

Similar cysts have not been recorded in ultrastructural studies of other histozoic species of Myxosporea. Current and his coworkers (1977, 1979) and Desser and Paterson (1978), for example, observed that plasmodia of *Henneguya exilis, Myxosoma funduli,* and *Myxobolus* sp., respectively, lay in direct contact with basal cells between the gill lamellae of the hosts. The formation of collagenous cysts around plasmodia of *T. nikolskii* is probably related to the localization of the parasite in the connective tissue of the fins.

The nature and origin of the plasmodium

The plasmodia were separated from the overlying cyst wall by a membrane with a closely-applied, outer, membranelike layer, coated with fine granular material. The origin of these "membranes" was impossible to determine in advanced plasmodia. Possibly the outer, coated, membranelike layer that separates the cyst from the plasmodium represents the modified basal lamina of the epidermis. The palisadelike folds in the young plasmodia and the channels and vesicles extending from the limiting membrane into the ectoplasmic layer of the plasmodia probably serve to facilitate in the uptake of nutrients.

The surface features of plasmodia of *T. nikolskii* differ from those of coelozoic species. The limiting membrane of plasmodia of *Sphaeromyxa* species, which occur in the gall bladder of their hosts, is thrown into microvilluslike folds that extend into the surrounding fluid (Lom, 1969; Grassé and Lavette, 1978).

Cytological studies on sporogenesis in the Myxosporea thus far have focussed on relatively advanced stages of plasmodial development, and it has not been possible to determine the origin of the plasmodium. Certain features of Myxosporean development, based on early light microscopic observations have been accepted as dogma. It is generally believed, for example, that the entire plasmodium is "parasite." This concept poses certain problems, one of which concerns the so called "vegetative" nuclei. For many years, on the basis of questionable evidence, these nuclei have been attributed the capacity of acquiring cytoplasmic organelles and limiting membranes to form the generative cells. Perhaps the plasmodium is a parasite-induced syncytium and the vegetative nuclei are host cell nuclei. Dramatic distortion of host cells by protozoan parasites has been well documented. *Leucocytozoon simondi* transforms phagocytic cells of the avian host into megaloschizonts up to 200 μm in diameter, whose hypertrophied nucleus and cytoplasmic components serve as a "metabolic factory" for the developing parasite (Desser and Fallis, 1967). Similar parasite-induced changes are seen in muscle and connective tissue cells infected with heteroxenous isosporoid par-

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cell; 2, 3, the flattened valvogenic cell; 4, the underlying sporoplasm cell. **27.** Dense material accumulates beneath the peripheral limiting membrane (arrow) of the valvogenic cell. **28.** The dense material becomes confluent. **29.** The cytoplasm of the valvogenic cell appears dense, and narrow, homogeneous bands (white arrows) are formed adjacent to the limiting membranes of the cell. Segments of dense material (arrows) are laid down above the peripheral dense band and beneath the now loosely-applied, limiting membrane of the valvogenic cell. At this stage the membrane of the envelope cell (Ec) still persists. **30.** In mature spores the two homogeneous bands formed in the valvogenic cell are broader and are appressed into a bilayer (arrow). A dense granular coat (Gc) overlies the bilayer. The spore wall is invested by the loose, limiting membrane of the valvogenic cell and sometimes persistent fragments of envelope cell membrane.

asites such as *Sarcocystis* and *Besnoitia* species (Dubey, 1977).

Although the precursor of the plasmodial stage of Myxosporea is not known there is some circumstantial evidence for a primary proliferative stage. In the case of *Myxosoma cerebralis,* for example, proliferative stages of a protozoanlike parasite were observed in the epithelium of young rainbow trout as early as 1 hr following their exposure to spores of this parasite (Daniels et al., 1976). Such primary stages are probably transported via the circulatory system to the target tissues where sporogenesis occurs. We suspect that a peculiar "sporozoanlike" parasite described from the peripheral blood of carp by Csaba (1976) may represent a precursor stage of *T. nikolskii.* In carp infected with *T. nikolskii,* stratum germinativum cells of the fin epidermis may be invaded by the plasmodial precursor, which could conceivably induce massive hypertrophy of the host cell and its nucleus with the resulting formation of a syncytial nurse cell, the plasmodium.

This hypothesis may apply to the majority of myxosporean species examined thus far in which plasmodium formation has been observed, but not in the case of *Sphaerospora angulata* where pansporoblasts occur in the lumen of renal tubules (Molnar, 1980). Perhaps in this species and in others where a plasmodium is lacking, preliminary sporogenic stages occur elsewhere in the host's tissue.

Early sporogenesis

It is generally accepted that sporogenesis is initiated by the association of two generative cells, one of which subsequently envelopes the other. Almost all of the ultrastructural studies of myxosporean sporogenesis to date contain illustrations of the presumed sequence of development including single, generative cells, two of the latter cells in close association, and a generative cell enclosed by an envelope cell. The presence of intercellular bridges between two generative cells of *T. nikolskii* (as illustrated by Fig. 7) suggests the possibility that sporogenesis may be initiated by the division of a single generative cell into the putative envelope and generative cells, the latter of which undergoes subsequent division to form the various spore-forming cells in the pansporoblast. Although intercellular bridges were observed often in early sporogenic stages, nuclear division was never seen, suggesting that the latter process occurs rapidly.

The fate of individual cells in the young sporonts was difficult to follow beyond the four-cell stage in thin sections. Analysis of many micrographs suggested that the development of the second capsulogenic cell of each sporont was abortive, resulting in the formation of only a single capsule-forming cell in each spore. This abortive development resulted in the formation of a dense, spherical body which was initially connected to the defective capsulogenic cell by an intercellular bridge (see Fig. 12) and which later persisted as a detached, membrane-bound, dense sphere. The defective capsulogenic cell subsequently degenerated. Our interpretation of these events differs from that of Debaissieux (1925) who believed that only a single capsulogenic cell is formed in *Thelohanellus* species.

Formation of the polar capsule and filament

Data from the present study indicate that the capsular primordium and external tubule are formed simultaneously in young capsulogenic cells. The reinforced terminal portion of the external tubule appeared to be anchored in position adjacent to an electron-dense cap, which subsequently formed the corklike stopper in the mouth of the mature polar capsule. Similar features have been described for stages of development of the polar capsules of other myxosporean species (Lom and de Puytorac, 1965; Lom, 1969; Desser and Paterson, 1978; Grassé and Lavette, 1978). Further elongation of the external tubule appears to occur in the nonreinforced portion which eventually coils about the expanding capsular primordium.

The microfilamentous girdle which surrounds the granular matrix of young capsular primordia was no longer apparent by the time immature polar filaments were observed in the capsule. If contractile in nature, this girdle could conceivably play a role in the incorporation of the external tubule into the capsular primordium. That the latter process occurs is supported by invagination of the walls of the polar capsule to form the walls of the internalized filament (see Fig. 20, inset).

The rudimentary polar filament is formed simultaneously within the capsular primordium and external tubule, possibly from the dense, granular material present in both stages. The process of invagination of the external tubule into the capsule could not be clearly determined from our micrographs, but must occur rapidly, be-

cause when the first rudimentary filaments were observed in the capsules, the external tubule (which was formerly so prevalent) was rarely seen. Once incorporated into the polar capsule the filament undergoes considerable modification. The diameter of its lumen increases considerably and it contains a homogeneous dense material. These changes are accompanied by the disappearance of the dense granular material which previously occupied the matrix of the capsular primordium.

The concentration of β-glycogen particles was greatest in the cytoplasm of sporogenic cells of *T. nikolskii*. A similar observation was made for *Myxobolus* sp. (Desser and Paterson, 1978). The spherical, dense bodies in the cytoplasm of sporoplasm cells are a common feature in all myxosporean species examined thus far. That these bodies (which according to Current and Janovy, 1977, contained stored metabolic products) were not carbohydrates, was indicated by their failure to stain by the PA–TSC–Ag method.

With the exception of the aforementioned abortive formation of the two capsulogenic cells, the general process of polar capsule formation in *T. nikolskii* closely resembles that described for other myxosporean species (Lom and de Puytorac, 1965; Lom, 1969; Current and Janovy, 1977; Grassé and Lavette, 1978).

Formation of the spore wall

The spore wall in *T. nikolskii* is formed from components synthesized in the valvogenic cells. In maturing sporoblasts electron-dense carbohydrate material accumulates beneath the limiting membrane and eventually occupies most of the cytoplasm. The narrow, homogeneous, dense layers subsequently appear adjacent to the proximal and distal limiting membranes of the flattened cells. These layers merge to form a bilaminate spore wall which is overlain by an additional covering of dense, granular material. The latter remains bound by the distended limiting membrane of the valve-forming cell.

The affinities of the Myxosporea have long been a subject of debate. Although myxosporean polar capsules resemble the nematocysts of certain coelenterates, it is generally accepted that the two groups of organisms are not related phylogenetically. More than 20 yr ago, Grassé (1960) suggested that because of the multicellular nature of their plasmodia, the "Myxosporidia + Actinomyxidia" should be transferred from the Protozoa to a new phylum, the Myxozoa. This suggestion has been accepted recently by an International Commission on Protozoan Nomenclature (Levine et al., 1980), which erected the new phylum Myxozoa containing classes Myxosporea and Actinosporea.

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