Fine structure of endogenous stages of *Eimeria turcicus* developing in gall bladder epithelium of the gecko *Hemidactylus turcicus*

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Transmission electron microscopic study of endogenous development of *Eimeria turcicus* Upton, McAllister and Freed, 1988 (Eimeriorina, Apicomplexa), in the gall bladder epithelium of the gecko *Hemidactylus turcicus* from Israel, revealed merogony and gamogony stages which induce hypertrophy and displacement of their host cell from the epithelial layer. Meronts yield over 70 merozoites. Microgametes are biflagellated. Macrogamonts contain both types of wall-forming bodies (WF1, 2). In the zygote, disaggregation of WF1s coincide with the onset of oocyst wall formation, while WF2s persist. A multilayered wall is formed at the first stage of the wall formation. Subsequently, 5 membranes (M1–5) are formed at the surface of the zygote like in other eimerine coccidia. The oocyst wall appears to be formed by M3 and M4.

Transmissie-elektronmikroskoopstudies van die endogene ontwikkeling van *Eimeria turcicus* Upton, McAllister en Freed, 1988 (Eimeriorina, Apicomplexa), 'n galblaasepiteelparasiet van die geitjie, *Hemidactylus turcicus* van Israel is uitgevoer. Hierdie studie het aangetoon dat daar stadiums van merogonie an gamogonie voorkom wat hipertrofie veroorsaak en 'n verplasing van gasheerselle uit die epiteellaag tot gevolg het. Meer as 70 merosoïete ontstaan per meront en die mikrogamete dra elk twee flagellums. Makrogamete bevat beide die WF1, sowel as WF2 wandvormende liggaampies. In die sigoot val disaggregasie van die WF1-liggaampies saam met die aanvang van oösist wandvorming, terwyl die WF2 wandvormende liggaampies bly voortbestaan. In die eerste stadium van wandvorming ontstaan 'n veelvuldige laagwand en vervolgens word vyf membrane (M1–5) op die oppervlak van die sigoot gevorm. Hierdie proses stem ooreen met dié by ander verwante Coccidia. Die oösistwand word waarskynlik deur die M3- en M4-membraan gevorm.

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Eimeria turcicus Upton, McAllister & Freed 1988 was described from the gall bladder of the East Mediterranean house gecko *Hemidactylus turcicus* Ruppel, 1835, recently introduced and established in Texas, USA. Only exogenous stages are reported in Upton, McAllister & Freed's (1988) taxonomic description. It is a common gall bladder parasite of *H. turcicus* in Israel.

The gall bladder is a common site for eimerian infections of reptiles. Such coccidia typically have an elongate elliptic or cylindroid oocyst without a micropyle and their sporocysts lack a stieda body (Bovee & Telford 1965; Cannon 1967; Vetterling & Widmer 1968).

At present, all except one of these species are classified as *Eimeria*. *E. flaviviridis* which according to Setna & Bana 1935, has a bivalved sporocyst wall has been transferred by Levine (1983) to the genus *Goussia,* which contains predominantly piscine species. Bivalved sporocysts were also reported in *E. turcicus* (Upton *et al.* 1988).

Accounts of light microscopy studies of endogenous development in the gall bladder epithelium mention hypertrophy and an associated protrusion of infected cells from the epithelial layer. Infected cells are eventually sloughed off into the gall bladder lumen, where oogony and sporogony are completed (Fantham 1932; Setna & Bana 1935; Brygoo 1963; Bovee & Telford 1965; Cannon 1967; Vetterling & Widmer 1968; Bovee 1969; Clark 1970).

Reptilian gall bladder eimerians, which have a distinct sporocyst structure and a unique relationship with their host cell during endogenous development, appear to be very different from eimerians of avian and mammalian hosts. This paper presents the first description of the fine structure of an eimerian from a reptilian host and of an endogenous 'proliferative' development in the epithelial cells of the gall bladder.

Materials and Methods

Gall bladders from four naturally infected house geckoes, *H. turcicus* from Israel were studied by transmission electron microscopy (TEM).

Infection in the geckoes was confirmed by the demonstration of oocysts in light microscope (LM) examinations of samples drawn both from the stools dissolved in a few drops of tap water and from the bile. Oocysts were measured with a calibrated ocular micrometer.

Infected gall bladders were fixed following Karnovsky's (1965) method for 24 h at 4°C, rinsed repeatedly in cacodylate buffer (0,1M; pH 7,4) and post-fixed in 1% osmium tetroxide in the same buffer for 1 h. After further rinsing in the same buffer the tissues were dehydrated in ethanol and embedded in epon. Thin sections cut by an LKB III ultratome with a diamond knife were stained on the grid with uranyl acetate and lead citrate and examined with a Joel 100CX TEM. Measurements

Key to abbreviations on figures

A:	Amylopectin granules.
a:	Apical complex.
ab:	Adnuclear body.
Cn:	Canaliculi.
c:	Centriole.
ed:	Electron dense globules.
er:	Endoplasmic reticulum.
f1–f2:	Flagellae.
G:	Golgi.
GC:	Granular cisterna.
g:	Microgamete.
gb:	Gall bladder epithelium.
H:	Host cell.
hn:	Host nucleus.
L:	Lipid vacuoles.
M1–5:	Pellicular envelopes of the oocyst.
MA:	Macrogamont.
MI:	Microgamont.
Mr:	Meront.
Mz:	Merozoite.
m:	Mitochondria.
mn:	Micronemes.
mp:	Micropore.
n:	Nucleus.
nu:	Nucleolus.
Ow:	Oocyst wall.
P:	Parasite.
pv:	Parasitophorous vacuole.
R:	Residual body of the microgamont.
RM:	Residual body of the meront.
r:	Rhoptries.
s:	Subpellicular band.
sp:	Sporocysts.
sz:	Sporozoites.
Un:	Uninfected host cell.
W1:	Wall-forming bodies type 1.
W2:	Wall Forming Bodies type 2.

were taken from the TEM photomicrographs.

Semithin sections for light microscopy studies were cut from the epon-embedded tissue with a glass knife and stained in toluidine blue.

Results

In light microscopy endogenous stages were seen inside hypertrophic cells which extend from the surface of the gall bladder epithelium (Figure 1). Unfixed oocysts from the gall bladder content (Figure 2) were oblong, 33–39 × 15–20 μm in size (n = 15) and contained either four 8–12 × 7–9 μm sporocysts, or free 9–10 × 3–4 μm sporozoites.

Changes in the infected host cell

Hypertrophy as well as emergence of the host cell from the epithelial layer occurred at an early stage of infection by either meronts or gamonts (Figure 3). With further parasite development, the host cell gradually became displaced from the epithelial layer to above the surface (Figures 1, 3, 4). The enlarged parasitized cell (to 10–24 × 10–15 μm) retained its microvillar surface. However, the density of the microvilli declined with increased hypertrophy of the host cell (Figures 3, 4).

The junction zone between the parasitized cell and its adjacent non-infected cells did not differ from that normally connecting non-infected cells and in both cases the membrane borders contained many desmosomes (Figure 5). The attachment area between the parasitized cell and the cells of the epithelial layer subsequently became reduced to a narrow peduncle (Figures 3, 4). The cytoplasm of the host cell retained its normal fine structure until late stages of merogony, microgamogony or early formation of the oocyst. Gradual degeneration of the cytoplasm was evident only during oogony; this appeared to coincide with the detachment of the host cell from the gall bladder epithelium. The parasitophorous vacuole contained variable amounts of a granular material at all stages of infection (Figures 3, 4). Infected cells usually contained a single parasite. Occasionally two parasites of different age occurred simultaneously in the same host cell (Figure 4).

Merogony

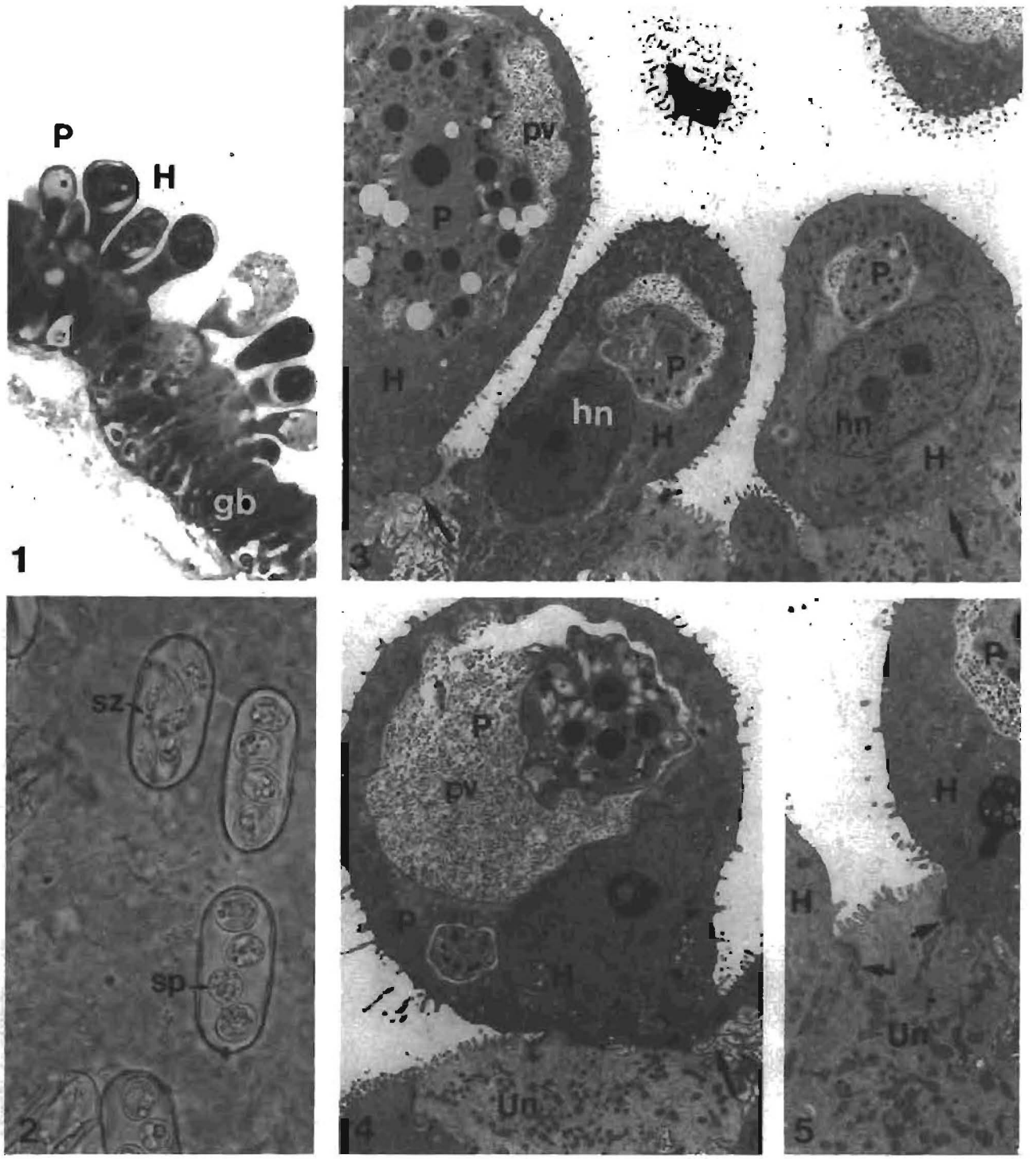
Merozoites entering host cells were 4,5–5,5 × 1,2–1,5 μm in size (Figure 6). Some young stages were showing one segment retaining merozoite features — three membrane pellicle, conoid, and rhoptries while the other segment was already bound, like a meront, by a single unit membrane (Figure 7).

Multinucleate meronts (19–22 × 10–12 μm) were distinguished from young microgamonts by the presence of a nucleolus in their nuclei (Figure 8). These meronts contained a dense network of smooth endoplasmic reticulum (ER), many tubular mitochondria (Figure 9), very few food vacuoles and numerous globules of high and medium electron density (Figures 8, 9). Nuclei were arranged at the peripheral zone of the meront and were accompanied by 'adnuclear' organelles: aggregates of electron lucent enclaves fringed at one end by an electron dense belt (Figures 8, 9).

Meronts yielded over 70 merozoites (Figures 10, 11), intermediate stages in the division process were not found. Both the residual meront cytoplasm and the merozoites contained amylopectin granules (Figure 11). In fully differentiated merozoites (7–8 × 1,5–1,8 μm) rhoptries and micronemes densely filled the area between the conoid and the nucleus (Figure 10).

Microgamogony

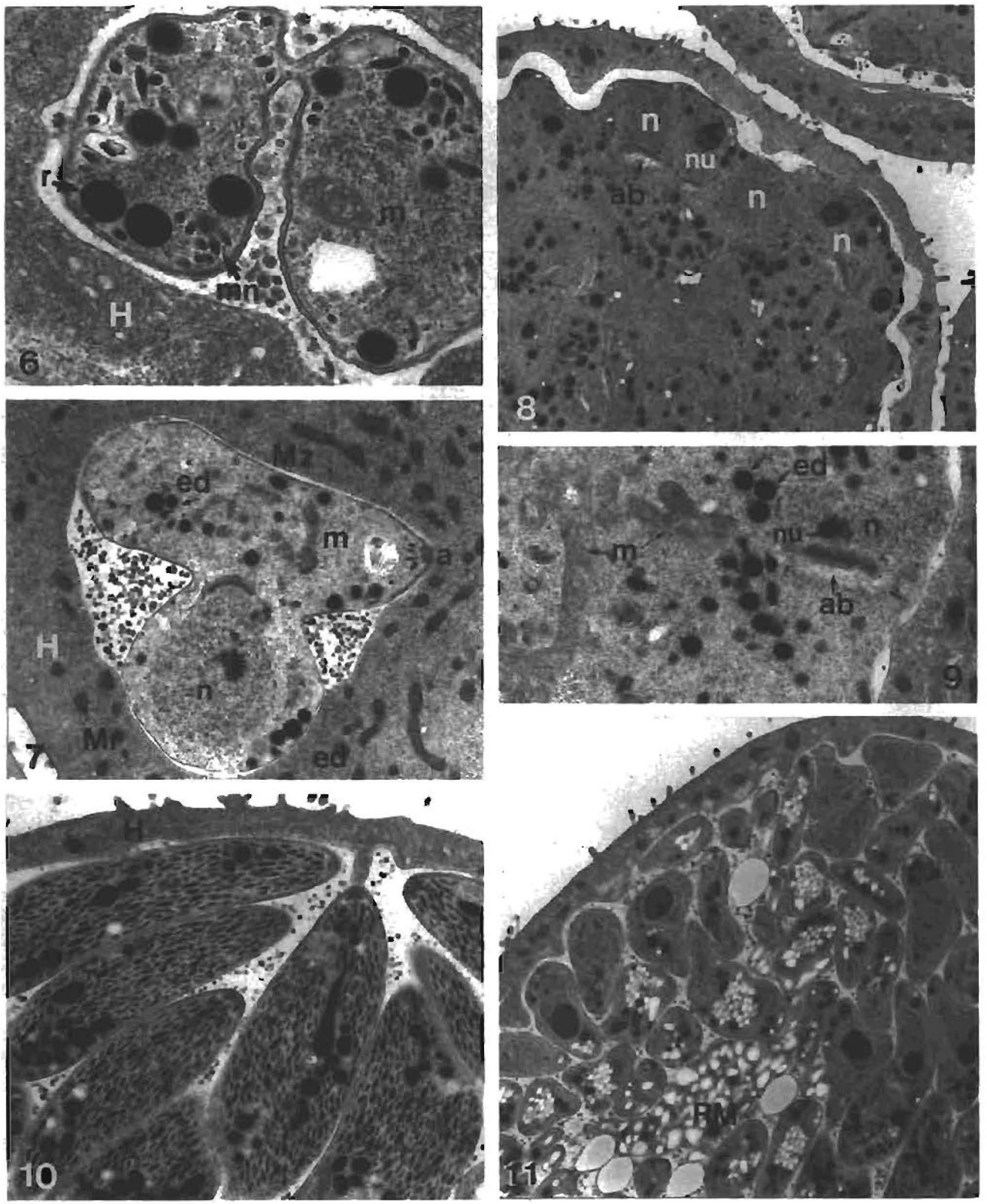
Immature microgamonts (reaching a size of 16 × 9 μm) (Figure 12) contained numerous peripherally arranged nuclei. In the ultrathin sections only one centriole could be demonstrated near each nucleus (Figures 14, 15). In the nuclei the nucleolus was absent and the chromatin was peripherally arranged (Figure 12). The plasma membrane near the centriole, next to the nucleus, was accompanied beneath by an electron dense band — the perforatorium anlage (Ferguson, Birch-Andersen, Hutchinson & Siim 1977a) (Figures 14, 15, 16). The microgamont's



Figures 1–5 1. LM view of infected gall bladder epithelium (semithin section stained Toluidine blue). ×940. 2. Oocysts of *G. cf. flaviviridis* from the gall bladder content. LM ×771. 3. Infected gall bladder epithelium cells, hypertrophic and displaced, in one cell attachment is reduced to narrow peduncle (arrow). TEM ×5400. 4. Displaced host cell with double infection (macrogamont and merozoite). TEM ×5100. 5. Junction zone between uninfected and infected host cells with desmosomes (arrows). TEM ×13200.

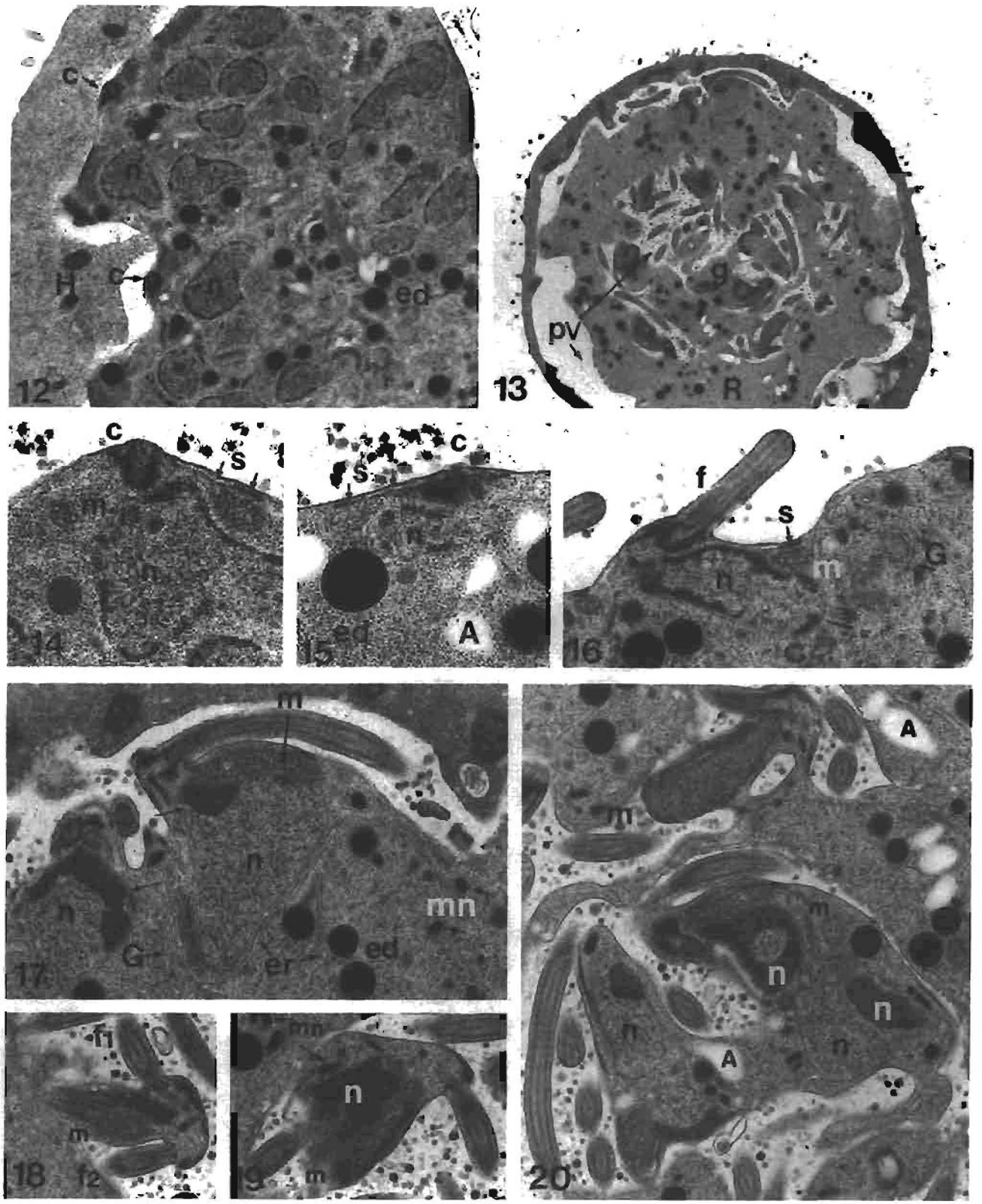
cytoplasm contained many electron dense globules and a few amylopectin granules (Figures 12, 15).

These inclusions were also present in the residual cytoplasm of the mature microgamont and in the forming microgamete (Figures 16, 17, 20). In the latter also occurred microneme-like organelles (Figures 17, 19). Mitochondria were tubular and were only seen in association with nuclei at the periphery of the microgamont (Figures 13, 15). Mature microgamonts were 14–16 µm in diameter. Deep infoldings increased the surface area of the differentiating microgamont (Figure 13). Flagella developed from the microgamete anlagen before the beginning of nuclear differentiation (Figure 16). A very distinct golgi apparatus occurred in the residual



Figures 6–11 6. Young bilobated merozoite. TEM ×22000. 7. Young bilobated meront with one segment retaining double membrane pellicule, conoid and rhoptries. TEM ×10700. 8. Multinucleate meront. TEM ×7900. 9. Enlarged sector of multinucleate meront, showing nucleus, adnuclear body, mitochondria and electron dense globules. TEM ×13150. 10. Divided meront with fully developed merozoites cut at their proximal part showing apical organ, rhoptries and micronemes. TEM ×10500. 11. Divided meront with fully developed merozoites cut at their median and distal parts and a residual body. TEM ×7200.

cytoplasm in association with differentiating nuclei (Figures 16, 17). The portion of the nucleus which extended into the protrusion was accompanied by a large, long mitochondrion (Figures 17–20). Condensation and separation of the microgamete nucleus from the residual nucleus occurred only at a more advanced stage in

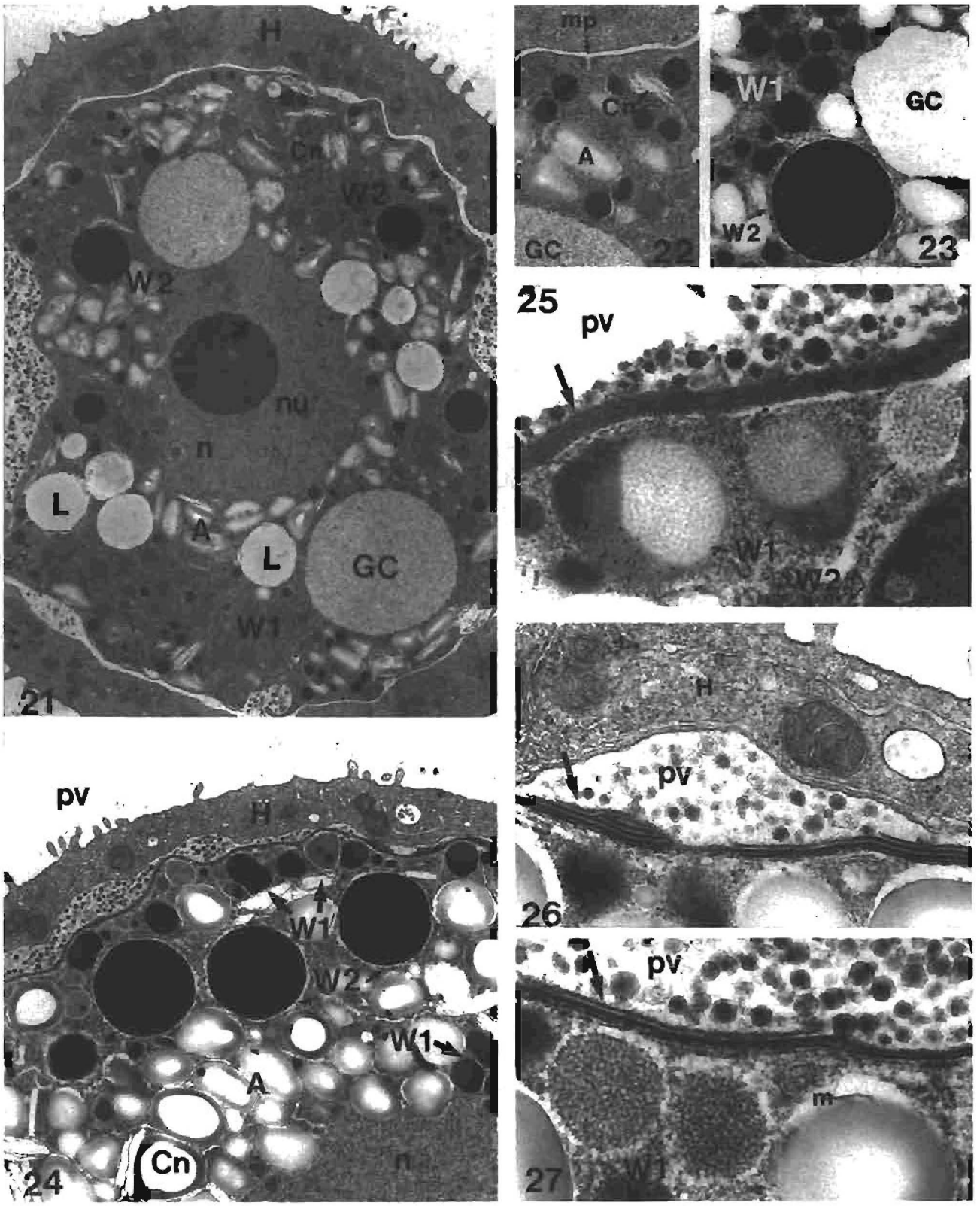


Figures 12–20 12. Young multinucleated microgamont with centrioles. TEM ×10700. 13. Differentiating microgamont with deep infoldings. TEM ×4750. 15 & 16. Centriole and an electron dense band positioned next to peripherally located nucleus and mitochondria. TEM ×27500. 16. A flagellum emerging next to peripheral nucleus accompanied by mitochondria and golgi complex. TEM ×19500. 17. Emerging microgamete; early condensation of the nucleoplasm (arrow). TEM ×17300. 18–20. Microgamete in different stages of maturation: with partly or completely condensed nucleus, amitochondrium, micronemes and two flagella. TEM ×12450 (Figure 18), ×15000 (Figure 19) and ×17300 (Figure 20).

microgamete development (Figures 17–20). Two flagella were identified in microgametes in the available ultrathin sections (Figures 18, 19).

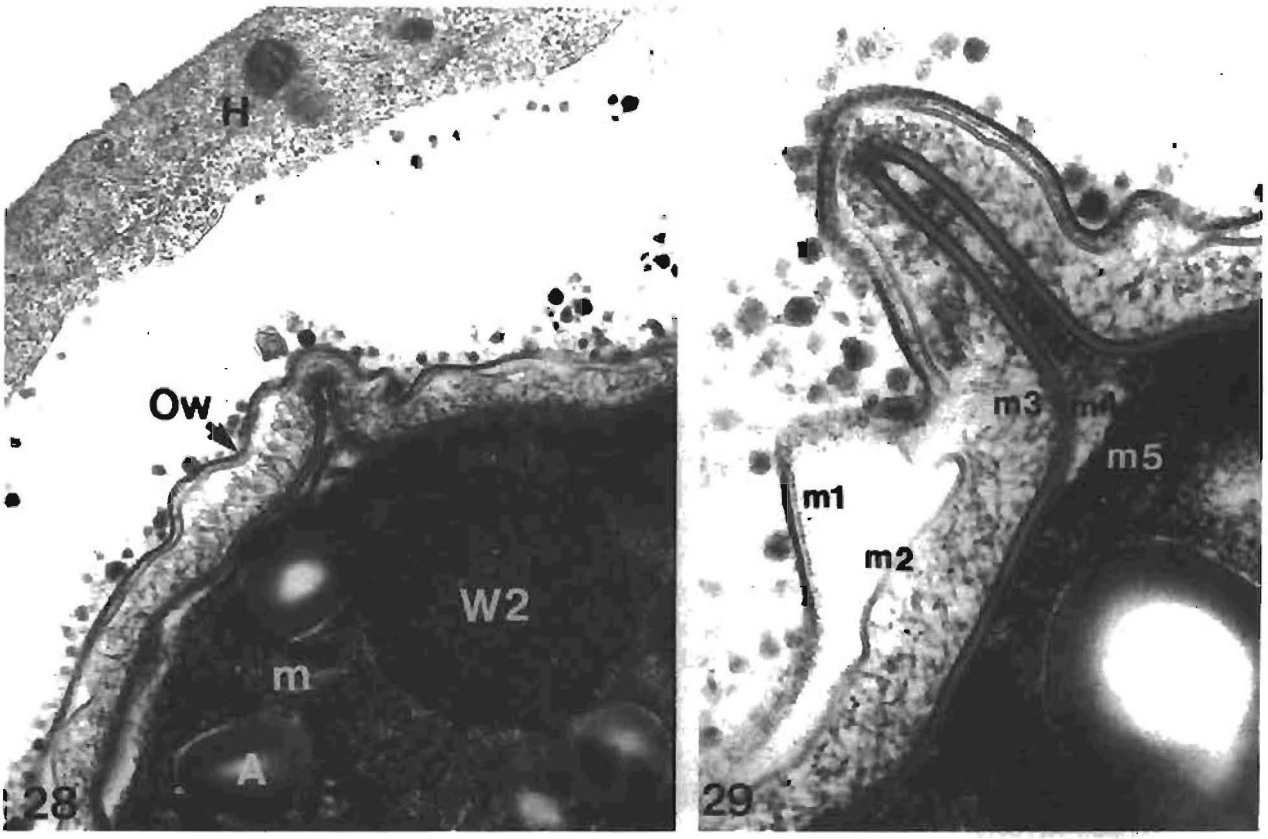
Macrogamonts

Mature macrogamonts (Figure 21) were spherical (10–15 μm in diameter) and bound by two membranes interrup-



Figures 21–27 21. Macrogamont. TEM ×7600. 22. Details of macrogamont's peripheral zone showing micropore. TEM ×17700. 23. Enlarged sector in a macrogamont at high contrast photomicrography showing the electron dense nature of the WF1s. TEM ×12170. 24. Zygote. TEM ×9040. 25. Multilayered wall of the zygote, WF1s in stages of disaggregation. TEM ×43000. 26. Multilayered wall of the zygote in fragmentation. TEM ×31300. 27. Zygote with fragmented wall and WF1s in advanced stage of disaggregation. TEM ×43000.

ted by some micropores (Figure 22). The centrally positioned nucleus contained a large dense nucleolus. Many small elongated mitochondria and a few large spherical mitochondria were located in the peripheral zone. The cytoplasm was transected by a loose network of rough ER (rER) and contained several large lipid vacuoles,



Figures 28–29 28. Young oocyst enclosed in membranous envelopes. TEM ×22000. 29. Enlarged view of M1–5 envelopes of the oocyst. TEM ×44000.

numerous amylopectin bodies of oblong shape, canaliculi and a few small food vacuoles (Figures 21, 22). Numerous variably sized (0,45–0,60 μm diameter), medium as well as high electron dense wall-forming bodies type 1 (WF1), were scattered throughout the cytoplasm (Figures 21, 23). A few large (0,8–1,6 μm diameter) electron dense wall-forming bodies type 2 (WF2) were located within rER cisternae (Figures 21, 23). Some macrogamonts also contained low electron dense, spherical and very expanded cisternae filled with a very fine granular substance (Figures 21, 22).

The zygote and the onset of the oocyst wall formation

The limiting membrane of the presumed zygote (13–15 μm in diameter) (Figure 24) was overlaid by a non-membranous electron dense band. This band was either homogenous, or consisted of 3–4 distinct layers which were either adherent (Figure 25) or in a process of detachment and fragmentation (Figures 26, 27). WF1s accumulated in the peripheral cytoplasm, while their dense contents gradually disaggregated into a granular substance of lower density (Figures 24, 25, 27). WF2s remained unchanged at this stage of zygote development (Figure 24, 25).

Young oocysts were enclosed by several membranes (Figures 28, 29):
M1 — an electron dense outer envelope or veil,
M2 — a membrane which consisted of two, bilaminated units separated by an electron lucent zone,
M3 and M4 — two electron dense envelopes separated from each other by a wide electron lucent gap and
M5 — a bilaminated membrane limiting the zygote cytoplasm.

Two delicate membranes were traced in the electron lucent gap between M3 and M4. The wide cytoplasmic zone between M2 and M3 contained reticular strands of fine granular material. The zone between M4 and M5 contained dense granular material (Figures 28, 29). At this stage all WF1s disappeared and only few WF2s, either intact or disaggregated into coarse granules were still present in the cytoplasm (Figure 28).

Discussion

It is assumed that the geckoes were parasitized by only one coccidian species because of the relatively uniform size of the oocysts encountered. Oocysts and sporocysts from the Israeli *H. turcicus* are identical in their dimensions to those described from the introduced population of *H. turcicus* from Texas (Upton *et al.* 1988).

Displacement of the infected epithelial cell to the surface of the epithelial layer, as revealed in this ultrastructural study of an eimerian infection of the biliary epithelium of geckoes, is unknown to occur among avian and mammalian coccidial infections, even in species such as *E. stiedai* which develop in the biliary epithelium (Scholtyseck 1979).

Displacement of the infected host cell to the surface of the epithelial layer occurs in *Goussia cichlidarum* infecting the swimbladder of fishes (Paperna, Landsberg & Feinstein 1986). Nevertheless there are several important fine structural differences between the presently described eimerian from the gecko gall bladder and *Goussia cichlidarum* or any other piscine eimerian thus far described (Paterson & Desser 1981a, b, 1984; Morrison & Hawkins 1984; Paperna *et al.* 1986).

Fine structural data demonstrate close affinity between our biliary eimeria, *Schellackia cf. agamae* (Lankesterellidae) from a reptilian host (Ostrovska & Paperna 1987) and eimerian coccidia of mammalian and avian hosts (Chobotar & Scholtyseck 1982): the presence in macrogamonts of WF1 and WF2, structurally and apparently also functionally similar to those described in eimerian coccidia of mammalian and avian hosts (Scholtyseck, Mehlhorn & Hammond 1971) and the formation of a hard oocyst wall. In *Goussia* from piscine hosts WF1 and WF2 are absent; organelles structurally reminiscent of either WF1 or WF2, if they do occur, do not appear to be involved in oocyst wall formation (Paterson & Desser 1981a, 1984; Paperna *et al.* 1986); the oocyst wall is soft and membranous and disintegrates at the end of oocyst formation (Dykova & Lom 1981; Paterson & Desser 1984; Paperna & Cross 1985).

On the strength of these observations it might be concluded, that this peculiar type of host parasite relationship evolved independently in coccidia of piscine and reptilian hosts.

The multilayered band formed over the early stage zygote is unknown from other coccidians. The succession of membranes forming around the zygote, on the other hand, is similar to that of the formation of membranes M1–M5 during oogony of *Eimeria* species of mammalian and avian hosts (Ferguson, Birch-Andersen, Hutchinson & Siims 1977b; Chobotar, Senaud, Ernst & Scholtyseck 1980; Sibert & Speer 1980; Pittilo & Ball 1984). The deposition of granular material between M2 and M3 and between M4 and M5 is reminiscent of the process of inner and outer wall formation between similar membranes in *Eimeria* species (Sibert & Speer 1980). However, the deposited material seems to disintegrate with further maturation of the oocyst. The oocyst wall seems to be formed from M3 and M4 alone. The electron lucent gap between M3 and M4 appears to be homologous with the gap which characteristically occurs between the outer and the inner oocyst walls in all hard-wall-forming eimerian coccidia (Ferguson *et al.* 1977b; Marchiondo, Duszynski & Speer 1978). Formation of additional fine lamellae within the gap were also reported in oocysts of *E. nieschulzi* (Marchiondo *et al.* 1978).

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