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Mémoire.

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ELECTRON MICROSCOPIC STUDIES OF *CARYOSPORA COLUBRIS* DEVELOPMENT IN THE SYRIAN BLACK SNAKE *COLUBER JUGULARIS*

I. PAPERNA

SUMMARY

Endogenous stages of *Caryospora colubris* Matuscha 1984 in the intestine of *Coluber jugularis* from Israel were studied by transmission electron microscopy. It is the first ultrastructural description of endogenous stages of *Caryospora*. The following stages are described: trophozoites, meronts microgamonts, macrogamonts and wall forming oocysts. The fine structure of the endogenous stages generally conforms with that of higher vertebrate *Eimeria* and *Isospora*. In the merozoites, however, the pellicle only lines the anterior tip, while the whole is bound by a single membrane with only fragments of an inner membrane. The wall formed around the oocyst, presumably the outer wall, is thick and contains a granular matrix. This stage of wall formation has been, thus far, not seen in other studied reptilian coccidia. Wall formation coincided with the disaggregation of type 1 wall forming bodies, while type 2 wall forming bodies remain unchanged.

RÉSUMÉ : **Étude ultrastructurale du développement de *Caryospora colubris* chez *Coluber jugularis*.**

La première étude ultrastructurale des stades endogènes du genre *Caryospora* est effectuée sur *Caryospora colubris* Matuscha, 1984, parasite de l'intestin de *Coluber jugularis* en Israël. Description des trophozoïtes, des mérontes, des microgamontes, des macrogamontes et de la formation des parois des oocystes. L'ultrastructure des mérozoïtes est conforme à celle des *Eimeria* et *Isospora* des vertébrés supérieurs, mais la pellicule limitante intéresse seulement l'extrémité antérieure, et l'ensemble de la cellule est entourée d'une membrane simple avec seulement des fragments de membrane interne. La paroi formée autour de l'oocyste, vraisemblablement la paroi externe, est épaisse et contient une matrice granuleuse. La formation de la paroi, qui n'avait pas encore été observée chez le coccidies de reptiles, s'effectue à partir de la désagrégation des initiales de type 1 (Wall Forming Bodies WF1), alors que les initiales de type 2 (WF2) restent inchangées.

C. colubris Matuschka 1984 was found in Israel in the Syrian black snake *Coluber jugularis*. A light microscopic (LM) description of this parasite's exogenous and endogenous developmental stages will be communicated elsewhere (Paperna and Finkelman, 1991). This is the first fine structural description of endogenous developmental stages of *Caryospora* in the intestine of its reptilian host. The only other available ultrastructural account of *Caryospora* is that of *C. bigenetica* caryocysts from tissues of experimentally infected mice (Sunderman and Linsday 1989).

MATERIALS AND METHODS

Transmission electron microscopic (TEM) studies of the endogenous stages were carried out on a laboratory infected juvenile (45 cm long) Syrian black snake. To ensure recovery of a wide variety of endogenous developmental stages the snake was orally inoculated with oocysts three times at nine-day intervals and was sacrified 43 days after the last inoculation, immediately following the recovery of oocysts in the faeces.

Small pieces of the anterior, middle, and hind gut were fixed in Karnovsky solution at 4C for 24 hours, washed three times in cacodylate buffer (0.1 M, pH 7.4) and post-fixed 1 hour in 1 % osmium tetroxide in the same buffer. After washing three times in the same buffer, the material was dehydrated in an ethanol series and embedded in Epon. Thin sections cut on a Reichert Ultracut ultratome with a diamond knife were stained on grid with uranyl acetate and lead citrate and then examined with a Jeol JEM 100CX TEM.

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RESULTS

Infections with all endogenous stages occurred mainly in the third quarter of the intestine.

MEROGONY STAGES

Oblong trophozoites (9-10 × 3-4 μm) inside the host cell *(fig. 1)* were bound by a single wavy membrane with a few fragments of additional inner membrane. Microtubules were traced beneath the boundary membrane *(fig. 2)*. Very

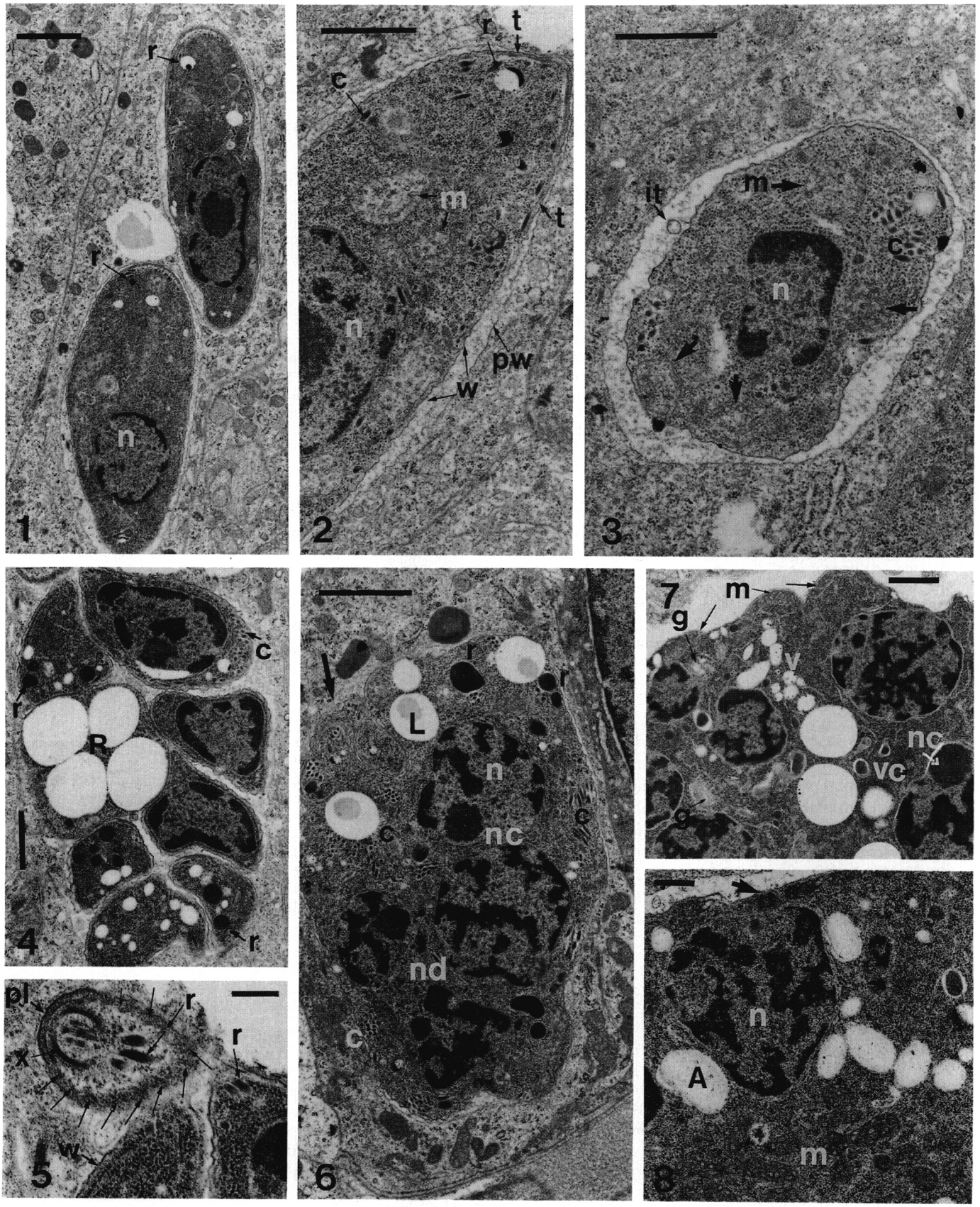
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PLANCHE 1.

few rhoptries, some with their contents extracted, were present; there were also only a few micronemes *(figs. 1, 2)*. The tubular mitochondria were large and conspicuous *(fig. 2)* and the nucleus contained peripherally aggregated chromatin and a large central nucleolus *(fig. 1)*.

Young meronts with a single nucleus in cross section, were bound by a single membrane. Their cytoplasm contained micronemes, several mitochondria and small vacuoles. One intravacuolar tubule was seen in the parasitophorous vacuole *(fig. 3)*.

Divided meronts contained eight merozoites and a vacuolated residual body *(fig. 4)*. The anterior end of the merozoite was lined with a pellicle while the whole was bound by a single membrane with only vague traces of a fragmented inner membrane *(figs. 4, 5)*. Microtubules extended from the apical complex *(fig. 5)* with a total of 24 counted beneath the cell wall of cross-sectioned merozoites. Merozoite transections revealed several rhoptries, micronemes, mitochondria and a nucleus with peripherally aggregated chromatin *(fig. 4)*.

Microgamonts

Young microgamonts (10-11 × 5-6 μm) with two large nuclei and one dividing nucleus were bound by a single membrane, had several lipid vacuoles and still contained many micronemes and a few rhoptries. Their surface formed several deep folds at one end *(fig. 6)*. Nuclei contained scattered strands of chromatin and a peripherally located nucleolus. All nuclei formed during microgamont differentiation were of similar structure *(figs. 7, 9)*.

The cytoplasm of the differentiating microgamonts was packed with ribosomes, and contained a dense network of endoplasmic reticulum (ER), vesicles or cisternae, which were either empty or contained a medium density granular substance, a variable number of lipid vacuoles and amylopectin granules, and golgi apparati *(figs. 7-9)*. Mitochondria were numerous, at the periphery of the gamont beneath the cell wall and also aggregated in the interior region *(figs. 7, 8)*. A centriole was seen *(fig. 8)* in a microgamont (16-18 × 10-11 μm in size) containing 14 nuclei in cross section *(fig. 9)*.

Only one mature microgamont with fully differentiated microgametes was found *(fig. 10)*. The residual cytoplasm contained many amylopectin granules.

Macrogamonts and oocysts

Macrogamonts (9-19 × 6-12 μm) were bound by a single, loosely apposed membrane and a plasmalemma. The cytoplasm contained a dense network of rough ER, large, peripherally arranged mitochondria, large rounded lipid vacuoles, smaller, oblong amylopectin granules, a few golgi elements, small, electron-dense type 1 wall forming bodies (WF1) and large type 2 wall forming bodies (WF2) surrounded by a concentric plexus of rough ER. Adnuclear organelles —inclusions containing a homogeneous, dense granular substance— occurred arround the nucleus *(fig. 11)*. In mature macrogamonts or zygotes the cytoplasm became filled with more amylopectin granules than lipid vacuoles, and contained arrays of canaliculi.

In the zygote the loose, outer wall membrane became doubled and the inner plasmalemma was gradually transformed into a detached membrane *(fig. 13)*, while a new plasmalemma was consolidating beneath it *(fig. 14)*. At this stage WF1 contents changed from high to medium electron density, while WF2 remained unchanged.

Young oocysts were bound by two bilayered membranes while their cytoplasm still contained WF1 *(fig. 15)*. Later stage oocysts were enclosed in a 0.5 μm thick wall *(fig. 16)* which consisted of an outer membrane and a median thick layer of granular matrix apposed to a basal double membrane. The oocyst cytoplasm was bound by a plasmalemma and contained residues of WF1, and large WF2 *(figs. 16, 17)*.

DISCUSSION

The fine structure of the endogenous stages, except for a few peculiarities, and the process of wall formation are both characteristic of eimerian coccidia from reptiles (Paperna 1989; Paperna and Landsberg 1989) and higher vertebrates (Chobotar and Scholtyseck 1982). However,

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Plate I. — *Fig. 1.* Oblong trophozoites, bar = 2 μm. *Fig. 2.* Higher magnification of an oblong trophozoite, anterior end, bar = 1 μm. *Fig. 3.* Young meront (mitochondria marked with arrows), bar = 1 μm. *Fig. 4.* Meront divided into merozoites, bar = 1 μm. *Fig. 5.* Enlarged view of anterior ends of merozoites (arrows point to microtubules extending from the apical complex), bar = 0.3 μm. *Fig. 6.* Young microgamont, bar = 2 μm. *Fig. 7.* Enlarged part of developing microgamont, bar = 1 μm. *Fig. 8.* Enlarged part of a premature microgamont seen in figure 9, showing a centriole (bold arrow) and arrays of mitochondria, bar = 0.5 μm.

Abbreviations : A, amylopectin granules; ad, adnuclear body; C, canaliculi; c, micronemes; er, endoplasmic reticulum; g, golgi aparatus; It, intravacuolar tubule; L, lipid vacuole; m, mitochondria; n, nucleus; nc, nucleolus; nd, dividing nucleus; p, plasmalemma; pl, pellicle; pw, parasitophorous vacuole wall; R, residual body; r, rhoptries; t, microtubules; v, vesicle; vc, vesicle with contents; w, parasite's cell wall; wf1, WF1; wf2, WF2; wo, forming oocyst wall; x, apical complex with conoid.

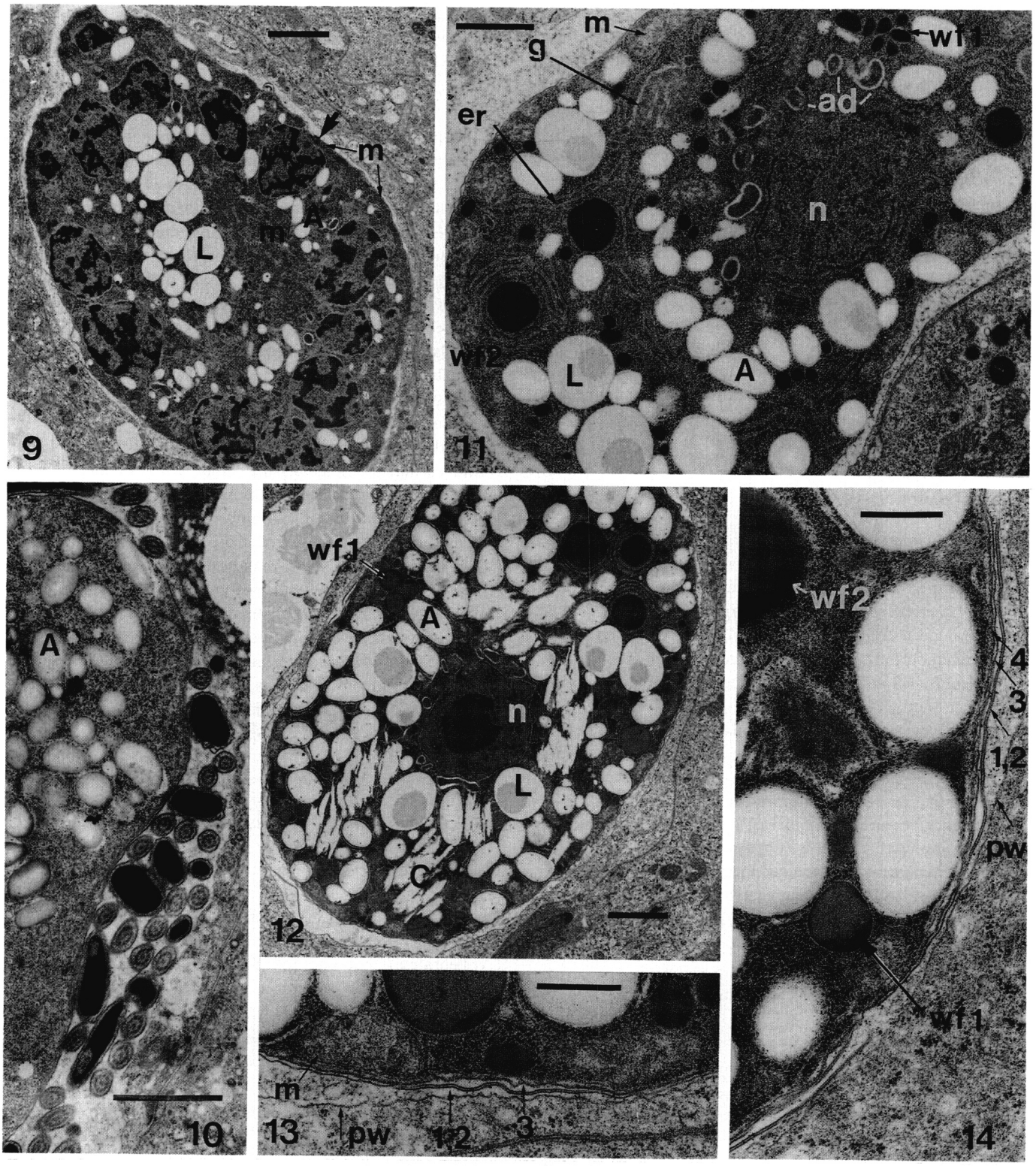

PLATE II. — *Fig. 9.* Premature microgamont, bar = 2 μm. *Fig. 10.* Mature microgamont with microgametes, bar = 2 μm. *Fig. 11.* Macrogamont, bar = 1 μm. *Fig. 12.* Late stage microgamont or a zygote, bar = 2 μm. *Figs. 13 and 14.* Peripheral zones of zygotes or young oocysts showing WF1 and the successively formed membranes (numbered 1-4), bar = 0.5 μm. Abbreviations: see legends of Plate I.

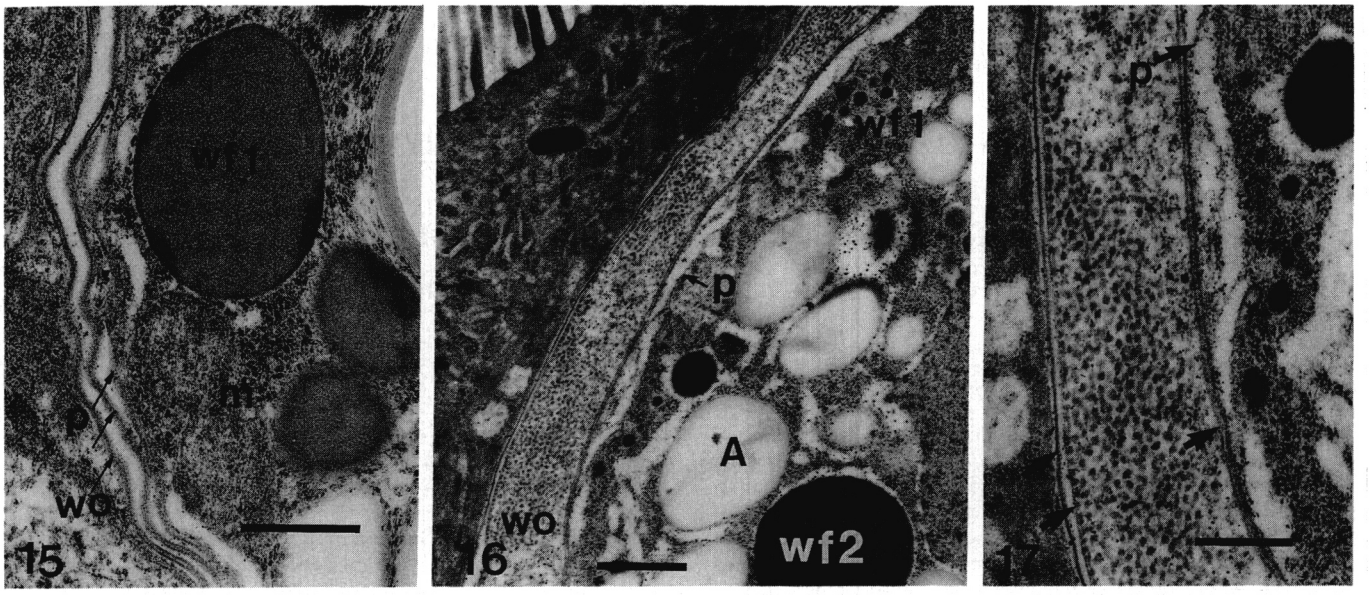

PLATE III. — *Fig. 15.* A further stage of oocyst wall formation showing two double membranes, bar = 0.5 μm. *Fig. 16.* A further stage in oocyst differentiation showing the appearance of a thick wall and degeneration of WF1; WF2 are still intact, bar = 1 μm. *Fig. 17.* Details of the thick (presumably outer) oocyst wall with a granular matrix (arrows point to the membranes involved), bar = 0.5 μm. Abbreviations: see legends of Plate I.

unlike other eimerian coccidia, in merozoites the second, inner membrane of the pellicle is thin and discontinuous, or altogether absent. The macrogamonts contain many adnuclear organelles resembling ER cisternae, the function of which is unknown. Until now they had only been seen in *Schellackia* cf. *agamae* macrogamonts (Ostrovska and Paperna 1987), and *Isospora* sp. from geckoes (Paperna, unpublished).

During the process of oocyst wall formation in eimerian coccidia of avian and mammalian hosts, the thin inner wall, claimed to be constructed with material from WF2, is formed at the same time or soon after the completion of the thick outer wall (Sibert and Speer 1980; Chobotar and Schotyseck 1980). TEM micrographs of some reptilian eimerian coccidia depict stages in which the outer wall is already formed while inner wall deposition between the fourth and the fifth membranes is only begining (Paperna 1989; Paperna and Landsberg 1989). In the wall-forming oocysts of *C. colubris* only four of the five membranes formed in other coccidia (Chobotar *et al.* 1980) were identified. The thick granular layer formed in this process apparently becomes the outer oocyst wall. Thus, the oocysts observed at a stage prior tp inner wall deposition. At this stage of development, WF1 in the oocyst cytoplasm has already disaggregated, while WF2 are still intact. LM observations demonstrated WF2's migration to and disaggregation at the periphery of walled oocysts prior or immediately after eviction into the gut lumen (Paperna and Finkelman, 1991). This must therefore be the stage at which inner wall formation occurs.

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