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Mémoire.

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DEVELOPMENT OF *CARYOSPORA COLUBRIS* (APICOMPLEXA: EIMERIIDAE) IN THE SYRIAN BLACK SNAKE *COLUBER JUGULARIS* L.

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SUMMARY

Syrian black snakes (*Coluber jugularis* L.) in Israel were found to be passing oocysts identical in size and shape to *Caryospora colubris* Matuschka, 1984. Attempts to infect mice with oocysts were unsuccessful. The endogenous developmental cycle of *C. colubris* is described from a Syrian black snake infected per os with oocysts obtained from natural infection. Meronts divide to eight merozoites and microgamonts form 18-25 microgametes. Macrogamonts contain both types of wall forming bodies, the first type very small and the second type large and conspicuously eosinophilic. The latter persist till the late stage of oocyst formation. Some adhere to the forming wall while the remaining coalesce into one or a few large inclusions. The significance of endogenous stage characteristics in *Caryospora* taxonomy and a possible origin of the sporozoite refractile body from the type 2 wall forming bodies are discussed.

RÉSUMÉ : **Développement de *Caryospora colubris* (Apicomplexa, Eimeridae) chez la couleuvre noire *Coluber jugularis* L.**

Des oocystes de taille et de forme identiques à ceux de *Caryospora colubris* Matuschka, 1984 sont excrétés par le serpent *Coluber jugularis* L. d'Israel. Des tentatives d'infection de Souris blanches avec des oocystes ont échoué. Le cycle de développement endogène du parasite de *C. colubris* est décrit chez un serpent infecté expérimentalement *per os* avec des oocystes provenant d'une infection naturelle. Les mérontes produisent 8 mérozoïtes, les microgamontes 18 à 25 microgamètes. Les macrogamontes contiennent les deux types de « wall forming bodies », le premier de très petite taille, le second grand et fortement éosinophile. Ce dernier persiste jusqu'à un stade avancé d'évolution de l'oocyste. Certains adhèrent à la paroi en cours de formation, d'autres fusionnent en une ou quelques inclusions de grande taille. L'intêret des caractères morphologiques des stades endogènes, pour la taxonomie des *Caryospora,* et l'origine éventuelle du cristalloide des sporozoites à partir des « wall forming bodies » secondaires sont discutés.

INTRODUCTION

Caryospora colubris has been described by Matuschka (1984) from oocysts in the faeces of *Coluber viridiflavus* from Sicily, Italy. The present communication reports *Caryospora colubris* infection in the Syrian black snake, *Coluber jugularis,* from Israel and describes its endogenous developmental stages.

MATERIALS AND METHODS

Oocysts were obtained from two naturally infected Syrian black snakes and from one laboratory-infected snake of the same species. Oocysts were extracted from faecal material by floatation in concentrated sugar solution. Sporulated oocysts were obtained either from faeces left at room temperature (24-28 C) for 48 hours, or after extraction, by incubation in a 1 % potassium dichromate solution for 48 hours at room temperature. Snakes were infected in the laboratory by oral inoculation of sporulated oocysts. Infected snakes were maintained at an ambient temperature of 25-30 C and fed young white mice. The possibility of *C. colubris* being a heteroxenous species was explored by feeding oocysts to 10 white mice and two starred lizards *(Agama stellio).* The mice were sacrificed for histological examination two months later, the lizards after one and three months. Histological 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 snakes was orally inoculated with oocysts three times, at nine days intervals and was sacrificed 61 days after the first ingestion, immediately following recovery of oocysts in the faeces. Samples from the gut tissue were fixed in 10 % neutral buffered formalin and embedded in glycol methacrylate (Lulham, 1979). Sections, 3-4 μm thick, were cut with a glass knife on a Sorval JB4 microtome and were stained with Meyer's haemalum, using eosin or eosin and phloxin as counterstains.

Histological preparations of paraffin wax embedded, haematoxylin eosin stained, *C. zuckermanae*-infected intestine of the type host-specimen *Coluber ravergieri nummifer* were available from Prof. G. Witenberg's collection, for comparison with the presently studied species.

RESULTS

NATURAL HOSTS AND LOCALITIES

Juvenile (< 50 cm long) and mature (> 100 cm long) Syrian black snakes, collected near Rehovot (Southern coastal plain), Israel.

LONGEVITY OF INFECTION

Faeces from naturally infected snakes contained oocysts for a period of 11 months; in one experimentally infected snake, oocysts appeared in the faeces up to seven months post-inoculation.

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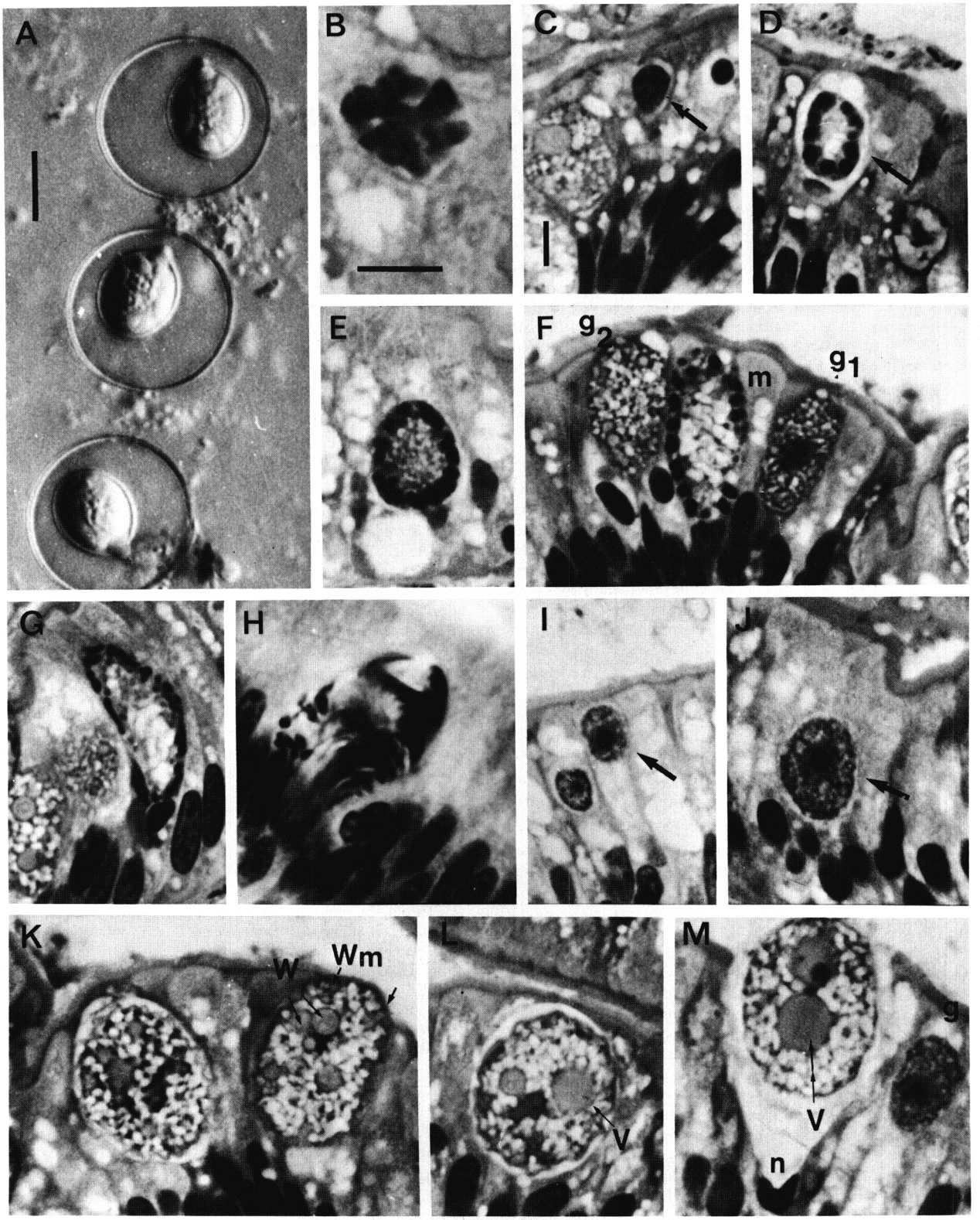


FIG. 1. — A: Oocysts, bar = 10 μm; B: developing meront, bar = 5 μm; C-E: young microgamonts; F: premature microgamont (m) and young oocysts (g); G: mature microgamont with young microgametes; H: mature microgamont with mature microgametes; I: young macrogamont; J: mature macrogamont; K: mature oocysts with WF2 coalesced into eosinophilic vacuoles (W) and marginally-distributed WF2 (Wm); L: wall-enclosed mature oocyst, with large central eosinophilic vacuoles (V); M: mature oocyst exiting from the host cell (n– host cell nucleus); bar = 10 μm; C-M: same magnification.

HETEROXENOUS STAGES

Oral inoculation of mice and *Agama stellio* lizards with *C. colubris* oocysts failed to yield tissue-stage infection.

DESCRIPTION

A — EXOGENOUS STAGES

Oocysts appeared in the faeces of laboratory-infected snakes a minimum of 18 days, after ingestion of infective oocysts. Sporulation was exogenous, after at least 48 hours incubation. Sporulated oocysts were spherical or subspherical *(fig. 1A)*, 16.8-26.0 (mean ± SD: 23.4 ± 2.7) μm long and 16.8-25.2 (21.9 ± 2.7) μm wide (n = 26). The wall was bi-layered with both layers being of equal density, and had a smooth surface. Micropile was present, whereas oocyst residuum and a polar granule were not seen. Sporulated oocysts > 20 μm in length comprised 76 % of the total measured; for both sporulated and unsporulated oocysts, modes were: length 25 μm and width 22.5 μm. Sporocysts were ovoid, 14-19.6 (15.4 ± 1.7)μm long and 8.4-14.0 (11.8 ± 1.2) μm wide (n = 30). Very distinct stieda and substieda bodies were present *(fig. 2)*, as well as sporocyst residuum. Unsporulated oocysts were almost spherical, 16.8-28.0 (22.7 ± 3.2)μm long and 15.4-28.0 (21.9 ± 3.2)μm wide (n = 31). Unsporulated sporocysts (or sporoblasts) were almost spherical, 11.2-16.9 (13.8 ± 1.7)μm long and 11.2-16.8 (12.8 ± 1.6)μm wide (n = 28). There were no morphometric differences between oocysts from natural and experimental infections. Mean length of oocysts from natural infections was 23.7 ± 2.5 μm (n = 22) and from experimental infections 22.4 ± 2.9 μm (n = 19) (Student t with 40 degrees of freedom = 0.16).

B — ENDOGENOUS STAGES

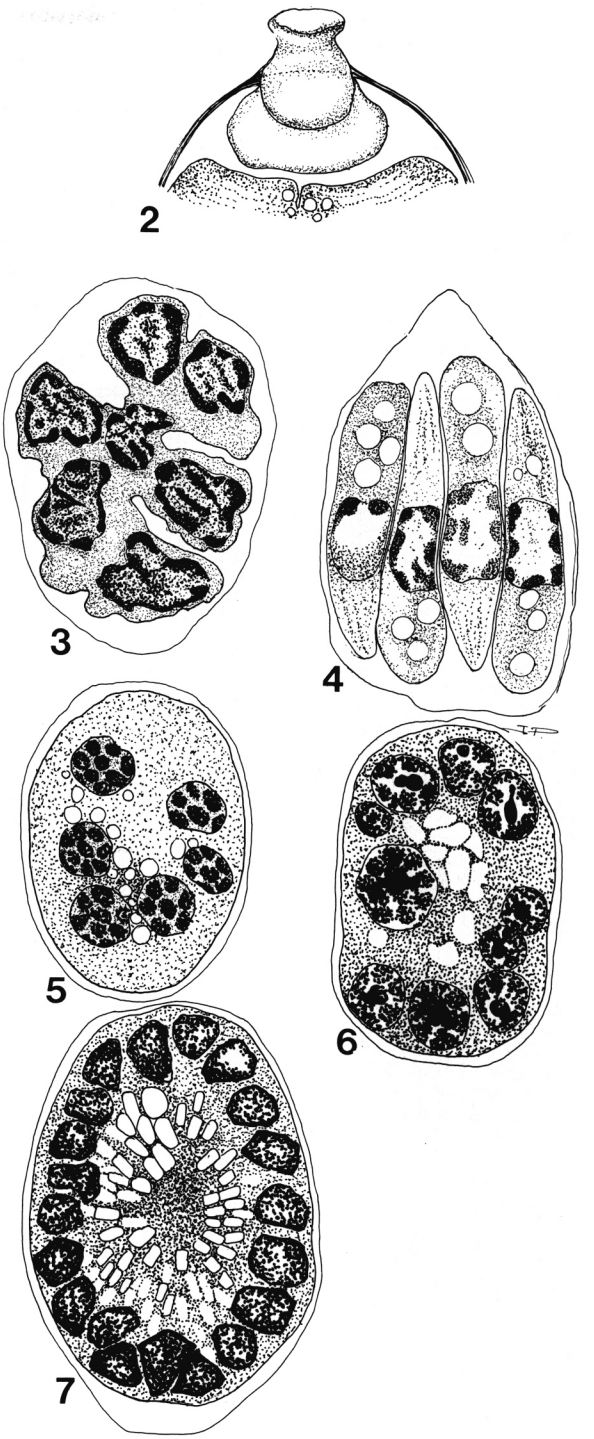
Infections with endogenous stages occurred mainly in the third quarter of the intestine with a few occurring in the second quarter. Only a few mature and dividing meronts were present in the examined material, while zygotes and young oocysts were the predominant developmental stage, and microgamonts and macrogamonts were also common.

Merogony stages

Invading merozoites and trophozoites in the gut epithelium were 4.1-8.1 × 2-2.4 μm in size and contained a large central nucleus. Developing meronts, 6-8 × 5-6 μm (n = 3) in size, contained up to eight nuclei *(figs. 1B, 3)*. Meronts, 8-13 × 7-10 μm (n = 4) in size, divided into up to eight, 7-8 × 1.2-2.0 μm merozoites, without leaving any residuum *(fig. 4)*.

Microgamonts

The earliest recognizable microgamonts 5-7 × 3-5 μm (n = 5) in size, contained four to six nuclei *(figs. 1C, 5)*. Sections of 7-11 × 7-10 μm (n = 6) microgamonts revealed 10-14 peripherally localized nuclei *(figs. 1D, 6)*. Through successive divisions the nuclei became smaller and their scattered chromatin denser. Vacuoles increased in numbers as microgamonts matured *(figs. 1E, 7)*. Sections of microgamonts,

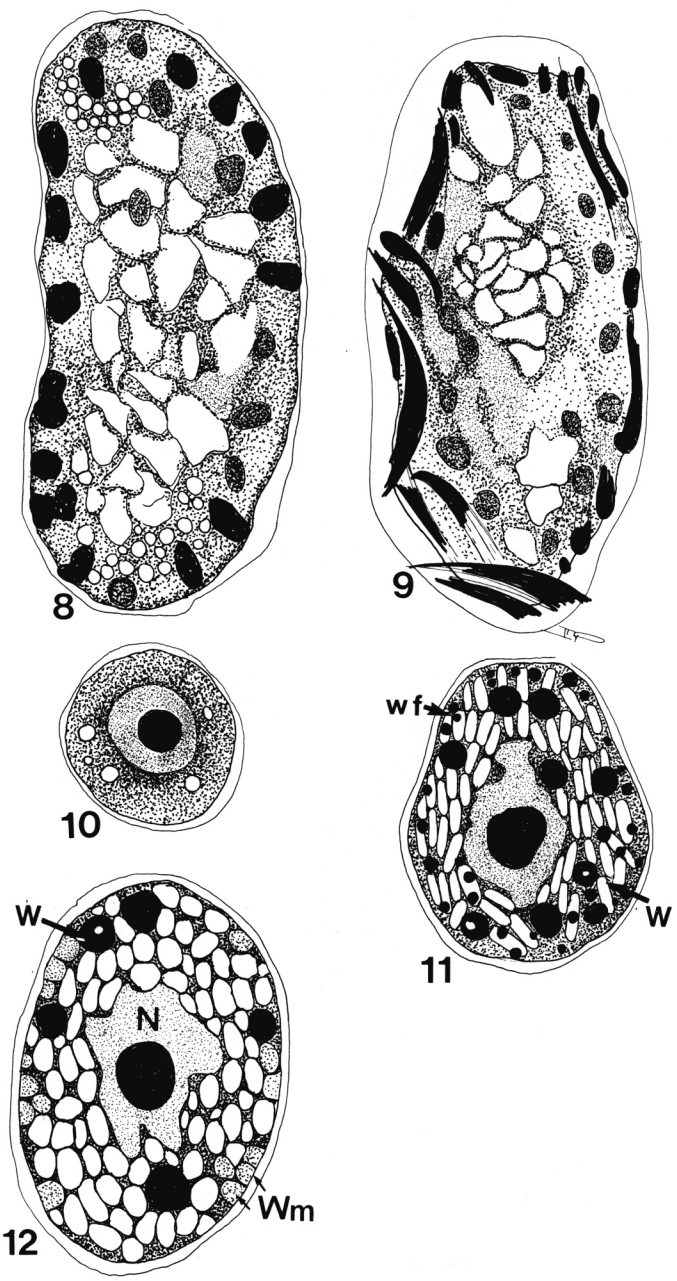


FIGS. 2 to 7. — *2*: Sporocysts stieda and sub-stieda bodies. *3*: Meront with divided nuclei. *4*: Formation of merozoites. *5-7*: Microgamont with 6, 10 and 20 nuclei, respectively, in cross section.

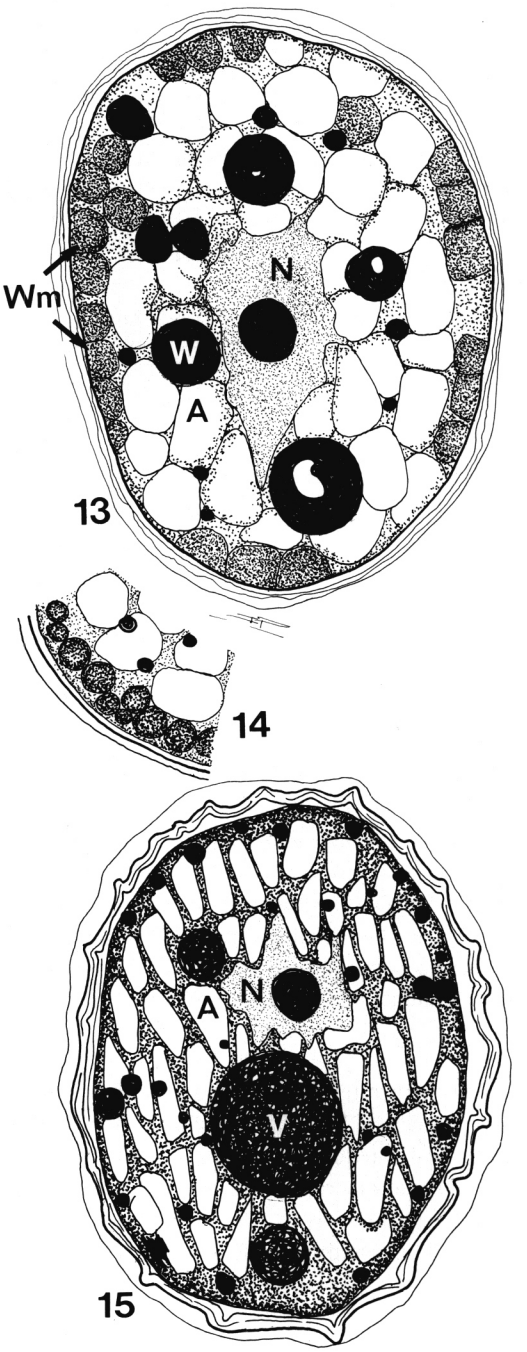
before microgamete differentiation were 12-17 × 7-10 μm (n = 14) in size, and contained 18-25 peripherally located nuclei *(figs. 1F, 7, 8)*. Sections of mature, 15-21 × 7-11 μm (n = 8) microgamonts revealed the same number of differentiating microgametes *(figs. 1G, 1H, 9)*.

Macrogamonts and oocysts

The youngest macrogamonts observed were round, 6 × 6 μm in size and had a very large (3 × 3 μm) centrally-located nucleus, a large nucleolus and a few vacuoles in the cytoplasm *(figs. 1I, 10)*. As they grew, macrogamonts became oval and loaded with amylopectin granules. The cytoplasm of 8-12 × 5-9 μm (n = 17) macrogamonts contained small basophilic and large eosinophilic and phloxinophilic wall forming bodies (WF1 and WF2, respectively) *(figs. 1J, 11)*. Macrogamonts 13-19 × 8-12 μm (n = 15)



FIGS. 8 to 12. — *8*: Mature microgamont before microgamete differentiation. *9*: Microgamont with microgametes. *10*: Young macrogamont. *11*: Mature macrogamont (wf: WF1, W: WF2). *12*: Young oocyst (W: central WF2, Wm: marginal WF2).



FIGS. 13 to 15. — *13*: Developed oocyst (A: amylopectin granules, N: nucleus, W: eosinophilic vacuoles, Wm: marginal WF2). *14*: Part of mature oocyst showing disaggregating marginal WF2). *15*: Mature walled oocyst.

were apparently fertilized *i.e.* zygotes or young oocysts. They contained a variable number of WF2, while WF1 were no longer detectable *(figs. 1F, 12)*. Some of the WF2 aggregated along the walls of the oocyst while either loosing their chromophilly, or becoming faintly basophilic. Full size oocysts, 20-30 × 12-20 μm (n = 20), contained large, rounded amylopectin granules *(figs. 1K, 13)*. The centrally located WF2 coalesced into larger eosinophilic-phloxinophilic vacuoles while the WF2 along the oocyst margin gradually disaggregated *(figs. 1K, 13, 14)*. In oocysts enclosed by a firm wall, amylopectin granules became somewhat smaller and angular, as though partly consumed. Central WF2 seemed to have coalesced into one or two large (up to 6 μm in diameter) vacuoles, while the peripheral bodies disaggregated into scattered residual granules *(figs. 1L, 15)*. The oocyst was evicted into the intestinal lumen when the parasitophorous vacuole rupture at the brush border of the host cell. The ruptured host cell still contained a nucleus *(fig. 1M)*.

DISCUSSION

Oocysts and sporocysts from *Coluber jugularis* from Israel conform in size and structural details to those from the type host species *C. viriflavus* from Sicily, Italy (Matuschka, 1984). In addition, the size distribution of the oocyst in both populations is similar: 90 % of the oocysts from *C. viriflavus* and 76 % of the oocysts from *C. jugularis* were larger than 22.3 μm in diameter. Despite the wide variation in their sizes, Matuschka (1984) assumed that the oocysts represented the same species. Similarly Bray (1960) found three size groups of oocysts in *Psammophis sibilans* from Liberia. He, however, assigned a separate specific epithet for each size category. In the infection from *P. sibilans,* size variability could also be demonstrated among the endogenous stages. Matuschka (1984) data were limited to exogenous stages. However, our measurements of endogenous stages from *C. jugularis* did not show any relevant size segregation. Oocysts of *Caryospora* from African *Psammophis* snakes (*C. legeri* Hoare, 1933, and *C. psammophi, C. hermae* and *C. weyerae* of Bray, 1960) grossly overlap in size and are also similar in structure to oocysts from *C. colubris* from *Coluber* species. Distinct differences may be demonstrated however, between endogenous stages: microgamonts of *C. colubris* yield a considerably smaller number of microgametes (< 30) than the ones from *Psammophis* snakes (< 50 and < 100). The number of merozoite progeny on the other hand are within the same range (6-10). In the only other species from *Coluber* snakes where endogenous stages were studied: *C. zuckermanae* Bray 1960, from *C. ravergieri nummifer* from Israel, oocysts are small (15-18 μm diameter), and similar to the species from *Psammophis* microgamonts yield numerous microgametes (< 60), while meronts yield about 30 merozoites. Variations in progeny number of microgametes and merozoites among *Caryospora* species could have been a criterion for species differentiation. However, a large part of the species is described only on the basis of oocyst characteristics (Upton, Current and Barnard, 1986).

Light microscopic descriptions of macrogamonts from various species of *Caryospora* report variable sizes and staining properties for organelles considered to be wall forming bodies (Hoare, 1933, Bray, 1960, Cannon, 1967, Wacha and Christiansen, 1982). This variation, in part at least, should be attributed to differences in processing and stains. In macrogamonts of *C. zuckermanae,* the small WF1 were strongly basophilic while the large WF2 were eosinophilic. The validity of the interspecific variability in wall forming bodies will only be resolved through fine structural studies.

Concurrent, not yet published, ultrastructural studies confirm that the small basophilic granules and the large eosinophilic or phloxinophilic granules seen in the macrogamonts are WF1 and WF2 respectively. The latter granules seem to include two functional types: the granules which move to the periphery of the oocyst appear to contribute to the forming oocyst wall. The others, which coalesce into larger central, conspicuously eosinophilic inclusions and persist in the oocyst till after their release from the tissue, seem to be the precursors of the sporozoite refractile body. We have observed a similar process in a current study of *Isospora* and *Eimeria* (s.l.) from reptilian hosts (unpublished).

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