Fine Structure of the Oocyst Walls of *Isospora serini* and *Isospora canaria* and Excystation of *Isospora serini* From the Canary, *Serinus canarius* L.

Clarence A. Speer  
*University of Montana - Missoula*

Donald W. Duszynski  
*University of New Mexico - Main Campus*

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SYNOPSIS. Oocysts of *Isospora serini* and *Isospora canaria*, from the canary *Serinus canarius*, were broken, added to a cell suspension, fixed in Karnovsky's fluid, and studied in the electron microscope. The oocyst wall of each species had an electron-lucent inner layer, a more osmiophilic middle layer and an outer layer of electron-lucent (*I. serini*) or electron-dense material interspersed with some electron-lucent material (*I. canaria*). A few, relatively large lipid-like bodies were present in the outer or middle layer of the oocyst wall of *I. canaria*. As many as 9 membranes were present in the oocyst wall of *I. canaria* and 3 in that of *I. serini*. When exposed to a trypsin-sodium taurocholate fluid, sporozoites of *I. serini* excysted from 5-month-old sporocysts in vitro, but not from sporocysts stored for more than 6 months. No excystation occurred in 15-month-old *I. canaria* sporocysts. Similarities and differences in excystation between *I. serini* and other *Isospora, Eimeria*, and *Sarcocystis* species are discussed.

**Index Key Words:** *Isospora serini; Isospora canaria; oocyst wall; excystation; electron microscopy.*

**MATERIALS AND METHODS**

Sporulated oocysts of *I. canaria* and *I. serini* were sent to us by Dr. Edith D. Box, Department of Microbiology, The University of Texas Medical Branch, Galveston, in March, 1974. Both groups of oocysts were kept in physiologic solution by Dr. Edith D. Box, Department of Microbiology, The University of New Mexico, Albuquerque, New Mexico 87131.

SYNOPSIS. Oocysts of *Isospora serini* and *Isospora canaria*, from the canary *Serinus canarius*, were broken, added to a cell suspension, fixed in Karnovsky's fluid, and studied in the electron microscope. The oocyst wall of each species had an electron-lucent inner layer, a more osmiophilic middle layer and an outer layer of electron-lucent (*I. serini*) or electron-dense material interspersed with some electron-lucent material (*I. canaria*). A few, relatively large lipid-like bodies were present in the outer or middle layer of the oocyst wall of *I. canaria*. As many as 9 membranes were present in the oocyst wall of *I. canaria* and 3 in that of *I. serini*. When exposed to a trypsin-sodium taurocholate fluid, sporozoites of *I. serini* excysted from 5-month-old sporocysts in vitro, but not from sporocysts stored for more than 6 months. No excystation occurred in 15-month-old *I. canaria* sporocysts. Similarities and differences in excystation between *I. serini* and other *Isospora, Eimeria*, and *Sarcocystis* species are discussed.

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**RESULTS**

*Isospora serini* (Figs. 3-12)

Sporulated oocysts of this species are spherical to subspherical and usually slightly smaller (cf. Figs. 1 & 3) than those of *I. canaria*, these findings being in agreement with those of Box (1).

The sporocysts (Figs. 3, 4) of this species, however, have a distinct substerial body which Box was unable to see. This substerial body is 3 μm deep and 5 μm wide, or almost 2X larger than that found in *I. canaria*.

Excystation did not occur in intact oocysts exposed to the trypsin-sodium taurocholate solution for 3 hr or in free sporocysts exposed to RPS for an equivalent time. After treatment with the trypsin-sodium taurocholate medium, sporozoites excysted from only some of the sporocysts obtained from 5-month-old oocysts, but not from oocysts stored for more than 6 months. The following sequence of events was usually observed during

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**Figs. 1-9.** [Photomicrographs. Figs. 1, 2, *I. canaria*; Figs. 3-9, *I. serini*. All Figs. X 1220.] 1. Oocyst in optical cross-section. 2. Sporocyst; note distinct Stieda (Sb) and substerial (Ss) bodies. 3. Oocyst in optical cross-section. 4. Sporocyst; note large substerial body. 5. Sporocyst after addition of trypsin-sodium taurocholate mixture; note that Stieda body has almost disappeared and substerial body has become more transparent. 6. Sporocyst after addition of excysting fluid; note disappearance of substerial body. 7. Sporozoite (Sp) excysting from sporocyst; note constriction (arrow) of sporozoite body at point of exit from sporocyst. 8. Intact empty sporocyst after sporozoites have excysted. 9. Free sporozoite; note the anterior (Ar) and posterior (Pr) refractile bodies.
The light-staining outer layer consists of a fine granular material, which is somewhat coarser than that of *I. canaria*. Indentations of the inner layer are rarely seen. Two closely applied unit membranes of the inner layer are adjacent to the osmiophilic middle layer (Fig. 13). The inner portion of the middle layer consists of 4 or 5 closely applied unit membranes; the middle portion has unit membranes interspersed with electron-dense spheroid bodies; and the outer portion consists of 2 closely applied unit membranes (Fig. 13). The 2 membranes of the outermost portion often separate from the remainder of the middle layer (Fig. 13). Areas of the middle layer not covered with these 2 membranes contain membrane-bounded vesicles and numerous electron-dense bodies. The latter (65-200 nm) are larger in size than those (15-30 nm) in intact walls (Fig. 13). In many preparations the inner layer separates from the middle layer, whereas the 2 outer layers remain intact (Figs. 14, 15). Usually, the outer layer consists of a homogeneous electron-dense material of irregular thickness (Figs. 14, 15). Occasionally, lightly staining spheroid bodies (50-180 nm, Fig. 14), similar in appearance to lipid droplets, are present. It is difficult to determine if these bodies are part of the outer or middle layer, since the most peripherally located membrane of the middle layer appears continuous with these bodies (Fig. 14). The electron-dense material of the outer layer is continuous over the surface of the spheroid, lipid-like structures, which were not seen on the surface of NaOCl-treated oocyst walls. In such specimens, the inner layer usually was separated from the middle one, whereas the 2 outer layers remained intact.

**DISCUSSION**

Several investigators have described in detail the excystation of sporozoites of various species of *Eimeria* and *Isospora* (2, 4-7, 14, 18), but only a few mentioned the length of time the oocysts were stored before being used for excystation studies. Only Box (1) working with *I. canaria* and *I. serini* and Speer et al. (18), working with *Isospora canis* Nemesi, 1959 from dogs stated the time that oocysts had been stored before their excystation experiments. In using relatively fresh oocysts (2-month-old), Speer et al. (18) experienced little difficulty in getting sporozoites to excyst. In experiments in this laboratory with *Isospora marquardti* Duszynski & Brunson, 1972 and 4 species of *Eimeria* from pikas (4, 5), the sporozoites of some species excysted while others did not when oocysts which had been stored for 12 months were used. In the present study, we found, as did Box (1), that sporozoites of *I. canaria* and *I. serini* would not excyst from oocysts stored for more than 6 months. For structural studies of *I. canaria* and *I. serini* sporozoites, Box (1) used sporozoites obtained from oocysts, which had been stored for less than 1 month and obtained numerous sporozoites. Most of the sporozoites appeared to have excysted after exposure to the excysting fluid.
fluid for 15 or more min (Box, personal communication). In the present study, we found that sporozoites excysted from only some of the sporocysts from 5-month-old oocysts of *I. serini*, and not at all from sporocysts obtained from 15-month-old *I. canaria* oocysts. These findings suggest that the ability of sporozoites to excyst decreases rapidly in oocysts of these species stored at 4°C in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution, even though all structures within the oocysts appear normal.

The details of excystation have been studied in only a few species of *Isospora*. Sporocysts of some species have Stieda bodies, those of others do not. During excystation of *I. canis* (18), *Isospora endocallimici* Duszynski & File, 1974 (6) and *Isospora bigemina* (Stiles, 1891) Löhle, 1906 (large form) (unpublished data) sporocysts, which lack Stieda bodies, the sporocyst walls collapse and the sporozoites escape randomly. In an electron microscope study, Speer et al. (18) found that the sporocyst wall of *I. canis* consists of 4 curved plates. They suggested that the trypsin-sodium taurocholate acts upon the sites of apposition between 2 plates resulting in rupture of the sporocyst wall and collapse of the sporocyst. The sporocyst wall of *Sarcocystis tenella* Railliet, 1886 (11) is structurally similar to that of *I. canis*. Excystation in *S. tenella* may, therefore, be very similar to that in *I. canis, I. endocallimici* and *I. bigemina*. Excystation of *I. serini* (present study) and *I. margaritii* (4) sporocysts, which have Stieda bodies, is similar to that observed in *Eimeria* spp. (2, 5, 7, 14). In these species, the sporozoites exit through a gap at one pole of the sporocyst created by dissolution of the Stieda body, which occurs in the presence of the trypsin-sodium taurocholate. Thus, 2 considerably different means of excystation are found within the genus *Isospora*. Therefore, it seems likely that even further variation in the excystation process may exist in other species of *Isospora* as well as in *Eimeria, Sarcocystis,* and *Toxoplasma*.

The fine structure of the oocyst wall has been described for only a few species of coccidia (3, 9-19). In most of these, the wall has at least 2 prominent layers, usually an electron-lucent inner and a more osmiophilic outer layer. Soon after fertilization of a macrogamete by a microgamete, these 2 primary layers are formed at the surface of the zygote by disaggregation and fusion of 2 types of wall-forming bodies (9, 17). Type I bodies form the outer, and type II bodies the inner layer. In some, *Eimeria tenella* (Railliet & Lucet, 1891) Fantham, 1909 (10), *Eimeria acerulina* Tyzzer, 1929 (9), *I. canis* (18) and *I. serini* and *I. canaria* (present study), a 3rd layer usually consisting of one or more membranes, is present on the outside. In *Eimeria perforans* (Leuckart, 1879) Shifer & Swelengrebel, 1912 (17), *E. acerulina* (9), and *E. tenella* (10), this outermost layer apparently represents the original limiting membrane of the merozoite. In most species, the innermost layer appears to be relatively consistent in thickness and appearance, being composed of fine granular electron-lucent material, whereas more variation in thickness and composition occurs in the outer 1 or 2 layers. Roberts et al. (14) found hexagonal columnar projections in the outer layer of *Eimeria callospermophili* Henry, 1932 oocysts, and in some specimens round electron-dense granules were scattered along the surface of the outer layer. In *I. canaria* and *I. serini,* we found similar spheroid electron-dense bodies in the middle layer. Electron-dense knoblike projections occur on the outer and middle layers of the oocyst walls of *Eimeria larimerensis* Vetterling, 1964 (14) and *I. canis* (18), respectively. We found relatively large electron-lucent bodies in the outer or middle layer of *I. canaria* oocysts, but not in those of *I. serini*.

Although the wall-forming bodies probably account for the formation of the bulk of the oocyst wall, membranes which have been seen in the walls of some species may also play an important role. Lee & Millard (9) found 2 external membranes in the wall of *E. acerulina* oocysts, the most peripheral one being the original membrane of the merozoite. A single membrane was found in the outermost portion of the inner layer in the oocyst wall of *E. callospermophili*, and in *E. larimerensis* a single membrane was observed on the outside of the oocyst (14). In *E. perforans*, Scholtyseck et al. (17) found 5 unit membranes in the oocyst wall. Vetterling et al. (19) reported that the oocyst wall of *Sarcocystis* sp. consisted almost entirely of 4 fused membranes which formed a dense outer layer, 6 nm thick, and an electron-lucent, thin inner layer, 7 nm thick. We found as many as 9 membranes in the oocyst wall of *I. canaria* and 3 in that of *I. serini*. These membranes may arise in part from the membranes surrounding the wall-forming bodies, which are incorporated into the wall during its formation.

According to Mehlhorn & Scholtyseck (11), unsporulated *S. tenella* oocysts were surrounded by a single electron-lucent layer, 0.25 μm thick, which shrank during sporulation to 0.1 μm. Scholtyseck & Voight (15) observed the outer layer of the oocyst wall of *E. perforans* was lost soon after wall formation was completed. These findings suggest that there may be considerable change in structure of the oocyst wall after its formation, particularly after the oocyst has left the host.

REFERENCES

