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Scanning and Transmission Electron Microscopy of the Oocyst Wall of *Isospora lacazei*

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Summary. The oocyst wall of *Isospora lacazei* from sparrows was studied with scanning (SEM) and transmission (TEM) electron microscopy. In TEM, the oocyst wall consisted of four distinct layers (L1-4). The innermost layer, L1, was moderately electron-lucent and 240–285 nm thick; L2 was electron-dense and 210–240 nm thick; L3 was moderately electron-lucent and 15–150 nm thick; L4, the outer most layer, was discontinuous and consisted of electron-dense discoid bodies which measured 180–220 nm × 320–840 nm. The discoid bodies of L4 as seen by TEM appeared spheroid in shape when observed by SEM. One or two membranes were situated on or between various layers of the oocyst wall. One such membrane occurred on the inner margin of L1, two closely applied membranes were interposed between L1 and L2, one membrane occurred between L2 and L3, and one membrane on the outer margin of L3.

Introduction

The ultrastructure of isosporan oocysts has been described only for *Isospora canis* (Speer et al., 1973) from dogs and *I. canaria* and *I. serini* (Speer and Duszynski, 1975) from canaries. The ultrastructure of the oocyst wall of *Isospora lacazei*, a parasite of sparrows, as determined by scanning and transmission electron microscopy is described herein.

Materials and Methods

Sporulated oocysts of *Isospora lacazei*, a parasite of sparrows, were prepared for study with transmission and scanning electron microscopy. For transmission electron microscopy studies, oocysts in minimal essentials medium or Hank's balanced salt solution (HBSS) were ground in a teflon-coated

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tissue grinder in order to break the walls of some oocysts. Oocysts were then added to a cell suspension which had been harvested with a trypsin-versene solution from cell monolayers in two 2-oz Brockway culture flasks. The suspension was centrifuged at 750 *g* for 5 min, the pellet fixed in Karnovsky's fixative (Karnovsky, 1965), or 3% or 15% glutaraldehyde in cacodylate buffer for 6-12 h at 22° C. The pellet was then cut into smaller pieces, post-fixed in OsO_4 for 2 h, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 or Spurr's medium (Spurr, 1969). Thin sections were placed on 200-mesh grids, stained with lead citrate and uranyl acetate, and examined with a Zeiss EM 9S 2 or Corinth 275 electron microscope.

For scanning electron microscopy, 2–3 drops of HBSS containing 100–300 oocysts were added to a 9 × 9 mm coverslip which had been coated with a solution containing 50 µg poly-l-lysine (No. P-1886, Type 1-B, Sigma Co.)/ml cacodylate buffer. The poly-l-lysine was necessary to ensure adherence of the oocysts to the coverslip (Stulting and Berke, 1973). After 1 h, oocysts on coated coverslips were fixed in 15% glutaraldehyde in cacodylate buffer for 18 h, rinsed in buffer, post-fixed in 2% OsO_4 for 2 h, dehydrated in ethanol, and critical point dried with CO_2 (31C at 1073 psi) in a Tousimis Samdri critical point drier. Coverslips were mounted on studs, coated with gold-palladium (60:40) in a vacuum evaporator or SPI sputter, and examined in an ETEC Autoscan or Zeiss Novascan 30 scanning electron microscope.

Results

When viewed with bright-field microscopy, the oocyst wall of *Isopora lacazei* appeared to consist of two layers with a slightly roughened surface. In SEM, oocysts were spheroid to ovoid in shape and the outer surface appeared to consist of numerous spheroid bodies and to be relatively free of extraneous debris (Figs. 1 and 3–5). When studied with the transmission electron microscope, the oocyst wall was found to consist of four layers (L1–4), each of which was covered or separated from an adjacent layer by one or two membranes (Fig. 2). The inner layer (L1) was moderately electron-lucent, 240–285 nm thick, and consisted of a fine granular matrix with a denser inner zone and a more lucent outer zone. The inner surface of L1 was limited by a single membrane, to which amorphous material was attached. A lucent line, about 23 nm thick, consisting of two closely applied membranes, was located between L1 and L2. Layer 2 consisted of a finely granular electron-dense material, 210–240 nm thick. A very electron-dense line, about 15 nm thick, was located on the outer margin of L2. A membrane which appeared as an electron-lucent line, about 10 nm thick, occurred between L2 and L3. Layer 3 was moderately electron-lucent and finely granular, and varied in thickness from 15 to 150 nm. Layer 4 was discontinuous and consisted of electron-dense bodies which appeared discoid in TEM (Fig. 2), but spheroid in SEM (Figs. 1 and 3–5). In TEM, the discoid bodies measured 180–220 nm in thickness and 320–840 nm in length and were closely applied to the outer margin of L3 immediately above the thinner areas (depressions) of L3. In SEM, some spheroidal bodies appeared to have joined at their margins resulting in the formation of irregularly shaped bodies (Fig. 3). Such areas in appropriately cut thin sections, in which the discoid bodies of L4 had fused, appeared in TEM as a continuous layer. Occasionally, a membrane was seen on the outer margin of L3, particularly in those areas not covered by the electron-dense bodies of L4 (Fig. 2). In some specimens studied with SEM, a small depression was observed on the outer surface of the oocyst (Fig. 3). A micropyle was not observed in SEM or TEM.

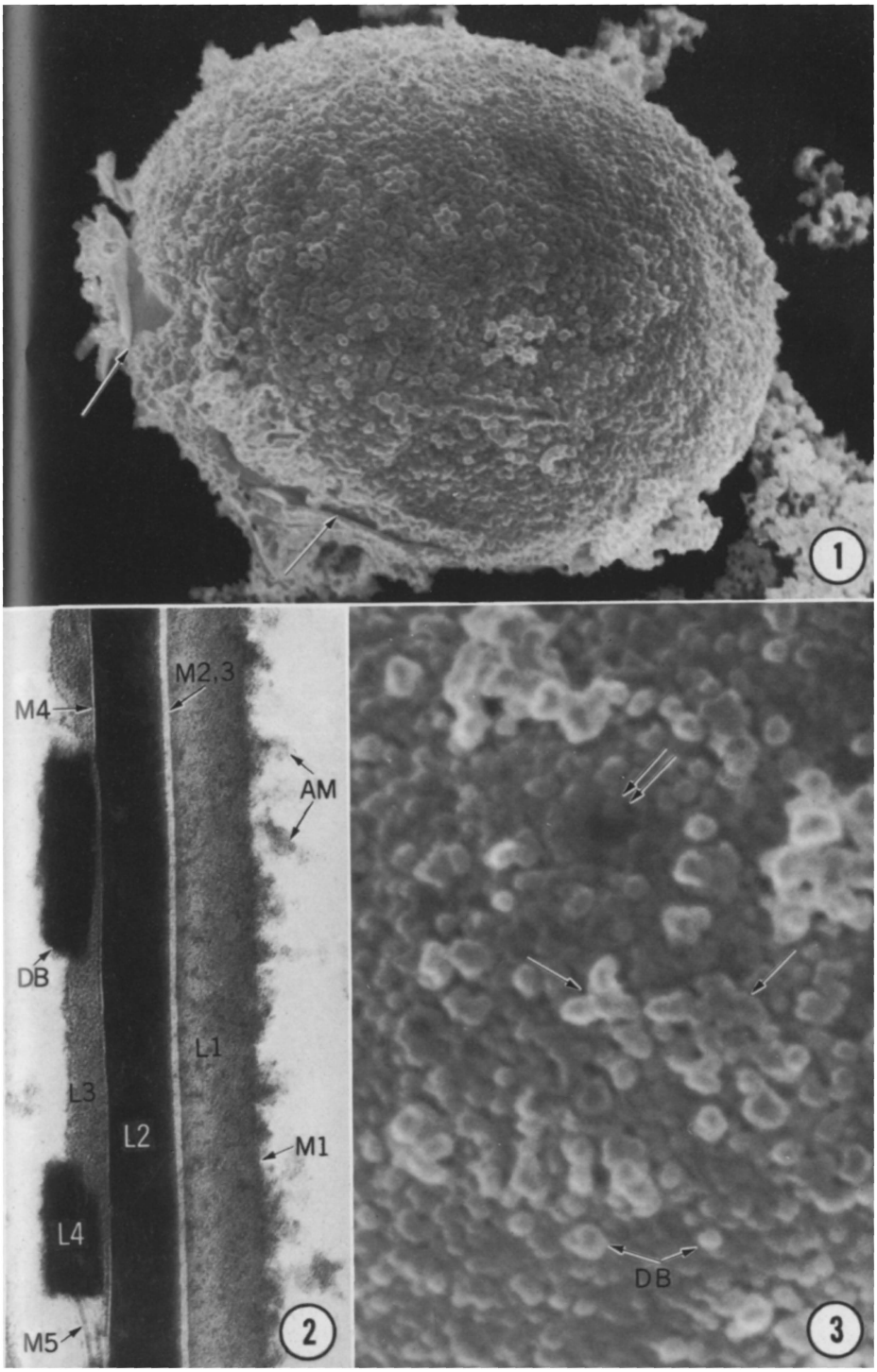

Fig. 1. Oocyst; note that oocyst wall has fractured at certain areas (*arrows*). ×4,800

Fig. 2. Cross section of oocyst wall. ×146,500

Fig. 3. High magnification of outer surface of oocyst; note spheroid bodies (DB) which appear fused in some areas (*single arrows*), and an area devoid of spheroid bodies (*double arrow*). ×12,800

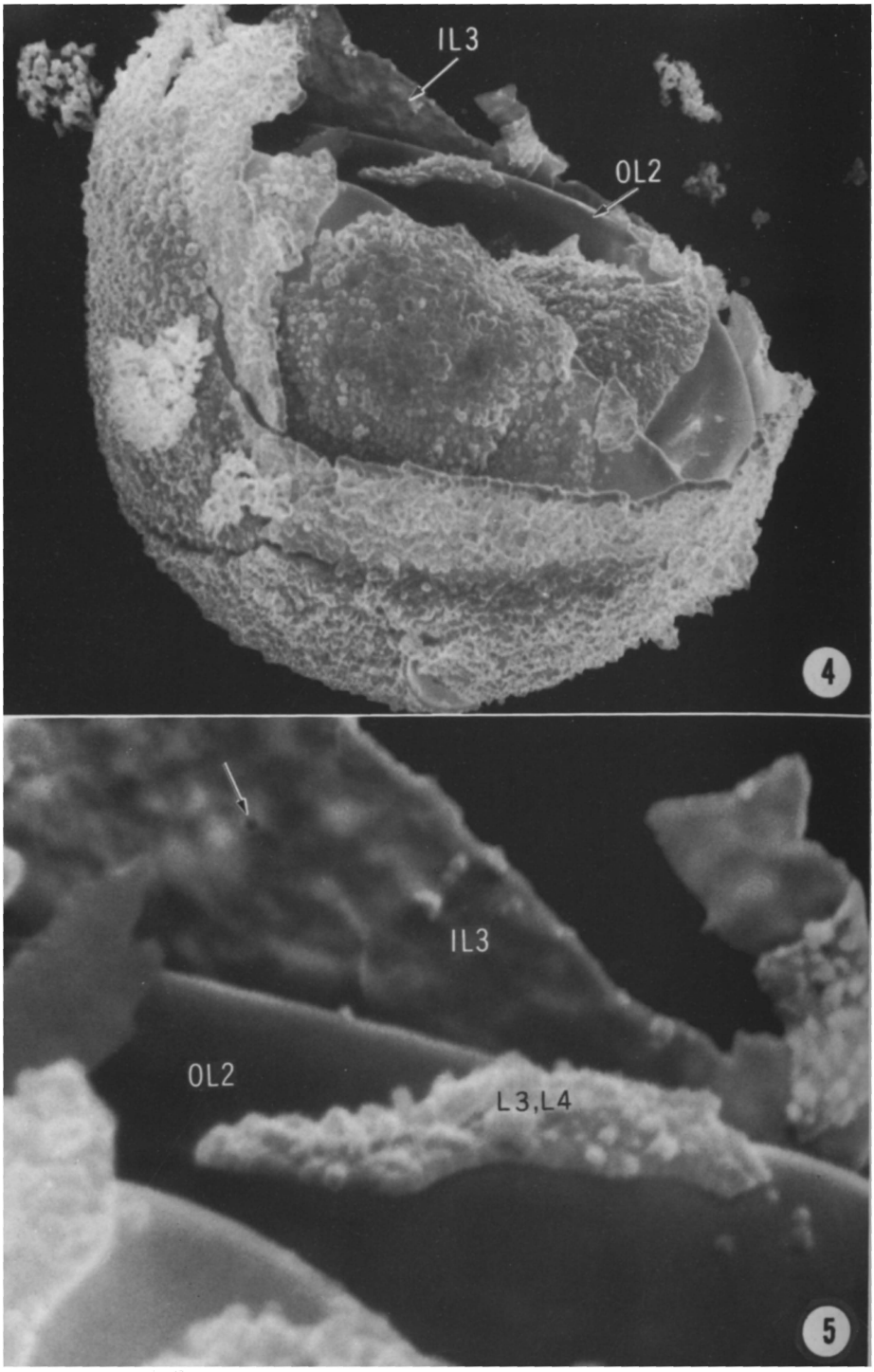

Fig. 4. Oocyst which collapsed during preparation, exposing the outer surface of L2 and the inner surface of L3. ×5,100

Fig. 5. Higher magnification of portion of specimen in Fig. 4; note perforation (arrow) in inner surface of layer 3. ×20,480

In some specimens, adjacent layers of the oocyst wall separated from each other (Figs. 1, 4 and 5), which evidently was a result of the preparation procedures used for TEM or SEM. In TEM, the separation occurred between L2 and L3, with L1 and L2, and L3 and L4 remaining attached to each other, respectively. Therefore, the separation of the layers of the oocyst wall which was observed in SEM (Fig. 1, 4 and 5) evidently occurred between L2 and L3, exposing the outer surface of L2 and the inner surface of L3. The outer surface of L2 appeared smooth, whereas the inner surface of L3 was slightly irregular in topography (Figs. 4 and 5). Occasionally, perforations were observed in L3 (Fig. 5).

Discussion

The ultrastructure of the oocyst wall has been described for mature oocysts of *I. canis* (Speer et al., 1973), *I. canaria* and *I. serini* (Speer and Duszynski, 1975), and *Sarcocystis tenella* (Mehlhorn and Scholtyseck, 1974), *Toxoplasma gondii* (Ferguson et al., 1975) and various *Eimeria* species (Dubremetz and Yvore, 1971; Duszynski et al., 1977; Ferguson et al., 1978; Marchiondo et al., 1978; Nyberg and Knapp, 1970a, b; Roberts et al., 1970). These reports have shown that the oocyst wall of various species of coccidia consists of one to three prominent layers. Among the species studied, the innermost layer appears to be relatively consistent in thickness and appearance, being composed of fine granular electron-lucent material. More variation occurs in the outer one or two layers. The oocyst wall of unsporulated oocysts of *S. tenella* consists of a single electron-lucent layer (Mehlhorn and Scholtyseck, 1974) similar in appearance to the innermost layer of other coccidian species which have two or more layers. Two prominent layers occur in the wall of immature oocysts of a *Sarcocytis* species from grackles which developed in cell culture (Vetterling et al., 1973). The oocyst wall of mature oocysts of *T. gondii* has an inner electron-lucent layer and an outer electron-dense layer (Ferguson et al., 1975). Oocysts of *E. brunetti* (Ferguson et al., 1977, 1978), *E. necatrix* (Dubremetz and Yvore, 1971), *E. tenella* (Nyberg and Knapp, 1970b), *E. nieschulzi* (Marchiondo et al., 1978), *E. crotalviridis* (Duszynski et al., 1977), *E. callospermophili,* and *E. larimerensis* (Roberts et al., 1970) have two prominent layers, an innermost electron-lucent and an outermost electron-dense layer. Three distinct layers occur in the oocyst walls of *I. canis* (Speer et al., 1973) and *I. canaria* and *I. serini* (Speer and Duszynski, 1975). The inner, middle, and outer layers are electron-lucent, dense, and lucent, respectively. In the present study, we found that the oocyst wall of *I. lacazei* has four prominent layers of which the outermost (layer 4) is discontinuous and consists of electron-dense spheroid bodies. Layers 1, 2, and 3 were electron-lucent, dense, and lucent, respectively.

The above studies have shown that each coccidian species studied thus far has a unique oocyst wall ultrastructure. On the basis of ultrastructural differences in the oocyst walls, Speer and Duszynski (1975) were able to distinguish between *I. canaria* and *I. serini* from canaries which appear nearly identical by light microscopy. Ultrastructural differences have also been observed in sporocysts

of *Eimeria* (Roberts et al., 1970) and *Isospora* species (Speer et al., 1973, 1976). If such differences could be detected by electron microscopy, then those species of coccidia (e.g. *Sarcocystis* species) which may be indistinguishable by light microscopy might be differentiated on the basis of ultrastructural differences in the sporocyst and/or oocyst walls.

Although there are numerous articles describing the ultrastructure of coccidian oocyst walls, only a few such reports (Nyberg and Knapp, 1970a, b; Roberts et al., 1970; Dubremetz and Yvore, 1971; Speer et al., 1973; Dubremetz et al., 1975; Speer and Duszynski, 1975; Duszynski et al., 1977; Ferguson et al., 1977, 1978; Marchiondo et al., 1978) actually studied completely formed oocysts. Reports which described the ultrastructure of incompletely formed coccidian oocyst walls were parts of studies of macrogametogony and oocyst wall formation (for review see Scholtyseck et al., 1971). These studies have shown that soon after fertilization of a macrogamete by a microgamete, Type I and II wall forming bodies which occur in the cytoplasm of the zygote form the outer and inner layers of the oocyst wall, respectively. During its formation, the oocyst wall eventually becomes resistant and essentially impermeable to fixatives and embedding media used for electron microscopy. Because of this, good quality specimens usually cannot be obtained after a certain stage of wall formation. This problem has been overcome somewhat by thin sectioning oocysts in frozen thick sections (Birch-Andersen et al., 1976). However, for the many species in which macrogametogony and oogony have been studied ultrastructurally, the events that occur late in oocyst wall formation have yet to be determined. Also, the mechanisms of oocyst wall formation have been studied only in those coccidian species which are known to have only two types of wall forming bodies and two prominent layers in the oocyst wall. Since *I. canis* (Speer et al., 1973), *I. canaria* and *I. serini* (Speer and Duszynski, 1975), and *I. lacazei* (present study) have three or four prominent layers in their oocyst walls, the mechanisms of wall formation in these and related species may be considerably more complex than in those coccidians which have only two layers.

Nyberg and Knapp (1970a) attributed the roughened outer surface of *E. tenella* oocysts as seen by SEM to debris or to artifacts caused by fixation. By comparing specimens prepared for TEM and SEM, Marchiondo et al. (1978) found that the roughened surface of *E. nieschulzi* oocysts was due to minute variations in thickness of the outer layer of the oocyst wall. In SEM in the present study, *I. lacazei* oocysts also had a roughened surface which was due to spheroid bodies in the outermost layer of the oocyst wall.

Abbreviations

AM amorphous material
DB electron dense (=spheroid) body
IL3 inner surface of layer 3
L1–L4 four layers of the oocyst wall
M1 membrane on inner surface of L1
M2,3 membranes 2 and 3 between layers 1 and 2
M4 membrane 4 located between layers 2 and 3
M5 membrane 5 on outer surface of layer 3
OL2 outer surface of layer 2

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