COCCIDIAN PARASITES (PROTOZOA: EIMERIIDAE) OF THE LESSER MOUSE-DEER *TRAGULUS JAVANICUS*

III. CYTOLOGICAL STUDIES OF THE SPOROZOITES OF *EIMERIA TRAGULI*

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INTRODUCTION

The purpose of this study is to describe certain cytological features of the sporozoites of *Eimeria traguli* and to compare them with the sporozoites of other *Eimeria* species.

MATERIALS AND METHODS

Oocysts of *E. traguli* were obtained and sporulated as described by Colley and Mullin (1971). They were cleaned and concentrated in Sheather's sugar solution by the method of Hammond *et al.*, (1968). Oocysts were washed in Ringer's solution several times to remove the sugar. Free sporocysts were obtained by mechanically breaking oocysts in a Thomas hand tissue grinder and were then placed in buffered Ringer's solution, pH 7.5, containing 0.25% trypsin and 0.75% sodium taurocholate to initiate excystation.

Living sporozoites in Ringer's solution were studied with bright field and phase contrast illumination under a Leitz Ortholux microscope with apochromatic objectives. Smears fixed in Schaudinn's or Bouin's fluid were stained by Heidenhain's iron haematoxylin, protargol (Honigberg and Davenport, 1954), and the bromphenol-blue method (Mazia *et al.*, 1953) for demonstration of protein. Giemsa and the periodic acid-Schiff (PAS) method were used to stain smears fixed in absolute alcohol. Control slides for the PAS method were treated with diastase.

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RESULTS

Two unexcysted sporocysts are shown in Figure 1. When sporocysts were treated with trypsin and sodium taurocholate, the sporozoites immediately became active. The Stieda body became indistinct, apparently dissolving, and the sporozoites glided through the opening. Excystation was completed approximately 5 minutes after addition of the excystation fluid.

Living sporozoites were vermiform, with a pointed anterior end and slightly curved body (Figs. 2, 3). Sporozoites moved by flexing and gliding. The anterior portion of the body bent towards the curved side (approximately 90° from the straight position) and then quickly straightened. While flexing, the sporozoite contracted, becoming broader and rounder. As the body straightened the sporozoite became more elongate and tapered at the anterior end. The elongate form glided forward for a short distance, then flexion was repeated. The sporozoites appeared to rotate during the gliding movement. All sporozoites examined had a large ellipsoidal refractile body near the posterior end and a smaller rounded refractile body anterior to the nucleus (Figs. 2, 3). The refractile bodies stained deep blue with Heidenhain's haematoxylin

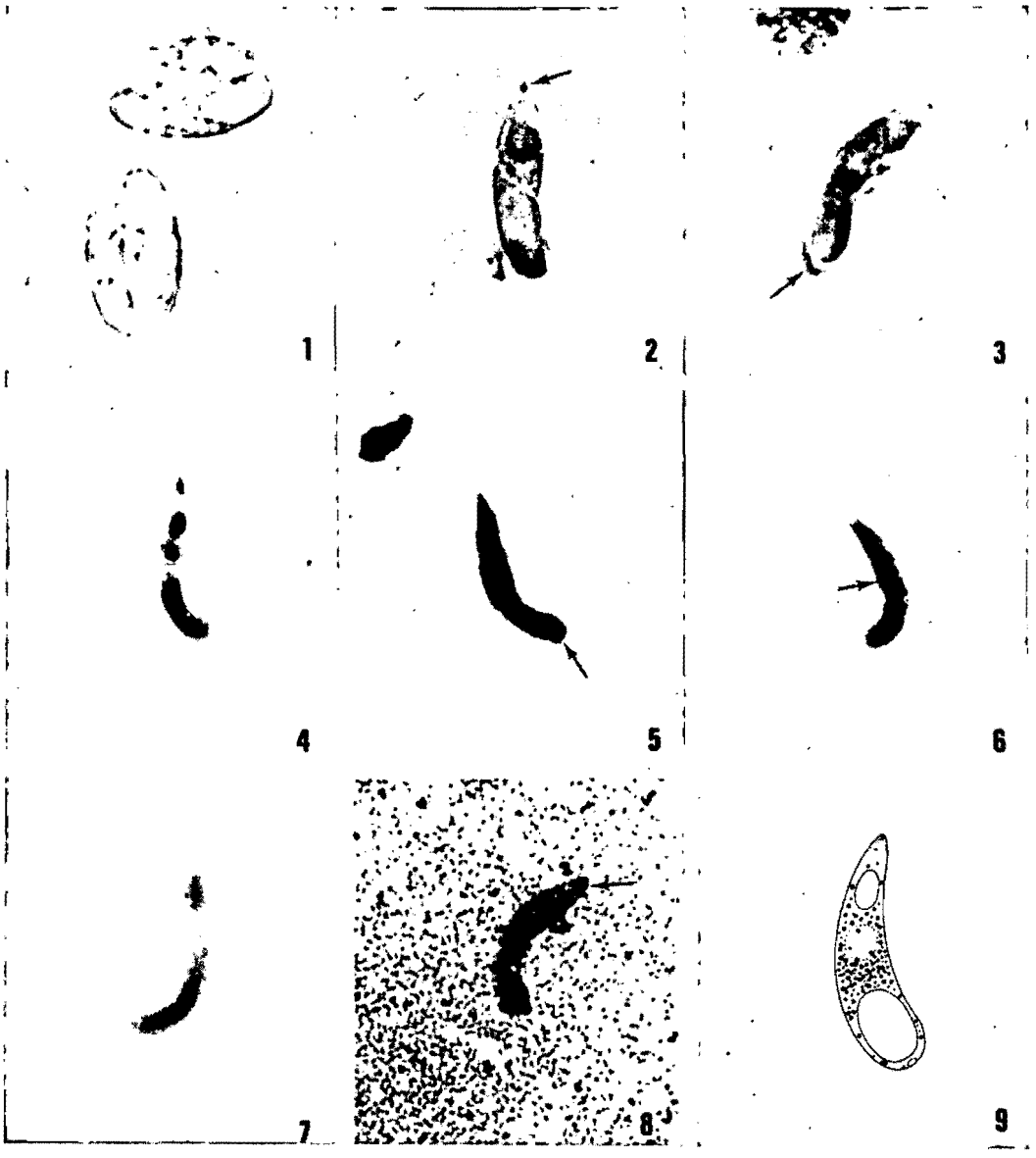

Fig. 1—Fresh unexcysted sporocyst of *Eimeria traguli*. X1500.
Fig. 2—Living sporozoite showing nipple-like projection at anterior extremity (arrow). X1450.
Fig. 3—Living sporozoite showing small refractile granule at posterior extremity (arrow). X1450.
Fig. 4—Sporozoite fixed in Bouin's fluid and stained with Heidenhain's haematoxylin. X1450.
Fig. 5—Sporozoite fixed in methanol and stained with Giemsa, showing concentration of granular material at the posterior end (arrow). X1450.
Fig. 6—Sporozoite fixed in methanol and stained with Giemsa, showing the nucleus surrounded with granular material (arrow). Note granules at the posterior end of the sporozoite. X1450.
Fig. 7—Sporozoite fixed in Bouin's fluid and stained with bromphenol-blue. X1450.
Fig. 8—Sporozoite fixed in Bouin's fluid and stained with protargol, showing 2 dense areas at the anterior tip (arrow), a median rod surrounded by granular material (middle), and dense granules at the posterior end. X1450.
Fig. 9—Line drawing showing location of PAS-positive granules. X2000.

(Fig. 4), pink with Giemsa (Figs. 5, 6), and pale blue with bromphenol blue (Fig. 7). A diffuse area at the anterior tip of the sporozoite usually stained blue with haematoxylin and bromphenol blue (Figs. 4, 7) and pink with Giemsa (Fig. 5). Twenty-five living sporozoites averaged 16 μ long and 5 μ wide.

Living sporozoites had a dark, nipple-shaped anterior tip (Fig. 2). Two small, dense areas appeared at the tip of protargol-stained specimens (Fig. 8). A few granules of different sizes were observed in the anterior and middle portion of the body of living sporozoites and a small refractile granule was seen in the extreme posterior end (Fig. 3). In Giemsa- and protargol-stained specimens the posterior body was usually surrounded by smaller granules (Figs. 5, 6, 8). In protargol-stained sporozoites a median rod-shaped organelle, surrounded by dense granules, was seen in the anterior third of the body (Fig. 8). Subpellicular tubules or fibrils were not observed.

In haematoxylin-stained sporozoites the nucleus usually appeared compact (Fig. 4), although a nucleolus was sometimes visible. The nucleus in Giemsa-stained specimens was similar but was usually surrounded by dense granules (Fig. 6).

With periodic acid-Schiff staining, many small PAS-positive granules were observed scattered throughout the cytoplasm of the sporozoite. They were most common in the middle and anterior portions of the body (Fig. 9).

Figure 10 is a line drawing of a typical sporozoite.

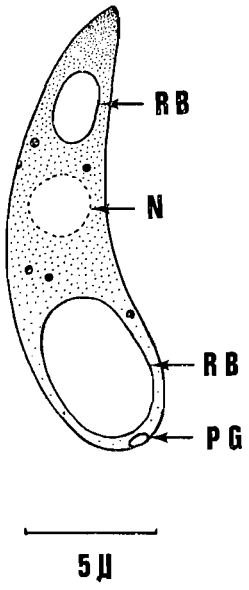

Fig. 10—Line drawing of typical living sporozoite of *Eimeria traguli*. N = nucleus; PG = posterior granule; RB = refractile body.

DISCUSSION

Hammond *et al.*, (1968) observed 4 different kinds of movement in *Eimeria* sporozoites. These were gliding, flexing, probing, and pivoting. Only the gliding and flexing movements were observed in our study of *E. traguli*. Hammond *et al.*, (1965) suggested that similar gliding movements of merozoites of *E. bovis* probably help them to enter the crypts of the intestine and invade host cells. The significance of the other movements is not yet known.

The distribution of PAS-positive granules in *E. traguli* corresponds to that observed by Hammond *et al.*, (1968) in sporozoites of *E. bovis* and by Cheissin (1958) in merozoites of *E. intestinalis*. In an electron microscope study of *E. perforans*, Scholtyseck (1964) recognized that these granules are different from metazoan glycogen and described them as "Coccidienglykogen". Ryley *et al.*, (1969) isolated the polysaccharide material from various stages of *E. brunetti* and *E. tenella* and characterized it by chemical and enzymatic methods as a typical amylopectin.

This polysaccharide provides energy for excystation and movement during penetration of host cells (Cheissin, 1958; Gill and Ray, 1954). Hammond *et al.*, (1968) observed that size of refractile bodies and number of polysaccharide granules are related to motility of sporozoites and merozoites of different *Eimeria* species, noting that the species with the largest refractile bodies had the smallest supply of polysaccharide. Further comparative studies of other *Eimeria* species are necessary to show the relationship between the polysaccharide granules and the refractile bodies and their function as an energy source for the sporozoite.

The nucleus of *E. traguli* is similar to the nuclei described in sporozoites of other species of *Eimeria* (Pattillo and Becker, 1955; Hammond *et al.*, 1968). The nucleus of merozoites of *E. bovis* (Hammond *et al.*, 1965) is also similar; however, no nucleolus was reported.

A spherical body was observed at the posterior tip of living sporozoites of *E. auburnensis* (Hammond *et al.*, 1968), *E. ellipsoidalis* (Roberts and Hammond, 1970) and a strongly argyrophilic posterior granule was reported in merozoites of *E. bovis* (Hammond *et al.*, 1965). The significance of this organelle is not yet known; however, the association with it of granular material in sporozoites of *E. traguli* indicates that it may have a secretory or excretory function.

The nipple-like projection seen at the anterior end of live sporozoites of *E. traguli* resembles the protrusion observed in *E. auburnensis* (Hammond *et al.*, 1968), *E. ellipsoidalis*, and *E. ninakohlyakimovae* (Roberts and Hammond, 1970). This protrusion and the dark areas seen at the anterior tip of protargol-stained specimens of *E. traguli* probably represent the conoid of the sporozoite. Electron microscope studies have shown that the conoid is a dense ring at the anterior tip of the sporozoite, which is thought to aid in penetration of the host cell.

Hammond *et al.*, (1965, 1968) observed a median rod-shaped organelle in sporozoites and merozoites of several *Eimeria* species. *E. traguli* has larger quantities of argyrophilic granular material surrounding the rod-like body than do other observed species. Probably the rod-shaped body represents the paired organelle and the granular material represents the micronemes of the sporozoite. These organelles have been described in electron microscope studies of several *Eimeria* species (Colley, 1967; Hammond *et al.*, 1968; Scholtyseck and Strout, 1968; Ryley, 1969; Roberts and Hammond, 1970; Roberts, Hammond and Speer, 1970; Roberts, Speer and Hammond, 1970). The function of these organelles is not completely known; however, it has been postulated that they may help to support the sporozoite or secrete some substance that helps it penetrate the host cell.

SUMMARY

Oocysts of *Eimeria traguli* from the lesser mouse-deer *Tragulus javanicus* were excysted *in vitro* using bile and trypsin. Sporozoites were studied in fresh and stained preparations. Living sporozoites moved by gliding and flexing. A relatively large refractile body was located near the posterior end of the sporozoite, and a smaller refractile body was present anterior to the nucleus. The staining reactions of these bodies indicate that they contain protein. PAS-positive granules were observed scattered throughout the sporozoite. The nucleus contained a dense nucleolus and was surrounded by granular material. A nipple-like projection was observed at the anterior tip of the sporozoite. In protargol-stained specimens a dense median rod surrounded by granular material was seen in

the anterior third of the body. A small refractile body, often surrounded by granules, was observed at the posterior end of the sporozoite.

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