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Scanning and transmission electron microscopy of host cell pathology associated with penetration by *Eimeria papillata* sporozoites

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Abstract. Scanning and electron microscopy was used to study the pathogenesis that occurred in mouse epithelial cells that had been penetrated by *Eimeria papillata* sporozoites. Optimal penetration of parasites injected into nonligated and ligated mouse intestine was found to occur at 4–15 min post-inoculation. During initial penetration, the parasite caused disruption of the microvilli of the intestinal cells, which led to detachment of the microvilli from the plasma membrane of the penetrated cell. Host cells penetrated by the parasite showed extensive destruction of the internal cellular organization together with blebbing of host-cell cytoplasm and release of internal organelles such as mitochondria. Ultimately, the penetrated cells completely broke down, leaving vacuolated areas next to ultrastructurally normal epithelial cells.

It has been reported that the sporozoites of *Eimeria* species have a dramatic effect on the host cells they infect, including hypertrophy of the nucleoli, nuclei, and cytoplasm coupled with a change in host-cell permeability (see review by Gregory 1990). These events are usually seen at 24–72 h after infection. Although numerous studies have examined the penetration of *Eimeria* sporozoites into host cells (see reviews by Chobotar and Scholtyseck 1982; Ball and Pittilo 1990), no information is available on the extent of cellular pathogenesis that occurs during initial invasion of the cells in vivo. In the present report, we describe the ultrastructural cellular pathogenesis seen in epithelial cells of ligated interestines of mice after their inoculation with excysted sporozoites.

Materials and methods

Oocyts from a pure culture of *Eimeria papillata* that was originally isolated and kindly supplied by Dr. J. Ernst were collected and cleaned and the sporozoites were excysted as previously described (Doran and Vetterling 1967; Hammond et al. 1968; Ernst et al. 1971). After two washes (1000 *g*) in Delbecco's phosphate-buffered saline (PBS), the sporozoites were resuspended in PBS and kept on ice until used.

In all, 12 Swiss-Webster mice (Charles River Laboratories, Wilmington, Mass.) were anesthetized with ether, an incision was made in the lower right side of the abdomen, and a portion of the small intestine was exposed. Eight of these mice were injected with approximately 1.5×10^7 sporozoites at a site located 2 cm below the pyloric valve. The abdominal wall was closed with wound clips and the mice were allowed to regain consciousness. Tissue from these mice was taken at 5, 10, 15, 30, and 45 min after inoculation. In the other mice, approximately 30 mm of the intestine was ligated by loosely tying the gut with suture material so as not to impede the blood flow. This ligated intestine was then injected with approximately 8×10^6 sporozoites. These mice were kept under anesthesia, and tissue from the ligated area was then removed at 5-, 10-, 15-, 20-, and 45-min intervals. All tissues were fixed in 2.5% phosphate-buffered glutaraldehyde for 2 h.

For scanning electron microscopy, the tissues were dehydrated in a graded series of ethanol, critical-point dried, coated with gold/palladium, and examined with an ISI 40 electron microscope. For transmission electron microscopy, the tissues were postfixed in 2% phosphate-buffered OsO_4, dehydrated in a graded series of ethanol, and embedded in Dow epoxy or Spurr's medium (Electron Microscopy Sciences, Fort Washington, Pa.). Specimens were then sectioned on an MT-2B ultramicrotome, stained, and examined in a Zeiss EM 9S2 or a Philipps 200B electron microscope.

Results

As determined by scanning electron microscopy, partial penetration of *Eimeria papillata* sporozoites into the epithelial cells was found to occur optimally at 4–15 min postinoculation (p.i.) in both nonligated and ligated tissue. Therefore, all observations by both SEM and transmission electron microscopy (TEM) were made during this period. Subsequent observations (30–45 min p.i.) revealed considerably fewer sporozoites in the process of invading host cells, although many parasites were seen free in the lumen of the intestine. At 45 min p.i., numerous sporozoites were located intracellularly as verified by TEM.

Initial penetration of the sporozoites into the host cells as viewed by SEM revealed displacement of the microvilli followed by disruption of these structures as

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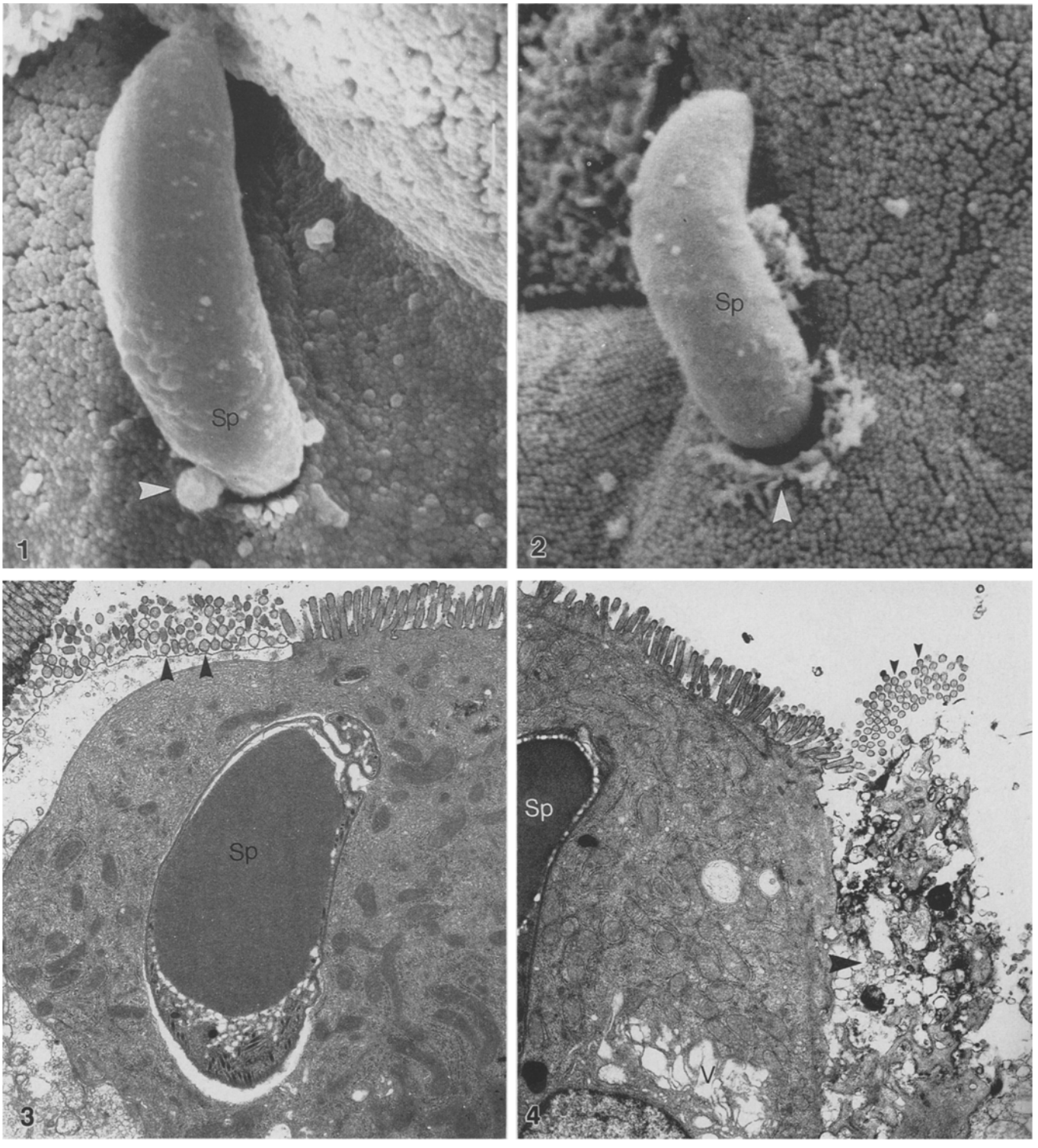

Fig. 1. Scanning micrograph of an *Eimeria papillata* sporozoite (*Sp*) at 4–15 min p.i. that is beginning to penetrate a mouse duodenal epithelial cell. Note the displacement of microvilli (*white arrowhead*). ×12000. **Fig. 2.** Scanning micrograph of an *E. papillata* sporozoite (*Sp*) at 4–15 min p.i. in the process of penetrating an epithelial cell and causing disruption of the microvilli (*white arrowhead*). ×11000. **Fig. 3.** Transmission micrograph of an intact host cell containing an intracellular *E. papillata* sporozoite (*Sp*) at 4–15 min p.i. Note that microvilli of the disrupted cell remain attached to the cell membrane (*black arrowheads*) and that they are undergoing repositioning from a longitudinal to a cross-sectional configuration. ×10000. **Fig. 4.** Transmission micrograph of an intact epithelial cell containing an intracellular *E. papillata* sporozoite (*Sp*) at 4–15 min p.i. Note that some vacuolization (*V*) has occurred in an area in which the sporozoite may have entered the cell from the adjacent, completely disrupted epithelial cell (*black head*) following its penetration by the sporozoite. ×10000

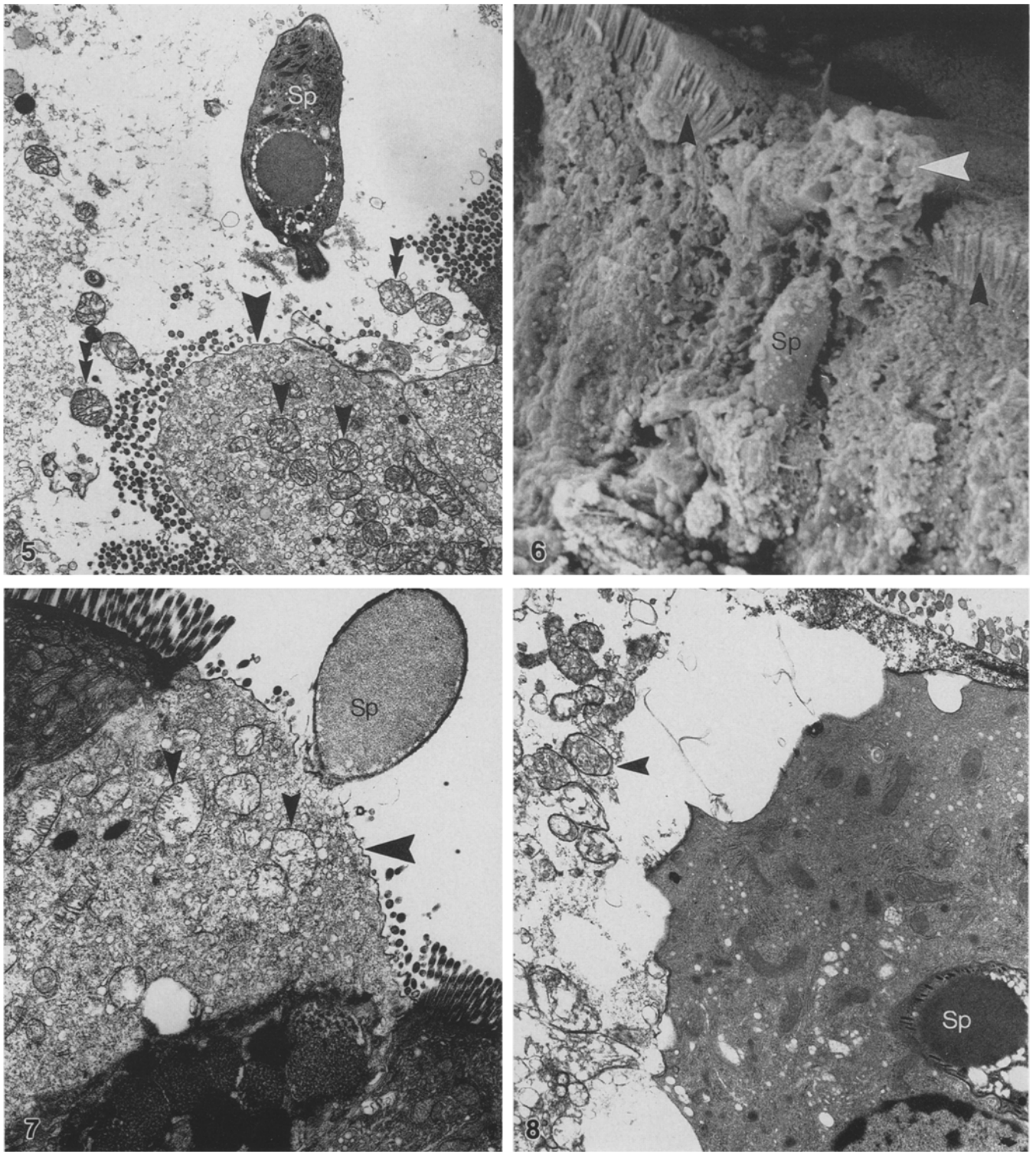

Fig. 5. Transmission micrograph of an extracellular *Eimeria papillata* sporozoite (*Sp*) at 8–15 min p.i. that is located next to epithelial cells that have lost their cytoplasmic consistency. Note the loss of microvilli (*large black arrowhead*), the degeneration of cell mitochondria (*small black arrowheads*), and the presence of degenerating extracellular epithelial cell mitochondria (*small, black double arrowhead*). ×7000. **Fig. 6.** Scanning micrograph of an *E. papillata* sporozoite (*Sp*) that has penetrated a cell 8–15 min p.i., showing disruption of the host cell with associated blebbing of cytoplasm (*large white arrowhead*) at the site of penetration. Note the intact microvilli (*black arrowhead*) on epithelial cells next to the penetrated cell. ×6000. **Fig. 7.** Transmission micrograph of an *E. papillata* sporozoite (*Sp*) penetrating a cell at 8–15 min p.i., resulting in almost complete degeneration of the epithelial cell (large black *arrowhead*). Note the loss of cytoplasmic consistency as compared with the intact adjacent epithelial cells on either side of the infected cell, the loss of microvilli, and the vascuolization of mitochondria (*small black arrowheads*). ×9000. **Fig. 8.** Transmission micrograph of a completely degenerated epithelial cell with vacuolated mitochondria (*black arrowhead*) at 8–15 min p.i., which is located next to a cell containing an intracellular *E. papillata* sporozoite (*Sp*). ×10000

more of the parasite's body passed into the epithelial cell (Figs. 1, 2). TEM observations of cells that had been penetrated by the parasite showed detachment of the microvilli from the plasma membrane of the epithelial cells (Figs. 3–5). In some cases, the microvilli remained attached to their own cell membrane, but they underwent reorientation into a cross-sectional rather than a longitudinal position (Figs. 3, 5). In addition, in some of the penetrated cells, no membrane was seen in association with the disrupted microvilli (Figs. 4, 5).

Continued cellular penetration by the parasite caused extensive disruption and damage to the individual cell but had little effect on the neighboring uninfected epithelial cells (Figs. 4–8). Neighboring cells that were infected with the parasite demonstrated some cytoplasmic vacuolization (Fig. 4). The passage of the parasite through the cell caused considerable alterations in the internal cellular organization, included cytoplasmic vacuolization (Figs. 4, 7), disappearance of microvilli (Figs. 5, 7), loss through blebbing of cytoplasm and internal organelles at the initial site of sporozoite penetration (Figs. 4–6), and degeneration of cell mitochondria and endoplasmic reticulum (Figs. 5, 7). These penetrated cells appeared to become nonfunctional as evidenced by their extensive vacuolation as compared ultrastructurally with uninvaded epithelial cells (Figs. 7, 8).

Discussion

The present shows that substantial ultrastructural damage, which can lead to complete cellular degeneration, occurs in individual intestinal epithelial cells during penetration by *Eimeria papillata* sporozoites. Part of this intracellular pathogenesis is caused by movement of the parasite through the cell. In previous in vitro work, it has been demonstrated that the flexing, gliding, bending, and exiting of sporozoites of *E. larimerensis* and *E. papillata* cause intracellular disruption and degeneration of the host cells penetrated (Speer et al. 1971; Long and Speer 1977; Danforth et al. 1984). As suggested by prior studies on *Eimeria*, *Toxoplasma*, *Plasmodia*, and *Sarcocystis* (Miller et al. 1983; Danforth et al. 1984; see reviews by Augustine 1989; Ball and Pittilo 1990), it is also possible that secretions by the parasite from either its surface or the rhoptry-microneme complex might have an effect not only on the host-cell membrane but also on the cytoplasm of the host cell. It has been reported that during host-cell invasion, the rhoptries empty their contents, and these secretions may be responsible for some of the vacuolization seen in the cell cytoplasm during the present study.

Previous studies based predominantly on in vitro work have led to conflicting reports as to whether the host-cell membrane is interrupted as the parasite enters the cell (see review by Augustine 1989). In the present study, an interruption of the cell membrane appeared to occur concomitantly with the initial in vivo penetration of *E. papillata* sporozoites. These parasites produce a path through the epithelial cells they penetrate, causing disruption of microvilli, blebbing of the cytoplasm out of the area of penetration, and complete destruction of the cell. In an in vivo scanning microscopy study (Pollari and Speer 1981), *E. falciformis* was found to displace the microvilli during early but not subsequent stages of penetration. No blebbing of the cytoplasm from the site of penetration was described by these authors, but they did report that the parasitized cells exhibited a partial to complete loss of microvilli at 2–7 days p.i.

Exactly why the parasite destroys these host cells is not known. Perhaps, as has been suggested, this, destruction is attributable to differences in the unique characteristics of the individual species involved (see review by Augustine 1989). A more plausible explanation may be that the parasites quickly escape the hostile environment of the gut by entering through the epithelial cells and then migrating to or penetrating into other epithelial host cells to continue their development. The invasion of these secondary epithelial cells probably involves a sealing off of the host-cell membrane at the site of penetration. Hence, intracellular parasites are usually seen either next to or in close proximity to the area of penetration in intact epithelial cells (Figs. 3, 4, 8).

The degree of pathogenesis caused in the host by this type of penetration is actually minimal, since the numbers of sporozoites that invade the gut tissue are quite low as compared with later stages of the parasite. It is therefore quite possible that this species of *Eimeria* demonstrates two forms of penetration of gut epithelial cells in vivo: one that destroys the cell and another during which the host cell is kept intact.

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