Parasitol Res (1993) 79:15–23

Parasitology Research

Ultrastructural observations of host-cell invasion by sporozoites of *Eimeria papillata* in vivo

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Accepted: 25 August 1992

Abstract. Scanning and transmission electron microscopy were used to study the invasion of mouse small-intestinal epithelium by sporozoites of *Eimeria papillata*. Some mice received oocysts by gavage and others received either sporocysts or sporozoites by direct injection into the small intestine. The highest concentration of invaded cells were found in ligated intestinal tissues studied at 5–45 min after the inoculation of sporozoites. Sporozoites actively invaded anterior end first, which resulted in extensive damage to the host cell. Such cells showed disrupted microvilli; protuberances of cytoplasm into the lumen, apparently the result of a disrupted plasma membrane; vacuolization of the cytoplasm; and damage to the mitochondria. These damaged cells were rapidly vacated as the sporozoite moved laterally into one or more adjacent intact host cells without entering the lumen. It is suggested that the host cell initially entered from the lumen becomes so severely traumatized that the parasite of necessity enters an adjacent cell as a prelude to further development. Various aspects of host-cell invasion by coccidia and malarial parasites are reviewed.

The survival and successful propagation of coccidian parasites involves their intracellular existence for a considerable portion of their life cycle. Such existence is initiated by the penetration of host cells by sporozoites and merozoites. Host-cell-parasite dynamics associated with the mode of entry have been the subject of numerous studies yielding varied and inconclusive results (see reviews by Doran 1973, 1982; Long and Speer 1977; Russell and Sinden 1981, 1982; Chobotar and Scholtyseck 1982; Russell 1983; Werk 1985; Augustine 1985, 1989).

A review of the literature shows that investigations dealing with cell invasion have relied primarily on the use of cultured cells. However, studies on this subject directly involving the host animal, in which the environment is much less variable and site and host specificity are more rigid, are lacking. Thus, valid comparisons of cell invasion in in vitro and in vivo systems have not been possible. Attempts to use host animals have been largely unsuccessful because inoculation of oocysts by gavage results in a nonsynchronous release of sporozoites, thus reducing their concentration over a relatively immense intestinal environment, even when large numbers of oocysts are inoculated. We have overcome this problem by inoculating sporocysts and sporozoites of *Eimeria papillata* directly into the intestine of host animals. In the present study we report the results of an ultrastructural investigation of host-cell invasion in the natural host.

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Materials and methods

Preparation of the parasites

Oocysts of *Eimeria papillata* were harvested, sporulated, cleaned, and stored according to the method of Hammond et al. (1968). Freshly sporulated (3 months or less) oocysts were washed four times to remove the potassium dichromate. After two washes in phosphate-buffered saline (PBS), the oocysts walls were crushed with a Teflon-coated tissue grinder (Tri-R Instruments). The sporocysts were washed twice in PBS and excysted in 2.0 ml excystation fluid at room temperature (22° C) for 5 min. The excystation fluid consisted of 0.05 g trypsin (Sigma) and 0.15 g taurocholic acid (Sigma) in 20 ml PBS (pH 7.4). The freed sporozoites were purified in a glass column assembly that had been packed with 1.0 g scrubbed nylon fiber. Purified sporozoites were concentrated in 1.0 ml PBS for injection into the mouse intestine.

In vivo experiments

Commercially purchased (Charles River) Swiss Webster outbred male mice (12–14 weeks old) were used for this study. Approximately 2×10^6 million sporulated oocysts were inoculated into the stomach of some mice. The small intestine was exposed through a small incision in the abdomen wall of other, anesthetized mice,

which were injected with approximately 3×10^6 million sporocyts by syringe directly into the upper duodenum or with 1.5×10^7 million sporozoites into a site located 2 cm below the pyloric valve. The abdominal wall was closed with clips and the mice were allowed to regain consciousness. Mice that had received oocysts were killed at 4, 8, 13, and 16 h after inoculation. The mice that had received sporocysts and sporozoites were killed at 5, 10, 15, 30, and 45 min after inoculation. Tissue samples were removed from these animals at 4-, 8-, 12-, 16-, 20-, and 24-cm levels of the small intestine for study by scanning (SEM) and transmission electron microscopy (TEM).

To increase the concentration of sporozoites per intestinal unit area, a 3-cm section of small intestine was lightly ligated with suture material at a site located 8–10 cm below the pyloric valve. Approximately 8 million sporozoites were injected by syringe directly into the ligated area, and tissue samples were taken at 5, 10, 15, 20, and 45 min after exposure. All tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h and then postfixed in 2% OsO_4 after two buffer washes. During the graded alcohol dehydration, tissues prepared for TEM were prestained en bloc with 1.0% uranyl acetate and 1.0% phosphotungstic acid in 70% ethanol. After dehydration, these tissues were cleared in propylene oxide and embedded in Dow epoxy resin or Spurr low-viscosity medium. Ultrathin sections were poststained with uranyl acetate and lead citrate and examined with a Zeiss EM 9S2 or a Philips 200 electron microscope. For SEM, the dehydrated tissues were critical-point-dried, coated with gold-palladium, and examined with an ISI 40 SEM. In addition, intestinal scrapings from several mice that had been exposed to sporozoites for 5, 10, and 15 min were used to prepare wet mounts (PBS) for observation of the interaction between host cells and sporozoites.

Results

The examination of tissues from mice that had been inoculated with either oocysts by gavage or with sporocysts by direct injection into the lumen of the upper duodenum proved to be unsatisfactory due to the paucity of parasites. More success was achieved in tissues from mice that had received excysted sporozoites. However, the highest number of parasites were observed in the ligated tissues.

Because the events associated with cell contact and progression of the parasite into the cell have been rather controversial, we wished to examine this phase rather carefully. Thus, when large numbers of sporozoites had been interiorized by 45 min after their inoculation into unligated intestine, we gradually decreased the time during which tissues were exposed to the sporozoites in the ligated tissue. The best results of early stages of invasion were observed at 5 and 10 min exposure. Even by this time, many sporozoites had completed cell entry, indicating that invasion is a rapid event.

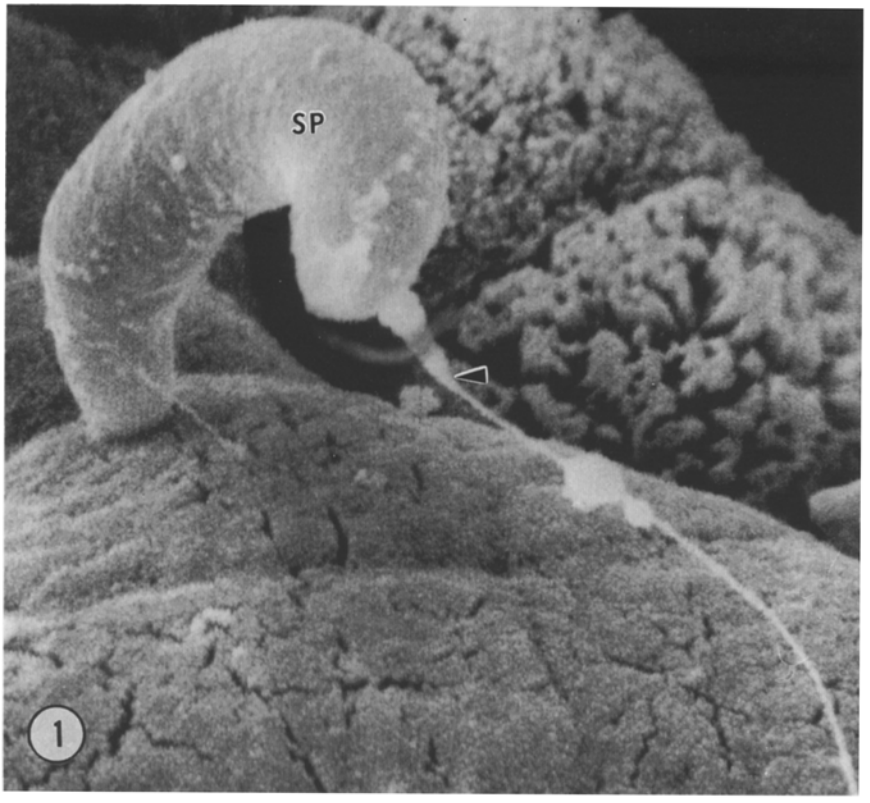
Light microscopic observations

Scrapings of intestinal tissues that had been exposed to sporozoites for 5, 10, and 15 min showed villi with numerous segments of intact epithelial cells. Many sporozoites had accomplished cell entry, but others were free among the tissues and were flexing, gliding, and twisting, and a number of these were observed to invade cells. Extension and retraction of the anterior apex of the sporozoites was common. Invasion always occurred anterior end first and proceeded so rapidly that interiorization was complete within 5 s in many cases.

There seemed to be little preliminary activity such as probing or site selecting; the sporozoite simply approached the cell while gliding and entered it immediately. Some leakage of contents from the entered cell occurred during the process of entry and immediately after the sporozoite was inside, but this ceased almost at once. When inside the cell, the sporozoite continued movements such as rotating on its longitudinal axis, limited flexing, and turning in tight circles while in a flexed position. A number of sporozoites continued these movements for a few seconds after cell entry and suddenly exited through the luminal side of the epithelial cells. Such cells usually showed evidence of considerable damage, including loss of cytoplasm and partial collapse of the cell. More commonly observed, however, was lateral displacement of the sporozoite from the cell initially entered, the process culminating in the sporozoite's entry into an adjacent epithelial cell. This usually resulted in the sporozoite's appearing to occupy two cells simultaneously. One sporozoite in particular was observed to enter and exit through a row of ten epithelial cells over a period of 3 min without entering the luminal area. This extensive passage through so many cells seemed to be an exception, as most other sporozoites stopped after passing through three of four cells. A constriction of the sporozoite similar to that observed during invasion from the lumen occurred during cell-to-cell passage. In all instances, sporozoite movement proceeded apical (conoid) end first.

During lateral passage from cell to cell, sporozoite movement and displacement was more sluggish, as if it were being retarded by the cytoplasm. Exiting one cell and entering another lasted 4–5 times (20–30 s) longer than entry from the lumen. When migration was about to cease, flexion of the sporozoite resulted in its repositioning within the last cell entered to conform to the longitudinal axis of the epithelial cell. In nearly every instance observed, the anterior end of the parasite pointed toward the lumen.

Fig. 1. Scanning electron micrograph of a sporozoite (*SP*) of *Eimeria papillata* entering an epithelial cell conoid end first. Note what appears to be a strand of mucus (*arrowhead*) extending from the sporozoite. Fixed at 5 min after inoculation. ×11000. **Fig. 2.** A sporozoite in the process of vacating a damaged cell (*DC*) that was entered initially. Note the protuberances and the lack of membranes on the cytoplasm of the damaged cell. Note also the apparent tight junction (*arrowheads*) at the sight of constriction as the parasite proceeds laterally into an adjacent cell. Fixed at 10 min after inoculation. ×20000. **Fig. 3.** Transmission electron micrograph of a specimen similar to that shown in Fig. 1. Note the dislocated microvilli and the invagination of the host-cell membrane (*arrowhead*). Fixed at 5 min after inoculation. ×20000. **Fig. 4.** Postinvasion morphological change in a host cell. Note the absence of microvilli and the bulging of the cytoplasm into the lumen (*arrowhead*). Note also the displacement of organelles as compared with the adjacent intact cell. Fixed at 10 min after inoculation. ×12000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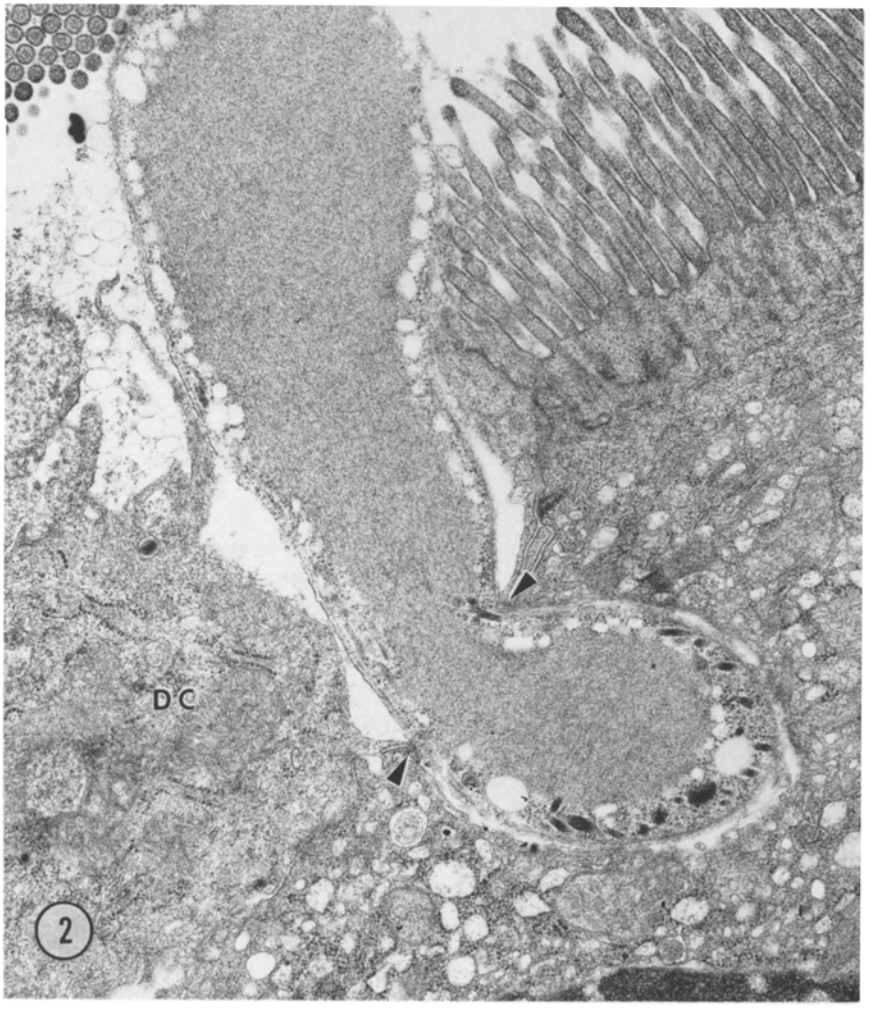
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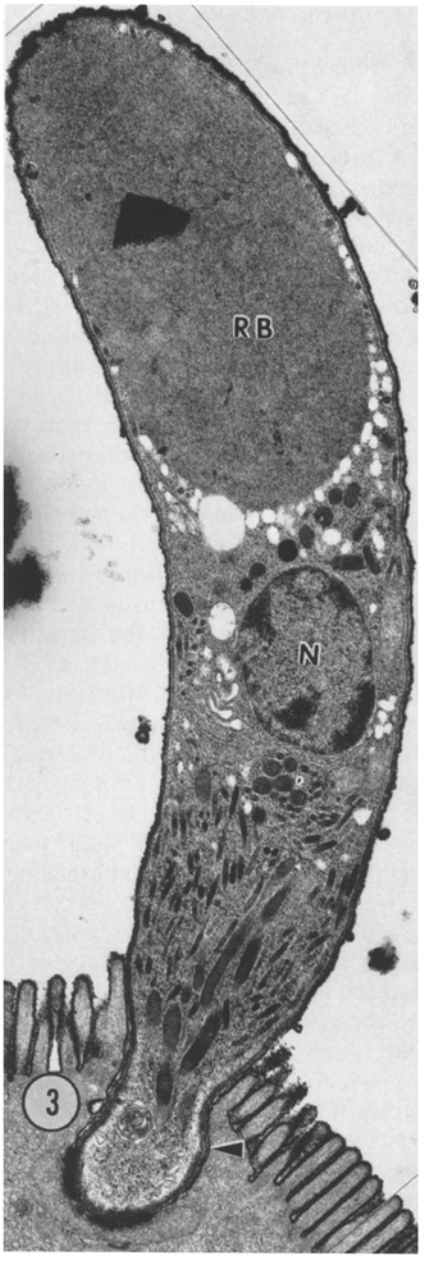
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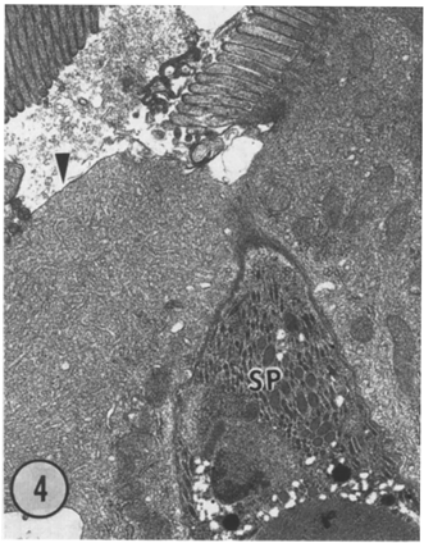
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Electron microscopic observations

The micromorphology of sporozoites of *Eimeria papillata* is nearly identical to that described by many other authors (Roberts and Hammond 1970; Scholtyseck 1973; Chobotar and Scholtyseck 1982) and is therefore not repeated in detail in this review.

Sporozoites in contact with and in the process of entering cells were positioned perpendicularly to the epithelial layer such that their posterior ends pointed into the lumen of the intestine (Figs. 1, 3). The tapered apex of the parasite with its extended conoid caused microvilli to part, making some of them appear fused (Fig. 3). A small constriction appeared at the lateral sides of the parasite (Figs. 2, 3). Inward movements led to a retraction of the conoid and a simultaneous stretching of the host-cell membrane, resulting in an invagination that conformed to the shape of the sporozoite. The area of closest contact between the host cell and the invading parasite was at the constriction, which was characterized by an increased electron density and represented a ring-like zone that moved over the parasite surface during invasion (Figs. 2, 3).

There was no apparent disruption of the invaginating membrane while the parasite was between one-fifth and halfway into the cell. In some locations, particularly at the anterior end, all membranes became indistinct (Fig. 5). This was interpreted to be the result of the plane of the section being tangential to the membrane, because no abrupt termination of membranes or membrane segments was observed, as would be expected if disruption were to occur. The host-cell membrane apparently became considerably stretched as the sporozoite progressed into the cell (Fig. 5). Constriction of the parasite continued at the entrance site until interiorization was complete at which time the sporozoite regained its normal shape (Figs. 6, 7, 9).

During the intermediate and late stages of entry and moments after the parasite was inside the cell, several changes became obvious with regard to the host cell and parasite. Such cells were considerably deformed as demonstrated by disruption of the normal palisade pattern of microvilli, and portions of the original plasmalemma of the host cell appeared to be completely detached from the entered cell. Protuberances of host-cell cytoplasm covered with one or two membranes projected into the lumen. Vacuolization of the host-cell cytoplasm and rearrangement of the mitochondria and endoplasmic reticulum were common. Some invaded cells appeared to be so badly damaged that the possibility of their recovery was in question (Figs. 2, 4).

In some areas, two or more adjacent cells were infected, and some cells contained more than one sporozoite. The parasites were found in a variety of positions with respect to the columnar configuration of the host cell, indicating that they had undergone considerable movement after cell invasion. Some sporozoites were fixed in a J-shaped position, with the concave side having a series of wave-like folds. Sporozoites that appeared to have been in motion at the moment of fixation were usually surrounded by fragments of host-cell membranes, if any membrane at all was present. The damage to host cells and their membranes was most evident in tissues that had been exposed to sporozoites for 5, 10, and 15 min. However, in some specimens, damage at the luminal surface of host cells was markedly reduced at 15 min and especially at 20 and 45 min. In these cells, the sporozoites were usually positioned parallel to the longitudinal axis of the cell, with the anterior end of the parasite nearly always being pointed toward the luminal surface of the epithelial cell, whose microvilli appeared normal, as did those of adjacent uninfected cells (Fig. 9). However, in nearly every case there was some evidence of disturbance of host-cell membranes and local cytoplasm at the cell-to-cell junction area either lateral to or below the sporozoite (Fig. 9). This suggested that the sporozoites had entered the present cell not from

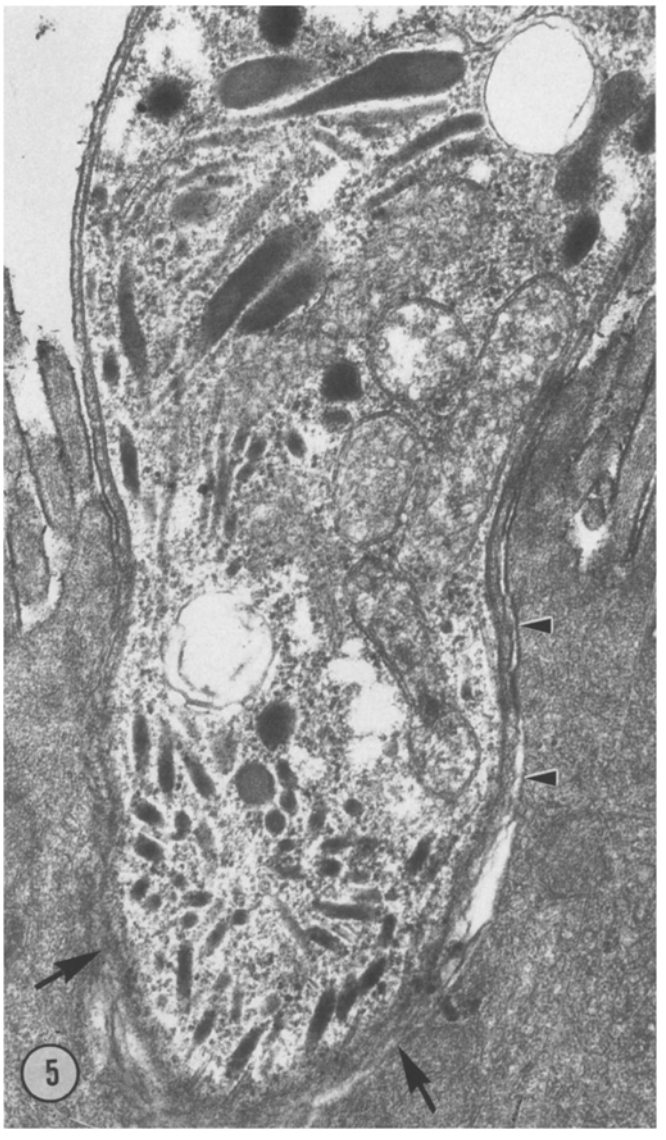

Fig. 5. Partial penetration of a host cell by a sporozoite. Note the constriction of the parasite and the invaginated host-cell membranes (*arrowheads*). Membranes of the parasite and host cell are not visible at the anterior end of the sporozoite due to a tangential cut through the membranes (*arrows*). Fixed at 10 min after inoculation. ×30000

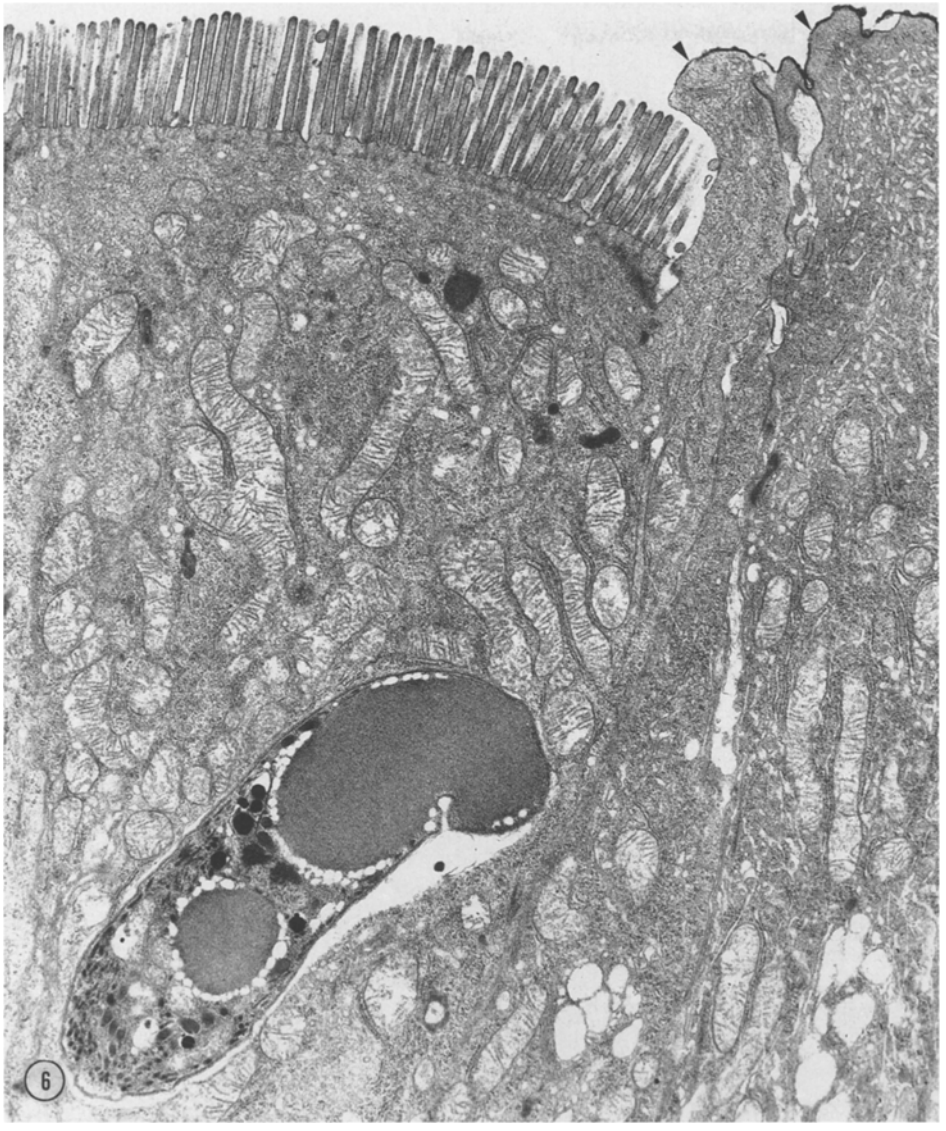

Fig. 6. Recently invading sporozoite fixed while apparently moving downward and to the left. Invasion appears to have occurred near the junction of two adjacent epithelial cells. Note the absence of microvilli and the bulging of the cytoplasm in the area of penetration (*arrowheads*). Fixed at 10 min after inoculation. ×14000

the luminal surface but laterally from an adjacent cell. These apparently no-longer-motile sporozoites were usually surrounded by an uninterrupted, closely apposed membrane, apparently the parasitophorous vacuole membrane.

Indeed, lateral passage of the sporozoites as observed by light microscopy was confirmed at the ultrastructural level (Figs. 6–8). Parasites in transit usually lay horizontal to the longitudinal orientation of the host cell and parallel to the lumen (Fig. 7). Longitudinal sections through such organisms revealed a constriction and some increase in electron density at the margins of the interface between the parasite and the membranes of the two adjacent epithelial cells. Whereas in light microscopy, the sporozoites appeared to occupy two epithelial cells simultaneously for a period during passage, electron micrographs revealed a somewhat different relationship: instead, the anterior portion of the parasite was invested with two membranes of host-cell origin, whereas the posterior part, usually that behind the constriction, was in contact with the cytoplasm with no epithelial-cell membrane covering (Figs. 7, 8).

Thus, the parasite actually remains in the cell it is vacating while superficially appearing in some electron micrographs to have up to two-thirds of its body length in the cell to be entered. The sporozoite therefore occupies a deepening invagination composed of the two membranes of adjacent epithelial cells that have become considerably stretched in the process. Because no sporozoite was observed to be covered with more than two host membranes, it is assumed that continued progression of the sporozoite eventually causes a breach in these membranes, permitting the parasite to enter the next cell. Except for some crowding and distortion in the shape of the mitochondria, the cells occupied by sporozoites in transit showed no unusual irregularity.

Discussion

The events of host-cell invasion at the ultrastructural and molecular levels in the Apicomplexa have proved to be one of the most challenging and conflicting problems encountered in the study of these organisms. Be-

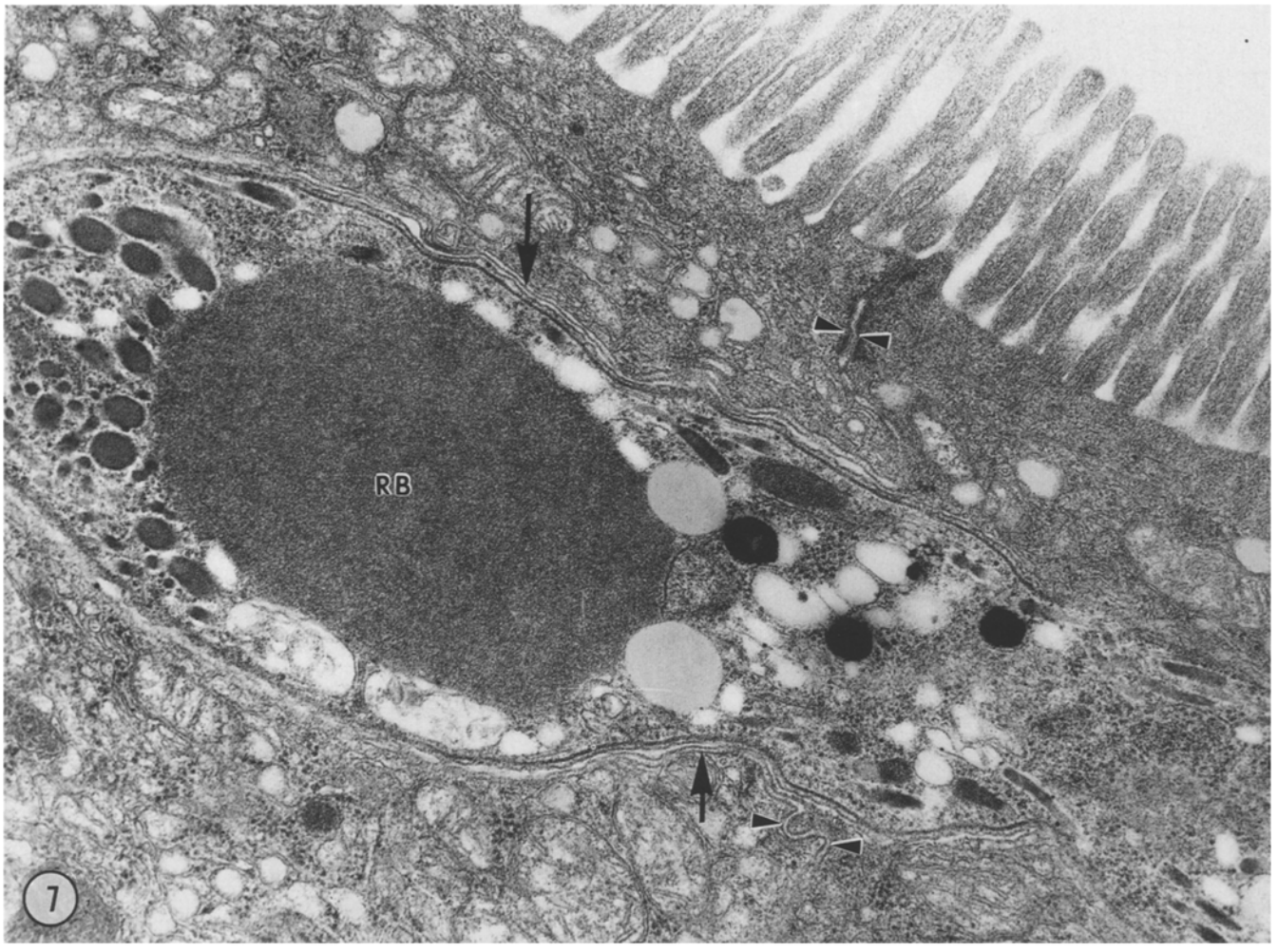

Fig. 7. Sporozoite in lateral displacement, moving from the right to the left from one epithelial cell to another. The boundaries between the two cells are clearly visible above and below the sporozoite (*arrowheads*). Note the stretched host-cell membranes that envelop the parasite toward the left of the cell-to-cell junction (*arrows*). Fixed at 15 min after inoculation. × 30000. **Fig. 8.** An enlarged parallel section of the specimen illustrated in Fig. 7, showing the relationship of the two adjacent host-cell membranes (*M1*, *M2*) to the membranes of the parasite. *M1* is the membrane of the epithelial cell on the *right* and *M2* is the membrane of the epithelial cell on the *left*. The plasma membrane (*P*) and the inner complex (*IC*) of the sporozoite (*SP*) are clearly evident. Fixed at 15 min after inoculation. × 38700

cause of the obvious morphological and biochemical intimacy between the host cell and the parasite as well as the subsequent pathogenesis that follows interiorization, a sufficient understanding of this process with the purpose of preventing entry is a highly desirable goal. To date, the majority of studies have involved a variety of primary and established cell lines grown in culture (see Doran 1982; Speer 1983). Such in vitro systems have proved to be useful because they can be easily manipulated and the host-cell-parasite interaction can be more readily monitored in culture than in vivo.

The ability of most species of coccidia to invade a wide variety of cells in culture, including those originating from an animal in a different taxonomic class, is

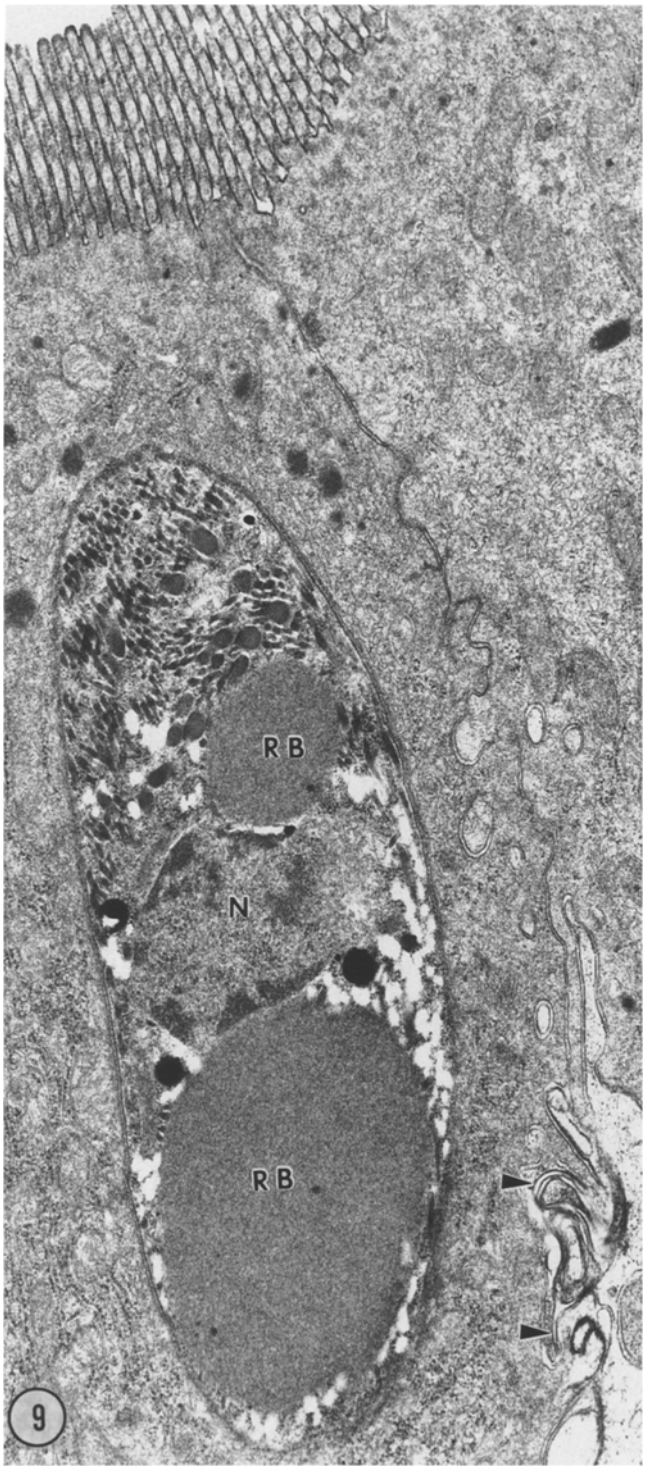

Fig. 9. Sporozoite positioned with its apex pointing toward the lumen in its orientation parallel with the long axis of the host cell. The parasite apparently entered this cell from a lateral rather than a luminal aspect as evidenced by the disturbance of the membrane in the *lower right* portion of the micrograph (*arrowheads*) and the intact microvilli. Fixed at 15 min after inoculation. ×20000

well established (see reviews by Doran 1982; Speer 1983; Werk 1985). This lack of specificity may account for the discrepancies among the descriptions of host-cell invasion. We therefore undertook a study of the tissues normally invaded by *Eimeria papillata* in vivo so as to provide more detailed information on the events that occur at the onset of coccidian infection.

The development cycle is completed throughout the small intestine of *Mus musculus*, with the highest concentration occurring in the middle one-third (Chobotar, unpublished data). By examining living and fixed cells, we established that interiorization of *E. papillata* is a consequence of the considerable motile activity of the parasite and that entry occurs conoid end first. The penetration is a relatively rapid event, with some parasites completing invasion within 5 s. Electron micrographs show an initially shallow, pocket-like invagination of the host-cell plasma membrane and a constriction of the parasite at the rim of the invagination. Continued inward displacement results in an enlarged membranous passage that appears to remain intact during the initial stages of entry.

The constriction zone represents an area of particularly close contact between the parasite and the host-cell membranes that is retained throughout the invasion. Although the constriction ring observed in the present study is not as pronounced as that in *Plasmodium*, it is similar to the "moving junction" described by Aikawa and co-workers in the latter species (1978, 1981) and by Entzeroth (1985) in *Sarcocystis* and to the "close junction" reported by Michel et al. (1980) in *Toxoplasma*. Aikawa et al. (1981) have proposed that the junction is a requirement for the invasion of erythrocytes by malarial parasites. The considerable alterations occurring in host-cell membranes at this contact zone has prompted Mitchell and Bannister (1988) to suggest that "the importance of the junction may be primarily to provide a stable area against which the motile apparatus of the merozoite can produce a shearing movement during interiorization." Although these junctions have not been studied in such detail as those of *Plasmodium*, their similarity in structure and consistent appearance in coccidia during invasion suggests a similar function.

From data obtained in many studies (Russell and Sinden 1981; Russell 1983; King 1988; Mitchell and Bannister 1988), it has been established that motility is an essential component of the invasion process in many members of the Apicomplexa; any interference with motility either reduces or inhibits invasion. The ability of eimerian sporozoites to cap surface ligands by a backward translocation from anterior to posterior, finally shedding the ligand into the medium, is considered by Russell and Sinden (1981) and Russell (1983) to be analogous to posteriad capping of the junction ring as the parasite "locomotes" into the cell. Such kinetic activity can result in complete interiorization of the parasite with 5–60 s (present study; Nichols and O'Connor 1981; Werk 1985; Mitchell and Bannister 1988).

In observations we have made over a period of 20 years, we have found that sporozoites of *E. papillata* show an unusually high level of movement and motive force as compared with other species. Flexing and gliding and twisting along the longitudinal axis are the most frequent movements observed in *E. papillata* and may be continuous for 8 h or more at room temperature (22° C) (Chobotar, unpublished data). The mechanical

force produced by such activity results in considerable alterations in cell monolayers and in damage to individual cells, including vacuolization of host-cell cytoplasm and disruption of mitochondrial and cell membranes by sporozoites of *E. papillata* (Danforth et al. 1984). More recently, in an in vivo study of host-cell invasion, Danforth et al. (1992) described extensive damage to mouse epithelial cells, including the release of cytoplasm and organelles.

In spite of the numerous studies thus far carried out, the fate of the target-cell membranes from the time of initial invagination until the complete interiorization of the parasite remains unsettled. The majority of authors agree that the host-cell membrane does not rupture (Jensen and Hammond 1975; Jensen and Edgar 1978; Werk 1985; Dubremetz 1988), yet other investigators have reported interruptions at the site of entry (Roberts et al. 1971; Scholtyseck and Chobotar 1975; Long and Speer 1977; Nichols and O'Connor 1981). Jensen and Edgar (1976) have rightly pointed out that some of the contradictions may be due to the methods used to interpret the position of membranes during the sectioning procedure. Stretching and variability in the orientation of the parasite through the plane of a section often results in cross and oblique sections through the same membrane. Thus, some membranes may be interpreted to have gaps in areas in which they have been cut along their length or at an oblique angle, whereas they appear as typical unit membranes when they have been cut in cross section. However, parasite membranes also "fade out" when sectioned obliquely; thus, Jensen and Edgar (1976) used the criterion of comparing adjacent host-cell and parasite membranes for distinctness.

A study of particular interest in this regard was conducted by Nichols and O'Connor (1981), who were able to distinguish between phagocytosis and parasite-mediated damage induced by *Toxoplasma* invasion by the invariable presence of disrupted membranes during the latter process. Despite the presentation of well-prepared specimens by Nichols and O'Connor (1981), a number of authors have stated that the broken membranes represent fixation artifacts. For example, in referring to this study, Silva et al. (1982) stated that "although the images presented are of excellent quality it is not possible to rule out the possibility that the plasma membrane of the macrophage at the region in close contact with the parasite was intact but somewhat modified and was not well preserved during fixation."

A possible reason for the resistance to accept the existence of broken membranes may be related to the basic structure and behavior of constituent molecules of membranes. A review of the chemicomolecular nature of the plasma membrane suggests that under normal conditions, portions of the bimolecular leaflets would not occur as loose fragments, even for short periods of time (Alberts et al. 1989). The tendency would be for any free edges resulting from a tear to form circular compartments or vesicles as a result of inward folding of hydrophobic molecules. Closed vesicles of this type are prominent adjacent to the invading toxoplasma, along with segments of broken host-cell membranes with free ends (Nichols and O'Connor 1981, Figs. 2–4). These fragments have sharply defined edges at the point of the apparent breaks and do not show the fading distinctness that would be expected as a result of an oblique cut. Thus, it would seem rather curious that this material would be subject to selective fixation, whereby optimal preservation would have been achieved throughout the cell with the exception of the area showing broken membranes.

Instead, we suggest that given the considerable mechanical force generated by some invaders, the tearing of target membranes should not be surprising. Furthermore, if the fixation process had "frozen" the fragments at the moment of rupture or immediately thereafter, it is possible that rejoining of the free ends would not have had time to occur and that the latter would thus appear as unattached pieces of membrane. This suggestion is supported by the presence of similar membrane segments in the present study and by previous observations of longitudinal membrane fragments adhering to the surface of *E. papillata* sporozoites that had exited after passing through potential host cells (Danforth et al. 1984, 1992). This question, which has generated a considerable amount of discussion, may be academic, as the parasites are capable of continuing propogation regardless of whether or not the target-cell membranes are ruptured; however, it is important in that it serves to distinguish between mechanisms of entry (active invasion versus phagocytosis, for example).

In later stages of entry, our findings provide cytological evidence of considerable alteration and disruption of host-cell membranes and loss of cytoplasm to the extent that the cell initially entered may not be suitable for further development of the parasite. Thus, the sporozoite vacates the damaged cell and passes into and through other cells with little inhibition (albeit with less kinetic motion than in the initial entry phase). Finally, after passage into one or more new cells, the sporozoite assumes an orientation parallel to the long axis of the epithelial cell and ceases movement, probably as a prelude to dedifferentiation and further development. The reduction in kinetic activity is reflected in the minimal intracellular disruption occurring during cell-to-cell passage.

The broad affinity of coccidian parasites for different host-cell types might imply that the kinetic component is primarily responsible for invasion, with little or no biochemical activity being involved. This process is much more precise in the malarial parasites that infect only erythrocytes for which they are highly selective according to host and nonhost origin. This selectivity is based on the presence of surface molecules that interact in a receptor-ligand arrangement (see reviews by Mitchell and Bannister 1988; Perkins 1989). Until recently, there has been little definitive information as to possible biochemical signals between coccidia and their target cells. However, the use of recently developed techniques has provided some lines of evidence to support the view that host-cell parasite recognition may be present. For example, data reported from several studies show that treatment of host cells and coccidial parasites with enzymes and/or monoclonal antibodies that either modify or block potential surface-receptor–ligand complexes prevents or reduces host-cell invasion (reviewed by Augustine 1989).

The study of this process in vitro brings along its own set of limitations. There is, of course, virtually no information as to the recognition factors that operate in vivo. Invasive stages of coccidia introduced parenterally subsequently migrate to, and develop in, their normal sites (Joyner 1982; Speer 1983). There is presently no indication of the chemotactic factors or homing mechanisms that direct the parasite to its proper location. Even more restrictive is host specificity, as evidenced by a lack or severe limitation of development in vitro, especially when nonhost cells are invaded (Doran 1982; Speer 1983). It is therefore clear that the biochemical factors that dictate site specificity and, especially, host specificity also determine cell specificity and further development in vivo.

The challenges involved in the study of a highly complex intracellular relationship in vivo are enormous and presently seem insurmountable. With reference to the malarial parasite, Perkins (1989) has noted that "the more we examine the biochemical events underlying the invasion process, the more complex and variable it seems to be." In our attempt to "standardize" host-cell invasion, we may have failed to recognize, as pointed out by Augustine (1989), that each species of coccidia may have its own set of requirements for successful invasion. Thus, there may in fact be as many variables as there are species of coccidia.

Acknowledgements. Special thanks are due the Alexander von Humboldt Foundation, which sponsored one of the authors (R.E.) through a Feodor Lynen postdoctoral fellowship during his stay at Andrews University. This work was also supported in part by grants from Andrews University and by a Faculty Development Grant from the Merck Foundation, Rahway, New Jersey. We also wish to acknowledge the expert assistance of Ms. D. Fisher and Ms. J. Messersmith.

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