THE INTERACTION BETWEEN *TOXOPLASMA GONDII* AND MAMMALIAN CELLS

I. Mechanism of Entry and Intracellular Fate of the Parasite*

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Toxoplasma gondii is a coccidian protozoan capable of infecting a wide range of vertebrate cells (1). Intracellular toxoplasmas, which appear to reside in cytoplasmic vacuoles, multiply with a generation time of 5–10 hr to form rosettes, leading eventually to cell rupture and liberation of the parasites. Extracellular multiplication of toxoplasmas has not been demonstrated.

Electron microscope studies have established the ultrastructure of toxoplasmas and the mechanism of their intracellular multiplication by an unusual process called endodyogeny (2, 3), but little or no information is available on the precise mechanism by which toxoplasmas enter the host cells, or on aspects of host cell physiology that allow the parasites to thrive. Phase-contrast motion pictures have demonstrated the entry of toxoplasmas into cells to be rapid, occurring within seconds of parasite-host cell contact, and the entry is furthermore characterized by a constriction of the parasite as it appears to traverse the cell membrane (4, 5). The term penetration has been widely used to describe this entry process.

We describe in this paper in vitro systems for studying the interaction between *Toxoplasma gondii* and various phagocytic and usually nonphagocytic mammalian cells. In order to study the penetration process by electron microscopy, a technique was devised for bringing parasites and host cells into contact at low temperatures. On warming, large numbers of toxoplasmas enter the cells in a short time; fixation of these specimens in the first few minutes after warming has allowed detailed observations to be made on the mechanism of parasite entry. Studies have also been made on the fate of toxoplasmas after their entry into macrophages, fibroblasts, and HeLa cells.

Materials and Methods

The Parasites.—The RH strain of *Toxoplasma gondii*, originally isolated by Sabin (6), was obtained from Dr. Ann Kimball of the Cornell University Medical Center. The parasite was passed intraperitoneally at 3- or 4-day intervals in NCS Swiss mice. Appropriate precautions were used to prevent infection of laboratory personnel. Parasite passage was accomplished by

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placing the toxoplasma-rich peritoneal exudate (usually 0.2–0.5 ml) from three infected mice into 1 ml of minimal essential medium (MEM)[1] (Microbiological Associates, Inc., Bethesda, Md.), diluting 1:20 in MEM and inoculating mice with 0.3 ml of this diluted suspension.

In order to recover toxoplasmas for experiments in vitro, the parasite-rich peritoneal fluid was diluted with 2 vol of MEM and centrifuged at 30 *g* for 5 min to remove cells and debris. The supernate was then centrifuged at 350 *g* for 10 min to deposit the free toxoplasmas. The pellet was resuspended in MEM, containing 20% heat-inactivated fetal calf serum (HIFCS, Sabin-Feldman dye test negative; Grand Island Biological Co., Grand Island, N. Y.), and the number of parasites was determined by counting in a hemacytometer using phase-contrast microscopy with a 40 × objective. The organisms were used within 1 hr after removal from the mouse peritoneal cavity. The yield of parasites was 4–10 × 10^7, 98% of which excluded trypan blue dye.

The Cells.—Mouse peritoneal macrophages were obtained from unstimulated peritoneal cavities of normal NCS mice by lavage with heparinized phosphate-buffered saline according to techniques described previously (7). These cells (1–2 × 10^6) in MEM and 20% HIFCS were allowed to adhere for 1 hr to round glass cover slips $\frac{5}{8}$ inches in diameter placed in 35-mm Falcon plastic dishes (Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.). The adherent cells were washed with MEM and maintained in MEM-20% HIFCS at 37°C in 5% CO_2 for 48 hr before use.

HeLa cells (Microbiological Associates, Inc.) were maintained in Falcon plastic flasks in MEM with 10% HIFCS and passed by trypsinization when the cell density approached a confluent monolayer. 2 days before use for the experiments, approximately 2 × 10^5 HeLa cells were placed on round cover slips $\frac{5}{8}$ inches in diameter in Falcon dishes and maintained at 37°C in 5% CO_2 and balanced air.

Mouse fibroblasts, L929 American Type Culture, were obtained from Dr. S. Silverstein. at The Rockefeller University. They were maintained in MEM-10% HIFCS in Falcon plastic flasks and passed by trypsinization when the cell density approached a confluent monolayer. They were plated on $\frac{5}{8}$ inch round cover slips 48 hr before each experiment as described for HeLa cells above.

Technique for Study of Parasite Entry.—In order to obtain specimens showing large numbers of toxoplasmas entering cells, a special procedure was required. Round cover slips with cell monolayers and a thin film of medium were carefully placed in flat-bottomed 19 × 65 mm glass cylinders (Titeseal 3-dr vials; Chemical Rubber Co., Cleveland, Ohio) and cooled to 4°C. A suspension containing 1–5 × 10^6 toxoplasmas/ml in MEM-20% HIFCS was cooled to 4°C. 1 ml of the cooled toxoplasma suspension was added to each vial, and they were then spun in a refrigerated centrifuge (model PR-2; International Equipment Co., Needham Heights, Mass.) at 4°C at 200 *g* for 5 min. Each vial was then carefully placed on ice and all but a trace of the supernatant medium was removed by aspiration. The vial was then transferred to a 37°C water bath. At appropriate short time intervals after warming the remaining medium was aspirated and the cover slips were rinsed once with 2.5% glutaraldehyde in sodium cacodylate buffer, pH 7.4, at room temperature, and then covered with a fresh aliquot of 2.5% glutaraldehyde for 10 min. During the first few minutes of this fixation, the specimen was maintained at room temperature, then it was transferred to 4°C for the remaining time. Near the end of the fixation period, cells were scraped with a plastic scraper into a small volume of 2.5% glutaraldehyde.

Electron Microscopy.—After initial glutaraldehyde exposure, the cells were fixed in a mixture of cold glutaraldehyde and osmium, were exposed to uranyl acetate, and were embedded

[1] *Abbreviations used in this paper:* HIFCS, heat-inactivated fetal calf serum; MEM, minimal essential medium.

in agar and then in Epon as described previously (8). Thin sections were stained with lead and uranyl solutions and were examined in a Siemens-Elmiskop IA (Siemens Corp., Iselin, N. J.) at 80 kv using a 50 μ objective aperture.

RESULTS

Preliminary observations were made on the morphology, the viability, and the infectivity of toxoplasmas under the conditions used in these experiments. As is illustrated in Table I, intraperitoneal infection of mice with the RH strain of *Toxoplasma gondii* was uniformly fatal in 6–12 days; the survival time was directly related to the number of organisms inoculated, and, within the limits of accuracy of counting procedures and serial dilutions, nearly all of the toxoplasmas were infectious. Toxoplasmas harvested from the perioneal cavity and

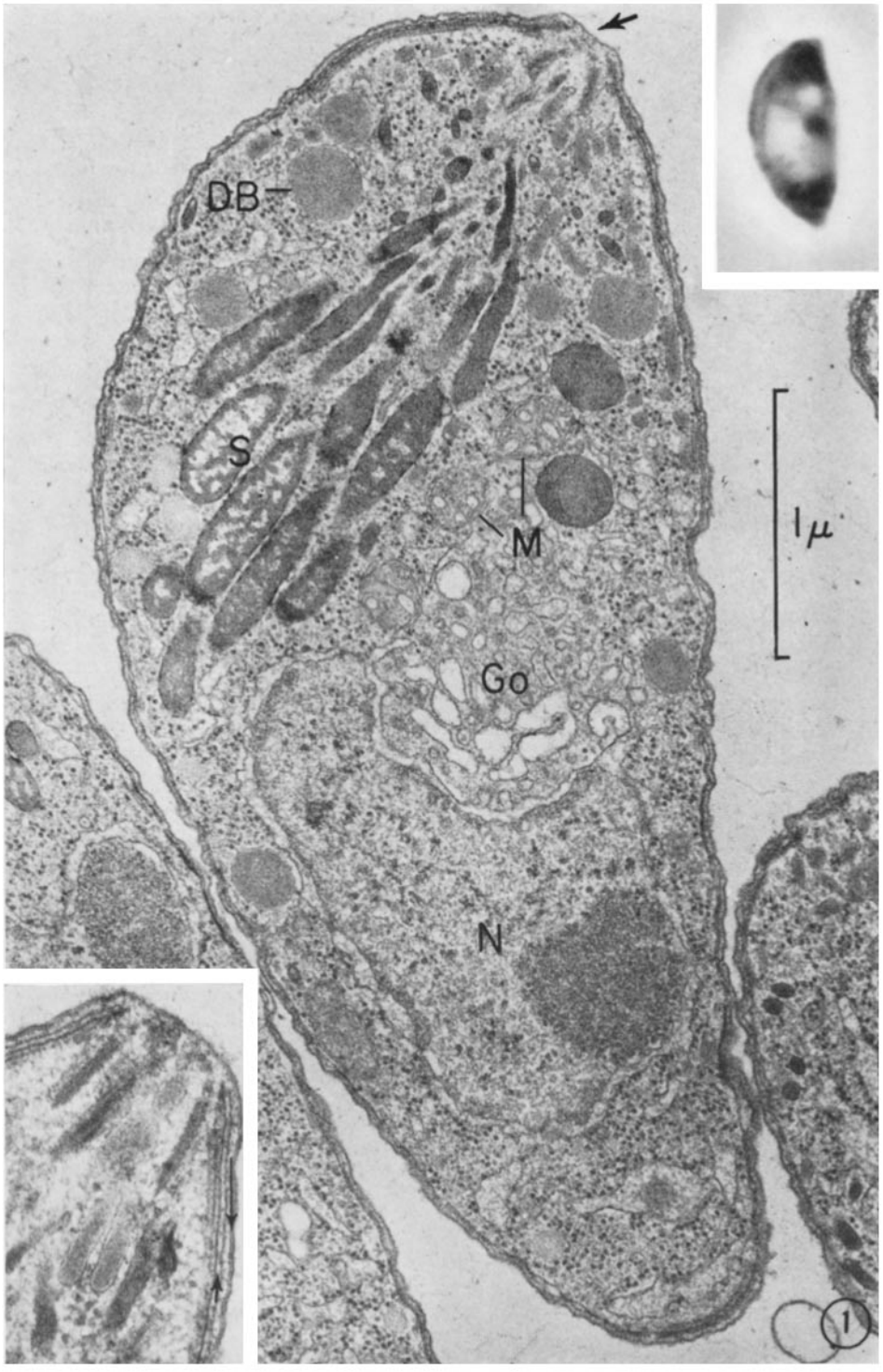
TABLE I

Relationship between Numbers of Toxoplasmas Inoculated Intraperitoneally and Duration of Survival of Mice

Time after inoculation	Approximate number of toxoplasmas given i.p.				
	10,000	1000	100	10	1
	No. of mice dead/No. of mice inoculated				
days					
6	0/12				
7	12/12	0/12	0/12	0/12	
8		12/12	8/12	2/12	
9			12/12	4/12	0/8
10				9/12	1/8
11				12/12	1/8
12					2/8

maintained at 37°C in MEM-20% HIFCS remained viable for at least 24 hr, as established by trypan blue dye exclusion and by titration of infectivity for mice. Injection of one or two toxoplasmas resulted commonly in fatal infection. There was, however, sometimes a decrease in the number of organisms present during a 24 hr storage period at 37°C, suggesting that variable numbers of parasites died and lysed during this time or that significant clumping of the toxoplasmas occurred. The per cent of toxoplasmas determined to be infectious for the tissue cultures was lower than that observed in the mouse infection model, and varied with the conditions (that is, confluency of monolayer, toxoplasma to cell ratio, volume of suspending medium, etc.). In most experiments 100–1000 toxoplasmas was the minimum number required to establish infection of the cultures. Infectivity of toxoplasmas for HeLa cells was not detectably different from that for macrophage cultures.

The morphology of the toxoplasmas under phase-contrast microscopy is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illustrated in the upper inset of Fig. 1. The parasite was oval or arc shaped, 2–4 × 5–7 μ in dimensions. The nucleus was relatively phase lucent and a nucleolus was often visible. The cytoplasm contained brownish granular material. A small nipple was often seen at one end. The parasites were usually nonmotile when observed in thin preparation on a heated stage, but occasionally for short periods they exhibited gliding and twisting movements. Phase-contrast motion pictures of toxoplasma-infected HeLa cells revealed after host cell rupture, locomotion of the liberated parasites and rapid entry into adjacent cells.

The ultrastructure of extracellular and intracellular toxoplasmas was similar. Fig. 1 shows an electron micrograph of an extracellular toxoplasma. Various structures were present typical of eukaryotic cells in general, including a nucleus, nucleolus, Golgi complex, ribosomes, rough endoplasmic reticulum, mitochondria, and dense granules. One notable morphologic feature of this protozoan was the apparently double outer membranes: an external typical ~9 nm unit membrane and a thick ~12–16 nm membrane beneath the unit membrane. The thick inner membrane was discontinuous at the region of the apical pole, leaving a gap bridged only by the outer unit membrane. Microtubules were present in the cytoplasm adjacent to the inner membrane. Unusual organelles seen in toxoplasmas were the saccular structures (termed paired organelles or rhoptries) which appeared to lead toward the apical pole. The

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FIG. 1. Morphology of *Toxoplasma gondii*. The inset at upper right shows a toxoplasma under phase-contrast light microscopy. The central nuclear area is phase lucent. Dense granules are present at the poles. × 4600.

The main photograph is an electron micrograph of a longitudinal section through a toxoplasma. The nucleus (*N*) appears amorphous and generally electron transparent, with finely granular deposits at the periphery and scattered in the nucleoplasm. The large round aggregate of dense granular material at right may represent a nucleolus. The perinuclear envelope appears somewhat dilated; ribosomes are attached to the outer membrane. The Golgi complex (*Go*) is made up of dilated saccules and small vesicles. Mitochondria (*M*) show small vesicular or saccular extensions from the inner membrane, rather than the cristae seen typically in mammalian cells. The peripheral cytoplasm contains membrane-bounded granules filled with amorphous electron-opaque material (dense bodies, *DB*), and strips of dilated, rough-surfaced endoplasmic reticulum (see lower pole). Abundant ribosomes also are scattered throughout the cytoplasmic matrix. The parasite is surrounded by two membrane layers, the inner membrane being interrupted at the apical pole (top right, *arrow*). Portions of microtubules are visible just beneath the inner membrane in some areas. Elongated pouches or saccules (*S*) containing electron-opaque material appear to extend long thin necks towards the apical pole; the nature and function of these so-called "paired organelles" is not known. Fixation: glutaraldehyde, mixture of glutaraldehyde and osmium, uranyl acetate. × 38,400.

The inset at lower left is a higher magnification of the apical pole region of another toxoplasma, showing the typical 9 nm trilaminar "unit" external membrane (*long arrow*), and the inner thick, or paired thin membranes, also having a trilaminar appearance (*short arrow*) but with over-all dimensions ranging from 12 to 16 nm. The outer membrane is seen to be continuous at the apical pole, whereas the inner membrane(s) is (are) interrupted. Thin extensions of the saccular or paired organelles are seen in the cytoplasm. × 65,000.

function of these saccular structures has not yet been established. The lower insert of Fig. 1 shows the apical pole region under higher magnification, illustrating the two membranes and the saccular structures in the apical pole region.

Entry of Toxoplasmas into Cells.—The early moments of toxoplasma entry

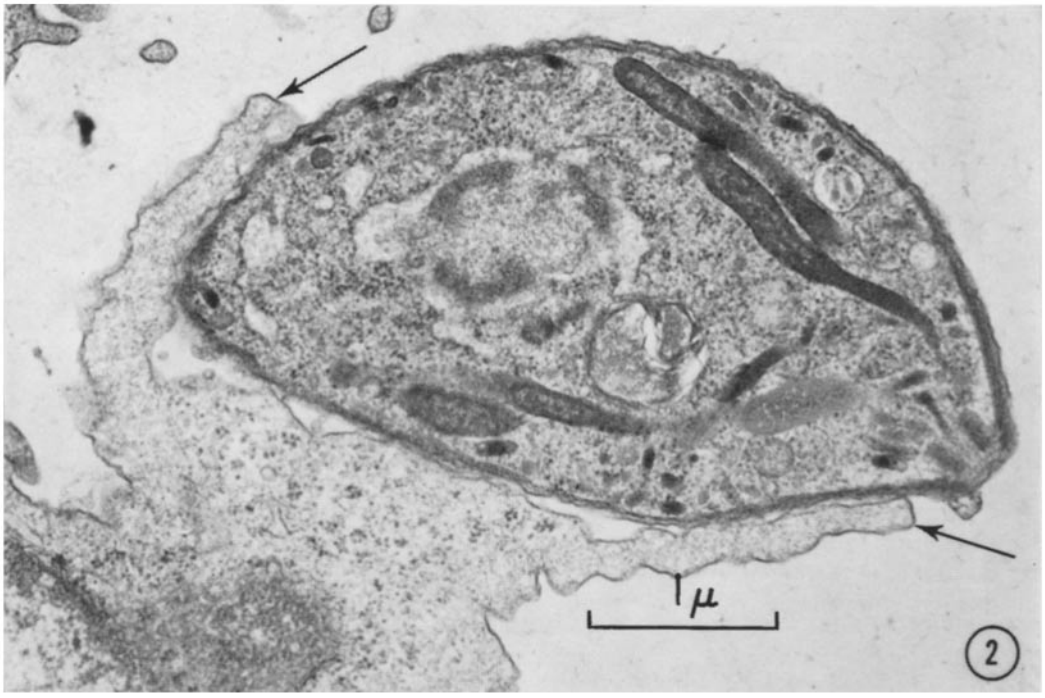

FIG. 2. Toxoplasma being ingested by a mouse fibroblast. The fibroblast has extended long micropseudopods (*arrows*) in a typical phagocytic response. Note that the apical pole of the toxoplasma is not in contact with the cell surface at the base of the phagocytic crypt. Specimen fixed 2 min after warming (see Materials and Methods). × 25,000.

into various types of host cells, phagocytic as well as usually nonphagocytic, was studied by cooling the parasites and cells, bringing them into close contact by centrifugation, then warming to stimulate entry. Phase-contrast light microscopy showed that toxoplasmas brought into cell contact by this technique did not enter cells while maintained at 4°C. After rapid warming to 37°C toxoplasmas were still on the cell surface after 1 min, but by 5 min many toxoplasmas had entered the cells. Therefore, specimens were fixed for electron microscopy between 1 and 4 min after warming.

Electron micrographs of these specimens revealed entry of the toxoplasmas into HeLa cells and fibroblasts by phagocytosis. Fig. 2 shows a fibroblast fixed

2 min after warming. Micropseudopods were seen partially surrounding the parasite. In no instance was there evidence of penetration through the plasma membrane and lodgement of the toxoplasma in the cytoplasmic matrix. Parasites seemed to be positioned in a random manner in the early phases of this endocytic process; there was no evidence to indicate that the apical pole or conoid first contacted the cell plasma membrane to initiate phagocytosis. Fig. 3 is a high magnification micrograph of a portion of a toxoplasma and a portion of the enveloping pseudopod of a HeLa cell fixed 2 min after warming

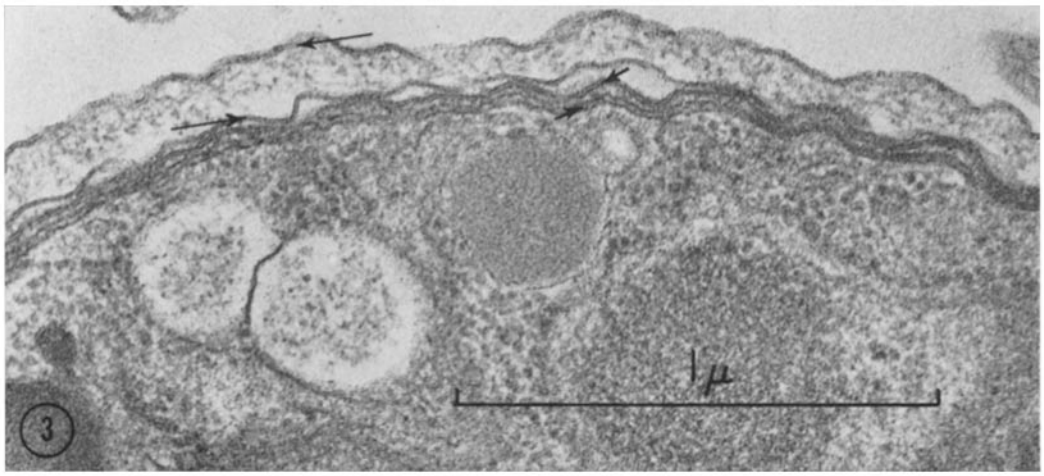

FIG. 3. High magnification electron micrograph of a toxoplasma (below) being enveloped by a micropseudopod (above) of a HeLa cell. The various membranes are well defined: inner and outer membranes of the toxoplasma (*short arrows*) and membranes of the HeLa cell micropseudopod (*long arrows*). The outer membrane of the toxoplasma has approximately the same dimensions as the HeLa cell plasma membrane, whereas the inner toxoplasma membrane is thicker. Specimen fixed 2 min after warming (see Materials and Methods). × 64,000.

illustrating the various membranes: the thick inner and thin outer parasite membranes (*short arrows*) and the membranes of the cell micropseudopod (*long arrows*). Toxoplasmas killed by heating or by glutaraldehyde fixation were not taken up by HeLa cells or fibroblasts.

Toxoplasmas also entered macrophages by phagocytosis. Fig. 4 shows a macrophage and an adherent parasite 1 min after warming. Micropseudopods partially surround the organism. Fig. 5 shows another toxoplasma which has been almost completely surrounded by the macrophage micropseudopods. This process of engulfment of a surface particle was indistinguishable morphologically from that of antibody-induced phagocytosis observed previously (7). Heat-killed or glutaraldehyde-fixed toxoplasmas were engulfed by macrophages in a fashion similar to that illustrated for living parasites here. Differences between

living and dead parasites in the macrophage responses subsequent to phagocytosis are considered in the accompanying paper (9).

Intracellular Fate of the Parasite.—In all cells studied a quiescent period approximately equal to the parasite generation time followed parasite entry; then division began. The parasites divided by a process well-described pre-

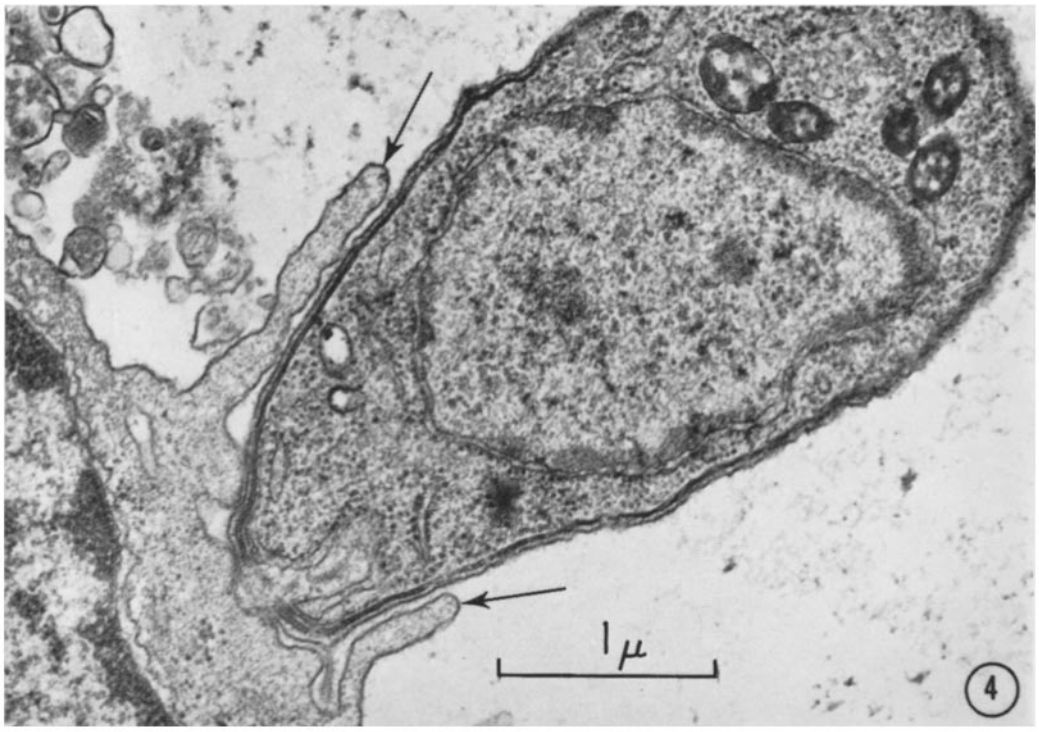

FIG. 4. Electron micrograph of an early stage in the phagocytosis of a toxoplasma by a mouse macrophage. Micropseudopods (*arrows*) are seen extending along the surface of the parasite. Specimen fixed 1 min after warming (see Materials and Methods). × 29,000.

viously (2, 3) and termed endodyogeny, characterized by formation of two daughter cells within the parent. All or nearly all of the parasites within HeLa cells divided. In contrast, in macrophages two approximately equal populations of toxoplasmas could be identified at 3 hr after infection. Under the phase-contrast light microscope one of these populations appeared rounded, somewhat swollen, and of medium phase density (Fig. 6 *a*); by 20 hr after infection, these organisms had usually divided three times (Fig. 6 *c*). Parasites in the second population in macrophages at 3 hr after infection were generally smaller, arc shaped, and more phase opaque (Fig. 6 *b*). These toxoplasmas were no

longer identifiable by 6 hr after infection. Disappearance of this second population of toxoplasmas was documented by counting the number of intracellular organisms in a macrophage culture at 30 min and 6 hr after infection and after washing to remove extracellular toxoplasmas (Table II). The parasite to cell

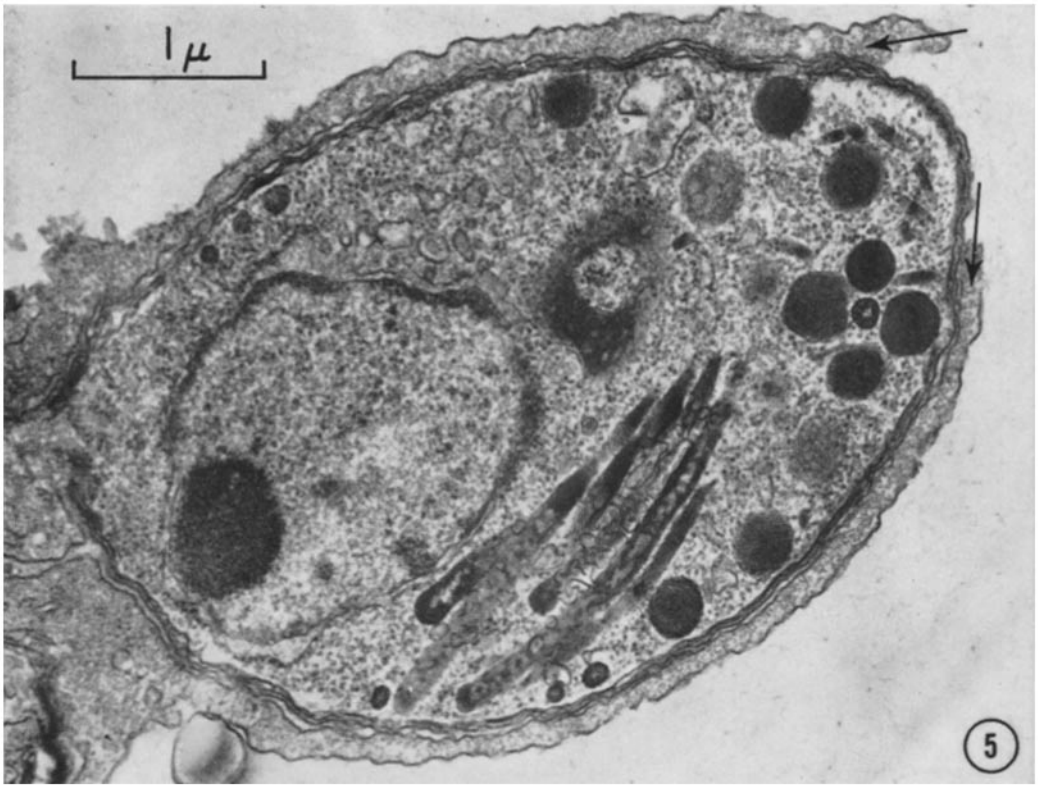

FIG. 5. Electron micrograph of phagocytosis of toxoplasma by a mouse macrophage, illustrating a later step of the envelopment by micropseudopods (*arrows*) than that seen in Fig. 4. The micropseudopods are closely apposed to the surface of the parasite and extend almost completely around it. × 25,000.

ratio at 6 hr was approximately half that at 30 min (0.190/0.373). Heat-killed parasites or parasites pretreated with antitoxoplasma antibody behaved in macrophages as did the second population above, i.e., they remained dense and arc shaped and were degraded within 6 hr to material no longer recognizable as a parasite.

When HeLa cells and macrophages were infected under similar conditions of cell density and parasite to cell ratio, the mean number of parasites per macrophage in the first hours after infection was higher (two to four times) than that

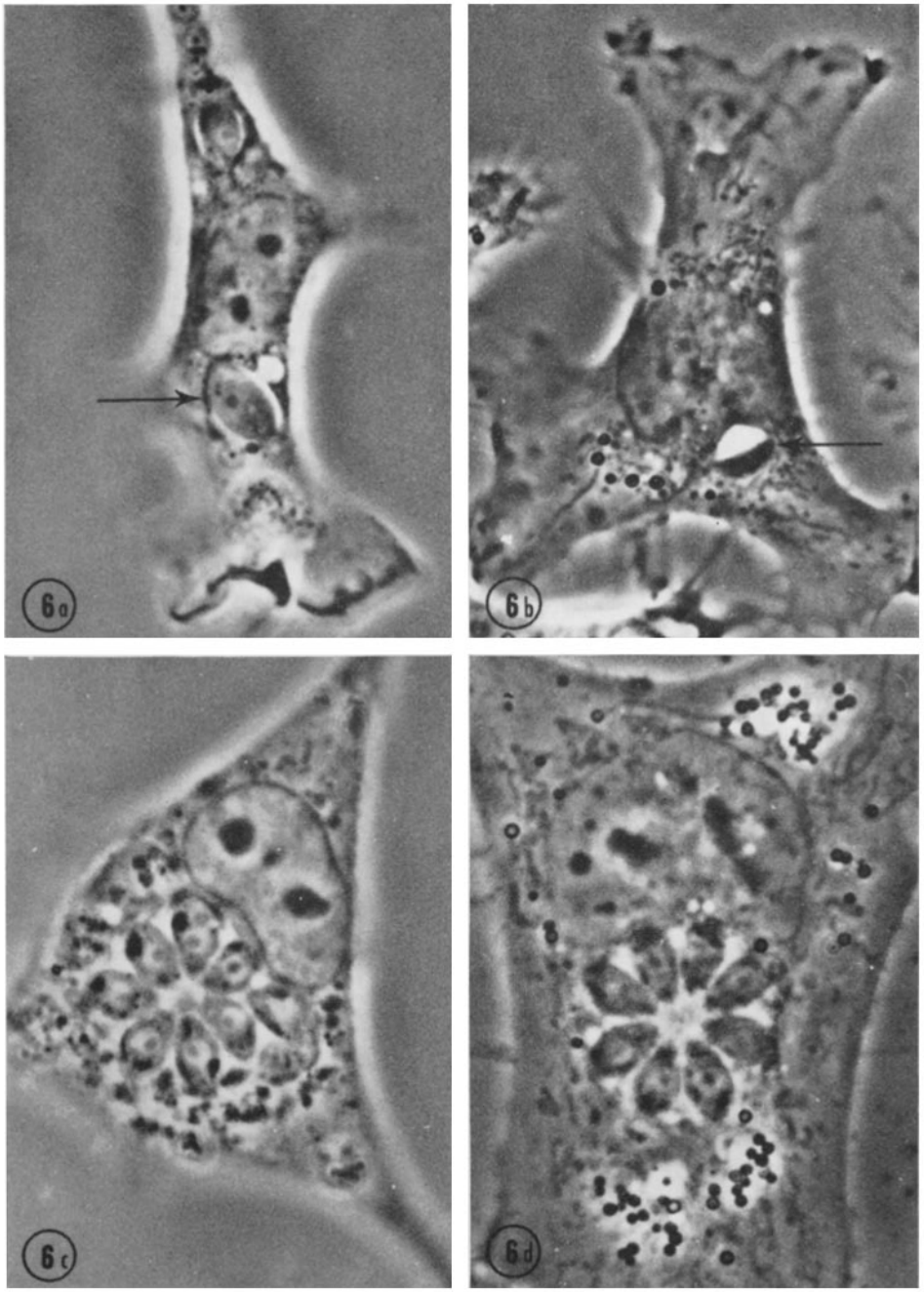

FIG. 6. Phase-contrast photomicrographs of toxoplasmas in macrophages and a HeLa cell. Figs. 6 *a* and 6 *b* illustrate the different appearance of the two populations of toxoplasmas seen in macrophages at 3 hr after entry of the parasites into the cells. The toxoplasma (*arrow*) in 6 *a* appears swollen and oval in shape, an appearance typical of the growing parasite; these organisms divided to form rosettes after approximately 1 day as illustrated in a macrophage (6 *c*) or in a HeLa cell (6 *d*). In contrast, the toxoplasma (*arrow*) in Figure 6 *b* is phase dense, contracted, and arc shaped; on repeated observation parasites with this appearance degenerated and were no longer distinguishable at 6 hr after their entry into macrophages. × 2000.

in HeLa cells, but by 6 hr the numbers of parasites in the two cell types was similar.

The mean generation time was calculated for the toxoplasmas by a method described previously (10). The generation time varied among the cell types under the culture conditions used here; 5½ hr in fibroblasts, 8 hr in macrophages, 9 hr in HeLa cells. Fig. 6 *d* shows a phase-contrast photomicrograph of a HeLa cell after three divisions of the toxoplasma, producing a rosette of eight parasites lying in a phase-lucent vacuole.

In all the cells studied, no detectable host cell toxicity was demonstrable early in the cultures with low parasite to host cell infection ratios; if large numbers of toxoplasmas were added (10:1 or higher toxoplasma to cell ratio), the macrophages rounded up and often detached from the glass. Host cell damage occurred only at the time of cell rupture, which appeared to be a mechanical process, occurring when the cells were overstuffed with dividing

TABLE II

Number of Intracellular Toxoplasmas at 30 Min and 6 Hr after Infection of a Macrophage Cell Culture

Time after infection	Macrophages/28 mm²	Intracellular toxoplasmas/28 mm²	Toxoplasmas/macrophage
30 min	268 ± 52*	100 ± 36	0.373
6 hr	231 ± 40	44 ± 19	0.190

* One standard deviation.

parasites. The macrophages tended to rupture after the fourth or fifth parasite division; HeLa cell rupture occurred somewhat later. During toxoplasma infection the macrophage nucleus often showed some swelling and an increase in the density of the nucleolus (Fig. 6 *c*). These nuclear changes were not seen in the HeLa cells or fibroblasts.

DISCUSSION

Toxoplasma gondii is an important cause of infection and disease in many vertebrates, including man. Approximately 40% of the U.S. population has been infected by age 50 as established in surveys on acquisition of antibody, detected by the Sabin-Feldman dye test. Infection is thought to be acquired by ingesting inadequately heated meats containing toxoplasma cysts, or by ingesting the oocyst found in cat feces (1). Usually the infection is asymptomatic; however, occasionally symptoms of fever and lymph node enlargement occur. When the fetus is infected transplacentally, a serious progressive necrosis of various organs results in the signs and symptoms of congenital toxoplasmosis. Recurrent attacks of retinochoroiditis are considered a late sequelae of congenital infection. Antibodies against a number of different toxoplasma antigens develop in response to the infection, and antibody plus heat-labile serum factors

can damage or kill the extracellular parasite, probably affording some protection against progressive infection (11). Evidence also suggests that the development of delayed hypersensitivity is important in control of the disease (12). Other cell-mediated effects determining the progress of the infection may be due to the influence of metabolic factors or interferon (13, 14) on the rate of toxoplasma multiplication. The diagnosis of toxoplasmosis may be made by following the changing antibody titers after infection or by isolation of the parasite. Infection induces lasting immunity against reinfection in the vertebrate host, but viable parasites can be isolated from muscle tissue or brain long after infection. Parasites at this stage of infection are within cysts. The factors leading to transformation of the infection from a rapidly necrotic process to encystment remain unknown. Precise understanding of the pathogenesis of toxoplasmosis and immunity directed against it awaits more information on the host-parasite interaction at the cellular level. Because of the probable role of the cellular immune mechanisms in protection against this disease, the interaction of toxoplasmas with lymphocytes and macrophages is of particular interest.

The studies described in this paper indicate that toxoplasmas enter macrophages, HeLa cells, and fibroblasts by phagocytosis under the in vitro conditions used. The mechanism of entry of toxoplasmas into host cells has been a subject of discussion for almost two decades. Pulvertaft et al. considered phagocytosis to be the most important mechanism for entry of toxoplasmas into a number of different cells (15). Vischer and Suter also felt that phagocytosis was the mechanism by which toxoplasmas entered peritoneal macrophages, but they were of the opinion that active penetration of the parasite occurred into nonphagocytic cells (16). During the past decade, the work of Lycke et al. and Norrby et al. also suggested a penetration process as the mechanism for entry of toxoplasmas into HeLa cells (17–19). Lycke and coworkers demonstrated that this penetration was enhanced in the presence of hyaluronidase or lysozyme (17), and Norrby et al. demonstrated by histochemical techniques at the light microscope level that toxoplasmas contain acid phosphatase-positive granules near the anterior part of the parasite and that the number of these granules decreases after penetration (19). From this work it has been proposed that release of hydrolytic enzymes from the parasite alters the cell membrane and allows the parasite to penetrate. Morphologic evidence in keeping with the concept of active penetration into nonphagocytic cells also comes from phase-contrast moving pictures taken by Bommer et al. and Lund et al. (4, 5). Bommer described parasite movement when in contact with the cell and showed a constriction of the parasite during passage through the host cell surface structures. The rapidity of this penetration process has made difficult its study at the ultrastructural level.

Our studies establish that the plasma membrane remains intact during entry of the parasite into host cells. An apparent constriction of the parasite during entry by phagocytosis is evident in some of our specimens, perhaps related to

resistance to deformation of the submembraneous filamentous layer of cell cytoplasm; in any event this appearance of constriction cannot be taken as meaningful evidence in favor of penetration rather than endocytosis. We would propose that the enzymatic or other factors postulated by others to be released by toxoplasmas to facilitate their penetration into host cells (19) may be acting to induce phagocytosis, even in cells not usually phagocytic, and not to lyse an area of plasma membrane. It is our opinion, therefore, that the term penetration, which gives the impression of passing through the membrane, is not appropriate. The terms engulfment or phagocytosis, when used unmodified, are incomplete since they emphasize the cellular process, but not the role of the parasite. Based on our studies, it is perhaps best to describe the entry of toxoplasmas into cells as induced or stimulated phagocytosis. Fibroblasts do not commonly engulf large particles, but under certain conditions they can be stimulated to take in particles such as glutaraldehyde-fixed red blood cells or latex (20, 21). Many other "nonphagocytic" cells such as tumor cells and endothelial cells have been shown to engulf large particles under certain circumstances (22, 23). To separate the phagocytosis observed in these cells from that of macrophages and polymorphonuclear leukocytes the terms professional and nonprofessional phagocytes have been used (24). Professional phagocytes are those cells, such as macrophages, that engulf at a rapid rate and display an enhanced appetite for particles coated with immunoglobulin. In contrast, nonprofessional phagocytes, such as fibroblasts, do not show an increased ingestion of red blood cells in the presence of opsonic antibody. Some differences have been demonstrated for the attachment and the ingestion phases of the phagocytic process (25) but little is known about the detailed determinants of this process, except perhaps in the case of antibody-stimulated uptake by the so-called professional phagocytes.

Phagocytosis has been shown to be the mechanism by which merozoites of the malaria parasite enter red blood cells (26), and many viruses also gain access to cells by endocytosis, as recently reviewed by Silverstein (27). As will be demonstrated in the following paper (9), the membrane surrounding toxoplasmas is preserved, whereas that surrounding some viruses is thought to disappear after virus uncoating (27).

Toxoplasmas in the proliferative stage multiply only inside of cells, but nothing is known of the metabolic or other contributions of the host cell to the parasite. Toxoplasmas are well endowed with glycolytic and respiratory systems for production of energy (28), and they are capable of nucleotide synthesis from small molecular precursors (29). From the morphologic point of view, toxoplasmas exhibit no deficiency of organelles or structures that might explain their dependence on a host cell. The parasite can divide in irradiated (30) or colchicine-treated (13) host cells, as well as in enucleated fibroblasts.[2]

[2] Jones, T. C. Multiplication of *Toxoplasma gondii* in enucleated fibroblasts. Manuscript in preparation.

Toxoplasma infection of macrophages frequently resulted in a change in the phase-contrast appearance of the macrophage nucleus, with decreased density of the nucleoplasma and prominence of the nucleolus and the nuclear margin. These alterations might have indicated some host cell toxicity, or they might have reflected activation of the macrophage nucleus as is seen in some instances after fusion of macrophages with other cell types (31). Studies done thus far on this point demonstrate no incorporation of tritiated thymidine, and no change in uptake of tritiated uridine into the macrophage nucleus in association with toxoplasma infection of the cell.

The present studies also demonstrated a difference in the survival of toxoplasmas in HeLa cells and in macrophages. All or nearly all the parasites which entered HeLa cells survived and divided, whereas in macrophages approximately half of the toxoplasmas were digested. This result suggested that macrophages engulfed a population of dead or defective parasites incapable of survival intracellularly in macrophages and unable to enter fibroblasts or HeLa cells. Attempts to identify this defective population were unsuccessful. Essentially all of the parasites added to the cultured cells were viable by trypan blue dye exclusion and incorporation of tritiated uridine, establishing that there was not a significant proportion of dead organisms. As an alternative explanation for these findings, it is possible that the toxoplasmas were all living and infectious, and that macrophages differed from HeLa cells in taking up parasites more rapidly, and in being capable of destroying approximately half of the toxoplasmas they engulfed.

In any event the presence in macrophages of these two populations of toxoplasmas, one thriving intracellularly and the other destroyed in a few hours, was an ideal experimental system for observing the ultrastructural changes associated with intracellular parasitism and to document possible mechanisms of survival of toxoplasmas in cells. These observations are presented in the accompanying paper.

SUMMARY

Macrophage, fibroblast, and HeLa cell cultures have been infected with *Toxoplasma gondii*, and observations have been made on parasite entry and fate.

A special procedure was devised for studying the entry of toxoplasmas by electron microscopy. Toxoplasmas were centrifuged onto the cells in the cold; fixation 1–3 min after warming yielded specimens showing numerous examples of parasites in the process of entering cells. The mechanism of entry into macrophages, fibroblasts, and HeLa cells was in all cases by phagocytosis. Micropseudopods were extended by the cells to envelop the attached parasites in a typical phagocytic vacuole. Apparently the toxoplasmas stimulated this response of HeLa cells and fibroblasts, cell types not usually phagocytic. No instance was seen of penetration of toxoplasmas through the cell membrane, or of parasites located free in the cytoplasm.

Essentially all of the toxoplasmas that entered HeLa cells divided with a generation time of 9 hr; the parasites formed large rosettes situated in vacuoles, eventually leading to host cell rupture. Macrophages took in larger numbers of toxoplasmas than did HeLa cells, but approximately half of the parasites inside of macrophages degenerated within a few hours. The surviving toxoplasmas in macrophages divided every 8 hr, forming rosettes and eventually rupturing the cells.

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