Use of Mouse Macrophage Cell Lines for *In Vitro* Propagation of *Toxoplasma gondii* RH Tachyzoites (43237)

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Abstract. **In most laboratories, *Toxoplasma gondii* is maintained in mice and is studied *in vitro* using nonlymphoid cell lines or primary mouse macrophages. In this study, three rapidly dividing mouse macrophage cell lines (J774 A.1, P388D1, RAW264.7) were evaluated for their suitability for studying the RH strain of *T. gondii*. For comparison, tachyzoites were also grown in two slowly dividing epithelial cell types: a rat lung cell line (L2) and a bovine turbinate cell line (BT). Various inocula of *T. gondii* were added to the above cells and tachyzoites were harvested from the culture supernatants after 2–8 days of infection. The mouse macrophage cell lines supported rapid growth of *T. gondii* RH allowing up to a 300-fold increase of the inoculum in 2–4 days. L2 and BT supported slower growth of *T. gondii* (10- to 90-fold increase of inoculum in 5 to 8 days) and, thus, may be more suitable for assessment of host cell-parasite interactions and drug activity. *Toxoplasma gondii* RH isolated from each of the cell cultures described were able to multiply in all cell types used. Protein profiles of whole tachyzoite isolated from mice or cell cultures and protein profiles of the corresponding soluble and membrane fractions of the intraphagosomal membrane network were similar as seen after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In mice, intraperitoneal injection of 10^6, 10^5, and 10^3 tachyzoites isolated from the cell cultures or from infected mice caused death after 4, 5, and 8 days, respectively, indicating that parasites grown *in vitro* retained virulence.** [P.S.E.B.M. 1991, Vol 197]

Toxoplasma gondii is an ubiquitous intracellular protozoan parasite of worldwide distribution. Toxoplasmosis is usually asymptomatic or subclinical in otherwise healthy adults. However, if a primary infection is acquired during pregnancy, transmission of *T. gondii* to the fetus often results in congenital and perinatal diseases of serious consequence. *Toxoplasma gondii* is also recognized as one of the most important opportunistic pathogens in immunocompromised individuals. It causes necrotizing encephalitis, pneumonitis, and myocarditis in AIDS and cancer patients and in transplant recipients (1–3). The renewed interest in the study of this obligate intracellular parasite has emphasized the need for better methods to maintain and/or cultivate *T. gondii*.

In most laboratories, the asexual proliferative stage of the parasite, the tachyzoite, is maintained by successive passages in highly susceptible animals such as mice. Large numbers of relatively pure tachyzoites are required for the isolation and biochemical characterization of parasite-specific cellular components. Large numbers of parasites can be harvested from mice; however, they are often difficult to separate from host cells and other debris in the peritoneal fluid. Thus, a more efficient *in vitro* cultivation procedure is needed for the production of large numbers of pure parasites. Tachyzoites have been grown in nonlymphoid cell lines (4–6) or primary macrophages (7), the latter still requiring the use of laboratory animals. Long-term mass cultivation of *T. gondii* in HeLa cells was also recently reported but requires specialized equipment that is not widely available (8).

We have studied the suitability of three mouse macrophage cell lines to support *in vitro* growth of *T.*

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gondii strain RH, and to produce large quantities of tachyzoites required for biochemical characterization of cellular components. The growth of *T. gondii* RH was also studied in the more slowly growing rat lung (L2) and bovine turbinate (BT) cell lines. Although it is understood that the *T. gondii* strain RH retained its virulence for mice while cultivated *in vitro*, little is known about possible differences in biochemical composition between *in vivo* and *in vitro* grown organisms. We have also compared protein profiles of whole tachyzoites grown *in vivo* and *in vitro* and the protein profiles of the intraphagosomal membrane network elaborated by *T. gondii* upon infection of host cells (9) to determine whether *in vitro* cultivation alters the protein composition of tachyzoites.

Materials and Methods

***Toxoplasma gondii* strain.** The virulent RH strain of *T. gondii* was obtained from Dr. Jack K. Frenkel and Dr. James L. Fishback (Department of Pathology and Oncology, College of Health Sciences and Hospital, The University of Kansas, Kansas City, KS), and maintained by intraperitoneal inoculation of 6-week-old CFW female mice (Charles River Breeding Laboratories, Inc., Wilmington, MA). Tachyzoites were harvested from mice by lavage of the peritoneal cavity with 3 ml of Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY) containing 10% fetal calf serum, 2 m*M* glutamine, 1 m*M* pyruvate, 100 units of penicillin/ml, 100 μg of streptomycin/ml, and 0.1 μg of Fungizone/ml (supplemented DMEM) or by *in vitro* cultivation as described below. Tachyzoites were separated from host cell debris by two subsequent filtrations through a 5- and 3-μm pore size polycarbonate membrane (Nucleopore Corp., Pleasanton, CA) and then counted using a hemacytometer. Viability of the partially purified tachyzoites was evaluated by trypan blue (Gibco) exclusion test. Tachyzoites were also obtained from the supernatant of the various cell cultures as described below.

Cell Lines. Three mouse macrophage cell lines (J774 A.1, ATCC TIB 67; P388D1, ATCC TIB 63; RAW264.7, ATCC TIB 71) were provided by Dr. S. Zuckerman (Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN). Two epithelial cell types were also used: a rat lung cell line (L2, ATCC CCL 149) and a bovine turbinate cell line (BT, ATCC CRL 1390). Cells were maintained in supplemented DMEM. The mouse macrophage cell lines were harvested using cell scrapers, washed with calcium/magnesium-free Hanks' balanced salt solution (HBSS), suspended in supplemented DMEM at a 1:100 split ratio, and added to tissue culture flasks. Confluent monolayers of BT and L2 cells were trypsinyzed, suspended in 30% fetal bovine serum, washed with HBSS, suspended in supplemented DMEM, and added to tissue culture flasks at a split ratio of 1:3. Before inoculation of parasites, host cells were incubated for 24 hr. All cells were maintained at 37°C in an atmosphere of 95% air and 5% CO_2, in 25-, 75-, and 150-cm^2 tissue culture flasks each containing 10, 30, or 60 ml, respectively.

Yield of Parasite Obtained *In Vitro*. Purified *T. gondii* RH tachyzoites obtained from mice or grown *in vitro* were added to 24-hr-old monolayers at a final concentration of 10^6, 5×10^6, or 10^7 in 25-, 75-, and 150-cm^2 tissue culture flasks containing 10, 30, or 60 ml of supplemented DMEM, respectively. After 24 hr of incubation at 37°C, the cell cultures were washed with HBSS, and fresh supplemented DMEM was added. The incubation was continued until the tachyzoites were released from host cells. Parasites were harvested, purified by filtration, and counted as described above. A total of five independent experiments were performed with each cell line, on different days and using each time freshly isolated tachyzoites.

Virulence. Groups of 10 mice were inoculated by intraperitoneal injection of various numbers of *T. gondii* RH tachyzoites isolated from mice or after three passages in each of the five cell lines described above. The number of deaths in each group was recorded daily.

***In Vitro* Drug Susceptibility Model.** L2 and BT cells were prepared as described above and grown in 24-well plates. Treatment groups of four wells containing different concentrations of pyrimethamine (Sigma Chemical Co., St. Louis, MO) in combination with sulfamerazine (Sigma) and 10^6 *T. gondii* RH tachyzoites (isolated *in vivo* or *in vitro*) were added to each well. After 24 hr of incubation at 37°C, the cells were fixed with methanol and stained with Giemsa. The number of infected host cells and the number of parasites per cell were determined by examination of two microscopic fields in four different wells; the four replicates of each treatment group.

Isolation of the Intraphagosomal Membrane Network. The intraphagosomal membrane network (IPM) elaborated by *T. gondii* upon infection of host cells was isolated. The IPM were prepared from tachyzoites isolated from mice or after three passages in all cell lines used by a modification of the method of Sibley *et al.* (9). Briefly, freshly isolated tachyzoites of *T. gondii* RH, grown in mice or in cell cultures were washed three times with Hanks' balanced salt solution (Gibco), suspended in Dulbecco's calcium-magnesium-free phosphate-buffered saline (Ca^{2+}/Mg^{2+}-free D-PBS) (Gibco, Grand Island, NY), pH 7.2, containing 10 units of heparin/ml, 0.1 m*M* EDTA, 0.1 m*M* dithiothreitol, 1 μg of phenylmethylsulfonyl fluoride/ml 10 μg of tosyl lysine chloromethyl ketone/ml (Sigma Chemical Co.) to a final concentration of 10^9 tachyzoites/ml, and incubated for 30 min at 4°C. Tachyzoites were then removed by slow-speed centrifugation at 1000 rpm for 10 min (Sovall H1000B rotor; Sorvall RT6000B refrig-

erated centrifuge). The supernatant was clarified by four subsequent centrifugations at 1000 rpm for 10 min (Sorvall H1000B rotor; Sorvall RT6000B centrifuge). The suspension was then centrifuged at 5000 rpm for 40 min (Rotor JA20; Beckman J2-21 centrifuge). The remaining supernatant was centrifuged at 45000 rpm for 2 hr (Rotor Ti50; Beckman L5-50B ultracentrifuge). The microsomal pellet was suspended in Ca^{2+}/Mg^{2+}-free D-PBS. The supernatant (soluble fraction) was concentrated by ultrafiltration in stirred cells using a membrane with a molecular weight cut-off of 10 kDa (Amicon, Danvers, MA). Protein concentration was determined according to the method of Lowry *et al.* (10).

To compare protein profiles obtained from tachyzoites grown in mice or in cell cultures, whole tachyzoites isolated from the cell cultures and from mice were washed three time with Dulbecco's phosphate-buffered saline (D-PBS) and suspended in D-PBS to a final concentration of 10^9 tachyzoites/ml. The tachyzoites were disrupted by sonication. Protein concentration of all isolated fractions was determined according to the method of Lowry *et al.* (10).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. The protein profiles of whole tachyzoites and isolated IPMs were obtained after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed according to the method of Laemmli and Favre (11). Protein samples were suspended in an electrophoresis sample buffer with 4% 2-mercaptoethanol. The samples were heated to 100°C for 5 min and were electrophoresed in 10% discontinuous SDS-polyacrylamide gels. Gels were stained with 0.1% Coomasie Brilliant Blue R or silver stained according to the method of Hitchcock and Brown (12).

Results

Kinetics of Release to *T. gondii* Tachyzoites in Cell Cultures. The kinetics of release of *T. gondii* RH tachyzoites from five different cell lines is shown graphically in Figure 1. The three mouse macrophage cell lines, RAW264.7, J774 A.1, and P388D1, supported a rapid parasite growth and numerous tachyzoites were released from the monolayers after only 2 days of incubation for RAW264.7 and P388D1 cells and after 3 days of incubation for J774 A.1 cells (Fig. 1A). Large numbers of tachyzoites were released from these cell cultures over the 3-day period and resulted in complete destruction of the host cells. Appearance of the RAW264.7 cell culture after 2 days of infection is shown in Figure 2. A large number of tachyzoites were present in the liquid medium and macrophages containing numerous parasites were also observed. Intracellular tachyzoites were freed mechanically by passing the cell suspension through a 27½-gauge needle, or were released spontaneously as incubation continued. More

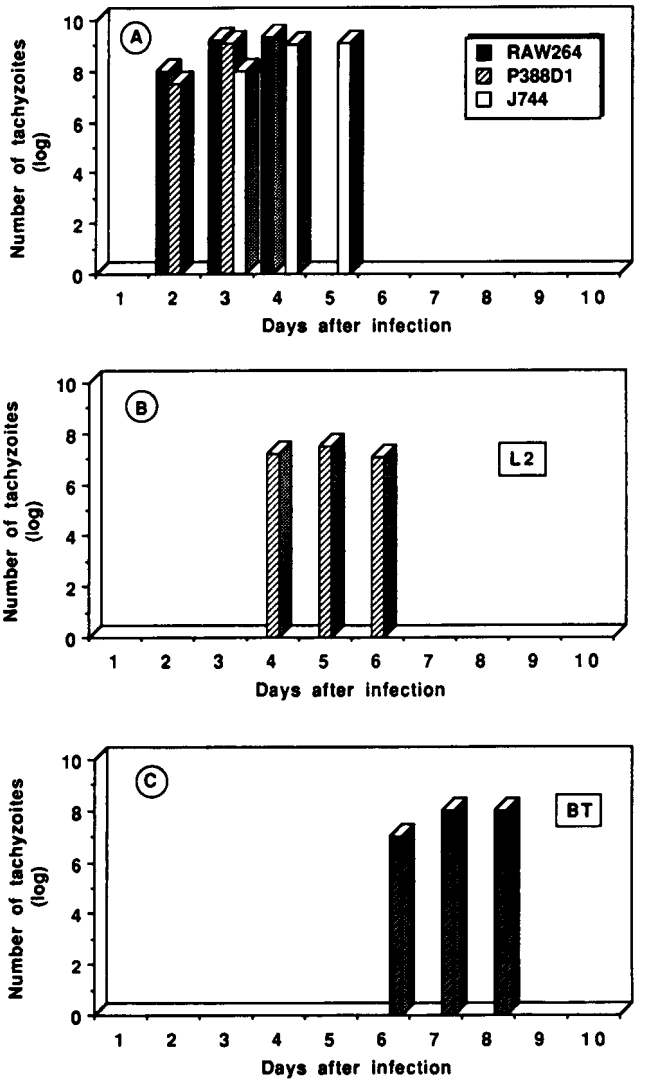

Figure 1. Kinetics of production of *Toxoplasma gondii* tachyzoites in cell cultures. *T. gondii* RH tachyzoites were added at a final concentration of 5×10^6 to 150-cm^2 tissue culture flasks (60 ml). Number of tachyzoites (log) obtained in the three mouse macrophage cell lines: RAW 264.7, P388D1, J774 A.1 (A), in the rat lung epithelial cell line: L2 (B), and in the bovine turbinate cell line: BT (C). Standard deviations were less than 1.5.

than 90% of the free parasites were viable, as demonstrated by the trypan blue exclusion test.

Parasites grown in L2 and BT cells were released from the monolayers after an incubation period longer than that necessary for the three infected mouse macrophage cell lines. Also, total numbers of tachyzoites obtained from L2 and BT cell cultures were not as large as those obtained from the macrophage cultures (Fig. 1B and C). This indicates that both L2 and BT cells supported a slower growth of *T. gondii* RH tachyzoites compared with the three mouse macrophage cell lines.

Figure 3 shows the subsequent events leading to the formation of plaques in L2 cells. The same phenomenon was also observed in BT cells, occurring only 4 days after infection of the monolayer (data not shown).

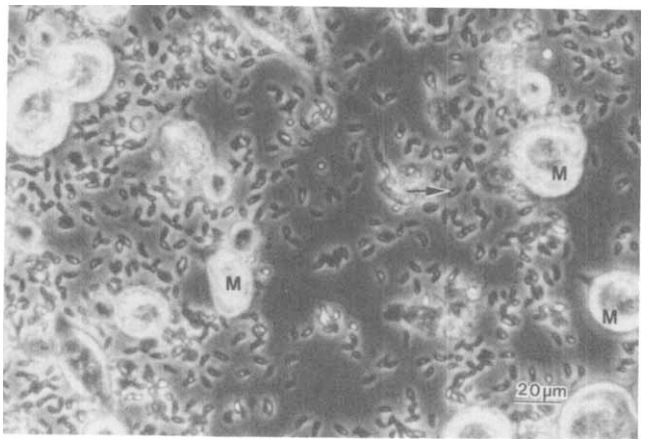

Figure 2. Phase contrast photomicrographs showing the production of *T. gondii* RH tachyzoites in the macrophage cell line RAW264.7 after 2 days of incubation at 37°C in a 5% CO_2 atmosphere. The host cells were grown in 150-cm^2 tissue culture flasks (60 ml) and inoculated with 10^7 tachyzoites. Arrows indicate the free tachyzoites, M indicates macrophages containing large numbers of tachyzoites which can be freed mechanically.

The results shown in Figure 1 were obtained by infection of the cell monlayers with 5×10^6 *T. gondii* RH tachyzoites isolated from infected animals. Equivalent results were obtained using tachyzoites isolated from three passages in each of the five cell lines studied. This indicates that parasites grown in any of the five cell lines retain the ability to infect and multiply in heterologous cell lines.

Parasites Yields. Various inocula of *T. gondii* RH tachyzoites were used to infect the five cell lines. Increases in tachyzoite numbers were determined at the time the parasites had destroyed the monolayers and are reported in Table I. Clearly, the mouse macrophage cell lines supported a rapid and abundant growth of *T. gondii* tachyzoites. In 150-cm^2 tissue culture flasks, an inoculum of 10^7 tachyzoites consistantly resulted in a 300-fold increase of partially purified parasites harvested from the medium. L2 and BT cells supported a moderate growth of *T. gondii* tachyzoites, and the numbers of parasites isolated from these cell lines were smaller than those obtained from the mouse macrophage cell lines. A larger inoculum did not increase the number of parasites isolated from any of the cell cultures (data not shown), indicating that in 150-cm^2 tissue culture flasks an inoculum of 10^7 tachyzoites (in 60 ml) results in maximum production of parasites.

In 25- and 75-cm^2 tissue culture flasks the number of parasites isolated from the cultures were not as impressive. This could be due to a parasites to host cells ratio that is inadequate to support maximum growth. The mouse macrophage cell lines were not grown in 25-cm^2 flasks because acidification of the medium was too rapid when parasites were growing in host cells. The data included in Table I were obtained using tachyzoites isolated from infected mice. However, similar data were

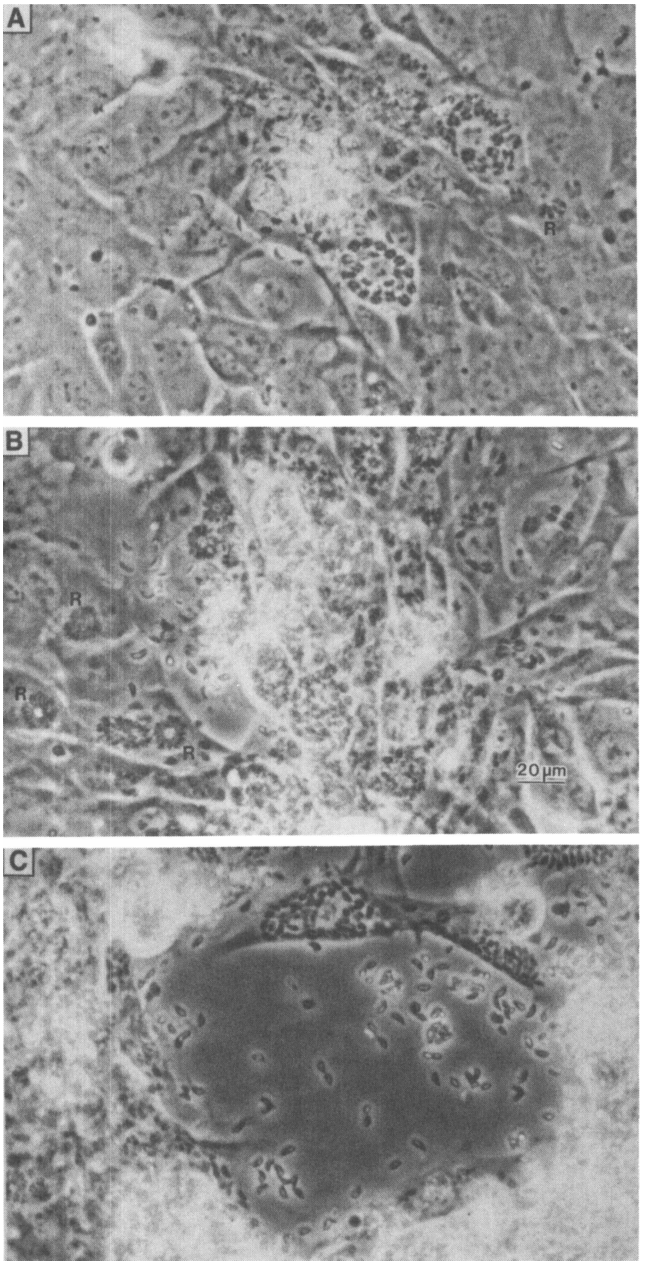

Figure 3. Phase contrast photomicrographs showing *T. gondii*-induced plaques in L2 cells. (A) After 2 days of incubation at 37°C. Rosettes (R) are small (4 tachyzoites). (B) After 4 days of incubation, a larger focus of cell lysis observed. Rosettes (R) contain 8–16 tachyzoites. (C) Plaques formed after 5 days of incubation.

obtained using tachyzoites harvested after three passages in cell cultures (data not shown).

***In Vitro* Drug Susceptibility Testing.** L2 and BT cells were evaluated for their suitability as host cells to test antimicrobial agents against *T. gondii* (Table II). Infected monolayers of L2 and BT cells support a moderate growth of *T. gondii* tachyzoites and can be easily fixed, stained, and observed microscopically.

Although the same inoculum was used, it seemed that a greater percentage of L2 cells were infected compared with the BT cells, whereas the number of

Table I. *Toxoplasma gondii* Tachyzoites Production Yield in Various Cell Lines

Flask size (cm^2)	Cell line	Days after infection	Fold increase of inoculum[a]		
			10^6	5×10^6	10^7
150	J774 A.1				
	P388D1	3	25.0 ± 2.5	30.0 ± 4.5	300.0 ± 14.5
	RAW264.7				
	L2	5	8.5 ± 1.3	10.0 ± 2.3	120.0 ± 12.4
	BT	8	6.0 ± 3.2	10.0 ± 0.5	90.0 ± 9.7
75	J774 A.1				
	P388D1	3	10.0 ± 2.0	10.3 ± 0.5	30.0 ± 3.0
	RAW264.7				
	L2	5	9.8 ± 1.8	10.4 ± 0.4	20.0 ± 2.0
	BT	8	10.0 ± 2.2	10.0 ± 0.0	20.0 ± 1.8
25	J774 A.1				
	P388D1		ND[b]	ND	ND
	RAW264.7				
	L2	5	10.2 ± 0.4	7.5 ± 0.7	9.7 ± 0.6
	BT	8	7.0 ± 0.8	10.0 ± 1.2	8.2 ± 0.5

[a] Means of five determinations ± SD. The fold increase was determined on the first day that parasites destroyed the host cell monolayer.
[b] ND, not determined.

Table II. Effect of Pyrimethamine in Combination with Sulfamerazine on the Development of *T. gondii* RH Tachyzoites in L2 and BT Cell Cultures after 24 hr of Incubation at 37°C

Treatment	Concentration (μg/ml)	% Infected cells[a]		No. of tachyzoites/cell[b]	
		L2	BT	L2	BT
Control	0/0	72.4 ± 4.7	53.9 ± 7.7	3.5 ± 0.9	2.6 ± 0.8
Pyrimethamine/ sulfamerazine	0.1/25	15.3 ± 0.2	14.5 ± 0.5	1.1 ± 0.5	1.7 ± 0.1
	0.05/12.5	15.5 ± 0.5	15.0 ± 1.0	1.0 ± 0.5	1.5 ± 0.5
	0.025/6.25	15.0 ± 0.5	21.3 ± 0.2	4.0 ± 1.0	2.3 ± 0.1
	0.0125/3.125	21.7 ± 0.3	33.0 ± 2.0	1.9 ± 0.5	1.8 ± 0.1
	0.006/1.6	35.0 ± 1.0	19.0 ± 1.0	2.9 ± 0.2	1.9 ± 0.1
	0.003/0.8	50.0 ± 3.0	25.5 ± 0.5	2.7 ± 0.1	2.3 ± 0.2

[a] Means of eight determinations ± SD.
[b] Means of eight determinations ± SD.

tachyzoites per infected cells were equivalent for both cell lines (Table II). Finally, the antimicrobial effect of combinations of pyrimethamine and sulfamerazine was similar in both cell lines, indicating that they are both suitable for antimicrobial agent susceptibility testing.

Virulence. Virulence of *T. gondii* RH tachyzoites was evaluated in a mouse model of acute infection. Figure 4A shows the mortality patterns for groups of 10 mice infected with various numbers of tachyzoites isolated from infected animals. In this model the infection is fatal and the time of death is inversely proportional to the number of tachyzoites injected intraperitoneally (Fig. 4B). No differences in animal survival were observed when tachyzoites used to induce the acute infection were harvested from infected mice or from cell cultures.

Protein Composition of Tachyzoites. In an effort to determine whether *in vitro* growth considerably altered the protein profiles of the parasite, whole tachyzoites or the intraphagosomal membrane network of tachyzoites were isolated from both infected mice and cell cultures and were compared after SDS-PAGE. There were no major differences in the protein profiles of whole tachyzoites isolated from mice or any of the three cell lines included (Fig. 5).

Protein profiles of the membrane and soluble fractions in the intraphagosomal membrane network of tachyzoites isolated from infected animals and obtained after SDS-PAGE and silver staining are shown in Figure 6. Membrane and soluble fractions of the intraphagosomal membrane network of tachyzoites grown in mouse macrophage cell lines, L2 cells, and BT cells did not show any major changes in their protein profiles (data not shown). This supports the concept that *in vitro* growth does not extensively alter tachyzoites of *T. gondii* RH.

Discussion

In most laboratories, the asexual proliferative stage of *T. gondii*, the tachyzoite, is maintained by successive

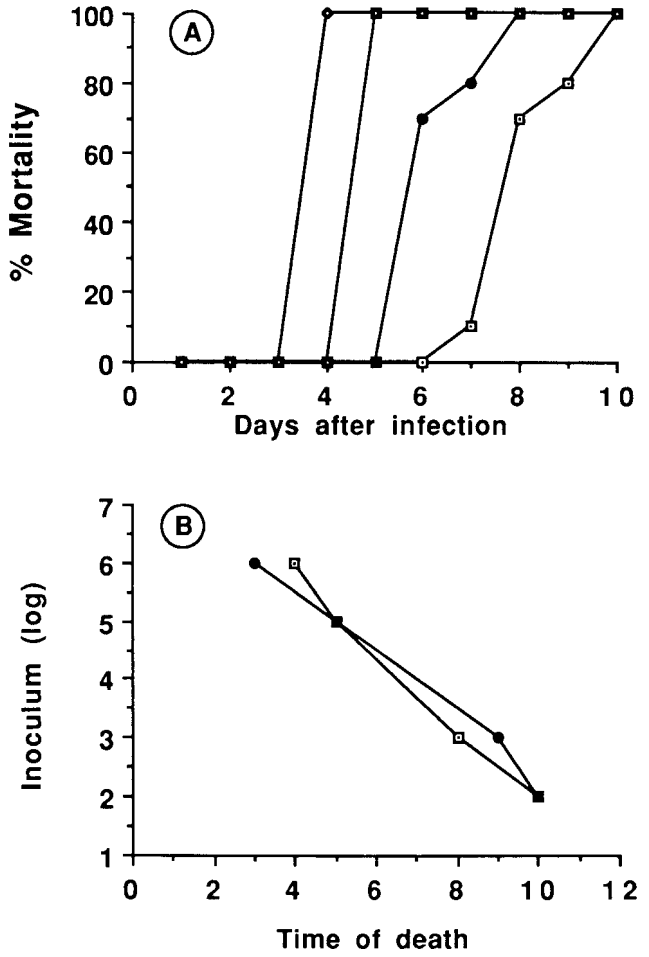

Figure 4. Virulence of *T. gondii* tachyzoites grown *in vitro* and *in vivo*. (A) Percentage of mortality in group of 10 mice inoculated intraperitoneally with 10^2 (⊡), 10^3 (●), 10^5 (■), and 10^6 (◇) *T. gondii* RH tachyzoites isolated *in vivo* from mice. (B) Relationship between the intraperitoneal inoculum of *T. gondii* RH tachyzoites isolated from infected mice (⊡) or from the cell culture of RAW264.7 (●) and the time of death of all animals (10) in each group. Standard deviations on measurements expressed in both A and B were not greater than 0.5 days.

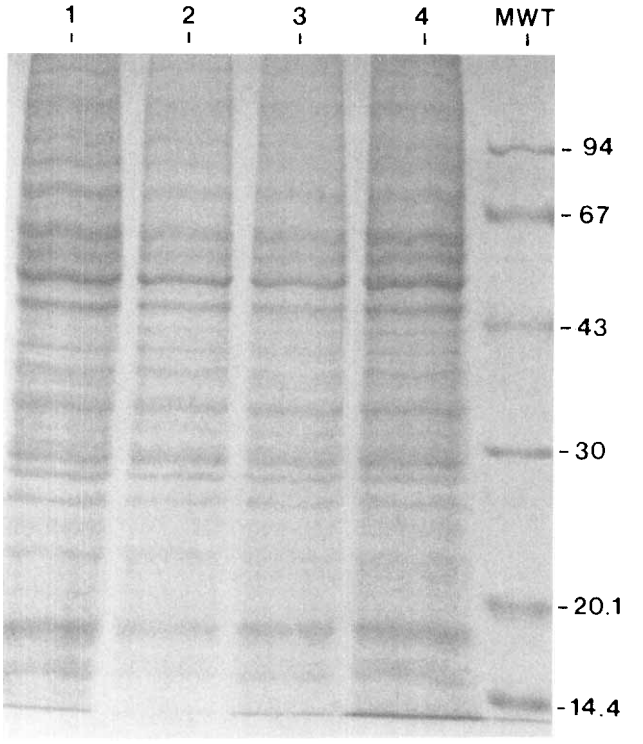

Figure 5. Protein profiles of whole *T. gondii* RH tachyzoites isolated from mice (Lane 1) and cell cultures (Lanes 2, BT; 3, RAW 264.7; Lane 4, J774 A.1). A polyacrylamide gel (10%) was loaded with 30 μg of protein/well and after electrophoresis, visualization was achieved with Coomasie Blue R stain. MWT, molecular weight ($\times 10^3$) of standard proteins indicated on the right.

passages in highly susceptible animals such as mice. Tachyzoites have been grown in nonlymphoid cell lines (4–6) or primary macrophages (7), the latter still requiring the use of laboratory animals. Long-term mass cultivation of *T. gondii* was also recently reported. This was achieved in suspension culture of HeLa cells in an automatically controlled fermenter and therefore requires specialized equipment that is costly and not widely available (8). Our studies demonstrate that three mouse macrophage cell lines, J744 A.1, RAW264.7, and P388D1, were able to support a rapid growth of *T. gondii* RH, allowing the production and simple isolation of a large number of tachyzoites in only 2–4 days. Using an inoculum of 10^7 tachyzoites in 150-cm^2 tissue culture flasks, the mouse macrophages cell lines were allowed to reach a relatively high production yield (up to 3×10^9/flask).

Several cell types have been used for the laboratory maintenance of *T. gondii* RH. These include YAC-1 tumor cell line (6), HF, CHO, 3T3 cells (13), MRC5, Vero cells (14), and HEp-2 cells (4, 5). It is difficult to compare the parasite yields reported in other studies to those we have obtained, since a wide variety of methods have been used to cultivate the parasite and to quantify parasite yields. For instance, HEp-2 cells grown in serum-enriched medium have allowed up to a 150-fold increase of the inoculum after extensive treatment with trypsin (4), while they supported only a 50-fold increase of the inoculum in a serum-free medium (5). We have found that the number of parasites isolated after cultivation on L2 cells was equivalent to that reported using HF, CHO, and 3T3 cell lines (13). The cultivation of *T. gondii* RH on YAC-1 tumor cells seemed to result in a parasite production equivalent to that we have obtained using L2 and BT cells (6), but we have shown that parasites were released from L2 and BT cells only 4 and 6 days after incubation, respectively. There is no information available about the time required for the natural release of tachyzoites from YAC-1 cell monolayers since in that study the cells were disrupted mechanically 2 days after infection (6). Human fetal lung cells (MRC5) and monkey kidney cells (Vero) have also been used to study the exo-antigens of *T. gondii*. There was no information given on the growth rate of *T.*

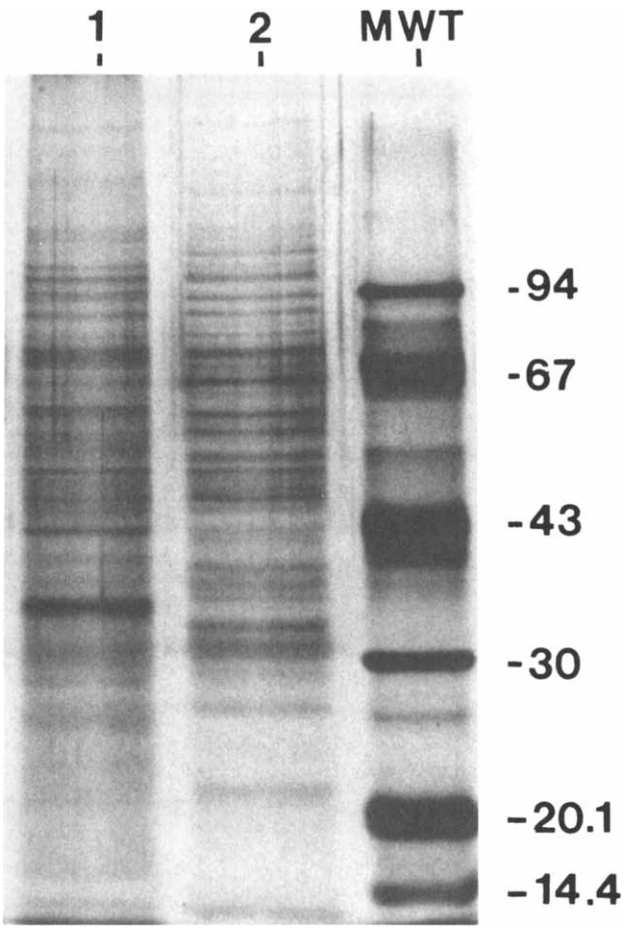

Figure 6. Protein profiles of fractions obtained during isolation of IPM from *T. gondii* tachyzoites grown in mice. Fractions were electrophoresed in a 10% polyacrylamide gel and the proteins were visualized by silver stain. Lane 1, membrane fraction; Lane 2, soluble fraction; MWT, molecular weight ($\times 10^3$) of standard proteins are indicated on the right.

gondii and the parasite yield attainable in such cell lines however, it was found that exo-antigens prepared from these two cell lines were different in their enzymatic and immunologic properties (14). Our study demonstrates that the use of mouse macrophage cell lines for the cultivation of *T. gondii* RH represent an improvement over other methods because it allows the rapid isolation of larger numbers of parasites.

The epithelial cells L2 and BT were also found to be suitable for *in vitro* development of *T. gondii*; however, these cell lines only supported a moderate growth of the parasite compared with that observed in the mouse macrophage cell lines. The L2 and especially the BT cell lines were found to be more suitable for microscopic visualization of the development of intracellular parasites and for the macroscopic visualization of growth seen as plaques in the monolayers. L2 and BT cells were also found suitable as host cells for the assessment of the activity of antimicrobial agents against *T. gondii*. BT cells were considered preferable because they require less maintenance than L2 cells. Although the *in vitro* anti-Toxoplasma activity of the combination of pyrimethamine and sulfamerazine was equivalent to that reported using HeLa cells by Chang and Pechere (7), both of our *in vitro* models resulted in a greater percentage of infected cells and apparently less tachyzoites per infected cells after 24 hr of incubation in the presence of the drug combination.

In an attempt to demonstrate that *in vitro* cultivation of *T. gondii* does not modify extensively the expression of major protein components, we compared the protein profiles of tachyzoites isolated from mice or from the five cell cultures used in this study. Protein profiles of whole tachyzoite isolated from mice or from cell cultures were similar as seen after separation by SDS-PAGE and staining by Coomasie Blue. An intraphagosomal membrane network seems to be elaborated by *T. gondii* upon infection of host cells. The biologic functions of such an organization is unknown but it is believed that proteins within the network interfere with the endocytic process (9, 15–20). The intraphagosomal membrane network from tachyzoites grown in the peritoneal cavity of mice and from tachyzoites grown *in vitro* in mouse macrophages or epithelial cells were separated by SDS-PAGE and revealed by silver staining. No major differences in protein profiles were found in the soluble and membrane fractions of the intraphagosomal membrane network of tachyzoites grown *in vitro* or in mice.

Our studies also demonstrate that *T. gondii* tachyzoites grown on any of the five cell lines used in our laboratory retain the ability to infect and multiply in heterologous cell lines. Finally, there was no difference in mortality rate and time of death of the mice inoculated with tachyzoites harvested from infected animals or from the various cell cultures. This indicates that *T. gondii* RH tachyzoites grown *in vitro* retained their full virulence. We conclude that *T. gondii* tachyzoites are not extensively modified by *in vitro* cultivation and that it would be appropriate to use such method of culture to produce large quantities of tachyzoites required for biochemical characterization of specific cellular components.

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