

## Phagocytosis of *Campylobacter jejuni* and Its Intracellular Survival in Mononuclear Phagocytes

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**In vitro phagocytosis and intracellular survival of *Campylobacter jejuni* strain 2964 in mononuclear phagocytes were studied. The following three types of mononuclear phagocytes were used: a J774G8 peritoneal macrophage line derived from BALB/c mice, resident BALB/c peritoneal macrophages, and human peripheral blood monocytes. When *C. jejuni* and mononuclear phagocytes were combined at a ratio of 75:1, light microscopy, fluorescent microscopy, and electron microscopy all indicated that *C. jejuni* cells were readily phagocytized. The majority of *C. jejuni* cells were spirals immediately following ingestion and were rapidly converted to the coccal form within 4 to 8 h. Conversion from the spiral form to the coccal form was complete in the presence of phagocytes within 96 h. In control preparations without phagocytes, conversion began after 24 h and was complete after 48 h. The extent of phagocytosis over time was determined by observing Giemsa-stained preparations and counting the number of intracellular bacterial colony-forming units after removal of extracellular *C. jejuni*. Human monocytes ingested *C. jejuni* more rapidly and vigorously than murine macrophages. Intracellular survival of *C. jejuni* was examined by measuring the number of *C. jejuni* colony-forming units associated with phagocytes after phagocytosis for 2 h and removal of extracellular bacteria. *C. jejuni* survived intracellularly for up to 6 to 7 days.**

Although certain species in the genus *Campylobacter* have been recognized as veterinary pathogens since the early 1900s, they have been established as common pathogens in humans only in the last decade. One species, *Campylobacter jejuni*, has recently been shown to be a major cause of human gastroenteritis (2). Although *C. jejuni* is currently isolated from approximately 4 to 9% of patients with diarrhea (2), little is known about its pathogenesis.

There is evidence which suggests that *Campylobacter* may invade the intestinal submucosa (9). In 3-day-old chickens, *C. jejuni* is found below the intestinal mucosal layer, causing intense infiltration of mononuclear cells. Light microscopy of these cells has indicated that macrophages may be the major phagocytic cells in the mucosa (21). This suggests that *C. jejuni* may be phagocytized by macrophages.

The purpose of this study was to examine phagocytosis of *C. jejuni* and its intracellular survival in a J774G8 peritoneal macrophage line derived from BALB/c mice (20), resident BALB/c peritoneal macrophages, and human peripheral blood monocytes. Although our data come from an in vitro system, it is possible that the capacity of *C. jejuni* to survive in cells for an extended period may contribute to its pathogenesis.

### MATERIALS AND METHODS

**Medium.** Unless otherwise specified, the medium used in these experiments was RPMI 1640 medium buffered with 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and sodium bicarbonate and supplemented with 20% heat-inactivated fetal calf serum (FCS; Sterile Systems, Inc., Logan, Utah). The FCS had ultralow levels of immunoglobulin (<31 µg/ml) and endotoxin (0.014 ng/ml) according to an analysis by the supplier. This medium is referred to below as RPMI-20% FCS medium. RPMI-20% FCS medium

was selected for use after preliminary experiments in which different serum concentrations and tissue culture media were examined. Survival of both *C. jejuni* and macrophages was optimal when they were incubated in this medium with 5% humidified CO<sub>2</sub> at 37°C.

**Collection and cultivation of murine macrophages.** Resident peritoneal macrophages were collected from BALB/c mice (Harlan Sprague Dawley, Indianapolis, Ind.) by using a modification of previously described methods (7). Cells were washed and cell viability was determined by trypan blue dye exclusion. Optimal adherence was obtained when  $1.7 \times 10^7$  viable peritoneal cells were cultured in 3 ml of RPMI 1640 medium containing 10% FCS in tissue culture dishes (60 by 15 mm). Peritoneal cells were allowed to adhere overnight, nonadherent cells were removed by washing with phosphate-buffered saline (PBS), and subsequently adherent cells were harvested from each dish by scraping with a rubber policeman in cold PBS containing 2.5 mM EDTA. This procedure provided a population of macrophages with relatively few contaminating polymorphonuclear leukocytes (19). Viable adherent cells were suspended in RPMI-20% FCS medium to a density of  $10^6$  cells per ml.

**Cultivation of J cells.** The J774G8 BALB/c mouse macrophage line (J cells) was cultured as monolayers in 25-cm<sup>2</sup> tissue culture flasks in RPMI-20% FCS medium at 37°C. The cells were passaged every 3 days.

**Collection and cultivation of human peripheral blood monocytes.** Heparinized human peripheral blood was diluted with an equal volume of RPMI 1640 medium. The diluted blood was layered over 10 ml of Lymphocyte Separation Medium (Litton Bionetics, Charleston, S.C.) in a 50-ml conical tube. This preparation was centrifuged at  $550 \times g$  for 25 min (3). The layer which contained mononuclear cells was collected and washed twice with RPMI 1640 medium. Mononuclear cells were then allowed to adhere to tissue culture dishes as described above.

**Collection and cultivation of *C. jejuni*.** A total of 10 strains

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of *C. jejuni* were provided by Raymond Kaplan, Section of Clinical Microbiology, Rush-Presbyterian St. Luke's Medical Center, Chicago, Ill. All of these isolates were from patients experiencing diarrhea. We identified the isolates by using 20 biochemical tests previously described by Hebert et al. (12). Each strain was then assigned a biotype. One isolate (strain 2964) was found to have a biotype corresponding to the most common biotype (biotype 1) found in patients in the United States with campylobacteriosis (12) and was chosen for our experiments. Strain 2964 was hippurase positive and is referred to below as *C. jejuni*. In some experiments, an additional hippurase-positive strain, strain 1702, which was isolated from an infant with enteritis, was also used. This strain was provided by John Bryner, National Animal Disease Center, Ames, Iowa.

*C. jejuni* cells were cultivated on blood agar plates under microaerophilic and capnophilic conditions in a 42°C incubator. These conditions were achieved by using an evacuation replacement system with a Torbal jar or plastic bags closed with rubber bands (13). In either case, a tank of gas containing 85% nitrogen, 10% carbon dioxide, and 5% oxygen (Matheson Gas, Joliet, Ill.) was used to fill the jar or bag. Pure cultures tended to flatten out and spread after approximately 10 subcultures, which indicated that a phenotypic change may have taken place after extended culture. We avoided this change by cryopreserving cells in FCS at -70°C and using them for no more than five subcultures.

At 2 days before use, frozen *C. jejuni* cells were thawed, streaked onto a blood agar plate, and incubated as described above. Organisms from the blood agar plate were then inoculated into brucella broth and incubated overnight under microaerophilic conditions. A suspension containing approximately  $7.5 \times 10^7$  viable *C. jejuni* cells per ml was prepared by adjusting the *C. jejuni* preparation to an optical density at 540 nm of 0.85. The actual number of viable *C. jejuni* cells in the suspension was determined by counting the number of colony-forming units (CFU). This was accomplished by preparing 10-fold serial dilutions of the *C. jejuni* preparation to  $10^{-4}$  in brucella broth; 20  $\mu$ l of each dilution was placed on a blood agar plate. The plates were then incubated as described above, and the numbers of colonies were determined.

**Direct observation of mononuclear phagocyte-*C. jejuni* interaction.** (i) **Giemsa-stained preparations.** One sterile 60-mm petri dish was used for each incubation time by placing three 15- to 18-mm<sup>2</sup> cover slips in each petri dish (6). Mononuclear phagocytes were then suspended to a density of  $10^6$  cells per ml of RPMI-20% FCS medium, and 100  $\mu$ l of this suspension was placed on each cover slip. The cells were allowed to adhere for 2 h at 35°C in a 5% humidified CO<sub>2</sub> atmosphere; the petri dishes were then flooded with 1 ml of prewarmed RPMI-20% FCS medium per cover slip. The dishes were incubated overnight, and the cover slips were removed from the dishes, rinsed with RPMI-20% FCS medium, and placed in a fresh petri dish. Then  $7.5 \times 10^6$  *C. jejuni* cells in 100  $\mu$ l of RPMI-20% FCS medium were placed on each cover slip, and the dishes were returned to the incubator. Thus, the ratio of *C. jejuni* cells to macrophages was 75:1.

The morphology of intracellular *C. jejuni* was studied at 0.5, 1, 2, 4, 8, and 24 h and every 24 h thereafter. After incubation for 0.5, 1, or 2 h, cultures were removed from the incubator, and cover slips were washed three times with warm PBS to remove any remaining extracellular bacteria. At the end of each time period, the cover slips were air dried, fixed with absolute methanol for 5 min, treated with 2% Triton X-100 for 3 min, rinsed three times with fresh

PBS, stained with Giemsa stain, and observed microscopically by using an oil immersion lens. In groups requiring longer incubation times, the cover slips were washed after 2 h to remove extracellular bacteria, and incubation was continued so that the morphology of the intracellular *C. jejuni* could be monitored later.

(ii) **Vital staining with acridine orange.** *C. jejuni* cells were prelabeled for 1 min with 0.014% acridine orange (Sigma Chemical Co., St. Louis, Mo.) in brucella broth. They were then washed three times with fresh RPMI-20% FCS medium and added to monolayers of mononuclear phagocytes. Ample acridine orange was carried into the macrophage cultures to permit visualization of both bacteria and mononuclear phagocytes. Samples were observed by using an epifluorescent Zeiss microscope equipped with a 495-nm excitation filter and a 519-nm emission filter. Under the conditions described above, it has been established that live bacteria stain green and dead bacteria and lysosomes are orange (11).

(iii) **Electron microscopy.** Phagocytic cells were infected as monolayers in tissue culture flasks, phagocytosis was allowed to proceed for 2 h, and extracellular bacteria were then removed by washing. Processing of samples for electron microscopy was carried out essentially as previously described (6, 17). Specimens were then examined by using a Zeiss model 109T electron microscope.

**Assay of phagocytosis and killing by mononuclear phagocytes.** A suspension containing  $10^6$  mononuclear phagocytes per ml and  $7.5 \times 10^7$  *C. jejuni* cells per ml of RPMI-20% FCS medium was prepared; 15 separate samples (100  $\mu$ l each) of this suspension were placed in separate wells of flat-bottom microtiter strips and incubated in 5% humidified CO<sub>2</sub> in air. Control wells containing only the *C. jejuni* suspension were also prepared to determine the viability of *C. jejuni* in the absence of macrophages.

The rate of phagocytosis was assessed by determining the number of CFU associated with adherent mononuclear phagocytes at 0.5, 1, 2, 4, and 8 h. To accomplish this, at the end of each incubation period, triplicate samples were processed as follows. Adherent cells were washed with RPMI 1640 medium to remove extracellular bacteria, and 100  $\mu$ l of fresh RPMI 1640 medium was added to each well. Macrophages were then lysed to release intracellular bacteria by adding 100  $\mu$ l of 0.2% Triton X-100 in PBS to each well. A 100- $\mu$ l portion of the resulting suspension was removed for assay at each time period, and the number of CFU was determined as described above. Controls in which *C. jejuni* was incubated in 0.2 to 2% Triton X-100 for 10 min showed that this detergent did not significantly affect the viability of the *C. jejuni*.

To determine whether phagocytized bacteria were killed, extracellular *C. jejuni* cells were removed after 2 h of phagocytosis by macrophages. This was designated zero time. Subsequently (0.5, 1, 2, 4, 8, and 24 h and every 24 h thereafter for 7 days), viable intracellular *C. jejuni* cells were enumerated. The macrophages were viable over this 7-day period, as determined by cell adherence.

Both phagocytosis of *C. jejuni* by macrophages and intracellular killing of *C. jejuni* were also examined in suspension cultures. Phagocytes and *C. jejuni* cells were suspended in a polypropylene conical tube and rotated on a Labquake rotator at 10 rpm. After the desired incubation period, 1.0 ml of the suspension was removed from the tube, and the remaining extracellular bacteria were removed by centrifuging the tube three times at  $200 \times g$  for 10 min prior to the CFU assay. The number of viable intracellular bacteria was determined as described above.

**Statistics.** Statistical comparisons between samples were made by using the Student paired *t* test.

## RESULTS

In all of the experiments described below, *C. jejuni* strain 2964 was used. Similar results were obtained in experiments done with strain 1702.

**Morphology of intracellular *C. jejuni*.** (i) **Giemsa-stained preparations.** After *C. jejuni* cells were combined with J cells on cover slips at a ratio of 75:1 under the conditions described above, Giemsa-stained preparations showed that internalized *C. jejuni* cells were converted from a spiral form to a coccal form; this began in the first 4 to 8 h, and 100% conversion occurred between 72 and 96 h (Table 1). In these preparations, the extent of infection increased for the first 24 h. Examination of these Giemsa-stained preparations and examination of preparations by using phase-contrast microscopy and scanning electron microscopy revealed that less than 1% of the *C. jejuni* cells were not ingested, indicating that the washing procedure used to remove adherent bacteria was effective. In controls prepared by using a suspension of *C. jejuni* cells in the absence of phagocytic cells, the morphological changes did not start until 24 h and were complete within 48 h.

(ii) **Electron microscopy.** Although light microscopy suggested that the *C. jejuni* cells were intracellular, electron microscopy was necessary to show the precise intracellular location. Electron microscopy of washed specimens processed after 2 h of phagocytosis showed unequivocally that most of the *C. jejuni* cells were intracellular in vacuoles; very few *Campylobacter* cells were not removed by the washing procedure used. Intracellular *C. jejuni* cells were found either exclusively as spiral forms inside individual vacuoles or as multiple single spiral and coccal forms inside large vacuoles (Fig. 1). These observations correlate well with what we observed in Giemsa-stained preparations. As shown in Fig. 1, the spiral form of *C. jejuni* was easily distinguished from the coccal form; the cell wall of the coccal form is separated from the rest of the cellular structure (4).

**Phagocytosis of *C. jejuni* as measured by the CFU assay.** After *C. jejuni* cells were combined with mononuclear phagocytes in a suspension at a ratio of 75:1 under the conditions described above, the phagocytes were then lysed, and the number of *C. jejuni* CFU associated with phagocytes was determined. In the continual presence of *C. jejuni*, phagocytosis by J cells, BALB/c resident peritoneal macrophages, and human monocytes increased steadily during the 8-h incubation period used (Table 2). Phagocytosis of *C. jejuni* by human monocytes was more rapid and vigorous than phagocytosis by murine cells. Most extracellular *C. jejuni* cells added were ingested by human monocytes over the 8-h time course used in this study. This 8-h time course also resulted in substantial ingestion of *C. jejuni* cells by mouse macrophages (30 to 50%).

**Intraphagocyte survival of *C. jejuni*.** In preliminary experiments, we found that acridine orange did not stain *C. jejuni* after phagocytosis by macrophages. When *C. jejuni* cells were labeled with acridine orange prior to phagocytosis and ingested by macrophages, the majority of the *C. jejuni* cells fluoresced green in orange-colored lysosomes. This suggested that *C. jejuni* cells were viable for up to 6 days following ingestion. Further evidence for intracellular survival of *C. jejuni* was obtained by determining the number of *C. jejuni* CFU after phagocytosis. Intraphagocyte survival was determined by removing extracellular *C. jejuni* after 2 h

TABLE 1. Morphology of *C. jejuni* after phagocytosis by J cells

Time (h) <sup>a</sup>	No. of <i>C. jejuni</i> cells per macrophage <sup>b</sup>	% of <i>C. jejuni</i> cells occurring as intracellular spirals <sup>c</sup>	% of <i>C. jejuni</i> cells occurring as spirals in the absence of macrophages
0.5	0.7 ± 0.8	94.2 ± 8.2	100.0 ± 0.0
1.0	3.4 ± 0.9	93.0 ± 7.1	100.0 ± 0.0
2.0	7.6 ± 3.2	93.0 ± 9.6	100.0 ± 0.0
4.0	6.5 ± 0.4	79.2 ± 18.4	100.0 ± 0.0
8.0	7.5 ± 1.9	70.4 ± 8.3	100.0 ± 0.0
24.0	13.9 ± 8.2	49.9 ± 11.1	100.0 ± 0.0
48.0	10.7 ± 1.0	21.7 ± 13.7	0.0 ± 0.0
72.0	14.3 ± 4.0	10.5 ± 7.5	0.0 ± 0.0
96.0	7.7 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
110.0	11.3 ± 2.6	0.0 ± 0.0	0.0 ± 0.0

<sup>a</sup> *C. jejuni* cells and J cells were placed on cover slips at a ratio of 75:1. After incubation for 0.5, 1, and 2 h, samples were washed to remove extracellular *C. jejuni* from the cover slips and Giemsa stained. For time periods longer than 2 h, the remaining extracellular *C. jejuni* cells were removed by washing, fresh medium was added, and the dishes were reincubated. After varying time periods, Giemsa-stained specimens were observed.

<sup>b</sup> Data are expressed as means ± standard deviations of three experiments; in each experiment 100 macrophages were examined.

<sup>c</sup> Data are expressed as means ± standard deviations and were calculated as follows: (number of spirals/total number of *C. jejuni* cells) × 100.

of phagocytosis. After further incubation in fresh RPMI-20% FCS medium, mononuclear phagocytes were lysed, and the number of intracellular *C. jejuni* CFU was determined. *C. jejuni* survived in mononuclear cells for at least 7 days (Fig. 2). Results obtained with control preparations of *C. jejuni* indicated that a significant loss of *C. jejuni* viability in the absence of phagocytes started to occur after 48 h; no viable *C. jejuni* cells were recovered after 4 days (Fig. 2).

Since other investigators have shown that there are significant differences in the metabolic activities of phagocytes cultured in suspension compared with phagocytes cultured as monolayers (15), we evaluated the short-term survival of *C. jejuni* in cells of the J cell macrophage line under these two different culture conditions. For such experiments, parallel monolayer and suspension cultures were prepared by using the same populations of J cells and *C. jejuni*. After 2 h of phagocytosis, extracellular bacteria were removed from monolayers by washing and from suspension cultures by centrifuging samples three times for 10 min at 200 × *g*. There was no significant difference between the levels of survival of *C. jejuni* in the two populations of J cells in suspension or in a monolayer culture, as evaluated by using the Student *t* test ( $P \leq 0.05$ ) (Table 3).

## DISCUSSION

During the course of human *Campylobacter* infections, organisms encounter phagocytic cells both in the intestinal mucosa (1, 9) and systemically during bacteremic conditions (5, 10, 16). Our overall goals are to determine whether macrophages or monocytes or both play a role in host defense against *Campylobacter*. In the in vitro experiments described above, we studied the ability of macrophages and monocytes to phagocytize *C. jejuni* by using the following three cell types: J774G8, a peritoneal macrophage line derived from BALB/c mice, resident peritoneal BALB/c macrophages, and human peripheral blood monocytes. We then examined intracellular survival of *C. jejuni* in these mononuclear phagocytes. Our results suggests that although *C. jejuni* cells are readily internalized by all three cell types,

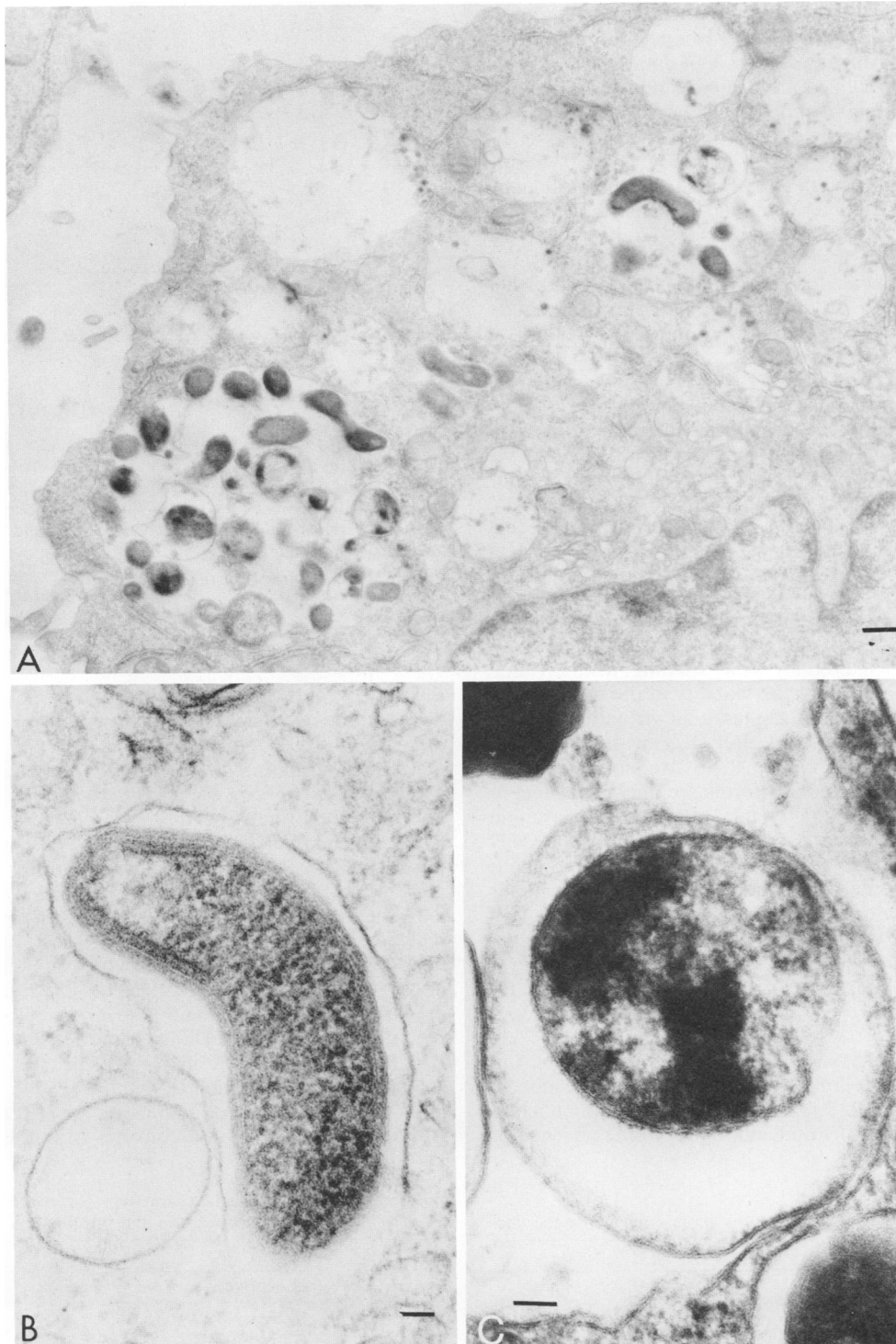


FIG. 1. (A) Electron micrograph showing *C. jejuni* individually or in groups in vacuoles of a J cell. Bar = 1  $\mu\text{m}$ . (B) Electron micrograph showing *C. jejuni* as a single spiral in a vacuole. Note the layer beneath the plasma membrane of *C. jejuni*, which may be the polar membrane described previously in related bacterial species (14). Bar = 0.1  $\mu\text{m}$ . (C) Electron micrograph showing intraphagocyte coccidial form of *C. jejuni*. The coccidial form is distinguishable from the spiral form by the separation of the cell wall from the remaining cellular structure. Bar = 0.1  $\mu\text{m}$ .

they are not rapidly killed following ingestion. The more rapid death of *C. jejuni* in the absence of phagocytic cells indicates that phagocytosis may actually promote the survival of *C. jejuni*.

Previous studies in which *Campylobacter fetus* subsp.

*fetus* was used showed that this bacterium possesses a glycoprotein which inhibits phagocytosis except in the presence of specific antiserum (8, 18). In our hands, *C. jejuni* was readily internalized in the absence of specific opsonins, as evidenced by the high number of intracellular bacteria

determined by a CFU assay and visualization of the organism inside phagocytes by light microscopy, fluorescent microscopy, and electron microscopy. Our data suggest that *C. jejuni* cells are internalized more rapidly and vigorously by human monocytes than by murine phagocytes (Table 2). It is possible that the apparent differences in the number of *C. jejuni* cells ingested by human monocytes and murine macrophages are due to differences in intracellular killing by the macrophages or bacterial replication instead of differences in ingestion rates. However, this is unlikely since the levels of intracellular survival of *C. jejuni* in the three types of mononuclear phagocytes were similar (Fig. 2). Further studies are in progress to determine the effects of antibody and complement on phagocytosis and intracellular survival of *C. jejuni*.

Intracellular *C. jejuni* cells are found primarily in the spiral form immediately following ingestion but convert rapidly from the spiral form to the coccal form. Electron microscopy demonstrated the presence of multiple spiral and coccal forms inside single vacuoles shortly after ingestion. Our observations confirm those of Buck et al. (4), who showed that the spiral form could be distinguished from the coccal form by electron microscopy since the cell wall of the coccal form is separated from the rest of the cellular structure. These authors proposed that the coccal form is a degenerative form of the spiral form. In our experiments, the majority of intracellular spiral and coccal forms were viable even after 6 days, as measured by vital staining with acridine orange. Further evidence for the viability of the intracellular coccal form was provided by the fact that virtually all of the intracellular forms were coccal after 48 h, without a corresponding change in viability. However, we cannot rule out the possibility that some coccal forms are nonviable since it is difficult to correlate the results of direct microscopic examination with the viability counts obtained by the CFU assay. The coccal forms of *C. jejuni* are extremely small, which makes accurate visual quantitation of intracellular forms difficult. Total *C. jejuni* counts which are obtained by quantitation of CFU are approximately 80% higher than estimates obtained by direct observation. Furthermore, Giemsa-stained preparations of intracellular organisms revealed spirals which were as many as three to six turns long. When macrophages are lysed and blended in a Vortex mixer, long spirals may be broken into multiple CFU. Since the morphological change from the spiral form to the coccal form occurs within 4 to 6 h after ingestion by mononuclear phagocytes and since this does not occur in the absence of

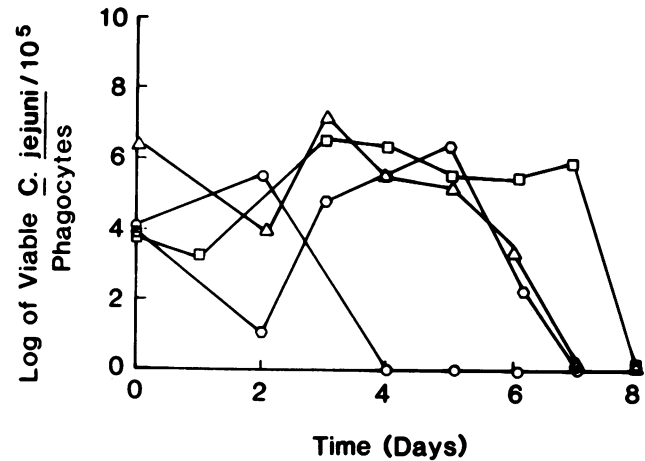


FIG. 2. Survival of *C. jejuni* in mononuclear phagocytes as determined by the CFU assay. Phagocytes were incubated with *C. jejuni* cells for 2 h; extracellular *C. jejuni* cells were then removed by washing, after which fresh RPMI-20% FCS medium was added to the mononuclear phagocytes. Experimental cultures of J cells (□), BALB/c macrophages (○), and human monocytes (△) were then incubated in 5% CO<sub>2</sub>. Duplicate cultures of *C. jejuni* without mononuclear phagocytes (○) were also prepared by incubating the original suspension of *C. jejuni* under the same conditions as the samples.

these cells until after 48 h, the mononuclear phagocytes appear to be responsible for the rapid morphological change of *C. jejuni*.

After determining that *C. jejuni* is phagocytized, we evaluated the capacity of *C. jejuni* to survive intracellularly. Our data show that *C. jejuni* survives better inside monocytes or macrophages than in control preparations without phagocytes. The apparent enhancement of survival of intracellular *C. jejuni* in mononuclear phagocytes cannot be explained without further study. Perhaps the mononuclear phagocytes provide a nutrient or other favorable environment or both for *C. jejuni* cells which prolongs their viability. In mononuclear phagocytes, *C. jejuni* cells survive for only 6 to 7 days rather than for several weeks, as is the case with more typical intracellular bacteria, such as *Salmonella typhimurium* or *Listeria monocytogenes*.

Our results indicate that *C. jejuni* is readily internalized by mononuclear phagocytes in the absence of specific opsonins. Although *C. jejuni* rapidly converts from the spiral form to

TABLE 2. Phagocytosis of *C. jejuni* by mononuclear phagocytes

Time (h)	% Phagocytosis by <sup>a</sup> :		
	J cells	BALB/c macrophages	Human monocytes
0.5	9.8 ± 1.0	11.4 ± 2.3	48.3 ± 4.7
1.0	13.2 ± 3.6	11.4 ± 2.3	62.7 ± 22.7
2.0	15.0 ± 7.4	22.4 ± 5.6	61.1 ± 8.1
4.0	19.9 ± 1.1	55.3 ± 4.5	87.1 ± 3.5
8.0	33.0 ± 2.8	44.9 ± 2.2	103.1 ± 30.6

<sup>a</sup> *C. jejuni* cells and mononuclear phagocytes were prepared in a suspension at a ratio of 75:1. After varying times, the percentages of phagocytosis of *C. jejuni* by three types of mononuclear phagocytes were determined by comparing the number of intracellular *C. jejuni* CFU with the total number of *C. jejuni* CFU added. Data are expressed as means ± standard deviations of triplicate cultures and were determined as follows: (number of CFU inside/number of CFU added) × 100. These data are from one of the three experiments performed.

TABLE 3. Comparison of short-term survival of *C. jejuni* in J cells in suspension and in monolayers<sup>a</sup>

Time (h)	No. of CFU in <sup>b</sup> :		
	Monolayers	Suspension	Control <sup>c</sup>
0.0	6.65 ± 0.16	6.28 ± 0.49	7.26 ± 0.24
0.5	6.25 ± 0.24	6.53 ± 0.20	7.36 ± 0.10
1.0	6.67 ± 0.05	6.50 ± 0.20	7.62 ± 0.15
2.0	6.74 ± 0.12	6.72 ± 0.05	7.52 ± 0.07
4.0	6.42 ± 0.49	6.16 ± 0.15	7.50 ± 0.20
8.0	6.42 ± 0.10	6.23 ± 0.40	7.48 ± 0.30

<sup>a</sup> J cells were washed free of extracellular *C. jejuni* after 2 h of phagocytosis (zero time), and the numbers of CFU were determined after varying time periods.

<sup>b</sup> Data, expressed as the log of CFU (means ± standard deviations of triplicate cultures) recovered from 10<sup>5</sup> cells, are from one of two experiments performed.

<sup>c</sup> The control was a preparation of *C. jejuni* cells without mononuclear phagocytes.

the coccal form after phagocytosis, the cells continue to survive intracellularly for at least 6 days. Therefore, phagocytosis may actually promote the survival of *C. jejuni*.

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