The Distribution and Persistence in Vivo of Corynebacterium parvum in Relation to Its Antitumor Activity

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SUMMARY

Killed Corynebacterium parvum was labeled with fluorescein isothiocyanate or 125I, and both preparations were shown to retain lymphoreticular stimulatory and antitumor activity. Large amounts of C. parvum injected i.v. were found in the liver, spleen, and lungs with less in bone marrow and lymph nodes. Apart from a rapid loss from the lungs within 24 hr, the persistence of killed C. parvum was striking, and some intact bacteria were still detectable in the liver and spleen at 15 days. (By contrast, the breakdown of an inactive C. parvum strain in the liver was considerably faster.) The blood clearance of 125I-labeled C. parvum injected i.v. into tumor-bearing mice was more rapid than in normal mice, and the absolute, but not the unit, amounts of C. parvum taken up by the spleen and tumor-draining node were increased. 125I-labeled C. parvum was found within the body of established solid tumors, but there was no correlation between the amount of C. parvum taken up by various mouse solid tumors after i.v. injection and their susceptibility to i.v. C. parvum therapy. The distribution and persistence of C. parvum injected into a tumor lesion was similar to that after s.c. injection. The bulk of the inoculum was retained at the injection site and draining lymph node. Contralateral nodes were unlabeled, and uptake in the liver and spleen was considerably less than after i.v. injection. Although no C. parvum was found in peritoneal cells after i.v. injection, the macrophages in this population became activated and were capable of nonspecifically inhibiting tumor cell growth in vitro.

INTRODUCTION

The bacterial vaccine Corynebacterium parvum is an effective antitumor agent in animals (reviewed in Ref. 23) and is currently undergoing clinical investigation (12, 20). The present studies using labeled C. parvum preparations were undertaken to delineate the distribution of injected C. parvum, which might provide a guideline in selecting the route and site of injection for given tumor situations and further information as to underlying antitumor mechanisms. This paper describes the preparation of both 125I- and fluorescein-labeled C. parvum and their distribution after either local or systemic injection in normal and tumor-bearing mice.

MATERIALS AND METHODS

Mice. Adult female CB6F1 and DBA/2 mice were maintained at Wellcome Research Laboratories, Beckenham, England. Adult female C3H/HeJ mice were from the specific pathogen-free breeding colony at the Section of Radiotherapy, M. D. Anderson Hospital, Houston, Tex.

C. parvum. A formal-killed suspension, Coparvax (Wellcome Reagents Ltd., Beckenham, Kent, England), Batch P401 (7 mg/ml) was used. In some experiments an inactive strain of C. parvum, CN5888, was used. It was grown and a formal-killed suspension was prepared as described in Ref. 1.

125I-labeled C. parvum. C. parvum (5 ml) was washed 2 to 3 times in 0.9% NaCl solution and resuspended in 0.4 ml phosphate-buffered saline (8.01 g NaCl, 1.42 g Na2HPO4, 0.41 g KH2PO4, and distilled water to 1.0 liter). 125I (2 mCi) (Radiochemical Centre, Amersham, England) was added, followed immediately by 0.4 ml chloramphenicol T (5 mg/ml). After 5 min, 0.4 ml sodium bisulfite (25 mg/ml) was added, followed immediately by 0.4 ml sodium iodide (10 mg/ml). The suspension was washed once by centrifugation in 0.9% NaCl solution and resuspended to its original volume. It was dialyzed for 5 days against daily changes of 2 liters of 0.9% NaCl solution at 4°C, by which time the radioactivity in the dialysate had stabilized. In different batches only 0.01 to 0.02% of iodine remained unbound, and no appreciable amount was released after incubating the labeled C. parvum for 24 hr at 37°C. One-tenth ml of 10% thiomersal preservative per 10 ml suspension was added. Suspensions were stored at 4°C. Concentration was determined by optical comparison with dilutions of unlabeled C. parvum vaccine of known concentration. Unless otherwise specified, injections were of 300 μg.

Gamma Counting. Organs or blood samples (50 μl) taken from 125I-labeled C. parvum-injected mice were placed directly into plastic tubes and counted using a Packard Tri Carb Automatic gamma counter. Results are expressed as percentage of total isotope injected. Means and standard errors were calculated for all experimental groups, and differences where p < 0.05 (Student’s t test) were considered significant.

Phagocytic Index (K). Mice received i.v. injections of 125I-labeled C. parvum and were bled repeatedly (50-μl samples) at recorded times over a 10-min period. The radioactivity of the samples was determined, and the phagocytic index was calculated as described previously (3).

FITC-CP.3 C. parvum was washed 3 times by centrifugation.
tion in 0.85% NaCl solution and resuspended to its original volume. Buffer was prepared by mixing Na2CO3 (53 mg/100 ml) and NaHCO3 (4.2 mg/100 ml) to pH 9.0. One volume of buffer was then added to 9 volumes of washed C. parvum. The suspension was set up on a stirrer, and FITC, 1 mg/ml suspension, was added very slowly while stirring at room temperature, and stirring continued for 1.5 hr. The suspension was washed 4 to 5 times in 0.85% NaCl solution to remove unbound FITC and resuspended to its original volume. Buffer was prepared by mixing Na2CO3 (53 mg/100 ml) with NaHCO3 (4.2 mg/100 ml) to pH 9.0. One volume of buffer was then added to 9 volumes of washed C. parvum. FITC-CP was stored in the dark at 4° with no noticeable loss of fluorescein into the supernatant during a 1-year period. Unless otherwise specified injections were of 300 μg.

Fluorescent Microscopy. Organs removed from FITC-CP-injected mice were immediately frozen using Polar Spray BPC (dichlorodifluoromethane; Aerosol Marketing and Chemical Co. Ltd., London, England). Cryostat sections were cut at —20°, fixed in methanol for 10 min, and mounted on Polarfluor B (Polaron Experiment Ltd., Watford, England). Blood smears were air dried before fixing and mounting. Peritoneal and bone marrow cells were washed and resuspended at 2 x 10⁶/ml in Eagle’s minimum essential medium. Aliquots (0.1 ml) were placed on coverslips, and the cells were allowed to settle for 1.5 hr at 37° in a 5% CO₂ atmosphere. The monolayers were then fixed and mounted as above. Slides could be stored in the dark at 4° for up to 2 weeks without marked loss of fluorescence. They were examined using a Leitz Ortholux II phase contrast/fluorescence microscope.

Tumors. CBA, methylcholanthrene-induced fibrosarcoma cells (M4) were maintained in vitro and prepared as described in Ref. 5. Similar details have been given for CBA T3 fibrosarcoma (7) and CBA RI leukemia cells (18). DBA/2 mastocytoma P185 (MA) was obtained in frozen form from Dr. A. C. Allison, Clinical Research Centre, Harrow, Middlesex, England. Cells were maintained by serial ascitic passage and obtained for injection by peritoneal lavage. C3H/Bu fibrosarcoma cells (Fsa) were maintained and prepared for injection as described in Ref. 16. Solid tumors were established by s.c. injection of cells into either the hind footpad or flank. Tumors growing in the flank were measured as 2 diameters at right angles, the size being expressed as the multiplicand.

In Vitro Assay for Nonspecific Inhibition of Tumor Growth. The technique has been described before (24). Peritoneal cells were mixed in vitro at a ratio of 10:1 with RI leukemia cells. [³H]Thymidine was added after 24 hr of culture, and its uptake by tumor cells was assessed 24 hr later.

RESULTS

Immunostimulatory and Antitumor Activity of Labeled C. parvum Preparations. Experiments were performed to confirm that both the FITC-CP and 125I-labeled C. parvum preparations retained their lymphoreticular stimulatory and antitumor activity. CBA mice received i.v. injections of FITC-CP, 125I-labeled C. parvum, or unlabeled C. parvum (Table 1), and, after 14 days, there were no significant differences among the splenomegalies induced by any of the C. parvum preparations (Table 1). They were also equally effective when injected into a tumor lesion, all treated tumors completely regressing with no regrowth during a 40-day period. By this time untreated mice were dying with large tumors (Table 1).

The Use of FITC-CP. FITC-CP preparations were highly fluorescent (Fig. 1a). After injection, fluorescence was clearly visible in tissue sections under ×20 magnification, and, using ×1000 magnification, individual bacteria could be resolved (Fig. 1b). Results obtained with FITC-CP provide unequivocal evidence of the presence of C. parvum organisms, but it is not possible to quantitate the amount of C. parvum in a tissue sample. For quantitative studies of the distribution and persistence of C. parvum in organs and other solid tissues, we have used 125I-labeled C. parvum, but all experiments were repeated using FITC-CP to confirm the presence or absence and the relative distribution of C. parvum. FITC-CP was particularly useful with cell populations (e.g., bone marrow, peritoneal) when FITC-CP-containing cells were readily identified and quantified.

Blood Clearance of 125I-labeled C. parvum and FITC-CP in Normal and Tumor-bearing Mice. M4 cells (10⁵) were injected s.c. into the hind footpad, and 3 days later C. parvum was injected s.c. into the tumor site.

### Table 1

<table>
<thead>
<tr>
<th>Injection i.v. at Day 0</th>
<th>Spleen Wt at Day + 14</th>
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<tbody>
<tr>
<td></td>
<td>(g ± S.E.; n = 4)</td>
</tr>
<tr>
<td>C. parvum (300)</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>FITC-CP (300)</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>125I-labeled C. parvum (300)</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>Intratumor injection at Day +3</td>
<td>Proportion of mice tumor free at Day +40</td>
</tr>
<tr>
<td>C. parvum (70)</td>
<td>6/6</td>
</tr>
<tr>
<td>FITC-CP (70)</td>
<td>6/6</td>
</tr>
<tr>
<td>125I-labeled C. Parvum (70)</td>
<td>6/6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, dose in μg.

* Significant difference from similarly designated results (p > 0.05).

* M4 fibrosarcoma cells (10⁵) were injected s.c. into a hind footpad, and 3 days later C. parvum was injected s.c. into the tumor site.

Intratumor injection, respectively

Intratumor injection at Day +3

Proportion of mice tumor free at Day +40

0/6

6/6

6/6

6/6

6/6

6/6

* Significant difference from similarly designated results (p > 0.05).

* M4 fibrosarcoma cells (10⁵) were injected s.c. into the hind footpad of CBA mice; 3 weeks later, when the footpad thickness was 7 to 8 mm, the mice received i.v. injections of 125I-labeled C. parvum, and the phagocytic index (K) was determined. The clearance of 125I-labeled C. parvum from the blood of tumor-bearing mice was significantly faster than normal; K = 0.25 ± 0.009 and 0.14 ± 0.007, respectively. Twenty-four hr after i.v. 125I-labeled C. parvum treatment in both normal and tumor-bearing mice, there was no detectable difference between the radioactivity of whole-blood samples and those in which cell and bacteria had been removed by high-speed centrifugation (10,000 x g for 15 min). This suggested that C. parvum had been completely cleared from the blood within 24 hr. It was confirmed by the complete absence of FITC-CP in blood smears taken 24 hr after i.v. FITC-CP treatment.
Distribution and Persistence of Labeled C. parvum Injected i.v. Into Normal and Tumor-bearing Mice. 

Distributors and identifications C. parvum was injected i.v. into normal and tumor-bearing CBA mice as above (Chart 1). At the time of injection, spleen weights in tumor-bearing mice (0.19 ± 0.2 g) were significantly larger than normal (0.10 ± 0.004 g), as were tumor-draining lymph nodes: 15.7 ± 0.5 and 1.4 ± 0.3 mg, respectively. There were no significant differences in lung and liver weights.

In normal mice large amounts of radioactivity (80.8% injected) were present in the liver after 1 hr. The levels then declined gradually with 10% still being detected after 15 days. The presence of the tumor did not modify the uptake or persistence of radioactivity in the liver. Radioactivity (2.8%) was present in normal spleen after 1 hr and persisted similarly to that in the liver. Significantly more radioactivity (4.8%) was found in tumors spleens after 1 hr; however, the uptake per g spleen was not significantly different: 30.4 ± 4.3% and 30.0 ± 3.0%/g for normal and tumor spleens, respectively. Normal lungs contained 3.8% radioactivity at 1 hr, and this declined rapidly over the next 24 hr (0.2%), but more gradually thereafter. Lungs from tumor-bearing mice were not significantly different. Significant levels of radioactivity were detectable in normal popliteal nodes only after 1 hr but persisted in tumor-draining nodes for 3 days. Despite the larger amounts of radioactivity in tumor-draining nodes at 1 hr (0.56 ± 0.003% compared with 0.009 ± 0.004%), compensating for their increased mass, the unit uptake (0.004 ± 0.001%/g), was significantly less than in normal and tumor-bearing mice. As noted in the previous section, radioactivity in the blood after 24 hr is in soluble form and most probably reflects the breakdown of 125I-labeled C. parvum in the various organs.

Parallel studies using i.v. FITC-CP confirmed the presence of intact FITC-CP in the various organs and their relative distribution (semi-quantitative estimate of numbers of FITC-CP per unit section). A correlation between the amounts of FITC-CP and radioactivity was particularly apparent from the marked loss of FITC-CP from the lungs between 1 and 24 hr. FITC-CP detected in the liver and spleen after 15 days appeared intact and was similar to that seen after only 1 hr (Fig. 1b). Intact FITC-CP have been detected in liver sections up to 28 days after injection.

Distribution and Persistence of Labeled C. parvum Injected s.c. into Normal Mice or Intralesionally into Tumor-bearing mice. Injection s.c. of C. parvum has been compared with injection of C. parvum directly into tumor tissue. The latter has been a particularly effective form of C. parvum therapy in many animal tumor models (13, 19, 23, 26) including the present M4 (see Table 1). Normal CBA mice received 125I-labeled C. parvum s.c. into a hind footpad. Another group of mice bearing a 3- to 4-mm M4 tumor in the hind footpad received 125I-labeled C. parvum s.c. directly into the tumor lesion (Chart 2). The bulk of the radioactivity (75%) was found at the injection site after both s.c. and intralesional injection. It persisted as shown with 0.4 and 0.6%, respectively, remaining at 15 days. The amounts of radioactivity and their persistence in liver and spleen were similar after both injections and were considerably less than after systemic injection (Chart 1). The radioactivity levels in the draining popliteal nodes after both injections were similar to each other and to those found in the spleen. No significant activity was detected in contralateral nodes. A consistent difference in the distribution of radioactivity after either s.c. or intralesional injection was found after 1 hr; with s.c. injection, peak levels in the liver, spleen, and draining node were delayed until 24 hr. In both cases these levels paralleled those in the blood. The presence or absence of C. parvum in the various organs at different times after both s.c. and intralesional injection was confirmed using FITC-CP.

Localization of i.v.-injected C. parvum within a Solid Tumor. Solid tumors were established in the flanks of CBA mice by s.c. injection of 106 M4 cells; 7 days later, when the tumors were 25 to 30 sq mm and still responsive to i.v. C. parvum (see Chart 3), FITC-CP was injected i.v. Twenty-four hr later the tumors were dissected out and carefully cleared of nontumorous tissue. Cryostat sections were prepared...
In vitro inhibition of RI leukemia cell growth by peritoneal cells from mice pretreated either i.v. or i.p. with FITC-CP. 

<table>
<thead>
<tr>
<th>Cells</th>
<th>[3H]Thymidine incorporation (cpm/culture ± S.E.)</th>
<th>cpm/culture (% RI alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI alone</td>
<td>32,996 ± 300</td>
<td>100.0</td>
</tr>
<tr>
<td>Normal peritoneal cells + RI</td>
<td>39,902 ± 3,759</td>
<td>120.9</td>
</tr>
<tr>
<td>i.v. FITC-CP peritoneal cells</td>
<td>734 ± 96c</td>
<td>2.2</td>
</tr>
<tr>
<td>+ RI (10:1)</td>
<td>860 ± 76c</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* Cultures of normal or FITC-CP-stimulated peritoneal cells alone incorporated insignificant amounts of [3H]thymidine.

Failure of an Inactive Strain of C. parvum to Persist in Vivo. Throughout these studies we were impressed with the relative resistance of C. parvum to degradation in vivo, some intact FITC-CP still being detectable more than a
month after injection. To determine whether the property may be related to the antitumor activity of *C. parvum*, the *in vivo* persistence of either 

\[ ^{125}I \]- or FITC-labeled inactive *C. parvum* (CN5888) was studied. This organism has no lymphoreticular stimulatory or antitumor effects (1) and did not acquire any after labeling. The results of i.v. injection of equivalent amounts of active and inactive 

\[ ^{125}I \]-labeled *C. parvum* into CBA mice are shown in Table 3. Considerably more radioactivity was lost from the liver and spleen during the 1st 24 hr after injection of inactive 

\[ ^{125}I \]-labeled *C. parvum* than with active 

\[ ^{125}I \]-labeled *C. parvum*. Studies using inactive FITC-CP again suggested a rapid breakdown of this organism *in vivo*: 24 hr after i.v. injection no intact bacteria could be resolved in spleen and liver sections, the only fluorescent material resembling debris (Fig. 1, c and d).

**DISCUSSION**

\[ ^{125}I \]- or FITC-labeled *C. parvum*, injected i.v. into normal mice, was found in high concentrations in the liver, spleen, and lung, and in lesser amounts in the lymph nodes and bone marrow cells. After s.c. administration, however, most of the bacteria were restricted to the site of injection, with high concentrations in the draining lymph nodes and none in the contralateral nodes. Amounts in the spleen, liver, and bone marrow were far less than after i.v. injection. Although *C. parvum* was rapidly lost from the lungs after i.v. injection, it was remarkably persistent elsewhere; this will be discussed later. The presence of *C. parvum* in the various tissues corresponds with reported biological modifications in them after injection of *C. parvum*, e.g., hepatomegaly, splenomegaly and lung weight increase (1), increased lymph node weights (8), augmented proliferative response of lymph nodes to mitogens (14) and antigens (25), and increased colony-forming capacity of bone marrow cells (30).

The restricted distribution of *C. parvum* after s.c. injection results in intense stimulation of the draining lymph nodes with only minimal splenomegaly and hepatomegaly compared with the i.v. route. This suggests that the degree of regional stimulation caused by *C. parvum* may be directly proportional to the amount of *C. parvum* reaching the tissue. The relative distributions of *C. parvum* after these routes of injection also parallel the *in vivo* antitumor activities induced. Whereas systemic *C. parvum* is capable of inhibiting the growth of tumors at various sites in the body (reviewed in Ref. 23), s.c. *C. parvum* is usually only effective when injected either directly into the tumor or so that it stimulates the tumor-draining nodes (13, 26). The same relationship holds for the generation of specific cell-mediated tumor immunity after local injections of *C. parvum* and irradiated tumor cells (5, 27). The immunity resulting from injecting irradiated tumor cells and *C. parvum* at separate sites having the same draining lymph node is as strong as when they are injected as a mixture, but is reduced by wide spatial separation of the injections (27).

The presence of a solid fibrosarcoma growing in the footpad increased the rate of clearance of i.v. *C. parvum* from the blood, and this elevated phagocytic activity of tumor-bearing mice has been noted previously (17). The amounts of *C. parvum* taken up by the spleen and tumor-draining node were also increased, but liver and lung uptake were not modified. That the increased splenic uptake was proportional to the tumor-induced splenomegaly suggests an increased nonspecific trapping of *C. parvum* rather than any selective uptake attributable to the tumor. This also applies to the increased uptake by the tumor-draining node where the unit uptake was actually decreased. However, the increased total amount of *C. parvum* in the tumor-draining node may contribute to the efficacy of i.v. *C. parvum* therapy, since stimulation of a tumor-draining node by *C. parvum* is known to result in specific cell-mediated anti-tumor immunity, whereas distant injections are ineffective (27).

The distribution of *C. parvum* after intralional injection resembled that after s.c. injection into normal animals. However, the systemic spread of *C. parvum* was more rapid after intralional than after s.c. injection, peak radioactivity being detected in the blood liver and spleen after 1 and 24 hr, respectively. This difference most probably reflects a better vascularization of the tumor bed compared with normal tissue.

The persistence of *C. parvum* at the tumor site after intralional injection may be a factor contributing to the efficacy of this form of *C. parvum* therapy against solid tumors (13, 19, 26). Recent studies by Tuttle and North (29) indicate that a major component of the mechanism of antitumor activity of intralional *C. parvum* is dependent on the generation of systemic immunity to *C. parvum* antigens which allows an immune-mediated inflammatory response to be focused at the site of *C. parvum* antigens (i.e., the tumor site). This local inflammation was shown to exert a powerful antitumor effect. The prolonged presence of intact *C. parvum* (*C. parvum* antigen) after intralional injection would be expected to prolong such local reactivity within the tumor site.

**Table 3**

| Table 3 | The relative in vivo persistence of active and inactive strains of 

\[ ^{125}I \]-labeled *C. parvum* during 24 hr after i.v. injection |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>Active CP*</td>
</tr>
<tr>
<td>1 hr</td>
<td>62.3 ± 3.8</td>
</tr>
<tr>
<td>24 hr</td>
<td>40.2 ± 3.2</td>
</tr>
<tr>
<td>% radioactivity lost during 24 hr</td>
<td>35.5</td>
</tr>
</tbody>
</table>

* CP, *C. parvum*.
# cpm/organ as a percentage of radioactivity injected.
The in vivo antitumor effects of systemic C. parvum are considered to be predominantly nonspecific and mediated by activated macrophages (reviewed in Ref. 23). Recent studies on the mechanism of macrophage activation by C. parvum implied that 2 activation pathways may operate in vivo: direct activation after the ingestion of C. parvum by the macrophage, and an indirect activation resulting from the interaction of C. parvum-sensitized lymphocytes with C. parvum antigen (6, 9). The present finding that peritoneal macrophages are fully activated 4 days after i.v. C. parvum treatment in the total absence of C. parvum-containing cells in the peritoneum supports the viewpoint that uptake of C. parvum by a macrophage is not a prerequisite for its activation. The data do not exclude the possibility that peritoneal macrophages expressing in vitro antitumor activity may be the progeny of cells that had taken up C. parvum elsewhere and digested it before migrating into the peritoneal cavity. It is likely that the source of such cells may be the bone marrow, since we have shown here that C. parvum is taken up by bone marrow cells, and others have shown that C. parvum stimulates macrophage production in the bone marrow (11, 30).

Mouse solid tumors have been shown to contain varying numbers of macrophages (10), and the amount of C. parvum that localizes within a solid tumor after i.v. injection is most probably a function of the number of these tumor macrophages and the degree of vascularization of the tumor. Overall, the strongest antitumor effects against solid tumors have been achieved by injecting the C. parvum directly in the tumor lesion (13, 19, 23, 26), and, on finding that some i.v. injected C. parvum localized within a solid tumor, it was considered that a component of the effects of i.v. C. parvum may be analogous to an intrallesional injection. However, the amounts of i.v.-injected C. parvum that localized within different solid tumors did not correlate with their sensitivities to i.v. C. parvum therapy. It would then seem that the amount of intratumor C. parvum found after i.v. injection does not make a critical or significant contribution to the overall antitumor effects of i.v. C. parvum therapy. A relevant consideration is our finding that C. parvum-activated macrophages, which have been considered to be the predominant effector cell after systemic C. parvum (reviewed in Ref. 23), need not contain C. parvum.

The in vivo degradation of killed C. parvum after both i.v. and s.c. injection is a gradual process, and intact FITC-CP were found consistently 2 weeks and more after injection. Interestingly, this resistance to digestion mirrors the chronic stimulation of hyperplasia in the spleen and liver which is detectable 2 to 3 days after i.v. C. parvum treatment and gradually increases, peaking around 14 to 16 days (1). Persistence of C. parvum has also been noted by Tuttle and North (29), who reported that 6 days after local C. parvum injection phagocytic cells at the injection site were still replete with intact bacteria. The data presented here show that "inactive" C. parvum (CN5888) was digested far more rapidly than "active" C. parvum. Rapid degradation of other inactive bacteria have been reported. Killed 131I-labeled Salmonella enteritidis were completely eliminated from the mouse liver within 24 hr of i.v. injection (4). Roberts (21) found that most of the heat-killed S. typhi phagocytosed by rabbit macrophages were completely digested within 6 hr, and Topley (28) showed that within 24 hr of being taken up by rabbit splenic histiocytes, S. paratyphi N was rendered nonantigenic. In contrast to these is a study with a yeast cell wall extract, zymosan, which is also a reticuloendothelial stimulant (2) with antitumor activity (17). **[C]Zymosan injected i.v. into mice and taken up by the liver persisted in relatively high amounts for a period of several weeks (22). Considering these points, it is interesting to speculate that the relative resistance of naturally occurring reticuloendothelial stimulants to intracellular degradation may contribute to their antitumor activity, and this is now under experimental consideration.

ACKNOWLEDGMENTS

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C. parvum Distribution in Vivo


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