

Survival of *Listeria monocytogenes* in Experimentally Infected Mice¹

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Physiological saline was found to be very detrimental to the viability of *Listeria monocytogenes*. The LD₅₀ value was substantially reduced when peptone was used as the suspending fluid rather than saline. Normal splenic tissue was not inhibitory to the survival of *Listeria*. In experimentally infected mice, *L. monocytogenes* survived for 8 days in the peritoneal cavity and for at least 11 days in the spleen.

Since mice are susceptible to infection with *Listeria monocytogenes* (4), they have been used in the study of the host-parasite relationship of this microorganism. Mackaness (7) injected mice intravenously with 0.1, 1, or 10 LD₅₀ dosages of *Listeria* and quantitated the number of organisms remaining viable in the liver and spleen for 10 consecutive days postinjection. However, his experimental data were limited because of the death of the infected animals at the higher dosages. Coppel and Youmans (2) quantitated the recovery of *Listeria* from the spleen for 7 consecutive days after an intravenous injection of 4.3, 43, or 430 MLD doses of *L. monocytogenes*. As a result of administering lethal doses of *Listeria*, many mice died before completion of the experiment. A better estimate of the ability of *Listeria* to survive in vivo should be obtained by exposing mice to bacterial concentrations well below the lethal level. The present investigation exposed mice to a sublethal dosage of *L. monocytogenes* and quantitated the in vivo survival of *Listeria* over a 32-day period postinjection.

MATERIALS AND METHODS

Culture and maintenance. *L. monocytogenes* strain 10403, serotype 1, obtained from the late M. L. Gray, Montana State University, was used in these studies. The culture was maintained on Brain Heart Infusion (BHI, Difco) semisolid agar at 4 C and examined periodically for purity during the course of this investigation.

Animal infectivity. Two inbred white mouse strains, Washington State University (WSU) and Swiss-Webster (SW), were maintained for experimentation.

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The two colonies were housed and bred in physically separated quarters.

L. monocytogenes was grown in 10 ml of BHI broth at 37 C for 16 hr. The cells were harvested and washed twice in physiological saline or 0.5% peptone (Difco) and resuspended in the same diluent. Mice were injected intraperitoneally (ip) with 0.2 ml of the desired bacterial concentration by using a tuberculin syringe (B-D plastipak) and a 25-gauge hypodermic needle (B-D Yale, disposable). One-month-old mice, weighing 16 to 20 g, were used throughout the investigation. All experimental mice, infected and controls, were held in animal quarters physically separated from the stock mouse colonies. Animals were provided with feed and water ad libitum.

LD₅₀ determination. The WSU and SW mice were injected ip with 10⁸ to 10⁹ viable *Listeria* cells. Groups of 10 to 20 mice, of mixed sexes, were injected with each bacterial concentration suspended in physiological saline or 0.5% peptone (Difco). The mice were held for 6 weeks before determining the LD₅₀ from the mortality data by the Reed-Muench method (9). Necropsy was performed on all mice which died.

Isolation and identification. Tissues were sampled for the presence of *Listeria* by making multiple incisions in the organs followed by smearing these specimens over an agar surface. Agar smears of the liver, spleen, kidney, and small intestine of mice which died from listeriosis or were sacrificed were made on McBride agar (Difco) and incubated at 37 C for 24 to 72 hr. Preliminary identification of the bacterial colonies was based on the Henry reflected light technique (6), in which *Listeria* colonies appear blue to blue-green. Confirmation of identification was made by the Gram stain, motility at room temperature, and fermentation reactions in glucose, sucrose, salicin, lactose, D-xylose, inulin, maltose, and trehalose.

Survival in splenic homogenate. The spleens from uninoculated mice were aseptically excised, homogenized with a tuberculin syringe, and placed into sterile Kahn tubes. A 16-hr BHI broth culture of *L. monocytogenes* incubated at 37 C was washed twice in peptone-Hanks balanced salt solution (HBSS) which was prepared by adding 10 ml of 10X HBSS (3) to

90 ml of 0.5% peptone (Difco). The resulting bacterial suspension was serially diluted in peptone-HBSS. A 0.1-ml volume of the final dilution was pipetted into sterile Kahn tubes with and without splenic homogenate. All tubes were incubated at 37 C and sampled daily on McBride agar.

Quantitative recovery. Mice which were injected ip with 1.6×10^8 viable *Listeria* cells were sacrificed over a 32-day period. The number of *Listeria* in the peritoneal cavity and spleen was determined for each of the 8 to 14 mice sacrificed per sample period. The peritoneal cavity was flushed with 1 ml of 0.067 M phosphate-buffered saline (PBS) at pH 7.2, followed by plating in duplicate 0.1 ml on McBride agar. Colony count determinations were made after 72 hr of incubation at 37 C. The spleen was aseptically excised, washed in PBS, and homogenized in 1 ml of PBS by using a tuberculin syringe. A 0.1-ml amount of the splenic homogenate was plated in duplicate on McBride agar followed by incubation at 37 C for 72 hr before determining the number of colonies.

RESULTS

LD₅₀ determination. Male and female mice from the two mouse colonies were injected ip with various concentrations of viable *Listeria* cells. The number of mice surviving the infection was determined 6 weeks postinjection. The LD₅₀ for mice injected with a saline-suspended *Listeria* preparation ranged from 3.4×10^6 to 3.2×10^7 viable cells. However, when *Listeria* was suspended in 0.5% peptone, the LD₅₀ ranged from 1.5×10^5 to 2.1×10^5 viable cells. The LD₅₀ values obtained with the saline prepa-

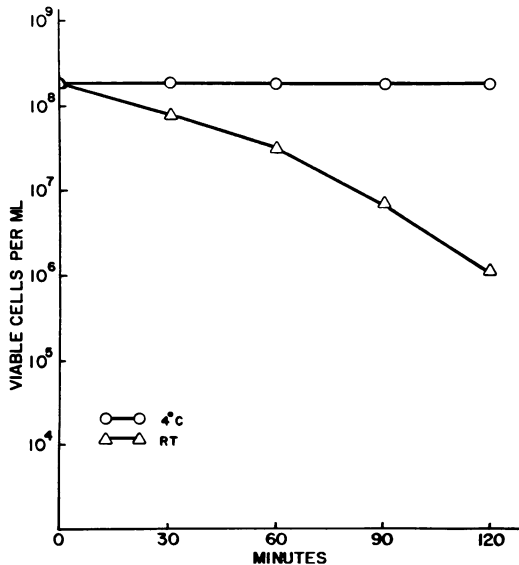


FIG. 1. Survival of *Listeria monocytogenes* in physiological saline at room temperature and at 4 C. RT, room temperature.

ration were invalid because of the detrimental effects of saline on the bacterial suspension. Physiological saline was found to reduce markedly the viability of *Listeria* when the preparation was held at room temperature for several hours (Fig. 1). This bacteriocidal effect was not observed if the saline suspension of *Listeria* was held at 4 C. With 0.5% peptone as the suspending fluid for *Listeria*, the titer before and after animal injection was constant. The average LD₅₀ for both sexes of the mouse colonies was 1.7×10^5 viable cells with peptone as the diluent. The length of the period postinjection that was found necessary to accurately determine the LD₅₀ was at least 5 weeks. The final mouse succumbed to the infection on day 33 postinjection.

Listeria viability in splenic homogenate. The quantitative recovery of *Listeria* from tubes with and without splenic homogenate inoculated with 44 *Listeria* cells was determined over a 4-day period (Fig. 2). The *Listeria* population increased 10^7 -fold in the presence of the splenic homogenate during the first 48 hr, whereas the control increased 10^5 -fold during the same period. The subsequent decline in the bacterial concentration in both the splenic homogenate and control tubes was attributed to desiccation.

Quantitative recovery in vivo. The survival of *L. monocytogenes* in the peritoneal cavity and

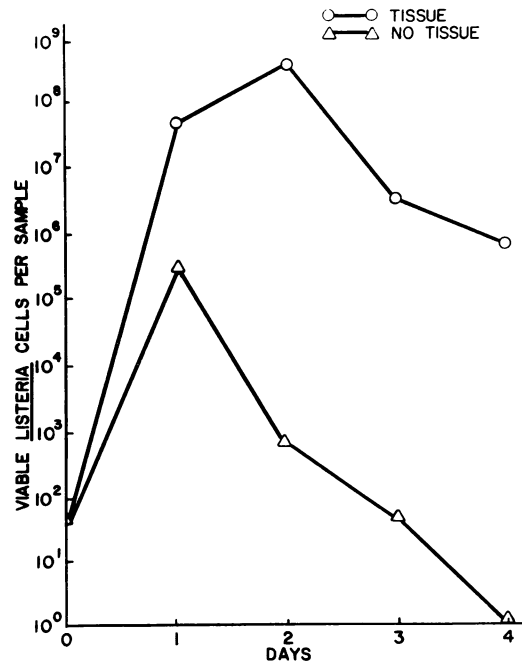


FIG. 2. Survival of *Listeria monocytogenes* in 0.1 ml of peptone-Hanks balanced salt solution with and without splenic homogenate at 37 C.

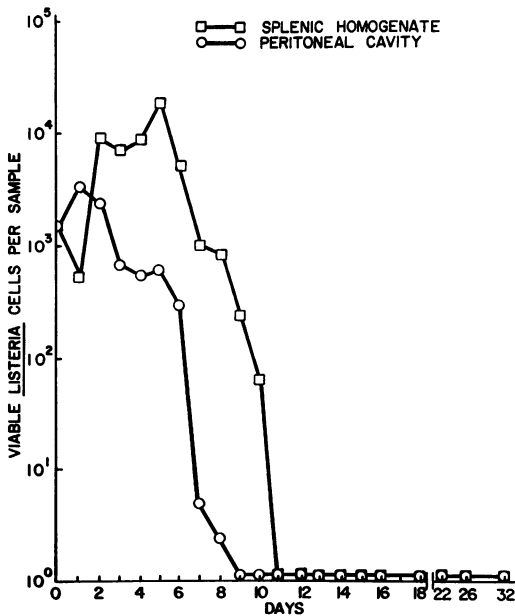


FIG. 3. Quantitative recovery of *Listeria monocytogenes* from the peritoneal cavity and spleen of mice injected intraperitoneally with 1.6×10^8 *Listeria* cells.

spleen of experimentally infected mice was determined over a 32-day period (Fig. 3). The population of *Listeria* cells in the peritoneal cavity was approximately double the inoculum titer for the first 2 days postinjection, followed by a 10-fold decrease in the *Listeria* cell concentration over the next 4 days. *Listeria* was not recovered from the peritoneal cavity after 8 days postinjection. The number of *Listeria* cells recovered from the spleen on day 1 postinjection was approximately one-third the original inoculum titer. During the next 4 days, the *Listeria* population in the spleen increased 40-fold followed by a gradual decline over the next 6 days. Only 30 to 120 *Listeria* cells were recovered from 1 of 14 spleens sampled on days 13, 14, and 16. *Listeria* was not isolated after day 16 postinjection.

DISCUSSION

Physiological saline was originally proposed as the diluent for the *Listeria* cell inoculum to be injected into mice. However, saline was found to reduce markedly the viability of *Listeria* when the preparation was held at room temperature for several hours. Gray and Killinger (4) pointed out that the use of saline in the recovery of *L. monocytogenes* from infected materials could be harmful especially if the concentration of the organisms was very low. The present study indi-

cates that saline is also a poor diluent for the preparation of a *Listeria* inoculum, since it could cause extreme variation in the number of viable organisms actually injected during a prolonged animal inoculation protocol. The use of saline as a suspending fluid for *Listeria* could account, in part, for the wide variation in the LD₅₀ reported for mice. Gray and Killinger (4), in reviewing the results of other investigators, found that the LD₅₀ for mice injected intraperitoneally ranged from 10³ to 10⁶ viable *Listeria* cells. Of course, this variation is also attributed to the difference in virulence of the culture and to the mouse strains employed. In the present investigation, the number of viable *Listeria* cells responsible for the LD₅₀ was substantially reduced when peptone was used as the diluent rather than saline.

Before attempting to isolate *Listeria* from experimentally infected mice, assurance had to be obtained that the spleen from uninoculated mice was not inhibitory to the survival of *L. monocytogenes*. Gray et al. (5) had originally postulated the presence of an inhibitory factor in the bovine brain which prevented the successful isolation of *Listeria*. However, they were unable to substantiate this hypothesis. Pittman and Cherry (8) postulated that a bacteriostatic agent in the brain tissue of feral animals prevented the successful recovery of *Listeria*. The present investigation showed that splenic tissue from normal mice actually promoted the growth of *Listeria*.

The administration of 0.01 LD₅₀ dose in mice resulted in the survival of *L. monocytogenes* for 8 days in the peritoneal cavity and at least 11 consecutive days in the spleen with sporadic recovery over the next 5 days. None of the experimentally infected mice died as a result of the injected dosage. The results of this investigation paralleled the observations of Mackness (7) and Coppel and Youmans (2) concerning the rise and fall of the *Listeria* population in the spleen during the first 7 to 10 days postinjection. Even though these investigators exposed mice to lethal dosages, the same general survival pattern of *Listeria* was observed. Apparently, the susceptibility of mice to listeriosis is due to the failure of the natural defenses of the host to prevent infection with *Listeria*. The longevity of *Listeria* in the peritoneal cavity would certainly support this conclusion. The leveling off and subsequent decline of the *Listeria* population in the spleen are associated with the development of the immune response. Coppel and Youmans (1) observed that the peak of immunity to *Listeria* infection in mice was obtained 7 days postinjection.

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