APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1986, p. 1398–1402
0099-2240/86/121398-05$02.00/0

Vol. 52, No. 6

Thermal Resistance of Intracellular *Listeria monocytogenes* Cells Suspended in Raw Bovine Milk

V. KELLY BUNNING,* RONALD G. CRAWFORD, JOE G. BRADSHAW, JAMES T. PEELER, JOHN T. TIERNEY, AND ROBERT M. TWEDT

Division of Microbiology, Food and Drug Administration, Cincinnati, Ohio 45226

Received 7 July 1986/Accepted 12 September 1986

The thermal resistance of *Listeria monocytogenes* associated with a milk-borne outbreak of listeriosis was determined in parallel experiments by using freely suspended bacteria and bacteria internalized by phagocytes. The latter inoculum was generated by an in vitro phagocytosis reaction with immune-antigen-elicited murine peritoneal phagocytes. The heat suspension medium was raw whole bovine milk. Both suspensions were heated at temperatures ranging from 52.2 to 71.7°C for various periods of time. Mean *D* values for each temperature and condition of heated suspension revealed no significant differences. The extrapolated $D_{71.7°C}$ (161°F) value for bacteria internalized by phagocytes was 1.9 s. Combined tube and slug-flow heat exchanger results yielded an estimated $D_{71.7°C}$ value of 1.6 s for freely suspended bacteria. The intracellular position did not protect *L. monocytogenes* from thermal inactivation.

Between 30 June and 30 August 1983, 49 people in Massachusetts contracted listeriosis. Forty-two cases occurred in immunosuppressed adults and seven occurred in neonates; mortality was 29%. Most of the *Listeria monocytogenes* isolates from these patients were serotyped as 4b. Epidemiological studies (6) showed that the disease was strongly associated with drinking a certain brand of pasteurized whole milk or milk containing 2% fat. Listeriosis was diagnosed in dairy cows at farms where the implicated milk was obtained. No evidence of faulty pasteurization was detected at the dairy plants where the milk was processed. These findings raised questions concerning the efficacy of pasteurization for eliminating *L. monocytogenes* in contaminated bovine milk. Another major outbreak of listeriosis has since occurred in a dairy product (cheese) in California, again raising similar concerns (M. J. Linan, L. Mascola, D. L. Xiao, V. Goulet, C. Salminen, S. Fannin, and C. V. Broome, Abstr. 9th Int. Symp. Probl. Listeriosis, abstr. no. 25-C3, 1985).

Thermal resistance studies of *L. monocytogenes* are few, but unusual heat resistance of this organism in whole milk (1, 4, 14; J. F. Fernandez, L. Dominguez, J. A. Vazquez, M. P. Echalecu, J. A. Garcia, and G. Suarez, Abstr. 9th Int. Symp. Probl. Listeriosis, abstr. no. 64-C8, 1985; I. Kovinicic, I. Vujicic, B. Stajner, B. Galic, and M. Vulic, Abstr. 9th Int. Symp. Probl. Listeriosis, abstr. no. 71-P4, 1985) and in meat (11) has been reported. *L. monocytogenes* suspended in skim milk at an inoculum of 10^5 bacteria per ml was shown to survive thermal processing during the production of cottage cheese (15) and nonfat dry milk (5). Heat studies at this inoculum level with strain Scott A, an isolate from the Massachusetts outbreak, indicated that the organism freely suspended in milk would not survive the pasteurization process (2).

The pathogenesis of *L. monocytogenes*, particularly the facultative intracellular nature of this organism, has caused epidemiologists to speculate that the intracellular position may protect *L. monocytogenes* from thermal inactivation during pasteurization (6). Somatic cell counts from mastitic cows are quite high compared with those of normal cows, and the phagocytic cells present (neutrophils and macrophages) could concentrate large numbers of organisms, obscuring bacterial CFU from milk samples (6, 10). This study was undertaken to determine if the intracellular position could protect *L. monocytogenes* from thermal inactivation.

(A preliminary report of this work has been presented [V. K. Bunning, R. G. Crawford, J. G. Bradshaw, J. T. Peeler, and R. M. Twedt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, P36, p. 281].)

MATERIALS AND METHODS

Bacterial culture and culture conditions. The *L. monocytogenes* culture associated with the Massachusetts outbreak was obtained from D. W. Fleming, Centers for Disease Control, Atlanta, Ga. This strain, Scott A, was isolated from blood culture and serotyped as 4b. Previous thermal resistance studies (2) established strain Scott A to be somewhat more resistant than other strains associated with the outbreak. This strain was selected for all subsequent intracellular heat studies.

Stock cultures were grown in Trypticase soy–0.6% yeast extract broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 24 h and maintained at 4°C with monthly transfers. Confirmation of pathogenicity was determined by lethality to mice.

Phagocyte elicitation. Nonimmune, random sex, 18- to 20-g outbred Swiss white mice (20 mice per experiment) were injected intraperitoneally with 10^5 CFU of an 18-h culture of *L. monocytogenes* Scott A grown at 37°C. The mice were kept for 6 days and given food and water ad libitum. At 24 h before phagocyte harvest, the mice were each given an intraperitoneal injection of 10^7 heat-killed (80°C for 10 min) *L. monocytogenes* Scott A cells to elicit peritoneal phagocytes.

Phagocyte harvest. The mice were killed by cervical dislocation, and the phagocytes were harvested by using a peritoneal lavage of 5 ml of phosphate-buffered saline–2 mg of bovine serum albumin per ml–0.05% disodium EDTA. The peritoneal phagocytes were washed twice in phosphate-buffered saline–2 mg of bovine serum albumin per ml–0.05% disodium EDTA at 900 × *g* for 5 min at 4°C and counted by

* Corresponding author.

trypan blue exclusion. A Wright-stained preparation showed that the cell population consisted of about 70% macrophages, 25% neutrophils, and 5% lymphocytes. The final pellet was suspended in 30 ml of minimal essential medium, giving approximately 10^7 phagocytes per ml.

Preparation of bacteria. *L. monocytogenes* Scott A was grown in 10 ml of Trypticase soy–0.6% yeast extract broth for 18 h at 37°C to an optical density at 625 nm of 0.8. At this absorbance, the bacterial count approached 10^9 organisms per ml. The suspension was centrifuged at 7,700 × *g* for 15 min at 4°C. The supernatant was decanted, and the pellet was suspended in 2 ml of normal mouse serum. The bacteria were opsonized at 37°C for 30 min. After opsonization, the final volume was adjusted to 50 ml by adding 48 ml of minimal essential medium (10^8 bacteria per ml).

Phagocytosis reaction. Twelve 50-ml Erlenmeyer flasks were labeled and grouped as follows. Group 1 contained flasks A, B, and C, each of which received 5 ml of phagocyte suspension; group 2 contained flasks D, E, and F, each of which received 5 ml of sterile minimal essential medium; group 3 contained flasks G_1, H_1, and I_1, each of which received 5 ml of phagocyte suspension; and group 4 contained flasks G_2, H_2, and I_2, each of which remained empty until initiation of the reaction. All flasks were equilibrated to 37°C in an incubator shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 70 rpm. Phagocytosis was initiated by inoculating each flask in each group except group 3 with 5 ml of the *L. monocytogenes* Scott A preparation. Reaction time was 60 min and was terminated by placing the flasks on ice for 15 min.

Phagocyte recovery. After the 15-min incubation on ice, each flask containing phagocytes was gently scraped with a rubber policeman to suspend any adhering phagocytes. The contents of each flask from groups 1 and 2 were then pipetted into 15-ml conical centrifuge tubes (Corning Glass Works, Corning, N.Y.) and placed on ice. The contents of the flasks from groups 3 and 4 were pipetted together, respectively (G_1 with G_2, etc.), into Corning tubes, giving a 10-ml volume, equivalent to other group flasks, and the samples are henceforth designated G, H, and I.

In summary, the final three groups of tubes generated were as follows. Group 1 included tubes A, B, and C, each containing phagocytes and free and intracellular *L. monocytogenes*; group 2 included tubes D, E, and F, each containing free *L. monocytogenes*; and group 3 included tubes G, H, and I, each containing phagocytes and free *L. monocytogenes*. Each tube was carried through three low-speed centrifugations and washings (International Equipment Co. CRU5000 centrifuge; 0°C at 900 × *g*, 300 × *g*, and 300 × *g*) to reduce the number of extracellular *L. monocytogenes* relative to the number of phagocytes.

Determination of viable intracellular bacteria. After the three centrifugations, all pellets were brought up in 1 ml of phosphate-buffered saline–2 mg of bovine serum albumin per ml–0.05% disodium EDTA. From tubes containing phagocytes (A to C and G to I), 10 μl was removed to 990 μl of 0.05% trypan blue in duplicate to determine final phagocyte counts (phagocyte recovery). A 2-ml volume of sterile raw milk was added to all tubes, and the contents were mixed; 100 μl was removed from each tube, spotted onto a Falcon tissue culture dish (35 by 10 mm; Becton Dickinson Labware, Oxnard, Calif.), and incubated at 37°C for 15 min to allow phagocytes to adhere. These preparations were stained with Wright stain to evaluate intracellularity.

Additional 100-μl portions were removed from these tubes (A to C, D to F, and G to I) and placed in 9.9 ml of sterile raw milk or sterile distilled water. The group diluted in sterile milk was plated at appropriate dilutions with Trypticase soy agar (BBL) supplemented with 0.6% yeast extract. The portions placed in water were allowed to sit at 4°C for 30 min; glass beads (0.45 to 0.52 mm) were added, and then each sample was vortexed vigorously before plating. All plates were incubated at 37°C for 48 h and then counted. This dilution-plating procedure was performed on the assumption that phagocytes harboring multiple *L. monocytogenes* cells would produce only 1 CFU when placed in an isotonic diluent (milk) but would be osmotically and mechanically lysed by dilution in water and vortexing with glass beads, respectively; the latter rise in CFU would indicate the number of viable intracellular bacteria. These data were statistically analyzed to confirm this assumption. Mechanical and osmotic lysis of phagocytes was confirmed by microscopic examination.

TABLE 1. Summary of means and variances for bacterial plate counts used in determining the number of viable intracellular bacteria[a]

Variable (X_i)	Triplicate samples (diluent)	Mean $\log_{10}$ CFU/ml	Replicate variance
1	A, B, C (milk)	5.1341	0.0029
2	D, E, F (milk)	4.7381	0.0043
3	G, H, I (milk)	4.7477	0.0010
4	A, B, C (water)	5.7746	0.0012
5	D, E, F (water)	4.7602	0.0084
6	G, H, I (water)	5.2237	0.0050
7	A, B, C (milk) + A, B, C (water)	5.8703	0.0010
8	G, H, I (milk) + G, H, I (water)	5.3546	0.0031
9	A, B, C (water) − G, H, I (water)	0.5509	0.0064

[a] For all variables, $n = 24$ and df = 16.

Preparation of *L. monocytogenes* for thermal resistance studies. Two suspensions were prepared for heating in sealed glass tubes: (i) *L. monocytogenes* within phagocytes (phagocytic reaction tubes A to C were pooled, and the final volume was adjusted to 40 ml by using low-count raw milk from individual cows) and (ii) freely suspended *L. monocytogenes* (an 18-h culture of *L. monocytogenes* grown at 37°C in Trypticase soy–0.6% yeast extract broth was diluted in low-count, raw milk [from individual cows] to approximately 10^5 bacteria per ml). Here, the final 40-ml volume contained 3 ml of phosphate-buffered saline, 2 mg of bovine serum albumin per ml, 0.05% disodium EDTA, and 6 ml of sterile raw milk, duplicating the medium conditions of the other suspension.

Thermal resistance studies and statistical analyses were performed by the procedure of Bradshaw et al. (2). After heating and before plating, all suspensions were vortexed for 15 s with glass beads to totally disrupt phagocytes.

Freely suspended bacteria were tested in a slug-flow heat exchanger (16) to compare with the sealed-tube data and obtain an estimated $D_{71.7°C}$ value.

RESULTS

Determination of viable intracellular bacteria. Statistical analysis was performed on CFU generated from dilution in milk and water during preparation for thermal resistance. Triplicate counts were recorded at the appropriate dilution and transformed to $X_i = \log_{10}$ counts (Y_i) to ensure normality (12). Table 1 identifies each variable used in the analysis and presents the mean and replicate error. Results of four comparisons made from these data are given below (12).

TABLE 2. Thermal resistance of intracellular *L. monocytogenes*

Temp (°C)	No. of freely suspended bacteria/ml	No. of intracellular bacteria/ml	No. of infected phagocytes/ml[a]	No. of bacteria/ infected cell	% Infected cells	*D* value (s) for:	
						Free bacteria	Intracellular bacteria
52.2	2.2×10^5	2.6×10^5	8.7×10^4	3.0	9.6	1,694.0	1,819.3
	1.5×10^5	3.0×10^5	7.4×10^4	4.1	23.1	2,564.1	3,380.3
	1.2×10^5	4.9×10^5	7.2×10^4	6.8	42.3	2,611.0	4,319.7
57.8	2.0×10^5	2.7×10^5	9.0×10^4	2.7		321.1	468.8
	6.9×10^3	2.7×10^5	6.5×10^4	4.2	8.3	520.9	410.4
	1.2×10^5	2.0×10^5	5.6×10^4	3.6	9.6	402.6	527.0
	1.7×10^4	1.2×10^4	2.6×10^3	4.6	0.5	538.3	556.4
63.3	2.1×10^5	3.4×10^5	8.6×10^4	4.0		44.2	43.1
	1.9×10^5	5.2×10^5	9.6×10^4	5.4	11.7	24.9	26.5
	2.0×10^5	1.9×10^5	6.4×10^4	3.0	18.3	33.7	32.6
68.9	2.2×10^5	8.6×10^5	8.4×10^4	10.2	8.8	7.3	7.3
	6.0×10^5	9.0×10^5	4.7×10^4	19.1	8.5	9.7	11.7
	1.8×10^5		1.1×10^5		2.1	6.1	2.9

[a] The range for the number of phagocytes recovered in each experiment was 1.7×10^5 to 1.0×10^6 cells per ml of milk, with an average of 5.7×10^5 cells per ml.

Counts from phagocytic reactions were significantly higher in water (X_4) than in milk (X_1) at the $\alpha = 0.05$ level. Comparison of freely suspended bacteria diluted in milk (X_2) and in water (X_5) showed that they did not differ significantly. Counts of freely suspended extracellular bacteria in the presence of phagocytes were significantly higher in water (X_6) than in milk (X_3). All tests represent the data observed from 8 of 10 thermal resistance experiments. The null hypothesis that $\log_{10}(Y_1 + Y_4) - \log_{10}(Y_3 + Y_6) = 0$ was rejected at the $\alpha = 0.05$ level. This rejection allowed the use of counts in water (X_4) as an indicator of the number of viable intracellular bacteria once the number of extracellular bacteria (X_6) was subtracted from this value. Significant levels of viable intracellular bacteria (10^5) were achieved in all thermal resistance experiments, i.e., the null hypothesis was rejected in each experiment in the positive direction (Table 2). Our method for determining intracellular viability was tested and was consistent with the following definitions. The number of infected phagocytes equaled $Y_1 - Y_3$ for each experiment. The average number of bacteria inside each infected cell equaled $(Y_4 - Y_6)/(Y_1 - Y_3)$ for each experiment. The percentage of infected cells was calculated from $(Y_1 - Y_3)/P$ for each experiment, where P is the number of phagocytes recovered from a phagocytosis reaction and subsequent differential centrifugation. There was essentially no variation in the number of phagocytes recovered from phagocytic reactions and extracellular bacterium suspensions for each separate experiment, thereby validating this calculation.

Thermal resistance of intracellular *L. monocytogenes*. The thermal resistance properties of *L. monocytogenes* Scott A suspended in raw whole milk and sealed in glass tubes was determined by heating bacteria and bacteria internalized by phagocytes at temperatures ranging from 52.2 to 68.8°C and calculating mean *D* values (Tables 2 and 3). The determination of initial counts for each condition is shown in Table 2.

TABLE 3. Mean *D* value estimates for intracellular[a] and freely suspended[b] *L. monocytogenes* Scott A in raw milk

Temp (°C)	Sample	*D* value[c] (no. of determinations)	Coefficient of variation (%)	*D* value[d] (no. of determinations)	Coefficient of variation (%)
52.2	Intracellular	3,171.8 (3)	39.8	ND[e]	
52.2	Free	2,289.3 (3)	22.5	ND	
57.8	Intracellular	490.1 (4)	13.2	ND	
57.8	Free	445.0 (4)	23.1	ND	
63.3	Intracellular	33.3 (3)	25.6	ND	
63.3	Free	33.4 (3)	29.6	29.7 (2)	0.7
66.1	Intracellular	ND		ND	
66.1	Free	ND		15.1 (2)	28.1
68.9	Intracellular	7.0 (3)	63.3	ND	
68.9	Free	7.2 (3)	27.9	5.3 (2)	5.3
71.7	Intracellular	ND		ND	
71.7	Free	ND		1.3 (2)	5.7

[a] z_D = 6.0°C.
[b] z_D = 6.1°C.
[c] Determined from small-volume samples (1.5 ml) in sealed borosilate glass tubes as described by Bradshaw et al. (2).
[d] Determined from large-volume samples (4 liters) in a slug-flow heat exchanger by the method of Stroup et al. (16).
[e] ND, Not determined.

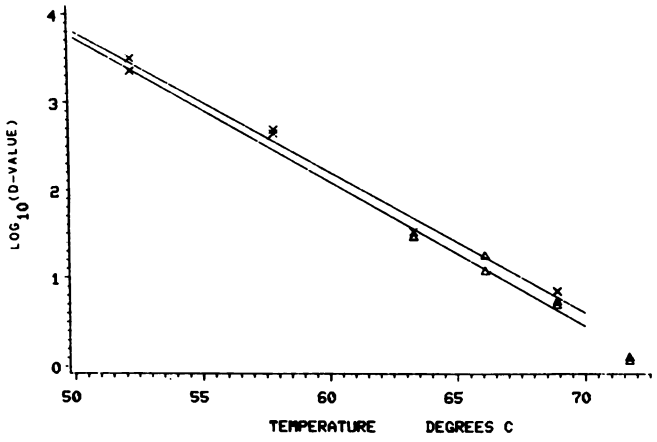

FIG. 1. Comparison of regression lines of D values obtained by heating in sealed glass tubes (×) and in a slug-flow heat exchanger (△).

The extrapolated $D_{71.7°C}$ value (161°F) for the internalized bacterium samples was 1.9 s with a z_D value of 6.0°C.

The comparison of results from testing freely suspended bacteria either in sealed tubes or in a slug-flow heat exchanger is given in Table 3. The regression lines generated by the separate methods did not differ significantly at the $\alpha = 0.05$ level (Fig. 1). The estimated $D_{71.7°C}$ value equaled 1.6 s and was obtained from the pooled regression line. The measured $D_{71.7°C}$ value was 1.3 s as shown in Table 3 and Fig. 1. The z_D value was 6.1°C.

DISCUSSION

Several methods for eliciting murine peritoneal phagocytes have been reported (3, 8, 9, 13, 17). Results from in vitro phagocytosis reactions of *L. monocytogenes* vary with the strain of mouse, the immunological status of the mouse (immune versus nonimmune), the elicitation procedure (antigen specific versus nonspecific), the ratio of bacteria to phagocytes in the reaction mixture, the time of reaction, the method of detecting viable intracellular bacteria (differential staining, chemiluminescence, osmotic or cold-shock lysis, and radioactive labeling), and the status of the phagocyte suspension (pooled phagocytes versus individual-mouse phagocytes). We used an immune-antigen-elicited protocol to generate a maximum number of peritoneal phagocytes that would, in turn, take up the most bacteria in the shortest incubation time so that the resulting suspension could be standardized and used in a raw-milk thermal resistance study. The composition of immune-antigen-elicited murine peritoneal cells compared with normal bovine milk cells is quite different, with the former having 70% macrophages and the latter having 70% neutrophils. The composition of cells in bovine milk from an *L. monocytogenes*-mastitic animal, however, has not been thoroughly examined. Significant levels of viable intracellular *L. monocytogenes* cells (10^5) were obtained after an incubation of 1 h, as determined by osmotic and mechanical lysis of phagocytes (Tables 1 and 2). Differential centrifugation, although not optimal in strict phagocytosis-bacteriocidal assays (8, 9, 17), was simpler and necessary for our purpose of establishing a standard suspension of viable intracellular *L. monocytogenes* cells for heat studies.

L. monocytogenes is a facultative intracellular parasite that can be shed within the milk of cows (6, 10). Cows with mastitis caused by *L. monocytogenes* shed 10,000 to 20,000 bacteria per ml of milk, as reported by Kampelmacher (10), with no inflammation of the quarters and normal-appearing milk. An on-site trip report from the United States-Yugoslavia Joint Board Program on listeriosis states that a range of 18,000 to 400,000 (mean, 100,000) somatic cells per ml of milk were found in cows with *Listeria* mastitis (J. T. Tierney, personal communication). A survey of raw milk for the incidence of *L. monocytogenes* in bulk tank samples from California and Ohio indicated that this organism is present in milk at low concentrations (1.0 organism per ml; J. Lovett, personal communication). Fleming et al. (6) suggested, after completing an epidemiological study of the Massachusetts outbreak, that intracellular *L. monocytogenes* may be protected from thermal inactivation during pasteurization. We studied the thermal resistance of intracellular *L. monocytogenes* relative to bacteria in free suspension, both at a level approaching 10^5 organisms per ml in raw milk, and found no differences as shown by D value consistency (Tables 2 and 3). Studies at the lower temperature of 52.2°C suggested that the number of bacteria per infected phagocyte and the percent infected phagocytes could promote variability in survival, at least at this temperature, although more experiments were required to statistically prove this phenomenon. Variations in the assay, as previously mentioned, made attempts to elaborate on these data uncontrollable and cumbersome. Nonetheless, at the highest temperature of 68.8°C, thermal inactivation was complete despite high infectivity. It should be noted that these data were derived from direct plating and that enrichments of the milk following heat treatment were not performed, compromising the ability to completely detect heat-injured *L. monocytogenes*. Under these experimental conditions, then, intracellular position did not protect *L. monocytogenes* from thermal inactivation at the pasteurization temperature. These data support the conclusion that the present pasteurization guidelines of the Food and Drug Administration (7) are adequate to inactivate *L. monocytogenes* in whole milk.

ACKNOWLEDGMENTS

We thank J. Corwin and J. Hunt for technical assistance and J. Williams and D. Redmond for typing the manuscript.

LITERATURE CITED

1. **Bearns, R. E., and K. F. Girard.** 1958. The effect of pasteurization on *Listeria monocytogenes*. Can. J. Microbiol. **4**:55–61.
2. **Bradshaw, J. G., J. T. Peeler, J. J. Corwin, J. M. Hunt, J. T. Tierney, E. P. Larkin, and R. M. Twedt.** 1985. Thermal resistance of *Listeria monocytogenes* in milk. J. Food Prot. **48**: 743–745.
3. **Czuprynski, C. J., P. M. Henson, and P. A. Campbell.** 1984. Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. J. Leukocyte Biol. **35**:193–208.
4. **Donker-Voet, J.** 1962. My view on the epidemiology of *Listeria* infections, p. 133–139. *In* M. L. Gray (ed.), Second Symposium on Listeric Infection. Montana State College, Bozeman.
5. **Doyle, M. P., L. M. Meske, and E. H. Marth.** 1985. Survival of *Listeria monocytogenes* during the manufacture and storage of nonfat dry milk. J. Food Prot. **48**:740–742.
6. **Fleming, D. W., S. L. Cochi, K. L. McDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404–407.
7. **Food and Drug Administration.** 1983. Grade A pasteurized milk ordinance. 1978 Recommendations. Publication 229 (revised

1983). Public Health Service, Washington, D.C.
8. **Harrington-Fowler, L., P. M. Henson, and M. S. Wilder.** 1981. Fate of *Listeria monocytogenes* in resident and activated macrophages. Infect. Immun. **33**:11–16.
9. **Hart, P. H., L. K. Spencer, P. J. McDonald, and J. J. Finlay-Jones.** 1985. Evaluation of intracellular killing of bacteria by enriched populations of mouse peritoneal exudate neutrophils. Aust. J. Exp. Biol. Med. Sci. **63**:361–370.
10. **Kampelmacher, E. H.** 1962. Animal products as a source of listeric infection in man, p. 146–156. *In* M. L. Gray (ed.), Second Symposium on Listeric Infection. Montana State College, Bozeman.
11. **Karaioannoglou, P. G., and G. C. Xenos.** 1980. Survival of *Listeria monocytogenes* in meatballs. Hell. Vet. Med. **23**: 111–117.
12. **Ostle, B., and R. W. Mensing.** 1975. Statistics in research, 3rd ed. Iowa State University Press, Ames.
13. **Ottendorfer, D., D. Bitter-Suermann, and U. Hadding.** 1985. An in vitro system to study listericidal capacity of macrophages from separate mice: resident macrophages exhibit different activation patterns. Infect. Immun. **49**:685–691.
14. **Potel, J.** 1951. Die Morphologie, Kultur und Tierpathogenität des *Corynebacterium infantisepticum*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **156**: 490–493.
15. **Ryser, E. T., E. H. Marth, and M. P. Doyle.** 1985. Survival of *Listeria monocytogenes* during manufacture and storage of cottage cheese. J. Food Prot. **48**:746–750.
16. **Stroup, W. H., R. W. Dickerson, Jr., and R. B. Read, Jr.** 1969. Two-phase slug flow heat exchanger for microbial thermal inactivation research. Appl. Microbiol. **18**:889–892.
17. **Wilder, M. S., and J. C. Edberg.** 1973. Interaction of virulent and avirulent *Listeria monocytogenes* with cultured mouse peritoneal macrophages. Infect. Immun. **7**:409–415.