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**EFFECT OF CHRONIC GAMMA RADIATION ON  
AIRBORNE INFECTION OF MICE WITH  
LISTERIA MONOCYTOGENES**

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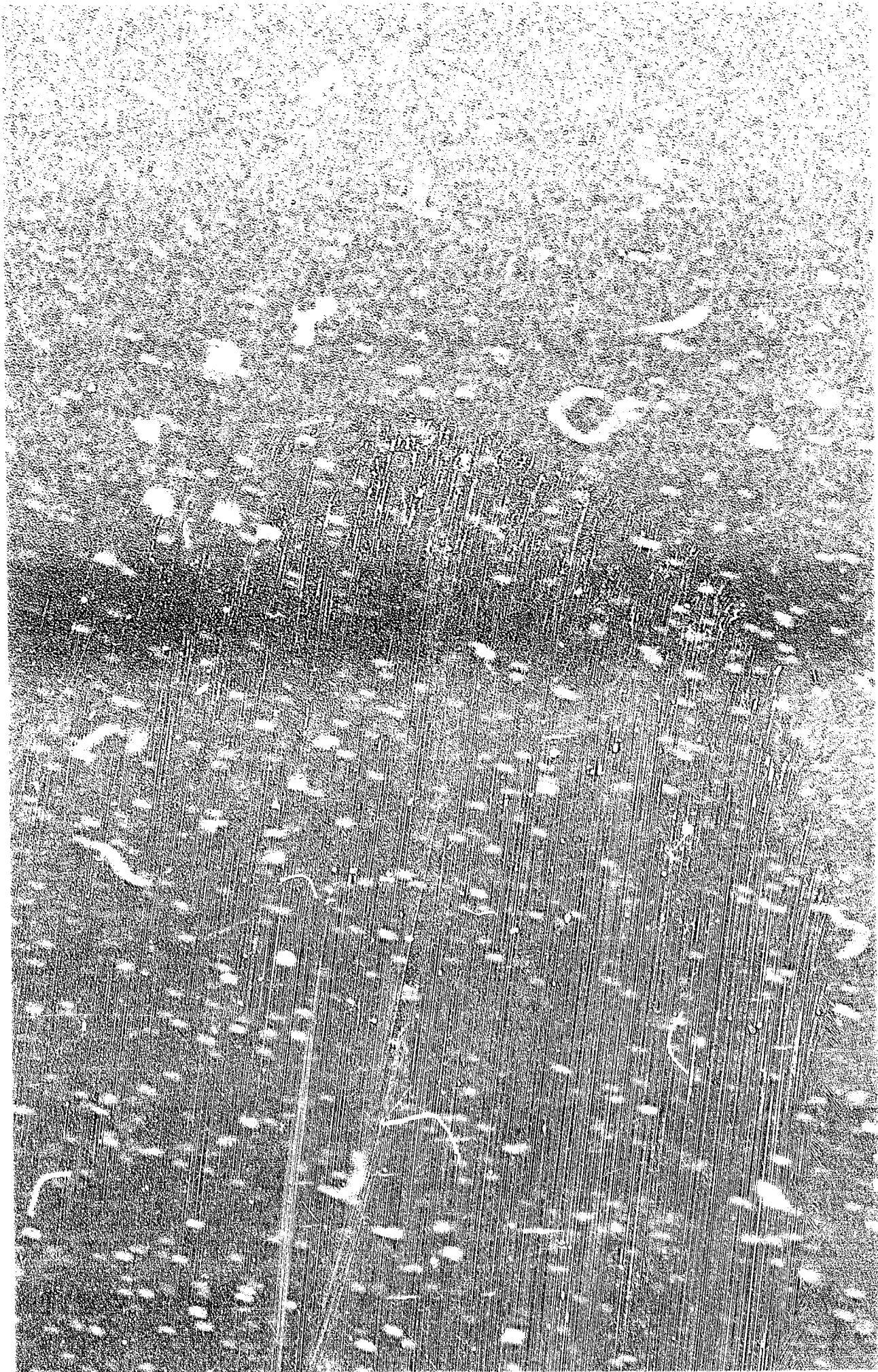
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ADMINISTRATIVE INFORMATION

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#### ABSTRACT

The susceptibility of mice to an airborne infection with Listeria monocytogenes increased following continuous exposure to  $\gamma$  radiation delivered at 1.0-1.5 rad/hour. The increase in susceptibility became greater, the larger the total radiation dose. The  $LD_{50/30}$  for non-irradiated mice was  $5.7 \times 10^5$  organisms, while after exposure to 500-1500 rads it dropped to  $1.8 \times 10^5$  and  $1.2 \times 10^5$ , respectively. Further exposure to 2000 rad decreased the  $LD_{50/30}$  to  $4.1 \times 10^4$ . After 2500 rad it was  $1.7 \times 10^4$ , a 33-fold increase in susceptibility compared to the non-irradiated mice.

The fate of inhaled L. monocytogenes in the lungs of irradiated (2000 rad) and non-irradiated mice was investigated at 4 hrs following infection. Irrespective of the aerosol challenge dose, the lungs of irradiated mice reduced bacterial numbers by 61% in this time compared to 80% for the non-irradiated animals.

Bacterial dissemination in the organs of mice challenged with sub-lethal and lethal doses of the organism was also determined at 2 day intervals. Following sublethal aerosol challenge, bacterial counts on homogenates from the lung, liver and spleen indicated that a more rapid proliferation of the organism occurred in the organs of  $Co^{60}$  irradiated mice. Furthermore, the ultimate disappearance of L. monocytogenes from the organs of irradiated mice was delayed in comparison

to the removal seen in non-irradiated animals. Following challenge with lethal numbers of L. monocytogenes, bacterial proliferation rates in the organs of irradiated and non-irradiated mice were comparable. However, deaths among irradiated mice occurred on days 3 and 4, whereas in the non-irradiated group they were recorded on days 5-6.

## NON-TECHNICAL SUMMARY

### The Problem

The effects of continuous exposure to low dose rate gamma radiation on susceptibility to infection has been reported by only a few investigators. This study was initiated to determine the effects of such exposure on mice challenged with airborne Listeria monocytogenes.

### The Findings

Following continuous exposure to low dose rate gamma radiation, the susceptibility of mice to airborne Listeria monocytogenes infection increased.

Impaired clearance (mechanical removal or killing) of L. monocytogenes from the lungs of irradiated mice, at a short interval after infection, was demonstrated. Also, the results indicated that both greater bacterial proliferation and delayed disappearance of the organism occurred in the organs of irradiated mice following sublethal aerosol challenge. Exposure to a lethal dose of the bacterial aerosol resulted in comparable proliferation rates in the organs of irradiated and non-irradiated mice. However, earlier deaths were observed in the irradiated animals.

## INTRODUCTION

It is well known that exposure to a single acute dose of total-body X-irradiation within the midlethal range markedly increases the mouse's susceptibility to experimentally induced bacterial infection. In comparison, a paucity of information exists regarding the effects of continuous exposure to low doses of gamma radiation. Hammond, et al. (1) studied the effects of continuous exposure to low doses of gamma radiation on the susceptibility of mice to Pseudomonas infection. Animals exposed at rates of 69 and 128 r/day demonstrated an increased susceptibility to intraperitoneal challenge of the bacteria. At these levels, accumulated doses of 3845 and 2695 r, respectively were obtained. Those exposed at 34 r/day, accumulated 2140 r but showed no significant increase in susceptibility. A few deaths from the radiation, alone, were observed in the groups receiving 69 and 128 r/day but none occurred among the mice exposed at 34 r/day. In a later study (2) the challenge inccula were graded by less than a 10-fold dilution, as previously used, in an attempt to detect smaller changes in susceptibility. Using this method it was shown that daily exposure to 15 r gamma radiation (1350 r accumulated) resulted in a slight but demonstrable increase in susceptibility to intraperitoneal inoculation of Pseudomonas.

In the present study, accumulated radiation doses up to 2500 rad were obtained by continuous exposure at 24-34 rad/day. These levels of radiation exposure were chosen because it was hoped that one could

observe effects on susceptibility to infection without encountering deaths from the radiation.

The microorganism used to study the effects of continuous exposure to low dose gamma radiation was Listeria monocytogenes. It has been shown (3) that susceptibility to infection with this organism is influenced by the state of general resistance and by many environmental and climatic factors. Lacking, however, are the effects of irradiation on susceptibility to infection. In this study the airborne route of infection was chosen because it could be quantitated conveniently and because it is a natural route of infection.

#### METHODS AND MATERIALS

##### Mice

Equal numbers of male and female LAF-1 (C57L ♀ X A/He ♂) mice from our laboratory colony were used in the experiments. Mice were 12-16 weeks old at time of exposure to bacterial aerosols.

##### Irradiation of Mice

Employing a dose rate of 1.0-1.5 rad/hr, mice were continuously exposed until accumulated doses of 500, 1000, 1500, 2000 and 2500 rad had been obtained. The range in dose rate was due to decay of the  $\text{Co}^{60}$  source during the time in which experiments were in progress. Plastic mouse cages were placed on curved wooden racks so that the center of each was equidistant from the 2.5 curie  $\text{Co}^{60}$  source. Dose measurements were made with a Philips dosimeter. The  $\text{Co}^{60}$  source was in



continuous operation except for 1 hr per week when the cages were changed. Fresh food pellets and water were also supplied at this time. No deaths occurred among the mice during the radiation exposure period.

### Listeria monocytogenes

Strain JHH\* was grown in Difco's brain heart infusion (BHI) for 18 hrs. The culture was mixed with 10% skim milk, distributed into several vials, and lyophilized. In each experiment a new vial of the lyophilized bacteria was used. The lyophilized organisms were re-suspended in 9 ml of 2.5% tryptose broth (Difco) and incubated at 37°C for 18 hrs. A loop of the culture was then transferred to a tryptose agar (Difco) slant and after 8 hrs incubation a second slant was streaked. After incubating the slant for 16 hrs, the bacteria were transferred to 2 Erlenmeyer flasks (250 ml) containing 50 ml of Difco's BHI and 3 glass beads. The flasks were kept in a 37°C waterbath on a reciprocating shaker (having a stroke of 2.6 cm and 90 excursions per min) for 16 hrs at which time the viable count ranged from 1.5-2.5 x 10<sup>9</sup> cells/ml. Bacterial numbers were estimated by plating 0.1 ml portions of the broth culture on tryptose agar in Petri dishes. The inoculum was spread with a curved glass rod and the plates were incubated overnight at 37°C. Depending upon the dosage level required for aerosol dissemination the BHI culture was either further concentrated by centrifugation or diluted by adding fresh BHI. In either

\*Serotype 4b. Obtained through the courtesy of Dr. Sidney J. Silverman, U. S. Army Biological Laboratories, Ft. Detrick, Frederick, Maryland.

case, 50 ml of the desired BHI suspension was consistently used for dissemination.

#### Exposure of Mice to Bacterial Aerosol

Mice were infected by exposure to aerosols of L. monocytogenes in a modified Henderson apparatus (4). Irradiated mice were exposed within 2 hrs after removal from the  $^{60}\text{Co}$  source. A titration of 6 doses, 3 of which encompassed the estimated  $\text{LD}_{50}$  for irradiated mice and 3 of which included the non-irradiated  $\text{LD}_{50}$ , was performed. Fifteen to 20 animals per dose were used. Desired doses were obtained by altering the concentration of the disseminating media or by varying the aerosol exposure time. The aerosol was sampled with impingers simultaneously with exposure of the animals. Calculations of the dose inhaled by the experimental animals were made from the data obtained on the concentration of cells collected in the impinger fluid and from the respiratory rate and volume of the animal according to the formula of Guyton (5).

Exposed animals were observed for 30 days. Most animals that succumbed were autopsied and examined for gross pathological changes. With very rare exception, the organism was readily isolated from the lung, liver and spleen.

#### Calculation of Bacterial $\text{LD}_{50}$

Several aerosol experiments performed at each accumulated radiation dose level indicated that results were quite reproducible. On this basis, the data from similar experiments were combined. By pooling

the data in these experiments it was possible to increase both the number of bacterial dose titrations and the number of animals responding.

The LD<sub>50</sub> values and the 95% confidence limits were determined by analysis of quantal response (carried out by computer) based on the maximum likelihood solution of the probit-log dose relationship (6).

#### Bacterial Enumeration in Lung, Liver and Spleen

In some experiments, bacterial proliferation rates in the lung, liver and spleen were determined. Mice were sacrificed at intervals by cervical dislocation. The organs were removed and the volume of each was determined by displacement. It was found that the lung generally displaced 0.4 ml, of 2.5% tryptose broth, the liver 1.4 ml, and the spleen, 0.1 ml. The organs were then homogenized with mortar and pestle in a measured amount of tryptose broth to which sand had been added. The tissue homogenate was diluted with tryptose broth and plated on tryptose agar plates. Liver homogenates were plated on tryptose agar containing 0.001% Bacto-Chapman Tellurite Solution (final concentration) to prevent growth of contaminants. Colony counts were made after 24 hrs incubation at 37°C. Results were expressed as number of organisms per organ.

#### RESULTS

Susceptibility to L. monocytogenes infection was evaluated by comparing the bacterial LD<sub>50</sub> of mice previously exposed to varying levels of accumulated gamma radiation to that of mice receiving no radiation.

The data presented in Table I and Figure 1 indicate that susceptibility to fatal listeriosis in mice increases following continuous exposure to low dose gamma radiation. The  $LD_{50}$  for non-irradiated mice was  $5.67 \times 10^5$  cells. Following exposure to an accumulated gamma radiation dose of 500 rad the  $LD_{50}$  dropped to  $1.80 \times 10^5$ . Exposure to 1000 rad and 1500 rad resulted in a slight decrease in the  $LD_{50}$  as compared with that observed at 500 rad. The  $LD_{50}$  at 1000 rad was  $1.15 \times 10^5$  and that at 1500 rad was  $1.44 \times 10^5$ . The  $LD_{50}$  of L. monocytogenes for mice receiving 2000 rad was  $4.06 \times 10^4$ . Exposure to an accumulated radiation dose of 2500 rad resulted in a further decrease in the  $LD_{50}$ . It was  $1.68 \times 10^4$ . From these data it is seen that the mean value ( $LD_{50}$ ) for any radiation dose lies far outside the 95% confidence limits for the non-irradiated mice. Among the irradiated mice there is evidence of increasing susceptibility with an increase in the accumulated radiation dose.

A ratio of the  $LD_{50}$  of non-irradiated mice/ $LD_{50}$  of irradiated mice was used to determine an index of increased susceptibility for each level of accumulated radiation. Using this index, it was observed that as the accumulated radiation dose increased, the susceptibility to infection increased. Mice which had received 2500 rad were over 30 times more susceptible to fatal listeriosis than those receiving no radiation.

TABLE I

LD<sub>50</sub> OF AIRBORNE LISTERIA MONOCYTOGENES FOR MICE FOLLOWING CONTINUOUS EXPOSURE TO LOW DOSE RATE  $\gamma$  RADIATION.

TOTAL RADIATION DOSE (RADS)	NUMBERS OF MICE USED	LD <sub>50</sub> OF <u>L. MONOCYTOGENES</u> (X 10 <sup>4</sup> )	95% CONFIDENCE LIMITS (X 10 <sup>4</sup> )	INCREASED SUSCEPTIBILITY*
0	756	56.7	51.3 - 63.3	- - -
500	153	18.0	12.0 - 24.5	3.1
1000	150	11.5	8.5 - 15.1	4.7
1500	204	14.4	11.0 - 19.6	4.1
2000	108	4.06	1.4 - 6.63	14.0
2500	100	1.68	0.35 - 2.9	33.9

\*LD<sub>50</sub> Non-irradiated

LD<sub>50</sub> Irradiated

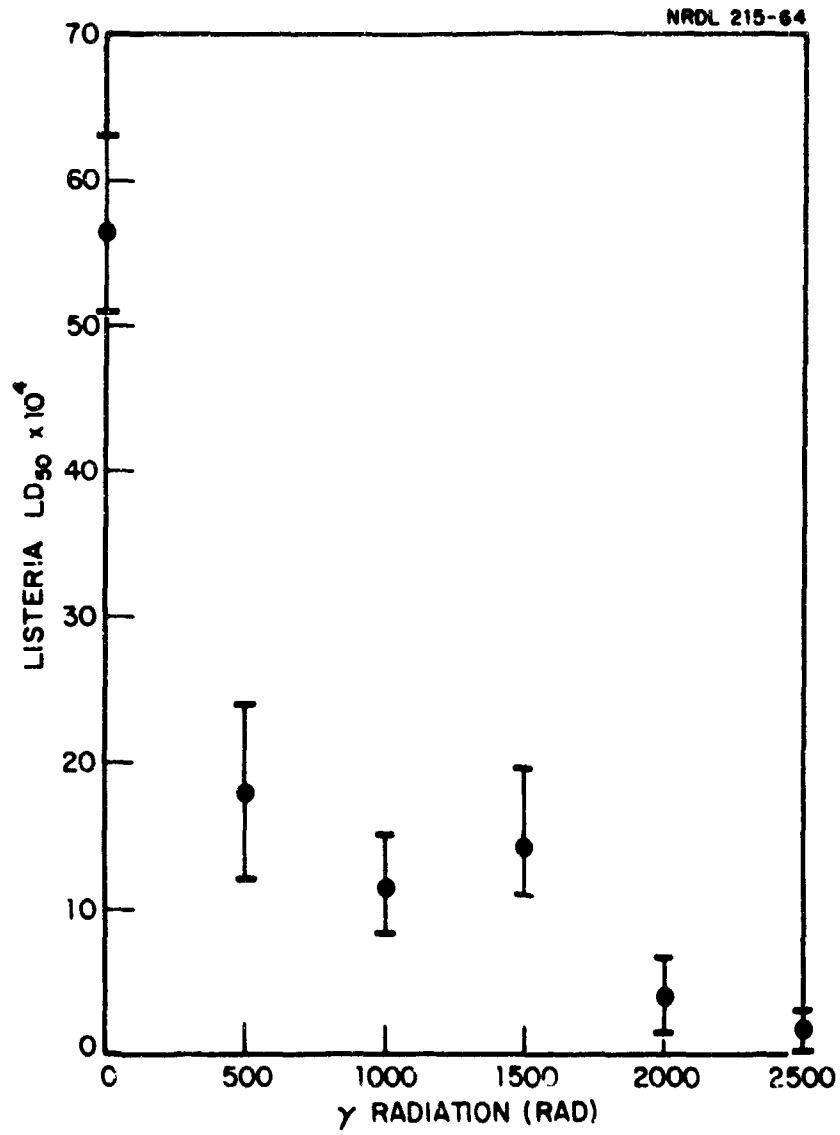


Figure 1. LD<sub>50</sub> of Airborne Listeria monocytogenes for mice following continuous exposure to low dose rate  $\gamma$  radiation. Confidence limits (95%) for each point are shown.

During the LD<sub>50</sub> experiments, mice usually died between the 5th and 10th days after exposure to the bacterial aerosol. However, a few deaths were occasionally recorded as long as 15 days after challenge.

A comparison of the time of death among irradiated and non-irradiated mice challenged with a similar dose of L. monocytogenes was not done in the LD<sub>50</sub> experiments as most challenge doses were adjusted to coincide with the estimated LD<sub>50</sub> of the two groups.

Since the LD<sub>50</sub> experiments had shown that continuous exposure to low dose gamma radiation results in increased susceptibility to L. monocytogenes it seemed of interest to study the pathogenesis of the organism in mice. These studies were performed with mice receiving 2000-2500 rad because these radiation levels had produced the most demonstrable effect on susceptibility.

The data presented in Table II show the fate of L. monocytogenes in the lungs of irradiated and non-irradiated mice 4 hrs after challenge with various doses of the organism. Mice were sacrificed within 5 min after aerosol exposure and the lungs were immediately homogenized in order to determine the number of organisms initially retained in the lungs. Four hrs later, the lungs were removed from animals similarly exposed and homogenized. In all cases, the percent reduction in number of inhaled organisms at 4 hrs was less in irradiated than in non-irradiated mice. In most cases the difference was approximately 17%. The percent reduction in 4 hrs was only slightly altered by varying

the aerosol challenge dose. This was encountered only in non-irradiated mice receiving a relatively small challenge. The lung homogenate counts of these mice indicated 85% reduction compared to 78% for the remainder of the non-irradiated animals. Variations in the accumulated radiation dose resulted in little difference in the percent reduction at 4 hrs among the irradiated mice.

The pathogenesis was studied further by sacrificing mice at intervals and determining the numbers of organisms in homogenates of the lung, liver and spleen. Heart's blood cultures were also done by streaking a loopful of blood on a tryptose agar plate.

The dissemination of L. monocytogenes was studied first in the organs of non-irradiated mice (Table III). Animals were challenged with a dose of  $4.3 \times 10^5$  cells. This was estimated to be an  $LD_{40-50}$ . Within 4 hrs the numbers of bacteria initially inhaled in the lungs had decreased by 77%. No organisms could be detected in the liver or spleen at the 4 hr determination. On day 2 large numbers of L. monocytogenes were recovered from all organs and 2 out of 5 heart's blood cultures were positive. Bacterial counts remained high through the 4th day and the largest number of positive blood cultures was obtained on this day. Bacterial numbers subsided in the spleen by day 6 and in the lung and liver by day 8. On the 10th day after infection a moderate number of bacteria remained in the liver but none could be detected in the lung and spleen homogenates. All blood cultures were negative on



TABLE II

FATE OF LISTERIA MONOCYTOGENES IN LUNGS OF IRRADIATED AND NON-IRRADIATED MICE FOUR HOURS AFTER AEROSOL CHALLENGE.

NON-IRRADIATED MICE

AEROSOL CHALLENGE DOSE	NO. LUNGS HOMOGENIZED	ZERO HOUR COLONY COUNT	4 HOUR COLONY COUNT	% REDUCTION
$3.5 \times 10^3$	3	$8.4 \times 10^1$	$1.1 \times 10^1$	87
$3.8 \times 10^4$	5	$1.2 \times 10^4$	$2.0 \times 10^3$	83
$3.3 \times 10^5$	12	$2.2 \times 10^4$	$4.9 \times 10^3$	78
$4.3 \times 10^5$	6	$2.9 \times 10^4$	$6.8 \times 10^3$	77
$1.3 \times 10^6$	8	$1.6 \times 10^5$	$3.5 \times 10^4$	78
$1.5 \times 10^6$	2	$1.3 \times 10^5$	$2.7 \times 10^4$	78
$1.8 \times 10^6$	3	$1.6 \times 10^5$	$4.5 \times 10^4$	78
$2.2 \times 10^6$	3	$2.4 \times 10^5$	$5.5 \times 10^4$	78

IRRADIATED MICE

AEROSOL CHALLENGE DOSE	RADIATION DOSE (RADS)	NO. LUNGS HOMOGENIZED	ZERO HOUR COLONY COUNT	4 HOUR COLONY COUNT	% REDUCTION
$3.5 \times 10^3$	2000	3	$7.3 \times 10^1$	$3.1 \times 10^1$	58
$2.2 \times 10^5$	1824	6	$4.4 \times 10^4$	$1.6 \times 10^4$	64
$3.3 \times 10^5$	2100	12	$2.2 \times 10^4$	$9.9 \times 10^3$	56
$8.0 \times 10^5$	2500	4	$6.8 \times 10^4$	$2.6 \times 10^4$	62
$2.2 \times 10^6$	2000	3	$2.6 \times 10^5$	$9.2 \times 10^4$	65

days 8 and 10. In this experiment, organ homogenate counts showed great variation among individual mice on days 4 and 6. As might be expected, mice sacrificed on these days were either potential survivors or would have eventually died. This may have accounted for the wide variation in number of recoverable L. monocytogenes from the organs of individual mice.

The next group of experiments consisted of comparing the pathogenesis among irradiated (2500 rad) and non-irradiated mice. Dissemination of the organism was studied in mice exposed to sublethal and lethal doses of L. monocytogenes. By employing aerosol doses which were either uniformly non-fatal or uniformly fatal it was possible to eliminate great variations in organ homogenate counts which inevitably occurred when mice were exposed to LD<sub>50</sub> doses.

The data presented in Table IV show the proliferation of L. monocytogenes in the organs of mice challenged with a sublethal dose of the organism. At 4 hrs the homogenate counts indicated that the lungs of irradiated mice were 29% less efficient in reducing the numbers of inhaled bacteria than those of non-irradiated animals. Organisms could not be recovered from the liver or spleen of either group at this time. On day 2, moderate numbers of bacteria were recovered from all organs of the irradiated mice. The organs of non-irradiated animals, on the other hand, contained fewer bacteria and in the liver, for instance, the difference was approximately 2 log. The organ homogenate counts from irradiated mice remained at the same level through day 6 and those

TABLE III

PROLIFERATION OF LISTERIA MONOCYTOGENES IN ORGANS OF NON-IRRADIATED MICE CHALLENGE WITH A MIDDLETHAL DOSE OF THE ORGANISM

COLONY COUNT PER ORGAN (GEOMETRIC MEAN)									
ORGAN	ZERO	TIME							
		4 HOURS	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10		
LUNG <sup>a</sup>	$2.9 \times 10^4$	$6.8 \times 10^3$	$7.1 \times 10^5$	$1.5 \times 10^6$	$4.8 \times 10^5$	$2.0 \times 10^2$	$2.0 \times 10^2$	$2.0 \times 10^2$	$< 3.0 \times 10^{1b}$
LIVER		$< 6.0 \times 10^1$	$1.8 \times 10^6$	$1.4 \times 10^7$	$2.0 \times 10^7$	$5.7 \times 10^4$	$5.7 \times 10^4$	$5.7 \times 10^4$	$3.5 \times 10^4$
SPLEEN		$< 2.0 \times 10^1$	$5.0 \times 10^4$	$1.8 \times 10^5$	$< 2.0 \times 10^3$	$3.0 \times 10^1$	$3.0 \times 10^1$	$3.0 \times 10^1$	$< 2.0 \times 10^1$
BLOOD		$0/5^c$	$2/5$	$4/5$	$1/5$	$0/5$	$0/5$	$0/5$	$0/5$

a. AEROSOL CHALLENGE WAS  $4.3 \times 10^5$  CELLS. FIVE MICE PER GROUP WERE USED.

b. NUMBER OF MICE DEAD IN MORTALITY CONTROL GROUP WAS 8/20 AT THIS TIME.

c. NUMBER OF ANIMALS SHOWING POSITIVE BLOOD CULTURES/TOTAL NUMBER WHEN ONE LOOPFUL OF HEART BLOOD WAS STREAKED ON A TRYPTOSE AGAR PLATE.

from non-irradiated animals continued at their lower level. By day 8, no bacteria could be recovered from the lungs or spleen of non-irradiated mice. Small numbers of L. monocytogenes persisted in the lung and spleen of irradiated mice at this time. The livers of irradiated mice had 3 log more bacteria than those of the non-irradiated. On the 10th day, bacteria were still present in the livers of non-irradiated mice. The lungs of irradiated animals contained a few organisms and the livers had greater numbers (2 log) than in the non-irradiated mice. None of the blood cultures were positive in either the non-irradiated or irradiated mice during the experimental period.

Table V presents the data obtained following challenge with a lethal dose of L. monocytogenes. The lungs of irradiated mice reduced bacterial numbers by 65% within 4 hrs, whereas the percent reduction for non-irradiated mice was 78. Liver and spleen homogenates contained no viable Listeria at 4 hrs. On the second day after infection the organs of both irradiated and non-irradiated mice contained high numbers of bacteria. Positive blood cultures were obtained in 2 out of 4 of the irradiated mice and in 1 out of 3 of the non-irradiated mice. A further increase in bacterial numbers was noted on day 4 and the organs of irradiated mice contained approximately 1 log more recoverable Listeria than those of animals receiving no radiation. Blood cultures were positive in 4 out of 4 of the irradiated animals and in 2 out of 3 of the non-irradiated ones on the 4th day. In this experiment irradiated mice died on days 3-4 whereas, the non-irradiated

TABLE IV

PROLIFERATION OF *LISTERIA MONOCYTOGENES* IN ORGANS OF IRRADIATED AND NON-IRRADIATED MICE CHALLENGED WITH A SUBLETHAL AEROSOL DOSE OF THE ORGANISM.

GROUP <sup>a</sup>	COLONY COUNT PER ORGAN (GEOMETRIC M <sup>a</sup> )						
	TIME						
	ZERO	4 HRS	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
<b>IRRADIATED<sup>b</sup></b>							
LUNG	$7.3 \times 10^1$	$3.1 \times 10^1$	$9.4 \times 10^3$	$7.3 \times 10^3$	$7.6 \times 10^3$	$1.1 \times 10^4$	$7.5 \times 10^{1d}$
LIVER		$< 6.0 \times 10^1$	$1.2 \times 10^5$	$4.6 \times 10^4$	$1.9 \times 10^5$	$2.6 \times 10^5$	$2 \times 10^5$
SPLEEN		$< 2.0 \times 10^1$	$1.0 \times 10^2$	$2.5 \times 10^4$	$8.1 \times 10^4$	$4.0 \times 10^2$	$< 2.0 \times 10^1$
BLOOD		0/3 <sup>c</sup>	0/3	0/3	0/3	0/3	0/3
<b>NON-IRRADIATED</b>							
LUNG	$8.4 \times 10^1$	$1.1 \times 10^1$	$3.2 \times 10^3$	$3.2 \times 10^3$	$1.8 \times 10^3$	$< 3.0 \times 10^1$	$< 3.0 \times 10^{1e}$
LIVER		$< 6.0 \times 10^1$	$4.4 \times 10^3$	$2.5 \times 10^3$	$8.0 \times 10^1$	$2.5 \times 10^2$	$1.5 \times 10^3$
SPLEEN		$< 2.0 \times 10^1$	$7.7 \times 10^1$	$2.3 \times 10^3$	$1.8 \times 10^3$	$< 2.0 \times 10^1$	$< 2.0 \times 10^1$
BLOOD		0/3	0/3	0/3	0/3	0/3	0/3

- a. Three mice per group were used. Aerosol challenge was  $3.5 \times 10^3$  cells for both groups.
- b. Accumulated dose of 2500 r.
- c. Number of animals showing positive blood cultures/total number when one loopful of heart blood was streaked on a Tryptone agar plate.
- d. Number of mice dead in mortality control group was 1/20 at this time.
- e. Number of mice dead in mortality control group was 0/20 at this time.

TABLE V

PROLIFERATION OF LISTERIA MONOCYTOGENES IN ORGANS OF IRRADIATED AND NON-IRRADIATED MICE CHALLENGED WITH A LETHAL AEROSOL DOSE OF THE ORGANISM.

GROUP <sub>a</sub>	COLONY COUNT PER ORGAN (GEOMETRIC MEAN)				
	TIME				
	ZERO	4 HOURS	DAY 2	DAY 4	DAY 6
IRRADIATED <sub>b</sub>					
LUNG	$6.8 \times 10^4$	$2.6 \times 10^4$	$4.9 \times 10^6$	$1.1 \times 10^8$	— <sup>e</sup>
LIVER		$< 6.0 \times 10^1$	$8.8 \times 10^6$	$2.4 \times 10^8$	
SPLEEN		$< 2.0 \times 10^1$	$1.8 \times 10^5$	$1.2 \times 10^7$	
BLOOD		0/4 <sup>d</sup>	2/4	4/4	
NON-IRRADIATED <sub>c</sub>					
LUNG	$1.6 \times 10^5$	$4.5 \times 10^4$	$4.0 \times 10^6$	$5.4 \times 10^7$	— <sup>f</sup>
LIVER		$< 6.0 \times 10^1$	$5.4 \times 10^6$	$9.2 \times 10^7$	
SPLEEN		$< 2.0 \times 10^1$	$2.1 \times 10^5$	$1.2 \times 10^6$	
BLOOD		0/3	1/3	2/3	

- a. Four irradiated mice and three non-irradiated mice per group were used.  
 b. Aerosol challenge was  $8.0 \times 10^5$  cells. Accumulated  $\gamma$  radiation was 2500 r.  
 c. Aerosol challenge was  $1.9 \times 10^6$  cells.  
 d. Number of positive blood cultures/total plated.  
 e. Number of mice dead in mortality control group was 25/25 at this time.  
 f. Number of mice dead in mortality control group was 16/18 at this time.

animals succumbed on days 5-6.

### DISCUSSION

These studies have shown that continuous exposure of mice to low dose rate gamma radiation increases their susceptibility to airborne L. monocytogenes infection. This increase in susceptibility was demonstrated by the depressant effect of radiation on the bacterial LD<sub>50</sub> for mice. As evidenced by the LD<sub>50</sub>'s, major damage to defense mechanisms occurred in mice receiving an accumulated dose of 500 rad. Thereafter, such a demonstrable effect of additional accumulated gamma radiation seemed to be less evident. There was, however, an indication that susceptibility to infection tended to increase as the radiation dose became greater.

Although the same strain of L. monocytogenes was employed in this study as in that of Kautter, et al. (7), the respiratory LD<sub>50</sub>'s for non-irradiated mice were quite different in the two studies. In the latter investigation it was  $2.4 \times 10^4$ , compared to  $5.6 \times 10^5$  in the former. This difference may be ascribed to the fact that the same strain and age of mice were not used in both investigations.

The results of this study also indicate that the ability of irradiated mice to reduce bacterial numbers in the lung during the early part of infection is impaired. It is not known whether this impairment reflects decreased ability to kill the inhaled bacteria or a lessened ability to remove the organisms from the lung. According to the observations of Green and Kass (8), bactericidal action of the

lung predominates over the mechanical removal process in achieving clearance of bacteria (Staphylococcus aureus and Proteus mirabilis) during the first 4 hrs of infection. In their study, bactericidal activity was attributed to the alveolar macrophages and it resulted in a 80-90% decline in viable organisms in 4 hrs. This compares favorably with the 80% reduction obtained with non-irradiated mice in the present study. Other studies (9) have indicated comparable clearance during the early hours following airborne infection.

A consistent difference between the theoretical dose and the actual numbers of bacteria recovered from the lung homogenate, immediately after aerosol exposure was noted in most experiments. The lung homogenates generally had 1 log fewer organisms than were theoretically determined for that group. This discrepancy was found both with irradiated and non-irradiated mice. Similar discrepancies have been reported (10) between the calculated and lung homogenates dose following aerosol challenge with Pasteurella multocida. It is difficult to implicate any single factor which may have resulted in the differences between the theoretical dose and the actual numbers of recoverable bacteria. Speculation, however, might lead to consideration of retention of bacteria by the upper respiratory tract or exhalation of a given number of organisms during and immediately after aerosol exposure. It is unlikely that pronounced bactericidal activity or removal of Listeria by the lung could have occurred during the exposure period or a few minutes after.



Dissemination of L. monocytogenes in the organs of non-irradiated mice challenged with a respiratory LD<sub>40-50</sub> is at variance with the observations of Silverman, et al. (11). In the latter study, larger numbers of bacteria were recovered from the organs during the experimental period and the ultimate disappearance of organisms from the host's tissues was delayed. These discrepancies may be accounted for on the basis of differences in strain and age of mouse used. Also, the same strain of Listeria was not employed in both studies.

Irradiated mice challenged with a sublethal dose of L. monocytogenes were less efficient in preventing multiplication of bacteria in their organs than were the non-irradiated animals. Also, ultimate removal of bacteria from the organs of irradiated mice was delayed in comparison to that observed in non-irradiated animals. Both of these effects in irradiated mice may be explained, in part, by the results obtained by Mackaness (12). He found that susceptibility to L. monocytogenes infection in normal mice is due to the capacity of the organism to survive and multiply in the host's macrophages. He further noted that an antibacterial mechanism appeared during the course of infection and that rapid inactivation of the organism occurred subsequent to the 4th day following challenge. This inactivation was attributed to a change occurring in the host's mononuclear phagocytes. It has been reported (13) that acute x-irradiation impairs the ability of phagocytes to ingest and digest bacteria. It is possible that chronic gamma radiation produces a comparable effect. On this basis it would be reasonable to

assume that the more rapid bacterial proliferation in irradiated mice and the delayed appearance of a demonstrable antibacterial activity may be related to alterations in the cellular defense mechanism.

The organs of irradiated and non-irradiated mice challenged with a lethal dose of L. monocytogenes were equally unable to control multiplication of the bacteria on days 2 and 4 after infection. It is possible that the overwhelming nature of the challenge prevented defense mechanisms from manifesting themselves at this time. Positive blood cultures were found in most animals on the 4th day after infection. Inability of the organs to cope with such large numbers of bacteria may account for this finding. Deaths in the irradiated mice occurred on days 3-4, whereas in the non-irradiated animals they were recorded on days 5-6. These earlier deaths in irradiated mice are obviously a reflection of damage to defense mechanisms.

In this study, increased susceptibility to airborne L. monocytogenes infection following continuous exposure to low dose rate gamma radiation was clearly demonstrated. It is less clear as to which defense mechanisms are altered to cause such a decrease in resistance. The results of this study suggest that lung clearance (mechanical removal or killing) is impaired and that ability of the organs to cope with bacterial proliferation during the course of infection is hindered.

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Bacterial dissemination in the organs of mice challenged with sublethal and lethal doses of the organism was also determined at 2 day intervals. Following sublethal aerosol challenge, bacterial counts on homogenates from the lung, liver and spleen indicated that a more rapid proliferation of the organism occurred in the organs of Co<sup>60</sup> irradiated mice. Furthermore, the ultimate disappearance of *L. monocytogenes* from the organs of irradiated mice was delayed in comparison to the removal seen in non-irradiated animals. Following challenge with lethal numbers of *L. monocytogenes* bacterial proliferation rates in the organs of irradiated and non-irradiated mice were comparable. However, deaths among irradiated mice occurred on days 3 and 4, whereas in the non-irradiated group they were recorded on days 5-6.  
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