

AD 637401

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 86, No. 3, pp. 355-362 September, 1963
Copyright © 1963 by The Williams & Wilkins Co.
Printed in U.S.A.

INFLUENCE OF ROUTE OF INFECTION AND OTHER FACTORS ON GROWTH AND DISTRIBUTION OF *LISTERIA MONOCYTOGENES* IN ORGANS OF MICE

SIDNEY I. SILVERMAN, LYNN P. ELWELL¹, AND JAMES F. DRAWDY²

S
Lal
LX
Infl
on
gen
196
the
was
intr
lung
tair
hr,
for
two
lung
and
the
the
Lis
gan
in s
inje
stra
resi
of f
witl
alor
the
wee
infe
intr
cov
sive
Hov
(tin

1
Uni
2
Fla

ing 7-
althy
terial
e two

been
r See-
anism
ly in-
1963)
dts of
n the
asure
ucible
ia the
ratio,

) ob-
steria,
IEF),
imals
isms.
other
terial

after
other
e fate
ervals
on, it
effect
rowth
wn to

it the
Ga.,
JHH,
t The

code 1, 20

CLEARINGHOUSE FOR FEDERAL SCIENTIFIC AND TECHNICAL INFORMATION			
Hardcopy	Microfiche		
\$ —	\$ — 1 —	8	PP
ARCHIVE COPY			

AD 632401

1041

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 86, No. 3, pp. 355-362, September, 1963
Copyright © 1963 by The Williams & Wilkins Co.
Printed in U.S.A.

INFLUENCE OF ROUTE OF INFECTION AND OTHER FACTORS ON GROWTH AND DISTRIBUTION OF *LISTERIA MONOCYTOGENES* IN ORGANS OF MICE

SIDNEY J. SILVERMAN, LYNN P. ELWELL¹, AND JAMES F. DRAWDY²
U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland

Received for publication 18 February 1963

ABSTRACT

SILVERMAN, SIDNEY J. (U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.), LYNN P. ELWELL, AND JAMES F. DRAWDY. Influence of route of infection and other factors on growth and distribution of *Listeria monocytogenes* in organs of mice. *J. Bacteriol.* **86**:355-362. 1963.—The growth of *Listeria monocytogenes* in the lungs, liver, spleen, brain, and blood of mice was observed after infection by the respiratory, intravenous, and intraperitoneal routes. The lungs of mice exposed to *Listeria* aerosols contained about 10⁶ infected cells per ml within 24 hr, and the number of organisms remained high for at least 9 days. After exposure by the other two routes, fewer organisms appeared in the lungs. The colony counts of organisms in spleen and liver homogenates were similar regardless of the route. Organisms were found sporadically in the brain and blood. When a cell extract of *Listeria* was injected simultaneously with the organisms, greater numbers of *Listeria* were found in spleen and liver than when the bacteria were injected alone. On the other hand, the BCG strain of *Mycobacterium tuberculosis* enhanced the resistance of the mice, as shown by the recovery of fewer organisms from tissues when compared with tissues from mice receiving *L. monocytogenes* alone. Prior administration of BCG also reversed the effect of the *Listeria* extract. Mice (5 to 6 weeks old) born of *Listeria*-infected mothers were infected with the homologous organism by the intraperitoneal route. Fewer organisms were recovered and the gross pathology was less extensive than in infected progeny of healthy females. However, there was no difference in the LT₅₀ (time to death for 50% of the animals) between

the two groups. In a similar experiment, using 7- to 8-week-old mice born of infected and healthy mothers, there was no difference in the bacterial counts, gross pathology, or LT₅₀ between the two groups.

Although the pathology of listeriosis has been studied by numerous authors (*see review by Seeliger, 1961*), the dissemination of the organism throughout the host has been observed only indirectly. In a recent report, Kautter *et al.* (1963) showed that differences occurred in the results of virulence titrations of *Listeria* strains when the route of infection was varied. Although exposure by the respiratory route resulted in a reproducible linear dose response, titrations performed via the intravenous or intraperitoneal routes gave erratic, nonlinear results.

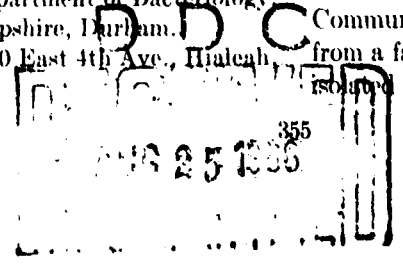
Silverman, Elwell, and Kautter (1961) observed that injection of a cell extract of *Listeria*, the so-called mortality-enhancing factor (MEF), reduced the resistance of mice and other animals to *Listeria* as well as to other microorganisms. *Mycobacterium tuberculosis* BCG, on the other hand, increased the resistance of mice to listerial infection and overcame the effect of MEF.

Because of the difference in response after aerosol exposure and after infection by the other routes, it seemed of interest to determine the fate of *Listeria* in the various organs and at intervals after exposure by these routes. In addition, it was considered desirable to determine the effect of the previous history of the host on the growth of the microorganism in various tissues known to be the loci of infection.

MATERIALS AND METHODS

L. monocytogenes strain A413, isolated at the Communicable Disease Center, Atlanta, Ga., from a fatal infection in a child, and strain JHH, isolated from a case of listerial meningitis at The

¹ Present address: Department of Bacteriology, University of New Hampshire, Durham, N.H.
² Present address: 1690 East 4th Ave., Hialeah, Fla.



Johns Hopkins Hospital, were used. When injected into Swiss-Webster mice by the intraperitoneal route, 4.8×10^2 cells of strain A4413 and 1.4×10^3 cells of strain JHH were required to kill 50% of the mice within 15 days (LD_{50}). By the respiratory route, the LD_{50} for A4413 was 3.5×10^3 cells and that for JHH was 2.1×10^4 cells. Brain Heart Infusion broth (Difco) was inoculated with the growth from an 8-hr Tryptose Agar (Difco) slant and incubated at 37 C for 16 to 18 hr on a reciprocating shaker (4- to 5-in. stroke, 100 excursions per min). Suitable dilutions were prepared in 1% Tryptose solution; the bacterial count of the inoculum was determined by quantitative plating on Tryptose Agar.

Swiss-Webster mice from the Fort Detrick colony were used. Animals were maintained and used in compliance with the principles established by the National Society for Medical Research (1961). Intravenous injections were performed via the caudal tail vein; 0.1 ml contained the challenge dose. Respiratory exposure was made in a modified Henderson apparatus according to the procedures described by Roessler and Kauter (1962). The usual volume injected for intraperitoneal challenge was 0.2 ml. Animals were killed at intervals after exposure by anesthesia either with chloroform or in an atmosphere of CO_2 . Blood was obtained from the jugular vein and cultured in Brain Heart Infusion broth; serial dilutions of the blood were prepared in this medium to determine the highest dilution at which growth would occur.

The spleens, livers, lungs, and brains were removed aseptically, and the volume of each was determined by displacement. In some experiments, similar organs from animals killed at the same time were pooled. In most cases, however, each organ was cultured separately. The tissues were ground aseptically with a glass rod and sand in a thick-walled tube. The tissue homogenate was diluted with 1.0% Tryptose and plated on Tryptose Agar plates by the method of Miles and Misra (1938). A portion (0.002 ml) of the dilution was dropped on the surface of dried Tryptose Agar from a 0.2-ml pipette with 0.01-ml gradations. After the drop was absorbed into the agar, the plates were inverted and incubated for 18 to 24 hr. Each dilution was replicated six times, and results were based on the mean count of the drops containing 10 to 40 colonies. Results were expressed as number of organisms per ml of tissue. In some experiments when the mean weight

of the organ was determined, results were expressed as number of colonies per mg of tissue. When individual organs were cultured, results were expressed as the geometric mean of counts obtained with three to five specimens.

When the effect of MEF of *Listeria* was studied, 2 ED_{50} doses of MEF (Silverman et al., 1961) were injected simultaneously with doses of the infecting organism. *M. tuberculosis* BCG was grown for 7 to 10 days in Dubos' medium. The cells were centrifuged, washed once with saline, and from 10^8 to 5×10^5 viable units were injected into the peritoneal cavity 4 to 7 days before infection with *Listeria*.

The LT_{50} (time to death for 50% of the animals) was calculated by the method of Litchfield (1949).

RESULTS

Mice, 2, 3, and 4 weeks old, were infected by the intravenous route with approximately 400 to 1,600 cells of strain A4413. Groups of three animals were killed at 24-hr intervals, and similar organs from the three animals were pooled for study. No difference was noted in the response of the three age groups, and this factor was not considered in the remainder of the study. The *Listeria* count in the lungs remained quite low (Table 1) but was not quantitatively reliable because of the heavy growth of other organisms in the low dilutions plated. However, few or no colonies of *Listeria* were present even on plates without contaminants.

Listeria strain A4413 multiplied more rapidly in the lungs after exposure by the respiratory route than after intraperitoneal injection. In replicate experiments, the maximal colony count after intraperitoneal injections ranged from 3.0×10^4 to 2.5×10^6 per ml and appeared between the first and sixth days. After respiratory exposure with this strain, the colony counts 1 day after exposure reached 1.9×10^6 to 3.7×10^6 per ml and persisted at a high level for the duration of the experiment. The maximal count (1.9×10^6) was reached at about 5 to 6 days and was greater than that observed after exposure by either the intravenous or intraperitoneal route.

The colony counts of *Listeria* obtained with homogenates of spleen or liver of infected mice were similar, regardless of the route of infection, within the wide range observed among the individual animals (Table 1).

The data in Table 2 indicate that less variation

TABLE 1. Distribution of *Listeria monocytogenes* A4413 in organs of mice infected by various routes

Exposure route*	Colony count per ml of tissue (geometric mean)								
	Day								
	1	2	3	4	5	6	7	8	9
Lung									
Respiratory	2.8 × 10 ⁶	1.7 × 10 ⁶	1.2 × 10 ⁷	3.1 × 10 ⁷	1.9 × 10 ⁶	9.8 × 10 ⁷	8.7 × 10 ⁶	2.4 × 10 ⁵	8.9 × 10 ⁶
Intraperitoneal	6.2 × 10 ²	9.0 × 10 ⁵	1.4 × 10 ⁵	2.2 × 10 ⁴	1.8 × 10 ⁴	9.1 × 10 ²			
Intravenous	0†	+	+	+	0	0	+	+	
Liver									
Respiratory	5.4 × 10 ³	10 ⁵	7.0 × 10 ⁴	4.7 × 10 ⁶	6.9 × 10 ⁷	1.3 × 10 ⁷	7.9 × 10 ⁵	<10 ³	5.8 × 10 ⁶
Intraperitoneal	1.2 × 10 ⁴	4.9 × 10 ⁵	4.7 × 10 ⁵	1.5 × 10 ⁵	5.4 × 10 ⁴	1.3 × 10 ³		5.7 × 10 ²	
Intravenous	3.3 × 10 ⁵	7.0 × 10 ³	1.8 × 10 ⁴	1.7 × 10 ⁶	1.1 × 10 ⁶	2.3 × 10 ⁴	8.1 × 10 ⁶	1.1 × 10 ⁵	
Spleen									
Respiratory	9.7 × 10 ²	7.8 × 10 ⁵	7.9 × 10 ⁶	9.4 × 10 ⁶	3.0 × 10 ⁷	6.0 × 10 ³	6.3 × 10 ³	4.0 × 10 ¹	10 ²
Intraperitoneal	3.4 × 10 ⁴	7.4 × 10 ⁶	5.2 × 10 ⁶	8.1 × 10 ⁵	1.5 × 10 ¹	6.4 × 10 ³		4.3 × 10 ²	
Intravenous	6.6 × 10 ⁵	5.9 × 10 ³	9.3 × 10 ⁵	6.5 × 10 ⁶	1.1 × 10 ⁵	1.3 × 10 ⁴	1.3 × 10 ³	4.0 × 10 ²	

* Respiratory dose was 1.2×10^3 cells, intraperitoneal dose was 1.5×10^3 cells, and intravenous dose was 1.6×10^3 cells. Five animals per group were used.

† Growth of other organisms in cultures from lungs made quantitative results unreliable.

TABLE 2. Variations in colony counts in tissues from individual animals infected by respiratory or intraperitoneal route with *Listeria monocytogenes* A4413

Route*	Organ	Colony count per ml of tissue (geometric mean)							
		Day							
		1		3		5		7	
		Mean	sd†	Mean	sd	Mean	sd	Mean	sd
Respiratory	Lung	3.0×10^6	2.4	1.9×10^6	1.1	1.9×10^7	2.3	1.3×10^6	11.1
	Liver	4.5×10^2	1.02	6.3×10^4	2.8	2.5×10^6	3.8	2.1×10^6	34.8
	Spleen	1.1×10^2	1.0	2.0×10^6	1.5	2.1×10^3	4.6	1.3×10^4	2.7
Intraperitoneal	Lung			6.1×10^4	4.7	3.6×10^4	46.4	1.6×10^3	8.6
	Liver			7.9×10^4	21.4	2.3×10^4	632.4	1.6×10^3	496.0
	Spleen			3.7×10^4	4.0	3.6×10^3	215.0	8.1×10^3	11.5

* Respiratory dose was 4.8×10^3 cells; intraperitoneal dose was 1.1×10^3 cells. Five animals per group were used.

† Standard deviation. Mean/sd = lower limit of distribution; mean × sd = upper limit.

was observed in the colony counts of tissues from mice exposed by the respiratory route than in those obtained from tissues from animals challenged by the intraperitoneal route. The standard deviation from the mean ranged from 1.0 to 34.8 colonies with cultures obtained from animals exposed by the respiratory route. With cultures of organs from mice exposed by the intraperitoneal route, the standard deviation was as high as 632.

In the experiments in which the mice were challenged via the intraperitoneal route, the *Listeria* counts in the organs of those mice receiving 1.5×10^5 cells of strain A4413 reached a maximum within 1 to 3 days (Table 3). The maximal counts in the organs of mice receiving smaller doses (2×10^2 or 1×10^3 cells) did not occur until the fifth or sixth day. There was some suggestion that the lower challenge doses resulted in a somewhat

higher maximal count than was obtained with larger doses.

Listeria were isolated from the brain homogenates sporadically, regardless of the route of infection. About 25% of the brains cultured contained *Listeria*. A few gave rise to colonies by the first day after injection, and *Listeria* were found through 7 days. The greatest number of positive cultures were obtained on the fourth day. The number of colonies ranged from 5.0×10^4 to 3.0×10^6 per ml of tissue.

Positive blood cultures were obtained from the first through the twelfth day. The greatest number of positive cultures were obtained from samples taken on the second through fourth days. Growth was obtained in dilutions as high as 10^{-4} at around the fifth day, although *Listeria* were generally found only at lower dilutions and during the early stages of infection.

Injection of the mortality-enhancing factor simultaneously with *L. monocytogenes* by the intraperitoneal route resulted in increased in vivo growth of the organism. The difference between the bacterial counts of animals receiving both MEF and *Listeria* and those receiving only the bacteria was apparent at 4 hr and persisted for the duration of the experiment (Table 4). Most animals that received MEF were dead by the fourth day.

BCG injected 6 days before challenge with *Listeria* increased the resistance of the mice so that the bacterial counts obtained from the tissue homogenates were lower than those obtained from the organs of animals receiving *Listeria* alone (Table 5). *Listeria* were not recovered from the blood of mice injected with BCG. Blood cultures were positive from most mice injected with *Listeria* only. BCG was also capable of over-

TABLE 3. Influence of size of challenge dose on numbers of *Listeria monocytogenes* in organs of mice after intraperitoneal challenge

Challenge cells per dose*	Maximal colony count per ml of tissue (geometric mean)					
	Lung		Liver		Spleen	
	Colonies per ml	Day	Colonies per ml	Day	Colonies per ml	Day
2.4×10^2	2.5×10^6	5	1.3×10^7	6	1.2×10^8	5
1.1×10^3	1.2×10^5	6	3.4×10^6	6	4.6×10^5	6
1.5×10^3	9.0×10^3	2	4.9×10^6	2	7.4×10^6	2
1.5×10^3	3.0×10^4	1	6.4×10^6	2	5.7×10^5	3

* Five mice per group were used.

TABLE 4. Effect of injection of mortality-enhancing factor (MEF) on growth of *Listeria monocytogenes* JHH in organs of mice

Inoculum	Colony count per ml of tissue (geometric mean)							
	Expt 1 (hr)				Expt 2 (hr)			
	4	12	24	24	48	72	96	
Liver								
JHH*	1.1	1.7	8.1	2.9	95	25	4.7	
JHH + MEF	0	200	57	8.2	3.5×10^4	2.7×10^4	$1.6 \times 10^{4\dagger}$	
Spleen								
JHH	1.1	2.0	14	3.0	43	100	6.8	
JHH + MEF	40	17	59	9.1	7.0×10^4	1.8×10^4	$6.5 \times 10^{3\dagger}$	

* Dose in experiment 1 was 4.5×10^3 cells; dose in experiment 2 was 4.6×10^3 cells.

† Only one animal survived at this time.

TABLE 5. Influence of prior injection of BCG on growth of *Listeria monocytogenes* JHH in organs of mice

Inoculum ^d	Colony count per mg of tissue (geometric mean)						
	Day						
	1	2	3	4	5	6	7
Lung							
JHH ^b	8.5×10^5	5.9×10^5	2.1×10^5	310	8.9×10^4	920	1,600
JHH + BCG ^c	<690	1,100	1,800	<300	<400	190	<340
Liver							
JHH	3.3×10^4	5.9×10^5	1.2×10^5	4,200	2.0×10^5	1.9×10^5	3,900
JHH + BCG	<200	460	2,700	90	<42	420	<81
Spleen							
JHH	1,600	1.2×10^7	1.3×10^6	7.7×10^4	3.6×10^5	1.5×10^5	2,000
JHH + BCG	<1,000	890	1.1×10^5	2,000	110	3,700	<360
Blood							
JHH	2/3 ^d	3/3	2/3	0/3	1/3	2/3	0/3
JHH + BCG	0/3	0/3	0/3	0/3	0/3	0/3	0/3

^a Three mice per group were used.

^b Inoculum: 7.3×10^4 cells per 0.2-ml dose.

^c Inoculum: 9.1×10^4 living BCG cells were injected into the peritoneal cavity 6 days before challenge with *L. monocytogenes*.

^d Number of animals showing positive blood cultures/total number when one loopful of heart blood was streaked on a Tryptose Agar plate.

TABLE 6. Effect of prior injection of BCG on in vivo growth in mice of *Listeria monocytogenes* JHH in the presence of mortality-enhancing factor (MEF)

Inoculum ^d	Colony count per ml of tissue (geometric mean)						
	Day						
	1	2	3	4	5	6	7
Liver							
JHH + MEF ^a	3.5×10^3	7.3×10^3	1.2×10^7	2.0×10^5	5.2×10^5	6.3×10^4	4.5×10^4
JHH + MEF + BCG ^c	4.8×10^4	2.7×10^4	4.0×10^4	8.2×10^3	8.7×10^3	1.1×10^3	3.0×10^3
Spleen							
JHH + MEF	1.2×10^4	5.1×10^4	6.1×10^7	5.3×10^6	2.0×10^5	2.0×10^4	3.8×10^4
JHH + MEF + BCG	7.0×10^4	1.5×10^4	2.8×10^3	4.4×10^3	1.2×10^3	4.8×10^3	1.4×10^3
Blood							
JHH + MEF	0/3 ^d	3/3	3/3	1/3	1/3	0/3	1/2
JHH + MEF + BCG	0/3	1/3	1/3	1/3	2/3	0/3	0/3

^a Three animals per group were used.

^b Strain JHH (6.7×10^4 cells) was injected by intraperitoneal route simultaneously with 2 equal amounts of MEF.

^c BCG (9.1×10^4 living cells) was injected into peritoneal cavity 6 days prior to challenge.

^d Number of positive blood cultures/total number plated.

TABLE 7. Comparison of growth of *Listeria monocytogenes* A4413 in organs of mice born of *Listeria*-infected mothers and of mice born of healthy mothers

Mouse litter	Organ	Colony count per mg of tissue (geometric mean)				
		Days				
		1	2	3	4	5
Mice 5 to 6 weeks old, from:*						
Infected mother	Liver	410	550	6,500	3,600	1,700
Normal mother	Liver	1.4×10^4	5.7×10^4	5.4×10^5	3.8×10^5	4.3×10^5 †
Infected mother	Spleen	910	380	440	6,100	1.2×10^4
Normal mother	Spleen	3.5×10^4	3.7×10^4	7.0×10^5	6.1×10^5	9.4×10^5 †
Mice 7 to 8 weeks old, from:†						
Infected mother	Liver	440	2.3×10^4	1.2×10^4	8.2×10^6	1.1×10^5
Normal mother	Liver	920	1.5×10^4	1.2×10^5	2.4×10^7	1.2×10^5 †
Infected mother	Spleen	730	2.0×10^4	5.1×10^4	2.9×10^6	3.2×10^4
Normal mother	Spleen	400	1.5×10^4	1.1×10^5	1.2×10^5	2.5×10^5 †

* Inoculum: 1.3×10^3 cells per dose; three mice per group.

† Inoculum: 5.4×10^2 cells per dose; four mice per group for infected progeny; two mice per group of controls.

‡ Single survivor.

coming the effect of MEF, so that the number of bacteria recovered from the tissues of animals that received *Listeria*, MEF, and BCG was somewhat less than that found in the organs of mice receiving only *Listeria* strain JHH (Tables 5 and 6).

Since in utero infection of the fetuses of females infected with *Listeria* has been reported by numerous investigators (see Seeliger, 1961), it was considered of interest to determine the fate of the organisms in surviving mice born of infected mothers and to determine the immunological state of these animals. Pregnant mice were infected by intraperitoneal injection of approximately 3×10^5 cells of strain JHH. Of the 49 progeny born to 5 females, 27 survived and, at 5 to 6 weeks of age, were infected in the peritoneal cavity with 1.2×10^2 cells of strain A4413. Although no significant difference in the LD_{50} was noted between the test group and mice of the same age from uninfected females, a marked difference was noted in both the extent of gross pathological changes and in the number of organisms recovered from spleens and livers. The LD_{50} for mice born of infected females was 4.5 days (95% confidence limits: 3.4 to 6.0 days); that for mice from an uninfected parent was 3.8 days (95% confidence limits: 3.0 to 4.8 days). Control animals, however, showed much more extensive pathology, and the cell counts per ml in spleen or liver were higher (Table 7). However, in a similar

experiment utilizing mice 7 to 8 weeks old, no difference was observed between the test group and the control group. The number of organisms per ml of spleen or liver tissue was essentially the same in both groups, and changes in cell population paralleled each other quite closely.

DISCUSSION

Kautter et al. (1963) reported extensive experiments which indicated the influence of a number of factors on the lethal effect of *L. monocytogenes*. Among these factors were the age and physiological state of the host, the host species, and the strain of organism used. A striking difference was observed among infections produced through different routes. Exposure by the respiratory route produced a linear dose-response curve, and the lethal dose obtained on repeated titrations was relatively constant. Exposure by the intraperitoneal or intravenous routes generally resulted in a lower lethal dose but the dose-effect response was nonlinear and erratic. The LD_{50} in repeated titrations extended over a wide range. This difference in response to infection by the various routes is reflected in the results presented here. Infection of mice by the respiratory, intravenous, or intraperitoneal routes produced the same amount of growth of *L. monocytogenes* in the spleen, liver, and brain. After aerosol exposure, however, the colony counts obtained from lung suspensions were high within the first 24 hr

after infection and remained at a high level for up to 9 days. Infection by the other routes not only resulted in a somewhat lower colony count but also in a more gradual increase in the number of *Listeria* in the lungs. The standard deviation from the geometric means of the colony count was more uniform and smaller with the cultures obtained from animals infected by aerosol than with cultures from mice infected by the other routes (Table 2).

The difference between the in vivo growth of *Listeria* after respiratory exposure and that after infection by the other routes also may be explained in part by unpublished observations by Kautter. He observed that, after introduction of *Listeria* into the lungs, there was an immediate increase in the number of organisms; 2 hr after exposure, the mean increase in *Listeria* was approximately 1.6 times the number present at 0 hr, and by 4 hr the bacterial count increased about fivefold. In the peritoneal cavity, however, the organisms disappeared rapidly for the first 6 hr and then began to increase (Silverman, unpublished data). In agreement with this, we have also observed that when *Listeria* were injected into the peritoneal cavity 24 hr after the injection of sodium caseinate, the accumulated polymorphonuclear cells rapidly ingested the organism so that within 10 to 15 min the leukocytes were completely filled with bacteria. This suggests that, in the lung, phagocytosis by cells lining the alveolar passages holds the organisms in situ and permits intracellular multiplication. The intracellular location of the *Listeria* may offer protection against the bactericidal activity of the blood observed in the guinea pig (Silverman, unpublished data). On the other hand, the organisms introduced into the peritoneal cavity or directly into the blood stream would be directly exposed to this hazard. Differences in response among the mice could reflect individual differences in the bactericidal activity of the blood such as has been observed in studies with sera from guinea pigs.

The infrequent occurrence of *Listeria* in brain homogenates is in accordance with the observations of various authors. Seeliger (1961) mentioned that only after intracranial or intrathecal injection was neuro-listeriosis consistently produced in rodents. He suggested that the short time interval between infection and death in septic listeriosis precludes infection of the brain.

The greater number of organisms recovered after injection of MEF was in accordance with

previous observations on the greater mortality that followed injection of the cell extract with the organism. The reverse effect induced by the prior injection of BCG and the neutralization of the effect of MEF by injection of the acid-fast organism suggests that MEF exerts its effect in part, at least, on the reticuloendothelial system. Lurie (1939) reported enhanced activity by the cells of this system during infection with *Mycobacterium*. The exact role of MEF in *Listeria* infections is yet to be elucidated. It may be, as with the toxin of plague (Meyer, 1958), that, as the host responds to the infection by destroying the organism, the cellular component (MEF) is liberated. Thus, the host indirectly enhances the infection. On the other hand, in view of the latent nature of *Listeria* infections (Seeliger, 1961), it may be that when resistance is reduced because of stress, and if *Listeria* are present in an inapparent infection, the host becomes more susceptible to the MEF produced by the organisms already present in the tissues, and an acute infection is initiated. These hypotheses require further investigation of the pathogenesis of the disease and of the role of cellular components of the parasite.

Increased resistance after injection of BCG is in accord with previous observations by Pullinger (1936), Henderson et al. (1956), Dubos and Schaedler (1957), and Sulitzanu et al. (1962) with *Brucella abortus*, *Bacillus anthracis*, *Staphylococcus aureus*, and *M. fortuitum*. Elberg, Schneider, and Fong (1957), studying monocytes obtained from animals exposed to *Mycobacterium*, also observed increased resistance toward infection with *Brucella*.

The comparative studies with progeny of *Listeria*-infected females and those of healthy animals indicate that prenatal exposure of *Listeria* produced a temporary degree of immunity rather than a state of immune tolerance. Rees and Garbutt (1961) reported immunity in mice infected in utero with *Mycobacterium*. They observed a shortened survival time in their control group. Although in utero infection failed to increase the size, the gross pathology and bacterial growth were less extensive in this group than in the control group. The temporary nature of the immunity suggests that it may have been passive in nature.

LITERATURE CITED

- DUBOS, R. J., AND R. W. SCHAEDLER. 1957. Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections.

- I. Protective effects. *J. Exptl. Med.* **106**:703-717.
- ELBERG, S. S., P. SCHNEIDER, AND J. FONG. 1957. Cross-immunity between *Brucella melitensis* and *Mycobacterium tuberculosis*. Intracellular behavior of *Brucella melitensis* in monocytes from vaccinated animals. *J. Exptl. Med.* **106**: 545-554.
- HENDERSON, D. W., M. C. LANCASTER, L. PACKMAN, AND S. PEACOCK. 1956. The influence of a pre-existing respiratory infection on the course of another superimposed by the respiratory route. *Brit. J. Exptl. Pathol.* **37**:597-611.
- KAUTTER, D. A., S. J. SILVERMAN, W. G. ROESSLER, AND J. F. DRAWDY. 1963. Virulence of *Listeria monocytogenes* for experimental animals. *J. Infect. Diseases* **113**:167-180.
- LITCHFIELD, J. T. 1949. A method for rapid graphic solution of time-per cent effect curves. *J. Pharmacol. Exptl. Therap.* **97**:399-408.
- LURIE, M. B. 1939. Studies on the mechanism of immunity in tuberculosis. The mobilization of mononuclear phagocytes in normal and immunized animals and their relative capacities for division and phagocytosis. *J. Exptl. Med.* **69**:579-605.
- MEYER, K. F. 1958. Pasteurella, p. 400-436. In R. J. Dubos [ed.], *Bacterial and mycotic infections of man*, 3rd ed. J. B. Lippincott Co., Philadelphia.
- MILES, A. A., AND S. S. MISRA. 1938. Estimation of the bactericidal power of the blood. *J. Hyg.* **38**:732-749.
- NATIONAL SOCIETY FOR MEDICAL RESEARCH. 1961. Principles of laboratory animal care. *Bio-med. Purview* **1**:14.
- PULLINGER, E. J. 1936. The influence of tuberculosis upon the development of *B. abortus* infection. *J. Hyg.* **36**:456-466.
- REES, R. J. W., AND E. W. GARBUTT. 1961. Development of immunity to tuberculosis in adult life in adult mice injected with tubercle bacilli during foetal life. *Immunology* **4**:88-93.
- ROESSLER, W. G., AND D. A. KAUTTER. 1962. Modifications to the Henderson apparatus for studying air-borne infections. Evaluations using aerosols of *Listeria monocytogenes*. *J. Infect. Diseases* **110**:17-22.
- SEELIGER, H. P. R. 1961. *Listeriosis*. Hafner Publishing Co., New York.
- SILVERMAN, S. J., L. ELWELL, AND D. A. KAUTTER. 1961. A mortality enhancing extract isolated from *Listeria monocytogenes*. *J. Immunol.* **86**: 669-674.
- SULITZEANU, D., A. BEKIERKUNST, L. GROTO, AND J. LOEBEL. 1962. Studies on the mechanism of non-specific resistance to *Brucella* induced in mice by vaccination with BCG. *Immunology* **5**:116-127.