FAILURE OF ANIMAL PASSAGE TO INCREASE THE VIRULENCE OF *LISTERIA MONOCYTOGENES*

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ABSTRACT

SILVERMAN, SIDNEY J. (U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.), JAMES F. DRAWDY, AND DONALD A. KAUTTER. Failure of animal passage to increase the virulence of *Listeria monocytogenes*. J. Bacteriol. **86**:92–94. 1963.—Three strains of *Listeria monocytogenes*, JHH, A4413, and 53P380, were serially passed through mice, guinea pigs, or monkeys via the intracerebral, intraperitoneal, and intraspinal routes, respectively. The first two strains had been maintained on laboratory medium for some time; strain 53P380, received in the brain of a sheep that had died of listeriosis, was never inoculated into laboratory media. The virulence of the strains was tested by the intraperitoneal or respiratory route. An apparent increase in virulence for mice of strain JHH was observed after animal passage. However, if the same strain maintained on Tryptose Agar and Brain Heart Infusion broth was injected in a suspension of normal mouse brain or spleen and liver, there was no difference in the virulence of the strain between animal-passaged and media-grown cells. An adjuvant effect was noted also with broth-grown cells suspended in mineral oil (Bayol F) or in alcohol or acetone extracts of normal mouse brain. A decrease in virulence for guinea pigs occurred with strain 53P380 after a series of passages by the intraperitoneal route in the same animal.

The restoration or enhancement of the virulence of microorganisms by passage through susceptible animals has been mentioned by various authors (MacCleod, 1958). Jones and Berman (1951) described the selection of smooth, virulent strains of *Brucella abortus* after the inoculation of the less virulent mucoid strains. Braun (1949) demonstrated the selection of smooth, virulent *Brucella* in the presence of sera from susceptible animals but not in the presence of sera from resistant animals. Although increased virulence of *Listeria monocytogenes* by animal passage has been suggested (Seeliger, 1961; Osebold and Inouye, 1954), no data seem to be available that indicate changes in virulence after animal passage. Serial passage in mice, guinea pigs, and monkeys was performed to determine changes in virulence of organisms maintained in this manner.

MATERIALS AND METHODS

L. monocytogenes JHH, isolated from a case of listerial meningitis at The Johns Hopkins Hospital, was received from L. E. Cluff. Strain A4413 was isolated at the Communicable Disease Center, Chamblee, Ga.; C. Olson, Jr. sent us this culture from the University of Wisconsin collection. Strain 53P380 was received from J. Orsborn of Colorado State University. It was received in the sheep brain taken at autopsy from a fatal case of listeriosis and was maintained in the brain at −20 C without growth on laboratory media.

Strains JHH and A4413 were cultured in Brain Heart Infusion broth for the first injection of the series. Subsequent passage was in homogenized brain or spleen and liver obtained from moribund animals.

A 16-hr culture of strain JHH was diluted to contain approximately five cells per 0.02-ml dose. Mice were injected by the intracerebral route. In 2 to 3 days, when the mice appeared moribund, they were killed, and brain, spleen, and liver were harvested. The organs were ground in a mortar and suspended in sufficient 1.0% Tryptose to give a 10.0% suspension (v/v). Spleens and livers were pooled, ground, and suspended together. The suspension was gently centrifuged to remove the larger particles. Two series of passages were

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maintained. Series A consisted of a brain-to-brain passage; in series B the spleen-liver homogenate was injected into the brain. The tissue suspensions were adjusted to contain approximately 10^3 cells per dose.

Virulence titrations were performed at intervals, using the tissue suspensions. The bacterial content of the tissue suspension was determined and the desired dilutions prepared in 1% Tryptose; the suspensions were kept at −20 C when not in use. At the time of injection, the number of organisms in the dilutions was determined again. Swiss-Webster mice, Hartley guinea pigs, and rhesus monkeys from the Fort Detrick stock were used. Animals were maintained and used in compliance with the principles established by the National Society for Medical Research (1961). The intraperitoneal or respiratory routes were used for virulence titrations, performed as described by Roessler and Kautter (1962).

The sheep brain containing strain 53P380 was homogenized and injected directly into mice by the intracerebral route. This strain was passed 24 times through mice and then through guinea pigs; the pigs were infected by the intraperitoneal route.

L. monocytogenes A4413 was used both for a series of intraspinal passages through rhesus monkeys and for respiratory passage through mice. After the first intraspinal injection of the Brain Heart Infusion culture, the inoculum for monkey passage was a brain suspension from the previous passage. The inocula varied from 2.8×10^3 to 5.6×10^7 cells. For the initial respiratory exposure in mice, slightly more than the respiratory LD_{50} (3.5×10^3 cells) was administered. The moribund animals were killed, and the spleens and livers were homogenized together and used for the succeeding passage. To obtain a tissue suspension satisfactory for passage through the nozzle of the Collison spray device (Roessler and Kautter, 1962), the tissue was first mixed in a Waring Blendor with sand and 0.1% Tryptose in saline. The homogenate was then centrifuged in an International PR2 centrifuge at $180 \times g$ for 60 min. The decanted supernatant fluid was filtered two times through a coarse sintered-glass filter and then two times through a medium filter. The filters were washed each time with Tryptose saline. Suspensions prepared in this way contained only particles capable of passage through the spray device.

TABLE 1. *Virulence for mice of Listeria monocytogenes maintained by serial passage in mice*

No. of passages	LD_{50}			Control in suspension of normal mouse tissue
	Series A*	Series B†	Control‡	
5	1.9×10^4	3.6×10^4	2.7×10^5	1.2×10^4
5				9.0×10^4
10	2.5×10^4		1.5×10^6	8.3×10^3
35	7.1×10^4	3.6×10^4		
57	10^4	3.8×10^4	5.2×10^4	

* Brain-to-brain passage.

† Infected spleen and liver tissue injected into brain.

‡ Grown in Brain Heart Infusion broth at 37 C for 16 hr.

RESULTS

Table 1 indicates an apparent tenfold increase in the virulence of *L. monocytogenes* JHH passed five or more times through mice, when compared with the same strain maintained on laboratory medium. However, when the cells of broth-grown *Listeria* were suspended in a 10% brain homogenate from normal mice, a similar decrease in the LD_{50} was observed. Suspension of *L. monocytogenes* in Bayol F, a light mineral oil, or in the acetone or alcohol extract of normal mouse brain also reduced the number of organisms necessary to kill 50% of the animals within 15 days. The LD_{50} for the broth suspension was 3.4×10^4 cells; for the cell suspension in oil, it was only 5.4×10^2 cells; for the two brain extracts, the LD_{50} was less than 10^3 cells. When the brain suspension of the tenth passage was used as an inoculum for a single passage through Brain Heart Infusion broth, the intraperitoneal LD_{50} for this culture was 3.4×10^5 cells. This was not significantly different at the 95% level from the LD_{50} for the culture maintained continuously on laboratory media (1.5×10^6 cells with 95% confidence limits of 3.4×10^5 to 6.1×10^6 cells). After 57 passages, no alteration in virulence was observed and the experiment was concluded.

The intraperitoneal LD_{50} for *L. monocytogenes* 53P380 for mice decreased from 1.7×10^4 cells to 1.5×10^2 cells after 24 intracranial passages in mice plus 16 passages through guinea pigs by

the intraperitoneal route. It is likely that this decrease was also the result of the homogenized tissue, since there was no alteration of the respiratory LD_{50} for mice.

During the intraperitoneal passage in guinea pigs, there appeared to be a decrease rather than an increase in virulence for this host. The incubation period became longer, and at autopsy the number of *Listeria* recovered from the tissues decreased. After the eighth serial passage, the pigs were apparently healthy at 14 days and no organisms were recovered from the tissues upon autopsy and culture. The virulence of this strain in the original sheep brain was compared with that in mouse brain after 24 intracranial passages and with the organisms in the spleen-liver suspension from the eleventh guinea pig passage via the peritoneal cavity. The LD_{50} values were 2.7×10^6, 1.7×10^6, and 5.0×10^6, respectively.

L. monocytogenes A4413 passed through monkeys 22 times by intraspinal injection also failed to show an alteration in virulence. No deaths resulted among the six monkeys exposed to doses ranging from 10^7 to 10^8 cells by the respiratory route. Fever, positive blood cultures during the first 24 hr, and the formation of antibodies against *Listeria* indicated that an infection had been established. However, similar findings were obtained with this strain maintained on laboratory media.

After 26 passages in mice via the respiratory route, no significant change had occurred in the respiratory LD_{50} of strain A4413. The LD_{50} of the mouse-passaged strains was 3.3×10^3 cells, whereas that of the culture maintained in laboratory medium was 4.3×10^3 cells.

DISCUSSION

It is apparent from these results that no increase in virulence occurred after rather extensive animal passage of strains of *Listeria*. Enhancement occurred for neither the host species used for passage nor for other animal species. Owen et al. (1961) experienced a similar failure to increase the virulence of *Pasteurella tularensis* by passage through laboratory animals. These same authors were unable to detect any increase in virulence of *P. tularensis* during the course of several natural epizootics.

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