

A More Malignant Course of Leprosy Infection in
Armadillos After Inoculation with Sonicated
Suspension of *Mycobacterium leprae*

TO THE EDITOR:

After 15 years of experience with the armadillo as a model for experimental leprosy, the rate of positivity and the yield of

Mycobacterium leprae from organs still represent a matter of concern.

As in most mycobacterial species, *M. leprae* form large aggregates in suspensions

THE TABLE. Yield of *M. leprae* from the armadillo tissue 24 months after inoculation.

Armadillo no.	Organ weight (g)		<i>M. leprae</i> concentration/g		Total harvest		Calculated ^a wet weight of <i>M. leprae</i>
	Liver	Spleen	Liver	Spleen	Tissue (g)	<i>M. leprae</i>	
56 ^b	128.4	17.3	5.1×10^9	1.8×10^{10}	145.7	9.6×10^{11}	0.96 g
58	188.5	89.2	2.0×10^{10}	3.3×10^{10}	277.7	6.7×10^{12}	6.7 g
61	93.1	21.5	1.5×10^{10}	3.3×10^{10}	114.6	2.1×10^{12}	2.1 g
23	111.4	27.0	2.7×10^{10}	5.1×10^{10}	138.4	4.3×10^{12}	4.3 g
55	213.5	69.7	4.8×10^{10}	3.9×10^{10}	283.2	1.2×10^{13}	12.0 g
57	138.4	35.3	1.5×10^{10}	4.2×10^{10}	173.7	3.5×10^{12}	3.5 g
53	184.0	96.3	9.6×10^9	6.3×10^{10}	280.3	7.8×10^{12}	7.8 g
60	102.5	38.4	4.8×10^8	6.2×10^9	140.9	2.8×10^{11}	0.28 g
54	95.2	28.6	3.2×10^8	7.2×10^8	123.8	2.9×10^{10}	29 mg
51	71.4	17.7	1.3×10^5	3.9×10^5	89.1	1.6×10^7	0.016 mg
52	69.5	15.4	6.0×10^5	3.3×10^5	84.9	4.6×10^7	0.047 mg
Total	1395.9	456.4			1852.3		37.669063 g

^a 10^9 *M. leprae* wet weight \cong 1 mg.

^b Sacrificed 18 months after inoculation.

prepared from infected tissue. In cultivable mycobacteria, the dispersion of cell aggregates enhances the count of colony forming units (CFU). According to Brown (¹), a brief exposure of BCG to ultrasonic radiation enhanced the count of CFU by 6.7 up to 15.3 times compared with nondispersed suspensions. When an immersion-type sonic bath was used for a period of 2 min, no obvious reduction of the viability of mycobacteria could be seen.

In our experiments, we used an immersion-type ultrasonic bath (SONOREX RK 102; Bandelin Electronic, Berlin), with the HF-frequency of 50 kHz and 120 W power, for the sonication of suspensions of *M. smegmatis* and *M. fortuitum*. In cultivation trials with suspensions treated for 1 to 5 min, an increase (15.9 times average) of CFU could be found compared with nontreated suspensions. The optimum sonication time was 3 min, with no significant differences between 1, 2, and 3 min of treatment.

An inoculum containing 8.8×10^8 *M. leprae* was treated for 1 min directly before intravenous inoculation in armadillos; a similar inoculum was used without treatment. A total of 13 nine-banded armadillos were infected with a sonicated suspension of *M. leprae* from spleen tissue (received from Drs. Dhople and Storrs, Medical Research Institute, Florida Institute of Technology, Melbourne, Florida, U.S.A.). Two of the infected animals died 2 to 3 months

later due to nonspecific causes; one animal was sacrificed 18 months after infection (advanced leprosy). The remaining 10 animals were examined 24 months after infection, and all of them had developed systemic leprosy (The Table).

In nine infected animals, a high count of *M. leprae* was found in liver and spleen. Especially in three of them (nos. 58, 55, and 53), immense enlargements of liver and spleen (over 300% and 1200%, respectively) were observed. The highest harvest from one animal (no. 55) was 1.2×10^{13} , or approximately 12 g *M. leprae* (wet weight). Six animals developed numerous cutaneous lepromas. In animal no. 56, which had to be examined at 18 months after infection, more than 70 cutaneous lepromas on the abdomen side and in the skin folds between the carapace bands were found. The collective weight of the larger lepromas was 56.8 g.

Armadillos infected with a nonsonicated suspension of *M. leprae* showed a yield and a positivity of infection comparable to those given in the literature. In the group of 6 animals, 4 were positive with an average harvest of 5.3×10^8 /g in the spleen and 2.3×10^8 /g in the liver tissue (data not shown).

As in other mycobacterial species, the viable count of *M. leprae* could apparently be enhanced by mild ultrasonic vibration. The single bacillus or aggregates of a few cells became better distributed in the blood, es-

pecially in small capillaries of the organs and the skin. This may explain the high yield and the dissemination of cutaneous lepromas in some infected animals. The viability of *M. leprae* was not negatively influenced by ultrasonic vibration. The increase of the viable count of *M. leprae* might have been expected due to similar findings in other pathogenic and saprosaprophytic mycobacteria. Accordingly, mild ultrasonic vibration is recommended for experimental infection in armadillos and other animal models for leprosy.

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Acknowledgment. This work was supported by the German Leprosy Relief Association.

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