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Prevention by Polymyxin B of Endotoxin Lethality in Mice

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Certain of the cyclic cationic polypeptide antibiotics, polymyxin B, colistin, and tyrocidine, prevent the lethal effect of endotoxin for chick embryos when admixed with the toxin prior to intravenous injection. In this system, 25 μg of polymyxin B sulfate prevented the toxicity of 0.217 μg (241 LD_{50}) of *Escherichia coli* endotoxin; an antibiotic-endotoxin weight ratio of 115:1. (D. Rifkind and J. D. Palmer, J. Bacteriol. **92**:815, 1966). The present study was undertaken to determine whether polymyxin B will also prevent endotoxin lethality in a mammalian host system.

E. coli 0111:B4 Westphal endotoxin (Difco), alone or mixed with polymyxin B sulfate (Burroughs Wellcome & Co., Tuckahoe, N.Y.), was injected intravenously, in a final volume of 0.2 ml in pyrogen-free saline, into 8- to 10-week-old female Swiss-Webster mice (Simonsen Laboratories, White Bear Lake, Minn.). Lethality was scored at 48 hr, and the LD_{50} was estimated by the Reed-Muench method (L. J. Reed and H. Muench, Am. J. Hyg. **27**:493, 1938). A 50-μg amount of polymyxin B was used, as this dose produced no lethality (0 of 20), whereas 100 μg resulted in a 33% (23 of 70) mortality. The LD_{50} of endotoxin alone was 36 μg, and this was not significantly decreased by the admixture of 50 μg of polymyxin B (21 μg, Table 1).

The antibiotic-endotoxin ratio which could be tested in the Swiss-Webster mouse system, was, however, relatively small as compared with that which provides protection in chick embryos (see above). As the mice were relatively sensitive to polymyxin B, the antibiotic-endotoxin ratio could be increased only by decreasing the resistance of mice to endotoxin. This was accomplished by two methods, the first of which was blockade of the reticuloendothelial system with lead acetate (H. Selye, B. Tuchweber, and L. Bertok. J. Bacteriol. **91**:884, 1966). A 1-mg amount of lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$, (Fisher Scientific Co., Fair Lawn, N.J.) was added to the endotoxin or endotoxin-polymyxin B mixture prior to injection. Sterile 5% dextrose in water was used as the diluent, since lead acetate precipitated in normal saline. Lead acetate decreased the endo-

TABLE 1. *Influence of polymyxin B on endotoxin lethality for mice*

Endotoxin	Lethality (dead/total)	
	Without polymyxin B	Polymyxin B, 50 μg
μg		
100	22/30 (73%)	10/10 (100%)
50	37/50 (74%)	18/20 (90%)
25	12/20 (60%)	12/20 (60%)
12.5	3/10 (30%)	3/10 (30%)
0		0/20 (0%)
LD_{50} (μg)	36	21

TABLE 2. *Influence of polymyxin B on endotoxin lethality for lead acetate-treated mice*

Endotoxin (μg)[a]	Lethality (dead/total)	
	Without polymyxin B	Polymyxin B, 50 μg
25	20/20 (100%)	9/10 (90%)
10	16/20 (80%)	9/10 (90%)
5	18/30 (60%)	7/10 (70%)
2.5	17/30 (57%)	4/10 (40%)
0		0/20 (0%)
LD_{50} (μg)	3.5	3.3

[a] In each instance, 1 mg of lead acetate was used.

TABLE 3. *Influence of polymyxin B on endotoxin lethality for adrenalectomized mice*

Endotoxin	Lethality (dead/total)	
	Without polymyxin B	Polymyxin B, 50 μg
μg		
0.4		3/5 (60%)
0.2		5/10 (50%)
0.1	15/15 (100%)	3/15 (20%)
0.05	14/17 (82%)	0/10 (0%)
0.025	9/10 (90%)	
0.012	2/4 (50%)	
0		0/5 (0%)
LD_{50} (μg)	0.02	0.19

toxin LD_{50} from 36 to 3.5 μg; however, there was no protection afforded by the addition of polymyxin B (Table 2). The second method of decreasing the resistance of the mice to endotoxin involved the use of adrenalectomized 8- to 10-week-old female CFW mice (Carworth Farms, Inc., New City, N.Y.). In these mice, the endotoxin LD_{50} was further decreased to 0.02 μg. When admixed with 50 μg of polymyxin B, the LD_{50} was 0.19 μg (Table 3). Thus, 50 μg of polymyxin B prevented the lethal effect of 0.17 μg of endotoxin; an antibiotic-endotoxin ratio of approximately 300:1.

These data demonstrate that polymyxin B prevents endotoxin lethality in adrenalectomized mice as well as in chick embryos, and suggest that the determining factor is the antibiotic-endotoxin ratio rather than the host system.

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