Bioassay of Endotoxin Inactivation in the Lead-Sensitized Rat[1] (34844)

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Lipopolysaccharides from gram-negative bacteria, *i.e.*, endotoxins, when administered parenternally, induce profound cardiovascular collapse and ultimately lethality in shock in various animals (1, 2). However, in comparison to other species, rats are highly resistant to the physiopathological effects of endotoxemia, *e.g.*, Zweifach (3) has compiled the following relative LD_{75} for circulatory collapse: cat, 1; rabbit, 2.5; dog, 8; guinea pig, 20; and the rat, 300. Thus, despite the great utility of rats in various experimental shock models, the high resistance to endotoxin has detracted from use of rats in analysis of the cardiovascular events in endotoxin shock.

Recently, Selye, Tuchweber, and Bertók (4) reported that a single intravenous injection of lead acetate markedly sensitized rats to lethal endotoxin shock and suggested use of this synergism as a convenient bioassay for minute amounts of endotoxin. The present study evaluated the use of the lead-treated rat in the bioassay of the ability of rat organ homogenates to inactivate endotoxin.

Methods. Male Sprague-Dawley rats of the Holtzman strain, with a body weight range of 300 ± 20 g, were maintained on Purina Chow and water *ad libitum* at a constant temperature of 24 ± 1° for 7–10 days prior to use. Endotoxin was purchased from Difco Laboratories, Detroit, Michigan, as the Boivin lipopolysaccharide preparation from *Salmonella enteritidis*. Endotoxin was prepared daily in 0.9% NaCl. Lead acetate (Mallinckrodt Chemical Works, St. Louis, Mo., No. 5684) was prepared daily in distilled water.

Organs for homogenates were obtained from rats sacrificed by a stunning blow to the head and exsanguinated by severing of the cervical blood vessels. One per cent (w/v) homogenates were prepared in pH 7.4 phosphate-buffered saline prepared by adding 1 vol of 0.2 *M* potassium phosphate buffer to 9 vol of 0.85% NaCl. Homogenizations were performed in a Teflon pestle-glass tube, Potter-Elvehjem type system (Size B, A. R. Thomas Co., Philadelphia, Pa.). The pestle was driven by a motor stirrer at 930 rpm, and temperature was maintained at 0–10°. Incubation of liver, spleen, lung, kidney, or brain homogenates was performed in 50-ml Erlenmeyer flasks containing 9 ml of the homogenate and 1 ml of 30 μg/ml of endotoxin in saline. The samples were gassed with 95% O_2–5% CO_2, stoppered, and incubated for 180 min at 37° in a Dubnoff Metabolic Shaker oscillating at 100 motions/min. Control samples were treated similarly, except that they were incubated at 4° until assay time.

All injections were made into the dorsal vein of the penis in rats under light ether anesthesia. A 1-ml endotoxin injection—either in saline or homogenate—was immediately followed by iv injection of 5 mg of lead acetate. In all the inactivation studies rat weights were kept uniform at 300 ± 20 g and a standardized endotoxin dose of 3 μg and lead acetate dose of 5 mg were used. For each homogenate inactivation trial, 8–10 rats were used to evaluate the inactivation. Lethality was followed for 48 hr—but most rats succumbed between 14–20 hr. Intergroup lethality was compared statistically by use of the chi-square test corrected for continuity by Yates' factor.

Results and Discussion. Effects of lead acetate treatment on lethality to endotoxin. As indicated in Fig. 1, lead acetate treatment resulted in an extreme sensitization to en-

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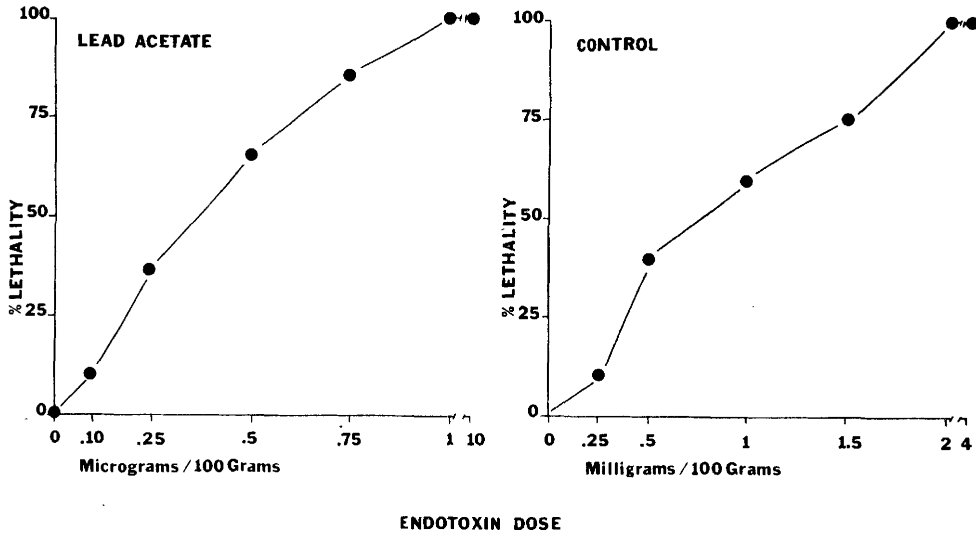

FIG. 1. Effect of lead acetate on lethality to endotoxin. Each point represents 20–25 rats per group. Endotoxin was administered iv immediately prior to 5 mg of lead acetate iv.

dotoxin; 2000 μg or 2 mg/100 g produced 100% lethality in control rats, whereas only 1 μg/100 g resulted in 100% lethality in the lead-treated group. The control group consists of rats which received 1 ml distilled water iv in lieu of the lead acetate and rats which received no second injection, *i.e.*, only the endotoxin. These control groups were combined since no differences in lethality were observed between them. In addition, lead acetate alone was evaluated for toxicity and was nonlethal at doses up to 25 mg iv. Autopsies revealed typical, severe gastrointestinal congestion in the control rats treated with endotoxin. In addition, the lead-treated rats, while also displaying marked intestinal congestion, had greater pulmonary hyperemia. Selye *et al.* (4) have also reported that the organ lesions—both gross and microscopic—were essentially the same in control and lead acetate-sensitized rats treated with endotoxin.

Effect of incubation with organ homogenates on toxicity of endotoxin. As shown in Table I, incubation of endotoxin with liver and spleen homogenates at 37° markedly re-

TABLE I. Effect of Incubation with Organ Homogenates on Toxicity of Endotoxin.

Organ homogenate	Incubation temperature (°C)	Mortality (deaths/no. rats in trial)				Totals	
		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Mortality	Per cent lethality
Liver	4	8/8	8/8	8/10	8/10	32/36	89%
	37	2/8	2/8	1/10	0/10	5/36*	14%
Spleen	4	8/8	8/8	7/8	—	23/24	96%
	37	1/8	1/8	0/8	—	2/24*	8%
Lung	4	8/8	7/8	8/8	—	23/24	96%
	37	6/8	5/8	6/8	—	17/24*	71%
Kidney	4	7/8	8/8	7/8	—	22/24	92%
	37	8/8	8/8	7/8	—	23/24	96%
Brain	4	8/8	7/8	8/8	—	23/24	96%
	37	8/8	6/8	7/8	—	21/24	88%

* $p < .001$ as determined by chi-square test.

duced toxicity as compared to control incubations performed at 4°. In contrast, homogenates of kidney and brain performed little or no inactivation of the endotoxin. Lung homogenate was only slightly active. Occasionally, injection of lung and kidney homogenates produced acute deaths and these rats were not included in the statistical analysis. The acute deaths usually followed very rapid iv injections and were easily eliminated by limiting the rate of injections to 1 ml in 15 sec.

Numerous theories of host-defense against endotoxin have centered on the ability of various tissues to inactivate endotoxin. Fine and colleagues (5, 6) have promulgated the idea that the macrophages of the hepatic and splenic components of the reticuloendothelial system (RES) play a pivotal role in shock in general, and endotoxin shock in particular. The present study confirmed the findings of Fine *et al.* (5, 6) and other investigators (7, 8) that the liver and spleen are active sites of endotoxin inactivation. Indeed, the relatively moderate capability of the lung to inactivate endotoxin may reflect its comparatively meagre reticuloendothelial constituent. In addition, this study has the added importance of employing the identical species, *i.e.*, the rat, for both the tissue donor and assay recipient. It is hoped that use of the lead-sensitized rat bioassay model will allow dissection of the cellular, subcellular, and molecular events in endotoxin inactivation. The mechanism of lead sensitization to endotoxin is unknown and merits additional study.

Summary. Inactivation of endotoxin by homogenates of rat liver, spleen, lung, brain, and kidney was bioassayed in the lead-sensitized rat. The assay was sensitive and reproducible in the range of 0.3–3.0 μg per rat of *S. enteritidis* lipopolysaccharide iv. Incubation of endotoxin with 1% (w/v) liver or spleen homogenates produced marked endotoxin inactivation. Slight inactivation was observed with lung homogenate. Kidney and brain homogenates were ineffective.

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