# Effects of Lead Acetate on Sensitivity to Shock, Intravascular Carbon and Endotoxin Clearances, and Hepatic Endotoxin Detoxification[1] (37048)

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Gram-negative bacillary lipopolysaccharide, *i.e.*, endotoxin, is a ubiquitous constituent of the intestinal flora and under diverse stresses has been shown to traverse the mucosal barriers, gain access to the circulation, and unleash cardiovascular degeneration which may eventuate in shock (1, 2). Based on the investigations of Fine (1), Caridis *et al.* (2) and others (3, 4) the primary mechanism of host defense against endotoxemia and the resultant endotoxicosis is thought to reside in the sequestering and detoxifying activities of the hepatic and splenic macrophage constituents of the reticuloendothelial system (1, 4).

Recently lead salts have been shown to induce a profound sensitization to endotoxicosis in rats (5–7), mice (8–10) and chicks (11). Furthermore, shock has been reported as an ominous complication in children with lead poisoning (12). The present study investigated the effects of lead acetate on the phagocytic and endotoxin detoxifying activities of the reticuloendothelial system. Specifically, the vascular clearance of colloidal carbon, the clearance of endotoxin, and the status of the hepatic endotoxin detoxifying system were measured in lead-treated rats. In addition, the ability of lead treatment to sensitize rats to four other types of experimental shock is presented.

*Materials and Methods. Endotoxin.* The lipopolysaccharide of *Salmonella enteritidis* prepared by the Boivin method was obtained from Difco Laboratories, Detroit, MI. For shock induction endotoxin was prepared daily in sterile 0.9% sodium chloride.

*Lead Acetate.* Lead acetate was obtained from the Mallinckrodt Chemical Works, St. Louis, MO, Code 5684. It was prepared daily in distilled, deionized water at a concentration of 5 mg/ml.

*Shock models.* Male rats of the Holtzman strain, 300 g body weight, were maintained on Purina Chow and water *ad libitum* at 76–78°F for 7–10 days prior to use. Endotoxin shock was produced by injection of endotoxin into the dorsal vein of the penis under light ether anesthesia. The endotoxin injection was immediately followed by either 5 mg of lead acetate or as a control 5 mg of sodium acetate. Lethalities were followed for 48 hr but most rats succumbed between 14 and 24 hr.

Traumatic shock was produced by 325 revolutions in a Noble Collip drum apparatus as previously published in detail (13). Lead or sodium acetate at a dose of 5 mg was administered under ether anesthesia 15 min prior to the tumbling stress. Hind limb ischemia was produced by the bilateral application of rubber band tourniquets for 270 min according to the procedure of Serkes, Lang and Pareira (14). Either 5 mg of lead acetate or sodium acetate were injected upon release of the tourniquets. Splanchnic ischemia shock was produced by 30 min occlusion of the superior mesenteric artery (15). Upon release of the occlusion, 5 mg of either lead or sodium acetate were administered iv. Anaphylactic shock was induced using horse serum injections according to the regimen of Adamkiewicz, Sacra and Ventura (16). Lead acetate or sodium acetate (5 mg/rat) was injected iv simultaneously with the eliciting dose of horse serum.

*Intravascular clearances of carbon and endotoxin.* The intravascular phagocytic removal

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TABLE I. Effect of Lead Acetate on Resistance to Experimental Shock.

| Shock model | Lead acetate 5 mg, iv | No. of rats | Percentage lethality |
|---|---|---|---|
| Endotoxin (1 μg/rat) | — | 24 | 0 |
| | + | 24 | 83[a] |
| Tumbling trauma (325 rev) | — | 24 | 8 |
| | + | 40 | 73[a] |
| Hind limb ischemia (270 min) | — | 14 | 14 |
| | + | 17 | 82[a] |
| Superior mesenteric artery occlusion (30 min) | — | 22 | 27 |
| | + | 25 | 88[a] |
| Horse serum anaphylaxis | — | 19 | 0 |
| | + | 20 | 40[a] |

[a] $p < .05$ as compared to control group which received 5 mg sodium acetate iv.

rate for colloidal carbon was evaluated as previously described in detail (17). In brief, colloidal carbon was injected iv at a dose of 8 mg/100 g body weight and half-time values for carbon clearance from the blood were determined from logarithmic plots of carbon concentration as determined densometrically vs time in minutes. Clearance rates are expressed as half-times in minutes.

Endotoxin clearances in normal rats were evaluated by sampling blood at 15, 30, 60, 120 and 240 min after iv injection of a standardized 0.5 mg test dose of endotoxin. The blood samples were diluted 40-fold in 0.9% saline and bioassayed for endotoxin content in lead-treated assay rats. The disappearance of toxicity from the blood as assayed in lead-treated rats presumably reflects the vascular clearance of endotoxin by the hepatic and splenic macrophages. In addition, as a critical test of the capability of the bioassay to detect alterations in endotoxin clearance, the clearance bioassay was performed in rats rendered tolerant to endotoxin. The tolerance regimen consisted of 1 mg doses of endotoxin on days 1, 3 and 5 prior to a 0.5 mg dose of endotoxin on Day 7.

*Assay of endotoxin detoxification.* The ability of liver homogenates to inactivate endotoxin was evaluated as previously described (3, 6). In essence, homogenates of rat liver were prepared in pH 7.4 phosphate buffered saline and were incubated for 180 min at either 4 or 37° with 1 μg/ml of endotoxin. The homogenates were then assessed for toxicity in lead-treated assay rats and the decrease in original endotoxin toxicity during incubation was noted and defined as detoxification.

*Analysis of data.* All lethality data were evaluated for statistical significance using the chi-square test corrected for continuity by the Yates' factor. Carbon clearance half-times were compared using Student's $t$ test. A 5% significance level was employed.

*Results. Lead sensitization to experimental shock.* In confirmation of earlier reports (5, 6) lead produced a marked sensitization to the lethal effects of iv endotoxin such that 1 μg/rat produced 83% lethality when injected with lead but was completely innocuous without lead co-treatment (Table I). Lead administration also increased shock lethality subsequent to tumbling trauma, 73 vs 8% lethality; hind limb ischemia, 82 vs 14% lethality; superior mesenteric artery occlusion, 88 vs 22% lethality, and anaphylactic shock, 40 vs 0% lethality. As previously reported a 5 mg dose of lead acetate alone is nonlethal in the nonstressed rat (6).

*Intravascular clearances of carbon and en-*

TABLE II. Effect of Lead Acetate on the Intravascular Phagocytic Clearance of Colloidal Carbon.[a]

| Experimental group | No. of rats | Intravascular half-time (min) |
|---|---|---|
| Control, sodium acetate | 46 | 17.7 ± 0.92 |
| Time (min) after lead acetate | | |
| 5 | 11 | 13.9 ± 1.10 |
| 15 | 8 | 33.3 ± 4.52[b] |
| 30 | 8 | 28.0 ± 1.70[b] |
| 60 | 8 | 32.6 ± 3.26[b] |
| 120 | 8 | 34.0 ± 1.98[b] |
| 240 | 8 | 35.0 ± 3.53[b] |

[a] Colloidal carbon was administered at a dose of 8 mg/100 g body weight. Lead acetate was injected iv at a dose of 5 mg/rat. Control rats received 5 mg of sodium acetate iv. Half-time values are expressed as the mean ± standard error.

[b] Signifies a $p < .05$ as compared to control group.

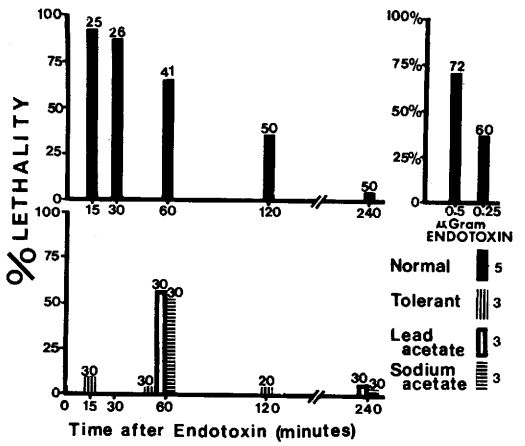


FIG. 1. Bioassay of the intravascular removal of blood endotoxin in rats. The numbers above each bar designate the number of assay rats/experiment. Normal rats ($N = 5$) were untreated prior to the endotoxin clearance challenge. Lead acetate ($N = 3$) was administered iv at a dose of 5 mg; sodium acetate ($N = 3$) was administered iv at a dose of 5 mg. All groups received 0.5 mg of endotoxin at time zero.

*dotoxin after lead treatment.* As indicated in Table II, the intravascular half-time for colloidal carbon clearance was increased within 15 min after lead treatment and remained prolonged for 240 min.

Figure 1 presents the bioassay of endotoxin clearance in the rat. As indicated by the two-point bioassay standards in the upper right panel, lead-treated rats given 0.5 μg and 0.25 μg of endotoxin had lethalities of 71 and 37%, respectively. In the upper left panel is depicted the bioassay of the vascular disappearance of a 0.5 mg dose of endotoxin in untreated, control male rats of 300 g body weight. As depicted, lethality in the bioassay groups declined progressively at 15, 30, 60, 120 and 240 min. The lower panel shows the blood bioassay data at 60 and 240 min after 0.5 mg of endotoxin in rats treated with either lead acetate or sodium acetate. No differences in blood endotoxin were detected at 60 and 240 min which suggests that lead did not influence the rate of endotoxin clearance. Also depicted in Fig. 1, is the bioassay of endotoxin clearance in rats rendered tolerant to endotoxin. Note that even at 15 min, as well as 60 or 120 min, no endotoxin was bioassayed in the blood. The rapid clearance of blood endotoxin in tolerant rats substantiates the sensitivity of the bioassay to detect altered clearance rates.

*Hepatic detoxification of endotoxin after lead.* The ability of lead acetate treatment to influence the capability of the liver to detoxify endotoxin was evaluated both *in vivo* (Table III) and *in vitro* (Table IV). As indicated in Table IV, 1% homogenates from control rats incubated with endotoxin reduced the lethality from 92 to 4%. When livers were removed at 30, 60, 180 and 480 min after 5 mg of lead acetate iv, no significant impairment of endotoxin inactivation was noted. In Table IV are presented data relevant to the ability of lead to influence the hepatic detoxification of endotoxin when added to homogenates *in vitro*. In these studies, 1 and 0.5% homogenates of rat livers were incubated with endotoxin in the presence of either 5 mg/ml lead acetate or sodium acetate. The homogenates were then bioassayed for endotoxin detoxification. As indicated, lead did not diminish the ability of either homogenate to detoxify endotoxin.

*Discussion.* Endotoxemia and the lethal syndrome accompaning endotoxicosis—generally termed "endotoxin shock"—is considered to underlie the clinical state of septic shock and indeed may be a common denominator in the pathogenesis of many forms of shock. In 1966 Selye, Tuchweber and Bertók (5) reported that lead among a host of heavy metals studied possessed the unique ability to markedly sensitize rats to endotoxin shock. Selye, Tuchweber and Bertók (5) and Filkins (3, 6) suggested that lead acts by inactivating the reticuloendothelial system (RES). Trejo and DiLuzio (18) subsequently evaluated the functional defects in the RES induced by lead, and presented data suggesting an enhanced phagocytic state as evaluated by carbon clearance and a depressed capacity of the liver and spleen to detoxify endotoxin. They suggested therefore that the mechanism of lead-induced hypersensitivity to endotoxin is due to enhanced phagocytosis of endotoxin and intracellular accumulation due to an impairment of the detoxification system.

The results of this investigation do not support the proposed mechanism of Trejo

TABLE III. Effect of Lead Acetate Administration on the Hepatic Detoxification of Endotoxin.[a]

| Liver donor treatment | incubation temp (°) | No. of experiments | No. of assay rats | Percentage lethality |
|---|---|---|---|---|
| Control, sodium acetate | 4 | 3 | 24 | 92 |
| | 37 | 3 | 24 | 4[b] |
| Time (min) after lead acetate | | | | |
| 30 | 4 | 3 | 24 | 96 |
| | 37 | 3 | 24 | 12[b] |
| 60 | 4 | 3 | 24 | 83 |
| | 37 | 3 | 24 | 16[b] |
| 180 | 4 | 3 | 24 | 88 |
| | 37 | 3 | 24 | 0[b] |
| 480 | 4 | 3 | 24 | 88 |
| | 37 | 3 | 24 | 4[b] |

[a] Homogenates (1%) were prepared in phosphate buffered saline (pH 7.4), and were incubated for 180 min with 1 μg/ml endotoxin.

[b] Signifies a $p < .05$ as compared to 4° group as determined by the chi-square test.

and DiLuzio (18). In contrast to their report of increased reticuloendothelial phagocytosis as evaluated by carbon clearance, our measurements indicate a profound depression of carbon clearing capability after lead treatment. Furthermore, direct measurements of endotoxin clearance using a sensitive bioassay system revealed no differences in the blood removal rate of endotoxin in lead treated vs control rats. This inability to correlate endotoxin sensitivity to blood clearance rates of endotoxin supports the dissociation of the endotoxin resistance and phagocytosis of endotoxin (4). Recent studies in mice have also indicated that lead acetate did not markedly alter the clearance of $^{51}$Cr-labeled endotoxin but did decrease the clearance rate of colloidal carbon (9).

Our findings on the inability of lead to modify the hepatic detoxification of endotoxin both *in vivo* or *in vitro* also do not support the contention of a depression in reticuloendothelial detoxification of endotoxin as a critical event in lead-induced hypersensitivity to endotoxin. It is likely therefore that lead's mechanism of action in undermining resistance to endotoxicosis resides in an interaction with the cellular or metabolic events

TABLE IV. Effect of Lead Acetate *in Vitro* on the Hepatic Detoxification of Endotoxin.[a]

| Incubation media | Incubation temp (°) | No. of experiments | No. of assay rats | Percentage lethality |
|---|---|---|---|---|
| 1% homogenate + 5 mg/ml sodium acetate | 4 | 3 | 24 | 88 |
| | 37 | 3 | 24 | 4[b] |
| 1% homogenate + 5 mg/ml lead acetate | 4 | 3 | 24 | 83 |
| | 37 | 3 | 24 | 12[b] |
| 0.5% homogenate + 5 mg/ml sodium acetate | 4 | 3 | 24 | 79 |
| | 37 | 3 | 24 | 0[b] |
| 0.5% homogenate + 5 mg/ml lead acetate | 4 | 3 | 24 | 71 |
| | 37 | 3 | 24 | 4[b] |

[a] Homogenates were prepared in 0.9% sodium chloride and were incubated for 180 min with 1 μg/ml endotoxin.

[b] Signifies a $p < .05$ as compared to 4° group as determined by the chi-square test.

which are the ultimate basis of shock pathogenesis. Further studies to support this suggestion are in progress. The ability of lead to sensitize to other forms of experimental shock also suggests that a central, common metabolic lesion in the compensatory adjustment to shock is the critical determinant of survival.

*Summary.* Lead acetate iv at 5 mg/300 g to male rats of the Holtzman strain sensitized to the lethal shock induced by endotoxin, Noble-Collip tumbling trauma, hind limb ischemia, bowel ischemia, and horse serum anaphylaxis. Lead treatment depressed the intravascular phagocytic clearance of colloidal carbon as evaluated at 15, 30, 60, 120 and 240 min. By use of an endotoxin bioassay based on lethality in lead-treated assay rats, the intravascular removal of a test dose of 0.5 mg of endotoxin was not altered by lead treatment as compared to normal and sodium acetate treated controls. The ability of liver homogenates to detoxify endotoxin was not altered by lead administration at 0.5, 1, 2, 3, or 8 hr prior to sacrifice. The *in vitro* addition of lead to homogenates incubated with endotoxin did not alter detoxification. It is suggested that the mechanism of lead sensitization to endotoxin probably does not reflect alteration in its rate of clearance or detoxification but rather may relate to lead's ability to alter metabolic responses during endotoxemia.

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