Impaired Detoxification as a Mechanism of Lead Acetate-Induced Hypersensitivity to Endotoxin[1] (35388)

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Lipopolysaccharides (LPS) obtained from gram-negative bacteria induce diverse physiological alterations such as changes in leukocyte levels, intravascular coagulation, body temperature, blood pressure, and immune reactivity when administered to experimental animals (1). Moreover, since shock and death may occur if endotoxins are present in sufficient quantity, the mechanisms of host defense by which endotoxins are rendered nontoxic has been the subject of investigation.

Since the major fraction of administered endotoxin is localized in cells of the reticuloendothelial system, particularly Kupffer cells and splenic macrophages (2–4), attempts have been made to define the role of liver and spleen in endotoxin detoxification. Waravdekar *et al.* (5) first described *in vitro* inactivation of endotoxin by rabbit liver homogenate as measured by alterations in the ability of endotoxin to elicit hemorrhagic necrosis in sarcoma implants in mice. Endotoxin inactivation has also been obtained using extracts of liver and spleen from various animal species, such as the dog (6), guinea pig (7), and rat (8).

Selye *et al.* (9) reported that a single, normally well-tolerated intravenous injection of lead acetate greatly enhances susceptibility of rats to the lethal effect of endotoxin. Although the mechanism of lead acetate enhancement of endotoxin shock is unknown, Filkins employed lead acetate to sensitize rats to endotoxin to ascertain the detoxification potential of various organs (8). Filkins reported that liver and spleen homogenates had the ability to detoxify endotoxin. Bertök (10) recently found that sulfhydryl compounds inhibit lead acetate-induced endotoxin hypersensitivity and suggested that lead acetate may inactivate sulfhydryl-containing enzymes necessary for inactivation or detoxification of endotoxin. Selye *et al.* (9) suggested that lead acetate might increase endotoxin sensitivity by inactivating the reticuloendothelial system (RES).

Since the delineation of the mechanism of lead acetate-induced hypersensitivity to endotoxin would contribute to defining host-defense mechanisms against endotoxin, the present study was undertaken to evaluate the mechanism of lead acetate-induced endotoxin hypersensitivity. A very sensitive, recently described endotoxin bioassay was employed to determine the ability of various tissues to detoxify endotoxin (11). Since RES stimulating agents have been demonstrated to enhance endotoxin sensitivity (12, 13) and Selye *et al.* (9) suggested lead acetate might enhance endotoxin shock by blockade of the RES, the influence of lead acetate on phagocytic function of the RES was also evaluated.

Materials and Methods. Endotoxin. The endotoxic polysaccharide of the Boivin type prepared from *S. enteritidis*, was obtained from Difco Laboratories, Detroit, Michigan. The endotoxin solution was prepared in sterile 0.9% sodium chloride.

Actinomycin D. Actinomycin D (Meractinomycin) was prepared in sterile 0.9% sodium chloride at a concentration of 500 μg/ml.

Lead acetate. Lead acetate (Fisher Scien-

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tific Co., New Jersey) was prepared in distilled water, at a concentration of 10 mg/ml. Normal rats received 5 mg of lead acetate as a single intravenous injection 8 hr prior to preparation of homogenates.

Endotoxin inactivation studies. Male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 280–320 g were maintained on Purina chow and water *ad libitum*. Homogenates of various tissues were prepared from ether anesthesized rats which were bled from the inferior vena cava. The liver was perfused with 50 ml of cold phosphate buffered saline solution. Organ homogenates (1%) were prepared in sterile 0.9% sodium chloride adjusted to a pH 7.4 with 0.01 *M* phosphate buffer. Homogenization was performed in a Teflon–glass tube, Potter-Elvehjem type, maintained at 1–5°.

Incubation of the various tissue homogenates was performed in 25-ml Erlenmeyer flasks. Each flask contained 4 ml of homogenate and 0.5 ml of a 2.0-μg/ml endotoxin solution. The flasks were gassed with 95% O_2 and 5% CO_2 and incubated at 37° for 3 hr in a Dubnoff metabolic shaking incubator at 100 cycles/min. Control samples either consisted of 4.0 ml of buffer incubated with 1.0 μg of endotoxin or homogenate preparations to which saline was added.

Bioassay. After incubation, 0.5 ml of actinomycin D solution (250 μg) was added to each flask, mixed, and 0.5 ml of the mixture was injected intraperitoneally into male mice (Carworth Farm) weighing 20–25 g. Following injection, the animals were maintained under observation and lethality was determined at 12-hr intervals. The designated mortality is that occurring within 48 hr following administration of the preparation.

Carbon clearances. The phagocytic activity of the RES was measured 2 hr after intravenous administration of 5 mg of lead acetate to normal rats. The colloidal carbon technique for measuring overt phagocytic activity has been described in detail elsewhere (14). Colloidal carbon (Gunther-Wagner, preparation C 11-1431a) was intravenously administered at a dose of 24 mg/100 g and the intravascular half-time was calculated (14).

Mortality data were analyzed using the chi-square test corrected by Yates' factor. Carbon clearance data were tested with the Student's *t* test with a 95% confidence interval to designate differences between the groups.

Results. The administration of 1000 μg of endotoxin to normal mice resulted in 100% lethality (Table I). In contrast 1.0 μg of endotoxin administered to actinomycin-treated mice induced 100% mortality. Ac-

TABLE I. Effect of Actinomycin D Administration on Lethality of *S. enteritidis* Endotoxin in Mice.[a]

Treatment	Endotoxin dose (μg/mouse)	Deaths/total	Mortality (%)	Endotoxin LD_{50} (μg)
Saline	1000	10/10	100	390
	500	6/10	60	
	250	3/10	30	
	125	1/10	10	
	100	0/10	0	
Actinomycin D	1.0	10/10	100	0.0039
	0.1	8/10	80	
	0.01	7/10	70	
	0.001	2/9	22	
	0.0001	0/5	0	
	—	0/5	0	

[a] Actinomycin D was administered in the amount of 25 μg ip simultaneously with the endotoxin. Mortality was determined at 12–24-hr intervals with the values recorded at the 48-hr period.

TABLE II. Effect of Incubation of Rat Tissue Homogenates[a] on the Lethality of *S. enteritidis* Endotoxin.

Homogenate	Endotoxin (0.1 μg)	Deaths/total	Mortality (%)	Protection (%)
Buffer	+	31/40	77.5	
Liver	+	11/38	28.9	62.7[b]
	—	0/23	0	
Spleen	+	10/25	40.0	48.4[b]
	—	1/15	6.7	
Lung	+	15/25	60.0	22.6
	—	0/17	0	
Kidney	+	7/12	58.3	24.8
	—	0/12	0	
Heart	+	8/14	57.1	26.3
	—	0/14	0	
Brain	+	8/11	72.7	6.2
	—	0/10	0	

[a] 4.0 ml of 1% tissue homogenates were incubated with 0.5 ml of 2 μg/ml endotoxin in saline at 37° for 3 hr. After incubation, 0.5 ml (250 μg) of actinomycin D was added, and 0.5 ml of the mixture was administered ip to normal mice.

[b] $p < .05$ as determined by chi-square test.

tinomycin D administration to normal mice in the dose of 25 μg did not induce lethality. The calculated LD_{50} dose of *S. enteritidis* endotoxin was profoundly reduced in actinomycin-treated mice reflecting an approximate 100,000-fold enhancement in endotoxin sensitivity. This increase is identical to that observed in actinomycin-treated mice which received *S. thyphosa* (11). It is therefore possible, by the use of actinomycin D to sensitize mice to endotoxin so that one can readily assay 0.001 to 1.0 μg of endotoxin.

In contrast to the approximate 78% mortality observed in the group which received endotoxin incubated in buffer, those mice which received endotoxin which was incubated with liver homogenates showed a 63% reduction in mortality ($p < .001$) (Table II). Spleen homogenates also possessed significant ($p < .01$) endotoxin detoxifying property. In contrast, lung, kidney, and heart homogenates were not able to detoxify LPS to a comparable extent as liver and spleen and the slight differences in mortality patterns were not significant ($p > .1$) from control values. Brain homogenates manifested no ability to detoxify endotoxin, as denoted by unaltered mortality pattern. In the absence of endotoxin addition, the administration of the various tissue homogenate preparations to mice did not induce lethality.

Lead acetate administered to normal animals altered the ability of liver, spleen, and lung to inactivate endotoxin (Table III). Lead acetate impaired the detoxification of endotoxin by liver homogenates as determined by an increase in mortality rate from 33% in the control group to 53% in the lead

TABLE III. Effect of Lead Acetate on Detoxification of *S. enteritidis* Endotoxin by Rat Liver, Spleen, and Lung Homogenates.

Pretreatment[a]	Tissue homogenate	Endotoxin (0.1 μg)	Deaths/total	Mortality (%)	Protection (%)
—	Buffer	+	28/38	73.7	
Saline	Liver	+	4/12	33.3	54.8
Lead acetate	Liver	+	8/15	53.3	27.7
	Liver	—	0/10	0	
Saline	Spleen	+	7/19	36.8	50.1
Lead acetate	Spleen	+	8/10	80.0	0
	Spleen	—	1/10	10.0	
Saline	Lung	+	12/19	63.2	14.2
Lead acetate	Lung	+	9/10	90.0	0
	Lung	—	1/10	10.0	

[a] Normal rats received 5 mg of lead acetate in distilled water, iv, 8 hr prior to preparation of tissue homogenates.

TABLE IV. Enhancement of the Intravascular Clearance of Colloidal Carbon by Lead Acetate Administration.[a]

Group	No.	Intravascular half-time[b] (min)
Saline	7	15.9 ± 0.71
Lead acetate	10	4.8 ± 0.40

[a] Lead acetate (5 mg) administered iv 2 hr before iv injection of colloidal carbon in the amount of 24 mg/100 g.

[b] Values are expressed as means ± standard error.

acetate-treated group ($p<.05$, $>.02$). Moreover, lead acetate produced a total impairment of endotoxin detoxification by spleen with mortality increasing from 37 to 80% in the untreated and lead-treated group, respectively. The slight degree of detoxification observed with lung homogenates was also eliminated by lead acetate administration.

Phagocytic activity of the reticuloendothelial system, as measured by the vascular clearance of colloidal carbon, showed a hyperphagocytic state in lead acetate-treated rats. This hyperfunctional state is indicated by a mean half-time ($t_{½}$) of colloidal carbon of 4.8 min opposed to a mean $t_{½}$ of 15.9 min in the control (saline) group (Table IV).

Discussion. The detoxification of endotoxin *in vitro* has been demonstrated to be a property of liver and spleen. These findings confirm recent observations by Filkins (8) who employed the lead acetate-sensitized rat to measure endotoxin inactivation. In our experimental conditions, liver and spleen, organs with major RE cell populations, possessed the highest endotoxin-detoxification activity. Comparative results also have been found by other investigators in rabbits (15), dogs (6), and rats (8) using different systems for endotoxin bioassay.

Lung, kidney, heart, and brain homogenates possessed little, if any, ability to inactivate endotoxin *in vitro*. The lack of detoxification of endotoxin by rat lung, kidney, and brain as noted in the present studies agrees with the observations of Smith *et al.* (6).

It is well known that endotoxins are removed from the circulation by the liver and spleen (2–4), organs which have significant endotoxin-detoxification ability. Since macrophages have been demonstrated to be the site of endotoxin detoxification (16), reticuloendothelial cells in liver and spleen appear to be the primary sites of initial host-response to endotoxin. This concept is also emphasized by previous observations which demonstrated that RES altering agents, such as methyl palmitate and glucan profoundly alter host response against endotoxins (12). Selye *et al.* (9) suggested the possibility that lead acetate might enhance endotoxin shock by blockade of the RES. The lead acetate-induced hyperphagocytosis of colloidal carbon does not support the concept of RES blockade as a contributing mechanism of endotoxin sensitivity.

The loss of endotoxin inactivation in lead acetate-sensitized rat supports the concept of Bertók (10) that lead acetate might inactivate enzyme(s) or other biologically active compound(s) necessary to endotoxin inactivation. Our finding that lead acetate stimulates phagocytosis of colloidal carbon also suggests, however, that the mechanism of lead acetate-induced endotoxin hypersensitivity may be more complex, since other RE stimulating agents which induce hyperphagocytic states also result in endotoxin hypersensitivity.

Weissmann and Thomas (19) and Janoff and Kaley (20) have found that endotoxin administration produces lysosomal instability. It is possible that lead acetate-induced hypersensitivity to endotoxin is related to the combined mechanisms of rapid accumulation of endotoxin in phagocytic cells due to hyperphagocytosis, as well as the absence of adequate detoxification mechanisms. These two events could cause release of large amounts of lysosomal hydrolytic enzymes into the cytoplasm, leading to functional impairment and/or destruction of cells by autophagia and/or release of 5-hydroxytryptamine and hydrolytic enzymes from platelets and leukocytes (21), leading to enhancement in endotoxin sensitivity in lead acetate-treated rats.

Summary. Actinomycin D was employed to profoundly enhance the sensitivity of mice

to endotoxin in order to bioassay submicrogram amounts of endotoxin. Homogenates of rat liver and spleen were able to detoxify endotoxin as bioassayed in actinomycin-treated mice. Lung, kidney, heart, and brain homogenate did not possess the ability to significantly detoxify endotoxin. Lead acetate, which profoundly sensitizes rats to endotoxin, enhanced the vascular clearance of colloidal carbon and also reduced detoxification of endotoxin by liver and spleen homogenates. Enhanced phagocytosis, resulting in accumulation of endotoxin in macrophages, coupled with the failure in endotoxin inactivation or detoxification, appears to be the mechanism of lead acetate-induced endotoxin hypersensitivity.

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