Influence of Altered Reticuloendothelial Function on Vascular Clearance and Tissue Distribution of *S. enteritidis* Endotoxin[1] (34288)

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The reticuloendothelial system (RES) was first implicated in host response to endotoxin by Beeson in 1947 (1). While a variety of studies have supported this basic hypothesis, they also have indicated that the relationship between host RES activity and endotoxin toxicity is complex (2–8). Intravenously administered endotoxin is localized in organs possessing significant RE cell populations, particularly the liver (2–4). "Blockade" of the RES has been demonstrated to render tolerant rabbits susceptible to the febrile and lethal effects of endotoxin (1, 5). In addition, RES stimulants such as BCG, triolein, and zymosan enhanced host susceptibility to endotoxin-induced mortality (6–8). The administration of a purified RES stimulant, glucan (9), induced RES hyperfunction and hypertrophy, as well as extreme sensitivity to endotoxin as indicated by a 40-fold decrease in the LD_{50} of rats to *Salmonella enteritidis* endotoxin (10). In direct contrast, RES depression, induced by methyl palmitate (11) administration, resulted in a highly significant resistance to lethal and supralethal doses of endotoxins (10). Heparin administration, which enhanced RES function (12), was associated with significant diminution in endotoxin lethality in rats (13). The protective effect of heparin administration was observed when heparin was administered prior to, as well as subsequent to, endotoxin administration (13).

In an effort to define the possible mechanisms by which RES functional alterations influence endotoxin lethality, the vascular clearance and tissue distribution of ^{51}Cr-labeled *S. enteritidis* endotoxin were determined in rats pretreated with either glucan, methyl palmitate, or heparin, agents which significantly alter RES activity (9–12).

Materials and Methods. Female Sprague-Dawley rats weighing from 200 to 225 g received glucan for 2 days in the amount of 1 mg/100 g to induce RES stimulation and hypertrophy (9); the control group received saline. Depression of the RES was induced by the intravenous administration of a methyl palmitate (11) emulsion in the amount of 100 mg/100 g for 2 days. The control group received the suspending solution, consisting of 0.1% Tween 20 in a 5% dextrose in water solution. Phagocytic clearances were measured in randomly selected rats in each group by the colloidal carbon technique (14), to determine the degree of RES stimulation or depression. In the study of possible effects of heparin on the clearance and distribution of endotoxin, heparin (Sigma) in the volume of 0.2 ml (1000 USP units) was administered intravenously 15 min prior to the administration of the labeled endotoxin (13). The *S. enteritidis* endotoxin (Difco, Bacto Lipopolysaccharide B) used in this study was labeled with $Na_2{}^{51}Cr\ O_4$ by the procedure of Braude *et al.* (15).

Labeled endotoxin was administered intravenously in a dose of 4 mg/100 g. At this dose, this endotoxin preparation induced a mean 70% mortality which was comparable to the degree of mortality previously observed in studies of the effectiveness of heparin to modify lethality (13). Blood samples were obtained from the tail vein at 2-min intervals

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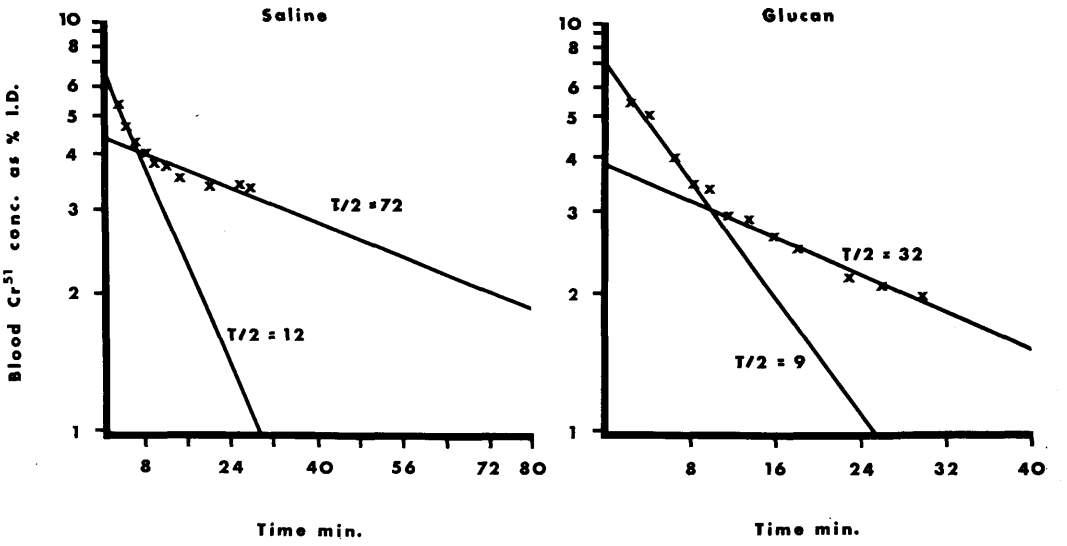

FIG. 1. Typical semilogarithmic plot of ^{51}Cr-labeled *S. enteritidis* endotoxin radioactivity in blood of a representative rat previously treated with either saline or the RES stimulant, glucan. The decrease in blood endotoxin concentrations as a function of time indicates the existence of a bimodal clearance rate in both control and RE-hyperfunctional rats.

after endotoxin administration for 20 min and then at 26 and 30 min postinjection. When blood radioactivity, as a percentage of the injected dose of radioactivity, was plotted on a semilogarithmic scale against time, a two-component curve was observed (Fig. 1). Both the initial, or rapid, phase and the secondary, or slow, phase of endotoxin clearance were calculated. Liver, lung, and spleen radioactivity were measured 30 min following endotoxin administration.

Blood and tissue radioactivity, which were determined by a Nuclear-Chicago crystal scintillation detector, were compared statistically using Student's *t* test; the level of confidence employed was 95%.

Results. Two injections of glucan led to a significantly enhanced vascular clearance of ^{51}Cr-labeled *S. enteritidis* endotoxin (Table I). The clearance of the endotoxin in glucan-treated rats revealed that both the rapid and slow components of endotoxin removal were significantly increased (Fig. 1). The initial rapid phase of endotoxin clearance was sig-

TABLE I. Vascular Clearance and Tissue Distribution of ^{51}Cr-Labeled *S. enteritidis*[a] Endotoxin in Saline and Glucan-Treated Rats.

Treatment	No.	Intravascular *T*/2 (min)		Tissue activity					
				Liver		Lung		Spleen	
		Fast	Slow	%ID/g	%ID/TO	%ID/g	%ID/TO	%ID/g	%ID/TO
Saline	8	15.3 ±2.6	55.3 ±4.8	3.0 ±0.21	22.8 ±1.37	2.0 ±0.14	3.1 ±0.25	7.0 ±0.49	4.1 ±0.33
Glucan	7	6.2 ±1.5	35.1 ±3.4	4.6 ±0.36	44.1 ±2.61	1.6 ±0.14	3.1 ±0.30	3.2 ±0.28	5.3 ±0.40

[a] *S. enteritidis* endotoxin was administered intravenously in a dose of 4.0 mg/100 g, and its tissue distribution was measured 30 min after injection. Values are expressed as mean ± SE. The tissue distribution data are expressed as percentage of injected dose per gram of wet weight (%ID/g) and total organ (%ID/TO).

TABLE II. Vascular Clearance and Tissue Distribution of ^{51}Cr-Labeled *S. enteritidis* Endotoxin in Tween and Methyl Palmitate-Treated Rats.[a]

Treatment	Intravascular $T/2$ (min)		Tissue activity					
			Liver		Lung		Spleen	
	Fast	Slow	%ID/g	%ID/TO	%ID/g	%ID/TO	%ID/g	%ID/TO
Tween	18.3 ±0.7	85.3 ±8.5	3.9 ±0.57	28.7 ±4.42	2.1 ±0.14	3.7 ±0.34	5.6 ±0.73	3.5 ±0.25
Methyl palmitate	21.3 ±2.0	67.0 ±3.3	2.7 ±0.15	24.4 ±1.70	2.4 ±0.23	4.1 ±0.30	0.8 ±0.22	0.6 ±0.13

[a] Values, expressed as means ± SE, are derived from 6 Tween control and 8 methyl palmitate-treated rats. The tissue distribution data are expressed as percentage of injected dose per gram of wet weight (%ID/g) and total organ (%ID/TO).

nificantly accelerated as denoted by the 59% decrease in the half time, whereas, the slow component of endotoxin removal was characterized by a 36% decrease.

The accelerated vascular disappearance of the endotoxin in the glucan group was associated with a 53 and 85% increase in hepatic uptake expressed on a gram and organ basis, respectively. Lung radioactivity was not significantly reduced in the glucan group. Splenic uptake was significantly decreased by 55% on a weight basis, although significantly increased 30% on total organ basis. The reduced activity of lung and spleen on a per gram basis reflected the glucan-induced hypertrophy of these organs which were increased in weight by 22 and 193% respectively.

The fast and slow components of ^{51}Cr-labeled endotoxin clearance rates were not significantly altered in the methyl palmitate group when compared to the Tween control group (Table II). Liver and lung uptake of labeled endotoxin were unaltered in the RES-depressed group. In contrast, the splenic localization of endotoxin in the methyl palmitate group was significantly diminished ($p < 0.001$) relative to its control on both an organ and per gram basis as indicated by an approximate 85% reduction in splenic activity.

Pretreatment of rats with heparin led to a significant inhibition in the rapid component of the endotoxin removal rate as revealed by a 56% prolongation in the half time (Table III). The slow component of the vascular clearance of endotoxin, however, was unaltered. The heparin-induced decrease in endotoxin removal from the blood was related to an 18 and 24% reduction in hepatic uptake on an organ and weight basis, respectively. The pulmonary and splenic localization of endotoxin was unchanged by heparin administration.

Discussion. The studies of Braude (15) and Chedid *et al.* (16) demonstrated that the $Na_2{}^{51}CrO_4$ method of labeling endotoxin is the method of choice for studies on the vascular clearance and tissue distribution of endotoxin. The biphasic clearance rate of labeled endotoxin, as observed in the present study, most probably reflected the heterogeneity in the size of the injected endotoxin, as suggested by studies with other colloids of varying particle size (18). The multiphasic nature of endotoxin removal has been noted previously (3, 7).

The present studies confirm previous observations that the RES, particularly the liver macrophage population, is the predominant factor in the vascular clearance of endotoxin (2–4, 17, 19).

In agreement with the observations of Stuart and Cooper (7) that there does not appear to be any correlation between endotoxin susceptibility and phagocytic capacity of the RES, the present studies indicate that the previously observed alterations in endotoxin susceptibility (10, 13) are not readily explained on the rate of vascular

TABLE III. Vascular Clearance and Tissue Distribution of ^{51}Cr-Labeled *S. enteritidis*[a] Endotoxin in Saline and Heparin-Treated Rats.

Treatment	Intravascular $T/2$ (min)		Tissue activity					
			Liver		Lung		Spleen	
	Fast	Slow	%ID/g	%ID/TO	%ID/g	%ID/TO	%ID/g	%ID/TO
Saline	17.6 ±1.1	59.4 ±11.8	2.5 ±0.16	20.5 ±0.75	2.1 ±0.11	3.1 ±0.22	5.8 ±0.42	3.3 ±0.15
Heparin	27.5 ±2.4	67.4 ±17.0	1.9 ±0.16	16.8 ±1.07	2.3 ±0.40	3.3 ±0.46	6.5 ±0.84	3.7 ±0.52

[a] *S. enteritidis* endotoxin was administered intravenously in a dose of 4 mg/100 g of body weight. Values, expressed as mean ± SE, are derived from six rats per group. Tissue distribution data are expressed as percentage of injected dose per gram of wet weight (%ID/g) and total organ (%ID/TO).

clearance of endotoxin. Methyl palmitate, which is a selective RES depressant agent (11), does not alter the vascular clearance of endotoxin from the blood while inducing a profound resistance to endotoxin lethality (10). The accelerated clearance of endotoxin induced by glucan appears not to be related to the profound enhancement of endotoxin lethality which characterized the glucan-treated group (10, 20).

Glycerol trioleate, which stimulates RES phagocytic function, as denoted by colloidal carbon clearance and ^{51}Cr-labeled endotoxin removal, enhanced susceptibility to lethal effects of endotoxin (7). Ethyl stearate-induced RES depression, which did not markedly alter endotoxin susceptibility of mice, further stresses the minor role of vascular clearance of endotoxin as a determinant in the degree of host endotoxin susceptibility (7).

Noyes *et al.* (19) demonstrated that mice which were treated with trypan blue, a so-called reticuloendothelial blocking agent which induces enhanced susceptibility to endotoxin, do not show an appreciable alteration in the hepatic localization of the endotoxin. Noyes *et al.* (19) reported that the lethality of endotoxin in mice could not be related to alterations in tissue distribution of the endotoxin. The present findings are in basic accord with this concept that endotoxin susceptibility is not related to initial tissue localization patterns of endotoxin.

The dissociation of vascular clearance and tissue localization of endotoxin as a fundamental factor in endotoxin lethality stresses cellular participation in endotoxin toxicity. A major area of consideration has been the possible involvement of lysosomes in endotoxemia (21–23). The RES stimulant, BCG, which promotes endotoxin lethality (6, 20, 21) also elevated lysosomal enzymatic activity (21, 22). The subsequent administration of endotoxin to RES-stimulated mice profoundly enhanced activity of lysosomal enzymes in plasma (21, 22). A correlation was suggested between the increase in plasma β-glucuronidase activity and mortality following endotoxin administration to BCG-treated animals (22). The development of tolerance in endotoxin-treated animals was associated with a decreased response of plasma β-glucuronidase activity which paralleled that of decreased mortality (22). Weissmann and Thomas (23) initially reported that a significant increase in the release of lysosomal enzymes occurred from liver homogenates prepared from rabbits which received an intravenous injection of endotoxin. The enzyme release from granular fractions of liver homogenates derived from animals which were made tolerant to endotoxin was significantly reduced. The studies of Weissmann and Thomas (23) and Saito and Suter (21, 22) indicated that endotoxins have a marked effect on the stability of lysosomes and that the modification in host response to endotoxin by the development of the tolerant state, as well as following administration of steroids

(23), may be mediated through the enhanced stability of the lysosomal granules. Since pretreatment of animals with cortison abolishes the increased toxicity to endotoxin which is caused by RE-stimulating agents (8), the possible role of lysosmal stability as a modifier in endotoxin toxicity is further supported.

Another possible mechanism by which RES-stimulating or -depressing agents might modify endotoxin toxicity involves an alteration in the detoxification potential of liver and spleen. Rutenburg *et al.* (24) reported the ability of liver and spleen to not only remove, but to rapidly detoxify bacterial endotoxins. It remains to be established whether RES-altering agents modify the detoxification capacity of liver and spleen.

The present studies dissociate the vascular clearance and tissue distribution of endotoxin from the previously characterized alterations in endotoxin lethality (10, 13). The influence of a macrophage mediated endotoxin response as a primary host-determinant to endotoxemia is suggested.

Summary. In an attempt to define the mechanism by which the reticuloendothelial stimulant, glucan, profoundly enhances susceptibility and conversely that the RES depressant, methyl palmitate, produces a resistance to intravenously administered endotoxins, the vascular clearance and tissue distribution of intravenously administered ^{51}Cr-labeled *S. enteritidis* endotoxin were studied. The vascular clearance of the labeled endotoxin preparation was biphasic consisting of an early, or fast, component followed by a slow component. The RES stimulant, glucan, enhanced both phases of removal and produced a significant increase in the hepatic localization of the endotoxin. Splenic and lung uptake were unaltered. In contrast, methyl palmitate exerted no influence on the vascular removal rate of endotoxin, but induced a significant decrease in splenic uptake. The administration of heparin, which has been previously demonstrated to modify endotoxin lethality, decreased the early phase of vascular clearance, but did not modify the tissue localization pattern. These studies dissociate the vascular clearance and tissue distribution of endotoxin from previously reported alterations in endotoxin lethality. The importance of a cellular-mediated response to endotoxemia as a major determinant to endotoxin induced lethality is suggested.

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