Decreased Shock Lethality in Rats with Surfactant-Treated Endotoxin[1] (35289)

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In an attempt to understand the molecular basis of endotoxic reactions, a variety of approaches toward modifying or inactivating endotoxin have been studied, *e.g.*, acid or alkaline hydrolysis, saponification, enzymatic degradation, chemical complexing, and immunochemical reactions (1). In addition, recent studies (2–7) have employed surfactants to disaggregate and/or modify endotoxin and thereby reduce its effectiveness in certain test systems, *e.g.*, pyrogenicity in rabbits or lethality in chicks. However, Nowotny (1) has noted that surfactant treatment of endotoxin did not modify either the local Shwartzman reaction in rabbits or lethality in mice. The current study substantiates the ability of various surfactants to inactivate endotoxin *in vitro* and extends the range of detoxification to amelioration of endotoxin shock in the rat. A sensitive detoxification test system based on the synergistic action of endotoxin and lead acetate was employed (8, 9).

Methods. Male rats of the Holtzman strain (Holtzman Company, Madison, Wisc.) in the range of 300 ± 20 g were fed Purina Chow and water *ad libitum* and maintained at 24 ± 1° for 7–10 days prior to experimentation. Endotoxin was purchased from Difco Laboratories, Detroit, Mich. as the Boivin lipopolysaccharide preparation of *Salmonella enteritidis*, Lot No. 172145. The surfactants used were Triton X100 (Rohm and Haas, Philadelphia, Pa.), Tween 20 (Vaughn, Inc., Memphis, Tenn.), sodium deoxycholate, sodium taurocholate, and sodium lauryl sulfate (Nutritional Biochemicals Corporation, Cleveland, Ohio). The endotoxin and surfactants were incubated in 0.02 *M* potassium phosphate buffered saline at pH 7.4 for 60 min at either 37 or 4° (9). The endotoxin content was 3 μg/ml of incubation media and each test rat received a standardized injection of 1 ml into the dorsal vein of the penis while under light ether anesthesia. The endotoxin injection was immediately followed by 1 ml of 5 mg/ml of lead acetate in water (Mallinckrodt Chemical Works, St. Louis, Mo.). Shock lethality occurred within 14–24 hr. Data were analyzed for significance by use of the chi-square test corrected with Yates' factor and probability values computed.

Results and Discussion. As indicated in Table I, the following concentations of surfactants resulted in almost complete inactivation of endotoxin lethality: Triton X100, Tween 20, and sodium deoxycholate at 0.05 and 0.01%; sodium taurocholate at 0.05%; and sodium lauryl sulfate at 0.5%. The dependence of inactivation on the ratio of surfactant to lipoplysaccharide has been observed previously (5, 7) and may explain Nowotny's failure to confirm the effect.

In all the studies the incubations at 37° were compared to control groups incubated at 4°. The inability of the 4° samples to reduce lethality negates a direct effect of the surfactants on the test recipient rat response to endotoxin. The temperature dependence of the surfactant inactivation of endotoxin was consistent in all groups and merits additional study in relation to the mode of surfactant–lipopolysaccharide interaction. In agreement with previous reports (8, 9) the sensitivity of the lead acetate model in evaluation of endotoxin in doses as low as 1 μg of lipopolysac-

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TABLE I. Effect of Surfactants on Endotoxin Shock Mortality in Lead-Treated Rats.[a]

Surfactant	Incubation conc (%; w/v)	Incubation temp (°)	Mortality	
			No. of deaths/ no. of test rats	(%)
None; only PBS	—	37	14/15	93
		4	12/12	100
Triton X100	0.05	37	0/12	0
	0.05	4	12/12[b]	100
	0.01	37	2/12	17
	0.01	4	11/12[b]	92
	0.001	37	10/12	83
	0.001	4	11/12	92
Tween 20	0.05	37	0/16	0
	0.05	4	15/16[b]	94
	0.01	37	2/16	12
	0.01	4	16/16[b]	100
	0.001	37	8/12	75
	0.001	4	12/12	100
Sodium deoxycholate	0.05	37	2/15	13
	0.05	4	10/12[b]	83
	0.01	37	3/15	20
	0.01	4	11/12	92
	0.001	37	12/15	80
	0.001	4	12/12	100
Sodium taurocholate	0.05	37	0/12	0
	0.05	4	11/12[b]	92
	0.01	37	10/12	83
	0.01	4	12/12	100
	0.001	37	11/12	92
	0.001	4	12/12	100
Sodium lauryl sulfate	0.5	37	2/15	13
	0.5	4	11/12[b]	92
	0.25	37	6/12	50
	0.25	4	11/12	92
	0.05	37	12/12	100
	0.05	4	13/14	93
	0.01	37	14/15	93
	0.01	4	13/14	93

[a] Endotoxin (3 μg/ml) was incubated for 60 min in phosphate buffered saline (PBS) (pH 7.4) containing a surfactant; then 1 ml of incubate plus 5 mg of lead acetate were injected iv.
[b] $p < .05$ compared to control group; calculated by the chi-square test.

charide/100 g of body weight recommends its utility for inactivation studies in the rat.

The ability of surfactants to inactivate endotoxin raises numerous questions regarding both the chemical nature of the inactivation and the relation of this phenomenon to the biological effects and physiological detoxification of endotoxin. Since disaggregation of endotoxin reportedly yields smaller endotoxin particles (2, 3), it is possible that modification of particle size *per se* is the crucial reaction. McIntire *et al.* (6, 7) have, however, contradicted the importance of disaggregation *per se* in detoxification and alterna-

tively suggested the key reaction is rather the formation of nontoxic molecular complexes.

Since endotoxin is sequestered by the reticuloendothelial system, some ill-defined relation between surfactant disaggregation or complex formation, and macrophage recognition and phagocytosis may be the crucial reaction. Furthermore, the probability that the biological mechanism of endotoxin inactivation may involve surfactant activity is worthy of further exploration. It is anticipated that further use of the lead acetate bioassay system may aid in delineating the mechanisms of endotoxin reactivity in rats and the precise action of surfactants.

Summary. Prior incubation of *Salmonella enteritidis* lipopolysaccharide with varying concentrations of Triton ×100, Tween 20, sodium deoxycholate, sodium taurocholate, and sodium lauryl sulfate markedly reduced lethality of the lead-sensitized rat in endotoxin shock. The inactivation process did not proceed at 4° in comparison to reduction in toxicity during 37° incubation. The possibility of a surfactant serving the physiological reticuloendothelial detoxifying system is presented.

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