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Blood Endotoxin Inactivation after Trauma and Endotoxicosis (39149)[1]

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Due to the landmark investigations of Beeson (1), the nature of physiological resistance, i.e., tolerance, to endotoxemia and the resultant endotoxic syndrome has been associated primarily with the sequestration and detoxification functions of the reticuloendothelial system (RES). In addition, Fine *et al.* (2) have accumulated vast support for their thesis that failure of RES function incident to trauma, hemorrhage, sepsis, or other stress is a pivotal event in the pathogenesis of irreversible shock.

While a cellular basis of endotoxin resistance which resides in the RES is of proven importance, numerous studies (3–9) have also suggested that a humoral or blood anti-endotoxin system exists. The physiological significance of the blood endotoxin detoxifying system in shock resistance is, however, essentially unknown.

The present report deals with the existence and temporal pattern of appearance of blood endotoxin detoxifying function after both experimental endotoxemia and trauma in rats. In addition, both passive transfer of protection in endotoxin shock and the loss of blood detoxifying function in lethal traumatic shock are described.

Materials and procedures. Male rats (320 ± 20 g) of the Holtzman strain (Holtzman Company, Madison, Wisc.) were either rendered endotoxic via administration of *Salm. enteritidis* lipopolysaccharide iv (Difco Laboratories, Detroit, Mich.) or subjected to Noble–Collip tumbling trauma (10). Saline-treated or untreated rats served as control blood donors. Blood was removed from all rats via cardiac puncture under light ether anesthesia. When prescribed by the protocol (see Results) the rats were administered 500 units heparin iv (Sigma Chemical Company, St. Louis, Mo.) prior to bleeding. The bioassay of blood endotoxin detoxifying activity was performed via shock induction in the lead-sensitized rat as described in detail previously (11). In essence, the media to be evaluated for detoxifying activity was incubated at either 4° or 37° with a LD_{90}/ml of endotoxin. After incubation, 1-ml samples were injected iv into assay rats and immediately followed by a sensitizing 5-mg dose of lead acetate iv. Decrements in lethality as compared to appropriate controls is the parameter of detoxification. Lethality data were tested for intergroup statistical significance at a confidence level of 95% using the chi-square test, corrected for continuity with the Yates' factor.

Results. Existence and time course of development of blood endotoxin detoxifying activity. Table I presents the bioassay of blood endotoxin detoxifying activity in three groups of blood donors: (i) control rats, either uninjected trauma controls, or administered 1 ml of saline iv, (ii) rats administered 0.1 mg of endotoxin 18–24 hr prior to bleeding, or (iii) rats subjected to 425 revs in a Noble–Collip tumbling apparatus 18–24 hr prior to bleeding. As indicated, when whole heparinized rat blood was employed in the detoxification test, no detoxification was observed in control blood as compared to incubation in pH 7.4 phosphate buffered saline (PBS). In contrast, whole rat blood from either post-endotoxic or post-trauma donors manifested marked detoxifying potential, i.e., lethalities of 13 and 10% in groups of 40 vs 92% in the PBS group (N = 50). Detoxifying activity also was not present in control blood cells, plasma, or serum. In contrast, either plasma or serum, but not blood cells, from post-endotoxic or post-trauma donors exhibited endotoxin detoxification.

The time course of the development of blood endotoxin detoxifying activity is presented in Table II. In contrast to little to no endotoxin detoxification in control blood, within 4–6 hr and definitely by 12 hr, either

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TABLE I. COMPARISON OF ENDOTOXIN DETOXIFICATION IN WHOLE BLOOD, BLOOD CELLS, PLASMA, OR SERUM OF CONTROL VS POST-ENDOTOXIN OR POST-TRAUMA DONORS.

	Experimental Donor Group					
	Control		Endotoxin[b]		Trauma[c]	
Assay media[a]	(N)[d]	(%)	(N)	(%)	(N)	(%)
Whole blood	60	92	40	13*	40	10*
Blood cells	30	93	30	83	30	97
Plasma	50	90	40	13*	40	8*
Serum	30	83	30	3*	30	10*

[a] The assay media consisted of 5 μg/ml of endotoxin in the specified detoxification test media and was incubated at 37° for 180 min in a Dubnoff metabolic shaker bath at 60 oscillations/min.

[b] Endotoxin was administered iv at 0.1 mg/rat, 18–24 hr prior to bleeding.

[c] Trauma was 425 revs in a Noble-Collip apparatus, 18–24 hr prior to bleeding.

[d] N = number of assay rats; % = percentage shock lethality of rats treated with 1 ml of assay media + 5 mg lead acetate iv.

* $P < .05$ as compared to phosphate buffered saline (pH 7.4) incubation at 37°, $N = 50$, lethality = 92%.

TABLE II. TIME COURSE OF DEVELOPMENT OF BLOOD ENDOTOXIN DETOXIFYING ACTIVITY.

	Experimental groups			
	Trauma (425 revs)		Endotoxin (0.1 mg/rat iv)	
Time of blood sampling (hr after treatment)	N[a]	Lethality (%)	N	Lethality (%)
0 (Controls)	30	100	30	93
1	30	100	30	100
2	30	93	30	93
4	30	50*	40	75
6	30	27*	40	30*
12	40	10*	30	0*
24	40	10*	40	10*

[a] N = number of assay rats.

* $P < .05$ as compared to 0 time control.

post-endotoxin or post-trauma blood possessed demonstrable endotoxin detoxifying activity.

Effect of varying the incubation time on endotoxin detoxification in blood. To further confirm the striking difference between control and post-endotoxic blood, the effects of varying incubation times for the detoxification test were evaluated. As depicted in Table III, control blood failed to

TABLE III. EFFECT OF VARYING INCUBATION TIME ON ENDOTOXIN DETOXIFICATION IN BLOOD.

	Experimental group			
	Control		Endotoxin	
Assay incubation time (min at 37°)	Assay N	Lethality (%)	Assay N	Lethality (%)
0 (4°)	30	100	30	93
60	30	93	30	67*
180	50	90	40	10*
360	30	87	30	3*

* $P < .05$ as compared to 0 min group.

detoxify endotoxin at 37° incubation periods of 60, 120, and 360 min as compared to a 4° control group. In contrast, post-endotoxic blood, while not manifesting detoxifying activity at 4°, showed some detoxification by 60 min and virtually complete detoxification by 180 to 360 min.

Passive transfer of blood endotoxin detoxifying activity. As a further test of the functional significance of the blood anti-endotoxin system, passive transfer experiments were performed (Table IV). Three groups of experimental rats served as blood donors for the passive transfer test: (i) untreated control donors, (ii) donors which received 1 ml of saline iv 18–24 hr prior, and (iii) donors which received 0.1 mg of endotoxin 18–24 hr prior. Two hours after receipt of 2 ml of the respective passive transfer blood, all rats, including non-transfer controls, received 5 μg of endotoxin plus 5 mg of lead acetate iv. As presented in Table IV, only rats which received a post-endotoxin blood transfer displayed a decreased lethality as compared to the nontransfer control group.

Table V illustrates the evaluation of the status of the blood detoxifying system of previously traumatized rats subjected to terminal traumatic shock. As indicated mild trauma (400 revs) 18 hr prior was associated with the capability to detoxify endotoxin. However, if rats subjected to mild trauma (400 revs) 18 hr prior, were subject to traumatic shock (475 revs, 60–120 min prior to bleeding) then the blood from the rats in agonic shock was unable to detoxify endotoxin.

Discussion. In accord with past studies

TABLE IV. PASSIVE TRANSFER OF BLOOD ENDOTOXIN DETOXIFYING ACTIVITY.

Experimental groups	Assay *N*	Lethality (%)
No Prior Blood Transfer	40	85
Passive transfer		
A. Control blood (2 ml)	40	77.5
B. Post-saline blood (2 ml)	40	82.5
C. Post-endotoxin blood (2 ml)	40	35*

* $P < .05$ as compared to any group above.

TABLE V. LOSS OF BLOOD ENDOTOXIN DETOXIFYING ACTIVITY IN TRAUMATIC SHOCK.

Experimental blood donor group	Incubation temperature (°)	Assay *N*	Lethality (%)
Trauma (400 revs) 18 hr prior	37	40	10
	4	40	90
Trauma (400 revs) 18 hr prior and traumatic shock (475 revs)	37	40	80*
60–120 min prior to bleeding	4	40	94

* $P < .05$ as compared to 37° preshock trauma group.

(3–9) the present work provides new insights into the existence of a potent blood anti-endotoxin system. While no detoxifying activity was demonstrable in control rat blood, the system was readily assessed after either mild endotoxemia or trauma. Both the ability to demonstrate a passive transfer and a loss incident to severe trauma suggests the functional significance of the blood anti-endotoxin system in the pathogenesis of shock. Since no detoxification was observed in washed blood cells, a role for the formed elements including leukocytes in the process is minimal. This finding is in basic agreement with our past study on the inability of leukocytes to inactivate endotoxin (12).

The mechanisms underlying the appearance of the anti-endotoxin system incident to either endotoxemia or trauma merit additional study. Since our previous work has implicated a macrophage lysosomal factor in endotoxin detoxification (13), current studies are evaluating the role of the RES in the elaboration of the blood system. The nature of the system is unknown; however, preliminary characterization studies (Table VI) have indicated that it is lost after barium sulfate deproteinization as well as heating to 65–80°. Heating to 56°, the conventional labile point for the complement system, did not alter detoxifying activity. Extensive *in vitro* studies of endotoxin detoxification have indicated that both the fifth and sixth components of complement are involved in the inactivation process (14); however, the mechanism by which the complement system participates in the detoxification of endotoxin is peculiarly different from the usual sequential pathway and remains obscure despite intensive investigation (15–17). It is anticipated that further effort will clarify both the essential character of the detoxifying system as well as its functional significance in shock resistance. The need for further understanding of the nature of physiological resistance to endotoxin is underscored by the realization that gram negative bacteremias may result in upwards of 100,000 hospital deaths per year (18).

Summary. In contrast to the inability to demonstrate endotoxin detoxifying activity in either control rat blood, blood cells, plasma, or serum, the induction of mild endotoxemia or trauma resulted in a prompt and marked detoxifying activity in blood plasma or serum, but not in the cellular elements. The functional significance of the blood anti-endotoxin system was dem-

TABLE VI. CHARACTERIZATION OF THE PLASMA ENDOTOXIN DETOXIFYING SYSTEM IN POST-ENDOTOXIN PLASMA.[a]

Plasma treatment	Detoxifying activity: Assay *N*	Detoxifying activity: Lethality (%)
Post-endotoxin	40	13
Barium sulfate deproteinization	30	83*
Heating		
56° for 60 min	30	7
65° for 60 min	30	97*
80° for 60 min	30	90*

[a] *N* = number of assay rats; % = percentage of assay lethality.

* $P < .05$ as compared to post-endotoxin pools.

onstrated by a capability for passive transfer and by its loss during severe traumatic shock. A role for the RES in elaboration of the blood anti-endotoxin system is postulated.

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