A LABILE SERUM FACTOR IN EXPERIMENTAL ENDOTOXIN SHOCK: CROSS-TRANSFUSION STUDIES IN DOGS

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Immediately after a lethal dose of endotoxin is injected intravenously into the dog, there is an abrupt onset of hypotension that is related to the pooling of blood in the liver and in the intestine (1). Congestion of the liver is associated with spasm of the hepatic venous system, and this can be correlated with an increase in the concentration of histamine in the hepatic vein (2). The most significant initial feature in the chain of hemodynamic alterations occurring in endotoxin shock is a marked vasoconstriction.

This report is concerned with studies on the initial effects of endotoxin on the vascular beds of the dog. As a preliminary step, *in vitro* experiments were carried out with isolated, canine, saphenous veins suspended in an oxygenated bath (3). The vessels were exposed to each of several physiologic solutions and endotoxin, and contraction took place only in the presence of *fresh* whole blood and endotoxin. A heat-labile serum or plasma factor, inactivated by heating at 56°C for 30 minutes, was essential. Vessel contraction induced by liberated histamine resulted from the interaction of endotoxin, fresh serum or plasma, and platelets. These results were extended to the intact animal, and the following experiments demonstrate that the initiation of the hemodynamic changes in endotoxin shock is dependent upon the interaction of a labile serum factor and endotoxin. The results suggest that an enzyme system may be involved in the initial stage of this type of shock.

Materials and Methods

A total of 78 adult mongrel dogs were used in five experimental studies. The dogs were anesthetized with Na pentobarbital (30 mg/kg). A standardized lethal dose of 0.55 mg/kg of *Escherichia coli* endotoxin was used throughout, prepared in a manner previously described (4). Hemodynamic alterations were measured over a period of 8 hours, and included continuous tracings of systemic blood pressure, and hourly recordings of blood pH and hematocrit values (5). Dogs were recorded as "survivors" if they lived 72 hours or longer postendotoxin.

Group 1 Experiments.—Ten control anesthetized animals were given the standardized lethal dose of endotoxin intravenously, and continuously observed for 8 hours, or until the animals expired.

Group 2 Experiments.—Blood was removed from the femoral artery of each of 10 donor animals and collected in sterile flasks containing 1 mg of heparin per 100 cc of blood. Immedi-

ately after withdrawal, plasma was separated from the cells under sterile conditions by centrifuging for 30 minutes at 1500 RPM. The blood was then reconstituted, warmed to body temperature, and infused into the femoral vein of a normal recipient animal, while an equivalent amount of blood was withdrawn from the contralateral femoral artery. Each dog received an average of 602 cc of reconstituted blood obtained from one donor. After the transfusion had been completed, there was an equilibration period of 1 hour, after which the lethal dose of endotoxin was intravenously administered.

Group 3 Experiments.—Blood was obtained from 10 donor animals in the same manner as described for the preceding group, centrifuged, reconstituted, and infused into 10 recipients. The essential difference in these experiments was that the donor plasma was heated for 30 minutes at 56°C before the blood was reconstituted and transfused into normal animals. Each dog received an average of 580 cc of blood.

Group 4 Experiments.—This group included 7 normal dogs that had been transfused with heparinized blood obtained from 7 donor animals treated in the following manner: each of the donors, under a protective regime described elsewhere (5–7), had survived a lethal dose of endotoxin. These animals were resistant to a lethal dose of endotoxin given 8 days after the initial dose. *Within 24 hours after receiving the second dose of endotoxin,* blood was withdrawn from these immune donors and transfused into each of the 7 normal animals. As in the previous experiments the same amount of blood was withdrawn from the recipient dogs as the amount infused. Each animal received an average of 340 cc of heparinized blood. After an equilibration period of 1 hour, each of the recipients was given 0.55 mg/kg of endotoxin.

Group 5 Experiments.—This group comprised 7 normal animals that had received heparinized whole blood under the same conditions as the animals in group 4, except *the blood from the donor animals was not withdrawn until 72 hours after the second dose of endotoxin.* In other words, animals in group 4 received blood from endotoxin-resistant donors that had been withdrawn within 24 hours after a second injection of endotoxin, while animals in the present group received blood from resistant donors that had been withdrawn 72 hours after the second injection. Each of the recipient animals received an average of 318 cc of blood. The purpose of the experiments in groups 4 and 5 was to determine if the amount of serum factor essential for endotoxin shock had been depleted for a given period of time, *i.e.* 24 hours, and if an elapse of 3 days or more was sufficient for the reappearance of enough serum factor to react with endotoxin and cause peripheral vascular collapse and death.

RESULTS

Group 1 Experiments. Endotoxin Shock in Control Animals.—Data recorded in Table I show that all the animals expired. Postendotoxin survival times ranged from 30 minutes to 28 hours. The characteristic features of irreversible endotoxin shock, as detailed elsewhere, were present and included progressive hypotension, oliguria and anuria, hemoconcentration, and acidosis (7).

Group 2 Experiments. Dogs Transfused with Unheated Plasma in Reconstituted Blood and Given Endotoxin.—As seen in Table II there were only 2 survivors of the 10 animals treated in this manner. All of the animals, except the 2 survivors, exhibited the usual pattern of progressive endotoxin shock.

Group 3 Experiments. Dogs Transfused with Reconstituted Blood Containing Plasma Heated to 56°C for 30 Minutes and then Given Endotoxin.—In contrast with the outcome in control animals (Table I), and animals transfused with reconstituted unheated blood (Table II), 9 out of 10 dogs transfused with an

average of 580 cc reconstituted heated blood survived a lethal dose of endotoxin (Table III). All of the animals in the latter group displayed a decline in blood pressure immediately following the injection of endotoxin. Except for 1 non-

TABLE I

Survival Time of Dogs Given 0.55 Mg per Kg of E. Coli Endotoxin

Dog No.	Weight	Sex	Survival time
	kg		
1	5.5	F	30 min.
2	9.5	M	48 "
3	8.2	M	16 hrs.
4	5.7	M	28 "
5	9.4	F	18 "
6	10.2	F	18 "
7	6.1	F	7 "
8	7.0	M	10 "
9	11.0	M	12 "
10	7.3	F	18 "

TABLE II

Survival Time of Control Dogs Given Transfusion of Whole Blood Reconstituted after Centrifugation with Unheated Plasma, and Then Injected with 0.55 Mg per Kg of E. coli Endotoxin

Dog No.	Weight	Sex	Amount of blood infused	Survival time
	kg		*cc*	*hrs.*
1	7.0	F	621	6
2	7.5	M	612	16
3	7.0	M	605	40
4	7.6	M	610	Permanent
5	4.8	F	532	"
6	5.4	F	590	16
7	9.0	F	737	2.5
8	6.4	F	600	3
9	4.1	M	516	62
10	6.5	F	600	16
Average			602	13

survivor, the blood pressure became stabilized within 2 to 3 hours post endotoxin. Hemoconcentration occurred in only 2 of the survivors and acidosis as measured by blood pH was not demonstrated in any of the animals. It is significant that this group of animals tolerated a large dose of endotoxin better than any group in our experience, including those given a lethal dose of endo-

toxin, and then protected with steroids, pressor agents, hydralazine or epsilon aminocaproic acid (5–7).

TABLE III

Survival Time of Dogs Given Transfusion of Whole Blood Reconstituted after Centrifugation with Plasma Heated to 56°C for 30 Minutes and Then Injected with 0.55 Mg per Kg of E. coli Endotoxin

Dog No.	Weight	Sex	Amount of blood infused	Survival time
	kg		*cc*	*hrs.*
1	8.9	F	623	Permanent
2	7.4	M	550	"
3	6.7	F	578	20
4	8.0	M	435	Permanent
5	9.0	F	583	"
6	7.5	F	605	"
7	8.4	M	660	"
8	8.0	M	620	"
9	5.0	F	620	"
10	6.7	M	523	"
Average			580	

TABLE IV

Survival Time of Dogs Given Infusion of Whole Blood

Donor blood obtained from animals 8 days after recovery from endotoxin shock and immediately after second injection of *E. coli* endotoxin.

Dog No.	Weight	Sex	Amount of blood infused	Survival time
	kg		*cc*	*hrs.*
1	9.0	M	300	Permanent
2	10.2	F	250	"
3	12.0	M	400	"
4	9.8	F	460	"
5	10.0	M	250	60
6	11.9	M	270	13
7	15.0	M	450	Permanent
Average			340	

Group 4 Experiments. Dogs Transfused with Blood from Immune Animals Given Second Dose of Endotoxin and Bled Within 24 Hours.—Five of the 7 recipient animals treated in this manner survived the lethal dose of endotoxin (Table IV). These animals all reacted more violently to the endotoxin than did those dogs in the group 4 experiments given reconstituted whole blood contain-

ing preheated plasma. However, hemoconcentration and acidosis were not prominent features in surviving animals.

Two facts emerge from these observations. First, dogs protected against an initial lethal dose of endotoxin survive a second lethal injection given 1 week later. Second, canine blood obtained from immune dogs within 24 hours after a second injection of endotoxin affords good protection for normal recipient dogs against the lethal effects of endotoxin.

Group 5 Experiments. Dogs Transfused with Blood from Immune Animals Given Second Dose of Endotoxin and Bled 72 Hours Post Endotoxin.—The fore-

TABLE V

Survival Time of Dogs Given Infusion of Whole Blood from Immune Animals That Had Survived Shock

Donor blood obtained 72 hours after injection of second dose of endotoxin.

Dog No.	Weight	Sex	Amount of blood infused	Survival time
	kg		*cc*	*hrs.*
1	10.9	M	250	15.0
2	9.0	M	250	16.0
3	10.6	M	380	7.5
4	6.2	M	250	4.0
5	7.9	M	375	Permanent
6	6.8	M	275	22.0
7	6.8	F	450	12.0
Average			318	12.7

going experiments suggested that a booster dose of endotoxin produced an immune response adequate to protect normal animals against a lethal dose of endotoxin. This protection was demonstrated when donor blood was obtained within 24 hours after the booster dose. However, only 1 of 7 dogs survived a lethal dose of endotoxin after being transfused with an average of 318 cc of blood drawn 72 hours after the booster dose from the immune donors (Table V).

The results in this experiment demonstrate that dogs surviving a lethal dose of endotoxin are protected against a second lethal injection of endotoxin. This immune state endures for several months. However, under the experimental conditions that were employed there is no evidence that this immunity could be passively transferred to other dogs.

DISCUSSION

These experiments on canine endotoxin shock have demonstrated that the initial hemodynamic alterations are induced by an interaction between endo-

toxin and a labile serum factor. This essential factor is heat-labile, suggesting that an enzyme or enzyme system is involved. Previous observations have indicated that endotoxin might activate a proteolytic enzyme system, since epsilon aminocaproic acid protected dogs against lethal endotoxin shock (7). This compound is an inhibitor of plasminogen activation, and the activation of plasminogen is associated with increased proteolytic activity in the serum.

It is intriguing to consider the possibility that an enzyme participating in endotoxin activity might involve complement. Janeway and Gitlin (8) have stated that children with agammaglobulinemia are unusually resistant to infections caused by Gram-negative organisms. The devastating effects of these bacteria are partly related to the liberation of endotoxin. It has been reported in a child with agammaglobulinemia that the first component of complement (C'1) was considerably reduced, although the hemolytic activity of the combined four components was normal (9). The first component is considered to be of the nature of globulin, and is presumed to exist as a precursor of the enzyme esterase. The possible role of complement in endotoxin activity is being explored further.

The present studies have also focused attention on a perplexing aspect of endotoxin immunity. Dogs surviving a lethal dose of endotoxin are protected over a period of several weeks against a second lethal dose of endotoxin. Similar observations have been made in mice (10). Although a considerable degree of active endotoxin immunity was established in dogs, this immunity could not be passively transferred to other dogs under the experimental conditions that were employed. However, passive immunity could be established in normal dogs against a lethal dose of endotoxin if the dogs were transfused with blood reconstituted with preheated plasma, or transfused with donor blood that had been removed from immune dogs within 24 hours after a second dose of endotoxin. In experiments not incorporated within this paper we have also protected dogs by infusing them with blood obtained from non-immune donor dogs given a sublethal dose of endotoxin within 24 hours before being bled.

These experiments lead to the hypothesis that in the dog resistance to endotoxin shock can be temporarily induced by the depletion of a serum enzyme or enzyme system. This refractory state can be accomplished in the normal dog through infusions of blood containing preheated plasma, or by infusion of blood obtained from dogs that had been bled within 24 hours after an injection of a large dose of endotoxin. It is further postulated that the enzyme system might involve complement. This concept of the essential nature of complement in endotoxin shock is supported by the many observations that have been made on the role of complement in anaphylactic reactions and in anaphylactoid shock. Weil and Spink (2) have pointed out that the pathophysiology of endotoxin shock simulates that described for anaphylactic shock. Schaedler and Dubos (11) have suggested that an immunologic reaction responsible for the

lethal action of endotoxin is based upon acquired hypersensitivity to Gram-negative bacilli. In support of this are the observations that mice with active brucella infection are much more susceptible to the lethal effects of brucella endotoxin than non-infected controls (12).

Our studies on endotoxin shock in several species of animals, including man, have clearly indicated the complexity of the problem. From the initial stages to either death or recovery there is a constantly changing spectrum of hemodynamic and metabolic activity. In an attempt to obtain more precise information on the nature of the vascular and humoral changes that occur the "trigger mechanism" has been explored in the dog. Similar studies have been made in other species, and the results would suggest that the initial vascular alterations have a common mechanism, but the subsequent vascular and tissue changes vary considerably.

SUMMARY

Peripheral vascular failure caused by endotoxin in the dog has an initial stage of vasoconstriction. Preliminary studies *in vitro* demonstrated that the constriction was due to the interaction of endotoxin with a heat-labile serum or plasma factor and platelets, resulting in the liberation of histamine. Further studies on the intact dog support and extend this concept.

A standardized dose of *Escherichia coli* endotoxin produced fatal shock in control adult mongrel dogs within 28 hours. The characteristic pattern of changes included progressive hypotension, oliguria and anuria, hemoconcentration, and acidosis.

Normal dogs were protected against endotoxin by transfusions of blood in which the essential serum factor was depleted in one of two ways. First, plasma separated from the blood of normal animals was heated at 56°C for 30 minutes, and the infused reconstituted whole blood protected normal dogs. Protection was not afforded by unheated reconstituted blood. Second, blood from immune dogs obtained within 24 hours after a second lethal dose of endotoxin protected recipient dogs. However, protection was not demonstrated with blood collected 72 hours after a second injection of endotoxin.

The nature of the serum factor essential for endotoxin activity is not known. It is postulated that an enzyme or enzyme system is involved, and the possible role of complement is discussed.

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