a test for low concentrations of trypsin as did the methods of Anson and Kunitz. However, because of its simplicity for assay of tryptic activity, the RIHSA method is considered to be of definite value when enzyme concentrations to be assayed are in excess of 2000 T.U. per ml.

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Received Nov. 14, 1960. P.S.E.B.M., 1961, v106.

Endotoxin Shock and the Coagulation Mechanism: Modification of Shock with Epsilon-Aminocaproic Acid. (26299)

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Injection of a lethal dose of endotoxin into the dog is followed by a characteristic pattern of hemodynamic changes(1,2). Within a minute there is a marked decline in systemic blood pressure and rise in portal vein pressure. Fifteen to 30 minutes later the blood pressure tends to approach pre-injection level, then a gradual decline occurs, accompanied by oliguria or anuria, acidosis, and hemoconcentration. Death takes place within 24 hours.

We have reviewed elsewhere our investigations on the mechanism of vascular alterations induced by endotoxin in the dog(2,3.4). Briefly, the initial vasoconstriction caused by endotoxin is probably due to histamine or a histamine-like substance, release of which is mediated through a component or components of blood. Contraction of an isolated canine vein occurred when the vessel was exposed to a bath containing endotoxin and fresh whole blood. Enzymatic participation in the reaction was suggested by the latent period that occurred before contraction took place; and by an activating serum factor that was dialyzable and heat-labile. Involvement of proteolytic activity was considered, possibly associated with the coagulation mechanism. Such a concept is suggested by other investigations. Unger and Mist(5) postulated that specific antigen-antibody reactions, peptones or bacterial polysaccharides caused liberation of histamine through increased proteolytic activity of the serum. Others have concluded(6,7) that the Shwartzman reaction in rabbits is a result of coagulation alterations caused by endotoxin. Deutsch and Elsner(8) have stated that administration of endotoxin results in activation of the plasminogen-plasmin system in which an initial state of hypercoagulability is followed by accelerated fibrinolysis. Gans and Krivit(9, 10) reporting on some significant comparative studies on endotoxin in rabbits and dogs as to effects on the coagulation mechanism, concluded that endotoxin did not activate the plasminogen-plasmin system in rabbits. Therefore, in this species endotoxin causes extensive thrombosis, which is a feature of the Shwartzman reaction. Endotoxin does not produce the Shwartzman reaction in the dog because plasminogen is activated and thrombolytic activity is prominent. Pertinent to the present study they found that endotoxin in the dog caused a marked increase in activity of plasminogen-activator, and a decline in plasminogen concentration.

Epsilon-aminocaproic acid (hereafter referred to as EACA) is a potent inhibitor of plasminogen activation(11). Sherry and his associates(12,13) have studied EACA intensively, both *in vitro* and in human subjects,

and have concluded that although the action of EACA on the coagulation mechanism is complex, the most striking effect is the inhibition of plasminogen activation.

If endotoxin shock in the dog is associated with activation of plasminogen, and the increase in proteolytic activity results in liberation of histamine, inhibition of plasminogen activation with EACA should lead to a reduction in severity of the vascular response to endotoxin. The present experiments demonstrate that pre-treatment of adult dogs with EACA results in survival of the majority of the animals injected with a lethal dose of endotoxin.*

Materials and methods. Endotoxin. A standardized amount of endotoxin, prepared from a strain of *Escherichia coli* according to methods described elsewhere(14), was given intravenously to dogs in a LD_{100} dose of 0.55 mg/kg. Animals expired within 24 hours from peripheral vascular collapse.

EACA. A sterile solution was slowly infused intravenously as a 1% solution in 200 ml of 5% dextrose and water.†

Animals. Adult mongrel male dogs were used in all of the experiments and housed in individual cages. Only water was permitted on day of experiments. Following each experiment surviving dogs were returned to the cages and offered food and water. "Survivals" included those animals that resumed their usual physical activities outside of the cages after recovery from the effects of endotoxin.

Experimental procedure. The dogs were anesthetized with 30 mg/kg body weight of sodium pentobarbital, and sufficient barbiturate was given subsequently to maintain a constant level of anesthesia.

Systemic arterial pressure was monitored continuously by means of a polyethylene catheter placed in the femoral artery, which in turn was connected to a Statham strain gauge, and tracings were made on a Sanborn Twin Viso recorder. The bladder was catheterized, and urine quantitated hourly. The pH of the femoral arterial blood was measured hourly with a Beckman pH meter, and hourly hematocrit determinations were also made.

Exp. 1. Twelve dogs were anesthetized and control observations were made over a period of 1 hour. Endotoxin was injected and continuous observations were carried out until the animals expired, or for at least 7 hours.

Exp. 2. The purpose of this experiment was to determine if pre-treatment of dogs with EACA would modify the severity of endotoxin shock. Ten dogs were anesthetized and control observations were made for 30 to 60 minutes, following which each of the dogs was given an infusion of 2 g of EACA over a period of 1 hour. During this time further measurements were made. At the end of the hour, endotoxin was injected, and the animals were continuously observed for a minimum of 7 hours.

Exp. 3. In this experiment the object was to determine if the severity of shock could be modified by giving EACA after injection of endotoxin. A group of 7 anesthetized animals were observed for a control period of 1 hour and then they received a lethal dose of endotoxin. Within 15 to 30 minutes after injection of endotoxin a sufficient amount of EACA was infused so that the blood pressure was stabilized, and a flow of urine occurred.

Exp. 4. Further to evaluate the post-endotoxin effect of EACA, 7 anesthetized animals were infused with a maximum of 2 g of EACA 40 to 80 minutes after receiving endotoxin.

Results. Control animals. Data on the 12 control dogs are shown in Table I. Mean survival time after injection of endotoxin was 12.6 hours. It will be noted that there was a precipitous drop in blood pressure in all of the animals, except 1 (Dog #9), within 15 minutes after injection of endotoxin. Over the succeeding hours a progressive hypotension developed, which was accompanied by oliguria and anuria. In addition (Fig. 1), the animals manifested an increase in hematocrit and a decline in blood pH.

Animals pre-treated with EACA. In a

* We are indebted to Dr. Sol Sherry, Washington Univ. Med. School, St. Louis, for the initial supply of EACA.

† Supplied by Merck Sharp and Dohme Research Laboratories, West Point, Pa.

TABLE I. Systolic Blood Pressure (BP) and Urine Output in cc (UO) in 12 Male Dogs Given a Lethal Dose of *E. coli* Endotoxin. Determinations recorded for a control period of 1 hr, then hourly after endotoxin.

Time of observation	Dog No. and wt (kg) 1 11.5 BP	UO	2 8.8 BP	UO	3 12.0 BP	UO	4 9.4 BP	UO	5 8.8 BP	UO	6 7.7 BP	UO
Control	150	8.8	140	9.2	115	10.9	150	10.0	215	9.9	145	2.0
15 min. post-endo.	40		35		30		25		40		50	
1 hr *idem*	40	12.4	30	11.1	50	0	80	18.6	40	12.0	50	0
2 " "	0	.9	0	0	50	0	75	2.3	0	0	45	0
3 " "					0	0	75	4.0			35	0
4 " "							85	5.0			0	0
5 " "							100	5.1				
6 " "							120	.6				
7 " "							70	.3				
Hr of survival post-endotoxin	2		2		3.5		18		2.5		4.0	

Time of observation	Dog No. and wt (kg) 7 13.8 BP	UO	8 8.8 BP	UO	9 11.0 BP	UO	10 8.0 BP	UO	11 7.0 BP	UO	12 11.0 BP	UO	Mean hourly value (dogs 1-12) BP	UO
Control	175	8.8	200	9.1	135	7.2	155	15.6	140	10.2	155	7.2	156	9.0
15 min. post-endo.	50		35		125		40		60		45		48	
1 hr *idem*	110	2.5	120	1.6	80	2.3	100	.5	110	11.4	65	8.1	73	6.7
2 " "	110	2.6	70	.3	110	2.4	50	.2	115	.8	65	.4	58	.8
3 " "	120	1.1	125	.5	135	4.8	70	.7	120	.4	110	1.1	66	1.2
4 " "	135	7.3	120	.4	135	4.8	0	0	120	.6	130	1.4	60	1.6
5 " "	110	14.8	0		135	4.1			120	.4	135	2.0	50	2.2
6 " "	110	14.0			125	4.3			120	0	120	.6	50	1.7
7 " "	110	14.6			120	5.0			80	0	105	0	40	1.7
Hr of survival post-endotoxin	56		5		18		4		12		13			

preliminary experiment rapid administration of 2 g of EACA resulted in a marked rise in blood pressure. For this reason, the EACA was slowly infused over a period of 1 hour. However, (Table II and Fig. 1), some hypertensive effect was still observed. The hypertension was not due to volume of fluid injected. Compared with the control group of animals the blood pressure became stabilized after injection of endotoxin. Seven of the 10 dogs survived. Severe oliguria and anuria did not occur in the majority of treated animals, and hemoconcentration and acidosis were minimal (Fig. 1).

Animals given EACA 15 to 30 minutes after endotoxin. A group of 7 dogs given a lethal dose of endotoxin manifested the initial decline in blood pressure, then within 15 to 30 minutes EACA was administered until the blood pressure became stabilized near pre-endotoxin levels. All 7 animals survived. One outstanding feature was the excellent output of urine manifested by all of the dogs (Table III) Amounts of EACA employed varied between 0.2 to 2 g, with an average of 1.2 g infused 25 minutes after endotoxin.

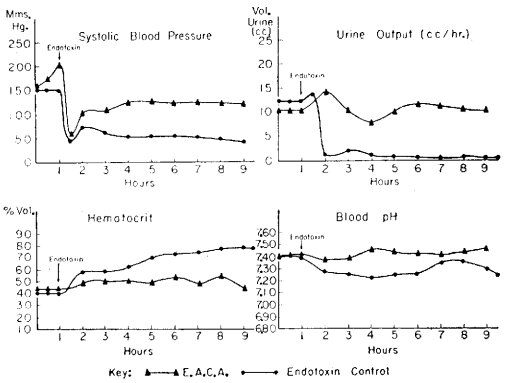

FIG. 1. Comparative mean hourly determinations of systolic blood pressure, urine output, hematocrit and blood pH in 10 control dogs given a lethal dose of endotoxin, and 10 dogs pre-treated with EACA. All of the control dogs died, while 7 out of the 10 treated animals survived.

TABLE II. Systolic Blood Pressure (BP) and Urine Output in cc (UO) in 10 Male Dogs Pretreated with 2 g Epsilon-Aminocaproic Acid and then Given Lethal Dose of *E. coli* Endotoxin. Determinations recorded for control period of 1 hr, 1 hr after EACA, then hourly after endotoxin.

	Dog No. and wt (kg)																					
	1		2		3		4		5		6		7		8		9		10		Mean hourly value	
Time of observation	9.5		12.0		12.7		10.7		9.0		11.0		12.4		11.6		9.1		12.0			
	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO
Control	150	10.6	130	9.8	140	8.8	150	11.2	145	7.2	175	12.4	215	8.1	45	4.2	180	11.1	165	8.5	150	9.2
1 hr post-EACA	285	11.0	175	10.4	155	9.6	160	10.1	205	7.0	185	13.9	290	10.0	155	5.4	265	10.8	230	9.5	211	9.8
15 min. post-endo.	70	—	60	—	35	—	50	—	45	—	130	—	36	—	90	—	45	—	45	—	61	—
1 hr *idem*	130	18.0	150	32.7	65	6.9	75	6.0	60	13.6	155	37.4	60	4.1	118	4.2	70	15.0	175	4.6	106	14.3
2 " "	90	00 0	125	13.4	80	12.6	95	5.9	70	.1	160	65.4	135	0	87	.2	100	6.5	130	2.5	107	10.7
3 " "	145	1.2	135	11.3	100	8.6	125	14.9	120	2.3	150	24.5	130	0	100	.2	125	.6	110	4.2	124	6.8
4 " "	125	.2	145	27.4	100	22.3	140	21.2	125	9.6	150	13.0	100	0	125	0	145	7.0	95	3.6	125	10.4
5 " "	110	.2	145	20.3	85	23.2	140	24.0	120	18.0	135	16.5	0	0	100	0	150	26.0	115	1.2	122	12.9
6 " "	110	.3	145	19.4	85	10.1	140	23.6	115	12.4	140	14.3	—	0	100	.4	150	28.0	120	3.6	123	11.2
7 " "	105	.4	140	18.5	85	0	135	22.1	120	6.8	135	15.8			95	.6	145	38.4	115	4.8	119	10.7
Outcome	Survived		Survived		Died 7 hr		Survived		Survived		Survived		Died 4.5 hr		Survived		Survived		Died 18 hr			

TABLE III. Systolic Blood Pressure (BP) and Urine Output in cc (UO) in 7 Male Dogs Given Lethal Dose of Endotoxin, then Treated with Epsilon-Aminocaproic Acid 15 to 30 Min. after Endotoxin. Measurements recorded for control period of 1 hr, then hourly after endotoxin.

	Dog No. and wt (kg)															
	1		2		3		4		5		6		7			
	11.4		11.2		9.0		14.0		10.2		12.0		9.0			
	Dose of EACA (g) and min. given after endotoxin														Mean hourly value	
Time of observation	1.0–20		0.2–30		1.2–15		1.0–15		2.0–15		1.6–22		0.8–25			
	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO
Control	210	8.8	135	6.4	175	10.8	195	9.6	140	10.8	210	7.8	170	8.0	176	8.9
15 min. post-endo.	45		25		45		50		45		50		65		46	
1 hr *idem*	225	26.4	105	5.7	60	8.7	185	50.0	110	8.0	100	6.8	85	4.1	124	15.6
2 " "	180	46.2	165	20.2	90	.5	90	8.2	140	14.6	105	8.0	130	2.0	129	14.2
3 " "	210	40.6	145	.7	105	3.2	125	1.6	145	12.1	155	11.9	140	4.5	146	10.6
4 " "	165	76.0	175	1.6	115	9.8	160	7.0	160	9.5	150	12.3	165	11.5	155	18.0
5 " "	145	45.0	180	5.0	105	9.6	170	4.6	150	16.0	160	11.0	170	14.0	154	15.0
6 " "	165	46.0	175	6.8	105	32.0	160	8.2	140	18.0	150	10.1	160	15.8	150	19.5
7 " "	150	50.0	170	8.9	100	63.0	170	7.3	140	19.5	140	9.0	150	18.8	145	25.2

TABLE IV. Systolic Blood Pressure (BP) and Urine Output in cc (UO) in 7 Male Dogs Given Lethal Dose of Endotoxin, then Treated with 2 g Epsilon-Aminocaproic Acid 45 to 80 Min. after Endotoxin. Measurements recorded for control period of 1 hr, then hourly after endotoxin.

	Dog No. and wt (kg)														Mean hourly value	
	1		2		3		4		5		6		7			
	11.0		10.0		10.5		7.3		8.6		5.9		6.1			
	Min. after endotoxin EACA given															
Time of observation	60		45		60		70		75		80		50			
	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO	BP	UO
Control	145	5.9	170	9.5	135	5.8	145	8.8	200	9.4	140	9.6	155	8.8	156	8.3
15 min. post-endo.	60		50		45		40		30		30		45		43	
1 hr *idem*	55	.2	65	2.8	45	2.0	30	20.0	175	3.2	135	5.7	135	4.9	91	3.0
2 " "	60	0	50	0	70	0	55	.8	170	4.8	170	30.9	140	11.8	102	6.9
3 " "	0				80	3.0	50	0	145	.9	143	11.2	190	14.0	87	4.2
4 " "					85	3.0	0		150	.4	145	4.9	185	15.0	81	3.3
5 " "					80	3.0			150	1.2	167	11.2	170	8.0	81	3.3
6 " "					75	3.0			145	.9	165	5.5	170	2.1	79	1.6
7 " "					70	2.8			140	1.0	160	3.0	165	0	76	1.0

Animals given EACA 45 to 80 minutes after endotoxin. In the preceding experiment dogs were given EACA during the initial phase of shock. In the present experiment 2 g of EACA were administered to a group of 7 dogs when the animals were in a more advanced stage of vascular collapse, but at a period when the shock could be reversed by other means, such as by a combination of a steroid and pressor agent(15). None of the 7 dogs survived. EACA given at the later stage of collapse did not correct the hypotension and oliguria (Table IV).

Discussion. These experiments have demonstrated that EACA, an inhibitor of plasminogen activation, protected dogs against lethal endotoxin shock, when EACA was infused either before or shortly after injection of endotoxin. This EACA-endotoxin time relationship is of interest in connection with observations that Sherry and his group(12) had carried out on the pyrogenic effect of endotoxin in human subjects who were given EACA. A hyperfibrinolytic state was induced with endotoxin (Pyrexal), then 1 hour after injection of endotoxin 10 g of EACA was administered. Although fibrinolysis was appreciatively decreased by this procedure, the endotoxin provoked the characteristic chills and fever. But no information is available as to whether pre-treatment with EACA would eliminate or modify the pyrogenic effect of endotoxin.

During these experiments, 2 consistent pressor changes occurred in dogs receiving EACA that are not readily explained. First, the infusion of EACA caused a significant rise in systemic blood pressure in normal anesthetized animals, particularly when concentrated solutions were used. The hypertension was detected because the pressure was monitored continuously. This phenomenon has not been reported by others. The pressor response was observed with 3 different sterile lots of EACA. Second, although EACA did have a pressor effect, pre-treatment of the dogs with the compound did not prevent, or apparently modify, the immediate hypotensive action of endotoxin that is unique for the dog. This initial hypotension, occurring within seconds after injection

of endotoxin, is due to intrahepatic venous constriction, which results in pooling of blood within the liver and walls of the intestinal tract. It is generally accepted that the vasoconstriction is due to histamine.

While it can be postulated that endotoxin activates the plasminogen-plasmin system with an increase in fibrinolytic activity and liberation of histamine, our experimental data are inadequate for the support of such a concept. It is possible that endotoxin activates proteolytic enzyme activity that is distinct from the plasminogen system. The foregoing results indicate that further information along several lines is needed. Quantitative plasminogen studies should be carried out in control animals, and in those pretreated with EACA, and quantitative histamine determinations should be made on the hepatic venous blood in similar groups of animals. The dose of both endotoxin and EACA should be varied in several groups of dogs.

If endotoxin does not activate the plasminogen-plasmin system in rabbits, as reported by others(10), it would be desirable to determine if pre-treatment of rabbits with EACA would protect them against a single lethal dose of endotoxin. Preliminary experiments in this laboratory indicate that EACA does not significantly protect this species.

The present experiments emphasize the complex nature of endotoxin shock. Incorporation of the coagulation mechanism into a concept on the pathogenesis of this type of shock requires more quantitative data in different mammalian species. The accumulating evidence suggests that several mechanisms may be responsible for the hemodynamic alterations caused by endotoxin, and there are important variations between species.

Summary. 1. Lethal shock was established with a standardized dose of *E. coli* endotoxin in adult mongrel dogs. In this species initiation of the peripheral vascular collapse involves liberation of histamine, which may be related to the activation of a proteolytic system by the endotoxin. 2. Pre-treatment of dogs with epsilon-aminocaproic acid (EACA), a potent inhibitor of plasminogen activator, protected the majority of animals against a lethal dose of endotoxin. Protection was also observed when EACA was given up to 30 minutes after injection of endotoxin. However, when EACA was administered beyond this time no protection occurred, and there was no evidence that the severity of the shock was modified. It is emphasized that these observations with EACA apply only to the dog. 3. The primary purpose of the present experiments was to determine if EACA protected dogs against lethal amounts of endotoxin. The results do not permit the conclusion that EACA modified the severity of shock by blocking plasminogen activation, and in this manner prevented liberation of histamine. There remains the possibility EACA blocked proteolytic activity that is independent of the plasminogen-plasmin system.

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