Effect of Endotoxin Shock on the Clotting Mechanism of Dogs *

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INTRAVENOUS injection of adequate amounts of endotoxin from gram-negative bacteria results in shock and death in the dog. The pathophysiology of shock so produced by endotoxin has been intensively studied. Consideration has been given to the effects on the central nervous,[13] gastro-intestinal [19] and reticuloendothelial [16] systems, and to the induction of generalized and/or localized vasoconstriction.[74]

Changes in the coagulation system have also been implicated in production of pathologic changes resulting from endotoxin. Good and Thomas [6] and Cluff and Berthrong [3] were able to prevent the general and local Schwartzman reaction of endotoxin by pretreatment with heparin. Later, McKay and Shapiro [10] used Warfarin successfully for the same purpose.

The present study was undertaken in order to determine the effect of endotoxin on the coagulation mechanism of dogs. Lillehei and McLean [9] had already noted thrombocytopenia and prolonged clotting times following endotoxin shock in the dog.

In this study the fibrinogen, and plasmin and plasminogen levels before and after the injection of endotoxin were measured. Subsequently, the fibrinogen levels as well were measured before and after injection of endotoxin in animals pretreated with heparin and Warfarin.

The present experiments indicate the profound alterations in the coagulation system produced by endotoxin shock in the dog. The decrease in fibrinogen is evidence of intravascular clotting. Moreover, when intravascular clotting is interfered with by heparin or Warfarin, death of the animals can be prevented.

Material and Methods

Healthy mongrel dogs, weighing from 20 to 30 kg. were anesthetized using Nembutal sodium, 1 cc. (1 grain) per 2.5 kg. body weight intravenously. A 15-gauge polyvinyl tube was introduced into the femoral artery and advanced approximately 20 to 30 cm. into the aorta. A three-way stopcock allowed for the injection of the various materials as well as the withdrawal of blood.

The endotoxin used was the purified commercial *E. coli* lipopolysaccharide (Difco, batch No. 0127: B8. Control 110733). Dosages used were 0.1 mg. and 1.0 mg./kg. given intravenously. Heparin sodium, 1,000 U.S.P. units per cc., was used in a dose of 0.6 to 1 mg./kg. body weight. This was repeated every two hours to maintain hypocoagulability. Warfarin 1 mg./kg. body weight was given daily intravenously or intra-arterially. Maintenance of hypocoagulability was obtained by keeping prothrombin times (Quick method) over 50 seconds.

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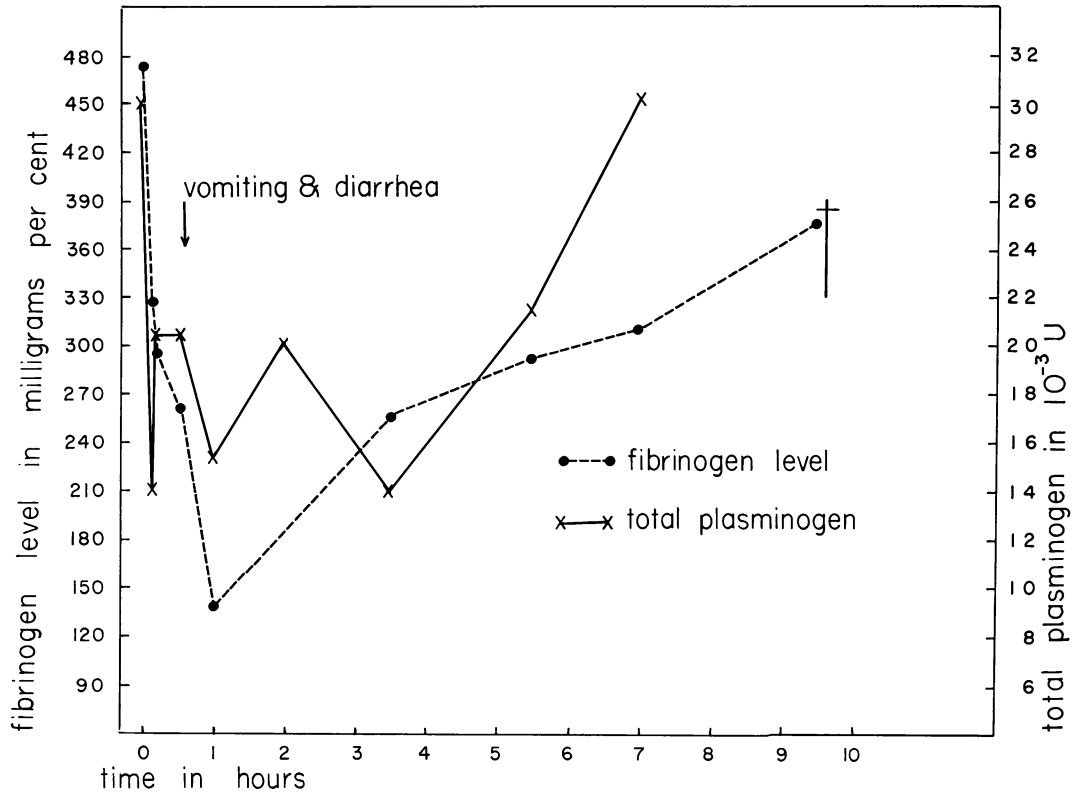

FIG. 1. Changes in fibrinogen level (in mg.%) and in the total plasminogen level (in 10^{-3} U) in dog #8. Following the administration of 0.1 mg. endotoxin per kg. body weight, there is a simultaneous fall of fibrinogen and total plasminogen.

Eight cc. of blood were allowed to flow freely from the stopcock into a tube containing 0.30 cc. of 10 per cent Sequestrene. Samples were taken for micro hematocrit determinations.† The blood was centrifuged immediately thereafter following which the plasma was transferred to lipvials and quick frozen at − 20° C.

Thawing and rewarming of plasma were done at 37° C. in a constant temperature waterbath. All samples from one dog were processed simultaneously.

The free plasmin activity was determined as follows: plasma, 0.5 cc. was incubated with 0.5 cc. of a four per cent casein solution (pH 7.4) for one hour. None of the constituents was diluted by any buffer solution. Subsequently, the plasma-casein mixture was precipitated with 10 per cent trichloracetic acid. The supernatant was read against an appropriate blank, in the Beckman U.V. spectrophotometer at 280 mu. The total plasminogen activity was determined as described by Norman.[12]

The platelet count was done by the Reese-Ecker technic.† The prothrombin time was done by the one-stage method of Quick. Lee-White clotting times were done using nonsiliconized and then later, siliconized tubes.

Fibrinogen determinations were done, as described by K. Jacobsson.[7] Also, the influence of antithrombin on the fibrinogen yield was determined according to Jacobsson's description. This influence was found to be negligible for all the experiments described in this paper.

† Will be reported in a separate paper.

Results

Fibrinogen: Endotoxin when tested *in vitro*, in the concentrations used in these experiments neither promotes clotting, nor decreases the fibrinogen yield.

Following the administration of endotoxin *in vivo*, the fibrinogen level shows a variable decline. As was noted in one of our dogs, this fall in fibrinogen was from 474 mg.% to 138 mg.% in one hour (Fig. 1). Fasting control levels of fibrinogen varied considerably. The results are therefore best compared by considering the original pre-endotoxin level as 100 per cent and changes thereafter as fractions of 100 per cent instead of in absolute mg.%. Thus, the above noted change represents a fall in fibrinogen to 27 per cent of the original value.

The fibrinogen levels of 10 dogs given endotoxin demonstrate a consistent rate of decline (Fig. 2). The average time for the maximum decrease is about one and one-half hours following endotoxin. Control dogs given saline instead of endotoxin showed no fall in fibrinogen (Fig. 3).

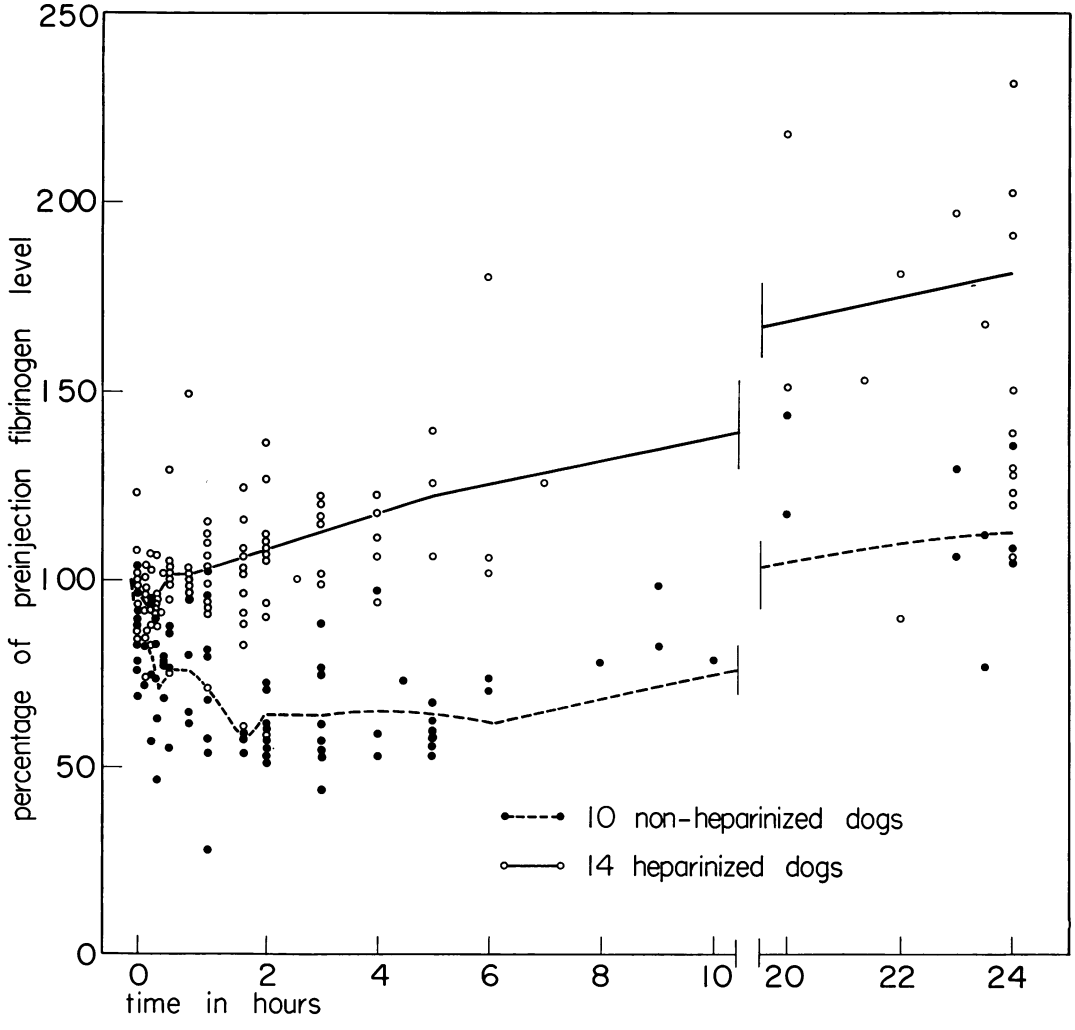

FIG. 2. Changes in the fibrinogen levels following endotoxin administration in ten nonheparinized and 14 heparin pretreated dogs. The fibrinogen levels are expressed in the percentage of the pre-endotoxin injection fibrinogen value.

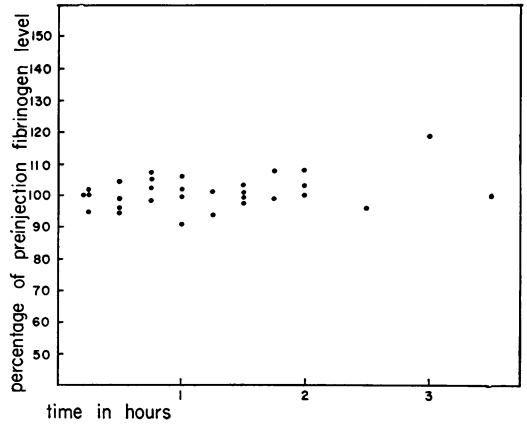

FIG. 3. Changes of fibrinogen levels in four control dogs receiving saline instead of endotoxin.

Fourteen dogs were heparinized prior to the injection of endotoxin. With the dosage of heparin used (0.6 to 1 mg./kg.) the clotting time was prolonged to 120 minutes with a total duration of one and one-half to two hours. Pretreatment with heparin before endotoxin injection regularly prevented the above cited decline in fibrinogen levels (Fig. 2).

The percentage change from the original fibrinogen levels and after endotoxin, were grouped into two time periods. The first was from 0 to one and one-half hours, the second was from after one and one-half to five hours. The statistical analysis in each time period revealed that there was a significant difference between the heparinized and nonheparinized fibrinogen levels (Fig. 4). In first time period $K = 3.5$ and $P < .0005$. In second time period $K = 7.4$ and $P < .0001$.

The dogs that were pretreated with Warfarin had prothrombin concentrations that were less than 5 per cent of normal. Following injection of endotoxin, these animals also revealed a minimal lowering of the fibrinogen levels (Fig. 5). The percentage of decrease in fibrinogen was significantly different from that noted in the animals who received only endotoxin.

Plasmin and Plasminogen: Repeated attempts to active the plasminogen-plasmin system *in vitro,* using endotoxin in different concentrations were unsuccessful. In dogs receiving saline I.V. no changes in the plasmin-plasminogen system were observed. Moreover, the measurement of plasmin did not reveal the presence of any free active enzyme in the dog.* This was found to be the same in all experimental groups so studied in this report.

In nine of the nonheparinized dogs there was a marked decline in the total plasminogen levels (Fig. 6). In nine of the heparinized dogs the values noted for the total plasminogen levels were quite variable. In some there was a marked rise and in others there was an immediate drop after the injection of endotoxin. In all pretreated with heparin, however, the average plasminogen value returned to normal within a relatively short period of time (approximately 45 to 60 minutes). A significant decline in plasminogen concomitant with fall in fibrinogen was obtained by slowly perfusing a dog with thrombin** over a 2-hour period (Fig. 7).

In the group of dogs receiving only endotoxin, without pretreatment with heparin, the mortality was 50 per cent. In the heparin pretreated group the mortality was 0%. Animals of both groups receiving 0.1 mg. of endotoxin per kg. body weight. This difference in the mortality is statistically significant. ($X^2 = 4$, $P < 0.046$.)

If lethal doses of endotoxin were given (1 mg./kg.) no such differences were evident between the two groups. Heparin therefore, only protected dogs against the lethal effect of endotoxin when less than overwhelming doses of toxin were used.

Discussion

The decrease in the fibrinogen level following the endotoxin requires explanation. One explanation would be an expanding blood volume. However, a rising

* This could be confirmed by using, besides Norman's method, the heated fibrin plate method (Astrup *et al.;* [18] Lassen [19]).

** Topical preparation; 5,000 NIH units (Parke, Davis Co.) dissolved in 300 cc. saline.

hematocrit during the early experimental time indicated an hypovolemia rather than an expanding blood volume.

Another explanation for the fall in fibrinogen would be the possibility that dogs possess a very active proteolytic (i.e. plasmin) enzyme system, which might be capable of lysing circulating fibrinogen, *in vivo*. However, our observation on the complete lack of free plasmin in all the dogs in the face of a rapidly falling fibrinogen value makes this latter explanation untenable.

An adequate explanation for the fibrinogen level changes must take cognizance of the lack of fall in fibrinogen in the heparinized and Warfarin-treated dogs. The only theory that remains tenable under all these circumstances is that endotoxin induces the fibrinogen to clot.

Intravascular clotting has been studied by a number of investigators. By using a slow infusion of thromboplastin, Ratnoff [15] caused afibrinogenemia in the dog. Also, Quick [14] infused thrombin slowly and noted a similar depletion of fibrinogen. There were no signs and symptoms of shock in these animals probably because these experiments were specifically done to produce only a gradual reduction of fibrinogen.

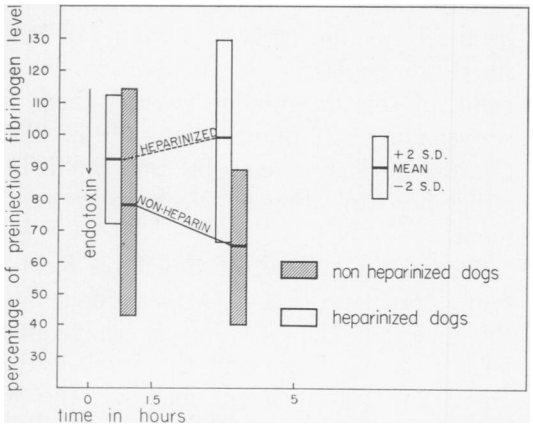

FIG. 4. Statistical analysis of the fibrinogen levels of the heparinized and nonheparinized group of dogs. The percentage changes of original fibrinogen levels are grouped in two time periods. The first is from 0–1½ hours, the second from after 1½ hours to five hours. The statistical analysis in each time period reveals that there is a significant difference between the two groups of dogs. (P is $< .0005$).

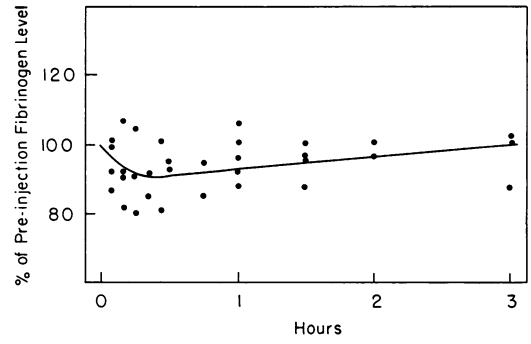

FIG. 5. Changes in fibrinogen levels following endotoxin administration in 5 Warfarin pretreated dogs.

In the rabbit, the documentation of intravascular clotting has been amply demonstrated during the Schwartzman phenomenon by Good and others.[6] McKay and Shapiro [10] moreover were able to demonstrate occasional fibrinoid deposits in vessels of the rabbit after *one* injection of endotoxin.

Therefore, there is ample morphological evidence that fibrin deposits are found in the rabbit after endotoxin injection. Only minimal evidence of any marked fibrin deposition was noted in the dog. Moreover, the dog does not develop the complex which is so characteristic for Schwartzman reaction. Rather, there is extensive hemorrhages in the lungs, gastro-intestinal tract and kidneys.

Both the fibrinoid deposition in the rabbit and hemorrhage in the dog possibly reflect the same basic phenomenon of intravascular clotting as a sequelae to endotoxin. In both instances, there is a considerable drop in fibrinogen after endotoxin. In both species pretreatment with heparin and Warfarin prevents death and fall in fibrinogen levels.

The differences in expression of these same phenomenon of defibrinogenation in the rabbit and dog may be found in the plasminogen system. The dog has been shown to have an active and potent plasminogen system. In the dogs that did not

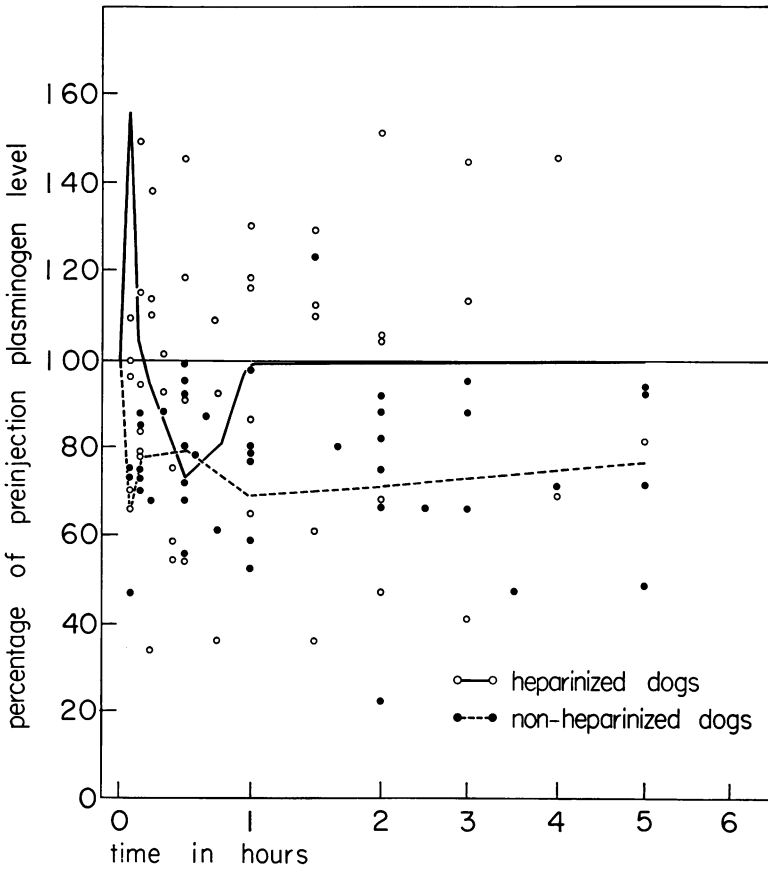

FIG. 6. Changes in total plasminogen levels following the administration of endotoxin in nine heparin pretreated and nine nonheparinized dogs.

receive heparin, there was a significant decrease in the plasminogen levels after injection of endotoxin. The decline of the plasminogen level can be interpreted as indicating that plasminogen had been absorbed to the fibrin formed following endotoxin injection. Evidence for an intimate attachment of plasminogen to fibrin has been presented by Back *et al.*[2] The plasminogen (precursor) is then converted to plasmin (active enzyme). Lysis of the fibrin reticulum occurs at the local site of plasminogen deposition. This concept thus would explain the fall in plasminogen, the lack of free circulating plasmin activity and the absence of morphological evidence for fibrin clot formation.

To explain the findings observed during these experiments the following sequence of events are postulated: First, endotoxin must somehow set the coagulation mechanism in motion by a secondary reaction since it cannot promote clotting of plasma *in vitro*. Such a secondary reaction might be the formation of large amounts of "tissue thromboplastin" in the plasma. As a result of this coagulation process there is conversion of fibrinogen into fibrin. The fibrinogen decline can be prevented by either heparinization or Warfarin pretreatment.

Second, as a result of the fibrin formation, there is adhesion of plasminogen to this material. The plasminogen is activated locally on the fibrin and the level of the circulating plasminogen declines. The fibrin desposits are rapidly lysed. As a result of the intrinsic vascular changes resulting from the lysis diapedesis of red cells and hemorrhage occurs.

That these effects of endotoxin shock may be explained by the pathogenesis of intravascular clotting is supported by the

TABLE 1

	Endotoxin Dose 0.1 mg./k.		Endotoxin Dose 1 mg./kg.	
	No. of Dogs	Deaths	No. of Dogs	Deaths
Heparin pretreated	10	0	5	4
Nonpretreated dogs	10	5	2	2

thrombin infusion study. The slow infusion of thrombin into a dog over a two-hour period resulted in nearly complete defibrinogenation of the blood. Parallel with the decline in fibrinogen there was a fall in plasminogen (Fig. 7). No clots were observed in this dog four days after the infusion. Since the thrombin acts primarily on the conversion of fibrinogen to fibrin, the plasminogen decline must be a secondary mechanism. The local plasmin activation so produced would be a defense against generalized clot formation.

Further evidence of a similar effect from shock has been noted by Turpini and Stefanini.[17] They noted a fall in fibrinogen and decline of plasminogen in the rabbits during hemorrhagic shock.

All the data obtained so far seem to confirm the impression that intravascular coagulation does occur during endotoxin shock but that in most instances the clots remain undetected because of their subsequent lysis.

The question whether a similar mechanism takes place in humans during endo-

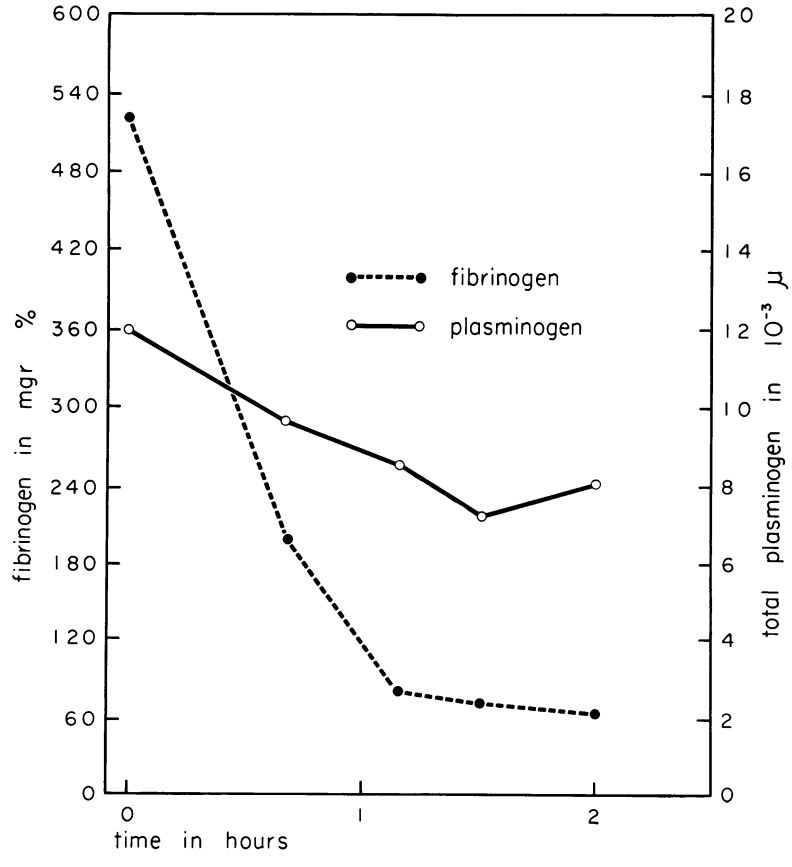

FIG. 7. Decline of fibrinogen and plasminogen levels in a dog receiving a slow infusion of 5,000 N.I.H. units of thrombin in 300 cc. saline over a two-hour period.

toxin shock has not yet been studied. However, it was observed that pyrogens, lipopolysaccharides derived from a variety of micro-organisms, activate the fibrinolytic system in the human to such an extent (Meneghini,[11] Eichenberger,[5] von Kaulla[18]) that pyrogens have been advocated by some in the treatment of thrombo-embolic phenomena.

Conclusions

1. In 10 nonheparinized dogs endotoxin caused a variable fall in fibrinogen. This fall in fibrinogen was prevented in 14 dogs pretreated with heparin and in five dogs pretreated with Warfarin.

2. Pretreatment of the dogs with heparin prevented death when 0.1 mg. of endotoxin was used. However, heparin did not prevent death when large doses were used.

3. No free plasmin activity was noted in any of the experiments. However, there is a definite decline of total plasminogen noted in the nonheparinized dogs. The dogs that received heparin had a variable response in plasminogen levels.

4. A possible explanation for the facts would be that endotoxin in the dogs causes intravascular clotting and that hemorrhage results from the rapid lysis of the clots so formed.

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