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# Effect of Endotoxin on the Clotting Mechanism: *

## II. On the Variation in Response in Different Species of Animals

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McKAY AND SHAPIRO have demonstrated that intravascular clotting follows endotoxin injection into rabbits. We previously have reported observations which indicate that intravascular clotting also occurs in dogs following the injection of endotoxin. No thrombi, however, could be demonstrated in dogs. It was therefore assumed, concomitant to their formation, that the clots were lysed.

Variations in the physiological response to endotoxin injection were further studied in dog and rabbit. The data obtained implicate the plasminogen-plasmin system as a protection of the dog against thrombosis; while, under similar conditions, this system fails to protect the rabbit from thrombosis after endotoxin injection.

### Methods and Materials

The experiments on rabbits were performed on two-kilogram albino rabbits. Each animal was anesthetized with sodium nembutal. The aorta was intubated at the trifurcation, directing the catheter proximally till the tip reached just below the take off of the renal arteries.

The experiments on dogs were performed on mongrel dogs weighing from 15–20 kilograms. After these animals were anesthetized with sodium nembutal, the femoral artery and vein were intubated.

The endotoxin used was the purified commerical *E. coli* lipopolysaccacharide (Difco, batch no. 0127:B 8. Control 110733). Dosage used: 0.1 mg./kg. body weight Whole blood (5 cc.) was withdrawn from either species before and after injection of endotoxin. These samples were collected in tubes containing 0.25 cc. of 10 per cent sequestrene.

The blood was centrifuged for 5 minutes and the plasma collected. Plasma samples were placed on the different fibrin plates (*vide infra*). The rest of the plasma was quick frozen. Thawing and rewarming of the samples was done at 37° C. in a constant temperature bath. All samples of one rabbit were processed simultaneously on the day of collection for their fibrinogen and plasminogen contents.

Fibrinogen concentrations were determined by the method of Kjell Jacobsson.[5] The plasma is diluted with buffer and clotted with a standard thrombin preparation. The clot is washed and dissolved in a 40 per cent urea solution. The solution is read in a Beckman spectrophotometer at 279, 320, and 360 mμ against a urea blank.

The plasminogen concentrations were determined by the method described by Norman.[9] In this method, the plasmin and plasminogen are freed from their inhibitors

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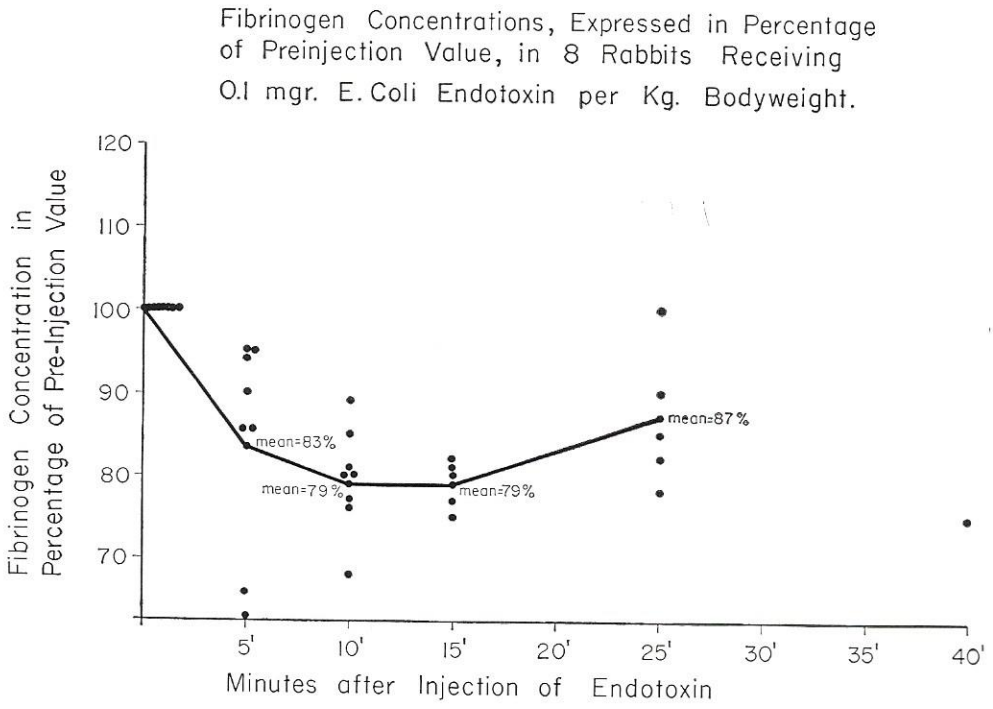


FIG. 1. Decline in the fibrinogen concentration in eight rabbits after injection of endotoxin.

by precipitating these components in the euglobulin fraction. The euglobulin solution is incubated with standard amounts of human proactivator, streptokinase, and casein for 30 minutes, and the amount of casein digestion determined by measuring the tyrosine content of the reaction mixture in the Beckman spectrophotometer at 280 mμ.

The plasminogen activator activity was determined by incubating 30 λ samples of plasma and euglobulin solution on different types of fibrin plates. The three fibrin plates used in these experiments were: those described by Astrup and Mullertz; [1] the heated fibrin plate described by Lassen; [6] and a plasminogen reinforced fibrin plate.[3] The plasminogen reinforced plate contained 0.1 cc. of a standard human plasminogen preparation (prepared ac-

TABLE 1. *Extent of Lysis of Fibrin Plates after Incubation (for 20 Hrs.) of Samples (0.03 cm.³) of Rabbit Euglobulin at 37° C.*

| | | Rabbit Number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Spec. No. | Time (min.) | 3 | 16 | 161 | 185 | 503 | 3 | 16 | 161 | 185 | 503 |
| | | Lassen Plate | | | | | Plasminogen Reinforced Fibrin Plate | | | | |
| 1 | pre-inj. | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 |
| 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 3 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| 5 | 25 | 0 | * | 0 | 0 | * | 0 | * | 0 | 25 | * |
| 6 | 40 | 0 | | 0 | 0 | | 0 | | 0 | 0 | |
| 7 | 60 | * | | * | * | | * | | * | * | |

* Animal expired.

TABLE 2. *Extent of Lysis (in mm.²) of Fibrin Plates After Incubation of Euglobulin Samples (0.03 cm.³) for 20 Hrs. at 37° C.*

| Spec. No. | Time (mins.) | Dog Number 109 | 110 | 111 | 112 | 113 | 109 | 110 | 111 | 112 | 113 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lassen Plate | | | | | Plasminogen Reinforced Fibrin Plate | | | | |
| 1 | pre-inj. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 | 16 | 49 |
| 2 | 5 | 0 | 0 | 0 | 0 | 0 | 4 | 25 | 180 | 16 | 144 |
| 3 | 10 | 0 | 0 | 0 | 0 | 0 | 20 | 90 | 330 | 33 | 100 |
| 4 | 15 | 0 | 0 | 0 | 0 | 0 | 225 | 49 | 690 | 16 | 156 |
| 5 | 25 | 0 | 0 | 0 | 0 | 0 | 360 | 80 | 396 | 72 | 225 |
| 6 | 40 | 0 | 0 | 0 | 0 | 0 | 308 | 100 | 468 | 165 | 110 |
| 7 | 60 | 0 | 0 | 0 | 0 | 0 | 500 | 168 | 396 | * | * |
| 8 | 90 | 0 | 0 | 0 | 0 | 0 | 400 | 0 | 540 | * | * |

* Not set out on plates.

cording to the Kline method) in addition to the normal constituents of the Astrup plate. The amount of lysis of the fibrin plates was determined as described by Astrup, i.e. by measuring the greatest diameters of the lysed area.

## Results of *in vivo* Experiments

### A. In the Rabbit

**Fibrinogen Concentrations.** Since the initial concentrations varied considerably from one animal to the next, the concen-

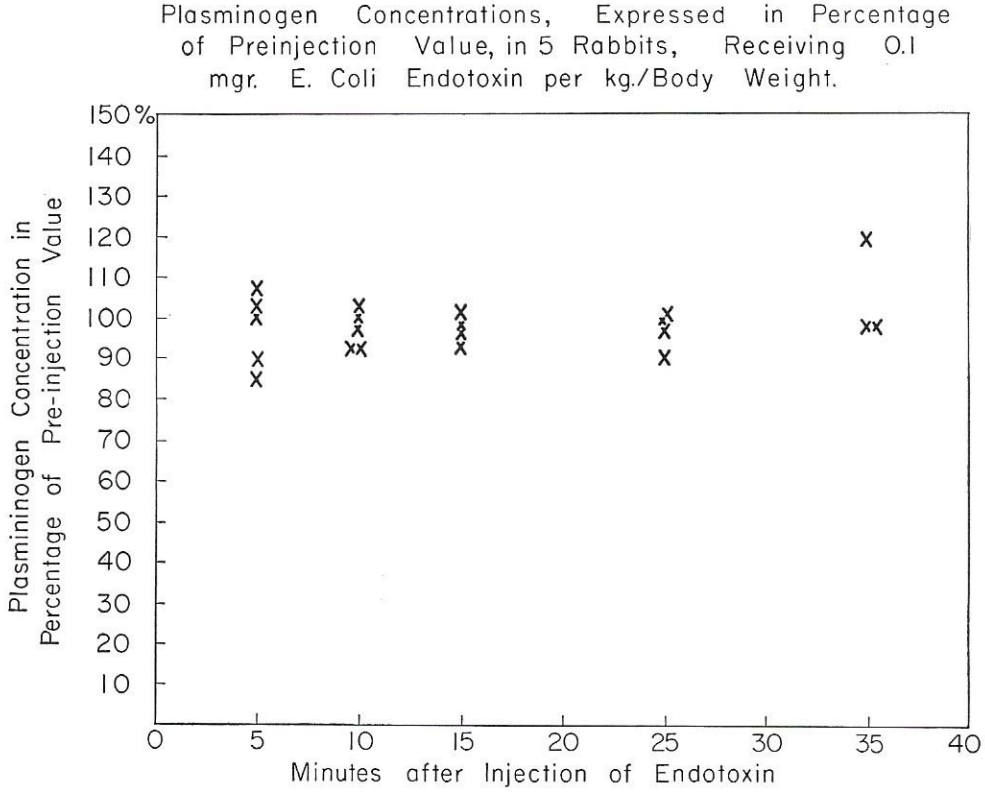


FIG. 2. No changes of any significance are observed in the plasminogen concentrations of five rabbits after injection of endotoxin.

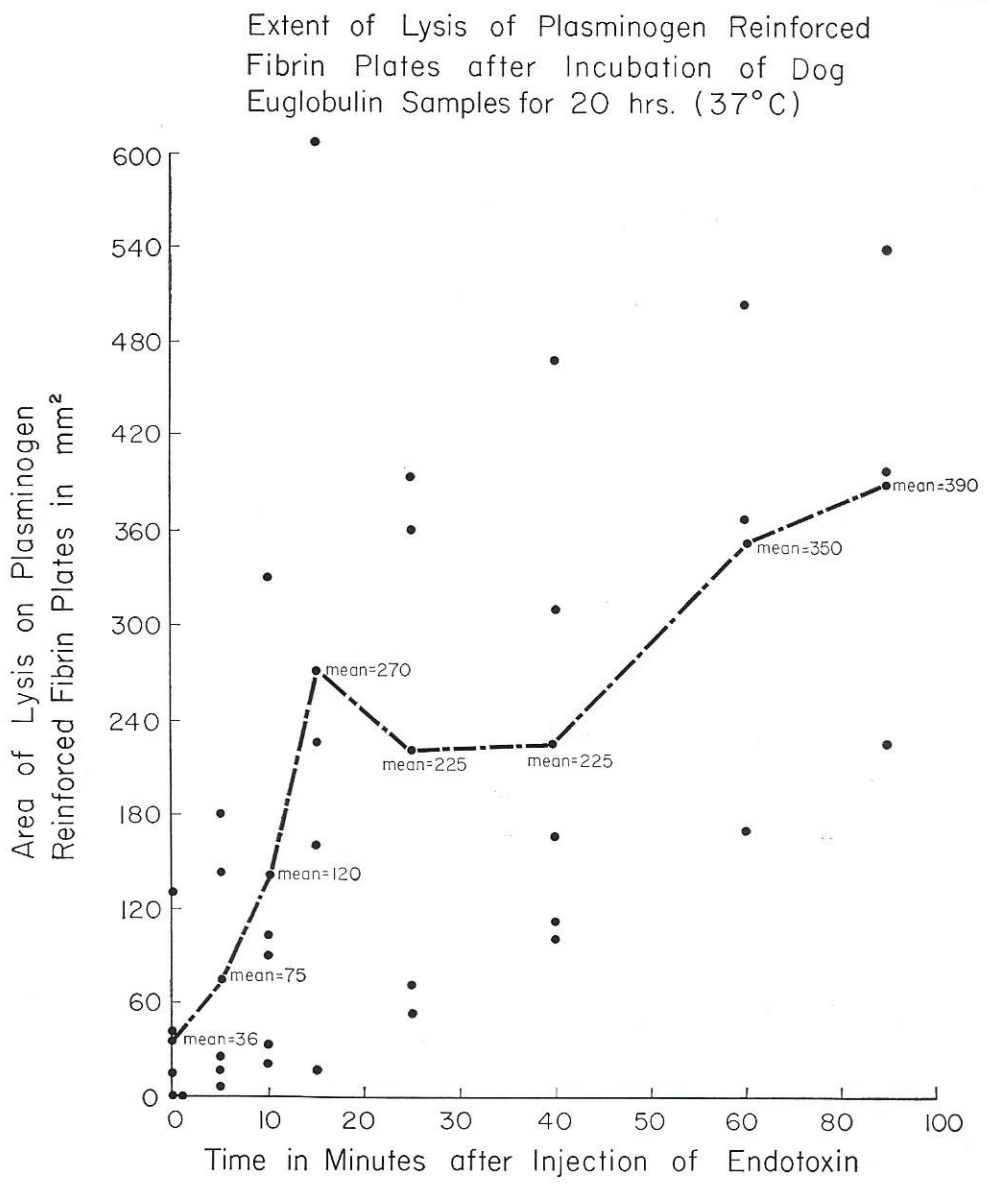


FIG. 3. Increase in plasminogen activator activity in the dog as determined by the extent of lysis of the plasminogen reinforced fibrin plates when dog euglobulin samples were incubated on these plates for 20 hours.

trations found after injection of endotoxin were expressed as a percentage of preinjection values.

Control animals which received saline instead of endotoxin showed no changes in their fibrinogen concentrations. After injection of endotoxin into eight rabbits a decline in the fibrinogen concentration invariably occurred (Fig. 1). The average decrease in fibrinogen value was found to be 21 per cent.

**Plasminogen Concentrations.** No changes of any significance were found in the control group of rabbits receiving saline. Nor were any changes in plasminogen concentrations observed after the injection of endotoxin (Fig. 2).

**Plasminogen Activator Activity.** Upon

incubating euglobulin samples on plasminogen reinforced fibrin plates, no consistent lysis was observed (Table 1).

### B. In Dogs

Fibrinogen and plasminogen concentrations decline after the injection of endotoxin, as previously described.[2] The mean fall in fibrinogen concentration was 45 per cent, that of the plasminogen nearly 30 per cent.

**Plasminogen Activator Activity.** Concomitant with the plasminogen decrease found after endotoxin injection, a substantial increase in plasminogen activator activity was noted. The increase in plasminogen activator activity was determined by the extent of lysis of the plasminogen reinforced fibrin plates when euglobulin samples were incubated on these plates for 20 hours (Fig. 3, 4 and Table 2).

## Results of *in vitro* Studies

**Demonstration of the Difference Between Fast and Slow Clot Lysis.** Addition of plasminogen activator (e.g., streptokinase) immediately *before* clotting blood with thrombin results in a rapid clot lysis. Addition of plasminogen activator immediately *after* blood has been clotted with thrombin, however, results in a greatly prolonged lysis time (Table 3).

## Discussion

Comparison in the response of the coagulation mechanism to endotoxin has been investigated in dog and rabbit. In both species the fibrinogen concentration declines after endotoxin injection. Prevention of this fibrinogen decline were reported by Good and Thomas [4] and by McKay and Shapiro [8] after endotoxin injection in the rabbit and similarly by ourselves in the dog.[2]

Widespread occlusive vascular phenomena in rabbits are observed during endotoxin induced Schwartzman reaction. In the dog, however, except for possible minimal changes in the intestinal wall (McLean *et al.*[7]), no marked thrombosis is observed.

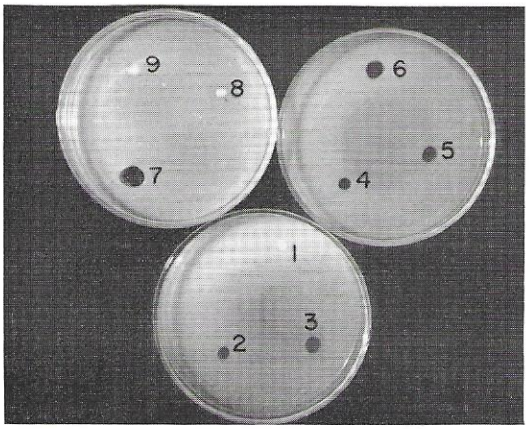


FIG. 4. Extent of lysis of plasminogen reinforced fibrin plates after incubation of euglobulin samples for 20 hrs. at 37° C. 1: prior to injection of endotoxin, 2: 5′ after injection, 3: 10′ 4: 15′, 5: 25′, 6: 40′, 7: 60′, 8: 90′, and 9: 120′ after injection of endotoxin into dog no. 110.

Despite the absence of histological evidence of thrombosis in the dog the decline in fibrinogen concentrations is interpreted here as indicating occurrence of intravascular coagulation. As intravascular coagulation takes place, a concomitant throm-

TABLE 3. *In Vitro Demonstration of the Difference Between the Fast and the Slow Type of Clots Lysis*

| Fast Lysis | Slow Lysis |
|---|---|
| Human pooled plasma* 1 ml. | Human pooled plasma* 1 ml. |
| S.K. in 0.1 ml. norm. saline (100 U.) | Norm. saline 0.1 ml. without S.K. |
| Thrombin 5 U. | Thrombin 5 U. S.K. in 0.1 ml. norm. saline (100 U.) |
| Lysis time: 2′ 55″ | Lysis time: After 1 hr. incubation at 37° C. approximately 30% of the clot is lysed |
| Occurrence *in* the clot | Occurrence *on* the clot |
| Plasminogen, absorbed on the fibrin—lysis of clots | |
| S.K. ↓↑ inhibitor ↑ plasmin | |

* Fibrinogen concentration 337 mg.%.
S.K. = Streptokinase.

bolysis occurs. Evidence for this concomitant thrombolytic activity is indicated by the decline in plasminogen concentration.

Further evidence of activation of the thrombolytic system is demonstrated by the actual increase in plasminogen-activator activity in the dog following endotoxin injection. In contrast, the rabbit does not have increased plasminogen-activator activity, nor is there a decrease in plasminogen concentration after the injection of endotoxin.

For the above theoretical interpretation to be valid, simultaneous and immediate lysis of the coagula formed must be demonstrated. Observations represented in Table 3 indicate that extremely rapid lysis can occur if clotting takes place in the presence of plasminogen activator. Thus in the dog these conditions are fulfilled since immediately after endotoxin injection plasminogen-activator activity increases. As indicated by the *in vitro* study this condition would favor rapid clot lysis.

These observations implicate the plasminogen-plasmin system as a possible means of protection against thrombosis following endotoxin injection into the dog. Since the rabbit does not demonstrate a similar role of the plasminogen-plasmin system after endotoxin injection, widespread thrombosis may occur.

An unexplained observation is that in anaphylactic shock in the rabbit the plasminogen-plasmin system is fully activated (Gans and Krivit [3]). This observation contrasts to the inability to activate the plasminogen-plasmin system in endotoxin shock. This difference in reaction possibly could be due to a different effect of these agents in releasing plasminogen-activator activity from the various shock organs, that is, in anaphylaxis the lung parenchyma may be more severely involved than during endotoxin shock. Work to elucidate the meaning of this difference is in progress.

## Summary

1. Evidence is presented that in the dog rapid activation of the plasminogen-plasmin system takes place after endotoxin injection. Plasminogen concentrations decline and plasminogen-activator activity increases.

2. Evidence is presented that in the rabbit no activation of the plasminogen-plasmin system takes place after endotoxin injection.

3. These observations are interpreted as reasons for the absence of thrombosis in the dog and for the presence of thrombosis in the rabbit.

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