Failure of Heparin, Epsilon Aminocaproic Acid, or Both Agents to Prevent Increased Fibrinogen Levels After Endotoxin in Rabbits[1] (37014)

L. J. LOGAN,[2] S. I. RAPAPORT, AND P. KUEFLER

Department of Medicine, University of Southern California School of Medicine, Los Angeles, California 90033

The intravenous injection of a single dose of gram-negative endotoxin into laboratory animals results in a delayed steady rise in plasma fibrinogen level (1, 2). The increased level stems from increased synthesis of fibrinogen (2). The agents mediating this increased synthesis are unknown. A single intravenous injection of endotoxin probably triggers an episode of intravascular clotting beginning shortly after the injection (3). Therefore, fibrinopeptides or clotting intermediates could conceivably stimulate subsequent increased fibrinogen synthesis. Moreover, endotoxin may activate fibrinolysis (3–5), and fibrinogen and fibrin degradation products have been reported to stimulate fibrinogen synthesis (6–8).

The experiments reported herein were designed to evaluate whether agents resulting from intravascular clotting, fibrinolysis, or both processes mediate the increased fibrinogen synthesis induced by endotoxin. Fibrinogen levels were measured before and 24 hr after injecting endotoxin into rabbits which were treated in one of four ways: with saline (controls); with heparin (to block clotting); with epsilon aminocaproic acid (EACA) (to block fibrinolysis); and with both heparin and epsilon aminocaproic acid (to block both clotting and fibrinolysis). The data suggest that neither intravascular clotting nor fibrinolysis is responsible for the increased fibrinogen synthesis induced by endotoxin.

Materials and Methods. Female albino rabbits between 1.5 and 2.0 kg in weight were used.

The endotoxin was *E. coli* lipopolysaccharide 0111:B4 (lot 463135 Difco Laboratories, Detroit), which was dissolved in isotonic saline just before use. In preliminary experiments an intravenous injection of 15 μg/kg of endotoxin was found consistently to cause an impressive rise in plasma fibrinogen level 24 hr later, and this dose was used for all subsequent experiments.

The heparin was Lipo-heparin (Riker, 5000 IU/ml). Heparin was given in two dosage schedules: either intravenously, 1500 IU/kg every 2 hr for 12 doses, or subcutaneously, 6500 IU/kg every 8 hr for 3 doses with a single supplemental intravenous injection of 2000 IU/kg at the time of the intravenous injection of endotoxin or isotonic saline (control for endotoxin).

Epsilon aminocaproic acid (EACA) powder (Calbiochem) was dissolved in sterile isotonic saline to concentrations of 25 or 50% (w/v) and passed through a Millipore filter for sterilization. EACA was given in two dosage schedules: either intravenously, 250 mg every 2 hr for 12 doses, or subcutaneously, 900 mg every 8 hr for 3 doses with a single supplemental intravenous injection of 150 mg at the time of the intravenous injection of endotoxin or isotonic saline.

The above dosage schedules were also used when animals were given both heparin and EACA.

Blood samples were drawn from the central artery of the ear. Nine parts of blood were added to 1 part of a buffered citrate anticoagulant (9) containing 0.02 *M* EACA and 10 units/ml of Trasylol (FBA Pharmaceuticals, New York, NY). In animals not

[1] Supported by grant number HE 05874-12 and HE 06128-11 from the National Heart and Lung Institute, National Institutes of Health.

[2] Present address: Letterman General Hospital, San Francisco, CA 94129.

TABLE I. Effect of Heparin, EACA, or Both Agents upon the Mean Percentage Change in Fibrinogen Level of Rabbits Given Endotoxin or Saline (Control).

Agent stimulating fibrinogen synthesis	No. of animals	Test procedure		Mean percentage change in fibrinogen level
		Agent	Route of admin	
Saline (control)	8	Saline	Intravenous	+14
	5	EACA	Intravenous	+6
	6	Heparin	Subcutaneous	−2
	6	Heparin–EACA	Intravenous	−6
Endotoxin	4	Saline	Intravenous	+154
	4	Saline	Subcutaneous	+182
	4	EACA	Intravenous	+150
	4	EACA	Subcutaneous	+72
	2[a]	Heparin	Intravenous	+295
	1	Heparin	Subcutaneous	+156
	3[b]	Heparin–EACA	Intravenous	+140
	4	Heparin–EACA	Subcutaneous	+235

[a] In one animal only 5 doses of heparin were given.

[b] In two animals only 5 doses of heparin–EACA were given.

given heparin, fibrinogen was measured by a method in which the plasma is clotted with calcium–thrombin (3). This method gave erratic results on plasma samples from rabbits given heparin even when the strength of the thrombin used was increased. Consequently, 0.1 ml of a calcium–Reptilase reagent—made by dissolving the contents of one vial of Reptilase-R (Pentapharm, Basel) in 1 ml of 0.025 *M* $CaCl_2$—was used instead of thrombin to clot all plasma samples from animals given heparin. Substituting this Reptilase reagent for thrombin did not affect the mean value for fibrinogen obtained on 10 plasma samples not containing heparin.

The experimental protocol may be summarized as follows: An initial dose of the test agent—isotonic saline (control), heparin, EACA, or both heparin and EACA—was administered either subcutaneously or intravenously. One hour later blood was drawn for the initial fibrinogen level, and either 15 μg/kg of endotoxin or an equivalent volume of isotonic saline (endotoxin control) was injected into a marginal ear vein. At this time animals receiving a subcutaneous test agent received one supplemental intravenous injection of the agent. Administration of the test agent was continued for 24 hr following which blood was drawn for the second fibrinogen determination. Histologic sections of the kidneys and lungs, stained with hematoxylin and eosin and with phosphotungstic acid hematoxylin, were examined from animals dying during the experiment or sacrificed after its completion.

Results and Discussion. The data from these experiments are summarized in Table I. Fibrinogen levels did not change in control animals injected with saline instead of endotoxin. Thus, neither the handling of the rabbits nor effects of the high doses of EACA and heparin altered fibrinogen levels over 24 hr. In contrast, every rabbit injected with 15 μg/kg of endotoxin had a striking increase in fibrinogen level 24 hr later. The administration of heparin, EACA or both agents failed to prevent this rise in fibrinogen.

The reduced number of animals in the endotoxin–heparin groups of Table I resulted from the death of most animals between 14 and 24 hr due to bleeding at needle puncture sites. In addition, three of six endotoxin-treated rabbits given both heparin and EACA intravenously died unexpectedly a few hours after the injection of the endotoxin. Areas of hemorrhage were noted grossly in their lungs, and microscopic examination demonstrated areas of hemorrhage interspersed between normal-appearing lung tissue. In two rabbits changes compatible with pulmonary edema were seen but artifact in fixation

could not be excluded. Fibrin was not detected on microscopic examination of the kidneys and lungs of rabbits from any of the treatment groups.

The high doses of heparin used in these experiments will prevent the deposition of fibrin in the glomeruli of young rabbits after the second, provocative dose of endotoxin used for the generalized Shwartzman reaction (10). Consequently, the failure of this heparin regimen to prevent fibrinogen levels from rising after endotoxin strongly suggests that neither intermediates of clotting nor fibrinopeptides mediate the rise. This finding confirms an earlier incidental observation from this laboratory that fibrinogen levels in rabbits rise after endotoxin despite prevention of intravascular clotting by granulocytopenia (11). Moreover, Takeda has recently reported (12) that heparin does not prevent fibrinogen levels from rising in calves given typhoid endotoxin. Thus, all evidence supports the conclusion that intravascular clotting after endotoxin and increased fibrinogen synthesis after endotoxin are independent phenomena.

If fibrinogen or fibrin degradation products were the major stimuli for endotoxin-induced fibrinogen synthesis, then the very large doses of EACA used in these experiments should have prevented the rise in fibrinogen level. The 3 g of EACA administered over 24 hr to a 2 kg rabbit would be equivalent on a weight basis to administration of 105 g (four times the maximal recommended dose) of EACA to a 70 kg human. Consequently, it seems reasonable to conclude that fibrinolysis after endotoxin and increased fibrinogen synthesis after endotoxin are also dissociated phenomena.

Summary. High doses of heparin, EACA, or both failed to prevent fibrinogen levels from rising after the intravenous administration of 15 μg/kg of *E. coli* endotoxin to rabbits. Apparently, endotoxin stimulates fibrinogen production by a mechanism independent of intravascular clotting, fibrinolysis or fibrinogenolysis.

The authors thank Major Robert Kovatch, V. C., for review of the histologic preparations.

1. McKay, D. G., and Shapiro, S. S., J. Exp. Med. **107**, 353 (1958).

2. Koj, A., and McFarlane, A. S., Biochem. J. **108**, 137 (1968).

3. Lerner, R. G., Rapaport, S. I., Siemsen, J. K., and Spitzer, J. M., Amer. J. Physiol **214**, 532 (1968).

4. Gans, H., and Krivit, W., Ann. Surg. **153**, 453 (1961).

5. Dinbar, A., Rapaport, S. I., Patch, M. J., Grant, W., and Fonkalsrud, E. W., Surgery **70**, 596 (1971).

6. Young, M. C., and Kolmen, S. N., Thromb. Diath. Haemorrh. **19**, 198 (1968).

7. Kropatkin, M. L., and Izak, G., Thromb. Diath. Haemorrh. **19**, 547 (1968).

8. Barnhart, M. I., Gress, D. C., Noonan, S. M., and Walsh, R. T., Thromb. Diath. Haemorrh. Suppl. **39**, 143 (1970).

9. Proctor, R. R., and Rapaport, S. I., Amer. J. Clin. Pathol. **32**, 212 (1961).

10. Good, R. A., and Thomas, L., J. Exp. Med. **97**, 871 (1953).

11. Lerner, R. G., Rapaport, S. I., and Spitzer, J. M., Thromb. Diath. Haemorrh. **20**, 430 (1968).

12. Takeda, Y., *in* "Plasma Protein Metabolism" (M. A. Rothschild and T. Waldman, eds.), Chap. 29. Academic Press, New York (1970).

Received June 1, 1972. P.S.E.B.M., 1973, Vol. 142.