Effect of Heparin on Endotoxin-Induced Thrombocytopenia[1] (35209)

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In vitro studies with rabbit platelets have shown that relatively small concentrations of heparin are capable of preventing thrombin induced-platelet injury whereas very large amounts of the anticoagulant are necessary to prevent similar damage by bacterial endotoxin (1). *In vivo* investigations have demonstrated that the usual anticoagulating amounts of heparin do not prevent the thrombocytopenia in the generalized Shwartzman reaction (gSr) (2) in rabbits and a similar phenomenon has been observed in humans with septic shock treated with heparin (3, 4). The gSr is elicited by giving rabbits two properly spaced intravenous injections of endotoxin and is characterized by the production of diffuse intravascular coagulation with the subsequent occurrence of bilateral renal cortical necrosis (5). It therefore provides an *in vivo* model for the study of both endotoxin- and thrombin-induced hematologic changes.

The purpose of this investigation was to evaluate the *in vivo* effect of large amounts of heparin on endotoxin-induced thrombocytopenia, leukopenia and hypofibrinogenemia using the gSr as the experimental model.

Material and Methods. E. coli 0127:B8 (Boivin type; Difco Laboratories, Detroit, Michigan) was prepared fresh daily in pyrogen-free normal saline solution.

Aqueous heparin, 20,000 units/ml, was purchased from the Upjohn Company, Kalamazoo, Michigan.

Rabbits, white hybrid of either sex weighing 1.0 kg were housed in an air-conditioned animal room and fed Purina rabbit pellet and water, *ad lib.*

The generalized Shwartzman reaction was produced in the classical manner (5). Animals received two intravenous injections of endotoxin (0.1 mg/kg) 24 hr apart. Twenty-four hr after the second endotoxin injection, they were sacrificed and the frequency of bilateral renal cortical necrosis noted.

Blood for white blood cell (WBC), platelet, and fibrinogen determination was obtained by two-syringe technique from the heart, and anticoagulated with 3.8% sodium citrate. Plasma was obtained by centrifuging the blood at 8000 rpm at 0° for 20 min. Blood was obtained immediately before (0 hr) and again 4 hr after the second (provocative) injection and the results were compared. WBC counts were done by standard technique, platelet counts by phase microscopy (6), and fibrinogen concentation by the method of Ratnoff and Menzie (7).

Results. Previous investigations had shown that rabbits are anticoagulated with concentrations of heparin of 5 mg (500 units)/kg or larger when administered intravenously (2). Des Prez reported that *in vitro* 5.0 mg of heparin/ml of platelet-rich plasma was necessary to inhibit the endotoxin effect on rabbit platelets (1). In these *in vivo* studies, concentrations of heparin ranging from 1000 units to 25,000/kg were used. The rabbits weighed 1.0 ± 0.1 kg, with hematocrits ranging from 42–45%.

All animals received the first injection of endotoxin. Twenty-four hr later they received either (a) endotoxin alone, (b) saline and no endotoxin or heparin, (c) heparin and no endotoxin, or (d) simultaneous intravenous injections of endotoxin and heparin. The significant results are shown on Tables I and II. Bilateral renal cortical necrosis, characteristic of the gSr, was produced in 72% of the

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TABLE I. Frequency of Renal Cortical Necrosis.

All animals were prepared with endotoxin and provoked (second injection) with either endotoxin alone, heparin alone, or simultaneous injections of endotoxin and varying amounts of heparin.

First injection endotoxin (mg/kg)	Second injection(s)		Renal cortical necrosis (positive/total)
	Endotoxin (mg/kg)	Heparin (units/kg)	
0.1	0.1	None	13/18
0.1	None	1500	0/10
0.1	0.1	1000	0/10
0.1	0.1	1500	0/10
0.1	0.1	25,000	0/10
0.1	None	None	0/10

animals who received two injections of endotoxin and no heparin. No rabbit developed this lesion when the heparin was administered simultaneously with the second endotoxin dose, as is shown on Table I. The data in Table II demonstrate that in those animals not given heparin significant ($p < 0.01$) reduction in the platelets, WBC, and fibrinogen concentration occurred 4 hr after the second endotoxin injection. Animals given an anticoagulating amount of heparin (1500 U/kg) or a very large dose (25,000 U/kg) [equivalent to >500 U/ml of plasma] with the endotoxin demonstrated significant reduction in the platelets and WBC but not in the fibrinogen concentration.

Discussion. Des Prez concluded from his *in vitro* studies that the platelet injury by endotoxin, although similar to thrombin, was mediated through some mechanism other than thrombin formation. However, he did show that a very large concentration of heparin (5 mg/ml of platelet-rich plasma) could inhibit this effect of endotoxin (1). Using the gSr as an *in vivo* system the data in this investigation indicate that even large amounts of heparin, equivalent to >500 U/ml of plasma, had no effect on the endotoxin-induced thrombocytopenia. The 4-hr interval following the second endotoxin injection was used because it is during this time that large amounts of thrombin are generated and the animals develop intravascular coagulation (5). In addition, heparin given at this time, and only this time, is capable of preventing the intravascular coagulation and subsequent renal cortical necrosis (2). In this model the leukopenia is known to be due to endotoxin and not thrombin and the fall in the fibrinogen concentration as due to thrombin. In those rabbits given endotoxin and heparin it was seen that the heparin, acting as an anticogulant (antithrombin), prevented the fibrinogen reduction but did not abolish the WBC–endotoxin interaction. Since the platelet response was similar to the WBC and not the fibrinogen, the thrombocytopenia must have been the result of a nonthrombin action, either by endotoxin directly or through some other endotoxin related mechanism. The contributing role of thrombin to

TABLE II. Results of the Platelet Count, WBC Count, and Fibrinogen Concentration Immediately Before (0 hr) and 4 hr After the Second Injection.

All rabbits were given endotoxin as the first injection 24 hr before the 0-hr studies.

	0 hr	4 hr				
Second injection [agent(s) used][a]	— —	E (0.1 mg) H (none)	E (none) H (1500 U)	E (0.1 mg) H (1500 U)	E (0.1 mg) H (25,000 U)	E (none) H (none)
Platelets ($\times 10^3$/mm^3)	266 ± 18[b] (113)[c]	70 ± 12 (18)	290 ± 53 (9)	91 ± 22 (10)	66 ± 11 (10)	297 ± 21 (10)
WBC (/mm^3)	8106 ±673 (58)	2246 ±320 (19)	— —	1067 ±173 (5)	2717 ±304 (10)	9966 ±1140 (10)
Fibrinogen (mg/100 ml)	351 ± 12 (114)	215 ± 26 (18)	574 ± 62 (9)	350 ± 32 (10)	305 ± 20 (10)	430 ± 34 (10)

[a] E = Endotoxin; H = Heparin.

[b] Mean ± SE.

[c] Number of animals is given in parentheses.

the thrombocytopenia in this model would therefore appear to be minor.

Summary. The generalized Shwartzman reaction in rabbits was used as the ***in vivo*** model for endotoxin- and thrombin-induced changes in the WBC, platelets, and fibrinogen concentration. Concentrations of heparin ranging from 1000 to 25,000 units/kg did not alter the effect of heparin on the changes in these hematologic constituents. Leukopenia and thrombocytopenia were produced but fibrinogen consumption did not occur in the presence of heparin. Since the change in the platelets was similar to the leukocytes and not affected by heparin, it was concluded that the mechanism of platelet injury was related to the endotoxin and not to thrombin generation.

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1. Des Prez, R. M., J. Exp. Med. **120**, 305 (1964).
2. Good, R. A., and Thomas, L. J., J. Exp. Med. **97**, 871 (1953).
3. Corrigan, J. J., Jr., Ray, W. L., and May, N., N. Engl. J. Med. **279**, 851 (1968).
4. Abildgaard, C. F., Corrigan, J. J., Seeler, R. A., Simone, J. V., and Schulman, I., Pediatrics **40**, 78 (1967).
5. Corrigan, J. J., Jr., Abildgaard, C. F., Vanderheiden, J. F., and Schulman, I, Pediat. Res. 1, 39 (1967).
6. Brecher, G., and Cronkite, E. P., *in* "Blood Coagulation, Hemorrhage and Thrombosis" (L. M. Toncantins and L. A. Kazal, eds.), p. 52. Grune and Stratton, New York (1964).
7. Ratnoff, O. D., and Menzie, C., J. Lab. Clin. Med. **37**, 316 (1951).

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