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Increased Fibrinogen Consumption Following Endotoxin Injection in Cortisone-Treated Rabbits[1] (37909)

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Treatment with cortisone prepares rabbits for the generalized Shwartzman reaction (GSR) after a single dose of endotoxin (1). Two mechanisms have been postulated to account for this preparatory effect of cortisone: impairment of reticuloendothelial cell function (2) and enhancement of the sensitivity of α-adrenergic receptor sites in the microcirculation (3). We report herein the results of experiments designed to evaluate the possibility that treatment with cortisone increases the extent of intravascular clotting produced by a single intravenous injection of endotoxin in the rabbit. Plasma fibrinogen consumption after endotoxin, calculated from plasma fibrinogen levels and disappearance of ^{125}I-fibrinogen radioactivity, was compared in rabbits treated with cortisone acetate for 4 days and in control rabbits treated with isotonic saline. The data indicate that treatment with cortisone significantly increases plasma fibrinogen consumption following a single intravenous injection of endotoxin in this species.

Materials and Methods. Male New Zealand rabbits weighing 1.9–2.8 kg were fed and given drinking water containing KI and NaCl as described earlier (4).

The endotoxin was Lipopolysaccharide B *E. coli* O111:B4 (Difco Laboratories, Detroit), which was dissolved in pyrogen-free isotonic saline in a concentration of 100 μg/ml just prior to use.

The cortisone was a sterile, aqueous 25 mg/ml suspension of cortisone acetate (Towne Paulson and Co., Monrovia, CA).

The radioactive fibrinogen was ^{125}I-fibrinogen prepared from purified rabbit fibrinogen as described earlier (4).

Blood sampling, measurement of plasma fibrinogen level, determination of radioactivity in plasma fibrinogen and in nonclottable plasma proteins, calculation of plasma volume, and calculation of plasma fibrinogen consumption were carried out as described in detail elsewhere (4, 5). The formula used to calculate plasma fibrinogen consumption was

$$\text{F/100 ml} = \frac{c_1 + c_2}{2} \times \frac{q_1 - q_2}{q_1} \times 100,$$

where F/100 ml equals mg of fibrinogen per 100 ml of plasma disappearing during the 6-hr period following injection of endotoxin, c_1 and c_2 equal plasma fibrinogen concentration (mg/ml) at the time of the injection of endotoxin and at 6 hr after the injection, q_1 and q_2 equal plasma fibrinogen radioactivity (% injected dose) at the time of injection of endotoxin and at 6 hr after the injection. Fibrinogen consumption was also expressed as mg/kg by substituting a value for plasma volume/kg for the value of 100 in the above formula. The plasma volume for each animal was calculated at the time of injection of ^{125}I-fibrinogen from the whole plasma radioactivity of a sample drawn 5 min after the injection.

The rabbits were divided into 2 groups. An experimental group of 12 rabbits were given 25 mg of cortisone acetate intramuscularly daily for 4 days. A control group of 13 rabbits were given 1 ml of isotonic

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saline intramuscularly for 4 days. On the second day each rabbit received 20–30 μCi of ^{125}I-fibrinogen intravenously. On the fourth day, 48 hr after the injection of ^{125}I-fibrinogen, each animal received an intravenous injection of 100 μg/kg of endotoxin. Blood samples were drawn in all animals at the following times: 5 min and 30 hr after the injection of ^{125}I-fibrinogen, just before the injection of endotoxin, and 3 and 6 hr after the injection of endotoxin. In addition, a blood sample was drawn 24 hr after endotoxin in all except 2 rabbits in the control group who died earlier and another blood sample was drawn in most rabbits at 48 hr. Animals were sacrificed and autopsied after the final blood sample and the kidneys examined grossly and microscopically (phosphotungstic acid-hematoxylin stain) for fibrin.

Results. Treatment with cortisone acetate resulted in weight gain, increase in plasma volume, and fall in hematocrit. Mean values for plasma volume on the second day of treatment (day of ^{125}I-fibrinogen injection) were: control group, 35.5 ml/kg; cortisone group, 43.0 ml/kg. Mean values for hematocrit on the fourth day of treatment were: control group, 39.0%; cortisone group, 31.5%.

The animals in both groups became ill after the injection of endotoxin, but the control animals appeared more severely affected. Two control animals died approximately 6 hr after the injection of endotoxin whereas all animals treated with cortisone survived the initial effects of the endotoxin.

Mean values for plasma fibrinogen concentration for the 2 groups of animals are plotted in Fig. 1. These data are as measured without correction for change in hematocrit and dilution by anticoagulant. When the foregoing corrections were made and the resultant values at 3 and 6 hr after injection of endotoxin were expressed as a percent of the fibrinogen level before injections of endotoxin, the following mean percentages were obtained: cortisone group, 3-hr value 96.7 ± 3.1% (mean ± SE) and 6-hr value 77.4 ± 5.6%; control group 3-hr value 104.7 ± 2.1% and 6-hr value

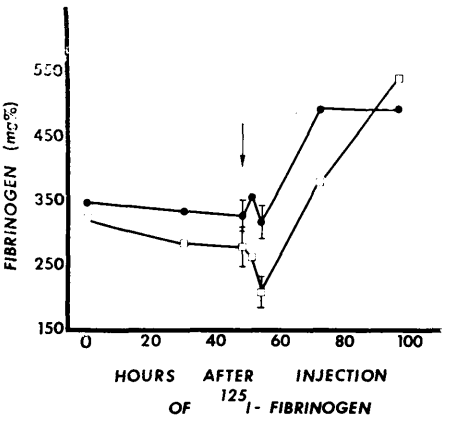

FIG. 1. The effect of injection of endotoxin upon mean fibrinogen levels in cortisone-treated rabbits (□) and in saline-treated control rabbits (●). In this and subsequent figures, the arrow indicates the time of injection of endotoxin. Means for the cortisone group are for 12 rabbits except that the mean at 96 hr is for 9 rabbits. Means for the control group are for 13 animals except that the mean at 72 hr is for 11 animals and the mean at 96 hr is for 9 animals.

98.2 ± 1.7%. The decrease in mean plasma fibrinogen concentration in the cortisone group observed 6 hr after endotoxin was not statistically significant for the data as measured but was statistically significant ($P < 0.001$) when the data were corrected and expressed as a percent of the mean value before endotoxin.

The mean and range for the plasma fibrinogen radioactivity curves and the mean for the plasma nonclottable protein radioactivity curves are plotted for the control animals in Fig. 2 and for the cortisone-treated animals in Fig. 3. With one exception, the dose of endotoxin we used failed to induce a measurable excess disappearance of plasma fibrinogen radioactivity in the control animals. In contrast, this dose of endotoxin resulted in excess disappearance of plasma radioactivity in every cortisone-treated animal. The extent of the excess disappearance of radioactivity varied considerably from animal to animal, as indicated by the wide range shown in Fig. 3. The injection of endotoxin was followed by a flattening of the plasma nonclottable pro-

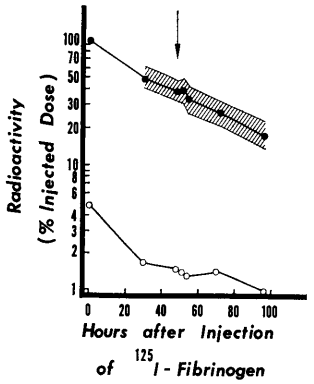

FIG. 2. The effect of injection of endotoxin upon the mean plasma fibrinogen radioactivity curve (●) and the mean plasma nonclottable protein radioactivity curve (○) for the control animals. The shaded area represents the range of the plasma fibrinogen radioactivity curves of the individual animals. Means are for the number of rabbits as given in the legend for Fig. 1.

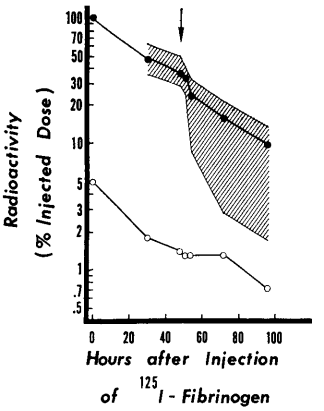

FIG. 3. The effect of injection of endotoxin upon the mean plasma fibrinogen radioactivity curve (●) and the mean plasma nonclottable protein radioactivity curve (○) for the cortisone-treated animals. The shaded area represents the range of the plasma fibrinogen radioactivity curves of the individual animals. The means are for the number of rabbits as given in the legend for Fig. 1.

tein radioactivity curve in both the control and cortisone-treated group, but an increase in plasma nonclottable protein radioactivity was not observed.

Mean values for plasma fibrinogen consumption over the 6 hr period after endotoxin are given in Table I. The mean value for the cortisone-treated animals, expressed as either mg% or as mg/kg of body weight, significantly exceeded ($P < 0.02$) the mean value for the control animals.

No gross or microscopic evidence of the GSR was found in the 13 control animals. In contrast, 2 of the 12 cortisone-treated animals had gross bilateral renal cortical necrosis and a third animal had microscopic evidence of the reaction consisting of deposits of fibrin in many glomeruli with associated hemorrhage and necrosis. Values for fibrinogen consumption over 6 hr after endotoxin for the animals with the GSR were as follows: for the 2 animals with gross renal cortical necrosis, 52.2 and 30.5 mg/kg; for the animal with the microscopic lesion, 79.7 mg/kg.

Discussion. The data presented above establish that rabbits treated with cortisone in the dose used to prepare for the GSR exhibit excess plasma fibrinogen consumption in the 6 hr following a single intravenous injection of *E. coli* endotoxin. As discussed elsewhere (5), plasma fibrinogen consumption provides a measure of the extent of intravascular clotting induced by endotoxin in this species. Consequently, one may conclude that a single injection of endotoxin causes significant intravascular clotting in rabbits which have been injected intramuscularly with 25 mg of cortisone acetate daily for 4 days.

TABLE I. Fibrinogen Consumption Over 6 Hr After Endotoxin.

Group	Number of animals	Fibrinogen consumption (mean ± SE) mg %	mg/kg
Cortisone treated	12	79.4 ± 14.8	33.9 ± 6.2
Control	13	37.4 ± 5.5	13.2 ± 1.9

In contrast, control animals given a single injection of endotoxin failed to exhibit significantly increased disappearance of plasma fibrinogen radioactivity. Their plasma fibrinogen consumption did not differ significantly from the plasma fibrinogen consumption of a group of animals injected with saline, instead of endotoxin, reported in an earlier study from this laboratory (5). Nevertheless, endotoxin appeared to produce more severe shock in the control animals than in the cortisone-treated animals, and 2 of the control animals died within hours after the endotoxin. Thus, the evidence continues to mount (2, 6) that shock and intravascular clotting are independent manifestations of endotoxemia in the rabbit.

How cortisone potentiates endotoxin-induced clotting remains to be clarified. As described earlier (3, 5), administration of cortisone resulted in retention of fluid, an increase in plasma volume, and a fall in hematocrit. However, we can not relate these changes to a mechanism enhancing clotting. Large doses of cortisone reportedly impede reticuloendothelial cell function (2). Conceivably, an impaired clearance of endotoxin or of activated clotting factors contributed to the increased clotting of the cortisone-treated animals.

In the present experiments and in earlier experiments from this laboratory utilizing the cortisone-treated rabbit (5), renal cortical necrosis after a single injection of endotoxin was found only in animals with extensive fibrinogen consumption over the 6 hr after injection of the endotoxin. Consequently, we conclude that the ability of cortisone to enhance endotoxin-induced clotting constitutes one important mechanism whereby cortisone could prepare the rabbit for the GSR. However, rabbits may be infused with thrombin (7) or thromboplastin (8) without development of renal cortical necrosis. Thus, an effect or effects of endotoxin other than its ability to trigger intravascular clotting must also contribute significantly to the pathogenesis of the GSR. The present data in no way rule out the possibility that cortisone also prepares by enhancing such other effects of endotoxin. Indeed, we suspect that preparation for the GSR by cortisone results from the summation of multiple, synergistic actions of the very large doses of cortisone used.

Summary. Rabbits prepared with prior injections of cortisone were given a single intravenous dose of *E. coli* endotoxin, Boivin type, at 100 μg/kg. The cortisone treatment potentiated intravascular clotting in these rabbits. Thus, excess disappearance of plasma ^{125}I-fibrinogen radioactivity was demonstrated after endotoxin in each of 12 rabbits treated with 4 daily intramuscular injections of 25 mg of cortisone acetate, whereas only 1 of 13 saline-treated control rabbits showed similar changes. Consumption of plasma fibrinogen in the 6 hr after endotoxin was calculated from plasma fibrinogen levels and plasma fibrinogen radioactivity. Mean values were as follows: cortisone group, 79.4 mg% or 33.9 mg/kg; control group, 37.4 mg% or 13.2 mg/kg. The GSR was found in 3 cortisone-treated rabbits but in no control rabbit. Enhancement of endotoxin-induced clotting represents one important mechanism whereby cortisone could prepare rabbits for the GSR.

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1. Thomas, L., and Good, R. A., J. Exp. Med. **95**, 409 (1952).

2. Thomas, L., *in* "Physiopathology of the Reticuloendothelial System" (B. N. Halpern, ed.), p. 226. Blackwell, Oxford (1957).

3. Latour, J.-G., Prejean, J. B., and Margaretten, W., Amer. J. Pathol. **65**, 189 (1971).

4. Shen, S. M-C., Feinstein, D. I., and Rapaport, S. I., Blood **42**, 509 (1973).

5. Shen, S. M-C., Rapaport, S. I., and Feinstein, D. I., Blood **42**, 523 (1973).

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

7. Lee, L., J. Exp. Med. **115**, 1065 (1962).

8. Rapaport, S. I., Hjort, P. F., Patch, M. J., and Jeremic, M., Scand. J. Haematol. **3**, 59 (1966).

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