Effect of Cortisone on Response to Endotoxin in Mature Rabbits. (21240)

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In previous studies in this laboratory of the effects of cortisone and other agents(1-6) on the response to endotoxin, small rabbits, 4-7 weeks in age and weighing between one and 1.5 kilo, were used in most experiments. In animals of this size, cortisone produced an effect which was analogous to one stage of the generalized Shwartzman reaction: a single intravenous injection of endotoxin, in cortisone-treated rabbits, resulted in bilateral cortical necrosis of the kidneys. The observations were interpreted to indicate an interference by cortisone with a protective mechanism, perhaps involving the reticuloendothelial system, against the vascular necrotizing action of endotoxin(6).

In view of the difference in the response to endotoxin in large and small rabbits demonstrated in the previous paper(7), an investigation of the effect of cortisone in large animals was undertaken. It was found that pretreatment with this material caused 2 separate effects. One was similar to that reported earlier with small rabbits: a single injection of endotoxin was followed by the development of renal cortical necrosis, but with an incidence lower than previously observed in small animals. The second effect was to prevent completely the reaction of early prostration and death produced by endotoxin in the older rabbits. By adjusting the doses of cortisone and endotoxin it was possible to dissociate the 2 effects, so that protection against the early lethal response without the subsequent development of renal necrosis could be demonstrated.

Material and methods. The rabbits were of the same stock as the "large" animals employed in experiments described in the preceding paper(7). Meningococcal endotoxin was prepared by a method described elsewhere(1). Cortisone was obtained from Merck and Co., in a preparation for intramuscular injection containing 25 mg/cc.

Experimental. Effect of cortisone on early lethal effect of endotoxin. A group of 24 large rabbits, weighing 2.5 to 3.0 kilos each, received cortisone each day for 3 days in an intramuscular injection of 10 mg per kilo. Twenty-four animals of the same size were used as untreated controls. On the third day, meningococcal endotoxin was injected intravenously in a dose known to be sufficient to cause prostration and death in the majority of large rabbits. The outcome is shown in Table I. The general appearance of the cortisone-treated animals was strikingly different from the untreated controls. They remained active and alert, continued to feed, and were not distinguishable from normal animals. In contrast, all of the untreated rabbits exhibited obvious weakness and lethargy beginning about an hour after the injection, and developed varying degrees of prostration, with diarrhea, labored respirations and inability to remain upright. Within less than 18 hours after the injection of endotoxin 18 of the untreated group were dead, as compared with 3 of the cortisone-treated animals.

Renal necrosis in cortisone-treated rabbits. On the day following endotoxin injection, the situation appeared to be reversed. The surviving rabbits of the untreated group, which had been severely prostrated during the early hours of the experiment, seemed to be entirely recovered. On the other hand, some of the cortisone-treated animals had become apathetic and disinclined to feed. At the end of

* This work was done during tenure of Fellowships with the Helen Hay Whitney Foundation and the National Research Council.

This work was done under sponsorship of the Commission on Acute Respiratory Diseases. Armed Forces Epidemiological Board, supported in part by the Office of the Surgeon General, Department of the Army, and by grants from the National Institutes of Health, Public Health Service, and the Minnesota Heart Association.

TABLE I. Effect of Pretreatment with Cortisone on Response to Meningococcal Endotoxin in Large Rabbits.

Group	No. of rabbits†	Death‡	Renal cortical necrosis
Cortisone-treated*	24	3	9
Untreated	24	18	0

* Cortisone 10 mg/kilo daily for 3 days prior to an intravenous inj. of meningococcal endotoxin.

† All rabbits were between 10 and 12 wk old, and weighed 2.5-3 kilos.

‡ Refers to No. dying within 24 hr after endotoxin. All animals were sacrificed at 24 hr.

24 hours after endotoxin, all were sacrificed and the kidneys examined. The results were included in Table I. Of the 24 treated animals, 9 had bilateral cortical necrosis of the kidneys. This lesion was not present in any of the untreated group of rabbits.

Protection by cortisone against death, without renal necrosis. In previous studies with small rabbits it had been learned that a pretreatment period of 3 days with cortisone was required for the consistent production of renal necrosis by a single dose of endotoxin(6). In order to determine whether a smaller dose of cortisone could protect against the early lethal effect of endotoxin without the subsequent occurrence of renal lesions, the following experiment was performed. Two groups of 6 large rabbits each were given an injection of 2 cc of a 1-20 dilution of meningococcal endotoxin. Six hours prior to this injection, one group received an intramuscular injection of 10 mg cortisone. The results are summarized in Table II. All of the untreated rabbits became prostrated within 1 to 2 hours, and 3 were dead within less than 12 hours. All of the cortisone-treated rabbits remained free of symptoms during the entire period of 24 hours after the injection, and when sacrificed at this time had no kidney lesions. The *results* indicate that a single dose of cortisone, 6 hours before endotoxin, is sufficient to provide protection against the early reaction of prostration and death. Renal cortical necrosis did not occur under these conditions. These findings suggest that the two effects of cortisone on the response to endotoxin may involve separate mechanisms. In *other experiments,* unsuccessful attempts were made to reverse the primary lethal reaction by injecting cortisone 1-2 hours after endotoxin, at a time when the rabbits were already beginning to show evidences of prostration. No effect on the symptoms or mortality could be demonstrated in this circumstance, with doses of cortisone as high as 25 mg per kilo.

TABLE II. Effect of a Single Injection of Cortisone 6 Hours before Endotoxin.

Group	No. of rabbits	Death	Renal necrosis
Cortisone-treated*	6	0	0
Untreated	6	3	0

* Cortisone, 10 mg, one inj. 6 hr prior to intravenous inj. of meningococcal endotoxin.

Effect of cortisone on lethal reaction to colloidal iron followed by endotoxin. In previous experiments it was found that the early lethal effect of endotoxin was much enhanced when rabbits were given an intravenous injection of colloidal iron, thorotrast, trypan blue, or colloidal carbon a few hours prior to the injection of endotoxin(3-5). In addition, it was observed that a high proportion of animals receiving such combinations of colloidal materials and endotoxin developed renal cortical necrosis. The effect of pretreatment with cortisone was studied in the following manner: Two groups of 10 rabbits each were employed. In one group cortisone was given for 3 days, in a daily intramuscular injection of 10 mg per kilo. On the third day, both groups received colloidal iron saccharate ("Proferrin") in a dose of 2 cc per kilo, followed 2 hours later by 2 cc of a 1-80 dilution of meningococcal endotoxin. The results are indicated in Table III.

In the 10 control rabbits, not treated with cortisone, death occurred within a few hours in 7. One animal which survived until the next day had bilateral cortical necrosis of the kidneys. In contrast, only 1 cortisone-treated rabbit developed early prostration and died, while 7 developed renal necrosis during the following 24 hours.

The results suggested, as in the preceding experiments, that the two effects of cortisone on the response to endotoxin may be separate events. The enhanced early lethal reaction was inhibited, but the development of renal cortical necrosis was not prevented. In fact,

TABLE III. Effect of Cortisone on Lethal Effect of Endotoxin following Colloidal Iron Saccharate.

Group*	No. of rabbits	Death	Renal necrosis
Cortisone-treated	10	1	7
Untreated	10	7	1

*All rabbits received 2 cc colloidal iron saccharate ("Proferrin") intrav., 2 hr before meningococcal endotoxin. The cortisone-treated group received 10 mg cortisone/kilo daily for the 3 preceding days.

the incidence of renal necrosis was observed in other experiments with colloidal iron and endotoxin to be consistently higher in cortisone-treated animals than in untreated controls. This was due in part to the protection by cortisone against early death, with a consequently greater proportion of rabbits surviving for a long enough period to develop the renal lesion, and in part to the previously demonstrated capacity of cortisone itself to bring about renal necrosis after a single injection of endotoxin.

Discussion. Two separate and perhaps independent effects of cortisone on the response to endotoxin in large rabbits have been demonstrated in these experiments. The first of these is to protect against the early reaction of prostration, shock and death which is produced by endotoxin in mature rabbits. The second, described in detail earlier(6), is to bring about the lesions characteristic of the generalized Shwartzman reaction with a single injection of endotoxin.

When cortisone is administered in a sufficient amount, with a pretreatment period of 3 days before endotoxin, both actions are simultaneously demonstrable in the same experiment. The animals withstand the early effects of endotoxin, but develop renal necrosis during the next 24 hours. But when only one injection of cortisone is given, 6 hours before endotoxin, protection against early death occurs without the subsequent appearance of renal lesions.

Both properties of endotoxin—the production of prostration and early death, and the development of renal necrosis—are exhibited when the injection of endotoxin is preceded by certain colloidal materials such as saccharated iron oxide. The occurrence of shock and early death is prevented by cortisone, but the subsequent development of renal cortical necrosis is not inhibited and is, in fact, to some extent augmented.

Studies by other workers have suggested that natural resistance to the lethal action of endotoxin in some way involves the adrenal gland. Lewis and Page(8) showed that adrenalectomized rats were extremely vulnerable to typhoid vaccine, and were protected by treatment with cortisone. In rabbits an antipyretic action of cortisone has been reported with several different endotoxins(9). Spink and Anderson(10) have shown that mice are protected against the lethal action of brucella endotoxin by cortisone; no renal lesions were encountered in this species.

On the other hand, the property of cortisone to bring about renal necrosis with a single injection of endotoxin cannot be interpreted to indicate that the adrenal cortex plays an essential role in the pathogenesis of the generalized Shwartzman reaction. In recent studies by Good and associates(11) it was found that this reaction could be produced by 2 injections of endotoxin in totally adrenalectomized rabbits, without supportive hormone therapy.

Summary. 1. Treatment with cortisone prevented the early reaction of prostration and death produced by an intravenous injection of endotoxin in mature rabbits. 2. In some of the rabbits pretreated for 3 days with large doses of cortisone, bilateral cortical necrosis of the kidneys occurred within 24 hours after endotoxin. However, when a single injection of cortisone was given 6 hours before endotoxin, protection against prostration and death was demonstrable without the subsequent development of renal necrosis. 3. The early lethal reaction caused by small amounts of endotoxin following an injection of colloidal iron saccharate was prevented by cortisone. Cortisone did not protect against the development of renal cortical necrosis in these animals.

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Received July 14, 1954. P.S.E.B.M., 1954, v86.

Cold-Precipitation by Heparin of a Protein in Rabbit and Human Plasma. (21241)

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The earliest pathological event in the generalized Shwartzman reaction, which is produced in rabbits by two intravenous injections of gram negative bacterial endotoxin, is the appearance of intravascular deposits of homogeneous, eosinophilic material with the staining properties of fibrinoid, within the lumen of glomerular capillaries and small vessels of the spleen, liver, and lungs, and in the walls of the coronary arteries(1,2). The morphological situation of the material indicates that it is derived from the circulating blood, and the observation that its deposition in the glomerular capillaries is prevented by heparin(3) suggests that the coagulation mechanism may be involved in its development. In the course of an investigation into possible precursors of fibrinoid, heparinized plasma was obtained from rabbits at various time intervals after an intravenous injection of endotoxin. It was noted that chilling of such plasma specimens at 4°C was followed within less than an hour by the precipitation of gelatinous, flocculant material resembling an incomplete plasma clot. Warming the plasma to 37°C resulted in rapid solution of the material, and chilling again caused precipitation to occur. No cold-precipitation occurred in citrated plasma or in serum. Heparin-precipitable material was not demonstrable in the plasma of a majority of normal rabbits. However, it was encountered in the plasma of normal human beings and, in greater amounts, in the plasma of patients with acute rheumatic fever.

The present paper is a preliminary account of the conditions under which the heparin-precipitable material is demonstrable, with certain observations suggesting that it may be derived from fibrinogen.

Materials and methods. Young hybrid albino rabbits, weighing 1 to 1.5 kilos, were used in all experiments. The animals were maintained on Purina rabbit pellets and water. The following endotoxins were employed: meningococcal "agar washings" toxin prepared as previously described(1) from a strain of meningococcus (44B) supplied by Dr. Gregory Shwartzman, Mount Sinai Hospital, New York, a polysaccharide endotoxin from *S. marcescens,* supplied by Dr. Murray Shear, National Cancer Institute, Washington, and a purified *Sh. paradysenteriae* endotoxin supplied by Dr. Walter Goebel, Rockefeller Insti-

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This work was done under sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, supported in part by the Office of the Surgeon General, Department of the Army, and by grants from the National Institutes of Health, Public Health Service, and the Minnesota Heart Assn.

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