Hepatic Detoxification of Endotoxin after Adrenalectomy[1] (36428)

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The physiology of host-defense against endotoxemia is under scrutiny by various laboratories since, as promulgated by Fine (1), endotoxin may be the final common pathway in the pathogenesis of irreversible cardiovascular shock ensuing hemorrhage, trauma, or infections. While a bewildering array of deviations in host homeostasis occur during endotoxemia and shock (2), it is widely recognized that among the primary determinants of host resistance to endotoxin are the functional status of the reticuloendothelial system (RES) and the adrenal glands (3). On the one hand, either impairment of RES activity or deprivation of glucocorticoids by adrenalectomy sensitizes various animal species to endotoxin shock (2). Conversely, albeit more controversially, either glucocorticoid therapy (4) or RES hyperfunction induced by an endotoxin pretreatment regimen (2, 5) is often associated with enhanced resistance or tolerance to endotoxin.

While the protective role of the RES in endotoxemia is thought to reflect its ability to sequester and detoxify endotoxin (6), the exact function of the adrenal glucocorticoids in deterring the homeostatic disruptions unleashed by endotoxin is essentially unknown. Recently the hepatic macrophages and especially the macrophage lysosomes have been implicated in endotoxin detoxification and thus in amelioration of the cardiovascular toxicity of endotoxin (7, 8). Since adrenal steroids are known to influence macrophage function (9) as well as lysosomal dynamics (10), the purpose of the present study was to evaluate if the deleterious consequences of adrenalectomy in endotoxin shock in the rat might be mediated by a defect in the hepatic mechanism for endotoxin detoxification.

Materials and Methods. Complete bilateral adrenalectomies and surgical control sham-operations were performed under ether anesthesia in male Holtzman rats in the weight range 300 ± 20 g. (Holtzman Company, Madison, WI). Adrenalectomized rats were maintained on Purina chow and 0.9% saline *ad libitum*. Endotoxin was purchased from Difco Laboratories, Detroit, MI as the Boivin lipopolysaccharide prepared from *Salmonella enteritidis*. The bioassay of endotoxin toxicity and detoxification was performed via shock induction in the lead-sensitized rat as described in detail previously (11). Briefly liver homogenates were prepared in phosphate buffered saline (0.02 *M* potassium phosphate buffer, pH 7.4, in 0.15 *M* NaCl) by the Potter-Elvehjem procedure and then were incubated for 180 min at either 37 or 4° with varying doses of endotoxin as prescribed by the experimental protocols. After incubation 1-ml samples of the incubation media were injected iv into assay rats and immediately followed by 5 mg of lead acetate iv. Death in endotoxin shock occurred within 14–24 hr. Lethality data were tested for intergroup statistical significance at a confidence level of 95% using the chi-square test corrected for continuity with the Yate's factor. LD_{50} determinations were measured by the graphic method of Miller and Tainter (12).

Results. Comparison of the sensitivity to endotoxin shock after acute vs. chronic adrenalectomy. As depicted in Fig. 1 acute adrenalectomy, *i.e.*, 60 min prior to endotoxin injection, or chronic adrenalectomy, *i.e.*, 7

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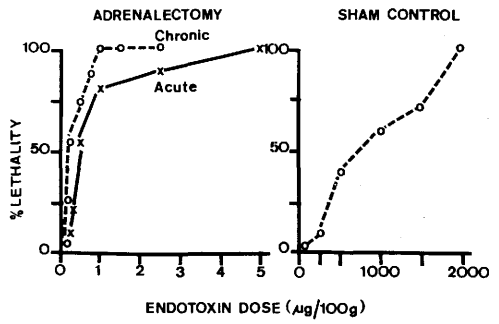

FIG. 1. Effect of chronic and acute adrenalectomy on endotoxin shock lethality. Each point represents 15–20 rats/group. Acute adrenalectomies were performed 60 min prior to endotoxin iv. Chronic adrenalectomies were performed 7 days prior to endotoxin iv. Surgical sham-controls were grouped since no differences were measured between acute and chronic groups.

days prior to endotxin injection, produced profound sensitization to endotoxin as compared to the combined surgical control, sham-operated groups. Thus, while the LD_{50} of the control group was 800 µg/100 g, acute adrenalectomy reduced the LD_{50} to 0.59 µg/100 g and chronic adrenalectomy reduced the LD_{50} to 0.25 µg/100 g.

In order to more accurately assess the rapidity and degree of sensitization to endotoxin shock after adrenalectomy, rats were adrenalectomized and then injected with endotoxin doses at varying times after the operation. As indicated in Table I the LD_{50} was reduced from 800 µg/100 g in the control group to 50 µg/100 g within 15 min after adrenalectomy. By 30 min, the LD_{50} was 10 µg/100 g and by 96 hr it approached 0.25 µg/100 g.

Bioassay of endotoxin detoxification by liver homogenates after adrenalectomy and in endotoxin shock. In order to bioassay endotoxin detoxification, the ability of varying percent weight/volume concentrations of control liver homogenates to detoxify a standard LD_{100} dose of endotoxin *i.e.*, 2 µg in a 300 g rat receiving 5 mg of lead acetate, was determined as depicted in Fig. 2. While homogenates at 0.01% did not detectably alter endotoxin toxicity, there was a progressive increase in detoxifying capacity as the homogenate concentration was increased from

TABLE I. Sensitization to Endotoxin at Varying Times After Adrenalectomy.[a]

Times after adrenalectomy	LD_{50} µg/100 g
0-min (Surgical controls)	800
15-min	50
30-min	10
60-min	0.59
24-hr	0.62
48-hr	0.50
96-hr	0.25

[a] LD_{50} determinations were graphically measured between probits 4.5 to 6.0 by the method of Miller and Tainter (12) using a minimum of three assay groups of 10–12 rats/group.

0.05% to 1.0%. In contrast, control incubations at 4° were uniformly 100% lethal. In further control studies, incubation of liver homogenates at either 4 or 37° without the addition of endotoxin were consistently nonlethal upon iv injection.

Based on the data in Fig. 2, liver homogenates from chronic adrenalectomy, acute adrenalectomy and surgical control groups at concentrations of 0.25% and 0.10% were incubated with 2 µg/ml of endotoxin prior to assay in lead cotreated rats. As seen in Fig. 3, no significant differences in detoxifying ability were apparent between control and

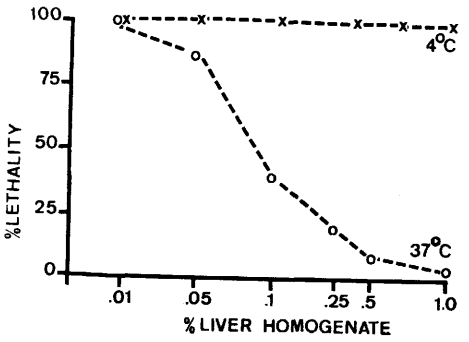

FIG. 2. Bioassay of endotoxin detoxification by liver homogenates using shock lethality in the lead-sensitized rat. Varying concentrations of liver homogenates were incubated at either 37°, (0- - -0) or 4°, (x- - -x) for 180 min with a standard LD_{100} dose of endotoxin, *i.e.*, 2 µg/ml. Number of rats were: 10 in 4° groups, and 15–20 in the 37° groups. Each assay rat received 1 ml of the incubation media plus 5 mg lead acetate iv.

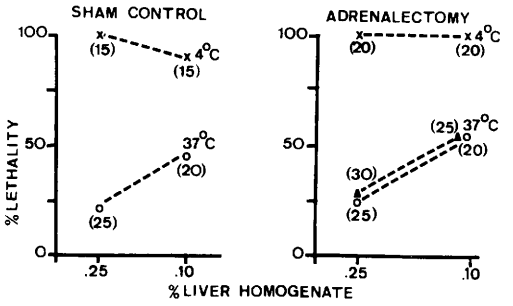

FIG. 3. Comparison of endotoxin detoxification by liver homogenates from chronic adrenalectomized, acute adrenalectomized and surgical control rats using the lead sensitized rat bioassay. Homogenates of 0.25 and 0.10% from acute adrenalectomized (△- - -△), chronic adrenalectomized O- - -O or combined surgical sham control groups were incubated at 37° or 4° with 2 μg endotoxin/ml. The 4° adrenalectomized controls were combined since no differences were evident. Each assay rat received 1 ml of the incubation media plus 5 mg lead acetate iv. Number of rats/group are indicated for each point.

adrenalectomized liver homogenates at either 0.25 or 0.10%. To further assess the ability of adrenalectomized rat liver to detoxify endotoxin, liver homogenates were prepared from either surgical controls or chronic adrenalectomized rats in the agonic stage of shock induced by either 2 mg/100 g or 2 μg/100 g of endotoxin iv, respectively. As shown in Fig. 4, neither control or adrenalectomized liver homogenates at 0.25 or .10% had any manifest inability to detoxify endotoxin.

Discussion. The complex sequence of reactions to endotoxin entering the blood vascular system includes (i) interaction of endotoxin with blood elements and plasma constituents, (ii) sequestration of endotoxin by the macrophages of the RES, especially in the liver and spleen sinusoids, and (iii) detoxification of endotoxin, either in the blood or in the RES. Thus, there are numerous loci where agents or treatments which modulate endotoxin's effects may operate.

Past studies of this laboratory (7, 8) have implicated the macrophages and their lysosomal apparatus in the detoxification of endotoxin. The present study evolved from an attempt to develop an experimental model in which the macrophage detoxification system could be altered by a physiologic regulatory mechanism. Since the adrenal gland is recognized as a prime determinant of RES activity and host-defense in endotoxemia, it was reasonable to postulate a role for the adrenal secretions in the control of macrophage endotoxin detoxification.

The experiments reported herein confirm and extend the exquisite sensitization to endotoxin imposed by either acute or chronic adrenalectomy (13–15). However, within the limits of the experimental model studied, no evidence for a defect in the hepatic endotoxin detoxifying system was manifest. Furthermore, neither adrenalectomized nor control livers had a detectable loss in detoxifying ability even when procured from rats in the terminal stages of endotoxin shock.

Since Fine (16) has amassed considerable evidence to indite a failure of the RES to detoxify endotoxin in shock, it is likely that the events susceptible to embarrassment in shock are centered around the macrophage sequestration of endotoxin, rather than the intracellular detoxification system *per se*. This notion is consonant with the early theory of Beeson (17) that tolerance to endotoxin resides in the rapid sequestration of endotoxin and the recent studies of Greisman *et*

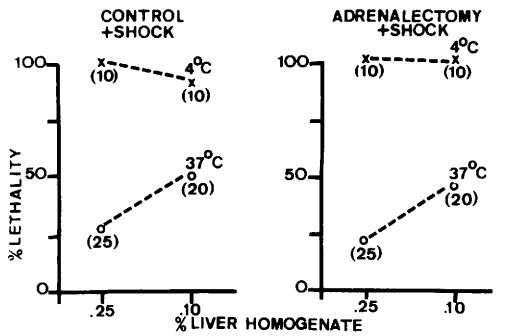

FIG. 4. Comparison of endotoxin detoxification by liver homogenates from chronic adrenalectomized or surgical control rats in endotoxin shock. Homogenates at 0.25 and 0.10% were prepared from adrenalectomized and surgical control rats in terminal endotoxin shock and were incubated at 37°, (O- - -O) or 4°, (x- - -x) with 2 μg endotoxin/ml. The incubation media were assayed by injecting 1 ml iv plus 5 mg lead acetate iv. Number of rats/group are indicated for each point.

al. (18) which impart a major role to an opsonic factor for endotoxin during tolerance. Further studies are needed to ascertain the influence of the adrenal gland on the phagocytic sequestration of endotoxin and the endotoxin opsonic factor. A similar analysis is also needed for the defect in endotoxin host-defense during shock pathogenesis.

Summary. Bilateral adrenalectomy of 300 ± 20 g male Holtzman rats resulted in extreme sensitization to endotoxin shock produced by iv *Salmonella enteritidis* Boivin lipopolysaccharide. Within 60 min after adrenalectomy, resistance to endotoxin as reflected in LD_{50} values decreased from 800 μg/100 g to 0.59 μg/100 g and by 168 hr after adrenalectomy the LD_{50} had diminished to 0.25 μg/100 g. Within 15 minutes after adrenalectomy endotoxin resistance had diminished to a LD_{50} of 50 μg/100 g. Liver homogenates from either acute (60 min) or chronic (168 hr) adrenalectomized and sham-operated control rats were assessed for endotoxin inactivating ability using a lead-sensitized rat bioassay. The ability of liver to detoxify endotoxin was not significantly altered by either acute or chronic adrenalectomy. Endotoxin inactivating ability also was measured in livers removed from either control or chronic adrenalectomized rats in the terminal phase of endotoxin shock; no appreciable loss of hepatic endotoxin inactivating ability was manifested in either group as compared to control or chronic adrenalectomized rats which were not exposed to endotoxin shock. The data suggest that the defect in host resistance to endotoxin imposed by adrenalectomy is probably not due to an impairment of the hepatic intracellular system for endotoxin inactivation.

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