Liver Carbohydrate Levels in Mice Treated with Endotoxin, Cortisone, and Elipten (34255)

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The marked increase in susceptibility of adrenalectomized animals to endotoxin shock has clearly shown the importance of adrenal function in endotoxemia (1). The protection against endotoxemic shock achieved by administration of cortisone further supports the need for adequately functioning adrenals (2, 3). A major supply of corticosteroids is eliminated by adrenalectomy, but this procedure also eliminates the source of a variety of other important substances as well. We found that several inhibitors of corticosteroid synthesis increased the susceptibility of mice to endotoxin, with α-(*p*-aminophenyl)-α-ethylglutarimide [Elipten, Ciba] being the most effective of those tested. The effect of Elipten on endotoxin shock was eliminated by administration of cortisone or certain other steroids (4).

Cortisone administered in pharmacologic doses also induces gluconeogenesis with deposition of glycogen in the liver while adrenalectomy and endotoxin shock are followed by a decrease in the glycogen level (5–7). Elipten alone failed to affect the glycogen level in the liver of normal mice, but in combination with cortisone appreciably decreased the deposition of glycogen in the liver. These observations, as well as the glycogen content of mouse liver after treatment with combinations of these drugs and endotoxin, are reported.

Materials and Methods. Mice. The ICR mice used in this study were 20–25 g males obtained from Rawley Farms (Plymouth, Michigan). The animals, housed on wire mesh in metal cages, were kept in the laboratory for 1 week before use. Food and water were freely available at all times.

Drugs. The endotoxin was prepared from *Salmonella enteritidis* by the method of Boivin (8) and the lyophilized endotoxin was rehydrated in sterile, pyrogen-free physiological saline. The endotoxin was administered to the animals intraperitoneally in 300 μg (1.25 LD_{50}) or 30 μg (0.15 LD_{50}) doses 18 hr before sacrifice. The inhibitor of steroidogenesis, Elipten, was dissolved in saline and 2.5 mg were given subcutaneously 20 hr before the animals were killed for assay. Cortisone (Sigma Chemical Co.) was suspended in saline containing a small amount of Tween 80 (2) and 5 mg were administered intraperitoneally immediately following the endotoxin or 2 hr following the Elipten.

Determination of liver carbohydrate. The test mice were killed by cervical dislocation and the livers were quickly excised and placed on ice. Approximately equal portions of liver from at least two mice receiving the same treatment were blotted on filter paper and after weighing were placed in a homogenizer tube (Tri-R Instruments, Jamaica, N.Y.) maintained at 4°. The samples were homogenized, deproteinized, and the liver glycogen plus glucose levels were determined by the method of Kemp and Kits van Heijningen (9). Livers from untreated mice were always included for control purposes.

Results and Discussion. As expected from the work of others (2, 7, 10), the carbohydrate content of livers of mice treated with endotoxin was markedly reduced. The glycogen plus glucose decreased to less than 5% of normal in livers of mice treated with 300 μg of endotoxin (Table I). Normal mice treated with cortisone showed a typical increase to greater than double the control level (2, 6). Treatment of endotoxicated animals with cortisone failed to maintain even normal carbohydrate levels. Thus, it would appear that the protection afforded by cor-

TABLE I. The Carbohydrate Level in Livers of Mice 18 hr after Treatment with Endotoxin, Cortisone, and Elipten.

Treatment (no. of determinations)	Carbohydrate level (mg of glycogen/g of wet tissue ± SD)
Normal	
Control (23)	44.7 ± 7.4
Cortisone (8)	117.8 ± 21.5
Elipten (6)	42.7 ± 14.6
+ cortisone (7)	53.2 ± 8.9
Endotoxin, 300 μg	
Control (15)	2.5 ± 0.6
Cortisone (6)	4.8 ± 2.9
+ Elipten (6)	4.3 ± 1.4
Endotoxin, 30 μg	
Control (5)	14.9
Elipten (6)	4.9 ± 3.9
Cortisone + Elipten (7)	49.9 ± 8.1

tisone against the lethal effects of endotoxin is not due to maintenance of normal carbohydrate reserves, although some minimal level may be required for survival (2).

Since previous studies showed that mice pretreated with Elipten became more susceptible to endotoxin (4) it was not surprising to find that animals treated with Elipten plus 300 μg of endotoxin rarely survived the required 18-hr incubation period. Therefore, a 30-μg dose of endotoxin was used when the combined effect of endotoxin and Elipten on liver carbohydrate was determined. Such treatment resulted in a reduction in the glycogen level to about one-third that observed with 30 μg of endotoxin alone. The relative reduction in carbohydrate level after this treatment was more severe than that encountered in the normal animals treated with Elipten in which little or no effect on the liver carbohydrate level was noted.

In mice treated with both Elipten and cortisone, the glycogenic effect of cortisone was essentially eliminated. The liver carbohydrate in the Elipten and cortisone-treated animal increased by 19% which is small when compared with the increase of more than 1.5-fold in the cortisone-treated mice. Despite this difference the increase in the store of carbohydrate in the liver was significant ($p < 0.01$ by the Student's t test).

Mice treated with the three substances, Elipten, endotoxin, and cortisone lost 90% of the liver carbohydrate store if the 300 μg dose of endotoxin were used. This effect was only slightly greater than in the animals treated with endotoxin and cortisone. The mice receiving the 30 μg dose of endotoxin with Elipten and cortisone maintained the same general carbohydrate level as the normal, the Elipten–cortisone treated animals, or the elipten treated animals.

The enhanced susceptibility of Elipten-treated mice to endotoxin indicates that fully functional adrenal glands are needed by the host to withstand the effects of endotoxin. Not only does the Elipten inhibit the synthesis of corticosteroids (11), but it also inhibits the induction of glycogen deposition by cortisone. This effect may extend to other synthetic systems mediated by corticosteroids, such as tryptophan oxygenase and tyrosine transaminase. Wogan and Friedman (12) reported that in rats treated with aflatoxin B, hydrocortisone similarly failed to induce either tryptophan oxygenase or tyrosine transaminase.

Summary. Mice treated with endotoxin 18 hr earlier had markedly reduced liver carbohydrate, but with cortisone under the same conditions the level was more than doubled. Elipten exerted no apparent effect on the carbohydrate levels in normal mice, but very nearly eliminated the deposition of glycogen after the mice were treated with cortisone. The effect of endotoxemia on the liver carbohydrate was enhanced by Elipten, and ameliorated slightly by cortisone alone or in combination with Elipten.

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