Inhibition of Hepatic Enzyme Induction as a Sensitive Assay for Endotoxin

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Bacterial endotoxins in mice reduced the induction by cortisone of two hepatic enzymes, tryptophan oxygenase, and phosphoenolpyruvate carboxykinase, they prevented the glyconeogenesis in liver induced by the same hormone, and they induced in intact animals the liver enzyme tyrosine-α-ketoglutarate transaminase, all in proportion to their LD_{50}. When cortisone was given in the least amount (100 μg), it resulted in near maximal induction of tryptophan oxygenase; a smaller amount of endotoxin reduced significantly the level of enzyme than that required when 5 mg of hormone was injected. The smallest amount of endotoxin that prevented tryptophan oxygenase induction was given intravenously to adrenalectomized mice in which 25 μg of cortisone was administered. The amount (0.01 μg) is 1/40,000th of the LD_{50}. The other metabolic processes subject to alteration by endotoxin required at least 100 to 400 times as much. This property of endotoxin can serve as a sensitive bioassay, although the dose-response curve is steep.

Several publications from this laboratory (2–6, 11, 12, 28), based on earlier reports (10, 13, 19, 21), have been devoted to an analysis at a metabolic level of how glucocorticoids protect against the biological effects of bacterial endotoxins. In mice, it has been shown that one dose of 5 mg of cortisone acetate (but not one dose of 1 mg) protects against lethality caused by an injection of endotoxin that kills 94% of the controls (6). Because an amount of hormone approximating the total weight of adrenal cortical tissue is necessary for the prevention of death from endotoxin, nearly all of the experiments reported until now have been carried out with doses of this magnitude.

The adrenal steroids induce a number of hepatic enzymes (14, 17, 23, 27, 29, 30, 32–34). Among these, at least four have half-lives of 2 to 3 hr (14, 23, 29, 33). If it is assumed that endotoxin exerts its biological effects through altered enzyme activity, then those enzymes with short half-life would most likely be involved because typical symptoms of endotoxin poisoning appear within a few hours after injection of the LD_{50} of endotoxin. Similarly, the ability to protect the mouse against lethality from endotoxin is lost when the hormone is given between 2 and 4 hr after the toxin (4). Events that occur in the first few hours postinjection are, therefore, obviously important. Three enzymes with short half-life have been studied in mice given the LD_{50} of endotoxin and 5 mg of cortisone acetate (3–5, and

unpublished data). The hormonal induction of two enzymes, tryptophan oxygenase and phosphoenolpyruvate carboxykinase, is strongly inhibited by endotoxin, whereas the third enzyme, tyrosine-α-ketoglutarate transaminase, is not inhibited. In intact mice, this enzyme is induced by endotoxin to approximately the same degree as it is by hormone, whereas only a limited but statistically significant induction occurs in adrenalectomized mice (5). The contrast in response of these three enzymes establishes the fact that endotoxin is not exerting its anti-inductive effect through general hepatotoxicity but, on the other hand, appears to have a selective action on certain enzymes. A fourth enzyme, pyruvate carboxylase, has not as yet been evaluated under these conditions.

Since tryptophan oxygenase in livers of mice has been found to increase in activity following an injection of as little as 100 μg of cortisone acetate (5), the minimal dose of hormone able to induce the enzyme and the minimal dose of endotoxin that prevents it have now been determined and form part of the material reported in this paper. In addition, the marked sensitivity of the mouse to endotoxin, as judged by this test, inspired an effort to compare different preparations of endotoxin to determine whether a correlation could be established between the toxicity of a given endotoxin and its ability to inhibit enzyme induction. Finally, because of the success of these studies with tryptophan oxygenase, it became

important to extend them to include measurements of tyrosine transaminase, phosphoenolpyruvate carboxykinase, and liver glycogen deposition under similar conditions. The data presented in this report are the results of such experiments.

MATERIALS AND METHODS

Endotoxin. A purified preparation of *Serratia marcescens* lipopolysaccharide obtained from lyophilized cells by the Boivin method was generously provided by A. Nowotny, Department of Microbiology, Temple University School of Medicine. This served as the primary material for most of the studies and is designated as "Etox." The same material detoxified by treatment with potassium methylate was also supplied by A. Nowotny and is indicated as "Mex." A heat-killed suspension of *Salmonella typhimurium* strain SR-11 in nonpyrogenic isotonic sodium chloride solution was prepared as previously described (3) and was used in some experiments. Other endotoxins employed were an *Escherichia coli* O2: K1, lot no. 3712: 92a, lipopolysaccharide, supplied by C. E. deWitt, The Upjohn Co., Kalamazoo, Mich., and a Boivin extract of *S. marcescens* prepared by E. R. Squibb & Sons, New Brunswick, N.J., and supplied by G. J. Miraglia. The latter is designated as *S. marcescens* lipopolysaccharide.

Enzyme assays. Tryptophan oxygenase activity was determined in whole liver homogenates by a modification of the method of Knox and Auerbach (26), as previously described (3). Results are expressed as micromoles of kynurenine produced per gram (dry weight) of liver per hour at 37 C. Tyrosine-α-ketoglutarate transaminase was assayed in whole liver homogenates by the method of Rosen et al. (31). Activities are expressed as micromoles of p-hydroxyphenylpyruvic acid formed per gram (dry weight) of liver per 10 min of incubation at 37 C. Phosphoenolpyruvate carboxykinase activity in whole liver

homogenate was determined by the method of Shrago et al. (32). Values are expressed as micromoles of phosphoenolpyruvic acid formed per gram (dry weight) of liver per 6 min. Liver glycogen was measured by the method of Kemp and Kits van Heyningen (22) and is given as percentage (wet weight) of liver.

Injections. Cortisone acetate (50 mg per ml) in stabilized suspension was purchased from United Research Laboratories, Philadelphia, Pa. By proper dilution with nonpyrogenic saline (Baxter Laboratories, Morton Grove, Ill.), the desired dose contained in 0.5 ml was injected subcutaneously in the interscapular region. Endotoxin was dissolved or suspended in nonpyrogenic saline and injected intraperitoneally in most experiments and intravenously in others.

Mice. Albino Swiss-Webster female mice were used in all experiments when they weighed 22 ± 2 g. Intact mice were purchased from Rockland Farms, Boyertown, Pa. The treatment and handling of these animals were the same as that previously described (4, 5). Adrenalectomized female CF 1-S mice were obtained from Carworth Farms, New City, N.Y. They were given 1% sodium chloride solution as drinking water and were tested for the completeness of the adrenalectomy by the water-retention test of Beatty et al. (1). They were given a day or two in the animal room before they were tested and a day or two of recovery before they were used experimentally.

RESULTS

Effect of Etox and Mex on tryptophan oxygenase. The effect of graded intraperitoneal doses of endotoxin on the activity of tryptophan oxygenase in livers of fasted mice 18 hr postinjection is presented in Table 1. Both the Etox and Mex preparations decreased the activity of the enzyme at each of the two larger doses tested, but the smallest amount (1 μg) failed to yield values sig-

TABLE 1. *Effect of Etox and Mex on tryptophan oxygenase activity in livers of fasted mice 18 hr postinjection*

Dose of endotoxin injected	Tryptophan oxygenase activity [μmoles of kynurenine per gram (dry weight) per hour] 18 hr after the injection of			Statistics control vs.	
	Control (no injection)	Etox[a]	Mex[b]	Etox	Mex
μg					
40	30.4 ± 1.6[c] (6)	10.8 ± 1.6 (6)	14.5 ± 1.7 (6)	$P < 0.01$	$P < 0.01$
4	27.6 ± 2.3 (18)	16.6 ± 1.4 (12)	18.9 ± 1.4 (12)	$P < 0.001$	$P < 0.01$
1	25.1 ± 1.9 (16)	20.6 ± 1.3 (18)	22.3 ± 2.8 (6)	N.S.[d]	N.S.

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Mex is detoxified endotoxin (LD_{50} about 4 mg).
[c] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[d] N.S. = not significant.

TABLE 2. *Effect of Etox and Mex on cortisone induction of tryptophan oxygenase in livers of mice fasted 6 hr postinjection*

Dose of endotoxin injected with 5 mg of cortisone	Tryptophan oxygenase activity [μmoles of kynurenine per gram (dry weight) per hr] 6 hr after the injection of			Statistics control vs.	
	Control (no endotoxin)	Etox[a]	Mex[b]	Etox	Mex
μg					
40	48.5 ± 3.7[c] (12)	21.1 ± 2.2 (12)	42.3 ± 2.3 (6)	$P < 0.001$	N.S.[d]
4	49.5 ± 4.3 (12)	31.8 ± 1.7 (12)		$P < 0.01$	
1	47.3 ± 2.6 (12)	43.5 ± 2.2 (12)		N.S.	

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Mex is detoxified endotoxin (LD_{50} about 4 mg).
[c] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[d] N.S. = not significant.

nificantly lower than that of the control, as calculated by the rank order test of White (35). No difference between the two preparations of endotoxin was evident, and their ability to lower the activity of this enzyme appears, therefore, to be independent of toxicity.

Effect of Etox and Mex on induction of tryptophan oxygenase after 5 mg of cortisone. A dose of 5 mg of cortisone acetate was used to determine the largest amount of Etox and Mex that failed to prevent the induction of tryptophan oxygenase. The results (Table 2) show that 4 μg of Etox (but not 1 μg) produced a significantly lower activity of the enzyme than that found for controls, whereas 40 μg of the less toxic Mex failed to alter the hormonal induction of the enzyme. This property of endotoxin is apparently related to toxicity of the preparation.

Effect of graded doses of cortisone acetate on tryptophan oxygenase induction in "fed," "fasted," and "fed-adrenalectomized" mice. The experiments reported in graphic form in Fig. 1 have relevance for several reasons. Since endotoxin in small amounts prevents ingestion of food (9), the appropriate control animal for endotoxin poisoning must be one that is fasted. The stress of starvation for an overnight period of about 18 hr elevated not only the base level of tryptophan oxygenase, probably through the release of endogenous adrenocorticoids, but also influenced the inducibility of the enzyme after injection of different amounts of exogenous hormone. These relationships seem evident by a comparison of the two curves in Fig. 1, designated "Fasted-Intact" and "Fed-Intact." The fed-adrenalectomized mouse, by contrast, has the lowest control level of tryptophan oxygenase of the three shown and the sharpest increase in enzyme activity following
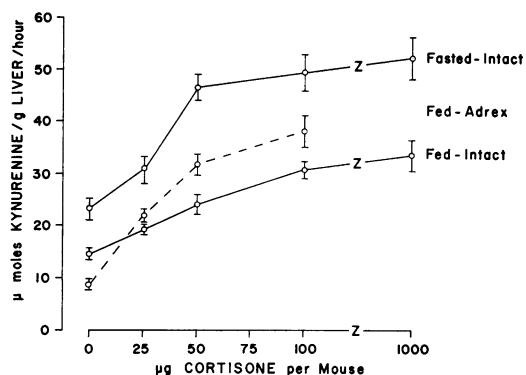

FIG. 1. *Tryptophan oxygenase activity in livers of mice 6 hr after the injection of cortisone acetate. Each point is the mean of from 6 to 26 separate determinations. The bars represent the standard error of the mean.*

administration of the least amount of hormone employed (25 μg). This may be observed by reference to the curve designated in Fig. 1 as "Fed-Adrex." The physiological and biochemical reasons for these relationships among the different groups of mice are not understood.

Since fasted-adrenalectomized mice were not used in these studies for any extended period of time, comparable data were not obtained. However, the activity of tryptophan oxygenase in fed and fasted-adrenalectomized mice was not significantly different (Table 3).

Effect of graded doses of Etox on induction of tryptophan oxygenase 6 hr after treatment with different amounts of cortisone acetate. These experiments were carried out with intact mice fasted for 18 hr at the time the injections were

administered; they were fasted for an additional 6 hr until they were sacrificed. The data presented (Table 4) established several facts. The most apparent of these was the ability of either 4 or 40 μg of endotoxin to lower tryptophan oxygenase activity to the same level independent of the dose of cortisone administered, at least over a 50-fold range (5 mg to 100 μg). The larger dose (40 μg) resulted in a greater decrease in activity than the smaller amount (4 μg). By contrast, the ability of 1 μg of endotoxin to alter tryptophan oxygenase level depended on the size of the dose of cortisone. No significant change occurred when 5 mg of hormone was given, but with 100 μg a significant decrease was produced.

Another important observation was the steep drop in activity of the enzyme that accompanied the injection of a small amount of endotoxin. This was evident when the change after 1 μg of endotoxin was given to mice that received 100 μg of cortisone is compared to the additional decrease that occurred after the dose of endotoxin was raised to 40 μg. The initial drop of activity of the enzyme after 1 μg of endotoxin was 48.8 to

30.7 = 18.1 units, whereas the added decrease after 40 μg was 30.7 to 19.8 = 10.9 units. The dose-response relationship is neither linear nor exponential.

An 0.1-μg amount of Etox in mice injected with 50 μg of cortisone acetate yielded a value of 19.4 ± 2.5 (Table 4). This dose of endotoxin in intact fasted mice yielded a statistically significant inhibition of hormonal induction of tryptophan oxygenase. This quantity of Etox is 1/4,000th of the LD_{50}.

Effect of route of injection of Etox on the hormonal induction of tryptophan oxygenase in adrenalectomized mice. Adrenalectomized mice were substituted for intact animals because they are more sensitive to endotoxin (7), and it was considered possible that smaller amounts would prevent the hormonal induction of tryptophan oxygenase. In addition, less cortisone acetate (25 μg) resulted in a significant induction of the enzyme in these animals (cf. Fig. 1). (In intact mice, variable results were obtained with 25 μg of cortisone, but the data are not presented.) Endotoxin was injected both intravenously and intraperitoneally (Table 5).

Endotoxin at a dose of 0.1 μg (but not of 0.05 μg) given intraperitoneally significantly inhibited tryptophan oxygenase induction. One-tenth of this amount (0.01 μg) depressed induction of the enzyme when it was given intravenously. For Etox, this amount represents 1/40,000th of the LD_{50}.

Effect of Etox and Mex on cortisone induction of phosphoenolpyruvate carboxykinase. Endotoxin prevents the hormonal induction of phosphoenolpyruvate carboxykinase similar to that of typtrophan oxygenase (*unpublished data*). Thus,

TABLE 3. *Effect of fasting for 18 hr on tryptophan oxygenase activity in adrenalectomized mice*

Nutritional status of mice	Tryptophan oxygenase activity [μmoles of kynurenine per gram (dry weight) per hour]
Fed mice............	6.1 ± 0.9[a] (6)
Mice fasted 18 hr.....	7.0 ± 1.0 (6)

[a] Each value is the mean ± standard error for the number of determinations shown in parentheses.

TABLE 4. *Effect of different doses of Etox on induction of tryptophan oxygenase 6 hr after treatment with different amounts of cortisone acetate*

Dose of cortisone acetate administered	Tryptophan oxygenase activity [μmoles of kynurenine per gram (dry weight) per hour] after an injection of Etox at a dose of			
	None	1 μg	4 μg	40 μg
5 mg	49.0 ± 4.0[a] (12)	43.5 ± 2.2 (12)	31.8 ± 1.7 (12)	21.1 ± 2.2 (12)
1 mg	52.0 ± 4.3 (6)		29.6 ± 1.6 (6)	22.2 ± 2.9 (5)
200 μg			31.7 ± 2.7 (12)	20.4 ± 2.1 (11)
100 μg	48.8 ± 3.6 (12)	30.7 ± 2.8 (6)	28.7 ± 1.5 (12)	19.8 ± 1.9 (6)
50 μg[b]	30.2 ± 2.4 (12)	17.7 ± 1.7 (12)		

[a] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[b] When 0.1 μg of Etox was given to mice in this group, a value of 19.4 ± 2.5 (12) was obtained. The *P* value versus the control is <0.01.

TABLE 5. *Effect of route of injection of Etox on induction of tryptophan oxygenase in adrenalectomized mice 6 hr after 25 µg of cortisone acetate*

Route of injection of endotoxin	Tryptophan oxygenase activity[a] [µmoles of kynurenine per gram (dry weight) of liver per hour] after an injection of Etox at a dose of			
	None	0.01 µg	0.05 µg	0.01 µg
Intraperitoneal...	22.8 ± 1.4[b] (12)		24.9 ± 1.6 (9)	12.6 ± 0.7 (9)
Intravenous......	24.7 ± 2.4 (9)	15.3 ± 2.3 (7)	9.4 ± 1.4 (9)	

[a] The activity of tryptophan oxygenase in adrenalectomized mice given no injection is 7.6 ± 0.5 (8).
[b] Each value is the mean ± standard error for the number of determinations given in parentheses.

TABLE 6. *Effect of Etox and Mex on induction of phosphoenolpyruvate carboxykinase 4 hr after cortisone*

Experimental treatment	Phosphoenolpyruvate carboxykinase activity [µmoles of phosphoenolpyruvate per gram (dry weight) of liver per 6 min]
Control, no injections..............	118 ± 10[a] (6)
Cortisone acetate, 5 mg..............	199 ± 9 (13)
Cortisone acetate (5 mg) + 40 µg of Etox..............	143 ± 15 (9)
Cortisone acetate (5 mg) + 40 µg of Mex..............	164 ± 19 (9)

[a] Each value is the mean ± standard error for the number of determinations shown in parentheses.

the effects of Etox and Mex on this enzyme were determined (Table 6). Intact mice fed up until the time of the injections were employed in these studies.

The ability of the more toxic Etox to significantly inhibit the hormonal induction of the carboxykinase ($P < 1\%$) is evident by a comparison of the data of lines 2 and 3 of Table 6. Cortisone injected alone resulted in a level of enzyme that was almost twice that of the control value ($P < 0.1\%$). The less toxic Mex, when given at the same time as cortisone, did not significantly decrease the activity of the enzyme below that obtained with cortisone alone. The large standard errors of the means attest to the fact that considerable variation in activity of phosphoenolpyruvate carboxykinase is encountered in the animals used in these experiments. Nevertheless, the data established that the inhibition of hor-

monal induction of this enzyme by endotoxin is dependent on the toxicity of the preparation employed. Carboxykinase is not, however, as suitable as tryptophan oxygenase for the detection of small amounts of endotoxin.

Effect of Etox and Mex on cortisone-induced glyconeogenesis. Mice given a subcutaneous injection of 5 mg of cortisone acetate and denied food for the succeeding 4 hr have 7.53% liver glycogen (Table 7). Mice similarly treated, but without an injection of cortisone, have 3.89% ± 0.25 glycogen, based on 11 separate determinations. The hormone, therefore, nearly doubles the amount of glycogen in liver. When 40 µg of either Etox or Mex was administered at the same time as the cortisone, the more toxic material, Etox, completely blocked glyconeogenesis because the amount of glycogen (3.87%) is identical to the amount observed in mice that were not injected (3.89%). Mex, on the other hand, did not alter the amount of liver glycogen from that obtained with hormone alone (7.52 versus 7.53%). When only 4 µg of Etox was given at the same time as the hormone, a significant decrease in liver glycogen (5.61%) compared to the control (7.53%) resulted (Table 7).

The data of Table 8 were obtained to determine whether the process of glyconeogenesis can be depressed with less endotoxin after a smaller amount of hormone is used. With 100 µg of cortisone alone, liver glycogen was 6.73%. This is lower by nearly 1% (Table 7) than that found after 5 mg of hormone. With the smaller amount of cortisone, 4 µg of Etox prevented glyconeogenesis; but with 1 µg of Etox, the amount of liver glycogen deposited was not significantly different from that in control mice. The process of glyconeogenesis was not as sensitive to endotoxin, therefore, as the induction of tryptophan oxygenase (Table 4).

Effect of Etox and Mex on the endogenous induction of tyrosine-α-ketoglutarate transaminase in livers of fasted mice 18 hr postinjection. The LD$_{50}$ of endotoxin induces the liver enzyme

Table 7. *Effect of Etox and Mex on glyconeogenesis in livers of mice fasted for 4 hr after the administration of 5 mg of cortisone and endotoxin*

Dose of endotoxin injected with 5 mg of cortisone	Liver glycogen content (percentage of wet weight) after the injection of			Statistics control vs.	
	Control (no endotoxin)	Etox[a]	Mex[b]	Etox	Mex
μg					
40	7.53 ± 0.40[c] (12)	3.87 ± 0.43 (10)	7.52 ± 0.11 (6)	$P < 0.001$	N.S.[d]
4	7.53 ± 0.40 (12)	5.61 ± 0.58 (8)		$P = 0.05$	

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Mex is detoxified endotoxin (LD_{50} about 4 mg).
[c] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[d] N.S. = not significant.

Table 8. *Effect of Etox on glyconeogenesis in livers of mice fasted 4 hr after the administration of 100 μg of cortisone and endotoxin*

Dose of endotoxin injected with 100 μg of cortisone	Liver glycogen content (percentage of wet weight) after the injection of		Statistics vs. control
	Control (no endotoxin)	Etox[a]	
μg			
4	6.73±0.50[b] (12)	3.44±0.44 (12)	$P < 0.001$
1	6.73±0.50 (12)	7.72±0.89 (6)	N.S.[c]

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[c] N.S. = not significant.

tyrosine-α-ketoglutarate transaminase in intact mice as much as an injection of 5 mg of cortisone acetate (5). The extent to which graded doses of Etox and Mex exert a similar effect was tested (Table 9). Etox (40 μg) strongly induced the enzyme, whereas the same amount of Mex did not. A smaller quantity of Etox (4 μg) resulted in a barely significant increase in the transaminase, but 1 μg did not. This particular effect of endotoxin was not elicited by a dose as small as some of the others described above.

Comparative effect of different endotoxin preparations on the hormonal induction of tryptophan oxygenase. The hormonal induction of tryptophan oxygenase was inhibited by a smaller dose of Etox than that required to prevent the induction of phosphoenolpyruvate carboxykinase. Larger amounts of Etox were also required to suppress glyconeogenesis and to induce tyrosine transaminase than to prevent the induction of trypto-

phan oxygenase. It seemed worthwhile to explore, therefore, the effect of graded doses of different preparations of endotoxin on the induction of tryptophan oxygenase with two questions in mind. First, is the inhibition by endotoxin of the hormonal induction of this enzyme related to the toxicity of the endotoxin and, second, can this inhibition serve as an assay for endotoxin? The results (Table 10) apparently answer both questions in the affirmative. With the five endotoxin preparations employed, the LD_{50} varied by more than 20-fold, but 1/400th of the LD_{50} significantly suppressed the induction of tryptophan oxygenase by 100 μg of cortisone acetate. This was true for heat-killed cells of *S. typhimurium*, a type of endotoxin that has been used in a number of studies from this laboratory and one that can be assumed to exist in a physical state distinct from that of the more highly purified lipopolysaccharides.

Discussion

The ability of endotoxin to suppress the hormonal induction of some hepatic enzymes and to induce another is based on a mechanism that is not understood at present. The apparent suppression of enzyme induction has not been proven to be the result of less enzyme synthesis. It may be caused by the presence of an inhibitor. Experiments now in progress may clarify the problem.

Since glycocorticoids are believed to increase enzyme synthesis because of an elevation in hepatic ribonucleic acid (RNA) synthesis (15, 16, 18, 20, 24, 25), endotoxin might be assumed to oppose the hormone through an effect of RNA metabolism. A sublethal dose of endotoxin given to adrenalectomized mice increased RNA synthesis (as judged by incorporation of ${}^{14}C$-orotic acid) more than an injection of cortisone (T. F. Shtasel, *in preparation*). A summation of these

TABLE 9. *Effect of Etox and Mex on tyrosine-α-ketoglutarate transaminase activity in livers of mice fasted 18 hr postinjection*

Dose of endotoxin injected	Tyrosine-α-ketoglutarate transaminase activity (μg of p-hydroxypyruvic acid per 10 min per mg of dry weight) after the injection of			Statistics control vs.	
	Control (no injection)	Etox[a]	Mex[b]	Etox	Mex
μg					
40	13.6 ± 1.1[c] (12)	30.0 ± 1.5 (5)	17.0 ± 1.4 (6)	$P = 0.001$	N.S.[d]
4	13.6 ± 1.1 (12)	17.6 ± 1.5 (6)		$P < 0.05$	
1	13.6 ± 1.1 (12)	15.9 ± 1.9 (6)		N.S.	

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Mex is detoxified endotoxin (LD_{50} about 4 mg).
[c] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[d] N.S. = not significant.

TABLE 10. *Comparative effect of different preparations of endotoxin on cortisone induction of tryptophan oxygenase in livers of fasted mice 6 hr postinjection*

Endotoxin preparation	LD50	Tryptophan oxygenase activity [μmoles of kynurenine per gram (dry weight) per hour] after the injection of 100 μg of cortisone plus indicated dose of endotoxin			
		Cortisone alone	Cortisone + 1/400 LD50	Cortisone + 1/100 LD50	Cortisone + 1/10 LD50
	μg				
Etox[a]	400	48.8 ± 3.6[b] (12)	30.7 ± 2.8 (6) $P < 0.01$[c]	28.7 ± 1.5 (12) $P < 0.001$	19.8 ± 1.9 (6) $P < 0.001$
Heat-killed *S. typhimurium*	750	55.4 ± 3.1 (20)	38.6 ± 3.2 (15) $P < 0.01$	37.4 ± 2.9 (13) $P < 0.01$	28.4 ± 2.7 (14) $P < 0.001$
E. coli (Upjohn) lipopolysac- charide......................	180	43.2 ± 2.1 (14)	33.8 ± 3.0 (15) $P < 0.05$	31.6 ± 2.1 (15) $P < 0.01$	25.7 ± 3.5 (7) $P < 0.01$
S. marcescens lipopolysac- charide......................	460	47.1 ± 1.8 (12)	35.0 ± 2.7 (8) $P < 0.01$	31.4 ± 4.1 (8) $P < 0.01$	23.6 ± 1.9 (8) $P < 0.001$
Mex[d]	4,000	45.7 ± 2.3 (16)	34.9 ± 1.8 (12) $P < 0.01$	32.9 ± 2.6 (19) $P < 0.01$	24.8 ± 1.7 (6) $P < 0.001$

[a] Etox is parent endotoxin (LD_{50} about 400 μg).
[b] Each value is the mean ± standard error for the number of determinations shown in parentheses.
[c] Probability value compared with the enzyme activity shown for cortisone alone.
[d] Mex is detoxified endotoxin (LD_{50} about 4 mg).

two effects, rather than an antagonism, was observed. Unless there is a high degree of specificity (which may exist), endotoxin is not likely to act by suppressing RNA synthesis.

The experiments described in this report establish quite clearly that endotoxin exerts its effect on hormonal induction of tryptophan oxygenase at comparatively low doses, especially when the quantity of cortisone is near the minimum required to elevate the activity of the enzyme. For a given weight of cortisone, the range in dose of endotoxin that gives a graded response of tryptophan oxygenase is narrow. Because of a steep dose-response curve, inhibition of enzyme induction is not an ideal type of assay. On the other hand, the least amount of endotoxin

that produces a significant reduction in the hormonally stimulated rise in tryptophan oxygenase is proportional to the toxicity (LD_{50}) of the endotoxin and is sufficiently sensitive to detect as little as 0.01 μg of a preparation that possesses an LD_{50} of about 400 μg. To achieve this degree of sensitivity, adrenalectomized mice must be administered the endotoxin intravenously. With intact mice, 0.1 μg of the same material can be detected intraperitoneally (Table 4). The method has the advantage of being independent of interference from tissue homogenates; thus, it should be possible to measure the quantity of endotoxin in organs or tissues of animals.

One of the major uncertainties in the method is the assay animal itself. The activity of tryptophan oxygenase may vary in mice injected with the same amount of cortisone or of cortisone plus endotoxin. At times, hardly any induction occurs. It is not known why induction is lost, but it may be the result of a gram-negative infection. Whatever the cause, it is necessary to include control mice each time an experiment is run. On the positive side and when proper control groups are included, it should now be possible to avoid some of the uncertainties shown recently by Cundy and Nowotny (8) that are inherent in the commonly employed bioassays for bacterial endotoxin.

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