EFFECTS OF BACTERIAL ENDOTOXINS ON METABOLISM

VII. Enzyme Induction and Cortisone Protection*

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It was suggested in the most recent paper of this series (1) that protection by cortisone against the lethal effect of bacterial endotoxin is related to the ability of this and other glucocorticoids to induce *de novo* synthesis of certain liver enzymes (2–4). The injection of selected intermediates along the metabolic pathway initiated by the enzyme tryptophan pyrrolase increased survivorship following endotoxin poisoning, indicating that tryptophan pyrrolase plays a protective role in the response of mice to bacterial lipopolysaccharide. It was recognized that other inducible enzymes also might promote reactions of equal or greater importance to the intoxicated animal. One of the significant findings in support of the concept that enzyme induction is involved in an animal's response to endotoxin was the failure of cortisone to induce tryptophan pyrrolase in mice when it was injected 4 hours after administration of the LD_{50} of endotoxin (1). Under the same conditions, cortisone is unable to increase the number of surviving mice. Apparently, therefore, endotoxin is able to block the synthesis of enzyme (protein) stimulated by cortisone.

In agreement with the ability of endotoxin to prevent enzyme induction are observations (5) that two known inhibitors of protein synthesis, actinomycin D and ethionine, when administered in sublethal amounts, significantly potentiate endotoxin toxicity in mice. These inhibitors also eliminate the protective effect of cortisone against endotoxin lethality.

The experiments described in this report extend these studies to include additional inhibitors of protein synthesis and correlate survival data with inducibility of tryptophan pyrrolase.

Methods

Endotoxins.—Heat-killed *Salmonella typhimurium*, strain SR-11, was suspended in nonpyrogenic isotonic sodium chloride solution (Baxter Laboratories, Morton Grove, Illinois) and stored at 5°C in screw-capped test tubes containing about 10 ml per tube. Each milliliter

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of suspension contained 6 mg dry weight of bacterial cells. All injections of this material were given intraperitoneally. With each set of experiments, results obtained with heat-killed cells were confirmed with a more highly purified lipopolysaccharide derived from *Serratia marcescens*.

Inhibitors.—Actinomycin D, kindly supplied by Dr. Vernon Bryson, Institute for Microbiology, Rutgers University, New Brunswick, New Jersey, was dissolved in 95 per cent ethanol (1 mg/ml) and then diluted in non-pyrogenic saline so that the desired dose, usually 10 μg per 22 gm mouse, was contained in 0.5 ml. Chloramphenicol was initially dissolved in alcohol and diluted to the desired concentration in non-pyrogenic saline. The other inhibitors were dissolved in 1.5 N sodium hydroxide, diluted with saline, adjusted to pH 8 with hydrochloric acid as judged by hydrion test paper, and finally diluted so as to have the desired amount in 0.5 ml. Saline at pH 8 injected intraperitoneally had no effect on the LD_{50} of endotoxin. Doses employed were as follows: DL-ethionine (Nutritional Biochemicals Corporation, Cleveland), 16 mg per mouse; 2-thiouracil (Mann Research Laboratories, New York), 8 mg per mouse; 8-azaguanine (Nutritional Biochemicals Corporation), 8 mg per mouse; and chloromycetin (chloramphenicol, Parke, Davis and Company, Detroit), 4 mg per mouse. All injections were administered intraperitoneally and the mice were selected so as to weigh 22 $\pm$ 1 gm.

Cortisone.—Cortisone acetate in stabilized aqueous suspension (United Research Laboratories, Philadelphia) was injected subcutaneously into the interscapular region, 5 mg per mouse contained in 0.5 ml. This is a pharmacological dose for mice.

Tryptophan Pyrrolase Assays.—Liver tryptophan pyrrolase was assayed according to the technique of Knox and Auerbach (2) as adapted to mice by Berry and Smythe (1). In all assays, hematin, the cofactor for tryptophan pyrrolase, was added *in vitro* in order to convert all enzyme into holoenzyme as shown by Feigelson and Greengard (6). For mouse liver it was experimentally determined that 20 μg of freshly prepared solution per reaction vessel was sufficient. The reaction vessels were incubated in a New Brunswick water bath shaker (New Brunswick Instrument Company, New Brunswick, New Jersey) in an atmosphere of pure oxygen for 1 hour at 37°C. All mice to be used for the assay were fasted at least 17 hours before sacrifice or prior to injection with cortisone, inhibitor, etc.

Protection Experiments.—In all experiments where mice were to be protected against or sensitized to the lethal effects of bacterial endotoxin, the two injections were given in rapid sequence. When a timed interval of 1 to 4 hours was to elapse between injections, ten animals in a given cage were handled without attempting to keep them in specific order. Survival for a period of 48 hours was taken as permanent survival.

Infection Experiments.—Mice were infected intraperitoneally with about 10^5 cells of *Salmonella typhimurium*, strain SR-11, contained in 0.5 ml of a saline dilution of an 18 hour brain-heart infusion broth (Baltimore Biological Laboratories, Baltimore) culture. Dilution was made on the established number of cells such a culture contains, 10^9 per ml. This infectious dose is known to produce first deaths on the 3rd day and to kill all mice after 7 to 10 days.

Statistics.—Significance of difference between groups was calculated by the non-parametric rank order test of White (7), the rank correlation method of Wilcoxon (8), or by the chi square test with Yates' corrected formula (9).

Mice.—Female Swiss-Webster mice, weighing 16 to 18 gm, were purchased weekly from Dierolf Farms, Boyertown, Pennsylvania. After 1 to 2 weeks, when they weighed 22 $\pm$ 1 gm, they were used experimentally. They were housed 10 per cage in stainless steel cages (some in plastic cages of similar size) with white pine shavings as bedding and water and pathogen-free mouse food (D & G, The Price-Wilhoite Co., Frederick, Maryland) available at all times, unless otherwise specified. The animal room and the experimental laboratory were maintained at 25 $\pm$ 2°C.

In some of the experiments, mice, on arrival from the dealer, were given drinking water containing tetracycline antibiotics (polyotic, American Cyanamid Company, Princeton, New Jersey) for 2 days. They were then put on tap water for a minimum of a week before they were used experimentally. This treatment was initiated in an effort to improve the reproducibility

of results. The antibiotic-treated animals gained weight more rapidly than controls and experimental findings seem to have been less variable. To the extent of our ability to judge, no undesirable consequences from this procedure were detected.

RESULTS

Influence of Delay on the Protective Effect of Cortisone against Endotoxin.— Table I summarizes the effect of cortisone on endotoxin lethality when given at the same time and 1, 2, and 4 hours after either the LD_{50} or twice the LD_{50} of endotoxin. The well known protective action of cortisone is clearly evident

TABLE I

Effect of Concurrent and Delayed Injection of Cortisone on Endotoxin Lethality in Mice

All injections were given intraperitoneally. Observations were terminated at 48 hours. *P* values were calculated by the rank correlation method (8).

Experimental treatment	LD_{50} endotoxin		2 × LD_{50} endotoxin	
	Alive / Total	*P* vs. Controls	Alive / Total	*P* vs. Controls
1. Control mice	9/20		2/20	
2. 5 mg cortisone at time of LPS*	20/20	<0.008	15/20	<0.008
3. 5 mg cortisone 1 hr. after LPS	16/20	0.057	8/20	N.S.‡
4. 5 mg cortisone 2 hrs. after LPS	12/20	N.S.	6/20	N.S.
5. 5 mg cortisone 4 hrs. after LPS	11/20	N.S.	4/20	N.S.

* LPS, lipopolysaccharide.

‡ N.S., not significant.

against each of the two doses of endotoxin when it was given at the same time as the heat-killed cells (line 2, Table I). Statistically, the degree of protection is approximately the same with each dose of endotoxin. When the hormone injection was given 1 hour after the endotoxin, statistically borderline protection was afforded mice given the smaller dose but not the larger dose of toxin (line 3, Table I). A delay of either 2 hours or 4 hours between administration of endotoxin and cortisone eliminated the ability of the hormone to protect the animals against endotoxin under the conditions of these experiments. It seems clear, therefore, that within an hour or 2, the mouse was changed by endotoxin in such a way that the metabolic events responsible for survival following an injection of cortisone no longer occurred. The possibility that these metabolic events are either dependent upon or related to the loss of ability of the hormone to induce liver enzymes was examined by evaluating the level of tryptophan pyrrolase under conditions comparable to those just described for survival.

Cortisone Induction of Liver Tryptophan Pyrrolase after Endotoxin.—Mice (1) and rats (10) injected with cortisone show a rise in liver tryptophan pyrrolase after a delay of 4 to 6 hours. Values for the enzyme derived from control mice and from animals 4 hours after administration of cortisone are presented in the first two lines of Table II. Under these conditions, the enzyme was about two and one-half times as active in hormone injected mice as in normal animals. However, if endotoxin was given at the same time as cortisone, there was no significant rise in enzyme activity in the hormone-treated animals (19.4 *vs.* 18.1, lines 3 and 1, Table II). A delay of 1 or 2 hours between the injection of cortisone and that of endotoxin permitted the enzyme activity to drop significantly below that seen in normal mice. The data are presented in lines 4 and 5, Table II.

TABLE II

Effect of Concurrent and Delayed Injection of Cortisone on Tryptophan Pyrrolase Induction in Endotoxin-Poisoned Mice

Assays were made 4 hours after cortisone. Animals had been fasted 17 to 21 hours at time of sacrifice. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Experimental treatment	Tryptophan pyrrolase activity (μM kynurenine/gm liver/hr.)
1. Control	18.1 ± 4.8 (12)
2. 4 hrs. after 5 mg cortisone	45.6 ± 6.7 (9)
3. 4 hrs. after 5 mg cortisone and LD_{50} of endotoxin	19.4 ± 5.5 (6)
4. 4 hrs. after 5 mg cortisone and 5 hrs. after LD_{50} of endotoxin	11.5 ± 4.7 (9)
5. 4 hrs. after 5 mg cortisone and 6 hrs. after LD_{50} of endotoxin	8.8 ± 3.2 (9)

On the basis of these observations, it seems clear that cortisone loses its ability to induce tryptophan pyrrolase in the presence of endotoxin and, indeed, it fails to maintain the normal enzyme level when administered 1 hour or 2 hours after the poison. Thus, under conditions where no enzyme induction occurs in response to cortisone, there is also no increase in survivorship, as the data of Table I established.

Of even greater possible significance to survival is the level of liver tryptophan pyrrolase activity 17 hours after endotoxin. This is the time when mice begin to die from the LD_{50} and it is also the time when the enzyme level was less than one-half normal (line 7 *vs.* line 1, Table III). Seventeen hours after an injection of cortisone alone, the enzyme activity was nearly triple that for control mice (lines 1 and 2, Table III), while concurrent injection of hormone and endotoxin resulted in a normal level of enzyme activity (line 3). A delay of 1, 2, or 4 hours in administration of hormone after the injection of endotoxin resulted in a level

of tryptophan pyrrolase activity significantly below that for control animals (lines 4, 5, and 6) and an activity only slightly greater than that observed when endotoxin alone was given (*cf.* line 7). Endotoxin, therefore, under the conditions just described, inhibits enzyme induction by cortisone and the inhibition is evident at both 4 hours (Table II) and at 17 hours (Table III) postinjection. At these times after injection of hormone alone, tryptophan pyrrolase was elevated two- to threefold.

Effect of Inhibitors of Protein Synthesis on Lethality of Endotoxin.—Five different inhibitors of protein synthesis were administered in sublethal amounts

TABLE III

Effect of Concurrent and Delayed Injection of Cortisone on Tryptophan Pyrrolase Induction in Endotoxin-Poisoned Mice

Assays were made 17 hours after the endotoxin injection or after cortisone alone. Animals were fasted 17 hours at time of sacrifice. Each value is the mean ± standard deviation of number of determinations shown in parentheses.

Experimental treatment	Tryptophan pyrrolase activity (μM kynurenine/gm liver/hr.)
1. Control	21.2 ± 6.0 (7)
2. 17 hrs. after 5 mg cortisone	54.4 ± 12.5 (11)
3. 17 hrs. after LD_{50} of endotoxin and 5 mg cortisone	20.1 ± 6.2 (20)
4. 17 hrs. after LD_{50} of endotoxin and 16 hrs. after 5 mg cortisone	10.6 ± 2.7 (8)
5. 17 hrs. after LD_{50} of endotoxin and 15 hrs. after 5 mg cortisone	11.0 ± 6.9 (9)
6. 17 hrs. after LD_{50} of endotoxin and 13 hrs. after 5 mg cortisone	9.9 ± 1.5 (8)
7. 17 hrs. after LD_{50} of endotoxin	6.2 ± 1.5 (8)

to mice at the same time they received an LD_{33} of endotoxin. Actinomycin D, ethionine, 2-thiouracil, and 8-azaguanine each potentiated the lethal action of endotoxin with a statistically significant difference from the control value in each case of less than 0.01 as calculated by the chi square test. Chloramphenicol, on the other hand, failed to alter significantly the number of surviving animals. The last observation is compatible with the finding that chloramphenicol exerts only limited inhibitory action in mammalian systems (11), in contrast to its effect in microbial organisms (12). The other inhibitors, however, are known to act in mammals as well as in bacteria.

Effect of Inhibitors of Protein Synthesis on the Protective Action of Cortisone against Endotoxin.—If it is assumed that cortisone protects mice against endotoxin lethality because of its ability to induce or maintain synthesis of certain enzymes, then inhibitors of protein synthesis given concurrently should block

TABLE IV

Effect of Sublethal Amounts of Inhibitors of Protein Synthesis on Lethality of Endotoxin

All injections were given intraperitoneally at the same time. Observations were terminated at 48 hours. *P* values were calculated by the chi square test (9).

Experimental treatment	Survivors / Total	*P vs.* controls
0.75 mg endotoxin	20/30	
0.75 mg endotoxin + 10 μg actinomycin D	2/30	<0.01
0.75 mg endotoxin + 16 mg ethionine	6/30	<0.01
0.75 mg endotoxin + 8 mg 2-thiouracil	1/30	<0.01
0.75 mg endotoxin + 8 mg 8-azaguanine	6/30	<0.01
0.75 mg endotoxin + 4 mg chloramphenicol	25/30	N.S.*

* N.S., not significant.

TABLE V

Effect of Sublethal Amounts of Inhibitors of Protein Synthesis on the Ability of Cortisone to Protect against Lethality of Endotoxin

All injections were given intraperitoneally at the same time. Observations were terminated at 48 hours. *P* values were calculated by the rank correlation method (8).

Experimental treatment	Survivors / Total	*P vs.* LPS + cortisone
3 mg endotoxin ($3 \times LD_{50}$)	0/20	<0.008
3 mg endotoxin + 5 mg cortisone	12/20	
3 mg endotoxin + 5 mg cortisone + 10 μg actinomycin D	0/20	<0.008
3 mg endotoxin + 5 mg cortisone + 16 mg ethionine	3/20	0.014
3 mg endotoxin + 5 mg cortisone + 8 mg 2-thiouracil	1/20	0.008
3 mg endotoxin + 5 mg cortisone + 8 mg 8-azaguanine	3/20	0.014
3 mg endotoxin + 5 mg cortisone + 4 mg chloramphenicol	14/20	N.S.*

* N.S., not significant.

the protective effect of cortisone. This prediction is confirmed by the data presented in Table V for the same four inhibitors that potentiated the lethality of endotoxin (see Table IV). Chloramphenicol, however, again proved to be the exception, probably because in mice it is not an effective inhibitor (see Tables VI and VII below).

Effect of Inhibitors of Protein Synthesis on Induction of Tryptophan Pyrrolase by Cortisone.—If the inhibitors employed in the experiments summarized by the data of Tables IV and V prevent the induction of enzymes by cortisone, then assays for tryptophan pyrrolase should make this apparent. The results pre-

TABLE VI

Effect of Inhibitors of Protein Synthesis on Cortisone Induction of Liver Tryptophan Pyrrolase in Mice

Assays were made 4 hours after cortisone. Animals had been fasted 17 to 21 hours at time of sacrifice. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Experimental treatment	Tryptophan pyrrolase activity (μM kynurenine/gm liver/hr.)
Control mice	18.1 ± 4.8 (12)
4 hrs. after 5 mg cortisone	45.6 ± 6.7 (9)
4 hrs. after 5 mg cortisone + 10 μg actinomycin D	27.4 ± 5.0 (12)
4 hrs. after 5 mg cortisone + 16 mg ethionine	9.6 ± 3.7 (8)
4 hrs. after 5 mg cortisone + 8 mg 2-thiouracil	17.8 ± 4.5 (7)
4 hrs. after 5 mg cortisone + 8 mg 8-azaguanine	27.9 ± 5.9 (7)
4 hrs. after 5 mg cortisone + 4 mg chloramphenicol	35.2 ± 6.0 (8)

sented in Table VI establish such a relationship. Four hours after the injection of cortisone alone the enzyme level nearly tripled but when a concurrent injection of actinomycin D, ethionine, 2-thiouracil, or 8-azaguanine was given, liver tryptophan pyrrolase activity was significantly below the control level in each instance. While chloramphenicol significantly lowered the activity of the enzyme below that measured in mice given cortisone alone, it was not to the same level as that observed with the other inhibitors. Of some potential relevance is the fact that all but chloramphenicol potentiated endotoxin and prevented the protective action of cortisone (see Tables IV and V).

Effect of Inhibitors of Protein Synthesis on the Ability of Cortisone to Maintain Tryptophan Pyrrolase Activity in Endotoxin-Poisoned Mice.—As stated in an earlier section of this paper, the level of tryptophan pyrrolase in the liver of mice 17 hours after an injection of endotoxin is believed to be a more reliable indication of the severity of the poisoning than values obtained after 4 hours.

Accordingly, the experiments summarized in Table VII were undertaken. Examination of the results makes it clear that endotoxin alone lowered tryptophan pyrrolase to about one-third the control level (lines 1 and 2). Cortisone alone raised the activity two- to threefold (line 3). When the two were combined, the

TABLE VII

Effect of Endotoxin and Inhibitors of Protein Synthesis on Cortisone Induction of Liver Tryptophan Pyrrolase in Mice

Assays were made 17 hours after the injections, all given at the same time. Animals had been fasted 17 hours at time of sacrifice. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Experimental treatment	Tryptophan pyrrolase activity (μM kynurenine/gm liver/hr.)
1. Control mice	21.2 ± 6.0 (7)
2. Endotoxin (LD_{50})	6.2 ± 1.5 (8)
3. Cortisone (5 mg)	54.4 ± 12.5 (11)
4. Endotoxin + cortisone	20.1 ± 6.2 (16)
5. Endotoxin + cortisone + actinomycin D (10 μg)	5.6 ± 1.2 (7)
6. Endotoxin + cortisone + ethionine (16 mg)	5.5 ± 2.6 (7)
7. Endotoxin + cortisone + 2-thiouracil (8 mg)	5.6 ± 2.1 (6)
8. Endotoxin + cortisone + 8-azaguanine (8 mg)	7.5 ± 3.0 (7)
9. Endotoxin + cortisone + chloramphenicol (4 mg)	20.0 ± 9.2 (6)

TABLE VIII

Effect of Infection with S. typhimurium, SR-11, on Cortisone Induction of Liver Tryptophan Pyrrolase

Assays were made 4 hours after cortisone at indicated times postinfection. Each value is the mean ± standard deviation for number of determinations shown in parentheses.

Experimental treatment	Tryptophan pyrrolase activity (μM kynurenine/gm liver/hr.) 4 hrs. after injection of	
	Saline	5 mg cortisone
1. Controls (no infection)	18.1 ± 4.8 (12)	45.6 ± 6.7 (9)
2. 18 hrs. postinfection	13.8 ± 4.3 (20)	29.1 ± 8.0 (10)
3. 42 hrs. postinfection	25.2 ± 8.1 (6)	55.8 ± 3.3 (6)

enzyme remained at the control level (line 4) while the added injection of actinomycin D, ethionine, 2-thiouracil, or 8-azaguanine resulted in the same suppression in tryptophan pyrrolase as that observed with endotoxin alone (lines 5 to 8). Chloramphenicol, however, produced no change in enzyme activity when it was injected concurrently with both endotoxin and cortisone

since the level of enzyme was almost identical with that found when the antibiotic was not given (lines 9 and 4). On the basis of the data of Table VII, it seems permissible to conclude, therefore, that the inhibitory effect of chloramphenicol is less intense and prolonged than that of the other inhibitors and its inability to alter the lethality of endotoxin in the presence or absence of cortisone may be tentatively explained in this way.

Induction of Tryptophan Pyrrolase by Cortisone in Mice Infected with Salmonella typhimurium.—If it is assumed that mice experimentally infected with a Gram-negative pathogen such as *Salmonella typhimurium*, strain SR-11, undergo metabolic alterations in part attributable to release of endotoxin, then one might predict for such animals an impairment in cortisone induction of liver tryptophan pyrrolase. Partial confirmation of this prediction is given in the results presented in Table VIII. The level of enzyme was significantly lower 18 hours postinfection than in uninfected control animals (lines 1 and 2). At the same time postinfection, cortisone caused an increase in level of enzyme activity but the absolute augmentation was not as high as that for control mice (line 1) or for mice 42 hours postinfection (line 3). Thus both the lower control value for tryptophan pyrrolase and the smaller induction with cortisone in mice 18 hours postinfection are consistent with the presence of endotoxin. Earlier results from this laboratory (13) also showed that mice with experimental salmonellosis behave as if endotoxin is present during the 1st day postinfection but not during the 2nd day under conditions comparable to those used in the present study. Quite different assays for endotoxin were employed in the earlier work. Until more is known about the requirements for enzyme induction in mammalian species, a conservative interpretation of these findings with infected animals is imperative.

DISCUSSION

The results described in this report when combined with work already published (1, 5), or in press (14) make it seem undeniably clear that endotoxin lowers the activity of liver tryptophan pyrrolase, cortisone raises it, and when the two are administered at the same time a normal level of activity is maintained. There are several conditions under which cortisone fails to increase the survival of endotoxin-poisoned mice. The best known situation is when the hormone injection is delayed for a few hours after the administration of endotoxin but to be added to this is the inclusion of an inhibitor of protein synthesis and exposure to stressful temperature of 5°C (14). Under all of these conditions, the common denominator seems to be failure to induce or maintain activity of tryptophan pyrrolase. While this is, at present, the only enzyme studied in this connection, it is not intended to suggest that it is the only one of importance. Other inducible enzymes, including those important in gluconeogenesis (15–17), may also be involved in some of the metabolic alterations previously described

and now associated with endointoxication (18, 19). This is especially true of the depletion in glycogen reserves in muscle and liver and the hypoglycemia that characteristically accompanies injection of toxic doses of endotoxin.

The promptness with which inducible liver enzymes increase or decrease following injection, respectively, of cortisone or inhibitor of protein synthesis (such as ethionine) is probably a reflection of their half-lives, at least this is the interpretation advanced by Goldstein *et al.* (20) for liver tryptophan pyrrolase in rats. From this point of view, tryptophan pyrrolase has a shorter half-life than the enzymes responsible for gluconeogenesis, the latter as judged by the data of Greengard *et al.* (16) and of Weber *et al.* (17) in rats. The sequence of metabolic changes that follows an injection of endotoxin may be dependent, at least in part, on relationships of this type, a concept that lends itself to direct experimental study.

One of the major questions yet remaining to be resolved is the primary effect and/or site of action of endotoxin. From the results presented above, it takes an hour or more for an LD_{50} to produce demonstrable inhibition of inducible enzymes. When this is balanced against the survival time of 18 to 36 hours for mice injected with this amount of endotoxin, it is now possible to detect events that occur in 5 per cent or less of the time required for the total sequence of changes leading to death. This, however, is longer in time than the reported release of lysosomal enzymes in livers of rats given a dose of endotoxin of comparable magnitude. The group at New York University (21) detects significant changes within as little as 5 minutes of an injection. This is dramatically rapid and one must consider that the cytological alterations accompanying intracellular exposure to the various hydrolases from lysosomes could soon produce, certainly within an hour, a block in inducibility of hepatic enzymes. It is, unfortunately, impossible at the present time to test unambiguously such an interpretation of sequential changes that follow endotoxin injection. The need for future investigations along these lines hardly requires comment.

SUMMARY

Cortisone acetate administered to mice at the same time as either the LD_{50} or $2 \times LD_{50}$ of endotoxin significantly protected against lethality. Delaying the injection of cortisone to 1, 2, or 4 hours after that of endotoxin resulted in loss of protection with the possible exception of a 1 hour delay with the LD_{50} of endotoxin. Associated with this loss of protection was the failure of the hormone to induce liver tryptophan pyrrolase. Normal mice given only cortisone showed an increase in enzyme activity nearly three times that of control values when assays were carried out either 4 or 17 hours after the hormone was given. Endotoxin-poisoned mice showed normal levels of enzyme activity with concurrent injection of cortisone but depressed levels of enzyme when the cortisone injection was delayed for only 1 hour or more. Apparently, therefore, enzyme induction (or maintenance) is related to survival in endotoxin poisoning.

In line with this hypothesis was the observation that inhibitors of enzyme (protein) synthesis were found to potentiate the lethal action of endotoxin and to prevent the protective effect of cortisone. The inhibitors employed were actinomycin D, ethionine, 2-thiouracil, and 8-azaguanine. Activity of liver tryptophan pyrrolase was lowered by endotoxin and elevated by cortisone. When the two were given concurrently, normal enzyme activity was maintained. Chloramphenicol, an active inhibitor of protein synthesis in microorganisms but with limited effect in mammals, was without observable influence in these respects.

Mice 18 hours postinfection with *Salmonella typhimurium*, strain SR-11, given at a level that caused first deaths on the 3rd day, had a lower than normal activity of liver tryptophan pyrrolase and responded to cortisone induction with a smaller increase in enzyme level than that found in control mice. Each is characteristic of endotoxin poisoning. Animals 42 hours postinfection were free of these signs of endointoxication, an observation in agreement with earlier experiments where other measures of endotoxin were employed.

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