JOURNAL OF BACTERIOLOGY, July, 1966
Vol. 92, No. 1

Printed in U.S.A.

Inhibition of Inducible Liver Enzymes by Endotoxin and Actinomycin D

L. JOE BERRY, DOROTHY S. SMYTHE, AND LOUISE S. COLWELL
Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania

Received for publication 24 March 1966

ABSTRACT

BERRY, L. JOE (Bryn Mawr College, Bryn Mawr, Pa.), DOROTHY S. SMYTHE, AND LOUISE S. COLWELL. Inhibition of inducible liver enzymes by endotoxin and actinomycin D. J. Bacteriol. **92**:107–115. 1966.—Bacterial endotoxin at the LD_{50} level lowers liver tryptophan pyrrolase in mice, it prevents for 16 to 20 hr the induction of the enzyme by a concurrent injection of cortisone, it lowers significantly but does not prevent substrate induction, and it reduces the enzymatic activity promptly and significantly when administered during the course of hormonal induction. The LD_{50} amount of actinomycin D has a similar effect on tryptophan pyrrolase, except that its inhibition of induction by cortisone persists for a longer period of time. Endotoxin in the intact mouse induces tyrosine-α-ketoglutarate transaminase almost as well as cortisone, but not in the adrenalectomized animal, a fact that suggests induction of this enzyme is due to release of endogenous adrenal hormones. Actinomycin D, on the other hand, has an effect on this transaminase similar to that on tryptophan pyrrolase. The site of action of endotoxin and actinomycin D would appear to be similar for one of the two enzymes studied and different for the other, a relationship that requires a specificity difficult to imagine for a material as complex as endotoxin.

An increasing body of evidence suggests that one of the metabolic effects of endotoxin poisoning is either a suppression of synthesis of certain inducible liver enzymes or else a release of an inhibitor(s) that reduces the activity of such enzymes (1–5). If an inhibitor is involved, it seems necessary to postulate its release from target cells, since endotoxin is not directly inhibitory under the in vitro conditions employed for the assay (see below). All previous experiments have been limited to studies with a single enzyme, liver tryptophan pyrrolase, but conceptually, at least, others may be involved. Tryptophan pyrrolase is inducible by substrate and by certain adrenocortical hormones, and its half-life in rats has been estimated at about 2.5 hr (11, 24, 29). This means that a comparatively high rate of synthesis and breakdown goes on concurrently, and a change in level within a comparatively brief interval of time would result from an alteration in either process. There is reason to believe that substrate induction of tryptophan pyrrolase is due to stabilization of enzyme by converting it into enzyme-substrate complex (6, 12, 17, 23). The basis for this belief is the lack of effect of actinomycin D on substrate induction. By contrast, induction of enzyme by cortisone is blocked by actinomycin D (10, 13), suggesting that augmented synthesis is responsible for the increase in tryptophan pyrrolase activity. More direct evidence based on immunological and isotopic techniques substantiate these interpretations (10, 13, 30). In addition, the effect of actinomycin D in blocking deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis (25, 26) implies that hormonal induction acts through some type of stimulation of RNA synthesis. Data from the literature are consistent with this concept (9, 14, 18).

Since most of the biochemical studies on induction of tryptophan pyrrolase have been done on rat liver enzyme, whereas those related to endotoxin poisoning have been carried out in mice, it is apparent that more detailed studies on the mouse enzyme are required. It is especially important to attempt to gain insight into the nature of the endotoxin suppression of tryptophan pyrrolase activity. Three sites of attack are possible. These are (i) an inhibition of synthesis at some point along the complex pathway of protein synthesis; (ii) an acceleration of enzyme breakdown (denaturation) through a direct or, more likely, mediated change; and (iii) a direct inhibition of

the enzyme either competitively or noncompetitively. Although the second and third processes might yield a similar net effect, the underlying basis of the change is quite distinct. The experiments described in this report are aimed at elucidating these relationships.

MATERIALS AND METHODS

Endotoxin. Heat-killed *Salmonella typhimurium*, strain SR-11, suspended in nonpyrogenic isotonic sodium chloride solution (Baxter Laboratories, Morton Grove, Ill.) was prepared as previously described (3). Injections were given intraperitoneally with the desired number of cells contained in 0.5 ml.

Actinomycin D. Injections of actinomycin D (generously provided by Vernon Bryson, Microbiological Institute, Rutgers University) were administered intraperitoneally at the LD_{50} level (25 μg) contained in 0.5 ml of solution. The solution was prepared as previously described (4), and the dose level employed (given specifically for each experiment) was 1 mg per kg of body weight.

Cortisone. Cortisone acetate in stabilized aqueous suspension (United Research Laboratories, Philadelphia, Pa.) was injected subcutaneously into the interscapular region, 5 or 0.1 mg per mouse contained in a volume of 0.5 ml. These are pharmacological doses for mice.

Tryptophan pyrrolase assays. Liver tryptophan pyrrolase was assayed according to the procedure of Knox and Auerbach (21) as described in earlier publications (3, 4). Mice were fasted for 17 hr prior to sacrifice in all instances. This was done because endotoxin-poisoned animals fail to eat, and hence all animals were made more comparable by this procedure. Hematin was added, unless otherwise specified, to convert all enzyme into holoenzyme (7, 11). Activity is expressed as micromoles of kynurenine formed per hour per gram (dry weight) of liver.

Tyrosine-α-ketoglutarate transaminase assay. The method of Rosen et al. (28) was used for the determination of tyrosine-α-ketoglutarate transaminase activity in livers of mice. All animals were fasted for 17 hr prior to sacrifice except when adrenalectomized animals were used. Since these animals cannot survive the stress of food withdrawal, food was available to them at all times. This assay is based on the conversion of tyrosine to *p*-hydroxyphenylpyruvic acid, a product of the transamination reaction. This substance is converted into a pigmented compound through the addition of the ammonium molybdate color reagent and read at 850 mμ in a Hitachi Perkin-Elmer spectrophotometer. The reaction is stopped after 10 min by the addition of trichloroacetic acid, and the activity of the enzyme is expressed as micrograms of *p*-hydroxyphenylpyruvic acid produced per gram (dry weight) of liver per 10 min.

Mice. Female Swiss-Webster mice purchased weekly from a local dealer (Dierolf Farms, Boyertown, Pa.) were used in all experiments. Animals weighing 22 $\pm$ 1 g were employed, and other handling procedures were the same as those previously described (4, 5). Adrenalectomized mice were tested for completeness of the operation, and were maintained postoperatively in a manner similar to that reported previously (3).

RESULTS

In vitro effect of endotoxin and actinomycin D on tryptophan pyrrolase. The addition of 1 LD_{50} of endotoxin directly to the assay flask containing liver homogenate and the other additives necessary for the assay resulted in no change in tryptophan pyrrolase activity. The mean value for six controls was 18.5 $\pm$ 2.6 (standard error) and for six flasks with endotoxin was 18.1 $\pm$ 3.1. The total volume of fluid in the assay flask was 8 ml. When the LD_{50} of actinomycin D (25 μg) was tested in the same way, nine control values yielded a mean of 18.4 $\pm$ 2.2, and those with the antibiotic gave a mean of 18.5 $\pm$ 2.1. Quite obviously, neither substance lowered tryptophan pyrrolase activity under in vitro conditions.

Rate of kynurenine formation by tryptophan pyrrolase in livers of normal and endotoxin-poisoned mice. In the assay for tryptophan pyrrolase, substrate is added at time zero. After 1 hr, the reaction is stopped and the amount of kynurenine accumulated is determined. The fact that livers from endotoxin-poisoned mice show less activity than those from normal mice might be the result of a progressive disappearance of enzyme under in vitro conditions rather than of the presence of less enzyme initially. To obtain information on this point, assays were carried out at more frequent intervals over a longer period of time than that normally employed. This was accomplished by pooling the homogenates of livers from four normal mice and from four mice 17 hr after an injection of the LD_{50} of endotoxin. Each homogenate was distributed to seven different flasks. All reactions were initiated at the same time. At intervals of 15 min for a total of 105 min, a flask containing liver from normal mice and from poisoned mice was removed from the water-bath shaker for assay.

A typical result is presented in Fig. 1. After an initial lag of approximately 30 min in control and in poisoned livers, a linear increase in production of kynurenine occurred for the next hour. During the last 15 min, a decline in rate appeared. This is similar to data reported for the rat liver enzyme (17, 31). The important observation, however, was the absence of decline in enzyme activity in livers of endotoxin-poisoned mice that might be attributable to an accelerated breakdown or inactivation of enzyme during the period of assay.

Effect of preincubation of liver homogenate in the absence of substrate on observed tryptophan pyrrolase activity. Not answered by the results described in the preceding section is the question of

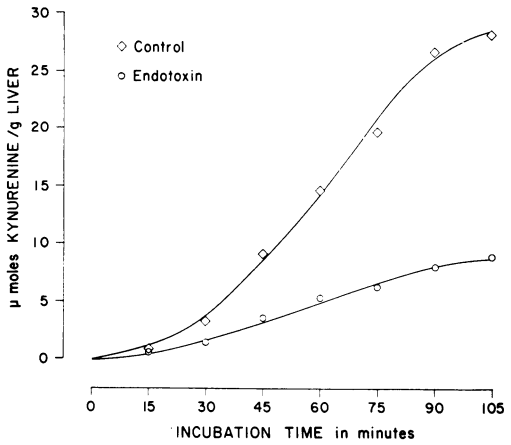

FIG. 1. *Rate of formation of kynurenine by liver homogenates from normal mice and from mice 17 hr after the injection of the* LD_{50} *of endotoxin. Conditions were the same as those used in the assay for tryptophan pyrrolase.*

whether endotoxin results in an increase in breakdown of apo-tryptophan pyrrolase rather than in holoenzyme. To gain information on this point, liver homogenates were incubated at 37 C without the addition of tryptophan for stated periods of time. The reaction was initiated by the introduction of substrate into the flasks, and assays were carried out as usual. Normal mice, mice 1 hr after an injection of the LD_{50} of endotoxin, and animals 4 hr after cortisone administration were studied. The results are shown in Fig. 2. Control values and those for endotoxin-poisoned animals declined at nearly identical rates. Incubation without substrate for only 15 min decreased the activity of tryptophan pyrrolase by about 50%. A similar percentage decrease occurred in homogenates prepared from livers of mice injected 4 hr previously with 5 mg of cortisone acetate. The initial enzyme level was nearly double that of normal animals, but the rate of loss of activity was approximately the same. Incubation of the liver homogenates for 45 min without substrate resulted in a near total loss of tryptophan pyrrolase in all groups, as the data show.

One must conclude from these results that cortisone does not stabilize tryptophan pyrrolase, nor does endotoxin increase its inactivation. The delay of 1 hr after endotoxin injection affords sufficient time for a measurable change in level of tryptophan pyrrolase, as shown in previously reported work (4). In comparing these results with those of Fig. 1, the stability of holoenzyme is seen to be greater than that of apoenzyme. This has also been reported for the rat liver enzyme (6, 12, 17, 23). The nature of enzymatic loss is not understood.

Effect of endotoxin and actinomycin D on tryptophan induction of tryptophan pyrrolase. The changing activity of mouse liver tryptophan pyrrolase with time after the intraperitoneal injection of 20 mg of L-tryptophan alone or in combination with either 25 μg of actinomycin D (the LD_{50}) or the LD_{50} of endotoxin is shown in Fig. 3. In each group of animals, the peak value was reached at 2 hr postinjection, followed by a decline in enzyme level that was almost as rapid as the rise. Neither inhibitor prevented induction completely, but each significantly lowered it. The *P* value at 2 hr for livers from mice given actinomycin D was 0.05 compared with controls, whereas for endotoxin-poisoned mice it was 0.01, as calculated by the rank order test of White (32), with six separate values in each group. If it is true that substrate stabilizes the enzyme, as the preceding data indicate, then the rise in activity could be explained accordingly. The rapid decline in activity after the peak of induction suggests an accelerated destruction, along with the possibility of a "feedback" inhibition of synthesis.

Effect of endotoxin and cortisone alone and in combination on induction of tryptophan pyrrolase. Experiments similar to those just described were carried out, but a subcutaneous injection of 5 mg of cortisone into the interscapular region was substituted for the intraperitoneal administration of

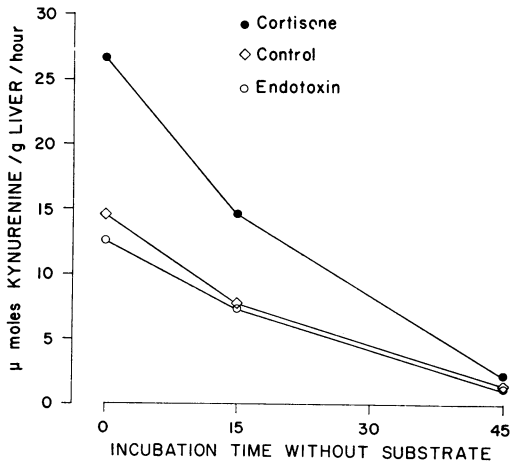

FIG. 2. *Loss of tryptophan pyrrolase activity prior to the addition of substrate from liver homogenates derived from normal mice, mice 4 hr after the injection of 5 mg of cortisone acetate, and mice 1 hr after the* LD_{50} *of endotoxin. Each point is the level of enzyme activity determined by the standard assay procedure initiated by the addition of substrate following the period of preincubation (at 37 C) indicated on the abscissa.*

tryptophan. The results are summarized in Fig. 4. The most obvious effect was the pronounced rise in enzyme after an injection of cortisone alone and the ability of endotoxin to prevent the increase for about 17 hr. Subsequent to this time, enzyme induction occurred, but it did not reach the level that followed hormone alone. Endotoxin suppressed the enzyme, but a significant "overshoot" was seen during the second 24-hr period.

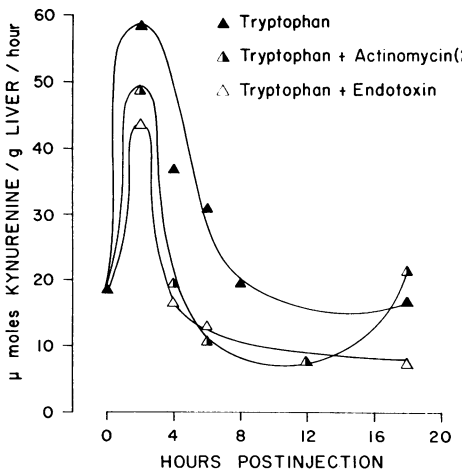

FIG. 3. *Change in liver tryptophan pyrrolase activity with time following an intraperitoneal injection of 20 mg of L-tryptophan alone or concurrent with either actinomycin D or the LD_{50} of endotoxin.*

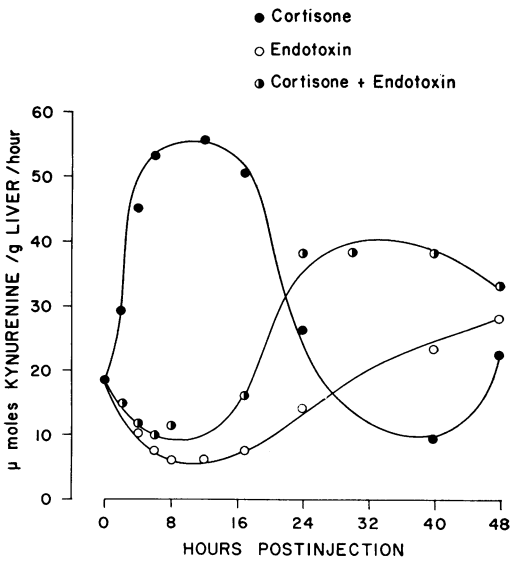

FIG. 4. *Change in liver tryptophan pyrrolase activity with time following an injection of 5 mg of cortisone acetate alone, the LD_{50} of endotoxin alone, or the two combined.*

The inductive response to cortisone as seen in Fig. 4 was more protracted than that reported for rats (4). The fact that it may be attributed to the large dose of hormone employed is shown in Fig. 5, where the change in tryptophan pyrrolase level after an injection of 0.1 mg of cortisone is presented graphically for comparison with the curve obtained after 5 mg of hormone. Under these conditions, the peak value with the smaller dose is about one-half that with the larger dose, and the enzyme returns to normal after 12 hr rather than 24 hr. The persistence of the effect of a large dose of hormone may account for the delayed induction of tryptophan pyrrolase in mice given both hormone and endotoxin, as shown in Fig. 4. The time at which the enzyme increased in these mice corresponds remarkably well with the time the increase was seen in mice given endotoxin alone. Possibly at this time endotoxin becomes significantly detoxified.

Effect of delayed injection of cortisone on induction of tryptophan pyrrolase in endotoxin-poisoned mice. Since it has been established that cortisone has no protective effect and fails to induce tryptophan pyrrolase when administered 4 hr after 1 LD_{50} of endotoxin (4), it was considered of interest to follow the time course of enzyme activity under these conditions. The results are presented graphically in Fig. 6. There was no evidence of hormonal induction of enzyme through 17 hr, and, at 24 hr, activity approached the original control value but it was not significantly above that for mice given endotoxin alone. This suggests

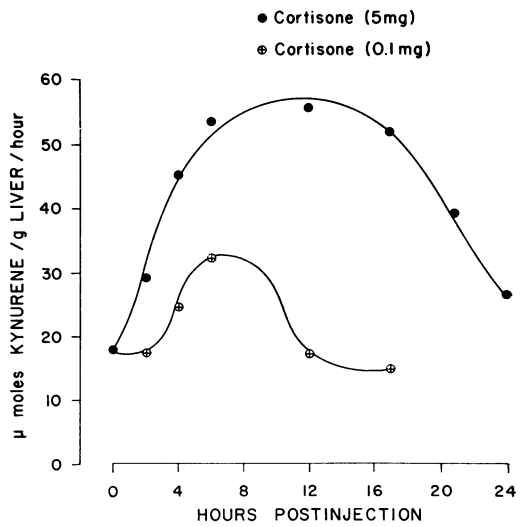

FIG. 5. *Change in liver tryptophan pyrrolase activity with time following an injection of either 5 or 0.1 mg of cortisone acetate.*

that the suppressive effect of endotoxin has begun to disappear by 16 to 20 hr postinjection, as was shown in Fig. 4. It should be emphasized, however, that most deaths from endotoxin occur prior to 24 hr.

Effect of delayed injection of endotoxin on cortisone induction of tryptophan pyrrolase. Cortisone was given at time zero, and the LD_{50} of endotoxin was then injected after 2, 4, or 17 hr. The level of tryptophan pyrrolase subsequent to endotoxin was determined, with the results shown in Fig. 7. Also included in the figure is the course of enzymatic change with time when cortisone alone was administered. Endotoxin lowered the activity of tryptophan pyrrolase after each injection, but the pattern of change varied with the stage of induction. Mice given endotoxin 2 hr after cortisone maintained about the same level of enzyme activity 4 hr later, but this was 25 units lower than that of mice injected with cortisone alone (54 units minus 29 units). When endotoxin was given 4 hr after cortisone, a decrease in activity of 17 units occurred during the next 4 hr (45 minus 28), as determined from the time of injection, but when this level is compared with that reached at the same time (8 hr from the beginning) in mice given cortisone alone, the decrease was 27 units (55 minus 28). Endotoxin administered at 17 hr produced a drop in activity of 25 units (50 minus 25), as measured from the time of injection, but, when compared with the corresponding value observed with cortisone alone, the decrease was only 14 units (39 minus 25). On the basis of these observations, endotoxin seems to cause a surprisingly constant decrease in activity so long as the value in control animals is not also diminishing.

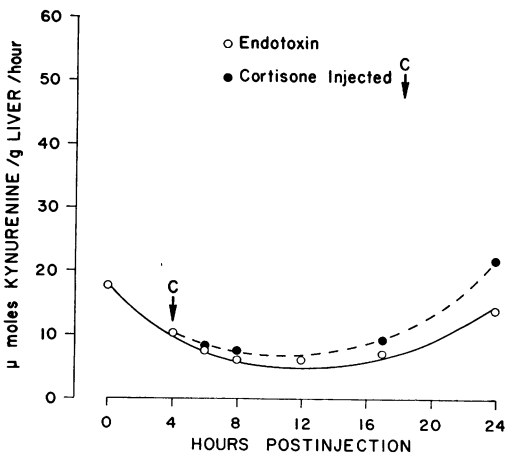

FIG. 6. *Change in liver tryptophan pyrrolase activity with time following an injection of the LD_{50} of endotoxin alone or in combination with an injection of 5 mg of cortisone acetate administered 4 hr after the endotoxin as indicated by the arrow.*

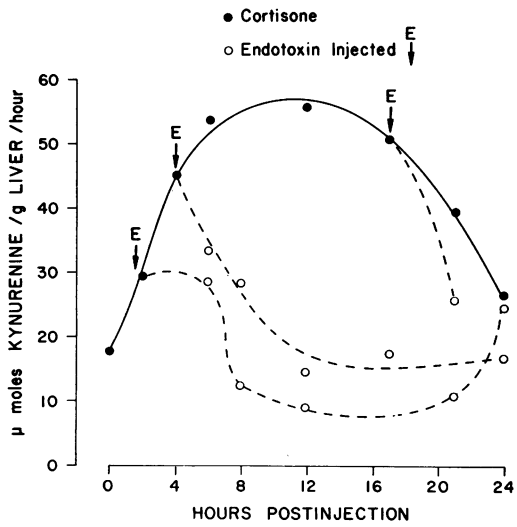

FIG. 7. *Change in liver tryptophan pyrrolase activity with time following an injection of 5 mg of cortisone acetate alone at time zero or in combination with the LD_{50} of endotoxin injected, 2, 4, or 17 hr after the hormone.*

The disappearance of the inhibitory effect of endotoxin on tryptophan pyrrolase 16 to 20 hr after its administration can be seen in the curve where endotoxin was given 2 hr after cortisone. The value measured at 24 hr indicates a beginning of induction similar to that observed previously in Fig. 4 and 7, when both cortisone and endotoxin were given.

Effect of actinomycin D on cortisone induction of tryptophan pyrrolase. To compare the effect of endotoxin on tryptophan pyrrolase induction with that of an inhibitor of protein synthesis, the mode of action of which is believed to be known (25, 26), actinomycin D was chosen. Results obtained with 5 mg of cortisone alone, with actinomycin D alone at the LD_{50} (25 μg per mouse), and with the two administered at the same time are presented in Fig. 8. Actinomycin D alone significantly lowered the enzyme within 2 hr. The minimal value was reached at about 8 hr postinjection, and it was approximately back to normal after 24 hr. When actinomycin D and cortisone were given concurrently, no induction occurred for 24 hr. This is the most complete and sustained suppression of induction observed under any of the conditions that have been employed.

Effect of delayed injection of actinomycin D on cortisone induction of tryptophan pyrrolase. When 25 μg of actinomycin D (the LD_{50}) was injected 4 or 17 hr after cortisone, the results summarized in Fig. 9 were obtained. Administration of the

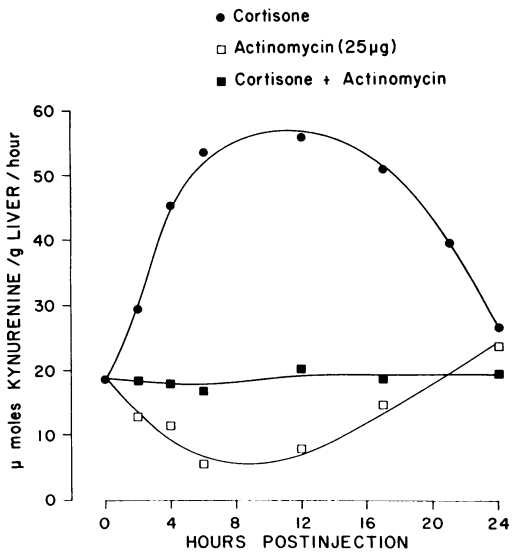

FIG. 8. *Change in liver tryptophan pyrrolase activity with time following an injection of 5 mg of cortisone acetate alone, actinomycin D (25 μg) alone, or the two combined.*

antibiotic 4 hr after cortisone resulted in immediate suppression of enzyme induction. After 4 hr more, the difference between the induced and inhibited values was 26 units (55 minus 29). This is nearly identical to the result obtained with 1 LD_{50} of endotoxin (Fig. 7). Injection of actinomycin D 17 hr after cortisone resulted in a prompt decrease in tryptophan pyrrolase activity. At 4 hr after the drug was given, the activity was 26.5 units lower (51 minus 24.5, the latter value estimated from the graph at hour 21 and not directly determined) but only 14.5 units (39 minus 24.5) below the value obtained at the same time (21 hr from the beginning) with cortisone alone. These observations are also in remarkable agreement with those found when endotoxin was injected (Fig. 7). On a comparative basis, it would be impossible to distinguish the inhibitory effect of endotoxin on tryptophan pyrrolase from that of actinomycin D. One might easily be led to conclude, and perhaps erroneously, as judged by results that follow, that the two inhibitors have a common site of action.

Effect of endotoxin and cortisone alone and in combination on tyrosine-α-ketoglutarate transaminase induction. Since the induction of tyrosine-α-ketoglutarate transaminase following an injection of hydrocortisone is known to parallel that of tryptophan pyrrolase in rat liver (16), its behavior in livers of mice in the presence of endotoxin was investigated. The results are presented in Fig. 10. The change in activity that accompanied an injection of cortisone is almost indistinguishable from that seen for tryptophan pyrrolase (Fig. 4). By contrast, however, endotoxin alone was almost as effective an inducer of the transaminase, even though the peak value was reached more slowly and was sustained for a longer period of time than with cortisone. This,

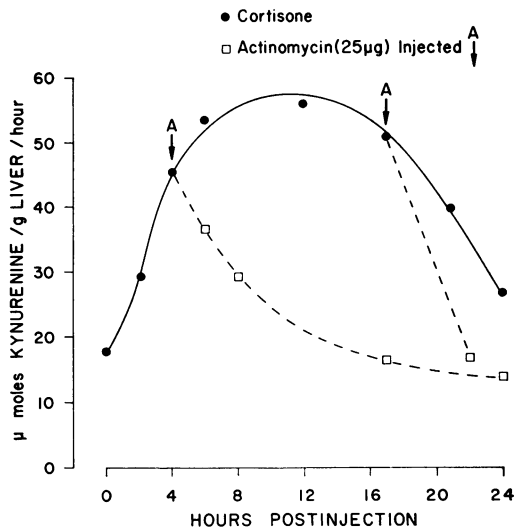

FIG. 9. *Change in liver tryptophan pyrrolase activity with time following an injection of 5 mg of cortisone acetate alone at time-zero or in combination with 25 μg of actinomycin D injected 4 or 17 hr after the hormone.*

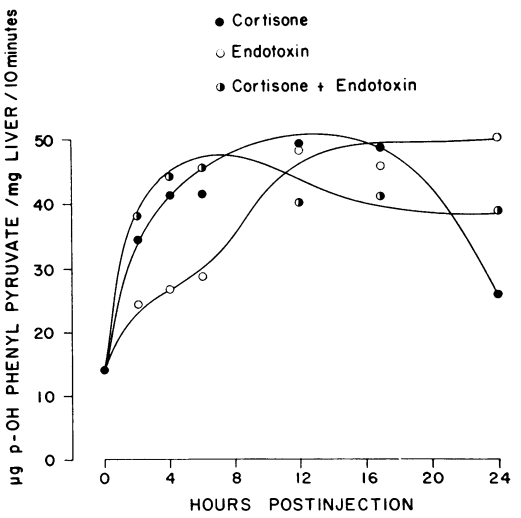

FIG. 10. *Change in liver tyrosine-α-ketoglutarate transaminase activity with time following an injection of 5 mg of cortisone acetate alone, the LD_{50} of endotoxin alone, or the two combined.*

presumably, is due to the release of endogenous adrenal cortical hormone in response to the endotoxin (see below). An injection of both cortisone and endotoxin resulted in a rise in activity of this enzyme more rapid than that seen with either alone, and the activity was maintained for 24 hr at an intermediate level. It was still above the control value at 48 hr postinjection.

Effect of endotoxin and cortisone on tyrosine-α-ketoglutarate transaminase activity in adrenalectomized mice. The failure of endotoxin to result in an increase in tyrosine-α-ketoglutarate transaminase activity in adrenalectomized mice comparable to that seen in control animals is made evident by the data presented in Table 1. A small dose of endotoxin ($LD_{50/250}$) resulted in a tripling of the transaminase in normal mice, as line 2 versus line 1 of the table shows. The same amount of endotoxin when injected into adrenalectomized mice caused a minimal, yet statistically significant, rise in the enzyme. This can be seen in lines 3 and 4 of the table. The basis for this small rise could be due either to a residuum of adrenal cortical tissue or to an unknown cause. An injection of cortisone in adrenalectomized mice gave full induction of the enzyme, as the results in line 5 of the table make evident. The transaminase level, it should be noted, was the same in normal mice and in adrenalectomized mice (lines 1 and 3).

Effect of actinomycin D and cortisone alone and in combination on tyrosine-α-ketoglutarate transaminase induction. Actinomycin D at a dose of 25 μg per mouse had little influence on the activity of the transaminase. This can be seen in the appropriate curve presented in Fig. 11. When the same amount of actinomycin D was administered concurrently with 5 mg of cortisone, induction of tyrosine-α-ketoglutarate transaminase was retarded but not completely suppressed for approximately 8 hr. A significant rise occurred at 17 hr postinjection, followed by a decline in activity paralleling that seen with cortisone alone.

TABLE 1. *Effect of adrenalectomy on induction of liver tyrosine-α-ketoglutarate transaminase in mice*

Experimental treatment	Tyrosine-α-ketoglutarate transaminase activity[a]	*P* value
1. Normal (fed) controls	10.8 ± 0.7 (10)	
2. Normal mice given $LD_{50/250}$ of endotoxin	33.3 ± 1.7 (5)	1 vs. 2 $P < 0.01$
3. Adrenex control	10.6 ± 0.8 (13)	1 vs. 3 NS[b]
4. Adrenex given $LD_{50/250}$ of endotoxin	13.7 ± 0.7 (10)	3 vs. 4 $P < 0.05$
5. Adrenex given 5 mg of cortisone	42.9 ± 1.3 (12)	3 vs. 5 $P < 0.01$

[a] Expressed as micrograms of *p*-hydroxyphenylpyruvic acid formed per 10 min per mg (dry weight) of liver at 4 hr after the indicated treatment. Each value is the mean ± the standard error of the number of determinations shown in parentheses.

[b] Not significant.

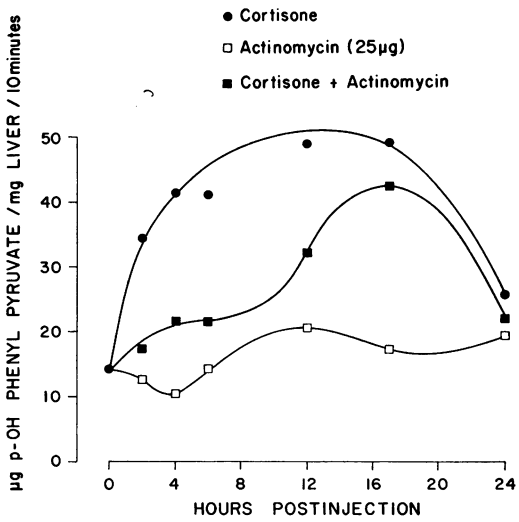

FIG. 11. *Change in liver tyrosine-α-ketoglutarate transaminase activity with time following an injection of 5 mg of cortisone acetate alone, 25 μg of actinomycin D alone, or the two combined.*

DISCUSSION

Several relationships are placed in clearer perspective by the foregoing observations. These may be summarized as follows. (i) Endotoxin does not appear to lower tryptophan pyrrolase activity by increasing its rate of destruction, nor does cortisone seem to increase its activity by preventing its destruction (Fig. 1 and 2). (ii) As judged by in vitro experiments, endotoxin and actinomycin D exert minimal influence on substrate induction of tryptophan pyrrolase, and the small but significant suppression accompanying their injection may be explained most obviously as a reduced synthesis during the 2-hr period required for the enzyme to reach its maximal level of activity (Fig. 3). (iii) Endotoxin at the LD_{50} level suppresses tryptophan pyrrolase for a period of 16 to 20 hr, and prevents its induction by cortisone for about the same period of time, but after the period of suppression a subsequent rise well above the control level reached by cortisone alone

occurs (Fig. 4 and 6). (iv) The magnitude and duration of change in tryptophan pyrrolase activity after an injection of cortisone is dose-dependent (Fig. 5). (v) The LD_{50} of actinomycin D has an effect similar to that of endotoxin, but the duration of suppression of induction of tryptophan pyrrolase is greater than that with endotoxin (Fig. 8). (vi) Injection of endotoxin at different stages of cortisone induction of tryptophan pyrrolase is followed by a remarkably constant decrease in activity, a finding commensurate with the postulate that enzyme synthesis is being impaired (Fig. 7). Actinomycin D, under similar conditions, yields nearly identical results (Fig. 9). (vii) Endotoxin has no inhibitory effect on cortisone induction of tyrosine-α-ketoglutarate transaminase, and when given alone induces the enzyme in the intact mouse but not in the adrenalectomized animal (Fig. 10 and Table 1). (viii) Actinomycin D inhibits the induction of the transaminase by cortisone in a manner similar to its effect on tryptophan pyrrolase induction (compare Fig. 11 and 8).

The above-listed similarities and differences between endotoxin and actinomycin D, as judged by their influence on inducible liver enzymes, permit several conclusions. Endotoxin suppresses tryptophan pyrrolase activity and its hormonal induction, but this effect cannot be extended to all protein synthesis since it is without influence on the transaminase system. Its action must be, therefore, either at a specific site of synthesis of the one enzyme (and perhaps others) or at target cells that, in turn, release specific inhibitors of certain enzymes, tryptophan pyrrolase among them. The possibility that endotoxin binds (chelates) or results in the removal of essential activators or cofactors cannot be excluded. That the reticuloendothelial system serves as a target for endotoxin is suggested by the fact that commonly used colloidal "blocking" agents lower tryptophan pyrrolase activity in a manner similar to that of endotoxin (3; Agarwal and Berry, *unpublished data*). Additional work is necessary to develop these relationships further.

The almost identical changes in tryptophan pyrrolase that occur during hormonal induction following an injection of endotoxin or actinomycin D strongly imply a common mode of action for these substances. The difference in their effect on tyrosine-α-ketoglutarate transaminase argues just as strongly for a different mode of action. If one accepts the experimental evidence that actinomycin D blocks DNA-dependent RNA synthesis (25, 26), then endotoxin would have to be capable of acting selectively against the synthesis of only that part of the DNA that codes for tryptophan pyrrolase and not the part that codes for tyrosine-α-ketoglutarate transaminase, an unlikely postulate. The apparent departures from the generally accepted site of action of actinomycin D occur at dose levels far in excess of those used here (27). Indeed, the results reported by Garren et al. (15), with rats treated in a manner similar to that shown in Fig. 9, were obtained with a dose of actinomycin D several times greater than that used in our experiments. Comparison is, therefore, impossible.

The behavior of mouse liver tryptophan pyrrolase after both substrate and hormonal induction and of tyrosine-α-ketoglutarate transaminase after hormone injection parallels closely the changes reported for the same enzymes in rat liver (19, 20). The activity of both is higher in the mouse than in the rat, and the duration of inductive changes are somewhat different, possibly because of the quantity of hormone employed and its route of administration, but these are matters of detail and not of trend. It should be mentioned, however, that livers of fasted control mice have a higher tryptophan pyrrolase activity than do those of fed animals. This is believed to be due to the release of endogenous adrenocortical hormones as part of the response to the stress of fasting. Should endotoxin be responsible for preventing the rise in enzyme activity associated with this endogenous hormonal induction, at least some of the findings in earlier reports (1–5) would be explained. This in no way alters their validity. The extent to which inducible enzymes and their control play a role in endotoxin poisoning, and more generally in an animal's response to stress, remains for future work to elucidate.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Science Foundation, by a Public Health Service training grant from the National Institute of Allergy and Infectious Diseases, and by a grant from the Infectious Disease Department, Smith Kline & French Laboratories, Philadelphia, Pa.

LITERATURE CITED

1. BERRY, L. J. 1964. Effect of endotoxins on the level of selected enzymes and metabolites, p. 151–159. *In* M. Landy and W. Braun [ed.], Bacterial endotoxins. Rutgers Univ. Press, New Brunswick, N.J.
2. BERRY, L. J. 1964. The reticuloendothelial system in tolerance to bacterial endotoxins. Proc. Intern. Symp. Reticuloendothelial System, Otsu, Japan, p. 273–279.
3. BERRY, L. J., AND D. S. SMYTHE. 1963. Effects of bacterial endotoxins on metabolism. VI. The role of tryptophan pyrrolase in response of mice to endotoxin. J. Exptl. Med. **118**:587–603.
4. BERRY, L. J., AND D. S. SMYTHE. 1964. Effects of

bacterial endotoxins on metabolism. VII. Enzyme induction and cortisone protection. J. Exptl. Med. **120**:721–732.

5. BERRY, L. J., AND D. S. SMYTHE. 1965. Some metabolic aspects of tolerance to bacterial endotoxin. J. Bacteriol **90**:970–977.
6. CIVEN, M., AND W. E. KNOX. 1960. The specificity of analogues as inducers, substrates, inhibitors, and stabilizers of liver tryptophan pyrrolase. J. Biol. Chem. **235**:1716–1718.
7. EAVES, G. N., M. K. TAYLOR, AND L. J. BERRY. 1966. Detection of apotryptophan pyrrolase activity in unfractionated liver homogenates. Proc. Soc. Exptl. Biol. Med. **121**:298–301.
8. FEIGELSON, P., T. DASHMAN, AND F. MARGOLIS. 1959. The half-lifetime of induced tryptophan peroxidase *in vivo*. Arch. Biochem. Biophys. **85**:478–482.
9. FEIGELSON, P., AND M. FEIGELSON. 1963. Studies on the mechanism of regulation by cortisone of the metabolism of liver purine and ribonucleic acid. J. Biol. Chem. **238**:1073–1077.
10. FEIGELSON, P., M. FEIGELSON, AND O. GREENGARD. 1962. Comparison of the mechanisms of hormonal and substrate induction in rat liver tryptophan pyrrolase. Recent Progr. Hormone Res. **18**:491–507.
11. FEIGELSON, P., AND O. GREENGARD. 1961. A microsomal iron-porphyrin activator of rat liver tryptophan pyrrolase. J. Biol. Chem. **236**: 153–157.
12. FEIGELSON, P., AND O. GREENGARD. 1962. Regulation of liver tryptophan pyrrolase activity. J. Biol. Chem. **237**:1908–1913.
13. FEIGELSON, P., AND O. GREENGARD. 1963. Immunologic and enzymatic studies on rat liver tryptophan pyrrolase during substrate and cortisone induction. Ann. N.Y. Acad. Sci. **103**: 1075–1082.
14. FEIGELSON, M., P. R. GROSS, AND P. FEIGELSON. 1962. Early effects of cortisone on nucleic acid and protein metabolism of rat liver. Biochim. Biophys. Acta **55**:495–504.
15. GARREN, L. D., R. R. HOWELL, G. M. TOMKINS, AND R. M. CROCCO. 1964. A paradoxical effect of actinomycin D: the mechanism of regulation of enzyme synthesis by hydrocortisone. Proc. Natl. Acad. Sci. U.S. **52**:1121–1129.
16. GOLDSTEIN, L., E. J. STELLA, AND W. E. KNOX. 1962. The effect of hydrocortisone on tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase activities in the isolated, perfused rat liver. J. Biol. Chem. **237**:1723–1726.
17. GREENGARD, O., AND P. FEIGELSON. 1961. The activation and induction of rat liver tryptophan pyrrolase *in vivo* by substrate. J. Biol. Chem. **236**:158–161.
18. GREENGARD, O., M. A. SMITH, AND G. ACS. 1963. Relation of cortisone and synthesis of ribonucleic acid to induced and developmental enzyme synthesis. J. Biol. Chem. **238**:1548–1551.
19. KENNEY, F. T., AND R. W. FLORA. 1961. Induction of tyrosine-α-ketoglutarate transaminase in rat liver. J. Biol. Chem. **236**:2699–2702.
20. KENNEY, F. T., AND F. J. KULL. 1963. Hydrocortisone-stimulated synthesis of nuclear RNA in enzyme induction. Proc. Natl. Acad. Sci. U.S. **50**:493–499.
21. KNOX, W. E., AND V. H. AUERBACH. 1955. The hormonal control of tryptophan peroxidase in the rat. J. Biol. Chem. **214**:307–313.
22. LIN, E. C. C., AND W. E. KNOX. 1957. Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. Biochim. Biophys. Acta **26**:85–88.
23. MORAN, J. F., AND T. L. SOURKES. 1963. Induction of tryptophan pyrrolase by α-methyltryptophan and its metabolic significance *in vivo*. J. Biol. Chem. **238**:3006–3008.
24. NEMETH, A. M. 1962. The effect of 5-fluorouracil on the developmental and adaptive formation of tryptophan pyrrolase. J. Biol. Chem. **237**: 3703–3706.
25. REICH, E. 1964. Actinomycin: correlation of structure and function of its complexes with purines and DNA. Science **143**:684–689.
26. REICH, E., R. M. FRANKLIN, A. J. SHATKIN, AND E. L. TATUM. 1961. Effect of actinomycin D on cellular nucleic acid synthesis and virus production. Science **134**:556–557.
27. REVEL, M., AND H. H. HIATT. 1964. Actinomycin D: an effect on rat liver homogenates unrelated to its action on RNA synthesis. Science **146**:1311–1313.
28. ROSEN, F., AND R. J. MILHOLLAND. 1963. Glucocorticoids and transaminase activity. VI. Comparison of the adaptive increases of alanine and tyrosine-α-ketoglutarate transaminases. J. Biol. Chem. **238**:3725–3729.
29. SCHIMKE, R. T., E. W. SWEENEY, AND C. M. BERLIN. 1964. An analysis of the kinetics of rat liver tryptophan pyrrolase induction: the significance of both enzyme synthesis and degradation. Biochem. Biophys. Res. Commun. **15**:214–219.
30. SCHIMKE, R. T., E. W. SWEENEY, AND C. M. BERLIN. 1965. The roles of synthesis and degradation in the control of rat liver tryptophan pyrrolase. J. Biol. Chem. **240**:322–331.
31. TANAKA, T., AND W. E. KNOX. 1959. The nature and mechanism of the tryptophan pyrrolase (peroxidase-oxidase) reaction of Pseudomonas and of rat liver. J. Biol. Chem. **234**:1162–1170.
32. WHITE, C. 1952. The use of ranks in a test for significance for comparing two treatments. Biometrics **8**:33–41.