Effect of Bacterial Endotoxin and Inhibitors on Tryptophan Oxygenase Induction in Mouse Liver Slices

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Tryptophan oxygenase activity in mouse liver slices maintained in culture medium, in Krebs-Ringer bicarbonate solution, or in homologous whole blood declined within 3 hr to about one-half the original level. Actinomycin D and puromycin accelerated the rate of decline, but endotoxin did not. Direct addition of tryptophan to the medium resulted in a higher than normal tryptophan oxygenase activity within 1 hr, and this was maintained well above that of control liver slices up to 6 hr. Triamcinolone, at a dose that doubles tryptophan oxygenase activity in vivo, had no effect on the enzyme in liver slices. Actinomycin and endotoxin did not alter the substrate induction of tryptophan oxygenase; however, puromycin did, but to a limited extent. Liver slices prepared from mice 4 hr after an injection of cortisone had a greater tryptophan oxygenase activity than those of controls. Either endotoxin or actinomycin D resulted in a more rapid decline of the enzyme when added to the slices than was observed in the controls.

For more than a decade, the major research effort in this laboratory has been aimed at an understanding of the metabolic effects of bacterial endotoxin in mice. This work has been predicated on the fact that cortisone antagonizes the action of endotoxin in many respects (3, 6, 20); hence, the two substances have been presumed to act in contrary ways. This concept was strengthened by the long-established ability of endotoxin to deplete carbohydrate reserves in mammals, whereas glucocorticoids were known to promote gluconeogenesis (6). More recently, the focus of attention has been on the interaction between hormone and endotoxin on selected inducible hepatic enzymes (2–5). Tryptophan oxygenase (formerly called tryptophan pyrrolose) has been studied more than other inducible enzymes, in part because of chance, but, more importantly, because its activity has been found to decrease in livers of endotoxin-poisoned mice and its induction by cortisone is suppressed by the bacterial substance. The relevance of these observations to an understanding of the metabolic basis for the antagonistic action of adrenocorticoids against endotoxin has been inferred but never proven (2, 3, 5).

Importance is attached to the apparent ability of endotoxin to interfere with enzyme induction because, if this occurs in vivo, it might account for at least some of the biological effects caused by this bacterial substance. This would be especially applicable to carbohydrate metabolism. If cortisone stimulates gluconeogenesis through the induction of limiting enzymes essential for the process, and if endotoxin inhibits or reduces in some way the activity of the same enzyme, then an explanation for the established effects of these substances would be available. If homeostasis, or the ability of the mammalian organism to maintain an internal milieu compatible with the continued existence of cells and tissues under extremes of environmental conditions or in the presence of noxious agents (such as endotoxin), depends to some degree on changes in activity of enzymes, then endotoxin, as a result of its interference with enzyme regulation, would upset the homeostatic balance. Several types of enzymatic adjustments are probably involved, including all of those responsive to cortisone. The most rapid changes, measured in minutes, probably occur in allosteric enzymes through feedback inhibition or activation. There is no
evidence, however, that endotoxin acts in this way. Over a period of 1 or 2 hr, enzymes with short half-life are able, in control animals, to manifest higher levels of activity as a result of increased rates of formation. The adrenocorticoids are, in part, responsible for these slower changes because they stimulate de novo synthesis of certain liver enzymes, probably through their effect on ribonucleic acid (RNA) synthesis (9, 14, 16, 27, 30). This property of cortical hormones is believed to explain, at a biochemical level, the long-recognized fact that the adrenal cortex is essential for the mammal's normal response to stress. If cortisone alleviates a normal of the toxic manifestations of endotoxin through its influence on enzyme induction, then it is necessary to establish an antagonism between the two substances at the enzymatic level, as previously cited work has suggested.

Even though earlier work (2-5) yielded data that show less tryptophan oxygenase activity in livers of endotoxin-poisoned mice compared to that in control mice or, especially, that in mice previously injected with cortisone, the necessity to administer these substances to intact animals makes it difficult to know how directly the enzyme is affected. The results that had been obtained could have been mediated through some metabolite formed elsewhere and transported to the liver via the circulation. It seemed desirable, therefore, to seek an in vitro method of further purifying the problem. Therefore, experiments with liver slices were undertaken. As an essential part of the experimental design, the manner in which endotoxin influenced the activity of tryptophan oxygenase in liver slices was compared to the effects produced by two widely used inhibitors, actinomycin D, which blocks protein synthesis by preventing deoxyribonucleic acid (DNA)-dependent RNA synthesis (19, 25), and puromycin, which inhibits amino acid incorporation in the growing peptide chain (22, 27, 28). The action of these compounds served as good indices of the types of synthesis that occurred in liver slices. The choice of this in vitro system was based on studies by Eisenstein and his collaborators (12, 13) and by Haynes (18), in which hormonal effects on liver slices were described. The results they obtained suggest that the hormone penetrated the cells in the slices and, apparently, enhanced transport of organic materials across the cells. Another encouraging lead was provided by the work of Saba and Di Luzio (26), who found that the Kupffer cells of liver slices ingested inert particles only when slices were bathed in whole blood, not in a complete tissue culture medium.

Although the results with liver slices did not wholly confirm earlier observations with intact mice, they were sufficiently in agreement to establish an antagonistic relationship between cortisone and endotoxin on tryptophan oxygenase induction that, if mediated, had to be from liver to liver.

MATERIALS AND METHODS

Endotoxin. Escherichia coli lipopolysaccharide 0127-B8 (Difco) was dissolved in the desired amount in nonpyrogenic, isotonic sodium chloride solution (Baxter Laboratories, Morton Grove, Ill.). Mice were injected intraperitoneally with 0.5 ml that contained the appropriate dose. Control mice were injected with saline alone.

Hormone. Either cortisone acetate in stabilized aqueous suspension (United Research Laboratories, Philadelphia, Pa.) or triamcinolone (E. R. Squibb & Sons, New York, N.Y.) was administered subcutaneously in the interscapular region with the desired amount contained in 0.5 ml. Baxter saline was used as diluent.

Metabolic inhibitors. Actinomycin D (generously supplied by Vernon Bryson, Microbiological Institute, Rutgers University) was dissolved as previously described (4) and added to the incubation medium. Puromycin (generously provided by J. S. Kiser, American Cyanamid Co., Princeton, N.J.) was added directly to the medium containing the liver slices.

Preparation of liver slices. Animals were sacrificed by cervical dislocation; the livers were removed rapidly and placed in iced Baxter saline. Slices were cut with a Stadie-Riggs (A. H. Thomas Co., Philadelphia, Pa.), tissue slicer weighed, and transferred to incubation vessels. The most consistent results were obtained with slices weighing from 70 to 120 mg.

Incubation of slices. Two methods of incubation were employed throughout these experiments. One method was designed along lines demonstrated by Anna Steinberger of the Albert Einstein Medical Center. This technique is a modification of the method of Trowell (29). Rectangular strips of 2% Special Agar (Noble, Difco) with 0.7% NaCl were placed across the top of stainless-steel wire grids placed inside 60- by 15-mm petri dishes. Slices were placed on top of the agar strip. Incubation medium was added to the dish so that it just contacted the under surface of the agar. Approximately 8 ml of medium was required to attain the correct level. For experiments of 6 hr or longer, all equipment was sterilized by autoclaving.

The petri dish containing the slices was placed in a plexiglass humidity chamber. Moisture in the chamber was maintained by damp filter paper and by beakers of water placed inside. The entire apparatus was housed in an incubator maintained at 37 C. Inlet and outlet pors in the chamber permitted the continuous passage of a washed mixture of 95% O2 and 5% CO2.

This procedure permitted the maintenance of slices for extended periods of time because the cells were
in contact with the medium by the process of diffusion through the agar, and they were exposed to the appropriate gaseous environment.

In the second procedure, slices were added directly to 1 ml of medium in plastic 5-ml Dispo beakers and placed in a metabolic shaker with 55 strokes of 2-cm amplitude per min. The temperature was maintained at 37°C; the atmosphere contained 95% O_2 and 5% CO_2.

Tissue culture medium. Eagles' minimal essential medium with Hanks' balanced salt solution was supplemented with 10% horse serum (Cultur Stat, BBL). In some experiments, the Cultur Stat base was used with 10% mouse blood substituted for the horse serum. Krebs-Ringer bicarbonate solution was used in a few test runs. Whole, heparinized, homologous mouse blood obtained from the hearts of donor mice was used in a number of experiments.

Tryptophan oxygenase assay. The assay used for measuring the activity of tryptophan oxygenase is a micromodification designed by Eaves and Berry (10) of the basic procedure of Knox and Auerbach (21), as adapted to mice by Berry and Smythe (3). To reaction vessels containing 0.9 ml of distilled water were added 0.2 ml of phosphate buffer (pH 7.0), 0.3 ml of 0.15 M L-tryptophan, 0.1 ml of hemat (6.5 mg/ml), and 0.5 ml of liver homogenate in 0.14 M KCl (1:7). The reaction was carried out at 37°C on a metabolic shaker with 120 strokes of 2-cm amplitude per min. The reaction was terminated with 1.0 ml of 15% metaphosphoric acid. The mixture was filtered, neutralized with 1.5 N NaOH, and the optical density was read at 360 nm in a Hitachi spectrophotometer.

Mice. Adult female mice of the Swiss-Webster strain were obtained from Dierolf Farms, Boyertown, Pa. The animals were housed in metal cages with food and tap water available at all times, except that the food was removed from the cages of experimental mice 17 hr before liver excision. Other handling procedures were the same as those previously described (4).

Statistical analyses. Results were analyzed statistically by the rank order test of White (31) and by the "t" test for correlated observations.

RESULTS

Effect of the culture medium on tryptophan oxygenase activity in liver slices. Tryptophan oxygenase activity in slices incubated in Krebs-Ringer bicarbonate solution in Cultur Stat with 10% mouse blood or in 100% whole mouse blood was compared with that found in slices incubated in Cultur Stat alone. During a 3-hr period of incubation, neither Krebs-Ringer solution nor Cultur Stat with 10% mouse blood was able to prevent the decline in enzyme activity that occurred during this interval of time (Table 1). Incubation in whole blood resulted in a significantly higher level of enzyme activity after 1 hr, but by 3 hr the difference was marginal compared to the control (Table 1). Although cofactor availability may contribute to the increased activity observed with whole blood, doubling the hematin concentration from 6.5 to 13.0 μM in the assay flask did not reproduce this effect.

Because of the variation in control values, all comparisons were made among slices taken from the same livers and incubated under the different experimental conditions. The reasons for the lack of reproducibility are not known, but some variations are believed to be seasonal in background and others may be related to the health of the mice.

Effect of inhibitors on normal enzyme activity. From the results presented above, liver slices are presumed to be able to support RNA and protein synthesis over a period of a few hours. This premise was subjected to indirect tests through

<table>
<thead>
<tr>
<th>Expt</th>
<th>Method</th>
<th>Medium</th>
<th>Tryptophan oxygenase activity in slices incubated for 3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Cultur Stat + Krebs-Ringer</td>
<td>20.9 ± 1.5 (4)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Cultur Stat + 10% mouse blood</td>
<td>18.5 ± 0.7 (4)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Cultur Stat + Whole blood</td>
<td>26.9 ± 8.5 (12)</td>
</tr>
</tbody>
</table>

* Each value is the mean ± the standard error for the number of separate determinations shown in parentheses.

In method 1, slices were incubated on wire grids; in method 2, they were immersed in 1 ml of medium.

Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).
the use of inhibitors. With 5 µg of actinomycin added to the medium, a significant decrease in enzyme activity was detected. After 3 hr, it was less than one-half that of the paired control. By 6 hr, the difference was borderline in significance, but control values were quite low; therefore, any difference was minimized (Table 2).

The addition of 5 mg of puromycin to the medium significantly reduced enzyme activity at 1 hr, but by 3 hr the difference had disappeared (Table 2). These results coupled with those for actinomycin support the concept that protein synthesis continues for at least 3 hr in the liver slices.

In vivo endotoxin, like actinomycin, is able to affect a decrease in tryptophan oxygenase activity when injected into normal, fasted mice (5). The addition of 0.75 mg of E. coli lipopolysaccharide to the incubation medium had no detectable effect on enzyme activity when slices were incubated on wire grids (Table 2). This amount of endotoxin killed 6 out of 10 mice when injected intraperitoneally. Since no evidence of diffusion of biologically active endotoxin through the agar was obtained, incubation by immersion (method 2) was attempted. Tryptophan oxygenase activity was unaffected by 1 mg of endotoxin added to the medium (Table 2).

**Effect of tryptophan.** Civen and Knox (9) reported substrate elevation of tryptophan oxygenase when rat liver slices were incubated in tissue culture medium, but not in Krebs-Ringer. The need for a medium capable of supporting some protein synthesis suggests, therefore, that this process, along with stabilization of the enzyme, is involved in the induction of this enzyme by its substrate.

The effect of tryptophan on tryptophan oxygenase activity in Cultur Stat is shown in Table 3. In 1 hr, enzyme activity was almost double that of the paired control, and a level well above that of the control was maintained for the entire 6 hr. The almost constant activity of the enzyme in tryptophan-treated slices from the first through the third hour, in contrast to the decline in control values, indicates that the substrate effect is clearly demonstrable under these conditions.

The tryptophan-induced increase in tryptophan oxygenase occurred in slices incubated in Krebs-Ringer as well as in Cultur Stat (Table 4). These results suggest that both activation and protection of the enzyme may play a role in the increases.

**Effect of glucocorticoids.** Although previous attempts at induction of tryptophan oxygenase in liver slices with glucocorticoids have been unsuccessful (1, 9), this problem was undertaken by means of the techniques described above. It is evident that no increase in tryptophan oxygenase activity resulted after the addition of 2 mg of triamcinolone directly to the medium, even though this amount of hormone was capable of doubling enzyme activity in vivo within 3 hr (Table 5). Substitution of 10% mouse blood for the horse serum in Cultur Stat did not alter the result. With 100% whole mouse blood, a significantly

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**Table 2. Effect of inhibitors and endotoxin on tryptophan oxygenase activity in mouse liver slices**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Method</th>
<th>Inhibitor</th>
<th>Tryptophan oxygenase activity in slices incubated for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Control Actinomycin (5 µg)</td>
<td>20.5 ± 2.5 (10)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Control Puromycin (5 mg)</td>
<td>12.1 ± 1.2 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Control Endotoxin (0.75 mg)</td>
<td>21.1 ± 1.9 (12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Control Endotoxin (1.0 mg)</td>
<td>11.9 ± 1.3 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each value is the mean ± the standard error for the number of separate determinations shown in parentheses.
* In method 1, slices were incubated on wire grids; in method 2, they were immersed in 1 ml of medium.
* Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).
* These values, compared to their corresponding controls, are significantly less (P < 0.005) by rank order test (31).
* These values, compared to their corresponding controls, are not significantly different (P > 0.05) by rank order test (31).
zyme activity. Studies with inhibitors or glucocorticoids that induce, distinguish between the results. Addition to Cultur Stat medium

None (controls) 26.1 ± 2.8 (16) 23.5 ± 2.1 (9) 16.5 ± 1.9 (14) 8.7 ± 1.9 (8)
Tryptophan (10 mg) 41.3 ± 1.9 (8) 40.1 ± 2.1 (16) 29.9 ± 3.6 (7)

Each value is the mean ± the standard error for the number of separate determinations shown in parentheses. Method 1 was employed, in which slices were incubated on wire grids.

Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).

Each value, compared to its corresponding control, is significantly greater (P < 0.005) by rank order test (31).

### Table 4. Effect of incubation medium on tryptophan induction of tryptophan oxygenase in mouse liver slices

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Tryptophan oxygenase activity in slices incubated for 6 hr</th>
<th>0 hr</th>
<th>1 hr</th>
<th>3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krebs-Ringer</td>
<td>20.9 ± 1.5 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krebs-Ringer + 10 mg of tryptophan</td>
<td>21.6 ± 1.7 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultur Stat</td>
<td>14.3 ± 1.7 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultur Stat + 10 mg of tryptophan</td>
<td>24.7 ± 4.7 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± the standard error for the number of separate determinations shown in parentheses. Method 1 was employed, in which slices were incubated on wire grids.

Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).

This value, compared with its control, is not statistically different by "t" test (11).

This value is significantly larger than its control (P < 0.01) by "t" test (11).

These values are significantly larger than their controls (P < 0.05) by "t" test (11).

lower enzyme activity, compared to the controls, was found after 1 hr in hormone-treated slices. In these experiments, the desired amount of triamcinolone dissolved in 0.5 ml of 95% ethyl alcohol was added to empty vessels. The alcohol was allowed to evaporate and the medium was then added. This procedure was used by Haynes (18).

These results clearly indicate that treatment with hormone under in vitro conditions fails to induce, and perhaps inhibits, the activity of enzyme. Since published studies (15) have shown that glucocorticoids induce tryptophan oxygenase in perfused livers but not in liver slices, one must conclude that the metabolism of liver slices is not qualitatively the same as that of the perfused organ.

Effect of inhibitors on tryptophan-induced enzyme activity. The initial elevation of tryptophan oxygenase in the presence of tryptophan may be the result of retarded degradation, increased synthesis, or the activation of an inactive form of the enzyme. Studies with inhibitors were designed to distinguish between these possibilities.

Actinomycin was without effect on the substrate induction of tryptophan oxygenase (Table 6). Activity of the enzyme was elevated and maintained with or without the inhibitor present (Table 6). Endotoxin, likewise, had no effect when it was added instead of actinomycin (Table 6).

Puromycin significantly reduced, but did not completely abolish, the tryptophan induction of the enzyme (Table 6). Activity of the enzyme in the presence of this inhibitor was greater than that of the controls, but it was below that found in the presence of tryptophan alone. These results suggest that, whereas some protein synthesis continues in liver slices during substrate induction of tryptophan oxygenase, some increase also occurs in enzyme activity not inhibited by puromycin. The latter is probably the result of enzyme stabilization.

In vivo-in vitro experiments on tryptophan oxygenase induction. This series of experiments was undertaken in an attempt to establish conditions under which an effect of endotoxin on the activity of tryptophan oxygenase could be observed. Enzyme activity was induced in mice by an injection of 5.0 mg of cortisone acetate 4 hr prior to excising and slicing the liver. Enzyme activity in these slices was significantly higher than that in control slices during the first hour in vitro, whether
slices were incubated in Cultur Stat or in whole blood. Slices derived from normal liver, however, had a higher tryptophan oxygenase level when the culture medium was whole blood rather than Cultur Stat.

Since the initial level of tryptophan oxygenase was higher in slices of liver derived from mice pretreated with cortisone, an attempt was made to detect decreases in enzyme activity affected by inhibitors. When either actinomycin D or endotoxin was added to the blood medium, a decrease in tryptophan oxygenase activity was observed when the data were compared to the appropriate paired control (Table 7). In addition, endotoxin treatment of these liver slices resulted in lower enzyme activity even when the slices were incubated in tissue culture medium (Table 7). This finding suggests that the effect of endotoxin is

**Table 5. Effect of triamcinolone on tryptophan oxygenase activity in mouse liver slices**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Methoda</th>
<th>Incubation medium</th>
<th>Tryptophan oxygenase activity in slices incubated forb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Cultur Stat</td>
<td>25.1 ± 2.7 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cultur Stat + triamcinalone (2 mg)</td>
<td>26.1 ± 1.7 (6)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Cultur Stat + 10% Mouse Blood</td>
<td>21.7 ± 1.9 (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cultur Stat + 10% mouse blood + triamcinalone (2 mg)</td>
<td>18.0 ± 0.9 (14)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Whole mouse blood</td>
<td>20.4 ± 2.7 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole mouse blood + triamcinalone (2 mg)</td>
<td>31.4 ± 1.7 (8)</td>
</tr>
</tbody>
</table>

a Each value is the mean ± the standard error for the number of separate determinations shown in parentheses.
b In method 1, slices were incubated on wire grids; in method 2, they were immersed in 1 ml of medium.
c Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).

**Table 6. Effect of inhibitors and endotoxin on tryptophan induction of tryptophan oxygenase in mouse liver slices**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Addition to medium</th>
<th>Tryptophan oxygenase activity in slices incubated forb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>14.1 ± 2.2 (6)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (10 mg)</td>
<td>26.1 ± 1.3 (6)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (10 mg)</td>
<td>25.5 ± 0.6 (6)</td>
</tr>
<tr>
<td></td>
<td>Actinomycin (5 μg)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tryptophan (10 mg)</td>
<td>16.8 ± 2.8 (8)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (10 mg)</td>
<td>21.4 ± 2.8 (7)</td>
</tr>
<tr>
<td></td>
<td>Endotoxin (0.75 mg)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>11.9 ± 2.7 (5)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (10 mg)</td>
<td>28.6 ± 2.0 (16)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (10 mg)</td>
<td>21.2 ± 2.0 (16)</td>
</tr>
<tr>
<td></td>
<td>Puromycin (5 mg)</td>
<td></td>
</tr>
</tbody>
</table>

a Each value is the mean ± the standard error for the number of separate determinations shown in parentheses. Method 1, in which slices were incubated on wire grids, and Cultur Stat medium were used in all experiments.
b Expressed as micromoles of kynurenine per hour per gram of liver (dry weight).
not mediated by the blood but rather is the result of a direct action on the liver slices.

**DISCUSSION**

Except in the presence of whole blood, the suspending medium had little influence on tryptophan oxygenase activity in liver slices. This activating effect of whole blood may be due to its content of methemoglobin and tryptophan similar to that observed by Knox and Piras (22, 23, 24) in rat liver. Incubation in whole blood may have activated a greater portion of total enzyme than occurs in other media. According to Civen and Knox (9), 5 to 10 μg of free tryptophan is present in 1 g of liver and in 1 ml of blood in normal rats. Using these figures for mice, about 0.5 μg of tryptophan would be present in the slice and another 5 μg in blood. Even with a small percentage of blood containing methemoglobin, enough of the two activators could have been present to account for at least some of the greater enzyme activity found in whole blood, or it may have been due to some factor similar to that recently described by Harris and Salter (17).

The role of protein synthesis in the maintenance of tryptophan oxygenase was assessed indirectly by the use of inhibitors. Both actinomycin and puromycin resulted in a more rapid decrease in enzyme activity than occurred in control slices. If the commonly accepted mode of action of actinomycin D (19, 25) is assumed, the results suggest that RNA and protein synthesis are involved in and may underlie the decline in tryptophan oxygenase activity in liver slices.

Puromycin, but not actinomycin or endotoxin, decreased the substrate induction of tryptophan oxygenase. This suggests that some protein synthesis is involved in this phenomenon. The failure of puromycin to abolish this type of induction suggests that some activation and stabilization of the enzyme occurs as well. A similar effect of substrate and puromycin was observed in vivo in rats by Schimke et al. (27, 28) and by Knox and Piras (22).

The data in this report differ from, but do not contradict, those of Civen and Knox (9), who found no elevation of tryptophan oxygenase in rat liver slices suspended in Krebs-Ringer for 1 hr and observed an increase after 3 hr when substrate was added to the tissue culture medium. The stabilization of the enzyme between the first and third hours, as observed in this study, would have gone undetected by Civen and Knox because of their experimental design. Although their conclusion regarding the contribution of protein synthesis to substrate induction was the same as ours, their work preceded the presently established importance of enzyme activation and stabilization in this process (7, 14, 16, 22).

All attempts to induce tryptophan oxygenase by addition of hormone to the medium were unsuccessful. The approximation of perfusion systems by incubation of slices in whole blood did not produce the desired effect. In the procedure used in these experiments, a slight decrease in enzyme activity was found in the presence of hormone, especially in slices incubated in whole blood. Civen et al. (8) found a similar decrease in tyrosine-α-ketoglutarate transaminase activity in liver slices incubated with hydrocortisone, but no explanation for this effect was offered.

When 5 mg of cortisone acetate was injected into mice 4 hr prior to their sacrifice and the preparation of liver slices, tryptophan oxygenase consistently increased during the first hour of synthesis is involved in this phenomenon. The failure of puromycin to abolish this type of induction suggests that some activation and stabilization of the enzyme occurs as well. A similar effect of substrate and puromycin was observed in vivo in rats by Schimke et al. (27, 28) and by Knox and Piras (22).

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When 5 mg of cortisone acetate was injected into mice 4 hr prior to their sacrifice and the preparation of liver slices, tryptophan oxygenase consistently increased during the first hour of
incubation. This increase could be inhibited by actinomycin and by endotoxin. From these results, it can be inferred that enzyme induction continues in vitro and is subject to regulation similar to that observed in vivo. This effect is similar to that reported by Weber et al. (30) for glucose-6-phosphatase and fructose-1,6-diphosphatase. The fact that endotoxin prevents this rise when added to slices suspended in either whole blood or a tissue culture medium indicates that it acts directly on the liver and not through the release of a mediator in blood.

A mediator rather than a direct effect of endotoxin is postulated, because it is difficult to believe that particles as large as cell wall fragments penetrate the parenchymal cells and act directly on tryptophan oxygenase. A number of studies, moreover, have indicated that only in Kupffer cells of the liver can endotoxin be seen. If phagocytic ingestion of endotoxin is required for subsequent changes in the activity of tryptophan oxygenase and probably other inducible hepatic enzymes, then some metabolite or cell product would be responsible. It has been shown in our laboratory that endotoxin stimulates both RNA and protein synthesis in mouse liver and spleen (T. Shtasel, unpublished data). Thus, the decrease in tryptophan oxygenase activity does not reflect a generalized anabolic paralysis. From this type of evidence, it is tentatively assumed that the mediator acts against specific enzymes, probably as a noncompetitive inhibitor. It remains to be determined whether the same mediator or a different one(s) is responsible for augmenting protein synthesis in general.

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LITERATURE CITED


