Induction of Tryptophan Peroxidase of Rat Liver by Insulin and Alloxan*

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Among the many important problems in biochemistry are those concerned with the mechanism of protein synthesis and hormone action. An interrelated subject is the mechanism of adaptive enzyme formation in mammals. The tryptophan peroxidase-oxidase system as described by Knox et al. (1-5) could be employed to good advantage in all three of these areas. The enzyme system is an adaptive one, whose synthesis in vivo reaches a maximum about 6 hours after administration of an active substance. The rapidity of TPO production gives hope that a system (in vitro) capable of protein synthesis can be established. In this work it has been found that the normal liver slice can, to some extent, be induced to form TPO in vitro.

The enzyme system is, at least partially, under hormonal control. Cortisone in vivo (6, 7), hydrocortisone (6), and adrenocorticotropic hormone (ACTH) (8, 9) increase, while growth hormone (10) and hypophysectomy (8, 11) decrease the production of TPO. In this work it has been found that insulin can induce the formation of TPO in vivo and this effect is independent of insulin shock or of the level of blood glucose. In addition, it has been found that the TPO level shows an 8- to 10-fold increase in alloxan diabetic rats.

The TPO system can be induced with a variety of substances other than the specific substrate, tryptophan. This coupled with the fact that the system is an adaptive one should permit manipulations leading to the mechanism of adaptation. It is conceivable that treatment for certain "inborn errors of metabolism" would consist of induction of the missing enzyme.

EXPERIMENTAL

Method of Assay—The albino rats used in this study were Wistar strain animals. At the start of each experiment they weighed between 200 and 300 gm. Both male and female rats were used and, as noted by Knox and Mehler (1), there were no differences in their TPO response. Unless otherwise mentioned they were fed water and Purina Laboratory Chow ad libitum.

The method was that of Knox and Mehler (1) with modifications. The liver was removed immediately after death and homogenized in the cold in a volume of fresh Krebs-Henseleit bicarbonate buffer (12) sufficient to make a 5 per cent homogenate. The buffer (which contains 0.2 per cent glucose) was also used as a solvent for various substances added to the incubation mixture. Additions were 1.0 ml. of homogenate, 1.0 ml. of a solution of 9 μmoles per ml. of L-tryptophan (omitted from the blank run) and buffer to make a total volume of 3.0 ml. The mixtures were incubated under 95 per cent O2-5 per cent CO2 in a Dubnoff shaker for 3 hours at 37.5°, and the kynurenine formation was determined by the increased absorption at 360 μm after deproteinization with 5 per cent zinc acetate (2.5 ml.) and 0.18 N NaOH (3.5 ml.). For the activity measurements the readings were calculated back to the μmoles of kynurenine formed per hour per gm. wet weight of liver tissue, using the value of 0.454 for the optical density of a 0.10 mM kynurenine solution. 1 activity unit of the enzyme is defined as the amount which produces 1 μmole of kynurenine per hour per gm. of liver. The low TPO activity in liver taken from normal rats and the high TPO activity from rats in which the enzyme had been induced by tryptophan in vivo, was linear to 3 hours of incubation. The concentration of tryptophan used in the incubation medium was optimal for the TPO activity occurring in both the normal liver and in the liver from induced rats.

Induction in Vivo of TPO—The rats were treated by intraperitoneal injection with the substance under investigation and killed 6 hours later. It required 6 hours for the maximal increase in TPO in response to a dose of tryptophan of 1 gm. per kilo of rat. It was felt that if the hormone or compound under study played a role in the induction of TPO, or was an inducer itself, this fact also should evince itself in a 6 to 8 hour period.

Liver Slice Experiments—In liver slices, as in homogenates, the optimal concentration of tryptophan in the incubation medium was 3 mM, and activity was a linear function of time. The 50 mg. slice obtained with a Stadie-Riggs slicer, in the cold, was incubated for 3 hours with tryptophan and buffer (total volume 3.0 ml.) or tryptophan and a solution of the test substance in the buffer. The slice was homogenized, in the cold, in its own incubating fluid. 2.5 ml. of this mixture were deproteinized with the proper volume of reagents, and kynurenine was determined at 360 μm.

Insulin in Normal, 48 Hour Fasted, and Adrenalectomized Rats—The hormone used was Lilly’s Iletin, a crystalline zinc-insulin preparation. The dosage of insulin was injected intra-
peritoneally into all three types of rats. The group permitted to drink and eat ad libitum showed no signs of shock or discomfort at dosages up to and including 100 units of insulin per 200 gm. rat. Rats denied food for 48 hours, but permitted water ad libitum, developed convulsions when given the injection of insulin. However, if these fasted rats were fed 10 per cent glucose at 15 to 20 minute intervals, starting immediately after injection of the insulin, they remained unaffected, even up to the 100 unit dosage level.

The adrenalectomized rats were extremely sensitive to the insulin, reacting to 1 to 3 units with convulsions, and sometimes death. Once again, if glucose was administered to these rats during the 6 hour induction period, they remained alert and free of convulsions at dosages up to 60 or 70 units of insulin per 200 gm. rat. Adrenalectomies were performed under ether anesthesia and the animals maintained on a 1 per cent sodium chloride solution. The rats were used 6 to 8 days after operation, and the completeness of the removal of the glands was verified by visual observation at autopsy.

Alloxan-Induced Diabetes—The effect of diabetes on the level of TPO was determined in rats treated with alloxan (Eastman Kodak Co.) according to the method of Kipnis and Cori (13). The rats were deprived of food but not water for 24 hours after which time 200 mg. of alloxan per kilo of rat was injected intraperitoneally. Glucose in the urine was estimated by Clinistix (14) purchased from the Ames Company and by Tes-Tape (15) purchased from Lilly.

RESULTS

TPO Induction with Tryptophan—For expository purposes, a dose of tryptophan of 1000 mg. per kilo of rat is considered the maximal dose, and the response obtained, i.e. 9-fold increase in TPO level, is considered the maximal response. Injection of 20 per cent of the maximal dose gave rise to 60 per cent of the maximal response. Consequently, an induction (in vivo) curve for TPO activity as a function of dosage of tryptophan injected was performed. Fig. 1 illustrates this plot. It is apparent from the figure that the activity response was not a linear function of dosage. From the curve it may be observed that small doses of tryptophan were far more efficient in inducing TPO, per mg. of tryptophan injected, than are large doses.

Induction in Vivo of TPO with Insulin and Survey of Other Hormones for Inductive Action—It was suspected that there must be at least one other factor in the blood of the rat that aids in the induction of TPO. Several hormones were therefore explored for their ability to induce TPO, the first of these being insulin. It was found that insulin could induce the formation of the enzyme in vivo although not significantly in vitro. In Fig. 2 the curve marked “normal” shows the increase in TPO activity of the normal rat as a function of insulin injected. Each open circle represents 3 to 4 rats. “Normal” is used here to indicate no prior treatment before the injection of insulin. The activity shows a 3- to 4-fold increase over the level in the normal rat. It must be remarked that these insulin-treated rats showed no signs of hypoglycemic convulsions but remained alert with no overt signs of stress.

Since fasted rats are more sensitive to insulin, animals fasted for 48 hours were tested for a more intense TPO production after insulin injection. This group will be referred to for

\[^2\] Insulin contains no tryptophan.
enzyme induction by insulin, a fresh series of fasted rats were fed 10 per cent glucose immediately after injection with insulin. In order to determine whether hypoglycemia played a role in the enzyme response to insulin, producing a 4- to 5-fold increase in the brevity henceforth as fasted rats. In Fig. 2 the curve marked “fasted” represents the TPO activity plotted as a function of insulin dosage. As can be seen, the fasted rats gave a greater response to insulin, producing a 4- to 5-fold increase in the enzyme.

The fasted rats developed hypoglycemic convulsions. In order to determine whether hypoglycemia played a role in the enzyme induction by insulin, a fresh series of fasted rats were fed 10 per cent glucose immediately after injection with insulin. These animals remained alert and free of convulsions. There were no differences in the activities of TPO produced by those fed glucose and those not given sugar. Results from both series were used to plot the curve in Fig. 2. Each triangle represents four to five rats.

From the above experiments it was inferred that the action of insulin might be independent of the stress reaction mediated through the adrenals. By stress reaction is meant the release of adrenal steroids, either directly or via adrenocorticotropic hormone (ACTH), in response to a stimulus. A test of the relationship between this insulin action and the stress reaction was performed by inducing TPO with insulin in adrenalectomised rats. Experimentally this was difficult, but the glucose-feeding technique helped to maintain the animals in good condition. Curve ADX in Fig. 2 illustrates the result of induction in the operated rats. The dark circles represent two to three rats and each square represents one rat. It may be seen that both the level of activity in the adrenalectomized control rat (not injected with insulin) and the activity of the injected rats decreased from the values obtained with the normal rats. The decrease in the control value of approximately 50 per cent corresponds closely to the value Knox (5) reported for adrenalectomised rats. However, the maximal increase in activity on injection of insulin in the operated animals is still 3- to 4-fold above that of the adrenalectomized control. Adrenalectomy causes approximately a 50 per cent decrease in both the control level and the insulin-induced level. It may be concluded that in an absolute sense the induction of TPO by insulin is partially dependent on the adrenals, but in a relative sense (relative to the adrenalectomized controls), the induction is independent of the stress reaction. The above data are summarized in Table I.

The control levels in the fasted but uninjected rat, and in the fasted rat injected with distilled water, were the same as the normal control. This agrees with the finding of Chiancone (16) that a fast of 8 or 13 days had no effect on the TPO level in noninduced rats. However, the control level doubled when these fasted rats were given 10 per cent glucose to drink. This is due probably to a “stress” mechanism since adrenalectomy abolishes the increase induced by glucose.

Another finding was that the induction of TPO by insulin is independent of the blood glucose level. The 48 hour fasted rats and the adrenalectomized rats when given insulin and fed the 10 per cent glucose showed, by 1es-Tape analysis, a positive glucose reaction in the urine (0.5 to 1.5 on our scale). These rats are thus hyperglycemic despite the insulin injected. Insulin, when given alone to the fasted animals, caused hypoglycemic convulsions, yet the TPO level induced was the same as that in the rats fed glucose.

Neither triiodothyronine (0.4 mg. per 200 gm. rat) nor diethylstilbestrol (50 mg. per 200 gm. male or female rat) affected significantly the TPO activity found in the liver 6 hours after intraperitoneal injection of the compound. However, testosterone (50 mg. per 200 gm. male or female rat) did double the TPO activity, but whether this effect was mediated through the adrenals via the “stress” reaction was not determined.

"Simultaneous" Experiments—In an attempt to obtain more information on the inducing action of insulin and cortisone, experiments were devised in which two test substances were injected at the same time into an animal. The first type of experiment involved the injection of insulin followed a few minutes later by an injection of tryptophan. It was known how much TPO could be induced by the insulin separately (Fig. 2) and by the tryptophan separately (Fig. 1). By appropriate summation of the data, the level could be compared to that produced in the animal receiving these materials by simultaneous injection. The tryptophan plus insulin could act synergistically, additively, or antagonistically.

The results shown in Table II indicate that insulin and tryptophan act additively.

From a comparison of Column 3 with Column 6, it can be seen that the TPO activity calculated on the basis of additivity (Column 6) is equal, within experimental error, to the experimentally determined value (Column 3). The calculation may be made in several ways. The method to be described compensates for two factors: (a) the nonlinear shape of the TPO induction curve (Fig. 1), and (b) the fact that the calculated value must be corrected for the use of two rats, each with the level of TPO normally present in the liver before induction. The experimental value is obtained by the use of only one animal. The value for TPO activity induced by 53 units of insulin per 200 gm. rat was 5.4 units as seen in Fig. 2. This activity was referred to Fig. 1 and from the curve it can be seen that the same level of activity was induced by 140 mg. of tryptophan per kilo of rat. The value of the tryptophan dosage actually given by injection to the experimental animal was then added to this insulin-equivalent value. For the ease of the first calculation in Table II the injected tryptophan was 200 mg., the total being 340 mg. The activity induced by 340 mg. of tryptophan was 9.0 TPO units. This was the value of the activity induced by insulin plus the increment due to the tryptophan injected and is the summed value appearing in Table II.

The second type of “simultaneous” experiment involved the use of insulin plus cortisone. The results in Table III show that the inductive effects of insulin and cortisone can best be

### Table I

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Control (not injected with insulin)</th>
<th>Maximal response (to insulin injection)</th>
<th>Per cent increase in activity over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.5 ± 0.3†</td>
<td>5.4 ± 1.1</td>
<td>360</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>0.8 ± 0.2</td>
<td>2.7 ± 0.5</td>
<td>340</td>
</tr>
<tr>
<td></td>
<td>0.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fed 10% glucose)</td>
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<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>1.6 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>3.2 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fed 10% glucose)</td>
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</table>

* All activities expressed in TPO units.
† Standard deviation.
From this it can be seen that 72 hours after administration of alloxan, the TPO level was elevated 8- to 9-fold over that of the normal rat. This increase is of the order obtained on injection of the maximal dose of tryptophan.

In addition, from the values given near the top of the figure it can be seen that the fluctuations in TPO observed were independent of the glucose level in the urine and hence presumably of the blood glucose concentration. The results obtained might also be independent of any endogenous tryptophan liberated during tissue breakdown (alloxanized rats after 24 hours lose 10 to 20 gm. of weight per day and show a negative nitrogen balance (17)). The weight loss at the 48th hour was of the same order of magnitude as that occurring at the 72nd hour. Yet the TPO activity at these two times was vastly different. However, it is still possible that the explanation for the second peak lies in the tryptophan liberated from endogenous tissue breakdown, which might stimulate the liver to produce TPO.

To determine whether the first peak was caused by endogenously liberated insulin, alloxan was given by injection to rats which had received 24 hours previously alloxan (insulin-free). The rats were killed 6 hours after the second injection. The

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**Table II**

Additivity of TPO induction caused by insulin plus tryptophan

| Treatment                        | No. of rats | TPO activity found | TPO activity due to tryptophan injected | TPO activity due to 53 units of insulin | TPO activity summed
<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>of maximal dose of tryptophan</td>
<td>4</td>
<td>10.0 ± 2.1$</td>
<td>8.1</td>
<td>5.4</td>
<td>9.0</td>
</tr>
<tr>
<td>200 mg. tryptophan per kg. rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 53 units of insulin per 200 gm. rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of maximal dose of tryptophan</td>
<td>4</td>
<td>8.0 ± 2.1$</td>
<td>3.9</td>
<td>5.4</td>
<td>8.4</td>
</tr>
<tr>
<td>100 mg. tryptophan per kg. rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 53 units of insulin per 200 gm. rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Activity in TPO units.
† From Fig. 1.
‡ From Fig. 2.
§ Column 5 plus increment due to tryptophan (from Fig. 1)—for calculation, see text.
¶ Standard deviation.

**Table III**

Additivity of TPO induction caused by insulin plus cortisone

| Treatment                        | No. of rats | TPO activity found* | TPO activity due to 1.67 mg. cortisone per 200 gm. rat | TPO activity summed
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>167 mg. cortisone$ per 200 gm. rat + 53 units of insulin per 200 gm. rat</td>
<td>3</td>
<td>9.5 ± 0.3$</td>
<td>4.0 ± 1.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* Activity in TPO units.
† Average of four rats so injected.
‡ Column 4 + increment due to insulin—for calculation, see text.
§ Cortisone (cortisone acetate) obtained from Merck and Co., Rahway, N. J.
¶ Standard deviation.

described as additive (compare Column 3 with Column 5). The calculation for the summation of the inductive effects of insulin and cortisone given separately was the same as that used in the preceding section. The equivalence of 53 units of insulin with the injection of 140 mg. of tryptophan which was shown in the previous discussion on calculation was also used here. It was added to the value of 4.0 units of activity by the aid of Fig. 1. These hormonal results will be evaluated more fully in the discussion.

**Alloxan Diabetes and the TPO Level**—A study was made of TPO activity in alloxanized animals as a function of time elapsed after the injection of alloxan. The results are illustrated in Fig. 3.
The following conclusions were drawn from the experiments listed in Table IV. (a) Adrenalectomy abolished the first peak, indicating that stress was its cause. The reason for the discrepancy between the results of this and the preceding experiment is not understood although it was necessary to administer glucose to this group by injection to keep them alive and they were moribund at the time they were killed. (b) The TPO-synthesizing system was not damaged by alloxan or by the short diabetic state. (c) The rate-limiting step in TPO synthesis manifested itself when the TPO level had increased 9-fold over that found in the normal rat. (d) Insulin tends to correct the state responsible for the increase in TPO; the activity would not be expected to return to normal levels because insulin is an active inducer at this dosage. (e) Insulin in a dosage too low to induce TPO and almost high enough to control the diabetes resulted in a return of TPO to normal levels.

**Production in Vitro of TPO**—Early in the work on the TPO system it was found that this enzyme could be induced, to a certain extent, in normal rat liver slices. Slices from normal rat liver were incubated for 3 hours in the presence of 3 mM tryptophan. As a control, a weighed portion of the same liver was homogenized and an amount of tissue (50 mg.) equivalent to that in the slice was incubated under the same conditions. This represents the amount of kynurenine formed by the TPO originally present in the normal liver. The activity remains linear over the 3 hour incubation period. It was found that the slice, on the average, showed 50 per cent greater activity than the homologous homogenate. This difference was significant by the *t* test (*P* < 0.02). In Table V are illustrated the values obtained for a series of five animals with the liver homogenate compared to the slice. That this difference was not due to destruction of TPO in the liver upon homogenization was indicated by the fact that homogenization in the presence of tryptophan (to serve as a protective agent for the TPO) did not alter the results. In another experiment, slices were incubated for 1 hour, homogenized and then incubated for 2 hours more. The resultant activity was close to that of the slice incubated for 3 hours and not that of the homogenate incubated for the same length of time.

Recently, Civen and Knox (18) reported that in their procedure TPO could be induced in normal rat liver slices when incubated in serum. The level of TPO was approximately double that of the homologous homogenate. Efimochkina (19) reported a large increase in TPO activity in liver sections incubated in the presence of L-tryptophan. The increase in activity was considerably greater than that obtained in our laboratory.

In other experiments it was found that the kynurenine formed by the liver slice diffused out freely and was to be found almost completely in the incubating fluid. A variety of substances was examined for their ability to stimulate synthesis of TPO *in vitro*. Adenosine triphosphate at 2 × 10⁻⁴ M, L-histidine at 10⁻³ M, histamine at 10⁻⁴ M, acetyl β-methyl choline chloride at 5 × 10⁻⁴ M, hydrocortisone at 10⁻⁴ M, insulin-zinc at 5.5 × 10⁻⁵ M and triiodothyronine at 10⁻⁴ M had no effect on the slice or homogenate. All concentrations were final concentrations in the incubation medium.

Several substances were tested for their effect on TPO itself. Homogenates were made from the livers of rats induced in vivo. These were incubated with tryptophan and the substance being tested. It was found that serotonin, carboxenzoxy L-tryptophan, and L-epinephrine (all at 3 × 10⁻³ M final concentration) were potent inhibitors of the TPO system. Varying the glucose concentration in the buffer from 0 to 5 times the amount usually used had no effect on the enzyme action.

**DISCUSSION**

**Action of Some Hormones**—It is possible that insulin is active in the TPO system by making more energy available to the enzyme-forming system. That this may not be the case, how-

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**Table IV**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Activity of slice</th>
<th>Activity of homog.</th>
<th>TPO activity in rats given only alloxan (Fig. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 5</td>
<td>7 days after adrenalectomy injury</td>
<td>6 1.3 ± 0.61</td>
<td>9.9</td>
</tr>
<tr>
<td>2 4</td>
<td>24 hrs. after alloxanization injury</td>
<td>30 13.0 ± 0.7</td>
<td>1.7</td>
</tr>
<tr>
<td>3 5</td>
<td>72 hrs. after alloxanization injury</td>
<td>78 10.8 ± 0.7</td>
<td>11.8</td>
</tr>
<tr>
<td>4 5</td>
<td>96 hrs. after alloxanization injury</td>
<td>102 5.0 ± 1.2</td>
<td>11.9</td>
</tr>
<tr>
<td>5 5</td>
<td>At 24, 48, and 72 hrs. after alloxanization injection 5 to 7 units of insulin§</td>
<td>96 1.7 ± 0.4</td>
<td>11.9</td>
</tr>
</tbody>
</table>

* Activity in TPO units.
† Alloxan: 40 mg./200 gm. rat.
‡ Standard deviation.
§ Urine showed 3+ glucose just before killing.

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**Table V**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Activity of slice</th>
<th>Activity of homog.</th>
<th>Percent increase of slice over homog.</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>1.06</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>2.64</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>1.89</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1.94</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>3.57</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.40 ± 0.70</td>
<td>1.60 ± 0.33</td>
<td>50</td>
</tr>
</tbody>
</table>

* Activity in TPO units.
† Standard deviation is used throughout.

TPO activity was found to be 4.0 ± 0.4 units*. This is to be compared to the maximal value for the peak of 9.9 ± 1.7 units (Fig. 3). Consequently, of the increment over the normal level as represented by the first peak, 60 to 70 per cent was due to endogenous insulin liberated by the alloxan and 30 to 40 per cent of this value was probably due to the stress reaction caused by alloxan injection. Since a dip occurs in the curve in the 24 to 48 hour period, stress cannot be the cause for the second peak.
ever, is indicated by the fact that insulin in the fasted rat produces a greater increase in TPO than in the normal, fed rat. If glucose utilization for energy were rate-limiting, it would be expected that upon injection of insulin the TPO level would be greater in the fed (greater glucose and oxygen levels) than in the fasted animal. As has been pointed out, just the reverse is found. In addition, the level of TPO in the 48-hour-fasted, un.injected animal is the same as in the normal, un.injected rat despite their differing glucose levels.

Another possibility is that insulin may make tryptophan available to the TPO enzyme-forming system. Insulin can not achieve this via hydrolytic or catabolic mechanisms. However, it could function by affecting the permeability of either the cellular or intracellular membrane to tryptophan. Sources of free tryptophan might be the amino acid pool of plasma or liver cell. This is essentially an extension of the concept of Levine and Goldstein, which suggests that insulin increases cell permeability to glucose.

The results of the simultaneous experiment in which insulin and tryptophan are injected at the same time indicate that their inductive powers are additive. This can be interpreted to signify that the tryptophan at the site of the enzyme-forming system comes from two sources; namely, the tryptophan injected and the tryptophan which is released from an endogenous pool by the insulin.

The action of cortisone can also be interpreted as affecting the amount of tryptophan available to the enzyme-forming system. It is known that cortisone causes a metabolic shift to protein catabolism resulting in the liberation of amino acids. The tryptophan thus produced might be available to the enzyme-forming system and induction would ensue. Knox and Auerbach also believe this to be a reasonable interpretation.

To summarize, hormonal induction may be a form of substrate induction in that certain hormones might affect the availability of tryptophan for the enzyme-forming system. The evidence for this is indirect and other mechanisms are possible, including the direct action of a hormone on the enzyme-forming system. If a change in tryptophan concentration occurs then the enzyme-forming system would have to be small for it has been shown by Lee (20) that TPO induction using tryptophan in vivo is not accompanied by gross changes in tryptophan concentration in the liver.

A matter of concern was the relatively high dose of insulin necessary to obtain induction of TPO. The rat is essentially a poor animal to use in a study of this hormone, since it is far less sensitive to the effects of the hormone than the mouse. Another factor is that induction of TPO with tryptophan requires relatively large doses of the amino acid, which makes the need for large doses of hormone less surprising.

Tryptophan Induction of TPO—If a rat were rapidly to consume, digest, and absorb 6 gm. of the average diet, he would ingest about 23 mg. of tryptophan. This amount of the amino acid is sufficient to cause a 3-fold increase in TPO when injected into a 200 gm. rat (Fig. 1). On this basis it is concluded that a 10-fold increase in TPO is obtained only under laboratory conditions.

Alloxan Diabetes and TPO Activity—As has been shown in this study, the level of TPO, a key enzyme in the metabolism of tryptophan, changes radically after diabetes is induced with alloxan. Possible interpretations of this information are: (a) this diseased state causes the production or liberation of large quantities of tryptophan; (b) alloxan blocks the formation of nicotinic acid from tryptophan and the TPO level increases due to the accumulation of tryptophan and/or its metabolites (kynurenine can also induce TPO but it does not accumulate in alloxan diabetes); (c) the diabetic state is caused by an excess of tryptophan or a metabolite thereof. It would appear that alloxan diabetes may be related in some manner to tryptophan metabolism.

SUMMARY

1. The induction of tryptophan peroxidase by tryptophan is not a linear function of dosage injected. Small doses are more efficient than large ones.

2. Insulin can induce formation of tryptophan peroxidase in vivo, and some of this induction is independent of the adrenals.

3. The inducing effects of tryptophan and insulin are additive, as are those of insulin and cortisone.

4. Tridiothyonine and diethylstilbestrol have no effect on tryptophan peroxidase under the experimental conditions used, but testosterone can induce a small increase in tryptophan peroxidase activity.

5. After injection of alloxan, the tryptophan peroxidase level shows a biphasic increase as a function of time elapsed after injection. The first peak is reached 6 hours postinjection; at 24 hours the TPO activity returns to normal levels; and at 72 hours the second peak is reached and maintained. The changes in tryptophan peroxidase level are independent of the glucose concentration in the urine.

6. Small increases in tryptophan peroxidase can be induced in liver slices from normal rats.

7. The hormonal relationships in the production of this adaptive enzyme are discussed.

REFERENCES

Induction of Tryptophan Peroxidase of Rat Liver by Insulin and Alloxan
Joseph M. Schor and Earl Frieden


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