In Paper I (1) it was reported that the amount of the liver enzyme, picolinic carboxylase, is greatly increased in diabetic rats, while other related enzymes are not changed greatly. In this paper some of the factors that influence the level of this enzyme in rat liver are described. A major role was found for adrenal steroids, which are required for the increase to occur. The increase in enzyme level is not a simple response to cortisone-like hormones, however, as the extent of the response is determined by other physiological factors. In general the changes of picolinic carboxylase parallel those reported for glucose-6-phosphatase (2). The finding of the same type of change in enzyme level with enzymes that have unrelated functions and occur in different cell fractions indicates that the effects of certain hormones may be to alter greatly the enzymatic composition of cells and not merely to influence the activities of individual enzymes.

Methods

The assays of enzymes were carried out as described in Paper I (1). Picolinic carboxylase activity is expressed as change in optical density at 360 μ per minute in the assay system; tryptophan peroxidase activity is expressed as the optical density difference at 360 μ between deproteinized mixtures incubated for 1 hour with and without tryptophan.

Hypophysectomized female rats were purchased from the Charles River Breeding Laboratories, Inc. At the conclusion of experiments involving these animals, the functional absence of pituitary hormones was confirmed by inspecting the uteri, which were atrophic. Rats were made diabetic with alloxan as described previously (3). Adrenalectomies were performed on diabetic rats only after 2 weeks of high water consumption and glycosuria. Adrenalectomized animals were given water containing 0.9 per cent NaCl ad libitum, and the completeness of the adrenalectomy was determined by the decrease in weight when tap water was substituted for the saline drinking water. Adrenalectomized animals were used in experi-
ments not less than 2 weeks after the operation. All animals were main-
tained on a commercial stock diet.

Results

The essential role of the adrenal hormones in establishing and maintain-
ing increased levels of picolinic carboxylase in diabetic animals was indi-
cated by the effect of adrenalectomy. In Table I are shown the levels of

Table I

Effect of Adrenalectomy and Cortisone on Liver Picolinic Carboxylase in Diabetic Rats

Diabetic adrenalectomized rats were given 1 mg. of cortisone acetate subcutane-
ously each day for 4 days preceding sacrifice. The diabetic controls were animals
given alloxan at the same time as some of the other animals represented in this

table. The values for picolinic carboxylase have not been corrected for the non-

enzymatic reaction, which has a value of about 0.015.

<table>
<thead>
<tr>
<th>Diabetic adrenalectomized</th>
<th>Diabetic controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cortisone</td>
<td>Cortisone</td>
</tr>
<tr>
<td>0.015, 0.016, 0.019, 0.032</td>
<td>0.084, 0.090, 0.175</td>
</tr>
</tbody>
</table>

Table II

Increase of Picolinic Carboxylase with Daily Administrations of Cortisone

Diabetic adrenalectomized rats were used several days after testing for adrenal

sufficiency. 0.1 mg. of cortisone acetate was injected subcutaneously into each rat

at the start of the experiment and at 24 hour intervals until the time of sacrifice.

<table>
<thead>
<tr>
<th>Hrs. after 1st dose</th>
<th>16</th>
<th>40</th>
<th>64</th>
<th>136</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picolinic carboxylase</td>
<td>0.029,</td>
<td>0.034,</td>
<td>0.125,</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>0.036</td>
<td>0.052</td>
<td>0.180</td>
<td></td>
</tr>
</tbody>
</table>

picolinic carboxylase in livers of animals adrenalectomized 7 to 21 days

following the establishment of diabetes. The control animals were made
diabetic at the same time as those eventually adrenalectomized. It is

seen that the adrenalectomized animals have enzyme levels reduced to the

very low levels characteristic of normal animals and that administration of
cortisone causes the levels to increase to those characteristic of diabetes.

In Table II is shown the time-course of the response to cortisone ad-

ministration to diabetic adrenalectomized rats. The response of the liver
to cortisone is slow. During the first 24 to 48 hours, the increase in enzyme

level is small or absent. After 3 days, however, with daily administrations

of the hormone, the picolinic carboxylase activity increases to very high
levels, equal to or exceeding the highest levels seen in livers of diabetic rats. No difference was detected in response to cortisone given at levels of 0.2 mg. to 10 mg. per animal per day.

The possibility that the action of cortisone is indirect was explored in experiments with hypophysectomized rats (Table III). In these, the level of picolinic carboxylase is within the normal range. When cortisone is administered, the level of enzyme increases to that found in diabetic animals, whereas similar doses of cortisone have little, if any, effect in normal animals.

**TABLE III**

*Picolinic Carboxylase in Livers of Hypophysectomized Rats*

Normal and hypophysectomized rats were given 1 mg. of cortisone acetate each of the 3 days preceding sacrifice.

<table>
<thead>
<tr>
<th>Hypophysectomized</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisone</strong></td>
<td><strong>No cortisone</strong></td>
</tr>
<tr>
<td>0.138, 0.170</td>
<td>0.028, 0.030</td>
</tr>
</tbody>
</table>

**TABLE IV**

*Differential Effect of Cortisone on Tryptophan Peroxidase (TP) and Picolinic Carboxylase (PC)*

<table>
<thead>
<tr>
<th>Hypophysectomized</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No cortisone</strong></td>
<td><strong>Cortisone</strong></td>
</tr>
<tr>
<td>TP</td>
<td>0.142</td>
</tr>
<tr>
<td>PC</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Cortisone had been shown previously to cause an increase in liver tryptophan peroxidase (4). Tryptophan peroxidase is an adaptive enzyme (5), and the time required for the level of this enzyme to increase is several hours, whether tryptophan or cortisone is the inducing agent. In cortisone-treated hypophysectomized or diabetic adrenalectomized animals, which have increased levels of picolinic carboxylase, tryptophan peroxidase was also found to be elevated, in contrast to 3-hydroxyanthranilic oxidase which remained at normal levels. Normal animals given cortisone for several days, however, showed inconsistent, modest increases in picolinic carboxylase, whereas tryptophan peroxidase was consistently elevated. The representative values are shown in Table IV.

Adrenalectomized diabetic animals do not exhibit the elevated blood
sugar levels or glycosuria of diabetic animals, but the glycosuria returns on administration of cortisone. The change in picolinic carboxylase, however, is not a result of changes in carbohydrate metabolism. This was shown in experiments in which cortisone was administered to normal animals. Small doses of cortisone, which produce modest increases in picolinic carboxylase in non-diabetic animals, do not cause glycosuria. Large doses (10 mg. per day) were given to rats for approximately 1 week, and the animals were force-fed to maintain body weight. The animals became glycosuric, but the levels of liver picolinic carboxylase ranged between 0.040 and 0.065, not significantly different from the levels produced by small doses of cortisone.

Fractionation of liver homogenates has shown that picolinic carboxylase is found only with soluble fraction. Livers were homogenized in Potter-Elvehjem type homogenizers (Teflon pestle in polyethylene tube) in 0.25 M sucrose and centrifuged for 10 minutes at 18,000 × g in the cold. The supernatant fluid was collected, an aliquot was saved for analysis, and the remainder was centrifuged at 50,000 × g at 0°. The sediment obtained in the second centrifugation (microsomes) was suspended in one-fourth the original volume of 0.25 M sucrose. Only a trace of activity was found in the unwashed particles, whereas all of the activity of the original extract was found in the final supernatant fluid. 3-Hydroxyanthranilic oxidase has a similar distribution. Therefore in the assay of picolinic carboxylase the microsome fraction was studied in the presence of added oxidase of rat liver acetone powder, which provided the intermediate substrate for the carboxylase. The changes in the level of picolinic carboxylase are thus seen to be restricted to the soluble fraction of liver protein. This is in contrast to the similar changes reported for glucose-6-phosphatase, which occur in microsomes (2).

DISCUSSION

The experiments reported in this paper indicate that several factors are involved in determining the levels of enzymes in the liver. Cortisone or similar steroids cause both picolinic carboxylase and tryptophan peroxidase to increase many fold, but there are many differences in the responses of the two systems to the hormone. The changes in tryptophan peroxidase are complete within a few hours, whereas several days are required for the changes in picolinic carboxylase. The increase in tryptophan peroxidase can be produced equally in normal, diabetic, hypophysectomized, or adrenalectomized animals, whereas only modest increases in picolinic carboxylase are produced in normal or adrenalectomized animals given cortisone, compared with the large changes produced in adrenalectomized diabetic or hypophysectomized animals. These findings do not imply
that the tryptophan peroxidase system is a more sensitive indicator of the hormonal activity, since diabetes alone causes a great increase in picolinic carboxylase, with only a small increase in tryptophan peroxidase.

It is not known whether either hormone, insulin or cortisone, produces its effect through direct action on the liver. Since the changes are produced slowly, there is time for many indirect actions to occur. It is apparent, however, that, in addition to its effect in stimulating protein breakdown, cortisone causes an increase of certain enzyme activities: tryptophan peroxidase, picolinic carboxylase, and glucose-6-phosphatase. These changes occur at different rates and in different cell constituents (soluble proteins and microsomes). The effect of insulin in countering the influence of cortisone can be seen with only two of the three enzymes assayed, and in the case of picolinic carboxylase it does not seem to be related to changes in blood sugar. Although the mechanism by which insulin affects liver enzymes has not been established, the lack of correlation between elevated blood sugar and picolinic carboxylase increase suggests that the control of liver enzyme levels is a separate function of insulin.

We wish to acknowledge the technical assistance of Mrs. Catherine J. Rhodes and Mr. William H. Mills.

SUMMARY
The increase in picolinic carboxylase in the livers of diabetic rats depends upon the presence of cortisone or related adrenal hormones. Cortisone administration to diabetic adrenalectomized or hypophysectomized rats causes the enzyme to increase for several days, but has much less influence in normal animals. Evidence is presented for at least two hormonal factors that influence the levels of liver enzymes.

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Alan H. Mehler, E. G. McDaniel and James M. Hundley


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