EFFECT OF HORMONES UPON THE PRODUCTION OF
KETONE BODIES BY RAT LIVER SLICES

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(Received for publication, February 17, 1950)

Numerous investigators have established the important rôle played by
the endocrine glands in the regulation of fat metabolism in the intact
animal. The administration to fasting rats of anterior pituitary growth
hormone and adrenocorticotropic hormone (ACTH) (1) has been shown
to increase both the concentration of ketone bodies in the blood and the
rate of their excretion. Adrenalectomy, on the other hand, tends to have
the opposite effect (2). Since these effects are believed to be due to
alterations of the rate of ketone body production by the liver (1), it ap-
peared desirable to investigate the effects of these endocrines upon the
ketone body metabolism of surviving liver slices.

Methods

Male Sprague-Dawley rats, weighing 70 to 250 gm., were anesthetized
with nembutal, and the livers were excised and immediately chilled in
Petri dishes set in ice and lined with filter paper dampened with the incu-
bation medium. Slices approximately 0.3 mm. thick were cut free-hand,
weighed on a torsion balance, and 100 ± 5 mg. (wet weight) were dis-
tributed in duplicate 25 ml. Erlenmeyer flasks containing 2.0 ml. of Krebs
phosphate-buffered medium at pH 7.4. The dry weight of similar samples
of liver slices was determined for each animal, and all values were referred
to this initial dry weight. The flasks were incubated in an atmosphere of
100 per cent oxygen in a Warburg water bath at 37°, with constant shaking
at a rate of 120 strokes per minute, for periods of 5 to 120 minutes. After
the incubation period, the contents of the flasks were treated with zinc
sulfate, followed by barium hydroxide, to precipitate the proteins. The
material was then decanted into a centrifuge tube. After centrifugation,
duplicate aliquots of the supernatant were taken for the determination of
ketone bodies by the method of Greenberg and Lester (3). Ketone body
determinations were also made by the same method with blood from the
tail vein or inferior vena cava. All the results given are the means of

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duplicate determinations on each of two flasks for each point, and represent the total ketone bodies (acetone plus acetoacetic acid plus β-hydroxybutyric acid) expressed as acetoacetate. In our hands, this method has had a standard deviation of 2.8 per cent, permitting the detection of differences of 7.7 per cent in sets of duplicate determinations, with a probability of less than 5 per cent.

The hypophysectomized rats used in this study were obtained from a commercial source. They were observed over a period of 3 to 4 months before being used. Animals which grew appreciably during this time were not used in the study; however, animals which failed to grow but still maintained normal testicular size were used. A comparison of such “partially hypophysectomized” animals with totally hypophysectomized rats failed to show any differences of ketone body formation in vitro.

Animals were adrenalectomized in a single operation by the lumbar route. They were maintained on saline drinking water, and were used within 2 weeks after adrenalectomy. A careful search in each case failed to reveal adrenal tissue after death.

Thyroidectomy was performed by the usual technique, and the rats were maintained on calcium gluconate-calcium chloride drinking water for at least 2 weeks after the operation. Animals which continued to gain weight at a rate of 1 gm. or more per day were excluded as being incompletely thyroidectomized.

The hormones administered included crystalline growth hormone, adrenocorticotropic hormone, and thyroxine. Growth hormone was given in a dosage of 5 mg. per 100 gm. intraperitoneally 2 hours before the rats were killed. ACTH was given intraperitoneally in a dosage of 5.0 mg. per 100 gm. 3 hours before the animals were killed. Thyroxine was given subcutaneously in a dosage of 20 μg per day for 7 days.

Results

It is of interest to compare the metabolic activity of tissues prepared by us with the results obtained by others. The oxygen uptake of these preparations was 8.9 ± 0.9 μl. per mg. (dry weight) per hour, as compared with Edson’s value of 10.5 ± 1.9 μl. per mg. (dry weight) per hour (5). The rate of ketone body production over a 2 hour incubation period was 4.4 ± 0.22 μM of acetoacetate per 100 mg. (dry weight) per hour. This may be compared with the rates of 17.8 μM per 100 mg. per hour obtained by Cohen (6), 7.5 ± 0.45 μM per 100 mg. per hour by Edson (5), and 4.0 ±

1 Hormone Assay, Inc., Chicago, Illinois.
2 Armour, lot 41-L-3, supplied through the courtesy of Dr. John R. Mote.
3 Supplied through the kindness of Dr. W. T. Salter, Department of Pharmacology, Yale University.
0.2 μM per 100 mg. per hour by Shipley (7) under comparable conditions. These values may not be an accurate measure of the rate of production of ketone bodies, because they are not corrected for the amounts of these substances present in the slices before incubation. In an effort to eliminate this potential error, the time course of formation of ketone bodies was studied and found to be constant from 5 minutes to 2 hours (Fig. 1). The lines representing the concentration of acetoacetate in the medium, extended back to zero time, intercept the ordinate at a positive value. This represents the preformed acetoacetate which diffuses rapidly from the slice into the medium, reaching equilibrium before 5 minutes. In these experiments, therefore, an initial observation of the ketone body concentration in the system was made after 15 minutes incubation, and a final observation after 120 minutes. The difference between the two is taken as a measure of the true rate of synthesis of ketone bodies by the liver slices. The second point was chosen at 120 minutes because the curves show no tendency to level off up to that time. It is thus possible to eliminate exhaustion of substrate or of oxygen as limiting factors in determining the final rates. The rate of ketone body production thus determined is expressed as micromoles of acetoacetate produced per hour per 100 mg. of dry tissue (Qacac).

The first series of animals studied was fasted for 18 hours before removal of the liver for slicing (Table I). Whereas hypophysectomy and thyroidectomy caused a highly significant depression of the Qacac, adrenalectomy
caused a moderate but significant rise in the rate of production of acetoacetate. The effects of hypophysectomy can be explained entirely by the depression of thyroid function. The administration of maintenance doses of thyroxine to hypophysectomized animals restored their $Q_{acac}$ to normal. No differences could be demonstrated in the blood ketone body concentration of normal, adrenalectomized, or hypophysectomized animals fasted for 18 hours (Table II).

Since we were unable to show a difference between adrenalectomized and normal rats either in the rate of formation of ketone bodies by liver slices, or in the blood ketone body concentration after an 18 hour fast, the experiment was repeated with animals fasted for 40 hours, in the hope that this might bring out differences not discernible after the shorter period of deprivation. The data are presented in Table III. The $Q_{acac}$ of normal rats was unchanged by prolonging the period of fasting. The rate appears slightly lower than normal in the livers of adrenalectomized rats, but the difference is not significant. The prolonged fast has, however, altered the $Q_{acac}$ of the adrenalectomized rat liver, for the differences between the rats fasted 18 hours and 40 hours are highly significant ($P < 0.01$).

Although removal of the adrenal gland does not alter the $Q_{acac}$, and the

### Table I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mean ± s.e.*</th>
<th>Compared to normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>3.4 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>7</td>
<td>1.3 ± 0.17</td>
<td>3.94 &lt; 0.01</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>7</td>
<td>1.5 ± 0.25</td>
<td>3.49 &lt; 0.01</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>6</td>
<td>4.7 ± 0.28</td>
<td>2.21 0.02 &lt; 0.05</td>
</tr>
<tr>
<td>Hypophysectomized + thyroxine</td>
<td>3</td>
<td>3.4 ± 0.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Hypophysectomized + 1.5 mg. growth hormone daily for 2 days</td>
<td>2</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

* S.e., $\sqrt{(Sd^2)/(n(n-1))}$.
† Student's formula, corrected for small numbers.
‡ From Fisher's tables.
removal of the pituitary appears to exert its effect entirely through a reduction of thyroid activity, it seemed possible that effects might be

TABLE II

Effect of Various Endocrine Manipulations on Blood Ketone Body Concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood ketone body formation after 18 hr.</th>
<th>Blood ketone body formation after 40 hr.</th>
<th>t*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>17.4 ± 2.3 mg. per 100 ml.</td>
<td>47.4 ± 3.2 mg. per 100 ml.</td>
<td>7.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>15.2 ± 1.1 mg. per 100 ml.</td>
<td>18.4 ± 2.7 mg. per 100 ml.</td>
<td>0.26</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>15.4 ± 0.4 mg. per 100 ml.</td>
<td>Concentration before treatment</td>
<td>Concentration after treatment</td>
<td>Change</td>
</tr>
<tr>
<td>Normal 18 hr. fast</td>
<td>49.1 ± 6.3 mg. per 100 ml./hr.</td>
<td>30.1 ± 4.4 mg. per 100 ml.</td>
<td>0.46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ 5 mg. growth hormone per 100 gm.</td>
<td>35.5 ± 4.2 mg. per 100 ml.</td>
<td>3.1 ± 2.8 mg. per 100 ml.</td>
<td>0.72</td>
<td>&gt;0.4</td>
</tr>
</tbody>
</table>

* See foot-note, Table I.

TABLE III

Effect of Various Endocrine Manipulations on Rate of Synthesis of Ketone Bodies by Surviving Rat Liver Slices in Krebs Phosphate Buffer, 40 Hour Fast

Techniques as outlined in Table I.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± s.e.*</th>
<th>Compared to normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.4 ± 0.23</td>
<td>1.90 0.1 &gt; 0.05</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>2.8 ± 0.30</td>
<td>0.59 &gt; 0.5</td>
</tr>
<tr>
<td>Normal + 5 mg. growth hormone per 100 gm.</td>
<td>3.3 ± 0.11</td>
<td>0.72 &gt; 0.4</td>
</tr>
<tr>
<td>Normal + 5 mg. ACTH per 100 gm...</td>
<td>3.8 ± 0.38</td>
<td></td>
</tr>
</tbody>
</table>

* See foot-note, Table I.

obtained by the administration of large amounts of growth and adrenocorticotropic hormones. Experiments were therefore performed upon ani-
mals fasted 40 hours, following the injection of adrenocorticotropic and of crystalline growth hormone. The administration of 5 mg. of these hormones per 100 gm. of body weight failed to alter the \( Q_{acac} \) in any way (Table III).

Both ACTH and growth hormone have a considerable effect upon the blood concentration of ketone bodies. Fasting from 18 to 40 hours causes a marked increase in the concentration of acetoacetate in the blood. Adrenalectomy prevents this increase (Table II). The administration of amounts of growth hormone and of ACTH which do not alter the \( Q_{acac} \) causes a significant increase in the blood ketone bodies (Table II). Growth hormone may be somewhat more effective than ACTH in this respect, since the increase following the administration of the latter is barely outside of the 5 per cent limits of probability.

In a few experiments it was found that growth hormones had no effect on ketone body production when incubated with liver slices from normal, 18 hour fasted hypophysectomized, 40 hour fasted hypophysectomized, and 40 hour fasted adrenalectomized animals.

**DISCUSSION**

These results are difficult to reconcile with previous observations of the effects of pituitary extracts in vitro and in vivo (1, 7, 8). Bennett et al. (1) have produced convincing evidence that one of the effects of growth hormone and of ACTH is to increase the production of ketone bodies in the intact animal. In the present experiments in vitro, however, the liver failed to respond as expected from the experiments on intact animals. Yet the potency of the hormone preparations and the accuracy of timing of the effects is attested by the fact that elevations of the blood ketone body concentration were produced both with growth hormone and with ACTH.

It is also difficult to explain the discrepancy between our results and those of Campbell and Davidson (8). It should be noted, however, that the increased \( Q_{acac} \) which they observed occurred 24 hours after the administration of a crude pituitary extract, whereas the present experiments tested the acute effects of highly purified hormone preparations, which were essentially free from extraneous physiological activity.

Shipley et al. (7) have reported an augmentation of the \( Q_{acac} \) after the addition of relatively crude pituitary extracts to liver slices incubated in rat serum. In their experiments smaller but definite effects were observed after the addition of boiled extract. This raises the possibility that they were observing an effect of some fraction other than those usually included among the pituitary hormone group. The failure of our liver slice preparations to respond to purified crystalline growth hormone in vitro suggests,
in any case, that the results observed by Shipley et al. may not have been due to this hormone.

SUMMARY

The effects of various hormonal manipulations upon the formation of ketone bodies by rat liver slices have been investigated. The following has been shown.

1. To obtain true rates of production, allowance must be made for the preformed ketone bodies in the liver slice.

2. Removal of the adrenal gland fails to depress the rate of formation of ketone bodies after 18 hours of fasting and may even raise it.

3. Removal of the hypophysis causes a reduction in the rate of formation of ketone bodies, which can be accounted for entirely on the basis of reduced activity of the thyroid gland. The effects of hypophysectomy can be returned to normal by the administration of thyroxine.

4. Adrenalectomy prevents the normal rise of blood ketone body concentration from the 18th to the 40th hour of fasting. During this time there is also a significant fall in the rate of production of ketone bodies by liver slices.

5. The administration of crystalline anterior pituitary growth hormone in vitro or in vivo and of adrenocorticotropic hormone in vivo does not alter the Qacet.
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