TISSUE COENZYME A IN NORMAL AND INDUCED STATES OF GROWTH

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Results of studies on pantothenic acid-deficient rats stimulated with anterior pituitary growth hormone (1–3) suggest involvement of pantothenic acid, or its metabolically active form, coenzyme A (4), in the mechanism by which growth hormone stimulates the growth process. Lotspeich (1), for example, describes the rapid production of symptoms of acute pantothenic acid deficiency as a sequel to the administration of growth hormone in adult rats maintained on a pantothenic acid-deficient diet. Beare, Beaton, and McHenry (2) note that pantothenic acid-deficient rats fail to respond anabolically to growth hormone, while Hazelwood, Bennett, and Nelson (3) have recently reported a reduction in the nitrogen-retaining effect of growth hormone in normal and adrenalectomized pantothenic acid-deficient rats.

These observations, and those of Greenbaum (5) and Greenbaum and McLean (6, 7) on adult rats, emphasizing the effects of growth hormone on fat metabolism, in which coenzyme A is known to be intimately involved, have prompted us to study coenzyme A concentrations in liver and kidney tissue obtained from rapidly growing immature normal rats, from immature rats in which the growth process has been arrested by hypophysectomy, from normal adult rats, and from animals in the last two groups in which the growth process has been stimulated with growth hormone.

EXPERIMENTAL

Animals—Female rats of the Sprague-Dawley strain, both normal and hypophysectomized, were used in all experiments. The hypophysectomized rats were 28 to 30 days old at the time of operation.

Diet—All animals were housed in separate cages and were maintained on a diet containing 67.5 per cent ground whole wheat, 15 per cent casein, 10 per cent whole milk powder, 0.75 per cent sodium chloride, 1.5 per cent calcium carbonate, and 5.25 per cent corn oil (8). The diet was supplemented with fresh lettuce and haliver oil.

Methods—In general, body weight and food intake were determined
daily for each rat for a period of sufficient duration to establish whether the animals were growing or in an arrested state of the growth process. The animals were then grouped and observations continued for a period of 10 days. During this period nitrogen storage and gain in body weight were induced in a group of the hypophysectomized rats and in a group of the normal adult animals by administering intraperitoneally a lyophilized preparation of purified growth hormone dissolved in 0.9 per cent saline. The hormone preparation was administered to the hypophysectomized rats in doses of 50 \( \gamma \) per day for 5 days and 100 \( \gamma \) per day for a subsequent 5 day period. In the experiments in which nitrogen storage was induced in normal adult rats, the hormone was administered in doses of 500 \( \gamma \) per day for the 10 day period. Upon termination of the 10 day period of hormonal stimulation, the treated rats and the untreated controls, both normal and hypophysectomized, were sacrificed by stunning and exsanguination. Liver and kidney were quickly excised, weighed, frozen on dry ice, and stored at \(-15^\circ\) to \(-20^\circ\) until convenient for coenzyme A determination. Coenzyme A concentrations in tissue were determined by the sulfanilamide acetylation procedure of Kaplan and Lipmann (9). Total nitrogen was determined by the procedure of Hiller, Plazin, and Van Slyke (10) on aqueous homogenates of tissue prepared for coenzyme A assay. Dry weights were determined on the same homogenates by drying an aliquot overnight at 115\(^\circ\).

Results

Data on each group of rats, showing the average food intake, initial body weight, and change in body weight over the 10 day period of observation, are presented in Table I. Liver coenzyme A is expressed in terms of Kaplan-Lipmann units of coenzyme A per gm. of wet weight, per 100 gm. of body weight, per 100 mg. of total homogenate nitrogen, and per 100 mg. of dry weight.

As has been previously observed, hypophysectomy greatly reduced the food intake of the immature rat, and such animals failed to gain weight over the 10 day experimental period. Hypophysectomized rats stimulated with growth hormone gained 15 gm. in body weight and increased their food consumption approximately 10 per cent. Normal adult rats gained 5 gm., while normal adult rats stimulated with growth hormone gained 14 gm.

The concentration of liver coenzyme A expressed on the basis of 100 gm. of body weight was found to be significantly lower in both hypophysectomized immature and normal adult rats than in rapidly growing

\footnote{We are indebted to the Armour Research Laboratories for a generous supply of purified growth hormone preparation, Somar M-10810.}
Immature normal rats. Stimulation of the normal adult rat with growth hormone resulted in a significant elevation in liver coenzyme A in this animal, while stimulation of the hypophysectomized immature rat with growth hormone resulted in a somewhat further but insignificant reduction in the level of liver coenzyme A in this animal.

**Table I**

*Liver Coenzyme A in Normal and Induced States of Growth*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats in group</th>
<th>Body weight at start of 10 day experimental period (gm.)</th>
<th>Change in body weight over 10 day period (gm.)</th>
<th>Daily food intake (gm. per day)</th>
<th>Terminal liver weight (gm.)</th>
<th>Liver coenzyme A, Kaplan-Lipmann units (mg. per 100 mg. body weight)</th>
<th>Per 100 mg. total homogenate N (mg.)</th>
<th>Per 100 mg. dry weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, immature</td>
<td>6</td>
<td>169</td>
<td>+19</td>
<td>13.1</td>
<td>7.18</td>
<td>150</td>
<td>(±26)</td>
<td>559</td>
</tr>
<tr>
<td>Hypophysectomized, immature</td>
<td>9</td>
<td>103</td>
<td>+0</td>
<td>5.2</td>
<td>3.27</td>
<td>127</td>
<td>(±22)</td>
<td>406</td>
</tr>
<tr>
<td>Hypophysectomized, immature, stimulated with growth hormone†</td>
<td>11</td>
<td>111</td>
<td>+15</td>
<td>5.8</td>
<td>4.11</td>
<td>119</td>
<td>(±15)</td>
<td>387</td>
</tr>
<tr>
<td>Normal, adult</td>
<td>6</td>
<td>281</td>
<td>+5</td>
<td>12.8</td>
<td>7.68</td>
<td>106</td>
<td>(±4.4)</td>
<td>284</td>
</tr>
<tr>
<td>&quot; &quot; &quot; stimulated with growth hormone†</td>
<td>6</td>
<td>310</td>
<td>+14</td>
<td>13.7</td>
<td>9.23</td>
<td>122</td>
<td>(±16)</td>
<td>347</td>
</tr>
</tbody>
</table>

* The values tabulated are averages calculated for each group of animals. The values in parentheses are standard deviations.
† Preparation and administration of growth hormone described under "Methods."

Results of coenzyme A determinations on kidney tissue obtained from the same series of rats are summarized in Table II. The concentration expressed on the basis of gm. of wet weight, per 100 mg. of total homogenate nitrogen, and per 100 mg. of dry weight was not significantly al-

2 Data expressed on the basis of units of tissue coenzyme A per 100 gm. of body weight were selected for statistical analysis, since it was felt that a better comparison of total coenzyme A concentration in animals of different weights would be obtained. The methods of Fisher (11) were used in analyzing the data, and *P* values <0.01 were considered significant.
tered by hypophysectomy of the immature rat, by cessation of growth in the normal rat as a result of attainment of the adult state, or by stimulation of the growth process in either the hypophysectomized immature or normal adult rat with growth hormone. Although coenzyme A concentration expressed on the basis of 100 gm. of body weight was found to be significantly higher in hypophysectomized immature rats and significantly lower in normal adult rats than in normal immature animals, there appears to be no relationship to the growth process. Stimulation of

### Table II

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats in group</th>
<th>Terminal kidney weight</th>
<th>Kidney coenzyme A, Kaplan Lipmann units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>Per 100 gm. wet weight</td>
</tr>
<tr>
<td>Normal, immature</td>
<td>6</td>
<td>0.689</td>
<td>49</td>
</tr>
<tr>
<td>Hypophysectomized, immature</td>
<td>9</td>
<td>0.557</td>
<td>(±9.8)</td>
</tr>
<tr>
<td>Hypophysectomized, immature, stimulated with growth hormone</td>
<td>11</td>
<td>0.632</td>
<td>51</td>
</tr>
<tr>
<td>Hypophysectomized, immature, stimulated with growth hormone</td>
<td>6</td>
<td>0.836</td>
<td>45</td>
</tr>
<tr>
<td>“ “ stimulated with growth hormone</td>
<td>6</td>
<td>0.942</td>
<td>47</td>
</tr>
</tbody>
</table>

* The values tabulated are averages calculated for each group of animals. The values in parentheses are standard deviations.

† Same series of animals described in Table I.

either hypophysectomized immature or normal adult rats with growth hormone produced no significant alteration in the level of kidney coenzyme A in these animals.

### DISCUSSION

Results of studies on the pantothenic acid requirement of the rat (12) seem to indicate that the normal adult requires less dietary pantothenic acid than the rapidly growing immature rat. This general observation and the finding that metabolically active pantothenic acid is largely bound in coenzyme A (4) suggest that the metabolic requirement for coenzyme A might also be similarly related to the growth process. The results of our studies on the concentration of coenzyme A in liver tissue...
obtained from rapidly growing immature normal rats, from normal adult rats, and from normal adult rats stimulated with growth hormone, as well as the results of studies on the concentration of liver coenzyme A in hypophysectomized immature rats, appear to be in complete agreement with this suggestion. Stimulation of the hypophysectomized rat with growth hormone, however, failed to alter the concentration of coenzyme A significantly in liver tissue obtained from this animal, even though the rat was known to be storing nitrogen and gaining body weight at a rate which approached that for rapidly growing immature normal animals. A priori, this anomaly in the otherwise direct relationship between liver coenzyme A and the growth process might be considered to be due to the lack of tropic hormones other than growth hormone. In this respect the work of Tabachnick and Bonnycastle (13) on the effects of thyroidectomy and thyroxine on the concentration of coenzyme A in liver tissue is particularly suggestive. Thyroidectomy resulted in a significant reduction in the level of liver coenzyme A, and stimulation of the thyroidectomized rat with thyroxine restored concentrations of liver coenzyme A to normal or above. Since the hypophysectomized rat is essentially hypothyroid due to the lack of thyrotropic hormone, the inability of the hypophysectomized immature rat stimulated with growth hormone to increase the concentration of coenzyme A in its liver tissue might well be due to thyroxine deficiency.

In view of the greatly reduced food intake of hypophysectomized immature rats stimulated with growth hormone in comparison with that of rapidly growing immature normal rats, it might also be suggested that a suboptimal dietary intake or perhaps an alternative metabolic channeling of pantothenic acid and amino acids required for coenzyme A biosynthesis could be considered explanatory of the negative effect of growth hormone on liver coenzyme A in the hypophysectomized rat.

Results of studies concerning the effects of pantothenate and amino acid supplementation on liver coenzyme A in hypophysectomized rats stimulated with growth hormone, as well as studies on similar rats supplemented with thyroxine, will form the basis of subsequent reports.

SUMMARY

Concentrations of liver coenzyme A were found to be significantly lower in both hypophysectomized immature and normal adult rats than in rapidly growing immature normal rats. Induction of nitrogen storage and gain in body weight in the normal adult rat as a result of stimulation with anterior pituitary growth hormone are accompanied by a significant elevation in the concentration of coenzyme A in the liver of this animal. Stimulation of the growth process in the hypophysectomized rat with
growth hormone does not significantly alter the level of liver coenzyme A in this animal. Concentrations of kidney coenzyme A do not appear to be significantly altered by hypophysectomy of the immature rat, by cessation of growth in the normal rat as a result of attainment of the adult stage, or by stimulation of the growth process in either the hypophysectomized immature rat or the normal adult rat with growth hormone. There appears to be a direct relationship between liver coenzyme A and growth in the normal rat.

**BIBLIOGRAPHY**

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