STUDIES ON CARBOHYDRATE METABOLISM IN SCORBUTIC GUINEA PIGS*

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Scurvy is associated with disturbed carbohydrate metabolism. A diminished glucose tolerance has been observed by several workers in scorbutic guinea pigs (1-4), in scorbutic monkeys (5), and in human subjects with low blood ascorbic acid level (6, 7). Several workers (2, 4, 8) have reported a decrease in the liver and muscle glycogen content of scorbutic guinea pigs. Banerjee and Ghosh (9) have observed a decreased liver and muscle hexokinase activity in scurvy. Lahiri and Banerjee (10) have further shown a decreased turnover rate of the phosphorylated intermediates of the carbohydrate metabolism in scurvy. Banerjee and his coworkers have also observed degenerative changes in the islets of Langerhans (11), and diminished insulin content of the pancreas in scurvy (4). According to them, the disturbance in carbohydrate metabolism in scurvy may be mainly due to diminished insulin production by the pancreas, a view not subscribed to by other workers (12, 13).

At present, there are increasing evidences that insulin is involved in the intermediate metabolism of carbohydrates at the level of the Krebs cycle (14-19). It is possible that in scurvy there may be a disturbance in carbohydrate metabolism at the level of the Krebs cycle, due either to diminished insulin production or to the direct effect of the lack of vitamin C on the enzyme systems concerned with the oxidation of intermediates through the Krebs cycle.

In the present paper, the effect of injections of insulin on glucose tolerance and on liver and muscle glycogen contents in scorbutic guinea pigs is reported. As a preliminary study of the metabolism of intermediates through the Krebs cycle, the tissue content of α-keto acids and of citric, malic, and lactic acids of normal, scorbutic, and insulin-treated scorbutic guinea pigs has also been presented.

EXPERIMENTAL

Female guinea pigs, weighing from 250 to 300 gm., were fed green grass, soaked gram, and the scorbutic diet (20) for 5 to 6 days. Those animals

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which grew well on this diet were selected and separated into several groups, each group consisting of one normal, one scurbutic, and one insulin-treated scurbutic guinea pig. The guinea pigs in each group were fed equal amounts of the scurbutic diet. The normal control was fed 5 mg. of ascorbic acid daily. All the animals were fed 2 drops of adexolin (Glaxo) (vitamins A and D) twice a week. Regular insulin (Lilly) was injected subcutaneously into the animal intended for insulin treatment with a dose increasing from 0.1 to 0.3 unit per 100 gm. of body weight per day from the beginning of the 2nd week. On the day of the experiment, insulin injection was stopped.

**Glucose Tolerance Test and Tissue Glycogen Content**—An oral glucose tolerance test was made in the 4th week of the regime with the scurbutic diet on eleven groups of animals after they were fasted for 15 to 18 hours. Blood was collected from the toe by cutting the nail just distal to the root. The animals were killed after the glucose tolerance test and liver and skeletal muscle were removed for the glycogen estimations. The glucose tolerance test was also carried out on three normal guinea pigs treated with the same dosage of insulin for the same period of time as the insulin-treated animals.

### Table I
**Glucose Tolerance Test of Eleven Normal, Eleven Scurbutic, and Eleven Insulin-Treated Scurbutic Guinea Pigs**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fasting blood sugar, mg. per cent</th>
<th>Blood sugar, mg. per cent</th>
<th>45 min.</th>
<th>90 min.</th>
<th>150 min.</th>
<th>180 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>111 ± 4.6</td>
<td></td>
<td>154 ± 11.6</td>
<td>181 ± 13.1</td>
<td>144 ± 10.4</td>
<td>123 ± 11.0</td>
</tr>
<tr>
<td>Scurbutic</td>
<td>124 ± 5.3</td>
<td></td>
<td>197 ± 17.0</td>
<td>244 ± 18.4</td>
<td>248 ± 18.8</td>
<td>227 ± 17.6</td>
</tr>
<tr>
<td>Insulin-treated scurbutic</td>
<td>128 ± 4.4</td>
<td></td>
<td>226 ± 16.8</td>
<td>242 ± 18.9</td>
<td>198 ± 7.9</td>
<td>161 ± 9.8</td>
</tr>
</tbody>
</table>

**Statistical analysis t values**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fasting 45 min.</th>
<th>90 min.</th>
<th>150 min.</th>
<th>180 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between normal and scurbutic</td>
<td>1.8</td>
<td>2.0</td>
<td>2.7</td>
<td>6.7</td>
</tr>
<tr>
<td>&quot; &quot; insulin-treated scurbutic</td>
<td>2.6</td>
<td>3.5</td>
<td>2.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Between scurbutic and insulin-treated scurbutic</td>
<td>0.5</td>
<td>1.2</td>
<td>0.07</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Blood sugar was estimated by the method of Hagedorn and Jensen (21), glycogen was precipitated by the method of Grattan and Jensen (22), and the reducing sugar in the hydrolyzed precipitate was determined by the method of Hagedorn and Jensen (21). The results of these analyses are shown in Tables I, II, and III.

**Table II**

*Glucose Tolerance Test of Insulin-Treated Normal Guinea Pigs*

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Fasting blood sugar, mg. per cent</th>
<th>Blood sugar in mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After glucose feeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 min.</td>
</tr>
<tr>
<td>1</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>116</td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

*Glycogen Content of Tissues of Normal, Scorbatic, and Insulin-Treated Scorbatic Guinea Pigs*

Glycogen is given in terms of glucose equivalent in gm. per 100 gm. of wet tissue.

| Tissue Content of α-Keto, Citric, Malic, and Lactic Acids—In the 4th week of the regime with the scorbatic diet, the guinea pigs were killed by a sudden blow on the head. They were decapitated and the tissues were immediately removed, wrapped in filter paper to remove the adherent blood, and then dropped into weighed beakers containing 10 per cent trichloroacetic acid. The beakers were weighed again to obtain the weight of the tissues taken. For the estimation of α-keto acids, blood from the
neck was collected in a bottle containing iodoacetate in a proportion so as to obtain a final concentration of 0.2 per cent. The blood was immediately deproteinized by being pipetted into 10 per cent trichloroacetic acid. The whole operation required no more than 1 minute. For the estimation of citric, malic, and lactic acids, blood was collected without any anticoagulant and was immediately deproteinized. Tissues taken for analyses were blood, liver, kidney, brain, and cardiac muscle.

$\alpha$-Keto acid was estimated by the method of Lardy (23) with sodium pyruvate (E. Merck) as the reference standard. Citric acid was determined by the method of Speck, Moulder, and Evans (24), malic acid by the fluorometric method of Hummel (25), and lactic acid by the method of Barker and Summerson (26). The results of these analyses are shown in Tables IV, V, VI, and VII.

DISCUSSION

Scorbutic guinea pigs showed a highly significant lowered glucose tolerance in comparison with normal controls. Insulin treatment of the scorbutic animals did not significantly alter the fasting blood sugar and the blood sugar values of samples of blood taken 45 and 90 minutes after the feeding of glucose. However, after 150 and 180 minutes there was a marked lowering of the elevated blood sugar in the insulin-treated animals although the sugar level was still significantly higher than that of the normal animal. The peak of the blood sugar value of the scorbutic pig was also shifted from the 150th minute period to the 90th minute period after glucose was fed under the action of insulin. In this respect it simulated the glucose tolerance pattern of normal controls (Table I).

Glycogen content of the liver and the skeletal muscle diminished in scorbutic guinea pigs in comparison with that of the normal controls, and the decrease was highly significant statistically. Treatment with insulin strikingly improved the glycogen content of the liver and the skeletal muscle of the scorbutic guinea pigs, though the values did not reach the normal level (Table III).

It was also observed that the dosage of insulin used in these experiments did not affect the glucose tolerance of normal guinea pigs (Table II).

These results indicate that treatment with insulin for a prolonged period can correct to a great extent the disturbed carbohydrate metabolism in scurvy.

It is reported that in diabetes there occurs an altered pyruvate metabolism characterized by a high concentration of blood pyruvate (27). This defect in pyruvate metabolism has been ascribed to the diminished formation of cocarboxylase (28). In scurvy, which simulates diabetes in many aspects of the carbohydrate metabolism, it was thought that an
alteration in the pyruvate metabolism might occur. Table IV shows that, except in blood, there was no significant difference in the concentration of \( \alpha \)-keto acids in the tissues of the scorbutic guinea pigs and of the normal controls. As \( \alpha \)-keto acids consist of the three keto acids, pyruvic, \( \alpha \)-keto-glutaric, and oxalacetic acids, any alteration in the tissue content of any one of these acids in scurvy cannot be specifically known from the data presented. However, it seems reasonable to assume that scurvy is not associated with any derangement in the metabolism of \( \alpha \)-keto acids. The observation that insulin treatment did not affect the tissue content of

### Table IV

**\( \alpha \)-Keto Acid Content of Tissues of Normal, Scorbutic, and Insulin-Treated Scorbutic Guinea Pigs**

The figures in parentheses denote the number of animals. The values are given in mg. per 100 gm. of wet tissue.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (12)</td>
<td>2.34 ± 0.20</td>
<td>2.26 ± 0.33</td>
<td>5.48 ± 0.36</td>
<td>2.53 ± 0.39</td>
</tr>
<tr>
<td>Scorbutic (12)</td>
<td>3.19 ± 0.03</td>
<td>2.44 ± 0.21</td>
<td>5.89 ± 0.25</td>
<td>2.56 ± 0.21</td>
</tr>
<tr>
<td>Insulin-treated scorbutic (10)</td>
<td>2.69 ± 0.19</td>
<td>2.45 ± 0.23</td>
<td>5.38 ± 0.30</td>
<td>2.54 ± 0.20</td>
</tr>
</tbody>
</table>

**Statistical analysis \( t \) values**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between normal and scorbutic</td>
<td>4</td>
<td>0.4</td>
<td>0.9</td>
<td>0.06</td>
</tr>
<tr>
<td>&quot; &quot; insulin-treated scorbutic</td>
<td>1.2</td>
<td>0.4</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>Between scorbutic and insulin-treated scorbutic</td>
<td>2.6</td>
<td>0.03</td>
<td>0.07</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\( \alpha \)-keto acids in scurvy shows that insulin presumably has no effect on their utilization. This agrees with the findings of other investigators that pancreatic diabetes does not seem to alter pyruvate metabolism (29, 30). Foster and Villee (31) have shown that the defect in pyruvate and acetate metabolism in alloxan diabetes is not due directly to a lack of insulin but rather to a lack of some cofactor which is essential to the oxidation of these substances.

Metabolism of citric acid gives a picture different from that of \( \alpha \)-keto acids in scurvy. Table V shows an increased content of citric acid in the tissues of the scorbutic animals in comparison with that of the normal controls. Treatment with insulin brought the level of citric acid to normal. This defect in citric acid metabolism may be due either to an accelerated
synthesis of citric acid or to the presence of a metabolic block below the level of citric acid in the Krebs tricarboxylic acid cycle. The first possibility is rather remote because so far there is no evidence of an acceleration of the reactions leading to citric acid formation in scurvy. It has also been observed that under conditions of increased citric acid formation, as induced by feeding of butyrate or citrate in normal guinea pigs, there is no change in the amount of citric acid excreted in the urine (32). This indicates that the excess citric acid is efficiently disposed of if there is no defect in the reactions involved in its utilization. The alternative explanation is the more probable. Takeda and Hara (32) observed an increased urinary excretion of citric acid and ketone bodies in scurbutic guinea pigs fed butyrate together with citrate or malate. They ascribed this finding to the lowered aconitase activity brought about by the deficiency of ascorbic acid which maintains ferrous ions, the cofactor of the enzyme, in the reduced state.

In our experiment, insulin as well corrected the defect in citric acid metabolism, possibly by activating the aconitase enzyme and thereby facilitating metabolism of citrate. This explanation is at variance with that of Takeda and Hara.

Table VI shows an increased malic acid content of tissues in scurbutic guinea pigs in comparison with that of the normal controls. Insulin

### Table V

**Citric Acid Content of Normal, Scurbutic, and Insulin-Treated Scurbutic Guinea Pigs**

The figures in parentheses denote the number of animals. The values are given in mg. per 100 gm. of wet tissue.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (12)</td>
<td>5.08 ± 0.30</td>
<td>6.47 ± 0.26</td>
<td>11.27 ± 0.59</td>
<td>7.01 ± 0.61</td>
</tr>
<tr>
<td>Scurbutic (12)</td>
<td>7.87 ± 0.17</td>
<td>10.65 ± 0.57</td>
<td>17.96 ± 0.85</td>
<td>12.01 ± 0.76</td>
</tr>
<tr>
<td>Insulin-treated scurbutic (8)</td>
<td>6.88 ± 0.70</td>
<td>6.18 ± 0.32</td>
<td>13.97 ± 0.92</td>
<td>7.60 ± 0.35</td>
</tr>
</tbody>
</table>

**Statistical analysis t values**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between normal and scurbutic</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>“ “ insulin-treated scurbutic</td>
<td>2.5</td>
<td>0.7</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Between scurbutic and insulin-treated scurbutic</td>
<td>1.5</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
treatment lowered the level of malic acid to normal in blood, liver, and cardiac muscle. The increase in malic acid content may be related to the

**Table VI**

**Malic Acid Content of Normal, Scorbutic, and Insulin-Treated Scorbutic Guinea Pigs**

The figures in parentheses denote the number of animals. The values are given in mg. per 100 gm. of wet tissue.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
<th>Cardiac muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8)</td>
<td>3.42 ± 0.34</td>
<td>20.32 ± 0.88</td>
<td>12.98 ± 0.95</td>
<td>13.43 ± 1.63</td>
<td>16.93 ± 1.91</td>
</tr>
<tr>
<td>Scorbutic (8)</td>
<td>8.12 ± 0.80</td>
<td>26.97 ± 2.09</td>
<td>26.60 ± 2.11</td>
<td>25.74 ± 1.67</td>
<td>24.03 ± 1.73</td>
</tr>
<tr>
<td>Insulin-treated scorbutic (6)</td>
<td>4.51 ± 0.37</td>
<td>17.55 ± 2.20</td>
<td></td>
<td></td>
<td>18.24 ± 1.24</td>
</tr>
</tbody>
</table>

Statistical analysis t values

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
<th>Cardiac muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between normal and scorbutic</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>“   ” “   ” insulin-treated</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Between scorbutic and insulin-treated scorbutic</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td>2.7</td>
</tr>
</tbody>
</table>

increased accumulation of citric acid. It has been shown by Stern et al. (33) that the following are reversible reactions.

I. Acetyl-S-CoA + oxalacetate = + H₂O ⇄ citrate = + HS-CoA + H⁺ (condensing enzyme)

II. L-Malate = + DPN⁺ ⇄ oxalacetate = + DPNH + H⁺ (malic dehydrogenase)

Sum: III. Acetyl-S-CoA + L-malate = + DPN⁺ + H₂O ⇄ citrate = + HS-CoA + DPNH + 2H⁺

They have further shown that the equilibrium position of Reactions I and III is far in the direction of citrate synthesis and that of Reaction II is markedly in favor of malate, but when there is an increased amount of citrate in the system, the malic dehydrogenase system effectively drives Reaction III towards malate formation. In scurvy we have observed an increased accumulation of citrate in the tissues. This might have caused shifting of the equilibrium position of Reaction III towards malate formation, resulting in the increased accumulation of malic acid in the tissues. This explanation is in harmony with our suggestion that accumulation of citrate in the tissues of the scorbutic animals may be due to the presence
of a metabolic block in the tricarboxylic acid cycle below the level of citrate. The observation that treatment with insulin lowers the levels of both citric and malic acids to normal level supports the conclusion that lack of insulin is an important factor in the carbohydrate metabolic derangement in scurvy.

Table VII shows a greatly increased lactic acid content of the tissues of scorbutic guinea pigs in comparison with that of the normal controls. Treatment with insulin lowered the lactic acid value even below normal. The accumulation of lactic acid in the scorbutic tissues may be due to decreased glycogen formation from lactic acid. We have shown that liver and muscle glycogen values were greatly reduced in scurvy and that prolonged treatment with insulin strikingly improved the glycogen content of the liver and muscle. It is, therefore, possible that glycogenesis is reduced in scurvy owing to the lack of insulin, and that exogenous insulin effectively promotes glycogen formation from several sources such as lactic acid.

It has been observed that neither adrenal medulla (34) nor thyroid (35) is concerned with the disturbed carbohydrate metabolism in scurvy. The activity of the adrenal cortex in scurvy seems not to be a specific function of vitamin C deficiency (36) and, as such, its relationship with the deranged carbohydrate metabolism is not clear. Bacchus and Heiffer (12), however, have shown that adrenalectomy has no effect on the typical pattern of carbohydrate metabolism in scurvy. Degeneration of the β...
cells (11) and decreased insulin content of the pancreas (4) seem to suggest that diminished insulin formation may be an important factor in the abnormal carbohydrate metabolism in scurvy. In view of the possibility that prolonged insulin deficiency may affect enzyme systems quantitatively and qualitatively, insulin treatment has been carried on in these experiments for a prolonged period with increasing doses. The ineffectiveness of insulin injection in improving carbohydrate metabolism, as observed by Bacchus and Heiffer (12) and by Murray (13), may be due to the fact that these workers used short term insulin injections. In the present study, it has been observed that both glucose tolerance and glycogenesis in scurvy are improved to a great extent by prolonged insulin treatment and it is concluded that insulin deficiency is a major cause of the abnormal carbohydrate metabolism in scurvy. The foregoing studies in the tricarboxylic acid cycle metabolism support this view and also agree with the suggestion of other workers that insulin is involved in metabolism at the level of the Krebs cycle.

SUMMARY

1. Glucose tolerance is lowered in scorbutic guinea pigs and is improved by prolonged treatment with insulin.

2. Liver and muscle glycogen levels are greatly decreased in scorbutic guinea pigs. Striking improvement of the glycogen values is observed in scorbutic guinea pigs treated with insulin for a prolonged period of time.

3. Except in blood, tissue content of α-keto acids does not change in the normal, scorbutic, and insulin-treated scorbutic guinea pigs.

4. Tissue content of citric, malic, and lactic acids is significantly increased in scorbutic guinea pigs. Treatment of the scorbutic animals with insulin for a prolonged period of time lowers the tissue content of these acids to the normal level.

5. The significance of these results in relation to the role of insulin in the abnormal carbohydrate metabolism in scurvy and in the Krebs tricarboxylic acid cycle metabolism has been discussed.

BIBLIOGRAPHY

STUDIES ON CARBOHYDRATE METABOLISM IN SCORBUTIC GUINEA PIGS
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