THE INFLUENCE OF THIAMINE DEFICIENCY ON CITRIC ACID EXCRETION

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According to current views on the intermediary metabolism of carbohydrate, both thiamine and citric acid are significant factors in the oxidation of this foodstuff. Thiamine in its phosphorylated form has been shown (Banga, Ochoa, and Peters, 1939) to be required for the oxidative removal of the pyruvic acid produced in the course of carbohydrate breakdown. Likewise, according to Krebs and Johnson (1937), some product (probably pyruvic acid) of carbohydrate metabolism reacts with oxalacetic acid to form citric acid which in turn yields carbon dioxide and a series of simpler dicarboxylic acids before the cycle is repeated. It might be expected, therefore, that the metabolic disturbance characteristic of thiamine deficiency would, in part, make itself felt in an alteration of the "citric acid cycle" and thus possibly in a change in the rate of excretion of citric acid in the urine, inasmuch as it has been shown that even with an adequate allowance of thiamine, dietary carbohydrate exerts a definite influence on the urinary citric acid (Smith and Meyer, 1939).

Studies have been carried out to determine whether or not a relationship between thiamine deficiency and the excretion of citric acid can be demonstrated. A marked increase in citric acid output in the urine of rats has been reported by Krusius and Simola (1938) and also by Krebs (1938). On the other hand Sober, Lipton, and Elvehjem (1940) indicate that severe thiamine deficiency is accompanied by a decrease in urinary citric acid, with a prompt rise of considerable magnitude when the experimental animals (rats) were realimented with thiamine. The data given in the present report have a bearing on this problem.
EXPERIMENTAL

Eighteen normal rats weighing from 44 to 58 gm. were housed in separate metabolism cages and given a thiamine-deficient diet consisting of sucrose 71, casein 18, salts 3, hydrogenated fat 5, cod liver oil 2, yeast 15. Factors of the vitamin B complex other than thiamine which are needed by rats were provided by a yeast preparation obtained according to Kline, Tolle, and Nelson (1938): 50 gm. of dried yeast were treated with 400 cc. of 0.1 per cent sodium sulfite and brought to pH 4.0 with sulfur dioxide. After standing at room temperature for 5 days, it was lyophilized and finally dried over P₂O₅ in a vacuum desiccator, and ground to a powder.

The funnels of the metabolism cages were washed down daily and the urine collections made in 3 or 4 day periods, at the end of which the rats were weighed and food intake determined.

Citric acid was determined in the urine by the method of Pucher, Sherman, and Vickery (1936), the final measurement being made photoelectrically with a color filter with maximum transmission at 4250 Å.

Six of the rats (Group II) were given in addition to the thiamine-deficient diet 30 μg of thiamine daily and their food intake was limited to that of a group of six rats (Group I) without thiamine for which they were paired, fed controls. At the end of seven periods (25 days) Group I was given 5 μg of thiamine daily and the experiment on Group II was discontinued. Six other thiamine-deficient rats (Group III) were at this time given 30 μg of thiamine daily and paired fed for four periods (14 days) with the six receiving 5 μg of thiamine daily.

Twice the daily indicated intake of thiamine, dissolved in 0.2 cc. of water, was placed on top of the food every 2nd day. In Group I after the 5 μg of thiamine were given, only slightly more food was put into the food cup than was consumed during the 2 day period.

1 Labco.
2 Hubbell, Mendel, and Wakeman (1937).
3 Crisco.
4 Northwestern Yeast Company.
5 Through the courtesy of Mr. Harvey Merker, Parke, Davis and Company.
to insure complete consumption of the vitamin. The response in food consumption and in increased body weight shows the effect of the thiamine.

Results

That the basal diet was deficient in thiamine was shown by the stationary body weights or losses in weight of the rats without thiamine after experimental Period 4, while the addition of 5 \( \gamma \) of thiamine \textit{per diem} in Group I and 30 \( \gamma \) in Group III was followed by prompt resumption of growth at rates of 1 to 3 gm. daily.

In Table I are shown the changes in excretion of citric acid in the various groups of experimental animals. In Groups I and III there is a progressive decrease in the excretion of citric acid by the kidneys as the period of deprivation of thiamine is prolonged. Furthermore, upon realimentation with 5 \( \gamma \) of thiamine in Group I and with 30 \( \gamma \) in Group III, the output of citric acid increases again. However, that the excretion is a function rather of food intake than of the presence or absence of thiamine is indicated by the parallelism in the amounts of citric acid eliminated by the

### Table I

**Average Excretion of Citric Acid**

The values are expressed in mg. per kilo of body weight \textit{per diem} based on body weights at the beginning of the indicated period. Each group contained six rats.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
<th>Period 5</th>
<th>Period 6</th>
<th>Period 7</th>
<th>Period 8</th>
<th>Period 9</th>
<th>Period 10</th>
<th>Period 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Thiamine-free diet*</td>
<td>86</td>
<td>101</td>
<td>49</td>
<td>42</td>
<td>29</td>
<td>20</td>
<td>19</td>
<td>25</td>
<td>29</td>
<td>65</td>
<td>38</td>
</tr>
<tr>
<td>II. Thiamine-free diet</td>
<td>74</td>
<td>72</td>
<td>56</td>
<td>67</td>
<td>60</td>
<td>28</td>
<td>17</td>
<td>25</td>
<td>29</td>
<td>65</td>
<td>38</td>
</tr>
<tr>
<td>+ 30 ( \gamma ) thiamine daily†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Thiamine-free diet†</td>
<td>64</td>
<td>105</td>
<td>64</td>
<td>58</td>
<td>42</td>
<td>37</td>
<td>29</td>
<td>21</td>
<td>62</td>
<td>55</td>
<td>27</td>
</tr>
</tbody>
</table>

* 5 \( \gamma \) of thiamine were fed daily in Periods 8 to 11.

† Paired with Group I as to food intake in Periods 1 to 7.

† Paired with Group I as to food intake in Periods 8 to 11 but 30 \( \gamma \) of thiamine in addition fed daily.
Thiamine Deficiency and Citric Acid

The observations herein discussed do not agree with those of Krusius and Simola (1938), whose experimental ration apparently was deficient in several factors (see Sober, Lipton, and Elvehjem (1940)), nor with those of Krebs (1938), who did not give details of his investigation. Likewise, the present data do not support the contention of Sober, Lipton, and Elvehjem (1940) that thiamine deficiency per se is the cause of a decreased output of citric acid. Their experimental procedure differed from that herein described with respect to the details of the diets, though both would appear to be lacking the same essential. Also, there is a difference in the degree of deficiency imposed, only one of the animals in the present study showing polyneuritic spasms. However, while their table is not entirely clear, it does not seem that a real difference exists between the preconvulsive and the convulsive states when such data on individual rats can be compared. A question may also be raised regarding the use of rats fasted 24 hours to provide controls for the effect of food intake on citric acid excretion. It has been our experience that following a change in dietary régime, the excretion of citric acid shows a definite lag over a period of several days, seldom stabilizing during the first 3 days (see Smith and Meyer (1939)). Another question which should be raised is the influence of the acidosis during polyneuritic convulsions; the excretion of citric acid is extremely sensitive to the acid-base balance in the organism (see Smith and Orten (1937)), even citric acid itself when administered orally causing a decreased excretion of citrate (Kuether, Meyer, and Smith, 1940). The result, in this case, would likewise be to depress the output.

SUMMARY

A deficiency of thiamine in the diet results in decreased excretion of citric acid in the urine. However, the results of paired feeding experiments indicate that this decrease is correlated with the diminished intake of food rather than with absence of thiamine per se.
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BIBLIOGRAPHY

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