On the Minimum Requirement and Treatment Dosis of Thiamine for Rats

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(Received for publication, February 28, 1961)

When vitamins are given to animals in excess, a great amount is decomposed or excreted, and only a very small portion is utilized by the body. But when they are given in small amounts, the body is very careful in utilizing them and does not waste them. Even though some vitamins are completely excluded from the diets, the rats do not show deficiency symptoms of the vitamins, as these are manufactured in their intestines by bacteria and absorbed and utilized within their bodies. The purpose of this work is to find out the effect of thiamine given in very small amounts orally to rats.

METHODS

1. Feedings—Young small rats strain were kept at first on normal diets. When their body weight became about 50 to 60 g., they were divided into 6 groups, the first a control normal group, the second a thiamine deficient group, the third a group to which 2.5 µg. of thiamine was given every day, the fourth a group to which 2.5 µg. of thiamine was given in the fourth or fifth week of thiamine deficiency, the fifth a group to which 5 µg. of thiamine was given in the fourth or fifth week of thiamine deficiency. The diet and the method of feeding are given in Table I.

2. Determination of Thiamine—The thiamine contents in the organs and in the gastrointestinal tracts of rats were measured by the fluorometric method of Fujiwara (1).

3. Electrocardiographic Examination—Unanesthetized rats were placed (2) on their abdomen on a board to which their feet were tied, and electrocardiographic recording were made. Electrodes were dipped in the electrode paste and these were taped to the feet soles of the rats with adhesive plaster. The faces of the rats were covered with gauze mask to keep them still. Electrocardiographic examination was started several minutes after the rats became quiet and standard limb and unipolar limb lead were recorded. The standardization used was the same as in men's electrocardiography (1 mvolt : 1 cm.). The room temperature was kept at about 20-25°C at all times during the examination.

<table>
<thead>
<tr>
<th>Basal diet</th>
<th>Salt mixture&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>Vitamin B&lt;sub&gt;2&lt;/sub&gt; group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein 20%</td>
<td>NaCl 20.2 µg.</td>
<td>Riboflavin 40 µg.</td>
</tr>
<tr>
<td>Starch 68</td>
<td>MgSO₄·7H₂O 63.5 µg.</td>
<td>Pyridoxine 50 µg.</td>
</tr>
<tr>
<td>Salt mixture&lt;sup&gt;1)&lt;/sup&gt; 4</td>
<td>K₂HPO₄ 111.5 µg.</td>
<td>Nicotinic acid 200 µg.</td>
</tr>
<tr>
<td>Soybean oil 5.5</td>
<td>Ca-lactate 28.7 µg.</td>
<td>Ca-Pantothenate 200 µg.</td>
</tr>
<tr>
<td>Cod liver oil 2.5</td>
<td>Na₂HPO₄·12H₂O 52.6 µg.</td>
<td>Choline-HCl 900 µg.</td>
</tr>
<tr>
<td>Water</td>
<td>Ca₃H₃(PO₄)₂·H₂O 111.6 µg.</td>
<td>Inositol 180 µg.</td>
</tr>
<tr>
<td>Fe-citrate 13.8</td>
<td></td>
<td>B&lt;sub&gt;12&lt;/sub&gt; 0.024 µg.</td>
</tr>
</tbody>
</table>

These are cooked and made into cakes.

<sup>1)</sup> The basal diet is given in a larger amount than that rats can eat. B<sub>2</sub> group suspension (0.1 ml.) is given orally daily. B<sub>1</sub> is given in various doses (or not given) orally, according to experimental conditions.
RESULTS

1. Thiamine Deficient Group—In the first week of thiamine deficiency, growth, appetite, vitality and stool of this group were the same as those of the normal group. From the middle of the second week, the appetite decreased gradually to the daily intake of about 15-20 g. of the diet, and the growth stopped, but vitality and stools were still good. In the third week appetite decreased to the daily intake of about 10-15 g. of the diet, that is, about one-half of the intake of normal control rats, and the weight loss appeared clearly. But in general there was no change in the vitality and character of the stools. In the fourth week the appetite decreased greatly and the daily food intake became only about 10 g. The body weight decreased markedly and showed a daily average loss of several grams. Most of rats lost their vitality and revealed paralytic symptom (in walking) and got diarrheas. Some rats died at the end of this week. In the fifth week the rats lost their appetite almost completely and daily food intake was only about 5 g. The body weight decreased extremely. Marked neuro-paralytic symptoms and ataxia were observed during this period. All the rats became enervated and had stinking black-brown diarrhea. Most of rats died during this week (Fig. 1). When the rats in the fourth or fifth week of thiamine deficiency were kept in cages without taking away their feces, most of the rats showed the fece in their stomachs, moreover, at times the fece was found even in the duodenum. In this stage, about 0.3 μg. of thiamine was found in the stomach and about 5 μg. of thiamine could always be found in the cecum. Furthermore, even though about

\[\text{FIG. 1. The progress of body weight of control normal rats and thiamine deficient rats.}\]

* by the administration of thiamine (5 μg.) orally daily.

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Condition} & \text{Stomach} & \text{Small intestine} & \text{Large intestine} & \text{Total} \\
\hline
\text{B1 deficiency group} & 0.25 (—) & 0.46 (0.16) & 5.39 (0.54) & 6.10 (0.70) \\
5 μg. of B1 (orally daily) group & 0.29 (0.08) & 1.18 (0.38) & 5.97 (0.71) & 7.44 (1.17) \\
10 μg. & (—) & 1.48 (0.48) & 6.92 (1.24) & 8.87 (1.82) \\
40 μg. & (—) & 4.31 (0.60) & 8.61 (1.86) & 13.44 (2.56) \\
\hline
\end{array}
\]

\text{TABLE II}

\text{Thiamine Content in the Gastrointestinal Tract of Rats}

(the average of thiamine content of 3 cases)

Determination of the thiamine contents was carried out 24 hours after the administration of thiamine.

The figures in the parenthesis show the free thiamine content.
5-6 µg. of thiamine could always be found in the gastrointestinal tract from the first stage of thiamine deficiency to the time just before the death, the rats lost their body weight and became enervated. And in this stage the ECG of rats showed various marked changes, such as bradycardia, arrhythmia (sinus arrhythmia, a-v block, interpolated nodal rhythm, and premature beat), increase of right axis deviation, ST changes (elevation and depression) and T changes (elevation and depression or negative) etc. (2).

2. Cases to Which 2.5 µg. of Thiamine Was Given Orally Daily—When the rats were given 2.5 µg. of thiamine orally daily, they grew rapidly. They gained 3-5 g. daily, but stopped growing when their body weight reached 80-90 g. (Fig. 6), but their general conditions were good. The amount of thiamine in the gastrointestinal tract was 5-6 µg., the same amount as in all stage of thiamine deficiency. The amount of thiamine in the principal organs of the rats was very little and it was found that the amount was the same as in the early stage of thiamine deficiency. There was no change in ECG. Judging from the above it is assumed that the minimum oral require ment of thiamine in rats to maintain the body weight at 70-80 g. is 2.5 µg.

In this group it can be assumed that the thiamine was completely absorbed and utilized by the body.

3. Cases to Which the 2.5 µg. of Thiamine Was Given Orally in the Fourth or Fifth Week of Thiamine Deficiency—When 2.5 µg. of thiamine was given orally to thiamine deficient rats, the rats regained vitality after 24 hours, but in this case their body weight decreased. The ECG abnormalities observed on the previous day improved to some extent (Fig. 2, 3, 4).

However, after 48 hours they returned to their former state (Fig. 5). There was no change in the amount of thiamine in the principal organs compared with the thiamine deficient rats.

4. Cases to Which 5 µg. of Thiamine Was Given Orally Daily—When 5 µg. of thiamine was orally given daily the rats kept growing evenly to the body weight of 140-160 g. (Fig. 6). Their general conditions were very good, they didn't eat their feces, and there was no change in ECG. On the other hand the amount of thiamine in the gastrointestinal tract was the same as in the thiamine deficient rats. There was no histological change in the principal organs.

5. Cases to Which 5 µg. of Thiamine Was Given Orally in the Fourth or Fifth Week of Thiamine Deficiency—When thiamine deficient rats in the fourth or fifth week were given 5 µg. of thiamine orally, they regained their vitality and gained weight after 24 hours. The ECG abnormalities observed on the previous day improved greatly, but the amount of thiamine in the principal organs showed no change, compared with the thiamine deficient rats.

### Table III

**Thiamine Content of the Organs of Rats**

<table>
<thead>
<tr>
<th>Organ</th>
<th>thiamine deficient rats (in the 4th week of deficiency) (average of 3 cases)</th>
<th>the rats given 5 µg. of thiamine orally daily (average of 4 cases)</th>
<th>Control normal rats given 40 µg. of thiamine orally daily (average of 4 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>repair</td>
<td>µg.</td>
<td>µg.</td>
<td>µg.</td>
</tr>
<tr>
<td>blood</td>
<td>6.9</td>
<td>1.15 (0.06)</td>
<td>92.2 (5.6)</td>
</tr>
<tr>
<td>liver</td>
<td>30.9 (3.1)</td>
<td>0.50 (0.05)</td>
<td>61.8 (18.5)</td>
</tr>
<tr>
<td>kidney</td>
<td>50.0 (4.7)</td>
<td>0.01 (0.04)</td>
<td>57.5 (2.1)</td>
</tr>
<tr>
<td>heart</td>
<td>22.3 (3.9)</td>
<td>0.87 (0.19)</td>
<td>100.3 (4.9)</td>
</tr>
<tr>
<td>brain</td>
<td>62.5 (7.4)</td>
<td>18.4 (3.7)</td>
<td>171</td>
</tr>
</tbody>
</table>

The figures in parenthesis show free thiamine contents.
The average of body weights of rats—thiamine deficient group, control group (I): 47 g., 5 µg. of thiamine group, control group (II): 150 g.
icient rats. They did not eat their feces. When 5 µg. of thiamine was administered every day, paralytic symptoms and diarrhea were cured within several days, and general state thereafter was similar to that of the normal group.

![Fig. 2. Electrocardiogram of a normal rat (body weight 179.5 g). Heart rate 530/min.](image1)

![Fig. 3. Electrocardiogram of a rat on the 20th day of thiamine deficiency. Arrhythmia (sinus arrhythmia and ventricular premature beat) and bradycardia were observed. Heart rate 300–375/min.](image2)

![Fig. 4. One day after oral administration of 2.5 µg. of thiamine. Arrhythmia disappeared and bradycardia improved. Heart rate 409/min.](image3)

DISCUSSION

Many studies hitherto have been made on the requirement of thiamine of the rats by Cowgill, et al. and many others, but few reports are found which deal with the
thiamine content in the gastrointestinal tracts of rats (3, 4). In the gastrointestinal tracts of thiamine deficient rats thiamine was always found and about 5 μg. of thiamine was found in the caecum from the early stage of deficiency until death. On the other hand after the third week of deficiency the body weight decreased steadily and they lost their vitality. At the fourth week and fifth week, the body weight fell down to the level of the beginning of deficiency and the rats died. But the rats which were given 2.5 μg. of thiamine orally everyday grew rapidly up to about 80 g. but at this stage the increase in the body weight stopped, and thereafter there was no change in weight but they maintained their health. On the other hand, when the daily dosis of thiamine given orally was increased to 5 μg., they gained weight rapidly to 140–160 g. Both of these groups showed no change in ECG. Judging from the above, with each 30 g. of increase of the body weight it is necessary to increase 1 μg. of the dosis of thiamine.

In the cases when 2.5 μg. of thiamine was given orally to rats in the first stage of thiamine deficiency, their appetite increased in 24 hours and their general conditions as well as ECG improved although their body weight decreased. The amount of thiamine in the gastrointestinal tract was almost the same as in the case of the thiamine deficient rats. And then, owing to the enhancement of catalysis of bacteria the rats lost weight despite the increase of food intake. The amount of thiamine in the gastrointestinal tract was the same, whether thiamine was administered or not. It might suggest that the thiamine in the bacteria is not absorbed as such in the intestines. The fact that there was no difference in the amount of thiamine in the organs suggests that the thiamine absorbed by the rats became completely utilized within a day.

When 5 μg. of thiamine was administered orally, the rats gained weight of about 5 g. and the ECG changes improved, but after 48 hours their conditions started to worsen again. However, when 5 μg. of thiamine was given orally daily, the ECG changes become normal again within four or five days, and the body weight increased daily. The amount of thiamine in the gastrointestinal tract of these rats was 5 μg., and the amount of thiamine in their principal organs was slightly more than in the case of rats that were not given thiamine orally at all. Judging from this, it is assumed that 5 μg. is the minimum amount as the curative dosis. The fact that acute human beriberi that was prevalent in the old days showed remarkable improvement with very little amount of thiamine has a very close resemblance to these findings on thiamine deficient rats.

**SUMMARY**

1. In the gastrointestinal tracts of thiamine deficient rats about 5 μg. of thiamine is always found until their death, but it is assumed that this is not utilized by the rats.
2. When 2.5 μg. of thiamine is administered orally daily, the rats grow up to about 80–90 g. and then stop growing. On the other hand, when 5 μg. is administered orally daily, they grow up to about 150 g. and then stop growing. In both cases 5–6 μg. of thiamine can be found in the gastrointestinal tracts, and the amount of thiamine in the vital organs is almost the same as when no thiamine is administered.

Daily administration of 2.5 μg. of thiamine maintains the health condition of the rats up to about 80 g. of body weight, and daily administration of 5 μg. thiamine maintains the health condition up to about 150 g.

3. The minimum amount of thiamine necessary for curative treatment of the rats in the fourth or fifth week of thiamine deficiency was found to be between 2.5 μg. and 5 μg.

The authors are deeply grateful for his encouragement to Prof. S. Tazaka, in whose Department this work was done. The authors are also grateful to Dr. T. Takagi for his helpful advice.

**REFERENCES**

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