THE METABOLISM IN VIVO OF dl-PHENYLALANINE IN THIAMINE DEFICIENCY*

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(Received for publication, August 20, 1945)

It is well established that phenylalanine and tyrosine are incompletely metabolized in scorbutic guinea pigs (15-17) and by premature infants (9, 10). Closs and Fölling (3) have reported that phenylpyruvic acid appeared in the urine of thiamine-deficient rats when extra phenylalanine was fed. The evidence in support of the latter claim was not conclusive, but such a finding would not be inconsistent with the abnormally great excretion of pyruvic acid in thiamine deficiency (14, 8) and the rôle of thiamine in the decarboxylation of both α-ketomonocarboxylic acids (11) and α-ketoglutarate (1, 7). Furthermore, there is some evidence to suggest that ascorbic acid synthesis by the rat and the dog is dependent upon an adequate supply of thiamine (19, 6, 4). Hence, it is conceivable that thiamine deficiency could lead to a secondary ascorbic acid deficiency in these species and, thereby, explain such findings as reported by Closs and Fölling.

In efforts to develop methods for the detection of vitamin deficiencies in man based upon the appearance of metabolic defects arising from the deficiency it is important to know whether the metabolic defect is specific for the condition under test. The question arises, therefore, whether thiamine lack as well as ascorbic acid deficiency might give rise to the appearance in urine of products of the incomplete metabolism of phenylalanine. The experiments here reported were carried out to investigate this point.

Methods

The thiamine-deficient diet used was of the following composition: vitamin-free casein (S. M. A. Corporation) 25, sucrose 64, cottonseed oil 8, and salt mixture (Osborne and Mendel (13)) 3, plus 5 mg. of riboflavin, 30 mg. of calcium pantothenate, 2.5 mg. of pyridoxine, 0.5 gm. of choline chloride, and 1 gm. of inositol per kilo of diet. Twice a week each rat received 2 drops of a mixture containing equal parts of cod liver oil and wheat germ oil. The control diet contained in addition 2.5 mg. of thiamine.

* This study has received financial support from the International Health Division of the Rockefeller Foundation and the Nutrition Foundation, Inc.

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per kilo of diet. The animals were kept in individual wire mesh cages and transferred to individual metabolism cages during the periods when urine was collected.

The urines were analyzed for total hydroxyphenyl compounds (12) by the Millon reaction as modified by Folin and Ciocalteu (5) and adapted for use with the Evelyn photoelectric colorimeter. After phenylpyruvic acid and other ether-soluble compounds had been extracted from the acid urine in the cold by shaking with an equal volume of ethyl ether, the non-extractable hydroxyphenyl compounds were determined by repeating the same procedure. The results are expressed as “tyrosine equivalents,” with no attempt to define the exact products responsible for the reaction.

The reduction of phosphomolybdic acid by the urines was taken as an estimate of aromatic keto acid excretion (18, 12, 10). This reduction was carried out by the Bodansky method for phosphorus (2) but with omission of the stannous chloride and addition of 1/15 KH₂PO₄. The intensity of the color was read in the Evelyn photoelectric colorimeter at the end of 3 hours and compared with a phosphorus standard which was prepared in the usual manner. No attempt was made to relate the values so obtained to the actual content of keto acids, as the interest was only in the comparative figures. The results are expressed as “phosphorus equivalents.” Each urine was also tested qualitatively with ferric chloride, which reacts with either phenylpyruvic acid (green) or homogentisic acid (blue).

EXPERIMENTAL

Albino rats, four females and one male, of the Fischer strain 4 weeks of age and weighing 36 to 53 gm. were placed alternately on the control diet (3 weeks), the thiamine-free diet (5 weeks), the control diet (4 weeks), and, again, the thiamine-free diet (8 weeks). All of the rats exhibited signs of thiamine deficiency such as marked loss of weight, typical hunched posture, and, in some cases, spasticity and paralysis while on the deficient regime, but they developed well during the control feeding. Two of the animals died during the period of deficiency.

During each period a number of 24 hour urine samples were collected in bottles containing 1 cc. of glacial acetic acid. The urine was preserved by deep freezing (-20°) for 1 to 5 days until the analyses were made. At intervals a solution of 100 or 200 mg. of dl-phenylalanine (Merck) dissolved in water with an equivalent amount of sodium carbonate was administered by stomach tube, and the urine was collected in the manner described during the subsequent 24 hour period.

The urinary excretion values during the various periods are summarized in Table I. The t test applied to these data reveals that the differences between the mean values for total phenols and for reducing substances
(phosphorus equivalent) for the first two experimental periods were statistically significant (P < 0.05). The findings suggest that more phenylpyruvic acid was excreted by the rats on the control diet than when they were deficient in thiamine. An increase of excretion of hydroxyphenyl compounds was again noted when the rats were returned to the control diet (Period III). Less marked differences were observed between the second control period and the following deficiency period. At no time did any of these urines give a color with ferric chloride.

**Table I**

Analyses of 24 Hour Urine Specimens of Rats Fed Alternately Diets with and without Thiamine

<table>
<thead>
<tr>
<th>Period No.</th>
<th>Diet</th>
<th>No. of urine specimens</th>
<th>Hydroxyphenyl compounds as tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>2.5-3.5 mg.</td>
</tr>
<tr>
<td>II</td>
<td>Thiamine-free</td>
<td>5</td>
<td>1.1-3.1 mg.</td>
</tr>
<tr>
<td>III</td>
<td>Control</td>
<td>3</td>
<td>4.0-7.4 mg.</td>
</tr>
<tr>
<td>IV</td>
<td>Thiamine-free</td>
<td>3</td>
<td>3.2-6.4 mg.</td>
</tr>
</tbody>
</table>

**Table II**

Analyses of 24 Hour Urine Specimens of Rats Fed Alternately Diets with and without Thiamine following Single Doses of 100 Mg. of Phenylalanine

<table>
<thead>
<tr>
<th>Period No.</th>
<th>Diet</th>
<th>No. of urine specimens</th>
<th>Hydroxyphenyl compounds as tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Thiamine-free</td>
<td>3</td>
<td>1.8-2.8 mg.</td>
</tr>
<tr>
<td>III</td>
<td>Control</td>
<td>3</td>
<td>4.7-7.1 mg.</td>
</tr>
<tr>
<td>IV</td>
<td>Thiamine-free</td>
<td>2</td>
<td>2.1-2.3 mg.</td>
</tr>
</tbody>
</table>

The data on the excretion of urine following the administration of test doses of dl-phenylalanine are given in Table II. It is again evident that there was a greater excretion of hydroxyphenyl compounds by the control rats than by the deficient animals. These differences were statistically significant. The ferric chloride test was positive in only one urine collected after feeding 100 mg. of dl-phenylalanine; this was in a deficient animal.

Two possible explanations of these results are apparent, (1) they may reflect the difference in the ages of the animals at the various periods or
(2) they may be due to variations in food intake during the two periods of feeding. This latter possibility is of considerable importance because of the high content of aromatic amino acids in casein.

In a second experiment twelve 30 to 50 gm. rats of the same strain were matched as to sex and divided into two equal groups. These were pair-fed. 24 hour urine specimens were again collected and analyzed before and after test doses of 200 mg. of dl-phenylalanine. The data are summarized in Table III. Statistical analysis of these results fails to reveal any significant differences between the animals on the two diets. It was concluded that any small differences which had been observed with the first group of animals had been due to variations in food intake and in the age of the animals at different stages of the experiment. The qualitative test with ferric chloride was negative in all urines except those following the administration of additional amounts of dl-phenylalanine. In the latter case an intense green was obtained with the urines from both the deficient animals and the pair-fed controls.

**DISCUSSION**

An examination of the data of Close and Fölling (3) reveals that the differences which they observed were based upon qualitative tests applied to urines of different concentrations and that the animals were not pair-fed. With the feeding régimes and the methods outlined here no evidence was obtained that thiamine-deficient rats were less able to metabolize dl-phenylalanine in either the amounts present in a diet containing 25 per cent casein or when additional single doses of 100 to 200 mg. of the amino acid were administered by stomach tube. Thiamine deficiency

<table>
<thead>
<tr>
<th>Table III</th>
<th>Analyses of 24 Hour Urine Specimens of Rats Pair-Fed on Diets with and without Thiamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>No. of animals</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>“+ 200 mg. phenylalanine”</td>
<td>4</td>
</tr>
<tr>
<td>Thiamine-free</td>
<td>6</td>
</tr>
<tr>
<td>“+ 200 mg. phenylalanine”</td>
<td>3</td>
</tr>
</tbody>
</table>
in the rat did not, therefore, lead to the appearance in the urine of an increased amount of abnormal metabolic products of the aromatic amino acids. These findings are in agreement with the experiences of Levine et al. (9) that supplementary vitamin B complex failed to reduce the excretion of products of tyrosine metabolism by premature infants. It does not appear that thiamine deficiency results in the development of a defect, which can be demonstrated in vivo, in the metabolism of phenylalanine in the rat.

**SUMMARY**

In young albino rats maintained on thiamine-free diets there was detected no increased excretion of abnormal metabolic products of tyrosine or phenylalanine. Following the administration by stomach tube of dl-phenylalanine to pair-fed deficient and control rats the observed increases in the output of all metabolites tested for were comparable.

The authors wish to express their appreciation to Dr. Albert Segaloff for the gift of the pure strain rats employed in this study and to acknowledge the technical assistance of Miss Caroline Ashley and Mrs. Naomi Dziewiatkowski and the valuable advice of Dr. Paul Densen in the statistical analyses of the results.

**BIBLIOGRAPHY**

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