Intestinal Absorption of Thiamin in Man Compared with Folate and Pyridoxal and Its Subsequent Urinary Excretion

Richard E. DAVIS, Graham C. ICKE, James THOM, and William J. RILEY

The Departments of Haematology and Biochemistry, Royal Perth Hospital, Western Australia

(Received December 3, 1983)

Summary The intestinal absorption of thiamin was compared with that of folate and pyridoxal in six healthy volunteers using an oral dose of each vitamin equivalent to ten times the recommended daily allowance. Folate and pyridoxal were rapidly absorbed and following the oral dose, serum concentrations rose from a mean basal level of 10.9 µg/liter and 17.5 µg/liter to 174.8 µg/liter and 315.2 µg/liter respectively — an increase of 1,500% for folate and 1,701% for pyridoxal. The mean serum level of thiamin rose only marginally from 5.1 µg/liter to 7.2 µg/liter, an increase of 42%.

One hour following the oral test dose of thiamin, the vitamin was actively excreted in the urine of all volunteers, with a mean creatinine/thiamin renal clearance ratio of 2.4. Active excretion continued for up to six hours. In contrast, folate was only actively cleared for a short period in two volunteers. Thiamin absorption appeared to be controlled and limited, and modest increases in the serum concentration were accompanied by active renal clearance.

Key Words vitamin absorption, folate, pyridoxal, thiamin, urinary excretion

The intestinal absorption of folate has been extensively studied in man. It has been shown that while absorption takes place mainly in the jejunum (1), absorption is not impaired in patients who have undergone substantial resection of the jejunum. It would seem therefore that this function can be exercised by other areas of the gut. Food folate is usually present in the form of a polyglutamate, and an intestinal conjugase reduces most of this to a monoglutamate. Whether folate absorption is an active or passive process has not been fully resolved; some studies have found evidence of an active process (2) while others favor passive absorption (3).
Few studies on the absorption of vitamin B6 (pyridoxal, pyridoxine or pyridoxamine) have been made. Booth and Brain (4) found that the absorption of labeled pyridoxine in rats increases in a linear fashion corresponding to the increases in the size of the oral dose, with no tendency for the pyridoxine to plateau even after a very large oral dose.

As with folate, the preferred site of pyridoxine absorption appeared to be the jejunum. In contrast to these findings, work on the absorption of thiamin has shown it to be subject to absorption control both in man (5) and animals (6). Other studies have shown that small doses of the vitamin are absorbed by an active transport mechanism (7).

Morrison and Campbell (7) found that the urinary excretion of thiamin expressed as a percentage of the dose decreased markedly when doses greater than 2.5 mg were given, and that increasing the dose from 2.5 to 20.0 mg resulted in an increase of only 0.2 mg in the amount of vitamin excreted. The purpose of the present study was to examine the apparent difference in absorption and subsequent urinary excretion of thiamin compared with folate and pyridoxal by measuring the absorption of all three vitamins simultaneously in six healthy volunteers.

**MATERIALS AND METHODS**

Six healthy laboratory workers between the ages of 22 and 43 years volunteered to take part in the study. There were three males and three females. All were known to be receiving nutritionally adequate diets. One female was taking oral contraceptive agents.

Oral loading doses of the vitamins were given on each of days five, four and three before the test pteroylmonoglutamic acid (4 mg), thiamin hydrochloride (11.12 mg) and pyridoxal hydrochloride (22.22 mg). This was equivalent to 10 mg of thiamin and 20 mg of pyridoxal. All vitamins were obtained from the Sigma Company. They were dissolved in a small volume of water and swallowed. No vitamins were given on the two days preceding the test. On the morning of the test a fasting blood sample was collected for the assay of serum and red cell vitamin levels and for the measurement of plasma creatinine. A sample of urine was collected at the same time for vitamin and creatinine measurements. Each volunteer was then given an oral dose of folate, thiamin and pyridoxal as described above. The vitamin concentrations were equivalent to ten times the recommended daily dietary allowance (8, 9). Normal dietary habits were resumed four hours after taking the test doses of the vitamins.

Blood samples were withdrawn at 0.5, 1.0, 2.0, 6.0 and 24 h intervals following the vitamin load. All urine passed over the following 24 h was collected at set intervals, volumes were recorded and an aliquot of the sample was retained for the measurement of vitamins and creatinine. Vitamins were measured using automated microbiological assays with *Lactobacillus casei* as the test organism for folate and pyridoxal (10, 11) and *Lactobacillus fermenti* for thiamin (12). The limits of de-

tection for these methods are 0.5 µg/liter for folate, 2 µg/liter for pyridoxal and 0.5 µg/liter for thiamin. The test organisms are sensitive to the various coenzyme forms of folate and thiamin but not to their metabolites. Under the conditions of the test, _L. fermenti_ was found not to respond to the presence of the pyrimidine or thiazole moieties at concentrations up to 1 mg per ml. The growth response of the test organism to thiamin monophosphate is similar to that of thiamin hydrochloride, however, the growth response of this organism to thiamin pyrophosphate is 35% greater than that obtained with equimolar amounts of thiamin (12). The findings of Thom (13) indicated that 20–30% of thiamin in normal plasma is protein bound and that this represents the thiamin pyrophosphate fraction of the total thiamin. _In vitro_, any thiamin pyrophosphate which is not protein bound is rapidly dephosphorylated by a potent plasma phosphatase which appears to be specific for thiamin. Hence plasma thiamin is predominantly in the free non-phosphorylated or monophosphate forms. The vitamin excreted in the urine is also in the free form and for this reason, thiamin hydrochloride standards were used. Erythrocytes on the other hand contain mainly thiamin pyrophosphate and this was used to prepare the standards when making red cell measurements (12, 13). Pyridoxal was dephosphorylated before being assayed using an acid phosphatase.

The methods as used routinely in our laboratory have a coefficient of variation of less than 5% for duplicate analyses.

Creatinine was measured using the method of Bonsnes and Taussky (14) modified for use on the Technicon S. M. A. 6/60. Urinary creatinine clearance ratios of folate and thiamin were calculated using the formula:

\[
\frac{A \times B}{C \times D}
\]

where _A_, urinary vitamin concentration; _B_, plasma creatinine; _C_, plasma vitamin concentration; _D_, urinary creatinine concentration.

A ratio exceeding 1.0 reflects active tubular secretion.

RESULTS

To enable the results of absorption of the three vitamins to be compared, increases in the serum and red cell concentrations were recorded as percentage rise above the baseline level. The results for the serum levels are shown in Fig. 1. Thirty minutes following the oral dose of the vitamins, the serum folate and pyridoxal concentrations had risen from a mean basal level of 10.9 µg/liter and 17.5 µg/liter to 66.7 µg/liter and 299.0 µg/liter respectively. Mean peak serum levels for folate and pyridoxal were 174.8 µg/liter and 315.2 µg/liter respectively, an increase above the baseline of 1,500% for folate and 1,701% for pyridoxal. The mean serum thiamin had risen only marginally at 30 min from 5.1 to 5.9 µg/liter and peaked at 7.2 µg/liter, an increase of 42%. Six hours following the test dose, the serum concentration of thiamin had fallen back to its basal level (5.2 µg/liter).

The red cell concentration of pyridoxal rose from a mean basal level of
Fig. 1. Serum levels of vitamins recorded as percentage increase above the baseline following an oral load. Folate —, pyridoxal ——, thiamin ----.

Table 1. 24 h urinary excretion of thiamin and folate following an oral dose of the vitamins.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Thiamin (µg)</th>
<th>Folate (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,013.4 (10.1)</td>
<td>2,109.7 (52.7)</td>
</tr>
<tr>
<td>2</td>
<td>957.7 (9.6)</td>
<td>2,455.1 (61.4)</td>
</tr>
<tr>
<td>3</td>
<td>873.8 (8.7)</td>
<td>2,097.0 (52.3)</td>
</tr>
<tr>
<td>4</td>
<td>726.5 (7.3)</td>
<td>1,948.6 (48.7)</td>
</tr>
<tr>
<td>5</td>
<td>878.7 (8.8)</td>
<td>2,084.3 (52.1)</td>
</tr>
<tr>
<td>6</td>
<td>556.7 (5.6)</td>
<td>1,776.4 (44.4)</td>
</tr>
</tbody>
</table>

Figures in parenthesis denote percentage of administered dose.

5.7 µg/liter to a peak of 76.9 µg/liter at 30 min, a rise of 1,249%, it then fell rapidly in a manner similar to the plasma vitamin. The red cell concentration of folate and thiamin showed only a small change. The 24-h urinary excretion of folate and thiamin is shown in Table 1. Very little urinary pyridoxal was detected (range 0.96–2.6, mean 1.74% of dose) because the vitamin is mainly excreted as the terminal metabolite 4-pyridoxic acid (15) and we were unable to measure this. A
mean total of 0.8 mg of thiamin equivalent to 8.3% of the oral dose, and 2.1 mg of folate equivalent to 52% of the oral dose, was excreted over the 24-h period. Folate and thiamin creatinine clearance ratios were calculated and the results are shown in Tables 2 and 3. The mean clearance ratios of thiamin and folate before the loading dose of the vitamins was 0.6 and 0.007 respectively.

Two hours following the dose, the mean clearance ratios were 2.4 and 0.7 and by 24 h they had fallen to 0.6 and 0.004 respectively. One volunteer showed a clearance ratio for thiamin greater than one in all samples; the reason for this is not clear. Two volunteers showed active excretion of folate in one sample.

**DISCUSSION**

Volunteers given an oral dose of pteroylmonoglutamic acid, pyridoxal hydrochloride and thiamin hydrochloride rapidly absorbed the pteroylmonoglutamate and pyridoxal, achieving peak mean serum levels of 174.8 µg/liter and 315.2 µg/liter respectively, representing an increase above the baseline concentration of 1,500 and
Thiamin appeared to be poorly absorbed and rose from a mean baseline concentration of 5.1 to a peak of 7.2 μg/liter, an increase of only 42%. It is unlikely that this was due to rapid uptake by tissues because the volunteers had been previously loaded with each of the vitamins. Because of the extensive work done previously on folate absorption which has been summarised by Chanarin (16), it was thought that a dose equal to ten times the recommended daily dietary allowance for each vitamin would be sufficient to adequately load the volunteers. It has been shown that approximately 50% of a test dose of folate is excreted in the urine using a wide range of test doses.

Only minor changes occurred in the red cell concentrations of folate and this related to the inability of pteroylglutamic acid to cross the red cell membrane. Thiamin is also unable to pass freely across the red cell membrane but in these studies the serum level of the vitamin did not increase sufficiently to test this. Pyridoxal on the other hand is able to move freely into and out of red cells and this is reflected in the rapid rise and fall in the erythrocyte concentration of the vitamin (17).

The purpose of this study was not to test the so-called active mechanism used for transporting small physiological quantities of thiamin (18, 19), but rather to look at the ability of the intestine to absorb larger quantities, using folate (as pteroylmonoglutamate) and pyridoxal, which appear to pass rapidly across the intestinal mucosa, for comparison.

A mean total of thiamin equivalent to 8.3% of the 10 mg dose was recovered from the urine compared with 52% of the 4 mg dose of folate. This is slightly more than that recovered by Hewitt and Levy (20) (mean 6.42%). However, they used a test dose of 26.9 mg of thiamin hydrochloride which was substantially larger than that used by us. It has been shown previously that increasing the dose of the vitamin results in a decreasing percentage recovery from the urine (7). The whole vitamin appears to be excreted rather than its metabolites; Icke (12) recovered 50% of a 10 mg intravenous dose from urine in two hours following injection.

Absorption of thiamin appears to be a controlled process and even at therapeutic doses only a small percentage of the vitamin is absorbed. Raising the serum level of the vitamin results in active urinary excretion on the basis of the creatinine clearance. Assuming the creatinine clearance to be an approximate measure of glomerular function, the ratio of vitamin clearance to creatinine clearance will give an estimate of the degree of retention or excretion of the vitamin by the renal tubules, a ratio of greater than 1.0 indicating active excretion. Before the test dose of the vitamins was given, both folate and thiamin (with one exception) were retained by the kidneys and folate in the main continued to be retained throughout the trial. In contrast, all volunteers actively excreted thiamin during the six hours following the oral vitamin load by which time the plasma level of the vitamin had fallen to the baseline. There appears to be a mechanism which is able to closely control the plasma concentration of thiamin. This is partially explained by Thom (13), who reported that 20–30% of plasma thiamin from normal adults was
protein bound, all of which appeared to be as pyrophosphate. This is not in accord with a previous report from this laboratory (21) where a higher level of thiamin pyrophosphate was found. This discrepancy is thought to be the result of improvements in methodology. Thom also demonstrated a potent enzyme in normal human plasma which rapidly dephosphorylated any unbound thiamin pyrophosphate, and suggested this may be a mechanism to facilitate excretion of an excess of the vitamin. The rapid dephosphorylation of thiamin pyrophosphate combined with the active renal clearance of the vitamin suggests close control on the plasma levels of this vitamin. The reason for this close control which, with the exception of riboflavin (7), does not occur with other water-soluble vitamins is not known. The active renal clearance of the vitamin is a new finding and requires further study.

This study was supported by a National Health and Medical Research Grant.

REFERENCES

15) Reddy, S. K., Reynolds, M. S., and Price, J. M. (1958): The determination of 4-


