TITLE: The absorption of Radioactive Sulphur-labelled Thiamine Hydrochloride in Control Subjects and in Patients with Intestinal Malabsorption.

SHORT TITLE: A test for the Absorption of Thiamine in Man

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SUMMARY:

(1) A method is described for investigating vitamin B₁ absorption in man by measuring the urinary radioactivity during the 24 hours following an oral dose of radioactive $^{35}$S-thiamine which is given along with a parenteral injection of non-radioactive thiamine hydrochloride.

(2) Analysis of small intestinal juice and characterisation of radioactive material in the urine indicate that the urine contains radioactive thiamine which was unchanged prior to absorption.

(3) In control subjects, the major period of thiamine absorption occurred within the first 2 hours and practically all of the oral dose absorbed was excreted during the first 24 hours.

(4) Eight patients with untreated primary malabsorptive disease (idiopathic steatorrhoea) showed a mean urinary excretion of radioactive thiamine significantly less than in the control group. The rate of excretion was markedly reduced but the period of maximal excretion occurred at approximately the same time as in control subjects. Thirteen patients with treated primary malabsorptive disease showed no significant difference from the control group.

(5) Ten patients who had undergone gastric surgery and two with resection of the terminal ileum excreted amounts which did not differ from the control group. Normal results were also found in four patients with pernicious anaemia.
A Test for the Absorption of Thiamine in Man

INTRODUCTION:

Disease of the small intestine may cause impaired absorption of certain water-soluble vitamins. Patients with primary malabsorptive disease (idiopathic steatorrhea), for example, frequently fail to absorb folic acid normally (Girdwood, 1953; Girdwood & Delamore, 1961; Chanarin, Anderson & Mollin, 1958; Cox, Meynell, Cooke & Gaddie, 1958). Although clinical evidence of deficiency of other water-soluble vitamins is rarely present, investigations using the tryptophan loading test have suggested subclinical deficiency of pyridoxine in patients with tropical sprue and primary malabsorptive disease (Kowlessar, Haeffner, Benson & Sleisenger, 1961; Sigler, Sheehy, Santini & Rubini, 1962). Baker & Sobotka (1962) have shown that patients with primary malabsorptive disease may have subnormal levels of serum pyridoxine and Brain & Booth (1964), using tritium-labelled pyridoxine, have demonstrated impaired absorption of pyridoxine in some of these patients.

Girdwood, (1956) using microbiological methods, studied the absorption of water soluble vitamins in patients with primary malabsorptive disease, but found no evidence of malabsorption of thiamine, riboflavin or pyridoxine in 10 patients with impaired absorption of folic acid. Except for this investigation, little has been published on the absorption of thiamine in patients with intestinal malabsorption.

The availability of $^{35}$S-labelled thiamine has allowed some of the limitations of the microbiological methods to be overcome. This paper presents a method for the measurement of thiamine absorption using radioactive thiamine and compares the results found in control subjects with those obtained in various groups of patients with gastro-intestinal disorders.

MATERIALS AND METHODS

Materials Used

All of the thiamine preparations were supplied as the hydrochloride. $^{35}$S-labelled thiamine was obtained from the Radiochemical Centre, Amersham. The radioactive material used orally had a specific activity of $17\,\mathrm{mc/gm}$ and was found to be radiochemically pure when tested in the three chromatographic systems described below. The solid material was dissolved in distilled water, $100\,\mu\mathrm{c}$ (10 doses) dispensed into each ampoule, freeze dried and stored at $-20^\circ\mathrm{C}$.
It was reconstituted by adding 10ml distilled water. In a few experiments radioactive thiamine was used intravenously. This material was supplied in aqueous solution pH5; it had a specific activity of 12.0μc/ml and analysis showed that it was more than 90% radiochemically pure. The non-radioactive thiamine was supplied in ampoules containing 100mg in one ml by Roche Products Ltd, England.

Method for the Study of Absorption of 35S-labelled thiamine

The radioactive material was diluted with non-radioactive thiamine so that each test dose contained 1.0mg and 10μc of radioactivity dissolved in 20ml of water. A quantity of the solution from which the test dose was prepared was used as a standard. The test dose was given orally to subjects after an overnight fast, and in some experiments a parenteral injection of non-radioactive thiamine was given immediately before the oral dose. The standard test referred to later includes both a 1.0mg oral dose of radioactive thiamine and a parenteral injection of 200mg non-radioactive thiamine given at the same time as the oral dose. Absorption was assessed by measuring the urinary radioactivity during the following 24 hours. The urine collections were removed after the intervals 0-5 hours, 5-12 hours, 12-24 hours, the volume measured and a sample stored at -20°C until the radioactivity was measured. The urine samples were counted in an Isotope Developments Ltd, Reading, Berkshire, liquid scintillation counter type 6012, using 0.8gm per cent of 2-5 diphenyloxazole and 0.005gm per cent of 1,4 - bis (2/5-phenyloxazolyl) benzene dissolved in toluene as the liquid scintillator. The samples were prepared by adding 1.0ml urine to 3ml hyamine chloride, 1.5M solution in methanol, and adding 10ml liquid scintillator. 10μc of the test dose was added as an internal standard to each sample.

Identity of the radioactivity present in intestinal contents and urine after oral administration of 35S-labelled thiamine; Urine was collected in a brown bottle surrounded by a freezing mixture of ice and solid carbon dioxide (Drikold, ICI Ltd), during the periods 0-1½ hours, 1½-3 hours, 3-12 hours. Following voiding, the urine was either analysed immediately or stored at -20°C. A modification of the phenol extraction procedures of Iacono & Johnson (1957) was used to extract the radio-metabolites from the urine. Over 90% of the radioactivity was removed by this method.
Descending chromatography was performed on Whatman No. 1 paper using N-propanol-water-acetate buffer pH 5(70:20:10), the upper phase of N-butanol/acetate acid/water (40:10:50), and the upper phase of sec-butanol/water as solvents. The papers were scanned in a BTL Radioactive Chromatogram Scanner and radioautographs were prepared with Gavert no-screen X-ray film; the film was exposed to the chromatogram for up to three months, depending on the amount of radioactivity.

Identification of Thiamine Compounds on Paper Chromatograms

A. Thiochrome Test. After drying the chromatograms at room temperature thiamine and its esters present on the paper were converted to the corresponding thiochromes by the method of Siliprandi & Siliprandi (1954); this test will detect 1.0 µg thiamine.

B. Use of Micro-organisms. Some chromatograms were not sprayed but were scanned and the radioactivity eluted with 3ml distilled water. The ability of the eluate to support growth of O. danioa which requires intact thiamine (Heinrich, 1955; Baker, Frank, Fennelly & Levey, 1964), was compared with a control consisting of an equivalent area cut from the same chromatogram. The method of Baker et al (1964) was used for thiamine assay. Following incubation, the material was centrifuged at 3,000 revolutions per minute for 30 minutes and subsequently washed by centrifuging after resuspension in two aliquots of 20ml sterile distilled water. The radioactivity could not be washed from the organisms. The organisms were finally suspended in gel-scintillator and the radioactivity present counted by well-scintillation counting.

C. Separation of Radioactive Compounds. To confirm that complete separation had been achieved, two chromatograms were run in each of the three solvent systems and the radioactivity corresponding to thiamine eluted as above. Each of the eluates was then re-run in a different chromatographic solvent system and produced a single spot at the same Rf as thiamine. The radioautographs also showed a single discrete spot.

Demonstration of Thiamine and its Metabolites in the Urine. Four patients were given 300mg of non-radioactive thiamine hydrochloride intravenously 48 hours before the experiment. On the day of the experiment, they received the standard test of 1.0mg oral radioactive thiamine hydrochloride and
200mg non-radioactive thiamine intravenously. In three subjects, over 90% of the radioactivity excreted during the 0-12 hour period had the same Rf as thiamine in all three solvent systems and gave a positive thiochrome test. The eluted radioactive substances supported the growth of O. danica and the radioactivity could not be washed from the cells. In the fourth subject, during the period 1\frac{1}{2}-3 hours after the dose 90% of the radioactivity was due to thiamine but this fell to 60% in the 3-12 hour period. No attempt was made to identify the other radioactive compounds.

**Extraction of Radio-metabolites from Small Intestinal Juice.** Two normal subjects were intubated using a 1.5mm bore polyvinyl tube weighted at one end. The duodenal-jejunal junction is approximately 90cm and the ileocecal valve 350cm from the nose in adults (Blankenhorn, Hirsch & Ahrens, 1955). Sampling in one subject was from points 105cm and 195cm from the nose on two successive days, i.e. from the jejunum and from the proximal ileum; in the other, sampling was only from the proximal ileum. The fasting subjects were fed a test meal consisting of "Humanised Trufood" (Trufood Ltd, The Creamaries, Wrenbury, Cheshire) and 5μg of 35S-thiamine with sufficient non-radioactive thiamine to give a final thiamine content of 1.0mg. The small intestinal juice was obtained by continuous sampling into a flask surrounded by a freezing mixture. The volume of intestinal juice decreased considerably after the third hour. Consequently, the observations relate to the 0-3 hour period. The pH of the intestinal juice was measured with a glass electrode and never rose above pH 6.1, i.e. it was always within the range at which thiamine is stable. At the end of the experiment, gastrografin was introduced into the tube to check the position of the sampling point.

Twenty ml of small intestinal juice were dialysed against three 100ml portions of distilled water left for 24 hours at 4°C. The dialysates were freeze-dried and then dissolved in 30ml absolute ethanol. The solution was concentrated in vacuo at 35°C to about 4ml and a sample chromatographed. It was found that over 90% of the radioactivity was identical, chromatographically, with thiamine.

**Subjects Studied**

Controls were sixty five convalescent, ambulant hospital patients free from gastro-intestinal, endocrine, collagen and malignant diseases. They had
normal hepatic and renal function and were not receiving drug therapy. They showed no signs of nutritional deficiency. Patients with primary malabsorptive disease included 10 cases who had not been treated with a gluten free diet. The diagnosis was established by jejunal biopsy and the results of the following absorption tests - folic acid absorption (Girdwood, 1953; Girdwood & Delamore, 1961), vitamin B₁₂ absorption (Schilling, 1953) xylose absorption (Fourman, 1948) and faecal fat excretion (Kamer, Bokkel, Huink, & Weyers, 1949). A sternal marrow was performed and serum folate, formiminoglutamic acid (FIGLU), and vitamin B₁₂ levels were measured. All patients had a barium meal and follow-through examinations. There were also 13 patients who had been treated with a gluten free diet for periods varying from 3 months to 5 years.

The other patients included 10 who had had gastric surgery and three patients with pernicious anaemia who had confirmed acid-fast achlorhydria in response to a maximum histamine test meal and were untreated at the time of investigation. Three patients had Crohn's disease and one who had had a sub-total proximal gastrectomy with an end-to-side oesophago-gastric anastomosis for an anaplastic carcinoma of the stomach 17 years previously, but there was no postmortem evidence of a recurrence when she died from a myocardial infarction some months after completion of the studies. Two of the patients with Crohn's disease had had a resection of the terminal ileum and a right hemicolectomy.

RESULTS

Investigation of Test Conditions. The excretion of radioactivity when ten control subjects were only given 1.0 mg of radioactive thiamine orally was 6.1% (SEM ± 0.64) but if the oral dose was preceded by giving 300 mg of non-radioactive thiamine intravenously 48 hours before the excretion of radioactive thiamine rose to 15.3% (SEM ± 2.63). This difference is highly significant. (t = 4.26, p < 0.01).

The Standard Test. The effect of varying the size of an intravenous dose of non-radioactive thiamine given at the same time as the oral dose of radioactive material was studied and the optimum amount for this intravenous flushing dose was found to be 200 mg. This dose ensured maximal excretion and was thereafter used in the standard test of thiamine absorption (Table 1). Giving thiamine to control subjects two days before they received the standard
### TABLE 1

**TWENTY FOUR HOUR URINARY EXCRETION OF RADIOACTIVE THIAMINE BY NORMAL SUBJECTS**

The effect of injecting varying amounts of non-radioactive thiamine at the time of administering 1.0mg radioactive thiamine by mouth on the excretion of radioactive thiamine in the urine.

<table>
<thead>
<tr>
<th>Intravenous dose of non-radioactive thiamine (mg)</th>
<th>Urinary excretion of radioactivity (% oral dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before loading</td>
<td>After loading **</td>
</tr>
<tr>
<td>0</td>
<td>6.1 ± 0.64 (10) 15.3 ± 2.64 (6)</td>
</tr>
<tr>
<td>25</td>
<td>35.0 ± 2.95 (5)</td>
</tr>
<tr>
<td>50</td>
<td>39.5 ± 3.18 (7)</td>
</tr>
<tr>
<td>100</td>
<td>51.7 ± 5.04 (9)</td>
</tr>
<tr>
<td>200</td>
<td>48.2 ± 4.54 (10) 54.1 ± 5.66 (10)</td>
</tr>
<tr>
<td>300</td>
<td>50.2 ± 3.66 (8)</td>
</tr>
</tbody>
</table>

* The results are expressed as the mean ± SEM (n). Urine was collected for 24 hours.

** Subjects given 300mg thiamine i.v. 48 hours before test, and a further dose of thiamine at the time of the test.
Fig 1. Cumulative urinary excretion of radioactivity after 1.0mg $^{35}$S-thiamine orally and 200mg non-radioactive thiamine intravenously in a control subject and in a patient with primary malabsorptive disease.
EFFECT OF VARYING THE TIME OF THE INITIAL FLUSHING
DOSE AND GIVING ADDITIONAL INTRAVENOUS FLUSHING DOSES

Subjects were given two tests: First* and Second** and received 300mg non-radioactive thiamine intravenously 4.8 hours before each test.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test</th>
<th>$^{35}$S- thiamine urinary excretion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>First</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>53.0</td>
</tr>
<tr>
<td>2</td>
<td>First</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>49.0</td>
</tr>
<tr>
<td>3</td>
<td>First</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>41.2</td>
</tr>
<tr>
<td>4</td>
<td>First</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>80.0</td>
</tr>
<tr>
<td>5</td>
<td>First</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>58.0</td>
</tr>
<tr>
<td>6</td>
<td>First</td>
<td>59.9</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>59.0</td>
</tr>
</tbody>
</table>

* First test = 1.0mg radioactive thiamine orally and 200mg non-radioactive thiamine flushing dose intravenously at the same time.

** Second test = Subjects 1-3 same as first test with additional 100mg flushing doses at 2, 6 and 12 hours after oral dose.

4 same as first test with additional 200mg at 4 hours
5 received 200mg i.m. only at time of oral dose
6 no flushing dose at time of oral dose but 200mg i.v. 1 hour after.
Fig. 2a  The radioactivity in serum and urine after administration of radioactive thiamine given intravenously along with 200mg non-radioactive thiamine.
The radioactivity in serum and urine after administration of radioactive thiamine intramuscularly on a different occasion to the same control subject as in Fig. 2a. 200mg non-radioactive thiamine were given intravenously along with the radioactive thiamine.

![Graph showing the radioactivity in serum and urine after administration of radioactive thiamine.](Image)
test did not alter the mean urinary excretion of radioactivity. \((t = 0.814; 0.2 \geq p < 0.5)\).

Figure 1 shows the cumulative excretion of radioactivity after 200mg flushing dose in a control subject who previously received 300mg thiamine intravenously 48 hours before. Excretion was maximal between 1-2 hours during which time 41% of the dose was excreted. By 12 hours, excretion was almost complete, 3% being added in 12-24 hours, and further small amounts thereafter (3.4% in 24-48 hours and 4.3% in the 48-96 hour period after which excreted radioactivity was too small to be measured accurately).

The decline in the rate of excretion of urinary radioactivity two hours after the standard test, may not have been due to a decrease in the absorption of oral thiamine but may have followed a fall in the blood level of the flushing dose due to the rapid excretion. Consequently, radioactive thiamine \((5.0\mu g)\) was incorporated in a 200mg intravenous injection on non-radioactive thiamine to investigate the duration of the flushing dose. This showed that 4.5% \((9.0mg)\) of the intravenous dose was still being excreted during the 4-5 hour period. Similarly, varying the time of the flushing dose or maintaining a high blood level of non-radioactive thiamine during the first 16 hours by repeated injection had a negligible effect on the total urinary recovery. (Table 2). When the standard test alone was repeated in four subjects, the following results were obtained: 46.0%, 38.4%; 49.0%, 54.0%; 58.4%, 54.7%; 60.0% and 62.7%.

Eighty eight per cent of the intravenous dose was recovered during the first 24 hours and 90% after the radioactive thiamine was given intramuscularly to the same subject (Figs 2a and 2b). Another subject excreted 93% of an intravenous dose in 24 hours. Consequently, it seems that practically all of the oral dose absorbed, once it reaches the blood stream, will probably be excreted during the first 24 hours, under the conditions of the standard test.

**STUDIES ON PATIENTS**

**Primary Malabsorptive Disease.** The excretion of eight patients with primary malabsorptive disease given 300mg intravenously 48 hours before the standard test was 26.7(SEM ± 5.11) which was significantly less than the control group \((t = 3.504; p<0.01)\). When the 300mg "loading" dose was omitted there was no decrease in the recovery of urinary radioactivity 28.1\%(SEM ± 4.75). The difference between these patients and the controls was not due to a decreased
The patient was given 300mg thiamine intravenously 48 hours before each test.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Urinary radioactivity after Standard Test</th>
<th>Time of additional 100mg i.v. flushing doses of thiamine</th>
<th>Urinary radioactivity after additional flushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2</td>
<td>0.3</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>2 - 4</td>
<td>7.4</td>
<td>4 hr</td>
<td>7.0</td>
</tr>
<tr>
<td>4 - 6</td>
<td>4.8</td>
<td>6 hr</td>
<td>6.1</td>
</tr>
<tr>
<td>6 - 12</td>
<td>6.4</td>
<td>12 hr</td>
<td>10.9</td>
</tr>
<tr>
<td>12 - 24</td>
<td>6.3</td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>

25.2 29.3
**TABLE 4.**

**THIAMINE ABSORPTION IN PATIENTS WITH PRIMARY MALABSORPTIVE DISEASE**

Standard test repeated in the same subject after giving 300mg thiamine intravenously 48 hours before the second test.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of subjects</th>
<th>Before loading</th>
<th>After loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>8</td>
<td>$26.1 \pm 4.75$</td>
<td>$26.7 \pm 5.11$</td>
</tr>
<tr>
<td>Treated</td>
<td>13</td>
<td>$36.9 \pm 4.33$</td>
<td>$40.8 \pm 3.84$</td>
</tr>
</tbody>
</table>

The results are expressed as the mean $\pm$ SEM (n)

Loading was achieved as before: (see Table 1)
Fig 3. Thiamine absorption in various clinical states.
ability to excrete thiamine as a patient was able to excrete an intravenous dose normally. The cumulative excretion of radioactivity in a "loaded" untreated patient showed delay in excretion and marked reduction in the total excreted 9.4% although maximal excretion occurred within the first 6 hours. Additional flushing doses given to three malabsorptive patients during the 0-24 hour period and to two others during the 24-48 hour period did not alter the pattern of excretion or significantly increase the total radioactivity excreted; the details of one patient are shown in Table 3.

In thirteen patients who had been treated with a gluten free diet and replacement therapy, previous loading again had no effect on the excretion of radioactivity (Table 4). The previously "loaded", treated patients did not differ from the control group \( t = 2.012; 0.5 < p < 0.1 \) but differed significantly from the untreated patients \( t = 2.231; 0.02 < p < 0.05 \).

Miscellaneous Conditions. The results of eight patients with gastroenterostomies are shown in Figure 3 and there is no evidence of abnormal absorption \( t = 0.395; p > 0.5 \).

One patient with a partial gastrectomy excreted 71.6% of the oral dose while another excreted only 3.8%; this second patient had an abnormal villous pattern which may have been associated with altered gastric function. He excreted 89% in the first 24 hours when the same dose was given intravenously. A patient who had had a sub-total gastrectomy 17 years previously excreted 65.5%

Three patients with Crohn's disease involving the terminal ileum, who had evidence of malabsorption of Vitamin B₁₂ and a reduced serum vitamin B₁₂ level, gave normal results. Four patients with untreated pernicious anaemia also showed normal results.

DISCUSSION

These investigations indicate that radioactive thiamine can be used to study the absorption of this vitamin in man. Analysis of small intestinal juice and extraction of the radioactivity present in the urine, shows that during the period when maximal absorption is taking place, little breakdown of thiamine occurs in the small intestine and that most of the radioactivity obtained in the urine is thiamine which was unchanged prior to absorption.

When a relatively large injection of non-radioactive thiamine is given intravenously, the percentage excretion of radioactivity rises from about
6.0% to over 50.0%. Since the non-radioactive parenteral thiamine promotes the urinary excretion of the labelled vitamin, it would seem that the radioactive thiamine behaves similarly to the non-radioactive thiamine during its passage through the body. Increasing the flushing dose to 300mg thiamine does not produce any further increase in the percentage urinary excretion of radioactive thiamine (Table 1; p<0.5) and it would therefore seem unlikely that the flushing dose is altering the normal physiological activity of the intestine.

If an absorption test is to be sensitive to physiological amounts of the test substance, the size of the test dose must be large enough to show that the subject is capable of absorbing his minimum daily requirement during the 24 hours but must not exceed the reserve capacity of the intestine in the control subject. The minimum daily requirement of thiamine for the adult human has been estimated at between 0.23mg/1000cal and 0.66mg/1000cal (Williams, Mason, Smith & Wilder, 1942; Oldham, Johnston, Kleiger & Hedderich-Arisendi, 1944; Daum, Tuttle & Wilson, 1949; Dick, Chen, Bert & Smith, 1958 and Ziporin, Nunes, Powell, Waring & Sauberlich, 1965). The work of Morrison & Campbell, (1960); Friedemann, Kmiecik, Keegan & Sheft (1948) and Schultz, Light & Frey (1938) suggests that little further absorption occurs when the dose of thiamine exceeds 4-5mg. Therefore, 1.0mg was chosen as a reasonable oral dose.

Although one control patient excreted 90.7% of the oral dose within the first 24 hours, the mean excretion for the group was only 54.1%(SEM ± 5.66) and prolonging the urine collection for nine days in one subject added only a further 7.5%. The total thiamine content of the human body has been estimated to be 30mg (Takeda, 1947). Approximately 0.05mg of radioactive thiamine is contained in the oral dose. An attempt was made to reduce any exchange with the body stores by previous loading of the patient and by the provision of additional flushing doses each of 200mg of non-radioactive thiamine to dilute the absorbed radioactive thiamine. However, giving additional flushing doses or variation in time or route of administration of the flushing dose, did not significantly increase the total 24 hour excretion of activity nor alter the pattern of excretion. Also Yano (1958) recovered approximately 0.5mg of a 2.0mg oral dose of thiamine from the stool. Consequently, it would seem
that most of the oral dose absorbed is excreted during the first 24 hours, under the conditions of the test. Haugen (1961) showed that excessive amounts of thiamine are rapidly excreted by the kidney and in the present investigations there was no evidence that at any time the kidneys were unable to excrete the load presented to them.

In control subjects the major period of absorption was in the first two hours after the oral dose (Fig. 1). The work of Middleton & Grice (1964) in the rat indicates that the site of maximum absorption for thiamine is in the duodenum and upper small intestine, and this agrees with the findings of Polin, Loukides, Wynosky & Porter (1964) in the chick. Three patients with Crohn's disease involving the lower small intestine and showing impaired absorption of vitamin B₁₂ excreted normal amounts of radioactivity after the standard test for thiamine, suggesting that absorption of vitamin B₁ in the human is mainly in the upper small intestine.

Wang & Harris (1939) and Brummer & Markkanen (1960) measured the daily urinary excretion of dietary thiamine in achlorhydric subjects found evidence of reduced excretion indicating depletion which might have been secondary to reduced absorption. During the present investigation, four patients with pernicious anaemia and three with histamine fast achlorhydria following gastric surgery, were all found to excrete normal amounts of radioactivity. If absorption is grossly impaired, malabsorption may become apparent when the test dose is small. A larger dose, however, may result in a greater difference between normals and patients or may demonstrate impairment which was not obvious with the smaller dose as occurs with xylose absorption. Consequently, the results in achlorhydria are not necessarily incompatible with the observations of Wang & Harris (1939) and Brummer & Markkanen (1960) although there may be other factors producing depletion in their patients.

The paired t-test showed no significant difference between malabsorptive patients who had been previously loaded and those who had not, either before, or after treatment. It was also shown that the mean of the untreated malabsorptive patients, with previous "loading" was significantly less than the treated patients. However, the mean of the treated malabsorptive patients, who were not given 300mg 48 hours before treatment, was low. Consequently, further statistical tests were applied. Seven of the eight differences in the untreated patients
were positive but the result of the Wilcoxon's sign test (Spiegel, 1956) confirmed the t-test findings. Similarly, in control subjects, previous "loading" did not significantly alter the result. Consequently, the average of the result before and after "loading" was taken in each malabsorptive patient and the resulting two groups compared with the control group considered as twenty independent observations. A one-way analysis of variance on these three groups was found to be highly significant confirming the difference between the untreated and control groups. $P[F] = 5.21;$ d.f.$[(2,38)] = 0.99$. The one-tailed $t$-test applied to the average of the treated and untreated malabsorptive patients, gave $t = 1.8$ with 19 d.f. and this was significant at the five per cent level. There was no correlation between the ability of the malabsorptive patients to absorb thiamine and other intestinal absorption tests or with the severity of the changes shown by jejunal biopsy. The results in these patients illustrate again the variation in the ability of individual patients to absorb different substances during the acute phase of the disease.
REFERENCES


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