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## OBSERVATIONS ON THE MECHANISM OF THIAMINE HYDROCHLORIDE ABSORPTION IN MAN

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### SUMMARY

1. The intestinal absorption of [<sup>35</sup>S]thiamine hydrochloride was investigated in healthy subjects, malnourished alcoholics and a patient with resection of the jejunum and ileum. Serum and urinary radioactivity was studied after administration of 10  $\mu$ Ci of [<sup>35</sup>S]thiamine hydrochloride in 1–50 mg of non-radioactive thiamine hydrochloride.

2. Results suggest that intestinal absorption of thiamine hydrochloride is rate-limited. Though the results provide only indirect information on intestinal transport rates, they are consistent with the Michaelis–Menten relationship used to describe enzyme–substrate reactions. Calculations by this model yielded a  $V_{\max}$  of  $8.3 \pm 2.4$  mg and a  $K_m$  of  $12.0 \pm 2.4$  mg for normal subjects with a significant decrease in  $V_{\max}$  in malnourished alcoholics and a patient with resected small intestine.

3. Intestinal absorption and the calculated value of  $V_{\max}$  for thiamine hydrochloride is increased in malnourished alcoholics after correction of malnutrition. These findings are consistent with the thesis that this vitamin is absorbed by a saturable mechanism and that the number of effective receptor sites may be reduced by malnutrition or intestinal resection.

**Key words:** thiamine hydrochloride absorption, malnutrition, intestinal resection.

Thiamine deficiency syndromes may occur despite oral intake of established minimum daily requirements of thiamine hydrochloride (Fennelly, Frank, Baker & Leevy, 1964). This is largely due to malabsorption of this vitamin, resulting from tissue changes induced by malnutrition (Thomson, Baker & Leevy, 1968), ethanol toxicity (Thomson, Baker & Leevy, 1970) or other factors. It is widely believed that interference with a rate-limited process may be responsible for defective absorption of thiamine hydrochloride. This view is supported by a decrease in absorption of [<sup>35</sup>S]thiamine hydrochloride in patients with coeliac disease, which

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returned to normal following a gluten-free diet (Thomson, 1966a). A similar decrease in thiamine hydrochloride absorption occurs in malnourished alcoholics, which also returns to normal with correction of nutritional deficiency (Thomson *et al.*, 1970).

Nevertheless, several workers have failed to demonstrate a rate-limited process for thiamine hydrochloride absorption in animals (Turner & Hughes, 1962; Spencer & Bow, 1964), although others claim to have demonstrated a saturable mechanism (Ventura, Ferrari, Theliadue & Rindi, 1969; Friedman, Kniecziak, Keegan & Sheft, 1948; Morrison & Campbell, 1960). Moreover, the validity of conclusions on studies of thiamine absorption in man has been questioned because of difficulty in identifying metabolites, and because of lack of information on intestinal breakdown, storage and utilization of the administered vitamin (Thomson, 1969). Absorption tests with [<sup>35</sup>S]thiamine hydrochloride indicate that the administered radioactivity is absorbed and excreted with negligible breakdown of the vitamin. This substance was used in the present studies to evaluate the kinetics of intestinal transport of thiamine hydrochloride.

## MATERIALS AND METHODS

### *Radioactive material*

[<sup>35</sup>S]Thiamine hydrochloride was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. It had a specific radioactivity of 356 mCi/g and was found to be radiochemically pure when tested by descending chromatography on Whatman No. 1 paper using *n*-propanol-water-1 M-sodium acetate buffer, pH 5 (7:2:1, by vol.) and by thin layer chromatography on silica gel using pyridine-acetic acid-water (10:1:40, by vol.). The solid material was dissolved in distilled water, 100  $\mu$ Ci was dispensed into each ampoule, freeze dried, and stored at -20°C. It was reconstituted by adding 20 ml of water and non-radioactive thiamine hydrochloride so that each test dose contained from 1.0-50 mg of thiamine hydrochloride and 10  $\mu$ Ci of radioactivity.

### *Studies on patients*

The absorption of [<sup>35</sup>S]thiamine hydrochloride was studied by previously described methods (Thomson, 1966a, b). Less than 10% of the thiamine was broken down before absorption or during passage through the body (Thomson, 1966a). Informed consent was obtained from both patients and normal subjects to the investigation of thiamine metabolism. After an overnight fast patients received a parenteral injection of 200 mg of non-radioactive thiamine hydrochloride, immediately after which the radioactive material was given orally. Arterial blood was collected via an indwelling catheter at 0, 3, 6, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min. Urine was collected hourly for the first 5 h and then after 12, 24, 48 and 72 h. Blood and urine radioactivity were determined in a Packard Tri-Carb liquid-scintillation counter, with 0.8 g/100 ml of 2,5-diphenyloxazole and 5 mg/100 ml of 1,4-bis-(5-phenyloxazol-2-yl) benzene dissolved in toluene as the liquid scintillator. The samples were prepared by adding 1.0 ml of urine or serum to 3 ml of methanolic 1.5 M-Hyaminate chloride, and 10 ml of liquid scintillator; 10 nCi of the test dose was added as an internal standard. The accuracy of the counting of radioactivity in the urine was determined by counting duplicate samples at three different concentrations of radioactivity. The following results were obtained:  $8.3 \pm 0.1$ ,  $8.3 \pm 0.1$  nCi/ml;  $0.76 \pm 0.1$ ,  $0.76 \pm 0.1$  nCi/ml;  $0.0408 \pm 0.00028$ ,  $0.0406 \pm 0.0003$  nCi/ml. Tests were undertaken to determine the effectiveness of the 200 mg flushing dose of non-radioactive

thiamine hydrochloride when larger oral doses of radioactive thiamine were tested. Twenty-four healthy subjects were given either 1.0, 5.0 or 20.0 mg of radioactive thiamine orally together with 200 mg of non-radioactive thiamine intravenously. The results were compared with those obtained in the same subject, given the same test as before, but with additional intravenous injections of 100 mg of non-radioactive thiamine 4 h, 9 h, 12 h and 24 h after the oral dose. Thirteen malnourished alcoholics were given the same tests using 5.0 mg of oral radioactive thiamine.

Absorption studies were conducted in sixty healthy subjects (ages 28–80; twelve females), twelve chronic alcoholics with clinical and laboratory evidence of thiamine deficiency, and a patient with resection of the ileum and jejunum (except for 10 cm). Six of the patients with thiamine deficiency had abducens palsy and evidence of Wernicke's encephalopathy, and the other six had peripheral neuropathy. Each of the patients had a significantly decreased blood thiamine concentration as measured by the *Ochromonas danica* technique (Baker & Frank, 1968). Intestinal biopsies obtained from the alcoholic patients appeared normal under light microscopy. A biopsy taken from the proximal end of the anastomosed intestine in the patient with a resected small intestine also revealed a normal villous pattern. Five healthy subjects were given 20–100 mg of non-radioactive thiamine 0.5–3 h before giving the 1.0 mg radioactive oral dose together with the 200 mg intravenous flushing dose.

The normal subjects, two alcoholics and the patient with intestinal resection were each given three different dose levels of thiamine orally, the order in which the various doses were given being randomized. Urine was collected in 0–5, 5–12, 12–24, 24–48 and 48–72 h samples.

### Calculations

The Michaelis–Menten formulation (Michaelis & Menten, 1913) was used to calculate maximum removal rate ( $V_{\max}$ ) by replacing reaction velocity and substrate concentrations in the original equation by thiamine excretion ( $v$ ) and dose ( $d$ ), respectively.

$$v = \frac{V_{\max} \cdot d}{K_m + d} \quad (1)$$

Regression analysis was performed to test the applicability of this formula to our data and to obtain the  $V_{\max}$  and the Michaelis constant ( $K_m$ ). For this  $d$  was treated as the independent variable and  $v$  as a normal randomly distributed variable with the expected value:

$$E(v/d) = \frac{V_{\max} \cdot d}{K_m + d} \quad (2)$$

and the variance:

$$\text{Var}(v/d) = \sigma^2 \quad (3)$$

Two or more elimination rates were determined using the same dose of thiamine hydrochloride to calculate confidence and estimate variation around the regression line. The 95% confidence limits were established for regression lines at their origin. The non-logarithmic transformation of Lineweaver & Burk (1934) was used to calculate the  $V_{\max}$  and  $K_m$ . The least-squares procedure of Wilkinson (1961) was used to obtain the standard errors of the intercepts of the plot.

## RESULTS

Normal subjects excreted  $33.0 \pm 2.7\%$  (SEM) of a 5.0 mg oral dose of [ $^{35}\text{S}$ ]thiamine hydrochloride whereas malnourished alcoholics excreted  $14.6 \pm 3.2\%$  (SEM) and the patient with intestinal resection  $8.5\%$  of the oral dose (Tables 1 and 2). After a 6–8 week period of a nutri-

TABLE 1. Thiamine absorption in normal subjects and malnourished alcoholics. Results are expressed as the mean % of the oral dose  $\pm$  SEM. Subjects were given 5.0 mg of [ $^{35}\text{S}$ ]thiamine hydrochloride orally together with 200 mg of intravenous non-radioactive dose. The loading was an additional 100 mg of intravenous non-radioactive thiamine hydrochloride at 4, 9, 12 and 24 h. The treated alcoholics were given 6–8 weeks of a nutritious diet with vitamin supplements.

Subjects	Loading	
	Before	After
Normal	$33.0 \pm 2.7$	$35.3 \pm 2.1$
Alcoholics:		
(a) Untreated	$14.6 \pm 3.2$	$16.3 \pm 5.7$
(b) Treated	$39.4 \pm 3.5$	$43.1 \pm 4.2$

TABLE 2. The 72 h cumulative urinary radioactivity in normal subjects and a patient with intestinal resection. Results are expressed as % of the oral dose.

Subjects	Oral dose of [ $^{35}\text{S}$ ]thiamine hydrochloride (mg)		
	1.0	5.0	20.0
Normal			
Mean	51.0	33.0	25.9
SEM	2.1	2.7	2.6
Intestinal resection*			
Mean	13.2	8.5	5.5

\* Duodenum, 10 cm of jejunum and descending colon only remaining.

tious diet and vitamin supplements, alcoholics excreted  $39.4 \pm 3.5\%$  of the administered radioactive thiamine. Additional intravenous flushing doses of non-radioactive thiamine did not increase the excretion of radioactivity in healthy subjects (Fig. 1). A one-way analysis of variance computed on the difference between the two tests showed that extra flushing doses did not significantly alter the excretion in healthy subjects  $\{P[F \pm 0.704; \text{degrees of freedom } (2, 22)] = 0.99\}$  or in malnourished alcoholics (mean in standard test =  $14.6 \pm 3.2\%$ ; with additional flushing =  $16.3 \pm 5.7\%$ ). When two groups of healthy subjects of mean ages 49 years and 82 years were compared using regression analysis at three different dose levels no correlation

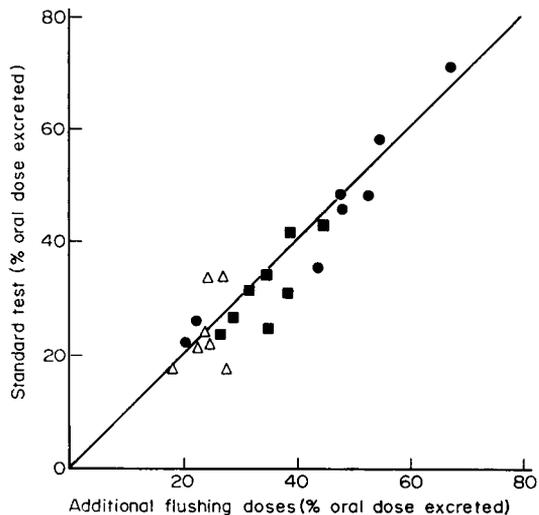


FIG. 1. Effect of giving additional intravenous flushing doses to control subjects receiving 1.0 mg (●), 5.0 mg (■) or 20 mg (Δ) of radioactive thiamine orally.

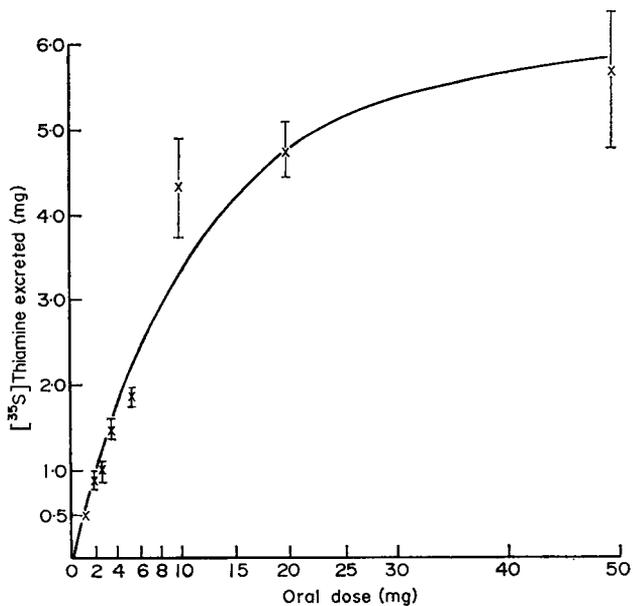


FIG. 2. Relationship between the dose of radioactive thiamine given orally and the cumulative 72 h urine radioactivity. Each point represents a mean value and the standard error is indicated. 200 mg of non-radioactive thiamine hydrochloride was given intravenously with each oral dose.

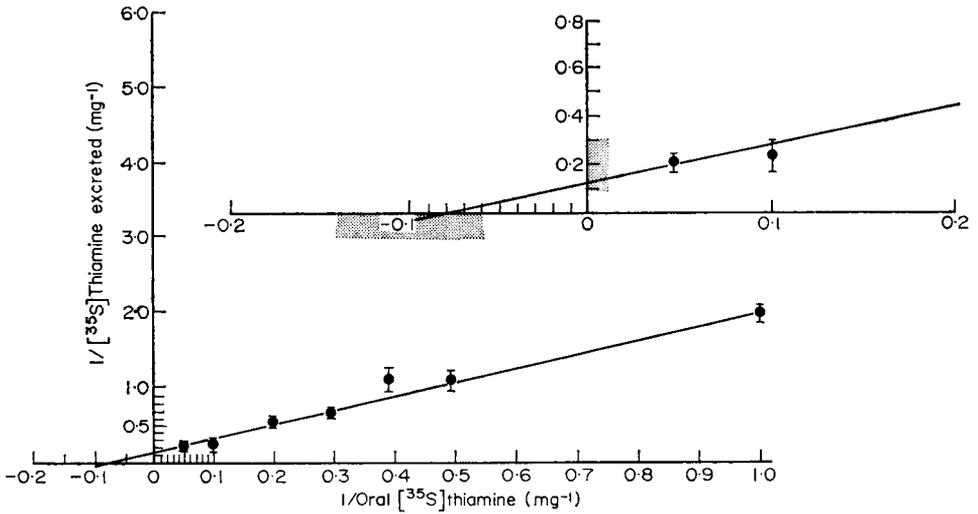


FIG. 3. Linear relationship between the reciprocal of the dose of radioactive thiamine given orally and the reciprocal of the cumulative 72 h urinary radioactivity. Each point represents a mean value; 200 mg of non-radioactive thiamine hydrochloride was given intravenously with each oral dose. The insert shows the values of  $V_{max}$  and  $K_m \pm$  two standard errors of the mean.

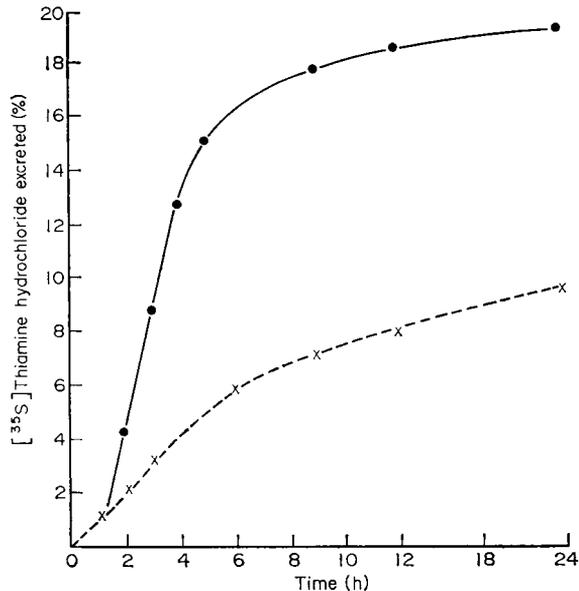


FIG. 4. Cumulative urinary excretion of radioactivity after 20 mg of [<sup>35</sup>S]thiamine hydrochloride was given orally together with a dose of 200 mg of thiamine hydrochloride intravenous non-radioactive flushing material (●). The test is repeated on the same subject 0.5 h after a 100 mg dose of non-radioactive thiamine hydrochloride was given orally (x).

between age and percentage of excretion of radioactivity could be demonstrated [At 1.0 mg oral dose/age  $t = 0.002$  (35 degrees of freedom)  $P > 0.5$ ; 5.0 mg,  $t = 0.687$  (27 degrees of freedom)  $P = 0.5$ ; 20 mg,  $t = 0.673$  (30 degrees of freedom)  $P > 0.5$ ].

The relationship between the administered and excreted radioactive thiamine in subjects receiving eight different dose levels from 1.0 to 50.0 mg of thiamine hydrochloride provides evidence of saturation (Fig. 2). A typical Michaelis-Menten curve fits the data if one excludes

TABLE 3. Urinary excretion of radioactivity in normal subjects with and without a prior non-radioactive oral loading dose. In the standard test the patient was given 20 mg of [ $^{35}\text{S}$ ]thiamine hydrochloride orally together with 200 mg of non-radioactive thiamine intravenously. An additional 100 mg of non-radioactive thiamine was given 1 h later. The urine was collected for 72 h.

Subject	Standard test (% of oral dose excreted)	Standard test with prior non-radio- active oral dose*		
		Non-radioactive oral dose		% of oral dose excreted
		Time before (h)	Amount (mg)	
E.C.	43.6	0.5	20	28.0
T.M.	40.7	1.0	20	24.8
P.H.	19.8	0.5	100	12.7
T.L.	21.8	1.0	100	11.5
N.B.	18.6	3.0	100	8.9

\* Test performed as above in the same patient but 20 mg or 100 mg of non-radioactive thiamine hydrochloride was given orally 0.5-3 h before the radioactive dose.

thiamine excretion values after the 10 mg dose of thiamine hydrochloride. A straight-line relationship in which the intercepts on  $X$  and  $Y$  axes are significantly different from zero was obtained when the data was plotted by the method of Lineweaver & Burk (1934). The mean calculated maximum amount of thiamine ( $V_{\max}$ ) absorbed after a single oral dose was  $8.3 \pm 2.4$  mg in healthy subjects and well-nourished alcoholics. The size of the oral dose ( $K_m$ ) required to produce half-maximum absorption was  $12.0 \pm 2.4$  mg when oral doses greater than twice this dose were given, and the amount excreted did not exceed the maximum predicted value when the 50 mg dose response is excluded (Fig. 3). Values were obtained by use of the weighted least-squares procedure (Wilkinson, 1961). Oral administration of 20-100 mg of non-radioactive thiamine to subjects 0.5-3 h before the 1.0 mg radioactive oral dose produced up to 50% decrease in the absorption of the radioactive dose (Table 3; Fig. 4).

Malnourished alcoholics and the patient with intestinal resection showed a considerable decrease in (a) serum of radioactivity amounts; (b) rate of rise in cumulative urinary excretion; (c) total amount of radioactivity excreted at all three dose values (Figs. 5 and 6). The Lineweaver-Burk plot was linear again (Fig. 7). The patient with intestinal resection had a  $V_{\max}$  of 1.1 mg. Assuming absorption of thiamine hydrochloride in alcoholic subjects still conforms

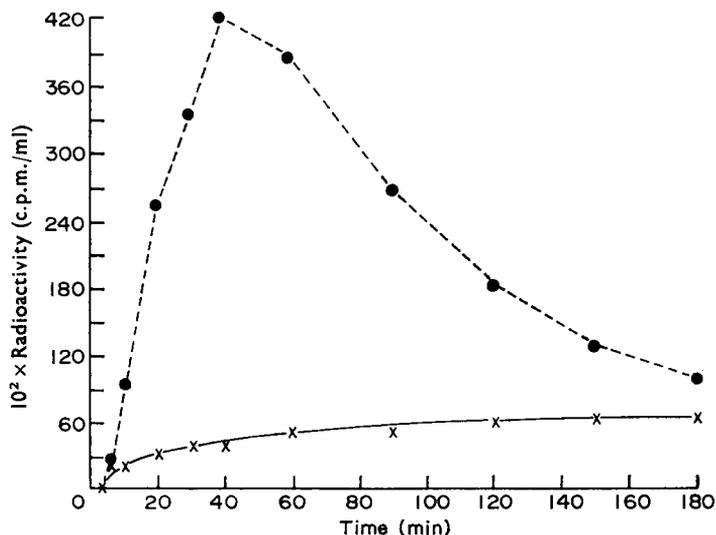


FIG. 5. The patterns of serum radioactivity seen in a normal subject (●) and a patient with an intestinal resection (×) after 1.0 mg of radioactive thiamine was given orally and a 200 mg flushing dose of non-radioactive thiamine hydrochloride.

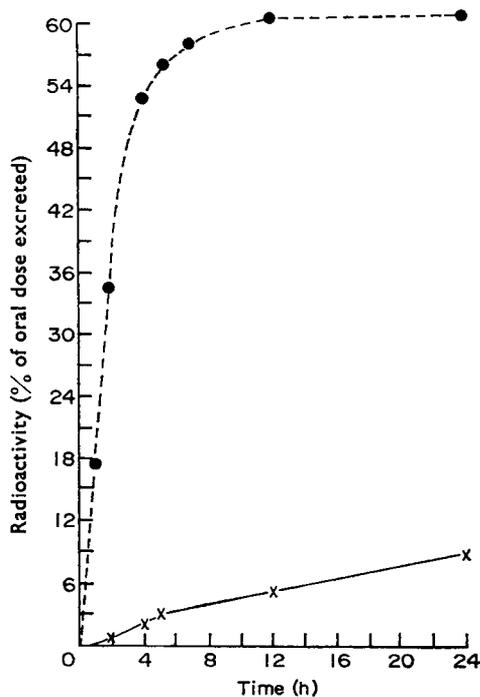


FIG. 6. Cumulative urinary excretion of radioactivity after 1.0 mg of radioactive thiamine was given orally and a 200 mg flushing dose of non-radioactive thiamine hydrochloride in a normal subject (●) and a patient with intestinal resection (×).

to Michaelis–Menten kinetics, thereby allowing use of three points serial study, a malnourished subject with fatty liver showed a  $V_{max}$  of 1.5 mg on admission; this value had increased to 8.0 mg after a 6 week period on a nutritious diet. In contrast with the marked decrease in

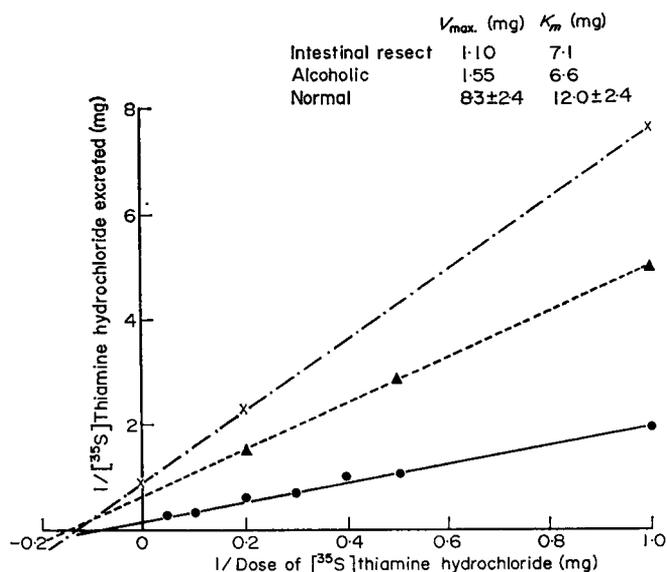


FIG. 7. Linear relationship between the reciprocal of the dose of radioactive thiamine given orally and the reciprocal of the cumulative 72 h urinary radioactivity in normal subjects (●) a malnourished alcoholic (▲), and a patient with intestinal resection (×).

$V_{max}$  in the malnourished alcoholic and the patient with intestinal resection, the  $K_m$  was not significantly altered, the  $K_m$  intercept for these patients falling within two standard errors of the intercept for healthy control subjects.

## DISCUSSION

Our data indicate that in normal man there is a maximum absorption of approx. 8.3 mg of thiamine hydrochloride after ingesting a single dose of this compound. Other workers have suggested that there is an upper limit to the absorption of thiamine in man. However, this view has been based on measurements of urinary excretion of thiamine without identification of metabolites or evaluation of diversion of administered thiamine to other areas (Friedman *et al.*, 1948; Morrison & Campbell, 1960). The use of radioactive thiamine in conjunction with a flushing dose of non-radioactive thiamine increases the reliability of the present observations. Additional intravenous flushing doses did not increase the excretion of radioactivity or alter the pattern of excretion in malnourished alcoholic patients or normal subjects (Fig. 1) and when labelled thiamine was given intravenously with the flushing dose, approximately 80% of the label was excreted within 4 h and about 90% within 24 h (Thomson, 1969). The amount of total body thiamine has been estimated to be 30 mg (Takeda, 1947) and the assumption that a large intravenous dose will allow practically all of any additional thiamine which may be

absorbed to be excreted in the urine appears to be well supported. Simultaneous measurements of urinary and faecal excretion of orally administered labelled thiamine given with a flushing dose show an inverse relationship (Tomasulo, Kater & Iber, 1968).

The maximum ability of the kidney to excrete thiamine was not exceeded during the tests (Thomson *et al.*, 1970) as is evident from the fact there was no significant change in the serum pattern of radioactivity in the presence of the additional flushing doses which could influence saturation. Absorption tests in the same subject before and after treatment show very similar patterns of serum and urinary radioactivity which differ only in the amount of radioactivity present at any one time. However, neither the comparative facility with which thiamine hydrochloride is absorbed from different areas of the intestine, nor the influence of intestinal motility on absorption is known. An earlier radioactive peak might have been expected had there been increased motility which altered absorption.

If one accepts the difficulties in quantifying the influence of multiple biological systems and the variable effects of flushing doses, the relationship between the dose of thiamine given and the amount recovered from the urine obeys Michaelis–Menten kinetics, suggesting that a rate-limiting step is involved. It has been assumed that thiamine hydrochloride is phosphorylated during its absorption (Linneweh & Muller, 1940; Tauber, 1937; Cerecedo, Eich & Bresnick, 1954; Rindi, Ventura, De Guiseppe & Sciorelli, 1966); however, this step does not account for the observed saturable phenomenon, since absorption of thiamine propyl disulphide which is also phosphorylated during intestinal transport is not rate-limited (Thomson, Frank, Baker & Leevy, 1971).

The suggestion that thiamine absorption follows Michaelis–Menten kinetics makes it possible to ascertain the mechanism whereby various factors interfere with its intestinal transport. The  $K_m$  value reflects the affinity of the transport system for thiamine and is equal to the dose of thiamine which is removed from the intestine at half maximum removal rate. An evaluation of the effects of disease on  $K_m$  and  $V_{max}$  values may be used to quantify and characterize the mechanism responsible for the decreased intestinal absorption. Both the patient with the intestinal resection and the alcoholic patients showed a marked decrease in  $V_{max}$ , without a significant decrease in  $K_m$ . This is found in non-competitive inhibition and is consistent with the hypothesis that there has been a decrease in the number of receptor sites available. The reversible decrease in  $V_{max}$  noted in the malnourished alcoholics suggests that receptor sites are temporarily damaged in these instances. The time-lapses required for restoration of normal thiamine absorption are attributable to the need for repair or regeneration of mucosal cells with adequate receptor sites.

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