The absorption of tritium-labelled pyridoxine HCl in control subjects and in patients with intestinal malabsorption

M. C. BRAIN AND C. C. BOOTH

From the Department of Medicine, Postgraduate Medical School of London¹

EDITORIAL SYNOPSIS It has been suggested that there may be subclinical deficiency of pyridoxine in patients with tropical and non-tropical sprue. This paper provides evidence of some failure of absorption of pyridoxine in idiopathic steatorrhoea.

Impaired absorption of certain water-soluble vitamins may be caused by disease of the small intestine. Patients with idiopathic steatorrhoea, for instance, almost invariably fail to absorb folic acid normally (Girdwood, 1953; Chanarin, Anderson, and Mollin, 1958; Cox, Meynell, Cooke, and Gaddie, 1958), and also suffer from folic acid deficiency. Clinical evidence of deficiency of other water-soluble vitamins, however, is rarely apparent and Girdwood (1956) was unable to demonstrate malabsorption of aneurine, riboflavine, or pyridoxine in 10 patients with impaired absorption of folic acid. Recently subclinical deficiency of pyridoxine in patients with tropical or non-tropical sprue has been suggested by reports that there is an increase in the urinary excretion of xanthurenic acid and other metabolites of l-tryptophan after an oral dose of this amino-acid (Kowlessar, Haefner, Benson, and Sleisenger, 1961; Sigler, Sheehy, Santini, and Rubini, 1962) and Baker and Sobotka (1962) have now shown that patients with non-tropical sprue may have subnormal levels of pyridoxine in their serum. These observations suggest that there may be a failure of absorption of pyridoxine in idiopathic steatorrhoea.

The successful labelling of pyridoxine with tritium has made it possible to study the absorption of the labelled vitamin directly (Booth and Brain, 1962; Brain, Stewart, and Booth, 1963) and its use overcomes the limitations of the microbiological method employed by Girdwood (1956). This paper reports a study of the absorption of ³H-labelled pyridoxine in control subjects and in patients with a variety of conditions associated with intestinal malabsorption.

METHOD

³H-LABELLED PYRIDOXINE This was prepared by the Radiochemical Centre, Amersham, from commercial pyridoxine hydrochloride (British Drug Houses). The resulting material had a specific activity of 1.6 mc. per mg. and was 97% pure by isotope dilution assay.

TEST DOSES Radioactive test doses contained between 1.0 and 100 mg. of pyridoxine hydrochloride and were prepared by diluting the labelled material with suitable amounts of non-radioactive pyridoxine. All test doses were adjusted so that 20 ml. contained the required amount of pyridoxine and 20 μ c. of radioactivity. A suitable amount of the solution from which the test dose was prepared was retained for use as a standard.

ABSORPTION OF ³H-LABELLED PYRIDOXINE This was studied by measuring the urinary radioactivity during the 24 hours following the test dose. The radioactive test doses were given to the subjects after an overnight fast, either alone or immediately after a parenteral injection of non-radioactive pyridoxine hydrochloride, a procedure which was found greatly to increase the urinary excretion of radioactivity. The urine passed during the 24 hours following the oral dose of labelled pyridoxine was usually collected in the following periods: 0 to 3 hours, 3 to 6 hours, 6 to 12 hours, and 12 to 24 hours. In some instances urine was collected at shorter intervals. The volume of each collection was measured and an aliquot was stored at -20°C. until the radioactivity was measured.

ASSAY OF URINE SAMPLES Radioactivity of the urine samples was assayed in a Packard Tri-Carb liquid scintillation counter, using 2,5-diphenyloxazole and 1,4-bis (2,5-diphenyloxazolyl) benzene with naphthalene, dissolved in a mixture of toluene, dioxane, and ethanol as the liquid scintillator. Volumes, each of 0.5 ml, of the urine to be counted were added to 10 ml. of the liquid

¹Postal address: Du Cane Road, London W.12.

scintillator and 0.05 ml. of the test dose was added as an internal standard to all samples.

SUBJECTS STUDIED

CONTROLS Control subjects were ambulant hospital patients in whom there were no clinical grounds for suspecting malabsorption.

PATIENTS Patients with intestinal malabsorption included 16 patients in whom the diagnosis of idiopathic steatorrhoea had been confirmed by jejunal biopsy (Table IV), and nine patients with steatorrhoea due to other causes. Four of these latter patients had had extensive resections of the distal small intestine and two others had Crohn's disease involving the ileum; there was also one patient with a duodeno-colic fistula, one who had steatorrhoea associated with a subtotal gastrectomy, and one with chronic pancreatitis.

RESULTS

URINARY EXCRETION OF RADIOACTIVITY AFTER 1.0 MG. ³H-PYRIDOXINE ORALLY IN CONTROLS Seven control subjects were given 1.0 mg. ³H-pyridoxine orally. Relatively little radioactivity was excreted when the dose was given in this way. The percentage of the oral dose excreted in 24 hours, shown in Table I,

TABLE I

EXCRETION OF RADIOACTIVITY (PER CENT) AFTER 1.0 mg. ³H-PYRIDOXINE HCl ORALLY BEFORE AND AFTER SATURATION¹ IN CONTROL SUBJECTS

Before Saturation	After Saturation
8.8	
13.8	21.5
10.0	18.5
9.9	14.3
19.2	16.6
12.3	13.3
9.6	
Mean 11.9	Mean 16.8
S.E. $\pm$ 1.21	S.E. $\pm$ 1.33

¹Subjects were 'saturated' with 300 mg. pyridoxine HCl intravenously, 48 hours before the test.

ranged from 8.8 to 19.2 (mean 11.9%). In five of these subjects the test was repeated 48 hours after 300 mg. non-radioactive pyridoxine hydrochloride had been given intravenously. Under these circumstances, the amount excreted ranged from 13.3 to 21.5% of the oral dose (Table I). Although the differences in excretion were variable in different patients, the mean percentage excretion after 'saturation' with pyridoxine (16.8%) was slightly greater than when no previous pyridoxine had been given and this difference is significant ($t = \pm 3.24$ $P = < 0.01$).

EFFECT OF SIMULTANEOUS PARENTERAL INJECTIONS OF NON-RADIOACTIVE PYRIDOXINE ON EXCRETION OF

RADIOACTIVITY AFTER ORAL DOSES OF ³H-PYRIDOXINE IN CONTROLS The administration of non-radioactive pyridoxine intravenously immediately before giving the oral dose greatly increased the percentage of the radioactivity excreted in the urine. Control subjects who had previously received 300 mg. pyridoxine intravenously 48 hours before the test were fed 1.0 mg. ³H-pyridoxine orally; parenteral injections of 25, 50, 100, or 200 mg. of non-radioactive pyridoxine were given intravenously at the time of the test. The percentages of the oral dose excreted when these injections were given are shown in Figure 1.

When the oral dose was given alone, the mean excretion was 16.8% of the oral dose. The mean percentage excretion rose as increasing amounts of non-radioactive pyridoxine were administered, reaching a maximum of over 50% when 100 mg. or more was injected. The increase in mean excretion observed with 100 mg. compared with 25 mg. is highly significant ($t = \pm 3.17$, $P < 0.01$) although the differences between the means with 50 mg., 100 mg., and 200 mg. are not ($P > 0.05$). Since maximal urinary excretion of the oral test dose was ensured if 100 mg. of pyridoxine was given as a 'flushing' dose, an injection of this amount was used in the standard test of absorption.

FIG. 1. Twenty-four hour urinary excretion of radioactivity after 1.0 mg. ³H-pyridoxine orally with varying amounts of pyridoxine HCl intravenously in control subjects.

EFFECT OF PREVIOUS 'SATURATION' WITH PARENTERAL INJECTIONS OF NON-RADIOACTIVE PYRIDOXINE IN CONTROLS The percentage excretion in nine subjects given 1.0 mg. ^3H -pyridoxine orally and 100 mg. non-radioactive pyridoxine intravenously who had previously received no pyridoxine, and in 14 subjects who had received 300 mg. pyridoxine 48 hours before the test, is given in Table II. Although the mean excretion after 'saturation' rose from 44.0% to 50.5%, this difference is not significant ($t = \pm 1.62$ $0.2 > P > 0.1$).

Four 'saturated' subjects were also given the 1.0 mg. test dose of ^3H -pyridoxine intramuscularly and a simultaneous injection of 100 mg. non-radioactive pyridoxine was given intravenously. The urinary excretion of radioactivity during the ensuing 24 hours ranged from 33.8 to 68.7% (mean 51.0%, S.E. $\pm$ = 7.8). These results are similar to those recorded when the same dose was given by mouth (Table II).

TABLE II

EFFECT OF SATURATION ON RESULTS OF STANDARD ORAL TEST¹ IN CONTROL SUBJECTS

Before Saturation	After Saturation
42.3	55.0
41.0	48.9
46.6	68.5
40.9	51.5
36.8	37.7
29.2	58.8
47.9	34.8
52.0	57.0
59.6	45.5
	61.3
	47.9
	45.1
	36.5
	59.3
Mean 44.03	Mean 50.55
S.E. $\pm$ 2.77	S.E. $\pm$ 2.59

¹Standard oral test = 1.0 mg. ^3H -pyridoxine orally, 100 mg. pyridoxine HCl intravenously. Saturation was achieved as before (see Table I).

RATE OF URINARY EXCRETION OF RADIOACTIVITY AFTER AN ORAL DOSE OF 1.0 mg. ^3H -PYRIDOXINE IN A CONTROL A control subject who had been 'saturated' 48 hours before the test was given 1.0 mg. ^3H -pyridoxine orally and 200 mg. pyridoxine as a 'flushing' dose to ensure maximum excretion of radioactivity. The urine was collected at intervals of half an hour for the first three hours, at hourly intervals for the next three hours, and at nine and 12 hours. The cumulative excretion of radioactivity is shown in Figure 2.

Of the oral dose, 4.9% was excreted in the first 30 minutes, and excretion continued at a similar rate for the next 30 minutes. Excretion was maximal between 60 and 90 minutes, during which time 15%

FIG. 2. Cumulative urinary excretion of radioactivity after 1.0 mg. ^3H -pyridoxine orally in a control subject; 200 mg. pyridoxine HCl was given intravenously.

of the dose was excreted. After two hours the rate of excretion began to diminish and by 12 hours excretion of radioactivity was virtually complete.

URINARY EXCRETION OF RADIOACTIVITY AFTER INCREASING ORAL DOSES OF ^3H -PYRIDOXINE IN CONTROLS Control subjects who had been previously saturated were then given successive doses of 1.0, 5.0, 10.0, 20.0, and 100.0 mg. ^3H -pyridoxine orally together with 200 mg. pyridoxine intravenously as a 'flushing' dose. The 24-hour urinary excretion, expressed as milligrams of ^3H -pyridoxine, is shown in Figure 3. For convenience, these results have been plotted on double logarithmic paper. There was a direct linear relationship between the amount of ^3H -pyridoxine given orally and the amount excreted in the urine.

IDIOPATHIC STEATORRHOEA The urinary excretion of radioactivity was measured in these cases under different conditions.

Urinary excretion of radioactivity after 1.0 mg. ^3H -pyridoxine orally In 16 patients with idiopathic steatorrhoea the urinary excretion of radioactivity was measured after 1.0 mg. ^3H -pyridoxine was given orally, together with a 100 mg. 'flushing' injection. In 13 of these patients, the test was repeated after previous saturation with 300 mg. of non-radioactive

FIG. 3. Twenty-four hour urinary radioactivity after varying oral doses of ^3H -pyridoxine in control subjects; 200 mg. pyridoxine HCl was given intravenously as a 'flushing' dose.

pyridoxine given intravenously. The results are given in Table III.

Before saturation, the percentage urinary excretion of radioactivity ranged from 19.0 to 50.0% (mean 29.95%). After saturation, the excretion was slightly greater, between 20.0 and 57.0% (mean 36.75%), but this difference is not significant ($t = \pm 1.79$, $0.1 > P > 0.05$).

If these results are compared with those obtained in control subjects (Table II) it is seen that six of the patients excreted less radioactivity than any of the control subjects, whereas in the other seven patients

TABLE III

STANDARD ORAL TEST BEFORE AND AFTER SATURATION¹ IN IDIOPATHIC STEATORRHOEA

Before Saturation	After Saturation
19.0	20.0
20.6	21.1
22.9	26.0
23.0	28.5
23.1	32.4
25.5	34.0
26.9	36.8
27.5	37.9
27.8	38.5
29.7	38.8
31.7	52.3
33.9	54.0
35.3	57.0
40.3	
42.0	
50.0	
Mean = 29.95	Mean = 36.74
S.E. ± 2.1	S.E. ± 3.27

¹Saturation was achieved as before (see Table I).

FIG. 4. Cumulative urinary excretion of radioactivity after 1.0 mg. ^3H -pyridoxine orally and 200 mg. pyridoxine HCl intravenously in a patient with idiopathic steatorrhoea (case 2); the results in a control subject are shown for comparison.

excretion was within the control range. The mean excretion after saturation in patients with idiopathic steatorrhoea shows a highly significant difference from the mean in saturated control subjects (Table II) ($t = \pm 3.38$, $P < 0.01$).

Rate of urinary excretion of radioactivity after 1.0 mg. ^3H -pyridoxine orally In Fig. 4 the cumulative urinary excretion of radioactivity in a patient with idiopathic steatorrhoea given an oral dose of 1.0 mg. of ^3H -pyridoxine, together with a flushing injection of 200 mg. of non-radioactive pyridoxine, is shown together with the results obtained in the control subject studied similarly (Fig. 2). In the patient with idiopathic steatorrhoea the total excretion in 12 hours was half that of the control subject, and the rate of excretion was consistently less. Nevertheless, the period of maximum excretion occurred at approximately the same time as in the control subject.

Urinary excretion of radioactivity after increasing oral doses of ^3H -pyridoxine One of the patients (case 11, Table IV), whose urinary excretion of radioactivity from the standard oral test dose was within the normal range, was given further oral

FIG. 5. Twenty-four hour urinary radioactivity after varying oral doses of ^3H -pyridoxine in a patient with idiopathic steatorrhoea (case 11); 200 mg. pyridoxine HCl was given intravenously. The shaded area represents the normal range.

test doses of 10 mg. and 100 mg. ^3H -pyridoxine together with a 200 mg. flushing injection. The urinary excretion of radioactivity has been calculated as milligrams pyridoxine and is plotted in Figure 5. The results at all three dose levels lie within the normal range and do not differ significantly from the regression equation calculated from the controls (Fig. 3) ($0.95 > P > 0.90$).

Correlation of pyridoxine absorption with other parameters of intestinal function and histological appearance of the jejunal mucosa In Table IV, the percentage of the oral dose of 1.0 mg. of ^3H -pyridoxine excreted in the urine by 13 patients

with idiopathic steatorrhoea studied after previous saturation is compared with the results of folic acid absorption, xylose excretion, faecal fat excretion, and the histological appearance of the jejunal mucosa. These results indicate that there was no correlation between pyridoxine absorption and any other parameter of intestinal function. For example, although the patients whose absorption of folic acid and fat was normal (cases 7 and 10) absorbed pyridoxine normally, another patient who had gross steatorrhoea (case 11) and total failure of folic acid absorption also excreted normal amounts of ^3H -pyridoxine. There was no clear difference in the result of pyridoxine absorption between those with 'partial' or 'subtotal' villous atrophy (Table IV).

INTESTINAL RESECTION Urinary excretion of radioactivity was studied in a small number of patients who had had varying amounts of small intestine resected.

Urinary excretion of radioactivity from an oral dose of 1.0 mg. ^3H -pyridoxine Four patients who had undergone extensive resection of varying amounts of the distal small intestine were given the standard oral test dose of 1.0 mg. ^3H -pyridoxine together with 100 mg. non-radioactive pyridoxine intravenously, all patients having been previously saturated with 300 mg. pyridoxine intravenously 48 hours before the test. One patient (case 17) received a further oral dose of 20 mg. ^3H -pyridoxine with a 200 mg. flushing injection. The urinary excretion of radioactivity, together with the estimated length of small intestine remaining, is given in Table V.

One patient (case 17) had only 4 feet of proximal small intestine remaining but excreted normal amounts of radioactivity after an oral dose of either 1.0 or 20.0 mg. The other three patients (cases 14,

TABLE IV

^3H -PYRIDOXINE ABSORPTION, FOLIC ACID ABSORPTION, XYLOSE AND FAECAL FAT EXCRETION, AND HISTOLOGY OF JEJUNAL MUCOSA IN IDIOPATHIC STEATORRHOEA

	Urinary Excretion of Radioactivity (%)	Level of Serum Folic Acid Two Hours after Oral Dose ($\mu\text{g./ml.}$)	5-Hour Urinary Xylose Excretion	Faecal Fat (g./day)	Intestinal Mucosa
1	20.0	2	1.8	11	Subtotal villous atrophy
2	21.1	15	2.7	13.7	Subtotal villous atrophy
3	26.0	—	3.0	20	Subtotal villous atrophy
4	28.5	9	2.5	17	Subtotal villous atrophy
5	32.4	16	1.8	7	Partial villous atrophy
6	34.0	28	4.8	5.6	Subtotal villous atrophy
7	36.8	56.5	1.8	4.5	Partial villous atrophy
8	37.9	10	1.9	—	Subtotal villous atrophy
9	38.5	5	1.6	16	Partial villous atrophy
10	38.8	88	2.5	4.4	Subtotal villous atrophy
11	52.3	0	1.1	70	Subtotal villous atrophy
12	54.0	0	3.6	9.8	Partial villous atrophy
13	57.0	—	1.3	—	Subtotal villous atrophy
Normal values	> 34.8	> 40	> 5.0	< 5.0	

TABLE V

STANDARD ORAL TEST IN FOUR PATIENTS WITH EXTENSIVE RESECTION OF DISTAL SMALL INTESTINE

Case No.	Operation	Excretion Radioactivity (%)	
		1 mg.	20 mg.
14	Resection of all but 6 ft. of jejunum	21.6	—
15	Resection of all but 8 ft. of jejunum	32.6	—
16	Resection of all but 6 ft. of jejunum	33.0	—
17 ¹	Resection of all but 4 ft. of jejunum	57.8	61.0
	Mean	37.6	
	S.E. $\pm$	3.7	

¹Case 17 also received 20 mg. ³H-pyridoxine orally and 200 mg. 'flushing' injection pyridoxine HCl.

15, and 16) had between 6 and 8 feet of jejunum remaining. In two (cases 15 and 16), the percentage urinary excretion was just below the normal range; in the third (case 14) the excretion was reduced to 21.6%.

OTHER CAUSES OF MALABSORPTION The standard oral test dose was given to the five other patients whose malabsorption was due to a variety of causes. The results of the test were normal in all the five patients (Table VI).

TABLE VI

STANDARD ORAL TEST IN FIVE PATIENTS WITH STEATORRHOEA OF MISCELLANEOUS AETIOLOGY

Case No.	Disease	Radioactivity Excreted (%)
18	Crohn's disease of terminal ileum	38.2
19	Crohn's disease of terminal ileum	34.4
20	Duodeno-colic fistula	40.0
21	Post gastrectomy	57.2
22	Chronic pancreatitis	33.8

DISCUSSION

The results reported in this paper indicate that ³H-pyridoxine may be used to study the absorption of this vitamin in man. When radioactive pyridoxine is given orally relatively little radioactivity is excreted in the urine during the following 24 hours. If, however, the oral dose is accompanied by a relatively large parenteral injection of non-radioactive pyridoxine, much larger amounts of radioactivity are excreted (Fig. 1). This technique is analogous to the Schilling test of vitamin B₁₂ absorption and has been successfully applied to the study of absorption of ³H-pyridoxine in the rat (Booth and Brain, 1962). The observation that the non-radioactive pyridoxine promotes the urinary excretion of the labelled vitamin further indicates that as in the rat the ³H-labelled pyridoxine behaves similarly to the unlabelled material and that the tritium label is not detached during passage through the body.

In control subjects ³H-pyridoxine was absorbed relatively rapidly, for the rate of urinary excretion following an oral dose suggested that the major period of absorption was the first two hours after feeding (Fig. 2). It seems likely that absorption takes place from the proximal small intestine in man, for patients with extensive resection of the distal small intestine clearly absorbed appreciable amounts of the test dose of ³H-pyridoxine, and in one instance normal absorption was achieved with only 4 feet of residual proximal small intestine (case 17, Table V). These results are in keeping with observations in the rat which have demonstrated that absorption of pyridoxine takes place in the jejunum (Booth and Brain, 1962).

The mechanism of absorption of pyridoxine has not been clearly established. Studies with everted sacs of small intestine in the rat have failed to reveal evidence for an active transport system (Brain, Stewart, and Booth, 1963). Current opinion favours the view that most of the water-soluble vitamins of the B group are absorbed by passive diffusion (Turner and Hughes, 1962; Wilson, 1962) and the linear relationship between oral dose and urinary excretion of ³H-pyridoxine demonstrated in this paper (Fig. 3) is compatible with a diffusion mechanism for the absorption of pyridoxine in man. Although only 50% of the dose was excreted in the urine in these experiments, it is likely that most if not all of the oral ³H-pyridoxine was absorbed, for a similar percentage was excreted when the labelled vitamin was given parenterally. Failure to excrete more of the administered radioactivity must reflect some alternative metabolic pathway than urinary excretion. A similar situation has been recorded in studies of the metabolism of ³H-pyridoxine in the rat (Cox, Murray, and Boone, 1962).

In six out of 13 patients with idiopathic steatorrhea there was evidence of impaired absorption of pyridoxine, for they excreted less radioactivity in the urine than any of the control subjects (Table III). The degree of impairment of pyridoxine absorption could not be correlated with results of other intestinal function tests in all cases. Many patients

with marked malabsorption (cases 1, 2, 3, and 4, for instance) failed to absorb pyridoxine normally, and there were two patients (cases 7 and 10) whose normal absorption of folic acid and fat was associated with a normal excretion of ^3H -pyridoxine. However, there were other patients who absorbed pyridoxine normally even when there was marked malabsorption of substances such as folic acid or fat (cases 9 and 11). The most striking example of this discrepancy was the patient (case 11) whose absorption of folic acid, xylose, and fat was grossly impaired yet who absorbed pyridoxine normally from oral doses of 1, 10, or 100 mg. (Fig. 5). This patient's jejunal biopsy showed an extreme degree of mucosal atrophy. There was also no correlation between the capacity of these patients to absorb pyridoxine and the severity of the changes in the jejunal biopsy. The results in idiopathic steatorrhea demonstrate the variability of absorption of different substances in different patients and provide a rational explanation for the very variable nutritional deficiencies which may occur in this condition. Malabsorption of pyridoxine in these patients also explains why abnormalities of tryptophan metabolism (Kowlessar *et al.*, 1961; Sigler *et al.*, 1962) and subnormal serum pyridoxine levels (Baker and Sobotka, 1962) may occur in idiopathic steatorrhea.

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