Factors Affecting the Absorption of Vitamin B_{12}

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PREVIOUS publications from this laboratory and others have described the variations in serum vitamin B_{12} levels, and in the absorption of vitamin B_{12} that are associated with advancing age,[1] pregnancy,[2] hypothyroidism,[3] ACTH and cortisone administration or excess secretion,[4] pyridoxine deficiency,[5] the administration of various intrinsic factor preparations,[6] and the administration of a multiple vitamin-lipotropic factor elixir containing sorbitol in the vehicle.[7] The general findings are summarized in Table I.

The purpose of this paper is to present our additional findings on the effects on the blood levels and absorption of vitamin B_{12} of (1) gastrectomy, (2) divided dose schedules, and (3) the physical state of the orally administered vitamin. We also present evidence further substantiating the effects of pyridoxine deficiency upon vitamin B_{12} absorption and tissue content.

METHODS FOR MEASURING VITAMIN B_{12} ABSORPTION

The absorption of orally administered radioactive vitamin B_{12} tagged with Co^{60} can be measured by the urinary[8] or fecal excretion[9] tests or by hepatic uptake test.[10] Absorption may be estimated from radiometric measurements of feces, scintillation counting of liver projections, or determination of urinary radioactivity estimated after injection of a massive dose of non-radioactive vitamin B_{12}.

In view of a general and understandable hesitancy on the part of investigators to allow the test subjects, particularly infants or pregnant women, to be exposed to the hazards of radioactivity, however safe this may be, the oral tolerance test[11] for estimating absorption is often preferred under such conditions. This test involves either the oral administration of a single dose of 1,000 μg or a daily dose of physiologic magnitude. Increase in serum vitamin B_{12} levels is estimated by a microbiologic assay and is taken as an index of absorption. When

TABLE I*

Variations in Serum Vitamin B_{12} Levels and in Absorption of Vitamin B_{12}

Condition or treatment	Effect on blood level of B_{12}	Effect on absorption of B_{12}
Aged	Decreased	Normal†
Pregnancy	Decreased	Increased
Hypothyroidism	Decreased	Decreased
ACTH or cortisone	Increased	Normal
Pyridoxine deficiency	—	Decreased
"Inhibitory" I. F.	Decreased	Decreased
"Non-inhibitory" I. F.	Increased	Increased
Liptril®	Increased	Increased

* These findings apply to subjects with gastric secretions containing endogenous intrinsic factor.

† Although elderly subjects were found to absorb vitamin B_{12} administered in the fasting state as well as young healthy adults, it was found that young adults responded to an injection of histamine with an increased absorption of vitamin B_{12}, whereas the elderly did not.[7a]

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a single large dose is given, the test involves the drawing of blood one to three hours after the administration. When a small daily dose is used, blood specimens are collected over a longer period of time (in weeks or months) depending on the efficacy of medication. The vitamin B_{12} content in sera is then determined and compared with that found before treatment. Although the oral tolerance test simulates the actual conditions of use, the application of the radiometric measurement has numerous advantages.

Since the details of the above mentioned methods of measurements have been published elsewhere, they will not be repeated in this communication.

RESULTS

Absorption of Vitamin B_{12} by Totally Gastrectomized Subjects: In these patients, the stomach had been removed in its entirety as attested to not only by the surgeons' notes, but also by the pathologist as well as by subsequent radiologic and endoscopic examinations. In some, the stomach had been removed because of malignancy and, in others, benign ulceration. All the subjects studied were between 50 and 70 years of age. Two of the subjects were given test doses of 3 mg, whereas the other two were given 1 mg. Sera samples were obtained shortly before, one and one-half and three hours after administration, and were then analyzed for vitamin B_{12} activity. It can be seen (Table II) that subjects with total gastrectomy showed no measurable vitamin B_{12} activity in the initial sera. However, the subsequent specimens contained high concentrations of vitamin B_{12}. (The average value for our group of healthy individuals is 210 ± 40 $\mu\mu$g/ml.) Therefore, absorption of the vitamin must have taken place.

TABLE II

Oral Tolerance Test of Vitamin B_{12}

Subject*	Years after gastrectomy	B_{12} given mg	Vitamin B_{12} serum levels ($\mu\mu$g/ml)		
			0 hr	1.5 hr	3 hr
J. W. (a)	8	3	0	198	262
E. W.	7	3	0	1280	1500
J. W. (b)	6	1	0	186	192
J. P.	9	1	0	810	700

* All subjects underwent total gastrectomy.

In the second experiment, another series of five subjects with total gastrectomy was used. The test dose of vitamin B_{12} was 1 mg in all instances. It can be seen again (Table III) that the administration of this dose of the vitamin by mouth brought about a definite increase within three hours, the exception being H. D. who did not show the standard response (an increase of 150 $\mu\mu$g/ml). Upon re-test, two weeks later, it was found that H. D. gave a very marked response with an increase of 350 $\mu\mu$g/ml in two hours. These results again demonstrate the low initial vitamin B_{12} serum level and the ability of totally gastrectomized subjects to respond to a 1,000 μg test dose of vitamin B_{12} given by mouth. It should be noted that the vitamin B_{12} serum level of the gastrectomized subjects returned to the original low values in about two weeks.

The absorption of vitamin B_{12} by gastrectomized subjects was determined with the urinary excretion test at two levels of oral intake, namely, 2 and 1,000 μg. Thus, subjects H. D. and W. H. C. were given 2 μg of cobalt^{60}-labeled vitamin B_{12} (specific activity = 180 μc/mg) by mouth followed by the injection of 1 mg of unlabeled vitamin B_{12} two hours later. It was found (Table IV) that the total amounts of radioactivity which appeared in the 24-hour urine specimen were 0.18 and 2.8 per cent, respectively; whereas, our experience[12] shows that healthy subjects with intact stomachs would excrete about 10 per cent of this orally administered dose. Thus, the gastrectomized subjects, like those with pernicious anemia, absorbed a small amount of the orally fed vitamin B_{12}. However, when 1,000 μg of this vitamin, prepared by mixing 2 μg of radioactive vitamin B_{12} with 998 μg of unlabeled vitamin B_{12}, was fed to four totally gastrectomized subjects (two of them received 2 μg radioactive vitamin B_{12} in the previous test), it can be seen that at the higher dose, as much as 36 μg (M. L.) of vitamin B_{12} representing 3.6 per cent of the administered dose, appeared in the urine. This amount represents only a small

TABLE III

Oral Tolerance Test for Vitamin B_{12} Absorption by Gastrectomized Subjects

Subjects	Dosage given *mg*	Vitamin B_{12} serum levels ($\mu\mu g/ml$)					
		0 *hr*	1.5 *hr*	3 *hr*	8 *days*	16 *days*	28 *days*
E. W.	1.0	35	1,450*	1,750*	193	97	47
H. D.	1.0	41*	52	122	41	29	—
J. P.	1.0	35	550*	580*	82	55	23
J. W. (a)	1.0	<50	198	262	70	58*	58*
W. J. C.	1.0	41	286	373	111	60	53

* = approximately.

fraction of the absorbed vitamin B_{12} and is considerably larger than that excreted by non-gastrectomized subjects under similar test conditions. These data, therefore, indicate that the rise in the microbial activity in serum following oral administration of a large dose is due to the increased absorption of the orally administered vitamin.

TABLE IV

Urinary Excretion Test of Radioactive Vitamin B_{12} Absorption by Gastrectomized and Non-gastrectomized Subjects

Subject (gastrectomized)	Administered radioactive vitamin B_{12}	Radioactivity in 24 hr urine	
	μg	$m\mu g$	%
H. D.	2	3.6	0.18
W. H. C.	2	55	2.8
H. D.	1,000	22,000	2.2
E. W.	1,000	12,000	1.2
M. L.	1,000	36,000	3.6
W. H. C.	1,000	27,000	2.7
(Non-gastrectomized)			
5 subjects	2	220 ± 15	11.0
5 subjects	1,000	3,600 ± 580	0.36

The Importance of Physical State and Chemical Substances with which Vitamin B_{12} Is Incorporated: (1) Effect of administration in divided dosages on urinary excretion. The cobalt^{60}-labeled vitamin B_{12} in various amounts (2.0 μg, 8.0 μg, and 50 μg)* was given by mouth to two groups of clinically healthy subjects. One group received one of the above mentioned quantities in one single dose in 20 ml of water with additional 50 ml water in five portions for rinsing. The second group received the same amounts of radioactive vitamin B_{12} in four divided doses at intervals of 15 minutes. The total water intake, including that used for rinsing purposes was the same for both groups. Results tabulated in Table V demonstrate that the administration of a total amount of 2 μg in divided doses resulted in a slight increase in the radioactivity in the 24-hour urine over the group receiving the same amount of radioactive vitamin B_{12} in single doses. The difference,

* The total radioactivity taken by each individual was 0.36 μc, regardless of the total dosage of vitamin B_{12}.

TABLE V

Effect of Administration in Divided Dosages on Urinary Excretion of Radioactive Vitamin B_{12}

Expt.	Total *mcg* administered	Doses	Number of subjects	$m\mu g$ of B_{12} in 24-hour urine*	P* value*
A	2.0	4	5	256 ± 30	>0.05
	2.0	1	5	220 ± 16	
B	2.0	4	8	244 ± 38.1	>0.05
	2.0	1	8	210 ± 21.3	
A	8.0	4	5	500 ± 51.2	<0.05
	8.0	1	5	335 ± 38.7	
B	8.0	4	10	486 ± 39.2	<0.01
	8.0	1	10	330 ± 18.1	
A	50.0	4	5	630 ± 42.1	<0.05
	50.0	1	5	502 ± 38.0	
B	50.0	4	8	721 ± 66.4	>0.05
	50.0	1	8	561 ± 60.2	
C	50.0	4	7	574 ± 42.2	<0.05
	50.0	1	7	454 ± 34.8	
D	50.0	4	7	602 ± 39.8	<0.02
	50.0	1	5	442 ± 28.2	

* Probabilities of differences in the means as determined by the Fisher test.

however, is not statistically significant. When the doses were increased to 8 or 50 μg, a significant increase in the urinary excretion was observed in six separate experiments involving the use of 70 healthy individuals, in favor of the divided doses. It was thought to be plausible that the effect of the divided doses of 8 μg or more may be due to the insufficiency of intrinsic factor needed for the absorption of this large amount of vitamin B_{12}. When a non-inhibitory intrinsic factor concentrate was used, actual enhancement in the urinary excretion was observed. Thus, one group of twelve subjects receiving 50 μg of radioactive vitamin B_{12} alone excreted on the average 512 ± 37 mμg of radioactive vitamin B_{12} in 24 hours, whereas another group of 12 subjects given 50 mμg of radioactive vitamin B_{12} plus four daily oral doses of an intrinsic factor concentrate (Neofactrin®)* gave a mean value of 628 ± 41 μg; the difference is statistically significant. In a like manner, it was found that when 25 μg of vitamin B_{12} were co-administered daily with non-inhibitory intrinsic factor† to 15 pregnant women from their third trimester to the time of delivery, the average vitamin B_{12} serum level was increased from 150 ± 21 μμg/ml to 185 ± 15 μμg/ml. However, when 25 μg of vitamin B_{12} alone was administered to another 15 pregnant women, the vitamin B_{12} serum level dropped from 168 ± 15 μμg/ml to 111 ± 12 μμg/ml.

(2) The physical state of vitamin B_{12} administered: Inasmuch as the site and the mechanism of absorption of vitamin B_{12} are poorly understood, we wished to ascertain whether the physical state in which the vitamin B_{12} is to be administered may play an important role in absorption. In experiment I, two groups of subjects were administered 2 μg of radioactive vitamin B_{12} in hard gelatin capsules (a) containing other vitamins, or in solution (b), respectively. Two μg of radioactive vitamin B_{12} was injected quantitatively with a syringe into the capsule, which was subsequently sealed with molten gelatin. The fluid intake, including that for rinsing, of both groups of subjects at the time of testing, was limited to 60 cc of water. Two hours after the administration of the radiovitamin, each subject received intramuscularly 1,000 μg of the unlabeled vitamin. The total radioactivity in the 24-hour urine specimen was measured by scintillation counting. It can be seen (Table VI) that two out of six subjects (group A, experiment I) receiving capsules excreted unusually small amounts of radioactivity in the urine, whereas those receiving the same amount of the radiovitamin in solution excreted uniformly pure. Three months afterwards, the same subjects were again used for testing, except that those who had previously received vitamin B_{12} in solution, now received it in capsules. A third group (C) of individuals was also used. They received, in solution, the same vitamins that group A received in the capsules, in order

TABLE VI

Effect of Capsule on Urinary Excretion of Radioactive Vitamin B_{12} in Six Subjects

	Experiment I		Experiment II		
	A*	B†	A*	B†	C‡
	226	240	180	276	194
	50	210	30	198	210
	40	170	200	208	246
	200	190	240	230	310
	176	220	36	176	188
	152	230	76	290	146
Mean	140.6 ± 31.9	210 ± 10.7	127 ± 37.1	229 ± 18.4	215.7 ± 23.1

All figures are mμg of radioactive vitamin B_{12} in the 24-hour urine.

Subjects used in group A (experiment I) were same as those in B (experiment II).

Subjects used in group B (experiment I) were same as those in A (experiment II).

Subjects used in group C (experiment II) were different subjects.

* A = capsule (Gevral + 2 mcg vitamin B_{12}) vitamin B_{12} was injected and sealed.

† B = 2 mcg vitamin B_{12}*.

‡ C = 2 mcg vitamin B_{12}* + content of Gevral® in suspension.

* Neofactrin was kindly supplied by the Stuart Company.

† We wish to thank Stuart Company for their supply of Prenatal capsules. The intrinsic factor preparation used contained intrinsic factor activity according to the standard U.S.P. test and would also aid absorption of orally administered vitamin B_{12} by clinically healthy subjects according to the urinary excretion test.

to be certain that the observed differences were not due to any reaction between vitamin B_{12} and some chemical substances. Three subjects who showed normal excretion patterns upon the receipt of vitamin B_{12} in solution now excreted small amounts of radioactivity in the urine. The results demonstrate that the vitamin B_{12} in these specific capsules was not absorbed uniformly well by the test subjects, possibly because the capsules did not dissolve with sufficient rapidity in some subjects.

TABLE VII
Composition of "Elixir"

Ingredients	Per 5 ml
Vitamin B_{12} (crystalline)	8.34 μg
Riboflavin	0.6 mg
Niacinamide	7.0 mg
Pyridoxine	2.0 mg
Betaine (anhydrous)	700.0 mg
Choline dihydrogen citrate	150.0 mg
Inositol	150.0 mg
Ferric pyrophosphate	35.0 mg
Caffeine citrate	65.0 mg
Alcohol (Sorbitol used as vehicle)	15 %

These findings on the relatively poor absorption of vitamin B_{12} provided in these specific capsules received additional experimental confirmation from another study with three groups of elderly subjects (clinically healthy and ambulatory residents of the Institute of Geriatrics in New York). Three groups of 12 subjects each received daily the following treatments:

Group A—100 μg of vitamin B_{12} in a hard gelatin capsule containing other vitamins.

Group B—100 μg of vitamin B_{12} in an aqueous solution together with a vitamin capsule of the same composition of that used in Group A, except vitamin B_{22} was absent.

Group C—25 μg of vitamin B_{12} in a lipotropic elixir[7] (Smith, Kline and French Laboratories*).

Serum specimens were obtained from the subjects in all three groups at regular intervals for the determination of the vitamin B_{12} activity. The results of this study are tabulated in Table VIII. The initial serum vitamin B_{12} levels in all three groups were low and statistically indistinguishable. One month after treatment, the serum vitamin B_{12} level of those receiving the elixir was elevated significantly. After four months, there was only a slight increase in group A, but marked increases in groups B and C. The elevation was more pronounced in Group C than in group B. Six months afterwards, the level of group A was essentially the same as that of group C after only one month of administration at one-quarter of the daily dose. Treatment with 100 μg of vitamin B_{12} in solution for six months resulted in an elevation equal to that of 25 μg of vitamin B_{12} in an elixir administered for four months.

Effect of Pyridoxine Deficiency: The effect of pyridoxine deficiency[5] on the absorption of vitamin B_{12} was studied with adult male and female rats. After ten weeks of feeding a pyridoxine-deficient diet, the male animals lost 18 g each, whereas those treated with pyridoxine gained 54 g each; thus, the algebraic difference between the changes in mean body weights of these two groups was 74 g. The mean body weight of the treated female controls remained unchanged after ten weeks, but was 33 g higher than those of pyridoxine-de-

TABLE VIII
Physical State of Administration of Vitamin B_{12}

Group	Vitamin B_{12}/day μg	Form of administration	Serum vitamin B_{12} levels in μμg months after treatment μμg/ml			
			0	1	4	6
A	100	Capsule	116 ± 21	—	192 ± 29	230 ± 36
B	100	Aqueous solution	112 ± 19	—	456 ± 38	662 ± 41
C	25	SKF elixir	120 ± 17	226 ± 15	675 ± 51	—

* See Table VII for composition of elixir.

ficient female rats. The results tabulated in Table IX demonstrate that the radioactivity in the fecal matter of the pyridoxine-treated male and female rats is consistently and significantly lower than those of the deficient rats, while the urinary excretion of the treated animals is higher than that of the deficient animals. The radioactivity in the target organs, such as liver and kidneys, is higher in the treated animals. It is of interest to note that radioactive vitamin B_{12} present in the gastrointestinal tract is highest among the deficient animals. These data taken as a whole suggest an impairment of vitamin B_{12} absorption related to pyridoxine deficiency.

If the impairment of vitamin B_{12} absorption elucidated above were due to pyridoxine deficiency, it may be expected that repletion with this vitamin will correct this defect unless the damage is irreversible. To this end, 12 young rats (group A) were placed on pyridoxine-deficient diets for a period of five weeks. A like number of animals (group B) were offered the same diet, but were treated with pyridoxine by injection. Five weeks later, six rats from group A, and an equal number from group B were randomly selected and given the oral test for vitamin B_{12} absorption with the procedure previously described. At the same time, the injection of pyridoxine to the remaining six rats in group A was started and was withdrawn from group B. This treatment was continued for eight weeks at which time the vitamin B_{12} absorption test was again applied. Our results (Table X) support our conclusion that pyridoxine deficiency impaired the absorption of vitamin B_{12}, and this can be fully corrected by treatment with pyridoxine.

TABLE IX

Effect of Pyridoxine Deficiency on Absorption of Vitamin B_{12}

Treatment	Radioactivity (per cent of oral dose)				
	Feces	Urine	Liver	Kidney	G.I. Tract
Adult male rats					
Pyridoxine deficiency	49.4 ± 3.4*	2.03 ± 0.24	6.2 ± 0.71	7.4 ± 0.40	10.8 ± 0.78
Pyridoxine treated	36.4 ± 4.2	3.25 ± 0.49	8.0 ± 0.60	8.8 ± 0.17	8.6 ± 0.65
Adult female rats					
Pyridoxine deficiency	59.8 ± 3.2	1.22 ± 0.12	6.2 ± 0.31	5.9 ± 0.49	10.1 ± 1.20
Pyridoxine treated	42.3 ± 2.7	1.92 ± 0.45	9.2 ± 0.45	10.5 ± 0.05	9.1 ± 1.01

* Standard error of the mean.

DISCUSSION

In spite of the availability of radioactive vitamin B_{12} little progress has been made in understanding the sites where the absorption of vitamin B_{12} can take place. While various methods have been proposed to estimate the absorption of vitamin B_{12}, each method has its own innate shortcomings which requires cautious interpretation of the results. For example, the interpretation of data obtained from the commonly used Schilling test,[8] as a measurement of vitamin B_{12} absorption, assumes equal retention of absorbed vitamin B_{12} by tissues of test subjects. Since the amount of vitamin B_{12} retained by the tissues is much greater than that excreted in the urine even after "flushing" by massive doses of unlabeled vitamin B_{12}, any small differences in tissue retention of vitamin B_{12} among different subjects may magnify the amount of urinary excretion. The fecal excretion procedure may appear to provide a direct measurement[9] of absorption of vitamin B_{12}. However, it ignores the possibility that absorbed vitamin B_{12} may be excreted through the bile and finally in the feces. This pathway of vitamin B_{12} elimination was demonstrated by Okuda *et al.*[13] The oral tolerance test is time-consuming and useful only for semiquantitative comparison. However, it need not involve the use of radioactive vitamins. Therefore, to understand the mechanism of absorption of vitamin B_{12} and the functions of various organs in the gastrointes-

tinal tract, the use of various types of patients may yield more informative data. For example, it was shown that feeding of radioactive vitamin B_{12} to totally gastrectomized subjects in small doses resulted in impaired absorption. On the other hand, if this vitamin is fed in large doses, the amounts of vitamin B_{12} appearing in serum or urine of gastrectomized subjects are higher than in normal subjects. From such data one may conclude that the absorption of vitamin B_{12} can take place in the absence of stomach, depending on the dose administered. Since absorption of vitamin B_{12} can take place, it is possible that the absorption of this vitamin could be affected by the divided dosage schedule and by the physical states in which vitamin B_{12} is administered. This belief is substantiated by the results of our studies in which it is shown that the amount of vitamin B_{12} absorbed is dependent on the physical state administered. Thus, vitamin B_{12} contained in at least one type of hard gelatin capsule is not uniformly absorbable; vitamin B_{12} in aqueous solutions is absorbed more easily.

TABLE X

Effect of Pyridoxine Repletion on Absorption of Vitamin B_{12} of Pyridoxine Deficient Female Rats

Group	Treatment	Average body weight			Radioactivity in per cent of administered dose			
		Initial g	End of 5 weeks g	End of 13 weeks g	Feces	Urine	Liver	Kidneys
A	Pyridoxine deficient	76 ± 2.1* (12)	120 ± 3.4	—	56.3 ± 3.4 (6)	1.22 ± 0.51	6.5 ± 0.54	6.3 ± 0.15
B	Pyridoxine treated	78 ± 1.4 (12)	164 ± 4.5	—	43.8 ± 4.1 (6)	1.85 ± 0.41	9.8 ± 0.61	8.4 ± 0.31
A	First 5 weeks (pyridoxine deficient) Second 8 weeks (pyridoxine injection)	—	—	204 ± 1.2 (6)	45.4 ± 3.1 (6)	1.91 ± 0.35	10.2 ± 0.41	8.5 ± 0.1
B	First 5 weeks (pyridoxine injection) Second 8 weeks (pyridoxine deficient)	—	—	173 ± 0.3 (6)	57.3 ± 4.5 (6)	1.16 ± 0.56	6.1 ± 0.39	6.1 ± 0

* Standard error of the mean.

Parentheses around numbers indicate number of rats used.

SUMMARY AND CONCLUSIONS

Data have been presented to show that absorption of vitamin B_{12} can take place in subjects without a stomach. Therefore, it must occur in the intestines or sublingually.

Absorption can be increased by the divided dosage schedule.

The absorption of vitamin B_{12} in clinically healthy subjects is partially dependent on the physical state in which it is administered. Vitamin B_{12} given in aqueous solutions to normal subjects is better absorbed than that given in a specific type of capsule.

Vitamin B_{12} absorption is also impaired by vitamin B_6 deficiency, and can be improved by subsequent administration of pyridoxine.

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