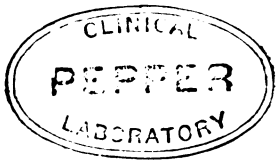

Enhanced Urinary Excretion of Co^{60} Vitamin B_{12} Produced by Delayed Release Capsules

By P. C. Johnson and E. S. Berger

DIETARY VITAMIN B_{12} requires intrinsic factor for its absorption from the gastrointestinal tract.[1] Even so, small amounts of vitamin B_{12} will be absorbed in the absence of intrinsic factor and therapeutic responses can be produced in pernicious anemia if sufficiently large amounts of vitamin B_{12} are ingested.[2] In the human, vitamin B_{12} is absorbed from the upper gastrointestinal tract. The colon probably does not absorb vitamin B_{12} in doses of 0.5 to 1.5 μg.[3] However, the administration of 3000 μg. of vitamin B_{12} rectally in pernicious anemia patients results in good hematologic responses.[4]

Co^{60}-labeled vitamin B_{12} has given us a convenient tool to measure the functional adequacy of intrinsic factor production. This may be done by measuring the proportion of the ingested dose that appears in the feces or the proportion of the absorbed dose that appears in the urine after a loading dose. Callender and Evans have demonstrated that after a loading dose of stable vitamin B_{12} urinary excretion is proportional to vitamin B_{12} absorption as measured by fecal excretion.[5] Within limits, a linear relationship has been shown between vitamin B_{12} absorption and intrinsic factor concentration.[6] Whether or not intrinsic factor plays any role in the metabolism of vitamin B_{12} after its absorption from the gastrointestinal tract is not yet established, but recent work with rat liver slices suggests that intrinsic factor may play a role in the absorption of vitamin B_{12} by a rat liver slice.[7]

Since vitamin B_{12} requires intrinsic factor for its absorption, one might postulate that vitamin B_{12} absorption would be maximal in the upper small intestine where the concentration of intrinsic factor might be greatest. In an attempt to test this, we have obtained delayed release capsules of vitamin B_{12} which are designed to release the vitamin B_{12} in one, four or seven hours after ingestion. In this way, the site of release of the maximal amount of vitamin B_{12} would occur in different areas of the intestine and stomach. By measuring fecal excretion and urinary excretion of vitamin B_{12} after a loading dose we would be able to obtain information about the effect of the rate of release of vitamin B_{12} on its absorption.

Materials and Methods

Fasting, hospitalized patients without known neurologic or hematologic abnormality were given 8 μg. of vitamin B_{12} labeled with 0.2 μc. of Co^{60} as a water solution or as a delayed release capsule. Simultaneously, one mg. of vitamin B_{12} was given subcutaneously and also 24 hrs. later. Two 24-hour urines were obtained. One-half Gm. of carmine red was given orally on the second day. Each patient was given 8 cc. of cascara on the second day. Complete stool collections were obtained daily until the

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majority of the carmine red appeared in the stool. In our study the carmine red appeared 4 to 10 days after the ingestion of the dye, and we have not found significant amounts of radioactivity after this time.

The patients were divided into 6 groups as follows:

Group 1 (Control Group). Given a water solution of vitamin B_{12}.

Group 2. Received capsules containing enteric-coated pellets that will not dissolve in acid but will release the vitamin B_{12} within one hour of the time they come in contact with alkali.* It is assumed that vitamin B_{12} would be released from these capsules in the upper small intestine.

Group 3. Received capsules containing pellets that would release the vitamin B_{12} within one hour of their ingestion.* It can be assumed the most of the B_{12} would be released in the stomach.

Group 4. Given capsules containing pellets that would dissolve over a 4-hour period.* The B_{12} would be released and distributed throughout the upper small intestine, especially in the jejunum.[8]

Group 5. Given capsules containing pellets that dissolve over a 7-hour period releasing vitamin B_{12} especially in the lower small intestine and colon.*

Group 6. Given a water solution in the same way as Group 1, except that they received in addition an extra loading dose of 1 mg. of vitamin B_{12} 8 hours before the tracer dose. This was done to test the effect preloading with vitamin B_{12} has on the absorption of the water solution. (This will be called the preloaded group.)

Group 7. Given a water solution as in Group 1, and treated in the same way as Group 1, except that the first loading dose of 1 mg. of vitamin B_{12} was given 2 hours after the oral dose. (This will be called the postloaded group.)

All capsules were counted in a scintillation well before administration. One capsule was kept for a standard. The water solution was made by dissolving capsules individually prior to administration. The standard capsules were dissolved and brought up to 5 ml. in a counting tube.

Three 20 ml. aliquots of each 24-hr. urine specimen were evaporated to 5 ml. in a counting tube. Pooled stool specimens were collected in a solution containing papain. These were homogenized and three 5 ml. aliquots of each were placed in counting tubes. The urine and stool aliquots were counted and compared to the counts of the standard. The percentage excreted was determined as follows:

$$\frac{\text{Net Standard Capsule Count}}{\text{Net Patient's Capsule Count}} = K$$

$$\frac{\text{Average Net Counts Urine} \times \frac{\text{Urine Vol.}}{20} \times 100 \times K}{\text{Net Count Standard Solution}} = \% \text{ in Urine}$$

$$\frac{\text{Average Net Count Stool} \times \frac{\text{Vol.}}{5} \times 100 \times K}{\text{Net Count Standard Solution}} = \% \text{ in Stool}$$

The contents of representative capsules of each type were chromatographed and found to contain over 90 per cent of their Co^{60} activity as vitamin B_{12} by chromatography using an aqueous 2, 4 lutidine system.[9]

Results

Table 1 shows that the mean fecal excretion of the various delayed release groups varied from 49.4 per cent for Group 4 to 66.4 per cent for Group 7.

*Assayed and furnished by Smith, Kline and French Laboratories, Philadelphia, Pa.

TABLE 1.—*Fecal Excretion of 8 μg. Vitamin B_{12}, 0.2 μc. Co^{60}*

Group No.	1 Control	2 Enteric-Coated	3 1-Hr. Release	4 4-Hr. Release	5 7-Hr. Release	6 Pre-loaded	7 Post-loaded
	47.9	57.4	56.9	40.0	48.6	60.7	45.9
	94.5	68.8	66.1	29.2	61.3	43.5	67.1
	76.8	73.7	32.4	72.6	84.8	70.9	
	70.1	52.4	48.1	43.5	70.9	62.2	96.0
	32.2	63.6	56.7	55.6	51.5	76.0	81.1
	60.3	65.9	25.6	47.7	63.0	63.8	15.8
	31.1	12.5	34.7	62.6	41.1	65.7	112.
	74.3	49.3	43.6	32.0	64.4	43.7	34.2
	67.1	59.8	81.8	75.7	70.1	69.8	79.4
	55.8	69.5	50.0	59.6	41.3	73.6	
		57.6	60.0	60.4		50.9	
			54.9	57.2		0.81	
				6.5		26.8	
						55.2	
						69.6	
Mean ±SE	61.0	57.3	50.9	49.4	59.7	55.5	66.4
	±6.01	±4.76	±4.37	±5.11	±4.23	±5.05	±10.7

Statistical analysis of these values showed no statistically significant differences among the various groups ($P > .10$) when these were analyzed by Student's 't' test.

In table 2 are presented the urinary excretions of vitamin B_{12} during the first 24 hours after ingestion of a labeled dose. The urinary excretion of each individual patient is presented in the same order that it was presented in table 1, so that a comparison can be made. The means of the seven groups of patients vary from 3.64 per cent to 7.12 per cent. There is a difference of statistical significance between the control group that received a water solution and Groups 4 and 5. These statistical differences are shown in table 3. The percentage in the urine tends to increase as the release time is prolonged. Table 4, presented in the same way as table 2, shows that these differences are magnified in the second 24 hours with urinary excretion of Groups 2, 4 and 5 being very much greater than the urinary excretion of the control. Table 5 shows the statistical analysis of these differences. There is no difference of statistical significance between the urinary and stool excretion of pre- and postloaded groups when they are compared to the control group.

DISCUSSION

It has been shown that the absorption of physiologic amounts of vitamin B_{12} is closely related to intrinsic factor activity.[6] This being so, one would expect then that the greatest absorption of vitamin B_{12} would occur after maximum contact with gastric juice. This was not proven in this experiment, since the fecal excretion of labeled vitamin B_{12} showed no statistically significant differences. The means of the groups varied from 49.4 per cent for Group 4 to 61.0 per cent for Group 1. This has occurred even though the

TABLE 2.—*0 to 24-Hour Urinary Excretion of 8 μg. Vitamin B_{12}, 0.2 μc. Co^{60}*

Group No.	1 Control	2 Enteric-Coated	3 1-Hr. Release	4 4-Hr. Release	5 7-Hr. Release	6 Pre-loaded	7 Post-loaded
	4.4	4.6	5.6	13.7	6.3	3.9	8.8
	2.9	6.9	3.5	8.4	4.5	7.5	5.7
	4.4	1.3	1.7	4.1	3.0	3.1	4.6
	1.8	2.3	11.	6.0	5.5	5.4	3.7
	1.4	5.4	4.8	5.1	5.2	1.7	5.2
	0.8	6.9	11.	3.0	14.	7.1	1.9
	4.6	2.2	1.9	5.4	8.5	2.8	6.0
	4.7	7.8	17.	5.1	5.3	1.3	1.4
	7.8	7.5	8.1	7.2	6.9	2.7	8.9
	4.6	3.9	8.4	7.1	12.	1.2	
		2.0	2.4	4.9		3.3	
			3.7	2.9		2.1	
				7.2		5.9	
						3.0	
Mean ±SE	3.74	4.62	6.59	6.16	7.12	3.64	5.13
	±0.63	±0.69	±1.29	±0.75	±1.03	±0.53	±0.65

labeled vitamin B_{12} given as a water solution would have been available for maximum contact with gastric juice. On the other hand, Group 4 and 5 capsules had the poorest chance to come in contact with gastric juice since most of the B_{12} would have been released in the lower small intestine with little or no B_{12} released in the stomach.

The urinary excretion, on the other hand, showed differences of statistical significance when the delayed release groups were compared to the control during the first 24-hour period after the loading dose. They ranged from 3.74 per cent for Group 1 to 7.12 per cent for Group 5. These differences are increased in the second 24 hours after a second loading dose of vitamin B_{12} with the excretion varying from 1.58 per cent for Group 1 to 8.80 per cent for Group 5.

There are several possible explanations for our finding an increased urinary excretion of vitamin B_{12} with the delayed release capsules. One explanation for this might be that since the loading dose had been given at the same time as the tracer dose, absorption of the water solution may have been decreased by high serum levels of vitamin B_{12}. The loading dose is given two hours after the tracer dose in the procedure described by Schilling.[10] However, other authors who obtain similar values have chosen to give the

TABLE 3.—*Percentage Co^{60} Vitamin B_{12} Excreted in 24-Hr. Urine*

Group	Percentage ±S.E.	"P" Value
1. Water Solution	3.74 ± 0.65	Control
2. Immediate in Alkali	4.62 ± 0.69	>0.4
3. One Hour	6.59 ± 1.29	>0.1
4. 4 Hours	6.16 ± 0.75	<0.05
5. 7 Hours	7.12 ± 1.03	<0.02
6. Preloaded	3.64 ± 0.53	>0.90
7. Postloaded	5.13 ± 0.65	>0.25

TABLE 4.—*24 to 48-Hour Urinary Excretion of 8 μg. Vitamin B_{12}, 0.2 μc. Co^{60}*

Group No.	1 Control	2 Enteric-Coated	3 1-Hr. Release	4 4-Hr. Release	5 7-Hr. Release	6 Pre-loaded	7 Post-loaded
	1.7	2.8	0.3	8.9	13.	17.	3.0
	1.3	3.0	2.4	13.	7.2	1.9	1.6
	1.4	4.8	0.1	1.6	3.6	1.7	0.9
	0.8	2.8	9.5	8.1	3.2	3.1	0.9
	1.3	2.1	0.2	4.6	3.4	2.1	3.9
	1.6	3.6	10.	1.3	17.	1.5	0.4
	2.0	10.	0.0	0.9	4.8	0.0	1.6
	2.4	5.4	13.	6.5	0.8	2.2	1.4
	1.3	3.3	2.1	9.1	18.		2.5
	2.0	2.5	4.4	5.1	17.	0.4	
		5.6	—	1.7		1.3	
			1.8	4.1		2.0	
				6.9		2.5	
						1.5	
						4.7	
Mean ±SE	1.58	4.17	3.98	5.52	8.80	2.99	1.80
	±0.15	±0.65	±1.34	±0.96	±1.99	±1.10	±0.36

loading dose simultaneously with the tracer dose.[5,11] Group 7, who received their loading dose two hours after the tracer dose, showed no statistically significant difference from Group 1 in stool and urinary excretion of the tracer dose, indicating that the timing of the loading dose probably is not the major cause of these differences in urinary excretion.

After a subcutaneous injection of vitamin B_{12}, maximum serum levels are reached in two hours. When one mg. or more of vitamin B_{12} is injected, the B_{12} binding-capacity of the serum, liver and kidney are saturated. All unbound vitamin B_{12} is quantitatively excreted by the kidneys. This fact is made use of in the loading dose of the Schilling test of urinary excretion of Co^{60} vitamin B_{12}.[10] Since the loading dose had been active for a greater length of time in the delayed release groups, one might postulate that relatively more of the absorbed Co^{60} vitamin B_{12} would remain unbound in the serum, allowing a greater percentage of it to be excreted in the urine. This second possibility was tested in Group 6 by loading the patients with one mg. of vitamin B_{12} eight hours before the tracer dose. This group was then given a water solution of vitamin B_{12} and loading doses as in Group 1. The urinary and fecal excretions of Group 6 when compared to Group 1

TABLE 5.—*Percentage Co^{60} Vitamin B_{12} Excreted in Second 24-Hr. Urine*

Group	Percentage ±S.E.	"P" Value
1. Water Solution	1.58 ± 0.15	Control
2. Immediate in Alkali	4.17 ± 0.65	<0.001
3. One Hour	3.98 ± 1.34	>0.1
4. 4 Hours	5.52 ± 0.96	<0.005
5. 7 Hours	8.80 ± 1.99	<0.005
6. Preloaded	2.99 ± 1.10	> .10
7. Postloaded	1.80 ± 0.36	> .60

showed no differences of statistical significance, suggesting that preloading is not an important factor in these differences.

As a third possibility, these differences might be explained by release in the gastrointestinal tract of the Co^{60} from the vitamin B_{12} molecule. Butanol extraction of the urines of Group 5 (6-hr. delayed release) according to the technic described by Maclean and Bloch[9] showed that over 90 per cent of the radioactivity was butanol soluble. Since ionic cobalt is not butanol soluble, one can assume that significant release of Co^{60} from vitamin B_{12} molecule had not occurred in this group.

The fourth possible explanation of the enhanced excretion after delayed release could be that the delayed release vitamin B_{12} was absorbed more completely than was the water solution. This would be possible if the delayed release capsules presented themselves to the intestine as multiple small doses which would be absorbed more efficiently than a large dose.[12]

It is generally accepted that measurement of fecal excretion of vitamin B_{12} is technically difficult and small differences in total absorption may be missed in groups of the size used here. However, Callender and Evans have shown that urinary excretion is proportional to fecal excretion of vitamin B_{12}.[5] The 0 to 48 hour urinary values of the seven-hour delayed release capsules are approximately three times greater than the control. However the average fecal excretion of the control is only slightly different from the seven-hour release group. If the urinary excretion is to remain proportional to the fecal excretion, greater differences in fecal excretion should have occurred.

The failure of the urinary excretion of the water solution of vitamin B_{12} to equal the excretion of the delayed release group might mean that the absorbed vitamin in Group 1 was bound to the serum proteins or the liver more efficiently than were the delayed release vitamin.

In favor of this theory is the difference in the urinary excretion of vitamin B_{12} after intramuscular and intravenous injection from the urinary excretion that occurs after oral absorption and a loading dose. Conley et al. have shown that doses of 500 μg. or more are nearly quantitatively excreted after parenteral injection.[13] However, after oral ingestion of tracer amounts of vitamin B_{12} more than 75 per cent of the tracer dose is absorbed in normal patients. Urinary excretion after a loading dose seldom reaches 40 per cent of the tracer dose, which is less than 60 per cent of the absorbed dose. This discrepancy would be explained if serum or tissue binding of vitamin B_{12} is influenced by absorption from the gastrointestinal tract. While this is a plausible explanation of the differences in urinary excretion seen here, the data do prove or disprove this theory.

Summary

1. Patients without known hematologic disease were given 8 μg. (0.2 μc. Co^{60}) vitamin B_{12} as a water solution or in various types of delayed release capsules.

2. All groups showed approximately equal fecal excretion. However, the cobalt^{60}-labeled vitamin B_{12} delayed release capsules showed a greater urinary

excretion after a loading dose of stable B_{12} than did the water solution of labeled vitamin B_{12}.

Summario in Interlingua

1. Patientes sin cognoscite morbo hematologic recipeva vitamina B_{12} marcate con Co^{60} in un dosage de 8 μg (continente 0, 2 μc de Co^{60}) in le forma de un solution aquose o de varie typos de capsulas a liberation retardate.

2. Omne le gruppos monstrava approximativemente equal valores de excretion fecal. Tamen, le capsulas a liberation retardate de vitamina B_{12} con Co^{60} se distingueva ab solutiones aquose de vitamina B_{12} con Co^{60} per monstrar un plus grande excretion urinari post le administration de un dose cargatori de B_{12} stabile.

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