

FEASIBILITY OF CONSECUTIVE-DAY SCHILLING TESTS

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The concept that parenteral vitamin B₁₂ inhibits intestinal absorption of orally administered vitamin B₁₂ has resulted in the policy that sequential Schilling tests be performed with a 3–4 day interval. The feasibility of performing repeat Schilling tests with shorter intervals was evaluated by studying 12 healthy volunteers. Four consecutive 24-hr collections were obtained following oral administration of labeled vitamin B₁₂; ⁵⁷Co on Days 1 and 4, and ⁶⁰Co on Day 2. The standard parenteral flushing dose of vitamin B₁₂ (1 mg) was given in conjunction with each test dose of labeled vitamin B₁₂. All subjects excreted normal quantities of the test dose on the control and test days with means of 16.9%, 14.2%, and 19.8%. The latter value was corrected to 17.0% for the contribution of ⁵⁷Co from the first day. There was no statistically significant difference between Days 1 and 2 when analyzed by the paired two-sided Student's t-test at a level of 0.05. The results of this study indicate that it is feasible to perform serial Schilling tests not only on alternate days but also on two consecutive days. Thus patients suspected of having pernicious anemia can be studied with the baseline Schilling test on one day and the repeat Schilling test with intrinsic factor on the following day.

The Schilling test has been utilized extensively since its introduction as an indicator of vitamin B₁₂ absorption (1). Chow and Okuda subsequently pointed out that prior large-dose vitamin B₁₂ administration could adversely affect the Schilling test (2). Ellenbogen, et al also found that massive doses of vitamin B₁₂ significantly decreased absorption and subsequent excretion of labeled vitamin B₁₂ (3). Mailloux and Streeto similarly demonstrated that the administration of large doses of vitamin B₁₂ during the 3–5 days prior to the Schilling test usually

decreased urinary excretion of the labeled vitamin B₁₂ (4).

In the above studies, the amounts of vitamin B₁₂ administered prior to Schilling testing ranged from 3 mg to 21 mg. There seems little doubt that large amounts of vitamin B₁₂ could invalidate the Schilling test; hence the policy in clinical laboratories has been to delay the repeat Schilling test with intrinsic factor for 3–4 days after the baseline study. Because of the increased clinical demands for more rapid patient evaluation, it would be advantageous to perform the Schilling studies with and without intrinsic factor with a shorter interval between tests. This study was designed to determine the effect of a 1-mg flushing dose of vitamin B₁₂ on the subsequent absorption of a test dose of vitamin B₁₂ 24 and 48 hr later.

MATERIALS AND METHODS

The study was composed of 12 apparently healthy volunteers ranging in age from 23 to 50 years. Ten were males and two were females. No subjects were taking medications known to affect vitamin B₁₂ absorption or excretion and none received vitamin B₁₂ preparations orally or parenterally during the previous 4 weeks.

At the initiation of the study, urine specimens were analyzed to determine background activity for ⁵⁷Co and ⁶⁰Co. Four 24-hr urine collections were obtained on four successive days. Urine creatinine determinations were made on all specimens. Standard Schillings tests were done on Days 1, 2, and 4 with labeled vitamin B₁₂ administered orally in the fasting state. Cobalt-57 was administered on Days 1 and 4 utilizing the same standard, and ⁶⁰Co was administered on Day 2. The standard flushing dose of 1 mg

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TABLE 1. URINE VOLUMES AND CREATININE EXCRETION

Pa- tient	Age	Sex	Day							
			1		2		3		4	
			Urine vol. (ml)	Creati- nine (gm)	Urine vol. (ml)	Creati- nine (gm)	Urine vol. (ml)	Creati- nine (gm)	Urine vol. (ml)	Creati- nine (gm)
GG	38	M	970	1.36	1155	1.34	1120	1.26	1720	1.46
RR	33	M	600	1.28	1145	1.24	615	1.22	790	1.26
SV	31	M	745	1.66	845	1.76	1155	1.10	1800	1.54
RH	41	M	1200	1.26	1320	1.30	1180	1.28	1400	1.32
RM	50	M	950	1.34	1050	1.34	890	1.44	805	1.38
FS	32	M	1225	1.54	1370	1.48	1320	1.60	1085	1.64
MH	40	F	1440	0.90	930	0.76	1130	1.00	850	0.92
CB	27	F	720	1.04	910	1.06	1235	1.04	1415	1.18
HS	45	M	780	1.28	790	1.28	920	1.32	780	1.32
CJ	41	M	570	1.84	650	1.78	585	1.58	670	1.84
WB	37	M	910	1.22	765	1.08	830	1.26	1105	1.22
DL	23	M	1050	2.00	1040	1.96	950	1.94	1025	1.92

of unlabeled vitamin B₁₂ was given intramuscularly immediately after the orally administered labeled vitamin B₁₂. Both ⁵⁷Co- and ⁶⁰Co-labeled vitamin B₁₂ were obtained from the same radiopharmaceutical laboratory. The dose of ⁵⁷Co was 0.56 μCi in 0.68 μg of vitamin B₁₂ and that of ⁶⁰Co was 0.51 μCi in 0.63 μg of vitamin B₁₂. The counting efficiency for ⁵⁷Co was 0.54 cps/dps and for ⁶⁰Co was 0.11 cps/dps. All precalibrated capsules were counted in our laboratory and an appropriate standard capsule was selected. The counting rate of all test capsules was within 0.4% of the standard. The standard capsule was completely dissolved and diluted with water to 1,000 ml. All 24-hr urine collections were diluted with water to 2,000 ml. Five-milliliter aliquots from the standard and urine collections were counted in a dual-channel auto-gamma counter with a 10% window. The subjects were studied in four groups of three. Each specimen was counted for 40 min and twice recycled for a total counting time of 120 min. Forty-minute background counts were measured every 160 min. Average background for ⁵⁷Co was 27 cpm and for ⁶⁰Co was 9 cpm. The percent counting error of the combined background, urine, and standard for ⁵⁷Co and ⁶⁰Co on Days 1 and 2 varied from 1.91 to 5.45 at the 95% confidence level. The highest percent counting error for ⁶⁰Co on Day 4 was 7.08. Six of the 12 subjects were done in duplicate with virtually identical results. The percent excretion of labeled vitamin B₁₂ was calculated as follows:

% excretion =

$$\frac{(\text{Urine cpm} - \text{background}) \times 400}{(\text{Standard cpm} - \text{background}) \times 200} \times 100$$

The ⁵⁷Co counts on Days 2 and 4 were corrected for the Compton contributions from the ⁶⁰Co decay.

This correction was made by counting the ⁶⁰Co standard in both channels. The ratio of the net counting rates was used as a correction factor. The normal excretion in this laboratory is greater than 7%.

RESULTS

The 24-hr urine volumes and total creatinine excretion are listed in Table 1. The baseline Schilling test for all 12 subjects was in the normal range with all values being greater than 7% (Table 2). The excretion of labeled vitamin B₁₂ on Day 2 was also in the normal range in all 12 subjects. The mean

TABLE 2. % EXCRETION OF LABELED VITAMIN B₁₂ (1 MG FLUSH)

Patient	Day			
	1	2	3	4
	⁵⁷ Co	⁶⁰ Co	⁵⁷ Co	
	% Excretion	% Excretion	% Excretion uncorrected	% Excretion* corrected
GG	19.6	14.4	15.7	13.3
RR	10.4	9.9	19.5	17.8
SV	11.2	9.9	18.0	14.5
RH	14.9	23.6	25.4	22.8
RM	17.5	23.3	20.3	18.1
FS	22.7	13.8	23.1	18.8
MH	22.9	13.9	24.2	21.2
CB	16.8	13.2	17.8	15.2
HS	22.5	18.3	21.4	18.5
CJ	18.6	12.2	26.2	21.6
WB	16.4	11.1	15.9	13.3
DL	9.8	7.2	10.4	9.4
Mean	16.9	14.2	19.8	17.0

* % Excretion corrected for estimated contribution from Day 1 (Table 4).

percent excretion of the group on Day 1 was 16.9%, and on Day 2 was 14.2%. The percent excretion of labeled vitamin B₁₂ in all subjects on Day 4 was also in the normal range with a mean of 19.8%. However, it should be noted that a portion of the radioactivity accumulated on Day 4 can be accounted for by the labeled vitamin B₁₂ administered on Day 1. Repeat flushing doses of vitamin B₁₂ result in a definite and significant excretion of the labeled vitamin B₁₂ on the second and third days (3). We have confirmed these findings in our laboratory and have observed this augmented excretion to occur as late as the fifth day following the standard Schilling test (Table 3). Considering this phenomenon and based on our data, approximately 28% of the ⁵⁷Co excreted on Day 2 would be anticipated to be excreted on Day 4 (Table 3). Therefore the mean of 19.8% for Day 4 would be corrected by a reduction of approximately 2.8% (Table 4) to 17.0% (Table 2).

The excretion data of Day 1 and Day 2 were compared for statistical difference by using a paired two-sided Student's t-test with 11 deg of freedom at a level of 0.05. The t value was 1.75 which was not significant. Because of the limited number of cases, the distribution of excretion rates of normal individuals cannot be determined. If we were to fit a normal distribution curve to the data of Day 2, the proportion of cases falling below 7% excretion would be 0.08 and below 5% excretion would be 0.036.

DISCUSSION

Measurements of fecal radioactivity following the oral administration of labeled vitamin B₁₂ suggest that the flushing dose of vitamin B₁₂ (1 mg) inhibits intestinal absorption (5). This finding was noted when the flushing dose was given at the time of oral administration of the labeled vitamin B₁₂ or 2 hr following. This absorptive inhibition does not negate the usefulness of the excretion test as a determinant of intestinal absorption because virtually no labeled vitamin B₁₂ is excreted without the flushing dose. Large prior doses of vitamin B₁₂ further impair absorption of labeled B₁₂ in the standard Schilling test. However, it cannot be concluded that the smaller standard 1-mg flushing dose of vitamin B₁₂ administered 24 or 48 hr previously would likewise invalidate the Schilling test.

In our series the mean excretion for the fourth day when corrected for the estimated contribution of radioactivity from the first day was virtually identical with the mean excretion of the first day. This would suggest that a standard flushing dose 48 hr previously would have essentially no effect on the intestinal absorption of the test dose of vitamin B₁₂.

TABLE 3. EFFECT OF INTERMITTENT B₁₂ FLUSHING (1 MG I.M.) ON % EXCRETION OF ⁶⁰Co-LABELED B₁₂

Patient	Day				
	2*	3	4	5	6
	Flush	No flush	Flush	†	†
GG	14.4	0.4	4.0	—	—
RR	9.9	0.2	5.2	—	—
SV	9.9	0.2	2.4	—	—
RH	23.6	0.3	3.9	1.2 (F)	0.7 (F)
RM	23.3	0.9	5.3	0.0 (NF)	1.2 (F)
FS	13.8	0.1	4.1	—	—
MH	13.9	1.0	3.9	0.1 (NF)	0.0 (NF)
CB	13.2	0.1	5.1	0.0 (NF)	0.1 (NF)
HS	18.3	0.4	4.8	0.1 (NF)	0.1 (NF)
CJ	12.2	0.1	3.2	—	—
WB	11.1	0.1	4.9	—	—
DL	7.2	0.2	1.6	—	—
Mean	14.2	0.3	4.0	—	—

* ⁶⁰Co-labeled B₁₂ was administered on Day 2 only.
 † Flush was variable as given in parentheses: F = flush; NF = no flush.

TABLE 4. EFFECT OF CONSECUTIVE-DAY B₁₂ FLUSHING (1 MG I.M.) ON % EXCRETION OF ⁵⁷Co-LABELED B₁₂

Patient	Day			
	1*	2	3	4†
	Flush	Flush	No flush	Flush
GG	19.6	8.6	0.2	2.4
RR	10.4	6.2	0.1	1.7
SV	11.2	12.4	0.1	3.5
RH	14.9	9.1	0.0	2.5
RM	17.5	7.7	0.2	2.2
FS	22.7	15.2	0.3	4.3
MH	22.9	10.7	0.2	3.0
CB	16.8	9.3	0.0	2.6
HS	22.5	10.4	0.0	2.9
CJ	18.6	16.2	0.1	4.5
WB	16.4	9.3	0.0	2.6
DL	9.8	3.5	0.1	1.0
Mean	16.9	9.9	0.1	2.8

* ⁵⁷Co-labeled B₁₂ was administered on Day 1.
 † Estimated % excretion based on 0.28 of the % excretion on Day 2.

Although the difference between the mean excretions on Day 1 and Day 2 was not statistically significant, there was less excretion on Day 2 in the majority of cases suggesting the possibility of a small systematic decrease secondary to impaired intestinal absorption. However, impairment of absorption of such a magnitude would no more negate the validity of the test than the absorptive inhibition associated with the flushing dose of the standard Schilling test. All subjects were in the normal range on the second day with a mean percent excretion

twofold greater than the lower limit of normal. It is concluded that a standard flushing dose of vitamin B₁₂ (1 mg) administered 24 hr previously would not invalidate a repeat Schilling test. This is particularly true because patients with pernicious anemia almost invariably have excretions less than 2% on the baseline test.

The clinical importance of this finding is readily apparent. Untreated patients suspected of having pernicious anemia can conveniently be studied on two consecutive days; the standard Schilling test being performed on the first day and the repeat Schilling test with intrinsic factor on the second day. It cannot be assumed that this technique would be valid for patients with renal insufficiency. There is not only delayed excretion of the labeled vitamin B₁₂ but more significantly the effect on intestinal absorption by the retained flushing dose is not known.

The mechanism whereby prior administration of large parenteral doses of vitamin B₁₂ impair intes-

tinal absorption of orally administered vitamin B₁₂ has not been established. More importantly, the minimum prior dose of vitamin B₁₂ necessary to produce significant absorptive inhibition has not been determined but certainly is greater than the standard 1-mg flushing dose received 24–48 hr previously.

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