SIMULTANEOUS MEASUREMENT OF FREE AND INTRINSIC FACTOR (IF) BOUND VITAMIN B_{12} (B_{12}) ABSORPTION: ABSOLUTE QUANTITATION WITH INCOMPLETE STOOL COLLECTION AND RAPID RELATIVE MEASUREMENT USING PLASMA B_{12} (IF): B_{12} ABSORPTION RATIO

Mathews B. Fish, Myron Pollycove, Ralph O. Wallerstein, Katherine Ka-Siu Cheng, and Mary Tono

University of California School of Medicine and San Francisco General Hospital, San Francisco, California

This report describes a procedure that involves the absolute and simultaneous measurement of free ^{58}Co-labeled B_{12} (^{58}Co-B_{12}) and IF-bound ^{57}Co-B_{12} [^{57}Co-B_{12}(IF)] using a nonabsorbable marker (^{60}Co microspheres) and incomplete stool collections. Absorption by this procedure in controls and megaloblastic folate deficient patients averaged 55% for B_{12} and 66% for B_{12}(IF) whereas absorption in megaloblastic patients with pernicious anemia averaged 7% of B_{12} and 34% of B_{12}(IF). These stool absorption data compared closely with simultaneous whole-body counter measurements. In addition, measurement and evaluation were made of a ^{58}Co-B_{12}(IF):^{57}Co-B_{12} ratio in a single plasma sample obtained 6–8 hr after test dose administration. This ratio was 1.1 in controls and folate deficiency, and 7.8 in patients with pernicious anemia. These plasma measurements are rapidly obtained and may furnish diagnostic information regarding possible intrinsic factor deficiency when quantitative results are equivocal or specimen collection is inadequate.

Since the availability of radiocobalt-labeled vitamin B_{12} (B_{12}), measurement of gastrointestinal absorption of this vitamin has continued to be a most useful and necessary procedure in the investigation of patients with megaloblastic anemia and patients with certain gastrointestinal or neurologic disorders (*1*). In addition, widespread measurement of serum B_{12} levels will undoubtedly result in the necessary performance of B_{12} absorption studies in individuals who exhibit subnormal B_{12} levels.

A great variety of techniques has been used to measure B_{12} absorption (*2–9*). The diversity of approaches reflects the advances and availability of instrumentation and illustrates the attempts to achieve certain theoretical, quantitative, or practical improvements in the performance of the test. Because intrinsic factor (IF) deficiency is by far the most common cause of decreased B_{12} absorption, the initial test is usually repeated after an appropriate period of delay by an absorption study that involves the simultaneous administration of radionuclide-labeled B_{12} and IF.

In an attempt to circumvent the problems of such sequential testing, studies involving the simultaneous administration of free B_{12} and gastric juice-bound B_{12}, each labeled with a different nuclide of cobalt, have been performed (*10–12*). These studies used Schilling's urinary excretion procedure (*2*).

This report describes a procedure that involves the absolute and simultaneous measurement of absorption of free ^{58}Co-labeled B_{12} (^{58}Co-B_{12}) as well as IF-bound ^{57}Co-B_{12} [^{57}Co-B_{12}(IF)] using a nonabsorbable radionuclide marker (^{60}Co microspheres) and incomplete stool collections. Results of this method are compared with simultaneous results using a whole-body radioactivity counter. In addition, simultaneous studies were performed that involved the measurement and evaluation of the B_{12}(IF):B_{12} ratio in a single plasma sample obtained 6–8 hr after test dose administration.

MATERIALS AND METHODS

Clinical material. In this study there were 36 patients who had been referred to the Nuclear Medi-

Received Dec. 11, 1972; revision accepted Mar. 6, 1973.

For reprints contact: Mathews B. Fish, Associate Director, Clinical Laboratories, Building 100, Room 163, San Francisco General Hospital, 1001 Potrero and 22nd Sts., San Francisco, Calif. 94110.

cine Laboratory, San Francisco General Hospital, for B_{12} absorption studies. All patients were carefully evaluated as to their hematologic and vitamin status by routine peripheral blood examinations, bone marrow aspirations, serum B_{12} (*13*), and folate levels (*14*). Other studies, including gastric analysis, uppergastrointestinal (GI) x-ray examination, and peroral biopsy of the small bowel, were performed when clinically indicated. Based upon the above studies, clinical findings, and course, the patients were divided into the following groups: (A) control group, consisting of 13 patients without hematologic or biochemical evidence of B_{12} or folate deficiency, whose diagnoses included chronic alcoholism, iron deficiency anemia, scurvy, psychosis, renal failure, cellulitis, and psychosomatic GI complaints; (B) folate deficient megaloblastic anemia group consisting of six patients; (C) intrinsic factor (IF)-lack B_{12}-deficient megaloblastic anemia group consisting of 15 patients with pernicious anemia (PA) in relapse and one patient who was 10 years postgastrectomy with B_{12} deficient megaloblastic anemia; and (D) small bowel B_{12} malabsorption group consisting of one patient with biopsy-proven sprue and B_{12} deficient megaloblastic anemia.

Measurements of GI absorption of B_{12} by stool and whole-body counter methods and determination of 6–8-hr plasma absorption ratio were performed after a single administration of test substances. After an overnight fast, subjects ingested one capsule containing 0.25 μg and approximately 0.8 μCi of ^{58}Co-B_{12}* and one capsule containing 0.25 μg and approximately 0.5 μCi of ^{57}Co-B_{12} bound to human gastric juice [^{57}Co-B_{12}(IF)]†; and an aqueous suspension of 100-micron-diam ^{60}Co-labeled microspheres, approximately 0.5–1.0 μCi‡.

Measurement of B_{12} absorption by stool method. Stool collections were obtained repeatedly in wide-mouth, screwcap plastic jars, from 1 to 7 days after administration of the test material. It was not necessary to homogenize and dilute the specimen before counting because the ratios of ^{57}Co:^{58}Co:^{60}Co counting rates remain quite constant, irrespective of the geometry of the stool, and ^{60}Co self-absorption under these circumstances is negligible. The content of ^{57}Co, ^{58}Co, and ^{60}Co in the specimens and appropriate standards for each nuclide were quantitated in a dual-crystal scintillation detector system with pulse-height analysis*. The spectrometer was adjusted to count each of the major photopeaks: for ^{57}Co, 0.025–0.200 MeV; for ^{58}Co, 0.700–0.950 MeV; and for ^{60}Co, 1.050–1.450 MeV. Counting efficiencies were as follows: ^{57}Co, 35%; ^{58}Co, 16%; and ^{60}Co, 11%. Using data derived from the standards, corrections were made for spectral overlap. All samples and standards were counted in similar containers and at the same geometry with respect to the two crystal detectors. Specimens were counted with at least 1% precision. The stool content of the individual nuclides was expressed as a fraction of the amount administered. The percent GI absorption of ^{58}Co-B_{12}(IF) and ^{57}Co-B_{12}(IF), corrected for incomplete stool collection by use of the excreted fraction of the nonabsorbable marker ^{60}Co microspheres, was calculated according to the following formula:

$$\%\ \text{GI absorption}\ [^{58}\text{Co-B}_{12}\ \text{or}\ ^{57}\text{Co-B}_{12}\text{(IF)}] = \left(1 - \frac{f^{57}\text{Co or } f^{58}\text{Co}}{f^{60}\text{Co}}\right) \times 100,$$

in which

f^{57}Co or f^{58}Co = fraction of administered dose of ^{57}Co or ^{58}Co in stool specimen,

and

f^{60}Co = fraction of administered dose of ^{60}Co in stool specimen.

Measurement of B_{12} absorption by whole-body-counter method. Quantitation of the whole-body content of ^{57}Co, ^{58}Co, and ^{60}Co was performed in a lead-shielded whole-body counter with an 11½-in.-diam scintillation crystal and a 400-channel analyzer. Channels selected for counting encompassed the energy ranges similar to those described above for the stool measurements. Counting efficiencies were as follows: ^{57}Co, 0.5%; ^{58}Co, 0.3%; and ^{60}Co, 0.2%. Counting of the patients was performed using 1-meter arc geometry. Counts were obtained to achieve a counting precision of approximately 1%. Corrections were made for individual patient backscatter and spectral overlap using data that were derived from counting each patient during the sequential administration of each nuclide as described below. On the initial day of the study (Day 0), after a background count was obtained, the patient was given the oral dose of ^{60}Co microspheres. The patient was then counted to determine the 100% count

* Dicopac Test Kit for Vitamin B_{12} Absorption; Amersham/Searle Corp., Arlington Heights, Ill. 60005.

† Ibid.

‡ Minnesota Mining and Manufacturing Co., St. Paul, Minn. 55119.

* Large sample gamma counting system with two opposed 5-in. NaI crystals (Tobor). Nuclear-Chicago Corp., Des Plaines, Ill. 60018.

for ^{60}Co and also to determine the contribution of ^{60}Co in the ^{57}Co and ^{58}Co major photopeak channels. The patient was then given the ^{58}Co-B_{12} and ^{57}Co-B_{12}(IF) capsules. On Day 7 the patient was again counted in the whole-body counter for ^{57}Co, ^{58}Co, and ^{60}Co content. Whole-body ^{60}Co content was an indicator of the maximum amount of nonabsorbed ^{58}Co-B_{12} and ^{57}Co-B_{12}(IF) remaining in the bowel. As the amount of whole-body ^{60}Co was negligible at Day 7 in all patients studied, the amount of nonabsorbed ^{58}Co-B_{12} and ^{57}Co-B_{12}(IF) remaining in the bowel can be assumed to be negligible. The patient then received an i.m. injection of ^{58}Co-B_{12}. This i.m. dose contained the same activity as the oral dose of ^{58}Co-B_{12}. After approximately 4 hr the patient was counted to determine the spectral overlap of ^{58}Co in the ^{57}Co channels. The patient was then given an i.m. dose of ^{57}Co-B_{12} equal to the previous oral dose of ^{57}Co-B_{12}(IF).

On Day 14 the patient was counted a final time for ^{57}Co, ^{58}Co, and ^{60}Co content. After making appropriate background, spectral overlap, and decay corrections, the GI absorption of ^{58}Co-B_{12} and ^{57}Co-B_{12}(IF) was calculated according to the following formula:

$$\%\ \text{GI absorption}\ [^{58}Co\text{-}B_{12}\ \text{or}\ ^{57}Co\text{-}B_{12}(IF)] = \frac{(^{57}\text{ or }^{58}Co\ \text{Day 7}) - (^{57}\text{ or }^{58}Co\ \text{Day 0})}{(^{57}\text{ or }^{58}Co\ \text{Day 14}) - (^{57}\text{ or }^{58}Co\ \text{Day 7})} \times 100,$$

in which

$(^{57}\text{ or }^{58}Co\ \text{Day 0})$ = patient background counts in ^{57}Co or ^{58}Co channels,

$(^{57}\text{ or }^{58}Co\ \text{Day 7})$ = ^{57}Co or ^{58}Co counts on Day 7,

$(^{57}\text{ or }^{58}Co\ \text{Day 14})$ = ^{57}Co or ^{58}Co counts on Day 14.

As no flushing dose of B_{12} is administered, the urinary excretion of the labeled B_{12} is negligible and therefore may be ignored in the above computation.

Plasma absorption ratio. At 6–8 hr after the oral administration of the ^{58}Co-B_{12} and ^{57}Co-B_{12}(IF) capsules, a 10-ml heparinized blood sample was obtained. This sampling time was selected because it is close to the time of maximal plasma radio B_{12} levels after administration of the oral test dose (*15*) and yet can be obtained in the usual working day. After centrifugation, 5 ml of plasma along with appropriate background and standard samples were counted in an automatic, dual-channel, well-type scintillation counter. The instrument was set to count each of the major photopeaks of the nuclides involved as described above. Using data derived from counting the standards, corrections were made for spectral overlap. Specimens and background samples were each counted repeatedly for 20-min periods for a total of 100–240 min/sample, to accumulate a total number of counts that would result in a net counting precision ranging from 5 to 20%, as estimated by the use of nomograms for standard error (*16*). In the 5-ml plasma sample, ^{57}Co and ^{58}Co were expressed as a percent of the oral dose.

The plasma absorption ratio B_{12}(IF):free B_{12} was then calculated according to the following formula:

$$\text{plasma absorption ratio} = \frac{\%\ ^{57}Co}{\%\ ^{58}Co},$$

in which

$\%\ ^{57}Co$ = amount of ^{57}Co in 6–8 hr plasma sample expressed as a % of the oral dose of ^{57}Co-B_{12}(IF),

$\%\ ^{58}Co$ = amount of ^{58}Co in 6–8 hr plasma sample expressed as a % of the oral dose of ^{58}Co-B_{12}.

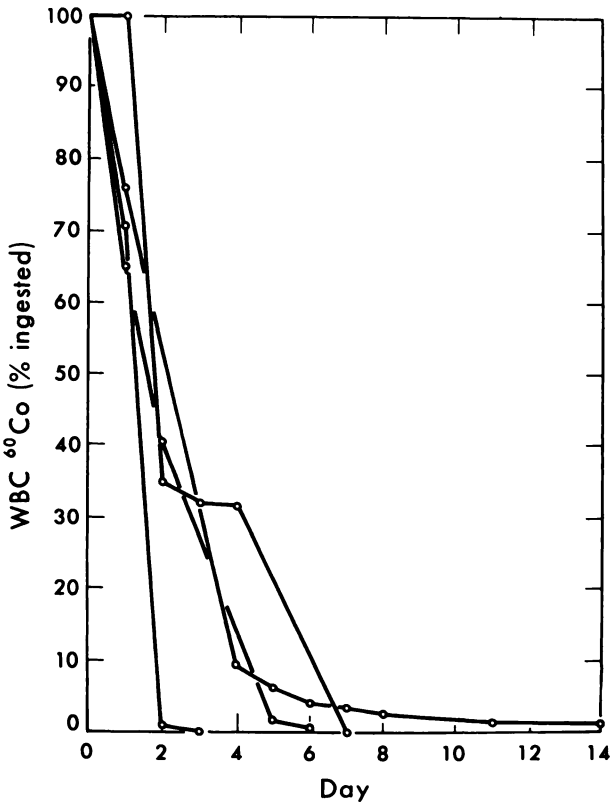

FIG. 1. Pattern of fecal excretion of ^{60}Co microspheres in four subjects. These results, obtained over 2 weeks by whole-body counting (WBC), are expressed as percentage of dose remaining in body.

TABLE 1. SIMULTANEOUS MEASUREMENT OF FREE AND INTRINSIC FACTOR (IF)-BOUND VITAMIN B_{12} ABSORPTION BY INCOMPLETE STOOL COLLECTION AND WHOLE-BODY COUNTER METHODS

Group	No.	% absorption			
		Stool		Whole-body counter	
		B_{12}	B_{12}(IF)	B_{12}	B_{12}(IF)
Control	13	57 (31–78)	66 (43–86)	52 (35–68)	64 (57–70)
Folate deficient megaloblastic anemia	6	53 (40–71)	67 (61–90)	58 (43–62)	69 (55–85)
Intrinsic factor lack vitamin B_{12} deficient megaloblastic anemia	16	7 (0–18)	34 (16–61)	12 (4–19)	42 (27–59)
Small bowel vitamin B_{12} malabsorption	1	0	0	6	8

RESULTS

The pattern of excretion of the nonabsorbable ^{60}Co microspheres in four subjects is shown in Fig. 1. These studies were performed by whole-body counting of the retained ^{60}Co and show a very rapid initial excretion of approximately 50% or more during the first 2 days after ingestion. Variation in bowel habits are reflected in the character of the retention curves. In three of four subjects, essentially all of the activity was eliminated by 1 week. In one subject a small amount (3.5%) was retained at 1 week and was followed by a progressive decrease to less than 1% by 14 days.

Measurements of gastrointestinal absorption of B_{12} and B_{12}(IF) by the incomplete stool collection and whole-body counter methods for the different patient groups are summarized in Table 1. As determined by the stool method, the absorption of B_{12} and B_{12}(IF) are quite similar in the control and folate deficient groups. A marked decrease in mean B_{12} absorption is noted in the B_{12} deficient groups, with no overlap evidenced by the other clinical groups. Improved, but somewhat subnormal, B_{12} absorption is noted with B_{12}(IF) in the IF-lack group. Excellent correspondence is noted between the absorption values obtained by the incomplete stool collection method and those determined by the whole-body counter method. The mean difference between these two methods is 5% with a range of 2–8% for all groups. In the 3 of the 13 control subjects whose absorption was measured simultaneously with and without IF by both methods, the range of differences was 0–5% (mean, 2.5%). In the three of the six folate deficient subjects whose absorption was measured simultaneously by both methods, the range of differences was 3–5% (mean, 3.3%). In the 6 of the 16 patients with intrinsic factor deficiency, the range of differences was 1–8% (mean, 5.1%). By counting a number of stool specimens separately from many of the patients, it was found that close agreement of the stool and whole-body counter methods was attained when greater than 50% of the stool specimen was collected; however, 30% of a complete collection is sufficient for diagnostic purposes.

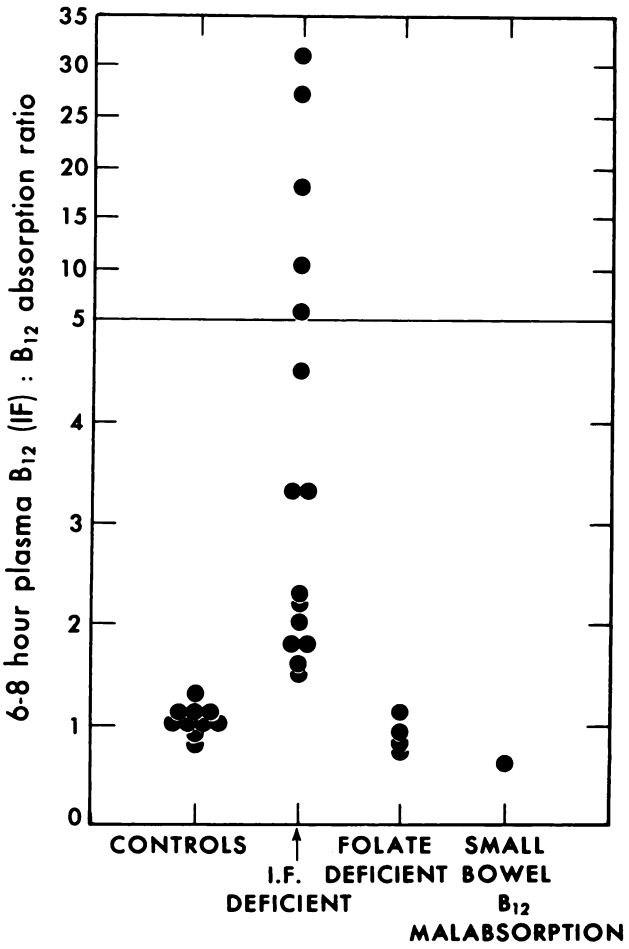

FIG. 2. Distribution of B_{12}(IF):B_{12} absorption ratios in all subjects studied as determined by measurement of plasma ^{57}Co:^{58}Co ratio 6–8 hr after ingestion of test material.

TABLE 2. SIMULTANEOUS MEASUREMENT OF FREE AND INTRINSIC FACTOR (IF)-BOUND VITAMIN B_{12} ABSORPTION: B_{12}(IF):B_{12} ABSORPTION RATIO

Group	No.	6–8-hr plasma ratio	Stool method ratio	Whole-body counter method ratio
Control	9	1.1 (0.8–1.4)	1.2 (0.9–1.9)	1.3 (1.0–1.6)
Folate deficient megaloblastic anemia	5	1.0 (0.7–1.3)	1.3 (1.2–1.4)	1.2 (1.1–1.3)
Intrinsic factor lack vitamin B_{12} deficient megaloblastic anemia	15	7.8 (1.5–31.0)	6.8 (1.9–31.0)	4.3 (1.7–7.4)
Small bowel vitamin B_{12} malabsorption	1	0.6 —	0 —	1.3 —

The distribution of B_{12}(IF):B_{12} absorption ratios in all subjects, as determined by the measurement of the plasma ^{57}Co:^{58}Co ratio 6–8 hr after ingestion of the test material, is shown in Fig. 2. For comparison, these plasma data are summarized for the different patient groups and are shown with corresponding absorption ratios derived from the stool and whole-body counter data in Table 2. Although the mean plasma ratio is approximately 1.1 in the control and folate deficient groups, it is markedly increased to 7.8 in the IF-lack B_{12}-deficient group. These plasma ratios correspond closely with ratios derived from stool data and reasonably well with those derived from whole-body counter data.

DISCUSSION

In the performance of absolute quantitation of B_{12} absorption by stool measurements, the use of a nonabsorbable stool marker obviates the necessary and most difficult task of complete stool collections. A number of different radionuclides in a variety of chemical forms have been used as nonabsorbable markers (*17–22*). The requirements for a nonabsorbable radioactive marker are: (A) that it is not absorbed; (B) that its appearance rate in stools is approximately similar to that of the nonabsorbed test substance; and (C) that its gamma-ray spectral emission allows for the simultaneous quantitation of marker and radiolabeled test substance by conventional instrumentation. A suspension of ^{60}Co microspheres was used as a nonabsorbable marker in the present study because of the character of its spectral emission and its appropriate behavior in the GI tract (Fig. 1).

The relative frequency of decreased B_{12} absorption due to an IF deficiency often necessitates a second B_{12} absorption study with administered IF. This second study must be repeated after an appropriate period of delay of 2–7 days, depending upon the technique used, in order to avoid the effect of overlap of the test doses. Aside from the difficulties to the patient and the necessity of repeating the entire procedure, such periods of delay often result in paired absorption measurements performed under different conditions. The ready availability of B_{12} labeled with different nuclides of cobalt has made possible the simultaneous administration of free and IF-bound B_{12}, thus circumventing the necessity for and the disadvantages of sequential studies (*10–12*). Preparation of IF-bound B_{12} in a relatively stable form for the simultaneous free B_{12} and B_{12}(IF) GI absorption studies has been achieved by use of normal human gastric juice as the source of IF (*10*). Complete binding of all IF sites is accomplished by exposure of the gastric juices to an excess of radioactive B_{12} followed by dialysis to remove the remaining radioactive B_{12} in the free form (*10*). The preparation used in the present study was similar to that described above, with the appropriate modifications for capsular dose.

In Table 3, the absolute GI absorption results of simultaneously administered free and IF-bound B_{12} by both the incomplete stool collection and whole-body counter methods in this study are compared with absolute absorption results obtained in other studies of sequentially administered free B_{12} and B_{12} with IF determined by whole-body counting, hepatic uptake, and complete stool collection techniques (*8,9,23–30*). Not only is there excellent agreement of values obtained by the two absolute methods used in this study, but also these values are in close correspondence with the majority of results obtained in other studies. This close correspondence of results, especially in the IF-deficient group, indicates that there was no significant in vivo exchange between the free and the gastric juice-bound B_{12} in this study. The somewhat decreased mean absorption of B_{12}(IF) in PA patients, as compared with mean absorption of B_{12}(IF) in normal subjects, is the result of malab-

sorption of B_{12} in the small intestine secondary to B_{12} deficiency per se (*31*).

Although the simultaneous administration of free and IF-bound B_{12} results in a number of logistical and theoretical advantages in the absolute quantitation of GI absorption as described above, it also affords the opportunity to determine an absorption ratio of IF-bound to free B_{12} in the same biologic specimen. The measurement of this ratio theoretically can be performed on stool, blood, or urine when the Schilling procedure is used and does not require the complete specimen collection that is necessary for quantitative studies.

The B_{12}(IF):B_{12} absorption ratio has been measured in previous studies using the Schilling urinary excretion test after the simultaneous administration of radiolabeled free B_{12} and gastric juice-bound B_{12} (*10–12*). Results of these studies are compared in Table 4 with the absorption ratios determined in plasma in the present study. A close agreement of absorption ratios is noted in all groups with sufficient IF (control, folic acid deficient, and intestinal malabsorption). Although similar in diagnostic specificity, a somewhat higher absorption ratio determined in plasma was noted in the IF-deficient group as compared with results obtained from urine measurements. There are two possibilities that could account for this difference in ratios. The difference could be real, reflecting the relatively limited number of patients studied by each of the investigators, or it could be related to the relatively low counting precision. However, the reasonably good internal agreement between plasma ratios and stool or whole-body counting ratios supports the validity of our higher ratios in the IF-deficient group.

Because collection and handling of the specimens are relatively less difficult with urine than with feces, the Schilling procedure remains the most widely used method. Aside from this obvious advantage, there may be some problems and limitations with the urine method. The problem incurred by incomplete urine collections is somewhat circumvented by the measurement of B_{12}(IF):B_{12} absorption ratio on the urine specimen. The limitation of the Schilling procedure is that the result is an index of absorption and not an absolute measurement of absorption. Furthermore, the necessity of giving a pharmacologic amount of B_{12} as a flushing dose will modify the patient's hematologic picture. While stool specimens are more difficult to obtain than urine, they do result in absolute measurements of absorption and do not require a flushing dose of B_{12}.

TABLE 3. COMPARISON OF ABSOLUTE MEASUREMENTS OF VITAMIN B_{12} ABSORPTION

		% of dose absorbed							
		Control		IF deficient		Folate deficient megaloblastic anemia		Small bowel malabsorption	
Author	Method	B_{12}	B_{12}(IF)	B_{12}	B_{12}(IF)	B_{12}	B_{12}(IF)	B_{12}	B_{12}(IF)
Turnbull (23)	Stool	69	—	12	—	—	—	—	—
		(53–86)	—	(0–26)	—	—	—	—	—
Arias, et al (24)	Hepatic	52	—	5	56	—	—	—	—
		(40–69)	—	(0–19)	(28–78)	—	—	—	—
Halsted, et al (25)	Stool	66	—	9	—	—	—	—	—
		(43–95)	—	(0–22)	—	—	—	—	—
Krevans, et al (26)	Stool	71	—	15	—	—	—	—	—
		(50–90)	—	(9–30)	—	—	—	—	—
Mollin, et al (27)	Stool	63	—	12	50	—	—	13	13
		(20–90)	—	(0–35)	(25–85)	—	—	(0–25)	(0–28)
Reizenstein, et al (8)	WBC	61	40	3	15	—	—	12	4
		(38–80)	(27–53)	(0–9)	(4–31)	—	—	(10–14)	(2–7)
Bozian, et al (28)	WBC	70	—	3	—	—	—	—	—
		(45–80)	—	(0–17)	—	—	—	—	—
Weisberg, et al (29)	Hepatic	72	72	4	50	—	—	—	—
		(45–99)	(50–90)	(0–11)	(30–69)	—	—	—	—
Finlayson, et al (30)	WBC	—	—	—	—	—	—	—	—
		(31–80)	—	(0–32)	—	—	—	—	—
Cottrall, et al (9)	WBC	49	—	10	44	32	47	16	—
		(30–80)	—	(0–22)	(27–56)	(13–50)	—	(1–28)	(0–33)
Fish, et al (present study)	WBC	52	64	12	42	58	69	6	8
		(35–68)	(57–70)	(4–19)	(22–59)	(43–62)	(55–85)	—	—
Fish, et al (present study)	Stool	57	66	7	34	53	67	0	0
		(31–78)	(43–86)	(0–18)	(16–61)	(40–71)	(61–90)	—	—

* Mean (range).

TABLE 4. COMPARATIVE MEASUREMENTS OF B_{12}(IF):B_{12} ABSORPTION RATIO

Author	Method	Control	IF deficient	Folate deficient megaloblastic anemia	Small bowel B_{12} malabsorption
Katz, et al (10)	Urine	1.0 (0.8–1.2)	2.6 (1.6–3.6)	—	1.0 (0.7–1.2)
Bell, et al (11)	Urine	— (0.8–1.5)	— (2.0–6.0)	—	—
Bell, et al (12)	Urine	1.2 (0.8–1.8)	3.7 (1.7–9.6)	—	—
Fish, et al (present study)	Plasma	1.1 (0.8–1.4)	7.8 (1.5–31.0)	1.0 (0.7–1.3)	0.6 —

The use of the nonabsorbable marker precludes the necessity of a complete stool collection and homogenization—two other features that have made stool methods difficult or less desirable. The use of the nonabsorbable marker also allows one to monitor accurately the adequacy of the collection. The decision as to whether to use the Schilling procedure or the incomplete stool collection method should depend upon the needs of the institution and facilities of the laboratory. In any case, the plasma B_{12}(IF): free B_{12} absorption ratio should serve as a valuable adjunct to quantitative absorption studies. The ratio can be determined relatively rapidly; it can serve as a control to the quantitative method; it can aid in the interpretation of borderline quantitative results; and it can furnish diagnostic information regarding possible intrinsic factor deficiency when specimen collection is inadequate.

ACKNOWLEDGMENT

These studies were carried out in part at the Clinical Study Center FR 83, provided by the Division of Research Facilities and Resources, USPHS. We wish to thank the Amersham/Searle Corporation, for their cooperation, and for making the Dicopac Test Kit for Vitamin B_{12} absorption available to us for this study.

REFERENCES

1. MOLLIN DL, WATERS AH: *The Study of Vitamin B_{12} Absorption Using Labeled Cobalamins.* Amersham, England, Radiochemical Centre, 1968

2. SCHILLING RF: Intrinsic factor studies II. The effect of gastric juice in the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12}. *J Lab Clin Med* 42: 860–866, 1953

3. GLASS GBJ, BOYD LJ, GELLIN GA, et al: Uptake of radioactive vitamin B_{12} by the liver in humans: Test for measurement of intestinal absorption of vitamin B_{12} and intrinsic factor activity. *Arch Biochem* 51: 251–257, 1954

4. POLLYCOVE M, Apt L: Absorption, elimination and excretion of orally administered vitamin B_{12} in normal subjects and in patients with pernicious anemia. *New Eng J Med* 255: 207–212, 1956

5. NELP WB, MCAFEE JG, WAGNER, HN: Single measurement of plasma radioactive vitamin B_{12} as a test for pernicious anemia. *J Lab Clin Med* 61: 158–165, 1963

6. MCINTYRE PA, WAGNER HN: Comparison of the urinary excretion and 8 hour plasma tests for vitamin B_{12} absorption. *J Lab Clin Med* 68: 966–971, 1966

7. COINER D, WALSH JR: Comparison of Schilling test and plasma level of cyanocobalamin Co^{57}. *JAMA* 215: 1642–1654, 1971

8. REIZENSTEIN PG, CRONKITE EP, COHN SA: Measurement of absorption of vitamin B_{12} by whole body gamma spectrometry. *Blood* 18: 95–101, 1961

9. COTTRALL MF, WELLS DG, TROTT NG, et al: Radioactive vitamin B_{12} absorption studies: Comparison of the whole-body retention, urinary excretion, and eight-hour plasma levels of radioactive vitamin B_{12}. *Blood* 38: 604–613, 1971

10. KATZ JH, DIMASE J, DONALDSON RM: Simultaneous administration of gastric juice-bound and free radioactive cyanocobalamin: Rapid procedure for differentiating between intrinsic factor deficiency and other causes of vitamin B_{12} malabsorption. *J Lab Clin Med* 61: 266–271, 1963

11. BELL TK, BRIDGES JM, NELSON MG: Simultaneous free and bound radioactive vitamin B_{12} urinary excretion test. *J Clin Path* 18: 611–613, 1965

12. BELL TK, LEE D: Evaluation of a dual radioisotope urinary excretion test in the diagnosis of pernicious anaemia. *Acta Haemat* 42: 183–187, 1969

13. LAU K, GOTTLIEB C, WASSERMAN L, et al: Measurement of serum vitamin B_{12} level using radioisotope dilution and coated charcoal. *Blood* 26: 202–214, 1965

14. HERBERT V: The assay and nature of folic acid activity in human serum. *J Clin Invest* 40: 81–91, 1961

15. DOSCHERHOLMON A, HAGEN PS: A dual mechanism of vitamin B_{12} plasma absorption. *J Clin Invest* 36: 1551–1557, 1957

16. GREENFIELD MA, KOONTZ RL: A universal function for computing counting performance. *Int J Appl Radiat* 8: 205–210, 1960

17. DEGRAZIA JA, RICH C: Studies of intestinal absorption of Calcium^{45} in man. *Metabolism* 13: 650–660, 1964

18. HAYES RL, CARLTON JE, NELSON B: Lienthanium-140 as a measure of the completeness of stool collections: Demonstration of delayed excretion of iron-59. *J Nucl Med* 5: 200–208, 1964

19. MACDOUGALL, L: Estimation of fat absorption from random stool specimens. Measurement by Zirconium 95 and Iodine 131. *Amer J Dis Child* 108: 139–143, 1964

20. GANATRA RD, SUNDARAM K, DESAI KB, et al: Determination of absorption of vitamin B_{12} by a double isotope tracer technique. *J Nucl Med* 6: 459–464, 1965

21. PEARSON JD: Use of Cr^{51}-labeled haemoglobin and Sc^{47} as inert faecal markers. *Int J Appl Radiat* 17: 13–16, 1966

22. SEIFE B, SHILS ME: The estimation of fat absorption in man with the use of double isotopes $^{131}BaSO_4$ and ^{131}Iodine-triolein. *J Lab Clin Med* 74: 119–128, 1969

23. TURNBULL A: Experiences with labeled vitamin B_{12}. *Proc Roy Soc Med* 47: 424–426, 1954

24. ARIAS IM, APT L, POLLYCOVE M: Absorption of radioactive vitamin B_{12} in nonanemic patients with combined-system disease. *New Eng J Med* 253: 1005–1010, 1955

25. HALSTED JA, LEWIS PM, HVOLBOLL EE, et al: An evaluation of the fecal recovery method for determining intestinal absorption of $cobalt^{60}$ labeled vitamin B_{12}. *J Lab Clin Med* 48: 92–101, 1956

26. KREVANS JR, CONLEY CL, SACHS MV: Radioactive tracer tests for recognition and identification of vitamin B_{12} deficiency states. *J Chronic Dis* 3: 234–252, 1956

27. MOLLIN DL, BOOTH CC, BAKER SJ: The absorption of vitamin B_{12} in control subjects, in Addisonian pernicious anemia, and in the malabsorption syndrome. *Brit J Haemat* 3: 412–428, 1957

28. BOZIAN RC, FERGUSON JL, HEYSSEL RM, et al: Evidence concerning the human requirement for vitamin B_{12}: Use of the whole body counter for determination of absorption of vitamin B_{12}. *Amer J Clin Nutrit* 12: 117–129, 1963

29. WEISBERG H, GLASS GBJ: A rapid quantitative method for measuring intestinal absorption of vitamin B_{12} in man using a double label hepatic uptake test. *J Lab Clin Med* 68: 163–172, 1966

30. FINLAYSON ND, SHEARMAN DJC, SIMPSON JD, et al: Determination of vitamin B_{12} absorption by a simple whole body counter. *J Clin Path* 21: 595–598, 1968

31. HERBERT V: Transient (reversible) malabsorption of vitamin B_{12}. *Brit J Haemat* 17: 213–219, 1969