Schilling Evaluation of Pernicious Anemia: Current Status

Lionel S. Zuckier and L. Rao Chervu

Albert Einstein College of Medicine, Bronx, New York

The Schilling examination remains a popular means of evaluating in vivo absorption of vitamin B_{12}. When absorption is abnormally low, the test may be repeated with addition of exogenous intrinsic factor (IF) in order to correct the IF deficiency that characterizes pernicious anemia. A dual-isotope variation provides a means of performing both stages of the test simultaneously, thereby speeding up the test and reducing dependence on complete urine collection. The dual-tracer test depends on no exchange of B_{12} moieties on the IF molecule. In vitro studies suggest that this exchange does take place, in a manner dependent on time, temperature, and pH. Furthermore, in vivo studies indicate that, when administered simultaneously, the absorption of unbound B_{12} is elevated, and IF-bound B_{12} is reduced, in pernicious-anemia patients, relative to the classic two-stage examination. A number of clinical studies indicate significant difficulty in resolving clinical diagnoses with the dual-tracer test. The potential weaknesses of the test discussed herein can be overcome by temporally separating the administration of the two B_{12} doses and by treating secondary malabsorption where it exists. An algorithm is offered for selecting the most suitable variation of the Schilling test to improve the accuracy of test results and the ease of performance.

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The Schilling examination, originally introduced in 1953 (*1*), remains the mainstay of in vivo evaluation of vitamin B_{12} absorption. The test, as conceived, involves the ingestion of a physiologic quantity of radiolabeled vitamin B_{12}, followed by administration of a pharmacologic parenteral flushing dose and quantification of subsequent urinary B_{12} excretion. When absorption of the test dose is abnormally low, the examination is repeated with administration of exogenous intrinsic factor (IF) in an attempt to correct the IF deficiency that characterizes pernicious anemia (PA) and other IF-deficiency states (e.g., after gastrectomy). An interval of 3–7 days before repeat administration is advocated in order to avoid interference by enterohepatic circulation of the first flushing dose.

While measurements other than urinary excretion have been introduced as alternative means of measuring vitamin B_{12} absorption, they all lack the precision and ease of administration of the Schilling test (Table 1). Nonetheless, difficulties exist in the performance of the Schilling examination, including critical dependence on complete urine collection and an extended period before completion of the two stages of the test. For the outpatient, the test requires multiple visits to the nuclear medicine department, and is often handicapped by poor compliance and an incomplete study.

In order to circumvent many of these problems, Katz et al. (*11*) in 1963 introduced a dual-tracer method using vitamin B_{12} labeled with Co-57 and Co-60, one administered free and the other bound as an IF-saturated complex. Quantification was performed much as in single-tracer tests, but the amount of each form of vitamin B_{12} was separately assessed and the ratio of IF-bound to free vitamin excretion calculated. This allowed simultaneous measurement of B_{12} absorption with and

For reprints contact L. Rao Chervu, PhD, Dept. of Nuclear Medicine, Albert Einstein College of Medicine, Bronx, NY 10461.

TABLE 1. TESTS FOR EVALUATION OF B_{12} ABSORPTION

	Hepatic† uptake	Stool‡ counting	Serum§ counting	Whole¶ body	Schilling** test
Duration* (days)	7	7	<1	7	1
Quantitative	no	yes	no	yes	no
Accuracy	++	+++	++	+++	+++
Availability	yes	yes	yes	no	yes
Sample handling	none	unpleasant	acceptable	none	acceptable

* Duration of single-stage of the test.
† Ref. *2,3.*
‡ Ref. *4,5.*
§ Ref. *6,7.*
¶ Ref. *8.*
** Ref. *1,9,10.*

without the influence of exogenous IF. Bell modified the test to its present form by substituting Co-58 for Co-60, resulting in a reduced radiation dose (*12*).

The dual-isotope Schilling test (DIST) offers several advantages over the classic single isotope Schilling test (SIST), including shortened study time and equivalence of urine collection for the two stages of the test. Nevertheless, the intricacies of preparing and purifying the vitamin B_{12}-IF complex prevented widespread clinical use before introduction of a commercial version (Table 2) that offered increased convenience, acceptance, and standardization of the technique (*13,14*). The kit currently consists of two capsules of radioactive B_{12}, one containing 0.25 μg of free Co-58-B_{12} (0.8 μCi), and the second containing 0.25 μg of Co-57-B_{12} (0.5 μCi) bound to human gastric juice.

In the DIST, three values are generated: percent excretion of IF-bound B_{12} (bound B_{12}), percent excretion of free B_{12} (free B_{12}) and the bound-to-free (B/F) ratio, which serves as an index of IF deficiency. While different workers have chosen varying criteria to differentiate between normal, PA, and IF-refractory malabsorption, the manufacturer's suggested ranges are given in Table 2. Although theoretically in the DIST the B/F ratio should be independent of renal excretion and completeness of urine collection, these remain desirable test prerequisites, since otherwise IF-mediated improvement in B_{12} absorption may be incompletely expressed. Furthermore, normal patients may have reduced bound and free B_{12} excretion, thus giving an erroneous malabsorption-like picture.

TABLE 2. INTERPRETATION OF DUAL-TRACER SCHILLING RESULTS*

	Normal	Pernicious anemia	Malabsorption
Free vitamin B_{12} excretion(%)	10–40	0–7	<6
Bound vitamin B_{12} excretion (%)	10–42	6–12	<6
Bound-to-free ratio	0.7–1.3	>1.7	0.7–1.3

* Manufacturer's suggested ranges (Amersham Corporation, Arlington Heights, IL 60005).

In addition to the quantification of urinary vitamin B_{12} excretion, the dual-tracer method of evaluating bound and free B_{12} absorption has also found application in whole-body counting, in plasma absorption tests, and stool analyses (*8,15–17*). The DIST remains the most convenient, reliable, and popular of all these test variations. An 8-hr serum sample is sometimes useful in conjunction with urine collection for clarification of cases with suspicion of incomplete urine collection or those with renal insufficiency (*16*). The present discussion focuses on use of DIST in evaluation of PA.

Our experience using the DIST (Fig. 1), along with that of others (*18*), has led us to conclude that there is a significant incidence of nondiagnostic test values leading to indeterminate diagnoses. While the results from some of these cases lie between the classic malabsorption and normal regions of the graph (B/F ratios approximating unity), these patients may be exhibiting minor degrees of malabsorption or have had incomplete urine collection. In other instances nondiagnostic values with elevated B/F ratios may reflect a physiologic "gray area" of gradation from normal to clearly deficient IF secretion (*5,19–22*) or may in fact represent spurious test results owing to experimental difficulties (vide infra).

THEORETICAL BACKGROUND AND IN VITRO VALIDATON

The inherent assumptions of the DIST are (a) that one of the isotopically labeled B_{12}s is administered completely bound to IF without presence of free vitamin, and

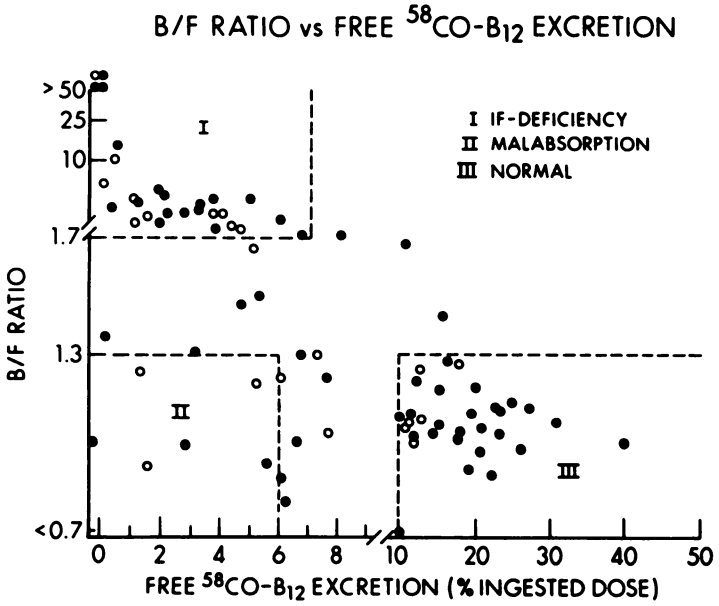

FIG. 1. Distribution of dual-isotope Schilling test results in 58 patients with 24-hr urine volume >750 cc (closed circles) and 23 patients with urine volume <750 cc (open circles) obtained over last 5 years at our institution. Numbered regions refer to manufacturer's suggested ranges. Large number (n = 16) of indeterminate results occur with this method.

(b) that, once ingested, there is no significant exchange between the bound and free forms of vitamin B_{12} (*23*). The first requirement is met by combining a given amount of IF with a surplus of B_{12}, followed by dialysis of the complex to remove any remaining unbound vitamin (*11*,*12*). The validity of the second assumption was established by studies on the relationship of IF-B_{12} binding to efficacy of IF in promoting absorption (Table 3). The relative absorption of IF-bound and free B_{12} was assessed in postgastrectomy and PA patients lacking endogenous IF, by comparing absorption of bound radioactive B_{12}, administered with an equal amount of free cold B_{12}, with the absorption of free radioactive B_{12} given with bound cold B_{12}. It was shown that B_{12} bound to gastric juice was preferentially absorbed over the unbound vitamin, which suggested that exchange between bound and free vitamin B_{12} was insignificant (*24–26*). Intrinsic factor does not bind dietary B_{12} in the stomach at acid pH, but rather this binding occurs after both moieties transit into the more alkaline small bowel.

More direct and quantitative studies have since been performed to determine the physicochemical affinity between B_{12} and IF (Table 3), with varying results. IF-bound Co-60 B_{12} was not significantly displaced by incubation for 1 hr with a fiftyfold excess of cold B_{12} at room temperature (*27*). At 4°C, less than 10% of the labeled vitamin was displaced by unlabeled B_{12} (20% of the maximally possible exchange) in 24 hrs (*29*), and at 37°C exchange was negligible (*30*). In 1963, Donaldson et al. did describe a time- and temperature-dependent

TABLE 3. PRELIMINARY AND IN VITRO STUDIES OF THE DUAL-TRACER SCHILLING TEST

Preliminary studies

Bishop (*24*) (1955)—Preferential absorption of B† to F‡ in a PG§ pt

Schilling (*25*) (1957)—Preferential absorption of B to F in a PG pt

Toporek (*26*) (1960)—Preferential absorption of B to F in 7 PG and 5 PA* pts

In vitro studies

Bunge (*27*) (1957)—B only slightly displaced by 50x excess of F

Donaldsopn (*28*) (1963)—Time- and temperature-dependent exchange of B_{12} onto IF

Highley (*29*) (1962)—<10% exchange of B and F at 4°C over 24 hr

Highley (*30*) (1964)—Negligible exchange of B and F at 37°C

Knudson (*16*) (1974)—High dissociation constant for IF-B_{12} complex

Fairbanks (*23*) (1983)—B_{12} exchange greater at low pH

* PA—Pernicious anemia.

† B—IF-Bound vitamin B_{12}.

‡ F—Free (unbound) vitamin B_{12}.

§ PG—Postgastrectomy.

B/F—Ratio of bound-to-free vitamin B_{12}.

TABLE 4. IN VIVO STUDIES OF EFFECTIVENESS OF THE DUAL-TRACER SCHILLING TEST

Group studies: comparison of DIST to SIST in comparable PA* pt groups
Bayly (*13*) (1971)—BX in the DIST† 70% of SIST** values. FX ↑
Briedis (*32*) (1973)—↓ BX‡ and ↑ FX†† with the DIST
Knudson (*16*) (1974)—↓ in B/F ratio with the DIST
Individual pt studies: comparison of DIST to SIST in the same individuals
Donaldson (*28*) (1963)—↓ BX and ↑ FX in 6 PA pts§ and 2 PG¶ rats
Briedis (*32*) (1973)—↓ BX and ↑ FX in 10/11 PA pts
Clinical correlation studies: comparison of DIST results with known pt diagnoses
Katz (*11*) (1963)—No overlap of FX or B/F ratios between 20 controls and 10 PA pts
Bell (*12*) (1965)—No overlap of FX or B/F ratios between 16 control, 4 PG & 13 PA pts
Bell (*31*) (1969)—1/38 PA pts with normal-range FX; 1 with normal B/F ratio
Bayly (*13*) (1971)—No overlap of FX or B/F ratios in early use of commercial kit
Payne (*14*) (1972)—Moderate overlap of FX; no intergroup overlap of B/F ratios in 40 pts
Briedis (*32*) (1973)—No overlap of FX or B/F ratios between 37 PA and 74 non-PA subjects
Knudson (*16*) (1974)—Some overlap of FX and B/F ratios between 26 control & 13 PA subjects
Pathy (*33*) (1979)—Mild intergroup overlap of FX & B/F ratios in 246 elderly with ↓ B_{12}
Domstad (*19*) (1981)—98% specificity and 83% sensitivity for diagnosing PA in 65 tests
Fairbanks (*23*) (1983)—High (46%) level of misdiagnosis in 28 PA patients

* PA—Pernicious anemia.
† DIST—Dual-isotope Schilling test.
‡ BX—IF-Bound vit. B_{12} excretion.
§ pt—Patient.
¶ PG—post-gastrectomy.
** SIST—Single-tracer Schilling test.
†† FX—Free (unbound) vit. B_{12} excretion.
B/F—Ratio of bound-to-free vit. B_{12}.

exchange of Co-60 B_{12} with Co-57 B_{12} on gastric juice (*28*). When free and bound vitamin B_{12} were mixed in equal amounts at 37°C and pH 6.8 to 7.0, 40% of the theoretically possible exchange occurred within 2 hr. When radioB_{12}, bound to gastric juice, was incubated with cobalt sulfate for 24 hr, the amount of bound activity did not decrease, indicating that the observed exchange between free and bound B_{12} involved the entire B_{12} moiety rather than the cobalt atoms. Fairbanks et al. (*23*) showed that exchange of vitamin B_{12} is dependent on the pH of the medium: in simulated acid gastric juice, Co-57 B_{12} equilibrated completely with IF-Co-58 B_{12} within 10 min, whereas in simulated intestinal or achlorhydric gastric juice (0.155% NaCl), exchange was only 64% or 38% complete, respectively, after 90 min. The dissociation constant for the IF-B_{12} complex at pH 7 and 25°C is reported as 0.005/sec; dissociation of the complex and subsequent exchange of the forms of B_{12} is anticipated under these conditions (*16*). In PA patients with achlorohydria, as well as in post-gastrectomy patients (groups that we tend to study for IF deficiency), higher gastrointestinal pH would tend to minimize the confounding effects of B_{12} exchange. We may now retrospectivelly understand why early studies performed in these groups did not suggest this phenomenon.

IN VIVO STUDIES

The clinical efficacy of the DIST method has not been thoroughly documented (*19*). Several existing studies pertain to overlapping patient groups (*12,13,31*). We have classified the published in vivo evaluations of the DIST into three subgroups (Table 4): (a) group studies where DIST results in a series of PA patients were statistically compared with classic SIST results in a comparable patient series; (b) individual patient studies comparing the results of the DIST with the SIST in individual patients; and (c) clinical studies where the DIST results were evaluated against independently determined clinical diagnoses.

Group studies. The ranges of bound and free vitamin B_{12} excretion by the SIST and DIST were studied in similar groups of patients. In the DIST method, the mean free B_{12} excretion was significantly higher, and the IF-bound B_{12} excretion conversely lower, than in the classic SIST method (*13,16,32*). Knudson and Hippe (*16*), using a double dose of the commercial test in 13 patients with PA, reported that 8-hr plasma levels of free B_{12} were also higher, and bound B_{12} levels lower, than those reported for the 2-stage test.

While lowering of the B/F vitamin B_{12} absorption ratio was statistically demonstrated in patients with PA,

it was not tested whether this decrease causes overlap with normal patient values. Comparison of test results in different and potentially nonequivalent populations is another limitation of these studies. Since as the comparison groups were either a previous cohort of patients (*32*) or a group described by other investigators (*16*), there is no assurance that variation in patient variables and experimental design did not cause the results noted.

Individual patient studies. A limited number of patients were studied by both the SIST and the DIST methods at an expense of increased cost, time and patient radiation dose (*19*). Donaldson and Katz (*28*) evaluated fecal excretion in two gastrectomized rats and urinary B_{12} excretion in six PA patients. Compared with levels in the SIST, simultaneous administration of the two tracers without prior incubation led to an increased absorption of free B_{12} and a corresponding decrease in the absorption of B_{12} bound to gastric juice. This phenomenon was enhanced by incubation of free and bound B_{12} at pH 6.8–7.0 at 37°C for 18–24 hr before administration. Using the DIST, Briedis et al. (*32*) showed relative elevation of free B_{12} levels, and depression of bound B_{12} absorption, in 10 out of 11 PA patients. The absorption of free Co-58-B_{12} alone was reassessed in an additional five patients following the initial DIST, and it dropped from a mean of 5% to only 2.3%. Since these two series are not randomized with regard to order of test performance, it is possible that prior administration of the SIST may have biased the subsequent DIST. Furthermore, as in the group studies, the diagnostic impact of these findings was not measured.

Clinical correlation studies. Accuracy of the DIST was assessed against independently confirmed clinical diagnoses. The ability of the dual-tracer test to resolve diagnoses represents the most relevant measure of its usefulness. Initially (*11–13,32*) it was thought that there would be a clear-cut separation of free-B_{12} excretion levels between normal patients and those with malabsorption (including PA), but subsequent investigators found this parameter unreliable due to intergroup overlap (*14,16,23,31,33*). The most useful value generated by the test was seen to be the B/F absorption ratio, which, in the setting of a decreased free B_{12} absorption, is an indicator for the presence of PA. While initial evaluation of this ratio was promising, with absent (*11–14,32*) or only occasional (*16,31,33*) overlap between clinical groups, greater overlap between controls and PA patients has since been described (*18,23*).

Several studies appear to use retroactively assigned cutoff values designed to optimize discimination between diagnostic groups (*14,16,32*). Other authors have directly evaluated readjustment of the cutoff levels in improving the test accuracy. Pathy et al. (*33*) were unable to demonstrate a sharp delineation between 71 PA and 175 other elderly subjects with low B_{12} levels. Using the manufacturer's suggested B/F cutoff ratio of 2.0, they found that 34% of the PA patients had B/F ratios below this value. By revising the cutoff to 1.4 they found only one false-negative and two borderline results. Of the 175 non-PA cases, nine (5%) gave false-positive results, but four of these had a free Co-58 excretion level high enough to make misdiagnosis unlikely. The fifth case represented an extreme situation of low Co-57 and Co-58 levels, possibly due to malabsorption, where the counting statistics did not allow determination of a valid ratio. Fairbanks et al. (*18*) described their experience with a large number of indeterminate examinations in 388 consecutive DISTs. While increasing the range of free-B_{12} excretion considered normal decreased the incidence of false positives, any potential advantage was lost in an increased false-negative rate.

As in the single-tracer test (*34–37*), 8-hr serum measurements of B_{12} absorption are shown to be inferior to 24-hr urine collections, with increased intergroup overlap of serum-free B_{12} levels and B/F ratios (*15,16,32*). Fish et al. (*17*) compared absorption of simultaneously administered Co-57 B_{12}-IF, free Co-58 B_{12}, and Co-60 microspheres by incomplete stool sampling, whole-body measurement, and 6–8 hr plasma values. The Co-60 microspheres (a nonabsorbable marker in the stool) served as an internal standard enabling calculation of absolute absorption with partial stool collection. Between PA patients and controls there was no overlap of the absorption ratios generated by either serum or whole-body counting methods. Normals and patients with PA had slightly overlapping B/F vitamin B_{12} absorption ratios when based on partial stool collection.

Two studies of dual-tracer B_{12} absorption using whole-body counting methods have been performed with a 1-hr delay between the administration of the B_{12} tracers, during which the patients drank water to aid in separating the B_{12} doses. No overlap of free B_{12} absorption (*8,38*), nor of B/F ratios (*38*), between clinical groups was reported, though the B/F ratios were found lower than in two-stage tests. A short pause between administration of the forms of vitamin B_{12} has been applied in urinary excretion tests as well. A 1-hr interval between B_{12} doses was used in an early study by McCurdy (*39*), with clear differentiation between PA patients and other subjects. Breidis et al. (*32*) repeated urinary dual-tracer studies in five patients, with a 2-hr interval between administration of the two labeled B_{12} moieties. In four of the five patients, the B/F ratio increased towards values obtained in the classic two-stage test.

DISCUSSION

The in vivo group and individual-patient studies discussed above show reduced B/F ratios in patients with PA using the DIST method compared with the SIST.

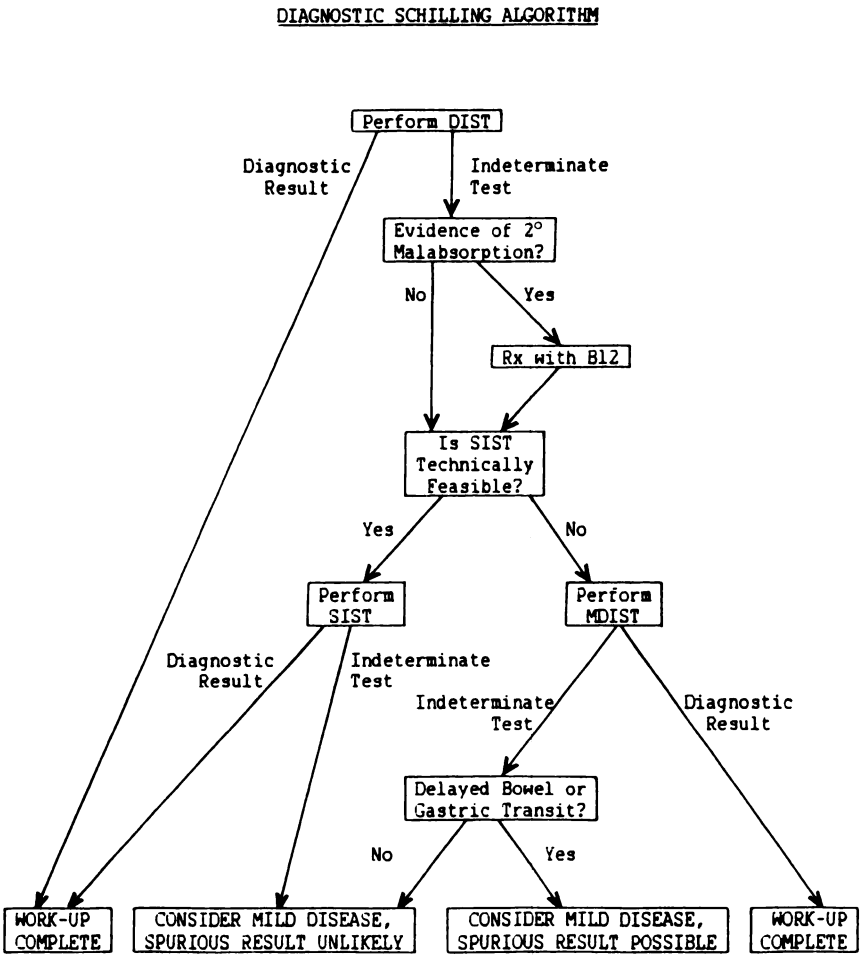

FIG. 2. Diagnostic algorithm for selecting most suitable variation of the Schilling test to retain greatest ease of administration and accuracy of results. MDIST refers to modified dual-isotope Schilling test with 2-hr pause before administration of second form of B_{12}. Algorithm assumes adequate urine collection and renal function.

Several clinical studies demonstrate significant difficulty in resolving diagnoses with this test. While the possibility of a physiologic "gray area" of absorption may be invoked to explain the presence of nondiagnostic test results (*5,19–22*), this does not explain the differences observed between the standard SIST and the DIST methods.

A number of authors cite the phenomenon of lability of the B_{12}-IF bond to explain these findings (*13,16,18,23,28,32*). In vitro data generally support the hypothesis of a time-, temperature-, and pH-dependent exchange of vitamin B_{12} bound to IF (Table 3). Since the majority of cases of elevated B/F ratios are expected in patients with elevated gastrointestinal pH (secondary to achlorohydria or gastrectomy), the incidence of B_{12} exchange would be reduced under these circumstances. Where gastric or intestinal transit are abnormally delayed, one would anticipate an increased incidence of this occurrence (*11,23*).

The presence of secondary malabsorption in patients with PA (*40–43*) could also lead to a reduced absorption of IF-bound B_{12} in the DIST relative to the SIST (*16*). In the standard single-tracer procedure, the flushing dose given in the initial portion of the test may act therapeutically to reverse, partially or totally, any secondary malabsorption that exists before initiation of the second stage of the test (*43*). In the DIST test, the two stages of the test are performed simultaneously, thereby precluding any therapeutic effect of the flushing dose on the absorption of the IF-bound vitamin B_{12}. PA patients with secondary malabsorption may therefore not correct their B_{12} absorption with exogenous IF, and hence might have atypical and nondiagnostic test results. In support of this hypothesis, Knudson and Hippe (*16*) described three patients with PA who had low IF-bound B_{12} absorption levels in the DIST, which increased on repeat testing after B_{12} treatment.

Though the DIST test will on occasion yield a nondiagnostic result, the classic SIST may also lead to uninterpretable or erroneous conclusions. In the latter, one may never be certain that the two halves of this test were carried out under comparable experimental and clinical conditions (*11*), and the incomplete collection of urine may not only lead to uninterpretable and invalidated

results but may simulate malabsorption or PA where it does not occur (*44,45*). In a DIST, even with partial loss of sample, the ratio of IF-bound to free vitamin B_{12} absorption remains preserved, thereby allowing differentiation of PA from normal [diagnosis of IF-independent malabsorption, with depressed B_{12} absorption but a normal B/F ratio, cannot be ascertained without complete and reliable collection of samples (*11,14*)]. Furthermore, decreased patient compliance and an increased incidence of incomplete tests has a detrimental bearing on the SIST performance. These not insignificant factors must be weighed against the decreased sensitivity of the DIST technique.

We have presented an algorithm (Fig. 2) that maintains the advantages of convenience, brevity, and congruity of samples in the DIST while minimizing the possibility of spurious results. The examination need be repeated only once in the event of an initially indeterminate test. Where the initial DIST yields a nondiagnostic result, repeat testing with a greater separation between administration of the two forms of B_{12} is advocated to minimize the possibility of exchange of vitamin on IF. If the SIST is not technically feasible, we suggest providing a 2-hr separation between the B_{12} doses in the manner of Breidis et al. (*32*), who proposed that a short interval between administration of the tracers improves the test resolution. On the other hand, Nickoloff has stated that regardless of which capsule is administered first, the first tracer will be preferentially excreted, perhaps because of the presence of a contaminant, the hydroxycobalamin analog of vitamin B_{12} (*46*). She maintains that the 2-hr capsule separation does not invariably clarify concerns about the simultaneous assay results. While delaying the administration of the second B_{12} will minimize the potential for B_{12} crossover, care must be taken to obtain complete urine collection, as any loss of sample will disproportionately affect the two B_{12} moieties. Where secondary malabsorption is suspected as having caused an indeterminate DIST response, a repeat study should not be performed until after vitamin B_{12} therapy.

A thorough understanding of the dual-tracer test is critial for the maximizing of its unique diagnostic usefulness and for avoiding its potential diagnostic pitfalls.

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Announcement from the Editor's Office

On January 1, 1985, Thomas P. Haynie, M.D. will assume the Editorship of *The Journal of Nuclear Medicine*. To ensure a smooth transition of duties, it is necessary that Dr. Haynie initiate manuscript processing in September, 1984. Beginning September 15, 1984, all manuscripts, original submissions, and revisions, should be forwarded to:

Thomas P. Haynie, M.D.
UT M.D. Anderson Hospital
6723 Bertner Avenue, Box 83
Houston, TX 77030

Frank H. DeLand, M.D.
Editor