Acta Med Scand 202: 419–422, 1977

Vitamin B_{12} Absorption Determined with a Double Isotope Technique Employing Incomplete Stool Collection

Reliability and Validity in Pernicious Anemia

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ABSTRACT. In 19 control patients and 10 patients with pernicious anemia (PA), the vitamin B_{12} (B_{12}) absorption was determined simultaneously with whole-body counting (FRB_{12}) and with a double isotope technique employing incomplete stool collection (FAB_{12}). The test dose was administered orally and consisted of 8 ml of a 10 ml solution containing 0.5 μg of ^{58}Co-B_{12} (approximately 0.4 μCi) and 2 mg of $^{51}CrCl_3$ (approximately 20 μCi). Two ml of the solution were used as standard. In order to follow the passage of the inabsorbable tracer, $^{51}CrCl_3$, the patients were given 25 radiopaque pellets and 4 carmine tablets (2 g) to swallow immediately after the test dose. Counting of a 3–4 ml specimen from one of the first two red stools was sufficient for calculating FAB_{12}. The findings correlated closely with the FRB_{12} values ($r=0.99$, $N=39$, $p<0.001$). In the control subjects, the FAB_{12} averaged 74% (range 37–88). In the patients with PA given intrinsic factor (IF), the FAB_{12} averaged 40% (range 22–59). When IF was not given, FAB_{12} averaged 2% (range 0–9). The test, therefore, is a valid indicator of pernicious anemia. *Reproducibility:* For the mean value of 74%, the standard deviation (S.D.) was 5%, corresponding to a coefficient of variation (CV) of 7%. In the patients not given IF, S.D. was 1% and CV 50%, and in those given IF, S.D. was 8% and CV 20%. This shows the test to be as reliable as whole-body counting. Carmine tablets proved to be a good indicator of isotope-containing stool. The test is easy to perform and requires only a scintillation well-counter. No co-operation on the part of the patients is necessary. The test is also suitable for outpatients. Furthermore, it is independent of kidney function and flushing with B_{12} is avoided.

The methods commonly used for determining vitamin B_{12} (B_{12}) absorption are the Schilling test, the double isotope urinary excretion test (Dicopac®), measurement of the plasma radioactivity, the hepatic uptake test, and whole-body counting. Of these tests, whole-body counting gives the best measure of the absorption of B_{12} (8), but it requires heavy equipment. A new double isotope technique employing incomplete stool collection seems very promising (3, 4, 5, 7). However, the reports give little information on the reliability, i.e. precision and accuracy, of the test. The purpose of this work was to evaluate the test when applied to control subjects and patients with pernicious anemia (PA).

STUDY POPULATION

The study population numbers 10 patients with PA in remission for several months, and 19 control subjects. The examination started not earlier than 14 days after an injection of B_{12}. Neither the controls nor the patients were given parenteral B_{12} during the examination period. Neither group included subjects with disorders of the thyroid gland, liver diseases, leukemia or renal diseases.

METHODS

Radiopharmaceuticals

Patients and controls fasted for 12 hours before and 4 hours after administration of ^{58}Co-B_{12} and $^{51}CrCl_3$. The radiopharmaceuticals were given orally in a preparation containing 0.5 μg of B_{12}, approximately 0.4 μCi ^{58}Co-B_{12}, 2 mg of $CrCl_3$ and approximately 20 μCi $^{51}CrCl_3$ dissolved in

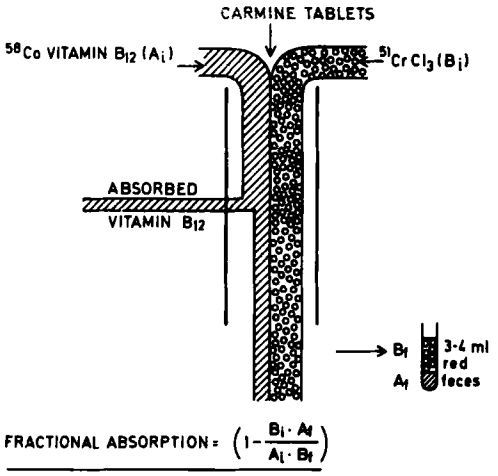

Fig. 1. The principle of the incomplete stool collection method for determining the fractional vitamin B_{12} absorption.

10 ml of deionized water. Two ml of this solution was used as standard. The rest plus 25 radiopaque pellets (5) and 4 carmine tablets containing 0.5 g each were given together with 80 ml deionized water. Intrinsic factor (IF) was given as hog-IF in a dose of 100 mg.

Fractional absorption of B_{12} (FAB_{12})

The principle of the test is shown in Fig. 1. A sample (3–4 ml) of stool is collected, and the amounts of the two isotopes in the sample and in the standards are measured with a well-type sodium iodide crystal connected to a gamma spectrometer using two separate spectrometer windows: ^{51}Cr was counted in the energy interval 0.290–0.350 MeV and ^{58}Co in the interval 0.760–0.860 MeV. Appropriate correction for the Compton scatter from ^{58}Co was made from counting of standards containing only ^{58}Co-B_{12} in the ^{51}Cr window. The stool specimens were not homogenized. It is assumed that $^{51}CrCl_3$ is not absorbed from the gut, that the two isotopes stay mixed during the passage through the gut, that this passage follows that of the intestinal contents, and that none of the absorbed ^{58}Co-B_{12} is excreted with the feces during the period of investigation. The fractional absorption is calculated from

$$FAB_{12} = 1 - \frac{B_i \times A_f}{A_i \times B_f}$$

where B_i and A_i are the amounts of $^{51}CrCl_3$ and ^{58}Co-B_{12}, respectively, given by mouth and B_f and A_f are the respective amounts in the stool specimen.

Fractional retention of B_{12} (FRB_{12})

FRB_{12} was measured with a whole-body counter as described by Hjelt et al. (7, 8) at a time when more than 98% of $^{51}CrCl_3$ and all the radiopaque pellets had been excreted (after 7 days in 27 patients, after 14 days in 2). In this way the presence of unabsorbed ^{58}Co-B_{12} on the last day of measurement was excluded. In the whole-body counter, correction for the Compton scatter from ^{58}Co in the ^{51}Cr window was made by measuring the count rate in the ^{51}Cr window from 3 control subjects and 7 patients with PA given ^{58}Co-B_{12} only.

Table I. *Percentages for fractional absorption of ^{58}Co-B_{12} (FAB_{12}) and for fractional retention of ^{58}Co-B_{12} (FRB_{12})*

	FAB_{12}		FRB_{12}	
	Mean	Range	Mean	Range
Control subjects ($N=19$)	74	37–88	72	39–87
Patients with pernicious anemia ($N=10$)	2	0–9	5	0–10
Patients with pernicious anemia given IF ($N=10$)	40	22–59	40	25–65

Procedure

The precision was calculated from duplicate determinations. The accuracy was assessed by comparing FAB_{12} with FRB_{12}. FAB_{12} was determined in three ways: 1) Counting of a sample of the first red stool in a well-counter. 2) Counting of the complete first red stool with the whole-body counter. 3) Counting of a sample of the second red stool in a well-counter.

Counting statistics

The coefficient of variation (CV) for the FRB_{12} values did not exceed 2%. The CV for FAB_{12} was maximum 5% when counting was performed with a well-counter, and 3% when the whole-body counter was used.

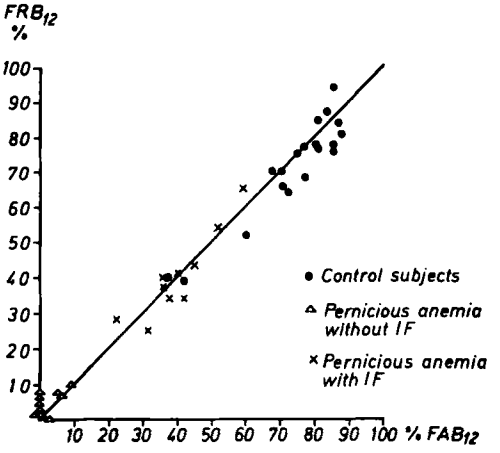

Fig. 2. Correlation of the fractional absorption of vitamin B_{12} (FAB_{12}) and the fractional retention of B_{12} (FRB_{12}) in 19 control subjects and in 10 patients with pernicious anemia given and not given intrinsic factor (*IF*).

RESULTS

The first red stool was passed 10–125 hours (mean 40 hours) after administration of the isotopes. All the specimens of red stools contained enough radioactivity for the results from counting in a well-counter to be within the above mentioned count rate error.

The results are given in Table I. In the controls, FAB_{12} averaged 74% (range 37–88) and FRB_{12} 72% (range 39–87). In the patients with PA given IF, FAB_{12} averaged 40% (range 22–59) and FRB_{12} 40% (range 25–65). When the patients were not given IF, FAB_{12} averaged 2% (range 0–9) and FRB_{12} 5% (range 0–10).

The reproducibility was assessed from double assays: For the mean value of 74%, the standard deviation (S.D.) was 5%, which corresponds to a CV of 7%. In the patients not given IF, S.D. was 1% and CV 50%, and in those given IF, S.D. was 8% and CV 20%. Fig. 2 shows a close correlation between FAB_{12} calculated from a sample of the first red stool and FRB_{12} ($r=0.99$, $N=39$, $p<0.001$). The other two methods of calculating FAB_{12} showed an equally good correlation to the FRB_{12}, the coefficients of correlation being 0.99 in both cases ($N=39$, $p<0.001$).

DISCUSSION

In his first communication about B_{12} absorption measured with a double isotope technique employing incomplete stool collection, Ganatra et al. (5) actually used the complete stool. In a later, short communication (4) it was stated that only "an aliquot of the stool sample is needed". Without giving any exact data, Fish et al. (3) stated that "30% of a complete collection is sufficient for diagnostic purposes", but "only close agreement of the stool and whole-body counter methods was attained when greater than 50% of the stool specimen was collected". We found close agreement between the two methods when no more than a 3–4 ml specimen from one of the first two red stools was used. Counting the complete first red stool in the whole-body counter did not change the correlation. Therefore, a standard scintillation well-counter is sufficient for performing the test.

Ganatra et al. (5) collected two samples of feces, the first 24 hours and the second 48 hours after administration of the test material, while Fish et al. (3) collected stools from day 1 to day 7. We used carmine tablets for colouring the feces. Though counting only a 3–4 ml specimen from one of the first two red stools, we obtained an excellent correlation with the results obtained with the whole-body counting technique. Hinton et al. (6) showed that the transit time for radiopaque pellets and powdered carmine correlated well. We have shown a good correlation between the passage of non-absorbed ^{58}Co-B_{12} and the pellets (8). In agreement with these results, our present investigation suggests that the carmine and the isotopes administered have similar transit times. In 30% of the B_{12} absorption studies, the first red stool arrived more than 72 hours after administration of the test material. In such cases, stools collected before the first 72 hours probably often contain too little radioactivity for counting! The fact that Ganatra et al. (5) had no trouble in collecting samples during the first 48 hours probably reflects a difference between their study population (from India) and our patients (2) with respect to gut transit time. We found that the best results were obtained by counting the first red stool, but that the next one will give almost identical values.

A new absorption study cannot be started until all the non-absorbed radioactive material has been excreted. As shown by Hjelt et al. (8), radiopaque pellets can be used as an indicator of the radioactivity, and an X-ray of the abdomen, therefore, will show whether the excretion of pellets is total.

The test is excellent in outpatients, and it is also very suitable in patients capable of no or minimal co-operation. In both these groups, the Schilling test is invalidated as it demands complete urine collection (1). Furthermore, the method is independent of kidney function, and flushing with B_{12} is avoided, leaving space for further hematological work-up of the patient.

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