Measurement of Absorption of Vitamin B₁₂ by Whole-Body Gamma Spectrometry

By P. C. Reizenstein, Eugene P. Cronkite and S. H. Cohn

Present methods of measuring intestinal absorption of radio-vitamin B₁₂ include measurements of the radioactivity (1) in the feces, (2) over the liver projection, (3) in the serum, or (4) of the fraction of the absorbed radioactivity excreted in the urine after the injection of a large amount of nonlabeled vitamin B₁₂. While all these methods give clinically valuable results, none gives a quantitative measurement of the amount of B₁₂ absorbed except the fecal excretion method, and this is clinically unpleasant, time-consuming and depends upon a complete fecal collection.

Absorption is determined most accurately by direct measurement of the amount remaining in the body after the unabsorbed radioactivity is excreted in the feces. Such a technic is described in this paper. It is more quantitative and simpler than the Schilling test and reduces the amount of radioactivity required per test.

There is an inherent uncertainty in repetition of Schilling-type B₁₂ absorption tests. The influence of the cold B₁₂ “flushing dose” and of the retained radioactivity on a second absorption test is not known. For this reason, to avoid perturbing the steady state by a large flushing dose and to increase the reliability, a direct method of measuring the retention of free vitamin B₁₂ and vitamin B₁₂ bound to intrinsic factor was developed. It avoids repetition of the Schilling test but still differentiates between intrinsic factor deficiency and malabsorption syndromes.

Materials and Methods

Vitamin B₁₂ labeled with either Co⁹⁹ or Cr⁵⁸, was diluted with unlabeled vitamin B₁₂ to a specific activity of 0.2 μC/μg. Absorption tests were performed on 10 patients with irrelevant diseases, serving as comparisons, and on 5 patients with deficient absorption of vitamin B₁₂, either due to intestinal malabsorption or to pernicious anemia.

The fasting patient was given 0.1 μC. (0.5 μg.) of Co⁹⁹B₁₂ orally. For one hour after administration of the first vitamin B₁₂ dose several glasses of water were consumed to assist in flushing the stomach free of radioactivity. Then 0.1 μC. (0.5 μg.) of Cr⁵⁸B₁₂, which had been incubated for one hour with 50 mg. of an intrinsic factor preparation, was given orally.*

In preliminary studies, whole body counts were performed at first every few hours and then daily in order to determine the best time and to minimize the ultimate number of measurements needed. The data is shown in figure 1 and table 2. The final test consists of a background count, a count after administration of a second dose of labeled B₁₂ which is taken as the 100 per cent value, and a third count seven days later to measure the amount of each isotope retained.

*Kindly donated by Dr. Robert Schilling, Madison, Wis.
Fig. 1.—The Brookhaven National Laboratory whole-body gamma spectrometer. At left, the exterior. From left to right, the steel room, the Penco 100-channel analyzer and the printout system. Inserted (upper right) is an enlargement of an oscilloscope gamma spectrum. At right, the interior, showing copper clad walls, a plastic phantom used for in vitro whole body counter studies of the effects of isotope distribution, etc., and, above, the phantom scintillation detector and photomultiplier tubes.

The Brookhaven National Laboratory whole-body counter consists of an 8" x 4" NaI (TI) crystal detector mounted with three 3" photomultiplier tubes. This detector is connected to a linear amplifier and a Penco (Model PA-4) 100-channel pulse-height analyzer (see fig. 1). The crystal detector is located in a shielded room, 6' x 7' x 9', constructed of 6" steel and lined with lead, cadmium and copper.

The patient is counted on an adjustable cot placed in a standard fixed position under the crystal.

Data from the spectrometer are in the form of pulse-height spectra obtained by analysis of the amplified output of the detector. The information plotted is the counting rate per 20 KEV energy interval. The radionuclides used in this study are identified by their distinctive energy spectra, and the number of pulses in a 100 KEV band embracing the photopeak of the predominant energy was taken as the quantitative measure of the amount present. Corrections were made for the appropriate background (predominantly K40 and Cs137 gamma activity) of the patient obtained before the injection of the tracer.

Method of Calculation

Since a double tracer was used in this study it was necessary to utilize the energy resolution capability of the spectrometer, i.e., its ability to distinguish between the γ quanta of the differing energies of Co60 and Co58. It is necessary, when using these two isotopes simultaneously, to resolve the full energy peaks of each isotope. Thus the higher energy Co60 photopeak (1.17 mev) must be corrected for the contribution of the Co58 photopeak, and similarly the count rate of the lower energy photopeak of Co58 (0.81 mev) must be corrected for the Compton continuum contribution of the Co60 photopeak. To do this it is necessary to obtain the calibrated pulse height distribution spectra of each Cobalt isotope individually, and ideally from a patient of the same size and weight. From these spectra, the contribution of each radionuclide to the photopeak of the other can be obtained mathematically, using the following matrix.
Table 1.—Characteristics of Whole Body Counter*

<table>
<thead>
<tr>
<th></th>
<th>Co58</th>
<th>Co60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integral counting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48.2 cm. distance†</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td>Integral counting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM distance †</td>
<td>0.3%</td>
<td></td>
</tr>
<tr>
<td>At predominant energy photopeak, IM distance †</td>
<td>0.053%</td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At predominant energy photopeak, IM distance. (10 minute count.)</td>
<td>2.98 mµc.</td>
<td>3.19 mµc.</td>
</tr>
<tr>
<td>Stability of counting equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variations in count rate with changing body position.†</td>
<td>1.8% of count</td>
<td></td>
</tr>
<tr>
<td>Variations in room background counts.</td>
<td></td>
<td>1.1% of count</td>
</tr>
</tbody>
</table>

*For methods of calculation, see reference 4.  
†Distance between crystal and patient in chair. Efficiency is calculated with isotope in the human body, immediately after administration.  
‡Repositioning, with chair fixed, same person.

\[ a_1, S \quad \text{where: } a_1 \text{ and } a_2 \text{ are, respectively, the observed counting rates for the } Co^{58} \text{ and } Co^{60} \text{ photopeaks. } S = \text{ the contribution fraction of lower, } Co^{58} \text{ energy peak (1.5 per cent) to the higher energy peak of } Co^{60}. \quad C = \frac{a_2}{a_1} \text{ fraction contribution of higher, } Co^{60} \text{ energy photopeak to the } Co^{58} \text{ photopeak (35 per cent).} \]

Thus:

\[ \text{Co}^{60} \text{ activity} = \frac{1 \times a_1 - S \times a_2}{1 \times 1 - C \times a_2} \]

The values for S and C showed slight variations from patients to patient: S between 1 and 2.5 per cent of a1, C between 32 and 38 per cent of a2. In some cases even higher values for S were found due to contamination of the Co58B12 labeled vitamin with Co60. This contamination was corrected for.

**Results**

The amounts of B12 absorbed by the various subjects are tabulated in table 2. The data correspond to absorption measured by other methods. The lowest amount absorbed by a comparison subject was 38 per cent of the ingested dose and the maximum absorbed by a pathologic case was 14 per cent. Thus, in the series to date, there is no overlapping in the absorption data.

Comparison subjects absorb less B12 complexed to intrinsic factor prior to ingestion than they absorb of free B12. This decreased absorption is not due to the prior administration of free B12 since reversal of the order of administration also results in reduced absorption of the bound B12. The diminution in absorption by prior complexing with intrinsic factor is in agreement with previous studies using the fecal excretion test but has not been observed with repeated Schilling tests.
Table 2.—Vitamin B₁₂ Absorption in Controls and in Patients with Deficient B₁₂ Absorption as Measured with the Whole-Body Counter (Means and Range)

<table>
<thead>
<tr>
<th></th>
<th>Number of tests</th>
<th>Absorption of free, Co⁵⁸-B₁₂</th>
<th>% of given dose</th>
<th>Absorption of intrinsic factor bound, Co⁴¹-B₁₂</th>
<th>% of given dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>10</td>
<td>61 (38–80)</td>
<td>40 (27–53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>3</td>
<td>3 (0–8.8)</td>
<td>15.3 (4°–31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal mal-</td>
<td>2</td>
<td>12.3 (10.3–14.4)</td>
<td>4.5 (1.8–7.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Patient had developed resistance to intrinsic factor during long-time oral therapy.

DISCUSSION

The errors inherent in this method are:

1. The statistical error of measurement of radioisotopes.
2. Changing counting geometry due to redistribution of the labeled vitamin B₁₂ within the body.
3. Continuing slow fecal excretion of the administered dose after seven days.

In Table 3 the standard deviation of the final count rate is plotted for 0.1 μc. administered. When 0.1 μc. is given, the statistical error due to the random nature of radioactive decay is acceptable.

One of the difficulties encountered in gamma spectrometry of subjects soon after administration of a dose of vitamin B₁₂ arises from the variation in the distribution of the labeled compound as a function of time. This difficulty is greatest within the first few hours or days following administration of the labeled compound, before it becomes fixed in the organs of ultimate deposition. At these early times, the vitamin B₁₂ may be chiefly in the intestinal tract, in the case of oral ingestion; while in the case of parenteral administration, it may be in the systemic circulation, generally distributed in the soft tissues, or in specific tissues or organs. The particular location of the isotope and its

Table 3.—Errors of the Present Method

<table>
<thead>
<tr>
<th>Nature of error</th>
<th>Magnitude of error</th>
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<tbody>
<tr>
<td>Standard deviation of counts:* at following levels of radioactivity in body</td>
<td>3.0% of final count</td>
</tr>
<tr>
<td>0.1 μc, Co⁵⁸</td>
<td></td>
</tr>
<tr>
<td>0.1 μc, Co⁴¹</td>
<td>3.2% of final count</td>
</tr>
<tr>
<td>0.01 μc, Co⁵⁸</td>
<td>13% of final count</td>
</tr>
<tr>
<td>0.01 μc, Co⁴¹</td>
<td>15.9% of final count</td>
</tr>
<tr>
<td>Variation due to isotope redistribution, average in 6 patients 1–8 hrs. after administration (mean and range)</td>
<td>-4.1 (-11.1 to +4.9) % of initial count</td>
</tr>
<tr>
<td>Variations due to possible loss of radioactivity in 7 patients between day 7 and day 22 after administration (mean and range)</td>
<td>-1.1 (-10 to 0)% of initial count</td>
</tr>
</tbody>
</table>

*Standard deviation due to random nature of disintegrations in 10-minute counts.
Fig. 2.—The pattern of whole-body counts after oral administration of radiovitamin B$_{12}$ to 2 patients. The initial fall and subsequent rise in the count rate is a result of isotope-redistribution in the body. The fall in counts between 1 and 7 days reflects the fecal excretion of the unabsorbed isotope, and the final slope shows the rate of turnover of the absorbed vitamin B$_{12}$. These 2 patients received only 1 isotope (10 µc Co$^{60}$).

changing distribution makes consecutive measurements made in this early period reflect anomalous changes in the counting rates. The apparent rise in activity noted following vitamin B$_{12}$ administration (fig. 2) reflects the altered geometry with respect to the relative position of the isotope concentration and the crystal detector position. This altered geometry of course results in a change in the counting efficiency. For these reasons it was found advantageous to count the patients at a distance of 1 meter even though this decreases the counting efficiency. The customary distance is 48.2 cm. Even at this distance a decrease in the initial counting rate of 4.1 per cent was found during the first few hours, probably corresponding to the passage of the material into the bowel. The ultimate geometry, with most of the radioactivity in the liver, corresponds closely to the initial geometry, when most of the radiovitamin is in the stomach. The general problem of radioisotope redistribution in the body and its effect on counting efficiency and error in whole-body counting is the subject of another study.$^5$

Continuing fecal excretion introduces another error. The rate of B$_{12}$ excretion has been studied after oral ingestion$^1$ and appreciable excretion was generally not apparent more than 7 days after ingestion. In this study, the average error due to excretion after 7 days was 1.1 per cent of the initial count (table 3).
For diagnostic purposes this error is negligible. For research purposes repetitive measurements after 7 days may be desirable.

The reasons for partial inhibition of absorption of B₁₂ by in vitro complexing of B₁₂ with heterologous intrinsic factor is not entirely clear. The failure to detect this with repeated Schilling test could be ascribed to residual activity from prior Schilling tests.*

The major disadvantages of performing B₁₂ absorption tests with the whole-body counter is the expense of the counter and the still relative inaccessibility of these devices for clinical medicine. However, these devices are being rapidly built throughout the United States frequently in association with medical centers and they do have major advantages in performing any absorption study when the substance is labeled by an appropriate gamma emitter. These advantages are:

1. One measures directly the entity in which one is interested—the amount retained in the body.
2. The method avoids the offensive, difficult and tedious collection and analysis of feces, which would require hospitalization.
3. Large flushing doses of parenterally injected B₁₂ are not needed, thus avoiding disturbance of the “steady state” equilibrium and permitting subsequent studies of undisturbed B₁₂ turnover, etc.
4. Renal disease cannot interfere with the results.
5. Uncertainty of repeated tests is avoided.
6. Tracer levels of Cobalt of only 0.1 μC or less are required.
7. The test is sensitive with clearly defined errors of measurement.
8. Distinction between intrinsic factor deficiency and intestinal malabsorption is accomplished without repetition of the test.

This method to measure intestinal absorption is being applied to other substances than vitamin B₁₂.**

**Summary**

A technic is described to measure directly the intestinal absorption of radiovitamin B₁₂ by using a whole-body gamma spectrometer. A double tracer technic is used, and the amounts of free and intrinsic factor bound vitamin B₁₂, respectively, retained in the body after final excretion of unabsorbed radioactivity, are measured. The results are in agreement with those previously obtained by other methods. The present method is simple and quantitative.

The primary advantage of the whole-body counter for measuring absorption of Cobalt-labeled B₁₂ is that it can measure less than 0.1 μC, with a high degree of accuracy. Whole-body counting substitutes a rapid and simple measurement for the difficult and tedious collection and radiochemical analysis of excreta.

**Summario in Interlingua**

Es describite un technica pro measurar directemente le absorption intestinal de radiovitamina B₁₂ con le uso de un spectrometro de radiation gamma ab le

*Similar studies are in progress elsewhere (H. C. Heinrich, personal communication).
VITAMIN B₁₂ ABSORPTION MEASUREMENT

corpore total. Un technica a tracitor duple es usate, e le quantitate de vitamina B₁₂ libere e ligate a factor intrinsec que es retenite in le corpore post le excretion final del non-absorbite radioactivitate es mesurate. Le resultatos se trova de accordo con illos previemente obtenite per altere methodos. Le presente methodo es simple e quantitative.

REFERENCES


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