Radioactive Vitamin B\textsubscript{12} Absorption Studies:
Comparison of the Whole-Body Retention, Urinary Excretion, and Eight-hour Plasma Levels of Radioactive Vitamin B\textsubscript{12}

By M. F. Cottrall, D. G. Wells, N. G. Trott, and N. E. G. Richardson

This paper reports the results of investigations designed to compare the whole-body retention, urinary excretion, and 8-hr plasma level of \textsuperscript{58}Co vitamin B\textsubscript{12} in some 80 absorption tests on 50 subjects. Residual levels of radioactivity in the body were measured using a whole-body counter. A proportional relationship is demonstrated between the 9-day whole-body retention, the 8-hr plasma radioactivity and the 48-hr urinary excretion of vitamin B\textsubscript{12}. The constants of proportionality are determined, enabling the results of absorption tests carried out by different methods to be compared. The mean values and the range of the results obtained are tabulated for subjects in each of four clinical categories. It is shown that approximately one third of the activity absorbed from a 1-\mu g dose is excreted in the urine following a single intramuscular injection of 1 mg nonradioactive vitamin B\textsubscript{12} at 3 hr. The administration of this flushing dose does not significantly alter the activity absorbed as represented by that retained together with that excreted in the urine. The plasma activity at 8 hr is increased by a factor of about 2 over that obtained without the flushing dose. Factors affecting the accuracy of the individual techniques have been studied and the poor reproducibility of successive tests is discussed.

IT WAS DEMONSTRATED by Schilling in 1953 that normal patients given an oral dose of radioactive vitamin B\textsubscript{12}, followed by a flushing dose of intramuscular nonradioactive vitamin B\textsubscript{12}, excreted a proportion of the radioactive vitamin B\textsubscript{12} in the urine.\textsuperscript{1} In patients with pernicious anemia little or no excretion of radioactive material occurred unless a potent source of intrinsic factor was also given with the oral dose. The percentage excretion of an oral dose of radioactive vitamin B\textsubscript{12} has proved a useful tool in the diagnosis of vitamin B\textsubscript{12} malabsorption.\textsuperscript{2}

Other tests of vitamin B\textsubscript{12} absorption, since introduced, include hepatic uptake, whole-body retention, and 8-hr plasma activity.\textsuperscript{3-6} Despite the extensive literature on these topics, the interrelationships between the quantities meas-
ured do not appear to have been fully studied. It was considered that it would be helpful in comparing the results obtained by different workers if the relationship between the results of the various measurements of absorption of vitamin B₁₂ were defined. Determination of the relationship between the whole-body retention at 9 days following a flushing dose of intramuscular vitamin B₁₂ and other parameters would be particularly useful, inasmuch as it would enable anomalous or borderline results of the 24-hr urinary excretion test or 8-hr plasma level to be checked by whole-body counting at 9 days.

The poor reproducibility of successive tests and the relatively large range of normal results have been the subject of some comment. It is pertinent to inquire whether this range is due to variation in the absorption itself, or in other factors that may affect the parameters being used as indices of absorbed radioactive vitamin B₁₂.

The observation by Forshaw and Harwood that a parenteral injection of stable vitamin B₁₂ increases the 8-hr plasma level of absorbed radioactive vitamin B₁₂ raises the question as to whether the total amount of radioactive vitamin absorbed through the gut wall is altered by the flushing dose.

**Materials and Methods**

This study was carried out while providing a routine diagnostic service for a busy general hospital. In addition to the radioactive absorption studies the following investigations were routinely performed to facilitate a definite diagnosis in patients with suspected vitamin B₁₂ malabsorption: routine hematological studies, serum vitamin B₁₂, serum folate, and red blood cell folate. Gastric antibody studies and tests of gastric acidity were also performed in some instances. Fat, glucose, xylose, and lactose absorption tests, together with radiological investigations, were carried out when a primary malabsorptive etiology seemed likely.

On the basis of the tests outlined above, the patients were divided into the following groups (parentheses indicating the number): (a) Normal (20). This group included patients who were initially investigated for suspected vitamin B₁₂ deficiency and who were ultimately proved to have no relevant disease. (b) Pernicious anemia (15). (c) Nutritional folate deficiency (10). (d) Malabsorption (4). (e) Undiagnosed (1).

The results of the absorption studies described below were obtained from more than 80 distinct absorption tests involving separate ⁵⁸Co-vitamin B₁₂ administration in 50 patients.

Absorption studies were carried out as follows:

**Schilling’s Tests**

Patients were kept without food overnight and in the morning given a measured oral dose carefully washed from a sealed ampule containing 1.1 μCi ⁵⁸Co-vitamin B₁₂, with activity approximately 1 μCi ⁵⁸Co, provided by the manufacturers (Radiochemical Centre, Amersham, Code CR1P). A dose of 1 mg nonradioactive vitamin B₁₂ (Cytamen) was given by a single intramuscular injection at 3 hr after the oral dose. During 48 hr, urine in two daily samples was collected and compared with a standard prepared from the administered dose in a ring of 16 Geiger-Müller counters. The calibration factor for this equipment was 180 cps/μCi with a background count rate of 24 cps. Patients were measured in a shielded multicrystal whole-body counter before the dose, about 30 min afterwards, and again on the 9th day. An energy band of 340–860 keV was used for counting, giving an in vivo calibration factor of 59 ± 5 (SD) cps/μCi with an in vivo background count rate of 10.8 ± 0.5 (SD) cps for the group of 50 subjects. The sum of the 48-hr excretion and the 9th-day retention was assumed to represent the proportion of the ⁵⁸Co-vitamin B₁₂ absorbed. The validity of this assumption is discussed later with reference to the results of successive Schilling and whole-body retention tests.
The procedure as described was repeated for patients suspected of having pernicious anemia with the addition of Hog intrinsic factor (1 U.S.N.F. unit) to the oral dose of vitamin B\textsubscript{12}.

**Whole-body Retention Tests**

The test described above was usually repeated after a 2-wk interval without the injection of the flushing dose of nonradioactive vitamin B\textsubscript{12}. Urine was not collected and the fraction of the dose retained at the ninth day measured by whole-body counting was assumed to represent the proportion of the \textsuperscript{58}Co-vitamin B\textsubscript{12} absorbed.

**8-hr Plasma Activity**

The radioactivity present in 4-ml aliquots of plasma from blood drawn at 8 hr after the administration of the dose was measured overnight using a 7.5-cm-diameter \times 7.5-cm-thick well NaI(Tl) crystal detector in a 2-in.-thick lead shield under 10-ft. chalk. The calibration factor using a 340–860-keV energy window was 8500 cps/\(\mu\)Ci with a background count rate of 1.5 cps.

**RESULTS**

**Successive Schilling and Whole-body Retention Tests**

The 14 sets of results available on successive Schilling and whole-body retention tests were considered, irrespective of the clinical category of the subjects; a regression line \(U = (0.34 \pm 0.10) X + 3.9 \pm 4.6\) fitted the observations of 0–48-hr urine activity (\(U\%\)) and the ninth-day whole-body retention (\(X\%\)) in subsequent tests. A significant linear correlation \((r = 0.68, 0.01 > p > 0.002)\) was found to exist between quantities \(X, U\).\textsuperscript{14} When the absorption (\(Y\%\)) obtained by adding the urine activity to that retained in the whole body at 9 days was compared with the retention (\(X\%\)) found during the subsequent retention tests, as shown in Fig. 1, a regression line was obtained with a higher degree of correlation \((r = 0.73, 0.002 > p > 0.001)\). The gradient and intercept of this line were not significantly different from 1 and 0 respectively. These results are compatible with the presumption that the absorption of radioactive vitamin B\textsubscript{12} during the Schilling test, determined by the method described, is equal to the retention determined during the whole body re-

![Fig. 1.—Comparison of successive tests of \textsuperscript{58}Co-vitamin B\textsubscript{12} absorption by (X) whole-body retention measurement at 9 days after administration and (Y) summation of 0–48-hr urinary excretion and retention at 9 days following flushing dose of parenteral vitamin B\textsubscript{12}.](image-url)
RADIOACTIVE VITAMIN B₁₂ ABSORPTION STUDIES

Fig. 2.—Comparison of plasma activities in successive tests of ⁶⁹Co–vitamin B₁₂ (Y) with and (X) without a flushing dose of parenteral vitamin B₁₂.

The results of 8-hr plasma activity measurements, which were available in 11 successive Schilling and whole-body retention tests, were compared, as shown in Fig. 2. The two variables were found to be significantly correlated \((r = 0.83, 0.002 > p > 0.001)\) and the flushing dose of nonradioactive vitamin B₁₂ appeared to elevate the plasma activity by a factor of about 2.

It should be noted that a single flushing dose was used for the Schilling test in these studies. The urinary activity would have been increased and that left in the body decreased if a second flushing dose of nonradioactive vitamin B₁₂ had been given after 24 hr, as has sometimes been recommended.¹⁵,¹⁶

Successive Schilling Tests

Some results of successive Schilling tests on the same individuals with and without intrinsic factor are given in Table 1. The quantities compared are the 0–48-hr urine activities expressed as fractions of the dose that was absorbed (0–48-hr urine + ninth-day whole-body retention). There is a significant correlation \((r = 0.89, 0.002 > p > 0.001)\) between the two fractions, and the regression line is consistent with the supposition that they are equal. It may be concluded that while the addition of intrinsic factor to the oral dose of vitamin B₁₂ increases the absorption of the vitamin, it does not appreciably alter the fraction of the amount absorbed that is excreted in the urine.

Of the radioactivity absorbed, approximately two thirds is retained and one third is excreted in the urine. However, an examination of Table 1 shows that there is considerable variation in the fraction excreted (0.15–0.49) between subjects. Urine activity is not likely, therefore, to be a reliable measure of the absorption in a single test. The data suggest that the fraction of the absorbed dose excreted is specific for an individual.
Table 1.—Comparison of Fraction of Absorbed Dose (≥3%) Excreted in 0–48-hr Urine in Successive Schilling Tests With and Without Intrinsic Factor

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test 1</th>
<th>Test 2 (with intrinsic factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67/57</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>67/58</td>
<td>0.49</td>
<td>0.48</td>
</tr>
<tr>
<td>68/33</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>68/42</td>
<td>0.35</td>
<td>0.34</td>
</tr>
<tr>
<td>68/45</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>68/50</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>69/13</td>
<td>0.38</td>
<td>0.34</td>
</tr>
<tr>
<td>69/17</td>
<td>0.45</td>
<td>0.35</td>
</tr>
<tr>
<td>69/31</td>
<td>0.40</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.35 ± 0.10</td>
<td>0.35 ± 0.07</td>
</tr>
</tbody>
</table>

Plasma and Urine Activity During the Schilling Tests

The relationship of the 8-hour plasma activity to the absorption in patients undergoing the Schilling test, irrespective of diagnosis, is shown in Fig. 3. The overall correlation coefficient was highly significant ($r = 0.82$, $v = 48$, $p < 0.001$). A significant relationship $Y = (0.061 ± 0.016) X + 0.036 ± 0.094$ ($r = 0.82$, $v = 49$, $p < 0.001$) was similarly found between the plasma (Y%/liter) and the urine activities (X%) in the Schilling test irrespective of diagnosis. Fig. 4 shows a comparison of the urine activity with the absorption in the Schilling test irrespective of diagnosis. The correlation was also highly significant ($r = 0.92$, $v = 56$, $p < 0.001$).

In addition, regression lines were calculated separately for absorption tests with and without the administration of intrinsic factor. The regression coefficients and intercepts obtained were compared and found not to be significantly different for any of the pairs of variables considered above.

The activity appearing in the 24–48-hr urine was usually negligible, but in
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Fig. 4.—Comparison of the 0–48-hr urine activity and the absorption of ⁵⁸Co–vitamin B₁₂ following a flushing dose of parenteral vitamin B₁₂. The absorption is measured by the summation of 0–48-hr urinary excretion and the retention at 9 days.

four subjects it exceeded 0.2% of the administered dose. In one of these the 24–48-hr excretion represented 30% of the total.

Plasma Activity During the Whole-body Retention Test

The relationship of the 8-hr plasma activity to the whole-body retention in the retention test, irrespective of diagnosis, is shown in Fig. 5. The correlation is significant, \( r = 0.64, v = 15, 0.01 > p > 0.002 \).

The gradient of the line \( (0.014 \pm 0.004) \) may be compared with that of Fig. 3 \( (0.027 \pm 0.003) \). These results confirm those obtained using successive absorption tests (Fig. 2) from which it was concluded that the flushing dose of nonradioactive vitamin B₁₂ increases the plasma levels of activity by a factor of about 2. Although every effort was made to ensure that patients were not given injections of nonradioactive vitamin B₁₂ during the course of these retention tests, it is possible that while under treatment from their local general practitioner, in addition to the investigations carried out at the hospital, some patients were, in fact, given injections in ignorance. Evidently, observations in such situations would give rise to erroneously high values of plasma activity and low values for the retention.

Fig. 5.—Comparison of the plasma activity at 8 hr and the whole-body retention at 9 days of ⁵⁸Co–vitamin B₁₂.
Table 2.—Summary of Results of Measurements

<table>
<thead>
<tr>
<th>Quantity Measured</th>
<th>Conditions of Test</th>
<th>Range of Results in Given Diagnostic Category</th>
<th>Mean ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Folate Deficient</td>
</tr>
<tr>
<td>Whole-body activity at 9th day, %</td>
<td>30–66</td>
<td>24–61</td>
<td>3–16</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(7)</td>
<td>(5)</td>
</tr>
<tr>
<td>With flushing dose of vitamin B₁₂</td>
<td>15–51</td>
<td>8–31</td>
<td>0–14</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(6)</td>
<td>(14)</td>
</tr>
<tr>
<td>With intrinsic factor and flushing dose of vitamin B₁₂</td>
<td>–</td>
<td>29</td>
<td>15–39</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(10)</td>
<td>(3)</td>
</tr>
<tr>
<td>Whole-body activity at 9th day + 0–48-hr urine activity, %</td>
<td>30–81</td>
<td>13–50</td>
<td>0–22</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(6)</td>
<td>(14)</td>
</tr>
<tr>
<td>With flushing dose of vitamin B₁₂</td>
<td>13–32</td>
<td>3–19</td>
<td>0–8</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(6)</td>
<td>(14)</td>
</tr>
<tr>
<td>With intrinsic factor and flushing dose of vitamin B₁₂</td>
<td>–</td>
<td>18</td>
<td>12–28</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(10)</td>
<td>(3)</td>
</tr>
<tr>
<td>0–48-hr urine activity, %</td>
<td>0.28–1.45</td>
<td>0.42–0.87</td>
<td>0–0.34</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(5)</td>
<td>(1)</td>
</tr>
<tr>
<td>With flushing dose of vitamin B₁₂</td>
<td>0.78–3.78</td>
<td>0.27–1.35</td>
<td>0.10–0.82</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(6)</td>
<td>(14)</td>
</tr>
<tr>
<td>With intrinsic factor and flushing dose of vitamin B₁₂</td>
<td>–</td>
<td>1.32</td>
<td>0.28–1.30</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(7)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

* Normal distribution assumed in calculations of standard deviations of the observations.

Dependence on Clinical Category

A summary of the results of the measurements for the subjects in the various clinical categories is shown in Table 2. The range of the ninth-day retentions and 48-hr excretions of vitamin B₁₂ for the group of patients with pernicious anemia showed marked differences from those for the normal group. However, there was some overlap between the groups in the results for the 8-hr plasma measurements.

Tests of vitamin B₁₂ absorption in the group of patients with primary malabsorption gave low values for each of the parameters observed. The range of results was such that in some instances it was not possible to differentiate those with a primary malabsorption and those with pernicious anemia.

In the small group of patients presenting with folate deficiency, two showed...
a very low absorption of vitamin B₁₂. In both instances they had suffered from a severe systemic illness prior to the test and had also received vitamin B₁₂ and folic acid therapy. One of them had an almost normal test result prior to the onset of pneumonia.

Corcino et al. (1970) have pointed out that for doses containing less than 2 μg, the absorption of radioactive vitamin B₁₂ is inversely proportional to the amount of carrier vitamin B₁₂ present. In considering the magnitude of the parameters given in Table 2 it should be remembered that a dose of 1.1 μg was administered and that larger values for these parameters may be expected for smaller administered doses.

Accuracy of Measurements

In order to assess the significance of the scatter in the values of the various parameters used as indices of absorption, the statistical counting errors were computed for the mean levels of activity found from the groups of subjects in the normal and pernicious anemia categories. The results have been published elsewhere and show that the statistical error (SD) in the whole-body counting was usually less than 3% even when the retention was only 10%, as in pernicious anemia. Errors of less than 9% were obtained for the corresponding measurements of 3.7% of the dose in the urine and 0.3%/liter in the plasma following a flushing dose of nonradioactive vitamin B₁₂. Errors of this magnitude are somewhat less than the scatter in the observed results.

DISCUSSION

The relationships between the 8-hr plasma level, 0–48-hour excretion and 9-day retention (or absorption) of vitamin B₁₂ are consistent with these parameters being in simple proportion for an individual. It is justifiable, therefore, to consider the use of any of them as a measure of absorption, although the method of choice will be dependent on the facilities available and the cooperation that may be expected from the patient. The 8-hr plasma level has much to recommend it, both in convenience and in providing an early report. However, even when a flushing dose of nonradioactive vitamin B₁₂ is given to enhance the plasma levels by a factor of about 2, and hence improve the statistical counting accuracy, there remains some overlap in the results between those in the pernicious anemia and normal groups. This is seldom a disadvantage if whole-body counting facilities are available, since an equivocal result can subsequently be checked by whole-body counting at 9 days, without further administration of radioactivity. This may be carried out even if a loading dose of vitamin B₁₂ has been given, inasmuch as a linear relationship persists between the whole-body retention at 9 days, the 24-hr urinary excretion of radioactive vitamin B₁₂, and the 8-hr plasma level. The flushing dose does not alter the total amount of vitamin B₁₂ absorbed through the gut wall, but only the percentage excreted.

The poor reproducibility of the results of successive absorption tests supports those of Finlayson and colleagues; they emphasize that absorption is dependent on several variables and that minor changes in vitamin B₁₂ absorption should be interpreted with caution. Herbert (1969) has reviewed the many
different factors that affect the absorption of radioactive vitamin B\textsubscript{12}.\textsuperscript{18}

The constancy of the fraction of the absorbed dose excreted in the urine for an individual indicates that it is the variation in the absorption, rather than variation in the excretion, that is mainly responsible for the poor reproducibility of measurements.

Intrinsic factor was found to alter the absorption of the vitamin in pernicious anemia, but not the relationship between any of the parameters. This result supports the validity of the use of the double isotope technique with \textsuperscript{57}Co-vitamin B\textsubscript{12} bound to human gastric juice and suggests that it may be used in conjunction with plasma, whole-body or urine assays.\textsuperscript{19}

ACKNOWLEDGMENT

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