Total Body Counting in the Assessment of Vitamin B\textsubscript{12} Absorption in Patients with Pernicious Anemia, Achlorhydria Without Pernicious Anemia and in Acid Secretors

By W. J. Irvine, D.R. Cullen, Laura Scarth, J. D. Simpson and S. H. Davies

Atrophic gastritis is a common occurrence in patients with autoimmune thyroid disease.\textsuperscript{1,2} The severity of the atrophic gastritis varies from achlorhydria with malabsorption of vitamin B\textsubscript{12} (Addisonian pernicious anemia) (P.A.), through achlorhydria with adequate vitamin B\textsubscript{12} absorption, to minimal or normal secretion of gastric acid and intrinsic factor. It was therefore considered that the study of thyroid patients supplemented by other patients who are known to have pernicious anemia should provide a good opportunity to determine critically the place of total body counting as a method of measuring vitamin B\textsubscript{12} absorption.

Patients Studied

A total of 124 patients were studied (Table 1). The patients classified as latent P.A. had not received vitamin B\textsubscript{12} or transfusion prior to establishing the diagnosis. The patients with frank and latent P.A. are grouped together unless otherwise stated. The 47 patients with achlorhydria but with normal absorption of vitamin B\textsubscript{12} by the Schilling test were obtained largely by screening the sera of thyroid patients for the presence of gastric parietal cell antibodies, which are a good indicator of the presence of atrophic gastritis,\textsuperscript{2} and then determining the acid secretion in the poststimulation hour in those that were positive. Table 2 shows the primary diagnosis in the 47 patients with achlorhydria but with normal vitamin B\textsubscript{12} absorption. The 47 patients who were capable of secreting gastric acid were obtained in the search for achlorhydric patients, but also included three patients with duodenal ulcer. None of the 124 patients were known to have small intestinal disease.

Methods

Whole body counting has been used for a number of years to measure the absorption of vitamin B\textsubscript{12} by means of very sensitive instruments.\textsuperscript{3,4} The whole body counter used in this study was of the “shadow shield” type\textsuperscript{5} consisting of two detectors one placed above and one below the patient. Each detector consisted of a 12.5-cm.-diameter by 9-cm.-thick sodium iodide crystal with a 12.5 cm. photomultiplier tube and was shielded by 10 cm. of lead.\textsuperscript{6-8} The detectors were suspended with their faces 83 cm. apart, and the patient lay on a couch.
ASSESSMENT OF VITAMIN B₁₂ ABSORPTION

Table 1.—Serum Vitamin B₁₂ Status and Gastric Function in the 124 Patients Studied

<table>
<thead>
<tr>
<th>Diagnostic Criteria</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pernicious Anemia</strong></td>
<td></td>
</tr>
<tr>
<td>Frank</td>
<td>25</td>
</tr>
<tr>
<td>Achlorhydria, megaloblastic marrow, serum vit. B₁₂ &lt; 50 pg./ml., abnormal Schilling test, Schilling test corrected by intrinsic factor and/or gastric intrinsic factor secretion &gt; 200 units in postpentagastrin hour.</td>
<td></td>
</tr>
<tr>
<td>Latent</td>
<td>5</td>
</tr>
<tr>
<td>Same as above but normoblastic marrow and serum vit. B₁₂ 50–170 pg./ml.</td>
<td></td>
</tr>
<tr>
<td><strong>Achlorhydria Without Pernicious Anemia</strong></td>
<td>47</td>
</tr>
<tr>
<td>Serum vit. B₁₂ &gt; 170 pg./ml. and intrinsic factor secretion &gt; 200 units in post-pentagastrin hour and/or normal or equivocal Schilling test.</td>
<td></td>
</tr>
<tr>
<td><strong>Acid Secretors</strong></td>
<td>47</td>
</tr>
<tr>
<td>0.01–35.4 mEq. HCl in gastric juice in postpentagastrin hour, serum vit. B₁₂ &gt; 170 pg./ml.</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.—Primary Diagnosis of the 47 Achlorhydric Patients Without P.A.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated Thyrotoxicosis</td>
<td>16</td>
</tr>
<tr>
<td>Primary Hypothyroidism</td>
<td>12</td>
</tr>
<tr>
<td>Hashimoto's Thyroiditis</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>4</td>
</tr>
<tr>
<td>Addison's Disease</td>
<td>2</td>
</tr>
<tr>
<td>Iron Deficiency Anemia</td>
<td>2</td>
</tr>
<tr>
<td>Post Partum Hypopituitarism</td>
<td>1</td>
</tr>
<tr>
<td>Other Disorders</td>
<td>3</td>
</tr>
</tbody>
</table>

which moved between the detectors over a total distance of 200 cm., the counting time being 12 minutes. A total body count was done before, immediately after and again seven days after an oral dose of 0.5 μGm. ⁵⁸Co-vitamin B₁₂ of specific activity 2 μCi./μGm. given to the overnight fasting patient and the results were expressed as the percentage retention of the isotope within the body at seven days. Patients were asked to ensure that they had regular and adequate bowel movements during this time with the use of aperients if necessary and to keep a record of the results. To allow for isotope decay and any counter instability, all measurements were related to the count rate obtained from a standard source. Twenty-seven patients with P.A., 35 with non-P.A. achlorhydria and 35 acid secretors were studied in this manner.

Vitamin B₁₂ absorption by total body counting was also measured following pentagastrin stimulation in 19 patients with P.A., in 38 non-P.A. achlorhydrias and in 38 acid secretors. The pentagastrin (6 μGm./Kg. body weight) was given 10 minutes before the oral dose of the vitamin, so that the peak secretion of intrinsic factor coincided with the administration of the isotope. Sixteen patients with P.A., 26 with non-P.A. achlorhydria and 26 acid secretors had total body counts done both with and without pentagastrin. The sequence of the tests with or without pentagastrin was random.

Gastric juice was collected from the fasting patient according to the procedure described by Makhlof et al.⁹ Pentagastrin in the same dose as above was used as the gastric stimulant. In this study achlorhydria was defined as pH greater than or equal to 6.0 in all samples before and after gastric stimulation. When the pH was less than 6.0, the acid content of the gastric juice secreted in the poststimulation hour was titrated to pH 7.0 without delay by an automatic titrator (type TTT 1c "Radiometer," Copenhagen) using 1.0 N NaOH. The intrinsic factor content of the gastric juice was assayed by a modification of the methods of Ardeman and Chanarin¹⁰ and Gottlieb et al.¹¹ as described by Irvine et al.⁷ Previous studies in our laboratory have shown that an intrinsic factor secretion in the poststimulation hour
Fig. 1.—$^{58}$Co Vitamin $B_{12}$ absorption measured by total body counting with (+P) and without (-P) prestimulation with pentagastrin. The results on the same patient are joined by a continuous line.

greater than 200 units is not compatible with a diagnosis of P.A. A unit of intrinsic factor is the amount that is capable of binding 1 nGm. vitamin $B_{12}$, this binding being amenable to inhibition by intrinsic factor antibody I.

An oral dose of 0.5 $\mu$Gm. (specific activity 1 $\mu$Ci./$\mu$Gm.) $^{58}$Co-vitamin $B_{12}$ followed by 1000 $\mu$Gm. Cytamen intramuscularly one hour later was used in the Schilling test. A 24-hour urinary excretion of more than 12.5 per cent of the oral dose was taken as normal, 7.5–12.5 per cent as being equivocal and less than 7.5 per cent as indicative of malabsorption of vitamin $B_{12}$. All patients included in the study had adequate renal function. When the Schilling test was repeated with intrinsic factor, an oral dose of at least 1000 units of hog intrinsic factor was used.

The serum vitamin $B_{12}$ level was determined microbiologically by the method of Girdwood.

In the majority of patients, these studies were completed within a period of six months.

RESULTS

The percentage retention at seven days of $^{58}$Co-vitamin $B_{12}$ as determined by total body counting without pentagastrin stimulation in 27 patients with frank or latent P.A., in 35 achlorhydric patients without P.A. and in 35 acid
Table 3.—Intrinsic Factor Content of Gastric Juice Aspirated During the Basal Hour and During the Hour Following Pentagastrin Stimulation in the Patients Included in Table 1

<table>
<thead>
<tr>
<th></th>
<th>P.A. Achlorhydria Without P.A. Acid Secretors</th>
<th>Mean</th>
<th>S.D.</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal hour I.F. secretion (units)</td>
<td>19 ± 25</td>
<td>465 ± 784</td>
<td>2023 ± 2283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poststimulation hour I.F. secretion (units)</td>
<td>18 ± 28</td>
<td>1015 ± 1286</td>
<td>7046 ± 6598</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The percentage retention of vitamin B₁₂ in the P.A. patients was between 0–25 per cent (mean 9.9 per cent SD ± 7.7 per cent), the values in the majority of patients in the non-P.A. achlorhydric and acid secreting groups being well above this. The five patients with latent P.A. (Table 1) had total body counts of 0, 4, 11, 13 and 20 per cent, respectively. The hemoglobin levels in all these five patients were between 13.3–14.6 Gm. per cent and the Schilling tests without intrinsic factor were between 0.4–2.8 per cent. The total body counting method did not distinguish between achlorhydric patients without P.A. and acid secreting patients, the range of ⁵⁸Co-vitamin B₁₂ retention in the respective groups being 22–79 per cent (mean 51.3 per cent SD ± 15.8 per cent) and 24–98 per cent (mean 64.3 per cent SD ± 18.8 per cent).

Table 3 shows that, in general, pentagastrin produces an increase in intrinsic factor secretion in achlorhydric patients without P.A. and in patients capable of secreting gastric acid, but not in patients with P.A. Figure 1 also compares the total body count results with and without prior stimulation with pentagastrin. The range of ⁵⁸Co-vitamin B₁₂ absorption was not altered by the use of pentagastrin in the patients without P.A. or in those with achlorhydria without P.A. In the acid secretors, however, there was an elevation in the lower limit of the retention of the labeled vitamin from 24 to 44 per cent.

There was a significant correlation between the total body count of vitamin B₁₂ absorption without pentagastrin stimulation and intrinsic factor secretion in the basal hour \((r = 0.57)\) and intrinsic factor secretion in the poststimulation hour \((r = 0.68)\) when all three groups of patients were considered together. The best correlation coefficient was between the total body count result for the vitamin B₁₂ retention with prior pentagastrin stimulation and intrinsic factor secretion in the hour following pentagastrin stimulation \((r = 0.76)\) (Fig. 2). It can be seen from Fig. 2 that, while none of the patients with P.A. had an intrinsic factor secretion of greater than 100 units in the poststimulation hour, as many as 10 of the 36 achlorhydric patients without P.A. had an intrinsic factor secretion of less than or equal to 100 units. The volumes of gastric juice in eight out of these 10 achlorhydric patients without P.A. were low and the consistency tenacious making it difficult to be certain of the completeness of gastric aspiration. Three of these 10 patients had equivocal Schilling tests although the serum vitamin B₁₂ levels were normal without previous therapy and before the Schilling tests were done. In spite of the diagnostic difficulties in strictly placing these three patients in their correct categories, it
Fig. 2.—Correlation between total body count (expressed as percentage retention of \(^{58}\text{Co}\) Vitamin \(B_{12}\)) with pentagastrin stimulation and intrinsic factor content of gastric juice aspirated during postpentagastrin hour measured by direct radioimmunoassay in 16 patients with P.A. (x), 36 patients with achlorhydria but without P.A. (○) and 38 acid secretors (▲).

Table 4.—Analysis of Total Body Count (TBC) Results

<table>
<thead>
<tr>
<th></th>
<th>No. Tests</th>
<th>Per cent Retention by TBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(± pentagastrin)</td>
<td>&lt; 21</td>
</tr>
<tr>
<td>P.A.</td>
<td>46 (30)</td>
<td>38 (25)</td>
</tr>
<tr>
<td>Non-P.A. Achlorhydria</td>
<td>73 (47)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Acid Secretors</td>
<td>73 (47)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>192 (124)</td>
<td></td>
</tr>
</tbody>
</table>

( ) indicates number of patients.

would appear that the total body counting method gives a clearer separation of achlorhydric patients with P.A. from those without P.A. than does the direct assay of intrinsic factor in gastric juice.

An analysis of the total count results is shown in Table 4. Taking a percentage retention of less than 21 as being indicative of vitamin \(B_{12}\) malabsorption, of 21–25 as being equivocal and of greater than 25 as being indicative of adequate vitamin \(B_{12}\) absorption, the total body count method agreed with the classification of the patients in 91 per cent of tests (89.5 per cent of patients) and gave an equivocal result in eight per cent of tests (10 per cent
patients) and a misleading result in 0.5 per cent of tests (0.8 per cent of patients).

**Discussion**

This study confirms the results of our preliminary communication\(^7\) that the upper limit of absorption of vitamin B\(_{12}\) in patients with pernicious anemia is 25 per cent. This is in general agreement with other workers\(^{14,15}\), some of whom obtained a slightly higher level of 30 per cent.\(^{16}\) Occasionally even higher values have been reported,\(^8\) the reason for which is obscure. It is of interest that the five patients with latent P.A. had total body counts scattered evenly throughout the range encountered in frank P.A. and were not grouped at the upper limit.

Previous workers have concentrated on the ability of total body counting to distinguish P.A. patients from acid secretors and little attention has been directed towards its ability to distinguish between achlorhydic patients with and without malabsorption of vitamin B\(_{12}\), which should provide a more critical test of a method for determining vitamin B\(_{12}\) absorption. Despite the presence of an equivocal zone between 21–25 per cent retention the method of total body counting, with or without pentagastrin, distinguishes patients with P.A. from those with achlorhydria without P.A. with 87 per cent accuracy and one per cent error and from acid secretors with 91 per cent accuracy and 0.5 per cent error.

On the data available it is not clear whether the use of pentagastrin stimulation improves the findings with the total body counting technic. Pentagastrin stimulation did not improve the separation of achlorhydic patients with and without P.A., but it did raise the lower limit of retention in the acid secretors. It might be anticipated that in normal subjects with equivocal results the total body counting technic should be able to show an increased absorption of vitamin B\(_{12}\) with gastric secretory stimulation in view of the increased secretion of intrinsic factor with gastric stimulation (Table 3).\(^{7,17-20}\) Studies on a larger number of patients may substantiate the impression that pentagastrin stimulation gives a better distinction between acid secretors and patients with P.A. Our findings with pentagastrin are in keeping with those of Finlayson et al.\(^8\) who have shown that improved results can be obtained in acid secreting patients if the labeled vitamin is given along with a vitamin B\(_{12}\)-free meal. Optimal results would possibly be obtained by using both pentagastrin stimulation and administration of the isotope with a vitamin B\(_{12}\)-free meal.

The total body counting method whether performed without or with pentagastrin stimulation is recommended as a method of determining the adequacy of vitamin B\(_{12}\) absorption since it dispenses with the inaccuracies entailed in the urine collections of the conventional Schilling test. It should be particularly useful in the study of the natural history of atrophic gastritis as it does not alter the level of vitamin B\(_{12}\) in the serum. Provided parenteral vitamin B\(_{12}\) has not been given during the previous 48 hours, there is no biologic reason why falsely low results should be obtained. Falsely high results with the total body counting technic may be avoided by the use of aperients. Some workers\(^5,21\) have recounted the patient at 10 days, but it has been shown\(^6\) that even with-
out a purgative only occasional patients have more than one per cent of the ingested dose of radioactivity in the stool on the seventh day. Disadvantages of the total body counting method include the limited availability of equipment in terms of expense and the relatively long delay in obtaining a result.

The correlation between vitamin B₁₂ absorption by total body counting using pentagastrin stimulation and the intrinsic factor secretion in the postpentagastrin hour was limited to 0.76 because of the sampling errors involved in completely aspirating tenacious gastric juice in patients with atrophic gastritis, the limitations in the reproducibility of the total body counting method, and almost certainly because of a nonlinear relationship between intrinsic factor secretion and vitamin B₁₂ absorption above a certain level, even if gastric and intestinal motility could be further controlled.

Patients with abnormal or equivocal vitamin B₁₂ absorption by the total body counting technic require further investigation using direct radioimmunoassay of intrinsic factor content of gastric juice aspirated during the postpentagastrin hour and, if necessary, by other procedures. The diagnostic usefulness of the total body counting technic is likely to be increased by studying vitamin B₁₂ absorption with and without oral intrinsic factor using a double isotope technic in a single procedure. It is suggested that the total body counting method is preferable to the direct radioimmunoassay of gastric intrinsic factor secretion as a screening procedure for vitamin B₁₂ absorption because of the difficulties already mentioned in aspirating completely all of the small volumes of gastric juice that may occur in atrophic gastritis with or without vitamin B₁₂ absorption and also on account of the inconvenience to the patient of gastric aspiration. Ideally, however, both procedures should be done to avoid the possibility of being misled by falsely high results in the total body counting method and falsely low results by the direct radioimmunoassay of intrinsic factor in gastric juice.

Summary

The absorption of ⁵⁷⁷Co-vitamin B₁₂ was assessed by the method of total body counting in 25 patients with frank P.A., five with latent P.A., 47 with achlorhydria but without malabsorption of vitamin B₁₂ and 47 acid-secreting patients. A total of 192 tests was done. With or without prior stimulation of intrinsic factor secretion by pentagastrin the upper level of absorption of vitamin B₁₂ in patients with frank or latent P.A. was 25 per cent. Taking an equivocal result to be 21–25 per cent inclusive, the total body counting method was able to distinguish P.A. patients from those with achlorhydria without P.A. with 87 per cent accuracy and one per cent error and from non-P.A. achlorhydric patients and acid secretors combined with 91 per cent accuracy and 0.5 per cent error.

Pentagastrin stimulation did not improve the separation between achlorhydric patients with and without P.A. but raised the lower limit of retention of vitamin B₁₂ from 24 to 44 per cent in the acid secreting patients.

The method of total body counting is recommended as a method of assessing vitamin B₁₂ absorption.
ACKNOWLEDGMENTS

We are grateful to our physician colleagues for allowing us to study their patients.

REFERENCES

Total Body Counting in the Assessment of Vitamin B₁₂ Absorption in Patients with Pernicious Anemia, Achlorhydria Without Pernicious Anemia and in Acid Secretors

W. J. IRVINE, D. R. CULLEN, LAURA SCARTH, J. D. SIMPSON and S. H. DAVIES