Intestinal Absorption of Radioactive Vitamin B₁₂ : A Comparison of Plasma, Faecal and Urinary Tests

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In order to assess the usefulness of plasma radioactivity measurements in determining the normality or otherwise of vitamin B₁₂ absorption, a comparison has been carried out in 55 individuals between the plasma measurement and the faecal balance on 110 occasions, and between the plasma measurement and the Schilling test on 55 occasions. Plasma measurements provided 5 per cent of false normal results, faecal balance studies 5 per cent of false normal results and the Schilling test 16 per cent of false low results. The measurement of the rise in plasma radioactivity 8 h. after the test dose is an easy test to carry out, demands minimal cooperation from the patient, requires least time of patient follow up and is at least as reliable as the faecal balance performed under ideal conditions. It is, therefore, the best test of ileal function for field population surveys.

Introduction

In studying the prevalence of intestinal disease in tropical communities, simple field tests of intestinal function, which require minimal patient cooperation, are necessary. Since vitamin B₁₂ is specifically absorbed in the ileum (Booth and Mollin, 1959), demonstration of normal absorption provides a useful parameter of normal ileal function. If other factors which may interfere with the absorption of the vitamin such as pernicious anaemia and bacterial overgrowth in the small intestine are excluded, the demonstration of abnormal absorption indicates ileal dysfunction.

Methods for measuring vitamin B₁₂ absorption such as the faecal balance (Heinicke et al., 1952) and urinary excretion (Schilling, 1953) depend on the adequate cooperation of the patient to ensure complete stool or urine collections and are not very satisfactory as field tests. The direct measurement of the amount of radioactivity retained in the body, either by the hepatic uptake technique (Glass, 1954) or by whole body counting (Heinicke, 1961) does not require so much patient cooperation, but does need more sophisticated counting apparatus which is not available in many centres. Furthermore, measurements can only be carried out some four to ten days after giving the test dose when all the unabsorbed vitamin has been excreted. The measurement of the rise in plasma radioactivity 8 h. after giving the oral dose, gives an indication of the amount of radioactivity absorbed (Booth and Mollin, 1956; Doscherholmen and Hagen, 1957) and would be applicable to field studies. This investigation was therefore undertaken.

*Welcome Trust in collaboration with the World Health Organization,*
to compare and contrast the results of the vitamin B_{12} absorption test using plasma radioactivity measurements with the faecal balance technique and the Schilling tests.

Material and Methods

Tests were carried out in 55 subjects: 40 patients with tropical sprue, 9 asymptomatic control subjects and 6 patients with secondary malabsorption. All subjects were admitted to a metabolic ward where every precaution was taken to ensure full collection of all urine and stool specimens.

The vitamin B_{12} absorption was tested twice in each subject at 10 to 14 day intervals. The test was carried out in the early morning after an overnight fast, and food was withheld till at least two hours after the test dose of the vitamin. In each case a one microgram dose of the vitamin labelled with 0.5 µC of Co^{58} was given. Faecal balance studies (Heimle et al., 1952) and plasma radioactivity measurements were carried out on both occasions in each subject. A specimen of blood taken immediately before the dose of radioactive vitamin B_{12}, served as a baseline. A second blood specimen was obtained eight hours after the test dose and the increase in radioactivity was taken as an index of the amount of the vitamin absorbed. At the time of the second test, an injection of 1000 µg. of non-radioactive vitamin B_{12} was given immediately after the oral dose and all urine collected for the subsequent 24 h. (Schilling, 1953). A further collection of urine from 24-48 h. after the test dose was also obtained. An absorption of more than 0.3 µg. in the faecal balance (Mollin et al., 1957) and 7 per cent or more excretion in the first 24 h. in the Schilling test (Schilling, 1955) were taken as indicating normal absorption.

All measurements of radioactivity were carried out in a Packard well scintillation counter with a sodium iodide crystal and pulse height analyser. Five ml. of each plasma specimen was counted in the well and 100 ml aliquots of stool and urine were counted in standardized containers placed on top of the crystal. All counts were carried on to an accuracy of 1 per cent or for a total of 50 min.

Results

Faecal and serum measurements: The results of the faecal balance and plasma measurement in the first test (Graph 1) show a correlation (r = 0.83, P < 0.01). By inspection, if a faecal absorption of more than 0.2 µg. is taken as normal, a plasma level of more than 0.2 per cent of the dose per litre of plasma is the best dividing point between normal and abnormal. By these criteria two subjects had a normal absorption by the stool balance but low absorption by the plasma measurement and three had normal plasma but low stool results.

The results of the faecal balance and plasma measurement in the second test (Graph 2) also show a correlation (r = 0.78, P < 0.01). The mean absorption by the faecal balance is the same for the two tests, but the plasma radioactivity is significantly

*Since the distribution of the values is not Gaussian the Rank correlation coefficient is used here and on the other reported analyses.
Radioactive Vitamin B_{12}

Graph 1
Fecal and plasma measurements in first test.

Graph 2
Fecal and plasma measurements in second test.

An injection of 1000 mg of unlabelled vitamin B_{12} was given immediately after the oral dose. Symbols as in Graph 1.
higher in the second test (Table I) indicating that the simultaneous administration of 1000 μg of unlabelled vitamin B₂ has raised the level of radioactivity in the plasma as compared with the level in the first test by a factor of 1·6. This suggests that the 'normal' in this case should be taken as more than 0·3 per cent per litre of plasma. In this second test there were 3 discordant results in which the plasma measurement gave a low absorption but the stool balance gave a normal absorption.

Table I. Mean, range and standard deviation for plasma and faecal measurements in first and second tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Plasma test % dose/litre plasma</th>
<th>Faecal test μg. absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>Mean</td>
<td>0·41</td>
<td>0·47</td>
</tr>
<tr>
<td>Range</td>
<td>0·1·2</td>
<td>0·2·9</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0·35</td>
<td>0·64</td>
</tr>
</tbody>
</table>

Faecal and urinary measurements: The results of the second faecal balance and the urinary excretion measurement are shown in Graph 3. There is an overall correlation

Graph 3
Urinary and faecal measurements in second test.

Symbols as in Graph 1.
between the two tests ($r=0.77; P<0.01$), but there were 12 subjects who had a low urinary excretion but normal absorption by the faecal balance.

**Serum and urinary measurements**: The results of the second plasma and the urinary excretion measurements (Graph 4) also show a correlation ($r=0.86; P<0.01$) but there were 9 subjects who had normal results by the plasma estimation and an abnormal urinary excretion.

**Graph 4**

*Urinary and plasma measurements in second test.*

Symbols as in Graph 1.
A number of investigators have used the rise in plasma radioactivity as a test of the intestinal absorption of vitamin B₁₂ (Dochsebolmen and Hagen, 1957; Nelp et al., 1963; McCurdy, 1965; Armstrong and Woodliffe, 1966; Coupland, 1966; Forshaw and Harwood, 1966; Workman and Rusche, 1966; Finney and Payne, 1968; Arkus et al., 1969; Donaldson et al., 1970; Coiner and Walsh, 1971). Different workers have used differing doses and various methods of expressing the results of the test. Some have simply given the result as counts per minute per unit of plasma (Dochsebolmen and Hagen, 1957). This is adequate in a given laboratory but is unsatisfactory for interlaboratory comparisons. The methods of expressing results as a percentage of the dose per litre of plasma eliminates variations due to differences in the amount of radioactivity given and of counting efficiencies. The suggested lower limit of normal in this study i.e. more than 0.2 per cent per litre of plasma, when no flushing dose is given, agrees well with the figure proposed by several other investigators (Workman and Rusche, 1966; Forshaw and Harwood, 1966; Coiner and Walsh, 1971).

Comparison of the plasma levels in tests one and two showed a significant rise in the mean plasma radioactivity without change in the mean faecal absorption (Table I). This confirms the findings of other (Coupland, 1966; Harwood and Forshaw, 1967; Armstrong and Woodliffe, 1969) that the administration of a parenteral flushing dose of non-radioactive vitamin B₁₂ raises the plasma levels of radioactivity and the lower limit of normality must therefore be higher. The flushing dose however did not significantly increase the sensitivity of the test.

Considering the faecal and plasma measurements in the first and second tests there was agreement as to the normality or otherwise of vitamin B₁₂ absorption in 102 instances and discordant results in 8 instances—in 5 the plasma measurements gave a low result and the stools a normal one and in 3 the plasma measurement gave a normal result and the stools a low one (Graphs I and 2). Without a whole body counter it is impossible to decide which of the two results give a better measure of absorption. It should be noted however that any error in stool collection will tend to give a falsely high estimate of the B₁₂ absorptive capacity. Since the difficulty in ensuring completeness of stool collections, even under ideal conditions, is well known, it is probable that in the 8 cases where plasma measurements were low, the apparently normal absorption in the faecal measurement was due to loss of stools. The 3 cases in which the plasma measurements were normal and the faecal low are more difficult to explain. This could be caused by high plasma or faecal counts due to contamination or counting errors. It is also theoretically possible that the faecal count could be higher due to increased enterohepatic circulation of the label, resulting in faecal excretion of some of the label already absorbed. However, in the absence of evidence to the contrary, we have designated these false normal plasma measurements. The only other published comparison of plasma and stool measurements seems to be that of Armstrong and Woodliffe (1966) who did plasma and faecal tests in 19 subjects and found good
correlation in all except one patient who had a normal plasma and equivocal faecal measurement.

Of the 55 Schilling tests done in this study, 9 (16 per cent) showed a low urinary excretion with normal faecal and plasma measurements. A further three subjects with low urinary excretion had a low plasma measurement and a normal stool measurement. It is probable that the discordant results in the latter 3 are due to failure of stool collections. If these 3 patients are excluded then in 16 per cent of patients the Schilling test gave a falsely low result. The reason for this large number of falsely low results is not clear. The patients had no abnormal findings on microscopy of the urine and no albuminuria and all had a normal blood urea. All collected an adequate urine volume but may nevertheless have failed to collect every specimen. The urine collected from 24-48 h after the test dose contained less than 1 per cent of the test dose in all except 5 cases. In 2 of these 5 the addition of the second day’s excretion to the first day’s brought the total excretion up to or beyond 7 per cent. It is possible that delay in intestinal transit which is seen in some cases of tropical sprue may have been responsible for this delayed excretion in these 5 subjects.

Several investigators have compared the results of plasma and urine measurements. Doscherholm and Hagen (1957) and McCurdy (1965) found good correlation between urine and plasma tests in 23 and 101 tests respectively. Armstrong and Woodliff (1966) found discordant results in 2 out of 20 (10 per cent) cases, the Schilling test being low and the plasma measurement being normal. Cramer and Walsh (1971) found 5 per cent of normal subjects had falsely low Schilling tests but normal plasma tests. In patients with renal disease the percentage of abnormal urine tests was 50 per cent. McIntyre and Wagner (1966) comparing the Schilling tests with the plasma measurements found discordant results in 30 out of 129 individuals (23 per cent). They concluded that of these 36 were unexplained, 12 were due to falsely low urine results and 12 were due to falsely normal plasma results. However, they failed to take into consideration the fact that giving a flushing dose of non-radioactive vitamin B₁₂ raises the level of plasma radioactivity at 8 h, which would have significantly reduced their number of falsely normal plasma measurements. Alexander et al. (1972) performed 270 paired plasma and urine tests, and found discordant results in 13 per cent. The exact distribution of these discordant results varies according as to what is considered the lower limit of normal. Alexander et al. used a serum level of 0.45 per cent of the dose per litre of plasma. The results reported here suggest this may be too high and if the limit of 0.5 per cent is applied to their data, all except one of their discordant results would be due to a falsely low Schilling result.

Comparison of the results of the two faecal and plasma measurements indicate that 8 out of the 55 subjects changed their B₁₂ absorptive status, as judged by both faecal and plasma tests, from normal to abnormal, or vice versa, in the 10-14 days between the tests. These 8 were all patients with intestinal malabsorption due to tropical sprue. Such spontaneous variations in vitamin B₁₂ absorption capacity have been observed previously in tropical sprue (Baker and Rao, 1962; Jeejeebhoy et al., 1968; Baker and
Mathan, 1971) and also in other intestinal disorders (Finlayson et al., 1968). In order to assess the physiological significance of a vitamin B₁₂ absorptive test in patients with intestinal disease it is therefore clearly desirable that the test should be done several times, since a persistent absorptive defect will have more serious consequences for the patient than an intermittent one.

Ultimately the most reliable method of measuring vitamin B₁₂ absorption is whole body counting, but when this is not available, tests employing simpler equipment have to be used. Of the three tests employed in this study all appear to produce some false results even when done under ideal conditions (Table II). The faecal and serum measurements have a similar percentage of false normal results while the Schilling test has a much higher percentage of false abnormal results. Clearly, when conditions are not ideal the number of false results in the faecal and urine measurements is likely to increase. Ditchburn et al. (1971) found that 48 per cent of patients on a general medical ward failed to produce a complete collection of stools. Under field conditions this percentage is likely to be much higher. It may therefore be concluded that in the absence of a whole body counter the measurement of the rise in plasma radioactivity 8 h after the oral dose gives the most reliable index of the normality or otherwise of vitamin B₁₂ absorption.

Table II. False results obtained by the different tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Criterion for normality</th>
<th>% False malabsorption, per cent</th>
<th>False normal, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal</td>
<td>0·3 μg.</td>
<td>..</td>
<td>5</td>
</tr>
<tr>
<td>Plasma</td>
<td>0·2 per cent/litre or 0·3 per cent/litre with flushing dose</td>
<td>..</td>
<td>3</td>
</tr>
<tr>
<td>Urine</td>
<td>7 per cent in 24 h.</td>
<td>16</td>
<td>..</td>
</tr>
</tbody>
</table>

Acknowledgment

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References

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