Vitamin B\textsubscript{12} absorption in some neurological and neuroendocrine disorders

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SYNOPSIS An assessment of vitamin B\textsubscript{12} absorption in neurological patients has been made by both serum and urine counting of \textsuperscript{57}Co cyanocobalamin during the conventional Schilling test. Although patients with pernicious anaemia, some with subacute combined degeneration of the cord, have been studied, emphasis in the discussion is placed on the significantly increased excretion of the radioactive vitamin in a group of patients with pituitary insufficiency. Results are also given for epileptic patients who have developed folate deficiency (as assessed by serum folate levels) coincidental with anticonvulsant therapy, as well as for some patients who have had neurological symptoms or signs following partial gastrectomy operations.

The Schilling test has been used for establishing the diagnosis of pernicious anaemia since 1953. It has also been a valuable laboratory investigation in patients with malabsorption syndromes. The parameters of normality have been accurately established both for urine and serum (Donaldson, Blight, and Lascelles, 1970), and the present communication describes applications of this technique to the investigation of certain neurological and neuroendocrine diseases.

Methods

COUNTING TECHNIQUE
This is described on page 559.

SELECTION OF PATIENTS
The following groups of patients were selected from an in-patient hospital population over a two-year period:

1 Patients with pernicious anaemia
Parameters used for studying this group included the following: full blood count; serum vitamin B\textsubscript{12} level (Lactobacillus leichmanii); serum folate level (Lactobacillus casei); augmented histamine test meal (in most patients); sternal marrow examination; and antibodies to gastric parietal cells and intrinsic factor (not examined in all patients).

These were all assessed before the Schilling test was performed. Table I shows the haemoglobin values at the time of diagnosis and indicates that many of the patients were only slightly anaemic. One patient (J.S., Table I) had coincidental hypopituitarism.

2 Patients with pituitary insufficiency
This state was as assessed by clinical and biochemical criteria. Part I Schilling tests were done on five patients in this group. Four of the patients had pituitary tumours removed (two before and two after the Schilling test), and were examined histologically. Three of these were reported to be chromophobe adenomas, and one was stated to be an adenoma. In the remaining patient the cause of the hypopituitarism had not been established. In addition there was one patient, not included in Table II, who had coincidental

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Haemoglobin Level (g/100 ml)</th>
<th>Serum Vitamin B₁₂ Level (μg/ml)</th>
<th>Schilling Test</th>
<th>24-Hour Urine Excretion (% of dose administered)</th>
<th>Eight-hour Serum Level (% of dose)</th>
<th>24-Hour Urine Excretion (% of dose)</th>
<th>Eight-hour Serum Level (% of dose)</th>
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<td>F</td>
<td>12.2</td>
<td>65</td>
<td></td>
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<td></td>
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<td>11-6</td>
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<td>—</td>
<td></td>
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<td>0-09</td>
<td>5-2</td>
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Table I Results of Schilling tests on patients with pernicious anaemia

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Hb Level (g/100 ml)</th>
<th>Serum Folate Level (μg/ml)</th>
<th>24-Hour Urine Excretion (% of dose administered)</th>
<th>Eight-hour Serum Level (% of dose)</th>
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<td>31-5</td>
<td>1-05</td>
<td>1-03</td>
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<td>SD</td>
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Table II Results of part I Schilling tests on patients with hypopituitarism

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Hb Level (g/100 ml)</th>
<th>Serum Folate Level (μg/ml)</th>
<th>24-Hour Urine Excretion (% of dose administered)</th>
<th>Eight-hour Serum Level (% of dose)</th>
</tr>
</thead>
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<td>D.T.</td>
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<td></td>
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<td>0-72</td>
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</table>

Table III Results of part I Schilling tests on folate-deficient epileptic patients on anticonvulsant drugs

3 Epileptic patients
All had been treated with anticonvulsant drugs, and except for one had folate levels of 3-0 mg/ml or less. Red cell folate levels were not arranged as a parameter for investigation in this group.

4 Patients after partial gastrectomy
The patients had had partial gastrectomy operations between one and 14 years previously. All the patients in this group had neurological symptoms and signs following the partial gastrectomy operation. In most the neurological involvement was of a type suggestive of vitamin B₁₂ deficiency, but the vitamin B₁₂ levels were in the normal range in a number of patients, possibly due to short-term vitamin B₁₂ administration before admission.

Five out of the seven patients had proved duodenal ulcers before operation. In two patients (R.S. and A.B., Table IV) the only information available is that they had peptic ulcers.

Serum vitamin B₁₂ was estimated by the microbiological method using Lactobacillus leichmanii, and serum folate was measured by the Lactobacillus casei method.

Results

Patients with pernicious anaemia
Table I shows the distribution of our data for urine and serum in patients with pernicious anaemia (for part 1 and for part 2 with intrinsic factor). In part 1 of the test the mean values for the urine excretion of ⁵¹Co vitamin B₁₂ were significantly lower than normal, and similarly for the serum levels, usually rising to normal levels when the test was repeated with the addi-
Ten normal drugs convulsant tests on normal patients

Ten normal controls and part 1 tests on patients with pernicious anaemia

Table IV Results of Schilling tests on patients following partial gastrectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Urine Values</th>
<th>Serum Values</th>
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<td>0.50 &gt;p &gt; 0.40</td>
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<td>Ten normal controls and part 1 Schilling tests on patients with hypopituitarism</td>
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<td>0.01 &gt;p &gt; 0.001</td>
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<tr>
<td>Ten normal controls and part 1 Schilling tests on epileptic patients with folate deficiency on anticonvulsant drugs</td>
<td>12</td>
<td>0.70 &gt;p &gt; 0.60</td>
<td>11</td>
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<tr>
<td>Ten normal controls and part 1 Schilling tests on gastrectomy patients</td>
<td>7</td>
<td>0.01 &gt;p &gt; 0.001</td>
<td>7</td>
</tr>
</tbody>
</table>

Table V Significance between results in different groups

except for two (J.S. and M.B., Table III) who had levels of 5.8 g/100 ml and 7.4 g/100 ml respectively.

STUDIES ON PATIENTS AFTER PARTIAL GASTRECTOMY

The data for this group of patients are given in Table IV. Parts 1 and 2 of the Schilling test were performed in each case except for the last patient in the list (R.D., Table IV).

Table V shows that there are no significant differences between the first and second parts of the test, either for urine or serum if the results are taken together, but individually it is seen that both R.S. and A.B. (Table IV) showed a significant rise. However, patients W.C. and A.W. both showed a fall in the second part of the test.

Discussion

Table I shows that there was clear demarcation between the part I Schilling test results (urine and serum levels) for patients with pernicious anaemia (including those with no evidence of anaemia) and those of normal controls, and that there was no overlap between the two groups, confirming the observations of many previous workers.

One patient (D.T., Table I) failed to show a rise in the urinary excretion of $^{57}$Co vitamin B$_{12}$ when intrinsic factor was given. This may be in keeping with the observations of several investigators that there is sometimes a malabsorption for vitamin B$_{12}$ in patients who have suffered deficiency of the vitamin for a considerable time (Haurani, Sherwood, and Goldstein, 1964; Brody, Estren, and Herbert, 1966; Carmel and Herbert, 1967). These authors have demonstrated that this specific malabsorption is reversible when patients are treated with vitamin B$_{12}$, and they
showed that the Schilling test part 2 became normal after periods ranging from one to 20 months. Some of the patients had only been re-investigated after an elapse of several months or more, and it is therefore possible that the time of response may have been considerably shorter. Goldberg, Bickel, and Fudenberg (1969) similarly described a patient who showed increased absorption of vitamin B\textsubscript{12} after six months of treatment; in addition these authors draw attention to the possibility that the presence of gastric antibody to intrinsic factor may be the cause of a low absorption of vitamin B\textsubscript{12} in the second part of the Schilling test.

It is of interest that the patient (D.T.) had the lowest haemoglobin in the group (5.8 g/100 ml) and therefore may have been deficient in vitamin B\textsubscript{12} for a long period. Unfortunately there was no opportunity to investigate this patient further. There was no clinical evidence of generalized malabsorption. Sauli, Astaldi, and Malossini (1963) studied the histopathology of the intestinal mucosa in patients with pernicious anaemia, before and after vitamin B\textsubscript{12} therapy. They showed that there were appreciable changes toward normality after one month of treatment with 500 gamma per day. Foroozan and Trier (1967) demonstrated that after two or more months of vitamin B\textsubscript{12} administration the small intestines of all their eight patients were histologically indistinguishable from those of the control subjects.

Apart from this one patient there was no difference between the mean value for part 2 of the Schilling test on patients with pernicious anaemia and the part 1 tests on normal controls (see Table V).

The five patients in the hypopituitary group all showed a urinary excretion of \textsuperscript{57}Co vitamin B\textsubscript{12} greater than 20-0%. Two of the patients had excrections greater than the upper limit of the normal range (using 95% confidence limits). In addition, two of the three remaining patients had serum levels of \textsuperscript{57}Co vitamin B\textsubscript{12} in excess of the normal range. Hence, four of the five patients would have been separable from the normal controls taking either the urine or serum values alone as significant.

From Table II it is noted that the highest two urinary excretions belonged to patients who were not receiving replacement therapy at the time of the test (W.M. and S.S., Table II). However, the excretion in the third patient not receiving therapy was lower than these, although the eight-hour serum level was the highest of the group.

It is possible that the physiological mechanism for this increased excretion of vitamin B\textsubscript{12} in hypopituitarism is in some way related to deficiency of certain hormonal factors, either the trophic hormones produced by the pituitary, or deficient production of hormones by the target organs for these trophic hormones, namely, the thyroid, adrenals, and the gonads.

Also to be considered is the possibility that the adenomatous tissue of the pituitary tumours themselves may be producing a substance which has an effect on vitamin B\textsubscript{12} metabolism. It is hoped that further work will establish whether or not pituitary adenomas are the necessary factor, or whether hypopituitarism \textit{per se}, whatever its cause, will produce the same effect.

It is established in humans that the thyroid gland influences xylose metabolism. Broitman, Bondy, Yachnin, Hoskins, Ingbar, and Zamcheck (1964) showed that although the absorption of xylose was normal in states of thyrotoxicosis and myxoedema, the urinary excretion was low in myxoedema and increased in hyperthyroidism. Furthermore, they showed that the excretory pattern was similar when the xylose was given intravenously. They calculated that there was a decreased rate of renal clearance and an apparently normal rate of extrarenal disposal in patients with myxoedema, and that there was a decreased rate of extrarenal disposal with a concomitant increase in renal excretion in patients with hyperthyroidism.

Also of importance in this connexion are experiments involving the use of rats in which there was shown to be increased absorption of vitamin B\textsubscript{12} when the animals were fed on large doses of desiccated thyroid together with a casein-containing diet, and a decreased absorption of the vitamin when the animals were thyroidectomized (Okuda, Steelman, and Chow, 1956).

It would appear, however, that these animal experiments were in conflict with our findings in hypopituitary patients if the thyroid were the factor responsible for the difference from the normal controls. One might thus expect a lowered excretion of vitamin B\textsubscript{12} if there was a degree of hypothyroidism secondary to hypopituitarism, instead of the increase observed. On the other hand, the aetiology of the hypothyroidism is different in the two examples, and this fact may be of importance in the interpretation of the results. The hypothyroidism of pituitary insufficiency is mediated through the pituitary, whereas that of the hypothyroid rats was induced by excision of the thyroid gland, in which case the pituitary gland in each animal of the series was intact. Experiments on vitamin B\textsubscript{12} absorption in patients with thyrotoxicosis and myxoedema are in progress.

The question arises whether the thyroid could have an effect on overall vitamin B\textsubscript{12} metabolism, or simply on transport across the intestinal mucosa. Thus it is established that the intracellular accumulation \textit{in vitro} of the amino acids cycloleucine, histidine, and valine, and the sugar \textalpha{}-methyl-D-glucopyranoside, were increased in the intestinal segments of rats made hypothyroid by thyroidectomy and radioactive \textsuperscript{131}I (London and Segal, 1967). When the hypothyroid animals were treated with triiodo-
thyroxine the accumulation of substrate fell toward the control level. The authors suggested that as this phenomenon was seen with an unutilizable amino acid and sugar, it strongly suggested an effect on transport rather than on metabolism.

So far we have considered only the hypothyroid element of the hypopituitary state. Another possible explanation to be considered is that the replacement therapy itself may be responsible for the increased vitamin B₁₂ absorption in the hypopituitary group. That this is not tenable is shown by the fact that three of the five patients were, at the time of the Schilling test, not on any form of therapy.

There is known to be an increased incidence of pernicious anaemia in myxoedema (Leithold, David, and Best, 1958; Forsell and Halunen, 1958; Ardeman, Chanarin, Kraefchik, and Singer, 1966; and Strickland, 1969), and also of low serum vitamin B₁₂ levels in hypothyroidism without pernicious anaemia (Tudhope and Wilson, 1962). Again this observation does not account for our hypopituitary observations. Ziffer, Gutman, Pasher, Sobotka, and Baker (1957), however, demonstrated that the whole-blood vitamin B₁₂ values were lower in a group of hypothyroid patients than in a normal control group and a myxoedematous group. The mean urinary excretion after the vitamin B₁₂ dose was considerably lower in hypothyroid patients than in the normal group, and the myxoedematous group showed an increased excretion. These authors suggested that vitamin B₁₂ turnover and demand is greater in hypothyroid patients and lower in myxoedema.

There have been reports of pernicious anaemia in Addison’s disease (Berlin, 1952; Strickland, 1969), but no literature is available regarding vitamin B₁₂ levels. Because of the increased absorption of vitamin B₁₂ in Schilling tests in which the dose is given to fasting patients, it does not necessarily follow that there is an increased absorption of vitamin B₁₂ from the food under physiological conditions in these patients.

In order to define the mechanism for the increased excretion of vitamin B₁₂ in the Schilling test we are currently investigating a further group of such patients, some of whom are on replacement therapy. We are also establishing the effect of ACTH, prednisone, and dexamethasone on the absorption of vitamin B₁₂ in a control group of patients, as well as studying the relationship of the thyroid function of the hypopituitary group to the problem. In addition, patients with non-pituitary cerebral tumours and raised intracranial pressure are being similarly investigated.

It is well established that patients who are treated with anticonvulsant drugs, such as phenobarbitone, phenytoin, and primidone, for epilepsy are liable to develop folate deficiency (Reynolds, Chanarin, Milner, and Matthews, 1966). It is also known that the metabolism of folate and vitamin B₁₂ is closely interdependent (Vilter, 1964). This can be seen by the changes in serum levels of the one in response to changes in serum levels of the other: for example, the tendency to high serum folate levels in pernicious anaemia (Waters and Mollin, 1961; Waters and Mollin, 1962; Waters and Mollin, 1963), and the reduction in the serum vitamin B₁₂ in epileptic patients who are treated with folic acid for folate deficiency induced by anticonvulsant therapy (Hunter, Barnes, and Matthews, 1969).

It was with these facts in mind that Schilling tests were done on a series of folate-deficient epileptic patients who had been treated with various combinations of the drugs mentioned above. Lees (1961) reported that the Schilling test indicated a lower absorption of vitamin B₁₂ in patients who had developed megaloblastic anaemia while on anticonvulsant drugs, and that in six of eight patients a considerable increase occurred when folic acid therapy was given.

In our series, however, there was no significant difference (see Table V) between the series of normal controls and the epileptic patients who were folate deficient. Only two of our series of 10 patients had a significant anaemia, and the explanation of the disparity between our results and those of Lees may lie here.

As shown in Tables III and V, there is no significant difference for the urine and serum levels of ⁵⁷Co vitamin B₁₂ in the Schilling test between the folate-deficient epileptic patients and the series of normal controls.

Table IV illustrates the importance of studying individual patients separately, as certain changes between part 1 and 2 tests do not show up when the group is studied as a whole.

It should be noted that none of these patients was given a vitamin B₁₂-free meal at the time the radioactive vitamin B₁₂ was given in the Schilling test. Deller, Germar, and Witts (1961) noted that when a vitamin B₁₂-free meal was given with the labelled vitamin B₁₂ in the Schilling test absorption was significantly enhanced in patients who had undergone partial gastrectomy. Turnbull (1967) observed similar results in a series of patients with anaemia after Polya partial gastrectomy, and suggested that the improved absorption of vitamin B₁₂ with food was likely to be due to stimulation of a small residual secretion of intrinsic factor.

These initial findings by the more precisely standardized Schilling test counting both urine and serum radioactivity confirm and extend the observations of others. The explanations for the mechanism of increased excretion of radioactive vitamin B₁₂ in patients with pituitary tumours and the fall in excretion in part 2 in some post-gastrectomy patients with neurological complications are not at present apparent and clearly need further investigation.

We wish to thank the physicians and surgeons
of the National Hospitals, Queen Square and Maida Vale, for permission to investigate patients under their care.

We are also grateful to Mrs Carol Pye at the Hospital for Sick Children, Great Ormond Street, for the serum folate assays.

References


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