A Study of Vitamin B₁₂ Requirements in a Patient With Pernicious Anemia and Thyrotoxicosis: Evidence of an Increased Need for Vitamin B₁₂ in the Presence of Hyperthyroidism

By Jack B. Alperin, Mary E. Haggard and Thomas P. Haynie

The response to small doses of vitamin B₁₂ was studied in a woman with pernicious anemia and thyrotoxicosis. No response to 1 μg per day occurred, but she did respond to 10 μg per day. Fourteen months later when she was euthyroid, the patient did respond to 1 μg per day. In another study, serum vitamin B₁₂ levels were significantly lower in a group of patients with hyperthyroidism than in a group of normal subjects. Seventeen of 20 patients with hyperthyroidism exhibited significantly higher serum vitamin B₁₂ levels 5–8 months after becoming euthyroid. The results of these studies provide evidence for increased vitamin B₁₂ requirements in the presence of hyperthyroidism. The greater need for vitamin B₁₂ appears to be related to increased utilization and/or accelerated turnover imposed by increased metabolism.

The minimum daily requirement for vitamin B₁₂ probably lies between 0.1 and 1.0 μg; however, 1 μg per day is recommended for therapeutic trials in patients with vitamin B₁₂ deficiency. Numerous studies suggest an increased requirement for vitamin B₁₂ in animals with hyperthyroidism. There are several reports of patients with pernicious anemia and thyrotoxicosis; however, none clearly shows an increased requirement for vitamin B₁₂ in hyperthyroidism. Our discovery of a woman with pernicious anemia and thyrotoxicosis provided a unique opportunity to evaluate her vitamin B₁₂ requirements before and after therapy with I³I. We describe here the investigations performed on this patient and the results of serum vitamin B₁₂ measurements in 34 other patients with hyperthyroidism.

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METHODS

The following laboratory tests were performed with established methods: hemograms, lobe average, serum levels of folic acid, vitamin B₁₂, iron, lactic acid dehydrogenase (LDH), and protein bound iodine (PBI); resin sponge uptake of ¹³¹I labeled triiodothyronine; and uptake of ¹³¹I by the thyroid gland. Following the ingestion of 15 Gm. l-histidine monohydrochloride monohydrate, forminoglutamic acid (FiGlu) in urine was quantitated by a modified electrophoretic technique. The accompanying table gives normal values for these tests.

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The acid content of gastric secretions was measured after maximal histamine stimulation and peroral biopsy of the gastric mucosa was accomplished with the instrument described by Crosby and Kugler. Vitamin B₁₂ absorption was evaluated with a modified Schilling test in which the patient ingests 0.5 µg. cyanocobalamin (CN-B₁₂) labeled with 0.6 µCi ⁵⁷Co. Normally, over 6 per cent of the ingested radioactivity is flushed into the urine in 24 hours.

CASE REPORT

First Admission

R.Y., a 67-year-old woman, entered the hospital complaining of weakness, heat intolerance and paresthesias in her hands and feet. An enlarged thyroid gland had been present for many years. Her appetite was poor and she had lost nearly 7 Kg. in the past 4 months. Blood pressure, respiratory rate and temperature were normal. The pulse was 118 beats per minute. Pallor of the skin and mucosal surfaces, lingual atrophy, widening of the palpebral fissure and lid lag were evident. The thyroid gland was diffusely enlarged and estimated to be three–four times normal size. A holosystolic murmur was heard over the entire precordium and a loud bruit was audible over the thyroid gland. Her spleen was palpable 3 cm. below the costal margin. Diminished vibratory sensation (C 256 tuning fork) in both ankles and wrists was evident; otherwise, no abnormalities were detected on neurological ex-

Table 1.—Results of Laboratory Studies

<table>
<thead>
<tr>
<th></th>
<th>First Admission*</th>
<th>First Outpatient Visit †</th>
<th>Second Outpatient Visit 1</th>
<th>Second Admission ‡</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Gm. %)</td>
<td>8.8</td>
<td>12.3</td>
<td>10.3</td>
<td>9.2</td>
<td></td>
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<tr>
<td>Red cells (10⁶/cu. mm)</td>
<td>2.64</td>
<td>4.31</td>
<td>3.52</td>
<td>3.10</td>
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<tr>
<td>Hematocrit (%)</td>
<td>27</td>
<td>49</td>
<td>34</td>
<td>31</td>
<td></td>
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<tr>
<td>MCV (µ₃)</td>
<td>103</td>
<td>93</td>
<td>96</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MCH (pg.)</td>
<td>34</td>
<td>26</td>
<td>29</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.2</td>
<td>0.4</td>
<td>0.9</td>
<td>0.2</td>
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<tr>
<td>Platelets (10⁹/cu. mm)</td>
<td>98</td>
<td>342</td>
<td>189</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (10⁹/cu. mm)</td>
<td>2.8</td>
<td>9.6</td>
<td>6.3</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Lobe average</td>
<td>5.1</td>
<td>3.3</td>
<td>4.1</td>
<td>4.9</td>
<td>3.1–3.5</td>
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<tr>
<td>Serum folate (ng./ml.)</td>
<td>12.4</td>
<td></td>
<td>6.8</td>
<td>3–14</td>
<td></td>
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<tr>
<td>Serum vitamin B₁₂ (pg./ml.)</td>
<td>54</td>
<td>98</td>
<td>68</td>
<td>200–900</td>
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<tr>
<td>FICl (mg./12 hours)</td>
<td>298</td>
<td></td>
<td>83</td>
<td>&lt; 25</td>
<td></td>
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<td>LDH (mU./ml.)</td>
<td>2932</td>
<td></td>
<td>824</td>
<td>80–200</td>
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<td>Serum Fe (µg. %)</td>
<td>210</td>
<td></td>
<td>142</td>
<td>75–140</td>
<td></td>
</tr>
<tr>
<td>PBI (µg. %)</td>
<td>18.7</td>
<td>6.4</td>
<td>4.8</td>
<td>3.9</td>
<td>3–8</td>
</tr>
<tr>
<td>T₃ resin sponge uptake (%)</td>
<td>40.3</td>
<td>27.6</td>
<td>24.8</td>
<td>25–35</td>
<td></td>
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<tr>
<td>24 hour thyroid uptake of ¹³¹I (%)</td>
<td>72</td>
<td>12</td>
<td>10–40</td>
<td></td>
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</tr>
</tbody>
</table>

* May 1964.
‡ June 6, 1965.
Fig. 1.—Hematological response to small quantities of CN-B$_{12}$ when the patient had hyperthyroidism.

Fig. 2.—Biochemical changes in response to vitamin B$_{12}$ supplements when the patient had hyperthyroidism. No vitamin B$_{12}$ supplement was given during the initial 12-day period of observation. From day 13 through day 24 the patient received 1 $\mu$g. vitamin B$_{12}$ per day; from day 25 through day 36 she received 10 $\mu$g. per day.

amination. Results of important laboratory studies performed within 72 hours after admission appear in Table 1. A Wright’s stained blood smear revealed many macroovalocytic red cells and hypersegmented neutrophilic leukocytes. Kingsley’s stained bone marrow smears disclosed typical megaloblastic changes. Chest X-ray showed moderate cardiac enlargement; ECG revealed a sinus tachycardia. Tests of renal and hepatic function were normal. Tests for malabsorption syndromes were negative.

A diagnosis of vitamin B$_{12}$ deficiency and thyrotoxicosis was rendered. Before treating the
patient with \( {^{131}}I \), we observed her response to small, titrated doses of CN-B\(_{12} \) (Figs. 1 and 2). Finally before leaving the hospital, she was given two larger injections of CN-B\(_{12} \), each consisting of 1000 \( \mu \)g. No additional vitamin \( B_{12} \) was administered until the second admission.

**Interim Examinations**

Four months after discharge, the patient was examined again. She felt much stronger and appeared clinically euthyroid. Her weight had increased almost 5 Kg. Neurological examination disclosed no abnormalities; the tip of the spleen was still palpable. Eight months later (1 year after leaving the hospital) she continued to feel well and appear euthyroid. Because of recurrent anemia (see Table 1), hospitalization was advised. She refused and another 2 months elapsed before she was readmitted.

**Second Admission**

At the time of the second hospital admission, her only complaints were weakness and paresthesias in her hands and feet of approximately 4 weeks duration. All vital signs were normal. Enlargement of the thyroid gland was no longer evident. Lingual atrophy and splenomegaly persisted. Absent vibratory sensation (C 256 tuning fork) was evident in her ankles and wrists, but no other neurological abnormalities were noted. Laboratory data obtained at the beginning of this admission appear in Table 1. The bone marrow again revealed megaloblastic erythropoiesis. Clinical and laboratory data showed the patient was now euthyroid, but vitamin \( B_{12} \) deficiency remained. Once more her response to a small dose of CN-B\(_{12} \) was determined (Figs. 3 and 4).

**RESULTS**

**Hematological Data**

During both hospitalizations, the patient ate a standard diet calculated to contain about 20 \( \mu \)g vitamin \( B_{12} \) per day. Figure 1 indicates the hematological response to CN-B\(_{12} \) while the patient had hyperthyroidism. During the initial 12 days of this study, supplemental vitamin \( B_{12} \) was not permitted and no improvement was seen. Similarly, no significant improvement appeared after

![Fig. 3.—Hematological response to small quantities of CN-B\(_{12} \) when the patient was euthyroid.](attachment:image-url)
The 1 and 10 µg. doses of CN-B₁₂ referred to in this report were quantitated by assay with *Euglena gracilis*.¹

**Biochemical Data**

Results of serial biochemical determinations appear in Figs. 2 and 4. Concentrations of iron, LDH and folate in serum and the urinary excretion of FICGlu remained elevated during each initial 12-day period of observation. When the patient was hyperthyroid, 1 µg. CN-B₁₂ per day for 12 days failed to significantly alter these parameters, but 10 µg. per day produced a significant decline in each. After the patient became euthyroid, a prompt fall in the levels
of iron, folate, FiGlu and LDH followed treatment with just 1 $\mu$g CN-B$_{12}$ per day. While the patient had hyperthyroidism, 1 $\mu$g of CN-B$_{12}$ daily failed to substantially elevate the serum vitamin B$_{12}$ level; however, a significant increase in the serum level occurred with 10 $\mu$g. per day. After the patient became euthyroid, the 1 $\mu$g. dose of CN-B$_{12}$ caused a significant increase in the serum vitamin B$_{12}$ level.

Studies of Thyroid Function

Thyroid function tests obtained at the beginning of the first hospitalization were diagnostic of hyperthyroidism (Table 1) and remained so until day 42 when the patient was treated with 5 mCi $^{131}$I. Subsequently, she appeared clinically euthyroid and tests of thyroid function performed during two clinic visits and during the second hospitalization were within normal limits.

Examinations of the Stomach and Measurements of Vitamin B$_{12}$ Absorption

During each hospitalization, the patient exhibited histamine-fast achlorhydria and peroral biopsy specimens of the gastric mucosa showed atrophic gastritis. Roentgenographic studies showed no abnormalities of the esophagus, stomach or small intestine. The Schilling test performed at the end of the first hospitalization revealed 1.1 per cent excretion of radioactivity without intrinsic factor and 24.2 per cent excretion with intrinsic factor. A repeat Schilling test during her second hospitalization gave almost identical results, i.e., without intrinsic factor 0.8 per cent excretion and with intrinsic factor 17.7 per cent excretion.

B$_{12}$ Measurements in Patients with Hyperthyroidism

Serum vitamin B$_{12}$ levels were measured in 34 patients with thyrotoxicosis which by history had been present at least 9 months. Each exhibited normal red cell morphology, a normal lobe average and normal leukocyte and platelet counts. Hemoglobin values varied from 9.8 to 13.1 Gm. per 100 ml. Thirty-two patients had normal serum vitamin B$_{12}$ levels; one had a level of 180 pg. per ml and the other, a level of 192 pg. per ml. The mean serum vitamin B$_{12}$ level for all patients with hyperthyroidism was 347 ± 144 pg. per ml. In a group of 54 normal subjects, the mean serum vitamin B$_{12}$ value was 572 ± 183 pg. per ml. Thus, vitamin B$_{12}$ levels were significantly lower in the hyperthyroid group than in the normal group ($p < 0.01$).

Each patient was treated with $^{131}$I. A second measurement of serum vitamin B$_{12}$ activity was performed in 20 patients 5–8 months after they became euthyroid. None received supplemental vitamin B$_{12}$ during the period of observation. All but three experienced an increase in the vitamin B$_{12}$ level; two had decreased values; and the third exhibited no significant change. The two patients whose original vitamin B$_{12}$ levels were < 200 pg. per ml. exhibited normal levels after they became euthyroid. The mean vitamin B$_{12}$ level in these 20 patients before therapy with $^{131}$I was 335 ± 144 pg. per ml. After they became euthyroid, the mean serum vitamin B$_{12}$ level measured 596 ± 171 pg. per ml. This difference represents a significant improvement ($p < 0.01$).
VITAMIN B12 REQUIREMENTS IN PERNICIOUS ANEMIA AND THYROTOXICOSIS

DISCUSSION

Vitamin B12 supplements may protect rats against weight loss and death from thyrotoxicosis. Furthermore, a number of metabolic and biochemical abnormalities occur in hyperthyroid rats which are corrected or prevented by treatment with vitamin B12. These abnormalities include uncoupling oxidative phosphorylation of mitochondria, decrease in serum proteins, rapid loss of vitamin B12 and glutathione from the blood and liver, decline in total content of soluble sulfhydryl-containing compounds in the liver and an increase in the hepatic content of lipid and coenzyme A. Methionine and betaine, given in place of vitamin B12, may also protect against toxic effects of hyperthyroidism. Both methionine and betaine have labile methyl groups and are important sources of one carbon fragments for intermediary metabolism. Since vitamin B12 plays a major role in the transport of one carbon moieties, it is tempting to speculate that thyrotoxicosis may also lead to a defect in the metabolism of one carbon fragments.

Each of 10 euthyroid patients with pernicious anemia that we studied exhibited an excellent clinical and laboratory response to 1 µg CN-B12 per day, in keeping with previous observations. In the case reported herein, a woman with hyperthyroidism and pernicious anemia was treated with small doses of CN-B12. While her hyperthyroidism remained untreated, no hematological or biochemical response to 1 µg CN-B12 per day occurred; however, response to 10 µg per day was excellent. After she became euthyroid, she exhibited a satisfactory response to 1 µg CN-B12 per day. These studies clearly indicate she needed more CN-B12 when she had hyperthyroidism. The body can not directly utilize CN-B12. It must first be converted to metabolically active forms, i.e., deoxyadenosyl-B12, methyl-B12, hydroxy-B12, etc. The possibility that hyperthyroidism in this patient interfered with the conversion of CN-B12 into metabolically active forms was not excluded, but seems unlikely. More likely she required more CN-B12 because vitamin B12 utilization and/or turnover accelerates in the presence of hyperthyroidism. Large amounts of vitamin B12 are presumably needed to satisfy increased metabolic needs which occur in hyperthyroidism.

Ziffer et al. found whole blood vitamin B12 levels before and after an injection of 50 µg of vitamin B12 significantly lower in patients with hyperthyroidism than in euthyroid subjects. Also, the urinary excretion of vitamin B12 after this injection was considerably less in the hyperthyroid group. The 34 patients with hyperthyroidism we studied showed significantly lower serum vitamin B12 levels than a group of normal men and women. Furthermore, 17 of 20 patients with hyperthyroidism exhibited significant increases in serum vitamin B12 levels after they became euthyroid. These data offer further evidence for an increased requirement for vitamin B12 in the presence of hyperthyroidism.

Decreased hepatic stores of folic acid in hyperthyroid rats and rapid clearance of intravenously injected folic acid in patients with thyrotoxicosis suggest that folic acid requirements are also increased in hyperthyroidism. Needed are reports of patients with hyperthyroidism and megaloblastic
anemia due to folic acid deficiency. Two patients with thyroid storm, folic acid deficiency and megaloblastic bone marrow have been examined in our laboratory; however, they were too ill to permit evaluation of their response to small doses of folic acid.34

Scores of investigations show a definite relationship between pernicious anemia and hyperthyroidism. A scholarly review of this subject appeared in a recently published textbook.12 There is an increased frequency of pernicious anemia in patients with hyperthyroidism. Conversely, patients with pernicious anemia have a higher incidence of hyperthyroidism. In a few instances, both diseases have been discovered simultaneously13-15 such as the patient described in this report. Further evidence for an association between pernicious anemia and thyrotoxicosis derives from determinations of autoantibodies. Parietal cell antibodies may be detected in the serum of about 85 per cent of patients with pernicious anemia and nearly 30 per cent of patients with hyperthyroidism. Thyroid antibodies in serum may be found in over half the patients with pernicious anemia and about 45 per cent of patients with hyperthyroidism. The incidence of these two antibodies in control sera does not exceed 15 per cent. About one-half of patients with pernicious anemia have intrinsic factor antibodies in serum, and pernicious anemia has been present in most instances wherein intrinsic factor antibodies were detected in patients with hyperthyroidism.12,28 A search for intrinsic factor and thyroid antibodies in our patient produced only negative results; we did not measure parietal cell antibodies.

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