Metabolic Interrelationship between Vitamin B₁₂ and Ascorbic Acid in Pernicious Anemia

By Sigmund Benham Kahn and Isadore Brodsky

With the technical assistance of Sandra A. Fein

SUBNORMAL PLASMA ascorbic acid concentration and rapid plasma ascorbate clearance have been found in patients with vitamin B₁₂ deficiency despite adequate intake of vitamin C.¹ ² Cox et al.² noted in their group of vitamin B₁₂-deficient patients that these abnormalities of ascorbate metabolism were still present 7–14 days following therapy with sufficient vitamin B₁₂ to cause clinical and hematological response. Prolonged vitamin B₁₂ therapy was necessary before ascorbate metabolism returned to normal.²

Recently we described a patient with scurvy and anemia in whom, during therapy with vitamin C, methylmalonate (MMA) excretion was detected.³ At this time, the hemoglobin concentration was rapidly rising but the plasma vitamin B₁₂ activity was normal. It was speculated that MMA excretion suggested depletion of body vitamin B₁₂ stores, since therapy with vitamin B₁₂ caused abolition of MMA excretion.⁴ ⁵ In this patient despite long term administration of high doses of vitamin C, the plasma ascorbate concentration did not become normal until MMA excretion was abolished.³ These data suggested that the plasma ascorbate concentration is related to the excretion of MMA and is directly or indirectly related to vitamin B₁₂ stores.

In the study reported herein, observations were made on three patients with vitamin B₁₂ deficiency associated with pernicious anemia with the purpose of correlating plasma ascorbic acid concentration with MMA excretion. Red blood cell (RBC) vitamin B₁₂ activity was measured serially in order to evaluate its sensitivity as an index of vitamin B₁₂ body stores when compared to MMA excretion. The data suggest an interrelationship between vitamin B₁₂ stores and plasma levels of ascorbic acid.

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MATERIALS AND METHODS

Blood was collected in sterile heparinized vacutainers (B-D. Company, Rutherford, N. J.) from patients in the fasting state. Within one hour of collection under sterile precautions, an aliquot of whole blood was removed for determination of routine blood counts, another aliquot was frozen (−20°C) for whole blood folate assay, and the plasma and RBC of the remainder separated and frozen for subsequent vitamin assays.

Blood counts were performed by routine methods. Platelets were counted by the method of Brecher and Cronkite and reticulocytes counted after staining with new Methylene blue. Bone marrow was aspirated from the posterior iliac crest and the specimen stained with Wrights stain. Specimens were stained for iron by the method of Dry. Iron stores were graded by the method of Gale, Torrance and Bothwell.

All vitamin assays were performed within a week of collection of the blood specimen. Plasma folate was assayed microbiologically utilizing L. Casei as described by Herbert. Whole blood (W.B.) folate was measured by adding 4 ml. of vitamin free distilled water to 1 ml. whole blood, centrifuging to remove red cell membranes and then diluting 1 ml. of the supernatant with 9 ml. phosphate buffer, 0.05 M, pH 6.1 containing 150 mg. per cent ascorbic acid and incubating for 90 minutes at 37°C. The mixture was then autoclaved at 108°C for 30 minutes. Following centrifugation, 0.1 ml. of the clear protein free supernatant was assayed for folate activity in a manner similar to plasma folate. Red blood cell (RBC) folate was then calculated by the following formula:

\[
\text{RBC fol. ng./ml.} = \frac{\text{W.B. fol. ng./ml.} - \text{Pl. fol. ng./ml.}}{\text{ml. RBC/ml. W.B.}} + \text{Pl. fol. ng./ml.}
\]

Normal values for plasma folate are 7–18 ng./ml. with borderline values of 4–6.9 ng./ml. Normal RBC folate ranges from 175–900 ng./ml.

Plasma vitamin B	extsubscript{12} activity was assayed utilizing E. Gracilis-Z by the method of Hutner, Bach and Ross. RBC vitamin B	extsubscript{12} activity was measured by adding to 1.0 ml. of packed RBC, 1.0 ml. of 0.4M acetate buffer pH 5.23, 0.2 ml., 0.1 per cent (W/V) KCN., and 8.0 ml. of vitamin free distilled water. Following autoclaving at 108°C for 30 minutes, the samples were centrifuged and an aliquot of the supernatant was assayed for vitamin B	extsubscript{12} activity in a manner similar to plasma. The normal plasma vitamin B	extsubscript{12} activity is 200–1000 pg./ml. with borderline values of 150–200 pg./ml. Normal RBC vitamin B	extsubscript{12} activity ranges from 200–600 pg./ml.

Plasma ascorbate concentration was measured by the method described by Natelson. This method utilizes dinitrophenylhydrazine and measures total plasma ascorbate. Normal values are 0.4–1.4 mg. per cent. In order to rule out possible interference in the measurement of plasma ascorbate by MMA or some metabolite in the plasma, a recovery experiment was performed using plasma obtained from W.E. An aliquot of plasma obtained during the pretreatment period was obtained and sufficient ascorbic acid added to raise the concentration of vitamin C to 0.5 mg. per cent. The results were as follows:

- Plasma alone < 0.1 mg. per cent
- Plasma and ascorbic acid 0.52 mg. per cent
- Water and ascorbic acid 0.5 mg. per cent

MMA was measured in 24 hour urine specimens collected under xylene. MMA was assayed by the method of Barness et al. In this method, 10 ml. of the 24 hour urine specimen is continuously extracted with ether. The ether is then evaporated and the residue redissolved in a small amount of ether and the total spotted on paper for chromatography. Results are expressed semiquantitatively. Normal urine does not contain detectable MMA by this method. A +1 result implies 6 mg./L concentration with MMA with ± indicating

*Assays performed in the laboratory of Dr. L. Barness, Hospital of the University of Pennsylvania, Phila., Penn.

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VITAMIN B₁₂ AND ASCORBIC ACID IN ANEMIA

Table 1a.—Hematological Data before Therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>HGB</th>
<th>HCT %</th>
<th>Platelet (10^{12}/\text{mm}^3)</th>
<th>Retic %</th>
<th>Marrow Cell.</th>
<th>Iron</th>
<th>Schilling Test without I.F.</th>
<th>Schilling Test with I.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. E.</td>
<td>52 M</td>
<td>6.8-7.5</td>
<td>20-23</td>
<td>3700-5250</td>
<td>0.3-0.6</td>
<td>ME = 1:1</td>
<td>3+</td>
<td>&lt;1.0%</td>
<td>6.4%</td>
</tr>
<tr>
<td>Y. Q.</td>
<td>68 F</td>
<td>5.5-6.4</td>
<td>17-20</td>
<td>3450-3750</td>
<td>1.1-1.3</td>
<td>ME = 1:1</td>
<td>2+</td>
<td>&lt;1.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>E. H.</td>
<td>68 F</td>
<td>6.0-9.2*</td>
<td>17-29*</td>
<td>3070-3500</td>
<td>0.5-1.3</td>
<td>ME = 1:1</td>
<td>3+</td>
<td>&lt;1.0%</td>
<td>17.0%</td>
</tr>
<tr>
<td>Normal</td>
<td>12-17</td>
<td>42-52</td>
<td>4500-10,000</td>
<td>150-450</td>
<td>0.5-1.5</td>
<td>3-7:1</td>
<td>2-4+</td>
<td>&gt;5.0% &gt;5.0%</td>
<td></td>
</tr>
</tbody>
</table>

*3 Units Packed Cells.
†All Marrows Megaloblastic.
‡I.F. = Intrinsic Factor.

Table 1b.—Biochemical and Dietary Data before Therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Vitamin B₁₂</th>
<th>Folate</th>
<th>M.M.A. (24 hr.)</th>
<th>Plasma Ascorbate</th>
<th>Dietary History</th>
</tr>
</thead>
</table>

Normal 200-1,000 200-600 7-20 175-900 NEG. 0 0.4-1.4

*After Transfusion.

RESULTS

Pretreatment

Pertinent hematological data prior to therapy are listed in Table 1a. Patients Y.Q. and E.H. had pancytopenia while W.E. had leukopenia and anemia. All peripheral smears revealed ovalomacrocytosis and hypersegmented polymorphonuclear neutrophiles. Bone marrow examination demonstrated hypercellu-
Table 2.—Hematological and Biochemical Data following Therapy

PATIENT Y.Q.

<table>
<thead>
<tr>
<th>Day of Therapy</th>
<th>HGB g. %</th>
<th>Retic %</th>
<th>Plasma $B_12$ pg./ml.</th>
<th>RBC $B_12$ pg./ml.</th>
<th>Plasma Vit. C mg. %</th>
<th>Urine MMA (24 hr.)</th>
<th>Vit. $B_12$ µg./day</th>
<th>Therapy</th>
<th>Vit. C mg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (3 days)</td>
<td>5.5-6.4</td>
<td>1.1-1.3</td>
<td>42-52</td>
<td>130-145</td>
<td>&lt;0.1-0.15</td>
<td>8+-10+</td>
<td>0</td>
<td>75 daily</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>2.0</td>
<td>78</td>
<td>135</td>
<td>2+</td>
<td>1* (day 1-11)</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.7</td>
<td>2.1</td>
<td>104</td>
<td>110</td>
<td>0.11</td>
<td>8+</td>
<td>1</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6.1</td>
<td>6.5</td>
<td>206</td>
<td>135</td>
<td>—</td>
<td>3+</td>
<td>1</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6.7</td>
<td>5.0</td>
<td>230</td>
<td>110</td>
<td>0.23</td>
<td>2+</td>
<td>1 (end 1 µg)</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>3.3</td>
<td>190</td>
<td>100</td>
<td>—</td>
<td>2+</td>
<td>10* (day 12-17)</td>
<td>200 (day 12-29)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.5</td>
<td>—</td>
<td>232</td>
<td>280</td>
<td>0.14</td>
<td>2+</td>
<td>10</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>6.2</td>
<td>4.7</td>
<td>392</td>
<td>250</td>
<td>—</td>
<td>2+</td>
<td>10 (end 10 µg)</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>7.1</td>
<td>5.5</td>
<td>240</td>
<td>260</td>
<td>0.11</td>
<td>1+ 900* (day 18-49)</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>8.6</td>
<td>4.4</td>
<td>192</td>
<td>310</td>
<td>0.18</td>
<td>1+</td>
<td>900</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>9.6</td>
<td>5.7</td>
<td>196</td>
<td>340</td>
<td>0.20</td>
<td>1+</td>
<td>900</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>13.2</td>
<td>0.6</td>
<td>212</td>
<td>290</td>
<td>0.37</td>
<td>0</td>
<td>900</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Normal 12-17</td>
<td>0.5-1.5</td>
<td>200-1000</td>
<td>200-600</td>
<td>0.4-1.4</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* Intramuscularly.
+ Oral.

larity with decreased myeloid-erythroid ratio, frankly megaloblastic erythropoiesis and adequate marrow iron stores in all three.

In Table 1b are listed the biochemical and dietary data obtained in these patients. Prior to therapy, all patients had low plasma and RBC vitamin $B_12$ levels, while plasma folate concentration was normal. RBC folate was low in W.E. and normal in the other patients. Interestingly, only W.E. excreted $\text{FiGlu}$.

All patients excreted MMA in their urine. The daily amount of MMA excreted was variable from day to day in individual patients and was dissimilar when each patient was compared to the other. This variation in daily excretion of MMA and the lack of correlation between the magnitude of MMA excretion and the clinical or hematological parameters of vitamin $B_12$ deficiency have been documented in prior studies.4,16

Plasma ascorbate concentration was below the normal range in all patients even though W.E. and E.H. were ingesting sufficient vitamin C to ensure normal plasma ascorbate levels.

Patient Y.Q. had anorexia and weight loss prior to admission and her initially low plasma ascorbate activity might have been related to lack of intake of vitamin C.

All patients had absolute achlorhydria even after Ewald stimulation. Other laboratory studies obtained included upper gastrointestinal x-rays, chest x-rays, liver function and renal function studies, which were normal in all three patients.

Schilling tests revealed malabsorption of vitamin $B_12$ due to the absence of intrinsic factor in all three patients. These data indicate that the cause of the vitamin $B_12$ deficiency demonstrated in these patients was pernicious anemia.
Table 3a.—Hematological and Biochemical Data following Therapy

PATIENT W. E.

<table>
<thead>
<tr>
<th>Day of Therapy</th>
<th>HGB g.%</th>
<th>Retic %</th>
<th>Plasma B12 ng./ml.</th>
<th>RBC B12 ng./ml.</th>
<th>Plasma Vit C mg.%</th>
<th>Urine MMA (24 hr.)</th>
<th>Vit B12 mg/kg/day</th>
<th>Therapy mg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (5 days)</td>
<td>6.8-7.5</td>
<td>0.3-0.6</td>
<td>58-102</td>
<td>170-200</td>
<td>0.1</td>
<td>1+-3+</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>7.0</td>
<td>0.7</td>
<td>130</td>
<td>190</td>
<td>0.1</td>
<td>2+</td>
<td>900 (day 1-103)</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>7.0</td>
<td>1.1</td>
<td>148</td>
<td>200</td>
<td>0.1</td>
<td>2+</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>6.8</td>
<td>2.7</td>
<td>148</td>
<td>180</td>
<td>0.18</td>
<td>2+</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>6.7</td>
<td>5.6</td>
<td>212</td>
<td>190</td>
<td>0.18</td>
<td>2+</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>21</td>
<td>10.4</td>
<td>1.4</td>
<td>190</td>
<td>380</td>
<td>0.14</td>
<td>1+</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>42</td>
<td>11.7</td>
<td>0.6</td>
<td>196</td>
<td>360</td>
<td>0.25</td>
<td>1+</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>68</td>
<td>12.4</td>
<td>—</td>
<td>196</td>
<td>280</td>
<td>±</td>
<td>—</td>
<td>900</td>
<td>75</td>
</tr>
<tr>
<td>103</td>
<td>13.7</td>
<td>—</td>
<td>380</td>
<td>290</td>
<td>0.56</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal 12-17</td>
<td>0.5-1.5</td>
<td>200-1000</td>
<td>200-600</td>
<td>0.4-1.4</td>
<td>0</td>
<td>—</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Table 3b.—Plasma and RBC Folate Activity following Therapy

PATIENT W. E.

<table>
<thead>
<tr>
<th>Day of Therapy</th>
<th>Plasma Folate ng./ml.</th>
<th>RBC Folate ng./ml.</th>
<th>Urine MMA</th>
<th>Plasma Vit C mg.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>8.0-13.5</td>
<td>100-140</td>
<td>1+-3+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>161</td>
<td>2+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>7</td>
<td>7.0</td>
<td>211</td>
<td>2+</td>
<td>0.18</td>
</tr>
<tr>
<td>21</td>
<td>7.4</td>
<td>264</td>
<td>1+</td>
<td>0.14</td>
</tr>
<tr>
<td>42</td>
<td>5.4</td>
<td>213</td>
<td>1+</td>
<td>0.25</td>
</tr>
<tr>
<td>68</td>
<td>8.8</td>
<td>344</td>
<td>±</td>
<td>—</td>
</tr>
<tr>
<td>103</td>
<td>12.0</td>
<td>—</td>
<td>0</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Post Treatment

Results obtained following therapy are given in Tables 2, 3, 3b, 4 and Fig. 1.

Table 2 lists data obtained on patient Y.Q. Initially this woman was given daily 1 µg. injections of vitamin B12. Reticulocytosis occurred and by the 9th day the plasma vitamin B12 activity became normal. On day 12, the daily dose of parenteral vitamin B12 was increased to 10 µg. By this time, the RBC vitamin B12 activity had not become normal. With continued therapy, it rose to normal by day 15. During this period the plasma ascorbate concentration remained sub-normal despite adequate intake of vitamin C and significant amounts of MMA were detected in 24 hour urine specimens. On the 18th day, daily oral vitamin B12 therapy was begun. In patients with pernicious anemia given large oral doses of vitamin B12, one may assume that 1 per cent of the ingested dose is absorbed. Therefore, in this and subsequent patients, the daily absorbed dose of vitamin B12 is assumed to be approximately 9 µg.

As therapy continued, the hemoglobin concentration became normal. However, MMA excretion continued and plasma ascorbate concentration remained low despite the fact that daily 200 mg. doses of vitamin C were given from day 12 through day 29. Plasma ascorbate reached the low normal range by day 49 and at this time MMA excretion was not detected.

In Table 3a are listed data obtained on W.E. This man was treated with...
daily oral 900 μg doses of vitamin B₁₂. The data demonstrate that the plasma ascorbate concentration remained low, despite adequate vitamin C intake, until sufficient vitamin B₁₂ had been absorbed to abolish MMA excretion. In a manner similar to the first patient, plasma and RBC vitamin B₁₂ activity returned to normal before MMA excretion was abolished. Table 3b lists changes in plasma and RBC folate concentration in this patient. Following institution

Fig. 1.—Hematologic and biochemical changes in patient E. H. following vitamin B₁₂ therapy.
of vitamin B\textsubscript{12} therapy, plasma folate levels fell and RBC folate activity rose. Both plasma and RBC folate activity returned to normal before vitamin C activity became normal.

Results of therapy in patient E.H. are listed in Table 4 and graphically presented in Fig. 1. Because of congestive heart failure secondary to hypertension and anemia, this patient was given 3 units (750 ml. total) of packed RBC prior to vitamin B\textsubscript{12} therapy. This caused the RBC vitamin B\textsubscript{12} activity to become normal although there was no significant change in plasma vitamin B\textsubscript{12} level. Following institution of oral vitamin B\textsubscript{12} therapy, reticulocytosis occurred and subsequently plasma vitamin B\textsubscript{12} activity became normal. By day 19, hemoglobin concentration, plasma and RBC vitamin B\textsubscript{12} activity were normal although MMA excretion persisted and plasma ascorbate concentration remained generally below normal. Significant excretion of MMA persisted throughout the 89 days of oral vitamin B\textsubscript{12} therapy. When oral vitamin B\textsubscript{12} therapy was stopped and the excretion of MMA checked 7 days later (day 96), significant quantities of MMA were detected. At this time, hemoglobin concentration, plasma and RBC vitamin B\textsubscript{12} activity were normal. One thousand \textmu g. of I.M. vitamin B\textsubscript{12} were given on day 96 and 124. Despite these injections of vitamin B\textsubscript{12}, MMA excretion persisted and on day 152 plasma ascorbate concentration was still below normal despite an adequate intake of vitamin C. On day 152, another parenteral injection of vitamin B\textsubscript{12} was given and when MMA excretion was checked 4 weeks later (day 180), only a faint trace of MMA was detected. At this time plasma ascorbate concentration was normal. Subsequently, daily 1000 \textmu g. parenteral injections of vitamin B\textsubscript{12} were given for 14 days. No further MMA excretion was found and plasma ascorbate concentration remained within the normal range.

\section*{Discussion}

The data obtained in this study document an interrelationship between the adequacy of vitamin B\textsubscript{12} stores and the maintenance of a normal plasma ascorbate concentration. Other investigators have demonstrated similar abnormalities of vitamin C metabolism in vitamin B\textsubscript{12} deficient patients.\textsuperscript{1,2} Cox et al.\textsuperscript{2} noted low plasma ascorbate levels and rapid ascorbate clearance in vitamin B\textsubscript{12}-deficient patients, most of whom were ingesting sufficient vitamin C to ensure normal plasma ascorbate levels and normal ascorbic acid clearance. These investigators also noted a delay in the return to normal of ascorbate metabolism following vitamin B\textsubscript{12} repletion therapy. The present study confirms the above observations and demonstrates that abnormalities of ascorbate metabolism persist in vitamin B\textsubscript{12}-deficient patients until MMA excretion is abolished.

MMA excretion has been detected in vitamin B\textsubscript{12}-deficient human beings\textsuperscript{4,5,14,18} and is the last abnormality to disappear in patients undergoing replacement therapy.\textsuperscript{4} Specifically, following institution of gradual repletion therapy, reticulocytosis, rise in hemoglobin concentration and plasma vitamin B\textsubscript{12} level are seen. Depending upon the rapidity of replacement therapy, MMA excre-
tion will persist for a period of time until further vitamin B₁₂ therapy causes its abolition.⁴

The data obtained in this study again document these findings. This suggests that the continued excretion of MMA indicates suboptimal replenishment of vitamin B₁₂ stores. Evidence in favor of this hypothesis is the fact that the most specific method of abolishing MMA excretion is the administration of vitamin B₁₂.⁴,¹⁶ It has also been shown that the repetitive administration of large doses of vitamin B₁₂ will abolish MMA excretion much more rapidly than will small dose gradual repletion therapy.⁴ However, further data are required which correlate MMA excretion with hepatic stores of vitamin B₁₂ before it can be definitively concluded that continued MMA excretion indicates suboptimal repletion of vitamin B₁₂ stores.

The study reported herein offers a possible explanation for the previously mentioned findings of Cox et al.² These investigators noted a delay of 7–14 days in the return of the ascorbate abnormalities following large I.M. doses of vitamin B₁₂. Since MMA excretion persists for periods of 2–15 days following administration of large doses of vitamin B₁₂,⁴ the data suggest that the ascorbate abnormalities would remain abnormal until MMA excretion is abolished by prolonged vitamin B₁₂ therapy. Our study indicates that subnormal plasma ascorbate persists until sufficient vitamin B₁₂ has been given to abolish MMA excretion.

There is no adequate explanation for the ascorbate abnormalities described. In otherwise normal human beings, a normal plasma ascorbate concentration is dependent upon an adequate intake and absorption of vitamin C.⁹ Two of our patients were ingesting enough vitamin C prior to and following vitamin B₁₂ therapy to maintain an adequate plasma ascorbate concentration. The only patient (Y.Q.) who had not been ingesting sufficient vitamin C prior to therapy was given a two week course of 200 mg. of vitamin C daily. Despite this therapy, her plasma ascorbate did not return to normal until vitamin B₁₂ therapy abolished MMA excretion. Inflammation, malignancy, stress, surgery and pregnancy may depress the plasma ascorbate level.²⁰ None of these phenomena were found in our patients. It might be suggested also that the presence of MMA in the plasma (which is inferred by its excretion in the urine) might interfere with the assay of vitamin C. Recovery experiments were performed and no interference detected. Impaired absorption of vitamin C or excessive excretion of the vitamin are also possible explanations for the findings. However, studies of absorption and excretion were not performed at the time of the original study. In no patient was there clinical evidence of any malabsorption. The recovery of the vitamin C level following vitamin B₁₂ therapy suggests that if excessive excretion of vitamin C were the mechanism of depression of plasma ascorbate level, then this is somehow related to MMA excretion. Further study of this possibility seems indicated.

Smith²¹ suggested an antioxidant property for vitamin B₁₂, postulating that deficiency of vitamin B₁₂ might lead to a rapid conversion of reduced ascorbate to oxidized ascorbate (dehydroascorbate). Since in our study total plasma ascorbate (oxidized and reduced) was measured and low levels detected, the
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antioxidant effect of vitamin B12 does not appear to explain the abnormal vitamin C metabolism noted in these patients. Cox et al. measured reduced and oxidized ascorbate separately and found no abnormal conversion of reduced to oxidized ascorbate in vitamin B12-deficient patients.

Rats made deficient in vitamin E and some premature infants with vitamin E deficiency excrete MMA. Vitamin B12 administration abolished the MMA excretion although administration of vitamin E reduces the excretion but does not completely abolish it. It is interesting that vitamin E and vitamin C are active in redox reactions. It is conceivable that the low plasma levels of vitamin C found in the patients herein reported may be related to excessive tissue utilization of vitamin C created in part by excessive production of MMA.

Despite the low levels of plasma ascorbate found in these patients, no evidence of scurvy was noted. In this study and the one of Cox et al., tissue levels of vitamin C were not measured. However, the plasma ascorbate level has been used by many investigators as an index of adequacy of vitamin C nutrition. This study suggests that plasma vitamin C levels might not accurately reflect the adequacy of vitamin C nutrition in patients with vitamin B12 deficiency. It may well be that these patients are actually vitamin C-deficient but this problem cannot be resolved until tissue levels of vitamin C are measured in vitamin B12-deficient patients.

Abnormalities in vitamin C metabolism have been demonstrated in patients with folate deficiency. Folate abnormalities were detected in one patient in our study (W.E.). This patient had a high normal plasma folate level, a RBC folate level below the lower limits of normal and excreted FICl following a histidine load. Similar abnormalities have been noted in vitamin B12-deficient patients by others. The actual cause of these folate abnormalities is speculative and debated. However, of three patients studied, only W.E. had a RBC folate level below the lower limit of normal and this patient excreted FICl while the others did not. Since not all patients with vitamin B12 deficiency excrete FICl, it might be interesting to correlate RBC folate activity with FICl excretion. However, in this patient absolute deficiency of folate did not exist in view of the normal plasma folate activity. Serial measurement of plasma and RBC folate concentration following vitamin B12 therapy in this patient and in the others revealed a fall in plasma folate and a rise in RBC folate before MMA excretion was abolished and plasma vitamin C levels became normal. These data imply that the low plasma ascorbate concentration was not directly related to abnormal folate metabolism.

One of the purposes of this study was the evaluation of RBC vitamin B12 activity as an index of body stores of vitamin B12 in patients undergoing replacement therapy. Herbert has demonstrated that as vitamin B12 deficiency develops, serum vitamin B12 activity falls first and is slowly followed by RBC vitamin B12 level. Kelly and Herbert also reported finding two patients with low serum vitamin B12 levels and normal RBC vitamin B12 activity. They suggest that RBC vitamin B12 activity in man may more accurately reflect tissue stores of vitamin B12 than does serum vitamin B12 activity. Similar conclusions are suggested by the data reported herein in the two patients in whom this
measurement was possible. In both Y.Q. and W.E., initially low plasma vitamin B₁₂ activity returned to normal before RBC vitamin B₁₂ levels. However, MMA excretion persisted despite normal RBC vitamin B₁₂ activity in these patients. These and other data⁴ lead to the suggestion that MMA excretion may be a more sensitive index of the adequacy of vitamin B₁₂ stores than the RBC or plasma vitamin B₁₂ levels. It may be that the deficiency of vitamin B₁₂ per se leads to apoenzyme deficiency of methylmalonate isomerase. Under these circumstances, continued MMA excretion would reflect apoenzyme deficiency and not deficiency of body stores of vitamin B₁₂. Until MMA excretion is correlated with hepatic vitamin B₁₂ content, this question must remain unanswered.

**Summary**

An interrelationship between vitamin C and vitamin B₁₂ was studied in three patients with vitamin B₁₂ deficiency associated with pernicious anemia. Subnormal plasma ascorbate concentrations were found prior to therapy confirming previous observations. Following vitamin B₁₂ administration and utilizing methylmalonate (MMA) excretion as a biochemical index of vitamin B₁₂ deficiency, low plasma ascorbate concentrations persisted until MMA excretion was abolished. In two patients, RBC vitamin B₁₂ activity was also serially measured in order to evaluate its sensitivity as an index of vitamin B₁₂ stores when compared to MMA excretion. The data demonstrate that in these two vitamin B₁₂-deficient patients undergoing slow repletion therapy, RBC vitamin B₁₂ activity returns to normal before MMA excretion is abolished.

Whether continued MMA excretion in these patients indicates a greater sensitivity of MMA excretion as an index of deficiency of vitamin B₁₂ stores than does RBC vitamin B₁₂ activity remains to be answered by future work.

**SUMMARIO IN INTERLINGUA**

Le relation inter vitamina C e vitamina B₁₂ esseva studiata in tres patientes con carentia de vitamina B₁₂ associate con anemia perniciose. Concentrationes infranormal de ascorbato plasmatic esseva trovate ante le therapia in confimation de previe observationes. Post le administration de vitamina B₁₂, e con le uso del excretion de methylmalonato (MMA) como indice biochimic del carentia de vitamina B₁₂, basse concentrationes plasmatic de ascorbato persisteva usque le excretion de MMA esseva abolite. In duo patientes, le activitate erythrocytic de vitamina B₁₂ esseva, in plus, mesurate serialmente, con le objectivo de evaluare su sensibilitate como indice del reservas de vitamina B₁₂ in comparation con le excretion de MMA. Le datos demonstra que in iste duo patientes a carentia de vitamina B₁₂ subjicite a un therapia a lente repletion. le activitate erythrocytic de vitamina B₁₂ retorne al norma ante que le excretion de MMA es abolite.

Si o non le excretion de MMA in iste patientes indica que le sensibilitate del excretion de MMA es plus grande que illo del activitate erythrocytic de vitamina B₁₂ como indice del carentia del reservas de vitamina B₁₂ es un question que debe esser resolvite per investigaciones futur.

**REFERENCES**


Metabolic Interrelationship between Vitamin B\textsubscript{12} and Ascorbic Acid in Pernicious Anemia

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