HEMATOLOGIC EFFECT OF 5,6-DIMETHYL-
BENZIMIDAZOXYL COBAMIDE COENZYME
IN A PATIENT WITH PERNICIOUS ANEMIA

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Since 5,6-dimethylbenzimidazolyl cobamide coenzyme (coenzyme B₁₂) as one of the
coenzyme forms of vitamin B₁₂ was reported by Barker et al. (1) for the first time in
1960, a good deal of works have been carried out on its biochemical and physiological
roles (2—8). The recent knowledge concerning the coenzyme has revealed that it is
an active form of vitamin B₁₂ (5,6-dimethylbenzimidazolyl cobamide cyanide, CN-B₁₂),
which has been applied to the clinical use for 15 years, and that CN-B₁₂ itself
does not participate in the enzymatic reactions unless converted into the coenzyme
form (1, 10), although its intestinal absorption mechanism is almost similar to that of
the cyanide form in terms of intrinsic factor dependency (4—8). It would be reason-
able that these observations have led us to the idea that the parenteral administration
of coenzyme B₁₂ must be one of the most effective way to treat the pernicious patient
and that it is worth-while trying to investigate the clinical effectiveness of coenzyme
B₁₂.

This paper deals with our experience on a case of pernicious anemia treated with
coenzyme B₁₂.

EXPERIMENTAL

Method and Materials

Crystalline 5,6-dimethylbenzimidazolyl cobamide coenzyme was dissolved aseptically in
the physiological saline solution under the dim light and made up to give the con-
centration of 0.5 mg/ml. The concentration of the coenzyme in this solution was
determined on the basis of molar extinction by means of Beckman type spectrophoto-
meter and the coenzyme activity was also checked by the enzymatic assay method
according to Abeles and Lee's method (11, 12). An aliquot of this stock solution
kept under —20°C was made up to contain 5 µg of coenzyme B₁₂ in 2 ml just before
use, and injected intramuscularly under the dim light.

The hematological, isotopic and laboratory tests were carried out according to
our routine method (13).

1 内野治人，矢切良雄，吉野俊昭，脇坂行一.
A patient with pernicious anemia in relapse belonging to our outpatient clinic was selected for the treatment.

Case — K. Ka., 62 years old, Japanese male. The patient complained of anemia, numbness of the extremities and burning sensation of the tongue since a couple of months. He had good appetite, but sometimes loose bowels.

Physical Examinations: He was well developed but ill-nourished. Puls was 72, regular and somewhat weak. The tougue was dry and atrophic, and had many rhagades. The heart was not enlarged but the heart sound was somewhat faint. There were no murmur and venous hum. The lung was clear. The liver was palpable two fingers width below the right costal margin and not tender. The spleen and kidney were not palpable. The abdominal reflexes were decreased at both sides. There was no edema of the extremities. The patellar reflexes were hypoactive but the Achilles reflexes were normal. No pathological reflexes were noted. The sensory examination was normal.

Laboratory Findings: Urinalysis showed that both albumin and urobilinogen tests were negative. Stools were found to be free from the ova of parasites and were negative for occult blood. Examination of the peripheral blood revealed 1.42 million of red blood cell count 3,900 of white blood cell count, 46% (Sahli) of hemoglobin content, 0.5% of reticulocytes, 1.6 of color index, 2.2 of volume index, 199 μ3 of mean corpuscular volume and 9.66 μ of mean corpuscular diameter. That was hyperchromic macrocytic anemia. Bone marrow examination could not be performed. The liver function test showed that the icteric index was 6, total serum bilirubin 1.5 mg/100 ml, direct serum bilirubin 0.7 mg/100 ml, thymol turbidity test 1 or 2 unit, serum colloid stability test as cobalt reaction 4 and cadmium reaction 7, bromsulfalein test 5% after 30 min, glutamic-oxaloacetic transaminase below 8, and glutamic-pyruvic transaminase below 5. The gastric juice analysis revealed no free hydrochloric acid before and after the stimulation with histamine injection. The gastric mucosa was found to be completely atrophic by the gastrocamera and the suction biopsy. Serum iron content was 157 μg/100 ml and serum copper 131 μg/100 ml. The examination of serum vitamin B12 content with Leichmannii assay method showed 110 μg/100 ml and the absorption test using the hepatic uptake method of Co60-labeled vitamin B12 showed the complete abolishment of absorption in 17 days without hog intrinsic factor administration, but 10.3% absorption in 10 days with intrinsic factor.

RESULTS

The patient was diagnosed to be pernicious anemia in relapse owing to these physical and laboratory findings. At first, the patient was intramuscularly injected with a single dose of 5 μg of coenzyme B₁₂, and then, after 17 days, injected twice with the single dose of 10 μg of coenzyme B₁₂ at the interval of a week.

The hematological examinations were used for the criteria of the effectiveness of the treatment. Table I shows the summarized hematological findings. The increase of reticulocytes began 3 days after the single administration of 5 μg of coenzyme B₁₂ and reached the maximal value after 7 days, amounting to 5%. The examination of the peripheral blood showed 2.25 million of red blood cell count, 64% of hemoglobin content, 1.4 of color index, 1.8 of volume index, 162 μ₃ of mean corpuscular volume.
Table I

Hematologic Effect of Coenzyme B₁₂ in a Patient with Pernicious Anemia in Relapse

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after administration</th>
<th>RBC</th>
<th>Hb</th>
<th>Ht</th>
<th>Ret</th>
<th>CI</th>
<th>VI</th>
<th>SI</th>
<th>MCH</th>
<th>MCV</th>
<th>MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 2</td>
<td>Before</td>
<td>1.42</td>
<td>46</td>
<td>28.0</td>
<td>0.5</td>
<td>1.6</td>
<td>2.2</td>
<td>0.8</td>
<td>53</td>
<td>199</td>
<td>30</td>
</tr>
<tr>
<td>Nov. 7</td>
<td>5 µg of coenzyme B₁₂ administered intramuscularly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 8</td>
<td>1 day</td>
<td>1.51</td>
<td>52</td>
<td></td>
<td>0.6</td>
<td>1.7</td>
<td></td>
<td></td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 10</td>
<td>3 days</td>
<td>1.60</td>
<td>58</td>
<td>30.0</td>
<td>1.6</td>
<td>1.8</td>
<td>2.1</td>
<td>0.8</td>
<td>56</td>
<td>187</td>
<td>30</td>
</tr>
<tr>
<td>Nov. 14</td>
<td>7 days</td>
<td>2.15</td>
<td>63</td>
<td></td>
<td>5.0</td>
<td>1.4</td>
<td></td>
<td></td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 24</td>
<td>17 days</td>
<td>2.25</td>
<td>64</td>
<td>36.5</td>
<td>0.8</td>
<td>1.8</td>
<td>0.8</td>
<td>45</td>
<td>162</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Dec. 6</td>
<td>10 µg of coenzyme B₁₂ administered intramuscularly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec. 8</td>
<td>2 days</td>
<td>2.92</td>
<td>83</td>
<td>41.0</td>
<td>1.6</td>
<td>1.4</td>
<td>1.6</td>
<td>0.9</td>
<td>44</td>
<td>141</td>
<td>26</td>
</tr>
<tr>
<td>Dec. 13</td>
<td>7 days</td>
<td>3.05</td>
<td>82</td>
<td>42.5</td>
<td>0.4</td>
<td>1.3</td>
<td>1.5</td>
<td>0.8</td>
<td>42</td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>Dec. 15</td>
<td>10 µg of coenzyme B₁₂ administered intramuscularly</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Dec. 20</td>
<td>2 days</td>
<td>3.07</td>
<td>76</td>
<td>39.0</td>
<td>1.4</td>
<td>1.4</td>
<td>1.0</td>
<td>0.8</td>
<td>37</td>
<td>127</td>
<td>30</td>
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<tr>
<td>Dec. 24</td>
<td>7 days</td>
<td>3.68</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan. 4</td>
<td>42 days</td>
<td>3.69</td>
<td>84</td>
<td>44.0</td>
<td>1.0</td>
<td>1.3</td>
<td>1.3</td>
<td>0.8</td>
<td>36</td>
<td>119</td>
<td>30</td>
</tr>
</tbody>
</table>

RBC, red blood cell count; Hb, hemoglobin content; Ht, hematocrit; Ret, reticulocytes; CI, color index; VI, volume index; SI, saturation index; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCC, mean corpuscular hemoglobin concentration.

As the hematological recovery was not enough after 2 weeks, the second administration of 10 µg of coenzyme B₁₂ was followed. The reticulocytes increased to 1.6% again 2 days after the second injection of 10 µg and red blood cell count reached to 3.08 million after 7 days. After the injection of 25 µg of coenzyme B₁₂ as a total amount the hematological findings disclosed almost normal values, namely, 3.69 million of red blood cell count, 84% of hemoglobin content, 1.1 of color index, 1.3 of volume index, and 119 µ₃ of mean corpuscular volume. Fig. 1 shows the change of red blood cell diameter of this patient before, during and after the administration of

![Fig. 1 Red Cell Diameter of a Patient with Pernicious Anemia in Relapse Before, During and After the Administration of Coenzyme B₁₂](image-url)

Subject: K.K., 62 years old, m:le.
Red cell diameter: ---, before, 9.66 µ; -----, during, 9.20 µ; ----, after, 8.04 µ.
coenzyme $B_{12}$. The mean red cell diameter, which was 9.66 $\mu$ before the treatment, decreased in the course of treatment, and showed 8.04 $\mu$ after the treatment. The clinical course of the hematological recovery was depicted in Fig. 2.

**DISCUSSIONS**

Wasserman et al. (2) stated in their short communication in 1960, when coenzyme $B_{12}$ was discovered and purified, that the daily administration of 1 $\mu$g of CN-$B_{12}$ as well as coenzyme $B_{12}$ to pernicious anemia patients resulted in almost the same hematological and clinical effectiveness. As a matter of fact, it appears difficult to compare the clinical effects of either forms of $B_{12}$ and demonstrate quantitatively whether coenzyme $B_{12}$ has the advantage over CN-$B_{12}$ or not. However, it is believed so far that coenzyme $B_{12}$ is approximately as effective on a molar basis as CN-$B_{12}$ (9). On the other hand, Campbell et al. (14) reported their diagram as to the relation between initial count, increase in red blood cells and dose of parenterally administered CN-$B_{12}$, according to their equation, $I_{15} = 0.912 - 0.313E + 0.65P$ ($I_{15}$ is the increase of RBC in 15 days, and $E$ the initial RBC and $P$ the logarithmic number of the parenteral dose in $\mu$g). As we described above, the increase of red blood cell count 2 weeks after the single injection of 5 $\mu$g of coenzyme $B_{12}$ was 0.83 million, while the expected increase with 4.06 $\mu$g of CN-$B_{12}$, corresponding to 5.0 $\mu$g of coenzyme $B_{12}$ because of molecular weight ratio (1660 vs. 1350), could be calculated to be 0.86 million according to the Campbell's equation. It seems likely that this finding gives the proof of the almost equivalent hematologic potency of coenzyme $B_{12}$ and CN-$B_{12}$ on a molar basis, although this conclusion was drawn based on the observation of only one case.
It has been well established that the conversion of CN-B₁₂ into the coenzyme form is required before vitamin B₁₂ plays its role in the intermediate metabolism at the biochemical level (10, 15). The etiology of pernicious anemia is so far understood to be the B₁₂ deficiency due to the impaired absorption caused by intrinsic factor defect in the gastric juice, and not to be any metabolic defect. Therefore, it appears reasonable that the parenteral administration of either CN-B₁₂ or coenzyme B₁₂ gave the almost same hematologic effect, because the disturbed conversion of CN-B₁₂ to coenzyme B₁₂ did not exist in the perniciousa patient. It is not yet known whether there is such a case or not, that can be treated only with coenzyme form of B₁₂, not with CN-B₁₂, namely, whether there is a case of B₁₂ deficiency due to the disturbance of conversion of B₁₂ into the active form or not. Further investigations are awaited.

SUMMARY

A patient with pernicious anemia in relapse was treated with coenzyme B₁₂. The red blood cell count increased significantly following the single administration of 5 µg of coenzyme B₁₂. The hematologic effect of coenzyme B₁₂ was compared with the effect of CN-B₁₂ according to the reported data and was found to be almost equivalent in each other on a molar basis. The discussions was extended to the clinical application of coenzyme B₁₂ in future.

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REFERENCES