Brief Report

B₁₂ Binding Protein Deficiency in Pernicious Anemia

By Christine Lawrence

Most of the vitamin B₁₂ in normal serum is bound to an alpha-1 globulin seromucoid.¹⁻⁵ When vitamin B₁₂ is added to normal serum in vitro, however, it is bound predominantly to beta globulin.⁶⁻⁹ In some patients with pernicious anemia, when B₁₂ is added to serum it is bound in less than normal amounts. It has been possible to attribute this to deficient binding by the beta globulin.

Case Report

Case 1 was a 29-year-old housewife whose father had pernicious anemia and whose great-grandfather had an illness suggestive of pernicious anemia. She experienced increasing fatigue and exertional dyspnea for 6 months before admission, and nausea and vomiting for 2 months. She had a 5-month episode of polyarthritis at age 14, diagnosed as rheumatoid arthritis. There had been no joint symptoms for 8 years before hospitalization. On examination she appeared pale, had 2 fingerbreadth splenomegaly, and normal joints. Her hemoglobin was 4.5 Gm./100 ml., packed cell volume 13 per cent, WBC 3100/cu. mm. and platelets 62,000/cu. mm. A smear of her marrow showed megaloblastic erythropoiesis, and she had histamine-fast achlorhydria. The latex fixation test was negative. Prothrombin time was 16 sec.; serum calcium 9.8 and inorganic phosphate 4.9 mg./100 ml.; albumin 4.7 Gm. and globulins 2.8 Gm./100 ml. Examination of the stool for fat was negative. The results of the first Schilling test were unexpectedly characteristic of a malabsorptive disorder in that she excreted only 1 per cent of the administered cobalt₁₆⁻³⁰⁻¹⁻¹ in her urine in 48 hours when the B₁₂ was given with intrinsic factor. Repetition of the second part of the Schilling test 3 weeks later showed only 3 per cent excretion of the 0.5 μg. administered. After 13 monthly injections of 1 mg. B₁₂, there was no urinary excretion of cobalt₁₆⁻³⁰⁻¹⁻¹ when given without intrinsic factor, but 11 per cent excretion when given with intrinsic factor. Thus, following treatment, the Schilling test result became characteristic of patients with pernicious anemia. Her hemoglobin and packed cell volume values became normal 5 weeks after the inception of parenteral B₁₂ therapy.

Methods

Cobalt₁₆⁻³⁰⁻¹⁻¹ (B₁₂⁻¹⁻¹) was added to serum, and the amount which was bound to protein was estimated after adsorption of free B₁₂⁻¹⁻¹ with charcoal.¹⁰ Two and three-tenths ml. of a solution containing 4000 μg. of B₁₂⁻¹⁻¹ (specific activity 4 μc./μg.) in buffered saline (NaCl, 135 mM/L.; KCl, 5 mM/L.; NaHCO₃, 0.7 mM/L.; pH 7.3) was added to 1.5 ml. of serum, giving a final concentration of 2867 μg./ml. serum. After incubation for 30 minutes at 37 C., 1 ml. was removed for measurement of total radioactivity, and 100 mg. of charcoal (Norite A) were mixed with the remaining solution. Unbound B₁₂⁻¹⁻¹ was adsorbed by the charcoal, which was then removed by centrifugation.

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at 34,000 g for 20 minutes. The residual radioactivity in 1 ml of charcoal-free supernatant solution was determined at the same time as the total radioactivity of the untreated sample. The percent retained per ml of serum was calculated from the formula

\[
\text{percent retained} = \frac{\text{residual radioactivity}}{\text{total radioactivity}} \times 100.
\]

Sixty \( \mu \)l of each adsorbed supernatant solution were applied to each of 8 strips of Whatman 3MM filter paper and subjected to electrophoresis at pH 8.6 in barbital buffer (0.05 M) in a Beckman-Spinco cell. Serum proteins were stained with Buffalo blue-black on 2 strips, and homologous segments of the other 6 strips were pooled for determination of the distribution of B12 among the serum protein fractions. The 6 strips were cut into 28 one-quarter inch segments from the beginning of the albumin zone through the gamma globulin fraction, and the homologous segments were counted together in a sodium iodide well counter.

Serum protein concentration was estimated by a biuret method. Electrophoresis of serum for determination of the protein fractions was performed at pH 8.6 in barbital buffer (0.075 M) on Whatman 1 paper in a Beckman-Spinco cell.

RESULTS

In 50 normal sera, an average of 39 per cent of the added 2667 \( \mu \)g./ml. of B12 was retained after adsorption with charcoal. The binding of B12 by the sera of 10 patients with untreated pernicious anemia varied from 10 to 54 per cent (Table 1). After electrophoretic separation of B12-enriched normal sera, almost all of the B12 was located in the beta globulin zone. The pattern in the right of Figure 1, though obtained in a specimen from a patient with treated pernicious anemia, was characteristic of the pattern in normal sera. In most pernicious anemia sera before treatment, there was also a small fraction bound in the alpha-1 globulin zone (left in Figs. 1 and 2).

The B12 binding capacity of serum was estimated in these patients with pernicious anemia before and after 4 or more months of treatment with at least 1 mg. B12 i.m. each month. The serum B12 binding capacity increased after treatment in 6 of the 10 patients (Table 1, Cases 1-6). A change in the distribution of B12 among the serum protein fractions accompanied this quantitative change (Figs. 1 and 2). Beta globulin binding of B12 increased after treatment in all of the 6 patients whose total serum B12 binding capacity rose. The pattern exhibited by the serum of case 1 (Fig. 2), showing virtually no binding of B12 by beta globulin before treatment, thus far has been unique in our series.

Electrophoresis of paired serum specimens concurrently (Table 2) showed that the concentration of beta globulins was below normal in all the untreated patients, as previously noted by Neill and Weaver and van Dommelen et al. This abnormality reverted to normal after treatment with B12.

DISCUSSION

There are at least two B12-binding proteins (BP) in serum: a beta globulin and an alpha-1 globulin. The beta globulin binds almost all B12 added to normal serum in vitro, presumably because the alpha-1 globulin is nearly saturated with endogenous B12. In pernicious anemia serum, with its very low level of endogenous B12, the alpha-1 globulin is capable of binding significant amounts of added B12.
The present studies demonstrated that the binding of B₁₂ by the beta globulin BP was abnormal in patients with pernicious anemia. The deficiency of binding by beta globulin was, in fact, greater than would appear from the values for total serum binding (Table 1), because binding by alpha-1 globulin accounted for a large proportion of the total in the pretreatment sera. Treatment with parenteral B₁₂ was accompanied by a restoration of the beta globulin binding protein toward normal. Heller et al.⁹ had previously observed that B₁₂-deficient sera bound less total B₁₂ than did normal serum. They reported, however, that the capacity of B₁₂-deficient serum to bind added B₁₂ was normal. The present studies indicate that this is not always so.
Table 1.—Serum $B_{12}$ Binding Capacity in Patients with Pernicious Anemia

<table>
<thead>
<tr>
<th>Normal Controls (50)</th>
<th>385 ± 109 (1 SD)</th>
<th>39 ± 12 (1 SD)</th>
<th>1041 ± 317 (1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pernicious Anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>10</td>
<td>256</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>35</td>
<td>453</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>29</td>
<td>765</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
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<tr>
<td>5</td>
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<td>45</td>
<td>1192</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>71</td>
<td>808</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>42</td>
<td>1120</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>43</td>
<td>1158</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>57</td>
<td>1440</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>40</td>
<td>1070</td>
</tr>
</tbody>
</table>

*PCV = packed cell volume.
†B = before treatment with $B_{12}$, and A = after treatment with $B_{12}$.
1Serum $B_{12}$ level was assayed microbiologically using *L. leishmannii* in only 20 of the 50 normal subjects.

Table 2.—Serum Protein Values in Gm./100 ml. in Patients with Pernicious Anemia before and after Treatment with $B_{12}$

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Total Protein</th>
<th>Albumin</th>
<th>Alpha-1</th>
<th>Alpha-2</th>
<th>Beta</th>
<th>Gamma</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>7.5</td>
<td>7.2</td>
<td>4.7</td>
<td>3.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>6.9</td>
<td>8.6</td>
<td>4.8</td>
<td>4.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>7.6</td>
<td>8.6</td>
<td>4.9</td>
<td>5.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>8.8</td>
<td>3.3</td>
<td>4.0</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>6.7</td>
<td>7.8</td>
<td>3.1</td>
<td>3.5</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>7.9</td>
<td>3.7</td>
<td>3.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>6.7</td>
<td>7.6</td>
<td>3.6</td>
<td>3.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>8.4</td>
<td>8.9</td>
<td>5.3</td>
<td>4.7</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>6.4</td>
<td>7.0</td>
<td>4.1</td>
<td>3.3</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>6.4</td>
<td>7.0</td>
<td>4.4</td>
<td>3.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Mean for Cases 1–6: 6.8 ± 0.2, 4.1 ± 0.4, 0.3 ± 0.5, 0.5 ± 1.0, 0.6 ± 1.1, 1.3 ± 1.7
Mean for Cases 7–10: 6.8 ± 0.7, 4.4 ± 0.3, 0.4 ± 0.4, 0.4 ± 0.9, 0.7 ± 1.1, 1.2 ± 1.6
Mean for Cases 1–10: 6.9 ± 0.9, 4.2 ± 0.7, 0.3 ± 0.5, 0.5 ± 0.9, 0.6 ± 1.1, 1.3 ± 1.6
Mean for 200 normal controls: 7.0 ± 0.8, 3.8 ± 0.4, 0.4 ± 0.8, 0.9 ± 1.1

*The values for statistical significance determined by applying the “t test” to concentrations before (B) and after (A) treatment in all patients with pernicious anemia are: total protein $p < 0.01$, albumin $0.4 > p > 0.2$, alpha-1 globulin $p < 0.001$, alpha-2 globulin $p < 0.001$, beta globulin $p < 0.001$, gamma globulin $p = 0.1$. 
The beta globulin has been shown by Hall and Finkler to have an important $B_{12}$ transport function in vivo. When they administered $^{57}Co B_{12}$ to normal subjects either orally or intravenously, the $B_{12}$ appeared to localize first in the beta globulin fraction of serum, and only some hours later in the alpha-1 globulin fraction.

Our case 1 exhibited two remarkable abnormalities. She had a “malabsorptive pattern” Schilling test when first examined and her serum beta globulin bound virtually no $B_{12}$. Her achlorhydria and family history suggested that she had pernicious anemia. There were no laboratory findings suggestive of a generalized malabsorptive disorder. Potency of this lot of intrinsic factor was assured by the characteristic Schilling test patterns it yielded in other patients with pernicious anemia. A malabsorptive pattern Schilling test in two patients with untreated pernicious anemia has also been reported by Haurani, Sherwood and Goldstein, who found that the pattern became characteristic of pernicious anemia after their patients received extended treatment with $B_{12}$. An intriguing possibility is that the sera of our Case 1 and their two patients were unable to bind $B_{12}$ and hence to transport it from its site of gastrointestinal absorption. With restoration of beta globulin $B_{12}$ binding capacity toward normal, this malabsorption of $B_{12}$ regressed.

The finding of decreased serum beta globulin concentrations in the untreated patients (Table 2) raises the possibility that this is the explanation for defective $B_{12}$ binding. However, the similarity of the levels in Cases 1–6 and 7–10 suggests that a more specific abnormality or deficiency is operative in the former group.

**SUMMARY**

When radioactive $B_{12}$ was added to normal serum, it was bound predominantly to beta globulin. Deficient binding was observed in the serum of five of ten patients with untreated pernicious anemia. Treatment of these patients with $B_{12}$ restored the beta globulin $B_{12}$ binding capacity toward normal.

In one of the five patients there was virtually no beta globulin binding of added $B_{12}$ before treatment. This patient exhibited a malabsorptive pattern Schilling test when first seen. Treatment with $B_{12}$ was attended by an increase of beta globulin binding capacity, and by return of the ability to absorb oral $B_{12}$ when given with intrinsic factor. It is possible that these two abnormalities are related in that the beta globulin may have an important role in the absorption and transport of $B_{12}$.

**SUMMARIO IN INTERLINGUA**

Quando radioactive vitamina $B_{12}$ esseva addite a sero normal, il esseva trovate que illo se ligava predominantemente a globulina beta. Defectivitate de iste processo ligatori esseva observate in le sero de 5 de 10 patientes con non-tractate anemia pernicioso. Le tractamento de iste patientes con vitamina $B_{12}$ restaurava le capacitate ligatori pro vitamina $B_{12}$ in globulina beta verso valores normal.
In un del 5 pacientes practicamente nulle ligation de vitamina B_{12} per globulina beta occurreeva ante le tractamento. Iste paciente produceva un configuration de malabsorption in un test de Schilling al tempore de su prime examine. Le tractamento con vitamina B_{12} esseva accompaniate de un augmentate capacitae ligatori del globulina beta e del retorno del capacitae de absorber oral vitamina B_{12} quando isto esseva administrate insimul con factor intrinsec. Il pare possibile que iste duo anormalitates es interrelateate per le facto que globulina beta ha un rolo importante in le absorption e le transporto de B_{12}.

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