Antibody to Transcobalamin II and B₁₂ Binding Capacity in Patients Treated With Hydroxocobalamin

By Arne P. Skouby, Erik Hippe, and Henrik Olesen

Thirty-two patients treated for B₁₂ deficiency with one or two initial depot series of five i.m. injections of 1 mg hydroxocobalamin on alternate days followed by i.m. injection of 1 mg hydroxocobalamin every third month for maintenance therapy were examined after more than 2 yr of treatment. Antibody to TC II demonstrable after agar gel electrophoresis was detected in 5 of 12 patients given two depot series 1–3 mo apart, while antibody to TC II detectable only after the more sensitive immunoelectrophoresis occurred in 2 of 20 patients given one initial depot series or two series 6 or 12 mo apart. No anti-TC II was observed in serum from untreated patients with pernicious anemia.

Vitamin B₁₂ deficiency is usually treated by injection of either cyanocobalamin or hydroxocobalamin. In order to avoid the frequent injections required with aqueous solutions of cyanocobalamin, suspensions with depot effect have been introduced reducing the requirement to four injections a year. However, protracted normalization of serum B₁₂ can be obtained by using aqueous solutions of hydroxocobalamin at similar intervals.¹ ²

With both of the latter forms of therapy the body stores may be replenished and the serum level kept inside the normal range for the last several weeks of the intervals between injections. In the first weeks after injection of 1 mg hydroxocobalamin, higher values are obtained because of a better retention in plasma.

In some patients both forms of therapy have caused an increase of the cobalamin level in serum to values considerably above the normal range throughout the interval between injections. Following injections of cyanocobalamin-tannate suspended in aluminum monostearate-sesame oil (Betolevex), one reason for this increase has been shown to be the occurrence of antibody to transcobalamin II (TC II), resulting in an increased level of TC II in serum.³ ⁴ ⁵ Following treatment with hydroxocobalamin similar high values for cobalamin and total B₁₂ binding capacity apparently were caused by other mechanisms in two of three patients examined, as antibody to TC II was demonstrable in serum from one of the patients only.

This present study was performed in order to examine the frequency for
the occurrence of antibody to TC II in patients treated with hydroxocobalamin as described.²

**MATERIALS AND METHODS**

Hydroxocobalamin (Vibeden®) for therapy was from Gea A/S (Copenhagen). The ampules contained 1 mg aqueous hydroxocobalamin in 1 ml stabilized isotonic solution pH 4.5. One depot series included five injections of 1 mg hydroxocobalamin given i.m. on alternate days. Maintenance therapy was 1 mg hydroxocobalamin given i.m. at intervals of 3 mo.²

Serum for study was obtained from 32 patients with classical pernicious anemia, megaloblastic anemia following subtotal gastrectomy, or nonmegaloblastic anemia with low values for serum cobalamin activity before treatment. The samples for analyses were obtained partly at random and partly at fixed times after the injection of 1 mg hydroxocobalamin during the maintenance therapy. Further, serum for control experiments was obtained from nine patients with untreated pernicious anemia and 14 normal subjects.

The sera were divided into the following four groups: Group A sera were from 12 patients that had been given two depot series at an interval of 3 mo or less, followed by maintenance therapy for 2 or 3 yr; group B sera were from seven patients given two depot series at an interval of more than 3 mo followed by maintenance therapy for 2–4 yr; group C sera were from 13 patients given one depot series followed by maintenance therapy for up to 4 yr; group D sera were from nine patients with pernicious anemia before treatment was initiated.

57Co-labeled cyanocobalamin with a specific activity of 20–100 μCi/μg was obtained from the Radiochemical Centre, Amersham, England. The activity was measured in a well-type scintillation counter.

Serum B₁₂ activity in samples examined for binding capacity was measured by a modification⁶ of the method of Lau et al.,⁷ except for five of the results incorporated in Table 1. These were determined by microbiological assay using Lactobacillus leichmannii. All five were inside the ranges given in Table 1. The microbiological measurements generally used for control of the therapeutic response were kindly performed by Professor E. Hoff-Jørgensen of the Department of Biochemistry, Royal Dental College, Copenhagen. Unsaturated B₁₂ binding capacity (UB₁₂BC) was determined by the method of Gottlieb et al.⁸

Antibody to TC II was detected by adding 400 pg 57Co-labeled cyanocobalamin to 100 μl of serum. In most cases this was about twice the unsaturated binding capacity. The mixture was separated by agar gel electrophoresis, and the dried plates were subjected to radioautography. In subjects without anti-TC II, the 57Co-activity has α₂-β-mobility (TC II) and γ-mobility (free vitamin) as seen in Fig. 1B. The presence of anti-TC II results in complexes consisting of IgG-globulin, TC II, and 57Co-labeled cyanocobalamin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Depot Therapy</th>
<th>Antibody to TC II Demonstrated by Radioautography</th>
<th>No. of Cases</th>
<th>Range for Serum Conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2 Depot series</td>
<td>Immunoelctrophoresis: 0</td>
<td>7</td>
<td>180–464, 984–2518</td>
</tr>
<tr>
<td></td>
<td>Interval ≤ 3 mo</td>
<td>Agar Gel Electrophoresis: +</td>
<td>5</td>
<td>768–5451, 4222–14750</td>
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<tr>
<td>B</td>
<td>2 Depot series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interval ≥ 6 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1 Depot series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>No treatment</td>
<td></td>
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</tbody>
</table>

*Values are observed ranges 3 mo after injection of 1 mg hydroxocobalamin.
Fig. 1.—A, agar gel electrophoresis of normal human serum. B, radioautography of electrophoresis of mixture of normal human serum and $^{57}$Co-B$_{12}$. $^{57}$Co-activity is between the $\alpha$- and $\beta$-regions (TC II) and in $\gamma$-region (free vitamin). C, radioautography of electrophoresis of mixture of serum with antibody to TC II and $^{57}$Co-B$_{12}$. $^{57}$Co-activity is in fast $\gamma$-globulin region (complex of $^{57}$Co-B$_{12}$, TC II, and antibody to TC II). D, radioautography of immunoelectrophoresis of mixture as in C. Polyvalent antihuman serum was used. $^{57}$Co-activity is located in anodal part of IgG-globulin precipitation line.
These complexes have fast y-mobility (Fig. 1C). Immunelectrophoresis was also used for separation. The use of rabbit antibody to whole human serum resulted in more than 15 precipitation lines with the mixture of $^{57}$Co-B$_{12}$ and serum. By radioautography $^{57}$Co-activity was detected only in the anodal part of the IgG-globulin precipitation line in some sera, indicating the presence of antibody to TC II (Fig. 1D).

**RESULTS**

Antibody to TC II was detected in serum from seven of 32 patients treated with hydroxocobalamin, while it was not demonstrated in serum from untreated patients (Table 1) or in serum from 14 normal subjects.

In group A, five sera out of the 12 contained antibody to TC II to be visualized by radioautography of both the immunelectrophoresis and the agar gel electrophoresis. In group C, two of the 13 sera had antibody to TC II, but in these sera antibody could be demonstrated only by radioautography of the immunelectrophoresis. In groups B and D, antibody was not demonstrated by the methods used.

*Serum B$_{12}$ and UB$_{12}$BC*

In the five patients with antibody to TC II in group A, the level of serum cobalamin 3 mo after an injection of 1 mg hydroxocobalamin was higher than in patients with no antibody to TC II, and the values for the UB$_{12}$BC were considerably above the ones recorded in other patients and normals. This was not so for sera from two patients in group C in whom the antibody was detectable only by radioautography of the immunelectrophoresis (Table 1). In Fig. 2 the variations with time for serum B$_{12}$ and UB$_{12}$BC are shown for four patients with antibody to TC II from Group A after cessation of therapy. In Fig. 3 values obtained during maintenance therapy are shown for the two patients from group C with antibody to TC II detectable only by immunelectrophoresis; they are similar to the ones for patients without antibody to TC II.

**DISCUSSION**

The present investigation indicates that an initial overloading of the organism with hydroxocobalamin can induce a production of antibody to TC II at nearly the same frequency as treatment with a depot preparation of cyanocobalamin (Betolvex). Thus, with Betolvex anti-TC II was detected in eight out of 15 patients, while it occurred in seven out of 32 patients in the present study. However, one depot series of hydroxocobalamin or two depot series with more than 3 mo interval followed by i.m. injection of 1 mg hydroxocobalamin every third month induced a production of antibody to TC II detectable by immuneelectrophoresis in only two out of 20 patients and by agar gel electrophoresis in none. Thus, the occurrence of antibody appeared to be correlated to the intensity of the initial depot treatment with hydroxocobalamin. Whether antibody to TC II can be entirely avoided by omitting the initial depot treatment remains to be studied, as does the possible effect of aqueous cyanocobalamin.

Abnormally high levels of serum cobalamin and UB$_{12}$BC occurred only in
serum from patients with antibody to TC II demonstrable by both of the methods used. This may be due to an overloading with hydroxocobalamin and/or production of antibody to TC II in a scale surpassing that obtained in other patients. Antibody to TC II produced during treatment with a depot preparation of cyanocobalamin (Betolvex) retained in the plasma pool cobalamin bound to TC II, leading to increased cobalamin levels and also causing elevated values for UB12BC. Similar experiments were not performed in the present study. However, repeated determinations of serum cobalamin and UB12BC during treatment with hydroxocobalamin in patients with antibody to TC II, demonstrable by agar gel electrophoresis, disclosed no definite correlation between values for serum cobalamin and the values for UB12BC measured in vivo (Fig. 2).

Furthermore, nothing indicated a hampered distribution of cobalamin from
the plasma pool during the weeks after injection of hydroxocobalamin or other untoward effects ascribable to an increased production of antibody to TC II.

REFERENCES


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