Hydroxocobalamin

IV. Biological Half-Life of Hydroxocobalamin in the Human Liver

By George B. Jerzy Glass and Duk Ho Lee

That vitamin B₁₂ has a long biological half-life in the human liver became apparent over 10 years ago, when it was observed that 3 months after injecting radioactive cyanocobalamin to man, the surface radioactivity over the liver only declined by 6–15 per cent.¹,² These findings, further expanded and confirmed by other workers³–⁵ and in our laboratory⁶–⁸ suggested that the biological half-life of cyanocobalamin was about one year,⁵,⁷,⁸ with individual variations ranging from 4.7 to 29.4 months.⁷ This prolonged half-life was also demonstrated by whole body counting⁶,¹⁰ and in studying the metabolic turnover of labeled and nonradioactive cyanocobalamin in man.¹¹,¹²

One of the cobalamin analogues, hydroxocobalamin (aquacobalamin, vitamin B₁₂₉) is more slowly absorbed from the site of injection,¹³–¹⁸ more slowly excreted in the urine,¹³–¹⁵,¹⁷–²¹ and builds much higher and more prolonged vitamin B₁₂ blood levels than cyanocobalamin.¹³,¹⁴,¹⁶,¹⁸,²²–²⁴ These "depot-like" features depend on the tighter binding of hydroxocobalamin to body proteins, including serum,²⁵–²⁸ liver¹⁵,²¹,²⁷ and muscle proteins.¹⁶ A preliminary report²⁹ indicates that the overall biological half-life of hydroxocobalamin is similar to that of cyanocobalamin, when measured by whole body counting. No data are available, to the best of our knowledge, on the biological half-life of hydroxocobalamin in the human liver, which does not necessarily have to be the same as in the rest of the body.³⁰

Materials and Methods

Hepatic Half-Life of Hydroxocobalamin

Five volunteers (Cases 1–5) were used for these studies. They ranged from 50–60 years of age, were hospitalized at Bird S. Coler Hospital and Home for sequelae of cerebrovascular accidents, or hip fracture, were in good health and free of cardiac, renal or hepatic disorders. At intervals of 1–10 days, each patient received an injection of a sterile solution of (a) Co⁵⁷-hydroxocobalamin (sp. act. 1.22–1.98 μC./μg.), and (b) Co⁹⁶-cyanocobalamin (sp. act. 0.78–0.85 μC./μg.). In 3 individuals, both injections were given intravenously; in 2 others, intramuscularly. Doses of both injected materials, measured precisely by means of tuberculin syringes, were similar and were 2.0–2.5 μg. in 4 individuals. Radiocobalamins were made available by the Merck Sharp & Dohme Research Laboratory, Inc., West Point, Pa.

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activity of Co\textsuperscript{57}-hydroxocobalamin injected in these 4 subjects was 2.4–4.0 μc., and that of Co\textsuperscript{60}-cyanocobalamin was 1.0–2.0 μc. In one subject, the dose of Co\textsuperscript{57}-hydroxocobalamin injected was only 0.13 μc. of high specific activity, and containing 2.0 μc. of Co\textsuperscript{57}.

Hepatic surface radioactivity was determined as previously.\textsuperscript{2,6–8} Three hepatic surface projections were scanned, and loci of maximal radioactivity were found in anterior mamillary, anterior-axillary and midaxillary lines. These were then marked by intracutaneous injections of 0.01 ml. sterile India ink, permitting exact duplication of the sites of hepatic counts over the entire period of this investigation. During counting procedure, patients were kept on a rigid board in left oblique supine position supported by sandbags, and counts were taken without collimation by a flash contact of the crystal parallel with the skin. Each of the 3 areas was counted until statistically significant counts were obtained (usually 10–15 minutes) with a thallium-NaI crystal (1½ by 1 in.), connected to a gamma spectrometer and scaler. The hepatic counts were averaged and air background was deducted. Co\textsuperscript{57} background counts were cut to 175–285 cpm, and Co\textsuperscript{60} to 30–40 cpm, by proper setting of the window and gain, with hepatic counts more than 10 times higher. Co\textsuperscript{57} does not count at the mev. setting of the Co\textsuperscript{60} peak, while Co\textsuperscript{60} is counted at the Co\textsuperscript{57} setting. Co\textsuperscript{57} counts had to be corrected, therefore, for Co\textsuperscript{60} contribution at Co\textsuperscript{57} mev. peak. The usual formula was applied with the use of Co\textsuperscript{60} and Co\textsuperscript{57} standards. This correction amounted to about 0.2 per cent of the Co\textsuperscript{60} counts at the Co\textsuperscript{60} setting. After all corrections, mean hepatic counts were recalculated per 1 μc. of material injected.

The overall duration of this study, for each subject, ranged from 31 to 59 weeks, from the time of injections. Hepatic counts were taken at intervals of 1 week, at the earlier, and 2 to 3 weeks at later stages of investigation.

The mean rate of clearance of Co\textsuperscript{57} and Co\textsuperscript{60} radioactivity from the liver was calculated in per cent per month as before: \textsuperscript{7} (a) from the initial and terminal corrected hepatic surface counts according to the formula:

\[ \frac{(A_1 - A_n) \times 100}{A_1 \times T} \]

where \(A_1\) and \(A_n\) represent the initial and terminal counts, and \(T\) represents the time in months; and (b) from the mean monthly hepatic counts calculated each month as the means of weekly counts over the liver according to the accumulative formula:

\[ \left[ \frac{(a_1 - a_2) \times 100}{a_1} + \frac{(a_2 - a_3) \times 100}{a_2} + \cdots + \frac{(a_{n-1} - a_n) \times 100}{a_{n-1}} \right] : t \]

where \(a_1, a_2, a_{n-1}\), and \(a_n\) represent consecutive corrected hepatic surface counts, and \(t\) represents the time in months of the entire observation period.

**RESULTS**

**Biological Half-Life of Hydroxocobalamin in the Liver**

The hepatic surface radioactivity in 5 individuals, over a period of time covering up to 59 weeks, is drawn in Figure 1. The counts have been corrected for physical decay and background, as well as for Co\textsuperscript{60} contribution in the Co\textsuperscript{57} hydroxocobalamin counting range, and the slopes represent clearance of Co\textsuperscript{57} (hydroxocobalamin) and Co\textsuperscript{60} (cyanocobalamin) from the liver. Since individuals received similar doses of hydroxo- and cyanocobalamin, these double-label data are comparable.

The initial counts of Co\textsuperscript{57}-hydroxocobalamin over the liver were about 1.5 to 2.2 times higher than those after Co\textsuperscript{60} cyanocobalamin, when calculated per 1 μc. of the isotope injected. This difference is not indicative of increased
Hepatic uptake of hydroxocobalamin, but is due to the greater efficiency of counting Co$^{57}$ than Co$^{60}$ with gamma spectrometer, despite lesser penetration of Co$^{57}$ through the tissues. This has been demonstrated in the as yet unpublished work of Weisberg from our laboratory, in which the ratio of the in vitro counts of Co$^{57}$B$_{12}$ to Co$^{60}$B$_{12}$ standards was compared with the ratio of the hepatic counts in vivo after intravenous administration of these standard doses.

Disappearance curves of Co$^{60}$-cyanocobalamin and Co$^{57}$-hydroxocobalamin from the liver were roughly parallel in cases 2 and 4 for the duration of study. In cases 1, 3 and 5, clearance curves of Co$^{57}$ hydroxocobalamin were slightly steeper than Co$^{60}$ cyanocobalamin curves.

Mean hepatic clearance data of Co$^{57}$-hydroxocobalamin and Co$^{60}$-cyanocobalamin are listed in Table 1, as calculated from the study of the 5 individuals just mentioned. The hepatic clearance of hydroxocobalamin is similar to that of cyanocobalamin both after intravenous and intramuscular administration of the cobalamins. However, during the late period of observation (between
Table 1.—Mean Hepatic Surface Radioactivity and Clearance Following Intramuscular or Intravenous Injection of $^{57}$Co-hydroxo- or $^{60}$Co-cyanocobalamin to 5 Normal Individuals

<table>
<thead>
<tr>
<th>Corrected cpm</th>
<th>Weeks</th>
<th>1</th>
<th>11</th>
<th>21</th>
<th>31</th>
<th>41</th>
<th>51</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{57}$Co-hydroxocobalamin</td>
<td>i.m. injection (mean of 2 cases)</td>
<td>701</td>
<td>662</td>
<td>627</td>
<td>567</td>
<td>567</td>
<td>536</td>
</tr>
<tr>
<td>$^{60}$Co-cyanocobalamin</td>
<td>314</td>
<td>308</td>
<td>296</td>
<td>263</td>
<td>245</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>$^{57}$Co-hydroxocobalamin</td>
<td>i.v. injection (mean of 3 cases)</td>
<td>1090</td>
<td>935</td>
<td>854</td>
<td>813</td>
<td>528</td>
<td>480</td>
</tr>
<tr>
<td>$^{60}$Co-cyanocobalamin</td>
<td>934</td>
<td>826</td>
<td>763</td>
<td>715</td>
<td>443</td>
<td>411</td>
<td>391</td>
</tr>
<tr>
<td>$^{57}$Co-hydroxocobalamin</td>
<td>i.m. or i.v. injection (mean of 5 cases)</td>
<td>100.0</td>
<td>94.4</td>
<td>89.4</td>
<td>80.9</td>
<td>80.9</td>
<td>76.5</td>
</tr>
<tr>
<td>$^{60}$Co-cyanocobalamin</td>
<td>100.0</td>
<td>98.1</td>
<td>94.3</td>
<td>83.8</td>
<td>78.0</td>
<td>68.8</td>
<td></td>
</tr>
</tbody>
</table>

Per Cent Clearance

<table>
<thead>
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<th>Weeks</th>
<th>1</th>
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<td>94.3</td>
<td>83.8</td>
<td>78.0</td>
<td>68.8</td>
</tr>
<tr>
<td>i.m. or i.v. injection (mean of 5 cases)</td>
<td>100.0</td>
<td>92.8</td>
<td>88.3</td>
<td>77.4</td>
<td></td>
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</tr>
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</table>

Table 2.—Mean Hepatic Clearance and Biological Half-Life in the Liver of $^{57}$Co-hydroxo- and $^{60}$Co-cyanocobalamin*

<table>
<thead>
<tr>
<th>Material</th>
<th>Mean Hepatic Clearance Per Week</th>
<th>Biological Half-life in Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{57}$Co-hydroxocobalamin</td>
<td>0.85%</td>
<td>0.52%</td>
</tr>
<tr>
<td>$^{60}$Co-cyanocobalamin</td>
<td>0.81%</td>
<td>0.71%</td>
</tr>
</tbody>
</table>

*Calculated according to cumulative formula.

the 30th and 52nd week) the clearance of hydroxocobalamin from the liver appears to be somewhat slower perhaps than that of cyanocobalamin, in 2 cases in which it was determined.

The average rate of discharge of Co$^{57}$ and Co$^{60}$ radioactivity from the liver, in the 5 individuals, is listed in Table 2. It was 0.85 per cent for Co$^{57}$ hydroxocobalamin and 0.81 per cent for Co$^{60}$ cyanocobalamin per week. The mean biological half-life of Co$^{57}$-hydroxocobalamin and Co$^{60}$-cyanocobalamin in the liver was, therefore, calculated to be 58.9 and 61.6 weeks, respectively, from data obtained during the 30-week observation period.

When these figures were calculated from data obtained during a year-long observation in 2 cases to whom cobalamins were injected intramuscularly, the mean discharge of cyanocobalamin from the liver between the 30th and the 52nd week was slightly slowed down to 0.75, while that of hydroxocobal-
HYDROXOCOBALAMIN

amin to about 0.2 per cent per week. This resulted in an increase of biological half-life of cyanocobalamin in these 2 cases to 70.4 and that of hydroxocobalamin to 96.1 weeks.

DISCUSSION

The average biological half-life of labeled hydroxocobalamin over the liver within the first 30 weeks after injection is similar to that of radioactive cyanocobalamin. This is in line with results obtained with whole body counting.26 However, when the liver is counted for longer periods of time, up to 1 year, it appears that the clearance of labeled hydroxocobalamin from the liver slows down perhaps more markedly than that of cyanocobalamin.

Hepatic clearance curves of cyano- and hydroxocobalamin seem thus to consist of at least 2 components. We refrained from a mathematical analysis of these curves, however, because of limitations of the hepatic surface counting technic,32 and a small number of observations.

The overall similarity of the biological half-life of cyanocobalamin and hydroxocobalamin may be due to cyanocobalamin's being converted into hydroxocobalamin in the liver.33,34 The slightly slower rate of hydroxocobalamin clearance may suggest, at a later date, that this conversion is not complete. Thus, the biological half-life of both cobalamins is initially (during the first 30 weeks) similar. However, it is probable, from our preliminary data, that later on hydroxocobalamin shows more prolonged biological half-life in the liver than cyanocobalamin. This may depend on the greater affinity of hydroxocobalamin to the liver tissue, as compared to the other tissues of the body35 or to the prolonged storage in the liver of cobalamin degradation products.36

SUMMARY AND CONCLUSIONS

The mean hepatic biological half-life of Co57-hydroxocobalamin injected to 5 normal human subjects was similar to that of Co60-cyanocobalamin, as shown by double-label hepatic surface counting during the first 30 weeks after intramuscular or intravenous injection of cobalamins. In 2 cases in whom the counting was extended over a year's period, the clearance of hepatic radioactivity following the intramuscular injection of hydroxocobalamin has slowed down as compared to that of cyanocobalamin, between the 30th and 52nd week after injection.

SUMMARIO IN INTERLINGUA

Le valor medie del biologic tempore de medie valor de hydroxocobalamina a Co57 in le hepate studiate in 5 normal subjectos human esseva simile a illo de cyanocobalamina a Co60 secundo le resultatos de contatation hepatosuperficial a duple marcage durante le prime 30 septimanas a partir del injection intramuscular o intravenose del duo compositos. In 2 casos in le quales le contation esseva continuare durante un periodo de un anno, le clearance de radioactivitate hepatic post le injection intramuscular de hydroxocobalamina ha devenite plus lente que illo post cyanocobalamina inter le trentesime e le cinquanta-secunde septimana a partir del application del compositos.
REFERENCES


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