Co\textsuperscript{58}B\textsubscript{12} ABSORPTION, PLASMA TRANSPORT AND EXCRETION IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS, SOLID TUMORS AND NON–NEOPLASTIC DISEASES *

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Several investigators have documented the fact that chronic myelocytic leukemia (CML) is associated with increases in serum vitamin B\textsubscript{12} concentrations which may be of the order of 20 to 40 times the normal value (1–4). Less striking and less consistent elevations have been found in other myeloproliferative disorders. These include acute myelocytic leukemia (1, 3, 4), myeloid metaplasia (2–4), polycythemia vera associated with myelocytic leukemia (4) and Di Guglielmo's disease (5). Elevations have also been reported in patients with solid tumors when there are metastases to the liver (3, 6). The present study was designed to investigate in vivo the role that gastrointestinal absorption, plasma transport and renal excretion of vitamin B\textsubscript{12} play in producing this abnormality.

Previous in vivo studies have dealt with the fate of a large intravenously administered dose of radioactive B\textsubscript{12} (7, 8). In the present studies a dose of approximately 0.5 \( \mu \)g of Co\textsuperscript{58}B\textsubscript{12} was chosen, since this is within the limits of the estimated daily requirement of B\textsubscript{12} (9). Oral administration was used so that gastrointestinal absorption could be evaluated and a physiologic pattern of plasma concentrations and excretion could be obtained. Twenty-four subjects have been studied, six of whom had CML.

MATERIALS AND METHODS

Materials. Co\textsuperscript{58}B\textsubscript{12} \(^{1}\) had an original specific activity of 3.0 \( \mu \)c per \( \mu \)g. The biologic activity of the material used was confirmed by microbiologic assay (Lactobacillus leichmannii) and found to agree within 93 per cent of that given by the manufacturer.

Dosage and administration. The dose administered ranged between 0.33 and 0.56 \( \mu \)g of Co\textsuperscript{58}B\textsubscript{12}. One subject, J.G., received 2.7 \( \mu \)g. Because of the relatively short 72 day half-life of Co\textsuperscript{58}, the calculated activity of the administered dose ranged between 0.3 and 1.4 \( \mu \)c, depending on the time of its administration. After an overnight fast, the subject drank the labeled vitamin along with 50 ml of distilled water. Breakfast was deferred for 1 hour.

Plasma concentrations. Ten ml of heparinized blood was drawn from an antecubital vein at 0, 3, 4.5, 6, 9, 12, 24, 36, 48 and 72 hours and then daily for at least 10 days. In a few studies, bloods were drawn at intervals up to 8 weeks. Plasma was separated by centrifugation and transferred to a 4 ml counting vial for radioassay.

Fecal excretion. Individual stools were collected in metal containers and were assayed for gross activity over the open well of a scintillation counter to evaluate the day-to-day pattern of excretion. Stools were then combined into 4-day pools and homogenized. A 100 g aliquot of each homogenized pool was placed in a 500 ml bottle and counted in the open well of a scintillation counter. A standard was diluted to 100 ml and counted in the same manner. The total fecal excretion over a 12 to 16 day period following administration of Co\textsuperscript{58}B\textsubscript{12} was determined and expressed as percentage of dose administered. This value subtracted from 100 per cent gave the percentage of dose absorbed.

Urinary excretion. Twenty-four-hour urines were collected for 14 days, 300 ml aliquots of each 24-hour specimen were counted in a manner similar to that used for fecal homogenates.

Radioassay. Plasma, feces and urine were counted in a thallium-activated sodium iodide well-type scintillation counter.\(^{2}\) Plasma samples were counted for a sufficient period of time to give a counting error of less than 5 per cent. Plasma activity was at a low level but at the time of peak activity was always 8 to 30 SD above background. The average background during the course of these studies was 183 cpm. Twelve cpm was equal to twice the SD of the average background. A suitably diluted 4 ml standard of Co\textsuperscript{58}B\textsubscript{12} was counted each day, and

\(^{1}\) Nuclear Chicago scaler, model number D181, and radiation analyzer, model number 1810.
the radioactivity of each plasma sample was converted to 
\( \mu g \) of Co\(^{58}\)B\(_{12}\) per ml plasma. Fecal specimens 
were counted with an error of less than 3 per cent. Urine 
specimens were counted for 20 minutes to demonstrate 
negligible radioactivity.

**Microbiologic assay.** Blood specimens from fasting 
patients were drawn on the morning of the study prior 
to administration of the tracer dose and assayed in 
quadruplicate for their B\(_{12}\) concentration by a modifica-
tion, described in detail elsewhere (3), of the USP method 
using *L. leichmannii*, A.T.C.C. 7830. With this method 
the mean serum B\(_{12}\) concentration for 31 normal subjects 
was 533 \( \mu g \) per ml of serum with a range of 260 to 850.

**Subjects.** The 24 patients chosen for this study, along 
with pertinent clinical data, are listed in Table I. All 
diagnoses were established both clinically and histolo-
gically. None of the patients studied had received oral 
or parenteral vitamins for at least 1 month prior to the 
study. All but 3 patients were studied during residency 
on the metabolism unit of the National Cancer Institute 
where they were fed diets of known composition. Their 
stool and urine collections were rigidly controlled ac-

Subjects have been classified according to the following 
diagnoses: non-neoplastic diseases, solid tumors, myeloid 
metaplasia, chronic myelocytic leukemia, hypersplenism 
and multiple myeloma. For the most part, subjects with 
non-neoplastic diseases and solid tumors displayed find-
ings similar to those noted by other investigators in 
normal subjects given a 0.5 \( \mu g \) oral dose of Co\(^{58}\)B\(_{12}\) and 
therefore in the discussion are referred to as control 
subjects.

**RESULTS**

**Serum B\(_{12}\) concentrations (Table I)**

Four of the five patients with non-neoplastic 
disease had serum B\(_{12}\) concentrations within 2 SD 
of the normal mean. The fifth patient, whose 
diagnosis was primary pulmonary hypertension 
had a moderately increased serum B\(_{12}\) concentra-
tion of 1,213 \( \mu g \) per ml.

The five patients with solid neoplasms had a 
mean serum B\(_{12}\) concentration of 577 \( \mu g \) per ml 
and a range of 330 to 890 \( \mu g \) per ml. Two of the 
four patients with myeloid metaplasia had moder-
ately increased serum B\(_{12}\) concentrations. The 
other two had normal concentrations. The mean 
concentration for this group was 1,129 \( \mu g \) per ml 
with a range of 420 to 2,100.

The seven patients with CML (including one 
diagnosed as eosinophilic leukemia) demonstrated 
striking increases in serum B\(_{12}\) concentration, the 
mean concentration being 8,550 \( \mu g \) per ml with 
a range of 1,800 to 17,700 \( \mu g \) per ml. Patient

![FIG. 1. Plasma Co\(^{58}\)B\(_{12}\) concentrations following 
oral administration to subjects with a normal serum B\(_{12}\).](image)

D.L. was in clinical and hematological remission 
and Patients J.L. and P.P. were in partial relapse 
at the time they were studied. The two patients 
with hypersplenism and pancytopenia had serum 
B\(_{12}\) concentrations at the lower limits of normal, 
195 and 200 \( \mu g \) per ml. The one subject with 
multiple myeloma had a normal serum B\(_{12}\) concen-
tration of 625 \( \mu g \) per ml.

**Gastrointestinal absorption**

Since all of the patients but J.G. received a dose 
of Co\(^{58}\)B\(_{12}\) in the 0.5 \( \mu g \) range, the percentage 
of oral dose absorbed has been used as a basis for 
comparisons between patients. The percentage 
absorbed by each of the 24 subjects is listed in 
Table I. The mean for all of the 24 subjects was 
66.8 per cent with a range of 30 to 81 per cent. No 
significant difference between the various disease 
groups studied was noted in terms of the percent-
age of dose absorbed. No correlation between a 
patient’s serum B\(_{12}\) concentration and the percent-
age of dose absorbed could be made.

J.G., who received a dose of 2.73 \( \mu g \), had an 
absorption of 71 per cent of the administered dose, 
a value not significantly different from that noted 
in the others.

**Plasma concentrations of Co\(^{58}\)B\(_{12}\)**

Curves of plasma radioactivity were obtained in 
19 of the 24 patients. For the purpose of this 
study, the rate of plasma disappearance has been 
evaluated by noting the \( T_1 \).\(^{3}\)

\(^{3}\) Since plasma curves had both an appearance phase 
as well as a disappearance phase, and since the disap-
pearance phase was not exponential, the use of this term
Non-neoplastic disease and solid tumor groups. A grossly similar pattern of plasma radioactivity was noted in four patients with non-neoplastic disease in the present context is somewhat unconventional. In the present paper “\( T_1 \)” has been arbitrarily defined as the time interval between peak plasma radioactivity and the time when plasma concentration of \( \text{Co}^{58}\text{B}_{12} \) falls to half of the peak concentration.

### TABLE 1
Clinical data on 24 subjects given an oral dose of \( \text{Co}^{58}\text{B}_{12} \); data obtained on gastrointestinal absorption and plasma concentration

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Clinical status</th>
<th>WBC/mm(^3)</th>
<th>( \mu g/\text{ml} )</th>
<th>µg</th>
<th>% ( \mu g/\text{ml} )</th>
<th>hrs</th>
<th>Peak concentration</th>
<th>Time of peak</th>
<th>&quot;T/1/2&quot;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.Q.</td>
<td>F</td>
<td>33</td>
<td>Partial resection of ileum</td>
<td>Stable</td>
<td>6,000</td>
<td>800</td>
<td>0.37</td>
<td>60</td>
<td>3</td>
<td>12</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>L.S.</td>
<td>M</td>
<td>22</td>
<td>Primary pulmonary hypertension</td>
<td>Stable</td>
<td>7,500</td>
<td>1,213</td>
<td>0.49</td>
<td>86</td>
<td>15</td>
<td>10</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>S.M.</td>
<td>F</td>
<td>32</td>
<td>Rheumatic heart disease</td>
<td>Stable</td>
<td>10,200</td>
<td>550</td>
<td>0.49</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>F</td>
<td>68</td>
<td>Subtotal gastrectomy</td>
<td>Weight loss</td>
<td>6,000</td>
<td>465</td>
<td>0.49</td>
<td>76</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>G.M.</td>
<td>M</td>
<td>42</td>
<td>Anorexia nervosa</td>
<td>Weight loss</td>
<td>8,200</td>
<td>790</td>
<td>0.56</td>
<td>76</td>
<td>27</td>
<td>12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>L.J.</td>
<td>F</td>
<td>16</td>
<td>Embryonal sarcoma</td>
<td>Stable</td>
<td>7,500</td>
<td>725</td>
<td>0.49</td>
<td>80</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>R.L.</td>
<td>M</td>
<td>20</td>
<td>Hepatoma</td>
<td>Partially resected</td>
<td>9,000</td>
<td>462</td>
<td>0.49</td>
<td>62</td>
<td>13</td>
<td>35</td>
<td>&gt;9</td>
<td></td>
</tr>
<tr>
<td>H.C.</td>
<td>M</td>
<td>52</td>
<td>Prostatic carcinoma</td>
<td>Bone metastases</td>
<td>3,000</td>
<td>330</td>
<td>0.48</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.S.</td>
<td>M</td>
<td>15</td>
<td>Embryonal sarcoma</td>
<td>Metastases</td>
<td>5,400</td>
<td>478</td>
<td>0.56</td>
<td>41</td>
<td>18</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>W.P.</td>
<td>M</td>
<td>54</td>
<td>Pancreatic carcinoma</td>
<td>Untreated</td>
<td>7,600</td>
<td>890</td>
<td>0.56</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.J.</td>
<td>M</td>
<td>61</td>
<td>Myeloid metaplasia</td>
<td>Untreated</td>
<td>4,000</td>
<td>2,100</td>
<td>0.33</td>
<td>80</td>
<td>15</td>
<td>48</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>S.P.</td>
<td>M</td>
<td>65</td>
<td>Myeloid metaplasia</td>
<td>Untreated</td>
<td>8,000</td>
<td>1,215</td>
<td>0.48</td>
<td>30</td>
<td>28</td>
<td>10d</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>J.G.</td>
<td>M</td>
<td>50</td>
<td>Myeloid metaplasia</td>
<td>Untreated</td>
<td>9,000</td>
<td>780</td>
<td>2.73</td>
<td>71</td>
<td>16</td>
<td>9</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>F</td>
<td>64</td>
<td>Myeloid metaplasia</td>
<td>Untreated</td>
<td>3,000</td>
<td>420</td>
<td>0.48</td>
<td>52</td>
<td>20</td>
<td>6d</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>D.L.</td>
<td>M</td>
<td>8</td>
<td>Chronic myelocytic leukemia</td>
<td>Remission</td>
<td>8,000</td>
<td>17,700</td>
<td>0.48</td>
<td>81</td>
<td>71</td>
<td>48</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>J.L.</td>
<td>F</td>
<td>23</td>
<td>Chronic myelocytic leukemia</td>
<td>Partial relapse</td>
<td>5,600</td>
<td>8,700</td>
<td>0.49</td>
<td>76</td>
<td>54</td>
<td>72</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>O.F.</td>
<td>M</td>
<td>48</td>
<td>Chronic myelocytic leukemia</td>
<td>Untreated</td>
<td>200,000</td>
<td>6,800</td>
<td>0.48</td>
<td>55</td>
<td>8</td>
<td>72</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>P.P.</td>
<td>M</td>
<td>36</td>
<td>Chronic myelocytic leukemia</td>
<td>Partial relapse</td>
<td>40,000</td>
<td>6,700</td>
<td>0.48</td>
<td>78</td>
<td>18</td>
<td>24</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>M.B.</td>
<td>F</td>
<td>37</td>
<td>Chronic myelocytic leukemia</td>
<td>Untreated</td>
<td>194,000</td>
<td>6,150</td>
<td>0.48</td>
<td>48</td>
<td>30</td>
<td>36</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>R.F.</td>
<td>M</td>
<td>52</td>
<td>Chronic myelocytic leukemia</td>
<td>Untreated</td>
<td>190,000</td>
<td>1,800</td>
<td>0.48</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.E.</td>
<td>M</td>
<td>25</td>
<td>Eosinophilic leukemia</td>
<td>Untreated</td>
<td>17,000</td>
<td>12,000</td>
<td>0.49</td>
<td>79</td>
<td>22</td>
<td>36</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>C.F.</td>
<td>M</td>
<td>40</td>
<td>Hypersplenism with pancytopenia</td>
<td>Untreated</td>
<td>2,500</td>
<td>195</td>
<td>0.48</td>
<td>81</td>
<td>14</td>
<td>72</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>P.K.</td>
<td>M</td>
<td>55</td>
<td>Hypersplenism with pancytopenia</td>
<td>Untreated</td>
<td>3,000</td>
<td>200</td>
<td>0.49</td>
<td>50</td>
<td>8</td>
<td>6</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>F</td>
<td>60</td>
<td>Multiple myeloma</td>
<td>Untreated</td>
<td>6,000</td>
<td>625</td>
<td>0.48</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See Footnote 3 in text.
was equivalent to a mean of 13 μg of Co$^{60}$B$_{12}$ per ml of plasma (range, 3 to 27 μg per ml). Subsequently, there was a gradual and quite irregular decline in plasma radioactivity during the next 10 to 14 days. The $T_1$ of plasma disappearance had a mean value of 4 days (range, 1.5 to 7 days). Three of these six patients had significant radioactivity in their plasma for as long as 10 days after administration of the Co$^{60}$B$_{12}$.

A seventh patient, R.L., whose diagnosis was hepatoma, had a peak concentration of 13 μg Co$^{60}$B$_{12}$ per ml of plasma. The peak, however, occurred at 35 hours. The plasma disappearance was also delayed with a $T_1$ of $>9$ days.

Chronic myelocytic leukemia. Figure 2 summarizes the plasma Co$^{60}$B$_{12}$ concentrations observed in seven patients with chronic myelocytic leukemia. (Subject L.E. with eosinophilic leukemia is included in this group because of his similar pattern.) The initial portion of their plasma appearance curves was approximately linear and had a slope similar to that of the previous group. At from 10 to 14 hours, however, instead of reaching a peak concentration, serial plasma samples continued to rise more slowly, resulting in a delay in reaching peak concentrations. The latter did not occur until a mean of 2 days (range, 1 to 3 days). In general, plasma radioactivity also reached much higher concentrations, being equivalent to a mean of 34 μg Co$^{60}$B$_{12}$ per ml of plasma (range, 8 to 71 μg per ml). In addition, there was delayed plasma disappearance with a mean $T_1$ of 10.7 days (range, 4 to 15 days). At the end of 10 days, four of the six patients retained in their plasma 56 to 88 per cent of their peak plasma concentration. A plasma sample drawn on M.B. at 6 weeks demonstrated significant residual radioactivity.

Myeloid metaplasia. Inspection of the plasma radioactivity curves for the four patients with myeloid metaplasia (Figure 3) reveals that, except for one subject, W.J., whose curve has the features noted for patients with chronic myelocytic leukemia, these patients had bizarre and irregular appearance and disappearance curves. They had a mean peak plasma concentration of 20 μg Co$^{60}$B$_{12}$ per ml of plasma (range, 15 to 28 μg per ml). Because of the very irregular plasma disappearance, $T_1$ could not be evaluated. In general, however, these patients demonstrated more residual Co$^{60}$B$_{12}$ in their plasma at the end of 10 days than did the non-neoplastic disease-solid tumor group.
Others, P.K., a patient with hypersplenism and pancytopenia, had a plasma CoB12 curve similar to the non-neoplastic disease and solid tumor groups. C.F., on the other hand, with hypersplenism and pancytopenia, had a delayed plasma peak occurring at 3 days. Plasma activity was not studied in the single case of multiple myeloma.

Urinary excretion of CoB12

There was no detectable urinary excretion of radioactive material in the unconcentrated urine specimens of any of the subjects studied. Calculations indicate that the method used in the present study would have detected excretion of more than 1 per cent of the absorbed dose per 24 hour urine specimen.

DISCUSSION

The studies indicate that gastrointestinal absorption of B12, as measured by the fecal excretion technique, is not increased in the patient with CML. The percentage of administered CoB12 absorbed had an over-all mean of 66.8, with a range of 30 to 81 for the 24 subjects studied. These figures are in good agreement with those reported by Halsted and colleagues (11) who found that with a 0.5 μg dose of radioactive B12, normal subjects absorbed 43 to 95 per cent of the dose, with a mean of 66 and a maximum variation within the same patient of 18 per cent. The absorption in seven subjects with CML in the present study did not differ from this range, nor was the amount absorbed abnormal in the two subjects with myeloid metaplasia and a high serum B12.

Heinrich and Erdmann-Oehlecker (12) have also found normal gastrointestinal absorption of B12 in one patient with CML given 1 μg of B12.

A corollary to these findings is that the serum concentration of B12 per se does not control the amount of B12 absorbed. Both Patient L.J., with a serum B12 of 725 μg per ml and Patient D.L., with a concentration of 17,700 μg per ml, absorbed approximately 80 per cent of the test dose. The data also indicate that the plasma concentration of "unsaturated B12-binding protein" does not greatly influence the amount of B12 absorbed, since plasma from patients with CML has a markedly increased unsaturated B12-binding capacity (13–16).

It would appear, therefore, that the high plasma B12 concentrations seen in CML are not due to excessive gastrointestinal absorption of the vitamin. In addition, it is unlikely that the abnormality is caused by impaired renal excretion, since the total daily urinary excretion of vitamin B12 is normally very small, viz., about 0.1 μg per day (17), and CoB12 was undetectable in the urine of both control and CML patients in the present study.

We believe that the high B12 content of CML plasma is due to a relative shift of B12 from tissue sites to plasma. It can be calculated from known data (18) that the transfer of only 3 per cent of the normal liver's content of B12 to the plasma compartment would be sufficient to produce the tenfold increase in plasma B12 seen in CML. This possibility would, of course, be excluded should studies reveal that CML is associated with a high tissue content as well as high plasma concentration of the vitamin. Data on tissue concentrations of B12 in CML are limited. In the single autopsied case studied by Mollin and Ross (2) the total B12 content of liver, spleen and muscle was within the normal range. Nelson has studied the B12 content of liver tissue obtained by needle biopsy in two cases of myelocytic leukemia associated with a high serum B12 and found it to be approximately 30 per cent below that of the average normal control (19).

The marked differences between control and CML subjects in plasma radioactivity curves following oral administration of CoB12 provide evidence for a disturbed kinetics of B12 in CML. An analysis of these curves necessitates a consideration of the factor involved in the absorption, plasma transport and distribution of B12. These are summarized in schematic form in Figure 4. Because of gaps in our present knowledge the scheme is tentative.

The delay in peak plasma concentration (9 hours in control patients) following an oral dose of CoB12 has been noted by other investigators (20, 21) and appears to be due to a delay in the movement of B12 across the small bowel mucosa (21–23). The initial slope of the plasma appearance curve for the CML patients appears to be comparable with that of the control patients (Figure 2) suggesting a normal rate of entry into the
plasma compartment. A greater than normal delay in peak plasma concentration (24 hours) was noted in the CML patients and is discussed below.

In both the control and CML subjects the plasma disappearance curves had an irregular downward slope. We believe that this is due to a complex recycling of B₁₂ through the plasma compartment. This is supported by the accumulating evidence for an enterohepatic circulation of B₁₂ (24, 25) as well as by the studies of Miller, Corbus and Sullivan (8) and Glass and Schaffer (22) which suggest that there is a transfer of absorbed B₁₂ from plasma to unidentified compartment(s) prior to its eventual deposition in the liver.

Because of the complex nature of the plasma radioactivity curves, in the present study T₁ is defined as the time following peak radioactivity when plasma radioactivity falls to and remains below half the peak concentration. The mean value for control and CML patients was 4 and 11 days, respectively. Employing an intravenous dose of 1.5 or 4 μg of radioactive B₁₂ Mollin, Pitney, Baker and Bradley (7) and Miller and colleagues (8) have demonstrated that in patients with CML there is a diminished plasma disappearance rate for injected B₁₂. The rates reported were much more rapid than those obtained in the present study and are probably due to the unphysiologic effects of administering a large dose of free B₁₂ intravenously. This is especially true in normal subjects who have a relatively limited plasma unsaturated B₁₂-binding capacity (see below) and, therefore, might be expected to have a rapid loss of free B₁₂ from the plasma.

The slower plasma disappearance rate combined with the prolonged influx at a normal rate into the plasma from the small bowel of the tracer dose of Co⁵⁸B₁₂ could, by a cumulative effect, account for the higher than normal but more delayed peak plasma concentration of Co⁵⁸B₁₂ seen in the patients with CML in the present study. In patients with CML, a given dose of Co⁵⁸B₁₂ enters a plasma B₁₂ pool which is approximately 10 times normal size. Therefore, the delayed plasma disappearance of the labeled vitamin may merely represent an isotope dilution effect. Since CML plasma has a high unsaturated B₁₂-binding capacity (13–16), an additional factor may be that as the tracer dose enters the plasma compartment a greater than normal fraction of it is tightly bound to plasma protein.

In both normal subjects and patients with CML, 80 to 90 per cent of total plasma B₁₂ is tightly bound to an α₁-globulin present in the seromucoid fraction of plasma (16). Since, within a few hours following an oral dose of Co⁵⁸B₁₂, the radioactive B₁₂ appearing in plasma is also in this form (13) it seems reasonable to assume that, once absorbed, an orally administered dose of Co⁵⁸B₁₂ rapidly equilibrates with the total plasma B₁₂. Using the previously mentioned disappearance rates it seems possible, therefore, to calculate the daily plasma turnover of vitamin B₁₂. For a control subject with a plasma B₁₂ of 0.5 mμg per ml, a plasma volume of 2,500 ml and a T₁ of four days, the plasma turnover would be 0.16 μg per day. Similar calculations for a patient with CML, assuming a plasma B₁₂ of 5 mμg per ml, a plasma volume of 2,500 ml and a T₁ of 11 days, give a plasma turnover of 0.57 μg per day.

If plasma turnover of B₁₂ is indeed increased in CML, the abnormality in B₁₂ metabolism may be more fundamental than merely a shift of tissue stores of B₁₂ to the plasma compartment resulting from an excess of plasma B₁₂-binding protein. Clearly, the assumptions underlying the calculations and the validity of the disappearance rates obtained bear further investigation.
1. Gastrointestinal absorption, plasma transport and urinary excretion of radioactivity following oral administration of 0.5 μg of Co3B12 have been evaluated in 24 patients, 7 of whom had chronic myelocytic leukemia.

2. The amount absorbed by all subjects, as measured by stool excretion, had a mean of 67 per cent. There was no correlation between diagnosis or serum B12 concentration and percentage of dose absorbed.

3. Subjects with non-neoplastic diseases as well as patients with solid tumors, all of whom had a normal serum B12 concentration, had a similar pattern of plasma clearance. Co3B12 reached a mean peak concentration of 13 μg per ml of plasma at 9 hours and fell to half the peak concentration at a mean of 4 days. In seven patients with chronic myelocytic leukemia, Co3B12 reached a mean peak plasma concentration of 33 μg per ml at 2 days and fell to half the peak concentration at a mean of 11 days.

4. No detectable urinary excretion of radioactive material in unconcentrated urine specimens was noted in any of the subjects studied.

5. The increased serum concentration of vitamin B12 seen in patients with chronic myelocytic leukemia does not appear to be due to increased gastrointestinal absorption or decreased urinary excretion of the vitamin. A relative shift of B12 from tissue sites to plasma appears to be the most likely mechanism.

6. Calculations based upon the plasma disappearance rates obtained in the present study suggest that, in addition to the increased concentration of B12 and B12-binding protein found in plasma of patients with chronic myelocytic leukemia, there is an increased plasma turnover of the vitamin in this disease.

REFERENCES
19. Nelson, R. S. Personal communication.