The Kinetics of Intravenously Injected Radioactive Vitamin B₁₂: Studies on Normal Subjects and Patients with Chronic Myelocytic Leukemia and Pernicious Anemia

By Eugene A. Brody, Solomon Estren and Louis R. Wasserman

Under normal conditions, vitamin B₁₂ (less than 2 μg.) is absorbed from the gastrointestinal tract through the mediation of “intrinsic factor” and enters the blood stream. It is transported in the plasma predominantly bound to the alpha globulins, but with a small portion “free” or perhaps loosely bound to the beta globulins. From the plasma, vitamin B₁₂ passes to the tissues for storage and utilization. The action of intrinsic factor in effecting absorption of B₁₂ is known to occur within the gastrointestinal tract, but the fate of intrinsic factor after it has effected this transfer is not known. Further, the precise mechanisms responsible for transfer of B₁₂ across the intestinal wall into the plasma, and from the plasma to the tissues, are also unknown.

In the course of studying the disappearance of intravenously injected vitamin B₁₂ from the plasma, observations were made which may be of significance in elucidating this transfer of vitamin B₁₂ from plasma to tissues.

Mollin et al. demonstrated that the rate of plasma clearance of intravenously injected radioactive B₁₂ was decreased in patients with pernicious anemia in relapse as compared to the normal, and that the rate was further diminished in patients with chronic myelocytic leukemia. These findings were ascribed to the degree of unsaturation of the particular plasma for vitamin B₁₂; i.e., the greater the degree of unsaturated binding capacity of the plasma for B₁₂, the slower the disappearance of injected B₁₂ from the plasma. Similar results in chronic myelocytic leukemia were reported by Miller et al., who suggested that a biochemical plasma abnormality was responsible for the increased binding of B₁₂ by the plasma proteins in this disease. Heinrich, using electrophoretic technics, noted the presence of increased B₁₂-binding proteins in the plasma of patients with chronic myelocytic leukemia. Weinstein et al. suggested that the B₁₂-binding substance found in the seromucoid fraction of the plasma was qualitatively similar in both the normal and in chronic myelocytic leukemia, with the increased binding capacity of the latter associated with an increase in normal seromucoid.

If the plasma clearance of vitamin B₁₂ were related to tissue concentration directly, in pernicious anemia in relapse one would expect that intravenously injected B₁₂ would be removed rapidly from the plasma to be transferred to the B₁₂-depleted tissues. Actually, however, the clearance is slower than anticipated, approaching the findings in chronic myelocytic leukemia. The work was supported in part by Grant A-3190 (C1), National Institute of Arthritis and Metabolic Diseases, and the Albert A. List, Frederick Machlin and Anna Ruth Lowenberg Research Funds.
present study was undertaken to examine further the clearance of vitamin B₁₂ from the plasma and to study some of the factors and diseases which modify this transfer to the tissues.

Experimental Technics

Materials and Methods

Vitamin B₁₂ (0.5 µg.) labeled with Ca¹⁹⁵ or Co⁶⁰ (1 µg., 1 µc.) was sterilized by heating to 60°C for two hours, made up into a volume of 1 to 2 ml. and rapidly injected into an antecubital vein with the use of a setup of two syringes and a three-way stopcock. The time required for injection, including three rinsings of the syringes with 3.8 per cent sodium citrate, was 20 seconds or less. A few patients received as much as 1.0 µg. of B₁₂.

Ten to 20 ml. samples of heparinized blood were withdrawn from the opposite arm via an indwelling needle at frequent intervals for the first two hours (8 to 14 samples), then at approximately 4, 8 and 24 hours, and daily or every other day thereafter for the following two weeks when possible. A 4 ml plasma aliquot of each specimen was assayed for radioactivity by the use of a well-type thallium-activated sodium iodide crystal. Microbiologic assay of the “free” and total vitamin B₁₂ utilizing Euglena gracilis as the test organism was performed on most of the plasma samples.

The normal range of total serum (or plasma) B₁₂ in our laboratory is 200 to 1000 µg. per milliliter. The per cent of the administered dose which was present in each plasma sample was determined by the formula:

\[
\text{% remaining in plasma} = \frac{\text{Plasma volume (ml.) \times \text{cps specimen}}}{\text{\(4 \times \mu g. injected into patient\)}} \times \text{100.}
\]

The plasma volume was estimated at 40 ml per kilogram of body weight.

In vivo counting by means 3 solid thallium-activated sodium iodide crystals was performed simultaneously over the liver, thigh and one of the following: spleen, sacrum, brain, heart, or left lower quadrant of the abdomen. Counting was begun just prior to injection of the test dose, continued uninterruptedly for two hours, and repeated at intervals corresponding to the drawing of plasma samples thereafter.

Subjects Studied

The patients studied were divided into four groups (table 1): (1) Normals: This group of 10 patients had disorders which, as far as is known, are not associated with any abnormality of vitamin B₁₂ metabolism. The range of serum B₁₂ levels in this group coincided with the range of normal B₁₂ levels (200 to 1000 µg. per milliliter). (2) Chronic Myelocytic Leukemia: Patients in this group had abnormally high serum concentrations of vitamin B₁₂. Two such patients were carefully studied and in addition, labeled B₁₂ incubated with chronic myelocytic leukemia plasma was injected into three “normal” patients, i.e., patients with normal serum vitamin B₁₂ levels. (3) Patients with abnormally low serum concentrations of vitamin B₁₂. This group comprised five patients with perni-
Table 1.—Serum Vitamin B₁₂ Concentrations of Patients in the 4 Groups Studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Pt.</th>
<th>Free</th>
<th>Total</th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal ganglia disease</td>
<td>96</td>
<td>230</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Migraine headache</td>
<td>92</td>
<td>270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerebral thrombosis</td>
<td>20</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain tumor</td>
<td>40</td>
<td>280</td>
<td>230-486</td>
<td>486</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sickle cell trait</td>
<td>84</td>
<td>320</td>
<td>1020</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukosarcoma</td>
<td>160</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No disease</td>
<td>48</td>
<td>480</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polycythemia vera</td>
<td>48</td>
<td>740</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma</td>
<td>200</td>
<td>840</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Multiple myeloma</td>
<td>344</td>
<td>1020</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chronic myelocytic leukemia</td>
<td>F. H.</td>
<td>330 (B)*</td>
<td>4100</td>
<td>1950-3483</td>
<td>3483</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. G.</td>
<td>88 (A)*</td>
<td>4400</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25 (A)*</td>
<td>1950</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td></td>
<td></td>
<td>W. W.</td>
<td>50 (A)†</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. L.</td>
<td>48 (C)†</td>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. L.</td>
<td>40 (B)‡</td>
<td>430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pernicious anemia in relapse</td>
<td>W. J.</td>
<td>0</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>W. U.</td>
<td>0</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. M.</td>
<td>0</td>
<td>45</td>
<td>12-67</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W. F.</td>
<td>0</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. P.</td>
<td>0</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malabsorption syndrome</td>
<td>T. A.</td>
<td>8 (A)‡</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. P.</td>
<td>5 (B)‡</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. L.</td>
<td>22 (C)‡</td>
<td>100</td>
<td>19-190</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. C.</td>
<td>8 (D)‡</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. O.</td>
<td>80 (E)‡</td>
<td>190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pernicious anemia in remission</td>
<td>B. A.</td>
<td>372 (A)§</td>
<td>855</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. J.</td>
<td>76 (B)§</td>
<td>940</td>
<td>855-932</td>
<td>932</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. K.</td>
<td>120 (C)§</td>
<td>1000</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. K.</td>
<td>22 (D)§</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total gastrectomy</td>
<td>F. Jo.</td>
<td>15 (E)§</td>
<td>220</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Plasma disappearance curves shown in figure 2.
†Plasma disappearance curves shown in figure 3.
§Plasma disappearance curves shown in figure 5.
||Normal patients who received labeled B₁₂ incubated with serum of patients with chronic myelocytic leukemia.

RESULTS

Clearance of radioactive vitamin B₁₂ from the plasma.—Certain similarities were noted in the plasma disappearance curves in all of the patients studied.

*The diagnosis in the patients in groups 3 and 4 had been or was subsequently established by means of the Schilling test or the Glass test.
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(figs. 1 to 7). Thus, following an initial rapid loss of plasma radioactivity during the first 30 minutes, there was a progressively slower loss of radioactivity in the next 30 to 90 minutes; with a final more horizontal component resembling a slow exponential clearance of radioactivity.

Although the appearance of the initial components of the curves of the four groups was similar, the extent of the disappearance of radioactivity from the plasma varied in a striking manner. Thus, patients exhibiting a shorter initial component as in chronic myelocytic leukemia retained more radioactivity in plasma at any given time, the final exponential decline having a half-time of about six days (fig. 7). This result is to be contrasted with the more rapid clearance noted in the normals and in pernicious anemia.

The maximum amount of radioactivity found in the plasma was 68 per cent of the injected dose in a normal and 85 per cent of the injected dose in a patient with pernicious anemia in relapse. In no patient was 100 per cent of the injected radioactivity found in the plasma, regardless of the time interval between injection of the dose and drawing of the first plasma sample.

NORMAL SUBJECTS. The total serum B₁₂ concentrations in these patients ranged from 230 to 1020 μg. per milliliter. The rate of disappearance of radioactivity from the plasma in each of these 10 patients was approximately the same (fig. 1). The initial rapid component had an average half time of disappearance (T₁/₂) of radioactivity of less than 3 minutes, with the final component having a T₁/₂ approaching 24 hours.

CHRONIC MYELOCYTIC LEUKEMIA. Two patients with chronic myelocytic leukemia were studied. Two tests were performed on one of these patients (D. G.) at an interval of 15 months (fig. 2, curves A and A¹), the total concentration of serum B₁₂ being 4400 and 1950 μg. per milliliter on the two occasions. In the second patient (F. H.), the serum B₁₂ was 4100 μg. per milliliter at the time of the test. The intersection of the initial rapid component (T₁/₂, 10 minutes) and the slower horizontal component occurred at a higher level of radioactivity than in the normal. Thus, the percentage of injected radioactivity remaining in the plasma at any given time was greater than in the normals (table 2). The difference in the two studies performed on D. G. was not striking. In the one case followed for five weeks, plasma radioactivity decreased progressively, with 2 per cent remaining after 35 days.

The increased capacity of leukemia sera to bind vitamin B₁₂ was studied by prior incubation for one hour at 37 C. of 0.5 μg. of labeled vitamin B₁₂ with 20 ml. of plasma obtained from a patient with chronic myelocytic leukemia. This mixture was then injected intravenously into a “normal” recipient. Three such experiments were performed with the use of three different leukemic sera into three different “normal” recipients (fig. 3). Curve “A” represents a normal subject who received Co⁸⁶B₁₂ incubated with plasma from patient D. G. (curve A of figure 2). Curve C is that of a normal patient who received Co³⁵B₁₂ incubated with plasma from a patient with chronic myelocytic leukemia whose serum B₁₂ level was 7400 μg. per milliliter. In each of these two experiments, incubation was performed with freshly drawn plasma. These two curves fall between the normal curves and those of patients with chronic myelocytic leukemia; i.e., the rate of plasma clearance was...
Fig. 1 (top, left).—Disappearance of radioactivity from plasma of 10 patients with normal serum B$_{12}$ concentrations after i.v. Co$^{65}$ or Co$^{60}$B$_{12}$.

Fig. 2 (top, right).—Disappearance of radioactivity from plasma of 2 patients with chronic myelocytic leukemia after i.v. Co$^{60}$B$_{12}$.

Curves A and A': same patient, 15 months apart.

Fig. 3 (bottom, left).—Disappearance of radioactivity from the plasma of 3 "normal" patients after i.v. Co$^{60}$B$_{12}$ incubated with plasma from 3 patients with chronic myelocytic leukemia.

<table>
<thead>
<tr>
<th>Curve</th>
<th>Norm. recip.</th>
<th>Serum B$_{12}$ (free total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>W. W.</td>
<td>50/190</td>
</tr>
<tr>
<td>C</td>
<td>M. L.</td>
<td>48/280</td>
</tr>
<tr>
<td>B</td>
<td>D. L.</td>
<td>40/480</td>
</tr>
</tbody>
</table>

Curves C. M. L. donor

Serum B$_{12}$ (free total): A D. G. 25/1950
C M. N. 420/7000
B P. H. 300/4100

Fig. 4 (bottom, right).—Disappearance of radioactivity from plasma of 5 patients with pernicious anemia in relapse after i.v. Co$^{60}$B$_{12}$. 

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Fig. 5 (top, left).—Disappearance of radioactivity from plasma of 5 patients with malabsorption syndrome (curves A–E) after i.v. Co$^{60}$ or Co$^{59}$B$_{12}$.

Fig. 6 (top, right).—Disappearance of radioactivity from plasma of 4 patients with pernicious anemia in remission (curves A–D) and 1 patient with relapse (curve E) after oral B$_{12}$ (curve F).

Fig. 7 (bottom, left).—Average rates of disappearance of radioactivity from plasma of normal patients and patients with chronic myelocytic leukemia and pernicious anemia in relapse after i.v. Co$^{60}$ or Co$^{59}$B$_{12}$.

Fig. 8 (bottom, right).—Liver uptake after i.v. Co$^{60}$ or Co$^{59}$B$_{12}$.
Table 2

<table>
<thead>
<tr>
<th>Radioactivity remaining in plasma</th>
<th>Time after injection</th>
<th>Normal (Gp. 1)</th>
<th>P.A. in remission (Gp. 4)</th>
<th>P.A. in relapse (Gp. 3)</th>
<th>Chronic myelocytic leukemia (Gp. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Min.</td>
<td>30 Min.</td>
<td>1 Hour</td>
<td>6 Hours</td>
<td>24 Hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/4 Radioactivity remaining in plasma</td>
<td>50</td>
<td>14</td>
<td>11</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

slower than normal. This result may be explained by the increased capacity of leukemic sera to bind vitamin B₁₂ in vitro as well as in vivo.

In the third experiment (curve B of figure 3), the plasma clearance fell within the normal range. A normal patient received Co⁵⁸B₁₂ incubated with plasma from patient F. H. (curve B of figure 2), but in this case the plasma had been frozen for two months before use. The freezing and thawing may well be responsible for the lack of binding as shown by the normal disappearance curve.

Pernicious Anemia in Relapse and Malabsorption Syndrome. Five patients with untreated pernicious anemia were studied. The total serum B₁₂ in these patients ranged from 12 to 67 μg. per milliliter (table 1). Each of these patients demonstrated a plasma disappearance intermediate between the normal and the chronic myelocytic leukemia (fig. 4), i.e., at any given time a greater percentage of the injected radioactivity was retained in the plasma by this group than by the normals, but a still greater percentage was retained by the leukemias (table 2). The average T₁/₂ for the initial component was 6 minutes, a value between the normals and the patients with chronic myelocytic leukemia.

Studies were also carried out on five patients with malabsorption syndrome (total serum B₁₂ 19 to 190 μg. per milliliter; table 1). In three of these, the plasma clearance was comparable to the lower limits of the patients with pernicious anemia in relapse (fig. 5). The total serum B₁₂ just prior to the test injection in these three patients was 48, 100 and 180 μg. per milliliter, respectively. The fourth patient with a total serum B₁₂ of 190 μg. per milliliter showed a disappearance curve intermediate between the normal and the pernicious anemia in relapse. The fifth patient (total serum B₁₂ of 19 μg. per milliliter) demonstrated a very rapid clearance of plasma radioactivity, more rapid indeed than any of the normals.

Pernicious Anemia in Remission.—Four patients with treated pernicious anemia were studied. Three of these had serum B₁₂ levels in the high range of normal (855 to 1000 μg. per milliliter; table 1). Each of these patients had been treated by intramuscular injections of vitamin B₁₂ for periods up to two years, the last dose having been administered nine days, seven days and one day prior to the test. In two of these patients, the disappearance curve lay between the relapse curves and the normal; in the third patient, the curve fell in the lower ranges of the relapse curve (fig. 6.)
The fourth patient with pernicious anemia had received 60 to 100 μg. of B₁₂ intramuscularly once a week for eight years before the experiment. Total serum B₁₂ in this patient immediately prior to the test was 180 μg. per milliliter, and his disappearance curve fell within the range of normal (Curve D, fig. 6).

A fifth patient had undergone a total gastrectomy for peptic ulcer four years prior to the development of a typical pernicious anemia syndrome. Treatment had consisted of 50 μg. of vitamin B₁₂ three times daily by mouth for one year prior to the intravenous test and had produced excellent reticulocytosis and complete clinical and hematologic remission. At the time of testing, the serum B₁₂ was 220 μg. per milliliter. The disappearance curve (E, fig. 6) fell in the lower range of the untreated pernicious anemia group.

The average half-time of disappearance of the radioactive B₁₂ from the plasma in this group was four minutes. At any time subsequent to the injection, the average percentage of radioactivity remaining in the plasma was intermediate between the normal and the patients with pernicious anemia in relapse (table 2).

**Urinary excretion of radioactivity.**—The percentage of injected radioactivity which appeared in the 24-hour urine was less than 4 per cent in all cases. Approximately one-half of this appeared in the first two hours following injection. There was no significant difference in any of the groups studied.

**Tissue uptake of radioactivity.**—In vivo probe counting revealed that almost all of the measurable radioactivity appeared in the liver simultaneously with the drop in plasma radioactivity. Small increases in radioactivity above body background were also noted over the sacrum, heart and brain in the first 24 hours following injection. There was no appreciable increase in radioactivity over the spleen in patients with splenomegaly. Postmortem studies in one patient ("normal") who had received Co⁶⁰B₁₂ one week prior to death revealed 50 per cent of the injected dose within the liver, and small amounts (1 to 2 per cent) in the bone marrow, kidneys, pituitary, adrenals and heart. The greatest concentrations of radioactivity per gram of tissue were present in the liver, adrenals, and kidneys.

Uptake in the liver was rapid in the first three to six minutes, slower in the next 30 minutes, and increased slowly but steadily during the next three to five days. In most patients, there was a leveling off and/or a gradual fall in liver radioactivity beginning with the third to tenth day. Liver radioactivity was detectable in vivo for at least one year after the test. There was no significant difference in hepatic uptake of radioactivity in the different groups studied (fig. 8), as measured by the technic used.

**Binding of injected vitamin B₁₂.**—Microbiologic assays of plasma for B₁₂ content were performed on the same specimens which were assayed for their radioactivity. The curves obtained were roughly parallel to those determined with the radioactive method. Determination of "free" and total serum B₁₂ in each sample of plasma showed that the bulk of the injected vitamin B₁₂

*In this single case, the injection was given intra-arterially.*
was rapidly bound to the serum proteins (within three minutes), and that the total serum B₁₂ fell rapidly toward the pre-test levels in the normals and in patients with pernicious anemia. In chronic myelocytic leukemia there was no apparent fall. However, it should be noted that the change of 400 µg. B₁₂ per milliliter (which is less than 10 per cent of the total B₁₂) is within the range of error of this microbiologic assay method and may not have been detected.

Modifying factors.—The plasma of five patients with normal B₁₂ levels was saturated by an intramuscular injection of 1000 µg. B₁₂ immediately prior to the intravenous injection of radioactive B₁₂. The rate of disappearance of radioactivity in these patients was greater than normal (fig. 10): up to 75 per cent of the injected dose appeared in the 24 hour urine, and uptake of radioactivity by the liver appeared to be less than in the normal.

The capacity of human serum albumin to bind vitamin B₁₂ was studied by incubating 0.5 µg. of labeled B₁₂ with 20 ml. of human serum albumin for one hour at 37 C. and injecting the mixture intravenously into two "normal"

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Fig. 9.—The serum vitamin B₁₂ concentration (free, bound and total) in patients with normal vitamin B₁₂ serum concentration, with pernicious anemia in relapse and with chronic myelocytic leukemia, after i.v. B₁₂. Note the rapid binding of the injected B₁₂ in each patient.

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Fig. 10.—Plasma and urinary radioactivity following i.v. Co⁶⁰B₁₂. Effect of 1000 µg. B₁₂ i.m.
Curve A, no i.m. B₁₂.
Curve B, i.m. B₁₂ (1000 µg.) 1 hour before i.v. B₁₂.
patients. The resultant plasma disappearance curves showed no difference from the normal group.\textsuperscript{11}

DISCUSSION

Three assumptions have been made: first, it is assumed that the average adult has 2500 ml. of plasma and 500 $\mu$g. $B_{12}$ per milliliter, with a total circulating vitamin $B_{12}$ of approximately 1.25 $\mu$g. Thus, the test dose utilized in these studies (0.5 $\mu$g. in most cases; 1.0 $\mu$g. in a few) is not a tracer dose, although it does represent only a small percentage of the total body $B_{12}$. The specific activity of our radioactive $B_{12}$ is such that a true tracer could not be used because the amount of radioactivity in such a dose would be insufficient for counting purposes.

Secondly, it is assumed that the injected radioactivity remained a part of the $B_{12}$ molecule throughout the test period, so that changes noted in the disappearance and uptake of radioactivity represent identical changes of the $B_{12}$ molecule. That this assumption is valid is confirmed by the similar curves of plasma clearance obtained with both the radioactive and microbiological methods.

Thirdly, as stated before, the plasma volume (used in the formula to determine the percentage of radioactivity remaining in the plasma after injection) was estimated at 40 ml. per kilogram of body weight. For patients with normal hematocrits, plasma volumes estimated in this way are comparable to those determined by the formula of Miller, Corbus and Sullivan\textsuperscript{\textsuperscript{2}}

$$\text{plasmavolume} = \frac{0.69 \times \text{Kg. body weight}}{100}$$

plasmavolume

(i.e., plasma volume estimated on the basis of 40 ml. per kilogram of body weight are lower than those calculated from the plasmavolume formula, and the per cent of radioactivity remaining in the plasma is correspondingly lower. Thus, the disappearance curves of patients with untreated pernicious anemia would have been displaced still further upward (away from the normal) if the plasmavolume formula had been used.

Vitamin $B_{12}$ in the body may be thought of as being present normally in a small plasma pool and a large tissue pool. The plasma pool approximates 1.25 $\mu$g. $B_{12}$; most of the tissue pool is located in the liver (approximately 1000 $\mu$g. $B_{12}$),\textsuperscript{12} but small amounts are probably present in all body tissues as well as other body fluids. $B_{12}$ exists bound to proteins both in the plasma and in the liver.

The serum proteins which are involved in binding $B_{12}$ under normal conditions are the alpha and beta globulins. Bound $B_{12}$ (as measured by microbiologic assay) is bound to the alpha globulins,\textsuperscript{4} primarily alpha-1\textsuperscript{14} or alpha-2.\textsuperscript{13,14} “Free” $B_{12}$ (i.e., immediately available to the test organism without heating the serum) is bound to the beta globulins.\textsuperscript{1,14} The normal amount of $B_{12}$ in the serum (or plasma) is insufficient to saturate the $B_{12}$-binding proteins, and there is available, therefore, a latent capacity of the protein to bind further amounts of $B_{12}$. This binding power of human blood has been
measured by microbiologic technics and has been found to be of the order of 700 to 1000 \( \mu \text{g.} \) of \( 
abla_2 \) per milliliter,\(^1\) both in the normal individual and in the patient with pernicious anemia.\(^2\) Under nonphysiologic conditions, e.g., following parenteral administration of large amounts of \( 
abla_2 \), normal human serum is capable of binding still greater quantities. This is apparently due to nonspecific binding to other serum proteins (albumin\(^1\) and gamma globulin\(^1\)) after the usual \( 
abla_2 \) binding proteins are saturated. Vitamin \( 
abla_2 \) exists in the liver in a loose combination with a protein resembling serum beta globulin.\(^2\)

The fate of intravenously injected vitamin \( 
abla_2 \) in the normal is seen to be initial rapid binding by the serum proteins and rapid uptake by the liver. There is also an early distribution of small amounts of \( 
abla_2 \) to other tissues and probably to interstitial fluids. Inasmuch as the hepatic radioactivity increases gradually after the plasma radioactivity has fallen to low levels (24 hours), it would seem that there is a shift of the \( 
abla_2 \) from other tissues to the liver after the initial period of the test. Very little of the injected \( 
abla_2 \) is lost in the urine in the normal or, indeed, in any of the four groups of patients studied, and no radioactivity could be demonstrated in the stool.

In chronic myelocytic leukemia, both the serum concentration and the in vitro binding capacity of vitamin \( 
abla_2 \) are increased.\(^2\) In this disease, the \( 
abla_2 \) is bound almost entirely to the alpha globulins.\(^1\) The binding capacity of chronic myelocytic leukemic serum has been found to be almost five times that of normal serum.\(^2\) Apparently the liver does not share this increased binding capacity, perhaps because the increased binding is due almost entirely to the alpha globulins. The total body pool of vitamin \( 
abla_2 \) may be pictured as greater than in the normal, with the increase in the plasma pool accounting for this. Intravenously injected \( 
abla_2 \) is distributed in the same manner as is other body \( 
abla_2 \) and is bound to an abnormally high extent to the serum proteins. With the increased serum binding and increased plasma pool, a greater percentage of the injected \( 
abla_2 \) remains in the plasma, and the rate of plasma clearance is decreased as compared to the normal.

When 1000 \( \mu \text{g.} \) of vitamin \( 
abla_2 \) were administered intramuscularly to a normal patient, the plasma and tissue proteins were presumably saturated. The plasma pool of \( 
abla_2 \) was markedly increased, and the tissue pool was somewhat increased, but unlike the situation in chronic myelocytic leukemia, there was no increase in the \( 
abla_2 \)-binding proteins. With only a minimal amount of \( 
abla_2 \)-binding protein (or perhaps none) available, the subsequent intravenous test dose disappeared rapidly from the plasma and appeared in the urine in large amounts. Similarly, there was a decreased uptake of the test dose in the liver.

The plasma \( 
abla_2 \) pool may thus be increased in two different ways. The plasma disappearance curves in the two cases lie on opposite sides of the normal curve. In the first (chronic myelocytic leukemia), the increased pool is due to an increase in the \( 
abla_2 \)-binding protein; the test dose of \( 
abla_2 \) disappeared from the plasma at a slower rate than in the normal. In the second type (presaturation with \( 
abla_2 \)), the increased pool is due to administered \( 
abla_2 \)
and is not associated with protein changes; the test dose of $\text{B}_12$ disappeared from the plasma more rapidly than in the normal.

The increased capacity of chronic myelocytic leukemia sera to bind vitamin $\text{B}_12$ was studied by pre-incubation of such sera with $\text{B}_12$ prior to injection into a "normal" patient. The resultant plasma disappearance curve resembled that seen in chronic myelocytic leukemia, i.e., the rate of plasma clearance was reduced. Similar experiments in which $\text{B}_12$ was pre-incubated with human serum albumin showed no change from the normal disappearance curve. It would appear that the alpha globulins in the 20 ml. of leukemic serum bound and retained sufficient $\text{B}_12$ to cause the alteration of the disappearance curve. We can only speculate as to whether freezing and thawing affected the alpha globulins of the one leukemic serum that showed no difference from the normal disappearance curve.

In contrast to chronic myelocytic leukemia, the reduced rate of plasma clearance seen in patients with untreated pernicious anemia as compared to normals is not readily explained on the basis of increased binding by serum proteins. In pernicious anemia in relapse, it has been suggested that (1) isotope dilution or (2) increased unsaturated binding capacity of serum could explain the abnormally slow clearance rates. In the first case, since both the plasma pool and the tissue pool are contracted in these patients, the radioactivity of any aliquot (e.g., the plasma) following the intravenous injection might be high merely by the laws of isotope dilution. Alternatively, Mollin' has suggested that the plasma clearance reflects a degree of unsaturation of the plasma for $\text{B}_12$, the unsaturated binding capacity of the serum proteins being greatest in chronic myelocytic leukemia, less in untreated pernicious anemia and least in the normal. The increase in unsaturation of the $\text{B}_12$ binding proteins in untreated pernicious anemia follows from the fact that, although the total binding capacity of normal and pernicious anemia sera are the same (some 700 to 1000 $\mu\text{g. B}_12$ per milliliter), the actual amount of $\text{B}_12$ is greatly diminished in the serum in pernicious anemia in relapse.

Of the five patients with malabsorption syndrome studied, three showed plasma clearance rates in the range of untreated pernicious anemia, and the fourth demonstrated a plasma disappearance rate slower than the normal but more rapid than the untreated pernicious anemia patients. The total serum $\text{B}_12$ levels in these four patients ranged from 48 to 190 $\mu\text{g.}$ per milliliter, reflecting the variable absorption state in this syndrome. The same postulates of isotope dilution or unsaturated binding capacity may be applied to explain the plasma clearance rates in this group. The fifth patient with malabsorption syndrome and a total serum $\text{B}_12$ concentration of 19 $\mu\text{g.}$ per milliliter demonstrated a plasma clearance rate more rapid than the normal.

Our findings in presaturating the normal patient with intramuscular $\text{B}_12$, thereby lowering the plasma disappearance curve below the normal, would tend to support either of the postulates (isotope dilution or unsaturated binding capacity). The large dose of intramuscular $\text{B}_12$ greatly expanded the total body $\text{B}_12$ pool (especially the plasma pool) for a short time and at the same time saturated the $\text{B}_12$ binding proteins.
Although there are perhaps too few patients in the various groups to eliminate completely the statistical possibility of overlap, the curves suggest a test for pernicious anemia in remission as well as relapse, i.e., to measure the radioactivity in a plasma sample drawn 6 hours after injection of the test dose. If this (fig. 7, table 2) is 10 per cent or more of the injected radioactivity, one should suspect pernicious anemia.

Three of the patients with pernicious anemia in remission had high normal serum $B_{12}$ levels (855 to 1000 $\mu$g. per milliliter) immediately prior to the intravenous disappearance test. In these patients the total body $B_{12}$ pool (and the plasma $B_{12}$ pool) was either normal or expanded. The degree of unsaturation of the serum proteins for $B_{12}$ was either equal to or less than the normal. Therefore, the failure of the plasma disappearance curves to fall within the range of normal in these patients cannot be explained by changes in isotope dilution or by increased latent binding capacity of the serum proteins. The shift of the disappearance curves toward normal in the treated pernicious anemia patient may indeed be due to one or both of these factors, or perhaps to the lack of some hypothetical acceptor substance in the tissues (liver) of the patient in relapse which returns incompletely with remission. Nevertheless, the fact that the curves remain above the normal range suggests the existence of some other mechanism governing the rate of disappearance of $B_{12}$ from the plasma and its transport to the tissues. It is not unlikely that a substance is present in the plasma whose function is to permit the transfer of circulating $B_{12}$ from the blood to the tissues. Such a hypothetical substance (a “$B_{12}$-transferase”), present in normal amounts in the normal person, would be diminished in the blood of patients with pernicious anemia, even in remission, and this diminution would account for the upward shift of the plasma disappearance curve in this disorder. Treatment of the patient with pernicious anemia in relapse would not restore this “$B_{12}$-transferase”; thus, although the $B_{12}$ pools were expanded and the serum proteins normally saturated, the plasma clearance rate would remain slower than the normal.

Although “$B_{12}$-transferase” has not yet been demonstrated to exist, it is interesting to speculate on the relationship to intrinsic factor, which may be present within the blood stream. If the two are identical, and if the sole site of intrinsic factor production is the gastric mucosa, then the lack of “$B_{12}$-transferase” in the blood of patients with pernicious anemia and total gastrectomy is readily understandable. Moreover, if intrinsic factor passes through the intestinal mucosa with $B_{12}$, intestinal mucosal defects such as in malabsorption syndrome would prevent its entering into the blood, and the plasma disappearance curves would be displaced upward. As noted before, four of the five patients with malabsorption syndrome showed a pernicious anemia type of disappearance curve. A variable absorption state may account for the rapid plasma clearance noted in the fifth patient.

The postulate of a “$B_{12}$-transferase” derived from intrinsic factor seems to explain the plasma disappearance curves noted in pernicious anemia in re-
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lapse and remission, total gastrectomy and malabsorption syndrome. Studies by Herbert and Miller lend support to this postulate; measurement of in vitro liver slice uptake of radioactive B₁₂ in the presence of intrinsic factor, normal human serum and serum from a patient with pernicious anemia suggested that "... there is a circulating intrinsic factor-like substance which may be involved in the selective deposition of vitamin B₁₂ in the liver."

SUMMARY

1. Studies of the fate of intravenously injected radioactive vitamin B₁₂ have been performed in patients with normal, low and high serum concentrations of vitamin B₁₂.

2. Abnormal plasma disappearance curves were noted in chronic myelocytic leukemia, pernicious anemia in relapse and in remission, total gastrectomy and malabsorption syndrome.

3. In chronic myelocytic leukemia, the slow clearance of plasma radioactivity may be explained by the increased binding capacity of the plasma proteins for vitamin B₁₂.

4. Plasma clearance of radioactivity is slower than normal in pernicious anemia, even in remission. The failure of the disappearance curve to return to normal in pernicious anemia in complete remission suggests the existence of a plasma "B₁₂-transferase," whose function is to transfer circulating B₁₂ to the tissues. The disappearance curves suggest that the amount of such "B₁₂-transferase" is diminished in pernicious anemia, total gastrectomy and certain cases of malabsorption syndrome.

5. A relationship between a hypothetical "B₁₂-transferase" and intrinsic factor is discussed.

SUMMARIO IN INTERLINGUA

1. Le destino de radioactive vitamina B₁₂ post injectiones per via intravenose esseva studiate in patientes con normal, basse, e alte concentrationes seral de vitamina B₁₂.

2. Anormal curvas de disparitio ab le plasma esseva notate in patientes con chronic leucemia myelocytic, anemia perniciose in recidiva e in remission, gastrectomia total, e syndrome de malabsorption.

3. In chronic leucemia myelocytic, le lente disparitio de radioactivitate ab le plasma pote esser explicate per le augmentate capacitate ligatori del proteinas del plasma pro vitamina B₁₂.

4. Le disparitio del radioactivitate ab le plasma es anormalmente lente in anemia perniciose, mesmo in casos in remission. Le factor que le curva de disparitio non retorna a un conformation normal in anemia perniciose in remission complete suggere que il existe in le plasma un transferase de B₁₂ que ha le function de transferer B₁₂ ab le circulation a in le histos. Le curvas de disparitio suggere que le quantitate de iste transferase de B₁₂ es reducite in casos de anemia perniciose, de gastrectomia total, e a vices de syndrome de malabsorption.
5. Es discutite le relation possibile inter le postulate transferase de B₁₂ e factor intrinsec.

REFERENCES


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The Kinetics of Intravenously Injected Radioactive Vitamin B₁₂: Studies on Normal Subjects and Patients with Chronic Myelocytic Leukemia and Pernicious Anemia

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