Surface Scintillation Measurements in Humans of the Uptake of Parenterally Administered Radioactive Vitamin B₁₂

By George B. Jerzy Glass, Linn J. Boyd and Gerald A. Gellin

In earlier work¹⁻² the metabolism of vitamin B₁₂ was studied in patients with pernicious and other nutritional anemias. The microbiological assay with E. coli mutant¹ and Euglena gracilis² was used for the quantitation of vitamin B₁₂ in urine and blood, following injection or oral administration of vitamin B₁₂ alone or with various gastric materials of human or animal origin, containing intrinsic factor. The output of vitamin B₁₂ in the urine, as well as its levels in the blood, were correlated in these studies with the hematologic status of patients studied and their hematopoietic responses to vitamin B₁₂ therapy. In the present work we approached the problem of the metabolism of vitamin B₁₂ by isotopic technic, using vitamin B₁₂ containing radioactive Co⁶₀.

In animals the radioactive vitamin B₁₂ was used for quantitation of the excretion of vitamin B₁₂ in the urine and feces³⁻⁸, as well as for the study of its absorption and storage in various organs of the body. The radioactivity counts were obtained on rats postmortem, and conclusions were drawn regarding the metabolic turnover of vitamin B₁₂ in the animal body.

On living human subjects radioactive vitamin B₁₂ was previously used only for determination of the excretion of vitamin B₁₂ in the feces in cases of pernicious anemia⁹⁻¹⁰, sprue¹¹, or following total gastrectomy¹², or in the urine¹³.

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Miss Rose Schneider, Ph.D., performed some of the radioactivity counts on the first two cases.

All patients who submitted themselves to these prolonged studies are gratefully remembered.
In this work we have measured the uptake of the radioactive vitamin B₁₂ by various areas of the human body, as determined by surface measurements of the radioactivity on cutaneous projections of underlying organs following parenteral administration of Co⁶⁰-B₁₂. The technic is similar to that employed for quantitation of the uptake of I¹³¹ or P³² by various organs of the body under pathological conditions. With the availability of the more potent radioactive vitamin B₁₂ preparations and with the advent of efficient scintillation detectors, it became apparent that this new technic could be fruitfully employed for the study of the metabolic turnover of vitamin B₁₂ in man. Moreover, the data could be compared with that obtained on oral administration of radioactive vitamin B₁₂.

**METHOD**

**Radioactive Material**

Radioactive vitamin B₁₂ containing Co⁶⁰ used in this study was of two specific activities: (a) 38 μc. per mg., (b) 90 μc. per mg. The stock solutions were diluted with sterile water or with a sterile solution of crystalline nonradioactive vitamin B₁₂ to readjust the ratio of radioactive and nonradioactive material in samples administered to patients, according to requirement of the test.

**Counting Equipment**

A wide-angle scintillation detector without collimator with a photomultiplier tube, thick window and a 1" x ½" sodium iodide thallium crystal shielded with ½” lead and a scintillation scaler without amplifier were used throughout this study.

**Counting Procedure**

Scintillation counts were performed at 1000 volts. At this voltage the calibration curves of our instrument with standards of radioactive vitamin B₁₂ have shown that in the range of counts used, the ratio of the B₁₂ counts to the background was optimal. With the thick window used, mainly gamma rays were counted.

Standards containing exactly measured amounts (0.01-0.04 μc.) of radioactive vitamin B₁₂ were used throughout this study to correct for the daily variations in the efficiency of the instrument and small deviations in the high voltage settings.

Standards were prepared for two stock solutions of radioactive vitamin B₁₂ having various specific activities; whereby each time a sample of some specific activity was used, a standard was prepared from the stock solution of the same specific activity. One to two ml. aliquots were transferred to cupped stainless steel planchets of 2 ml. volume, 1 inch diameter and 5 ½ inch height, and evaporated under infra-red light with usual precautions and avoiding boiling. After drying of the material planchets containing standards were covered with parafilm and stored. To eliminate possible losses standards were prepared anew every 1-2 months. The usual radioactivity of standards was 0.01-0.02 μc. per planchet. Counts obtained at close approximation of the detector varied from about 2000 to 2400 c.p.m. per 0.02 μc. standard, depending on the actual efficiency of the instrument, whereby the usual background counts were in the range of 50-70 c.p.m. A maximum standard deviation between triplicate standard planchets did not exceed 70 c.p.m., which gave a coefficient of variation which did not exceed 3.6 per cent.

A correction factor 

\[
\frac{S_{T_s}}{S_T}
\]

was determined and used through the study to compensate for variation in the efficiency of the instrument. In this correction STₚ represents the mean initial standard value calculated from counts taken on duplicate, triplicate or quadruplicate standard planchets at the beginning of the experiment, i.e. prior to the administration of the radioactive material. ST is the mean standard count calculated from the multiple
counts of the same standard planchets taken before, during, and immediately after measurements of the surface counts on each of the days of the study. The correction factor was calculated daily, or twice daily, and it varied in this study from 0.85 to 1.15, but was usually in the range of 0.9 to 1.1.

A straight line relationship was obtained between the number of counts per minute above background and the contents of the radioactive vitamin \( B_2 \) in the standard planchets. The straight line correlation was obtained for the activities ranging from 0.0001 to 0.1 microcuries. Depending on the specific activity of the radioactive material used, this straight line relationship corresponded to amounts of radioactive vitamin \( B_2 \) ranging from 0.002 to 4.4 \( \mu g \). Thus, the straight line correlation covered the entire range of values encountered in the present study.

**Surface Counts**

Surface measurements of the radioactivity were performed on skin projections of various organs by means of the direct application of the scintillation detector to the skin. The counts were taken under similar basal conditions throughout this study, at least two hours apart from meals, with the patients in recumbent position and the tube of the detector perpendicular to the plane of the skin. The pressure exerted on the tube did not exceed the weight of the tube, and particular care was taken to avoid any additional pressure. This prevented the changes in the approximation of the detector crystal to the underlying internal organs, the site of the radioactivity storage.

The projections of the liver on the skin were determined with the use of palpation and percussion. Dye markings, ink spots, pencil marks, as well as circular hand-aids were placed on the selected skin areas to facilitate the task of duplication of the positioning of the tube during subsequent surface measurements.

Various topographic areas of the human body were used throughout this study for surface count measurements, as follows: (1) upper epigastrium, on xyphoid process; (2) mid-epigastrium, half-way between the tip of the xyphoid process and the umbilicus; (3) mid-abdomen, on the umbilicus; (4) lower abdomen, half-way between the umbilicus and the symphysis; (5) left hypochondrium (stomach projection) three inches to the left of the mid-line and one inch below the left costal margin; (6) left hypochondrium (spleen and splenic flexure) in the left anterior lateral line in the 8th or 9th intercostal space; (7) anterior liver projection, over the ribs, in the right mamillary line in the 6th or 7th intercostal space; (8) anterolateral liver projection, in the anterior lateral line, in the 7th, 8th or 9th intercostal space, depending upon the patient; (9) lateral projection of the liver, in the right mid-lateral line, in the 9th or 10th intercostal space; (10) left iliac crest, 1 inch to the rear of the iliac spine, on the bone; (11) left lumbo-dorsal area (left kidney projection), left mid-scapular line in the 10th or 11th intercostal space; (12) left upper calf, in the upper \( \frac{1}{2} \) of the lower leg, on gastrocnemius muscle; (13) left forearm, in the upper \( \frac{1}{2} \) of the forearm on its medial aspect; (14) injection site (when radioactive \( B_2 \) had been administered intramuscularly), upper lateral quadrant of the left gluteal area.

In order to obtain a better uniformity of surface counts over certain areas, such as the liver or the rest of the abdomen, several projection areas were combined into one larger territory and all areas in this territory were averaged.

All the individual surface counts were taken for 4 minutes (2 minute counts in duplicate) each with one minute interval between consecutive counts. At least 10 or 12 positions were taken in each series of surface counts on each individual, as well as initial and final measurement of the air background, surface background, and duplicate or triplicate standards. In some of the experiments, the series of surface counts was repeated in rotation every hour for six consecutive hours. The period of observation ranged from 5 weeks to 9 months for each of the particular cases (see below).

Each of the surface counts was calculated above the mean surface background. This was used instead of the usual air background because of the difference observed between the background count taken on the skin prior to the experiment, and the air background. Counts taken over the skin (before experiment was started) gave counts 10-15 c.p.m., lower than the air background obviously due to shielding effect of the human body.
fore, if the surface counts were measured following the administration of the radioactive material, somewhat higher radioactivity increments would be obtained. Because of this consideration instead of air background counts, surface background counts were determined as follows:

At the beginning of the experiment, prior to the administration of the radioactive material, surface counts of all the projections tested were taken, and averaged, and termed “initial mean surface background count” \((SB_0)\). The standard deviation was calculated from individual surface background counts obtained on various areas of the human body under similar basal conditions and in the same individual. As a rule, it was below 5 c.p.m. for the usual mean surface background of 50-60 c.p.m. In addition to the initial mean surface background \((SB_0)\) also the air background count was measured in duplicate for 8-10 minutes each, and the value obtained was averaged to obtain the mean initial air background \((B_0)\). Moreover, the average initial standard count \((ST_0)\) was determined, as described above.

After the administration of the radioactive material the surface background count could no longer be determined directly for obvious reasons, but it was calculated indirectly from the mean air background count \((B)\) taken together with the series of surface measurements. Each of these background counts was determined in duplicate for 4-10 minutes, before, during, and at the end of the measurements. This was done on each day of the experimental period. Using the previously determined figures for the initial air background \((B_0)\) and initial mean surface background count \((SB_0)\), the actual mean surface background count \((SB)\), was calculated as follows:

\[
SB = \frac{SB_0 \times B}{B_0}
\]

After deduction of the above surface background count \((SB)\) from the surface count \((SC)\) and correcting this value for the efficiency of the machine by multiplying it by the correction factor \(\left(\frac{ST_0}{ST}\right)\) the corrected surface count above surface background was obtained \((R_a)\):

\[
R_a = SC \times \frac{ST_0}{ST} - \left(\frac{SB_0 \times B}{B_0} \times \frac{ST_0}{ST}\right)
\]

Thus, the surface counts obtained during the experimental period became comparable with each other, as well as with the baseline values at the start of the experiment, and were not influenced by the efficiency of the instrument.

The percentual accuracy of our measurements \((S)\) has been calculated according to the usual formula:

\[
S = \frac{67.4 \times \sqrt{\frac{R}{T_R} \times \frac{B}{T_B}}}{R - B}
\]

where \(R\) is the surface count, \(B\) is the background count, and \(T_R\) and \(T_B\) represent the respective times during which surface and background counts were taken.5,6

This calculation shows that with four minute counts over the organs, and 8-10 minute background counts (60-70 counts per minute), the percentual accuracy was 1.2 per cent at 1,000 counts per minute above background, 1.9 per cent at 500 counts per minute, 2.9 per cent at 200 counts per minute and 4.7 per cent at 100 counts per minute. The calculated standard error of our measurements was therefore usually between 5 and 10 counts per minute. Only at a low counting rate, such as we found over the extremities, the error of percentual accuracy increased to 9.7 per cent at 40 counts per minute and to 17.8 per cent.
at 20 counts per minute, i.e., to about 4–5 counts per minute, which was significant at this low counting rate.

The increments in the radioactivity of the tissues following consecutive administration of multiple doses of radioactive material do not depend upon the initial levels of radioactivity and follow the additive pattern. It is therefore possible to repeat several experiments on the same individual and to calculate each time the increments of the radioactivity above the base line, whereby the last count taken before the administration of the new dose of radioactive material is considered as the base line. The prerequisite for this method is: (a) The attainment of a steady state of radioactivity in the area tested; this is reached about one week after injection and usually lasts for a few weeks during the experimental period; (b) A duplication of the increments in the radioactivity in each area tested when similar doses of radioactive material are injected repeatedly; this was the case in regard to the hepatic uptake of radioactivity.

We attempted to calculate the total dose of radiation in terms of roentgen equivalent physicals (rep) delivered to the critical tissues as result of the parenteral administration of radioactive Co$^{60}$-B$_2$. Since the liver is the critical target organ where the largest amount of Co$^{60}$-B$_2$ is stored, we calculated the dose of gamma radiation delivered to the liver.

According to the formula of Evans the tissue dosage rate (R) in rep per minute is represented as follows:

$$R = 42.4 \times C \times E_{av}$$

C in this equation denotes local concentration of the isotope in the critical organ calculated in millicuries (mc) per Gm. of tissue. Since the fraction of the entire radiation delivered to the body which becomes deposited in the liver is assumed to be 0.68 and the average weight of the liver is 1700 Gm., C would equal

$$\frac{0.925 \times 0.68}{1700 \times 1000} = 3.7 \times 10^{-5}$$

millicuries per 1 Gm. liver following injection of 0.925 mc. Co$^{60}$-B$_2$. E$_{av}$ in this equation represents the averaged radiation energy per disintegration which equals 1.19 Mev. Therefore the total tissue dosage rate of radiation delivered to the liver per minute will be equal to:

$$R = 42.4 \times 3.7 \times 10^{-7} \times 1.19, \text{ i.e., } 1.867 \times 10^{-4} \text{ rep per minute or}$$

$$\text{(after multiplication with } 60 \times 24 \times 7) = 0.19 \text{ rep per week.}$$

If, instead of the averaged energy of disintegration (E$_{av}$), the effective energy of disintegration is used in this equation, i.e., (2bE) resulting from disintegration of both the radioactive isotope in question and its daughters and which equals 0.72 Mev, the dose delivered to the liver will be much less and will amount to 0.11–0.12 rep per week only. This dose of radiation corresponds to about $1/4$ of the allowable dose of 0.3 rep per week in human studies.

If the same calculation is done according to the formula devised by Marinelli, Quimby, and Hine, then the total tissue dose (D$_{t}$) equals:

$$D_{t} = K_{t} \times C \times g.$$  

In this equation D$_{t}$ stands for the total microcuries of gamma rays emitted by the isotope in the liver, C represents the concentration of the isotope in the liver in microcuries per 1 Gm. of tissue, K$_{t}$ represents the number of roentgen equivalent physicals (rep) delivered at 1 cm. distance in the air from 1 microcurie of gamma ray emitter destroyed, and g represents the geometrical factor depending on size and shape of tissue mass, in which the isotope is distributed. Since K$_{t}$ equals 900, C equals $3.7 \times 10^{-7}$ in millicuries (see above), i.e., $3.7 \times 10^{-4}$ microcuries and g for liver of 1700 Gms. weight and 10 cm. diameter equals about 90, the total dose of radiation delivered to the liver will be:

$$D_{t} = 900 \times 3.7 \times 10^{-4} \times 90, \text{ i.e., } 30.0 \text{ rep.}$$

Since this amount represents the total radiation delivered to the liver from complete disintegration of the material injected, and F$_{d}$, which represents the fraction of the material disintegrated per day with Co$^{60}$, equals $3.5 \times 10^{-5}$, the total dose delivered per week to
the liver from 0.925 microcuries injected will be: $30.0 \times 3.6 \times 10^{-4} \times 7$; i.e., 0.0756 rep per week. This is $\frac{1}{4}$ of the allowable dose of 0.3 rep per week in humans.$^{16}$

**Human Material**

Five cases were studied:

1. Case 1, R. T., m., age 25, convalescent from sub-acute bacterial endocarditis without any bacteriological or clinical evidence of activity of the disease.
2. Case 3, R. C., m., age 18, rectal parasitism, no other disease.
3. Case 11, M. Mc., f., age 65, pernicious anemia at the beginning of a relapse with red cell count of 2,703,000 and hemoglobin of 9 grams. The patient was a known, typical case of pernicious anemia described in greater detail elsewhere.$^{22}$ She had been successfully treated a few months previously with a potent oral preparation of vitamin B$_{12}$ with intrinsic factor concentrate, but during the last two months had had no medication.
4. Case 2, J. J., m., age 55, had a total gastrectomy one and one-half years earlier for a lesion in the distal part of the stomach which proved on pathological examination to be due to benign peptic ulcer. One year earlier he had received treatment preventively with vitamin B$_{12}$ parenterally, and did not show any signs of anemia during the experimental period.
5. Case 4, P. M., m., age 71, sprue, was treated successfully a few months earlier with vitamin B$_{12}$ parenterally and showed no evidence of anemia.

**Dosage of Radioactive vitamin B$_{12}$**

The following doses of radioactive material were injected to the above subjects:
- **Case 1**: 10 $\mu$g. radioactive vitamin B$_{12}$ containing 0.925 $\mu$C., i.m.
- **Case 3**: 5 $\mu$g. radioactive vitamin B$_{12}$ containing 0.185 $\mu$C., i.m.
- **Case 11**:
  - a) 2 $\mu$g. radioactive vitamin B$_{12}$ containing 0.185 $\mu$C., i.m.
  - b) 2 $\mu$g. radioactive vitamin B$_{12}$ containing 0.185 $\mu$C., i.v.
  - c) 10 $\mu$g. radioactive vitamin B$_{12}$ containing 0.925 $\mu$C., i.m.
- **Case 2**: 10 $\mu$g. radioactive vitamin B$_{12}$ containing 0.925 $\mu$C., i.m.
- **Case 4**: 2 $\mu$g. radioactive vitamin B$_{12}$ containing 0.425 $\mu$C., i.m.

**Duration of observation periods and number of serial counts taken on each subject tested:**

- **Case 1**: 14 series during the 92 day experimental period
- **Case 3**: 10 series during the 63 day experimental period
- **Case 11**: 21 series during the 138 day experimental period
- **Case 2**: 23 series during the 238 day experimental period
- **Case 4**: 6 series during the 163 day experimental period

A 1 cc. tuberculin syringe and a 20 gauge needle 1 inch long were used for the intramuscular administration of radioactive vitamin B$_{12}$. The depth of penetration of the needle and its site were presumably the same in all subjects. The intravenous injection was done in the right cubital vein also with a tuberculin syringe. Utmost care was given to a complete discharge of the syringe content.

Parenteral doses of radioactive material given in this study were well below the permissible limit of 3 $\mu$c. of Co$^{60}$ per individual.$^{19}$

**Results**

The results of these studies are shown in figures 1-7. The presence of secondary and back scattered radiation as well as absorption of both primary and secondary radiation in the tissues makes the quantitative interpretation of radioactivity detected by external body scanning difficult. These
factors complicate comparisons of measurements over different body sites and over the same sites from one subject to another. However, the evaluation of patterns of distribution of radioactivity over various organs of the body is entirely feasible and appears to be of great interest.

The comparison of maximal radioactivity counts obtained over various organs after parenteral administration of radioactive vitamin B<sub>12</sub> shows that in all cases the highest counts were obtained over the liver, then over the spleen and kidney area, then over the iliac crest, and the lowest values were obtained over the extremities (calves or forearms).

Data on three cases listed in table 1 indicate that the maximal counts over the hepatic area are 2.5–3.5 times higher than over the kidney, 1.9–4.0 times higher than over the spleen, 4.5–7.0 times higher than over the iliac crest, and 16 to 33 times higher than over the calf muscle.

Table 2 lists the range of peak values of radioactivity counts over various organ projections during the first week after injection of 10 µg. Co<sup>60</sup>-B<sub>12</sub> (0.925 µc. Co<sup>60</sup>) in three subjects.

It can be concluded that the distribution of radioactivity following parenteral administration of Co<sup>60</sup>-B<sub>12</sub> is different over various areas of the human body. Moreover, the uptake of radioactivity by various organs of the human body, both under normal and pathological conditions follows different patterns. This is shown by the relative radioactivity values which are listed in table 3.

Since most of the radioactivity is accumulated in the liver, the scattered radiation around this organ makes it difficult to interpret the uptake of radioactivity by organs close to the liver. However, data listed in table 3 leave no doubt that the counts over the spleen, kidney or the iliac crest cannot be interpreted as ex-

**Table 1.**—Mutual Ratio of Peaks of Radioactivity over Various Areas of the Human Body to that over the Liver = 1.0 following Intramuscular Injection of 10 µg. Radioactive B<sub>12</sub> Containing 0.925 µc. Co<sup>60</sup>

<table>
<thead>
<tr>
<th>Areas of the body</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver projection</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Left kidney projection</td>
<td>0.40</td>
<td>0.28</td>
<td>0.38</td>
</tr>
<tr>
<td>Spleen projection</td>
<td>0.40</td>
<td>0.25</td>
<td>0.52</td>
</tr>
<tr>
<td>Left iliac crest</td>
<td>0.14</td>
<td>0.22</td>
<td>0.16</td>
</tr>
<tr>
<td>Left mid-calf</td>
<td>0.06</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Table 2.**—Range of Peak Counts of Radioactivity Per Minute above Background over Various Areas of the Body in Three Individuals (cases 1, 2 and 3) following Intramuscular Injection of 10 µg. Radioactive Vitamin B<sub>12</sub> Containing 0.926 µc. Co<sup>60</sup>

<table>
<thead>
<tr>
<th>Areas of the body</th>
<th>Counts per minute above background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver projection</td>
<td>650–1190</td>
</tr>
<tr>
<td>Spleen projection</td>
<td>300–350</td>
</tr>
<tr>
<td>Left kidney projection</td>
<td>245–330</td>
</tr>
<tr>
<td>Left iliac crest</td>
<td>105–255</td>
</tr>
<tr>
<td>Left calf</td>
<td>22–47</td>
</tr>
<tr>
<td>Area of the body</td>
<td>Case</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Site of injection (left upper gluteal area)</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100.0</td>
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<tr>
<td>2</td>
<td>100.0</td>
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<tr>
<td>3</td>
<td>100.0</td>
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<tr>
<td>11a</td>
<td>100.0</td>
</tr>
<tr>
<td>11b</td>
<td>100.0</td>
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<tr>
<td><strong>Averaged liver projection</strong></td>
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<tr>
<td>1</td>
<td>2.6</td>
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<tr>
<td>2</td>
<td>11.7</td>
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<td>4</td>
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<tr>
<td>1</td>
<td>29.3</td>
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<tr>
<td>11a</td>
<td>89.4</td>
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<td><strong>Spleen projection</strong></td>
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<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>11a</td>
<td></td>
</tr>
<tr>
<td>11b</td>
<td></td>
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<tr>
<td><strong>Mid-sternum</strong></td>
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<td>2</td>
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<td>11a</td>
<td>100.0</td>
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<td>11b</td>
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<tr>
<td><strong>Left iliac crest</strong></td>
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<td>1</td>
<td>44.0</td>
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<tr>
<td>2</td>
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<tr>
<td>11b</td>
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<tr>
<td><strong>Left mid-calf</strong></td>
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<tr>
<td>1</td>
<td>2.1</td>
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<tr>
<td>2</td>
<td></td>
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<td>3</td>
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<td>11a</td>
<td></td>
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<tr>
<td>11b</td>
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</table>
UPTAKE OF RADIOACTIVE VITAMIN B₂ IN A NORMAL SUBJECT

PARENTERAL DOSE: 10⁴ - 0.925 μc.

Fig. 1.—Uptake of radioactive vitamin B₂ in a normal subject (case 1) following intramuscular injection of 10 μg. radioactive vitamin B₂ containing 0.925 μc. of Co⁶⁰.

UPTAKE OF RADIOACTIVE VITAMIN B₂ IN A NORMAL SUBJECT

PARENTERAL DOSE: 10⁴ - 0.925 μc.

Fig. 2.—Uptake of radioactive vitamin B₂ in a normal subject (case 1) following intramuscular injection of 10 μg. radioactive vitamin B₂ containing 0.925 μc. of Co⁶⁰.
Fig. 3.—Uptake of radioactive vitamin B₁₂ in a normal subject (case 3) following intramuscular injection of 5 μg. radioactive vitamin B₁₂ containing 0.185 μc. of Co⁶⁰. (The average liver count on the 63rd day not shown in the figure was 101 c. p. m. above background).

Exclusively derived from scattered radiation of the liver and they indicate that the changes in radioactivity over various areas of the body follow their own distribution patterns. Thus, in case 2, two or two and one-half months after the injection the counts over the liver dropped to about 1/2, as compared to peak values noted at the end of the first week, while those over the kidney remained almost stationary, and those over the spleen declined much less. At the same time the counts over the iliac crest showed a large increment (fig. 5). Similar divergence of trends was observed in case 11 B (see table 3 and fig. 6).

The site of injection (left lateral gluteal area): The radioactive vitamin B₁₂ disappears rapidly from the site of intramuscular injection (figs. 1 and 4). Over 96 per cent of radioactivity deposited by injection is absorbed within the first three to four hours following injection, whereby only some small part of the injected dose (0.5–3.5 per cent) remains in the site of the injection for a week or two. This rapid absorption of radioactive vitamin B₁₂ from the site of the intramuscular injection obviously facilitates the rapid uptake of radioactivity by various tissues of the human body under these circumstances.

The amount of radioactivity detected in the gluteal area immediately after injection represented only from 4.9 to 16.4 per cent and, on the average, only 11.7 per cent of that which an equal amount of radioactive material showed when counted in planchets outside of the body. While 1 μc. of Co⁶⁰ gave, on the average, 120,000 counts per minute in planchets, the same amount of material when measured over the gluteal area immediately after injection produced only from
OF INJECTION UPTAKE OF RADIOACTIVE VITAMIN B₂ IN A PATIENT AFTER TOTAL GASTRECTOMY

PARENTERAL DOSE: 10 µg radioactive vitamin B₂ containing 0.925 µc. of Co₆₀.

5,100 to 17,510 counts per minute. The reasons for this decrease in counts in vivo appear to be: (1) Increase of the distance between the material in the tissue and the crystal of the scintillation tube from about one to several centimeters; this decreases the counts in square to the increase of distance. (2) The self-absorption of the radioactive material in the tissues. (3) Very fast absorption of radioactive material from the tissue into the blood which follows the hyperbolic curve (figs. 1 and 3) and which results in the departure of a large part of the deposited material from the site of the injection within a few minutes following its administration.

Left kidney and spleen projections: The data listed in table 3 indicate a rapid increase of radioactivity over skin projections of these two organs following intramuscular injection of Co₆₀-B₂. At the end of one to four hours, radioactivity uptake reaches a peak over these organs (figs. 1, 4 and 6). A slight and gradual decline in radioactivity follows thereafter, so that at the end of 2–3 weeks 50 to 70 per cent, and 2–5 months after injection about 25 to 45 per cent of the peak radioactivity remained over the spleen, and 50–85 per cent and 50–65 per cent of peak radioactivity remained over the left kidney after 2–3 weeks or 2–3 months respectively. It should be noted that in case 11 (pernicious anemia, fig. 6) and case 4 (anemia in sprue, table 3) a second peak of radioactivity over the kidney and spleen was observed 4–6 days after injection.

Liver area: An initial sharp rise in the radioactivity over the liver was observed in all cases tested within two hours after injection. (Table 3, figs. 1, 4 and 6.)
continued at a slower pace through the next week to reach a peak between the third and seventh day in all our cases. The high radioactivity counts over the liver showed a plateau-like leveling during the second to fourth week after injection, and a slight and gradual decline thereafter so that in normals 65 to 86 per cent of the peak radioactivity was still maintained over the liver area two to three months later whereby in some cases (cases 4 and 11) as much as 60–85 per cent was retained for five months, and as much as 38 per cent for eight months after injection (case 2). This decline includes the normal decay of Co⁶⁰ which is about 1.0 per cent per month. In the patient with total gastrectomy (case 2) a transient drop in the counts on the lateral aspect of the liver was observed for about one week following the liver biopsy. This probably was related to the formation of a hematoma somewhere between the liver and skin, which might have created a buffer zone to cause a drop in the surface counts.

**Iliac crest:** The initial peak level of radioactivity was built over the iliac crest during the first hour after injection in all cases (table 3). This was usually retained for a long time (figs. 1, 2, 4–6) so that a month after injection from 47 to 72 per cent of the peak radioactivity was still observed over this area. In the patient with total gastrectomy a secondary rise in the radioactivity counts over the iliac crest was observed to the double of its previous value at the end of the second month. This was coincident to the decline in the radioactivity over the liver (fig. 5).
FIG. 6.—Uptake of radioactive vitamin B₂ in a patient (case 11) with pernicious anemia following intramuscular injection of 10 μg. radioactive vitamin B₂ containing 0.925 μc. of Co⁶⁰. (The average liver count on the 153rd day not shown in the figure was 507 c. p. m. above background).

Averaged abdominal area (beyond the liver) was counted in the control subject (case 3, fig. 3) and patient with sprue (case 4). At the end of the first week the count amounted to 20–25 per cent, at the end of the fourth week to about 20 per cent and at the end of the five months to about 13 per cent of the averaged hepatic count at that time.

Left mid-calf: The uptake of radioactivity over the calf was very low (figs. 1–6). The peak values were usually observed 24 hours after injection. This was followed by a progressive gradual decline to about 21–57 per cent from the initial peak values at the end of two to three weeks. In view of very low radioactivity counts over the extremities, which implies great margin of error, the calculation of the percentual changes of radioactivity over the calf (table 3) cannot be considered reliable.

Left forearm: The counts over the forearm were slightly higher than those over the calf, but still were too low to permit reliable conclusions.

Uptake of radioactivity after intravenous injection of Co⁶⁰-B₂ was measured in one patient (case 11, pernicious anemia in remission). The data obtained are listed in table 4, and compared with that registered in the same patient after intramuscular administration of the same dose of radioactive material a few weeks before.

The range of radioactivity over various organs was grossly similar in both instances (table 4). In view of a low dosage of Co⁶⁰ in this case (0.185 μc.) the
Table 4.—Increments in Radioactivity in Counts Per Minute following Intramuscular and Intravenous Injection of 2 μg. Radioactive Vitamin B₁₂ Containing 0.185 μc. of Co⁶⁰ in a Patient with Pernicious Anemia in Remission (Case II)

<table>
<thead>
<tr>
<th>Areas of the body</th>
<th>Kind of injection</th>
<th>Time after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>½ hour</td>
</tr>
<tr>
<td>Liver projection</td>
<td>i. m.</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>57</td>
</tr>
<tr>
<td>Left kidney projection</td>
<td>i. m.</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>56</td>
</tr>
<tr>
<td>Spleen projection</td>
<td>i. m.</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>57</td>
</tr>
<tr>
<td>Mid-sternum projection</td>
<td>i. m.</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>15</td>
</tr>
<tr>
<td>Left iliac crest</td>
<td>i. m.</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>19</td>
</tr>
</tbody>
</table>

Fig. 7.—Radioactivity counts over the liver in four individuals following intramuscular injection of 2 and 10 μg. radioactive vitamin B₁₂ containing 0.185 and 0.925 μc. Co⁶⁰.

Some significance may be attached, however, to peak of radioactivity over the kidney after intravenous injection about two hours earlier than after intramuscular injection (figure 7). This might reflect the faster elimination of vitamin B₁₂ through the kidneys following intravenous injection.  

Differences between both sets of data are within the margin of error of the method.
DISCUSSION

The method of surface measurements as applied to the study of radioactive vitamin B₁₂ distribution in the body has many limitations. Counts taken on skin projections of various internal organs are the resultants of radiation not only of the underlying, but also of the superimposed or neighboring organs, the radiation from which interferes to some extent with the radiation of the organ studied. Inferences concerning the uptake of radioactivity by small organs, which are surrounded by the large neighboring organs, are unreliable. This may be partly remedied by exact location of the detector, by taking of counts on several projections of the same organs, and repeating them on several consecutive days in order to obtain an average from all these multiple counts. Vice-versa, the study of the uptake by a large massive organ such as the liver is facilitated. In view of the above, the inferences drawn from the actual study must be very limited, and restricted mainly to the findings regarding the distribution, uptake, and length of storage of radioactivity by various areas of the human body and especially the liver.

Our data on the uptake of radioactive vitamin B₁₂ following its parenteral administration in small doses of 2–10 μg. as measured by surface counts, roughly correspond to data of Barbee and Johnson and Rosenblum et al., obtained on rats; they also found the greatest accumulation of radioactive material in the liver, kidney and spleen after parenteral administration of Co⁶⁰-B₁₂.

The persistence of the storage of radioactive vitamin B₁₂ in the liver for many months after parenteral administration, which is also found following oral administration of Co⁶⁰B₁₂, raises the problem whether this residual radioactivity is due to the deposition of radioactive Co⁶⁰ containing vitamin B₁₂ as such, or rather to the storage of Co⁶⁰ after its detachment from the radioactive vitamin B₁₂ molecule during its metabolism in the liver. No definite opinion on this subject is possible at the present time; however, the results of animal studies speak rather in favor of radioactive vitamin B₁₂ staying as such in the liver. Moreover, according to Hevesy, when Co⁶⁰ is injected alone (not coupled to B₁₂), 65 per cent is excreted in the urine and 35 per cent in the feces within a few days.

It is probable, therefore, that the radioactivity detected over the liver many months after injection is due to the presence of Co⁶⁰-B₁₂ in this organ. In this case the slow decline of radioactivity in the liver would point to a great ability of the liver to store vitamin B₁₂ and reflect the well-known high vitamin B₁₂ content of the liver. Moreover, if we assume that the long persistence of radioactivity in the liver is due to the storage of the whole Co⁶⁰-B₁₂ molecule, and not to the storage of Co⁶⁰ alone after its detachment from B₁₂ in the liver, this might explain the prolonged time needed to deplete stores of vitamin B₁₂ and for development of macrocytic anemia after total gastrectomy, as well as long periods of remission observed in pernicious anemia following therapy. This problem requires further study, and the data presented in this paper need amplification.

The strict proportion of the increased dosage of radioactive material to increased uptake of radioactivity by the liver requires further comment. The averaged liver counts in a control subject (case 3) was equivalent to 19 per cent of the peak count over the liver observed in another control subject (case 1): 795 versus 151 counts per minute above background. This almost exactly reflected the dose
of radioactivity which was 20 per cent of that given to the first subject. Also in
the patient with pernicious anemia, the uptake of radioactive vitamin B\textsubscript{12} by the
liver was 3.35 times as great at its peak as when the five-fold larger dose of the
radioactive material (0.925 \(\mu\)c.) was injected initially (0.185 \(\mu\)c.): 047 versus 121
counts per minute. This tends to indicate the applicability of the isotope technic
for the study of the extent of the storage of radioactive vitamin B\textsubscript{12} by the liver.

Only limited inferences are permissible in comparing the uptake of radioac-
tivity by various individuals. Since the differences in the body weight and struc-
ture affect the distance of the underlying organ to the detector tube, they have
an important effect upon the counts obtained. Consequently, only obviously out-
standing and statistically significant differences in uptake by various individuals
who do not differ much in their weight and body build are significant. Moreover,
the metabolic state of the patient also influences the results, especially the states
of saturation or desaturation in respect to vitamin B\textsubscript{12}, which may influence the
tissue retention and urinary elimination of the vitamin.

Keeping these limitations in mind, it would appear from figure 7 that the up-
take of the radioactivity by the liver at the same parenteral dosage of Co\textsuperscript{60}-B\textsubscript{12}
was highest in the individual with the total gastrectomy. The normal control
subject and patient with pernicious anemia in partial remission had an uptake at
a lower level. Similarly with a lower dosage of Co\textsuperscript{60}-B\textsubscript{12}, the uptake of radioactiv-
ity by the liver was almost identical in the control subject 3, and patient with
pernicious anemia in partial remission. The uptake by the kidney and spleen was
slightly higher in the patient with pernicious anemia than in the normal subject
1, while on the other hand the radioactivity over the iliac crest showed a faster
decline during the course of study than in normals. It is possible that in a patient
with total gastrectomy the turnover of the radioactive vitamin B\textsubscript{12} was acceler-
ated. This might be indicated by the occurrence of the peak uptake by the liver
two days earlier, a 1\(\frac{1}{2}\) times higher hepatic and 2\(\frac{1}{2}\) times higher uptake over the
iliac crest than in control subject, and a sharp drop of the radioactivity level over
the liver to one-half of its value at the end of 10 weeks after injection. Whether
this should be considered as the evidence of a greater unsaturation of vitamin B\textsubscript{12}
stores in the patient because of total gastrectomy, subsequent to the defective
intestinal absorption of vitamin B\textsubscript{12} cannot be decided in view of limitations of the
method.

**Summary and Conclusions**

1. The distribution of radioactive vitamin B\textsubscript{12} in humans was studied by scin-
tillation counting of radioactivity over various skin projections of underlying
organs in five individuals, following parenteral administration of radioactive
vitamin B\textsubscript{12}. The results of these investigations showed that the scintillation sur-
face measurements of the radioactivity following parenteral administration of
radioactive vitamin B\textsubscript{12} may be profitably applied to the study of the metabolic
turnover of vitamin B\textsubscript{12} under normal and pathological conditions in humans.

2. In two young normal control subjects over 96 per cent of the parenterally
administered radioactive vitamin B\textsubscript{12} (5 and 10 \(\mu\)g, containing 0.925 \(\mu\)c. of Co\textsuperscript{60})
disappeared from the site of the intramuscular injection within three to four
hours, and during that time radioactivity rose to its peak value over the areas
corresponding to kidney, spleen and iliac crest, and somewhat later over the mus-
cles of the extremities. Subsequently, a gradual decline of radioactivity was observed over the spleen, kidney and extremities, so that at the end of three months in the normal subject from $\frac{1}{3}$ to $\frac{1}{2}$ of radioactivity as compared to peak values was observed over the spleen and left kidney and about $\frac{1}{4}$ over the calf muscle.

The projection areas of the liver differed from all the other areas of the body in requiring about five to six days to build up the peaks of radioactivity. These exceeded counts over the kidney and spleen about $2\frac{1}{2}$ times, counts over the iliac crest approximately 7 times, and those over the calves about 17 times. The decline of radioactivity over the liver and iliac crest was very slow and small.

3. The patterns of the uptake of injected radioactive vitamin B$_{12}$ by the totally gastrectomized patient were grossly similar to those observed in normal controls, except for possibly slightly faster absorption of the injected material from the site of injection, and somewhat faster and larger accumulation, but also faster discharge of radioactivity from the liver area.

4. In a patient with pernicious anemia in partial remission who received three parenteral doses of 2 respectively 10 $\mu$g. radioactive vitamin B$_{12}$ (0.185-0.925 $\mu$C. Co$^{60}$), the uptake of the radioactive vitamin B$_{12}$ by the kidney and spleen area was slightly higher than that of the normal controls, that of the liver similar to the normal, and that over the iliac crest showing a significantly faster decline than in normals during the course of study. The uptake of radioactive vitamin B$_{12}$ in the same patient following intravenous injection of the same dose of radioactive vitamin B$_{12}$ was grossly similar to that following intramuscular injection of the same dose of radioactive material, except for the faster accumulation of radioactivity over the kidney area.

5. From 65 to 86 per cent of the peak radioactivity persisted over the liver 2–3 months after parenteral administration of radioactive vitamin B$_{12}$ to two control subjects. After five months 60 per cent of the initial peak of radioactivity was still observed over the liver in the patient with anemia of sprue in remission, 85 per cent in a patient with pernicious anemia in partial remission, and at the end of eight months still 35 per cent of the peak liver count in a patient with total gastrectomy without anemia. This does not take into account the normal decay of Co$^{60}$ which is about 1 per cent per month.

6. If the presence of Co$^{60}$ in the liver indicates the deposition of Co$^{60}$-B$_{12}$ in this organ, which is most probable, then the long storage of vitamin B$_{12}$ in the liver may explain the long time needed in humans for depletion of hepatic stores of vitamin B$_{12}$ as well as for long remissions observed in pernicious anemia following parenteral treatment with liver extracts or vitamin B$_{12}$.

Summario e Conclusiones in Interlingua

(1) Le distribution de radioactive vitamina B$_{12}$ in humanos esseva studiate per medio de mesurationes scintillatori del radioactivitate supra varie projectiones al pelle in le regiones de certe organos de cinque individuos a qui radioactive vitamina B$_{12}$ habeva essite administrate parenteralmente. Le resultatos de iste investigationes indica que le metodo del mesuration scintillatori superficial pote applicar se profitabilemente al studio del metabolismo de vitamina B$_{12}$ in humanos sub conditiones normal e pathologie.

(2) In duo juveme e normal subjectos de controlo plus que 96 pro cento del
112 INTRINSIC FACTOR AND VITAMIN B₁₂

dosages parenteral de vitamina B₁₂ (5 e 10 µg con un contenuto de 0,925 µg Co₆₀) dispareva ab le sito del injection intramuscular intra tres a quatro horas. Durante iste periodo le radioactivitate aceseseva a valores maximal supra le regiones de ren, splen, e crista iliac e un pauc plus tarde supra le musculos del extremidades. Subsequentemente un reduction progressive del radioactivitate esseva observate supra splen, ren, e extremidades. Al fin de tres menses le radioactivitate in subjectos normal esseva inter 1/₅ e 1/₆ del valor maximal supra splen e ren e circa 1/₄ del maximo supra le muscolo sural.

Le areas de projection supra le hepate differeva de omne le altere regiones del corpore in tanto que illos requireva circa cinque a sex dies pro attinger lor maximos de radioactivitate. Iste maximos excedeva le contationes supra ren e splen per circa 2/₃ vices, illos supra le crista iliac per circa 7 vices, e illos supra le suras per circa 17 vices. Le regression del radioactivitate supra hepate e crista iliac esseva lentissime e multo parve.

(3) Le schema del absorption de injectiones de radioactive vitamina B₁₂ in patientes a gastrectomia total esseva grossiermente simile al schemas observate in subjectos de controlo normal, excepte possibilemente que le injicite materia esseva absorbite un pauc plus rapidemente ab le sito del injection, que le accumulation esseva levemente plus rapide e plus grande, sed etiam que le discarga de radioaetivitate ab le region hepatic esseva plus rapide.

(4) In un patiente con perniciose anemia in remission partial, qui recipeva tres doses parenteral de 2 e 10 µg de radioactive vitamina B₁₂ (0,185-0,925 µc Co₆₀), le absorption per le areas del ren e del splen esseva levemente plus alte que in le controlos normal. In iste caso le absorption del region hepatic esseva simile al norma, e illo del crista iliac monstrava un regression significativemente plus rapide que in subjectos normal. In le mesme patiente le absorption de radioactive vitamina B₁₂ administrate per injection intravenose esseva grossiermente simile al absorption de un dose identic administrate per injection intramuscular, excepte que le accumulation de radioactivitate supra le ren esseva plus rapide.

(5) In duo subjectos de controlo, inter 65 e 86 pro cento del radioactivitate maximal resultante del administration parenteral de radioactive vitamina B₁₂ persisteva supra le hepate pro 2 a 3 menses. Post cinque menses ancora 60 pro cento del maximo initial esseva observate supra le hepate del patiente con anemia de sprue in remission e 85 pro cento in un patiente con perniciose anemia in remission partial, sed post octo menses le valor correspondente in un patiente con gastrectomia total esseva 35 pro cento. Iste valores non considera le decadentia normal de Co₆₀ que es circa 1 pro cento per mense.

(6) Si le presentia de Co₆₀ in le hepate indica le deposition de Co₆₀-B₁₂ in iste organo (lo que es probabilissime), alora le longe immagazinage de vitamina B₁₂ in le hepate pot explicar le longe tempore requirite in humanos pro depler le stock hepatic de vitamina B₁₂ e etiam le longe remissiones que ha essite observate in casos de perniciose anemia post tractamento parenteral con extractos hepatic o vitamina B₁₂.

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Surface Scintillation Measurements in Humans of the Uptake of Parenterally Administered Radioactive Vitamin B₁₂

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