ESTIMATION OF THE TOTAL BODY VITAMIN B₁₂ IN THE LIVE SUBJECT

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SUMMARY

1. Values for total body vitamin B₁₂ were calculated for eighteen patients by giving a tracer dose of radioactive cyanocobalamin and measuring the radioactivity and microbiological activity in liver biopsies obtained at laparotomy.

2. The values for total body vitamin B₁₂ ranged from 960 to 5984 µg with a mean of 2528 µg. Five values were greater and thirteen less than the mean.

3. Significant correlations were found between the serum vitamin B₁₂ and total body vitamin B₁₂, the serum vitamin B₁₂ and the hepatic vitamin B₁₂ and the hepatic B₁₂ and the total body vitamin B₁₂.

Work published to date on the vitamin B₁₂ content of the body relies mainly on calculations based on microbiological analysis of tissues obtained post mortem from patients with a wide variety of diseases. Mean values of 2689 µg, 3900 µg and 5000 µg with ranges of 1634 to 3475 µg, 790 to 11,100 µg and 3480 to 10,950 µg have been suggested by Kurlov (1961, 1962), Gräsbeck, Nyberg & Reizenstein (1958) and Heinrich (1964) respectively, and Adams (1962) reported a mean of 2221 µg with a range of 953–4304 µg from a combined isotope dilution and microbiological assay procedure.

The only report on the vitamin B₁₂ content of the body in the living subject is one by Reizenstein, Ek & Matthews (1966) in which an average value of 3030 µg was obtained by kinetic analysis of values for whole body retention, faecal excretion and plasma clearance after parenteral radioactive vitamin B₁₂. We are not aware of any other estimates of total body vitamin B₁₂ in the living subject and so we report here our experience with a procedure by which approximate values were obtained by relatively simple methods in this situation. Although limited in scope and application we believe it has a place in studies of vitamin B₁₂ metabolism in man.

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MATERIALS AND METHODS

The basic principle is that of isotope dilution which assumes that, at a finite time after administration, a tracer dose of radioactive material is distributed throughout the body in proportion to the endogenous non-radioactive material. Previous work suggests that equilibration of an intravenously administered dose of 100 ng radioactive cyanocobalamin with body stores of vitamin B₁₂ occurs 5 to 10 days after injection and that the loss of radioactive material from the body between the time of injection and equilibration is of the order of 5% (Adams & Boddy, 1968). Thus the total amount of vitamin B₁₂ in the body can be calculated by measurement of radioactivity and vitamin B₁₂ content of a suitable tissue obtained after equilibration and by allowing for the loss of radioactive material from the body.

On this basis patients referred for elective surgery were appraised of the nature of the study and, after a sample of blood had been obtained for serum vitamin B₁₂ estimation, were given 50 ng 5.0 μCi [⁵⁷Co]cyanocobalamin in 5.0 ml water intravenously. At operation, which was performed 6–29 days later, a wedge of liver was obtained, usually from the antero-inferior aspect of the left lobe, and haemostasis secured by sutures before the major procedure. The tissue was weighed in a Stanton analytical balance and stored at -20° if necessary before homogenization with water in a Potter Elvehjem or Silverson microhomogenizer to a volume of 20 ml. The activity was measured in a well-type scintillation counter IDL type 663 with a thallium activated sodium iodide crystal, 5.5 cm. diameter and 6.9 cm. deep, shielded by 10-0 cm lead and connected to an IDL 1700 automatic scaler using three standards each containing 1.0% of the dose in 20 ml water. At least 10,000 counts were obtained from every sample. The homogenate was then further homogenized with water in a Waring Blender to suitable dilutions for microbiological assay by the method of Hutner, Bach & Ross (1956) using Euglena gracilis Z strain as the test organism, and commercially available medium (Difco Laboratories Inc.). The homogenate and serum sample from each patient were assayed together on at least three occasions.

[⁵⁷Co]cyanocobalamin was obtained from the Radiochemical Centre, Amersham, two batches being used in the study. The material was dissolved in distilled water and dispensed in 5 ml aliquots in dark glass ampoules after sterilization by Millipore filtration. The ampoules were stored at +4°. Before use of a batch an ampoule was taken at random and the purity of the solution tested by column chromatography using carboxy-methyl-cellulose and diethylamino-ethyl-cellulose. The final ampoule in each batch was tested similarly. The rationale of these procedures, described in detail by Kennedy & Adams (1965) & Kennedy (1967) is that cyanocobalamin, being neutral, is not retained by either material whereas hydroxocobalamin, which results from photolysis of cyanocobalamin (Smith, 1965), is retained by the cation exchanger carboxy-methyl-cellulose and the ‘red acids’, which result from radiochemical decomposition of cyanocobalamin (Smith, 1965), are retained by the anion exchanger diethylamino-ethyl-cellulose.

RESULTS

Eighteen patients were studied, the relevant information on each being given with the essential results in Table 1. There was no morbidity in the series. There was no evidence, by the methods used, that the [⁵⁷Co]cyanocobalamin solutions tested had deteriorated during storage.
**TABLE 1. Showing details of patients and essential results**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Serum $B_{12}$ (pg/ml)</th>
<th>Disease and operation</th>
<th>Time interval between injection and biopsy (days)</th>
<th>Mass (g)</th>
<th>Liver biopsy activity (% dose)</th>
<th>Assayable $B_{12}$ (µg)</th>
<th>Calculated 'Total Body $B_{12}$' (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>M</td>
<td>463</td>
<td>Duodenal ulcer—partial gastrectomy</td>
<td>29</td>
<td>3.7210</td>
<td>0.2389</td>
<td>6.1305</td>
<td>2438</td>
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<tr>
<td>2</td>
<td>47</td>
<td>F</td>
<td>—</td>
<td>Gastric ulcer—partial gastrectomy</td>
<td>25</td>
<td>5.4507</td>
<td>0.3832</td>
<td>6.7689</td>
<td>1678</td>
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<tr>
<td>3</td>
<td>59</td>
<td>M</td>
<td>433</td>
<td>Gallstones—cholecystectomy</td>
<td>18</td>
<td>4.1903</td>
<td>0.1979</td>
<td>3.3255</td>
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<td>4</td>
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<td>191</td>
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<td>5.0027</td>
<td>0.2184</td>
<td>3.1650</td>
<td>1377</td>
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<tr>
<td>5</td>
<td>74</td>
<td>F</td>
<td>511</td>
<td>Gallstones—cholecystectomy</td>
<td>6</td>
<td>4.4167</td>
<td>0.2867</td>
<td>7.7263</td>
<td>2560</td>
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<tr>
<td>6</td>
<td>64</td>
<td>M</td>
<td>490</td>
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<td>11</td>
<td>2.0411</td>
<td>0.1017</td>
<td>1.0526</td>
<td>593</td>
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<tr>
<td>7</td>
<td>57</td>
<td>M</td>
<td>444</td>
<td>Duodenal ulcer—gastroenterostomy and vagotomy</td>
<td>15</td>
<td>2.5576</td>
<td>0.1247</td>
<td>2.0680</td>
<td>1375</td>
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<tr>
<td>8</td>
<td>53</td>
<td>M</td>
<td>353</td>
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<td>14</td>
<td>2.3975</td>
<td>0.0644</td>
<td>1.6156</td>
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<td>9</td>
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<td>F</td>
<td>403</td>
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<td>3.0041</td>
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<td>2.5530</td>
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<td>33</td>
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<td>46</td>
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<td>535</td>
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<td>2.2592</td>
<td>0.0634</td>
<td>2.1439</td>
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<td>12</td>
<td>52</td>
<td>F</td>
<td>617</td>
<td>Cholecystitis—cholecystectomy</td>
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<td>3.9550</td>
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<td>4.5601</td>
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<td>26</td>
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<td>670</td>
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<td>2.7906</td>
<td>0.0715</td>
<td>4.5040</td>
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<tr>
<td>14</td>
<td>54</td>
<td>F</td>
<td>585</td>
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<td>22</td>
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<td>0.0874</td>
<td>1.8972</td>
<td>2062</td>
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<tr>
<td>15</td>
<td>43</td>
<td>F</td>
<td>286</td>
<td>Crohn's disease—resection of intestinal stricture</td>
<td>20</td>
<td>2.2201</td>
<td>0.0896</td>
<td>0.9058</td>
<td>960</td>
</tr>
<tr>
<td>16</td>
<td>52</td>
<td>M</td>
<td>607</td>
<td>Duodenal ulcer—pyloroplasty and vagotomy</td>
<td>29</td>
<td>2.2922</td>
<td>0.0535</td>
<td>3.3145</td>
<td>5836</td>
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<tr>
<td>17</td>
<td>56</td>
<td>M</td>
<td>333</td>
<td>Gastric ulcer—partial gastrectomy</td>
<td>28</td>
<td>2.8689</td>
<td>0.1029</td>
<td>3.3508</td>
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</tr>
<tr>
<td>18</td>
<td>37</td>
<td>M</td>
<td>350</td>
<td>Cholecystitis—cholecystectomy</td>
<td>24</td>
<td>2.1527</td>
<td>0.0541</td>
<td>1.8018</td>
<td>3164</td>
</tr>
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</table>
The amount of radioactivity in the liver biopsies ranged from 0.0535 to 0.3832% of the dose and the microbiological activity expressed as cyanocobalamin ranged from 0.9058 to 7.7263 pg per biopsy and from 0.41 to 1.75 µg/g tissue, mean 1.06 µg/g. The total body vitamin $B_{12}$ values were calculated from the values for radioactivity and vitamin $B_{12}$ in the biopsies assuming retention of 95% of the dose and ranged from 960 to 5984 pg with a mean of 2528 pg; five values were greater than the mean and thirteen less than the mean. Serum vitamin $B_{12}$ values were estimated in seventeen subjects (collection of the sample from one patient having been omitted by accident) and ranged from 191 to 670 pg/ml, mean 459 pg/ml.

Significant correlations were found between the values for serum vitamin $B_{12}$ in pg/ml ($x$) and total body vitamin $B_{12}$ in µg ($y$) in seventeen patients the regression equation being:

$$y = 6.0634 x - 207.8946 \quad (r = 0.54; \quad P < 0.05)$$

and between the values for serum vitamin $B_{12}$ in pg/ml ($x$) and hepatic vitamin $B_{12}$ in µg/g of tissue ($y$) in seventeen patients the regression equation being:

$$y = 0.001674 x + 0.2357 \quad (r = 0.53; \quad P < 0.05)$$

and between the hepatic vitamin $B_{12}$ in µg/g tissue ($x$) and the total body vitamin $B_{12}$ in µg ($y$) in eighteen patients the regression equation being:

$$y = 2250.4866 x - 237.0602 \quad (r = 0.63; \quad P < 0.01).$$

**DISCUSSION**

The validity of the results depends on three premises. First, that the $^{57}$Co cyanocobalamin had equilibrated with the non-radioactive vitamin $B_{12}$ in the body when the liver biopsy was obtained. Second, that the microbiological assay procedure using cyanocobalamin standards measured the liver vitamin $B_{12}$. Finally that the use of an arbitrary value, in this case 95%, for dose of tracer retained in the body at the time of the liver biopsy is acceptable for calculation of total body vitamin $B_{12}$ values.

Probably the most contentious of these premises is the first. There is controversy as to whether orally or parenterally administered radioactive vitamin $B_{12}$ does, or does not, equilibrate with the non-radioactive stores in the body. Reizenstein, Matthews & Ek (1964), Reizenstein, Matthews & Ek (1966) and Schiffer, Cohn, Price & Crunkite, (1968) do not consider that equilibration occurs within a finite time and the first two groups of workers link this opinion with the concept of at least three pools in the body. Heinrich (1964) while also favouring a multipool system accepts that equilibration occurs but only after 240–300 days after administration of radioactive vitamin $B_{12}$. Others have concluded from studies in animals that equilibration occurs more rapidly (Cooperman, Luhby, Teller & Marley, 1960; Grasbeck, Ignatius, Jarnefelt, Linden, Mali & Nyberg, 1961; Newman, O’Brien, Spray & Witts, 1962) and similar conclusions have been reached from studies in man (Bozian, Ferguson, Heyssell, Meneely & Darby, 1963; Heyssel, Bozian, Darby & Bell, 1966; Boddy & Adams, 1968; Adams & Boddy, 1968). With the exception of the time scale suggested by Heinrich (1964) the differences of opinion about the time taken for equilibration are less contentious being related, in part at least, to the mass of the dose given and possibly also to the species studies. In humans given 5000 µg doses parenterally the loss of whole body radioactivity did not occur at a steady rate, which was taken as evidence of equilibration, for several weeks (Boddy & Adams, 1968), whereas with doses of 100 ng parenterally the rate of loss was steady after 5–10 days (Adams & Boddy 1968).

We feel that the measurement of liver vitamin $B_{12}$, which is mainly coenzyme $B_{12}$ (Toohey &
Total body vitamin $B_{12}$ in the live subject

Barker, 1961; Ståhlberg, Radner & Nordén, 1967), by microbiological assay using cyanocobalamin standards is acceptable, our opinions being based on the results of recovery studies with coenzyme $B_{12}$ added to liver homogenates in vitro. The values for vitamin $B_{12}$ are greater than those found by Pitney, Beard & Van Loon (1955) and Jhala & Gadgil (1960) but are comparable to those reported by Blum & Heinrich (1957), Ross & Mollin (1957), Pitney & Onesti (1961), Adams (1962), Joske (1963), Anderson (1965) and Ståhlberg et al. (1967) also using Euglena gracilis as the test organism.

The use of an arbitrary value for the proportion of dose retained in the body at the time of liver biopsy was based on data reported by Adams & Boddy (1968). In this study the loss of whole body radioactivity by normal subjects given 100 ng $[^{57}Co]$cyanocobalamin intravenously was initially rapid but after 5–10 days, by which time about 5% had been lost, settled to a rate of 0.1–0.2% per day. We felt, therefore, that a suitable overall allowance for loss in the time between administration of the smaller dose used in this study and liver biopsy would be 5%. The ideal procedure, of course, would be to use a whole body monitor to obtain a 100% value after administration of the tracer dose and to repeat the measurement on the day of operation to obtain a value for retained dose. Consideration of the values involved, however, makes it doubtful if the results would be materially affected by such a procedure even given a monitor with the sensitivity and performance required in such a situation. In view of the doubts, however small, which must always attend the use of arbitrary values we regard it as prudent to regard the estimates of total body vitamin $B_{12}$ obtained by this method as approximate values.

Of the correlations between serum, hepatic and total body vitamin $B_{12}$ which we report, two are novel and one, that between serum and hepatic vitamin $B_{12}$, complements the finding by Anderson (1965) of a correlation between these measurements in a large series of vitamin $B_{12}$ deficient patients and the circumstantial evidence for a relationship adduced by Chanarin (1969) from selected data on vitamin $B_{12}$ deficient and vitamin $B_{12}$ replete subjects studied by Joske (1963), Anderson (1965) and Ståhlberg et al. (1967). In his own results Joske (1963) did not find a correlation probably because, as he states, the results were obtained mainly from patients with parenchymal liver disease which may upset the balance between the serum and hepatic vitamin $B_{12}$. We think it unwise at present to draw any conclusions from the correlations other than the general consideration that the serum vitamin $B_{12}$ per unit of volume, the hepatic vitamin $B_{12}$ per unit of mass and the total body vitamin $B_{12}$ would appear to be related to each other.

Whether the values for total body vitamin $B_{12}$ we report can be regarded as representative of normality is conjectural. Certainly we have doubts about regarding a patient with Crohn's disease as normal from the point of view of vitamin $B_{12}$ metabolism and the fact that patients with duodenal ulcers have a higher than normal output of intrinsic factor in response to histamine (Rødbro, Christiansen & Schwartz, 1965) might raise doubts about this group. In this connection it may be relevant to note that the mean total body vitamin $B_{12}$ for the seven patients with duodenal ulcers was 3288 µg and for the six patients with gall stones was 1867 µg. These values are significantly different when analysed by the Mann-Whitney 2 tail test ($P = 0.014$) but the mean value for the ulcer patients is heavily loaded by inclusion of two very high results and when these are excluded the significance disappears ($P = 0.53$). Within these limitations and the obvious problems associated with the study of normal subjects we feel that the results at least provide material of relevance and interest in studies of vitamin $B_{12}$ metabolism in man.
ACKNOWLEDGMENTS

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REFERENCES


Total body vitamin \textit{B}_{12} in the live subject


