Vitamin B12 Levels and Age

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Vitamin B12 concentration was determined by radioassay in 179 healthy volunteers between the ages of 20 and 93 years in order to determine whether vitamin B12 levels decline with advancing age. The authors found no statistically significant decline in vitamin B12 levels in older individuals, nor a difference between males and females. A review of previous reports identifies potential reasons for controversy regarding the normal concentration of vitamin B12 in the elderly. (Key Words: Vitamin B12; Aging; Geriatrics) Am J Clin Pathol 1986; 85: 32-36

OVER THE YEARS, we have encountered a number of hospitalized and outpatient elderly individuals with mild to moderate reductions in the serum concentration of vitamin B12 without clinical signs of B12 deficiency. Controversy has existed for 30 years concerning normal serum B12 levels in various age groups.2-5,7-9,10,12,13,15,16,18-20,24-29,32,33,36,40,45,46 From our clinical experience and a review of the literature, three important questions relating to vitamin B12 and aging need clarification: (1) Do older individuals have a lower normal range of serum vitamin B12 concentration? (2) Are assay results of serum B12 a valid measure of total body B12? (3) Should asymptomatic patients with borderline low B12 levels be treated? The present study addresses the first question and assesses the change of B12 levels with aging as measured by radioassay in 179 healthy subjects.

Materials and Methods

Two hundred sixty-three individuals were screened by means of a questionnaire from which 179 healthy subjects ranging in age from 20 to 93 years were chosen. The questionnaire included medical, dietary, and drug information. No subjects were institutionalized, and all were ambulatory. Those who had a history of vitamin B12 abnormalities or problems associated with deficient serum B12 levels were eliminated from the study. Other subjects not included in the analysis were those with a history of anemia (including pernicious anemia), cancer, multiple myeloma, sprue, Crohn’s disease, active peptic ulcer disease, severe gastritis, or gastrectomy. Anyone taking oral contraceptives, vitamin C, chloral hydrate or anti-convulsants also were excluded, as were pregnant women and strict vegetarians.

A 4-mL blood sample was drawn from each individual by routine venipuncture between 9 A.M. and 3 P.M., clotted for approximately 2 hours, and centrifuged, and the serum was aliquoted in duplicate and frozen at −70 °C until all samples were collected. Samples were assayed at room temperature using the Corning IMMO PHASE® radioassay for vitamin B12. This radioassay was chosen because it was the assay in use when the clinical questions arose. Furthermore, the hospital laboratory selected it because of its use of purified intrinsic factor as a binding protein, its reproducibility, its correlation with other radioassay systems, and its cost. Based upon the initial standardization of the assay, the intrasample coefficient of variation for B12 values in a low range (357-562 pg/mL) was 10%; for values in a medium range (823-1,000 pg/mL), 3.5%; and for values in a high range (1,093-1,397 pg/mL), 3%. Each sample was assayed in duplicate, and those that fell above or below the reference range provided by the company (171-953 pg/mL) were reassayed (11 samples).

The average of duplicate readings of B12 levels for each individual was used to compute the mean and standard error of the mean. Pearson’s correlation coefficient was calculated to determine the correlation between age and B12 levels for all subjects and for males and females. Analysis of variance was used to compare B12 levels among age groups by decade and between sexes. The Student’s t-test was used to compare B12 levels between subjects younger than 70 years old and older than 70 years old.

Results

Figure 1 graphically displays the measured serum vitamin B12 concentration in each subject and indicates a random distribution (r = −0.0069). Table 1 shows the mean and standard error of the mean for B12 concentration by sex and by decade. Subjects younger than 70 also are combined and compared with those older than or...
equal to 70 years of age. There are no statistically significant differences in B12 concentration among any of the groups. The reference range determined by our laboratory using the Corning IMMO PHASE radioassay for vitamin B12 is 441 ± 13 pg/mL.

Discussion

This study shows no difference in the serum concentration of vitamin B12 in a population of healthy ambulatory individuals from 20 to 93 years of age. Previous studies comparing normal vitamin B12 levels between older and younger individuals have reported contradictory results, we believe these contradictory results are attributable to either variations in the patient population studied or in the methods used for determining vitamin B12 concentration.

Many of the past studies have used institutionalized subjects either from hospitals, prisons, psychiatric institutions, or geriatric homes. Many of the past studies have used institutionalized subjects either from hospitals, prisons, psychiatric institutions, or geriatric homes. In the present study, these patients were excluded because of the association of mental illness with vitamin B12 deficiency and the potential for dietary inadequacies. All of our subjects were noninstitutionalized and ambulatory. Most of those from ages 70 to 93 were participants in daily city-sponsored meal programs that provided a well-balanced diet. In the past, not all investigators screened subjects to eliminate individuals with conditions associated with B12 deficiency. In this study, subjects receiving medications such as ascorbic acid, oral contraceptives, or biguanides, were excluded.

The first reported measurements of serum vitamin B12 were done by microbiologic assay using two test organisms, Euglena gracilis and Lactobacillus leichmanni. Some studies using E. gracilis reported a decrease in serum vitamin B12 with increasing age, while others reported no significant change. Most studies done with L. leichmanni found a lower level of B12 in older subjects. Other methods of measurement also have been employed. McEvoy and associates found a decrease in vitamin B12 levels with age using a whole body retention method of measurement, and Hyams found no change with a fecal excretion and hepatic uptake method.

Assay techniques have evolved over the past 30 years from the microbiologic assays to the currently used radioassays. Since their first introduction in 1961, radioassays have been refined and are now used routinely, although not without criticism. Fairbanks and Munasinghe found a significant decrease in vitamin B12 with age using a radioassay technic, whereas Magnus and Bailey found no significant decrease in B12 levels. Table

Table 1. Mean Serum Vitamin B12 Concentration by Decade and by Gender

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>n (male/female)</th>
<th>Mean B12 value (pg/mL) + SEM</th>
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<tbody>
<tr>
<td>20–29</td>
<td>30</td>
<td>15/15</td>
<td>452 ± 27</td>
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<tr>
<td>30–39</td>
<td>25</td>
<td>6/19</td>
<td>431 ± 27</td>
</tr>
<tr>
<td>40–49</td>
<td>19</td>
<td>3/16</td>
<td>444 ± 45</td>
</tr>
<tr>
<td>50–59</td>
<td>26</td>
<td>5/21</td>
<td>390 ± 27</td>
</tr>
<tr>
<td>60–69</td>
<td>23</td>
<td>9/14</td>
<td>517 ± 46</td>
</tr>
<tr>
<td>70–79</td>
<td>34</td>
<td>11/23</td>
<td>426 ± 29</td>
</tr>
<tr>
<td>80–89</td>
<td>17</td>
<td>9/8</td>
<td>436 ± 46</td>
</tr>
<tr>
<td>90–99</td>
<td>5</td>
<td>2/3</td>
<td>375 ± 77</td>
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<tr>
<td>&lt;70 years</td>
<td>123</td>
<td>38/85</td>
<td>446 ± 15</td>
</tr>
<tr>
<td>≥70 years</td>
<td>56</td>
<td>22/34</td>
<td>430 ± 25</td>
</tr>
<tr>
<td>All ages</td>
<td>179</td>
<td>60/119</td>
<td>441 ± 13</td>
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</tbody>
</table>

Fig. 1. Serum vitamin B12 concentration by age and sex.
Table 2. Summary of Studies of Serum Vitamin B$_{12}$ Levels in Elderly Subjects

<table>
<thead>
<tr>
<th>Year/Reference</th>
<th>Assay</th>
<th>Characteristics of Subjects</th>
<th>Number</th>
<th>Age</th>
<th>B$_{12}$ Concentration</th>
<th>Decrease with Age</th>
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<td>EG</td>
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<td>65</td>
<td>15-40</td>
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<td>31</td>
<td>71-93</td>
<td>259</td>
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<tr>
<td>1957$^{25}$</td>
<td>EG</td>
<td>Screened hospitalized patients</td>
<td>26</td>
<td>20-29</td>
<td>570/408</td>
<td>No</td>
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<td>49</td>
<td>40-49</td>
<td>489/449</td>
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<td></td>
<td></td>
<td>12</td>
<td>60-69</td>
<td>562/487</td>
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<td>12</td>
<td>70-79</td>
<td>518/400</td>
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<td>1959$^{13}$</td>
<td>EG</td>
<td>Screened normals</td>
<td>15</td>
<td>20-29</td>
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<td>30-39</td>
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<td>44</td>
<td>70-79</td>
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<td>20</td>
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<td>18</td>
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<td>Demented</td>
<td>18</td>
<td>68-92</td>
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<td>Ambulatory, living independently</td>
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<td>15-60</td>
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<td>56</td>
<td>&gt;60</td>
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<td>1965$^{26}$</td>
<td>EG</td>
<td>Agricultural and industrial community</td>
<td>890</td>
<td>15-75</td>
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<td>1967$^{20}$</td>
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<td>Healthy younger subjects</td>
<td>44</td>
<td>20-60</td>
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<td>Healthy elderly</td>
<td>64</td>
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<td>Geriatric inpatients</td>
<td>36</td>
<td>65-92</td>
<td>350</td>
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<tr>
<td>1971$^{16}$</td>
<td>EG</td>
<td>Miners/nonminers</td>
<td>78/79</td>
<td>35-44</td>
<td>299/354</td>
<td>+ Trend†</td>
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<td></td>
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<td>Miners/nonminers/women</td>
<td>59/49/104</td>
<td>55-64</td>
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<td>1971$^{16}$</td>
<td>EG</td>
<td>Community residents</td>
<td>M229/F304</td>
<td>65-90</td>
<td>230/255</td>
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<td>1977$^{12}$</td>
<td>EG</td>
<td>Screened patients</td>
<td>40</td>
<td>≤60</td>
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<td>42</td>
<td>&gt;60</td>
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<td>1955$^{9}$</td>
<td>LL</td>
<td>State hospital males</td>
<td>108</td>
<td>42 (mean)</td>
<td>580</td>
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<td>State hospital females</td>
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<td>State school females</td>
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<td>37 (mean)</td>
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<td>Ambulatory male blood donors</td>
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<td>1956$^{19}$</td>
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<td>107</td>
<td>20-29</td>
<td>270</td>
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<td></td>
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<td>inmates, volunteers and ambulant residents of a</td>
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<td>68</td>
<td>80-89</td>
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<tr>
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<td>Method</td>
<td>Description</td>
<td>Participants</td>
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<tr>
<td>1956</td>
<td>LL</td>
<td>Students, technical assistants and prison inmates</td>
<td>17 (29 mean) 231</td>
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<td></td>
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<td>Prison inmates and rest home residents</td>
<td>48 (23 mean) 268</td>
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<td>13 (77 mean) 153</td>
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<td>26 (75) 152</td>
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<td>93 (78 mean) 148</td>
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<td>3 (66 mean) 181</td>
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<td>1957</td>
<td>LL</td>
<td>Residents of Marion County home</td>
<td>2 (31-40) 650</td>
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<td>8 (41-50) 540</td>
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<td>39 (51-60) 610</td>
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<td>37 (61-70) 480</td>
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<td>54 (71-80) 450</td>
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<td>40 (81-90) 444</td>
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<td>1 (91-100) 360</td>
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<tr>
<td>1957</td>
<td>LL</td>
<td>Prison inmates</td>
<td>20 (27 mean) 297</td>
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<td>Rest home residents</td>
<td>21 (72 mean) 160</td>
<td></td>
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<tr>
<td>1967</td>
<td>LL</td>
<td>Newly admitted hospital patients</td>
<td>100 (60-80) 359</td>
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<tr>
<td></td>
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<td>Control subjects</td>
<td>106 (10-59) 425</td>
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<td>62 (60+) 429</td>
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<tr>
<td>1967</td>
<td>RIA</td>
<td>Geriatric center patients</td>
<td>M61/F123 (79/80 mean) 337/405</td>
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<tr>
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<td>Reference population</td>
<td>— (mean) 465</td>
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<td>1964</td>
<td>RIA</td>
<td>Fecal excretion and hepatic uptake</td>
<td>10 (28-50) 567</td>
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<td>Hospitalized men</td>
<td>13 (73-90) 618</td>
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<td>1980</td>
<td>RIA</td>
<td>Patients with reduced acid secretion</td>
<td>76 (total) 512</td>
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<td>&lt;50 529</td>
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<td>51-60 449</td>
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<td>61-70 449</td>
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<td>≥71 436</td>
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<td>1980</td>
<td>RIA</td>
<td>Noninstitutionalized elderly blacks and Spanish Americans</td>
<td>111 (60-87) 700</td>
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<td>Geriatric homes, 1980</td>
<td>217 (—) 300</td>
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<td>Hospital employees, 1975</td>
<td>248 (M35/F36 mean) 389</td>
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<td>Hospital employees, 1980</td>
<td>200 (M38/F34 mean) 290</td>
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<td>1983</td>
<td>RIA</td>
<td>Healthy subjects</td>
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<td>F190-765</td>
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</table>

* M = male, F = female.
† Positive trend toward a decrease of B<sub>12</sub> with age, but not significant.
2 summarizes reports of serum vitamin B12 levels in older subjects using the various assays. By using purified intrinsic factor, the Corning Vitamin B12 Radioassay* used in the present study overcomes the problem of falsely elevated values attributable to nonspecific binding of cobalamin analogs.11,17,21,31

Discrepancies from the past concerning changes in vitamin B12 levels with aging may be due to biases in sample selection or assay technics. The present results support the conclusion that serum vitamin B12 levels do not decrease with age in healthy, nutritionally stable adults.

References