SUMMARY

Vitamin B\textsubscript{12} (cobalamin) functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine and for a separate reaction that converts 1-methylmalonyl-coenzyme A (CoA) to succinyl-CoA. The Recommended Dietary Allowance (RDA) for vitamin B\textsubscript{12} is based on the amount needed for the maintenance of hematological status and normal serum vitamin B\textsubscript{12} values. An assumed absorption of 50 percent is included in the recommended intake. The RDA for adults is 2.4 µg/day of vitamin B\textsubscript{12}. Because 10 to 30 percent of older people may be unable to absorb naturally occurring vitamin B\textsubscript{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B\textsubscript{12} or a vitamin B\textsubscript{12}-containing supplement. Individuals with vitamin B\textsubscript{12} deficiency caused by a lack of intrinsic factor require medical treatment. The median intake of vitamin B\textsubscript{12} from food in the United States was estimated to be approximately 5 µg/day for men and 3.5 µg/day for women. The ninety-fifth percentile of vitamin B\textsubscript{12} intake from both food and supplements was approximately 27 µg/day. In one Canadian province the mean dietary intake was estimated to be approximately 7 µg/day for men and 4 µg/day for women. There is not sufficient scientific evidence to set a Tolerable Upper Intake Level (UL) for vitamin B\textsubscript{12} at this time.
BACKGROUND INFORMATION

Cobalamin is the general term used to describe a group of cobalt-containing compounds (corrinoids) that have a particular structure that contains the sugar ribose, phosphate, and a base (5, 6-dimethyl benzimidazole) attached to the corrin ring. Vitamin B₁₂ can be converted to either of the two cobalamin coenzymes that are active in human metabolism: methylcobalamin and 5-deoxyadenosylcobalamin. Although the preferred scientific use of the term *vitamin B₁₂* is usually restricted to cyanocobalamin, in this report, B₁₂ will refer to all potentially biologically active cobalamins.

In the United States, cyanocobalamin is the only commercially available B₁₂ preparation used in supplements and pharmaceuticals. It is also the principal form used in Canada (B. A. Cooper, Department of Hematology, Stanford University, personal communication, 1997). Another form, hydroxocobalamin, has been used in some studies of B₁₂. Compared with hydroxocobalamin, cyanocobalamin binds to serum proteins less well and is excreted more rapidly (Tudhope et al., 1967).

**Function**

B₁₂ is a cofactor for two enzymes: methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase requires methylcobalamin as a cofactor for the methyl transfer from methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate. L-Methylmalonyl-CoA mutase requires adenosylcobalamin to convert L-methylmalonyl-CoA to succinyl-CoA in an isomerization reaction. In B₁₂ deficiency, folate may accumulate in the serum as a result of slowing of the B₁₂-dependent methyltransferase. An adequate supply of B₁₂ is essential for normal blood formation and neurological function.

**Physiology of Absorption, Metabolism, Storage, and Excretion**

Small amounts of B₁₂ are absorbed via an active process that requires an intact stomach, intrinsic factor (a glycoprotein that the parietal cells of the stomach secrete after being stimulated by food), pancreatic sufficiency, and a normally functioning terminal ileum. In the stomach, food-bound B₁₂ is dissociated from proteins in the presence of acid and pepsin. The released B₁₂ then binds to R proteins (haptocorrins) secreted by the salivary glands and the gastric mucosa. In the small intestine, pancreatic proteases partially de-
grade the R proteins, releasing B₁₂ to bind with intrinsic factor. The resulting complex of intrinsic factor and B₁₂ attaches to specific receptors in the ileal mucosa; after internalization of the complex, B₁₂ enters the enterocyte. Approximately 3 to 4 hours later, B₁₂ enters the circulation. All circulating B₁₂ is bound to the plasma binding proteins—transcobalamin I, II, or III (TCI, TCII, or TCIII). Although TCI binds approximately 80 percent of the B₁₂ carried in the blood, TCII is the form that delivers B₁₂ to the tissues through specific receptors for TCII (Hall and Finkler, 1966; Seetharam and Alpers, 1982). The liver takes up approximately 50 percent of the B₁₂ and the remainder is transported to other tissues.

If there is a lack of intrinsic factor (as is the case in the condition called pernicious anemia), malabsorption of B₁₂ results; if this is untreated, potentially irreversible neurological damage and life-threatening anemia develop.

The average B₁₂ content of liver tissue is approximately 1.0 µg/g of tissue in healthy adults (Kato et al., 1959; Stahlberg et al., 1967). Estimates of the average total-body B₁₂ pool in adults range from 0.6 (Adams et al., 1972) to 3.9 mg (Grasbeck et al., 1958), but most estimates are between 2 and 3 mg (Adams, 1962; Adams et al., 1970; Heinrich, 1964; Reizenstein et al., 1966). The highest estimate found for an individual’s total body B₁₂ store was 11.1 mg (Grasbeck et al., 1958). Excretion of B₁₂ is proportional to stores (see “Excretion”).

Absorption

Studies to measure the actual absorption of B₁₂ involve whole-body counting of radiolabeled B₁₂, counting of radiolabeled B₁₂ in the stool, or both. No data are available on whether B₁₂ absorption varies with B₁₂ status, but fractional absorption decreases as the oral dose is increased (Chanarin, 1979). Total absorption increases with increasing intake. Adams and colleagues (1971) measured fractional absorption of radiolabeled cyanocobalamin and reported that nearly 50 percent was retained at a 1-µg dose, 20 percent at a 5-µg dose, and just over 5 percent at a 25-µg dose. The second of two doses of B₁₂ given 4 to 6 hours apart is absorbed as well as the first (Heyssel et al., 1966). When large doses of crystalline B₁₂ are ingested, up to approximately 1 percent of the dose may be absorbed by mass action even in the absence of intrinsic factor (Berlin et al., 1968; Doscherholmen and Hagen, 1957).
Absorption from Food. The approximate percentage absorption of B\textsubscript{12} from a few foods is presented in Table 9-1. These values apply to normal, healthy adults. No studies were found on the absorption of B\textsubscript{12} from dairy foods or from red meat other than mutton and liver. The absorption efficiency of B\textsubscript{12} from liver reportedly was low because of its high B\textsubscript{12} content. Although evidence indicates that a B\textsubscript{12} content of 1.5 to 2.5 µg/meal saturates ileal receptors and thus limits further absorption (Scott, 1997), absorption of as much as 7 µg in one subject (18 percent) was reported from a serving of liver paste that contained 38 µg of B\textsubscript{12} (average absorption was 4.1 µg or 11 percent) (Heyssel et al., 1966).

Assumptions Used in this Report. Because of the lack of data on dairy foods and most forms of red meat and fish, a conservative adjustment for the bioavailability of naturally occurring B\textsubscript{12} is used in this report. In particular, it is assumed that 50 percent of dietary B\textsubscript{12} is absorbed by healthy adults with normal gastric function. A smaller fractional absorption would apply, however, if a person consumed a large portion of foods rich in B\textsubscript{12}. Different levels of absorption are assumed under various conditions, as shown in Table 9-2. Crystaline B\textsubscript{12} appears in the diet only in foods that have been fortified with B\textsubscript{12}, such as breakfast cereals and liquid meal replacements.

Enterohepatic Circulation

B\textsubscript{12} is continually secreted in the bile. In healthy individuals most of this B\textsubscript{12} is reabsorbed and available for metabolic functions. El Kholti et al. (1991) demonstrated that the secretion of B\textsubscript{12} into the bile averaged 1.0 ± 0.44 nmol/day (1.4 µg/day) in eight cholecystectomized patients, and this represented 55 percent of total corrinoids. If approximately 50 percent of this B\textsubscript{12} is assumed to be available for absorption, it is observed that the net absorption from liver is 5.5 percent.

### Table 9-1 Percentage Absorption of Vitamin B\textsubscript{12} from Foods by Healthy Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Food</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heyssel et al., 1966</td>
<td>Mutton</td>
<td>65</td>
</tr>
<tr>
<td>Heyssel et al., 1966</td>
<td>Liver</td>
<td>11</td>
</tr>
<tr>
<td>Doscherholmen et al., 1975</td>
<td>Eggs</td>
<td>24–36</td>
</tr>
<tr>
<td>Doscherholmen et al., 1978</td>
<td>Chicken</td>
<td>60</td>
</tr>
<tr>
<td>Doscherholmen et al., 1981</td>
<td>Trout</td>
<td>25–47</td>
</tr>
</tbody>
</table>

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TABLE 9-2  Assumed Vitamin B\textsubscript{12} Absorption under Different Conditions

<table>
<thead>
<tr>
<th>Form of Vitamin B\textsubscript{12}</th>
<th>Normal Gastric Function (%)</th>
<th>Pernicious Anemia(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naturally occurring(^b)</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Crystalline, low dose (&lt; 5 µg)(^b)</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Crystalline, high dose (≥ 500 µg) with water(^c)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Crystalline, high dose with food(^c)</td>
<td>0.5</td>
<td>≤ 0.5</td>
</tr>
</tbody>
</table>

\(^a\) A disorder in which lack of intrinsic factor severely limits the absorption of vitamin B\textsubscript{12}.

\(^b\) Heyssel et al. (1966).

\(^c\) Berlin et al. (1968).

reabsorbed, the average loss of biliary B\textsubscript{12} in the stool would be 0.5 nmol/day (0.7 µg/day). Research with baboons (Green et al., 1982) suggests that the form of B\textsubscript{12} present in bile may be absorbed more readily than is cyanocobalamin, but the absorption of both forms was enhanced by intrinsic factor. Both Green and colleagues (1982) and Teo and coworkers (1980) reported data suggesting that bile enhances B\textsubscript{12} absorption. However, in the absence of intrinsic factor, essentially all the B\textsubscript{12} from the bile is excreted in the stool rather than recirculated. Thus, B\textsubscript{12} deficiency develops more rapidly in individuals who have no intrinsic factor or who malabsorb B\textsubscript{12} for other reasons than it does in those who become complete vegetarians and thus ingest no B\textsubscript{12}.

**Excretion**

If the circulating B\textsubscript{12} exceeds the B\textsubscript{12} binding capacity of the blood, the excess is excreted in the urine. This typically occurs only after injection of B\textsubscript{12}. The highest losses of B\textsubscript{12} ordinarily occur through the feces. Sources of fecal B\textsubscript{12} include unabsorbed B\textsubscript{12} from food or bile, desquamated cells, gastric and intestinal secretions, and B\textsubscript{12} synthesized by bacteria in the colon. Other losses occur through the skin and metabolic reactions. Fecal (Reizenstein, 1959) and urinary losses (Adams, 1970; Heinrich, 1964; Mollin and Ross, 1952) decrease when B\textsubscript{12} stores decrease. Various studies have indicated losses of 0.1 to 0.2 percent of the B\textsubscript{12} pool per day (Amin et al., 1980; Boddy and Adams, 1972; Bozian et al., 1963; Heinrich, 1964; Heyssel et al., 1966; Reizenstein et al., 1966) regardless of the size of the store, with the 0.2 percent value generally applicable to those with pernicious anemia.
Clinical Effects of Inadequate Intake

Hematological Effects of Deficiency

The major cause of clinically observable B12 deficiency is pernicious anemia (see “Pernicious Anemia”). The hematological effects of B12 deficiency are indistinguishable from those of folate deficiency (see Chapter 8). These include pallor of the skin associated with a gradual onset of the common symptoms of anemia, such as diminished energy and exercise tolerance, fatigue, shortness of breath, and palpitations. As in folate deficiency, the underlying mechanism of anemia is an interference with normal deoxyribonucleic acid (DNA) synthesis. This results in megaloblastic change, which causes production of larger-than-normal erythrocytes (macrocytosis). This leads first to an increase in the erythrocyte distribution width index and ultimately to an elevated mean cell volume. Oval macrocytes and other abnormally shaped erythrocytes are present in the blood. Typically, as with folate deficiency, the appearance of hypersegmentation of polymorphonuclear leukocytes precedes the development of macrocytosis. However, the sensitivity of this finding has recently been questioned (Carmel et al., 1996). By the time anemia has become established, there is usually also some degree of neutropenia and thrombocytopenia because the megaloblastic process affects all rapidly dividing bone marrow elements. The hematological complications are completely reversed by treatment with B12.

Neurological Effects of Deficiency

Neurological complications are present in 75 to 90 percent of individuals with clinically observable B12 deficiency and may, in about 25 percent of cases, be the only clinical manifestation of B12 deficiency. Evidence is mounting that the occurrence of neurological complications of B12 deficiency is inversely correlated with the degree of anemia; patients who are less anemic show more prominent neurological complications and vice versa (Healton et al., 1991; Savage et al., 1994a). Neurological manifestations include sensory disturbances in the extremities (tingling and numbness), which are worse in the lower limbs. Vibratory and position sense are particularly affected. Motor disturbances, including abnormalities of gait, also occur. Cognitive changes may occur, ranging from loss of concentration to memory loss, disorientation, and frank dementia, with or without mood changes. In addition, visual disturbances, insomnia, impotency, and impaired bowel and bladder control may devel-
The progression of neurological manifestations is variable but generally gradual. Whether neurological complications are reversible after treatment depends on their duration. The neurological complications of B$_{12}$ deficiency occur at a later stage of depletion than do the indicators considered below and were, therefore, not used for estimating the requirement for B$_{12}$. Moreover, neurological complications are not currently amenable to easy quantitation nor are they specific to B$_{12}$ deficiency.

**Gastrointestinal Effects of Deficiency**

B$_{12}$ deficiency is also frequently associated with various gastrointestinal complaints, including sore tongue, appetite loss, flatulence, and constipation. Some of these complaints may be related to the underlying gastric disorder in pernicious anemia.

**SELECTION OF INDICATORS FOR ESTIMATING THE REQUIREMENT FOR VITAMIN B$_{12}$**

Search of the literature revealed numerous indicators that could be considered as the basis for deriving an Estimated Average Requirement (EAR) for vitamin B$_{12}$ for adults. These include but are not limited to hematological values such as erythrocyte count, hemoglobin concentration or hematocrit, and mean cell volume (MCV), blood values such as plasma B$_{12}$, and the metabolite methylmalonic acid (MMA).

**Indicators of Hematological Response**

Measurements used to indicate a hematological response that could be considered as indicative of B$_{12}$ sufficiency have consisted of either a minimal but significant increase in hemoglobin, hematocrit, and erythrocyte count; a decrease in MCV; or an optimal rise in reticulocyte number.

In the earliest studies, MCV was a calculated value that was derived from relatively imprecise erythrocyte counts. Although MCV is now directly measured and precise, the response time of this measurement to changes in dietary intake is slow because of the 120-day longevity of erythrocytes. Consequently, the MCV is of limited usefulness. The erythrocyte count, hemoglobin, and hematocrit values are all robust measurements of response. Again, however, the response time is slow before an improvement in B$_{12}$ status leads to a return to normal values. Partial responses are of limited value.
because they do not predict the ultimate completeness or maintenance of response.

The reticulocyte count is a useful measure of hematological response because an increase is apparent within 48 hours of $B_{12}$ administration and reaches a peak at 5 to 8 days.

**Serum or Plasma Vitamin $B_{12}$**

The concentration of $B_{12}$ in the serum or plasma reflects both the $B_{12}$ intake and stores. The lower limit is considered to be approximately 120 to 180 pmol/L (170 to 250 pg/mL) for adults but varies with the method used and the laboratory conducting the analysis. As deficiency develops, serum values may be maintained at the expense of $B_{12}$ in the tissues. Thus, a serum $B_{12}$ value above the cutoff point does not necessarily indicate adequate $B_{12}$ status (see the section “Vitamin $B_{12}$ Deficiency”) but a low value may represent a long-term abnormality (Beck, 1991) or prolonged low intake.

**Methylmalonic Acid**

The range that represents expected variability (2 standard deviations) for serum MMA is 73 to 271 nmol/L (Pennypacker et al., 1992). The concentration of MMA in the serum rises when the supply of $B_{12}$ is low. Elevation of MMA may also be caused by renal failure or intravascular volume depletion (Stabler et al., 1988), but Lindenbaum and coworkers (1994) reported that moderate renal dysfunction in the absence of renal failure does not affect MMA values as strongly as does inadequate $B_{12}$ status. MMA values tend to rise in the elderly (Joosten et al., 1996); in most cases this appears to reflect inadequate $B_{12}$ intake or absorption. Lindenbaum and coworkers (1988) reported that elevated serum MMA concentrations are present in many patients with neuropsychiatric disorders caused by $B_{12}$ deficiency. Pennypacker and colleagues (1992) found that intramuscular injections of $B_{12}$ reduced the elevated MMA values in their elderly subjects. The reduction of elevated MMA values with $B_{12}$ therapy has also been reported in other studies (Joosten et al., 1993; Naurath et al., 1995; Norman and Morrison, 1993). Increased activity of anaerobic flora in the intestinal tract may increase serum MMA values; treatment with antibiotics decreases the serum MMA concentration in this situation (Lindenbaum et al., 1990). Because the presence of elevated concentrations of MMA in serum represents a metabolic change that is highly specific to $B_{12}$ deficiency, the serum MMA concentration is a preferred indicator
of B\textsubscript{12} status. However, data were not sufficient to use MMA as the criterion on which to base the EAR in this report. Serum MMA values from older studies may not be comparable with those obtained recently because of improvements of methods over time (Beck, 1991; Green and Kinsella, 1995). More importantly, no studies were found that examined directly the relationship of B\textsubscript{12} intake and MMA concentrations.

**Homocysteine**

Serum total homocysteine concentration is commonly elevated in elderly persons whose folate status is normal but who have a clinical response to treatment with B\textsubscript{12} (Stabler et al., 1996). Because a lack of folate, vitamin B\textsubscript{6}, or both also results in an elevated serum and plasma homocysteine concentration, this indicator has poor specificity and does not provide a useful basis for deriving an EAR.

**Formiminoglutamic Acid, Propionate, and Methylcitrate**

Although most patients with untreated B\textsubscript{12} deficiency excrete an increased amount of formiminoglutamic acid (FIGLU) in the urine after an oral loading dose of histidine, FIGLU excretion is also almost invariably increased in folate deficiency as well. The test, therefore, lacks specificity for the diagnosis of either vitamin deficiency. Concentrations of propionate, the metabolic precursor of methylmalonate, also rise with B\textsubscript{12} deficiency. Propionate may be converted to 2-methylcitrate, serum and cerebrospinal fluid concentrations of which also rise in B\textsubscript{12} deficiency (Allen et al., 1993). However, the measurement of either propionate or methyl citrate offers no advantages over serum MMA for the detection of B\textsubscript{12} deficiency.

**Holotranscobalamin II**

Among the three plasma B\textsubscript{12} binding proteins, transcobalamin II (TCII) is responsible for receptor-mediated uptake of B\textsubscript{12} into cells. However, only a small fraction of the plasma B\textsubscript{12} (10 to 20 percent) is present as the TCII-B\textsubscript{12} complex. This fraction, termed holoTCII, may provide a good indication of B\textsubscript{12} status, and methods have been described to measure this fraction (Herzlich and Herbert, 1988; Vu et al., 1993). These methods are currently considered to be insufficiently robust for routine clinical use.
VITAMIN B\textsubscript{12} 315

METHODOLOGICAL ISSUES

Vitamin B\textsubscript{12} Content

The two primary microbial organisms used to determine the vitamin B\textsubscript{12} content of serum, urine, and stool are \textit{Euglena gracilis} and \textit{Lactobacillus leichmannii}. Although either organism will yield essentially similar results, \textit{L. leichmannii} is the preferred method for reasons of convenience (Chanarin, 1969). Microbiological assays have been largely supplanted by radioligand binding assays. Until 1978 radioligand binding assays frequently gave higher results; the binding protein for B\textsubscript{12} used in these assays would also bind analogues of B\textsubscript{12} (Beck, 1991; Russell, 1992). Since 1978 the use of purified intrinsic factor as the binder in commercial radioisotope dilution assay kits has resulted in serum concentrations of B\textsubscript{12} comparable with those obtained from microbiological assays. More recently, nonisotopic serum B\textsubscript{12} assays have been introduced, which has resulted in cutoff levels for B\textsubscript{12} deficiency again rising. Care must be taken in comparing studies because much variation has been noted across laboratories, and different cutoff points have been used to identify deficiency (Beck, 1991; Green and Kinsella, 1995; Miller et al., 1991; Rauma et al., 1995; WHO, 1970; Winawer et al., 1967).

The serum B\textsubscript{12} value may be misleading as an indicator because it includes all the B\textsubscript{12} regardless of the protein to which it is bound. Transcobalamin II (TCII) is the key transport protein, and it has been proposed that only the TCII-bound fraction of the serum B\textsubscript{12} (holoTCII) is important in relation to B\textsubscript{12} nutritional and metabolic status (Herzlich and Herbert, 1988; Vu et al., 1993). However, at this time, there is no reliable method to determine holoTCII.

Retention

Studies of the retention of parenterally administered B\textsubscript{12} indicate that percentage retention depends on the dose and the route of administration (intramuscular [IM] or intravenous). The expected percentage retention of IM cyanocobalamin is shown in Table 9-3. These values, which vary from 15 to 100 percent, are useful when IM doses of B\textsubscript{12} are used to estimate the B\textsubscript{12} requirement.
TABLE 9-3  Change in Percentage Retention of Vitamin $B_{12}$ with Increasing Intramuscular Dose

<table>
<thead>
<tr>
<th>Vitamin $B_{12}$ Dose (µg)</th>
<th>Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>25</td>
<td>95</td>
</tr>
<tr>
<td>40</td>
<td>93</td>
</tr>
<tr>
<td>1,000</td>
<td>15</td>
</tr>
</tbody>
</table>


**DIAGNOSIS**

*Vitamin $B_{12}$ Deficiency*

Early detection of vitamin $B_{12}$ deficiency depends on biochemical measurements. Lindenbaum and colleagues (1990) reported that metabolites that arise from $B_{12}$ insufficiency are more sensitive indicators of $B_{12}$ deficiency than is the serum $B_{12}$ value. This was found in patients with pernicious anemia or previous gastrectomy who experienced early hematological relapse: serum methylmalonic acid (MMA), total homocysteine, or both were elevated in 95 percent of the instances of relapse whereas the serum $B_{12}$ value was low (less than 150 pmol/L [200 pg/mL]) in 69 percent. Similarly, serum $B_{12}$ was found to be an insensitive indicator in a review of records of patients with clinically significant $B_{12}$ deficiency. Five deficient individuals had neurological disorders that were responsive to $B_{12}$ and had elevated serum MMA and homocysteine values even though their serum $B_{12}$ values were greater than 150 pmol/L (200 pg/mL) and anemia was absent or mild. In a recent series of 173 patients, 5.2 percent of those with recognized $B_{12}$ deficiency had serum $B_{12}$ values in the normal range. Similar findings were reported elsewhere (e.g., Carmel, 1988; Pennypacker et al., 1992; Stabler et al., 1996). At present, the techniques developed to measure serum MMA and homocysteine (capillary gas chromatography and mass spectrometry) are costly and may be beyond the scope of routine laboratories. Conditions that may warrant assessment of $B_{12}$ status because they may result in $B_{12}$ deficiency are summarized in Table 9-4.
TABLE 9-4 Conditions That May Result in Vitamin B_{12} Deficiency

<table>
<thead>
<tr>
<th>Cause</th>
<th>Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary deficiency</td>
<td>Insufficient B_{12} intake, as seen in complete vegetarians</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>Lack of intrinsic factor</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>Lack of intrinsic factor</td>
</tr>
<tr>
<td>Atrophic gastritis</td>
<td>Inability to digest protein-bound B_{12} and bacterial uptake and/or conversion</td>
</tr>
<tr>
<td>Bacterial overgrowth of the small intestine</td>
<td>bacterial uptake and/or conversion of B_{12}</td>
</tr>
<tr>
<td>Infection with <em>Diphyllobothrium latum</em></td>
<td>Uptake of B_{12} by the parasite</td>
</tr>
<tr>
<td>Terminal ileal disease or resection</td>
<td>Inability to absorb B_{12}</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>Inability to digest protein-bound B_{12}</td>
</tr>
</tbody>
</table>

Pernicious Anemia

Pernicious anemia is the end stage of an autoimmune disorder in which parietal cell autoantibodies against H^{+}K^{+}-adenosine triphosphatase cause loss of gastric parietal cells. The loss of parietal cells reduces and then completely prevents production of intrinsic factor. In addition, blocking autoantibodies can bind to the B_{12} binding site for intrinsic factor and prevent the formation of the B_{12}-intrinsic factor complex. Deficiency of intrinsic factor gradually results in B_{12} deficiency (see “Clinical Effects of Inadequate Intake”).

The prevalence of undiagnosed, untreated pernicious anemia was recently estimated to be approximately 2 percent in a nonrandom sample of free-living elderly aged 60 years or older in Southern California (Carmel, 1996). Rates were higher for white and black women than for Latin American or Asian women and for all men. These estimates are consistent with the 2.9 percent prevalence of intrinsic factor antibody in individuals older than 60 years (Krasinski et al., 1986). Earlier studies reported a higher prevalence of anti-intrinsic factor antibody in blacks with pernicious anemia than in whites with pernicious anemia (Carmel, 1992) and an earlier onset of pernicious anemia in blacks (Carmel et al., 1987; Houston et al., 1985) and Hispanics (Carmel et al., 1987). Approximately 20 percent of relatives of patients with pernicious anemia also have pernicious anemia (Toh et al., 1997). Pernicious anemia carries an excess risk of gastric carcinoma (1 to 3 percent) and of gastric carcinoid tumors (Hsing et al., 1993).
A flow sheet for the diagnosis of pernicious anemia appears in Figure 9-1. Autoantibodies to gastric parietal cells should be measured along with intrinsic factor. The demonstration of circulating intrinsic factor autoantibodies is almost diagnostic of type A gastritis and pernicious anemia (Toh et al., 1997).

FACTORS AFFECTING THE VITAMIN B₁₂ REQUIREMENT

Aging

Plasma vitamin B₁₂ tends to decrease and serum methylmalonic acid (MMA) concentration tends to increase with age. These changes may represent a decline in B₁₂ status. Factors that may contribute to these changes include a decrease in gastric acidity, the presence of atrophic gastritis and of bacterial overgrowth accompanied by food-bound B₁₂ malabsorption, severity of atrophic gastritis, compromised functional and structural integrity of the B₁₂ binding proteins, and a lack of liver B₁₂ stores (van Asselt et al., 1996). Percentage absorption of crystalline B₁₂ does not appear to decrease with age (McEvoy et al., 1982). In a study of 38 healthy subjects each 76 years old taken from a larger cohort study (Nilsson-Ehle et al., 1986), cyanocobalamin absorption was found to be comparable with that reported in eight other studies of healthy younger people.

Studies of absorption in the elderly have yielded somewhat contradictory results. van Asselt and coworkers (1996) found no significant difference in cobalamin absorption (either free or protein bound) between subjects younger than 64 years (median 57) and those 65 years and older (median 75 years). These investigators could not explain the high prevalence of low cobalamin values in the elderly by either the aging process or the occurrence of mild-to-moderate atrophic gastritis. In contrast Krasinski and coworkers (1986) demonstrated that although a small proportion of the elderly with atrophic gastritis have a low serum concentration of B₁₂ (less than 88 pmol/L [120 pg/mL]), those with lowest serum B₁₂ values tend to have severe atrophic gastritis. Scarlett and colleagues (1992) reported a reduction in dietary B₁₂ absorption with age that was associated with elevated serum gastrin, which indicates reduced gastric acidity.

Prevalence of Atrophic Gastritis

Large differences in the prevalence of atrophic gastritis in the elderly, ranging from approximately 10 to 30 percent, have been
Symptoms and signs of cobalamin (Cbl) deficiency: paresthesias; gait disturbance; mental status changes; vibratory/position sense impairment; absent ankle reflexes; extensor plantar responses

Cobalamin assay

Radioassay* (170–900 pg/ml)

<200

Homocysteine, methylmalonic acid

+ – 

Intrinsic factor antibody

Homocysteine, methylmalonic acid

– + 

False-positive Cbl assay: repeat in 1 year

Pernicious anemia

1,000 µg IM B$_{12}$ × 5 days, then 500–1,000 µg IM every month; consider oral replacement at 500–1,000 µg/d

Part II

Consider false negative: Food-bound malabsorption due to achlorhydria (part IV)

True negatives: Dietary deficiency (vegan)

Cbl binding-protein abnormality

Consider false positives due to mucosal megaloblastosis

Part III (after antibiotic)

Ileal malabsorption or defective intrinsic factor in test

Blind loop syndrome

Algorithm for cobalamin (Cbl) deficiency diagnosis and treatment. *A higher normal reference range should be applied if chemiluminescence or other nonradioisotopic ligand-binding assays are used. **If the clinical picture is suggestive of possible Cbl deficiency, patients with serum Cbl >300 pg/ml should be investigated similarly. IM = intramuscular.

reported in Australia (Andrews et al., 1967), Missouri (Hurwitz et al., 1997), Scandinavia (Johnsen et al., 1991), and Boston (Krasinski et al., 1986). In the general elderly population, many cases of atrophic gastritis may remain undiagnosed.

Food-Bound $B_{12}$ Malabsorption

Testing of individuals who have low serum $B_{12}$ values but who do not have pernicious anemia reveals a substantial proportion with malabsorption of protein-bound $B_{12}$ (Carmel et al., 1987, 1988; Jones et al., 1987). More importantly, Carmel and coworkers (1988) found that 60 percent of those with neurological, cerebral, or psychological abnormalities malabsorbed food-bound $B_{12}$. Food-bound malabsorption is found in persons with certain gastric dysfunctions (e.g., hypochlorhydria or achlorhydria with an intact stomach, post-gastric surgery such as Billroth I or II, and postvagotomy with pyloroplasty) and in some persons with initially unexplained low serum $B_{12}$ (Carmel et al., 1988; Doscherholmen et al., 1983). Suter and colleagues (1991) reported that subjects with atrophic gastritis absorb significantly less $B_{12}$ than do healthy control subjects but that the difference disappears after antibiotic therapy.

Miller and colleagues (1992) studied the absorption of radio-labeled $B_{12}$ in patients who had not had gastric surgery but who had low $B_{12}$ values. All patients with elevated serum gastrin levels absorbed food-bound $B_{12}$ poorly compared with 21 percent of all those with normal serum gastrin values. In this study normal values were specified as greater than 12 percent absorption of food-bound $B_{12}$ and greater than 33 percent absorption of free $B_{12}$ as measured by direct body radioactivity measurements. Control subjects with normal serum $B_{12}$ values (median 173 pmol/L [234 pg/mL], range 125 to 284 pmol/L [170 to 385 pg/mL]) absorbed 12 to 39 percent of food-bound $B_{12}$ and 54 to 97 percent of free $B_{12}$ (median 75 percent). The median age of this group was 61 years (range 49 to 69 years). Available evidence does not indicate that aging or atrophic gastritis increases the amount of $B_{12}$ that must actually be absorbed to meet the body’s needs.

Smoking

The high cyanide intake that occurs with cigarette smoking may disturb the metabolism of $B_{12}$. In a study of healthy adults (Linnell et al., 1968), mean urinary $B_{12}$ excretion was significantly higher in the 16 smokers than in the 16 nonsmokers (81.2 ± 8.7 [standard
error] and 60.3 ± 7.9, respectively, \( p < 0.02 \), and urinary thiocyanate excretion (an index of the exogenous cyanide load) was inversely associated with serum \( B_{12} \). Similarly, in a study of pregnant women, the distribution of values of serum \( B_{12} \) was slightly lower for smokers than for nonsmokers. However, in a cross-sectional study, differences in \( B_{12} \) concentrations of smokers and nonsmokers were not significant in multivariate analyses. The effect of smoking on the \( B_{12} \) requirement thus appears to be negligible.

**Gender**

In a cross-sectional study of 77 young men and 82 young women (Fernandes-Costa et al., 1985), the women were found to have significantly higher serum \( B_{12} \) values and unsaturated cobalamin binding capacity than did the men \((p < 0.001 \text{ and } 0.05, \text{ respectively})\). Subjects were excluded if they were taking vitamin supplements, oral contraceptive agents, or other medications other than patent analgesics. Mean serum \( B_{12} \) values were 477 and 604 pmol/L (647 and 819 pg/mL) for men and women, respectively—well above the cutoff of adequacy. Other investigators have reported similar findings (Low-Beer et al., 1968; Metz et al., 1971). Studies that have found no difference in mean \( B_{12} \) values were smaller and less well-controlled for other factors that could influence \( B_{12} \) values (Rosner and Schreiber, 1972; Scott et al., 1974). Taken together, these studies do not provide sufficient evidence on which to quantitate a difference in \( B_{12} \) requirements by gender.

**Nutrient-Nutrient Interactions**

**Folate with \( B_{12} \)**

Although adequate or high folate intake may mitigate the effects of a \( B_{12} \) deficiency on normal blood formation, there is no evidence that folate intake or status changes the requirement for \( B_{12} \).

**Vitamin C with \( B_{12} \)**

Low serum \( B_{12} \) values reported in persons receiving megadoses of vitamin C are likely to be artifacts of the effect of ascorbate on the radioisotope assay for \( B_{12} \) (Herbert et al., 1978)—and thus not a true nutrient-nutrient interaction.
Other Food Components

Although it is clear that protein-bound $B_{12}$ is less well absorbed than crystalline $B_{12}$, the effect varies greatly with the specific protein and may be modified by gastric factors (see “Food-Bound $B_{12}$ Malabsorption”). Data on absorption from different types of diets (e.g., high in dairy products or beef) are not sufficient to use as a basis for adjusting the estimated requirement for $B_{12}$.

No evidence was found that a high-fiber diet increases the amount of $B_{12}$ that should be consumed. A single study (Doi et al., 1983) was found that examined the effect of dietary fiber (specifically, konjac mannan, or glucomannan) on the absorption of $B_{12}$. A 3.9-g dose of the fiber with a meal did not change the rate of $B_{12}$ absorption in either normal subjects or those with diabetes mellitus.

Genetic Defects

Underutilization of $B_{12}$ has been reported in individuals with genetic defects that involve deletions or defects of MMA-CoA mutase, transcobalamin II, or enzymes in the pathway of cobalamin adenosylation (Kano et al., 1985; Rosenberg and Fenton, 1989).

FINDINGS BY LIFE STAGE AND GENDER GROUP

Infants Ages 0 through 12 Months

Methods Used to Set the Adequate Intake

An Adequate Intake (AI) is set for the recommended intake for infants. The AI reflects the observed average vitamin $B_{12}$ intake of infants fed principally with human milk.

Reported values for the concentration of the vitamin in human milk vary widely, partly because of differences in methods of analysis and partly because of differences in maternal $B_{12}$ status and current intake. Despite high intraindividual diurnal variability within a group of lactating women, no consistent effect on $B_{12}$ concentration of time of day, breast, or time within a feed has been demonstrated. Thus, casual samples of human milk can be used to represent concentrations for the group (Trugo and Sardinha, 1994). However, the wide intraindividual variability may lead to inaccuracies in reported mean values if the number of individuals sampled is small. Median values are substantially lower than average values (Casterline et al., 1997; Donangelo et al., 1989). Acceptable meth-
ods of analysis include *Euglena gracilis* after pretreatment with papain to release the vitamin from the R protein in milk and radioassays in which the vitamin is released by heating (Areekul et al., 1977; Trugo and Sardinha, 1994). Studies used for estimating the concentration of the vitamin in human milk are limited to those that used one of these two methods.

The single longitudinal study of the change in B\textsubscript{12} concentration in human milk over time (Trugo and Sardinha, 1994) suggests somewhat higher concentrations in colostrum than in mature milk (≤ 21 days postpartum) but little change after the first month of lactation.

**Ages 0 through 6 Months**

The AI for infants ages 0 through 6 months is based on the B\textsubscript{12} intake of infants fed human milk. B\textsubscript{12} deficiency does not occur in infants fed milk from mothers with adequate B\textsubscript{12} status. In samples collected from nine well-nourished Brazilian mothers who were not taking supplements and whose infants were receiving human milk exclusively, the average concentration of the vitamin was 0.42 μg/L at 2 months; this decreased to an average of 0.34 μg/L at 3 months (Trugo and Sardinha, 1994). Milk collected at least 2 months postpartum from 13 unsupplemented American mothers who were vegetarians was lower in B12 content, averaging 0.31 μg/L (Specker et al., 1990). The B\textsubscript{12} content of milk in a large group of low-income Brazilian mothers (n = 83) who had received prenatal supplements containing B\textsubscript{12} was much higher, averaging 0.91 μg/L after 1 month of lactation (Donangelo et al., 1989). Given that the average concentration at 2 months postpartum of well-nourished mothers whose infants received exclusively human milk was higher than those on vegetarian diets, the higher value of 0.42 μg/L is chosen in order to be sure adequate amounts are available. Using the average human milk volume of 0.78 L/day during the first 6 months and the higher average B\textsubscript{12} content of 0.42 μg/L, the AI for B\textsubscript{12} for the infant 0 through 6 months of age fed human milk would be 0.33 μg/day, rounded up to 0.4 μg.

**Maintenance of Normal Methylmalonic Acid Concentrations.** Data on methylmalonic acid (MMA) excretion is also available for infants. An infant may be born with low B\textsubscript{12} stores and may consume human milk that is low in B\textsubscript{12} if its mother is a vegan (a person who avoids all animal foods) or has untreated pernicious anemia. Such infants begin to show clinical signs of B\textsubscript{12} deficiency at about 4 months.
postpartum. In the study of 13 vegan mothers and their infants, Specker and colleagues (1990) found increased urinary MMA in 2- to 14-month old (mean 7.3) infants predominantly fed human milk when the $B_{12}$ concentration in human milk was below 0.49 $\mu$g/L. Assuming an average volume of human milk consumption of 0.78 L/day during the first 6 months, the infant of a vegan mother would be receiving an average of 0.24 $\mu$g/day of $B_{12}$ (0.31 $\mu$g/L $\times$ 0.78 L/day).

In these infants, urinary MMA concentrations were strongly correlated with those of their mothers and inversely related to maternal plasma $B_{12}$ concentrations, supporting the assumption that the elevations in infant urinary MMA were caused by poor maternal $B_{12}$ status (Specker et al., 1988). Although these infants were probably born with depleted stores of the vitamin, the data suggest that a mean intake of 0.24 $\mu$g/day is inadequate to maintain $B_{12}$ balance in infants.

Clinical signs of $B_{12}$ deficiency are usually seen if the mother has been a strict vegetarian for at least 3 years. The $B_{12}$ status of the infant is clearly abnormal by about 4 to 6 months of age. In case studies of infants born to strict vegetarians who were identified because of clinical signs of $B_{12}$ deficiency, human milk concentrations have been reported to be 0.02 (Hoey et al., 1982), 0.037 (Gambon et al., 1986), 0.032 and 0.042 (Jadhav et al., 1962), 0.051 (Johnson and Roloff, 1982), and 0.085 (Kuhne et al., 1991) $\mu$g/L. If clinical signs appear within 9 months in infants consuming milk containing 0.085 $\mu$g/L of $B_{12}$, this intake (approximately 0.07 $\mu$g/day) cannot support $B_{12}$ requirements of the infant during the first year. However, from these data it cannot be determined how far this estimate falls below the average requirement for these infants.

Rate of Depletion of Stores. The liver of a well-nourished newborn infant contains 18 to 22 pmol (25 to 30 $\mu$g) of $B_{12}$ (Baker et al., 1962; Loria et al., 1977; Vaz Pinto et al., 1975). There are no data on liver $B_{12}$ content at birth in full-term infants born to depleted mothers, only data for two infants who died prematurely (Baker et al., 1962); thus, the utilization of $B_{12}$ by infants remains speculative.

Summary. The AI for infants ages 0 through 6 months is 0.33 $\mu$g/day based on the average concentration of $B_{12}$ in the milk of mothers with adequate $B_{12}$ status. This value is rounded up to 0.4 $\mu$g/day. The adequacy of this intake is supported by evidence that it is above the intake level that has been associated with increased urinary MMA excretion.
Ages 7 through 12 Months

If the reference body weight ratio method described in Chapter 2 to extrapolate from the AI for B\textsubscript{12} for infants ages 0 through 6 months is used, the AI for B\textsubscript{12} for the older infants would be 0.5 µg/day after rounding up. This is a somewhat lower value than that obtained from the second method (see Chapter 2) by extrapolating down from the Estimated Average Requirement (EAR) for adults and adjusting for the expected variance to estimate a recommended intake, which results in an AI for B\textsubscript{12} of 0.6 µg/day.

In one study of three infants exclusively fed human milk who had clinically observable B\textsubscript{12} deficiency caused by low maternal consumption of animal products, one infant was treated parenterally with B\textsubscript{12} whereas two infants were treated with small oral B\textsubscript{12} doses (Jadhav et al., 1962). At 9 months of age, 0.1 µg/day of oral B\textsubscript{12} normalized bone marrow within 5 days in one of the two infants given oral doses and produced profound improvements in behavior by 18 days (after a total of 1.8 µg of B\textsubscript{12} had been given). The mother’s milk contained 0.032 µg/L. In the second infant, who was 7 months old, 0.1 µg/day of B\textsubscript{12} caused abnormal pigmentation to disappear and “an adequate hematologic response.” His mother’s milk contained 0.042 µg/L. Although evidence of sustained recovery was not provided, it appears from these limited data that 0.1 µg/day may be adequate to improve clinical and hematological signs of deficiency in infants at this age. However, it is not known whether this level of intake is adequate to sustain normal plasma B\textsubscript{12} and MMA concentrations or hematological response.

In the study of infants of vegan mothers (Specker et al., 1990) the mean age of infants was 7.3 months (ranged 2 to 14 months). As discussed, a mean intake of 0.23 µg/day was not adequate to maintain B\textsubscript{12} balance in this group as determined by urinary MMA excretion.

\textbf{B\textsubscript{12} AI Summary, Ages 0 through 12 Months}

\begin{center}
\begin{tabular}{|l|c|}
\hline
\textbf{AI for Infants} & \\
\textbf{0–6 months} & 0.4 µg/day of vitamin B\textsubscript{12} ≈ 0.05 µg/kg \\
\textbf{7–12 months} & 0.5 µg/day of vitamin B\textsubscript{12} ≈ 0.05 µg/kg \\
\hline
\end{tabular}
\end{center}

\textit{Special Considerations}

Infants of vegan mothers should be supplemented with B\textsubscript{12} at the AI from birth on the basis of evidence that their stores at birth are
low and their mother’s milk may supply very small amounts of the vitamin.

**Children and Adolescents Ages 1 through 18 Years**

*Method Used to Estimate the Average Requirement*

Only one study is available to provide data regarding $B_{12}$ status and intake in young children. Plasma MMA was elevated in 11- to 22-month-old (mean 16.8 months) infants of Dutch vegan mothers. The sensitivity of plasma MMA to distinguish the group of infants born to macrobiotic mothers from those born to omnivorous mothers was 85 percent (Schneede et al., 1994). The average intake of $B_{12}$ by these infants, who were exclusively fed human milk for a mean of 4.8 months and then at least partially fed human milk for $13.6 \pm 6.6$ (standard deviation) months and fed macrobiotic foods, was $0.3 \pm 0.2 \mu g/day$ for the first 6 to 16 months compared with $2.9 \pm 1.2 \mu g/day$ for well-nourished control infants (Dagnelie et al., 1991). These data suggest that an intake of $0.3 \mu g/day$ of $B_{12}$ between 6 and 16 months of age was inadequate to prevent elevated plasma MMA concentrations of infants born to vegan mothers.

No other direct data were found on which to base an Estimated Average Requirement (EAR) for $B_{12}$ for children or adolescents. In the absence of additional information, EARs and RDAs for children and adolescents have been estimated by using the method described in Chapter 2, which extrapolates down from adult values, and rounded up.

**$B_{12}$ EAR and RDA Summary, Ages 1 through 18 Years**

<table>
<thead>
<tr>
<th></th>
<th>1–3 years</th>
<th>4–8 years</th>
<th>9–13 years</th>
<th>14–18 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EAR for Children</strong></td>
<td>0.7 $\mu g/day$ of vitamin $B_{12}$</td>
<td>1.0 $\mu g/day$ of vitamin $B_{12}$</td>
<td>1.5 $\mu g/day$ of vitamin $B_{12}$</td>
<td>2.0 $\mu g/day$ of vitamin $B_{12}$</td>
</tr>
<tr>
<td><strong>EAR for Boys</strong></td>
<td>1.5 $\mu g/day$ of vitamin $B_{12}$</td>
<td>2.0 $\mu g/day$ of vitamin $B_{12}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EAR for Girls</strong></td>
<td>1.5 $\mu g/day$ of vitamin $B_{12}$</td>
<td>2.0 $\mu g/day$ of vitamin $B_{12}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The RDA for $B_{12}$ is set by assuming a coefficient of variation (CV) of 10 percent (see Chapter 1) because information is not available on the standard deviation of the requirement for $B_{12}$; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of
97 to 98 percent of the individuals in the group (therefore, for $B_{12}$ the RDA is 120 percent of the EAR).

<table>
<thead>
<tr>
<th>Age Group</th>
<th>RDA for Children</th>
<th>RDA for Boys</th>
<th>RDA for Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 years</td>
<td>0.9 µg/day of vitamin $B_{12}$</td>
<td>1.8 µg/day of vitamin $B_{12}$</td>
<td>1.8 µg/day of vitamin $B_{12}$</td>
</tr>
<tr>
<td>4–8 years</td>
<td>1.2 µg/day of vitamin $B_{12}$</td>
<td>2.4 µg/day of vitamin $B_{12}$</td>
<td>2.4 µg/day of vitamin $B_{12}$</td>
</tr>
<tr>
<td>9–13 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–18 years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adults Ages 19 through 50 Years

Method Used to Estimate the Average Requirement

No single indicator was judged to be a sufficient basis for deriving an EAR for adults. It was not deemed appropriate to base the EAR on an examination limited to studies that provided data on mean cell volume (MCV) or serum $B_{12}$ or any other single laboratory value. Data on men and women were examined together because of small numbers. Three general approaches were considered to derive the EAR for adults: determination of the amount of $B_{12}$ needed to maintain adequate hematological status (as measured by stable hemoglobin value, normal MCV, and normal reticulocyte response) and serum $B_{12}$ values in persons with pernicious anemia or with known intakes that were very low in dietary $B_{12}$; use of daily $B_{12}$ turnover to estimate the amount of $B_{12}$ needed to maintain body stores at a specified level; and estimation of the dietary $B_{12}$ intake by healthy adults that corresponds to adequate serum values of $B_{12}$ and of MMA.

The first approach was chosen as the primary method for deriving an EAR because it is the only approach for which there are sufficient and reliable data for estimating need. A low serum $B_{12}$ value in persons with pernicious anemia was assumed to indicate incomplete response to treatment.

Primary Criterion: Maintenance of Hematological Status and Serum $B_{12}$ Values. The primary method used to derive the EAR for adults estimates the amount of $B_{12}$ needed for the maintenance of hematological status and serum $B_{12}$ values, primarily by using data derived from patients with pernicious anemia in remission. Data from studies of vegetarians were also examined to determine whether they
BOX 9-1 Assumptions Made in Estimating the Amount of Vitamin B\textsubscript{12} Needed for Maintenance of Hematological Status and Serum Vitamin B\textsubscript{12} Values

- Maintenance of hematological status requires a relatively stable hemoglobin value upon administration of B\textsubscript{12} and a normal mean cell volume, not just a reticulocyte response.
- Normal serum B\textsubscript{12} is $\geq 150$ pmol/L (200 pg/mL).
- Because B\textsubscript{12} is not absorbed from the bile, the estimated extra loss of B\textsubscript{12} by a person with pernicious anemia in remission is 0.4 nmol/day (0.5 $\mu$g/day) based on data from Bozian et al. (1963), El Kholty et al. (1991), Heyssel et al. (1966), and Reizenstein (1959).
- The average fractional absorption of B\textsubscript{12} from food by healthy individuals is approximately 50 percent (see “Absorption”).

provided information on levels of B\textsubscript{12} intake needed to maintain hematological status. In some cases, neurological manifestations may be the earliest clinical sign of low B\textsubscript{12} values (Beck, 1991; Karnaze and Carmel, 1990; Lindenbaum et al., 1988; Martin et al., 1992). Assumptions that were integral to the application of this method are shown in Box 9-1.

In brief, this method involves estimating the amount of B\textsubscript{12} required daily to maintain hematological and serum B\textsubscript{12} status of individuals with pernicious anemia in remission; subtracting the amount of endogenous B\textsubscript{12} lost from the bile in excess of that lost by a healthy individual; and, because the value is to be used for individuals with normal ability to absorb B\textsubscript{12} from food, correcting for bioavailability. The result is shown in Box 9-2.

BOX 9-2 Steps Used to Estimate the Vitamin B\textsubscript{12} Requirement by Using Data Obtained from Subjects with Pernicious Anemia

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Estimate the average intramuscular requirement for maintenance of person with pernicious anemia</td>
<td>1.5 $\mu$g/day</td>
</tr>
<tr>
<td>2</td>
<td>Subtract estimate of extra losses due to lack of reabsorption of biliary B\textsubscript{12}</td>
<td>$-0.5$ $\mu$g/day</td>
</tr>
<tr>
<td>Subtotal</td>
<td>Estimate average requirement of normal person for absorbed B\textsubscript{12}</td>
<td>1.0 $\mu$g/day</td>
</tr>
<tr>
<td>3</td>
<td>Correct for bioavailability (50 percent)</td>
<td>$\div 0.5$</td>
</tr>
<tr>
<td>Result</td>
<td>Average requirement of normal person for B\textsubscript{12} from food: Estimated Average Requirement (EAR)</td>
<td>$2.0$ $\mu$g/day</td>
</tr>
</tbody>
</table>
The following studies provide the basis for the estimate used in Step 1. These studies do not provide ideal data on which to base an EAR, but they bracket the requirement by providing values that are obviously too low or too high to meet the needs of 50 percent of the individuals in an age group.

*Studies of Patients with Pernicious Anemia.* Darby and coworkers (1958) studied the effects of various intramuscular (IM) doses of $B_{12}$ in 20 subjects with pernicious anemia who had not previously been treated or who were in relapse. The diagnosis of pernicious anemia had been based on the clinical history and on the findings of macrocytic anemia, megaloblastic hyperplasia of bone marrow, histamine-fast achlorhydria, and a negative radiological examination of the gastrointestinal tract. These diagnoses were not made based on results of the Schilling test, first published as a method in 1953 (Schilling, 1953). The extent of the disease differed among the subjects; 14 had neurological manifestations. Of the 18 subjects who received doses of 1 $\mu$g/day of $B_{12}$ or less for 2 weeks, 5 or fewer responded satisfactorily according to the standards used for erythrocytes (Isaacs et al., 1938) and reticulocytes (Isaacs and Friedman, 1938). At $B_{12}$ dosages of less than 0.5 $\mu$g/day, no patient met those standards. Dosages used for maintenance were increased to 1 to 4 $\mu$g/day for a period of months to years. MCVs greater than 100 were considered macrocytic. No reticulocyte counts or serum $B_{12}$ values were reported. According to the authors' interpretation, the data indicated that subjects achieved and maintained maximum erythropoiesis as indicated in Table 9-5. Approximately half (4 of 7) did so at a $B_{12}$ intake of 1.4 $\mu$g/day IM.

<table>
<thead>
<tr>
<th>Daily $B_{12}$ Dose, Intramuscular ($\mu$g)</th>
<th>Number of Subjects Achieving Maximum Erythropoiesis ($n=7$)</th>
<th>Cumulative Number Achieving Maximum Erythropoiesis ($n=7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1.4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>4.0</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

SOURCE: Darby et al. (1958).
Results of other studies of patients with pernicious anemia are presented in Table 9-6. The short-term study by Hansen and Weinfeld (1962) used relatively high B₁₂ doses to restore normal status but did not assess maintenance requirement. The long-term studies by Bastrup-Madsen et al. (1983) and Lindenbaum et al. (1990) used different dosages and methods of reporting that make it impossible to draw precise conclusions. Nonetheless, the results indicate that 0.8 to 1.0 µg/day of B₁₂ IM will maintain normal hematological, serum B₁₂, and serum metabolite status in nearly half of the individuals over time and that 1.7 µg will maintain it in all individuals. The study conducted by Best and colleagues (1956) was designed to determine the effective dosage of intrinsic factor concentrates, not to estimate the B₁₂ requirement, but it suggests that 1.4 µg of B₁₂ exceeds the requirement for absorbed B₁₂ in most of the subjects tested. The often-cited study of Sullivan and Herbert (1965) was interpreted as providing evidence that 0.1 µg/day of B₁₂ was not sufficient for treating pernicious anemia and maintaining adequate B₁₂ status. Similarly, the 0.6 to 0.7 µg/day of B₁₂ supplied IM in the study by Will and coworkers (1959) was also judged too low to maintain a normal serum B₁₂ concentration.

The study by Darby and colleagues (1958), which indicates an average requirement in such patients of approximately 1.5 µg, is supported by the supplementary data from the other studies described in Table 9-6. These studies provide support for a physiological average requirement of 1.0 µg/day of B₁₂ after adjustment for the extra loss of B₁₂ by subjects with pernicious anemia (0.5 µg/day) (Step 2 in Box 9-2). Adjusting for incomplete absorption of B₁₂ from food of 50 percent (Step 3) converts this value to an EAR for B₁₂ of 2.0 µg/day.

Studies of Individuals with Low B₁₂ Intake. Studies of individuals with low B₁₂ intake were examined to determine whether these reports (Table 9-7) supported the findings for subjects with pernicious anemia. Because B₁₂ is not a component of plant foods, diets containing little or no animal food may lead to B₁₂ deficiency. Deficiency develops slowly because of efficient reabsorption of biliary B₁₂. It is also possible but not certain that vegans consume some B₁₂ from animal products that contaminate plant food or from bacterial action. Studies of vegetarians generally have not analyzed the B₁₂ content of the food, and accurate data are not available for some of the foods (e.g., certain algae) consumed by vegetarians. Without actual analyses it is not clear what B₁₂ content should be assumed for vegans.
The studies covered by Table 9-7 suggest that the B$_{12}$ requirement is higher than the amounts reported to be consumed by the subjects and more than that provided by the treatments that were described. In three studies (Baker and Mathan, 1981; Jathar et al., 1975; Winawer et al., 1967), all adults required more than 1 µg/day of B$_{12}$ by mouth. Two studies (Narayanan et al., 1991; Stewart et al., 1970) give evidence that 1.5 µg/day of dietary B$_{12}$ is not sufficient to maintain hematological status and serum B$_{12}$ in half of the subjects studied. The meager data provided by the studies of vegetarians indicate that the B$_{12}$ average requirement should probably be at least 1.5 µg/day, but a higher average requirement is not ruled out.

**Supportive Data: Maintenance of B$_{12}$ Body Stores**

Various studies have indicated losses of 0.1 to 0.2 percent/day of the B$_{12}$ pool (e.g., Amin et al., 1980; Boddy and Adams, 1972; Heyssel et al., 1966; Reizenstein et al., 1966) regardless of the size of the pool. A loss of 0.2 percent appears to be typical for individuals who do not reabsorb biliary B$_{12}$ because of pernicious anemia (Boddy and Adams, 1972). A person with a B$_{12}$ pool of 1,000 µg and a loss of 0.1 percent would excrete 1 µg of B$_{12}$ daily, and a person with a 3,000-µg pool would excrete 3 µg daily. If only 50 percent of dietary B$_{12}$ is absorbed, the amounts required daily to replenish the pools are 2 and 6 µg of B$_{12}$, respectively. The higher value would lead to less efficient use of B$_{12}$, but the larger store of B$_{12}$ would cover a longer period of inadequate B$_{12}$ intake or absorption.

With a 0.1 percent loss, the period of protection afforded by the B$_{12}$ pool can be estimated if the lowest pool size consistent with health is also known. If it is assumed that this value is 300 µg (derived from Bozian and coworkers [1963]), there is no absorption of B$_{12}$ from food or supplements, and the enterohepatic circulation is intact, then stores of 1 mg would be expected to meet the body’s needs for 3 years, 2 mg for about 5 years, and 3 mg for about 6 years. A 1.5 percent loss would reduce these estimates to 2, 3.6, and 4 years (see Appendix N for the method used to obtain these values).

The extent of the supply of reserve B$_{12}$ may be an important consideration when persons approach the age of 50 and the risk increases for food-bound B$_{12}$ malabsorption secondary to atrophic gastritis (see “Factors Affecting the Vitamin B$_{12}$ Requirement” and section “Adults Ages 51 Years and Older”). Because the absorption of B$_{12}$ from fortified foods, oral supplements, or the bile does not
## Table 9-6 Other Studies of Subjects with Pernicious Anemia Considered in Setting the Estimated Average Requirement for Vitamin B₁₂ for Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of Subjects</th>
<th>Age Range (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hansen and Weinfeld, 1962</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Suggested IM&lt;sup&gt;a&lt;/sup&gt; requirement &gt; 2.0 µg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bastrup-Madsen et al., 1983</td>
<td>112</td>
<td>33–78</td>
</tr>
<tr>
<td>Lindenbaum et al., 1990</td>
<td>44</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Suggested IM requirement of 1.0–2.0 µg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best et al., 1956</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Will et al., 1959</td>
<td>40</td>
<td>NA</td>
</tr>
<tr>
<td>Sullivan and Herbert, 1965</td>
<td>8</td>
<td>46–86</td>
</tr>
</tbody>
</table>

<sup>a</sup> IM = intramuscular.

<sup>b</sup> 1,000 µg × 0.15 retention/90 d.

<sup>c</sup> NA = not available.

<sup>d</sup> 1,000 µg × 0.15 retention/182 d.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–5 µg of B₁₂ IM for 8–15 d.</td>
<td>Five persons who were given 3 µg/d of B₁₂ for 15 d had a reticulocyte response that was not followed by a further response to more B₁₂. This amount allowed restoration of status.</td>
</tr>
<tr>
<td>1 mg of slow-release B₁₂ IM every 2 or 3 mo for at least 8 y. The less-frequent dose was equivalent to 1.7 µg of B₁₂/d.</td>
<td>Serum B₁₂ values were well above the cutoff of 180 pmol/L (250 pg/mL) early in the study and complete hematological remission occurred in all.</td>
</tr>
<tr>
<td>35 received 1 mg of B₁₂ IM every 5–6 mo, 6 received it every 3–4 mo, 3 received it every 2 mo. Smallest and most frequent dose was equivalent to 0.8–1.0 µg of B₁₂/d.</td>
<td>From total group analyses, 14 subjects had mild hematological relapse on 42 occasions; 34 subjects had at least one abnormal serum B₁₂ or metabolite value on 146 occasions when there was no evidence of hematological relapse.</td>
</tr>
<tr>
<td>2.0 µg oral dose of B₁₂ Co₆₀ given with intrinsic factor.</td>
<td>With 70% absorption, complete hematological response, and adequate plasma B₁₂ concentration, 1.4 µg of absorbed B₁₂ met the requirements of two-thirds of the subjects.</td>
</tr>
<tr>
<td>10 µg of B₁₂ given IM every 2 wk or 20 µg of B₁₂ given IM monthly for 10 y (equivalent average of 0.7 µg/d).</td>
<td>None of the subjects maintained serum B₁₂ concentration above the 180 pmol/L (250 pg/mL) lower limit of normal for the Lactobacillus leichmannii method.</td>
</tr>
<tr>
<td>0.1 µg/d of cyanocobalamin IM for 10 d; 0.1 µg/d of coenzyme B₁₂ IM for 10 d.</td>
<td>Posttreatment serum B₁₂ was 85 pmol/L (60 pg/mL) (range, 20–200 pmol/L [14–139 pg/mL]); 6 of 8 had reticulocyte response, but macrocytosis persisted in all and hypersegmentation did in many. In some, neurological abnormalities progressed until at least 1 µg of B₁₂ was given daily. All but one were later given higher doses of B₁₂.</td>
</tr>
</tbody>
</table>
### TABLE 9-7  Studies of Individuals with Low Vitamin B₁₂ Intake Considered in Setting the Estimated Average Requirement for B₁₂ for Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
<th>Dietary B₁₂ Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suggested dietary B₁₂ average requirement &gt; 1.5 µg/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart et al., 1970</td>
<td>1 Hindu woman with megaloblastic anemia</td>
<td>0.5 µg/d (analyzed homogenate)</td>
</tr>
<tr>
<td>Narayanan et al., 1991</td>
<td>10 subjects with serum B₁₂ values below the 2.5 percentile (&lt; 120 pmol/L [162 pg/mL]) not caused by disease or vegetarianism</td>
<td>1.5 ± 0.4 (SD) µg/d of B₁₂ (range 0.6–1.9)</td>
</tr>
<tr>
<td><strong>Suggested dietary B₁₂ average requirement &gt; 1.0 µg/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winawer et al., 1967</td>
<td>1 64-y-old vegan with B₁₂-deficient megaloblastic anemia, gastritis on biopsy, and normal gastric acidity</td>
<td>Assumed to be negligible</td>
</tr>
<tr>
<td>Jathar et al., 1975</td>
<td>7 East Indian lactovegetarians</td>
<td>0.3–0.8 µg/d of B₁₂ from milk, assuming that it was not boiled</td>
</tr>
<tr>
<td>Baker and Mathan, 1981</td>
<td>4 East Indians with B₁₂ deficiency anemia secondary to diet</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p.o. = by mouth.
<sup>b</sup> Based on USDA data (URL http://www.nal.usda.gov/fnic/foodcomp/).

appear to be impaired, the combination of stores and absorbed crystalline B₁₂ may cover needs for an extended period.

The estimates above for the period of protection afforded by body stores are consistent with the periods required to develop overt signs of B₁₂ deficiency after a total gastrectomy; for example, megaloblastic anemia has been typically diagnosed 2 to 5 years after a total gastrectomy (Chanarin, 1990).
Possible Ancillary Method: Maintenance of a Serum B<sub>12</sub> Concentration That Is Consistent with a Normal Circulating MMA Value

Several investigators have urged the use of the serum MMA concentration as the most sensitive indicator of B<sub>12</sub> status (Lindenbaum et al., 1990; Moelby et al., 1990; Savage et al., 1994b; Stabler et al., 1996). This indicator could not be used as the criterion for setting
the EAR for $B_{12}$ because of a lack of direct data. At least one study (Lindenbaum et al., 1994) relates serum $B_{12}$ to circulating MMA values. None link MMA with $B_{12}$ intake. Moreover, although MMA is a metabolite that accumulates abnormally when the $B_{12}$ supply is low, studies have not yet convincingly demonstrated that elevated MMA caused by insufficient $B_{12}$ intake has adverse health consequences. However, because MMA values hold promise as a criterion for estimating the $B_{12}$ requirement in the future, an indirect approach was used to estimate a requirement for $B_{12}$ as a means of confirming or refining the EAR value derived by using the primary approach. For example, because the serum $B_{12}$ value of 150 pmol/L (200 pg/mL) appears to be the level at which half the population would have an elevated MMA value (Lindenbaum et al., 1994), one could select the dietary intake that would maintain this value in healthy individuals in that population.

In a study of 548 surviving members of the original Framingham Heart Study cohort, aged 67 to 96 years, and 117 healthy control subjects younger than 65 years, Lindenbaum and colleagues (1994) reported on serum $B_{12}$, MMA, and homocysteine values (Table 9-8). These investigators used a cutoff value equal to or greater than 260 pmol/L (350 pg/mL) of $B_{12}$ as adequate; more than 15 percent of subjects below the cutoff value had elevated MMA concentrations whereas fewer than 10 percent of subjects above the cutoff did. Serum creatinine was elevated in 10 of those with both increased MMA and low $B_{12}$ values, which would indicate confounding abnormal renal function. Slightly more than 40 percent of the 70 elderly

### TABLE 9-8 Vitamin $B_{12}$ Status: Occurrence of Low Serum Values for Two Age Groups

<table>
<thead>
<tr>
<th>Serum Values of Subjects</th>
<th>Healthy Younger Control Subjectsa (Number [%])</th>
<th>Elderly Subjects from Framingham Studyb (Number [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin $B_{12} &lt; 148$ pmol/L (201 pg/mL)</td>
<td>2 (1.7)</td>
<td>29 (5.3)</td>
</tr>
<tr>
<td>Vitamin $B_{12} &lt; 258$ pmol/L (351 pg/mL)</td>
<td>21 (17.9)</td>
<td>222 (40.5)</td>
</tr>
<tr>
<td>Methylmalonic acid &gt; 376 nmol/L</td>
<td>Not available</td>
<td>82 (15.0)</td>
</tr>
</tbody>
</table>

NOTE: 1 pmol/L of $B_{12} = 1.36$ pg/mL.

a Aged < 65 years; $n = 117$.
b Aged 67–96 years; $n = 548$.

SOURCE: Lindenbaum et al. (1994).
subjects with serum $B_{12}$ less than 150 pmol/L (200 pg/mL) had an elevated MMA concentration.

Studies of $B_{12}$ intake and serum $B_{12}$ concentration provide very limited information on the relationship of the two. In Finland, vegans consuming an uncooked ("living food") diet were estimated to consume a mean of 1.8 $\mu$g/day of $B_{12}$ (range 0 to 12.8 $\mu$g) (Rauma et al., 1995), but the accuracy of the dietary intake data is uncertain. The 16 vegans who ate seaweed (the main source of $B_{12}$ reported) had $B_{12}$ concentrations twice as high as those not eating seaweed (mean of 220 pmol/L [300 pg/mL] compared with 105 pmol/L [142 pg/mL]). On this diet 57 percent of the vegans had serum $B_{12}$ concentrations less than 200 pmol/L (270 pg/mL). A study by Draper and colleagues (1993) provided dietary data on vegans that were not sufficient for drawing conclusions about diet-$B_{12}$ relationships. Neither Garry and coworkers (1984) nor Sahyoun and colleagues (1988) separated data with regard to supplement use, so their data are not interpretable for setting EARs. A study of a macrobiotic population (Miller et al., 1991) revealed that more than half of the adults had low serum $B_{12}$ concentrations and nearly one-third were excreting high amounts of MMA, but dietary information from the study was not sufficient for drawing conclusions. Moreover, studies need to be conducted in younger persons in whom $B_{12}$ absorption is more likely to be normal.

$B_{12}$ EAR and RDA Summary, Ages 19 through 50 Years

On the basis of hematological evidence and serum $B_{12}$ values, the EAR for $B_{12}$ is estimated to be 2 $\mu$g/day for men and women ages 19 through 50 years. Sufficient data were not available to enable differences in requirements to be discerned for men and women in these age groups.

<table>
<thead>
<tr>
<th></th>
<th>19–30 years</th>
<th>31–50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EAR for Men</strong></td>
<td>2 $\mu$g/day of vitamin $B_{12}$</td>
<td>2 $\mu$g/day of vitamin $B_{12}$</td>
</tr>
<tr>
<td><strong>EAR for Women</strong></td>
<td>2 $\mu$g/day of vitamin $B_{12}$</td>
<td>2 $\mu$g/day of vitamin $B_{12}$</td>
</tr>
</tbody>
</table>

The RDA for $B_{12}$ is set by assuming a coefficient of variation (CV) of 10 percent (see Chapter 1) because information is not available on the standard deviation of the requirement for $B_{12}$; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of
97 to 98 percent of the individuals in the group (therefore, for $B_{12}$ the RDA is 120 percent of the EAR).

### RDA for Men
- 19–30 years: 2.4 µg/day of vitamin $B_{12}$
- 31–50 years: 2.4 µg/day of vitamin $B_{12}$

### RDA for Women
- 19–30 years: 2.4 µg/day of vitamin $B_{12}$
- 31–50 years: 2.4 µg/day of vitamin $B_{12}$

#### Adults Ages 51 Years and Older

**Evidence Considered in Estimating the Average Requirement**

Because 10 to 30 percent of people older than 50 years are estimated to have atrophic gastritis with low stomach acid secretion (Andrews et al., 1967; Hurwitz et al., 1997; Johnsen et al., 1991; Krasinski et al., 1986), they may have decreased bioavailability of $B_{12}$ from food. Therefore, because of the high prevalence of this condition, 50 percent bioavailability of dietary $B_{12}$ (see Box 9-2) cannot be assumed for this age group, and the EAR would be higher than 2.0 µg. Similarly, 2.4 µg of $B_{12}$, which is the RDA for younger adults, might not meet the needs of 97 percent of this large age group. There is not sufficient information on which to base a bioavailability correction factor for persons with atrophic gastritis who obtain their $B_{12}$ from animal foods. However, because the bioavailability of crystalline $B_{12}$ is not altered in people with atrophic gastritis, the same EAR and RDA would apply if the dietary sources of $B_{12}$ were foods fortified with $B_{12}$, supplements, or a combination of both.

### $B_{12}$ EAR and RDA Summary, Ages 51 Years and Older

The EAR and RDA for $B_{12}$ for adults ages 51 years and older are the same as for younger adults but with the recommendation that $B_{12}$-fortified foods (such as fortified ready-to-eat cereals) or $B_{12}$-containing supplements be used to meet much of the requirement.

#### EAR for Men
- 51–70 years: 2 µg/day of vitamin $B_{12}$
- > 70 years: 2 µg/day of vitamin $B_{12}$

#### EAR for Women
- 51–70 years: 2 µg/day of vitamin $B_{12}$
- > 70 years: 2 µg/day of vitamin $B_{12}$
The RDA for B$_{12}$ is set by assuming a coefficient of variation (CV) of 10 percent (see Chapter 1) because information is not available on the standard deviation of the requirement for B$_{12}$; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97 to 98 percent of the individuals in the group (therefore, for B$_{12}$ the RDA is 120 percent of the EAR).

**RDA for Men**
- 51–70 years: 2.4 µg/day of vitamin B$_{12}$
- > 70 years: 2.4 µg/day of vitamin B$_{12}$

**RDA for Women**
- 51–70 years: 2.4 µg/day of vitamin B$_{12}$
- > 70 years: 2.4 µg/day of vitamin B$_{12}$

*It is advisable for most of this amount to be obtained by consuming foods fortified with B$_{12}$ or a B$_{12}$-containing supplement.

**Pregnancy**

**Evidence Considered in Estimating the Average Requirement**

**Absorption and Utilization of B$_{12}$.** There is some evidence that the absorption of B$_{12}$ may increase during pregnancy. An increase in the number of intrinsic factor–B$_{12}$ receptors was observed in pregnant mice and found to be regulated by placental lactogen (Robertson and Gallagher, 1983). A greater absorption of oral B$_{12}$ was reported from the single study of pregnant women (Hellegers et al., 1957), but the methods used do not permit quantification of the increase.

Serum total B$_{12}$ concentrations begin to decline early in the first trimester. In a longitudinal Dutch study of 23 subjects, serum B$_{12}$ fell significantly by the end of the first trimester, more than could be accounted for by hemodilution (Fernandes-Costa and Metz, 1982). There were further decreases through the sixth month to about half of nonpregnancy concentrations. Some of the later decrease was due to hemodilution. However, transcobalamin I and III increase during the second and third trimesters, and transcobalamin II increases sharply in the third trimester to about one-third more than in nonpregnant, nonlactating control subjects (Fernandes-Costa and Metz, 1982).

**Transfer to the Fetus.** The serum B$_{12}$ concentration of the newborn is twice that of the mother, decreasing to adult concentrations at about 6 to 7 months postpartum (Luhby et al., 1958). The placenta
concentrates $B_{12}$, which is then transferred to the fetus down a concentration gradient. Fetal and maternal $B_{12}$ serum concentrations are quite strongly correlated (Fréry et al., 1992). It appears that only newly absorbed $B_{12}$ is readily transported across the placenta and that maternal liver stores are a less important source of the vitamin for the fetus (Luhby et al., 1958). This implies that current maternal intake and absorption of the vitamin during pregnancy have a more important influence on the $B_{12}$ status of the infant than do maternal $B_{12}$ stores. The importance of adequate maternal intake during pregnancy is supported by the appearance of $B_{12}$ deficiency in infants at 4 to 6 months when their mothers have been strict vegetarians for only 3 years (Specker et al., 1990).

**Fetal Accumulation.** The human fetus accumulates an average of 0.07 to 0.14 nmol/day (0.1 to 0.2 µg/day) of $B_{12}$, a range based on three studies of the liver content of infants born to women who were adequate in $B_{12}$ (Baker et al., 1962; Loria et al., 1977; Vaz Pinto et al., 1975) and an assumption that the liver contains half the total body $B_{12}$ content. Placental $B_{12}$ is negligible (0.01 nmol/L [14 ng/L]) (Muir and Landon, 1985). The low body content of $B_{12}$ in the newborn implies that pregnancy is unlikely to deplete maternal stores.

**$B_{12}$ EAR and RDA Summary, Pregnancy**

On the basis of a fetal deposition of 0.1 to 0.2 µg/day throughout pregnancy and evidence that maternal absorption of the vitamin becomes more efficient during pregnancy, the EAR is increased by 0.2 µg/day during pregnancy. No distinction is made for the age of the mother.

<table>
<thead>
<tr>
<th>EAR for Pregnancy</th>
<th>µg/day of vitamin $B_{12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14–18 years</td>
<td>2.2</td>
</tr>
<tr>
<td>19–30 years</td>
<td>2.2</td>
</tr>
<tr>
<td>31–50 years</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The RDA for $B_{12}$ is set by assuming a coefficient of variation (CV) of 10 percent (see Chapter 1) because information is not available on the standard deviation of the requirement for $B_{12}$; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97 to 98 percent of the individuals in the group (therefore, for $B_{12}$ the RDA is 120 percent of the EAR).
Lactation

Evidence Considered in Estimating the Average Requirement

As described earlier, the average amount of B\textsubscript{12} secreted in the milk of mothers with adequate B\textsubscript{12} status is approximately 0.33 μg/day during the first 6 months of lactation. During the second 6 months, the average amount of B\textsubscript{12} secreted is slightly less: 0.25 μg/day.

The concentration of B\textsubscript{12} in milk is usually similar to that in maternal plasma. In some studies, human milk and maternal plasma concentrations are strongly correlated (Srikantia and Reddy, 1967) but in others they are not (Casterline et al., 1997; Donangelo et al., 1989). The correlation appears to be stronger when maternal B\textsubscript{12} status is marginal (Fréry et al., 1992).

Current maternal intake of the vitamin may have an important influence on secretion of the vitamin in milk. In several studies of infants with clinical signs of B\textsubscript{12} deficiency caused by low maternal intake or absorption of the vitamin, maternal plasma concentrations of the vitamin were found to be normal or low normal, suggesting that maternal B\textsubscript{12} stores are less important than current maternal intake (Hoey et al., 1982; Johnson and Roloff, 1982; Kuhne et al., 1991; Sklar, 1986). This is also indicated by the observation that the length of time that mothers had been strict vegetarians was not correlated with the urinary MMA concentrations of their infants (Specker et al., 1988).

Low B\textsubscript{12} concentrations in human milk occur commonly in two situations involving inadequate intake: when the mother is a strict vegetarian and in developing countries where the usual consumption of animal products is low. When the B\textsubscript{12} status of the mother is marginal, further maternal depletion may occur as reflected in decreasing concentrations of maternal plasma B\textsubscript{12} (Black et al., 1994; Shapiro et al., 1965).

B\textsubscript{12} EAR and RDA Summary, Lactation

To estimate the EAR for lactation, 0.33 μg/day of B\textsubscript{12} is added to the EAR of 2 μg/day for adolescent girls and adult women; the result is rounded up.
The RDA for B<sub>12</sub> is set by assuming a coefficient of variation (CV) of 10 percent (see Chapter 1) because information is not available on the standard deviation of the requirement for B<sub>12</sub>; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97 to 98 percent of the individuals in the group (therefore, for B<sub>12</sub> the RDA is 120 percent of the EAR).

**RDA for Lactation**

- **14–18 years**: 2.8 µg/day of vitamin B<sub>12</sub>
- **19–30 years**: 2.8 µg/day of vitamin B<sub>12</sub>
- **31–50 years**: 2.8 µg/day of vitamin B<sub>12</sub>

**Special Considerations**

Persons with any malabsorption syndrome will likely require increased amounts of B<sub>12</sub>. Patients with pernicious anemia or Crohn’s disease involving the terminal ileum and patients who have had a gastrectomy, gastric bypass surgery, or ileal resection will require B<sub>12</sub> under a physician’s direction. Persons who are positive for human immunodeficiency virus with chronic diarrhea may also require either increased oral or parenteral B<sub>12</sub>.

Patients with atrophic gastritis, pancreatic insufficiency, or prolonged omeprazole treatment (Bellou et al., 1996; Gueant et al., 1990; Suter et al., 1991; Termanini et al., 1998) will have decreased bioavailability of food-bound B<sub>12</sub> and will require normal amounts of crystalline B<sub>12</sub> (either in foods fortified with B<sub>12</sub> or as a supplement).

**INTAKE OF VITAMIN B<sub>12</sub>**

**Food Sources**

Ordinarily, humans obtain vitamin B<sub>12</sub> from animal foods. Unlike other B vitamins, B<sub>12</sub> is not a normal constituent of plant foods except for certain algae (Ford and Hutner, 1955). B<sub>12</sub> is not supplied by commonly eaten plant foods unless they have been exposed to bacterial action that has produced the vitamin; contaminated with soil, insects, or other substances that contain B<sub>12</sub>; or fortified with B<sub>12</sub> (e.g., fortified ready-to-eat breakfast cereals and meal replacement formulas).

Data obtained from the 1995 Continuing Survey of Food Intakes...
by Individuals (CSFII) indicate that the greatest contribution to \( B_{12} \) intake of the U.S. adult population comes from the category of mixed foods (including sandwiches) with meat, fish, or poultry as the main ingredient (Table 9-9). For women, the second category contributing the most \( B_{12} \) is milk and milk drinks, whereas beef is the second category of \( B_{12} \) for men. Fortified ready-to-eat cereals contribute a greater proportion of dietary \( B_{12} \) for women than for men. The foods that are the richest sources of \( B_{12} \)—shellfish, organ meats such as liver, some game meat, and a few kinds of fish (see Table 9-9)—are not a regular part of many people’s diets.

Analyses of CSFII 1994 to 1995 intake data for food fortified with \( B_{12} \) for adults aged 51 through 70 years and older than 70 years were provided by the U.S. Department of Agriculture (A. Moshefegh, Agricultural Research Service, U.S. Department of Agriculture, personal communication, 1997). Because of the higher bioavailability of synthetic \( B_{12} \) than of protein-bound \( B_{12} \) for a substantial proportion of older adults, these results were examined to determine whether fortified foods contributed differently to the \( B_{12} \) content of the diet for different age groups (Table 9-10). These cross-sectional data suggest that fortified foods provide a larger proportion of the \( B_{12} \) consumed by older than by younger adults, especially men.

Few studies report cooking losses. However, Stewart and coworkers (1970) tested one sample and found that boiling milk for 10 minutes reduced its \( B_{12} \) content by about 50 percent. Reconstituted evaporated milk contains only about 25 percent of the \( B_{12} \) content of fluid whole milk (USDA, 1997). Such cooking losses may seriously limit \( B_{12} \) intake by vegetarians. Boiling milk, for example, was described as a common cooking practice among Hindu women in the United Kingdom (Stewart et al., 1970). With a \( B_{12} \) content of 0.4 mg/100 mL (0.9 mg/8 oz), fresh pasteurized fluid milk may be an important source of \( B_{12} \) for vegetarians.

Dietary Intake

Because a generous intake of animal foods is common in the United States and Canada, median \( B_{12} \) intake from food is well above the EAR. For example, in the United States the median daily intake from food by young adult men has been reported to be approximately 4 to 5 \( \mu \)g and by young adult women, 3 \( \mu \)g (Appendixes G and H). In one Canadian province, the mean dietary intake was reported as approximately 7 \( \mu \)g/day for men and 4 \( \mu \)g/day for women (Appendix I).
### Table 9-9: Food Groups Providing Vitamin B₁₂ in the Diets of U.S. Men or Women Aged 10 Years and Older, CSFII, 1995

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Contribution to Total B₁₂ Intake (%)</th>
<th>Foods Within the Group that Provide at Least 1 µg of B₁₂ per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td><strong>Food groups providing at least 5% of total vitamin B₁₂ intake</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed foods&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.5</td>
<td>16.4</td>
</tr>
<tr>
<td>Beef</td>
<td>15.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Milk and milk drinks</td>
<td>10.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Shellfish</td>
<td>9.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Mixed foods, main ingredient is grain</td>
<td>7.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Processed meats&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Organ meats</td>
<td>5.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Ready-to-eat cereals</td>
<td>4.7</td>
<td>8.2</td>
</tr>
<tr>
<td>Finfish</td>
<td>3.4</td>
<td>5.7</td>
</tr>
<tr>
<td><strong>Vitamin B₁₂ from other food groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb, veal, game, and other carcass meat</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Soy-based supplements and meal replacements</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> CSFII = Continuing Survey of Food Intakes by Individuals.

<sup>b</sup> Contribution to total intake reflects both the concentration of the nutrient in the food and the amount of the food consumed. It refers to the percentage contribution to the American diet for both men and women, based on 1995 CSFII data.

<sup>c</sup> 1 µg represents 20% of the Recommended Daily Intake (6.0 µg) of B₁₂—a value set by the Food and Drug Administration.

<sup>d</sup> Includes sandwiches and other foods with meat, poultry, or fish as the main ingredient.

<sup>e</sup> NA = not applicable. Mixed foods were not considered for this table.

<sup>f</sup> Whole, low fat, and nonfat.

<sup>g</sup> Includes frankfurters, sausages, lunch meats, and meat spreads.

TABLE 9-10  Contribution of Fortified Foods to the Vitamin B<sub>12</sub> Intake of U.S. Men and Women by Age Group, CSFII, 1995<sup>a</sup>

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Contribution of Food Group to Total B&lt;sub&gt;12&lt;/sub&gt; Intake&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adults ≥ 19 Years</td>
</tr>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Ready-to-eat cereals</td>
<td>4.7</td>
</tr>
<tr>
<td>Soy-based supplements and meal replacements</td>
<td>0.7</td>
</tr>
<tr>
<td>Milk-based supplements and meal replacements</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>5.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> CSFII = Continuing Survey of Food Intake by Individuals.

<sup>b</sup> Refers to the percentage contribution to the American diet for both men and women, based on 2-day weighted 1995 CSFII data.


Intake by the elderly continues to be high relative to the EAR and RDA (Appendix F); however, quantitative data are not available on the amount of B<sub>12</sub> provided by fortified foods. In a study of Boston elderly aged 60 to more than 90 years (Russell, 1992), median B<sub>12</sub> intake by males who were not taking supplements was 3.4 µg/day. The median plasma B<sub>12</sub> concentration for this unsupplemented group was 286 pmol/L (388 pg/mL). For females not taking supplements, the median B<sub>12</sub> intake was 2.6 µg/day and the median plasma B<sub>12</sub> concentration was 272 pmol/L (369 pg/mL). B<sub>12</sub> intake was correlated with serum levels, but the actual correspondence of intake with plasma values was not determined.

Quinn and Basu (1996) reported on the dietary B<sub>12</sub> intake estimated from 3-day (nonconsecutive) food records of 156 elderly males and females aged 65 to 77 years residing in Northern Alberta, Canada. Supplement users were excluded from the sample. The mean daily B<sub>12</sub> intake by males was 3.7 ± 0.3 (standard error of the mean) µg and by females was 4.3 ± 1.0 µg. Mean plasma B<sub>12</sub> was 286 ± 24 pmol/L (388 ± 33 pg/mL) for males and 335 ± 37 pmol/L (454 ± 50 pg/mL) for females, which is consistent with the difference in reported dietary intake. None of the males and 7 percent of the females had estimated intakes of less than 1.3 µg/day.
Intake from Supplements

Information from the Boston Nutritional Status Survey on supplement use of B₁₂ by a free-living elderly population is given in Appendix F. For those taking supplements, the fiftieth percentile of supplemental B₁₂ intake was 5.0 µg for men and 6.0 µg for women. Approximately 26 percent of all adults reported taking a B₁₂-containing supplement in 1986 (Moss et al., 1989).

TOLERABLE UPPER INTAKE LEVELS

Hazard Identification

Adverse Effects

No adverse effects have been associated with excess B₁₂ intake from food or supplements in healthy individuals. There is very weak evidence from animal studies suggesting that B₁₂ intake enhances the carcinogenesis of certain chemicals (Day et al., 1950; Georgadze, 1960; Kalnev et al., 1977; Ostryanina, 1971). These findings are contradicted by evidence that increased B₁₂ intake inhibits tumor induction in the human liver, colon, and esophagus (Rogers, 1975). Some studies suggest a possible association between high-dose, parenterally administered B₁₂ (0.5 to 5 mg) and acne formation (Berlin et al., 1969; Dugois et al., 1969; Dupre et al., 1979; Puissant et al., 1967; Sherertz, 1991). However, the acne lesions were primarily associated with hydroxocobalamin rather than cyanocobalamin, the form used in the United States and Canada. Furthermore, iodine particles in commercial B₁₂ preparations may have been responsible for the acne. In conclusion, the evidence from these data was considered not sufficient for deriving a Tolerable Upper Intake Level (UL).

Studies involving periodic parenteral administration of B₁₂ (1 to 5 mg) to patients with pernicious anemia provide supportive evidence for the lack of adverse effects at high doses (Boddy and Adams, 1968; Mangiarotti et al., 1986; Martin et al., 1992). Periodic doses of 1 mg are used in standard clinical practice to treat patients with pernicious anemia. As indicated earlier, when high doses are given orally (see “Absorption”) only a small percentage of B₁₂ can be absorbed from the gastrointestinal tract, which may explain the apparent low toxicity.
**Special Considerations**

B₁₂-deficient individuals who are at risk for Leber’s optic atrophy should not be given cyanocobalamin to treat the B₁₂ deficiency. Leber’s optic atrophy is a genetic disorder caused by chronic cyanide intoxication (present in tobacco smoke, alcohol, and some plants). Reduced serum B₁₂ concentrations have been associated with a reduced ability to detoxify the cyanide in exposed individuals (Foulds, 1968, 1969a, b, 1970; Wilson and Matthews, 1966). Cyanocobalamin may increase the risk of irreversible neurological damage (from the optic atrophy). Hydroxocobalamin is a cyanide antagonist and therefore not associated with adverse effects when given to these individuals.

**Dose-Response Assessment**

The data on adverse effects of B₁₂ intake were considered not sufficient for a dose-response assessment and derivation of a UL.

**Intake Assessment**

In 1986 approximately 26 percent of adults in the United States took a supplement containing B₁₂ (Moss et al., 1989). Although no UL can be set for B₁₂, an exposure assessment is provided here for possible future use. Based on data from the Third National Health and Nutrition Examination Survey (see Appendix H), the highest median intake of B₁₂ from diet and supplements for any life stage and gender group was for males aged 31 through 50 years: 17 µg/day. The highest reported intake at the ninety-fifth percentile was 37 µg/day for pregnant females aged 14 through 55 years.

**Risk Characterization**

On the basis of the review of data involving high-dose intakes of B₁₂, there appear to be essentially no risks of adverse effects to the general population even at the current ninety-fifth percentile of intake noted above. Furthermore, there appear to be no risks associated with intakes of supplemental B₁₂ that are more than two orders of magnitude higher than the ninety-fifth percentile of intake. Although there are extensive data showing no adverse effects associated with high intakes of supplemental B₁₂, the studies in which such intakes were reported were not designed to assess adverse effects.
RESEARCH RECOMMENDATIONS FOR VITAMIN B$_{12}$

High-Priority Recommendations

Priority should be given to three topics of research related to vitamin B$_{12}$:

- The prevalence of B$_{12}$ deficiency as diagnosed by biochemical, neurological, or hematological abnormalities (e.g., methylmalonic acid and holotranscobalamin II).
- Improved, economical, and sensitive methods to detect B$_{12}$ malabsorption and deficiency before adverse neurological and hematological changes occur.
- Effective methods to reduce the risk of suboptimal B$_{12}$ status resulting from B$_{12}$ malabsorption or vegetarian diets. For elderly persons with food-bound malabsorption, research is needed on the form and amount of B$_{12}$ that can normalize and maintain B$_{12}$ stores. For vegetarians, information is needed about the absorption of B$_{12}$ from dairy products, algae, and fortified food products.

Other Research Areas

Two additional topics also merit attention:

- The feasibility and potential benefits and adverse effects of fortification of cereal grain foods with B$_{12}$, considering stability, identity of any degradation products, and bioavailability for normal individuals and those who malabsorb protein-bound B$_{12}$.
- The contribution of bacterial overgrowth to elevated serum methylmalonic acid.

REFERENCES


VITAMIN B₁₂


VITAMIN B\textsubscript{12} 351


Georgadze GE. 1960. Effect of vitamin B\textsubscript{1} and B\textsubscript{12} on induction of malignant growths in hamsters. \textit{Vopr Onkol} 6:54–58.


Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline


