9

Vitamin B_{12}

SUMMARY

Vitamin B_{12} (cobalamin) functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine and for a separate reaction that converts L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA. The Recommended Dietary Allowance (RDA) for vitamin B_{12} is based on the amount needed for the maintenance of hematological status and normal serum vitamin B_{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_{12}. Because 10 to 30 percent of older people may be unable to absorb naturally occurring vitamin B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B_{12} or a vitamin B_{12}-containing supplement. Individuals with vitamin B_{12} deficiency caused by a lack of intrinsic factor require medical treatment. The median intake of vitamin B_{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_{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_{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 B_{12} 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 B_{12}* is usually restricted to cyanocobalamin, in this report, B_{12} will refer to all potentially biologically active cobalamins.

In the United States, cyanocobalamin is the only commercially available B_{12} 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 B_{12}. Compared with hydroxocobalamin, cyanocobalamin binds to serum proteins less well and is excreted more rapidly (Tudhope et al., 1967).

Function

B_{12} 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-Methymalonyl-CoA mutase requires adenosylcobalamin to convert L-methymalonyl-CoA to succinyl-CoA in an isomerization reaction. In B_{12} deficiency, folate may accumulate in the serum as a result of slowing of the B_{12}-dependent methyltransferase. An adequate supply of B_{12} is essential for normal blood formation and neurological function.

Physiology of Absorption, Metabolism, Storage, and Excretion

Small amounts of B_{12} 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 B_{12} is dissociated from proteins in the presence of acid and pepsin. The released B_{12} 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 B_{12} to bind with intrinsic factor. The resulting complex of intrinsic factor and B_{12} attaches to specific receptors in the ileal mucosa; after internalization of the complex, B_{12} enters the enterocyte. Approximately 3 to 4 hours later, B_{12} enters the circulation. All circulating B_{12} is bound to the plasma binding proteins—transcobalamin I, II, or III (TCI, TCII, or TCIII). Although TCI binds approximately 80 percent of the B_{12} carried in the blood, TCII is the form that delivers B_{12} 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 B_{12} 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 B_{12} results; if this is untreated, potentially irreversible neurological damage and life-threatening anemia develop.

The average B_{12} 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 B_{12} 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 B_{12} store was 11.1 mg (Grasbeck et al., 1958). Excretion of B_{12} is proportional to stores (see "Excretion").

Absorption

Studies to measure the actual absorption of B_{12} involve whole-body counting of radiolabeled B_{12}, counting of radiolabeled B_{12} in the stool, or both. No data are available on whether B_{12} absorption varies with B_{12} 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 B_{12} given 4 to 6 hours apart is absorbed as well as the first (Heyssel et al., 1966). When large doses of crystalline B_{12} 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_{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_{12} from dairy foods or from red meat other than mutton and liver. The absorption efficiency of B_{12} from liver reportedly was low because of its high B_{12} content. Although evidence indicates that a B_{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_{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_{12} is used in this report. In particular, it is assumed that 50 percent of dietary B_{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_{12}. Different levels of absorption are assumed under various conditions, as shown in Table 9-2. Crystalline B_{12} appears in the diet only in foods that have been fortified with B_{12}, such as breakfast cereals and liquid meal replacements.

Enterohepatic Circulation

B_{12} is continually secreted in the bile. In healthy individuals most of this B_{12} is reabsorbed and available for metabolic functions. El Kholty et al. (1991) demonstrated that the secretion of B_{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_{12} is assumed to be

TABLE 9-1 Percentage Absorption of Vitamin B_{12} from Foods by Healthy Adults

Reference	Food	Absorption (%)
Heyssel et al., 1966	Mutton	65
Heyssel et al., 1966	Liver	11
Doscherholmen et al., 1975	Eggs	24–36
Doscherholmen et al., 1978	Chicken	60
Doscherholmen et al., 1981	Trout	25–47

TABLE 9-2 Assumed Vitamin B_{12} Absorption under Different Conditions

Form of Vitamin B_{12}	Normal Gastric Function (%)	Pernicious Anemia[a] (%)
Naturally occurring[b]	50	0
Crystalline, low dose (< 5 μg)[b]	60	0
Crystalline, high dose (≥ 500 μg) with water[c]	1	1
Crystalline, high dose with food[c]	0.5	≤ 0.5

[a] A disorder in which lack of intrinsic factor severely limits the absorption of vitamin B_{12}.
[b] Heyssel et al. (1966).
[c] Berlin et al. (1968).

reabsorbed, the average loss of biliary B_{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_{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_{12} absorption. However, in the absence of intrinsic factor, essentially all the B_{12} from the bile is excreted in the stool rather than recirculated. Thus, B_{12} deficiency develops more rapidly in individuals who have no intrinsic factor or who malabsorb B_{12} for other reasons than it does in those who become complete vegetarians and thus ingest no B_{12}.

Excretion

If the circulating B_{12} exceeds the B_{12} binding capacity of the blood, the excess is excreted in the urine. This typically occurs only after injection of B_{12}. The highest losses of B_{12} ordinarily occur through the feces. Sources of fecal B_{12} include unabsorbed B_{12} from food or bile, desquamated cells, gastric and intestinal secretions, and B_{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_{12} stores decrease. Various studies have indicated losses of 0.1 to 0.2 percent of the B_{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 B_{12} deficiency is pernicious anemia (see "Pernicious Anemia"). The hematological effects of B_{12} 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 B_{12}.

Neurological Effects of Deficiency

Neurological complications are present in 75 to 90 percent of individuals with clinically observable B_{12} deficiency and may, in about 25 percent of cases, be the only clinical manifestation of B_{12} deficiency. Evidence is mounting that the occurrence of neurological complications of B_{12} 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-

op. 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_{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_{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_{12} (Stabler et al., 1996). Because a lack of folate, vitamin B_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_{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_{12} deficiency. Propionate may be converted to 2-methylcitrate, serum and cerebrospinal fluid concentrations of which also rise in B_{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_{12} deficiency.

Holotranscobalamin II

Among the three plasma B_{12} binding proteins, transcobalamin II (TCII) is responsible for receptor-mediated uptake of B_{12} into cells. However, only a small fraction of the plasma B_{12} (10 to 20 percent) is present as the TCII-B_{12} complex. This fraction, termed *holoTCII,* may provide a good indication of B_{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.

METHODOLOGICAL ISSUES

Vitamin B_{12} Content

The two primary microbial organisms used to determine the vitamin B_{12} content of serum, urine, and stool are *Euglena gracilis* and *Lactobacillus leichmannii*. Although either organism will yield essentially similar results, *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_{12} used in these assays would also bind analogues of B_{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_{12} comparable with those obtained from microbiological assays. More recently, nonisotopic serum B_{12} assays have been introduced, which has resulted in cutoff levels for B_{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_{12} value may be misleading as an indicator because it includes all the B_{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_{12} (holoTCII) is important in relation to B_{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_{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_{12} are used to estimate the B_{12} requirement.

TABLE 9-3 Change in Percentage Retention of Vitamin B_{12} with Increasing Intramuscular Dose

Vitamin B_{12} Dose (μg)	Retention (%)
3	100
10	97
25	95
40	93
1,000	15

SOURCE: Chanarin (1969).

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

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

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 B_{12} REQUIREMENT

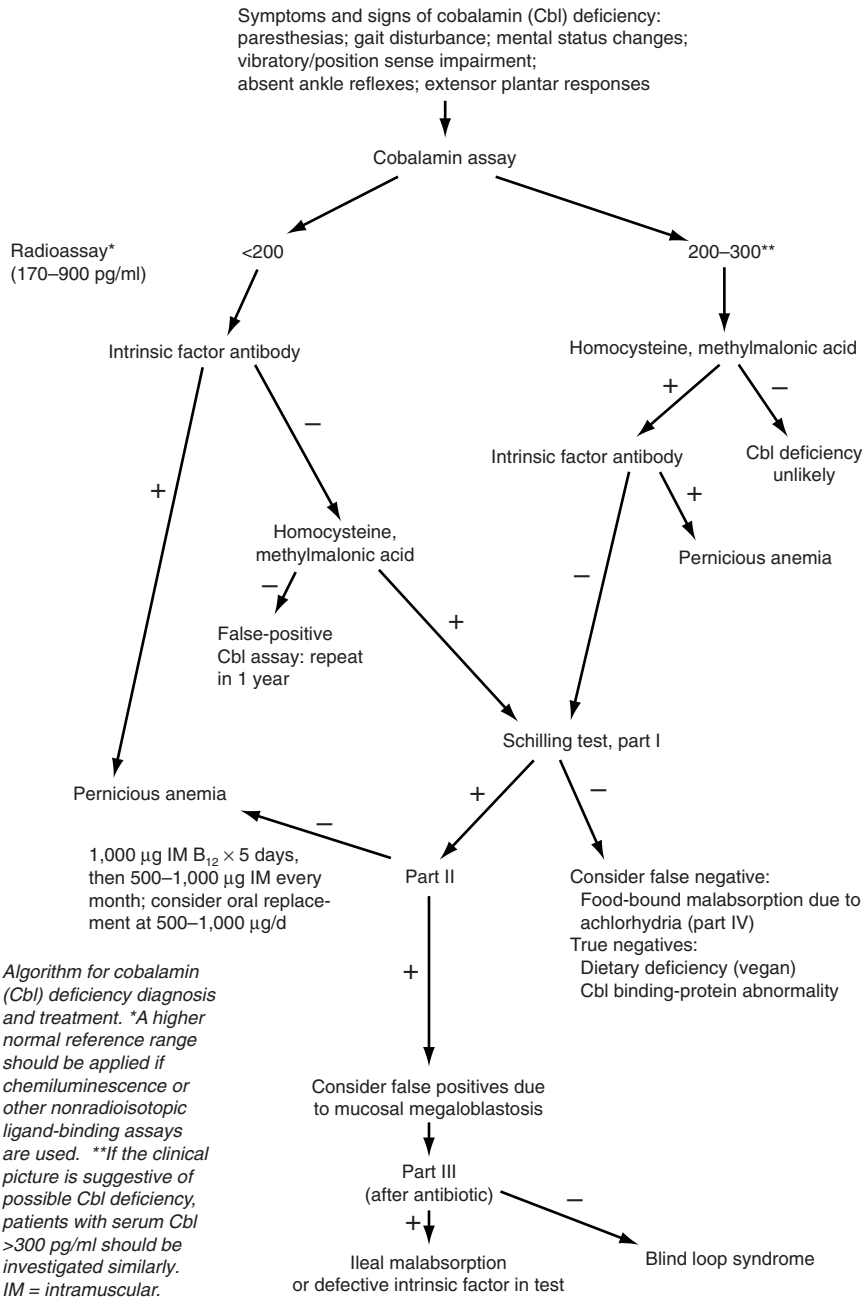
Aging

Plasma vitamin B_{12} tends to decrease and serum methylmalonic acid (MMA) concentration tends to increase with age. These changes may represent a decline in B_{12} 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 B_{12} malabsorption, severity of atrophic gastritis, compromised functional and structural integrity of the B_{12} binding proteins, and a lack of liver B_{12} stores (van Asselt et al., 1996). Percentage absorption of crystalline B_{12} 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 B_{12} (less than 88 pmol/L [120 pg/mL]), those with lowest serum B_{12} values tend to have severe atrophic gastritis. Scarlett and colleagues (1992) reported a reduction in dietary B_{12} 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



*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.*

FIGURE 9-1 The diagnosis of pernicious anemia. Reprinted with permission from Green and Kinsella (1995). Copyright 1995 by Lippincott-Raven Publishers.

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, postgastric 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 radiolabeled 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 ($p < 0.001$ and 0.05, 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_{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_{12} intake of infants fed human milk. B_{12} deficiency does not occur in infants fed milk from mothers with adequate B_{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 B_{12} content, averaging 0.31 μg/L (Specker et al., 1990). The B_{12} content of milk in a large group of low-income Brazilian mothers (n = 83) who had received prenatal supplements containing B_{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_{12} content of 0.42 μg/L, the AI for B_{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_{12} stores and may consume human milk that is low in B_{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_{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 μg/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 μg/day of B_{12} (0.31 μg/L × 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 μg/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) μg/L. If clinical signs appear within 9 months in infants consuming milk containing 0.085 μg/L of B_{12}, this intake (approximately 0.07 μg/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 μg) 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 μg/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 μg/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_{12} for infants ages 0 through 6 months is used, the AI for B_{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_{12} of 0.6 μg/day.

In one study of three infants exclusively fed human milk who had clinically observable B_{12} deficiency caused by low maternal consumption of animal products, one infant was treated parenterally with B_{12} whereas two infants were treated with small oral B_{12} doses (Jadhav et al., 1962). At 9 months of age, 0.1 μg/day of oral B_{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_{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_{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_{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_{12} balance in this group as determined by urinary MMA excretion.

B_{12} AI Summary, Ages 0 through 12 Months

AI for Infants		
0–6 months	**0.4 μg/day of vitamin B_{12}**	**≈0.05 μg/kg**
7–12 months	**0.5 μg/day of vitamin B_{12}**	**≈0.05 μg/kg**

Special Considerations

Infants of vegan mothers should be supplemented with B_{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 ± 6.6 (standard deviation) months and fed macrobiotic foods, was 0.3 ± 0.2 μg/day for the first 6 to 16 months compared with 2.9 ± 1.2 μg/day for well-nourished control infants (Dagnelie et al., 1991). These data suggest that an intake of 0.3 μg/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

EAR for Children	**1–3 years**	**0.7 μg/day of vitamin B_{12}**
	4–8 years	**1.0 μg/day of vitamin B_{12}**
EAR for Boys	**9–13 years**	**1.5 μg/day of vitamin B_{12}**
	14–18 years	**2.0 μg/day of vitamin B_{12}**
EAR for Girls	**9–13 years**	**1.5 μg/day of vitamin B_{12}**
	14–18 years	**2.0 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 Children	**1–3 years**	**0.9 μg/day of vitamin B_{12}**
	4–8 years	**1.2 μg/day of vitamin B_{12}**
RDA for Boys	**9–13 years**	**1.8 μg/day of vitamin B_{12}**
	14–18 years	**2.4 μg/day of vitamin B_{12}**
RDA for Girls	**9–13 years**	**1.8 μg/day of vitamin B_{12}**
	14–18 years	**2.4 μg/day of vitamin B_{12}**

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_{12} Needed for Maintenance of Hematological Status and Serum Vitamin B_{12} Values

- Maintenance of hematological status requires a relatively stable hemoglobin value upon administration of B_{12} and a normal mean cell volume, not just a reticulocyte response.
- Normal serum B_{12} is ≥ 150 pmol/L (200 pg/mL).
- Because B_{12} is not absorbed from the bile, the estimated extra loss of B_{12} by a person with pernicious anemia in remission is 0.4 nmol/day (0.5 μg/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_{12} from food by healthy individuals is approximately 50 percent (see "Absorption").

provided information on levels of B_{12} intake needed to maintain hematological status. In some cases, neurological manifestations may be the earliest clinical sign of low B_{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_{12} required daily to maintain hematological and serum B_{12} status of individuals with pernicious anemia in remission; subtracting the amount of endogenous B_{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_{12} from food, correcting for bioavailability. The result is shown in Box 9-2.

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

Step 1	Estimate the average intramuscular requirement for maintenance of person with pernicious anemia	1.5 μg/day
Step 2	Subtract estimate of extra losses due to lack of reabsorption of biliary B_{12}	– 0.5 μg/day
Subtotal	Estimate average requirement of normal person for absorbed B_{12}	1.0 μg/day
Step 3	Correct for bioavailability (50 percent)	÷ 0.5
Result	Average requirement of normal person for B_{12} from food: Estimated Average Requirement (EAR)	2.0 μg/day

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 μg/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 μg/day, no patient met those standards. Dosages used for maintenance were increased to 1 to 4 μg/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 μg/day IM.

TABLE 9-5 Effectiveness of Intramuscular Vitamin B_{12} Doses for Maintenance of Maximum Erythropoiesis

Daily B_{12} Dose, Intramuscular (μg)	Number of Subjects Achieving Maximum Erythropoiesis (n = 7)	Cumulative Number Achieving Maximum Erythropoiesis (n = 7)
0.5	1	1
1.0	2	3
1.4	1	4
2.0	2	6
4.0	1	7

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 B_{12} 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 B_{12} IM will maintain normal hematological, serum B_{12}, 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 B_{12} requirement, but it suggests that 1.4 μg of B_{12} exceeds the requirement for absorbed B_{12} 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 B_{12} was not sufficient for treating pernicious anemia and maintaining adequate B_{12} status. Similarly, the 0.6 to 0.7 μg/day of B_{12} supplied IM in the study by Will and coworkers (1959) was also judged too low to maintain a normal serum B_{12} 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 B_{12} after adjustment for the extra loss of B_{12} by subjects with pernicious anemia (0.5 μg/day) (Step 2 in Box 9-2). Adjusting for incomplete absorption of B_{12} from food of 50 percent (Step 3) converts this value to an EAR for B_{12} of 2.0 μg/day.

Studies of Individuals with Low B_{12} Intake. Studies of individuals with low B_{12} intake were examined to determine whether these reports (Table 9-7) supported the findings for subjects with pernicious anemia. Because B_{12} is not a component of plant foods, diets containing little or no animal food may lead to B_{12} deficiency. Deficiency develops slowly because of efficient reabsorption of biliary B_{12}. It is also possible but not certain that vegans consume some B_{12} from animal products that contaminate plant food or from bacterial action. Studies of vegetarians generally have not analyzed the B_{12} 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 B_{12} 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 B_{12} for Adults

Reference	Number of Subjects	Age Range (y)
Suggested IM[a] requirement > 2.0 μg		
Hansen and Weinfeld, 1962	14	
Suggested IM requirement of 1.0–2.0 μg		
Bastrup-Madsen et al., 1983	112	33–78
Lindenbaum et al., 1990	44	NA[c]
Other Studies		
Best et al., 1956	6	NA
Will et al., 1959	40	NA
Sullivan and Herbert, 1965	8	46–86

[a] IM = intramuscular.
[b] 1,000 μg × 0.15 retention/90 d.
[c] NA = not available.
[d] 1,000 μg × 0.15 retention/182 d.

Treatment	Results
2–5 μg of B_{12} IM for 8–15 d.	Five persons who were given 3 μg/d of B_{12} for 15 d had a reticulocyte response that was not followed by a further response to more B_{12}. This amount allowed restoration of status.
1 mg of slow-release B_{12} IM every 2 or 3 mo for at least 8 y. The less-frequent dose was equivalent to 1.7 μg of B_{12}/d.[b]	Serum B_{12} values were well above the cutoff of 180 pmol/L (250 pg/mL) early in the study and complete hematological remission occurred in all.
35 received 1 mg of B_{12} 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 B_{12}/d.[d]	From total group analyses, 14 subjects had mild hematological relapse on 42 occasions; 34 subjects had at least one abnormal serum B_{12} or metabolite value on 146 occasions when there was no evidence of hematological relapse.
2.0 μg oral dose of B_{12} Co^{60} given with intrinsic factor.	With 70% absorption, complete hematological response, and adequate plasma B_{12} concentration, 1.4 μg of absorbed B_{12} met the requirements of two-thirds of the subjects.
10 μg of B_{12} given IM every 2 wk or 20 μg of B_{12} given IM monthly for 10 y (equivalent average of 0.7 μg/d).	None of the subjects maintained serum B_{12} concentration above the 180 pmol/L (250 pg/mL) lower limit of normal for the *Lactobacillus leichmannii* method.
0.1 μg/d of cyanocobalamin IM for 10 d; 0.1 μg/d of coenzyme B_{12} IM for 10 d.	Posttreatment serum B_{12} 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 B_{12} was given daily. All but one were later given higher doses of B_{12}.

TABLE 9-7 Studies of Individuals with Low Vitamin B_{12} Intake Considered in Setting the Estimated Average Requirement for B_{12} for Adults

Reference	Description	Dietary B_{12} Intake
Suggested dietary B_{12} average requirement > 1.5 µg/d		
Stewart et al., 1970	1 Hindu woman with megaloblastic anemia	0.5 µg/d (analyzed homogenate)
Narayanan et al., 1991	10 subjects with serum B_{12} values below the 2.5 percentile (< 120 pmol/L [162 pg/mL]) not caused by disease or vegetarianism	1.5 ± 0.4 (SD[c]) µg/d of B_{12} (range 0.6–1.9)
Suggested dietary B_{12} average requirement > 1.0 µg/d		
Winawer et al., 1967	1 64-y-old vegan with B_{12}-deficient megaloblastic anemia, gastritis on biopsy, and normal gastric acidity	Assumed to be negligible
Jathar et al., 1975	7 East Indian lactovegetarians	0.3–0.8 µg/d of B_{12} from milk, assuming that it was not boiled
Baker and Mathan, 1981	4 East Indians with B_{12} deficiency anemia secondary to diet	NA[d]

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

appear to be impaired, the combination of stores and absorbed crystalline B_{12} 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 B_{12} deficiency after a total gastrectomy; for example, megaloblastic anemia has been typically diagnosed 2 to 5 years after a total gastrectomy (Chanarin, 1990).

Treatment	Comments
1 μg/d of B_{12} p.o.[a]	Serum B_{12} rose to 121 pmol/L (164 pg/mL) (said to be normal) and hemoglobin stabilized at 10.7 g/100 mL
1 pint/d of fresh milk (≈1.5 μg of B_{12})[b]	Serum B_{12} maintained at 100 pmol/L (134 pg/mL)
Not specified	Seven fulfilled at least one criterion for tissue B_{12} deficiency
1 μg/d of B_{12} p.o.	Serum B_{12} rose to 64 pmol/L (87 pg/mL), well below normal; gastritis may have decreased absorption of any B_{12} inadvertently present in the food
None	Half had serum B_{12} values < 74 pmol/L (100 pg/mL)
0.07–0.25 μg/d of B_{12}	Judged inadequate
0.3–0.65 μg/d of B_{12}	Hematological responses seen but serum B_{12} ≤ 74 pmol/L (100 pg/mL) in all Interpretation complicated by transfusions and intramuscular injections

[c] SD = standard deviation.
[d] NA = not available.

Possible Ancillary Method: Maintenance of a Serum B_{12} 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_{12} 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

Serum Values of Subjects	Healthy Younger Control Subjects[a] (Number [%])	Elderly Subjects from Framingham Study[b] (Number [%])
Vitamin B_{12} < 148 pmol/L (201 pg/mL)	2 (1.7)	29 (5.3)
Vitamin B_{12} < 258 pmol/L (351 pg/mL)	21 (17.9)	222 (40.5)
Methylmalonic acid > 376 nmol/L	Not available	82 (15.0)

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 μg/day of B_{12} (range 0 to 12.8 μg) (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 μg/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.

EAR for Men	**19–30 years**	**2 μg/day of vitamin B_{12}**
	31–50 years	**2 μg/day of vitamin B_{12}**
EAR for Women	**19–30 years**	**2 μg/day of vitamin B_{12}**
	31–50 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	**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.

EAR for Pregnancy	**14–18 years**	**2.2 μg/day of vitamin B_{12}**
	19–30 years	**2.2 μg/day of vitamin B_{12}**
	31–50 years	**2.2 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 Pregnancy	**14–18 years**	**2.6 μg/day of vitamin B_{12}**
	19–30 years	**2.6 μg/day of vitamin B_{12}**
	31–50 years	**2.6 μg/day of vitamin B_{12}**

Lactation

Evidence Considered in Estimating the Average Requirement

As described earlier, the average amount of B_{12} secreted in the milk of mothers with adequate B_{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_{12} secreted is slightly less: 0.25 μg/day.

The concentration of B_{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_{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_{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_{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_{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_{12} status of the mother is marginal, further maternal depletion may occur as reflected in decreasing concentrations of maternal plasma B_{12} (Black et al., 1994; Shapiro et al., 1965).

B_{12} EAR and RDA Summary, Lactation

To estimate the EAR for lactation, 0.33 μg/day of B_{12} is added to the EAR of 2 μg/day for adolescent girls and adult women; the result is rounded up.

EAR for Lactation	**14–18 years**	**2.4 μg/day of vitamin B_{12}**
	19–30 years	**2.4 μg/day of vitamin B_{12}**
	31–50 years	**2.4 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 Lactation	**14–18 years**	**2.8 μg/day of vitamin B_{12}**
	19–30 years	**2.8 μg/day of vitamin B_{12}**
	31–50 years	**2.8 μg/day of vitamin B_{12}**

Special Considerations

Persons with any malabsorption syndrome will likely require increased amounts of B_{12}. 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_{12} 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_{12}.

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_{12} and will require normal amounts of crystalline B_{12} (either in foods fortified with B_{12} or as a supplement).

INTAKE OF VITAMIN B_{12}

Food Sources

Ordinarily, humans obtain vitamin B_{12} from animal foods. Unlike other B vitamins, B_{12} is not a normal constituent of plant foods except for certain algae (Ford and Hutner, 1955). B_{12} 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_{12}; or fortified with B_{12} (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. Moshfegh, 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 μg and by young adult women, 3 μg (Appendixes G and H). In one Canadian province, the mean dietary intake was reported as approximately 7 μg/day for men and 4 μg/day for women (Appendix I).

TABLE 9-9 Food Groups Providing Vitamin B_{12} in the Diets of U.S. Men or Women Aged 10 Years and Older, CSFII, 1995[a]

	Contribution to Total B_{12} Intake[b] (%)		Foods Within the Group that Provide at Least 1 µg of B_{12}[c] per Serving	
Food Group	Men	Women	1–2 µg	> 2 µg
Food groups providing at least 5% of total vitamin B_{12} intake				
Mixed foods[d]	18.5	16.4	NA[e]	NA
Beef	15.0	12.0	Beef	—
Milk and milk drinks	10.6	14.6	Plain and flavored yogurt[f]	—
Shellfish	9.4	4.9	Crayfish and scallops	Clams, oysters, mussels, crab, and lobster
Mixed foods, main ingredient is grain	7.1	5.7	NA	NA
Processed meats[g]	7.0	5.0	—	—
Organ meats	5.5	6.9	—	Liver, kidney, heart, brains, and tongue
Ready-to-eat cereals	4.7	8.2	Moderately fortified	Highly fortified
Finfish	3.4	5.7	Catfish, pike, whiting, perch, swordfish, carp, porgy, and flounder	Herring, sardines, trout, mackerel, salmon, and canned tuna
Vitamin B_{12} from other food groups				
Lamb, veal, game, and other carcass meat	0.8	0.8	Lamb and veal	Venison, rabbit, and squirrel
Soy-based supplements and meal replacements	0.7	0.2	Soy-based meat substitutes	—

[a] CSFII = Continuing Survey of Food Intakes by Individuals.

[b] 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.

[c] 1 µg represents 20% of the Recommended Daily Intake (6.0 µg) of B_{12}—a value set by the Food and Drug Administration.

[d] Includes sandwiches and other foods with meat, poultry, or fish as the main ingredient.

[e] NA = not applicable. Mixed foods were not considered for this table.

[f] Whole, low fat, and nonfat.

[g] Includes frankfurters, sausages, lunch meats, and meat spreads.

SOURCE: Unpublished data from the Food Surveys Research Group, Agricultural Research Service, U.S. Department of Agriculture, 1997.

TABLE 9-10 Contribution of Fortified Foods to the Vitamin B_{12} Intake of U.S. Men and Women by Age Group, CSFII, 1995[a]

	Contribution of Food Group to Total B_{12} Intake[b] (%)					
	Adults ≥ 19 Years		Ages 51–70 Years		Ages 70+ Years	
Food Group	Men	Women	Men	Women	Men	Women
Ready-to-eat cereals	4.7	8.2	7.8	10.3	10.9	11.9
Soy-based supplements and meal replacements	0.7	0.5	0.9	0.5	1.2	0.3
Milk-based supplements and meal replacements	0.2	0.2	0.2	0.3	0.5	0.3
Total	5.6	8.9	8.9	12.1	12.6	12.5

[a] CSFII = Continuing Survey of Food Intake by Individuals.

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

SOURCE: Unpublished data from the Food Surveys Research Group, Agricultural Research Service, U.S. Department of Agriculture, 1997.

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_{12} provided by fortified foods. In a study of Boston elderly aged 60 to more than 90 years (Russell, 1992), median B_{12} intake by males who were not taking supplements was 3.4 μg/day. The median plasma B_{12} concentration for this unsupplemented group was 286 pmol/L (388 pg/mL). For females not taking supplements, the median B_{12} intake was 2.6 μg/day and the median plasma B_{12} concentration was 272 pmol/L (369 pg/mL). B_{12} 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_{12} 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_{12} 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_{12} 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 B_{12} by a free-living elderly population is given in Appendix F. For those taking supplements, the fiftieth percentile of supplemental B_{12} intake was 5.0 µg for men and 6.0 µg for women. Approximately 26 percent of all adults reported taking a B_{12}-containing supplement in 1986 (Moss et al., 1989).

TOLERABLE UPPER INTAKE LEVELS

Hazard Identification

Adverse Effects

No adverse effects have been associated with excess B_{12} intake from food or supplements in healthy individuals. There is very weak evidence from animal studies suggesting that B_{12} 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 B_{12} intake inhibits tumor induction in the human liver, colon, and esophagus (Rogers, 1975). Some studies suggest a possible association between high-dose, parenterally administered B_{12} (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 B_{12} 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 B_{12} (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 B_{12} can be absorbed from the gastrointestinal tract, which may explain the apparent low toxicity.

Special Considerations

B_{12}-deficient individuals who are at risk for Leber's optic atrophy should not be given cyanocobalamin to treat the B_{12} deficiency. Leber's optic atrophy is a genetic disorder caused by chronic cyanide intoxication (present in tobacco smoke, alcohol, and some plants). Reduced serum B_{12} 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 B_{12} 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 B_{12} (Moss et al., 1989). Although no UL can be set for B_{12}, 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 B_{12} 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 B_{12}, 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 B_{12} 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 B_{12}, 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.

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