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Chapter 60

Neurologic aspects of cobalamin (B_{12}) deficiency

NEERAJ KUMAR*
Department of Neurology, Mayo Clinic, Rochester, MN, USA

INTRODUCTION

Optimal functioning of the central and peripheral nervous system is dependent on a constant supply of appropriate nutrients. Neurologic signs occur late in malnutrition. Deficiency diseases such as kwashiorkor and marasmus are endemic in underdeveloped countries. Individuals at risk in developed countries include the poor and homeless, the elderly, patients on prolonged or inadequate parenteral nutrition, individuals with food fads or eating disorders such as anorexia nervosa and bulimia, those suffering from malnutrition secondary to chronic alcoholism, and patients with pernicious anemia (PA) or other disorders that result in malabsorption such as sprue, celiac disease, and inflammatory bowel disease. Of particular concern in the developed world is the epidemic of obesity. The rising rates of bariatric surgery have been accompanied by neurologic complications related to nutrient deficiencies. The preventable and potentially treatable nature of these disorders makes this an important subject. Prognosis depends on prompt recognition and institution of appropriate therapy.

Particularly important for optimal functioning of the nervous system are the B group vitamins (vitamin B_{12}, thiamin, niacin, pyridoxine, and folic acid), vitamin E, and copper. Not infrequently multiple nutritional deficiencies coexist. This review deals with neurologic aspects of vitamin B_{12} deficiency and attempts to highlight recent developments. A prior edition of *Handbook of Clinical Neurology* contains a more comprehensive account of historical and clinical aspects of the neurology of cobalamin (Cbl) deficiency (Cole, 1998). This chapter is biased toward more recent references. The interested reader is directed to some recent review articles and book chapters for detailed bibliographies (Tefferi and Pruthi, 1994; Green and Kinsella, 1995; Cole, 1998; Carmel, 2000, 2008; Ward, 2002; Carmel et al., 2003a; Alpers, 2005; Kumar, 2007; Dali-Youcef and Andrès, 2009; Quadros, 2009; Kumar, 2010).

Formulation of liver extract to treat pernicious anemia led to Minot, Murphy, and Whipple being awarded the Nobel Prize for Physiology/ Medicine in 1934 (Chanarin, 2000). Subsequent elucidation of the crystalline structure of vitamin B_{12} led to Dorothy Hodgkins being awarded the Nobel Prize for Chemistry in 1964 (Chanarin, 2000).

COBALAMIN

TERMINOLOGY

Vitamin B_{12} refers to a specific group of cobalt-containing corrinoids with biological activity in humans. Cobalt is responsible for the red color of this water-soluble vitamin. This group of corrinoids is also referred to as cobalamins. The main cobalamins in humans and animals are adenosylCbl, methylCbl, and hydroxoCbl. Food Cbl is hydroxoCbl. AdenosylCbl and methylCbl are the active coenzyme forms. In all tissues adenosylCbl is the predominant intracellular form and is located in the mitochondria. MethylCbl has a cytosolic localization. MethylCbl is a minor component of intracellular Cbl but is the major form of Cbl in plasma and is the form that is disproportionately reduced in Cbl deficiency. CyanoCbl is a stable synthetic pharmaceutical that also has to be converted to adenosylCbl or methylCbl to become metabolically active. Even though vitamin B_{12} refers specifically to cyanoCbl, the terms Cbl, B_{12}, and vitamin B_{12} are generally used interchangeably.

REQUIREMENT FOR AND SOURCES OF COBALAMIN

The recommended dietary allowance of Cbl for adults is 2.4 μg/day and the median intake from food in the US is

*Correspondence to: Neeraj Kumar, M.D., Department of Neurology, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA. E-mail: kumar.neeraj@mayo.edu

3.5 µg/day for women and 5 µg/day for men. No adverse effects have been associated with excess Cbl intake. Cbl is synthesized solely by microorganisms. Ruminants obtain Cbl from the foregut. Foods of animal origin such as meat, eggs, and milk are the major dietary sources. The richest sources of Cbl include shellfish, organ meats such as liver, some game meat, and certain fish. In some countries Cbl-fortified cereals are particularly efficient sources.

FUNCTIONS AND KINETICS

In the stomach, Cbl bound to food is dissociated from proteins in the presence of acid and pepsin (Fig. 60.1). The released Cbl binds to haptocorrins (HC, encoded by the *TCN1* gene). The HC have been referred to in the literature as R proteins or R-binder or transCbl I and III. The HC are secreted by many cell types including glandular cells (salivary glands, gastric mucosa, and others). In the small intestine, pancreatic proteases partially degrade the Cbl-HC complex at neutral pH and release Cbl which then binds with intrinsic factor (IF, encoded by the *GIF* gene). IF is a Cbl-binding glycoprotein secreted by parietal cells in the fundus of the stomach. The Cbl-IF complex binds to a specific receptor in the ileal mucosa called cubilin (CUB, encoded by the *CUBN* gene) and is then internalized (Christensen and Birn, 2002). The internalization of cubulin with Cbl-IF is facilitated by amnionless (AMN, encoded by the *AMN* gene), an endocytic receptor protein that directs sublocalization and endocytosis of CUB with its Cbl-IF complex (Fyfe et al., 2004). The megalin receptor (MAG, encoded by the *LRP-2* gene) may play a role in the stability of the cubilin/AMN complex. Like MAG, the receptor-associated protein (RAP) can interact with

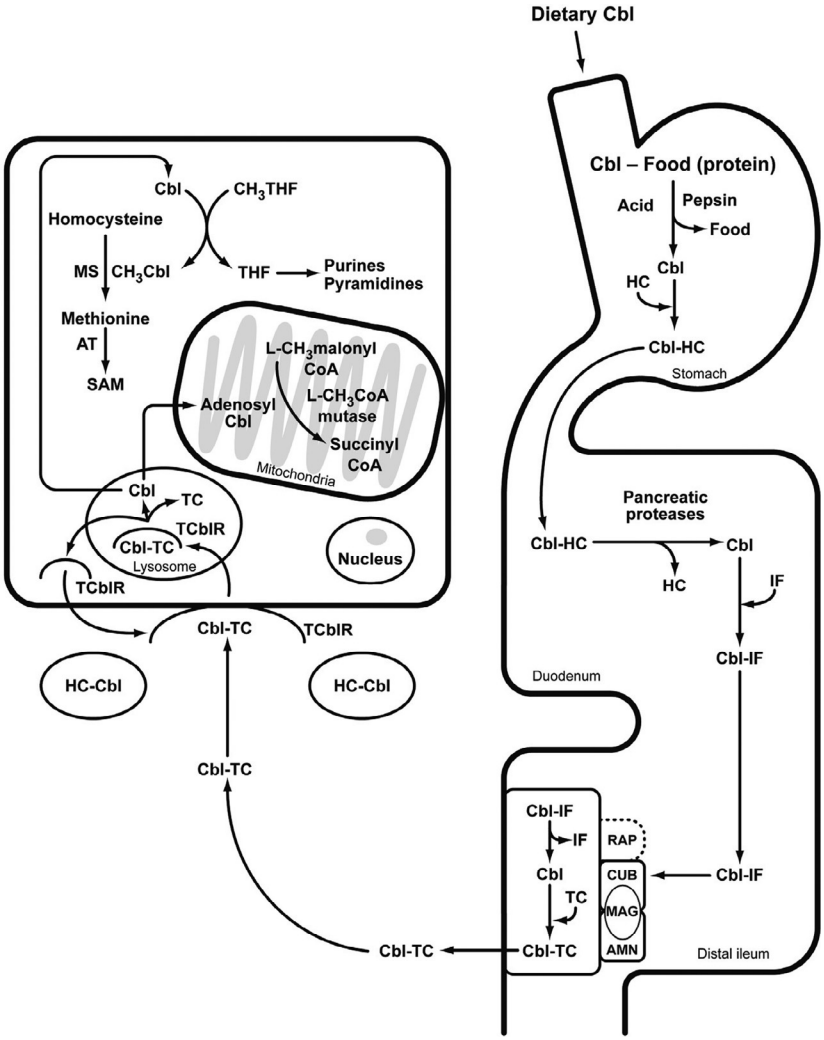

Fig. 60.1. Cbl absorption and metabolism. Cbl, cobalamin; HC, haptocorrin; IF, intrinsic factor; CUB, cubilin; AMN, amnionless; MAG, megalin; RAP, receptor-associated protein; TC, transcobalamin; TCblR, transcobalamin receptor; CH3, methyl; THF, tetrahydrofolate; MS, methionine synthetase; AT, adenosyl transferase; SAM, *S*-adenosylmethionine; CoA, coenzyme A.

CUB, but the precise role of these proteins in CUB-mediated Cbl-IF absorption has not been determined. The Cbl-IF complex enters the ileal cell where IF is destroyed. In addition to the IF-mediated absorption of ingested Cbl, there is a nonspecific absorption of free or crystalline Cbl that occurs by passive diffusion at all mucosal sites. This is a relatively inefficient process by which 1–2% of the ingested amount is absorbed.

TransCbl (TC, encoded by the *TCN2* gene) is a nonglycosylated plasma protein that carries 10–30% of the total Cbl. TC has been referred to in the literature as transCbl II. TC-bound Cbl (holotransCbl, holoTC) represents the active form of Cbl (Refsum et al., 2006). TC binds to and transports the newly absorbed Cbl in the distal ileum to cells throughout the body where it is internalized by receptor-mediated cellular uptake (Quadros et al., 2009). The gene encoding the transCbl receptor (TCblR), *CD320*, was identified from the human genome databank (Quadros et al., 2009). Following internalization, the Cbl-TC complex is degraded by the lysosome and the receptor is recycled to the plasma membrane. Intracellular lysosomal degradation releases Cbl (hydroxoCbl) for conversion to methylCbl in the cytosol or adenosylCbl in the mitochondria (Tefferi and Pruthi, 1994). TC reflects rapidly turning over B_{12}, while B_{12} attached to HC in circulating plasma reflect tissue levels of B_{12}.

MethylCbl is a cofactor for a cytosolic enzyme, methionine synthase, in a methyl-transfer reaction which converts homocysteine (Hcy) to methionine. Methionine is adenosylated to *S*-adenosylmethionine (SAM), a methyl group donor required for neuronal methylation reactions involving proteins, nucleic acids, neurotransmitters, myelin, and phospholipids. Decreased SAM production possibly leads to reduced myelin basic protein methylation and white matter vacuolization in Cbl deficiency. The biologically active folates are in the tetrahydrofolate (THF) form. MethylTHF is the predominant folate and is required for the Cbl-dependent remethylation of Hcy to methionine. During the process of methionine formation methylTHF donates the methyl group and is converted into THF, a precursor for purine and pyrimidine synthesis. Methionine also facilitates the formation of formylTHF which is involved in purine synthesis. Impaired DNA synthesis could interfere with oligodendrocyte growth and myelin production. Methylation of deoxyuridylate to thymidylate is mediated by methyleneTHF. Impairment of this reaction results in accumulation of uracil which replaces the decreased thymine in nucleoprotein synthesis and initiates the process that leads to megaloblastic anemia. AdenosylCbl is a cofactor for mitochondrial L-methylmalonyl coenzyme A (CoA) mutase which catalyzes the conversion of L-methylmalonyl CoA to succinyl CoA in an isomerization reaction. Accumulation of methylmalonate and propionate may provide abnormal substrates for fatty acid synthesis.

Between 0.5 and 5.0 μg of Cbl enters the bile each day. This binds to IF. Most of the Cbl secreted in the bile is reabsorbed along with Cbl derived from sloughed intestinal cells. Reabsorption of biliary Cbl is intact in vegetarians. Hence, Cbl deficiency develops more rapidly with malabsorption than in vegetarians. The estimated daily losses of Cbl (mainly in the urine and feces) are minute (1–3 μg) compared with body stores (2–3 mg). The body does not have the ability to degrade Cbl. Hence, even in the presence of severe malabsorption, 2–5 years may pass before Cbl deficiency develops (Green and Kinsella, 1995).

CAUSES OF COBALAMIN DEFICIENCY

An acidic environment in the stomach is required for Cbl to be released from food protein. The incidence of atrophic gastritis increases with age. Atrophic gastritis is accompanied by hypochlorhydria. Cbl deficiency is particularly common in the elderly and is most likely due to the high incidence of atrophic gastritis and associated achlorhydria-induced food-Cbl malabsorption (Pennypacker et al., 1992; Carmel, 1995, 1997, 2000; Andrès et al., 2005). Other causes of Cbl deficiency (e.g. *Helicobacter pylori* infection, antacid therapy) may coexist (Andrès et al., 2005). Food-bound Cbl malabsorption does not affect free Cbl, including recycled biliary Cbl (Carmel, 1995). Food-Cbl malabsorption is insidious in onset and rarely associated with overt clinically significant deficiency. Though controversial, there has been recent concern that low Cbl levels in the elderly might cause nervous system damage, but studies specifically in the elderly have not consistently demonstrated improvements in neurologic function following therapy. This concern has led to the development of the controversial concept of subclinical or subtle Cbl deficiency (Carmel, 2000; Carmel et al., 2003b). The low Cbl levels commonly seen in elderly patients can be accompanied by elevated methylmalonic acid (MMA) and Hcy.

Many patients with clinically expressed Cbl deficiency have IF-related malabsorption such as that seen in pernicious anemia (Pruthi and Tefferi, 1994; Toh et al., 1997). Pernicious anemia is associated with IF antibodies. The literature suggests that it is more common in African Americans and in people with a northern European background. Onset is often after age 60, but may be earlier in African American and Hispanic women.

Cbl deficiency is commonly seen following gastric surgery (gastrectomy and bariatric surgery) (Juhasz-Pocsine et al., 2007). This may result from inadequate intake, impaired hydrolysis of vitamin B_{12} from dietary

protein, IF loss, or due to abnormal IF and vitamin B_{12} interaction.

Acid reduction therapy such as with H_2 blockers and prolonged use of drugs such as metformin can also cause Cbl deficiency (Marcuard et al., 1994; Ting et al., 2006). Cbl deficiency has also been reported in association with oral therapy with the multitargeted tyrosine kinase inhibitor sunitinib (Gillessen et al., 2007). Cbl malabsorption has been rarely reported with some other drugs but this is generally not clinically significant.

Other causes of Cbl deficiency include conditions associated with malabsorption such as ileal disease or resection, intestinal tuberculosis or lymphoma, celiac disease, Whipple's disease, inflammatory bowel disease, radiation enteritis, graft-versus-host disease, pancreatic disease, and tropical sprue (Carmel, 2000). Bacterial overgrowth can occur in jejunal diverticulosis, enteroanastomosis, strictures, fistulas, and operative procedures and result in Cbl malabsorption. The high acidity associated with the Zollinger–Ellison syndrome causes inactivation of pancreatic trypsin and prevents Cbl release from HC. *H. pylori* infection of the stomach may be associated with mucosal atrophy, hypochlorhydria, and impaired splitting of bound Cbl from food proteins. Competition for Cbl secondary to parasitic infestation by the fish tapeworm *Diphyllobothrium latum* may cause Cbl deficiency. This is not uncommon in the Baltic states, Finland, and Russia.

Certain hereditary enzymatic defects and mutations in genes encoding endocytic receptors involved in ileal absorption and cellular Cbl uptake can also manifest as disorders of Cbl metabolism (Alpers, 2005; Dali-Youcef and Andrès, 2009). Mutations in the gene encoding for the gastric IF (*GIF*) can cause hereditary Cbl deficiency (Tanner et al., 2005). Inborn errors of intrinsic factor are rare and range from a total lack of intrinsic factor to a nonfunctional protein. Mutations in *CUBN* and *AMN* genes have been associated with selective Cbl malabsorption and proteinuria (Imerslund–Gräsbeck syndrome) (Aminoff et al., 1999; Tanner et al., 2003; Fyfe et al., 2004). Low serum Cbl levels can be seen with HC deficiency but this is not clinically significant (Carmel, 2003). Mutation in *TCN2* leading to TC deficiency is clinically significant (Qian et al., 2002; Namour et al., 2003). Congenital abnormalities of TC include complete absence of TC, immunoreactive TC that does not bind to Cbl or does not bind to the receptor. Additional genetic defects of Cbl metabolism involve intracellular processing and utilization of Cbl and include lysosomal release of free Cbl and enzymes involved in synthesis and utilization of Cbl cofactors. Disorders involving the synthesis of Cbl cofactors are identified as cblA to cblG based on the order in which they were discovered (Coelho et al., 2008; Quadros, 2009). These disorders are rare and generally present in childhood with multisystem clinical abnormalities, including developmental, hematologic, and neurologic findings with methylmalonic aciduria or homocystinuria.

Increased prevalence of B_{12} deficiency has been recognized in HIV-infected patients with neurologic symptoms but the precise clinical significance of this is unclear (Kieburtz et al., 1991; Robertson et al., 1993). In AIDS-associated myelopathy the Cbl and folate-dependent transmethylation pathway is depressed and cerebrospinal fluid and serum levels of SAM are reduced (Di Rocco et al., 2002). Despite low B_{12} levels in many AIDS patients, Hcy and MMA levels are normal and Cbl supplementation fails to improve clinical manifestations.

Nitrous oxide (N_2O, "laughing gas") is a commonly used inhalational anesthetic that has been abused because of its euphoriant properties. N_2O irreversibly oxidizes the cobalt core of Cbl and renders methylCbl inactive. Clinical manifestations of Cbl deficiency appear relatively rapidly with N_2O toxicity because the metabolism is blocked at the cellular level. They may, however, be delayed up to 8 weeks (Marie et al., 2000). Postoperative neurologic dysfunction can be seen with N_2O exposure during routine anesthesia if subclinical Cbl deficiency is present (Kinsella and Green, 1995). N_2O toxicity due to inhalant abuse has been reported among dentists, other medical personnel, and university students (Ng and Frith, 2002).

Vitamin B_{12} deficiency is only rarely the consequence of diminished dietary intake. Strict vegetarians may rarely develop mild Cbl deficiency after years. The low vitamin B_{12} level noted in vegetarians is often without clinical consequences. Clinical manifestations are more likely when poor intake begins in childhood wherein limited stores and growth requirements act as additional confounders.

Not infrequently the cause of Cbl deficiency is unknown (Carmel, 2000; Andrès et al., 2005).

CLINICAL MANIFESTATIONS OF COBALAMIN DEFICIENCY

Neurologic manifestations may be the earliest and often the only manifestation of Cbl deficiency (Lindenbaum et al., 1988; Healton et al., 1991; Carmel et al., 2003a). The severity of the hematologic and neurologic manifestations may be inversely related in a particular patient. Relapses are generally associated with the same neurologic phenotype. The commonly recognized neurologic manifestations include a myelopathy with or without an associated neuropathy, optic neuropathy (impaired vision, optic atrophy, centrocecal scotomas), and paresthesias without abnormal signs.

The best characterized neurologic manifestation of Cbl deficiency is a myelopathy that has commonly been referred to as "subacute combined degeneration." The neurologic features typically include a spastic paraparesis, extensor plantar response, and impaired perception of position and vibration. Accompanying peripheral nerve or rarely optic nerve involvement may be present. Asymmetry should prompt search for other causes. Copper deficiency can cause a myeloneuropathy identical to the subacute combined degeneration seen with Cbl deficiency (Kumar et al., 2004).

Neuropsychiatric manifestations of Cbl deficiency include decreased memory, personality change, psychosis, emotional lability, and rarely delirium or coma (Kosik et al., 1980; Lindenbaum et al., 1988; Healton et al., 1991). A concomitant encephalopathy may obscure a coexisting myelopathy (Kosik et al., 1980). Cbl-responsive neuropsychiatric manifestations may be seen in patients without hematologic manifestations and in some patients with a low-normal Cbl level (Lindenbaum et al., 1988). In an individual patient with dementia and Cbl deficiency, the response of the cognitive complaints to Cbl administration is variable and may relate to duration of deficiency (Andrès and Kaltenbach, 2003; Andrès et al., 2005).

Epidemiologic data on Cbl deficiency and cognitive impairment is complex and often contradictory (Clarke, 2008; Vogel et al., 2009). The studies done (cross-sectional surveys, longitudinal studies, intervention studies) are heterogeneous in terms of design and populations studied. Variables include the basis on which Cbl deficiency and cognitive impairment are defined. Additional variables in intervention studies, mostly uncontrolled, include the dose, duration, and route of Cbl supplementation. Many, but not all, studies have shown a relationship between cognitive decline or cognitive deficits and Cbl deficiency. This relationship has been studied not only with vitamin B_{12} levels but also with Hcy or MMA levels, holoTC levels, and vitamin B_{12} intake. Some studies have also looked into rate of brain volume loss and white matter hyperintensities (Vogiatzoglou et al., 2008; de Lau et al., 2009). Despite these observations, the bulk of evidence suggests that vitamin B_{12} supplementation does not result in improved cognition or slowed cognitive decline despite normalization of Hcy or B_{12} levels (Vogel et al., 2009; Ford et al., 2010).

Unusual, and therefore poorly characterized, reported neurologic manifestations possibly related to Cbl deficiency include cerebellar ataxia, leukoencephalopathy, orthostatic tremors, myoclonus, ophthalmoplegia, catatonia, vocal cord paralysis, a syringomyelia-like distribution of motor and sensory deficits, and autonomic dysfunction (Eisenhofer et al., 1982; Kandler and Davies-Jones, 1988; Benito-Leon and Porta-Etessam, 2000; Berry et al., 2003; Celik et al., 2003; Morita et al., 2003; Ahn et al., 2004; Puri et al., 2004; Puri et al., 2006; Akdal et al., 2007). The pediatric literature makes note of involuntary movements and severe neurologic findings including hypotonia and developmental regression with delayed myelination and cerebral atrophy (Avci et al., 2003). Symptoms like fatigue, irritability, and lethargy are nonspecific but not uncommonly reported in the older literature.

Clinical, electrophysiologic, and pathologic involvement of the peripheral nervous system has been described with Cbl deficiency (McCombe and McLeod, 1984; Saperstein et al., 2003). In most cases the clinical features of a Cbl deficiency polyneuropathy are similar to those of a cryptogenic sensorimotor polyneuropathy. Clues to possible B_{12} deficiency in a patient with polyneuropathy included a relatively sudden onset of symptoms, findings suggestive of an associated myelopathy, onset of symptoms in the hands, concomitant involvement of upper and lower limbs, macrocytic red blood cells, and the presence of a risk factor for Cbl deficiency.

Serum Cbl can be normal in some patients with Cbl deficiency and serum MMA and total Hcy levels are useful in diagnosing patients with Cbl deficiency (Allen et al., 1990; Lindenbaum et al., 1990; Stabler et al., 1990; Savage et al.,1994; Green and Kinsella, 1995; Stabler, 1995). The sensitivity of the available metabolic tests for Cbl deficiency has facilitated the development of the concept of subclinical Cbl deficiency (Carmel, 2000; Carmel et al., 2003b). This refers to biochemical evidence of Cbl deficiency in the absence of hematologic or neurologic manifestations. These biochemical findings should respond to Cbl therapy if Cbl deficiency is their true cause (Stabler et al., 1990). If it is unclear whether an elevated MMA or Hcy is due to Cbl deficiency, the response to empirical parenteral B_{12} replacement can be assessed. The frequency of subclinical Cbl deficiency is estimated to be at least 10 times that of clinical Cbl deficiency and its incidence increases with age (Lindenbaum et al., 1994; Metz et al., 1996; Carmel et al., 2003b). The cause of subclinical Cbl deficiency includes food-bound Cbl malabsorption but is frequently unknown; the course is often stationary (Elwood et al., 1971; Waters et al., 1971). Some of these individuals may have subtle neurologic and neurophysiologic abnormalities of uncertain significance that may respond to Cbl therapy (Karnaze and Carmel, 1990; Carmel et al., 1995). The presence of a low Cbl in the association with neurologic manifestations does not imply cause and effect or indicate the presence of metabolic Cbl deficiency. The incidence of cryptogenic polyneuropathy, cognitive impairment, and Cbl deficiency increases with age and the latter may be a chance occurrence rather than causative (Lindenbaum et al., 1994). Further, though Cbl levels are frequently low in the elderly, up to a third are

falsely low by clinical and metabolic criteria, and many of the remainder are clinically innocent (Lindenbaum et al., 1994; Carmel, 1997, 2008; Carmel et al., 1999). The clinical impact of subclinical Cbl deficiency and its appropriate management are uncertain.

INVESTIGATIONS

Serum Cbl determination has been the mainstay for evaluating Cbl status (Green and Kinsella, 1995; Snow, 1999; Carmel, 2008). The older microbiological and radioisotopic assays have been replaced by immunologically based chemiluminescence assays. Though a widely used screening test, serum Cbl measurement has technical and interpretive problems and lacks sensitivity and specificity for the diagnosis of Cbl deficiency (Lindenbaum et al., 1990; Moelby et al., 1990; Stabler et al., 1990; Lindenbaum et al., 1994; Matchar et al., 1994; Savage et al., 1994; Green and Kinsella, 1995; Stabler, 1995; Snow, 1999; Carmel, 2000; Carmel et al., 2003a; Solomon, 2005). A proportion of Cbl deficient patients may have Cbl levels that are on the lower side of the normal range (Lindenbaum et al., 1990). A proportion of patients with low Cbl levels are not Cbl deficient (Stabler et al., 1990; Matchar et al., 1994). Levels of serum MMA and plasma total Hcy are useful as ancillary diagnostic tests (Allen et al., 1990; Lindenbaum et al., 1990; Moelby et al., 1990; Stabler et al., 1990; Savage et al., 1994; Green and Kinsella, 1995; Stabler, 1995). They too have significant limitations (Chanarin and Metz, 1997). The specificity of MMA is superior to that of Hcy. Though Hcy is a very sensitive indicator of Cbl deficiency, its major limitation is its poor specificity. Table 60.1 indicates causes other than Cbl deficiency that can result in abnormal levels of Cbl, MMA, and Hcy (Snow, 1999; Carmel, 2000; Ward, 2002; Carmel, 2003; Carmel et al., 2003a). Low serum Cbl levels can be seen with HC deficiency but this is not clinically significant (Carmel, 2003). The highest levels of serum B_{12} reflect concomitant systemic disease in some individuals. Some authors suggest that low Cbl and increased MMA or Hcy levels may not be sensitive markers of Cbl-responsive

Table 60.1

Common causes, other than Cbl deficiency, for abnormal Cbl, MMA, and Hcy levels

Cbl	MMA	Hcy
Decrease (falsely low)	*Increase*	*Increase*
Pregnancy (third trimester)	Renal insufficiency	Renal insufficiency
Haptocorrin deficiency (also seen in sickle cell disease)	Volume contraction (possible)	Volume contraction
Folate deficiency	Bacterial contamination of gut (possible)	Folate deficiency
Other diseases: HIV infection and myeloma (abnormalities in Cbl binding proteins)	MMCoA mutase deficiency	Vitamin B_6 deficiency
Drugs: anticonvulsants, oral contraceptives, radionuclide isotope studies	Other MMA-related enzyme defects	Other diseases: hypothyroidism, renal transplant, leukemia, psoriasis, alcohol abuse
Idiopathic	Infancy, pregnancy	Inappropriate sample collection and processing
Increase (falsely normal)	*Decrease*	Drugs: isoniazid, colestipol, niacin, L-dopa, diuretics
Renal failure	Antibiotic-related reductions in bowel flora	Enzyme defects: cystathionine β-synthase deficiency, MTHFR deficiency
Intestinal bacterial overgrowth (measurement of biochemically inert B_{12} analogs)		Increased age, males, caffeine consumption, increased muscle mass
Increase haptocorrin concentration (seen in liver disease and myeloproliferative disorders such as polycythemia vera, chronic myelogenous leukemia, chronic myelofibrosis)		*Decrease*
		Drugs: estrogens, tamoxifen, statins

Cbl, cobalamin; MMA, methylmalonic acid; Hcy, homocysteine; HIV, human immunodeficiency virus; MTHFR, methylene tetrahydrofolate reductase.

disorders and MMA and Hcy may be normal in some patients with neurologic or hematologic abnormalities responsive to Cbl (Solomon, 2005). Further, short-term fluctuations of Cbl, MMA, and Hcy may obscure Cbl deficiency and lead to erroneous conclusions regarding response to therapy (Solomon, 2005). Measuring MMA and Hcy is also useful in patients with N_2O toxicity and some inherited disorders of Cbl metabolism. In these conditions vitamin B_{12}-dependent pathways are impaired despite normal vitamin B_{12} levels.

Vitamin B_{12} bound to TC (the Cbl-TC complex, also called holoTC) is the fraction of total vitamin B_{12} available for tissue uptake. HoloTC concentration or TC saturation (holoTC:total TC) have been proposed by some as potentially useful alternative indicators of vitamin B_{12} status (Pennypacker et al., 1992; Herbert, 1994; Hvas and Nexo, 2005; Morkbak et al., 2005; Miller et al., 2006; Refsum et al., 2006; Clarke et al., 2007; Brady et al., 2008). Its levels appear to fall before those of B_{12} as measured by standard methods. A major limitation had been availability of sensitive and reproducible methods of detecting holoTC levels. Some recently published determination methods hold promise (Morkbak et al., 2005; Brady et al., 2008). The test is not available for clinical use and the clinical utility of the measurement awaits further studies (Carmel, 2002). Increase in urinary MMA after an oral dose of one of its precursors, usually valine, can indicate Cbl deficiency but this test is cumbersome and has limited sensitivity (Chanarin et al., 1973).

A rise in the mean corpuscular volume may precede development of anemia. The presence of neutrophil hypersegmentation may be a sensitive marker for Cbl deficiency and may be seen in the absence of anemia or macrocytosis. Megaloblastic bone marrow changes may be seen. The deoxyuridine suppression test measures the synthesis of thymidine and its incorporation into DNA by bone marrow cells. The incubation of nucleated hematopoietic cells with excessive deoxyuridine reduces the uptake of subsequently added titrated thymidine into DNA. This suppression is subnormal in patients with B_{12} or folate deficiency. It is not available for clinical use.

In order to determine the cause of Cbl deficiency tests directed at determining the cause of malabsorption are undertaken. Concerns regarding cost, accuracy, and radiation exposure have led to a significant decrease in the availability of the Schilling test (Carmel, 2007). Further, the Schilling test is based on absorption of crystalline Cbl (with and without intrinsic factor) and does not detect food-Cbl malabsorption. Tests of food-cobalamin absorption using cobalamin bound to animal protein (eggs, salmon, trout, chicken serum) have been devised (Carmel, 2000; Andrès et al., 2003, 2005). The disparity between the abnormal results of these tests and the normal results with the Schilling test defines the disorder of food-cobalamin malabsorption. An elevated serum gastrin and decreased pepsinogen I is seen in 80–90% of patients with pernicious anemia but the specificity of these tests is limited (Carmel, 1988). Elevated gastrin levels are a marker for hypochlorhydria or achlorhydria which are invariably seen with pernicious anemia. Elevated serum gastrin levels may be seen in up to 30% of the elderly (Hurwitz et al., 1997). Elevated serum gastrin levels are approximately 70% specific and sensitive for PA (Miller et al., 1989). Anti-intrinsic factor antibodies are specific (over 95%) but lack sensitivity and are found in approximately 50–70% of patients with pernicious anemia (Carmel, 1992). Studies suggest that antiparietal cell antibodies may not be seen as commonly as was earlier believed and therefore have limited utility (Carmel, 1992). Further, false-positive results for the gastric parietal cell antibody are common. They may be seen in 10% of people over age 70 and are also present in other autoimmune endocrinopathies. A common approach is to combine the specific but insensitive intrinsic factor antibody test with the sensitive but nonspecific serum gastrin or pepsinogen level in patients with Cbl deficiency (Carmel, 2008).

Electrophysiologic abnormalities include nerve conduction studies suggestive of a sensorimotor axonopathy, and abnormalities on somatosensory evoked potentials, visual evoked potentials, and motor evoked potentials (McCombe and McLeod, 1984; Hemmer et al., 1998; Saperstein et al., 2003). Somatosensory evoked potential abnormalities may commonly be seen in patients with a Cbl-deficiency neuropathy and indicate a subclinical myelopathy. Quantitative sensory testing abnormalities are commonly seen but are not specific.

Magnetic resonance imaging (MRI) abnormalities in Cbl deficiency include a signal change in the posterior and lateral columns and less commonly subcortical white matter (Murata et al., 1994; Hemmer et al., 1998; Vry et al., 2005) (Fig. 60.2). Similar spinal cord MRI findings are seen with nitrous oxide toxicity (Ng and Frith, 2002). Contrast enhancement involving the dorsal or lateral columns may be present (Locatelli et al., 1999). The dorsal column may show a decreased signal on T1-weighted images (Locatelli et al., 1999). Other reported findings include cord atrophy and anterior column involvement (Bassi et al., 1999; Karantanas et al., 2000). Treatment may be accompanied by reversal of cord swelling, contrast enhancement, and signal change (Hemmer et al., 1998; Locatelli et al., 1999; Karantanas et al., 2000). Also reported are increased T2 signal involving the cerebellum (Katsaros et al., 1998; Morita et al., 2003). Rarely striking diffuse

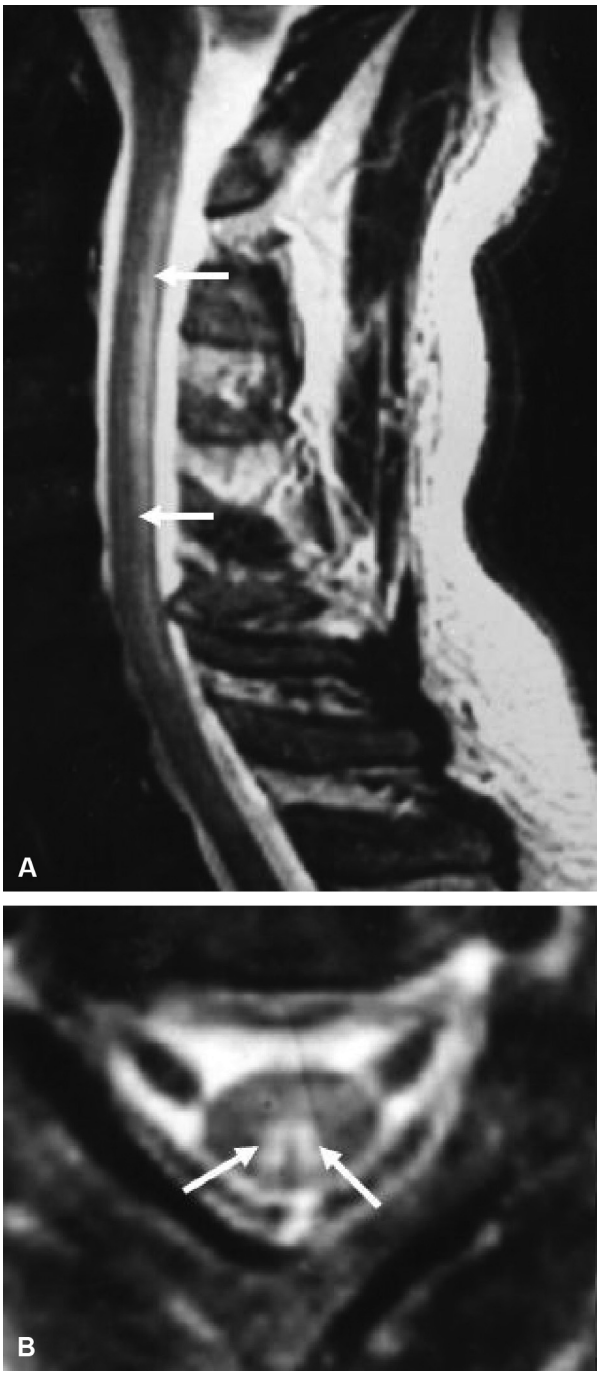

Fig. 60.2. Magnetic resonance imaging (MRI) in cobalamin deficiency. T2-weighted sagittal (**A**) and axial (**B**) MRI of the cervical spinal cord from a patient with myelopathy due to cobalamin deficiency showing increased signal involving the dorsal column. (Adapted from Hemmer et al., 1998, with permission.)

white matter abnormalities (supratentorial and very rarely infratentorial) suggestive of a leukoencephalopathy may be seen (Stojsavljevic et al., 1997; Su et al., 2000; Morita et al., 2003). Brain T2 hyperintensities seen in Cbl deficiency may show significant improvement with vitamin B_{12} replacement (Stojsavljevic et al., 1997; Su et al., 2000; Morita et al., 2003).

PATHOLOGY

The most severely involved regions in Cbl deficiency-related myelopathy are the cervical and upper thoracic posterior columns. Changes are also seen in the lateral columns. Involvement of the anterior columns is rare. The earliest change is in the dorsal columns and involves splitting and swelling of the myelin sheath which histologically manifests as vacuolization. There is myelin loss followed by axonal degeneration and gliosis. Nerve biopsies show evidence of axonal degeneration (Kosik et al., 1980; McCombe and McLeod, 1984).

MANAGEMENT

The goals of treatment are to reverse the signs and symptoms of deficiency, replete body stores, ascertain the cause of deficiency, and monitor response to therapy. With normal Cbl absorption, oral administration of 3–5 μg of vitamin B_{12} may suffice. In patients with food-bound Cbl malabsorption due to achlorhydria 50–100 μg vitamin B_{12} given orally is often adequate (Verhaeverbeke et al., 1997). More recent studies have shown blunted metabolic responses in elderly persons with subclinical deficiency until oral doses reached 500 μg or more (Eussen et al., 2005). Patients with Cbl deficiency due to achlorhydria-induced food-bound Cbl malabsorption show normal absorption of crystalline B_{12} but are unable to digest and absorb Cbl in food due to achlorhydria.

The more common situation is one of impaired absorption where parenteral therapy is required. A short course of daily or weekly therapy is often followed by monthly maintenance therapy. A common regimen is 1000 μg intramuscular injections for 5–7 days followed by monthly 500–1000 μg intramuscular injections (Green and Kinsella, 1995).

If the oral dose is large enough, even patients with an absorption defect, including pernicious anemia, may respond to oral vitamin B_{12} (Kuzminski et al., 1998; Bolaman et al., 2003; Butler et al., 2006; Andrès et al., 2010). The daily requirement for vitamin B_{12} is 1–2 μg, and approximately 1% of orally administered vitamin B_{12} can be absorbed by patients with pernicious anemia. Consequently an oral vitamin B_{12} dose of 1000–2000 μg/day could suffice. This has been confirmed in clinical trials (Kuzminski et al., 1998; Bolaman et al., 2003; Butler et al., 2006). The role of oral therapy in patients with severe neurologic disease has not been well studied (Andrès et al., 2010).

Patients with pernicious anemia have a higher risk of gastric cancer and carcinoids and therefore should get an endoscopy (Kokkola et al., 1998). Patients with pernicious anemia also have a higher frequency of thyroid disease, diabetes, and iron deficiency and should be screened for these conditions (Carmel and Spencer, 1982; Carmel et al., 1987). A clinical relapse in pernicious anemia after interrupting vitamin B_{12} therapy takes approximately 5 years before it is recognized.

Patients with Cbl deficiency are prone to develop neurologic deterioration following N_2O anesthesia. It is preventable by prophylactic vitamin B_{12} given weeks before surgery in individuals with a borderline B_{12} level who are expected to receive N_2O anesthesia. Intramuscular B_{12} should be given to patients with acute N_2O poisoning. Methionine supplementation has also been proposed as a first-line therapy (Stacy et al., 1992). With chronic exposure, immediate cessation of exposure should be ensured.

In AIDS-associated myelopathy possible benefit of administration of the *S*-adenosyl methionine precursor, L-methionine, was suggested by a pilot study but not confirmed in a subsequent double-blind study (Di Rocco et al., 2004).

Response to treatment may relate to extent of involvement and delay in starting treatment (Healton et al., 1991). Remission correlates inversely with the time lapsed between symptom onset and therapy initiation. Response of the neurologic manifestations is variable, may be incomplete, often starts in the first week, and is complete in 6 months (Healton et al., 1991; Carmel, 2008). Approximately 2% of patients show a "coasting" phenomenon wherein sensory symptoms show an initial worsening (Healton et al., 1991). The neuropathy may be slow to respond or may not respond at all (McCombe and McLeod, 1984; Saperstein et al., 2003). This is not unexpected given the underlying axonal degeneration. Response of the hematologic derangements is prompt and complete. Reticulocyte count begins to rise within 3 days and peaks around 7 days. Red blood cell count begins to rise by 7 days and is followed by a decline in mean corpuscular volume, with normalization by 8 weeks. MMA and Hcy levels normalize by 10–14 days. If it is unclear whether an elevated MMA or Hcy indicated Cbl deficiency, empirical vitamin B_{12} replacement therapy can be given and metabolite levels repeated after a few weeks. If an elevated MMA or Hcy is due to Cbl deficiency, these values will normalize after 1–2 weeks of replacement therapy (Stabler, 1995). Cbl and holoTC levels rise after injection regardless of the benefit. Hence MMA and Hcy are more reliable ways for monitoring response to therapy. In patients with severe Cbl deficiency, replacement therapy may be accompanied by hypokalemia due to proliferation of bone marrow cells that utilize potassium. The clinical significance of this hypokalemia is unproven (Carmel, 2008).

HydroxoCbl is commonly used in parts of Europe. It is more allergenic but has superior retention and may permit injections every 2–3 months (Skouby, 1987). Compared with hydroxoCbl, cyanoCbl binds to serum proteins less well and is excreted more rapidly. Intranasal administration of hydroxoCbl has been associated with fast absorption and normalization of Cbl levels (Slot et al., 1997). Advantages of delivering Cbl by the nasal or sublingual route are unproven. Oral preparations of intrinsic factor are available but not reliable. Antibodies to intrinsic factor may nullify its effectiveness in the intestinal lumen.

For unclear reasons, neurologically affected patients with Cbl deficiency may have high folate levels (Carmel et al., 2003b; Quinlivan, 2008). Further, serum B_{12} levels may be lowered in patients with established folate deficiency. Anemia due to Cbl deficiency often responds to folate therapy, but the response is incomplete and transient. Anecdotal evidence suggests that inappropriate folate therapy in patients with Cbl deficiency-related anemia may delay recognition of the Cbl deficiency and cause neurologic deterioration (Kosik et al., 1980). This is controversial, and it is unclear if routine folate supplementation may compromise the early diagnosis of the hematologic manifestations or worsen the neurologic consequences (Dickinson, 1995). Folate exposure has increased after food fortification but studies suggest that this has not resulted in masking of Cbl deficiency (Mills et al., 2003).

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