

REVIEW ARTICLE

The Neurology of Cobalamin

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ABSTRACT: The following review indicates that the impact of cobalamin on neurologic disease extends far beyond the traditional myelopathy of classical pernicious anemia. The delineation of a broad spectrum of inherited disorders of cobalamin processing has served to illustrate and precisely define each step in the normal absorption, transport and intracellular metabolism of this essential vitamin. Recent clinical work has extended the boundaries of acquired cobalamin deficiency to encompass a variety of neuropsychiatric disturbances without identifiable concomitant hematologic derangements and emphasized the utility and sensitivity of new laboratory tests. These findings will demand increased vigilance from clinicians so that atypical and subtle cobalamin deficiency states will be readily diagnosed. The wide range of neurologic dysfunction observed in both inherited and acquired disorders of cobalamin metabolism challenges basic scientists to delineate cobalamin's presumed important role in the normal development and homeostasis of the nervous system.

RÉSUMÉ: La neurologie de la cobalamine. Cette revue fait ressortir clairement que l'impact de la cobalamine sur la maladie neurologique s'étend bien au-delà de la myélopathie traditionnelle de l'anémie pernicieuse. La définition d'un large spectre de maladies héréditaires du métabolisme de la cobalamine a servi à illustrer et à définir précisément chaque étape de l'absorption normale, du transport et du métabolisme intracellulaire de cette vitamine essentielle. Des travaux cliniques récents ont repoussé les frontières de la déficience en cobalamine pour inclure diverses maladies neuropsychiatriques sans anomalie hématologique concomitante identifiable et ont souligné l'utilité et la sensibilité des nouvelles épreuves de laboratoire. Les cliniciens devront exercer une vigilance accrue afin de diagnostiquer promptement les déficits atypiques et subtils en cobalamine. La grande variété de dysfonctions neurologiques observées dans les anomalies congénitales ou acquises du métabolisme de la cobalamine présente un défi pour les chercheurs qui devront définir le rôle présumé important de la cobalamine dans le développement normal et dans l'homéostasie du système nerveux.

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The history of a role for cobalamin (vitamin B₁₂) in disorders of the nervous system begins with the original observation of a progressive myelopathy occurring in association with pernicious anemia made by Lichtheim¹ in 1887. Russell, Batten and Collier² coined the term "subacute combined degeneration" to describe the observed pathology and Minot and Murphy³ developed the first successful treatment of this devastating illness. The "anti-pernicious anemia" factor of liver (cobalamin) was first isolated in 1948⁴ and its three dimensional structure deciphered by X-ray crystallographic techniques by Hodgkin and co-workers.⁵

The traditional neurological view of cobalamin has focused on the various manifestations of pernicious anemia as exhaustively detailed in the monograph of Pant, Asbury and Richardson.⁶ However over the past twenty years attention has focused on an increasing number of inherited disorders of cobalamin transport and intracellular metabolism.⁷ Though rare, these

disorders have served to greatly further our understanding of normal cobalamin absorption, transport and metabolism. In addition, recent articles have highlighted the atypical subtle cobalamin deficiency states and their broad range of associated neurological and psychiatric disorders.⁸

COBALAMIN CHEMISTRY

Cobalamin is a complex corrinoid compound (Figure 1) that consists of a central cobalt atom, of variable oxidation-reduction state, surrounded by a planar corrin ring composed of four pyrroline rings with a nucleotide, phosphoribo-5,6-dimethylbenzimidazole, attached to both one of the pyrroline rings and the central cobalt atom.⁵

A variety of different ligands may be attached to the central cobalt atom including; -CN, -OH, -SH, -SO₃, glutathione, -CH₃ and 5'-deoxyadenosyl. Only methylcobalamin and adeno-

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ABSORPTION

sylcobalamin are known to act as specific intracellular cofactors in mammalian systems.⁹ The oxidation-reduction state of the cobalt atom is a critical determinant of functional status. The cobalt atom is trivalent [Cob(III)alamin] in hydroxycobalamin and must be reduced by specific intracellular reductases to first a divalent [Cob(II)alamin] then a monovalent [Cob(I)alamin] state prior to the addition of either a methyl or 5' adenosyl group to yield methylcobalamin or adenosylcobalamin respectively.⁹

Cobalamin is a water soluble vitamin synthesized by bacteria and fungi that is widely distributed in animal tissues.¹⁰ Dietary cobalamin is derived exclusively from animal sources.¹¹ The average North American diet contains approximately 30 µg/day of cobalamin¹¹ with the World Health Organization presently recommending a minimum daily dietary intake of 1 µg for adults, 1.4 µg for pregnant women and 0.1 µg for infants.¹²

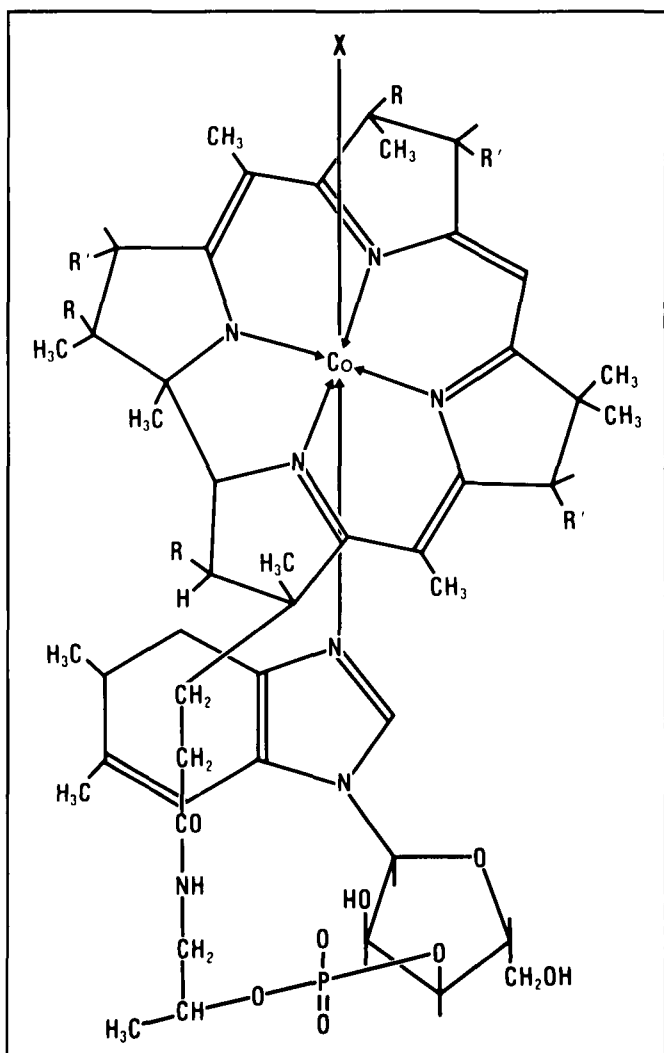


Figure 1 — The structure of cobalamin. $R = \text{CH}_2\text{CONH}_2$; $R' = \text{CH}_2\text{CH}_2\text{CONH}_2$; $X = \text{CH}_3$, 5'-Deoxyadenosyl, OH , CN (Reprinted with permission from Rosenblatt DS, Cooper BA. *Inherited disorders of vitamin B₁₂ metabolism. Blood Reviews* 1987; 1: 177-182).

The absorption of dietary cobalamin (Cbl) is outlined in Figure 2 and depends on the combined interaction of gastric, pancreatic and ileal components.¹³ Within the acidic environment of the stomach cobalamin is first released from dietary protein. The free cobalamin released initially binds to a group of glycoproteins known as "R" binders (Transcobalamin I, Transcobalamin III, cobalophyllin, hepatocorrin) that are of salivary and possibly gastric origin and have a high affinity for cobalamin. With passage into the upper portion of the duodenum, pancreatic trypsin digests the "R" binders and the free cobalamin then binds to another glycoprotein known as Intrinsic Factor (IF). Intrinsic factor is of gastric origin and is secreted by the parietal cells. Specific ileal receptors, located on the brush border of enterocytes are the site of uptake of the IF-Cbl complex via a calcium dependent, probably endocytic, transport mechanism.¹⁴ Within the enterocyte, cobalamin is released from intrinsic factor and then binds to Transcobalamin II.¹⁵ All newly absorbed cobalamin will be found bound to Transcobalamin II (TC-II) which is free of carbohydrate¹⁶ and has been shown to be required for the intestinal uptake of cobalamin.¹⁷ TC-II is the serum transport protein for newly absorbed cobalamin and the source of all physiologically active cobalamin.¹⁸

TC-II represents only a portion of total plasma cobalamin binding activity. Approximately 75% of total endogenous cobalamin is found bound to plasma "R" binders such as Transcobalamin I and Transcobalamin III.¹⁹ Biologically inactive cobalamin analogues are also found bound to plasma "R" binders²⁰ and it is postulated that plasma "R" binders' physiological role is to function as a transport system for the removal of potentially toxic cobalamin analogues.²¹

CELLULAR UPTAKE & INTRACELLULAR METABOLISM

The circulating TC II-Cbl complex is recognized by specific high affinity cell surface receptors that are widely distributed.²² TC II enhances and facilitates the uptake of cobalamin at the level of the cell, however some tissues demonstrate TC II independent uptake of free unbound cobalamin if the extracellular concentration of the unbound cobalamin is particularly high.²³ The TC II-Cbl complex is initially internalized²⁴ and then undergoes lysosomal processing (Figure 3) which leads to degradation of TC II and the release into the cytosol of free cobalamin.⁷

Cobalamin is released from the lysosome presumably in a trivalent [Cob(III)alamin] state. A poorly characterized reductase then reduces the central cobalt atom to a divalent state [Cob(II)alamin].²⁵ The divalent cobalamin may then remain in the cytosol and proceed to methylcobalamin synthesis or enter the mitochondria and proceed to adenosylcobalamin synthesis.⁷

It is thought that divalent cobalamin may bind to cytosolic methionine synthase and is then acted upon by a specific reducing system that yields monovalent cobalamin [Cob(I)alamin].²⁶ The methylation of methionine synthase bound monovalent cobalamin to yield methylcobalamin, occurs with S-adenosylmethionine providing the initial methyl group.²⁷ Transfer of the methyl group of methylcobalamin to homocysteine leads to the formation of methionine. 5-Methyltetrahydrofolate then provides the subsequent methyl groups for the methylation of methionine synthase-bound monovalent cobalamin.²⁷ Apparently, the monovalent cobalamin undergoes an eventual spontaneous oxidation to

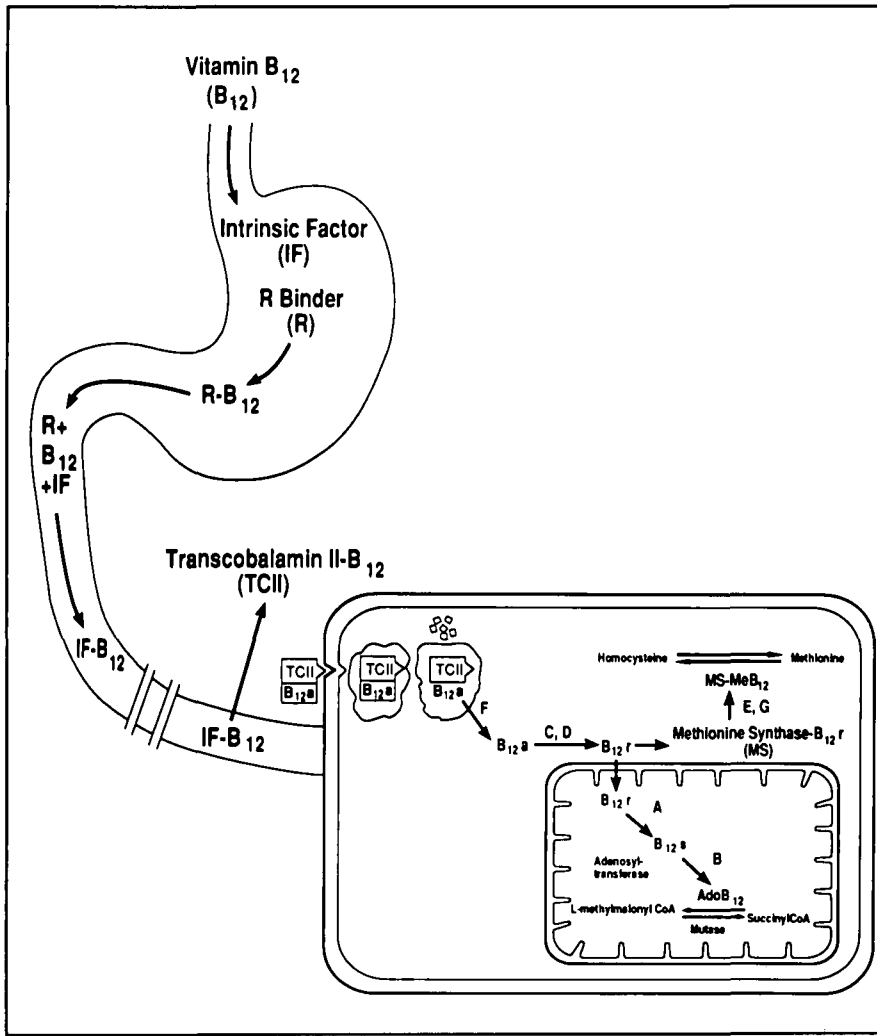


Figure 2 — Cobalamin absorption, transport and intracellular metabolism (Reprinted with permission from Rosenblatt DS, Cooper BA. Inherited disorders of vitamin B₁₂ metabolism. Blood Reviews 1987; 1: 177-182). [B_{12a} = Cbl³⁺, B_{12r} = Cbl²⁺, B_{12s} = Cbl¹⁺]

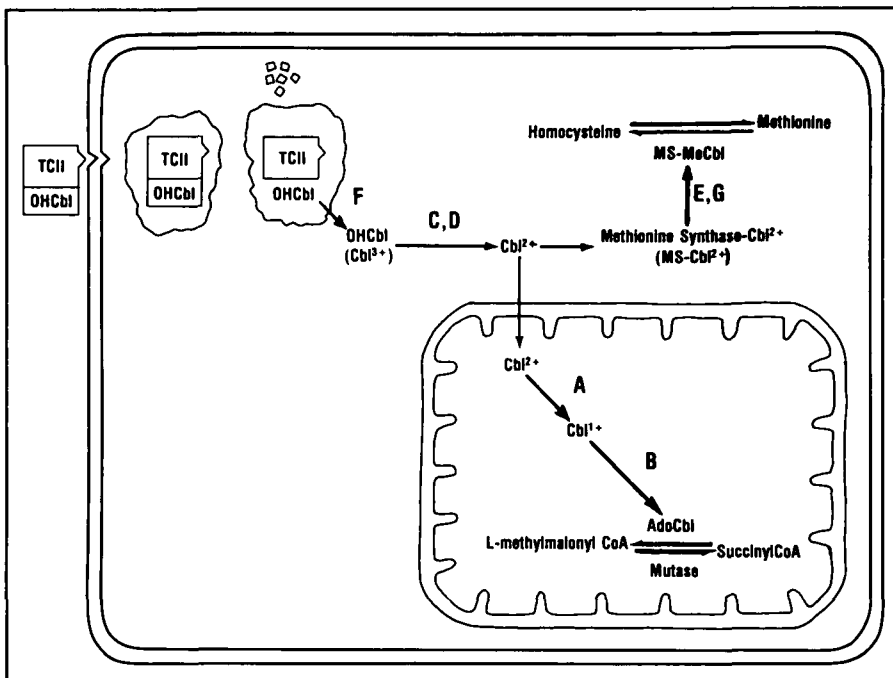


Figure 3 — Inborn errors of intracellular cobalamin metabolism. The letters A-G refer to the presumed sites of the blocks that have been identified by complementation analysis. Lysosomal, cytoplasmic and mitochondrial compartments are indicated. MeCbl = Methylcobalamin, AdoCbl = Adenosylcobalamin, MS = Methionine synthase, Mutase = Methyl-malonylCoA mutase, TCII = Transcobalamin II, OHCbl = Hydroxycobalamin (Reprinted with permission from Rosenblatt DS, Cooper BA. Inherited disorders of vitamin B₁₂ utilization. Bioessays 1990; 12: 331-334).

divalent cobalamin and must then require both the reducing system and S-adenosylmethionine for the re-formation of methylcobalamin.²⁶

Alternatively cobalamin may enter the mitochondria and be acted upon by one or two reductases to yield monovalent cobalamin [Cob(I)alamin].²⁸ Together with ATP, mitochondrial monovalent cobalamin acts as a co-substrate for adenosyltransferase to yield 5' adenosylcobalamin.²⁸

More than 95% of intracellular cobalamin is bound to either methionine synthase or methylmalonyl-CoA mutase, enzymes which catalyze the two reactions for which cobalamin is an essential cofactor.¹⁸ Only reduced cobalamin binds to these two enzymes thus necessitating the sequential reduction of trivalent cobalamin to retain intracellular cobalamin and to permit enzyme action.⁷ Methionine synthase is a cytosolic enzyme that catalyses the methylation of homocysteine to methionine and methylcobalamin is the cofactor for this reaction.²⁹ Methylmalonyl-CoA mutase is a mitochondrial homodimer that permits the isomerization of methylmalonyl-CoA to succinyl-CoA which is a necessary step in the catabolism of propionate. Adenosylcobalamin is the essential cofactor for this reaction.^{30,31}

LABORATORY EVALUATION OF COBALAMIN DISORDERS

Acquired Disorders

The traditional hallmark of acquired cobalamin deficiency has been the finding of megaloblastic anemia.^{32,33} This occurs because the demethylation of methyltetrahydrofolate to tetrahydrofolate is via the methionine synthase reaction which is dependent for its activity on adequate methylcobalamin cofactor.³⁴ Functional deficiency of methionine synthase leads to accumulation of methyltetrahydrofolate and inadequate production of tetrahydrofolate and other reduced folates that are essential for the synthesis of purines and pyrimidines.³⁴

The classical notion held that neurologic dysfunction occurred late in the course of acquired cobalamin deficiency, after hematologic manifestations were well established.³⁵ It is now clear that clinically apparent neurologic abnormalities often occur in the setting of normal hematological parameters.^{36,36A} These have been labeled "subtle" or "atypical" cobalamin deficiency states.^{8, 37}

Serum cobalamin levels can be assayed directly using radioassays employing purified intrinsic factor, a cobalamin specific binding protein that does not bind cobalamin analogues.³⁸ Less commonly a microbiological assay using *Lactobacillus leichmannii* can be employed.³⁹ "Normal" serum cobalamin levels are greater than 150-200 pg/ml or 110-147 pmoles/L in SI units.⁴⁰ The Schilling test, though often used as a diagnostic test for cobalamin deficiency, is more properly considered an evaluation of an individual's ability to absorb orally administered crystalline cobalamin. It delineates the precise mechanism by which a patient has become cobalamin deficient and is diagnostic of pernicious anemia, the most common etiology for acquired cobalamin deficiency.⁴¹ Similarly the egg yolk-cobalamin absorption test is capable of providing the diagnosis of food cobalamin malabsorption, an increasingly recognized cause of acquired cobalamin deficiency.⁴²

Subtle cobalamin deficiency states may occur in the setting of "normal" measured serum cobalamin values in the absence of

any anemia.⁴³ A clinical response to exogenously administered cobalamin or a serum measurement of elevated methylmalonic acid and total homocysteine by gas chromatography/mass spectrometry techniques is necessary to diagnose a cobalamin deficiency in such a situation.⁴³ Reference values for serum methylmalonic acid (73-271 nmol/L) and total homocysteine (5.4-16.2 nmol/L) have been established.⁴⁴ Elevated methylmalonic acid occurs in acquired cobalamin deficiency as a result of the function impairment of methylmalonyl-CoA mutase and the subsequent block in propionate catabolism.⁴⁴ Elevated total homocysteine occurs as a result of the failure to convert homocysteine to methionine, that is, the byproduct of functional impairment of methionine synthase is due to inadequate cobalamin cofactor synthesis.⁴⁴ Recent studies have indicated that serum methylmalonic acid and total homocysteine elevations are reliable, sensitive and early indicators of acquired cobalamin deficiency.^{44,45}

Inherited Disorders

Cell culture techniques are employed to precisely identify, classify and study the various inherited disorders of cobalamin metabolism. Cultured fibroblasts are incubated in a medium containing labelled cyanocobalamin which has been pre-incubated in human serum as a source of TC II.⁷ In this setting, the total cellular uptake of cobalamin can be measured as well as the distribution of free and unbound forms. Polyacrylamide gel electrophoresis distinguishes protein bound and free cellular cobalamin. Cofactor (i.e. methylcobalamin, adenosylcobalamin) distribution determination requires the initial removal of cobalamin from protein and the HPLC measurement of the two cofactors.⁴⁶

Cobalamin-dependent enzyme activity can be directly assayed independently in cell extracts grown in medium with and without added exogenous cobalamin.⁷ Methionine synthase activity can be assayed by measuring the incorporation of labelled methyltetrahydrofolate into acid precipitable material, while methylmalonyl CoA mutase activity can be assayed by measuring the incorporation of labelled propionate.⁷

Cell complementation analysis has been used extensively to define subsets of inherited disorders that are frequently indistinguishable either clinically or biochemically.⁷ Equal numbers of cultured fibroblasts from different patients are co-incubated in the presence or absence of polyethylene glycol (PEG), an agent that induces cell fusion. If cell fusion results in increased uptake of either propionate and/or methyltetrahydrofolate then the cell lines belong to different complementation classes which implies mutations at different and distinct genetic loci.

In the appropriate clinical setting (see below) an inherited disorder of cobalamin metabolism is initially suspected by screening for megaloblastic anemia, homocystinuria or methylmalonic aciduria. Secondary acidosis and ketosis along with the accumulation of various propionate catabolites occur when methylmalonyl CoA mutase activity is compromised.⁴⁷ Serum cobalamin, TC II, cobalamin absorption (Schilling test) and intrinsic factor antibody are all easily assayed.

Measurement of cellular cobalamin uptake, cofactor distribution and uptake of labelled propionate and methyltetrahydrofolate in cultured fibroblasts provide confirmation of a defect in cobalamin metabolism. A definitive diagnosis is then made by complementation studies employing a panel of cells with known disorders. Using these techniques, inherited disorders of

cobalamin processing have been identified at almost every step in the absorption, transport and intracellular metabolism of this essential vitamin.⁷

ANIMAL MODELS

Several animal models, utilizing either dietary manipulation or selective toxins, have been developed to approximate in the laboratory setting, acquired cobalamin deficiency, thus permitting investigators to explore both the sequence and pathogenesis of neurologic dysfunction. Unfortunately, none of these models exactly replicate the clinical and pathological features of human cobalamin deficiency.

Beck and Abeles through dietary restriction induced a severe cobalamin deficiency in piglets. While these animals were unable to ambulate, no hematological changes were seen.⁴⁸ Agamanolis and colleagues with prolonged dietary deprivation (less than 500 pg/day of cobalamin for up to 5 years) of rhesus monkeys noted optic atrophy after 33-45 months in all animals and myelopathy after 37-52 months in 4 of 9 animals.⁴⁹ Detailed light microscopic and ultrastructural studies⁵⁰ revealed CNS lesions indistinguishable from those seen in human sub-acute combined degeneration. However no hematological changes were ever noted. The South African cape fruit bat (*Rousettus aegypticus*), when fed an all fresh fruit diet that is pest free with fresh water supplied, consistently develops a cobalamin deficiency manifested by a myeloneuropathy that resembles sub-acute combined degeneration on autopsy.⁵¹ Again no hematological changes were observed.

Exposure to nitrous oxide (N₂O), an agent which oxidizes monovalent [Cob(I)alamin] to trivalent [Cob(III)alamin] cobalamin and selectively inhibits the cobalamin-dependent methionine synthase reaction⁵²⁻⁵⁴ has been used in a variety of animals to rapidly induce cobalamin deficiency. These include the rat⁵⁵⁻⁵⁷ with or without underlying dietary cobalamin deficiency, the cape fruit bat,⁵⁸⁻⁶⁰ pig⁶¹ and the rhesus monkey.⁶²⁻⁶⁴ No clinical or pathological derangement was noted in the rat following exposure to nitrous oxide, however, in the fruit bat, pig and rhesus monkey a myelopathy without megaloblastic anemia was observed. Cycloleucine, an analogue of methionine that inhibits the cellular transport of methionine, produces neurological changes in mice that are indistinguishable from human subacute combined degeneration.⁶⁵⁻⁶⁶

With the exception of a recently described dog model for defective cobalamin transport by enterocytes (Imerslund-Grasbeck syndrome),⁶⁷ no animal models have yet been delineated for the various inherited disorders of cobalamin transport and intracellular metabolism. The dog (giant schnauzers) model for Imerslund-Grasbeck features autosomal recessive inheritance and is characterized by failure to thrive and chronic inappetence beginning at 6-12 weeks of age.⁶⁷ Megaloblastic bone marrow changes, low serum cobalamin concentrations, elevated serum methylmalonic acid and total homocysteine are evident.⁶⁷ A selective defect in cobalamin absorption was demonstrated and immunoelectron microscopic study of ileal biopsies revealed an absence of IF-Cbl complex receptors.⁶⁷ No specific neurological or neuropathological findings have yet been described in this animal model.

PATHOGENESIS

The precise role of cobalamin in the normal development and homeostasis of the intact nervous system is not yet understood, thus there has been considerable, often conflicting, speculation concerning the precise pathogenesis of neurological dysfunction in acquired and inherited disorders of cobalamin metabolism. While early attention was directed at the presence of an accumulating potential toxin such as methylmalonic acid⁶⁸ or the action of physiologically inactive cobalamin analogues that inhibit cobalamin-dependent enzymes,⁶⁹ present theories of pathogenesis largely focus on the two cobalamin-dependent enzymes themselves.⁷⁰

Impairment of methylmalonyl CoA mutase, which is a critical step in propionate catabolism by cobalamin deficiency, results in the accumulation of propionylCoA which is thought to replace succinylCoA and acetylCoA as substrates in the synthesis of fatty acids leading to the formation of odd chain and anomalous fatty acids.⁷¹ Frenkel⁷² using radiolabelled propionate and *in vitro* cultures of sural nerve biopsy specimens from patients with pernicious anemia demonstrated decreased fatty acid content and the presence of C15 and C17 odd chain fatty acids not found in normal neural tissue. This combination was thought to result in altered myelin integrity and renewal and the pathological finding of the splitting of lamellae. Support for this hypothesis is found in the dietary cobalamin-deficient fruit bat in which there is an alteration of the fatty acid composition of neural tissue⁷³⁻⁷⁴ and the prevention of neurological dysfunction by valine supplementation.⁷⁵ However, inherited disorders of cobalamin metabolism that selectively impair mutase activity, either due to inadequate adenosylcobalamin formation or an apoenzyme defect do not result in any apparent myelinogenic or myelinoclastic changes.⁷ In these situations, neurological dysfunction is now attributed to the systemic effects of secondary acidosis and ketosis.

Other investigators have focused on derangement of methionine synthase activity and its role in essential methylation reactions. The key observation is the prevention of myelopathy in nitrous oxide-exposed rhesus monkeys⁶⁴ and pigs⁶¹ by simultaneous supplementation with methionine. The putative mechanism is the drop in S-adenosylmethionine supply (Figure 4) which serves as a methyl donor in a variety of essential methylation reactions involved in the synthesis of proteins and neurotransmitters.⁷⁰ This theory is supported by the observation of cerebral myelination defects in patients with inherited disorders of cobalamin metabolism selectively affecting methionine synthase activity.⁷⁰ While a fall in tissue S-adenosylmethionine (SAM) and rise in S-adenosylhomocysteine (SAH) has been observed in the pig⁶¹ and rhesus monkey⁶⁴ leading to an altered SAM/SAH ratio, no such tissue alteration has been documented in the fruit bat.⁶⁰ Furthermore, methylation of myelin basic protein and of myelin lipids remains unchanged in the nitrous oxide exposed bat despite a reduction in methionine synthase activity.⁷⁶⁻⁷⁷ A putative toxic role for S-adenosylhomocysteine is not supported by the experimental observation that nitrous oxide-exposed rats accumulate large amounts of S-adenosylhomocysteine without developing a myelo-neuropathy.⁷⁸

Chanarin and colleagues⁷⁹ have recently advanced the hypothesis that a decline in formate synthesis may be the critical

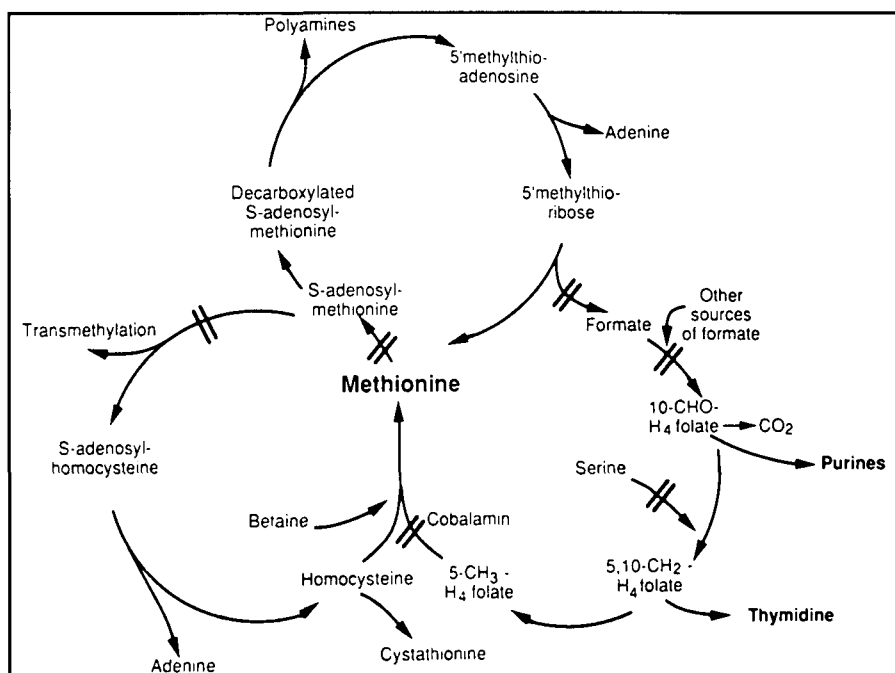


Figure 4 — Cobalamin, folate and methionine interaction. Double lines indicate points at which cobalamin deficiency ultimately results in impaired functional activity (Reprinted with permission from Chanarin I, Deacon R, Lumb M, Perry J. Cobalamin-folate interrelations. *Blood Reviews* 1990; 4: 211-215)

event underlying the neurologic dysfunction of cobalamin deficiency. This hypothesis focuses on the complex cobalamin-folate interactions outlined in Figure 4. Decreased methionine formation results in a fall in the amount of S-adenosylmethionine entering polyamine pathways and a decline in folate production. In situations of cobalamin deficiency, formate is not linked to tetrahydrofolate and is unavailable in its essential role as a source of one carbon units.⁷⁹ Of note, providing formyltetrahydrofolate (i.e., folinic acid) has been observed to reverse the various biochemical pathways impaired by cobalamin inactivation in animal models.⁸⁰

It appears that important species differences in the metabolism of cobalamin underly the conflicting results and varying interpretations outlined above. The pathogenesis of the neurologic dysfunction that is such a prominent feature of cobalamin deficiency in humans, is poorly understood.

INHERITED DISORDERS OF COBALAMIN METABOLISM

Cobalamin Absorption and Transport Defects

“R” Binder Deficiency

Since the original case report,⁸¹ a total of seven patients have been described with “R” binder deficiency. Not all have florid neurologic dysfunction. The initial description involved two brothers, one asymptomatic, the other with a progressive neurologic disorder characterized by optic atrophy, myelopathy and dementia.⁸¹ Carmel further reported four patients, in two of whom myelopathy was limited to posterior column involvement which was manifested by the late onset of mild impairment of position and vibration sense.⁸² While the remaining two patients had documented “R” binder deficiency, co-existing alcohol

abuse or cerebrovascular disease were plausible explanations for the observed neurological symptoms.⁸² The most recent case report featured a severe myelopathy clinically and a post mortem examination of the spinal cord revealed extensive non-inflammatory destruction of myelin, most prominent in the posterior and lateral columns, with secondary axonal loss.⁸³ The original finding of “R” binder deficiency in siblings⁸¹ leads to a suspicion that this may be an inherited disorder. The pathogenesis of symptoms is possibly related to the “R” binders’ physiological role in the removal of potentially toxic cobalamin analogues.

Defective Intrinsic Factor

In these patients, absorption of cobalamin can be corrected to normal by mixing with normal gastric juice as a source of intrinsic factor. Etiology is heterogeneous and several different groups of defective intrinsic factor have been delineated. These include a failure to secrete any immunologically detectable intrinsic factor⁸⁴ and the production of intrinsic factor with reduced affinity for cobalamin/ileal receptor sites⁸⁵ or increased susceptibility to proteolytic degradation.⁸⁶ Clinically these patients present in early childhood (1-5 years) with megaloblastic anemia, global developmental delay and myelopathy.⁸⁶⁻⁸⁷ Serum cobalamin levels are low, gastric function and morphology normal and auto-antibodies to intrinsic factor agent.⁸⁶

Imerslund-Grasbeck Syndrome (defective cobalamin transport by enterocytes)

This syndrome is characterized by decreased serum cobalamin levels with normal intrinsic factor and Transcobalamin II levels and no apparent intrinsic factor antibodies.⁸⁸ The absorption defect is *not* corrected to normal by mixing with normal

gastric juice suggesting that intrinsic factor is functionally normal and that the defect lies at the level of the enterocyte's handling of cobalamin.⁸⁸ Heterogeneous abnormalities have been identified including defective Cbl-IF receptor formation, receptor internalization and transfer of absorbed cobalamin to TC II.⁸⁹ More than 60 individuals with this disorder have been identified. Clinical features include early childhood onset (1-5 years), megaloblastic anemia, failure to thrive, global delay and myelopathy.^{88,90} As noted above, a dog model for Imerslund-Grasbeck syndrome has recently been described featuring autosomal recessive inheritance and evidence for absent ileal IF-Cbl complex receptor formation.⁶⁷

Transcobalamin II Deficiency

Transcobalamin II is absent on protein electrophoresis or gel filtration fractionation, while serum cobalamin levels are normal or minimally decreased.⁹ Since TC II facilitates the uptake of cobalamin at the cellular level, these patients are symptomatic in early infancy when maternally derived cobalamin stores are initially exhausted.⁹ Affected infants are typically severely ill with megaloblastic anemia, failure to thrive and global delay.⁹¹ Neurologic symptoms are often absent at presentation and are observed to develop after prolonged disease with inadequate cobalamin treatment.^{91A} Co-existing combined immunological deficiency has been observed in some patients.⁹² The rare survivors will develop childhood myelopathy.⁹

Disorders of cobalamin absorption and transport outlined above should theoretically lead to reduced synthesis of methylcobalamin and adenosylcobalamin and subsequent functional impairment of the methionine synthase and methylmalonylCoA mutase reactions that are dependent on these cobalamin cofactors. While homocystinuria and methylmalonic aciduria have been demonstrated in some patients with absorption and transport defects, levels observed are much less than those seen in the cobalamin utilization disorders.¹³ In the Imerslund-Grasbeck dog model, elevated serum methylmalonic acid and total homocysteine were both observed.⁶⁷ Neurologic dysfunction in Imerslund-Grasbeck patients is thought to be the result of altered 1-carbon metabolism which is the byproduct of disruption of methionine/homocysteine balance and folate metabolism.⁷⁰

Megaloblastic anemia, developmental delay and decreased serum cobalamin levels are the hallmarks of cobalamin transport disorders.⁹ The infant's early use of pinocytic, instead of receptor mediated, intestinal transport mechanisms, is thought to explain the early childhood presentation after a symptom free interval.⁹

Treatment consists of pharmacologic doses of hydroxycobalamin or cyanocobalamin with the aim of maintaining serum cobalamin at high levels (1000-10,000 pg/ml).¹⁸ The megaloblastic anemia may be corrected by folic acid supplementation, however, this should always be coupled to cobalamin therapy.¹³ A wide range of neurological outcomes is possible with the extent of recovery dependent on the duration of symptoms prior to instituting therapy.⁹ Parenteral cobalamin reversed the hematological and clinical derangements observed in the dog model for Imerslund-Grasbeck.⁶⁷

Autosomal recessive inheritance for these disorders is suggested by reports of affected sibships (including identical twins),

equal numbers of affected males and females and lack of vertical transmission.¹³ Ethnic clustering of Imerslund-Grasbeck syndrome in Finns and Sephardic Jews has been noted. The TC II gene has been linked to the long arm of chromosome 22,⁹³ and the rat intrinsic factor⁹⁴ and the porcine gastric haptocorrin⁹⁵ gene have been cloned and sequenced. These two physiologically diverse cobalamin binding proteins share remarkable similarity with respect to amino acid sequence suggesting the possibility of a common evolutionary origin.

Intracellular Cobalamin Utilization Defects

Detailed biochemical and cell complementation studies have thus far identified seven distinct disorders, labelled cblA-cblG in order of their discovery, that are the result of a failure of target cells to properly utilize intracellular cobalamin. These seven disorders are properly organized into 3 groups (Figure 3); i) impaired methylcobalamin and adenosylcobalamin synthesis (cblC/cblD/CblF) ii) impaired methylcobalamin synthesis only (cblE/cblG) iii) impaired adenosylcobalamin synthesis only (cblA/cblB).⁷ The laboratory features of the various cobalamin utilization defects are summarized in Table 1.

Impaired Methylcobalamin and Adenosylcobalamin Synthesis

Blocks at sites common to the intracellular biosynthetic pathways of both methylcobalamin and adenosylcobalamin, following the endocytosis of circulating TC II-Cbl and its lysosomal hydrolysis, result in both homocystinuria and methylmalonic aciduria. This group includes at least three distinct complementation classes; cblC, cblD and cblF.⁷

The defect in cblF is presumed to be a defect in lysosomal exiting with the demonstration of the accumulation of non-metabolized, non-protein-bound cobalamin in secondary lysosomes.⁹⁶ A defect in the cytosolic reductase(s) responsible for the reduction of trivalent cobalamin [Cob(III)alamin] to divalent cobalamin [Cob(II)alamin] is thought to underly the cblC and cblD disorders.⁹⁷ Mutations at two different genetic loci are probable since cell lines from cblC and cblD patients complement with each other.⁹⁸

Patients with this group of disorders have both homocystin-

Table 1: Cbl Utilization Defects (Summary)

	Complementation Group (cbl)						
	A	B	C	D	E	F	G
Megaloblastic Anemia	-	-	+	+	+	±	+
Homocystinuria	-	-	+	+	+	+	+
Methylmalonic Aciduria	+	+	+	+	-	+	-
% AdoCbl	D	D	D	D	N	D	N
% MeCbl	N	N	D	D	D	D	D
Propionate Fixation	D	D	D	D	N	D	N
MTHF Fixation	N	N	D	D	D	D	D
Lysosomal Efflux of Cbl	N	N	N	N	N	D	N
[⁵⁷ Co] CN-Cbl Uptake	N	N	D	D	N	I	N

+ = Present
 - = Absent
 D = Decreased
 N = Normal
 I = Increased
 Cbl = Cobalamin
 AdoCbl = Adenosylcobalamin
 MeCbl = Methylcobalamin
 MTHF = Methyltetrahydrofolate
 [⁵⁷Co] CN-Cbl = Cobalt labelled cyanocobalamin

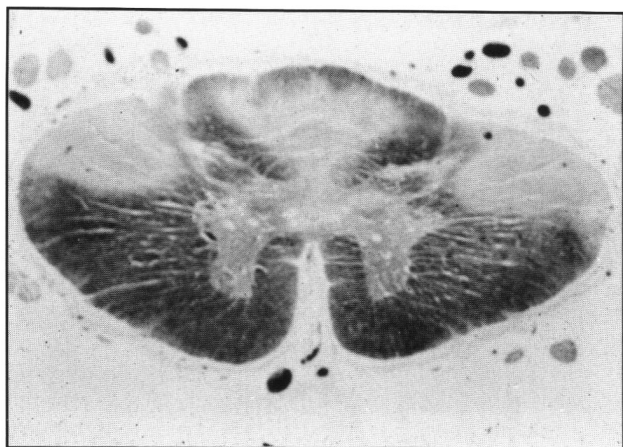


Figure 5 — Confluent demyelination in the dorsal and lateral columns in a patient with pernicious anemia and myelopathy. (Heidenhain's Stain, x 6)

uria and methylmalonic aciduria and usually megaloblastic anemia with normal serum cobalamin and TC II levels.⁷ *In vitro* cellular uptake of labelled cyanocobalamin is reduced in cbIC and cbID but increased in cbIF. There is a reduction in ability to convert labelled cyanocobalamin to methylcobalamin or adenosylcobalamin. In all three classes there is a decreased incorporation of labelled propionate and methyltetrahydrofolate. Direct assay of methionine synthase and methylmalonylCoA mutase enzyme activity in cell extracts is low.

The cbIC complementation class represent the most common of the cobalamin utilization defects. The majority of patients present in infancy with megaloblastic anemia, feeding difficulty, failure to thrive and global developmental delay with seizures and microcephaly.⁹⁹ Progressive visual impairment with retinal degeneration and "salt and pepper" retinopathy with predominantly peri-macular depigmentation has been described in some cbIC patients.⁹⁹⁻¹⁰¹ Electroretinogram study in one patient suggested isolated cone cell dysfunction.⁹⁹ A subset of cbIC patients have a later mode of presentation in childhood or adolescence with prominent delirium, psychosis and spasticity.^{99,102} Neuroradiological findings are sparse, however cerebral atrophy has been described.⁹⁹

The only report of cbID complementation class is limited to a single sibship of two brothers.¹⁰³ The eldest was mildly mentally retarded with behavioural difficulties when first brought for medical evaluation in early adolescence, while the younger sibling was asymptomatic though biochemically affected. The eldest developed recurrent cerebrovascular thromboemboli in early adulthood.⁹

There are only two case reports of cbIF complementation class in two non-related females.¹⁰⁴⁻¹⁰⁵ While both patients had methylmalonic aciduria only one had demonstrable macrocytosis or elevated plasma homocysteine.¹⁰⁵ The first reported patient had glossitis, dextrocardia, minor facial anomalies and prominent neurological symptoms that included neonatal seizures and hypotonia, abnormal extra-ocular movements and psychomotor delay.¹⁰⁴ A cranial CT scan demonstrated diffuse cerebral atrophy. A second patient died suddenly at 5 months after initially presenting with mild facial anomalies, hypotonia and failure to thrive. An autopsy revealed no CNS pathology.¹⁰⁵

Treatment for cbIC patients consists of parenterally administered hydroxycobalamin (up to 1 mg IM daily) titrated to control the observed homocystinuria and methylmalonic aciduria.⁹ Clinical and *in vitro* studies have shown hydroxycobalamin to be more effective than cyanocobalamin.¹⁰⁶ Oral administration of hydroxycobalamin was found to be ineffective.¹⁰⁶ Adjunctive therapies can include moderate protein restriction, carnitine supplementation which enhances organic acid excretion, folic acid supplementation and betaine administration (250 mg/kg/day). Betaine functions as an alternative hepatic methyl donor to homocysteine and has synergistic effects with parenterally administered hydroxycobalamin in normalizing methionine and homocysteine levels.¹⁰⁶ Carnitine supplementation increased propionylcarnitine excretion but did not consistently decrease serum methylmalonic acid.¹⁰⁶

Biochemical improvement is usual with hydroxycobalamin supplementation, however, clinical outcome depends on the treatment lag. Mild to moderate retardation in surviving patients has been observed despite metabolic control.^{9,13}

The mode of inheritance is presumed to be autosomal recessive and pre-natal diagnosis has been accomplished in cbIC and cbIF using amniocytes.

Impaired Methylcobalamin Synthesis only

Megaloblastic anemia, homocystinuria and hypomethioninemia without methylmalonic aciduria are the biochemical signatures of these disorders suggesting a block in the biosynthetic pathways unique to methylcobalamin.⁹ There is reduced methylcobalamin formation¹⁰⁷ with direct assay of methionine synthase demonstrating decreased activity either under special assay (cbIE) or standard assay conditions (cbIG) with concomitant reduction in the incorporation of labelled methyltetrahydrofolate.¹⁰⁸ It is postulated that cbIG patients have a defect in the methionine synthase apoenzyme itself since even under optimal assay conditions enzyme activity is reduced.¹⁰⁷ A subset of these patients demonstrated a lack of binding of cofactor to enzyme in fibroblasts incubated in labelled cyanocobalamin.¹⁰⁹ cbIE patients appear unable to reduce divalent cobalamin [Cob(II)alamin] to monovalent cobalamin [Cob(I)alamin] or to actively maintain the reduced state.¹¹⁰

These patients usually present with vomiting, lethargy and poor feeding in the first two years of life (70% prior to age 3 months).¹¹¹ Psychomotor delay, hypotonia and frequent seizures, without a specific EEG abnormality, characterize the prominent neurological dysfunction observed.¹¹¹ Neuroimaging studies reveal cortical atrophy in a majority⁷⁰ and delayed cerebral myelination with magnetic resonance scanning has been documented.⁷⁰ A single patient with adult onset symptoms of a progressive myelopathy, initially misdiagnosed as multiple sclerosis, has been diagnosed with a cbIG disorder.¹¹² Clinically and biochemically cbIE and cbIG patients are phenotypically similar with differentiation established by complementation analysis.¹¹¹

Treatment consists of exogenous hydroxycobalamin in pharmacologic amounts (1 mg/day-1mg/week) while monitoring metabolic parameters.¹³ As in the cbIC, cbID and cbIF disorders, a variety of adjunctive therapies (folic acid, methionine, betaine, pyridoxine and carnitine) have been tried in individual patients with varying results. Cobalamin supplementation results in biochemical and hematologic amelioration, however, neurologic

outcome appears to be a function of the treatment lag with cblG patients appearing to have a slightly worse prognosis.¹¹³

Reports of sibships with either cblE or cblG suggest an autosomal recessive mode of inheritance.¹⁰⁷ Prenatal diagnosis, coupled with *in utero* and post-natal cobalamin supplementation in a single patient has resulted in a good outcome with only a slight speech impediment evident at the 5 year follow-up.¹¹⁴

Impaired Adenosylcobalamin Synthesis

Methylmalonic aciduria with secondary acidosis, ketosis, hypoglycemia and hyperammonemia without homocystinuria or megaloblastic anemia characterizes this group of patients which comprise two complementation classes; cblA and cblB.⁹ The observed hypoglycemia is the result of inhibition of pyruvate carboxylase,¹¹⁵ while inhibition of intramitochondrial glycine cleavage results in hyperammonemia.¹¹⁶

Cobalamin uptake is normal in fibroblasts derived from these patients while there is a reduction in the adenosylcobalamin fraction¹¹⁷ and labelled propionate incorporation.¹¹⁸ MethylmalonylCoA mutase activity is reduced but is responsive to exogenously added cobalamin.¹¹⁹ Complementation between cell lines derived from cblA and cblB patients suggests that the underlying genetic defects are at different points in the biosynthetic pathway of adenosylcobalamin, which results in a decrease in the functional activity of methylmalonylCoA mutase and a resulting block in propionate catabolism.¹²⁰ Presumably, cblA patients have a decreased ability to reduce intramitochondrial divalent cobalamin [Cob(II)alamin] to monovalent cobalamin [Cob(I)alamin],⁷ while a defect in the adenosyltransferase enzyme itself appears to underly the cblB complementation class,¹²¹ since these patients' fibroblasts are unable to synthesize adenosylcobalamin in an appropriate reducing environment. Of note, a single patient with the clinical and biochemical phenotype of cblA whose fibroblasts complemented with cblA, cblB and mutase cell lines has been recently described. This patient was postulated to have defective penetration of cobalamin into the mitochondria.¹²²

An autosomal recessive mode of inheritance is presumed with early and late onset presentations possible.¹²³ Early onset features include a severely ill infant with failure to thrive, lethargy, recurrent vomiting, profound dehydration and hypotonia.¹²³ Childhood, or late, onset may present insidiously with developmental delay or acutely with coma.¹²³

Decreased, but not normal, levels of methylmalonic acid excretion can be achieved by supplementary hydroxycobalamin in pharmacologic amounts and dietary protein restriction.^{9,13,124} Phenotypic differences are apparent between cblA and cblB patients in that 90% of cblA patients respond biochemically while only 40% of cblB patients respond biochemically.¹²³ Outcome is also better in the cblA patients with 70%, as opposed to 30% of the cblB patients, well at 14 years of age.¹²³ Enhanced metabolic and clinical control was demonstrated in the single cblB patient thus far reported given metronidazole to reduce the significant contribution of propionate from enteric anaerobic bacteria.¹²⁵ Treatment lag once again is the important determinant of neurologic outcome with a broad range of intellectual impairment observed.⁹ Prenatal diagnosis, with hydroxycobalamin supplementation begun antenatally¹²⁶ or at birth,¹²⁷ has resulted in a normal outcome in both cblA and cblB patients.

ACQUIRED DISORDERS OF COBALAMIN METABOLISM

Pernicious Anemia

Until recently, pernicious anemia and its associated neurological complications was the major focus of the neurology of cobalamin. Due to easily obtainable screening tests, earlier diagnosis and treatment as well as the widespread use of cobalamin supplements for vague complaints, the classical neurological syndrome of sub-acute combined degeneration is now a rarely observed clinical entity. The extent of neurological dysfunction secondary to cobalamin deficiency tends to be milder in current clinical practice.^{127A}

As noted, the original description of a progressive myelopathy occurring in association with pernicious anemia was made by Lichtheim in the latter part of the nineteenth century.¹ Pernicious anemia is classically the result of a defect in cobalamin absorption that is due to a failure of gastric parietal cell secretion of intrinsic factor that is caused by an autoimmune disorder involving antibody formation against gastric parietal cells and lymphocytic infiltration of the gastric mucosa.¹²⁸ The resulting cobalamin deficiency leads to an impairment of DNA synthesis¹²⁹ and accounts for the observed hematologic abnormalities of megaloblastic anemia which includes elevated mean corpuscular volume, elevated mean corpuscular haemoglobin and both hypersegmented neutrophils and macroovalocytes on smear with megaloblastic, erythroid and granulocytic hyperplasia on bone marrow examination.¹³⁰ A positive Schilling test and demonstration of intrinsic factor antibody confirms the diagnosis.⁴¹ Acquired malabsorption of cobalamin may also occur following gastric or ileal resection¹³¹ and in the setting of a wide range of gastrointestinal disorders including fish tapeworm infestation, Crohn's disease, celiac disease, jejunal diverticulosis and tuberculous enteritis with the same resulting hematologic and neurologic abnormalities.⁶

Originally it was thought that the neurologic dysfunction was a late complication of longstanding pernicious anemia occurring after hematologic derangements were well established.³⁵ It is now clear that there is a lack of parallelism between hematologic and neurologic dysfunction.^{36A,132} Furthermore, the onset, progression and severity of neurologic symptoms does not co-relate with the degree or duration of the observed megaloblastic anemia¹³² and indeed may be inversely related.^{127A} The majority of patients (67%) with pernicious anemia will have some neurological symptoms¹³³ and often multiple neurological syndromes are seen in a single patient.^{127A}

The myelopathy of pernicious anemia, the neurological manifestation most emphasized historically, is the result of sub-acute combined degeneration of the spinal cord involving the symmetrical loss of myelin sheaths most evident in the posterior and lateral columns.¹³⁴ The initial pathologic changes occur in the thoracic portion of the cord and spread laterally, superiorly and inferiorly.¹³⁴ The thickest myelinated fibres are the first affected.¹³⁴ Superior extent is limited to the medulla oblongata with no reported pontine or midbrain involvement.¹³⁴ A lesser, secondary loss of axons is also observed within the cord.¹³⁴

The myelopathy typically presents initially as distal paresthesias involving the legs. Gait is invariably affected, characterized by stiffness and unsteadiness (ataxia). Examination usually reveals a loss of vibration sense and a lesser impairment of position sense, reflecting dorsal column disease, with weakness,

spasticity and extensor plantar responses in advanced cases reflecting corticospinal tract involvement.¹³⁵ Findings are symmetrical with a parallel involvement of motor and sensory symptoms. With progression the upper limbs are involved as well, but usually to a lesser extent than the lower limbs.¹³⁵ In a minority of patients, upper limb paresthesias precede lower limb paresthesias.^{127A} Significant central inter-peak latencies on SER testing have been documented in myelopathic pernicious anemia patients, thus demonstrating the physiological impact of spinal cord disease.¹³⁶

Peripheral neuropathy is an often overlooked common feature of pernicious anemia.¹³³ First suggested by Ungley in 1949,¹³⁷ it was the most common neurologic abnormality observed by Shovron et al.¹³³ in their survey of patients with megaloblastic anemia secondary to cobalamin deficiency. Pathologic examination of autopsy and biopsy samples reveals a loss of axons in excess of myelin destruction.¹³⁸ Clinically the peripheral neuropathy is characterized by symmetrical distal impairment of superficial sensation and the loss of tendon reflexes.¹³² Electrophysiologic studies of peripheral nerves in cobalamin deficient patients document a reduction in both sural action potential amplitude and peroneal action potential amplitude, the former more severely affected suggesting a preferential involvement of sensory fibres.^{136,138,139} Distal denervation on electromyography, preservation of conduction velocity and only mild delay of F responses also suggest an axonopathy predominantly.¹³⁶ When observed, the reduction in nerve conduction velocity in the setting of severe axonal loss was attributable to secondary demyelination.¹⁴⁰ Furthermore, sub-clinical neuropathy on electrophysiological testing was a common finding.¹³³

Visual impairment in the setting of pernicious anemia was originally described by Putnam and Taylor.¹⁴¹ Symmetrical centrocecal scotomata can be found on visual field testing with optic atrophy evident on examination.¹³⁵ Pathologic examination on autopsy has revealed degeneration of the optic nerves anterior to the optic chiasm.¹⁴² Visual evoked response (VER) testing has revealed a high frequency of abnormality in cobalamin deficient patients in the absence of clinically evident visual impairment.^{136,143} The VER abnormality is suggestive of primary axonal loss with secondary patchy demyelination.³⁶

Dementia, featuring confusion, memory impairment and cognitive deterioration has been observed with some frequency in patients with pernicious anemia.^{132,135} Shovron et al. in their survey found 1/3 of pernicious anemia patients have evidence for an organic brain syndrome.¹³³ Affective disorders, both depression and mania, have also been documented to some extent¹⁴⁴⁻¹⁴⁶ as well as acute psychosis.^{127A} Lesser mental changes such as irritability, apathy and emotional instability are common findings.¹³³ These manifestations are attributed to scattered foci of cerebral white matter pathology that may yield generalized slowing on EEG.^{142,147,148}

In the absence of treatment, neurologic impairment in pernicious anemia is relentlessly progressive. Treatment with lifetime parenteral intramuscular hydroxycobalamin or cyanocobalamin results in an arrest of disease progression and some degree of partial reversal of existing symptoms¹³² with complete recovery possible in almost half of the patients.^{127A} Sufficient cobalamin is administered to restore depleted reserves and to meet ongoing needs.³³ There is some evidence to suggest that hydroxycobalamin is superior to cyanocobalamin,¹⁴⁹ however, this superiority

was demonstrated in tobacco amblyopia and has not yet been shown in pernicious anemia secondary to cobalamin deficiency. The severity and duration of symptoms prior to diagnosis and instituting proper therapy are the major determinants of eventual outcome.^{127A, 135}

Subtle/Atypical Cobalamin Deficiency

Over the past several years, the concept of atypical or subtle expressions of cobalamin deficiency has evolved.³⁷ The clinical parameters and natural history of this heterogeneous entity has not yet been fully clarified.³⁷ However, it is suggested that this may be as frequent in occurrence as the classical hematologic and neurologic symptoms of pernicious anemia.³² As conceptualized by Carmel,³⁷ this entity is defined by either "subtle" expression of an underlying cobalamin deficiency (i.e., neuropsychiatric symptoms in the absence of hematological derangement) and/or an "atypical" cause of cobalamin deficiency (i.e., a normal Schilling test).

It is now clearly established that significant neurological disease can occur secondary to cobalamin deficiency without concomitant hematologic changes.^{36A} In an extensive series of consecutive patients with neuropsychiatric abnormality due to cobalamin deficiency, Lindenbaum and colleagues⁸ found 28% without anemia or macrocytosis. Carmel³⁶ found 20% of his patients with serum cobalamin deficiency and pernicious anemia (abnormal Schilling test or Intrinsic Factor antibodies) did not have anemia (despite its name, pernicious anemia is essentially defined as an autoimmune gastrointestinal disorder) and 33% did not have macrocytosis, the two hematologic clues commonly used to suspect cobalamin deficiency. Of the 14% of patients in this sample with neither anemia nor macrocytosis, 60% had significant neurologic symptoms.³⁶ In both these series, a broad range of neurologic disorders was encountered including myelopathy/dorsal column dysfunction, cognitive/memory changes and peripheral neuropathy.^{8,36} Karnaze and Carmel³² also demonstrated that evoked response testing (somatosensory and visual) detected electrophysiologic evidence of central processing abnormalities in "subtle" cobalamin deficient patients.

Cobalamin deficiency in these patients is established by a variety of means including low serum cobalamin levels, elevated serum methylmalonic acid or total homocysteine and an abnormal deoxyuridine suppression test.^{32,45} The use of serum methylmalonic acid and total homocysteine assays have redefined the scope of cobalamin deficiency.⁴⁰ Relative and clinically important cobalamin deficiency can now be reliably diagnosed in non-anemic individuals with "normal" serum cobalamin values if there is an elevation of either of these substrates for the two cobalamin-dependent intracellular enzymes.⁴⁰ Serum methylmalonic acid and total homocysteine now represent an essential mode of possible diagnosis in the non-anemic individual with a diverse range of unexplained neuropsychiatric symptoms and a normal serum cobalamin level.^{8,40,44} This is not merely of academic interest since clinical response has been demonstrated in these patients with cobalamin supplementation.^{8,37}

The deoxyuridine suppression test is perhaps the most sensitive metabolic indicator of cobalamin deficiency⁴² as it assesses the ability of deoxyuridine added *in vitro* to suppress the subsequent incorporation of radiolabelled thymidine into DNA. Since

it requires a bone marrow aspirate and is currently performed in a few reference laboratories, its clinical use is restricted.

A possible association between atypical cobalamin deficiency and primary degenerative dementia was suggested by the largely retrospective study of Karnaze and Carmel.¹⁵⁰ Of the seventeen patients with primary degenerative dementia, often resembling Alzheimer's disease with predominant spatial disorientation and language disturbances, five had low serum cobalamin. In the two patients so tested, an abnormal deoxyuridine suppression test was also documented. In their group of patients with dementia attributable to a specific cause (secondary dementia) none had a low serum cobalamin. Of note, none of the patients with low serum cobalamin and dementia had any hematologic abnormality evident. Cole and Prchal found 30% of patients with Alzheimer's type dementia to have subnormal cobalamin.¹⁵¹ This is in contrast to a 3-7% rate for low serum cobalamin levels in an out-patient ambulatory geriatric population.^{152,153} Furthermore, an autopsy study of demented patients has documented decreased cobalamin content in the frontal and temporal lobes.¹⁵⁴ A prospective causal link between low serum cobalamin and dementia has not yet been shown. The possibility of prevention through early detection and treatment has raised the spectre of screening the elderly population for subtle cobalamin deficiency.¹⁵⁵

The neurologic sequelae of cobalamin deficiency may occur in the setting of a normal Schilling test thus suggesting an "atypical" cause for the low serum cobalamin. Atypical causes³⁷ include food-cobalamin malabsorption, dietary insufficiency, nitrous oxide exposure and infants born to a vegan mother (the latter two entities are considered separately below). In food-cobalamin malabsorption, there is an apparent failure to absorb cobalamin bound to food proteins while the absorption of free cobalamin is unimpaired.¹⁵⁶⁻¹⁵⁷ The egg yolk cobalamin absorption test is the means of diagnosing this condition.⁴² Actual dietary cobalamin insufficiency is rarely observed outside of infancy.³⁷

Nitrous Oxide

Nitrous oxide (N₂O) oxidizes cobalamin from an active monovalent [Cob(I)alamin] state to an inactive trivalent [Cob(III)alamin] state.⁵³ As noted above, it is commonly used in a variety of animal models to experimentally mimic cobalamin deficiency.⁵⁵⁻⁶³ Not surprisingly, prolonged use of nitrous oxide (> 6 hours) as an inhalational anaesthetic has been recognized to induce megaloblastic anemia that is readily reversible after discontinuation of inhalation.¹⁵⁸ However in the setting of a patient with borderline or overt cobalamin deficiency, intra-operative use of nitrous oxide has been noted to result in a non-transitory post-operative myeloneuropathy with neurologic decompensation attributable to inadequate marginal cobalamin stores.^{159,160}

Recreational abuse of nitrous oxide on a prolonged daily basis can result in a myeloneuropathy resembling sub-acute combined degeneration.¹⁶¹ This toxic exposure has been clustered in dentists who have easy access to nitrous oxide.^{162,163} Other commercial sources of nitrous oxide for potential abuse include aerosols, whipped cream dispensers and gas cylinders/charges.¹⁶⁴ In addition to recreational abuse, toxic exposure may occur by working in poorly ventilated dental surgeries.¹⁶³ Symptoms in both these situations tend to resolve by discontinuation of exposure and cobalamin supplementation.¹⁶¹⁻¹⁶⁴

Infantile Nutritional Cobalamin Deficiency

Since dietary cobalamin is derived exclusively from animal sources, a strictly vegetarian diet is deficient in cobalamin.¹¹ The offspring of vegan mothers are at risk for infantile nutritional cobalamin deficiency that may result in a severe and progressive neurological disorder characterized by marked developmental delay.^{134,165,166} Once thought to be confined to India, this entity was reported several years ago in a European child who presented at 18 months of age with pronounced psychomotor retardation.¹⁶⁷ The child was an exclusively breast fed offspring of a vegan mother. EEG showed general slowing with multifocal sharp waves and the cranial CT scan demonstrated moderate cortical atrophy.¹⁶⁷ Treatment with high doses of cobalamin resulted in clinical, electroencephalographic and neuroradiologic improvement.¹⁶⁷

AIDS and Cobalamin Deficiency

Low serum cobalamin levels have been documented in individuals with the acquired immunodeficiency syndrome at a significant frequency (15%) both in the presence¹⁶⁸ and absence of clinically evident bowel dysfunction.¹⁶⁹ The cobalamin deficiency appears attributable to cobalamin malabsorption (abnormal Schilling test, abnormal food cobalamin absorption test) secondary to biopsy evidence for chronic gastrointestinal inflammation.¹⁶⁹ Asymptomatic HIV-I positive individuals also have evidence for negative cobalamin balance and low serum cobalamin levels.¹⁷⁰ Low holotranscobalamin-II levels appear to be the earliest serum marker for negative cobalamin balance.¹⁷¹ Negative cobalamin balance and low serum cobalamin levels appear to correlate significantly with measures of cognitive function in asymptomatic HIV positive individuals¹⁷⁰ and be a frequent finding (20%) in those referred for neurologic dysfunction such as peripheral neuropathy and myelopathy.¹⁷² The likelihood of cobalamin deficiency has been found to increase with disease progression.^{171,172} Some response to replacement therapy has been noted¹⁷² and routine evaluation of cobalamin status in AIDS has been recommended.^{169,172} To date no convincing case of neurological dysfunction in AIDS that can be solely attributed to cobalamin deficiency has been described.

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