# The Coenzyme Forms of Vitamin B12: Toward an Understanding of their Therapeutic Potential

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## Abstract

Although cyanocobalamin and hydroxycobalamin are the most commonly encountered supplemental forms of vitamin B12, adenosyl- and methylcobalamin are the primary forms of vitamin B12 in the human body, and are the metabolically active forms required for B12-dependent enzyme function. Evidence indicates these coenzyme forms of vitamin B12, in addition to having a theoretical advantage over other forms of B12, actually do have metabolic and therapeutic applications not shared by the other forms of vitamin B12. This article will provide an overview of the metabolism and function of adenosyl- and methylcobalamin, and will discuss the potential therapeutic relevance of the coenzyme forms of vitamin B12 in a variety of clinical conditions, including anemia, anorexia, cancer, HIV, and liver and sleep disorders.
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## Introduction

Cyanocobalamin (CN-Cbl) is the most commonly supplemented form of vitamin B12, but it is present in the body in trace amounts and its biochemical significance remains uncertain. Although the amount of cyanide is considered toxicologically insignificant, humans must remove and detoxify the cyanide molecule, reduce the cobalamin to its usable +1 oxidation state, and then enzymatically convert the cobalamin into one of two metabolically active coenzyme forms. Nutritional inadequacies, enzyme defects, and pathological changes to tissues can all contribute to a reduced ability of the body to accomplish the synthesis of the active forms of vitamin B12 from CN-Cbl.

The two forms of vitamin B12 having activity in B12-dependent enzymes within the human body are adenosylcobalamin (AdeCbl) and methylcobalamin (MetCbl). AdeCbl is occasionally referred to as coenzyme B12, cobamamide, cobinamide, or dibencozide. In some biochemical or therapeutic situations, the clinical utilization of either AdeCbl or MetCbl (alone or in combination) can produce results not found with the supplementation of either CN-Cbl or hydroxycobalamin (OH-Cbl).

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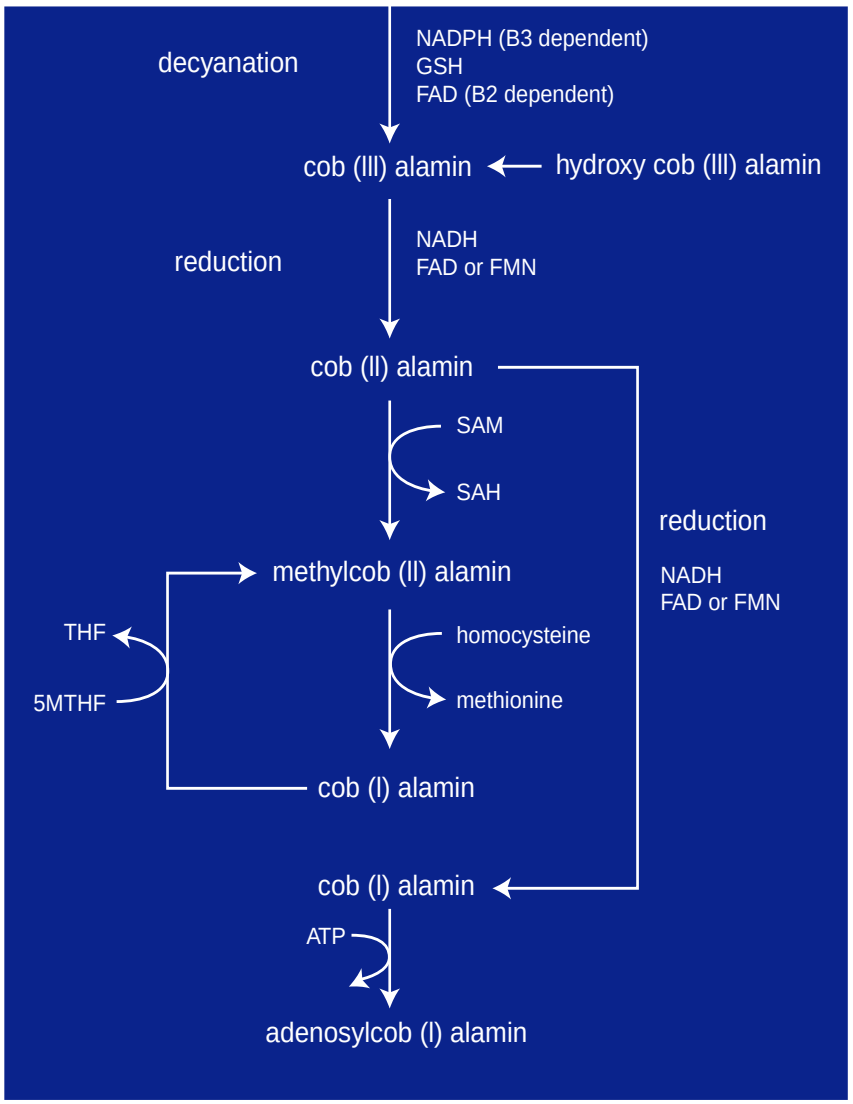
Figure 1. Synthesis of coenzyme forms of B12.




## Biochemistry, Metabolism, and Enzyme Functions

Cobalamin is a very complex molecule, containing cobalt surrounded by five nitrogen atoms. Surrounding this central cobalt is a corrin ring, which structurally resembles the porphyrin ring found in hemoglobin, the cytochromes, and chlorophyll. The use of cobalt in the coenzyme forms of cobalamin is the only known function of this metal in biological systems.

In humans, the cobalt in the coenzyme forms of vitamin B12 exists in a univalent (+1) oxidative state, designated as cob(I)alamin. Cobalamin molecules can also contain cobalt in a +3 (cob(III)alamin) or +2 (cob(II)alamin) oxidative state; however, in these forms the cobalt must be reduced prior to having enzyme activity.

The compound most commonly referred to as vitamin B12 is CN-Cbl; however, this molecule does not occur naturally in plants, micro-organisms, or animal tissues.[1] CN-Cbl has a cyanide molecule at the metal-carbon position and its cobalt atom exists at an oxidative state of +3, not the biologically active +1 state. In order to be utilized in the body, the cyanide molecule must be removed and eliminated through phase II detoxification. It is thought that glutathione (GSH) might be the compound performing the function of decyanation *in vivo*, since glutathionylcobal-amin (GS-Cbl) has been isolated from mammalian tissue.[2] If, in fact, GSH is needed as a cofactor to activate CN-Cbl to the coenzyme forms of vitamin B12, clinical situations characterized by decreased tissue levels of GSH might be expected to result in a functional deficiency of vitamin B12, even in the presence of adequate plasma or tissue levels of the cobalamin moiety (typically labs are looking only for a cobalamin moiety and do not differentiate between CN-Cbl and the active forms of vitamin B12).

Humans are incapable of synthesizing the corrin ring structure, and so are completely dependent upon dietary sources of vitamin B12. The ultimate source of all vitamin B12

occurring in the diet is bacteria, with animal products providing the majority of the dietary intake. It had been proposed that humans could absorb vitamin B12 formed by colonic flora; however, this appears to be untrue since no significant amount of cobalamin can be absorbed in the colon.[1]

The optimal absorption of dietary vitamin B12 requires the formation of a complex between dietary cobalamins and R-proteins, and the secretion, by the stomach parietal cells, of intrinsic factor. The cobalamin-R-protein complex is digested by pancreatic enzymes in the small intestine, and the released cobalamin molecule binds with intrinsic factor and is absorbed in the distal ileum. Cobalamin is then detached from intrinsic factor in the enterocyte cells of the small intestine, and is bound to transcobalamin II for transport into tissues.

Although the basic cobalamin molecule is only synthesized by micro-organisms, all mammalian cells can convert it into the coenzymes AdeCbl and MetCbl. OH-Cbl, MetCbl, and AdeCbl are the three forms of cobalamin most frequently isolated from mammalian tissue. However, only MetCbl and AdeCbl actually function as cofactors in human enzymes. AdeCbl is the major form in cellular tissues, where it is retained in the mitochondria. MetCbl predominates in blood plasma and certain other body fluids, such as cerebral spinal fluid, and, in cells is found in the cytosol.[3]

AdeCbl functions in reactions in which hydrogen groups and organic groups exchange places. In humans, AdeCbl is required for the enzyme methylmalonyl-CoA mutase which is used in the catabolic isomerization of methylmalonyl-CoA to succinyl-CoA (used in the synthesis of porphyrin) and as an intermediate in the degradative pathway for valine, isoleucine, threonine, methionine, thymine, odd-chain fatty acids and cholesterol.[1] Deficiencies in this coenzyme form of vitamin B12 result in increased amounts of methylmalonyl-CoA and generally in an increase in glycine.

MetCbl's only known biological function in humans is as a cofactor in the enzyme methionine synthase. The methionine synthase enzyme is located in the cytosol of cells and participates in the transfer of methyl groups from 5-methyltetrahydrofolate to homocysteine, resulting in the subsequent regeneration/remethylation of methionine.

Pezacka et al have proposed that at least four steps are required to convert supplementary CN-Cbl to the coenzyme forms of vitamin B12. These are: (i) decyanation; (ii) reduction of the +3 and +2 forms; (iii) synthesis of MetCbl in the cytosol; and (iv) synthesis of AdeCbl in the mitochondria. The initial step of decyanation is probably dependent on GSH, possibly in combination with NADPH and FAD.[2] This results in the formation of cob(III)alamin. OH-Cbl is also a cob(III) form but has an advantage over CN-Cbl since it bypasses the need for decyanation. The next step required is the reduction of cob(III)alamin to cob(II)alamin. This reduction is probably dependent upon NADH and possibly either FAD or FMN.[2] Once cob(II)alamin is formed, a similar reduction can shunt it into the formation of cob(I)alamin and subsequently, with ATP, AdeCbl. An alternate pathway can, with the donation of a methyl group from S-adenosylmethionine (SAM), result in the formation of MetCbl from cob(II)alamin. MetCbl becomes cob(I)alamin after donating its methyl group; however, MetCbl can be regenerated, by accepting a methyl group from 5-methyltetrahydrofolate, for reuse in methionine synthase (see figure 1.).

Evidence indicates alpha-tocopherol protects against a reduction in AdeCbl in oxidatively stressed cells.[4] Experimental evidence suggests alpha-tocopherol might be needed for formation of AdeCbl; however, further studies are required to clarify this relationship. If alpha-tocopherol is used in the reducing steps, a deficiency would be expected to decrease the formation of both AdeCbl and MetCbl.[5]

It is important to be aware that nitrous oxide inactivates the coenzyme forms of vitamin B12 by oxidizing cob(I)alamin to either cob(II)alamin or cob(III)alamin. Nitrous oxide also interferes with the activity of methionine synthase.[6]

## Absorption

Evidence indicates cobalamin from MetCbl is utilized more efficiently than CN-Cbl to increase the levels of coenzyme forms of vitamin B12. Although free MetCbl is not very stable in the gastrointestinal tract, and considerable loss of the methyl group can take place under experimental conditions, in physiological situations intrinsic factor probably partially protects MetCbl from degradation. Paper chromatography of digested ileal mucosa has demonstrated unchanged absorption of MetCbl following oral administration. The quantity of cobalamin detected following a small oral dose of MetCbl is similar to the amount following administration of CN-Cbl; but, significantly more cobalamin accumulates in liver tissue following administration of MetCbl. Human urinary excretion of MetCbl is about one-third that of a similar dose of CN-Cbl, indicating substantially greater tissue retention.[7]

In humans, about 35 percent of AdeCbl appears to be absorbed intact following oral administration, and about 77 percent of the absorbed oral dose is retained in body tissues. Although a higher percentage of CN-Cbl appears to be absorbed, only 50 percent is retained in tissues, and assuming an adequate supply of necessary cofactors is available, probably is converted to the coenzyme forms of vitamin B12 over a period of 1-2 months.[8]

Although individuals with pernicious anemia do not produce the intrinsic factor needed for vitamin B12 absorption, high doses of oral vitamin B12 (above 1000 mcg) have been shown to be an adequate treatment of B12 deficiency and pernicious anemia, indicating there is some mechanism of absorption independent of intrinsic factor.[9, 10] It is likely that with supra-physiological doses of the coenzyme forms of vitamin B12, some of the absorption is also independent of intrinsic factor.

## Clinical Implications

**Anemia:** The use of the coenzyme forms of vitamin B12 will be useful in some types of anemia and might offer an advantage over supplementation of the non-biologically active forms of vitamin B12. Under experimental conditions, poisoning of rabbits with phenylhydrazine results in the development of hemolytic hyperchromic anemia and impairment of hematopoiesis in the bone marrow. A decrease in the MetCbl content of the blood serum is observed during spontaneous recovery from this experimentally induced anemia. Administration of MetCbl results in a complete normalization of some blood and hematopoiesis patterns, as well as a restoration of total cobalamin content, and an improved ratio of the spectrum of cobalamin forms. AdeCbl, although somewhat effective, exhibited a distinctly lower effect on the patterns studied.[11]

A 50-day treatment with a ferritin preparation combined with folinic acid and AdeCbl was well tolerated and demonstrated efficacy in normalizing various hematological parameters (hemoglobin, hematocrit, red cell count, mean corpuscular volume, iron, and transferrin iron binding capacity) in pregnant women.[12] Granese et al similarly report a positive result from the supplementation of a ferritin-AdeCbl-folinic acid preparation to 40 women during pregnancy. A progressive increase in hematological parameters was demonstrated and a complete normalization of red cell morphology was observed.[13]

**Anorexia:** Carnitine and AdeCbl were shown to promote cerebral mass growth, increase neocortical layer thickness and pyramidal neuron volume, and fully restore

normal structure of the neocortex in an experimental model of anorexia nervosa. In patients with anorexia nervosa, carnitine and AdeCbl accelerate body weight gain and normalization of gastrointestinal function. Latent fatigue was reported to disappear and mental performance increase under this treatment regimen.[14] Korkina et al report the combined use of carnitine and AdeCbl eliminate fluctuations in the work rate and improve the scope and productivity of intellectual work in patients with anorexia nervosa in the stage of cachexia. Latent fatigue in the population studied was not fully removed.[15]

Children with infantile anorexia were divided into two groups. One group of children was given 2000 mcg of AdeCbl and 1000 mg of carnitine, while the other group was given cyproheptadine, an anti-histamine used to stimulate appetite. The results of using the AdeCbl and carnitine mixture were judged good by the authors, were comparable to the effects of the pharmaceutical agent, and were produced with no side-effects.[16]

**Cancer:** While information is very limited, both AdeCbl and MetCbl might eventually be shown to have a supportive role in the prevention or treatment of cancer. A significant body of experimental evidence suggests a deficiency of vitamin B12 can enhance the activity of various carcinogens.[17] Experimental results also indicate a link between alterations in the intracellular metabolism of cobalamin and the increased growth of human melanoma cells.[18]

A methyl group-deficient diet (MGDD) has been shown to result in hypomethylation of DNA and tRNA, and to promote cancer in the liver of rats in as short a period of time as one week. Results of experiments conducted by Wainfan and Poirier support the hypothesis that intake of a MGDD, by causing depletion of SAM pools, results in DNA hypomethylation, and subsequently leads to changes in gene expression.[19] Although many of the MGDD-induced alterations in methylation and gene expression occur rapidly, Christman et al have demonstrated they are essentially reversible.[20]

It is not surprising that MetCbl, because of its ability to donate a methyl group and because of its role in the regeneration of SAM, the body's universal methyl donor, might be protective against cancer. Cell culture and *in vivo* experimental results indicate MetCbl can inhibit the proliferation of malignant cells.[21] Experimental results also indicate MetCbl can enhance survival time and reduce tumor growth following inoculation of mice with Ehrlich ascites tumor cells.[22] Both of the coenzyme forms of vitamin B12 have been shown to increase survival time of leukemic mice. Under the same experimental conditions, CN-Cbl was inactive.[23]

Although more research is required to verify findings, MetCbl might also enhance the efficacy of methotrexate. MetCbl appears to stimulate the rate of 3H-methotrexate influx into tumors in experimental animals. Miasishcheva et al have suggested, based on kinetic analysis, a dose of 0.01 mg/kg of MetCbl might be an optimal dose for improving the antitumor drug action of methotrexate.[24]

Heimburger et al have reported that in a preliminary study, four months' treatment with 10 mg of folate plus 500 mcg of OH-Cbl resulted in a reduction of atypia in male smokers with bronchial squamous metaplasia.[25] Since folate and cobalamin interact in re-methylation, it is possible MetCbl would have worked as well or better than the OH-Cbl.

**Diabetic Neuropathy:** Yaqub et al conducted a double-blind study on the clinical and neurophysiological effects of MetCbl administration in 50 patients with diabetic neuropathy. Each patient in the active group was given 500 mcg of MetCbl orally three times per day for four months. Individuals

receiving MetCbl reported subjective improvement in somatic and autonomic symptoms (parasthesias, burning sensations, numbness, loss of sensation, and muscle cramps), and regression of signs of diabetic neuropathy (reflexes, vibration sense, lower motor neuron weakness, and sensitivity to pain). However, motor and sensory nerve conduction studies showed no statistical improvement after four months. MetCbl was well tolerated by the patients and no side-effects were encountered.[26]

Power spectral analysis of heart rate variability is a means of detecting the relative activity and balance of the sympathetic/parasympathetic nervous systems, and has been suggested to be a good qualitative method of evaluating sub-clinical diabetic autonomic neuropathy. Yoshioka et al have shown for individuals with NIDDM, oral administration of 1500 mcg/day of MetCbl produces improvements in several components of heart rate variability.[27]

**Eye function:** Experiments indicate chronic administration of MetCbl protects cultured retinal neurons against N-methyl-D-aspartate-receptor-mediated glutamate neurotoxicity. Kikuchi et al suggest the action is probably due to alteration in the membrane properties mediated through methylation by SAM. In their experiments, an acute exposure to MetCbl was not effective in protecting retinal neurons.[28] Results also indicate MetCbl enhances the ability to evoke a field potential in rat suprachiasmatic nucleus slices. CN-Cbl had no activity in this experimental model.[29]

Iwasaki et al studied the effect of MetCbl on subjects with experimentally induced deterioration of visual accommodation. The authors report the deterioration of accommodation following visual work was significantly improved in individuals receiving MetCbl.[30]

**Genital-Urinary:** Administration of 2g/kg of di(2-ethylhexyl)-phthalate (DEHP) induces severe testicular atrophy, reduction of testicular specific lactate dehydrogenase activity, and decreased zinc, magnesium and potassium concentrations in rats. Co-administration of AdeCbl with DEHP is reported to prevent these changes. MetCbl, when co-administered with DEHP, was unable to prevent the testicular atrophy induced by DEHP under similar experimental conditions.[31]

Thirty-nine patients with diagnosed oligozoospermia were divided into two groups and administered MetCbl at a dose of either 6 mg or 12 mg per day for 16 weeks. MetCbl appeared to be transported to seminal fluid very efficiently, and no dose-dependent difference between vitamin B12 concentrations in the serum or seminal fluid was observed between groups. The efficacy rate for the group receiving 6 mg per day was 37.5 percent and for the group receiving 12 mg per day was 39.1 percent.[32]

MetCbl was administered daily (1,500 micrograms/day, for 4-24 weeks) to 26 infertile male patients. Patients with azoospermia were excluded from the trial. Sperm concentration increased in 10 cases (38.4%), total sperm count increased in 14 cases (53.8%), sperm motility increased in 13 cases (50.0%), and total motile sperm count increased in 13 cases (50.0%). Serum luteinizing hormone, follicle stimulating hormone, and testosterone were unchanged.[33]

**HIV:** It has been observed that human immunodeficiency virus (HIV) seropositive individuals have decreased levels of metabolites involved in methylation, and that low serum vitamin B12 levels are associated with an increased risk of progression to AIDS; however, the effect of supplementation of coenzyme forms of vitamin B12 on disease progression is unknown.

May has proposed that the replication of HIV might be, in part, modulated by DNA methylation, and has suggested

hypermethylation of the HIV provirus might suppress viral replication and play a role in the establishment of latency. Because of its central role in methylation, MetCbl, as well as SAM and methyltetrahydrofolate, might have potential as therapeutic agents in HIV-infected individuals.[34]

Evidence is beginning to suggest low serum vitamin B12 concentrations might precede disease progression in individuals positive for HIV. Tang et al have reported the risk of progression to AIDS is increased in individuals with low serum vitamin B-12 concentrations (RH = 2.21, 95% CI = 1.13-4.34).[35]

Weinberg et al investigated cobalamins to determine their ability to modify HIV-1 infection of hematopoietic cells *in vitro*. Their results indicate, under experimental conditions, OH-Cbl, MetCbl, and AdeCbl inhibit HIV-1 infection of normal human blood monocytes and lymphocytes. They suggest that because of the relative ease with which high blood and tissue levels of cobalamins can be achieved *in vivo*, these agents "should be considered as potentially useful agents for the treatment of HIV-1 infection."[36]

**Homocysteinemia and Methylmalonic Acidemia:** Elevated levels of homocysteine and methylmalonic acid can be metabolic indications of decreased levels of the coenzyme forms of vitamin B12, or the presence of a genetic enzyme defect.

Propelled by evidence that elevated concentrations are associated with an increased risk for a variety of chronic clinical conditions, homocysteine has received a tremendous amount of emphasis in the scientific literature. Because MetCbl is a potential donor of the methyl group required to regenerate methionine from homocysteine, a theoretical argument can be used to justify this coenzyme form of vitamin B12 as a part of the nutritional protocol for lowering homocysteine. Araki et al have demonstrated that elevated homocysteine levels are reduced following parenteral treatment with MetCbl. In their trial, ten diabetic patients with elevated plasma levels of homocysteine were administered 1000 mcg of MetCbl i.m. daily for three weeks. Following treatment, the plasma levels of homocysteine decreased from a mean value of 14.7 to 10.2 nmol/ml ($P < 0.01$).[37]

Methylmalonic acidemia is generally the result of an inherited metabolic defect, although it is possible to have elevated levels of this metabolite due to a functional deficiency of AdeCbl in the absence of an inherited defect. Bhatt et al have suggested a transient response to OH-Cbl might be misleading and might subsequently impair the therapeutic response to AdeCbl. They further suggest AdeCbl be the cobalamin therapy of choice for individuals with biochemically uncharacterized methylmalonic acidemia.[38]

**Liver Disease:** AdeCbl and MetCbl appear to offer a theoretical advantage over either CN-Cbl or OH-Cbl in the treatment of liver disorders. Although high blood levels of vitamin B12 have been reported in patients with hepatitis, cirrhosis, and other liver disease, it is not unusual to actually have a correspondingly low liver tissue concentration of vitamin B12 and its coenzymes. Glass et al proposed this observation might be due to an impaired ability of the liver to absorb vitamin B12 from the portal circulation.[39]

Because a vitamin deficiency can persist during liver disease despite oral vitamin supplementation, Leevy et al have suggested the liver's ability to convert vitamins into metabolically active forms might be compromised.[40] It is possible, during these pathological conditions, the liver will not contain adequate supplies of the needed cofactors to optimally form coenzyme analogues of vitamin B12. Because of these factors, Iwarson et al suggested that vitamins used in the treatment of liver disorders should be given in their metabolically active form, thereby eliminating the need for conversion to occur in damaged liver cells.[41]

In experimentally induced lipid peroxidation of liver microsomes resulting from poisoning of rabbits with phenylhydrazine, MetCbl and AdeCbl were shown to modulate the activity of the monooxygenase system. MetCbl appeared to induce the system, and AdeCbl seemed to repress the system. Administration of MetCbl into poisoned rabbits stimulated the activities of dimethyl aniline N-demethylase, aniline p-hydroxylase, NADPH-cytochrome P-450, and NODH-cytochrome b5 reductases as compared with normal state, while AdeCbl inhibited the reduction of all the monooxygenase system patterns studied. Although the therapeutic relevance of these actions of the coenzyme forms of vitamin B12 on the monooxygenase system is open to debate, the authors observed that both of these coenzymes contributed to normalization of lipid peroxidation in liver microsomes of poisoned rabbits.[42] AdeCbl also exerts hepato-protective activity after carbon tetrachloride-induced hepatitis in rabbits. The normalization of results from the sulfobromophthalein test and the normalization of activity of sorbitol dehydrogenase and alanine aminotransferase indicate AdeCbl enhanced the recovery process.[43]

In an experimental model, a low protein choline-deficient diet, although it did not change total cobalamin content in the liver of rats, significantly decreased total and non-protein sulfhydryl (SH)-group levels as well as GSH transferase activity in the liver. MetCbl (but not AdeCbl) administration restored non-protein SH-group levels and GSH transferase activity, and administration of both MetCbl and AdeCbl normalized total SH-group content.[44]

AdeCbl appears to be a useful supplement for support of patients with hepatitis A. Two groups of patients from the same hepatitis A epidemic received either AdeCbl or OH-Cbl. Patients were given 1 mg per day i.m. for the first 12 days and then received 1 mg orally for the next 23 days. The group treated with AdeCbl had a quicker return to normal of serum aminotransferase levels.[41] Fossati reported improvements in body weight and appetite in adults with liver disease and chronic pulmonary tuberculosis following supplementation with 6 mg/day of AdeCbl for three months.[45]

Medina et al treated 37 people suffering from viral hepatitis with either AdeCbl or CN-Cbl. Their observations indicate the AdeCbl was significantly more efficacious than CN-Cbl in normalizing total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase values. The AdeCbl was administered i.m. at a dose of 1 mg per day for the first 12 days and then orally for the next 12 days. After 24 days total bilirubin was normal in 13/18, SGOT in 15/18, SGPT in 10/18, and alkaline phosphatase in 18/18 subjects receiving AdeCbl.[46]

Resta et al have reported a combination of AdeCbl, along with liver extract, adrenal cortex extract, and nucleosides, is effective in normalizing SGOT, SGPT, and total bilirubin values in patients with a variety of acute liver diseases. In their study, one group of patients received the extracts (E) and another group of patients received the extracts plus AdeCbl (E + C). After 21 days of supplementation, total bilirubin, SGOT and SGPT were normalized in 18 of 20 patients in the E + C group. Corresponding values in the group receiving E alone were 15/20, 13/20, and 12/20.[47] Teti et al have similarly reported improvements in parameters of liver function following administration of a complex containing 3 mg of AdeCbl.[48]

**Sleep Disturbances:** The use of MetCbl in the treatment of a variety of sleep-wake disorders is very promising. Although the exact mechanism of action is not yet elucidated, it is possible MetCbl is needed for the synthesis of melatonin, since the biosynthetic formation of melatonin requires the donation of a methyl group. Based on

available information, MetCbl appears to be capable of modulating melatonin secretion, enhancing light-sensitivity, and normalizing circadian rhythm.

Uchiyama et al have reported that intravenous injections of MetCbl increased rectal temperature in the later hours of the daytime and correspondingly improved alertness, as assessed with a visual analog scale, during the same time interval. They suggest these observations were mediated by an effect of MetCbl on the circadian clock.[49]

Tomoda et al report a case of a 13-year-old male with adrenoleukodystrophy who had developed a sleep-wake disorder subsequent to his complete loss of vision. His sleep-wake cycle had been 25 hours; however, following administration of MetCbl, his sleep-wake rhythm was normalized. After MetCbl therapy, circadian rhythms in his plasma melatonin and beta-endorphin levels approximated those of healthy volunteers, and his peak cortisol time shifted backward.[50]

Yamada et al have reported the successful treatment of a 32-year-old male patient, who had suffered from recurrent hypersomnia for 12 years, with administration of MetCbl. During this period of time, the individual had experienced several episodes of hypersomnia, lasting a few days at a time, reoccurring each year. The individual had also reported the frequency of these episodes had increased during the past two years. MetCbl was administered for six months, during which time no episodes of hypersomnia were experienced. After cessation of treatment, over a follow-up observation period of 17 months, no episodes of hypersomnia were noted.[51]

Ohta et al report that two adolescent patients suffering from persistent sleep-wake schedule disorders appear to have responded to treatment with MetCbl. In this report, a 15-year-old girl diagnosed with delayed sleep phase syndrome (DSPS) and a 17-year-old boy with free-running sleep-wake rhythm (hypernychthemeral syndrome), had consistently complained of not being able to attend school despite trials of several different medications. Immediately following administration of 3 mg/day of MetCbl, an improvement of both sleep-wake rhythm disorders was observed. Serum concentrations of vitamin B12 during treatment were in the high range of normal or above normal. The duration of the sleep period of the DSPS patient decreased gradually from 10 hours to 7 hours, and the time of sleep onset advanced from 2 a.m. to midnight. The period of the sleep-wake cycle of the hypernychthemeral patient was 24.6 hours before treatment and 24.0 hours after treatment. Neither of these patients had shown any laboratory or clinical evidence suggestive of vitamin B12 deficiency prior to the therapy.[52]

Mayer et al investigated the effects of MetCbl and CN-Cbl on circadian rhythms, well-being, alertness, and concentration in healthy subjects. Six women and 14 men were randomly assigned to receive either 3 mg of MetCbl or 3 mg of CN-Cbl for 14 days. All individuals were initially observed for nine days prior to beginning either supplementation regime. Activity from 2300-0700 hours increased significantly with supplementation of both forms of vitamin B12. However, sleep time was only significantly reduced in the group receiving MetCbl. In this group, improvements in subjective parameters of "sleep quality," "concentration," and "feeling refreshed," as determined by a visual analog scale, were correlated with vitamin B12 plasma levels during the first week of MetCbl supplementation. No observed changes in either cortisol excretion or temperature were noted in individuals receiving either form of vitamin B12. The authors concluded that, "...only methylcobalamin has a positive psychotropic alerting effect with a distribution of the sleep-wake cycle toward sleep reduction."[53]

Eight young males were subjected to a single-blind cross-over test to determine the effects of MetCbl on the phase-response of the circadian melatonin rhythm to a single bright light exposure. MetCbl (0.5 mg/day) was injected intravenously at 1230 hours for 11 days. Starting on day 12, this regimen was superseded by oral administration of MetCbl (2 mg tid) for seven days. The melatonin rhythm before the light exposure showed a smaller amplitude in the individuals treated with MetCbl than in those receiving the placebo. The light exposure phase-advanced the melatonin rhythm significantly in the MetCbl group, but not in the placebo group, indicating MetCbl enhanced the light-induced phase-shift in the human circadian rhythm.[54]

**Miscellaneous:** A combination of a coenzyme complex combining AdeCbl, pyridoxal phosphate, and phosphaden appears to be efficacious in the treatment of patients with infectious allergic myocarditis. Mazurets et al report a corrective action of this metabolic therapy on myocardial enzymatic status. Antiarrhythmic and cardiotonic actions of the coenzyme complex were also noted.[55]

Jaludin et al included sixty patients with Bell's palsy in an open randomized trial. Patients were assigned to one of three treatment groups: steroid, MetCbl, or MetCbl + steroid. The quickest time required for complete recovery of facial nerve function occurred in the group receiving MetCbl alone (mean of 1.95 +/- 0.51 weeks); however, the mean recovery time of the group receiving MetCbl and steroid treatment was similar (2.05 +/- 1.23 weeks). Individuals receiving only steroid treatment had a mean recovery time of 9.60 +/- 7.79 weeks). The authors also noted the facial nerve score after 1-3 weeks of treatment was significantly better in individuals receiving MetCbl than in those only receiving steroid therapy. The improvement of concomitant symptoms was also better in the groups treated with MetCbl.[56]

Katsuoka et al reported a case of a 48-year-old woman with a positive response to MetCbl. Her initial complaint was gait disturbance; however, by the time she was evaluated, her symptoms had progressed to motor weakness, sensory disturbances in her limbs, and dementia. She also had widespread coarse hair. In response to injections of 500 mcg of MetCbl every other day, the patient's paresthesia resolved, hand grip strength improved, and her dementia was evaluated as reduced. Her gait also improved, until she was able to walk on tiptoe, and her hair texture returned to normal.[57]

## Dosage and Toxicity

A therapeutic dose for conditions requiring MetCbl would be a minimum of 1500 mcg and a maximum of 6000 mcg per day. No significant therapeutic advantage appears to occur from dosages exceeding this maximum dose; however, it is likely that beneficial physiological effects occur at dosages as low as 100 mcg per day, especially if this dose is given repetitively over time.

A therapeutic dose for AdeCbl is 1000-6000 mcg per day. Similarly, some physiological benefits are likely to occur at repetitive doses far below this therapeutic range.

Both MetCbl and AdeCbl have been administered orally, intramuscularly, and intravenously; however, positive clinical results have been reported irrespective of the method of administration. It is not clear whether any therapeutic advantage is gained from non-oral methods of administration.

MetCbl and AdeCbl have usually been administered in divided doses three times daily. These supplements have excellent tolerability and no known toxicity. AdeCbl has been administered safely during pregnancy. No rationale exists to suspect MetCbl would not also be safe during pregnancy.

## Conclusion

AdeCbl and MetCbl are the coenzyme forms of vitamin B12 utilized in the vitamin B12-dependent enzymes in humans. Because the coenzyme forms bypass several of the enzymatic reactions required for the formation of the functional forms of vitamin B12, they offer a theoretical advantage in cobalamin supplementation. Both AdeCbl and MetCbl are retained in the body better and increase tissue concentrations of cobalamin better than CN-Cbl. Additionally, the coenzyme forms of vitamin B12 demonstrate a range of activity and clinical results not shown by the other supplemental forms of vitamin B12.

It is important to remember that circulating levels of vitamin B12 are not always a reflection of tissue levels, and that even if an adequate supply of cobalamin appears in the circulation, a functional deficiency of the coenzyme forms might coexist in tissues and other body fluids. Although CN-Cbl will usually increase circulating levels of cobalamin, its ability to increase tissue levels of the active forms of vitamin B12 can be limited in a range of sub-clinical and clinical conditions. Even in a best case scenario, the activation of CN-Cbl to either AdeCbl or MetCbl does not occur instantly, possibly occurring over 1-2 months, and requires the interaction of GSH, reducing agents, possibly alpha-tocopherol, and in the case of MetCbl, SAM and the active form of folic acid.

The use of either AdeCbl and/or MetCbl offers a significant biochemical and therapeutic advantage over other existing forms of vitamin B12, and should be considered as a first-line choice for correcting vitamin B12 deficiency and treating conditions shown to benefit from cobalamin administration.

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