

## **Spirulina Pacifica as a Source of Cobalamin Vitamin B-12**

Vitamin B-12 is actually a family of derivatives, some forms being active for humans and other forms which are not. The active form, cobalamin, is of significant interest to strict vegetarians because this particular vitamin is essential for normal maturation and development of blood cells (erythrocytes), but it is not normally obtained by consuming pure plant foods. The ultimate source of all vitamin B-12 is derived at the microbial production level. Although plants and animals cannot synthesize or store vitamin B-12, it is effectively passed through the food chain, accumulated and recycled by animals. Therefore, vitamin B-12 is abundant within animal products such as fish, meat, eggs and milk and lactoovovegetarians or lactovegetarians ingest an ample supply for normal functions (Herbert 1994).

The discovery of vitamin B-12 was from studies of a previously incurable disease, pernicious anemia. The condition is nearly identical to folate deficiency but leads to irreversible degeneration of the nervous system if left untreated. Two Harvard physicians, George Monot and William Murphy, found in 1926 that symptoms of the disease could be alleviated by feeding patients large amounts of raw liver, they described this active “liver factor” as vitamin B-12. In 1948 vitamin B-12 was isolated and crystallized, but it was not until 1957 that a combination of chemical methods and x-ray diffraction was utilized to ascertain the precise molecular structure. Subsequently, it has been shown that vitamin B-12 is required for two key enzymatic steps in mammalian metabolism, synthesis of methionine from homocysteine (methionine synthetase) and the isomerization of methylmalonyl-CoA to succinyl-CoA (methylmalonyl-CoA mutase). The latter reaction is involved the catabolism of odd-chained fatty acids and several amino acids. Because of reduced flux through the methylmalonyl CoA mutase reaction, abnormalities in fatty acid metabolism likely cause the subsequent neurological tissue damage.

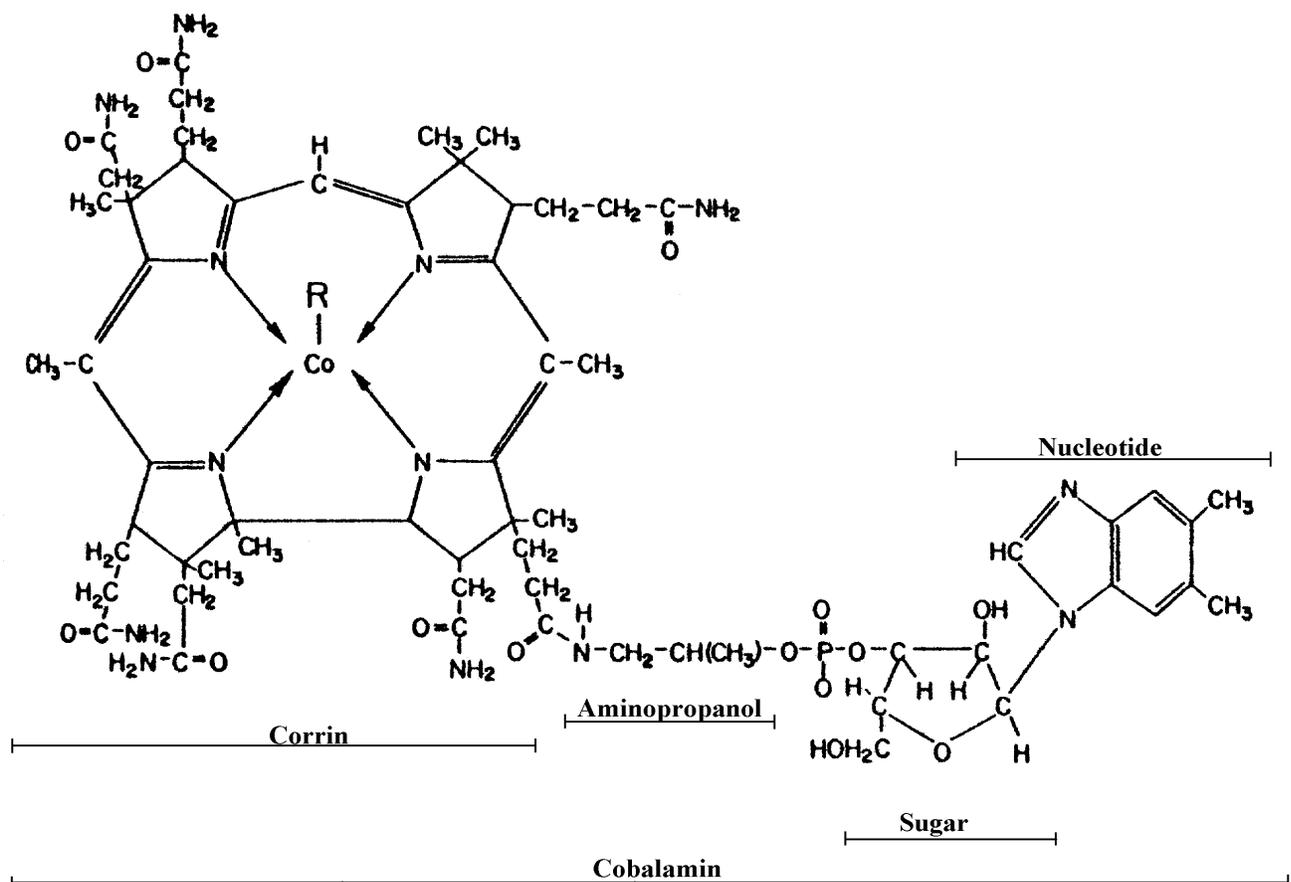
Pernicious anemia is a disease of the stomach that initiates as megaloblastic anemia. Normally, the gastric tissue secretes a glycoprotein called “intrinsic factor” which complexes with ingested vitamin B-12 in the digestive tract and helps promote its absorption through the small intestine and into the bloodstream. The affliction of pernicious anemia results from the lack of secreted intrinsic factor and resulting deficiency of vitamin B-12 absorption. Uncomplexed vitamin B-12 can be absorbed, but with such low efficiency that massive doses must be administered to prevent or cure the disease. Ulcer patients or those that have undergone stomach removal often have special requirements to prevent pernicious anemia.

Not all forms of vitamin B-12 produced by microbes are metabolically active for mammals. Some forms are termed “analogs” or “corrinoids” because they are sufficient for enzymatic reactions and the growth of microorganisms, but do not fulfill the specific roles in humans. The vitamin B-12 molecule has four characteristic components that determine whether it is “human-active” or not. The largest constituent and core of vitamin B-12 is the corrin ring system which is somewhat similar to the porphyrin ring system of hemoglobin. At the heart of the corrin ring is cobalt, which can be bound to various adducts and still remain human-active (depicted as an “R”). The “R” adducts can be one of two types, either a methyl group as in methylcobalamin or 5'-deoxyadenosyl known as coenzyme B-12 and provide human activity. Additionally, humans have the enzymatic machinery to convert other “R” groups such as a

hydroxocobalamin and others into one of the two types of active cobalamin adducts. The dominant forms in meat are adenosylcobalamin and hydroxocobalamin, whereas dairy products (including human milk) contain primarily methylcobalamin and hydroxocobalamin (Herbert 1987). Because cyanide stabilizes the cobalamin molecule so well, cyanocobalamin is the chemical form of the molecule most often produced in commercial formulations, usually by microbial fermentation. This form is water-soluble, heat stable and when taken orally it is converted to forms nutritionally active to humans by exchange of the cyanide group.

The other three vital components of the cobalamin structure are aminopropanol, a sugar group, and a nucleotide (Figure 1). Microbes synthesize various combinations of these four components, however all four units must be present to be active for humans. Thus, the vitamin B-12 family encompasses both the analog “corrinoids” that contain corrin and some of the side chains which can be utilized by only bacteria and algae, and the specific human-active form with corrin and all three side chain components, specifically called “cobalamin”.

**Figure 1-Components of Cobalamin Vitamin B-12**



A majority of scientific papers and nutrition information lists total vitamin B-12 activity as the sum of both human-active and the analog values. This is a result of the current official assay that is used to report vitamin B-12 activity in foods and feeds. The standard US Pharmacopeia (USP) method employs a water extract of the test sample that is fed to a special B-12-deficient strain of the bacterium, *Lactobacillus leichmannii*. The amount of vitamin B-12 in the sample can then be determined by the amount of growth from the bacterium. The underlying flaw of the assay is that what is active for the bacterium is not necessarily active for humans. That is, *L. leichmannii* responds to non-cobalamin corrinoids, which in turn leads to erroneously high vitamin B-12 measurements.

Clearly, a new standard assay is needed other than the current USP standard to accurately determine human-active vitamin B-12 from analog corrinoids. One assay has been developed using a technique called differential radioassay (Herbert 1984;Herbert 1985). This procedure uses the test mixture to first measure the total content of corrinoids by using a binder that attaches to the corrin nucleus. Then, the human-active fraction is assayed by using a substance that attaches to both ends of the cobalamin molecule, the corrin end and the nucleotide end. By subtracting the cobalamin fraction from the corrin fraction one can determine the amount of analog and human-active in a mixture, thus the term, “differential radioassay of analogs”. Other radioisotope dilution assays (RIDA) have been developed and commercialized into kits, but the diagnostic credibility of RIDA has been discredited because of inconsistent sensitivities to metabolically inactive B-12 cobalamins (Kolhouse 1978;LeFebre 1980;Oxley 1984)

Fortunately, an alternate microbial growth assay has been verified which is highly specific for human-active vitamin B-12. It has been demonstrated that an isolate of the microorganism, *Ochromonas malhamensis*, requires vitamin B-12 for growth, but responds only to the human-active cobalamin form unlike *L. leichmannii*. As in the USP assay, a water extract of a test sample is fed to this organism, and the cobalamin concentration can be accurately calculated from the growth curve. Thus, the *O. malhamensis* assay may be the “gold standard” for metabolically active B-12 that researchers have been seeking (Baker, 1986;Ford, 1953). Although this procedure is the most specific assay for “human-active” cobalamin B-12, it has not been adopted as the USP standard yet.

The normal omnivorous American diet contains 5-15 micrograms of vitamin B-12 per day of which the excess is continually accumulated and stored in the liver throughout life (Herbert 1987). Normal humans have the ability to reabsorb vitamin B-12 very efficiently through the recycling of bile, which also serves the function of removing inactive forms of vitamin B-12 from the active forms. About 5-10 micrograms of vitamin B-12 may be secreted in the bile each day, but 3-5 micrograms of that is resorbed to the body through enterohepatic recirculation. This reabsorption of bile vitamin B-12 accounts for the fact that it takes about 20-30 years to deplete stores of vitamin B-12 after one discontinues dietary B-12 consumption, whereas those with absorption deficiencies or metabolic dysfunction may deplete in 3 years. As body stores begin to decline and daily bile output of B-12 falls, reabsorption efficiency rises to nearly 100%. A vegetarian may at times obtain more cobalamin from enterohepatic recirculation than from dietary sources (Kanazawa and Herbert, 1983; Herzlich and Herbert, 1984;Herbert 1988). An additional

non-dietary source of absorbable vitamin B-12 may be from bacteria in the small intestine of humans (Herbert 1984, Albert 1990). Intestinal bacteria can produce 5 ug of cobalamins and 95 ug of B-12 analogues per 24 hours. Thus, vitamin B-12 deficiency can be attained by inadequate ingestion over decades, defective absorption or utilization (metabolic defects), increased requirement (pregnancy, hyperthyroidism), increased excretion (alcoholism), or increased destruction as by megadoses of vitamin C (Herbert, 1994). The most common cause of omnivore vitamin B-12 deficiency is a defect in gastric/pancreatic metabolism, small intestinal dysfunction or age-dependent loss of gastric secretory activity (Herbert 1984).

Studies of normal patients with no stores of cobalamin have shown that only 1 microgram per day is required to quickly reverse early pernicious anemia. A dramatic increase in young red blood cells and reticulocytes and a rise to normal hemoglobin and hematocrit was observed within days. The minimum daily requirement (MDR) for cobalamin appears to be even lower, 0.2-0.25 micrograms per day absorbed from food is adequate for normal people (Herbert 1987).

It has been found that a significant percentage of the activity in 'B-12 enriched' foods are inactive analogs. Hamburger, cottage cheese and boiled eggs averaged about 10% analogs while milk products (whole, evaporated, nonfat) averaged about 30%, whereas nearly 100% is inactive from tempeh. A typical 'VA lunch' consisting of potato soup, cottage cheese, lettuce, peaches, crackers, butter and milk was analyzed and found to contain 40% inactive analogs (Herbert 1984b). This is not a problem for normal people, as it has been established that inactive B-12 analogs exist in human liver, red blood cells, brain and mineral and vitamin supplements (Kanazawa 1983; Herbert 1982). Normal humans are able to discriminate between the active and non-active forms as both have always been in nature and in foods. For example, the role of the plasma transport proteins transcobalamins I and III are to deliver non-functional B-12 analogs to the liver for discard in the bile (Burger, 1975, Jacob 1980, and Kanazawa 1983b). Moreover, an effective enterohepatic circulation recycles the vitamin from bile and other intestinal secretions accounting for its long biological half-life. During this process, vitamin B-12 analogues are preferentially excreted while human-active cobalamins are largely resorbed (Kanazawa 1983). However, those with genetic defects in vitamin B-12 metabolism or absorption deficiencies may have special requirements to supplement diets with pure cobalamin. Such patients should be under physician treatment and guidance to closely monitor cobalamin levels.

The microalgae, *Spirulina*, has often been mentioned as source of vegetarian vitamin B-12. *Spirulina Pacifica* is a spray-dried powder or tablet produced from the bacterial microalgae, *Spirulina platensis*. *Spirulina* has also been consumed for centuries as a major source of nutrition and protein by the Kanembu people who live along the shores of Lake Chad in Africa. *Spirulina* is collected from the waters edge in fine-woven baskets, transferred to clay pots or gourds, and dried under the sun into small biscuits called "dihe". Dihe' is combined into the majority of sauces and is eaten in about 70% of their meals, amounting to about 10-12 grams per person. In times of famine, dihe' is a main ingredient of their diets (Ciferri O., 1983; Furst P.T., 1978). *Spirulina* has been marketed and consumed as a human food in over 60 countries, and approved as a food for human and/or animal consumption by most governments, health agencies and associations (Henrickson, 1989). *Spirulina* has been subjected to extensive safety studies, independent feeding studies in France, Mexico and Japan demonstrated no undesirable results or toxic effects on

humans, rats, pigs, chickens, fish and oysters. There have been no negative effects reported for acute toxicity, chronic toxicity or reproduction (Takemoto K., 1982; Atatsuka, 1979; Chamorro-Cevallos, 1980). *Spirulina* has been classified by The Bergey's Manual of Determinative Bacteriology (Ninth Edition) as in Table 1.

**Table 1-Classification of Spirulina**

Group 11	Oxygenic Phototropic Bacteria
Family	Cyanobacteria
Order	Oscillatoriales (subgroup 3)
Genus	Spirulina
Species	Platensis
Strain	Pacifica

Assays of vitamin B-12 in *Spirulina Pacifica* using the standard US Pharmacopeia (USP) method to measure total corrinoids reveals an average activity of about 7 micrograms per 3 grams of *Spirulina* (one serving size). Using the *O. malhamensis* assay in parallel to specifically measure human-active cobalamins the assay exhibits an average activity of 2.5 micrograms per 3 grams of *Spirulina*. These figures demonstrate that about 36% of the total corrinoid vitamin B-12 activity in *Spirulina* is human active. An additional non-dietary source of low amounts of absorbable vitamin B-12 may be obtained from bacteria in the small intestine of humans (Albert, 1980). *Spirulina* is not an animal source, but rather a vegetarian source of cobalamin B-12 amongst many other nutrients and antioxidant carotenoids. Normal healthy vegetarians should be able to attain sufficient levels of cobalamin to fulfill their requirements with a few serving sizes daily. As before, those with metabolic defects or absorption difficulties should always consult medical advice and monitor their condition closely.

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