Role Of Cyanocobalamin In Nutrition Ruminant: A Review

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Abstract: Vitamin B_{12}, vitamin B12 also called cobalamin, is a water soluble vitamin by prominent effects in the normal functioning of the brain and nervous system, and for the structure of blood. It is one of the eight B vitamins. It is normally include in the metabolism of every cell of the human body, particularly affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis. Vitamin B_{12} regular of a class of chemically-related compounds, all of that have vitamin activity. It contains the biochemically rare element cobalt. Biosynthesis of the basic structure of the vitamin in nature is only accomplished by simple organisms such as some bacteria and algae, but conversion between different forms of the vitamin can be accomplished in the human body. A normal synthetic form of the vitamin, cyanocobalamin, doesn't occur in nature, but is used in many pharmaceuticals and supplements, and as a food additive, thus of its stability and lower cost. In the body it is converted to the physiological forms, methylcobalamin and adenosylcobalamin, leaving behind the cyanide, albeit in minimal concentration. More lately, hydroxocobalamin, methylcobalamin, and adenosylcobalamin can also be found in more expensive pharmacological products and food supplements. Intrinsic factor is crucial for the normal absorption of B_{12}, so a lack of intrinsic factor, as seen in pernicious anemia, causes a vitamin B_{12} deficiency. Many other subtler kinds of vitamin B_{12} deficiency and their biochemical effects have since been elucidated.
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1. Introduction

Vitamins are defined as a group of complex organic compounds current in nominal amounts in natural foodstuffs that are essential to normal mal metabolism and lack of which in the diet causes deficiency diseases. Vitamins consist of a mixed group of chemical compounds and are not related to each other as are proteins, carbohydrates, and fats. Their classification together depends not on chemical characteristics but on function. Vitamins are differentiated from the trace elements, also present in the diet in small quantities, by their organic nature.

Vitamins are required in trace amounts in the diet for health, growth, and reproduction. Omission of a single vitamin from the diet of a species that requires it will produce deficiency signs and symptoms. Many of the vitamins function as coenzyme others have no such role, but perform certain essential functions. Some vitamins deviate from the preceding definition in that they don't always need to be constituents of food. Certain substances that are considered to be vitamins are synthesized by intestinal tract bacteria in quantities that are often adequate for body needs. However, an obvious distinction is made between vitamins and substances that are synthesized in tissues of the body. Ascorbic acid, for example, can be synthesized by most species of animals, except when they are young or under stress conditions. Likewise, in most species, niacin can be synthesized from the amino acid tryptophan and vitamin D from action of ultraviolet light on precursor compounds in the skin. Thus, under certain conditions and for specific species, vitamin C, niacin, and vitamin D would not always fit the classic definition of a vitamin.

Classically, vitamins have been divided into two groups based on their solubility's in fat solvents or in water. Thus, fat-soluble vitamins include A, D, E, and K, while vitamins of the B-complex and C are classified water soluble. Fat-soluble vitamins are found in foodstuffs in association with lipids. The fat-soluble vitamins are absorbed along with dietary fats, apparently by mechanisms similar to those involved in fat absorption. Conditions favorable to fat absorption, such as adequate bile flow and good micelle formation, also favor absorption of the fat-soluble vitamins (Scott et al., 1982).

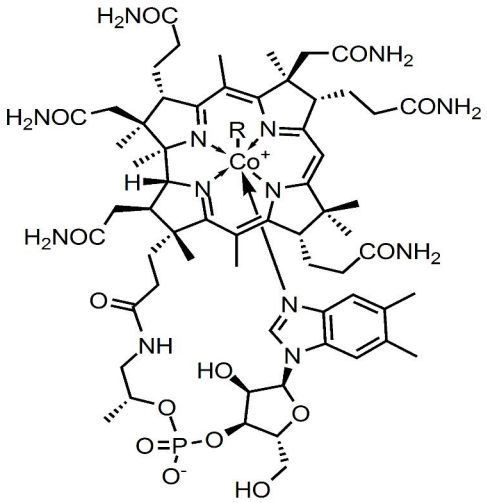
2. Chemical structure

Vitamin B_{12} is a collection of cobalt and corrin ring molecules that are defined whit their special vitamin serve in the body. All of the substrate cobalt-corrin molecules from that B_{12} is made, must be synthesized whit bacteria. Thus, after this synthesis is complete, the body has a limited power to alter any form of B_{12} to another, whit means of enzymatically removing certain prosthetic chemical groups from the cobalt atom. The various forms of B_{12} are all deeply

red colored, due to the color of the cobalt-corrin complex.

Cyanocobalamin is one such "vitamer" in this B complex, because it can be metabolized in the body to an active co-enzyme form. therefore, the cyanocobalamin form of B_{12} doesn't occur in nature normally, but is a byproduct of the fact which other forms of B_{12} are avid binders of cyanide (-CN) that they pick up in the process of activated charcoal purification of the vitamin after it is made by bacteria in the commercial process. Thus the cyanocobalamin form of B_{12} is easy to crystallize and isn't sensitive to air-oxidation, it is normally used as a form of B_{12} for food additives and in many common multivitamins. therefore, this form is not perfectly synonymous with B_{12}, in as much as a number of substances have B_{12} vitamin activity and can properly be labeled vitamin B_{12}, and cyanocobalamin is but one of them. Pure cyanocoblamin possesses the deep pink colour related by most octahedral cobalt (II) complexes and the crystals are well formed and easily grown up to milimetre size.

Hydroxocobalamin is another form of B_{12} normally encountered in pharmacology, but that isn't commonly present in the human body. Hydroxocobalamin is sometimes denoted B_{12a}. This form of B_{12} is the form produced whit bacteria, and is what is changed to cyanocobalmin in the commercial charcoal filtration stride of production. Hydroxocobalamin has an avid affinity for cyanide ion and has been used as an antidote to cyanide poisoning. It is supplied normally in water solution for injection. Hydroxocobalamin is thought to be altered to the active enzymic forms of B_{12} more easily than cyanocobalamin, and since it is little more expensive than cyanocobalamin, and has longer retention times in the body, has been used for vitamin replacement in situations where added reassurance of activity is desired. Intramuscular administration of hydroxocobalamin is also the preferred treatment for pediatric patients by intrinsic cobalamin metabolic diseases, for vitamin B_{12} deficient patients with tobacco amblyopia, and for treatment of patients by pernicious anemia who have optic neuropathy. B_{12} is the most chemically complex of all the vitamins. The structure of B_{12} is based on a corrin ring, that is similar to the porphyrin ring found in heme, chlorophyll, and cytochrome. The central metal ion is cobalt. Four of the six coordination sites are provided by the corrin ring, and a fifth whit a dimethylbenzimidazole group. The sixth coordination site, the center of reactivity, is variable, being a cyano group (-CN), a hydroxyl group (-OH), a methyl group ($-CH_3$) or a 5'-deoxyadenosyl group, respectively, to yield the four B_{12} forms mentioned above. Historically, the covalent C-Co bond is one of first examples of carbon-metal bonds to be discovered in biology. The hydrogenases and, whit necessity, enzymes associated with cobalt utilization, involve metal-carbon bonds. Vitamin B12 is a dark red, crystalline, hygroscopic substance, freelysoluble in water and alcohol but insoluble in acetone, chloroform, or ether. Cyanocobalamin has a molecular weight of 1354 and is the most complex structure and heaviest compound of all the vitamins. Oxidizing and reducing agents and exposure to sunlight tend to destroy its activity. Losses of vitamin B12 during cooking are usually not excessive (Fig 1 and 2).



R = 5'-deoxyadenosyl, Me, OH, CN

Figure 1: Chemical structure of Vitamin B_{12} from www.wikipedia.org/wiki/Vitamin_B12

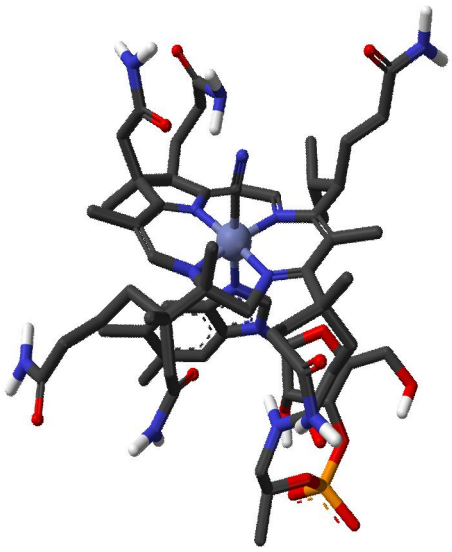


Figure 2: Aspect 2 chemical structure of Vitamin B_{12} from www.wikipedia.org/wiki/Vitamin_B12

3. Metabolism

Digestion and Absorption

Passage of vitamin B12 through the intestinal wall requires intervention of certain transporter compounds able to bind the vitamin molecule. Vitamin B12 in the diet is bound to food proteins. In the stomach, the combined effect of gastric acid and peptic digestion releases the vitamin which is then bound to a nonintrinsic factor–cobalamin complex (Toskes et al., 1973).

The nonintrinsic protein that is secreted in the saliva has been named cobalophilin, formerly known R-proteins, because of their rapid electrophoretic mobility compared by other cobalamin-binding proteins. Vitamin B12 is bound preferentially to cobalophilin in the acid medium of the stomach rather than to intrinsic factor. The B12 remains bound to cobalophilin in the immencly alkaline environment of intestine until pancreatic proteases particularly degrade the cobalophilin protein and thereby enable B12 to become bound exclusively to intrinsic factor. Thus, patients by pancreatic insufficiency absorb B12 poorly (Jorgensen et al., 1991).

This mal absorption is completely corrected whit administration of pancreatic enzymes or purified trypsin.

A prerequisite for intestinal absorption of physiological amounts of cobalamin is binding to intrinsic factor. Intrinsic factor is a glycoprotein synthesized and secreted by parietal cells of the gastric mucosa. Atrophy of the fundus, where intrinsic factor is produced, and lack of free HCl are usually related by pernicious anemia (Behrns et al., 1994).

Gastric juice defects are responsible for most cases of food-vitamin B12 mal absorption (Carmel, 1994).

The formation of this intrinsic factor complex protects the vitamin from bacterial utilization and degradation as it traverses the lumen of the small intestine to the terminal ileum, where absorption occurs (Ellenbogen and Highley, 1970).

The intrinsic factor–B12 complex is transiently attached to an ileal receptor. The proximal small intestine doesn't have the ability to enhance absorption of the vitamin—only the ileum has this property. In the ileum, the intrinsic factor moiety of the intrinsic factor–B12 complex binds to a specific receptor protein on the microvillus membrane of brush borders of intestinal epithelial cells. Next there is transport of vitamin B12 from the receptor intrinsic factor–B12 complex through the epithelial cell to portal blood.

The absorption of vitamin B12 is limited by the numerous of intrinsic factor–vitamin B12 binding sites in the ileal mucosa, that not more than about 1 to 1.5 μg of a single oral dose of the vitamin in humans can be absorbed (Bender, 1992).

The absorption is also slow; apex blood Concentrations of the vitamin isn't achieved for some 6 to 8 hours after an oral dose. When B12 enters the portal blood, it isn't longer bound to intrinsic factor but to specific carry proteins called transcobalamins. 3 binding proteins have been identified in normal human serum and are designated as Trans cobalamin I, II, and III. The transcobalamins are synthesized by several tissues, including intestinal mucosa and liver, and have been shown to deliver B12 to diverse tissues, such as liver, kidney, Spleen, heart, lung, and small intestine (Rothenberg and Cotter, 1978).

Transcobalamin II emerges to be primarily concerned by transport of vitamin B12, therefore transcobalamin I is include in storage of the vitamin.

The function of transcobalamin III is to provide a mechanism for returning vitamin B12 from peripheral tissues to the liver, as well as for clearance of other corrinoids without vitamin activity, which may arise either from foods or from the products of intestinal bacterial action and be absorbed passively across the lower gut (Bender, 1992).

These corrinoids are then secreted into the bile, bound to cobalophilins. Like dietary vitamin B12 bound to salivary cobalophilin, the biliary cobalophilins are hydrolyzed in the duodenum, and the associated vitamin B12 binds to intrinsic factor, permitting reabsorption into the ileum.

To summarize, B12 absorption for most species studied requires the following: (1) adequate quantities of dietary B12, (2) normal stomach for breakdown of food proteins for release of B12, (3) normal production of cobalophilin secreted in saliva, (4) normal stomach for production of intrinsic factor for absorption of B12 through the ileum, (5) normal pancrease (trypsin) required for release of bound B12 prior to combining the vitamin by the intrinsic factor, and (6) normal.

Ileum with receptor and absorption sites. Additional factors which diminish vitamin B12 absorption included deficiencies of protein, iron, and vitamin B6; thyroid removal; and dietary tannic acid (Anonymous, 1984).

Intrinsic-factor concentrates prepared from one animal's stomach don't in all cases increase B12 absorption in other species or in humans. There are structural differences in the B12 intrinsic factor among species. Likewise, species differences exist for B12 transport proteins (Polak et al., 1979).

Intrinsic factor has been demonstrated in the human, monkey, dog, pig, cow, rabbit, hamster, fox, lion, tiger. It hasn't been detected in the guinea pig, horse, sheep, chicken, or a number of other species. The dog stomach produces only small amounts of intrinsic factor, with larger amounts produced with the pancreas (Simpson et al., 1989).

Absorption of vitamin B12 doesn't completely depend on active intervention of the intrinsic factor. Both active and passive mechanisms exist for absorption of B12 (Herbert, 1990).

The passive mechanism, simple diffusion, has low efficiency and is operative

Throughout the digestive tract; it becomes practically important only in the presence of large quantities of the vitamin, in excess of those present in most foods.

About 3% of ingested cobalt is altered to vitamin B12 in the rumen. Of the vitamin B12 produced, only 1 to 3% is absorbed. In the rumen, iron interacts by cobalt so that iron deficiency enhances cobalt absorption (Keen and Graham, 1989).

As in most strains, the absorptive zone for ruminants is the lower portion of the small intestine. Substantial amounts of B12 are secreted into the duodenum and then reabsorbed in the ileum.

4. Function

Vitamin B12 is an essential part of several enzyme systems that carry out a number of very basic metabolic functions. Specific biochemical reactions in that cobalamin coenzymes participate are of 2 types: (1) those that contain 5´-deoxyadenosine linked covalently to the cobalt atom and (2) those that have a methyl group attached to the central cobalt atom. A number of vitamin B12-dependent metabolic reactions have been identified in microorganisms; however, only three vitamin B12-dependent enzymes have been discovered in animals: methylmalonyl CoA mutase and leucine mutase, that each requires adenosylcobalamin, and methionine synthetase, which requires methylcobalamin. Most reactions requiring adenosylcobalamin can be classified as rearrangement reactions of the carbon skeleton of several metabolic intermediates; a hydrogen atom moves from one carbon atom to an adjacent one in exchange for an alkyl, acyl, or electronegative group that migrates in the opposite direction (El- lenbogen and Cooper, 1991).

In all these rearrangement reactions, adenosylcobalamin is an intermediate hydrogen carrier. The reactions requiring methylcobalamin involve transfer or synthesis of one-carbon units, for example, methyl groups.

Vitamin B12 is metabolically related to other essential nutrients, such as choline, methionine, and folacin (Savage and Lindenbaum, 1995).

Though the most important tasks of vitamin B12 concern metabolism of nucleic acids and proteins, it also functions in metabolism of fats and carbohydrates. A summary of B12 functions would include (1) purine and pyrimidine synthesis, (2) transfer of methyl groups, (3) formation of proteins from amino acids, and (4) carbohydrate and fat metabolism. A general function of B12 is to promote red blood cell synthesis and to maintain nervous system integrity that is functions noticeably affected in the deficient state.

Vitamin B12 is necessary in reduction of one-carbon compounds of formate and formaldehyde, and in this way it participates by folacin in biosynthesis of labile methyl groups. Formation of labile methyl groups is necessary for biosynthesis of purine and pyrimidine bases that represent essential constituents of nucleic acids. Disorders of nucleic acid synthesis in vitamin B12 deficiency are connected by this. The purine bases as well as thymine are constituents of nucleic acids, and by folacin deficiency there is a reduction in biosynthesis of nucleic acids essential for cell formation and function. Hence, deficiency of either folacin or B12 leads to impaired cell division and alterations of protein synthesis; these effects are most noticeable in rapidly growing tissues.

Deficiency of B12 will induce folacin deficiency with blocking utilization of folacin derivatives. A vitamin B12-containing enzyme removes the methyl group from methylfolate, thereby regenerating tetrahydrofolate, from that is made the 5,20-methylene-THF required for thymidylate synthesis. Because methylfolate returns to the body's folacin pool only via the vitamin B12-dependent step, vitamin B12 deficiency results in folacin being "trapped" as methylfolate, and thus becoming metabolically useless. The "folate trap" concept explains why hematological damage of vitamin B12 deficiency in indistinguishable from that of folacin deficiency with alleging that in both instances the defective synthesis of DNA results from the same final common pathway defect, namely, an inadequate quantity of 5,10-methylene-THF to participate adequately in DNA synthesis (Herbert and Zalusky, 1962).

Vitamin B12 is a metabolic essential for all animal strains studied, and vitamin B12 deficiency can be induced by the addition of high dietary levels of propionic acid. Therefore, metabolism of propionic acid is of particular interest in ruminant nutrition because large quantities are produced during carbohydrate fermentation in the rumen. A propionate production proceeds normally, but in cobalt or vitamin B12 deficiency, its rate of clearance from blood is depressed, and methylmalonyl-CoA accumulates. These results in increased urinary excretion of methylmalonic acid and in loss of appetite because impaired propionate metabolism leads to higher blood propionate levels, that are inversely correlated to voluntary feed intake (MacPherson, 1982).

Injection of cobalt-deficient animals by vitamin B12 produces overnight improvement in appetite, whereas oral dosing with cobalt takes 7 to 10 days to

produce the same effect.

A further important function of vitamin B12 in intermediary metabolism consists of maintaining glutathione and sulfydryl groups of enzymes in the reduced state (Marks, 1975).

The reduced activity of glyceraldehyde-3-phosphate dehydrogenase, that needs glutathione as a coenzyme, is possibly responsible for carbohydrate metabolism being impaired in vitamin B12 deficiency. Vitamin B12 also influences lipid metabolism via its effect on the thiols.

Effects of Deficiency

Ruminants

Vitamin B12 deficiency can occur in young ruminants as long as the microflora of the forestomachs isn't yet far enough developed and hence unable to furnish sufficient amounts of the vitamin.

Lassiter et al. (1953) demonstrated vitamin B12 deficiency in calves less than 6 weeks old which received no dietary animal protein. Clinical signs characterizing the deficiency included poor appetite and growth, muscular weakness, demyelination of peripheral nerves, and poor general condition. Young lambs, if weaned early, likewise had a need for dietary vitamin B12 (NRC, 1985b).

In vitamin B12-deficient lambs, there was a sharp decrease of vitamin B12 concentrations in blood and liver before signs like anorexia, loss of body weight, and a decrease in hemoglobin concentration were observed. Sheep were more sensitive to cobalt deficiency than cattle, and Mburu et al. (1993) suggested which sheep are likewise less resistant to low dietary cobalt than goats, based on blood parameters.

As cobalt is required for biosynthesis of vitamin B12, lack of cobalt may cause deficiency of the vitamin in adult ruminants. Cobalt deficient soils occur in large areas of many countries; therefore grazing ruminants may be particularly affected by the deficiency. By the exception of phosphorus and copper, cobalt deficiency is the most extensive mineral limitation to grazing livestock in tropical countries (McDowell, 1997).

Cobalt deficiency signs aren't specific, and it is often difficult to distinguish between an animal by cobalt deficiency and malnutrition due to low intake of energy and protein and an animal which is diseased or parasitized. Acute clinical signs of cobalt deficiency include lack of appetite, rough hair coat, thickening of the skin, anemia, wasting away, and eventually death, if the animals aren't moved to “healthy” pastures or if cobalt supplements are not made available.

Cobalt deficiency has been reported to reduce lamb survival and increase susceptibility to parasitic infection in cattle (Ferguson et al., 1988). Cobalt deficiency was associated by photosensitization of lambs, characterized with a swollen head (Hesselink and Vellema, 1990). The condition responded to 2 injections of vitamin B12 3 weeks apart. Cobalt-deficient ewes produced fewer lambs and had more stillbirths and neonatal mortality than cobalt-sufficient controls (Fisher and MacPherson, 1991). Lambs from deficient ewes were also slower to start suckling. Auricular myocardial necrosis was reported in cobalt-deficient sheep.

At necropsy, the body of a severely affected cobalt-deficient animal presents a picture of extreme emaciation, often by a total absence of body fat. The liver is fatty, the spleen hemosiderized, and in some animals there is hypoplasia of the erythrogenic tissue in the bone marrow (Filmer, 1933).

The anemia in lambs is normocytic and normochromic, but the mild anemia isn't responsible for the core signs of cobalt deficiency. In appetence and marasmus invariably precede any considerable degree of anemia. Clinical symtoms of cobalt deficiency are identical to those of simple starvation, and may indicate that the symtoms observed by adequate cobalt may be simply due to the in appetence caused with the lack of cobalt. The first discernible response to cobalt feeding or parenteral vitamin B12 is swift improvement in appetite and body weight.

Two other conditions attributed to cobalt deficiency are ovine white liver disease and Phalaris staggers (Graham, 1991; McDowell, 1992). Ovine white liver disease is characterized with hepatic lipidosis and emaciation. At necropsy, affected lambs had pale, swollen, and friable fatty livers and showed accumulation of lipofuscin (Kennedy et al., 1994b).

Alteration in choline synthesis presumably leads to mended lipid mobilization, but white liver disease may be complicated with other factors. Similarly, the persistent neural effects of *Phalaris* spp., inducing Phalaris staggers, have been shown to be preventable by oral cobalt supplementation, but not by B12.

Prior to the recognition of cobalt deficiency in livestock in many parts of the world, cattle could be maintained on deficient pastures only if they periodically were relocated to so-called healthy ground. Cobalt deficiency can be prevented with moving animals for a few months every year to a healthy region, preferably during the rainy season. An example of the necessity of periodically moving animals was illustrated in a disease condition known as “togue” in Espirito Santo, Brazil (Tokarnia et al., 1971).

The disease was observed when animals stayed for longer than 60 to 180 days on certain pastures. Sick animals isolated themselves from the rest of the herd; were apathetic; showed loss of appetite, rough hair coat, and dry feces; and lost body condition. If the

animals were not moved from the pasture, they died, but if they were taken to a pasture where the disease didn't occur, the animals recovered quickly.

Cobalt subclinical deficiencies or borderline states are extremely common and are characterized with low production rates unaccompanied with clinical manifestations or visible signs (McDowell, 1985).

Subclinical deficiencies often go unnoticed, thereby resulting in great economic losses to the livestock industry. No estimate can be made of the effect of cobalt sub deficiency on animal performance in general, but in many areas of the world, it is one of the major causes of poor production.

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