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# Biologically Active Vitamin $B_{12}$ Compounds in Foods for Preventing Deficiency among Vegetarians and Elderly Subjects

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**ABSTRACT:** The usual dietary sources of vitamin $B_{12}$ are animal-source based foods, including meat, milk, eggs, fish, and shellfish, although a few plant-based foods such as certain types of dried lavers (nori) and mushrooms contain substantial and considerable amounts of vitamin $B_{12}$, respectively. Unexpectedly, detailed characterization of vitamin $B_{12}$ compounds in foods reveals the presence of various corrinoids that are inactive in humans. The majority of edible blue-green algae (cyanobacteria) and certain edible shellfish predominately contain an inactive corrinoid known as pseudovitamin $B_{12}$. Various factors affect the bioactivity of vitamin $B_{12}$ in foods. For example, vitamin $B_{12}$ is partially degraded and loses its biological activity during cooking and storage of foods. The intrinsic factor-mediated gastrointestinal absorption system in humans has evolved to selectively absorb active vitamin $B_{12}$ from naturally occurring vitamin $B_{12}$ compounds, including its degradation products and inactive corrinoids that are present in daily meal foods. The objective of this review is to present up-to-date information on various factors that can affect the bioactivity of vitamin $B_{12}$ in foods. To prevent vitamin $B_{12}$ deficiency in high-risk populations such as vegetarians and elderly subjects, it is necessary to identify plant-source foods that contain high levels of bioactive vitamin $B_{12}$ and, in conjunction, to prepare the use of crystalline vitamin $B_{12}$-fortified foods.

**KEYWORDS:** *cobalamin, vitamin $B_{12}$ food sources, inactive corrinoids, bioactive vitamin $B_{12}$, vitamin $B_{12}$ deficiency*

## INTRODUCTION

Vitamin $B_{12}$ ($B_{12}$) or cyanocobalamin belongs to the group "corrinoids," which is a group of compounds having a corrin macrocycle. $B_{12}$ (molecular weight = 1355.4) is stable in an aqueous solution of pH 4−7 and can be heated at 120 °C without significant loss.[1] Naturally occurring $B_{12}$compounds include methylcobalamin, which functions as a coenzyme for methionine synthase (EC 2.1.1.13) involved in methionine biosynthesis, and 5′-deoxyadenosylcobalamin, which functions as a coenzyme for methylmalonyl-CoA mutase (EC 5.4.99.2) involved in amino acid and odd-chain fatty acid metabolism in mammalian cells.[2,3] Corrinoids carrying a base other than 5,6-dimethylbenzimidazole as the lower ligand (cobalt-coordinated nucleotide) are also found in nature.

$B_{12}$ is synthesized only by certain bacteria[4] and is primarily concentrated in the bodies of predators higher in the food chain. Thus, foods derived from animals, that is, meat (approximately 9.4 $\mu$g/100 g), milk (approximately 0.4 $\mu$g/100 g), eggs (approximately 1.3 $\mu$g/100 g), fish (approximately 8.9 $\mu$g/100 g), and shellfish (approximately 52.4 $\mu$g/100 g), are considered to be the major dietary sources of $B_{12}$.[5] Although $B_{12}$ is well-known as the sole vitamin absent in plant-source foods, certain dried algae [*Porphyra* sp. (approximately 77 $\mu$g/100 g), *Chlorella* sp. (approximately 200 $\mu$g/100 g), etc.] contain substantial amounts of $B_{12}$.[6] It has long been unclear whether algae have an absolute requirement for $B_{12}$ for growth and why algae that have no $B_{12}$ requirement for growth contain substantial amounts of $B_{12}$. However, recent biochemical and bioinformatics studies have accurately defined the $B_{12}$ requirements of various algae and have suggested possible physiological functions of algal $B_{12}$.[7,8]

Gastrointestinal absorption of dietary $B_{12}$ is a complex process in humans.[9] $B_{12}$ released from protein in foods is first bound to haptocorrin (salivary $B_{12}$-binding protein) in the stomach. After proteolysis of the haptocorrin−$B_{12}$ complex by pancreatic proteases in the duodenum, the $B_{12}$ released binds to intrinsic factor (IF; gastric $B_{12}$-binding protein) in the proximal ileum. The IF−$B_{12}$ complex can enter mucosal cells in the distal ileum by receptor-mediated endocytosis. However, it is unclear why humans require such a complex system for the gastrointestinal absorption of $B_{12}$ from foods.

Strict vegetarians (vegans) are at higher risk of $B_{12}$ deficiency than nonvegetarians.[10] They must consume $B_{12}$-fortified foods or $B_{12}$-containing dietary supplements to prevent $B_{12}$ deficiency. Furthermore, in developing countries, childhood $B_{12}$ deficiency is increasing because of low consumption of animal-source foods.[11] Similar results have been reported among low-income populations in developed countries.[12] $B_{12}$ fortification of flour is considered for the high-risk population because of the high prevalence of $B_{12}$ deficiency worldwide.[13,14]

A considerable proportion of elderly subjects with low serum $B_{12}$ levels without pernicious anemia have been recently reported to have malabsorption of protein-bound $B_{12}$ (food-bound $B_{12}$ malabsorption).[15] Food-bound $B_{12}$ malabsorption is found in patients with certain gastric dysfunctions, such as atrophic gastritis with decreased stomach acid secretion.[16] Because the bioavailability of crystalline $B_{12}$ is normal in patients with atrophic gastritis,

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the Institute of Medicine recommends that adults aged 51 years and older should obtain the majority of their recommended dietary allowance (RDA) of $B_{12}$ (2.4 $\mu$g per day for adults in the United States, Canada, and Japan) by consuming foods fortified with crystalline $B_{12}$ or $B_{12}$-containing supplements.[17,18] The objective of this review is to present up-to-date information on various factors that can affect the bioactivity of $B_{12}$ in foods and identifies $B_{12}$-rich plant-source foods.

## FACTORS AFFECTING BIOLOGICAL ACTIVITY OF $B_{12}$ IN FOODS

**Inactive Corrinoid Compounds in Foods.** *Edible Cyanobacteria.* Some species of cyanobacteria, including *Spirulina*, *Aphanizomenon*, and *Nostoc*, are produced by food and pharmaceutical industries at worldwide annual rates of 500–3000 tons.[19] Tablets containing *Spirulina* sp. are sold as dietary supplements, because *Spirulina* sp. are known to contain large amounts of $B_{12}$.[20] We found that commercially available spirulina tablets contain 127–244 $\mu$g of $B_{12}$/100 g weight and that the values obtained by the microbiological assay method are 6–9-fold greater than those determined by the chemiluminescence method.[21] Characterization of two corrinoid compounds from spirulina tablets identified the major (83%) and minor (17%) compounds as pseudo-$B_{12}$ (adeninyl cobamide) and $B_{12}$, respectively (Figure 1). It has been reported that pseudo-$B_{12}$ is barely absorbed by the mammalian intestine, and it has low affinity for IF.[22] Furthermore, it has been reported that *spirulina* $B_{12}$ may not be bioactive in mammals.[23]

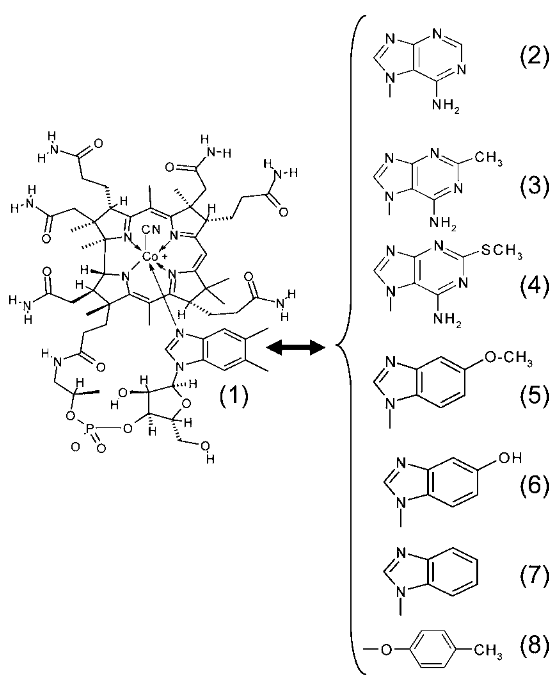


**Figure 1.** Structural formula of vitamin $B_{12}$ and partial structures of $B_{12}$ related compounds showing the portions that differ from vitamin $B_{12}$: (**1**) vitamin $B_{12}$ or cyanocobalamin; (**2**) pseudovitamin $B_{12}$ or factor IV; (**3**) factor A (2-methyladenylcobamide); (**4**) factor S (2-methylmercaptoadenylcobamide); (**5**) factor IIIm (5-methoxybenzimidazolylcobamide); (**6**) factor III (5-hydroxybenzimidazolylcobamide); (**7**) BIA (benzimidazolylcobamide); (**8**) pCC (*p*-cresolylcobamide).

All edible cyanobacteria tested, including *Aphanizomenon flos-aquae*,[24] *Aphanothece sacrum*,[25] *Nostoc commune*,[26] *Nostoc flagelliforme*,[27] and *Nostochopsis* sp.,[28] contained substantial amounts of pseudo-$B_{12}$. These results indicate that edible cyanobacteria often contain large amounts of pseudo-$B_{12}$, which is known to be biologically inactive in humans.[22] Therefore, cyanobacteria are not suitable for use as a source of $B_{12}$ for high-risk populations such as vegetarians and elderly subjects.

*Edible Shellfish.* Various shellfish are widely consumed by humans. Shellfish, which siphon large quantities of $B_{12}$-synthesizing microorganisms from seawater and fresh water, are known to be excellent sources of $B_{12}$.[29] The $B_{12}$-synthesizing microorganisms can also synthesize various other corrinoids, including corrinoid compounds that are inactive in humans, with different bases as the lower ligand. The $B_{12}$ contents of certain shellfish have been determined by the microbiological assay method to be several-fold greater than those determined by the chemiluminescence method.[30] When corrinoid compounds from popular shellfish such as oysters, mussels, and short-necked clams were isolated and characterized, each was identified as $B_{12}$.[30,31] However, our recent study[32] and unpublished data (Y. Tanioka et al.) using liquid chromatography–electrospray ionization/multistage mass spectrometry (LC/ESI-MS/MS) analysis indicated that certain edible shellfish (abalones, ark shells, corb shells, whelks, etc.) contain substantial amounts of pseudo-$B_{12}$, factor A, factor S, and/or factor IIIm, as well as $B_{12}$ (Figure 1). Therefore, these shellfish are not adequate sources of $B_{12}$. Further detailed analysis of the corrinoid compounds in various animal-source foods using LC/ESI-MS/MS is required.

**Heat Degradation Products of $B_{12}$ from Cooking and Food Processing.** Japanese people receive approximately 84% of their daily $B_{12}$ intake from fish and shellfish.[33] Fish is a good source of minerals, vitamins, high-value proteins, and unsaturated lipids containing eicosapentaenoic acid and docosahexaenoic acid.[34] Many studies have suggested that regular fish intake prevents atherosclerosis, thrombosis, and cardiac diseases.[35,36] Therefore, fish consumption is increasing throughout the world. However, the $B_{12}$ content of round herring meats decreases by up to approximately 62% by grilling, boiling, frying, steaming, or microwaving.[37] Our results indicate that loss of $B_{12}$ is dependent on the temperature and time of conventional cooking and is further affected by other food ingredients.[37] Although there is no loss of $B_{12}$ during vacuum-packed pouch cooking,[37] there is not much detailed data on the nutritional aspects of vacuum-packed pouch cooking; thus, it is difficult to evaluate whether the vitamin preservation is because of the vacuum-packaging or solely because of the lower temperature used.[38] Some vitamins (C, $B_6$, and folate) sensitive to oxidation have higher stability in vacuum-packed pouch cooking than in conventional or traditional cooking.[39] Retention of $B_{12}$ in vacuum-packed pouch-cooked meat and fish has been reported to be 100% for veal, lamb, and pork, 87% for beef, 92% for salmon, and 72% for cod.[40]

Microwave ovens are widely used for cooking and food processing and show equal or better retention of various vitamins ($B_1$, $B_2$, $B_6$, C, and folic acid) compared with conventional heating.[41,42] However, appreciable $B_{12}$ loss occurs due to degradation of the $B_{12}$ molecule by microwave heating.[43] Microwave heating of hydroxocobalamin, which is predominant in foods, results in the formation of several degradation products.[43,44] These compounds may result from the elimination of the base portion, a change in the sugar moiety of the lower ligand, and/or skeletal alterations. Thus, the decreased $B_{12}$ content of various cooked foods appears to be because of the destruction of $B_{12}$ by heating (Figure 2).


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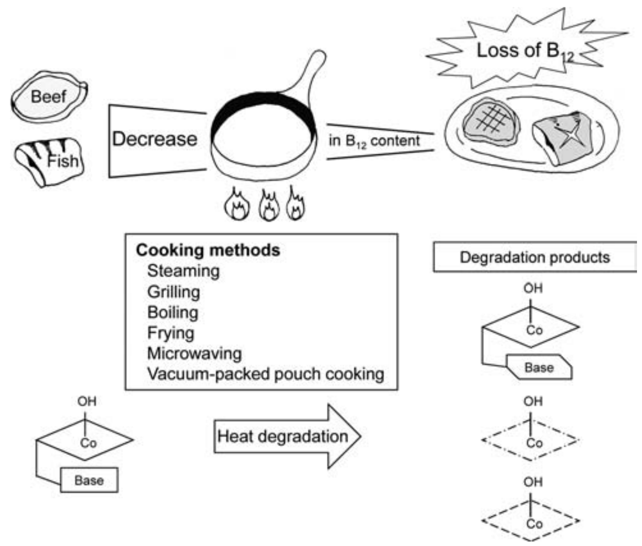


**Figure 2.** Loss of vitamin $B_{12}$ during cooking and its heat degradation products.

**$B_{12}$ Degradation and/or Transformation Products by Chemical Treatment and Light Exposure.** *Radical Reactions.* Milk is the most important source of vitamins $B_2$ ($B_2$) and $B_{12}$ in the human diet. Photodegradation of $B_2$ and color change during storage of milk in light have been studied extensively.[45−47] On exposure to light, $B_2$ produces free radicals (superoxide anion radical and singlet oxygen) that accelerate the oxidation of tryptophan and other amino acids. Amino acid oxidation is involved in the color change in milk.[48] To evaluate the loss of $B_{12}$ in milk during light-induced $B_2$ degradation, various milk samples were exposed to fluorescent light for 24 h at 4 °C.[49] The $B_{12}$ concentration decreased by 1−27%, depending on the type of milk tested. These results indicate that storage in light for a long period can induce degradation of both $B_2$ and $B_{12}$ in various types of liquid milk. Light irradiation of $B_{12}$ in an oxygen-saturated solution indicates that the corrin macrocycle of $B_{12}$ is degraded by singlet oxygen.[50] These observations suggest that $B_{12}$ in milk is significantly degraded by free radicals formed during light-induced $B_2$ degradation.

An appreciable loss of $B_{12}$ also occurs in $B_{12}$-containing multivitamin/mineral supplements[51] because of degradation by addition of a substantial amount of vitamin C (VC) in the presence of copper.[52] VC or metal ion alone does not degrade $B_{12}$. However, the combination of VC and copper ion significantly destroyed $B_{12}$ with formation of large amounts of $B_{12}$ degradation products, as indicated by ladder-like red spots on silica gel thin-layer chromatography.[52] Some of these $B_{12}$ degradation products have been reported to block $B_{12}$ metabolism in mammalian cells.[51] Degradation of $B_{12}$ was reduced significantly by the addition of certain antioxidants such as carnosine or anserine,[52] presumably because of free radicals generated by VC in the presence of copper.

*Organochlorides and Other Chemicals.* Chloramine-T (*N*-chloro-*p*-toluenesulfonamide) is an active chlorine compound with strong oxidative activity and is widely used as a biocide.[53] For a long time, chloramine-T was believed to be nontoxic. It is used in the food industry as a disinfectant for plant sterilization.[54] It is nowadays used in cosmetics but not foodstuffs. Chloramine-T at 200 ppm for 5−20 min is effective against three virulent Gram-positive bacteria without causing human fibroblast damage in vitro.[55] Bonnet et al. found that at pH 4.0, 1 mol of chloramine-T, which is a source of electrophilic chlorine (equivalent to $Cl^+$), produces a lactone form (1 mol) of $B_{12}$, $B_{12}$[*c*-lactone].[56] Our unpublished results indicate that certain foods (shiitake mushrooms) contain considerable amounts of $B_{12}$[*c*-lactone]. Although potential harmful effects of $B_{12}$[*c*-lactone] on mammalian cells have not been investigated, it may block mammalian $B_{12}$ metabolism because the similar compound, $B_{12}$[*c*-lactam], is a well-known $B_{12}$ antagonist.[57]

Formation of a blue corrinoid from partial degradation of $B_{12}$ in aqueous bicarbonate has been reported recently.[58] The corrin macrocycle of $B_{12}$ is degraded (B-ring-opening), with the formation of 7,8-*seco*-$B_{12}$.

## PREVENTION OF $B_{12}$ DEFICIENCY IN VEGETARIANS

Strict vegetarians are at higher risk for developing $B_{12}$ deficiency because of low intake of animal-source foods.[10] Thus, it is necessary to identify plant-source foods containing high $B_{12}$ to prevent vegetarians from developing $B_{12}$ deficiency.

**Fermented Beans and Vegetables.** The $B_{12}$ content of soybeans is low or undetectable; however, tempe, a soybean-fermented food, contains a large amount of $B_{12}$ (0.7−8 $\mu$g/100 g).[59] Contamination by certain bacteria during tempe production may contribute to its increased $B_{12}$ content.[60] Natto, another soybean-fermented food,[61] and Korean soybean products (doenjang, chungkookjang, and kochujang)[62] contain minute amounts of $B_{12}$ (0.1−1.9 $\mu$g/100 g). Fermented Korean vegetables (kimuchi) contain traces (<0.1 $\mu$g/100 g) of $B_{12}$.[62,63] Various fermented tea leaves contain very small amounts of $B_{12}$ (0.1−0.5 $\mu$g/100 g dry weight).[64,65] The juice of fenugreek (*Trigonella foenum graecum*) leaves can be enriched with $B_{12}$ (12.5 $\mu$g/100 mL) by certain lactic fermentations.[66] The addition of *Propionibacteria* to cabbage during sauerkraut production results in higher concentrations of $B_{12}$ (7.2 $\mu$g/100 g).[67]

**Edible Algae.** Various types of edible algae are consumed by humans worldwide. Dried green (*Enteromorpha* sp.) and purple (*Porphyra* sp.) lavers (nori) are the most widely consumed edible algae and contain substantial amounts of $B_{12}$ (approximately 133 $\mu$g/100 g dry weight).[68,69] However, edible algae other than these two species contain no $B_{12}$ or only trace amounts.[6] Because the biological activity of algal $B_{12}$ in humans is unclear, we characterized corrinoid compounds to determine whether dried purple and green lavers and eukaryotic microalgae (*Chlorella* sp. and *Pleurochrysis carterae*) used in human food supplements contain $B_{12}$ or inactive corrinoids.[70,71]

To measure the biological activity of $B_{12}$ in lyophilized purple laver (*Porphyra yezoensis*), the effects of laver feeding were investigated in $B_{12}$-deficient rats.[72] Within 20 days of initiating a diet supplemented with dried purple laver (10 $\mu$g of $B_{12}$/kg diet), urinary methylmalonic acid excretion became undetectable, and hepatic $B_{12}$ (especially coenzyme $B_{12}$) levels increased significantly. These results indicate that $B_{12}$ from the purple laver is bioavailable in rats.

Nutritional analysis of six vegan children who had been on vegan diets including brown rice and nori (dried laver) for 4−10 years suggests that consumption of nori (dried laver) may prevent $B_{12}$ deficiency in vegans.[73]

**Edible Mushrooms.** Wild mushrooms are increasing in dietary importance because of their nutritional and medicinal characteristics.[74,75] Many species of wild mushrooms are widely consumed. Six wild edible mushroom species are popular among vegetarians in European countries. Zero or trace levels (0.01−0.09 $\mu$g/100 g dry weight) of $B_{12}$ were measured in porcini mushrooms (*Boletus* sp.), parasol mushrooms (*Macrolepiota procera*), oyster mushrooms (*Pleurotus ostreatus*), and


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black morels (*Morchella conica*). In contrast, black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) mushrooms contain considerable levels of $B_{12}$ (1.09−2.65 μg/100 g dry weight).[76] To determine whether *C. cornucopioides* or *C. cibarius* contains $B_{12}$ or other corrinoid compounds that are inactive in humans, we purified a corrinoid compound using an immunoaffinity column and identified it as $B_{12}$ by LC/ESI-MS/MS.

Consumption of approximately 100 g of dried black trumpet (approximately 1 kg of fresh mushroom with 90% moisture content) could provide the RDA for adults (2.4 μg/day), although ingestion of such large amounts would not be feasible. Nevertheless, moderate mushroom intake may slightly contribute to prevention of severe $B_{12}$ deficiency in vegetarians.

**$B_{12}$-Enriched Vegetables.** Many studies have measured the $B_{12}$ content of various vegetables. Only trace amounts of $B_{12}$(<0.1 μg/100 g of wet weight of an edible portion) are found in broccoli, asparagus, Japanese butterbur, mung bean sprouts, tossa jute, and water shield.[77] Mozafar demonstrated that addition of an organic fertilizer such as cow manure significantly increases the $B_{12}$ content of spinach leaves (17.8 ng/g dry weight).[78] As sheep fed a cobalt-deficient diet rapidly develop $B_{12}$ deficiency,[79,80] microbiological synthesis of $B_{12}$ in the lumen or intestinal tract is an important $B_{12}$ source in herbivorous animals. However, our recent[81] and unpublished results indicate that most organic fertilizers, particularly those prepared from animal manures, contain considerable amounts of inactive corrinoid compounds as well as $B_{12}$.

Hydroponic cultivation is an emerging technology that enables better control of water and nutrient supplies, improves plant productivity, and reduces the use of pesticides.[82] Attempts have been made to obtain certain $B_{12}$-enriched vegetables by treatment with solutions containing high levels of $B_{12}$.[83,84] Mozafar and Oertli[83] reported that uptake of $B_{12}$ by soybean leaves does not reach saturation even with an extremely high $B_{12}$ concentration in the nutrient solution (3.2 mmol/L). It has been shown that when soybean seedlings are placed in a solution containing 10 μmol/L of $B_{12}$ for 24 h, the leaves contain a significantly higher level of $B_{12}$ (9.8 μg/g fresh weight). Japanese radish sprouts (kaiware daikon) also show significant increases in $B_{12}$ content (1.28 μg/g fresh weight) after the seeds had been soaked for 6 h in a solution containing 200 μg/mL of $B_{12}$.[84] Recently, we produced $B_{12}$-enriched lettuce leaves cultivated using hydroponics.[85] The majority of $B_{12}$ present in the leaves (86%) was recovered in the free $B_{12}$ fractions, suggesting that $B_{12}$-enriched lettuce leaves are an excellent source of free $B_{12}$. These results suggest that $B_{12}$-enriched vegetables may be of special benefit for vegetarians.

## PREVENTION OF $B_{12}$ DEFICIENCY IN ELDERLY SUBJECTS

A considerable proportion of people with low serum $B_{12}$ levels who do not have pernicious anemia exhibit food-bound $B_{12}$ malabsorption.[15] Food-bound $B_{12}$ malabsorption occurs with certain gastric dysfunctions, especially atrophic gastritis with low stomach acid secretion, which is prevalent in elderly subjects.[15] In this section, "true " $B_{12}$ defines $B_{12}$ compounds that are biologically active in mammals including humans, but not inactive corrinoid compounds such pseudo-$B_{12}$. Furthermore, "free" $B_{12}$ means the protein-unbound $B_{12}$.

**Free $B_{12}$-Containing or -Fortified Foods.** Several investigators have evaluated the efficacy of oral $B_{12}$ therapy for the treatment of $B_{12}$ malabsorption in elderly people with $B_{12}$ deficiencies.[86,87] Ready-to-eat cereals fortified with $B_{12}$ constitute a large proportion of the dietary $B_{12}$ intake in the United States.[17] It has been reported that breakfast cereals fortified with folic acid, $B_{12}$, and $B_6$ increase blood concentrations of these vitamins and decrease plasma total homocysteine concentrations in elderly populations.[88] The effects of $B_{12}$ supplementation with a milk product on $B_{12}$-deficient elderly people have also been studied.[89] Thus, fortified foods have become a particularly valuable source of $B_{12}$ for elderly people.

**Bonito and Clam Soup Stocks and Extracts.** The natural food chain would suggest that the meat of larger fish (e.g., tuna and bonito) contains high amounts of $B_{12}$. Tuna and bonito are popular food items in various countries, and our previous studies[90,91] have demonstrated that muscles of these fish contain substantial amounts of true $B_{12}$. In Asian countries, especially Japan, various types of fish soup stocks (primarily dried bonito or small dried sardines) and extracts are manufactured for use as seasoning or flavoring. If the fish extracts and soup stocks contain substantial amounts of free $B_{12}$, they would be excellent natural sources of free $B_{12}$ for elderly people with food-bound $B_{12}$ malabsorption. Although the $B_{12}$ content of powdered- and granule-type soup stocks is very low (approximately 0.8 μg/L), some liquid-type soup stocks contain considerable amounts of $B_{12}$ (>5.0 μg/L).[92] Among the fish extracts tested, the $B_{12}$ content of the extract made of raw bonito muscle was the highest (40.9 ± 5.5 μg/100 g).[92] Selected fish extracts (bonito, salmon, and tuna) contained considerably high amounts of true free $B_{12}$.[92]

Various types of shellfish extracts are also manufactured for use as seasonings and flavorings. The $B_{12}$ contents of scallop and freshwater clam extracts were zero or very low (0.1 μg/100 g), respectively, whereas short-necked clam extract contained substantial amounts of true $B_{12}$ (131.8 μg/100 g).[93] Most of the $B_{12}$ in the clam extract was free $B_{12}$. These results indicate that the fish or shellfish extracts are natural sources of free $B_{12}$ for elderly people with food-bound $B_{12}$ malabsorption.

Fresh clams and their processed food products (e.g., canned clams) are popular food items in various countries. Canned clams are the most readily accessible clam products. The broth of canned clams (boiled plain) contains considerable amounts of free $B_{12}$ (approximately 2.7−14.1 μg/100 g or 1.3−6.7 μg/can),[94] suggesting that it is also an excellent natural source of free $B_{12}$ for elderly people with food-bound $B_{12}$ malabsorption.

## CONCLUDING REMARKS

When corrinoids were purified and characterized from various foods, some foods were unexpectedly found to contain inactive corrinoids as well as $B_{12}$. Because intestinal bacteria synthesize various corrinoid compounds with different bases in the lower ligand, animal manures and human feces[95] contain substantial amounts of inactive corrinoid compounds (Figure 1). Certain bacteria (as biofertilizers), fish viscera, fish meal, and animal manures are generally used as organic fertilizers for vegetables and grain. Furthermore, $B_{12}$ is partially converted to a biologically inactive $B_{12}$ analogue by the reactions of certain chemical reagents such as the biocide chloramine-T. Intact $B_{12}$, naturally occurring inactive corrinoids, and chemically modified inactive $B_{12}$ compounds exist in the natural environment. Thus, $B_{12}$ and other related compounds are concentrated primarily in predators higher on the natural food chain. Foods derived from animal products are the major dietary sources of $B_{12}$, although $B_{12}$ is significantly degraded during the cooking and storage of foods (Figure 2). Thus, humans have evolved an IF-mediated gastrointestinal absorption system to select biologically active $B_{12}$ from among the various corrinoid compounds and/or $B_{12}$ degradation products. In addition, the intestinal bacteria of mammals, including humans, synthesize substantial amounts of various inactive corrinoids as well as $B_{12}$. As shown in Figure 3, these

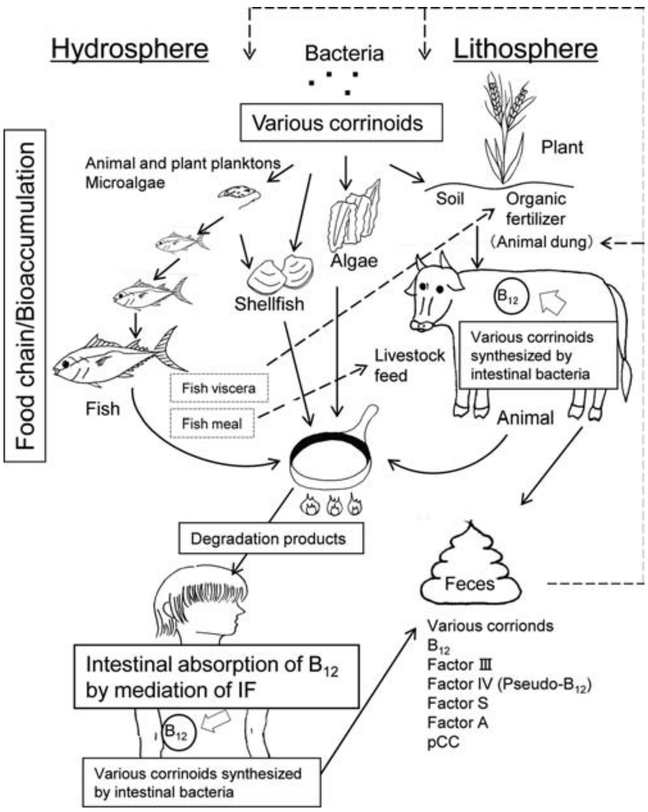


**Figure 3.** Circulation of corrinoid compounds in the natural environment.

corrionids are rapidly returned to the soil or water and recirculate in the natural environment.

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### Author Contributions

All authors equally contributed to the preparation of the manuscript and have approved the final version.

### Notes

The authors declare no competing financial interest.

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