

# Vitamin B12:

## Are You Getting It?

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(Table of Contents located at the end.)

### 1. Introduction

The overwhelming consensus in the mainstream nutrition community, as well as among vegan health professionals, is that plant foods do not provide vitamin B12. Despite this, some vegan advocates still believe that plant foods provide all the nutrients necessary for optimal health and, therefore, do not address vitamin B12 when promoting the vegan diet. Other vegan advocates acknowledge the need for B12, but only as an afterthought. The result is that many vegans do not eat B12 fortified foods or supplements. Many have developed classic neurological symptoms of a B12 deficiency. In some cases, the symptoms have cleared up after taking B12 supplements, but not everyone has been so lucky.

While many current vegans report feeling better on a vegan diet, the most common complaint I hear from ex-vegans is that they didn't feel healthy. This seems logical: The people who feel good on the diet stick with it. The people who feel bad, don't. Could it be that some of the people who go back to eating animal products are feeling the effects of a reduced B12 status? Many vegans would not consider this a possibility, because humans need very little B12 and new vegans usually have a healthy store which can last months or years.

The fact that vegans tend to have lower B12 levels than lacto-ovo vegetarians or nonvegetarians is often countered with, "Few vegans have ever shown signs of B12 deficiency." However, most vegans appear to supplement their diet with B12 (often unknowingly through fortified foods), which could explain why most vegans never show B12 deficiency. As for vegans whose diets are not supplemented, I disagree that they rarely show signs of B12 deficiency. As the reader will soon see, there have certainly been plenty of vegans who have suffered from B12 deficiency, and it is time that there were no more. Vegans can ensure optimal B12 status, reducing their risk for many diseases, by following the recommendations in Table 1.

This article is a thorough review of the scientific literature about vitamin B12 and the vegan diet, including every relevant study on vegans and vitamin B12 published since 1980. Recommendations are summarized in Table 1. Vegan advocates who may otherwise not be interested in the details of vitamin B12 are encouraged to read the Conclusion.

### From a Long-term Vegan:

For the last few months, I was feeling sluggish, had to lie down a couple of times a day, found it difficult to work evenings and to exercise for long periods. Under [my vegan, medical doctor's] guidance, I was taking protein powder, creatine, testosterone, nystatin, etc., all to no avail. I was taking nutritional yeast every day, so I knew it wasn't B12 deficiency.

Then, one day, I came across your B12 article by sheer accident. I wasn't going to read the whole thing, but I glanced through it and was struck by your insistence that none of the usual sources are adequate. I still didn't believe it, but I had some old B12 pills in the fridge, so I popped one.

The effect was almost immediate and remarkable. I have been taking them almost every day, my stamina and energy level are up, and I feel middle-aged again instead of a tired old man.

### Table 1: Recommendations for Vegans

See Appendix L for how these recommendations were formulated.

In foods, B12 is measured in *micrograms* (aka  $\mu\text{g}$  or mcg.).  $1,000 \mu\text{g} = 1 \text{ mg}$ .

**Step 1.** If you have not had a regular source of B12 for some time, buy a bottle of sublingual B12. The following are vegan:

Solgar Sublingual 1,000 mcg nuggets  
[www.solgar.com/online\\_reference/b\\_b\\_complex/](http://www.solgar.com/online_reference/b_b_complex/)

Veg Life Sublingual 1,000 mcg lozenges  
Sold in stores (website has a store locator).  
[www.nutraceutical.com/search/view\\_product.cfm?section=search&product\\_index=74345](http://www.nutraceutical.com/search/view_product.cfm?section=search&product_index=74345)

Freeda Vitamins 500 mcg lozenges  
[www.freedavitamins.com/shop/fsearch07.asp](http://www.freedavitamins.com/shop/fsearch07.asp)

Nature's Bounty Sublingual 2500 mcg  
[http://www.naturesbounty.com/Pages/sf\\_3860.html](http://www.naturesbounty.com/Pages/sf_3860.html)

Place 2,000  $\mu\text{g}$  under your tongue until the tablet(s) has dissolved, once a day, for 2 weeks. Then follow the advice under Step #2. (Note: you can break the remaining tablets in half or quarters for Step #2. It's okay to take more than recommended.)

**Step 2.** If you have had a regular source of B12, skip Step 1. *One* of the following daily recommendations should maximize B12 status:

**fortified foods** (in  $\geq 2$  servings,  $\geq 6$  hours apart): 3-5  $\mu\text{g}^{\text{A}}$   
**1 supplement:** 10-100  $\mu\text{g}^{\text{A,B}}$

**2 supplements** (spaced  $\geq 6$  hours apart): 5  $\mu\text{g}$

<sup>A</sup>Lower limit based on minimum recommendations in *What Every Vegan Should Know about Vitamin B12* (<http://veganoutreach.org/health/b12letter.html>).

<sup>B</sup>In a single dose, B12 absorption drops to 1-1.5% for the amounts above 5  $\mu\text{g}$ .

### Notes

#### Fortified foods:

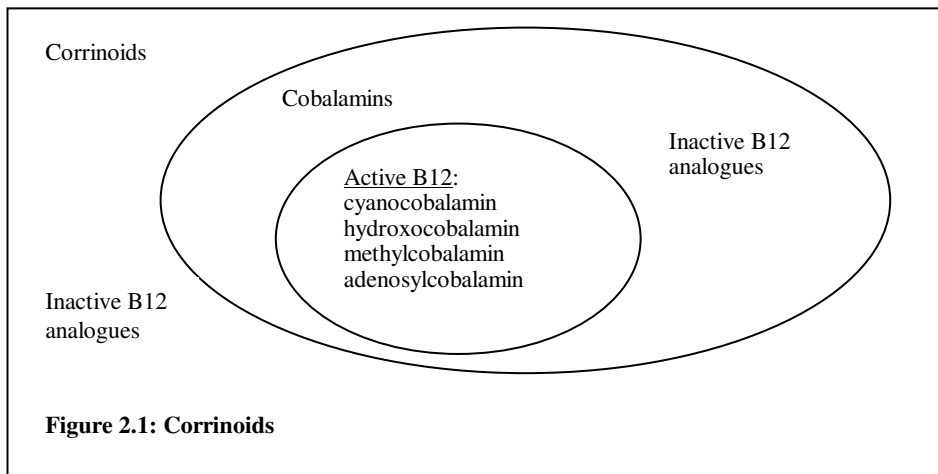
Amounts listed on a nutrition label are based on 6  $\mu\text{g}/\text{day}$ .  
For example, 25% of the Daily Value =  $.25 * 6 \mu\text{g} = 1.5 \mu\text{g}$ .

**Do Not** rely on any seaweed (e.g., algae, nori, spirulina), brewer's yeast, tempeh, or "living" vitamin supplement that uses plants as a source of B12. Do not rely *solely* on one type of fortified food such as Red Star Nutritional Yeast.

**Vegan Infants:** The Institute of Medicine recommends that infants of vegan mothers be supplemented with B12 from birth because their stores at birth and their mother's milk supply may be low.<sup>206</sup>

**Exceptions:** People with digestive or malabsorption diseases (such as pernicious anemia), B12 metabolism defects, kidney failure, or cyanide metabolism defects should consult a bona fide health professional. See Appendix M.

**Cigarette smokers** should consider a non-cyanocobalamin source of B12. See Appendix O.



## 2. Vitamin B12: A Pesky Molecule

Vitamin B12 deficiency in industrialized countries is rare.<sup>55</sup> B12 is a complicated vitamin with a unique absorption mechanism, wide array of deficiency symptoms, and a number of inactive analogues (molecules that appear to be active B12, but actually are not) that possibly interfere with its function (see Appendix H for more information).

### 2.1 The B12 Molecule

B12 is a coenzyme: it is needed for enzymes to do their job of changing

one molecule into another. As vitamins go, B12 is large. One part of its structure is known as the *corrin* nucleus, which holds an atom of cobalt. The corrin resembles the heme of hemoglobin which holds an atom of iron. Any molecule that contains a corrin nucleus is considered a *corrinoid*.

The corrin plus other atoms make up the *cobalamin* part of B12. There are many different cobalamins and they are named after their attachments. For example, *methylcobalamin* is cobalamin with a methyl group attached.

Only two cobalamins are active as coenzymes in the human body: adenosylcobalamin and methylcobalamin. The body has the ability to convert at least some other cobalamins into one of these active forms. Cyanocobalamin (a cyanide molecule attached to a cobalamin) is the form most often found in supplements and fortified foods because it is the most stable form of B12. (Is this amount of cyanide safe? See Section 7.5.1) Most people readily convert cyanocobalamin into one of the B12 coenzymes.<sup>47</sup> Hydroxocobalamin is also common in foods and the body; it can be converted into a B12 coenzyme.

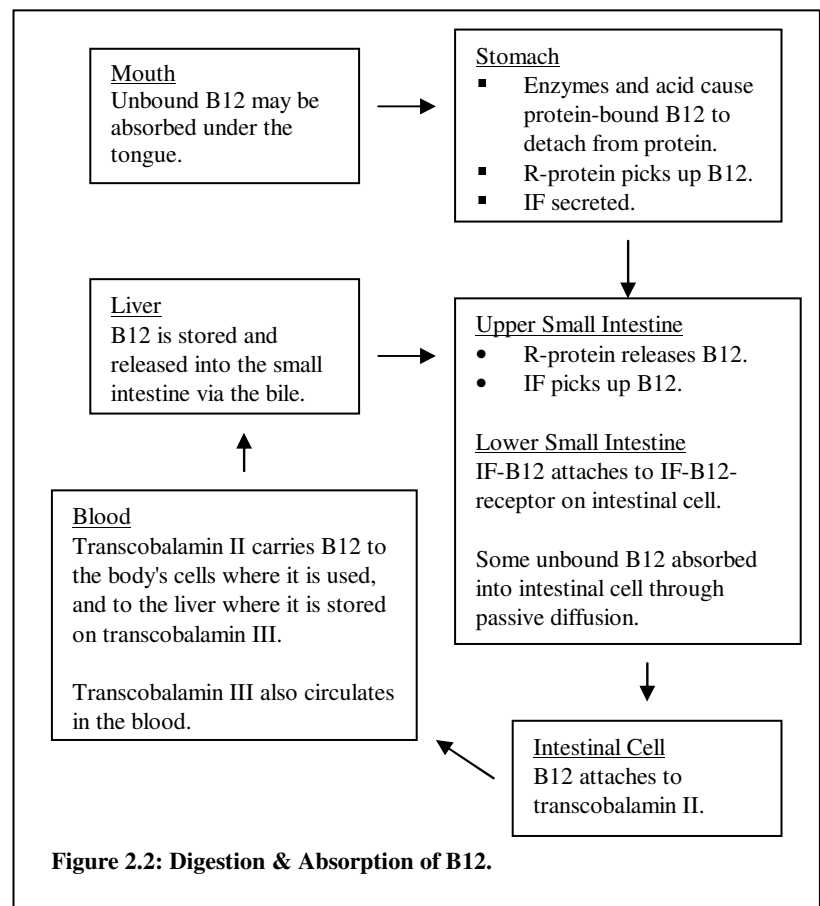
All corrinoids are considered *B12 analogues*. Many corrinoids, and possibly even some cobalamins, are not useable by human B12 enzymes. These are considered *inactive B12 analogues*. About 1/3 of the corrinoids in the typical person are inactive analogues, while the rest are active B12.<sup>48</sup> **In this article, unless otherwise noted, “B12” refers only to active B12 analogues.**

#### 2.2.1 Digestion & Absorption of Protein-bound B12

Microorganisms, primarily bacteria, are the only organisms known to manufacture B12. These bacteria are thought to live in water, soil, and the digestive tracts of animals. In animals, B12 is normally attached to a protein either for transport or storage.

B12 is generally found in all animal foods (except honey). When humans eat animal foods, the B12 is protein-bound. When the protein-B12 complex reaches the stomach, the stomach secretes acids and enzymes that detach the B12 from the protein. Then, in a process unique to B12, another protein, *R-protein* (aka *cobalophilin*, *haptocorrin*, and *transcobalamin I*<sup>197</sup>) picks up the B12 and transports it through the stomach and into the small intestine. R-protein is found in many fluids in the human body including saliva and stomach secretions. In addition to B12, R-protein can pick up any corrinoid.<sup>45</sup> The stomach cells also produce a protein called *intrinsic factor* (IF), which travels to the small intestine.

When the corrinoid-R-protein complex gets to the small intestine, the corrinoid is liberated from the R-protein by enzymes made by the pancreas.<sup>35</sup> Of the liberated corrinoids, only the cobalamins (most of which are, normally, active B12) attach to intrinsic factor. Intrinsic factor then carries the cobalamins to the last section of the small intestine, the ileum. The cells lining the ileum contain receptors for the



cobalamin-IF complex. The cobalamin-IF complex protects the cobalamin against bacterial and digestive enzyme degradation.<sup>64</sup> The IF-receptor also ensures that cobalamins will be given priority for absorption over non-cobalamin corrinoids. In addition to the IF mechanism, *passive diffusion* normally accounts for 1-3% of B12 absorbed when obtained through normal food sources.<sup>35</sup> Some inactive B12 analogues are most likely absorbed through passive diffusion.

### 2.2.2 Digestion & Absorption of Unbound B12

In supplements, B12 is not bound to protein, and therefore does not need digestive enzymes or stomach acid to be detached from a protein. Stomach acid is needed to dissolve some B12 tablets, especially if not chewed. When taken in large enough doses, unbound B12 can overcome intrinsic factor defects because so much can be absorbed through passive diffusion. There is some preliminary evidence that B12, especially when combined with an absorption enhancer, can be directly absorbed through the membranes under the tongue at higher rates than through passive diffusion in the digestive tract.

### 2.3 Enterohepatic Circulation

The average nonvegetarian stores 2,000-3,000 µg B12,<sup>206</sup> while losing only about 3 µg/day.<sup>103</sup> About 60% of the total amount of B12 in the body is stored in the liver and 30% is stored in the muscles.<sup>64</sup> The body has a special circuit between the digestive tract and the liver. Bile, which is made in the liver and needed to digest fat, is secreted into the beginning of the small intestine. It is then reabsorbed at the end of the small intestine and taken back to the liver where it is used again. This circuit is called *enterohepatic circulation*. People normally secrete 1.4 µg/day into their small intestines via their bile.<sup>206</sup> Consequently, healthy people can reabsorb about .7µg B12/day from their bile.<sup>206</sup> It is thought that in states of low B12 intake, absorption increases which can delay B12 deficiency, sometimes for 20-30 years.<sup>48</sup> For vegans who do not supplement, slight differences in enterohepatic circulation may determine how long one can go before developing B12 deficiency symptoms.<sup>121</sup>

One study has looked at changes in serum B12 (sB12) levels in new vegans. Crane et al.<sup>18</sup> (1994, USA) had 13 students change from a lacto-ovo vegetarian to a vegan diet:

- All 4 with sB12 in the 600-900 range fell to below 500 pg/ml in 2 months.
- 10 students followed the diet for 5 months and their average sB12 went from 417 ± 187 to 276 ± 122 pg/ml.
- After 5 months, 2 went from normal to below normal.

### 2.4 Transport in the Blood

After B12 is absorbed into the intestinal cells, it attaches to *transcobalamin II* (TC2). Transcobalamin II is made in the intestinal cells<sup>48</sup> where it picks up B12 and transports it to all body tissues through the blood and cerebrospinal fluid.<sup>197</sup> Cyanocobalamin appears in the blood no longer than 5 hours after ingestion.<sup>52</sup> Once the B12-TC2 complex arrives at the cell where it is needed, B12 is released from TC2 in the form of hydroxocobalamin. It is then turned into methylcobalamin or adenosylcobalamin<sup>35</sup> and used for their respective enzymes. Transcobalamin II also transports B12 to the liver for storage on *transcobalamin III*.

### 2.5 Pernicious Anemia

Without intrinsic factor, very little B12 is absorbed. People with intrinsic factor defects who do not get treatment eventually develop a very serious, pernicious (destructive) anemia. More recently, *pernicious anemia* (PA) has become the term referring to people with intrinsic factor defects. PA requires medical treatment. Most doctors will prescribe B12 injections, although there is evidence that oral B12 is adequate (see Oral B12 for People with Malabsorption). Studies on people with PA have provided a great deal of insight into the role of B12 and amounts needed by humans.

## 3. Coenzyme Functions of Vitamin B12

In the cells of mammals,<sup>197</sup> B12 performs two different functions according to its form:<sup>25</sup>

- Methylcobalamin is used by the enzyme *methionine synthase* to turn homocysteine into methionine.
- 5'-deoxyadenosylcobalamin is used by the enzyme *methylmalonyl-CoA mutase* in converting methylmalonyl-CoA to succinyl-CoA.

### 3.1 Homocysteine Clearance

Methionine is an essential amino acid provided by the diet. Some methionine is turned into homocysteine. Homocysteine (HCY) appears to be a nerve and vessel toxin, promoting cardiovascular disease (CVD) at elevated levels. HCY is thought to cause CVD via oxidative and vessel wall damage.<sup>132</sup> The body normally turns HCY into other molecules, one of which is back into methionine. If this pathway is blocked, HCY levels increase. Methylcobalamin (B12) is needed by the enzyme that

#### Table 2.6 B12 In Older Adults

10-30% of people over 50 may have protein-bound B12 malabsorption from either decreased enzyme production or decreased stomach acid.<sup>206</sup> Decreased stomach acid reduces enzyme action on protein-bound B12 and/or allows bacterial overgrowth in the stomach and small intestine. Some types of bacteria use B12 for themselves.<sup>140</sup>

Approximately 2% of older adults do not produce enough intrinsic factor to prevent pernicious anemia.<sup>206</sup> In order to know whether someone suffers from such a problem, elderly people should have their B12 status tested (preferably their B12-TC2 levels<sup>48</sup>) every 5 years starting at age 55.

Because older adults (vegan or not) may not produce much intrinsic factor or gastric acid, chewable tablets and sublingual supplements may be the best way for them to obtain B12. People over 55 years should consider occasionally taking a sublingual B12 supplement of 500-1,000 µg.

converts HCY into methionine. Thus, if someone is B12 deficient, HCY levels will increase.

Elevated HCY can also happen with deficiencies in vitamin B6 or folate.<sup>48</sup> Herbert reports that RDA amounts of the deficient vitamin can reduce HCY levels to normal when a vitamin deficiency is the cause.<sup>48</sup>

### 3.2 Anemia, DNA, and Folate

The vitamin folate (aka folic acid) affects the anemia symptoms of B12 deficiency. Folate is needed to turn uracil into thymidine, an essential building block of DNA.<sup>37</sup> DNA is needed for new red blood cell production and division. B12 is involved in this process because in creating methylcobalamin (used in the HCY to methionine reaction), B12 produces a form of folate needed to make DNA. If there is no B12 available, this form of folate can become depleted (known as the *methyl-folate trap*) and DNA production slows.<sup>74</sup> See Figure 3 (below) for a description of this pathway.

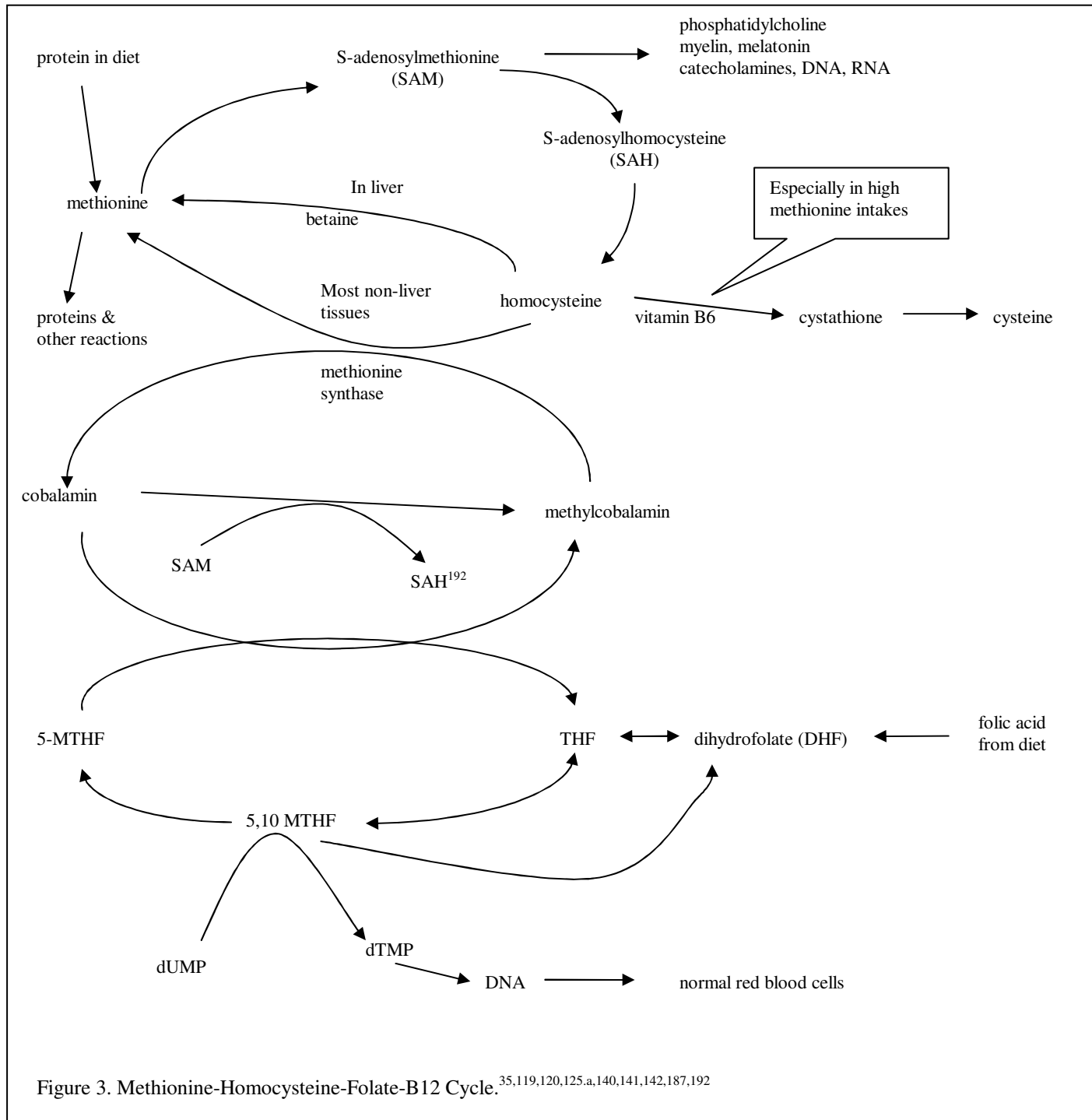


Figure 3. Methionine-Homocysteine-Folate-B12 Cycle.<sup>35,119,120,125.a,140,141,142,187,192</sup>

Only RNA is needed to produce the hemoglobin found in the red blood cells. Unlike DNA, RNA does not require thymidine. Therefore, if there is not adequate folate, the new red blood cells (which start out as large cells called *reticulocytes*) divide slowly, as they are dependent on DNA for division. At the same time, their hemoglobin is only dependent on RNA and it is produced at a normal rate. This causes large red blood cells known as *macrocytes*.<sup>35,37</sup> If enough of these macrocytes accumulate, the result is *macrocytic anemia*. Macrocytic anemia is determined by the average volume of the red blood cells, known as mean corpuscular volume (MCV). Macrocytic anemia is called *megaloblastic anemia* when determined by looking at the size of individual red blood cells.

If there are large amounts of incoming folate from the diet, the body does not need to rely on regeneration of folate from the B12 cycle. Instead, it can use the extra dietary folate to produce DNA, thus preventing macrocytic anemia (see Figure 3, bottom right-hand portion). This is why high intakes of folate are said to "mask" a B12 deficiency.

To add insult to injury, an iron deficiency (which results in small red blood cells from inadequate hemoglobin synthesis) can counteract the macrocytic cells, making it appear as though the blood cells are normal in the face of multiple nutritional deficiencies.<sup>46</sup>

Intestinal cells are also rapidly dying and being replaced using DNA. A B12 deficiency can make itself worse because it can prevent the production of the intestinal cells needed to absorb B12.

### 3.2.1 Macrocytic Anemia: Not an Adequate Measure of B12 Status

Traditionally, the existence of macrocytic anemia was relied on to indicate a B12 deficiency. However, neurological disorders due to B12 deficiency commonly occur in the absence of a macrocytic anemia. Lindenbaum et al.<sup>60</sup> (1988, USA) examined 141 cases of neurological problems due to B12 deficiency. 40 (28%) had no macrocytic anemia (iron deficiency may have contributed to a lack in 6 patients, and folate therapy could account for 2 others). These 40 had very high levels of sMMA (R .76-187  $\mu\text{mol/l}$ , 78% > 2  $\mu\text{mol/l}$ ) and HCY (23-289  $\mu\text{mol/l}$ , 45% > 100  $\mu\text{mol/l}$ ). Characteristic features of patients with B12 deficiency but without macrocytic anemia included: sensory loss, inability to move muscles smoothly (ataxia), dementia, and psychiatric disorders. They also had borderline (and sometimes normal) sB12 (Table 3.2.1). One patient died during the first week of treatment, but the other 39 benefited from B12 therapy. Some patients had residual abnormalities after years of treatment.

### 3.3 Methylmalonic Acid (MMA)

The second coenzyme form of B12, adenosylcobalamin, takes part in the conversion of methylmalonyl-CoA to succinyl-CoA. When B12 is not available, methylmalonyl-CoA levels increase. Methylmalonyl-CoA is then converted to methylmalonic acid (MMA) which then accumulates in the blood and urine. Since B12 is the only coenzyme required in this pathway, MMA levels are the best indicators of a B12 deficiency.

High MMA level can also (but rarely) be caused by genetic defects, kidney failure, low blood volume, gut bacteria changes, pregnancy, and thyroid disease.<sup>126,127</sup> See Appendix B for a brief discussion of slightly elevated MMA levels.

## 4. Serum B12 Level: Not a Reliable Measure of B12 Adequacy

A sB12 level below the normal range indicates that B12 levels are becoming depleted. *However, a sB12 level in the normal range does not ensure that B12 levels are healthy.* This is well known by people who study B12. Unfortunately, medical practitioners still use serum B12 to evaluate levels, even of vegans.

**Table 3.4 Values Related to B12**

Serum B12 (sB12) is measured in both pmol/l and pg/ml. **All sB12 values in this article are in pg/ml** (using a conversion factor of 1 pg/ml = 1.35 \* pmol/l<sup>98</sup>).

Average daily dietary intake of B12 for adults in the USA:<sup>206</sup>

men: 5  $\mu\text{g}$ ; women: 3.5  $\mu\text{g}$

normal serum B12

range, newborns<sup>118</sup> 160-1300 pg/ml

range, adults<sup>a</sup> 200-900 pg/ml

average 450 pg/ml

<sup>a</sup>sB12 does not change more than 7% from week to week under normal conditions (i.e. consistent intake, no disease).<sup>117</sup>

breast milk<sup>C,55</sup>

180-300 pg/ml

<sup>C</sup>Numbers vary widely depending on measurement methods and differences in maternal stores and intake.

normal MCV range<sup>95</sup>

80-96 fl

normal serum MMA (sMMA)

.07-.27  $\mu\text{mol/l}$ <sup>56,147,160</sup>

sMMA cutoff in diagnosing

B12 deficiency .376  $\mu\text{mol/l}$ <sup>147</sup>

normal urine MMA (uMMA)

.58-3.56  $\mu\text{mol/mmol Cr}$ <sup>213</sup>

normal serum HCY

2.2-13.2  $\mu\text{mol/l}$ <sup>192</sup>

Pre-treatment ranges in infants and children who have serious neurological problems due to genetic defects in B12 metabolism:<sup>129</sup>

sMMA 65-246  $\mu\text{mol/l}$

urine MMA

820-11,292  $\mu\text{mol/mmol Cr}$

HCY

trace-89  $\mu\text{mol/l}$

**Table 3.2.1. sB12 Levels in Neurological Patients Without Macrocytic Anemia**

# of patients	sB12
2	> 200
16	100-200
22	< 100

#### 4.1 Seaweeds Can Falsely Inflate sB12 Levels

Methods for determining sB12 levels rarely distinguish between B12 and inactive B12 analogues. Seaweeds contain a variety of inactive B12 analogues. Someone who is eating large amounts of seaweed may have serum B12 levels well above normal, but much of it could be inactive B12 analogues that may actually be interfering with B12 function (see Appendix H).

#### 4.2 Measuring Transcobalamin II

Transcobalamin II (TC2) transports B12 to tissues. TC2 normally contains about 20% of B12 in the blood.<sup>48</sup> TC1 and TC3 are the proteins that normally store the other 80% of the B12 in the blood.<sup>48</sup> When absorption of B12 via the intestines slows, B12-TC2 levels fall rapidly.<sup>48</sup> If TC2 lacks B12, the vitamin will not be delivered to tissues, regardless of whether the total sB12 is low, normal, or high.<sup>46</sup> TC2 is depleted of B12 within days after absorption stops.<sup>48</sup> Thus, the tissues are not getting B12 even though sB12 appears normal.

#### 4.3 B12-Deficient Nerve Damage with Normal sB12 and No Macrocytosis

Some people with normal sB12 levels and without macrocytosis suffer from B12-deficient nerve damage, elevated homocysteine, and elevated MMA acid levels. See Section 3.2.1 for more details.

### 5. Symptoms of B12 Deficiency

#### 5.1 Neurological Symptoms

Neurological symptoms, often referred to as subacute combined degeneration (SCD), are the biggest concern regarding B12 deficiency. The damage can be irreversible if not caught early enough. SCD affects peripheral nerves and the spinal cord, and is normally different in children than adults.<sup>197</sup> There are 3 main theories as to how this happens, listed in Table 5.1.

#### 5.2 Early, Noticeable Symptoms of B12 Deficiency:<sup>18</sup>

- unusual fatigue
- faulty digestion
- no appetite
- nausea
- loss of menstruation

#### 5.3 Other symptoms of B12 Deficiency:

- numbness and tingling of the hands and feet<sup>18</sup>
- nervousness<sup>18</sup>
- diarrhea<sup>60</sup>
- mild depression<sup>18</sup>
- striking behavioral changes<sup>18</sup>
- paranoia<sup>18</sup>
- hyperactive reflexes<sup>18</sup>
- fever<sup>171</sup>
- frequent upper respiratory infections<sup>19</sup>
- impotence<sup>49</sup>
- impaired memory<sup>49</sup>
- infertility<sup>55</sup>
- sore tongue<sup>60</sup>
- enlargement of the mucous membranes of the mouth, vagina, and stomach<sup>117</sup>
- macrocytic anemia
- low platelet count<sup>157,171</sup> and increased bleeding<sup>171</sup>
- neutropenia<sup>171</sup>

There are some serious diseases that have similarities to B12 deficiency, including Guillian-Barre syndrome, Lyme neuropathy, heavy metal intoxication, and lupus myelopathy.<sup>178</sup> Anyone who develops symptoms of nerve damage should see a doctor *immediately* for treatment.

#### 5.5 Ways to Get B12 Deficiency

The two main ways people get vitamin B12 deficiency are inadequate dietary intake and inadequate absorption from loss of intrinsic factor or lack of stomach acid. Other, much less common ways are listed in Appendix K.

### 6. Unusually High B12

Some diseases can cause sB12 levels to increase higher than normal in some patients. These include kidney, liver, cancer, and duodenal ulcers,<sup>117</sup> diabetes, obesity,<sup>118</sup> and cyanide metabolism defects (see Appendix M).<sup>52</sup> B12 deficiency can be hidden by alcoholism because excess B12 is released into the blood from the damaged liver.<sup>48</sup>

<p>1. B12 deficiency produces a lack of methionine for conversion into S-adenosylmethionine (SAM).<sup>35</sup> SAM is required for the production of phosphatidylcholine<sup>36</sup> which is part of the myelin (the fatty material that insulates many nerves).<sup>36</sup> (See Figure 3.)</p> <p>2. The inability to convert methylmalonyl-CoA (a 3-carbon molecule) to succinyl-CoA (a 4-carbon molecule) results in an accumulation of propionyl-CoA (a 3-carbon molecule). Fatty acids are normally made by adding 2 carbons at a time to an even numbered carbon molecule. In an overabundance of 3 carbon molecules, large amounts of unusual 15-carbon and 17-carbon fatty acids may be produced and incorporated into nerve sheets, causing altered nerve function.<sup>103</sup></p> <p>3. Nerves are damaged by different hormone-like molecules (cytokines, tumor necrosis factor, and epidermal growth factor) which become unbalanced in the nerve tissue in B12 deficiency.<sup>197</sup></p>
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## 7. Sources of B12 for Vegans

### 7.1 Myth about How Often Someone Needs B12

When some vegans hear other vegans saying things such as "B12 is stored in the body for years," they sometimes take it to mean you only need B12 once every few years. (I do not blame people for thinking this; such statements are irresponsibly misleading.) You cannot keep your B12 status healthy by simply eating a small amount of B12 every few weeks. The body is constantly losing small amounts of B12 and

the length of time that any given molecule of B12 will stay in the body will vary based on intake levels, absorption efficiency, and which cell the B12 ends up in.

### 7.2 Small Amounts of Animal Products

There is evidence that B12 function cannot be restored to optimal levels by adding small amounts of animal products into the diet.<sup>102</sup> See Table 7.2.2 for details.

### 7.3 Fortified Foods

*Streptomyces griseus*, a bacterium once thought to be a yeast, was the commercial source of vitamin B12 for many years.<sup>52,222</sup> The bacteria *Propionibacterium shermanii* and *Pseudomonas denitrificans* have now replaced *S. griseus*.<sup>224</sup> At least one company, Rhone Poulenc Biochimie of France, is using a genetically engineered microorganism to produce B12.<sup>223</sup>

There are many vegan foods fortified with B12. They include non-dairy milks, meat substitutes, breakfast cereals, and one type of nutritional yeast.

The "Daily Value" for B12 found on food labels is based on 6 µg, which was the RDA in 1968. How to calculate the amount of B12 from a food label is explained in Table 1 under Fortified Foods.

#### 7.3.1 Brewer's and Nutritional Yeasts

Brewer's and nutritional yeasts do not contain B12 unless they are fortified with it. At least one vegan B12-fortified yeast is currently on the market: Red Star Vegetarian Support Formula (also known as Red Star Yeast T6635+). Unfortunately, there are some drawbacks to relying solely on B12-fortified nutritional yeast for B12:

1. Nutritional yeast usually comes from bins in health food stores. If not careful, it would be easy for a store employee to order the wrong nutritional yeast out of the distributor catalogs which often list many yeasts. It would also be easy to accidentally put the wrong yeast into the Vegetarian Support Formula bin.
2. B12 is light sensitive. Nutritional yeast is likely to be exposed to the light because it is often stored in clear bins or plastic bags.
3. At least one vegan who thought he was getting B12 from nutritional yeast developed B12 deficiency symptoms that cleared up upon taking a B12 supplement (see the sidebar From a Long-Term Vegan on p. 1).

If you are using Red Star Vegetarian Support Formula for B12, make sure you are actually getting what you think. It is also best to keep it in the refrigerator or freezer, out of the light.

Please note: Red Star Vegetarian Support Formula nutritional yeast has many other nutrients and I eat it myself, but vegans shouldn't rely on it for their sole source of B12.

#### 7.3.2 Cooking Foods

Cooking may destroy the B12 found naturally in animal foods. Cyanocobalamin, the form in fortified foods, may be more stable during cooking. For example, in an acid medium (pH 4-7), cyanocobalamin can withstand boiling at 120° C.<sup>116</sup>

**Table 7.2.1 What is Statistical Significance?**

A statistically significant difference between 2 groups normally means that the difference has a 5% or less chance of being due to random chance.

**Table 7.2.2 Moderate Amounts of Animal Products Do Not Replenish B12 Stores**

van Dusseldorp et al.<sup>102</sup> (1999, Netherlands) investigated whether moderate consumption of animal products is sufficient for achieving normal B12 function in 73 adolescents who had been strict macrobiotics (MAC) until 6 years old and then switched to a lacto-ovo vegetarian (LOV) or nonvegetarian (NV) diet. 94 people who had never been macrobiotics or vegetarian were controls. Dairy supplied an average of ~ 1 µg B12/day for the MACs. They also ate fish, red meat, or chicken 2-3 times/week.

		#	sB12 pg/ml	sMMA (R) µmol/l	% > .41 µmol/l	HCY > 12.8 µmol/l
MAC	Boys	37	288 <sup>A</sup>	.29 (.09-.93) <sup>C</sup>	24%	8%
	Girls	36	389 <sup>B</sup>	.25 (.09-.70) <sup>D</sup>	17%	11%
Control	Boys	39	653 <sup>A</sup>	.15 (.06-.43) <sup>C</sup>	5%	5%
	Girls	55	618 <sup>B</sup>	.17 (.07-.40) <sup>D</sup>		

<sup>A,B,C,D</sup> Statistically significant difference between groups with same letters. R - range.

Thus, moderate consumption of animal products was not enough to restore normal B12 status.

## 7.4 Multivitamins

There are some concerns about vegans relying solely on multivitamins that contain only small amounts of B12 (less than about 10 µg):

- Herbert et al.<sup>44</sup> (1982, USA) reported that vitamins B1, B3, C, and E, and copper and iron can damage B12. They tested 15 multivitamin preparations used daily by approximately 100 million Americans for inactive B12 analogues and all preparations contained some (6-27% of total corrinoids). In fact, vitamin C in doses of 500 mg or more taken with meals or within one hour after a meal, may diminish B12 availability or destroy the B12.<sup>35</sup>
- Many multivitamins cannot be chewed, which is very important for B12 absorption in some people.

That said, if a multivitamin is chewable and has 10 µg of B12 (as cyanocobalamin) or more, and taken daily, it is most likely adequate.

## 7.5 Supplements

### 7.5.1 Safety

In 1988, Herbert cautioned that large amounts of B12 may eventually be found to be harmful.<sup>47</sup> In contrast, Hathcock & Troendle<sup>40</sup> (1991) point out that there appears to be little or no question that B12 intakes of 500-1000 µg/day are safe. The cobalt and the cyanide contribution of 1000 µg/day of cyanocobalamin are considered toxicologically insignificant.<sup>40</sup> The Institute of Medicine has not set an Upper Tolerable Limit for a daily vitamin B12 intake.<sup>206</sup> People with cyanide metabolism defects, chronic kidney failure, and probably smokers should take a different form of B12.

### 7.5.2 Chew or Dissolve Supplements Under the Tongue

Crane et al.<sup>18</sup> (1994, USA) noted that tablets of one vitamin company dissolved slowly in water and acid. They then conducted a study to see if vegan patients who had not responded to oral B12 (described below) tablets swallowed whole could improve their B12 response by chewing the tablets. 7 people chewed the tablets of 100 µg (once a week for 6 weeks) and their average sB12 levels went from 116 to 291. Of the 9 who didn't chew, theirs increased from 123 to only 139. These 9 then chewed 500 µg/day for 10 days and their levels rose to normal with a final average of 524 ± 235. Three participants could not raise their levels orally and required B12 injections to maintain sB12 above 300. Crane et al. recommend that vegans chew, or let dissolve in the mouth, a 100-500 µg B12 tablet once a week or more often.

### 7.5.3 Light

B12 supplements should not be left in the light as prolonged light damages cyanocobalamin.<sup>89,52</sup>

### 7.5.4 Twinlab B12 Dots

Twinlab B12 dots are not vegetarian. See Appendix G.

### 7.5.5 Non-cyanocobalamin Supplements

In addition to cyanocobalamin, there are oral supplements available for methylcobalamin, adenosylcobalamin (known in the supplement industry as *dibenzozide* and *coenzyme B12*), and to a lesser extent, hydroxocobalamin. See Appendix N. As mentioned above, these forms of B12 are probably preferable for vegan smokers.

S-adenosylmethionine (aka SAM and SAME) is another supplement that has received attention. It is found in the homocysteine-methionine pathway, and some people think it may be relevant to B12 status in people who have been B12 deficient. More information is in Appendix N.

## 7.6 Oral B12 for People with Malabsorption

Some recent studies have shown that taking large amounts of sublingual B12 can normalize B12 status even in people with pernicious anemia. For more information, see Appendix L.1.1.

## 8. The B12 Status of Vegans

### 8.1. Infants

During pregnancy, B12 is actively transported by the placenta to the fetus, which can reduce the mother's stores of B12 if she has none in her diet.<sup>55</sup>

In contrast to the adult's normal storage of 2-3,000 µg B12, newborn infants (of mothers with normal B12 stores) have body stores of only 25 µg. Studies have shown that colostrum and/or milk during the first week of life contains larger amounts of B12 (as much as 2421 pg/ml) than later milk.<sup>94</sup> The B12 in mother's milk is more related to current B12 intake than to

#### Table 7.7 B12 in Animal Foods

In animal foods, B12 is protein-bound and partially light-protected.<sup>52</sup>

It is mostly in the form of adenosylcobalamin and hydroxocobalamin. In cow's milk it is mainly adenosylcobalamin, but in dried milk it is mostly hydroxocobalamin with a trace of cyanocobalamin. Egg yolks and sardines contain a substantial proportion of methylcobalamin.<sup>52</sup>

#### Table 8.1 Infancy: A Critical Time

Weaning may be a critical time to ensure that vegan infants have plenty of B12, as low sB12 at weaning was correlated to retarded growth in one study of vegan, macrobiotic children.<sup>88</sup>



mother's B12 stores.<sup>206</sup> B12 stores in infants at birth are normally adequate to last the first several weeks of life,<sup>55</sup> after which they must get it from breast milk or other sources. Serum B12 levels of healthy, nonvegetarian infants decrease progressively until 6 months,<sup>64</sup> after which they start to increase again.

If the mother is B12 deficient during pregnancy, the baby may have low B12 levels and some have developed clinical signs of deficiency as young as 2 weeks of age.<sup>10</sup> At birth, these newborns typically have higher sB12 than their mothers and usually show no deficiency symptoms.<sup>64</sup>

Minet et al.<sup>126</sup> (2000, Switzerland) found that among apparently healthy infants, low sB12 (and serum folate) was correlated with increased homocysteine levels; many infants in the lower range of normal sB12 had elevated homocysteine levels. Breast-fed infants had significantly lower sB12 levels and significantly higher homocysteine levels than infants using a formula fortified with B12. This does not mean that mothers should choose formula over breast milk, as there are many advantages to breast-feeding. Rather, it indicates that even among the nonvegetarian population, B12 can be a problem in infants, and breastfeeding mothers should consider a B12 supplement for themselves and/or their infants.

In rare cases, some infants cannot convert cyanocobalamin to an active form and thus cannot rely on cyanocobalamin supplements.<sup>47</sup> These infants require medical treatment.

### 8.1.1 Infants of Vegan Mothers Who Do Not Use B12 Supplements

Since 1980, and excluding the Black Hebrews described below, there have been 30 reports of very serious B12 deficiency in vegan mothers' infants whose main or only food was breast milk. This happened only when the mother did not supplement her own or the baby's diet with B12. In many cases, the mother belongs to a subculture which does not believe in supplementation. Lack of B12 in the mother's diet during pregnancy has been shown to cause a severe lack of myelin in nerve tissue.<sup>61</sup> Appendix C lists the cases since 1981 in which infants of vegan mothers have suffered from B12 deficiency. In all cases, the infants are healthy until about 1-12 months of age after which they failed to thrive and showed developmental regression. They are lethargic, lose their ability to use their muscles adequately, and sometimes cannot sense properly. They normally have macrocytic anemia, which is unusual in childhood and normally due to nutritional deficiency rather than to congenital disease.<sup>27</sup>

### 8.1.2 Correction of B12 Deficiency in Infants

Although B12 supplementation has been shown to result in rapid lab value improvements in these infants, concern has been raised about their long-term development. von Schenck<sup>103</sup> (1997) reviewed 25 reports of infant B12 deficiency which had appeared in the scientific literature. Among the 25, vegan mothers were associated with 13 of the infant B12 deficiencies (9 of which occurred since 1979 and are included in Appendix C). Of the 7 vegans that were followed for a certain amount of time, 5 had abnormal neurological development at their final follow-up (26 months, 26 months, 2 years, 5 years, and 12 years after diagnosis). 2 were normal at their respective final follow-up (13 months and 2 years). von Schenck says, "Efforts should be directed therefore to preventing deficiency in pregnant and breast-feeding women on vegan diets and their infants.... If dietary changes are not acceptable to parents, vitamin B12 supplements are essential."

#### Table 8.3.1.1 Case Study: 36 y.o. Vegan Not Supplementing with B12

Li & McKay<sup>59</sup> (2000, UK) reported a woman who was vegan for many years. She had megaloblastic anemia due to folate deficiency 6 years earlier. Her B12 levels and absorption were normal at that time. During her current visit to the clinic, she reported decreased vision during the previous week. Again, she had signs of megaloblastic anemia, but this time with low sB12 (130 pg/ml) and low folate. She was treated with oral folate and iron, and a B12 injection. Her vision returned to normal within days. Again, there were no features of malabsorption and Li & McKay concluded that her vitamin deficiencies were of dietary origin.

Grattan-Smith et al.<sup>36</sup> (1997, Australia) reports the cases of 3 infants of vegan mothers who developed muscle twitching and/or seizure-like symptoms upon treatment with B12 in doses of 500 µg or more. Other infants have developed tremors at doses of 300 µg. Grattan-Smith et al. state that in the cases of dietary deficiency in infants, it seems unnecessary to give such high doses of B12.

Goraya<sup>33</sup> (1998, India) reported that in India, many infants have "infantile tremor syndrome." This occurs in exclusively breast-fed infants from low socioeconomic conditions. Documented B12 deficiency, megaloblastic anemia, and response to B12 therapy was observed in some but not all patients.

#### Table 8.1.3 A Commitment to Dogma

The reason the Black Hebrew leaders gave for withholding vitamin supplements:

If the organism of the cow subsists by eating plant foods only, why should the human organism need artificial vitamins, animal foods, and drugs?

### 8.1.3 Black Hebrews

Zmora et al.<sup>110</sup> (1979, Israel) reported severe nutritional deficiencies in 4 infants from a vegan religious community, the Black Hebrews. The Black Hebrews originated in the USA. The infants received breast milk until the age of 3 months; thereafter, breast milk was supplemented with, or replaced by, extremely low caloric preparations. All of the infants had profound protein-calorie malnutrition, severe rickets, osteoporosis, and B12 and other deficiencies. One infant died, while 3 others recovered after treatment. After discharge of the infants from the hospital, the community

responded well to a modification of the infants' diet which did not violate their vegetarian philosophy. However, they refused to give their infants B12 on a regular basis.

Shinwell & Gorodischer<sup>90</sup> (1982, Israel) also reported on a Black Hebrew religious community. Infants were breast-fed for 3 months and then fed mainly a dilute, homemade soymilk from 3 months to 1 year. 25 infants showed evidence of protein-calorie malnutrition, iron and B12-deficient anemia, rickets, zinc deficiency, and multiple recurrent infections. 3 of the infants were dead on arrival to a hospital. 5 more died within a few hours of admission despite treatment. Serum B12 levels were low in 9 of 15 cases (undetectable in 3). Shinwell & Gorodischer said, "In spite of tactful but perseverant contact with the community health leaders during this time, no change in feeding habits of infants was achieved."

#### 8.1.4 Vegan Infants Taking B12 Supplements

In stark contrast, Sanders<sup>86</sup> (1988, UK) studied the growth and development of 37 vegan children. All were breast-fed for their first 6 months and in most cases well into their second year. The majority of these children grew and developed normally. They tended to be smaller in stature and lighter in weight than the general population. Energy, calcium, and vitamin D intakes were usually below the recommended amounts. Their diets were generally adequate with a few children having low intakes of riboflavin and B12. Most parents knew to supplement the diet with B12. Sanders concluded that provided sufficient care is taken, a vegan diet can support normal growth and development. Sanders<sup>87</sup> (1995, UK) points out that many potential hazards of vegan diets can be avoided by the use of soymilks fortified with calcium and B12 in the post-weaning period.

Fulton et al.<sup>111</sup> (1980, USA) studied 48 preschool children between 2-5 years old, who had followed a vegan diet since birth. They lived at The Farm, a vegan commune in Tennessee, where soymilk was fortified with B12 at a rate of 6.25 µg per 8 oz of milk. They also supplemented with nutritional yeast containing 2.0 µg B12 per tablespoon, which they used as a flavoring agent in many foods. B12 status was not assessed, but there were no cases of overt B12 deficiency reported.

##### **Table 8.3.1.2 Case Study: 57 y.o. Vegan Not Supplementing with B12**

Carmel<sup>13</sup> (1978, USA) reported on a man admitted to the hospital in 1976 with trouble walking. He had been in good health until 1972 when he retired because of fatigue. He had been almost vegan, eating small amounts of dairy, for 25 years. He had siblings who ate a similar diet, but who took B12 and did not develop problems. In 1974, a chiropractic nutritionist told him his problems were not related to a B12 deficiency. He then replaced the dairy in his diet with a raw egg yolk every other day. He improved for a short time but then got worse. Two weeks before admission, he began taking 100 µg B12/day. Upon admission, he had decreased sensation in the hands and below the knees, with slight megaloblastic anemia. His sB12 levels were 146 pg/ml. He was injected with 1000 µg/day of B12 for 3 weeks which caused rapid improvement. He then received B12 injections once a month for 6 months (by which time his only neurological problem was in his feet) and then he started 100 µg/day orally. He also started eating fish, dairy products, and eggs. His sB12 stabilized at about 300. He had slightly depressed intrinsic factor secretion and reduced stomach acid, which was apparently not due to the B12 deficiency as it did not improve with treatment. His problems were probably due to a combination of low B12 intake and decreased absorption.

#### 8.2 Children & Teenagers

##### 8.2.1 Vegan Children & Teenagers Not Supplementing with B12

Ashkenazi et al.<sup>6</sup> (1987, Israel) reported a 14-year-old girl who had been vegetarian (and apparently vegan) for 8 years after witnessing the slaughter of a cow. She had been healthy previously and seemed well nourished. She had neurological problems including an unstable walk, unsteady standing with eyes closed, some impaired sensations, mildly reduced muscle strength, and reduced ankle-jerk reflex. Her sB12 was 50 pg/ml. Absorption was normal. She had not been taking B12 supplements as her parents were unaware of the need and her doctor was unaware of her diet. Injections and supplements were given, and the girl followed advice to begin eating fish and dairy products. (This was unnecessary, and could have been avoided if she had been supplementing the entire time.) A rapid improvement was noted with a complete neurological recovery after 4 months.

##### **Table 8.3.1.3 Case Study: 56 y.o. Vegan Not Supplementing with B12**

Murphy<sup>73</sup> (1981, UK) reported a male vegetarian of 40 years. He had been vegan for the previous 4 years since developing angina. He had been treated for the previous 8 years with phenobarbital for epileptic seizures. He was admitted for an aortic valve replacement and found to have low hemoglobin, a 98 fl. MCV, and 50 pg/ml sB12. Folate was normal. A deoxyuridine suppression test showed B12 deficiency. He was treated with 5 µg B12/day, orally, for one month. His sB12 levels rose to 205 and his hemoglobin returned to normal.

Chiron et al.<sup>153</sup> (2001, France) reported a 15-year-old boy hospitalized because of lameness and jaundice. He had B12-deficiency anemia as well as rickets. A diet supplemented with calcium, vitamin D, and B12, and orthopedic treatment stabilized the bone lesions. The anemia was cured by a B12 injection(s). The authors state, "The adolescent and his brother were victims of a diet imposed by a cult and a lack of care due to their parents refusing that a vegan diet was the cause of the deficient pathology." The article was in French and no further information was given in the abstract.

More studies on vegan children are listed in Appendix E.

### 8.2.2 Vegan Children & Teenagers Supplementing with B12

Sanders & Purves<sup>85</sup> (1981, UK) assessed the nutritional status of 23 vegan children (1-5 yrs old), contacted through The Vegan Society (UK). All the children had been breast-fed for at least the first 6 months of life and in most cases well into the second year. The majority of children were growing normally but tended to be shorter and lighter than the standards. Energy, calcium, and vitamin D intakes were usually below those recommended. Their diets were generally adequate with a few children having low intakes of vitamin B2 and B12. All parents were aware of the need for B12 supplementation and provided it through yeast extract, soymilk, TVP, or B12 syrup. B12 intake was  $2.7 \pm 0.63$ , (range .3-15.2  $\mu\text{g/day}$ ). The parents were very receptive to advice. Sanders concluded that, provided sufficient care is taken, a vegan diet can meet the nutritional requirements of the preschool child.

### 8.3 Adult Vegans

#### 8.3.1 Studies on Adult Vegans Not Supplementing with B12

Australians have a lifestyle similar to North Americans, but with limited B12 fortified foods. Hokin & Butler<sup>50</sup> (1999) examined Australian Seventh-day Adventist ministers, aged 22-80, who did not take B12 supplements. Results (Table 8.3.1.5) showed vegans to have significantly lower sB12 levels.

Areekul et al.<sup>4</sup> (1988, Thailand) found a significant difference between B12 levels ( $62 \pm 78$  pg/ml) in 29 apparently healthy vegetarians and 60 omnivores ( $629 \pm 160$ ). 8 vegetarians had undetectable B12 levels, while only 2 had levels over 200. The researchers did not state whether any of the vegetarians were taking B12 supplements, but they appeared not to be doing so.

**Table 8.3.1.6 sB12 levels in 4 vegans with history of nerve-related problems.**

subject	1	2	3	4
age	56	64	40	57
serum B12	65	84	89	90

Table 8.3.1.6). 3 of the 4 were followed and showed substantial clinical improvement after B12 injections which increased their B12 levels to over 200.

Tungtrongchitr et al.<sup>99</sup> (1993, Thailand) studied 132 Thai adult vegetarians (64 males, 68 females) and 47 healthy nonvegetarians. The vegetarians apparently ate no animal products. Serum B12 levels are listed in Table 8.3.1.7. There were some blood cell differences between vegetarians and nonvegetarians. Serum B12 decreased as years as a vegetarian increased. Groups practicing vegetarianism for  $\geq 6$  years resulted in particularly low average sB12 levels (83-135 pg/ml).

**Table 8.3.1.8 Case Study: Rastafarian Community**

Campbell et al.<sup>12</sup> (1982, Jamaica) reported 10 Rastafarian men (age 18-49) with B12 deficiency. They had been vegan 2-20 years. 8 had neurological symptoms. B12 malabsorption was ruled out in all cases. 8 had moderate to severe macrocytic anemia. 6 were jaundiced, 3 had a swollen tongue, 2 had anorexia, 1 had vomiting, 1 had abdominal pain, and there were other symptoms. Their sB12 range was 10-130 pg/ml, with only 2 over 75. Their blood completely responded to cyanocobalamin injections. Of 3 who had subacute neurological degeneration, one recovered completely. Another improved considerably but had residual motor defects after several months of treatment. The third patient died of a heart attack 11 days after admission. Campbell stated, "Our patients were not very cooperative in taking oral vitamin B12 or attending the clinic for vitamin B12 injections...."

**Table 8.3.1.4 Case Study: 33 y.o. Vegan Not Supplementing with B12**

Milea et al.<sup>67</sup> (2000, France) reported a male vegan of 13 years who appeared to have a poor diet given deficiencies of vitamins A, B1, B12 (154 pg/ml), C, D, E, and folate, and zinc and selenium. He had been vegan for "improved health," and did not smoke or drink alcohol. He was found to have severe optic nerve disease, sensory nerve problems, reduced hemoglobin, and a 110 fl. MCV. He had no signs of malabsorption. After a multivitamin and B12 injections of 1000  $\mu\text{g/day}$  for 1 week, his hemoglobin and sensory nerve problems disappeared, but his vision didn't recover. The authors concluded that "The optic neuropathy in our patient was apparently related to deficiencies of B12 and B1, but other associated deficiencies may have had a role."

**Table 8.3.1.5 Results of Hokin & Butler<sup>50</sup>**

	#	sB12 (range)	Malabsorption / IF deficiency
NV	53	$394^A \pm 196$ (181-973)	NR
LOV	234	NR <sup>B</sup>	20% / 10%
Vegans	11	$224^A \pm 100$ (99-420)	NR
Total	298		

<sup>A</sup>Statistically significant difference between groups with same letters.

<sup>B</sup>Not significantly different from the NV. NV – nonvegetarians.

Bar-Sella et al.<sup>8</sup> (1990, Israel) compared 36 vegans (5-35 years on diet) to 36 nonvegetarians. None of the vegans used supplements. Vegans had significantly lower levels of B12 (164 vs. 400 pg/ml). No nonvegetarian was deficient in B12, but 2 were borderline. No subjects had blood abnormalities. 4 vegans had a history of muscle pain, abnormal sensations in the legs, and difficulty concentrating (sB12 levels listed in

**Table 8.3.1.7 Tungtrongchitr et al.<sup>99</sup>**

	sB12	sB12 range
Male NV	490	176-825
Female NV	500	270-1400
Male Veg	117	31-730
Female Veg	153	22-460

**Table 8.3.1.9 Case Study: 80 y.o. Vegan Not Supplementing with B12**

Bernstein<sup>9</sup> (2000, USA) describes a man in his eighties who had been in excellent health, and was a runner. He had been vegan for 38 years and attributed his ability to outperform younger people to his diet. In the span of a few weeks, mental disturbances began to set in. He cried, was confused, got lost, was incontinent, lost control of his bowels, and lost motor skills to the point where he could barely stand with help. He was diagnosed with “senile dementia.” A blood test showed slightly large red blood cells. Further blood tests revealed that his sB12 was undetectable. He was given an injection of 1000 µg of B12. The next morning he could sit without help. His bladder control returned within 48 hours. By the end of the week, he could play simple card games, read get-well cards, and talk on the phone. He still cried easily and his attention span was too short to go back to work. Bernstein concludes, “[A] diet free of animal protein can be healthful and safe, but it should be supplemented periodically with vitamin B12.”

**Table 8.3.1.10 Results of Crane et al.<sup>18</sup>**

	#	sB12 < 200	sB12 < 100	sB12 range
no FF or SUP for 1 yr	76	47 (62%)	19%	41-615
fortified soymilk for 1 yr	20 <sup>A</sup>			304-540

<sup>A</sup>8 were children; FF -fortified foods; SUP - supplements

Crane et al.<sup>18</sup> (1994, USA) measured the sB12 of healthy adult vegans (1-28 years on the diet) who had not used B12 supplements or fortified foods in the previous year or more. Results are in Table 8.3.1.10.

Participants with low sB12 were given oral B12. The sB12 of some of these participants did not increase, which led to the study about chewing B12 tablets mentioned earlier in section 7.5.2.

Crane et al.<sup>18</sup> (1994, USA) examined urinary MMA levels in 29 vegan adults who had not used B12 supplements or fortified foods in the previous year:

- 11 had sB12 levels < 200. Their average MCV (95.9 ± 5.5 fl) was significantly higher than those with higher sB12.
- 7 of these 11 had high MMA.
- None with normal B12 levels had elevated MMA.
- One vegan of 5 years had no symptoms of B12 deficiency despite a sB12 of 90. However, after 1 month of oral B12, he noticed that his chronic indigestion after meals had disappeared.

Crane et al.<sup>19</sup> (1998, USA) studied 2 families (9 people) who were vegan for over 1 year and who did not regularly take B12 supplements or fortified foods:

- They ate food from their gardens or local grocery stores.
- sB12 was below 200 in 8 members; average sB12 was 190 ± 65.
- The only patient over 200 (331) was also the only one with signs of deficiency (mild numbness in one hand and easy fatigue). These cleared up after starting oral B12, so her high levels might be attributable to inactive B12 analogues.
- 8 had high uMMA.
- Homocysteine levels were in the normal range, but dropped after B12 therapy.
- The subjects were given 500 µg B12/day, which they chewed before swallowing. After 2 months:
  - Average red blood cell count increased.
  - Average total cholesterol decreased by 10.3% and LDL cholesterol decreased by 19.6%. (Note: This is the only study that observed a cholesterol reduction in vegans because of B12 supplementation.)
  - Serum MMA levels dropped dramatically (from .65 ± .61 to .13 ± .06 µmol/l).
  - Average sB12 rose to 553 ± 113 pg/ml.

Crane et al. write:

The laboratory evidence in these two families is too strong to believe that they had an adequate amount of [B12]. It is remarkable that they had been on a total vegetarian diet for so long, yet with little or no clinical symptoms or signs of an insufficiency of cobalamin. In this study none of the family members were aware of symptoms of easy fatigability, tingling in the extremities, or frequent upper respiratory infections.

### 8.3.2 Vegans Taking B12 Supplements

Sanders et al.<sup>78</sup> (1978, UK) compared adult vegan (no animal products  $\geq$  1 year; average 8 yrs) members of The Vegan Society (UK) to age and sex matched NV. Results (Table 8.3.2.1) indicate that supplementing vegans had higher sB12 levels than non-supplementing.

Alexander et al.<sup>3</sup> (1994, New Zealand) looked at 18 vegetarians. sB12 levels listed in Table 8.3.2.2. Other findings were as follows:

- 6 had sB12 levels below the reference range: 82, 86, 182, 190, 197 (vegan or LOV wasn't specified). None had macrocytosis. The woman with the lowest value was found to have an intrinsic factor deficiency.
- 7 vegetarians took B12 supplements and only one had low B12 levels, but their levels were not significantly different than those who did not take supplements.
- The vegans actually had a higher average sB12 than the LOV. This is generally not the case.

Harman & Parnell<sup>39</sup> (1998, New Zealand) compared 24 adult vegetarian Seventh-day Adventists (SDA) (including some vegans; number not specified) to 23 nonvegetarian SDAs. Some vegetarians were taking B12 supplements and injections. sB12 did not differ significantly between groups. See Table 8.3.2.3 for results.

Haddad et al.<sup>38</sup> (1999, USA) compared vegans to nonvegetarians. Results are in Table 8.3.2.4.

**Table 8.3.2.1 Results of Sanders et al.<sup>78</sup>**

	sB12
22 Vegans	289 R 120-675
22 NV:	371 R 250-775

5 vegans were not taking B12 supplements or fortified foods:

yrs as vegan:	2	4	6	6	13
sB12	200	225	230	180	120

**Table 8.3.2.2 sB12 levels in Alexander et al.<sup>3</sup>**

	#	yrs on diet	sB12
vegan	5	6.6 (1-12)	277
LOV	13	> 5	229

**Table 8.3.2.3 Results of Harman & Parnell<sup>39</sup>**

	sB12
Male Vegetarians	220 $\pm$ 103
Female Vegetarians	282 $\pm$ 132
Male NV	331 $\pm$ 142
Female NV	331 $\pm$ 167

**Table 8.3.2.4 Results of Haddad et al.<sup>38</sup>**

	#	yrs on diet	# taking B12 supplmnts <sup>A,B</sup>	sB12	# with sB12 < 203	sMMA > .38 $\mu$ mol/l	had 1 indicator of B12 deficiency
vegans	25	4.2 (1-25)	9	421 $\pm$ 169	3	5	10
NV	20			423 $\pm$ 134	0	0	

<sup>A</sup>Per correspondence with author. <sup>B</sup>Some vegans ate B12 fortified foods.

There were no differences in homocysteine between the groups. There was a significant association between B12 supplementation and sB12 but no relation with sMMA. In private correspondence, Haddad suggested this was because some vegans did not regularly take the supplements and some had only recently begun. Blood will reflect recent higher B12 intake while MMA levels take longer to change.

More studies on vegans' B12 status are located in Table 11.2, in *Vegans, B12, Homocysteine, & Cardiovascular Disease*.

### 8.4 Elderly Vegetarians & Vegans

Brants et al.<sup>11</sup> (1990, Netherlands) and Lowik et al.<sup>112</sup> (1990, Netherlands) compared the B12 status of elderly (65-97 yrs) lacto-ovo vegetarians to nonvegetarians (Table 8.4.1). They concluded that a lacto-ovo or lacto vegetarian diet can be adequate in old age, with positive impacts on heart disease risks, provided that it is carefully planned, especially with respect to iron, zinc, and B12.

Woo et al.<sup>106</sup> (1998, Hong Kong) compared 106 elderly Chinese LOV and vegan women to 229 NV:

- All were older than 65 yrs (avg. 81); all were apparently healthy.
- Vegetarians had been on the diet > 10 yrs.
- Low sB12 (< 203 pg/ml) occurred in 53.8% of the vegetarians (data not given for nonvegetarians).
- 16 vegetarians had B12-deficient anemia compared to 1 NV.
- Vegetarians had a lower prevalence of a history of smoking and heart disease.

**Table 8.4.1 Results of Brants et al.<sup>11</sup>:**

	#	sB12	< 186.3
NV males	54	NR	4%
NV females	54	NR	6%
LOV males	17	317 $\pm$ 277	35%
LOV females	23	247 $\pm$ 96	13%

NR - not reported.

## 8.5 Raw Foodist Vegans

Dong & Scott<sup>29</sup> (1982, USA) examined 83 subjects from an American Natural Hygiene Society conference. They tended to follow natural hygiene diets consisting of whole raw fruits, vegetables, nuts, and seeds, with a minimal intake of grains and legumes. They considered this to be a natural primate diet and believed their bodies received B12 through small intestinal bacteria which live only in the intestines of those who follow whole raw food diets. Table 8.5.1 shows the results among subjects who did not supplement with B12.

**Table 8.5.1 Results of Dong & Scott.<sup>29</sup>**

	#	sB12 < 200	sB12 < 100
vegans	13	92%	53%
LV	28	64%	
LOV	15	47%	13%
SV	10	20%	

LV - lactovegetarians; SV - semivegetarians

Macrocytosis among the vegetarians was minimal. One 63-year-old vegan with a sB12 of 117 (MCV = 86 fl) had a nerve-related disorder. For males who did not take B12 supplements, there was a correlation between length of time as a vegetarian and lower sB12. Among subjects who had taken B12 or multivitamins, all had sB12 levels above 200 pg/ml. Dong & Scott concluded that there is no indication that natural hygiene vegetarian diets contribute to higher sB12 levels than other vegetarian diets.

Rauma et al.<sup>78</sup> (1995, Finland) examined the B12 status in long-term adherents of a strict, uncooked (raw) vegan diet called the “living food diet.” They assumed their large intake of bacterially fermented foods (about 2 kg/day in this study) would provide plenty of B12 as well as modify their intestinal bacteria to provide more B12.

In Part 1 of the study, food consumption data were collected and blood samples were taken from 9 vegan “living food eaters” (LFE) (1 male, 8 females), 2 years apart. Six of the 9 vegans showed slow, consistent deterioration of B12 status over this period, indicating that the supply of B12 from the “living food diet” was inadequate to maintain the sB12.

In Part 2, sB12 of LFE were compared to nonvegetarians. Blood values (MCV, hemoglobin) of LFE did not differ

significantly from the nonvegetarians’ nor did they correlate with sB12 levels. In the vegan group, B12 analogue intake (through nori and chlorella) correlated with sB12. The Finnish eaters of the living food diet participating in this study started to supplement their diet after finding out their low vitamin B12 status. Results in Table 8.5.2.

**Table 8.5.2 Rauma et al., Part 2.<sup>78</sup>**

	#	sB12	< 203
LFE	21	261 <sup>A</sup> R 47-551	6
consuming seaweed	16	298 R 101-551	
not consuming seaweed	5	142 R 47-340	
NV	21	420 <sup>A</sup> R 177-651	1

<sup>A</sup>Statistically significant difference between groups with same letters.

Donaldson,<sup>121</sup> (2000, USA) studied people following the Hallelujah Acres’ diet, a vegan diet consisting mostly of raw foods with small amounts of cooked whole grains and root vegetables.

- Subjects in the study did not take B12 supplements. Some ate small amounts of nutritional yeast resulting in an average intake of  $5 \pm 11 \mu\text{g}$  B12/month, with a median intake of  $.7 \mu\text{g}$ /month. Results are in Table 8.5.3.
- 19 subjects had normal sB12 levels with elevated uMMA levels. Subjects with elevated (and one with borderline) uMMA were divided into four groups to receive different treatment:

- Sublingual B12: 500  $\mu\text{g}$  Twinlab sublingual B12 3x/wk
- Nutritional yeast: 5  $\mu\text{g}$  B12/day via 1 tablespoon of Red Star Vegetarian Support Formula nutritional yeast
- Probiotic Formula intestinal bacteria: 2 capsules/day which contain 5 bacteria species:

*Lactobacillus plantarum*  
*Lactobaccillus salivarius*  
*Lactobacillus acidophilus*  
*Bifidobacterium bifidus*  
*Bacillus subtilis*

- Flora Food intestinal bacteria: 2 capsules/day which contain 2 bacteria species:

*Lactobacillus plantarum*  
 variant OM  
*Lactobacillus salivarius*

**Table 8.5.3 Results of Donaldson.<sup>121</sup>**

# of subjects	49
sB12 < 347	44
sB12 < 200	6
sB12 < 160	0
uMMA $\geq 4 \mu\text{g}/\text{mg}$ creatinine	23

**Table 8.5.4. Results of treatment in Donaldson.<sup>121</sup>**

	Sublingual B12	Nutritional yeast	Probiotic Formula	Flora Food
Total number	8	8	4	4
uMMA $\geq 4.1 \mu\text{g}/\text{mg}$ creatinine after 3 months	1 uMMA = 4.1	5 All improved	4 3 improved	4 1 improved

The results after 3 months are in Table 8.5.4. Donaldson suggests that the small improvement from taking intestinal bacteria could be from:

- The bacteria producing B12 while still in the supplement.
- The bacteria taking up residence in the digestive system and producing B12.

In either case, these probiotics were not enough to normalize B12 status and are not recommended for vegans to rely on in improving their B12 status.

More studies on vegans' B12 status are located in Table 11.2, in *Vegans, Homocysteine, & Cardiovascular Disease*.

### 9. Lacto-ovo Vegetarians (LOV)

In general, LOV have lower sB12 levels than nonvegetarians.<sup>64</sup> Appendix D is not an exhaustive review of the literature on LOV and B12, but rather a report of studies accumulated in the process of research on vegans. The studies show that LOV's B12 status could often be improved. If B12 supplements or fortified foods are available, they should be used regularly to ensure optimal B12 status.

### 10. Macrobiotics

A macrobiotic diet typically consists of 50-60% whole cereal grains, 5% soups, 20-25% vegetables, and 5-10% beans and sea vegetables. Occasionally, small quantities of other foods, such as seafood, are included. Meat is avoided and little or no dairy or eggs are eaten. Vitamin supplements generally are not taken.<sup>68</sup> Infants are normally breast-fed until whole foods are added (i.e., infants are not fed formula). As can be seen from Appendix F, many macrobiotics who do not supplement their diets with B12 are found to be deficient. There is growth retardation in some macrobiotic children due to low B12 intake.<sup>88</sup>

### 11. Vegans, B12, Homocysteine & Disease

#### 11.1 Background on Homocysteine

Homocysteine levels > 10 µmol/l are considered to increase one's risk of heart disease.<sup>62</sup> High homocysteine levels have also been associated with stroke, Alzheimer's Disease, age-related hearing loss, neural tube defects, recurrent pregnancy loss, and overall mortality.

Levels of homocysteine in typical Western populations are about 12 µmol/l.<sup>143</sup> Keeping homocysteine at levels associated with lower rates of disease requires both adequate B12 and folate status. In most nonvegetarians with elevated homocysteine, reduced folate status is more of a problem than is reduced B12 status. In vegans who do not supplement with B12, the problem is normally due to reduced B12 status as vegan diets are typical high in folate.

Does elevated homocysteine cause cardiovascular disease? Some vegans are skeptical that elevated homocysteine will increase a vegan's risk for cardiovascular disease given that vegans have lower cholesterol levels. For example, in the Oxford Vegetarian Study,<sup>135</sup> vegans had the lowest total and LDL cholesterol (Table 11.1.1).

Because some vegans are skeptical that homocysteine increases their risk for heart disease, Appendix I examines the evidence as to whether elevated homocysteine is simply a byproduct, or rather a cause, of various diseases with which it is associated. Appendix I.2.3.1 includes information about one study in which reducing homocysteine levels actually reversed plaque in the carotid arteries.

#### 11.2 Studies on Homocysteine in Vegans and Vegetarians not Supplementing with B12

Table 11.2 summarizes the studies looking at vegetarians' homocysteine levels.

Mann et al.<sup>63</sup> concluded:

1. In habitual diets where folate intake is adequate, low B12 intake leads to depleted B12 levels with an increase in HCY.
2. The benefits of a vegan diet for vascular disease probably outweigh the increased homocysteine, but vegan foods should be fortified with B12.

Mezzano et al.<sup>65</sup> recorded significantly faster blood clotting time (a risk factor for heart disease) for the vegetarians, probably due to their lower levels of omega-3 fatty acids (see *Staying a Healthy Vegan* at [www.veganoutreach.org](http://www.veganoutreach.org) for more information on vegans and omega-3s). They said that the increased clotting function and homocysteine found in these vegetarians may counteract the known cardiovascular health benefits of a vegetarian diet.

**Table 11.1.1 Cholesterol levels in the Oxford Vegetarian Study.**<sup>135</sup>

	#	Total cholesterol mg/dl	LDL cholesterol mg/dl
vegans	114	166	88
vegetarians	1550	188	106
fish eaters	415	193	111
meat eaters	1198	205	122

Vegetarians ate no meat or fish but did eat dairy products, eggs, or both.  
Fish eaters ate fish but no meat.

**Table 11.1.2 Plant Food Sources of Folate<sup>1</sup>**

RDA for folate is 400 µg for people over age 13.

Food	amount	µg
cooked lentils	1/2 Cup	179
cooked black beans	1/2 Cup	128
romaine lettuce	1 1/2 Cup	114
orange juice	1 Cup	109
cooked spinach	1/2 Cup	103
canned refried beans	1/2 Cup	106
cooked garbanzo beans (aka chickpeas)	1/2 Cup	80
cooked navy beans	1/2 Cup	82
cooked broccoli	1 Cup	78
sunflower seeds	1/4 Cup	76
cooked pinto beans	1/2 Cup	72
cooked kidney beans	1/2 Cup	63

In their second study, Mezzano et al.<sup>124</sup> gave the vegetarians a 1,000 µg B12 injection. Homocysteine normalized in all 11 subjects whose homocysteine was initially high.

Herrmann et al.<sup>119</sup> said their results indicated that MMA is a more sensitive indicator of early B12 deficiency than is elevated homocysteine. Elevated MMA was found in subjects with sB12 up to 486. In contrast, only 3 of the 5 people with sB12 less than 211 pg/ml (the lower end of normal in this study) had elevated homocysteine or MMA. This is evidence that sB12 is an inaccurate indicator of poor B12 status (although levels ≤ 100 pg/ml, indicate that B12 status is well on its way to being compromised). They suggested that transcobalamin II and MMA may be better markers for determining B12 deficiency.

Hung et al.<sup>172</sup> noted that in Taiwan, vegetarian diets include few dairy products and consist mostly of rice, vegetables, fruits, and soy products. They determined that differences in sB12 and folate accounted for only 28.6% of the variation in homocysteine levels within the vegetarian group. B12 intake (rather than sB12) may have better predicted homocysteine levels, but this was not tested.

### 11.3 Conclusion

Unless there is a study that follows vegans with elevated homocysteine to see if or how it affects them, we will not know the effects with 100% certainty. Because of the enormous cost, such a study will probably not be conducted. It would also take many years and we do not have that long to wait before taking action. (Furthermore, it is my hope that all vegans will recognize the benefits of taking B12 and begin doing so, making such a study impossible.)

Appendix I shows that there is significant evidence that elevated homocysteine is associated with heart disease, stroke, Alzheimer's Disease, age-related hearing loss, neural tube defects, recurrent pregnancy loss, and overall mortality. In contrast, there is no evidence that vegans are protected from the possible damage of elevated homocysteine.

The studies in Table 11.2 show that without supplementing with B12, vegans have higher homocysteine levels than lacto-ovo vegetarians and nonvegetarians. B12 treatment succeeds in normalizing these vegans' homocysteine levels. There is no reason to put yourself at risk.

## 12. B12 & Cancer

Recently, there has been some research concerning a link between low B12 status and cancer. It is thought that since B12 is needed for proper DNA production, a lack of B12 could have an effect on cancer through the incorporation of uracil into DNA. This can cause chromosome breakage resulting in a cancerous cell.<sup>183</sup> The same can be said of folate.<sup>183</sup>

Fenech<sup>187</sup> studied folate and B12 levels and intake in respect to DNA damage in white blood cells (lymphocytes) which has been shown to be a good marker for future cancer. They found that sB12 > 405 pg/ml and a supplemental intake of 7 µg of B12/day was optimal for reducing DNA damage. The subjects were not vegetarian.

**Table 11.2 Vegetarians and Homocysteine Levels.**

	#	sB12 pg/ml	HCY µmol/l			
<b>Mann et al.<sup>63</sup> 1999, Australia</b>						
vegans	18	196 <sup>A</sup> ± 92	19.2 <sup>B</sup> ± 10.7	No subjects took SUP. Folate levels were not related to HCY. Vegans had higher folate levels.		
LOV	43	285 ± 132	15.8 ± 9.3			
Mod meat < 285 g meat/d	60	452 ± 134	11.0 ± 2.5			
High meat	18	544 <sup>A</sup> ± 228	11.6 <sup>B</sup> ± 2.7			
<b>Mezzano et al.<sup>65</sup> 1999, Chile</b>						
V (3 vegans)	26	166 <sup>A</sup> ± 89 21 < 200 7 < 100	13.5, <sup>B</sup> 8.0-70	Serum folate was higher in vegetarians. HCY was negatively related to sB12. Dairy & eggs did not cover the B12 needs of most LV & LOV.		
NV	26	401 <sup>A</sup> ± 202	9.6, <sup>B</sup> 5.5-27			
<b>Mezzano et al.<sup>124</sup> 2000, Chile</b>						
LOV 1 wk after 1,000 µg B12 I	18	149 <sup>A</sup> ± 63  532 ± 204	12.4 <sup>B</sup> ± 4.7  7.9 ± 3.1	No subjects took SUP in previous 6 mos. 11 had HCY above 11.5 for women, 14.5 for men.		
NV	40	NR <sup>A</sup>	8.4 <sup>B</sup> ± 2.3			
<b>Krajcovicova-Kudlackova et al.<sup>125</sup> 2000, Slovak Republic</b>						
vegans	32	189 ± 7	15.8 <sup>A</sup> ± .9	53% of vegans had HCY > 15.0 In vegans, low sB12 was related to higher HCY.		
LOV	62	290 ± 7	13.2 <sup>A</sup> ± .3			
NV	59	464 ± 11	10.2 <sup>A</sup> ± .3			
<b>Herrmann et al.<sup>119</sup> 2001, Germany</b>						
vegans	7	293 (207/591) <sup>C</sup>	15.2 <sup>A</sup>	No subjects took SUP. MMA & HCY were related. MMA levels showed B12 deficiency in 25% of the 3 veg groups. Decreased sB12 found only in subjects with HCY > 8.		
LOV/LV	34	342 (207/508)	11.0			
SV	19	324 (159/447)	11.8			
NV	44	373 (232/548)	9.8 <sup>A</sup>			
<b>Hung et al.<sup>172</sup> 2002, Taiwan</b>						
	#	sB12	HCY	B12 intake	Folate intake	
vegans	6	280 ± 172 <sup>A</sup>	11.2 ± 4.3 <sup>B</sup>	.42 µg/d	329 µg/d	See notes below.
LV	39					
NV	45	544 ± 188 <sup>A</sup>	8.6 ± 2.1 <sup>B</sup>	7.12 µg/d	156 µg/d	

<sup>A,B</sup> Statistically significant difference between groups with same letters within the same study.  
<sup>C</sup> Avg (5<sup>th</sup>/95<sup>th</sup> percentiles). HCY – homocysteine; I – injection; LOV - lacto-ovo vegetarian; LV - lacto vegetarian; MMA - methylmalonic acid; NR - Not Reported; SUP – supplements; SV - Ate poultry or fish about 1-2/week; V - lacto-, ovo-, or vegan



## 12.1 B12 & Breast Cancer

In the first prospective study looking at B12, folate, B6 and breast cancer, Wu et al.<sup>107</sup> (1999, USA) found no association between serum folate or B6. There was a small increased risk for the postmenopausal women in the lower one-fifth of B12 levels (averaging 280 pg/ml) in one of the two groups studied. In the other group, where lower levels of B12 were not associated with increased risk, the women in the lowest one-fifth of sB12 levels averaged 312 pg/ml.

There is one study that slightly contradicts Wu's study on breast cancer. Vachon et al.<sup>191</sup> (2000, USA) performed a cross-sectional study looking at diet and breast tissue density. The percentage of breast tissue that is dense is thought to be a risk factor for future breast cancer. Studies have shown that some women whose breast tissue is 50% dense have 3-5 times more risk of breast cancer than those whose breast tissue is < 10% dense. Vachon et al. found that of postmenopausal women taking B12 supplements, those in the highest one-fourth of B12 intake averaged 2% more dense tissue than those in the lowest one-fourth. In my opinion, this should not be of much concern as:

- 1) The increase in risk is very small and barely significant (P = .05).
- 2) This was a cross-sectional study and did not follow people with this B12 intake to see what would happen to them.
- 3) The idea that B12 increases breast cancer is contradicted by the other evidence (above).

In reviewing the literature on B12 and cancer, Choi<sup>16</sup> (1999, USA) points out that although early links between breast cancer and per capita animal fat consumption were seen, prospective studies looking at the association between a woman's daily total fat intake and breast cancer risk did not show a clear association. Most case-control studies have suggested a higher intake of fiber, vegetables, and fruit is protective. The fiber is thought to reduce estrogen levels. Despite this, vegetarians have been shown to have the same mortality rates from breast cancer as nonvegetarians with similar lifestyles.<sup>54</sup> Could improved B12 status make vegetarians' rates lower?

## 13. Measuring B12 in Plant Foods: Why the Confusion?

Since inactive B12 analogues can interfere with the function of active B12, simply measuring the amount of B12 in a particular plant food cannot tell us anything about that food's ability to maintain or improve B12 status. Because attempts to measure B12 in plant foods have led to many misconceptions, these methods will be discussed here.

**Table 13.1 Test Organisms for B12 Microbiological Assays**

Organism	Organism <sup>151</sup>	Notes <sup>89</sup>
<i>Escherichia coli</i> mutant 113-3 <sup>89</sup>	bacteria	Not recommended because it responds to so many B12 analogues. <sup>89</sup>
<i>Lactobacillus leichmannii</i> 326 <sup>89</sup>	bacteria	May utilize some non-B12 corrinoids. <sup>89</sup>
<i>Euglena gracilis</i> Z-alga <sup>89</sup>	protozoa	May utilize some non-B12 corrinoids. <sup>89</sup>
<i>Ochromonas malhamensis</i> <sup>89</sup>	protozoa	Most specific for cobalamins. <sup>89</sup>
<i>Lactobacillus delbrueckii</i> ATCC 7830 <sup>152</sup>	bacteria	No information at this time.
<i>Arthrobacter</i> Lochhead 38	bacteria	A 1959 study showed it to be similar to <i>O. malhamensis</i> . <sup>214</sup>

### 13.1 Microbiological Assay

Traditionally, B12 has been measured by "feeding" a food to certain bacteria and measuring how well they grow. This is known as *microbiological assay*. Various test organisms for measuring B12 have been used (see Table 13.1).<sup>89</sup> Some or all of these bacteria thrive on various inactive B12 analogues, making them unreliable for measuring the B12 content of plants, which often contain a variety of inactive B12 analogues. Despite this, many laboratories, especially those of private companies who want to market their product as containing B12, still use the less reliable of these methods when measuring the B12 content of plant foods and seaweeds.

### 13.2 Radioassay

B12 has also been measured through *radioassay* (which has other names, such as *competitive binding assay*) by seeing whether it binds to R-protein or intrinsic factor (IF). It is now known that both R-protein and IF can bind to inactive B12 analogues.

### 13.3 *Ochromonas malhamensis* Fares Better than an Intrinsic Factor Assay

Baker et al.<sup>151</sup> compared *Ochromonas malhamensis* to an IF assay and found that IF gave a 44% higher reading than *Ochromonas malhamensis*. The authors suggest that *O. malhamensis* may be the most effective of the various assays at measuring metabolically active B12. Unfortunately, only one study on B12 in plant foods has used *O. malhamensis*.

### 13.4 Paper Chromatography

For simplicity, *paper chromatography* is the term used here to refer to an array of methods that when combined can provide a much more precise determination of the exact structure of a molecule. These methods are more reliable than those mentioned above, but are difficult to perform. Furthermore, they cannot tell the actual B12 activity of a given plant food as a whole.

### 13.5 Methylmalonic Acid Reduction: The Gold Standard

Because inactive B12 analogues can interfere with the function of active B12, the "gold standard" for determining the B12 activity of a plant food is to feed it to people and see if their methylmalonic acid (MMA) levels go up or down. If they go down, then that particular food (or batch of foods) can be considered to be a source of B12. (An alternative is to feed the food to people with macrocytic anemia and see if the anemia improves. Unfortunately, this can be confounded by the presence of folate. It is also possible that some B12 analogues which are active for red blood cells may be inactive for nerve tissue. See Appendix H.)

### 13.6 Plant Foods Tested According to the Gold Standard

The only foods which have been tested using the gold standard of lowering MMA are dried and raw nori from Japan. Dried nori made MMA status worse, indicating that it can reduce B12 status and can possibly harm people who are B12-deficient. Raw nori kept MMA levels about the same, indicating that it didn't harm B12 status, but it did not help either.

No food in Europe or the U.S. has been tested for lowering MMA levels. Thus, the discussion about whether Western vegans can get B12 from plant foods can, and probably should, end here (until proper research is conducted). Because so many plant foods have *failed* tests that do not measure up to the gold standard, and because there are so many false rumors being passed around, the studies of B12 in plant foods are examined in Appendix A.

## 14. Are Intestinal Bacteria a Source of B12?

### 14.1 Bacteria in the Large Intestine

It has long been assumed that B12 is produced by bacteria in the large intestine (aka the colon), but since B12 is produced below the ileum (where B12 is absorbed), it is not available for absorption. This theory is reinforced by the fact that many species of totally or primarily vegetarian animals eat their feces. It is surmised that eating feces allows them to obtain B12 on their diets of plant foods. Although I believe this to be true, it has not been proven beyond a doubt.

The best evidence I have found for this theory is reported by Herbert.<sup>47</sup> He reports a study in the 1950s in England where vegan volunteers with B12 deficiency (megaloblastic anemia) were fed B12 extractions made from their own stools and it cured their deficiency. He said it proves that the colon bacteria of vegans produce enough B12 to cure a deficiency, but that the B12 produced by the bacteria in the colon is excreted rather than absorbed. This appears to be convincing evidence. However, the study Herbert cites as the source (Callender ST, Spray GH. Latent pernicious anemia. *Br J Haematol.* 1962;8:230-40) does not mention this experiment. There is another study by Callender and Spray that sounds like it could be the one Herbert is describing: Preparation of hematopoietically active extracts from faeces. *Lancet* 1951(June 30):1391-2. This study was not performed on vegans, but rather on people with pernicious anemia (lack of intrinsic factor). Because these people were ingesting B12, the B12 in their stool could have been from the B12 they were eating. On the other hand, according to *Lactobacillus lactis* Dorner and *Lactobacillus leichmannii* assays, there were fairly substantial amounts of B12 analogue found in the feces (e.g., 5 µg per 10 ml (2 teaspoons)). This seems like too much to have been provided by only the diet and enterohepatic circulation. Apparently, enough of this B12 analogue was active to improve these patients' anemia. Thus, this study provides good evidence that there is active B12 produced by bacteria in the colon of some humans.

Another variable to consider is that there are over 400-500 species of bacteria in the average human's colon and these bacteria have not all been delineated. It is plausible that some humans have B12-producing bacteria in significant amounts while other humans do not. Some bacteria in the digestive tract absorb B12 for their own use, further complicating this situation.

### 14.2 Bacteria in the Small Intestine

B12 deficiency has been found with relatively high frequency among vegetarian Indian immigrants in England, while it is supposedly uncommon among native Indians with identical dietary patterns.<sup>2,157</sup> Healthy Indian subjects have a more extensive amount of bacteria in their small intestine than people in the West.<sup>2</sup> Albert et al.<sup>2</sup> (1980) measured B12 production of bacteria in the small intestines of people in India using a *Euglena gracilis* Z assay. Results were confirmed by an *Ochromonas malhamensis* assay, which is thought to be specific for active B12. They determined that some active B12 was produced by members of the bacteria genera *Klebsiella* and *Pseudomonas*. Further confirmation using chromatography and bioautography showed a molecule with similar properties to cyanocobalamin. Albert et al. speculated that when Indians migrate to the West, their digestive tracts become like those characteristic of people in Western countries: with little or no bacteria in their upper small intestines.

An article in *Nutrition Reviews*<sup>76</sup> (1980) suggested some alternative causes of Indian immigrants to Britain having more B12 deficiency than Indian natives:

- In India, water is contaminated with various bacteria, including those from human and animal feces.
- The practice of defecating in open fields and lack of proper sewage.

- The mode of toilet hygiene where water is used instead of toilet paper.

It should also be noted that there are few vegans in India and there is also evidence that B12 deficiency is not so uncommon there (see Table 14.2),<sup>157</sup> especially in lower economic, lacto-ovo vegetarians.<sup>171</sup>

### 14.3 Conclusion

Given that many lacto-ovo vegetarians in India develop B12 deficiency and that many vegans also develop B12 deficiency when not supplementing their diets with B12, intestinal bacteria cannot be relied upon to prevent B12 deficiency in vegans. Are raw foodists or people who eat fermented foods exceptions? No. See Section 8.5.

**Table 14.2 B12 Status of a Group of Indians age 27-55.**<sup>157</sup>

	#	sB12	sB12 < 203	MMA > .26 µmol/l	HCY > 15 µmol/l
NV <sup>B</sup>	36	216	46%	70%	81% <sup>C</sup>
LOV <sup>A</sup>	27				

<sup>A</sup>1 person was vegan. <sup>B</sup>Tended to eat only small amounts of animal products. <sup>C</sup>A low folate status could have contributed to the high HCY levels.<sup>158</sup> LOV - lacto-ovo vegetarian; NV – nonvegetarian.

## 15. Organic Produce & Soil as a B12 Source for Vegans

It is common in vegan circles to hear that if your produce has soil on it and you do not wash the produce before eating it, bacteria that lives in the soil and on the produce will provide B12. It is also claimed that in today's world, our food supply is very sanitized whereas in the past, vegan humans would have received plenty of B12 from the unsanitized produce. What is the evidence for these claims?

### 15.1 B12 Analogue-Producing Bacteria in the Soil

There is a one paragraph report often cited in vegan literature for showing that B12 is found in the soil. Robbins et al.<sup>83</sup> (1950, New York Botanical Gardens) used *Euglena gracilis* var. bacillari as a microbiological assay for vitamin B12 "or its physiological equivalent." A considerable proportion of bacteria and actinomycetes (molds) in the soil were found to synthesize B12 analogues. B12 analogues were also found in the roots of plants (.0002-.01 µg B12/g of fresh material). Some stems had some B12 analogue, but leaves and fruit generally did not. B12 analogue was also found in pond water and pond mud. There was no indication in the report as to how many different soils were tested but the impression was that it was all in one local area. There is no way to know whether these molecules were active B12 or inactive analogues.

### 15.2 Organic Produce Using Human Manure as Fertilizer

Herbert<sup>47</sup> (1988) reports that in 1959, some Iranian vegans were found to be growing plants in night soil (human manure). The vegetables were eaten without being carefully washed and the amount of B12 was enough to prevent deficiency. However, for this information, Herbert cites Halstead et al. (1959),<sup>117</sup> who do not mention these Iranians in their paper. Stephen Strauss, science reporter with the *Globe and Mail* newspaper in Toronto, tried to track down this story. He spoke with Halstead's son, who said his father never performed such a study.<sup>190</sup> Thus, at this time, this anecdote should be considered unsubstantiated.

### 15.3 Organic Produce Using Cow Manure as Fertilizer

#### 15.3.1 Soybean Plants Absorb B12 when Added to the Soil

Mozafar & Oertli<sup>70</sup> (1992, Switzerland) added cyanocobalamin to the soil of soybean plants in amounts ranging from 10 to 3200 µmol/l. Using an intrinsic factor assay, 12-34% of the B12 was absorbed by the plants. 66-87% of the absorbed vitamin remained in the roots and the rest was transported to the various other parts, mainly the leaves. Mozafar points out that the concentrations of B12 in the soil used in this study were many times higher than the reported vitamin concentration in soil solution (.003 µmol/l) measured by Robbins<sup>83</sup> (mentioned above in section 15.1).

#### 15.3.2 Plants Absorb B12 Analogue when Added to the Soil via Cow Dung

In light of the above results, Mozafar<sup>71</sup> (1994, Switzerland) then studied how the B12 levels in plants are affected by adding cow dung to the soil. An assay using pig intrinsic factor was used to measure the B12. The study looked at the B12 content of both organically fertilized soil and plants.

Two samples were taken from soil that had been treated with organic fertilizer every 5 years over the previous 16 years. These were compared to soil that had only synthetic fertilizer applied. Results are in Table 15.3.2.1.

**Table 15.3.2.1 B12 Analogue in Soil**

	Sample 1 µg/kg	Sample 2 µg/kg
synthetically fertilized soil	9	5
organically fertilized soil <sup>A</sup>	14	10

<sup>A</sup>Treated with organic fertilizer once every 5 years.

**Table 15.3.2.2 B12 Analogue (ng/g) in Plants**

	nothing added	"organic" 10 g dry cow manure added
soybeans	1.6	2.9
barley kernels	2.6 <sup>A</sup>	9.1 <sup>A</sup>
spinach	6.9 <sup>B</sup>	17.8 <sup>B</sup>

<sup>A,B</sup>Statistically significant difference between groups with same letters.

Soybean, barley, and spinach plants were then grown in pots of 2.5 kg of soil. 10 g dry cow manure was added to each pot. Plant parts were thoroughly washed to remove any soil before B12 was measured. Results are in Table 15.3.2.2.

Further analysis showed that most or all of the B12 analogue in the plants was unbound. Mozafar concluded that plant uptake of B12 from the soil, especially from soil fertilized with manure, could provide some B12 for humans eating the plants, and may be why some vegans, who do not supplement with B12, do not develop B12 deficiency.

Does this mean that organic foods are a good source of B12? No. These studies show that when B12 analogues are placed in the soil, plants can absorb them.

#### 15.4 Conclusion about *Organic Produce & Soil as a B12 Source for Vegans*

To date, there has been no research looking at B12 on unwashed produce. Until unclean produce is shown to lower MMA levels, it is unjustified to claim that B12 can be obtained in such a manner, or to claim with certainty that humans have ever relied on it as a source of B12.

To be considered a reliable source of B12, organic foods from various regions must consistently be shown to improve B12 status by lowering serum MMA levels. Only until organic foods are chosen randomly from markets and grocery stores throughout the country (or world) and are consistently shown to decrease MMA levels will someone not be taking a considerable risk in relying on organic foods for B12. It should be noted that this article contains many vegans suffering from B12 deficiency, and it is probably safe to assume that many of these vegans consumed significant amounts of organic produce and other foods.

Additional note: The vegan movement is typically not aiming for a world where there are enough cows to produce a significant amount of manure for fertilizer.

#### **Table 15.4 Cobalt is Necessary for Bacterial Synthesis of B12**

The availability of B12 for animals who rely on bacterial synthesis of B12 (rather than getting it from animal foods) is dependent on cobalt levels in the soil. Citing an article from the *Annals of the New York Academy of Science* (1964;112:735-55), Crane et al.<sup>18</sup> point out that some soils in Australia, New Zealand, Britain, Canada, Ireland, Germany, Holland, Kenya, Poland, South Africa, Sweden, Russia, and the USA have insufficient cobalt for adequate B12 formation. They state, "This is a major concern of ours because vegans commonly seem to hold to the concept that all essential nutrients will be supported in foods from non-animal sources. They fail to realize that plants can grow readily in soil that is too low in cobalt for bacterial action to supply animals with sufficient B12."

## 16. B12 in Non-Human Animals

Some have suggested that vegans do not need to fortify their diets with B12, because other herbivorous animals do not do so. For this reason, I have included the following short discussion on the subject.

Cows are ruminants (as are bison, buffalo, goats, antelopes, sheep, deer, and giraffes).<sup>200</sup> Ruminants have a four-chambered stomach and a rich supply of bacteria in their rumen (the first chamber that their food enters).<sup>200</sup> These bacteria produce B12 in amounts normally sufficient to meet their needs.<sup>201</sup>

#### **Table 16.1 B12 Analogue in Insects**

Wakayama et al.<sup>104</sup> (1984) measured the amounts of B12 analogue in insects using an intrinsic factor assay. They determined that bacteria in the insects' digestive tracts were responsible for its production. Some insects, such as the housefly, had no detectable B12 analogues. Other insects had some, such as one cockroach species (.004 µg/g); and 5 termite species produced large amounts of B12 (.455-3.21 µg/g). Thus, certain insects could be a source of B12 in animals (including humans who eat insects), if this B12 is actually active.

Horses, elephants, zebras, rabbits, hares, and many rodents have large cecums in their digestive tracts, located between the small and large intestine,<sup>200</sup> where bacterial fermentation takes place. Some sources say that all non-ruminant herbivores require some B12 fortification of their feeds,<sup>201</sup> but at least one source says that bacteria in a horse's digestive tract are able to produce enough B12 to prevent a dietary need.<sup>202</sup>

Many wild herbivores, such as elephants,<sup>203</sup> ingest soil on a regular basis. Hares, rabbits, and some rodents eat their fecal pellets, which provide an opportunity to obtain vitamins produced by bacteria in their digestive tracts.<sup>200</sup>

Non-human primates typically eat small amounts of eggs, insects, and small vertebrates and/or soil.<sup>204</sup> Gorillas, possibly the closest to vegan of all the species closely related to humans, eat insects,<sup>204</sup> and sometimes feces.<sup>205</sup>

Thus, there are many ways that mostly, or completely, herbivorous animals can potentially obtain B12 which are not available to vegans living in Western

society.

## 17. Immerman: The Exception

The lone peer-reviewed article published that downplayed, rather than emphasized, the need for vegans to supplement their diets with B12 was by Immerman<sup>51</sup> (1981).

Immerman reviewed 13 case studies in which a vegan was found to have B12 deficiency. He lists a number of criteria which he claims must be used to determine a true B12 dietary deficiency (versus a different problem; see Table 17.1). None of these studies met all of his criteria. Immerman was particularly critical of the idea that a dietary B12 deficiency exists if B12 symptoms improve upon B12 injections rather than oral tablets. Because the authors of earlier case studies were interested in helping the patients rather than proving whether vegans should take B12, they used injections, the common method of clearing up B12 deficiency quickly. The injections helped their patients.

#### **Analysis of Immerman's Paper**

A more rigorous analysis of Immerman's paper appears online at <http://www.beyondveg.com/billings-t/comp-anat/comp-anat-7b.shtml>.

**Table 17.1 Factors that can cause B12 deficiency listed by Immerman:<sup>51</sup>**

- Malabsorption via inadequate protein, calories, or folate; radiation, drugs and toxins (neomycin, colchicine, para-amino salicylic acid, alcohol); pancreatic tumor; failure of the small intestine to contract and move food associated with bacterial overgrowth; oral contraceptives.
- Increased excretion due to liver or kidney disease.
- Tobacco smoking.
- Vitamin B6 or iron deficiency.

Immerman says that low sB12 levels should not be used to determine a B12 deficiency. By this criterion, he says “[I]t would be necessary to characterize not only most Western vegetarians but also much of the population of India and other Eastern countries as deficient; this is clearly unacceptable.”

Immerman says that cooking destroys significant amounts of B12 and that consuming primarily raw foods could increase one’s intake of B12. Future studies by Dong & Scott<sup>29</sup> (1982), Rauma et al.<sup>78</sup> (1995), and Donaldson<sup>121</sup> (2000) showed that vegan raw foodists do not appear to have an advantage over other vegetarians. Immerman states, “[M]any studies have found apparently adequate B12 status in individuals consuming [a vegetarian or vegan] diet, although the investigations in some of these studies have been incomplete. It must

be concluded, therefore, that pure dietary B12 deficiency in all classes of vegetarians is the exception rather than the rule.”

Immerman was writing in 1981, before the great majority of the studies included in this review were published. While it is true that the worst cases of B12 deficiency listed here were sometimes aggravated by other health problems, it seems likely that had these vegans been taking oral B12, the problems never would have become so severe, and in many cases, never would have occurred at all. In many of the cases, B12 deficiency was immediately cleared up through oral B12. Additionally, numerous studies presented here show that much of the world’s vegetarian population who do not supplement with B12 could improve their B12 status resulting in health benefits.

In the end, even Immerman suggests that vegetarians have their B12 levels checked every 4-5 years. It would be more prudent, and easier, to take B12 supplements or eat fortified foods. Why wait 4-5 years for a doctor to tell you what you should have been doing all along?

## 18. Conclusion

The longer a vegan does not supplement with B12, the lower their active B12 levels will drop. It is unlikely that most (or possibly any) vegans can achieve optimal health for a considerable length of time without supplementing with B12. In Western society today, it is easy to ensure an adequate B12 intake. In fact, vegans who supplement with B12 can have superior B12 status to nonvegetarians who do not supplement. I would encourage vegan advocates to make achieving superior B12 status for all vegans one of our goals. As such, all new vegans should be told to ensure an adequate supply of B12 by the people or organizations who encourage them to change their diet.

### 18.1 Can a *Natural* Diet Require Supplements?

Some vegans wrote me after reading earlier versions of *Vitamin B12: Are You Getting It?*, saying that by implying vegans need to take a supplement, I am portraying the vegan diet as unnatural. One person said, "All the vegans I know are healthy and they neither take vitamin B12 supplements nor eat foods fortified with vitamin B12." As pointed out earlier, it is true that many vegans do not supplement with B12 and remain apparently healthy for many years. These vegans normally have no idea what their homocysteine levels are nor what chronic diseases such elevated levels might be causing. They also do not know if they are suffering from unnoticeable nerve damage. You are taking a big chance by assuming you have transcended a need for a typical B12 intake.

Is the vegan diet natural? Whether any prehistoric humans were vegan cannot be concluded from the research presented here. In the interest of disclosure, and because knowledge and truth can only help the vegan cause, I recommend an article that examines the subject of the naturalness of a vegan diet: *Comparative Anatomy and Physiology Brought Up to Date: Are Humans Natural Frugivores/Vegetarians, or Omnivores/Faunivores?* by Tom Billings (at: <http://www.beyondveg.com/billings-t/comp-anat/comp-anat-1a.shtml>). After an extensive review of the research, Billings concludes that humans are not naturally vegetarians or vegans. Despite this, he says:

I am both pro-vegetarian and pro-[eating raw foods as a large portion of the diet]. Readers should be aware that I am a long-time vegetarian (since 1970), a former long-time (8+ years) fruitarian (also a former vegan),.... However, I am definitely not a promoter of, or a "missionary" for, any specific diet. In reality, I am tired of seeing raw and veg\*n [vegan/vegetarian] diets promoted in negative ways by extremists whose hostile and dishonest behavior is a betrayal of the positive moral principles that are supposedly at the heart of veg\*ism.

Billings goes on to say:

You really don’t need the naturalness claim to be a veg\*n! That is, moral/spiritual reasons alone are adequate to justify following a veg\*n diet (assuming the diet works for you, of course). Further, if the motivation for your diet is moral and/or spiritual, then you will want the basis of your diet to be honest as well as compassionate. In that case, ditching the false myths of naturalness presents no problems; indeed, ditching false myths means that you are ditching a burden.

Readers may also be interested in the article *Humans are Omnivores*, adapted from a talk by John McArdle, PhD, at <http://www.vrg.org/nutshell/omni.htm> (originally published in the May/June 1991 edition of the *Vegetarian Journal*).

The suffering endured by the majority of animals raised in contemporary animal agriculture far outweighs any desire of mine to eat the same as my prehistoric ancestors. But, even if the animals' suffering were of no consequence, these assumptions are dubious:

1. There is one prehistoric or natural diet.
2. This diet can reasonably be approximated today.
3. This diet is optimal for human health in today's world.

Today's commercial plant foods and meats are different than the foods available in prehistoric times. We eat hybrids of plants and we feed foods to animals that they would not normally eat. We keep them confined so that they do not exercise. We cook animal products to make them palatable and to kill pathogens. We cook vegetable foods that would otherwise be inedible. The U.S. food supply is routinely fortified with a host of vitamins and minerals (such as vitamin D in milk), and most people who turn to what they consider to be a more natural diet as adults have often benefited from this supplementation.

## 18.2 The Medical Community: Future of Research on Vegans

Some vegans dislike the medical community. By refusing to accept the scientific evidence in favor of the need to supplement with B12, the vegans who dislike the medical community are providing a steady flow of vegans with poor health for the medical community to study. If you do not like the medical community, the best thing you can do is ensure that you do not develop B12 deficiency and will never be used by them to say that a vegan diet is unhealthy.

I tend not to believe that the medical community is one big conspiracy against veganism, and am glad that research has been done on vegans who do not supplement with B12. But enough is enough. I am tired of seeing study after study looking at vegans who do not supplement with B12. It is the vegan community's responsibility to stop this flow of research subjects. When a researcher decides to do a study looking at the various health problems of vegans who do not supplement their diets with B12, it would be best if they simply could not find any.

## 18.3 Encourage New Vegans to Concern Themselves with B12 Supplements

All vegan advocates should be aware of the symptoms of B12 deficiency.

As pointed out earlier (see *Transport in the Blood*), adequate transport of B12 to the tissues ceases within days after B12 absorption stops. B12 intake through the diet is not necessary for B12 absorption (see *Enterohepatic Circulation*), because the body can adjust to the lower intakes by reabsorbing B12 secreted into the digestive tract. But these reabsorption mechanisms may take time to adjust to a sudden drop in B12 intake. Additionally, these mechanisms alone cannot keep someone in B12 balance (where one is taking in as much B12 as they are losing).

Some people who try a vegan diet may already have low B12 levels or hampered absorption mechanisms. Some of these factors could cause a new vegan to feel badly and go back to eating animal products. Therefore, it is prudent for new vegans to follow the recommendations in Table 1. This will prevent any potential lapse in adequate B12 delivery to tissues.

## 18.4 Daily or Weekly Supplementation?

Throughout human history, people have ingested B12 on a daily basis, and most often throughout the day. This has given people a steady supply of B12 to their tissues (above that reabsorbed through the enterohepatic circulation). While there is no research on whether a vegan is better off taking B12 on a daily vs. weekly basis, I would err on the side of caution and supplement daily.

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If you found the information in this article important for the spread of veganism, please consider a donation to Vegan Outreach at [www.veganoutreach.org](http://www.veganoutreach.org).

## Appendix A. B12 Analogue in Tempeh, Seaweeds, and Other Plant Foods

It would be great to find a reliable plant source of B12 for vegans. One might get the opposite impression given my level of critique of some of these studies. My skepticism comes only from the potential harm that can be caused by vegans eating a food they believe contains active B12 that, instead, might actually contain inactive B12 analogues that interfere with B12, making a B12 deficiency even worse.

There has been a long history of misconceptions about which, if any, plant foods are sources of B12. Much of this stems from the methods of measuring B12 analogue. Other confusion stems from the fact that different bacterial contamination may occur in some samples of certain foods but not in others. Please see *Measuring B12 in Plant Foods: Why the Confusion?* (Section 13) for an explanation of the methods for determining B12 analogues in plant foods.

Most, if not all, plants do not require B12 for any function, and therefore have no active mechanisms to produce or store B12. Many seaweeds have been shown to have B12 analogues. (Note: Most seaweeds are algae, which are technically not plants.) It is not clear whether the algae make the B12 analogues or whether they absorb the B12 analogues from their environment.<sup>195</sup>

During the 1970s, two enzymes in plants (potatoes and bean seedlings) were found to respond to the addition of adenosylcobalamin.<sup>198,199</sup> One explanation is that adenosylcobalamin provides some factor that is usable by these enzymes, but that adenosylcobalamin is not required by these plants for growth. Thus far, these plants have not been shown to counteract B12 deficiency symptoms (though I am not aware of any well-designed attempts as it is assumed that they do not contain B12). My guess is that many vegans who have developed severe B12 deficiency ate potatoes and beans.

There are some rumors that if you let organic produce, such as carrots, sit at room temperature for a few hours, bacteria on the surface of the carrots will produce B12. For this to happen, specific species of bacteria would be required, as would cobalt. Until there is research showing that such a method can lower MMA levels, this method should not be considered to provide B12.

### Acronyms:

PC - paper chromatography  
 IF - intrinsic factor  
 ND - none detected

### Table A.1.1 Foods with No Detectable B12 Analogue

amesake rice<sup>101</sup>  
 barley miso<sup>101</sup>  
 miso<sup>7</sup>  
 natto<sup>4</sup>  
 rice miso<sup>101</sup>  
 shoyu<sup>101</sup>  
 tamari<sup>101</sup>  
 umeboshi prunes<sup>101</sup>  
 various fruits, vegetables, nuts, seeds, & grains<sup>4</sup>

### A.1 Foods with No Detectable Amount of B12 Analogue

Various studies have tested the food in Table A.1.1. for B12 analogues and found none. To my knowledge, other than in Mozafar's studies (section 15.3) in which B12 and cow dung were carefully added to the soil of potted plants, no published study has shown any B12 analogues in any of these foods.

### A.2 Foods with Detectable B12 Analogue in Some Studies

Table A.2 shows the B12 analogue content of various plant foods. As you can see, there are very small amounts, if any. Since the amounts are so small, any inactive analogues should not significantly interfere with an individual's active B12 from other sources, and if the analogue is active B12, it will not provide much. Thus, these foods should neither add to, nor detract from, a vegan's B12 status.

**Table A.2 B12 Analogue Content (µg/30 g) of Various Foods**

Country	Netherlands <sup>101</sup>	Thailand <sup>4,5</sup>
Assay	IF	IF or R-protein <sup>A</sup>
fermented soybean		.15
barley malt syrup	.006-0.1	
sourdough bread	Only info given.	
parsley		
shiitake mushrooms		
dried fermented soybean		.01
tofu	ND	.02
soybean paste		.03
soy sauce		.01 <sup>B</sup>

<sup>A</sup>Used an assay method by Lau et al.<sup>37</sup> (1965) which uses R-protein or IF.  
<sup>B</sup>µg/30 ml.

### A.3 Tempeh

For a long time, tempeh has been said to be a source of B12. Table A.3 shows the results of measuring B12 analogue in various tempehs.

The studies in the USA and in The Netherlands showed little to no B12 analogue.

In contrast, Areekul et al.<sup>5</sup> (1990, Thailand) found more significant amounts of B12 analogue. Tempeh production requires molds belonging to the genus *Rhizopus*. These were found not to produce B12 analogues. A bacterium, identified as *Klebsiella pneumoniae*, was isolated from the commercial tempeh starter and determined to be the source. This confirmed Albert et al.'s<sup>2</sup> (1980) finding that the *Klebsiella* genera could produce B12 analogues. In Albert's study, the analogue was thought to be active B12. Whether the analogues found by Areekul et al. were the same as in Albert's study is not known. Given that *K. pneumoniae* is not required for tempeh production, this was a case of

**Table A.3 B12 Analogue Content (µg/30 g) of Tempehs**

Country	Netherlands <sup>101</sup>	USA <sup>93</sup>	Thailand <sup>4,5</sup>
Assay	IF	IF	IF or R-protein <sup>A</sup>
tempeh	ND	.02 <sup>C</sup>	.054-1.2 <sup>B</sup>

<sup>A</sup>Used an assay method by Lau et al.<sup>37</sup> (1965) which uses R-protein or IF.  
<sup>B</sup>10 commercial tempeh samples purchased from various markets in Jakarta, Indonesia. <sup>C</sup>Cooked for 60 minutes.

bacterial contamination (though apparently common in Indonesia). Tempeh in Europe and the U.S. cannot be relied on as a source of B12. Until tempeh in Thailand and Indonesia are shown to reduce MMA levels, it should not be relied upon there, either.

#### A.4 Seaweeds

##### A.4.1 Various Seaweeds: Dulce Warrants Further Study

Table A.4.1 shows the B12 analogue content of arame, dulce, hijiki, kelp, kombu, and wakame per 30 g of seaweed. Please note that 30 g is a lot of seaweed. A serving size would be closer to 3 grams. Seaweeds also tend to be very high in iodine, which can cause problems at high intakes. So, consuming mass quantities of seaweed is unadvisable.

The only seaweed in this list that warrants further study is dulce (also spelled "dulce"), which contains .3 to .39 µg of B12 analogue per 3 g serving. Unless dulce is eventually shown to lower MMA levels, it should not be considered a source of active B12.

**Table A.4.1 B12 Analogue Content (µg/30 g) of Various Seaweeds.**

Country	Netherlands <sup>101</sup>		USA <sup>93</sup>
	IF	L. leich.	IF
aramé			.042
dulce ( <i>Palmaria palmata</i> )	3.9	3	
hijiki	< .006	< .006	
kelp	1.2	.12	
kombu	.84	.018	.57-1.3 <sup>A</sup>
wakame	1.4	.009	1.29 <sup>B</sup>

<sup>A</sup>Range of 5 samples of 3 different brands, with 3 samples cooked for 60 minutes. <sup>B</sup>Cooked for 60 minutes.

##### A.4.2 Aphanizomenon Flos-aquae

Cell Tech and some other companies market algae from Klamath Lake in Oregon. Cell Tech markets a species, *Aphanizomenon flos-aquae*, they call Super Blue Green Algae (SBGA). The company claims that this algae contains active B12. On February 28, 2002, Cell Tech's site, <http://www.celltech.com/resources/technical/FAQ.asp#CONTENT7>, stated:

"Is the vitamin B12 in SBGA bioavailable and bioactive?"

"Yes. The Super Blue Green Algae (SBGA) strain, *Aphanizomenon flos-aquae*, has been tested by Lancaster Labs for B12 analog levels using microbiological testing methods that are comparable to methods 952.20 and 960.46 of the Association of Analytical Chemists (AOAC).

"Unlike other plant foods such as Spirulina, which contain corrinoids with virtually no vitamin B12 activity, *Aphanizomenon flos-aquae* is a reliable source for vegetarians seeking to supplement their diets with a bioactive form of this important nutrient."

However, test methods 952.20 and 960.46 use *Lactobacillus leichmannii*,<sup>7</sup> which can measure non-B12 corrinoids.<sup>89</sup> Thus, it can only be concluded that Cell Tech's SBGA contains B12 analogues whose activity is yet to be determined.

##### A.4.3 Chlorella

Pratt & Johnson<sup>185</sup> (1968, USA) studied numerous batches of chlorella and occasionally found amounts of B12 analogue that were in the range of error for the test method. In other words, they were not able to detect appreciable amounts. They noted that their extraction processes might not have been adequate though they used many different methods. They also noted that their synthetic medium on which the chlorella was grown might have interfered with B12 analogue synthesis.

**Table A.4.3 B12 Analogue Content (µg/30 g) of Chlorella**

Country	USA <sup>185</sup>
Assay	<i>E. gracilis</i> & <i>O. malhamensis</i>
<i>Chlorella vulgaris</i>	ND
<i>Chlorella pyrenoidosa</i>	ND

##### A.4.4 Spirulina

Table A.4.4 shows the B12 analogue content (µg/30 g) of various spirulina batches. It appears that spirulina has a wide variety of analogues, many of which are inactive, and some of which may interfere with B12 activity in humans.

**Table A.4.4 B12 Analogue Content (µg/30 g) of Spirulina**

Country	Netherlands <sup>101</sup>		USA <sup>43</sup>		Japan <sup>195</sup>		
	IF	L. leich.	IF	L. leich.	L. leich.	IF	PC
spirulina	14.5	67	36.7	193.1	73	2.5	.44
spirulina			6.0	35.3	38	1.9	.32
spirulina			1.67	8.7	44	5.2	.88

##### A.4.5 Nori

Table A.4.5.1 shows the B12 analogue content of various nori types and batches. Various batches of nori were found to contain significant amounts of B12 analogue. One study verified the molecular weight through paper chromatography, indicating that there is a good chance that some of this B12 is active. Yamada et al.<sup>108</sup> (1996, Japan) determined that nori contains what they considered to be active B12 analogues using various assays and methods (results not reported here).



However, in light of Dagnelie's<sup>21</sup> results (discussed below), they decided to test the nori to see if it could reduce methylmalonic acid levels (1999, Japan),<sup>109</sup> the gold standard for determining the B12 activity of a food. Raw nori was purchased within 48 hours of harvesting. Dried nori was purchased from a store. Inactive vs. active B12 was determined by IF assay and confirmed by paper chromatography. 10 people (all nonvegetarian) were then studied. The results are in Table A.4.5.2. These results indicate that B12 in raw nori can be changed into harmful inactive B12 analogues by the drying process and that the dried nori decreases B12 status. Yamada et al. thought that although *dried* nori cannot be used as a source of B12, in small amounts it is not harmful. However, they believe that *raw* nori is an excellent source of genuine B12.

I disagree with their conclusion that raw nori is an excellent source of active B12. Even though the patients' uMMA levels did not increase enough to be statistically significant while eating the raw nori, they did increase 5%. In my opinion, this study merely shows that this batch of raw nori did not have enough inactive B12 analogue versus active B12 to be considerably detrimental, but it did not prove any benefit.

This study was further confounded by adding valine (an amino acid that can be converted into methylmalonic acid when B12 is deficient) to the subjects' diet in order to increase MMA levels so that a difference could be seen. The valine did not appear to do this when given without the nori, and no control groups were included, making the results even more difficult to interpret.

#### A.4.6 Coccolithophorid Algae

Coccolithophorid algae (*Pleurochrysis carterae*) is being used in Japan as a calcium supplement. Miyamoto et al.<sup>152</sup> (2001, Japan) analyzed it for B12 analogue content (results in Table A.4.6). They tried to verify whether it is an active form of B12 through liquid chromatography, and thought that it was. This algae deserves further attention to see if it can lower MMA levels in humans.

#### A.5 Plant Sources of B12 Analogues Studied in Humans

Miller et al.<sup>68</sup> (1991, USA) found that sB12 appeared to be unrelated to consumption of wakame, kombu, other sea vegetables, or tempeh in macrobiotic children and adults.

Dagnelie et al.<sup>21</sup> (1991, Netherlands) quickly changed the thought that intrinsic factor assays can predict the contribution of foods to B12 status. Macrobiotic toddlers (14-26 months old) with B12 deficient anemia (sB12 < 184) were given foods found to have some B12 analogue using IF assays. According to Dagnelie, as little as 0.1 µg/day of B12 can lead

**Table A.4.5.1 B12 Analogue Content (µg/30 g) of Nori**

Country	Netherlands <sup>101</sup>		Japan <sup>186</sup>		Japan <sup>109</sup>		
Assay	IF	L. leich.	L. leich.	IF	<i>E. Coli</i> 215	IF	PC
nori ( <i>P. umbilica</i> )	3.6						
nori ( <i>P. tenera</i> )	5.4-12.9 <sup>A</sup>						
nori (purple, <i>Porphyra</i> sp)			9.7	7.5			
nori (green, <i>Enteromorpha</i> sp)			19.1	21			
nori ( <i>P. tenera</i> )	20.1	20.1					
dried nori ( <i>P. tenera</i> )					4.3	< 4.3	1.5
raw nori ( <i>P. tenera</i> )					3.8	~ 3.8	2.7

<sup>A</sup>Range of 3 different samples.

**Table A.4.5.2 Yamada et al.'s<sup>109</sup> Study of Nori's Impact on urine MMA Levels**

	#	B12 found to be analogue	amount	duration	uMMA
dried nori	6	65%	40 g (20 sheets) <sup>A</sup>	6-9 days	increased 77% <sup>B</sup>
raw nori	4	27%	320 g/day <sup>A</sup>	3-6 days	increased 5% <sup>C</sup>

<sup>A</sup>Equivalent amounts. <sup>B</sup>Statistically significant. <sup>C</sup>Not statistically significant.

**Table A.4.6 B12 Analogue Content (µg/30 g) of Coccolithophorid Algae**

Country	Japan <sup>152</sup>	
Assay	IF	<i>L. delbrueckii</i>
coccolithophorid algae	37.6	37.6 <sup>A</sup>

B12 analogue remained stable for 6 months of storage.

<sup>A</sup>Study said the amount was "identical" to that found with IF; the number was not actually given.

**Table A.5.1 Results of Dagnelie et al.<sup>21</sup>**

subject	µg/day of "B12" given	"B12" source	anemia (MCV)
vegans			
1	2.7	spirulina, nori	worse
2	2.1	spirulina, nori	worse
3	1.5	nori	worse
4	.3	nori	worse
5	.1	sourdough bread, kombu, barley malt syrup	worse
non-vegans			
6	1.5	algae	better
	.5	fish & milk	
7	.3	fish	better
8	.2	fish	better
9	.2	fish, milk	better
10	.15	fish, nori	worse
11	.2	supplement, fish, nori	worse

to a full therapeutic response in 1 month.<sup>21</sup> Despite eating foods that supposedly contained B12, 4-6 months later, their sB12 levels had increased, but the anemia got worse in the vegan children. See Table A.5.1.

A likely explanation for the poor response is that nori, spirulina, and kombu either contained no active B12 or that they contained enough inactive B12 analogue that it overcame the active B12, producing an overall negative effect. Dagnelie et al. say, "It seems unjustified to advocate algae and other plant foods as a safe source of vitamin B12 because its bioavailability is questionable."

It should be noted that, based on a more recent study, intakes as high as .3 µg/day for infants 6-16 months old are probably not enough to prevent B12 deficiency (based on MMA levels).<sup>206</sup> Thus, some of the patients in Dagnelie's study above may have needed more B12 for a positive response.

Specker et al.<sup>93</sup> (1988, USA) reported a macrobiotic mother of an infant with a uMMA of 146 µg/mg who modified her diet by increasing her consumption of seaweeds and fermented foods. The infant's uMMA dropped to 27 µg/mg in 2 months and to 13 µg/mg in 4 months. It was later discovered that this mother had also eaten fish and clam broth which were probably responsible for the improvement rather than the seaweeds and fermented foods.<sup>21</sup> Specker et al. stated, "The vegetarian community we worked with believed fermented foods in their diet contained adequate amounts of vitamin B12." However, on analysis, the fermented foods were shown not to have B12.<sup>93</sup>

Suzuki<sup>97</sup> (1995, Japan) studied 6 vegan children eating a genmai-saishoku (GS) diet based on high intakes of brown rice. The GS diet contains plenty of sea vegetables, including 2-4 g of nori per day ("dried laver"), as well as hijiki, wakame, and kombu. The foods are organically grown and many are high in cobalt (buckwheat, adzuki beans, kidney beans, shiitake, hijiki). B12 levels are shown in Table A.5.2. None of the many measurements between the vegans and 4 nonvegan controls were significantly different, including sB12, MCV, and iron indicators. MMA and HCY were not measured. Some suggestions as to how the vegans got their B12:

- From nori or the other seaweeds. The nori was most likely dried.
- Small amounts of B12 from B12 uptake or contamination of plants grown in manure.
- B12 from their mother's stores.

These results are both interesting and perplexing. The sB12 levels are easy to explain as possibly being inactive B12 analogues. On the other hand, it is particularly impressive that the eight-year-olds were doing well given that their mothers had been vegan for some time, supposedly without fortified foods or supplements. Unfortunately, many vegan children have not had the same positive results and until more is known about these children's diets, this study should be considered an unsolved mystery. If these children were my own, I would make sure they got at least a modest B12 supplement to ensure their good health.

**Table A.5.2 Results of Suzuki.<sup>97</sup>**

age	yrs vegan	s B12
7.1	4.4	520
7.7	4.4	720
8.6 <sup>A</sup>	8.6	480
8.8 <sup>A</sup>	8.8	300
12.7	10.0	320
14.6	10.0	320
average		443 (±164)

Exclusively breast-fed until 6 months old. Mothers had been vegan for 9.6 & 6.5 yrs prior to conception. Both mothers consumed 2 g of nori per day.

#### A.6 Conclusion

Of all the foods studied above, only tempeh in Indonesia or Thailand, dulse, raw nori, and coccolithophorid algae (*Pleurochrysis carterae*) warrant much further attention for providing B12. Unless these foods are shown to correct B12 deficiency consistently, vegans should not rely on them as a B12 source.

#### Appendix B. Slightly Elevated MMA Levels

In a study of nonvegetarian, older adults with slightly elevated methylmalonic acid (MMA) levels (.29-3.6 µmol/l), higher sMMA levels did not predict neurological problems.<sup>127</sup> However, these individuals were not compared to people with normal sMMA levels. Because there was no control group, we cannot say that people with slightly elevated sMMA are not at risk for neurological problems. We can only suggest that increasing sMMA from .29 to 3.6 may not do any further, measurable neurological harm.

Lindenbaum et al.<sup>60</sup> showed that people with very high levels of sMMA get neurological problems (see section 3.2.1).

In another study, older adults with slightly elevated MMA levels (.27-2.00 µmol/l) were treated with cyanocobalamin injections: MMA levels decreased 66% and homocysteine levels decreased 23%. Patients with MMA in the range of .60-2.00 µmol/l had neurological improvements after B12 therapy.<sup>128</sup>

These studies indicate:

- Slightly increasing sMMA levels from .29 to .60 µmol/l may not increase one's risk for neurological problems.
- People with MMA levels above .27 µmol/l may have elevated homocysteine which can benefit from B12 therapy.
- People with sMMA levels above .60 µmol/l may have neurological problems that can benefit from B12 therapy.

## Appendix C. Vegan Infants & Toddlers with Serious B12 Deficiency

Study sex, age, sB12	Additional Symptoms <sup>A</sup> Tx: Treatment	Results/Notes
normal sB12 for newborns: <sup>118</sup>	160-1300 pg/ml	
Davis et al. <sup>27</sup> 1981, USA M, 6 m	heart failure Tx: B12 I's	Mother's sB12 was 278. By day 28, all blood values were normal. Infant then given 50 µg B12 twice per w.
Close <sup>17</sup> 1983, Dominica M, 12 m	vomiting Tx: oral B12 for 42 d	Full recovery.
Hellebostad et al. <sup>41</sup> 1985, Norway F, 2 y, 54	Tx: B12 injections vitamin D, calcium, iron	Also suffered from rickets. and low protein. The girl ate small amounts of milk, eggs, and cod liver oil. Her symptoms of rickets and B12 deficiency resolved after 2 w. Mother was pregnant and her sB12 was 149. She was given B12 injections and a MV and she and the baby have both done well.
Sklar <sup>91</sup> 1986, USA M	Swelling of hands, feet, & abdomen. Tx: blood transfusion, 1000 µg B12 injections for 5 d. Child & Mother: 1 m of 50 µg B12/day 4 m of MV	Improved in 48 hrs. Discharged on 4 <sup>th</sup> d. Was doing much better after 6 m. The parents had been vegan for 6 years. 2 w prior to admission, a naturopath suggested they take him to a hospital. The parents refused, but the mother agreed to begin taking MV.
Gambon et al. <sup>32</sup> 1986, Switzerland M, 11 m	Tx: Admitted to hospital and given three 500 µg B12 injections in 6 d.	Full recovery. Discharged with an oral vitamin supplement containing 6 µg B12 /d and oral iron. Mother did not follow the advice to give iron. Child was readmitted 2 m later showing iron deficiency. He was put on an iron supplement and blood value returned to normal in 1 m. No relapse of B12 deficiency was noted after oral supplementation. The infant was a twin. Other twin was not reported to have problems.
Stollhoff & Schulte <sup>96</sup> 1987, Germany M, 18 m, 63	Progressive nerve disorder since 6 m of age. Tx: 1000 µg/day	sMMA & uMMA were not elevated. At 5 w, anemia gone & nerve problems dramatically improved. At 26 m, functioning at a 12-month-old level with exaggerated reflexes. Continued to make psychomotor progress. Stollhoff thought the normal MMA levels were possibly due to the small amounts of animal products prior to hospital admission.
Cheron et al. <sup>189</sup> 1989, France F, 6 m, 45	Hemoglobin 1.9 g/dl Tx: B12 I(s).	Parents' sB12: 110 and 105. B12 in mother's milk 12 pg/ml. Treatment was successful.
Bar-Sella et al. <sup>8</sup> 1990, Israel 14 m	Severe nerve damage. Tx: B12 therapy	Partially corrected.
Michaud et al. <sup>66</sup> 1992, Canada F, 2.5 m, 104	Had no symptoms. Tx: Oral B12	uMMA started at 537µmol/mmol Cr, but dropped to 10.9 after 2 w. Mother's sB12 was 81.
Graham et al. <sup>34</sup> 1992, Australia		Reviewed the cases of B12 deficiency seen during the previous 10 y at Prince of Wales Children's Hospital to obtain long-term follow-up. 3 of the 6 patients were infants of vegetarian mothers. 1 had borderline intellectual development at age 5.
Kuhne et al. <sup>55</sup> 1991, Switzerland F, 9 m, 28	Optic nerve atrophy. Tx: 1000 µg injection for 4 d 1000 µg orally on 5 <sup>th</sup> d	uMMA and plasma HCY were initially very high. All muscle problems vanished after 10 d. Girl was discharged and parents were given an oral vitamin B complex including B12. Parents agreed to add eggs & dairy to her diet. By 15 <sup>th</sup> m, her physical measurements and lab values were normal.
Monfort-Gouraud et al. <sup>188</sup> 1993, France 15 m, very low	Severe megaloblastic anemia. No growth. Lack of muscle tone & psychomotor development. Tx: Injections and possibly a blood transfusion.	Very low levels of B12 in milk of mother who had been vegan for 10 y. At 4 y, patient's growth and psychomotor development were normal.
Drogari et al. <sup>10</sup> 1996, Greece 3 M, 1 F, 2-5 w, very low	Low hemoglobin, no megaloblastic anemia. Vomiting in 3/4. Elevated uMMA (NR). Tx: Appropriate treatment	Mothers also had elevated uMMA. All children doing very well after 4-5.5 y.
Lovblad et al. <sup>61</sup> 1997, Switzerland F, 14 m, 124	Severe brain atrophy. Tx: MV, iron, folic acid, trace elements, 1 ng B12 every other d.	Responded well and at 20 months could sit alone, crawl, walk with help, and speak simple words. The "1 ng" was probably a typo and was actually "1 mg" (1,000 µg).

Continued

<b>Study sex, age, sB12</b>	<b>Additional Symptoms<sup>A</sup> Tx: Treatment</b>	<b>Results/Notes</b>
von Schenck et al. <sup>103</sup> 1997, Germany M, 14 m, ND	Severe nerve problems. Comatose at hospital admission. Severe brain atrophy. Tx: 1 250 µg B12 injection., 25 µg oral B12/d plus supplemented soy formula.	uMMA was initially very high. Mother had been vegan for 6 y, and vegetarian for 8 y prior. She ate a high proportion of raw foods. The infant had been exclusively breast-fed for 9 months and then ate small amounts of fruit. Regained consciousness in hours. By day 3 able to walk, eat, drink, and was discharged. Parents agreed to give soy formula & oral B12. Cranial MRI 10 w later showed all structural brain abnormalities had disappeared, but he continued to show nerve damage. At 2 y, still showed psychomotor retardation, was agitated, and had poor concentration. Could not speak any words. 3-year-old sister had sB12 of 139. Mother had sB12 of 149 which became normal after oral SUP.
Grattan-Smith et al. <sup>36</sup> 1997, Australia M, 10 m, 47	Intermittent tremors that had started at 4 m. Tx: 1000 µg B12/d injection for 6 d	Developed tremors (after treatment) which lasted for 6 w. By 23 m, motor skills were normal, but intellectual development appeared to be slow for his age.
Grattan-Smith et al. <sup>36</sup> 1997, Australia F, 12 m, 50	Irritable, lethargic, & constipated. Tx: 1500 µg B12 injections	After 2 <sup>nd</sup> injection, she developed tremors which eventually went away and she returned to normal.
Grattan-Smith et al. <sup>36</sup> 1997, Australia M, 18 m, 202	Stopped developing at 1 y. Tx: 500 µg B12/day injections.	Mother's diet showed a B12 intake of .3 µg/day and sB12 of 202. She also had B12 malabsorption thought to be due to a temporary small intestine problem caused by B12 deficiency. After treatment, child's twitching developed into episodes more like seizures as his neurological status gradually improved.
Renault et al. <sup>81</sup> 1999, France M, 12 m, ND	Low level of red & white blood cells & platelets. Tx: 1,000 µg/d	Had increased HCY and MMA in urine. Suffered muscle tremors during 1 <sup>st</sup> 3 d of treatment. Normal after 3 w.
Renault et al. <sup>81</sup> 1999, France M, 10 m, ND	Regurgitation, edema. Tx: 300 µg/d	Suffered muscle tremors during 1 <sup>st</sup> 4 d of treatment. Normal after 2 m. Mother had a sB12 of 109 with normal intrinsic factor function.
Wiley et al. <sup>105</sup> 1999, Australia		In screening 137,120 newborns for inborn error of metabolism, they found 2 B12-deficient babies of vegan mothers. No further information was given; "B12-deficient" was not defined.
Fogarasi et al. <sup>155</sup> 2001, Hungary NR, 9 m	Repetitive vomiting, trouble swallowing, tremor. Tx: B12 injections	Improved dramatically in days. Blood was normal by 6 w. Article in Hungarian. Only the abstract was available.
Smolka et al. <sup>156</sup> 2001, Czech Republic F, 13 m, low	Brain atrophy. Tx: B12 supplements.	Fed only vegetables after 9 m old. Elevated MMA. Condition improved after treatment. Article in Czech. Only the abstract was available.
Smolka et al. <sup>156</sup> 2001, Czech Republic M, 8 m, low	Tx: B12 supplements.	Fed breast milk and fruit juice only after 6 m. Elevated MMA & HCY. Mother's B12 was low. Improved after treatment but still had problems including language delay.
Ciani et al. <sup>179</sup> 2000, Italy M, 22 m, 75	pH 6.95 (severe acidosis) Tx: hydroxocobalamin 1,000 µg/d, biotin, and carnitine, and low protein all via injection.	Mother's sB12: 162. Complete recovery in 3 m. Lack of elevated HCY led researchers to determine the boy had a mild case of genetic methylmalonyl-CoA mutase deficiency which was exacerbated by a low B12 intake.

<sup>A</sup>Unless otherwise noted, all infants were healthy until about 6-12 m after which they failed to thrive and showed developmental regression. All became lethargic and lost their ability to use their muscles adequately. Some could not sense properly and most had macrocytic anemia. "Additional Symptoms" only lists symptoms in addition to those just described.

d - days; f - female; HCY - homocysteine; I - injection; m - male; m - months; MV - multivitamin; ND - non-detectable; NR - not reported; sB12 - serum B12; sMMA - serum methylmalonic acid; SUP - supplement, Tx - treatment; uMMA - urinary methylmalonic acid; w - week; y - years.

## Appendix D. B12 Levels in Lacto/Lacto-ovo Vegetarians

Study Subjects	sB12	Notes	
Matthews & Wood <sup>114</sup> (1984, UK)			
22 adult Hindu vegetarians w/ B12-deficient anemia.	9 had < 50	Symptoms were weakness, weight loss, nausea, vomiting, diarrhea, and abdominal pain. No neurological problems were detected. Many had other nutritional deficiencies.	
Chanarin et al. <sup>15</sup> (1985, UK)			
95 adult Hindu LV w/ B12 -deficient anemia	R 0-170 5 < 30	34% had fat malabsorption. 47 treated with oral B12; all responded. 48 treated with injections. 5 women had been attending an infertility clinic, but conceived after B12 treatment. 1 had hair pigment loss which normalized after B12 therapy.	
Faber et al. <sup>123</sup> (1986, South Africa)			
19 female SDA LOV	287 ± 91 <sup>A</sup>	Age of subjects: 18-40 yrs. LOV had been on diet > 2 yrs; average of 8.6 yrs No subjects took SUP.	
14 male SDA LOV	273 ± 69		
12 female NV	397 ± 167 <sup>A</sup>		
10 male NV	354 ± 71		
Helman & Darnton-Hill <sup>42</sup> (1987, Australia)			
120 adult LOV from a meditation center or clinic. All were healthy adults.	350 ± 171 <sup>A</sup> 16% < 200	22% used vitamin SUP No vegetarians demonstrated B12 deficiency symptoms.	
53 NV	490 ± 179 <sup>A</sup>	30% used vitamin SUP	
Reddy & Sanders <sup>80</sup> (1990, UK)			
18 adult white LOV	197	All subjects were women. 2 took a MV and had an average sB12 of 391.	
21 adult Indian vegetarians	199		
22 adult white NV	341		
Solberg et al. <sup>92</sup> (1998, Norway)			
63 adult LOV of an average of 17 yrs (R 10-23)	374	20 LOV took a MV w/ 2-4 µg of B12. They did not have significantly higher sB12.	
63 adult NV hospital employees	394	Authors conclude that Norwegian LOV are not at risk of developing B12 deficiency.	
Pongstaporn & Bunyaratavej <sup>77</sup> (1999, Thailand)			
68 adult vegetarians 4 vegans	202 <sup>B</sup>	No info on SUP. B12 deficiency found in 23 vegetarians (40%) & 4 vegans. sB12 was not correlated with years of vegetarianism.	
30 adult NV	471 <sup>B</sup>		
Leung et al. <sup>122</sup> (2001, Hong Kong)			
Buddhist and SDA children		Had been vegetarian for > 1 year.	
10 (5.9 ± .8 yrs old)	590 ± 316		
24 (9.3 ± 1.1 yrs old)	566 ± 297		
22 (12.3 ± .7 yrs old)	423 ± 136		
Millet et al. <sup>69</sup> (1989, France)			
11 M Veg	313 ± 130 <sup>A</sup>	6% < 203	Included 3 vegans. Excluded people taking supplements. Subjects were middle-aged.
26 F Veg	301 ± 150 <sup>B</sup>	17% < 203	
33 M NV	356 ± 174 <sup>A</sup>	0 < 203	
36 F NV	328 ± 176 <sup>B</sup>	0 < 203	

<sup>A,B,C</sup> Statistically significant difference between groups with same letters. f - female; LV - lacto vegetarian; m - male; LOV - lacto-vegetarian; MV - multivitamin; NV - nonvegetarian; sB12 - serum B12; SUP - supplements.

## Appendix E. Vegan or Vegetarian Children with Serious B12 Deficiency

Study sex, age, sB12	Symptoms Tx: Treatment	Results/Notes
Licht et al. <sup>178</sup> 2001, USA M, 14 y, 281	Fatigue, daytime sleeping, aching calf. Stumbling. Small for age. Heart murmur. Withdrawn. Tx: 1,000 µg B12 IMI/day	MCV 108 fl, sMMA 2.5 µmol/l, HCY 64.4 µmol/l. No anemia. Mother considered him a "picky eater." Treatment resulted in normal gait, but after 18 m still had nerve problems.
Cornejo et al. <sup>154</sup> 2001, Spain M, 10 y	Serious neurological problems.	Researchers described the boy as "a member of a religious community who were strict vegetarians." Article in Spanish; no further information was given in the abstract.

HCY - homocysteine; IMI - intramuscular injection; m - month(s); M - male; MCV - mean corpuscular volume; sB12 - serum B12; sMMA - serum methylmalonic acid; y - year.

## Appendix F. B12 Levels in Macrobiotics

Study Subjects	sB12	Notes
Dwyer et al. <sup>31</sup> (1982, Boston, MA)		
39 V & SV children 27 were MAC (9 MAC were vegan)	489, R 100-700 All were normal.	Only 2 of the vegans used SUP. Avg. age was 4.0 y (R 0.8 - 8.4).
Specker et al. <sup>93</sup> (1988, USA)		
17 vegan MAC mothers 6 NV mothers	56% had < 200 NR	Infants had significantly higher uMMA, which dropped after 7-10 d of B12 therapy.
Dagnelie et al. <sup>20</sup> (1989, Netherlands)		
50 MAC infants	201 50% < 188 10% < 107	Authors stated, "Our findings indicate that the plasma B12 concentration in macrobiotic infants is sufficiently low to have physiological consequences raising concerns about neurological development."
57 NV infants	545	
Schneede et al. <sup>88</sup> (1994, Netherlands)		
41 MAC infants 50 NV infants	191 R 79-459 538 R 261-1108	HCY & sMMA were increased in the MAC and were inversely related to sB12.
Dagnelie & van Staveren <sup>23</sup> (1994, Netherlands)		
(Follow-up article to Schneede et al. <sup>88</sup> ) Growth in MAC infants & children was retarded between ages of 6-18 m, and did not catch up (by age 10) unless animal products were added to the diet.		
Specker et al. <sup>94</sup> (1990, USA)		
13 MAC (mostly vegan), no SUP	B12 in breast milk: 312 ± 127 pg/ml	B12 in breast milk decreased as time on MAC diet increased. Infant uMMA inversely related to milk B12 levels below 489.
6 NV, 5 took SUP	510 ± 101 pg/ml	
Miller et al. <sup>68</sup> (1991, New England USA)		
110 MAC adults 42 MAC children	entire group: 202	51% of adults had sB12 < 200 30% of adults sampled had high uMMA sB12 negatively associated w/ time on diet & uMMA. Several had low sB12 after a short time as a MAC (they may have had poor status before beginning the diet). 55% of children had high uMMA. Children were short in stature. Decreased stature was related to high uMMA

d - days; m - months; MAC - macrobiotic; NR - not reported; NV - nonvegetarian; sB12 - serum B12; SV - semi-vegetarian (ate some meat); SUP - supplements; V - lacto-ovo vegetarian or vegan.

## Appendix G. Note about Twinlab B12 Dots

Although Twinlab does not list any animal products in the ingredients on the bottle, the tablets actually contain gelatin. The following is a reply to a product information request placed with Twin Laboratories, Inc. on Wednesday, May 30, 2001:

"Our B-12 Dots contain gelatin derived from beef and pork, which would not make them suitable for vegetarians."

## Appendix H. Inactive Analogues: Worse than Useless

Carmel et al.<sup>14</sup> (1988, USA) examined the medical records of 364 patients with low B12 levels. R-protein and human intrinsic factor were used to measure patients' B12 levels: Because active B12 analogues would be detected using either method, any difference between the two methods would indicate inactive B12 analogues.

- Patients with primarily neurological problems had significantly higher inactive B12 analogue levels (as shown by a difference between assays), than did patients with primarily blood problems.
- 33 of the 76 patients with neurological symptoms had a normal sB12 when measured with R-protein; when measured with intrinsic factor, many of these patients had much lower sB12 levels. In contrast, the R-protein assay was a reliable indicator of B12 deficiency in patients whose symptoms were primarily blood-related.

This study indicates that one of the following is probably true:

1. Some B12 analogue may be harmful to the nervous system.
2. Some B12 analogue may have B12 activity in bone marrow (which produces blood cells) but not in the nervous tissue.

## Appendix I. Homocysteine & Disease

### I.1 Background

Table 3.4 lists the normal serum HCY range as 2.2-13.2  $\mu\text{mol/l}$ . Although that range is “normal,” it is not necessarily healthy.

#### I.1.1 Measuring Homocysteine

It should be noted that there are some differences in homocysteine (HCY) measurements between laboratories,<sup>148</sup> which detract from comparing numbers between studies. However, the *changes* in HCY levels should be fairly comparable between studies.

#### I.1.2 Vitamin Supplementation Can Confound Study Results

HCY levels were recorded at the beginning of these studies. Vitamin supplementation which started after the study began could affect the results.<sup>160,162</sup> If HCY is a cause of disease, this vitamin supplementation would have biased the results against finding HCY to be an independent risk factor. This is because people with high HCY at the beginning of the study would have subsequently lowered their HCY while still being included in the “high HCY” groups during follow-up.

### I.2 Homocysteine & Cardiovascular Disease

Cardiovascular disease (CVD) includes the diseases listed in Table I.2.1.

Elevated homocysteine is most definitely *associated* with cardiovascular disease. The question is whether elevated HCY is an *independent risk factor* (i.e., a cause) of CVD.<sup>162</sup> Another possibility is that elevated HCY is not an independent risk factor for acquiring CVD, but can exacerbate existing CVD.<sup>162</sup>

**Table I.2.1 Common Cardiovascular Diseases**

Ischemic heart disease (IHD) - heart attack
Coronary artery disease (CAD) - plaque obstruction of the coronary arteries to the heart
Coronary heart disease (CHD) - same as CAD
Cerebrovascular disease - mainly stroke

The original evidence that elevated HCY might cause CVD came from studies of people who have genetic defects in the homocysteine metabolizing enzyme *cystathionine synthase*. Bostom et al.<sup>136</sup> report that despite the absence of any traditional CVD risk factors, 50% of untreated children and young adults with this defect experience a major CVD event by age 30. On the other hand, Cleophas et al.<sup>159</sup> report that only 10 out of 629 people with a genetic disease causing elevated HCY had CAD. Cleophas et al. note that this genetic defect often causes severe peripheral (away from the center of the body) vessel disease. What about in the adult population without such obvious genetic problems?

#### I.2.1 Recent Reviews of the Studies on Elevated Homocysteine and CVD

In a review of the literature (Booth et al.,<sup>133</sup> 2000), the Canadian Task Force on Preventative Care determined that 5-10% of the general population, and as high as 30-40% of the elderly, have elevated HCY levels (above 15  $\mu\text{mol/l}$ ); and that elevated HCY may be responsible for 10% of heart attacks. They noted that caffeine is associated with high levels of HCY.<sup>133</sup> Smoking is also associated with higher HCY.<sup>134</sup> Booth et al. recommended meeting the RDA for folate, B12, and B6 and that a vitamin deficiency should be investigated in cases of elevated HCY. They said there was not enough evidence to determine if folic acid could reduce heart attacks.

In another review of the literature, Cleophas et al.<sup>159</sup> (2000) suggested that elevated HCY may be an expression of unhealthy lifestyles, rather than a cause of CVD. They did not think it was an indication of low folate or B6 intake. Their analysis showed that there may be a bias in that studies showing a positive link between HCY and CVD are more likely to be accepted for publication than those not showing an association. They concluded that:

[H]omocysteine may, indeed, not be as harmful for the heart as it seems, despite studies to the contrary. At the same time, however, homocysteine may be an indicator for unhealthy lifestyles, and therefore, an important variable for cardiologists to take into account when assessing patients for [coronary artery disease].<sup>159</sup>

However, Cleophas et al.'s analysis only included 9 of the 20 prospective studies contained in this Appendix. It did not include the study by Hackam et al.<sup>132</sup> (described below) in which folate, B6, and B12 supplements resulted in reducing fatty plaques.

Brattstrom & Wilcken<sup>174</sup> (2000) wrote a review "Homocysteine and cardiovascular disease: cause or effect?" In it, they point out:

- The vascular disease caused by the genetic deficiency in cystathionine synthase is much different than typical CVD in that it affects mostly the veins without increasing plaque.
- People with a particular defect in HCY metabolism (the TT genotype) do not have a higher risk of CVD even when they have low folate status.
- Vascular disease is not a known complication of folate and B12 deficiency despite moderately ( $> 30 \mu\text{mol/l}$ ) or even greatly elevated homocysteine ( $> 100 \mu\text{mol/l}$ ).

- Two studies have shown an increased risk of mortality or CHD in subjects with elevated sB12. These studies are discussed in section I.2.4, *Elevated sB12 as an Increased Risk Factor for Disease*. Ridker et al.<sup>163</sup> (1999, USA) found lower HCY levels in multivitamin users (10.9 vs. 13.1  $\mu\text{mol/l}$ ) but not lower risks for heart attack or stroke.

*Note:* Ridker et al.'s study is listed in Table I.2.2.1, but the analysis of multivitamins did not give information as to what amounts, which vitamin, or how often. Additionally, the statistical methods applied to the multivitamin versus the non-multivitamin users were not helpful. Without more information, these results do not mean much.

- Brattstrom & Wilcken previously performed a meta-analysis that showed that moderately elevated HCY is not even an independent cause of vein (or artery) thrombolism.

Despite making the above points, Brattstrom & Wilcken are not clearly of the opinion that elevated HCY is not an independent risk factor for CVD.

In the same issue of the *American Journal of Clinical Nutrition*, Ueland et al.<sup>173</sup> also wrote a review, "The controversy over homocysteine and cardiovascular risk." They provide some evidence to counter points made by Brattstrom & Wilcken:

- There has not been enough data on the genetic defects in HCY metabolism to contradict the hypothesis that HCY is an independent risk factor for CVD.
- There is evidence that people with the TT genotype may have adaptations that counteract damage by elevated HCY.
- 3 intervention trials suggest that B vitamins have a protective effect. (Folate is a B-vitamin.)
- Betaine (which converts homocysteine to methionine in the liver, see Figure 3) supplementation has had a dramatic effect of nearly preventing the occurrence of vascular events in people with elevated HCY.
- If HCY causes CVD, adjusting for factors such as age, sex, smoking, exercise, kidney function, and blood pressure could lead to a HCY risk underestimation.

Ueland et al. conclude that elevated HCY, in isolation, probably confers minor risk for CVD. However, it probably further increases risk when it occurs in combination with other factors that provoke vessel damage; therefore, it is a strong risk factor in subjects with underlying disease and predicts the short-term outcome in such individuals.

### I.2.2 Prospective Studies on Homocysteine and Cardiovascular Disease

In reviewing the data below on elevated HCY as an independent risk factor for CVD, I have used only prospective studies (or case-control studies nested within prospective studies) which are the most relevant studies for answering this question. Only studies of people who started out without CVD are included. In most of the studies, there was a strong, statistically significant correlation between HCY and CVD before adjustments. After adjusting for factors such as age, smoking, and other CVD risk factors, the statistical significance often disappeared, although the trend often remained towards higher disease. Numbers are listed only after adjusting for other CVD risk factors. The studies are summarized in Tables I.2.2.1-4.



**Table I.2.2.1 Homocysteine and Increased Risk of CVD.**

HCY $\mu\text{mol/l}$	Increased risk of disease	Notes
Evans et al. <sup>162</sup> (2000, USA, Multiple Risk Factor Intervention Trial) 712 men aged 35-57 after avg. 20 years of follow-up		
For death from heart disease or for heart attack:		
3.7-9.5	controls	Adjusted for: age, smoking, hypertension, cholesterol, triglycerides.
9.6-11.8	3% <sup>NS</sup>	
11.9-14.9	-16% <sup>NS</sup>	
15.0-80.4	-18% <sup>NS</sup>	
Bots et al. <sup>161</sup> (1999, Netherlands, Rotterdam Study)		
For heart disease: 637 people aged 55- ~85 (avg. 68.5) after avg. 2.7 years of follow-up		
< 12.0	controls	Adjusted for: age, sex, smoking, hypertension, cholesterol, diabetes, previous heart attack, previous stroke.
12.0-13.7	95% <sup>NS</sup>	
13.8-15.5	85% <sup>NS</sup>	
15.6-18.5	63% <sup>NS</sup>	
$\geq$ 18.6	110% <sup>NS</sup>	
For stroke: 653 people aged 55- ~80 (avg. 69.5) after avg. 2.7 years of follow-up		
< 12.0	controls	Adjusted for: age, sex, smoking, hypertension, cholesterol, diabetes, previous heart attack, previous stroke. Associations were strong for both hemorrhagic and cerebral infarction stroke.
12.0-13.7	61% <sup>NS</sup>	
13.8-15.5	-11% <sup>NS</sup>	
15.6-18.5	38% <sup>NS</sup>	
$\geq$ 18.6	90% <sup>NS</sup>	
Ridker et al. <sup>163</sup> (1999, USA, Women's Health Study) 366 women aged 56 years (average) after a 3 year follow-up For cardiovascular events (stroke, heart attack, etc.):		
< 9.54	controls	After adjustment for baseline differences in other coronary risk factors, each 5 $\mu\text{mol}$ increase in HCY had a SS association with a 24% increase in risk.
9.54-11.19	10% <sup>NS</sup>	
11.20-13.26	20% <sup>NS</sup>	
> 13.26	130% <sup>SS</sup>	
Stehouwer et al. <sup>138</sup> (1998, The Netherlands, Zutphen Elderly Study) 878 men aged 64-84 after a 10 year follow-up		
For heart disease:		
6-12	controls	Adjusted for: age, BMI, BP, cholesterol, diabetes, smoking.
13-16	23% <sup>NS</sup>	
17-97	58% <sup>NS</sup>	
For cerebrovascular disease:		
6-12	controls	Adjusted for: age, BMI, BP, cholesterol, diabetes, smoking.
13-16	23% <sup>NS</sup>	
17-97	58% <sup>NS</sup>	
For cerebrovascular disease among 505 men with normal BP:		
6-16	controls	Adjusted for: age, BMI, BP, cholesterol, diabetes, smoking.
17-97	518% <sup>SS</sup>	
Ubbink et al. <sup>164</sup> (1998, UK, Caerphilly Cohort) 2290 men aged 50-64 after a 5 year follow-up For heart attack:		
avg HCY		Adjusted for: age, social class, BMI, smoking, BP, diabetes, cholesterol, prevalent IHD. The negative association of HCY with B12, folate, and B6 intake was SS. HCY level was determined not to be an independent risk factor for IHD in this study.
11.7	2136 controls	
12.2 <sup>NS</sup>	154 cases	
Bostom et al. <sup>136</sup> (1994, USA, Framingham Study) 1933 people age 59-91 after 10-13 years of follow-up. Death from CVD:		
< 14.26	controls	Adjusted for: age, sex, diabetes, smoking, BP, cholesterol.
> 14.26	52% <sup>SS</sup>	

BMI - body mass index; BP - blood pressure; IHD - ischemic heart disease; NS - not statistically significant; SS - statistically significant.

**Table I.2.2.2 Results of Christen et al.'s<sup>134</sup> Review of Studies on HCY & Heart Disease Pre-September 1998.**

HCY (μmol/l)	Increased risk	Notes
Alfthan et al., 1994; Tromso Study; 460 people age 40-60		
> 95 <sup>th</sup> percentile	30% <sup>NS</sup>	Adjusted for: age, sex.
Arnesen et al., 1995; 600 people age 12-61		
NR	NR	Those developing heart disease had higher HCY (12.7 vs. 11.3). <sup>SS</sup>
Chasen-Taber et al., 1996; 666 people age 40-84		
> 15.8	70% <sup>NS</sup>	Adjusted for: age, smoking, diabetes, angina, aspirin, BP, BMI, cholesterol, follow-up time.
Verhoef et al., 1997; 298 people age 40-84		
> 15.8	10% <sup>NS</sup>	Adjusted for: age, smoking, diabetes, aspirin, BMI, cholesterol, alcohol, fasting time, follow-up time.
Evans et al., 1997; 712 people age 35-57		
> 15.0	-10% <sup>NS</sup>	Adjusted for: age, race, smoking, BP, TG, cholesterol, field clinic, study group.
Wald et al., 1998; 1355 people age 35-64		
≥ 15.17	190% <sup>SS</sup>	Adjusted for: age, BP, apolipoprotein B, duration of storage of blood sample.
Folsom et al., 1998; 769 people age 40-84		
≥ 11.5	30% <sup>NS</sup>	Adjusted for: age, sex, race smoking, diabetes, cholesterol, BP, field center.

BMI - body mass index; BP - blood pressure; NS - not statistically significant; SS - statistically significant; TG - triglycerides.

**Table I.2.2.3 Results of Christen et al.'s<sup>134</sup> Review of Studies on HCY & Cerebrovascular Disease Pre-September 1998.**

HCY (μmol/l)	Increased risk	Notes
Alfthan et al., 1994; 343 people age 40-60		
> 95 <sup>th</sup> percentile	NR	Those developing cerebrovascular disease had a higher HCY (10.3 vs. 9.6). <sup>NS</sup>
Verhoef et al., 1997; 536 people age 40-84		
> 16.6	-20% <sup>NS</sup>	Adjusted for: age, smoking, diabetes, BP, BMI, aspirin, cholesterol, fasting time, follow-up time.
Perry et al., 1995, British Regional Heart Study; 225 people age 40-59		
≥ 15.4	370% <sup>SS</sup>	Adjusted for: age, town, social class, BMI, hypertension, diabetes, smoking, alcohol, cholesterol, packed cell volume, creatinine, forced expiratory volume.

BP - blood pressure; BMI - body mass index; NS - not statistically significant; SS - statistically significant.

**Table I.2.2.4 Moller et al.'s<sup>160</sup> Review: One Study on HCY and Cerebrovascular Disease Not in Christen et al.'s<sup>134</sup>**

HCY (μmol/l)	Increased risk	Notes
Israelsson et al., 1992; 34 men		
> 16.9	821% <sup>NS</sup>	No adjustments noted.
Included studies by Alfthan, Bots, and Verhoef reported above. Moller et al. <sup>160</sup> calculated an overall, SS increased risk of 274% for cerebrovascular disease in those having a HCY level higher than 95% of the people who did not develop the disease.		

NS - not statistically significant; SS - statistically significant.

### I.2.3 Treatment of High Homocysteine with Folate, B12, & B6 in Nonvegetarians

Evans et al.<sup>162</sup> (2000) said, "It is unlikely that further case-control or prospective studies will resolve issues concerning homocysteine as an independent risk factor for coronary artery disease...." They added, "Several groups are proposing secondary prevention trials. The trials (even if successful in reducing CHD) will not prove that homocysteine is a risk factor. It is possible that increased folic acid intake will lower homocysteine concentration and reduce CHD but that the homocysteine/CHD relationship is not casual."

The British Medical Journal published an analysis<sup>143</sup> of 12 studies on the effectiveness of reducing HCY levels with folic acid and vitamin B12 and/or B6 supplements for 3-12 weeks. They concluded that folic acid in the range of 500-5,000 μg/day reduced HCY by 25%, and that B12 supplements (average intake of 500 μg/day) reduced it a further 7%. B12 supplements were also suggested for avoiding the masking of a B12 deficiency with high levels of folate. Vitamin B6 supplements (average of 16.5 mg/day) did not reduce HCY further.

500 μg B12/day is probably more than necessary. In one study reported in the BMJ, only 100 μg B12/day (combined with folate and B6) was successful in reducing HCY from 7.2 to 5.8 μmol/l.<sup>143</sup> In another, only 20 μg B12/day (combined with folate and B6) resulted in reducing HCY from 11.9 to 7.8 μmol/l.<sup>143</sup> There was no evidence that 20 μg/day B12 is not a sufficient amount to recommend.

(Note: Homocysteine levels of people with B6 metabolism problems or deficiencies would possibly benefit from a B6 supplement, and B6 supplements might be necessary for the beneficial effects in the study by Hackam et al.<sup>132</sup> below.)

#### I.2.3.1 Reduced Plaque in Carotid Arteries

Hackam et al.<sup>132</sup> (2000, Canada) treated

**Table I.2.3.1 Results of Hackam et al.<sup>132</sup>**

	HCY ≤ 14	HCY > 14
HCY range (μmol/l)	4.7-13.9	14.3-69.4
duration of vitamin therapy (yrs)	1.9 ± .9	1.8 ± .7
progression before vitamin therapy (cm <sup>2</sup> /year)	.13 ± .24 <sup>A</sup>	.21 ± .41 <sup>B</sup>
progression after vitamin therapy	-.024 ± .29 <sup>A,C</sup>	-.049 ± .24 <sup>B,C</sup>
#	50	51

<sup>A,B</sup>Statistically significant difference between groups with same letters.

<sup>C</sup>Not a statistically significant difference between groups with same letters.

patients with rapidly progressing atherosclerosis who had not responded well to a program of diet, exercise, smoking cessation, or drug treatment for high cholesterol and triglycerides. The treatment consisted of 2.5 mg folic acid, 25 mg of vitamin B6, and 250 µg B12/day. The results are shown in Table I.2.3.1. Vitamin therapy actually reversed the amount of plaque in some of the patients, including some with a HCY level < 14 µmol/l.

### I.2.3.2 Improved Exercise Test, Ankle-brachial Pressure, and Arterial Stenosis

Vermeulen et al.<sup>216</sup> gave patients 5 mg of folic acid and 250 mg of vitamin B6 per day for 2 years. The therapy resulted in reducing homocysteine levels by 50%. Those in the treatment group were about 60% less likely to have an abnormal exercise electrocardiography test (though the statistical significance disappeared after fully adjusting for variables). There were no differences between the treatment and placebo groups in likelihood of having an abnormal ankle-brachial pressure index or in detecting an arterial stenosis.

### I.2.3.3 Improved Endothelial Function

The endothelium is the tissue that lines the blood vessels. Endothelial dysfunction is the first detectable physiological abnormality in patients with atherosclerosis.<sup>218</sup>

Chambers et al.<sup>219</sup> (2000, UK) studied 89 men with CHD. The treatment group received 5 mg of folate and 1,000 µg of B12/day. After 8 weeks, brachial artery dilation had significantly improved in the treatment group. After controlling for many variables, the brachial artery dilation improvement showed an inverse relationship with free plasma homocysteine, while there was no relation to total or protein-bound homocysteine. Thus, free homocysteine may be a more accurate marker for homocysteine damage than is total homocysteine.

Thambyrajah et al.<sup>218</sup> (2001, UK) studied 90 patients with CAD and elevated homocysteine. The treatment group received 500 mg of folate/day for 12 weeks. In the folate group, homocysteine levels fell from 11.7 to 9.3 µmol/l. Endothelial-dependent dilation of the brachial artery improved in both groups, but more so in the treatment group. The difference between the groups was not quite enough to reach statistical significance. The authors said that their study was set up to measure a larger change in dilation, but that this smaller change could possibly have benefits. Other markers of endothelial function, serum nitrate/nitrite and plasma von Willebrand factor, did not improve with therapy. This was in contrast to improvements found in earlier studies on patients without heart disease. The authors speculated that once someone has heart disease their endothelium may be too damaged to respond as well to homocysteine-lowering and/or folate therapy.

Title et al.<sup>220</sup> (2000, Canada) studied 75 patients with CHD and homocysteine > 9 µmol/l. There were three groups:

- Group 1: 5 mg of folic acid/day
- Group 2: 5 mg of folic acid, 2 g of vitamin C, 800 IU of vitamin E/day
- Group 3: Placebo.

After 4 months, brachial artery dilation in Group 1 had significantly improved and the improvement correlated to a reduction in homocysteine levels. There was a trend toward improvement in Group 2, but it was not statistically significant. Side effects (abdominal cramps, diarrhea, and rash) were noted in one patient in Group 1, one in Group 2, and two in Group 3. The degree of improvement in Group 1 was similar to improvements seen with drug therapy using statins and ACE (angiotensin-converting enzyme) inhibitors. The authors speculate that the improvement was due partly to folate's reducing homocysteine and partly due to a direct effect of folate on the endothelium.

## I.2.4 Elevated sB12 as an Increased Risk Factor for Disease

Two studies have shown an increased risk of mortality or CHD in subjects with elevated sB12:

1. Pancharuniti et al.<sup>182</sup> (1996) performed a retrospective study in which sB12 levels did not differ significantly between those with CAD and matched controls. However after controlling for homocysteine levels and other CAD risk factors, there was a barely significant association between sB12 and increased risk of CHD (rr = 1.5; CI: 1.0, 1.8). The authors thought this could be due to the fact that B12 is found in foods that increase one's risk of heart disease such as red meats. Folate was linked more with higher HCY in this study than was sB12 – as is the case for most studies on a nonvegetarian population.
2. Zeitlin et al.<sup>207</sup> (1997) followed 488 people aged 75-85 for an average of 6 years. Average sB12 levels were 515 pg/ml. In measuring death, stroke, heart attack, coronary heart disease, and cardiovascular disease events, there was no significant difference in sB12 between those who did (569 ± 479 pg/ml) and did not (491 ± 267 pg/ml) have an event. However, each increase of 1 pg/ml in sB12 was associated with a .07% increase in risk of death (P = .041). This means that someone with a 100 pg/ml higher sB12 has a 7% higher chance of death. Zeitlin et al. suggest that the higher risk of death could be because B12 is a marker for animal product intake, and not from B12 itself. But, they also speculate that high B12 stores could cause liver damage, contributing to death. This is purely conjecture as there are no reports of liver toxicity due to high B12 stores.

The two studies above showed only a small increased risk with higher sB12. As reported throughout this article, most studies have shown B12 levels on the higher end of normal to be healthy. Thus, the overwhelming evidence is that high B12 levels do not cause disease.

### I.3 B12, Homocysteine, & Alzheimer’s Disease

Approximately 6-8% of all people older than 65 have Alzheimer’s Disease (AD).<sup>113</sup> At least two recent literature reviews<sup>113,140</sup> and a letter to the editor<sup>115</sup> note a connection between elevated HCY levels, elevated MMA levels, reduced B12 and folate status, and Alzheimer’s Disease. Possible causes are:

- B12 deficiency may lead to reduced availability of methyl groups needed for the metabolism of myelin (which protects nerve tissue and assists in nerve impulse conduction), neurotransmitters (e.g., acetylcholine), and membrane phospholipids.
- B12 deficiency can lead to increased HCY levels which may damage blood vessels in the brain and/or nerve cells.

Recently, Seshadri et al.<sup>180</sup> (2002, USA) published a prospective study of 1092 elderly people in which a 5 µmol/l increase in HCY increased the risk of AD by 40%. However, the data is somewhat mixed,<sup>115,167</sup> and in some cases B12-deficient dementia may be misdiagnosed as AD.<sup>168</sup>

### I.4 B12, Folate, Homocysteine, & Age-Related Hearing Loss

*Age-related hearing loss* is hearing loss that occurs with age and with no other known cause. There have been two studies on B12 and age-related hearing loss.

In a cross-sectional study of 55 women aged 60-71, Houston et al.<sup>169</sup> (1999, USA) showed that women with age-related hearing loss had lower sB12 and red blood cell folate levels, as well as lower dietary intakes than those without hearing loss (Table I.4.1). However, because red blood cell folate and sB12 were highly correlated, it was not possible to determine which nutrient, if either, had more of an effect. As for vitamin intake, low folate intake was more associated with hearing loss than B12 intake. The authors suggested this might be because B12 malabsorption might have affected the results.

**Table I.4.1 Results of Houston et al.<sup>169</sup>**

	Normal hearing	Impaired hearing
number	38-44	10-11
sB12	513 ± 208 <sup>A</sup>	319 ± 203 <sup>A</sup>
B12 intake from food	4.2 ± 2.3 <sup>B</sup>	2.4 ± 1.3 <sup>B</sup>
B12 intake from food and supplements	40 ± 156 <sup>C</sup>	9.1 ± 15 <sup>C</sup>

<sup>A</sup>Statistically significant difference between groups with same letters after adjustment for age. <sup>B</sup>P-value = .05 after adjustment for calories (i.e., on the border of statistical significance). <sup>C</sup> Not statistically significant after adjustment for age.

**Table I.4.2 sB12 & Supplementation in Houston et al.**

	#	sB12
Taking supplements	12	674 <sup>A</sup>
Not taking supplements	6	500

<sup>A</sup> Calculations based on information provided in the study.

Through my own calculations, I was able to determine the sB12 levels of those taking vitamins and compare them to the levels of those who did not. See Table I.4.2.

A second cross-sectional study looking at 35 males and 56 females, aged 67-88 (Berner et al.<sup>170</sup> 2000, Denmark), found age-related hearing loss was not associated with B12 or folate levels. A weak association was found with HCY levels and age-related hearing loss. The study did not include any people without some age-related

hearing loss for comparison, and B12 *intakes* were not examined. The sB12 levels in this study were 320 (R 107-1566) and HCY levels were 11.1 (7.0-33.7).

Based on these results, it can be tentatively concluded that an impaired B12 status may contribute to age-related hearing loss.

### I.5 B12, Folate, Homocysteine, & Birth Defects

Folic acid intake in the weeks before and after conception has been shown to decrease the number of neural tube defects (NTD), such as spina bifida, in at least four studies.<sup>141</sup> The basis for this is not yet completely clear. It could be that when HCY cannot be converted back into methionine, there is not enough methionine available for normal closure of the neural tube. Some evidence for this is a case-control study in which women with the lowest methionine intakes had a higher rate of having a baby with a NTD (although this study was not conclusive).<sup>165</sup> While the focus has been primarily on folate, there has been rising interest in B12’s part in preventing NTDs.

In a prospective study, Kirke et al.<sup>142</sup> (1993, Ireland) studied folate and B12 in pregnant women, and the development of NTDs in their infants. There was a statistically significant difference between sB12 levels of women who had an infant with a NTD and those who did not (results in Table I.5.1). The average difference was not large (53 pg/ml).

**Table I.5.1 Results of Kirke et al.<sup>142</sup>**

	#	sB12
Mothers with NTDs	81	243 <sup>A</sup> (R 102-759)
Controls	247	296 <sup>A</sup> (R 88-757)

<sup>A</sup>Statistically significant difference between groups with the same letters.

**Table I.5.2 Results of Wald et al.<sup>181</sup>**

sB12	Increased risk of NTD	
	Highest 3 <sup>rd</sup> of serum folate	Lowest 3 <sup>rd</sup> of serum folate
< 239	20%	350% <sup>SS</sup>
239-368	0	150%
≥ 369	-20%	110%

SS - statistically significant. Statistical significance for other numbers was not reported.

Afman et al.<sup>217</sup> (2001, The Netherlands) found that low B12-TC2 levels increased the risk of having a baby with a NTD.

Wald et al.<sup>181</sup> (1996, UK) studied 135 women, 27 of whom had a baby with a NTD. The women who had children with NTDs had sB12 levels an average of 38 pg/ml lower in the first trimester than controls. However, after adjusting for folic acid levels, there was no independent association for B12 and NTDs (results in Table I.5.2).

Bronstrup et al.<sup>144</sup> (1998, Germany) studied 150 nonvegetarian women aged 20-34. HCY levels were negatively correlated with plasma folate and B12, but not with vitamin B6. The subjects were divided into 3 groups and vitamin treatment was given for 4 weeks (results in Table I.5.3). They found that at higher initial HCY levels and lower initial serum folate levels, the reductions in HCY were greater from the treatment.

Considering Bronstrup<sup>144</sup> and the BMJ<sup>143</sup> (see section I.2.3 *Treatment of High Homocysteine with Folate, B12, & B6 in Nonvegetarians*), it appears that somewhere between 6 and 20 µg should be adequate for reducing HCY levels for most people who start off with relatively

Group	#	Treatment (per day)	Pre-treatment HCY	Post-treatment HCY	Pre-treatment sB12	Post-treatment sB12	Change in sB12
A	51	400 µg folic acid	8.1 ± 2.1	7.2 ± 1.6 <sup>A</sup>	339 ± 138	350 ± 140	+ 11
B	49	400 µg folic acid, 6 µg B12	8.2 ± 2.4	6.8 ± 1.4 <sup>B</sup>	423 ± 150	501 ± 169	+ 78
C	50	400 µg folic acid, 400 µg B12	8.1 ± 1.9	6.6 ± 1.1 <sup>A</sup>	358 ± 147	549 ± 213	+ 191

<sup>A</sup>Statistically significant difference between groups with same letters. <sup>B</sup>Statistically significant difference from Group A when adjusted for pre-treatment HCY and serum folate levels.

normal sB12 and a ~ 3.5-5 µg daily intake from their diet (based on averages for nonvegetarians in the USA, see Table 3.4). Interestingly, among vegan mothers who were not supplementing with B12 during pregnancy, none on record had a baby with a NTD, despite other severe B12-related problems occurring after birth in their infants.

Conclusion: Ensuring adequate B12 status in the weeks before and after conception may reduce the chances of NTDs.

#### I.6 Homocysteine and Recurrent Early Pregnancy Loss

A number of retrospective, case-control studies have been performed looking at elevated homocysteine (HCY) levels and recurrent first trimester pregnancy loss. Nelen et al.<sup>166</sup> (2000) conducted a meta-analysis of the studies performed from 1992 to 1999. They found that women with elevated HCY levels (fasting levels > 10-18.3) had a statistically significant, 170% greater chance of two or more pregnancy losses in the first trimester. They were not able to determine whether elevated HCY is a marker or a cause of disease. No clinical intervention studies have been conducted as of this writing.

## I.7 Homocysteine and Mortality

In reviewing the data below on elevated HCY as an independent risk factor for mortality, I have used only prospective studies (or case-control studies nested within prospective studies), which are the most relevant studies for answering this question. Only studies of people who started out without pre-existing disease are included. There appears to be a strong, statistically significant correlation between HCY and mortality, after adjustments, in homocysteine levels greater than 12-15  $\mu\text{mol/l}$  when compared to lower levels (Table I.7.1).

**Table I.7.1 Homocysteine and Mortality.**

HCY $\mu\text{mol/l}$	Increased mortality rate	Notes
Vollset et al. <sup>131</sup> (2001, Norway, Hordaland Homocysteine Study) 4766 people age 65-67 after a median of 4.1 years follow-up		
5.1-8.9	controls	For those with no history of a heart attack, stroke, angina, diabetes, or high blood pressure treatment; and adjusted for total cholesterol, blood pressure, smoking, body mass index, physical activity, age, and gender. After adjustments there was a 49% increase in mortality for every 5 $\mu\text{mol/l}$ increase in HCY. This included a statistically significant ~ 47% increase in heart disease and a 76% increase in cerebrovascular disease.
9.0-11.9	9% <sup>NS</sup>	
12.0-14.9	63% <sup>NS</sup>	
15.0-19.9	110% <sup>SS</sup>	
20.0-137	164% <sup>SS</sup>	
Hoogveen et al. <sup>137</sup> (2000; The Netherlands, Hoorn Study) 811 people age 50-70 (at entry) after 5 years follow-up		
< 14.0	controls	In subjects without Type II diabetes. However, it appears from data given that if subjects with > 14 were compared to subjects with < 10, there would have been a SS difference.
> 14.0	34% <sup>NS</sup>	
Kark et al. <sup>139</sup> (1999, Israel, Kiryat Yovel Community Health Study) 1788 people age $\geq 50$ after 9-11 years follow-up		
$\leq 8.52$	controls	Adjusted for: age, BP, serum glucose, health status, serum creatinine. A SS association between plasma homocysteine and increased risk of mortality was found for all deaths, CVD, CHD, and all other causes, but not for cancer.
8.53-10.11	42% <sup>NS</sup>	
10.12-11.89	28% <sup>NS</sup>	
11.90-14.69	53% <sup>SS</sup>	
$\geq 14.7$	97% <sup>SS</sup>	
Bostom et al. <sup>136</sup> (1994, USA, Framingham Study) 1933 people age 59-91 after 10-13 years follow-up		
< 14.26	controls	Adjusted for: age, sex, diabetes, smoking, BP, cholesterol
> 14.26	54% <sup>SS</sup>	

BP – blood pressure; NS – not statistically significant; SS – statistically significant.

## I.8 Homocysteine & Kidney Disease

Homocysteine levels are often highly elevated in those with kidney disease. See Appendix J for more information.

## I.9 Low Methionine Intakes May Exacerbate High Homocysteine Levels in B12-Depleted States

Some studies have shown vegetarians to have lower methionine intakes than nonvegetarians (see Table I.9). There is a hypothesis that lower methionine intakes combined with B12 deficiency can increase HCY levels by slowing down the HCY to cystathione pathway (see Figure 3). This has not been fully studied and the evidence appears mixed.<sup>149</sup>

**Table I.9 Methionine Intake (g/day) in Various Diets**

	NV	LOV	Vegans
Krajcovicova-Kudlackova et al. <sup>125</sup> 2000, Slovak Republic	1.46 $\pm$ .32	.9 $\pm$ .02	.77 $\pm$ .03
Hung et al. <sup>172</sup> 2002, Taiwan	1.3	1.1 (6 vegans, 39 LV)	

LOV - lacto-ovo vegetarians; LV - lacto vegetarians; NV - nonvegetarians

## I.10 One Week of Vegan Diet (with B12) & Other Lifestyle Changes Lower Homocysteine

DeRose et al.<sup>120</sup> (2000, Oklahoma) placed 40 people with various diseases (heart disease, diabetes, hypertension, high cholesterol) on a vegan-diet-based lifestyle program for 1 week. The program included:

- No B vitamin supplements. However, the subjects had an average intake of .4  $\mu\text{g}$  of B12/day. Personal communication with the author verified that this was from fortified foods.
- Moderate physical exercise.
- Stress management and spirituality enhancement sessions.
- No caffeine, alcohol, or tobacco.

HCY levels fell 13%, from 8.66  $\pm$  2.7 to 7.53  $\pm$  2.12  $\mu\text{mol/l}$ . The researchers estimated that the subjects' folate intakes had gone from 301  $\mu\text{g}$  for men and 226  $\mu\text{g}$  for women (based on typical U.S. intakes) to 480  $\mu\text{g}$ . The researchers did not think this could explain the entire HCY reduction. Other aspects of the diet and lifestyle changes might explain the remaining reduction.

Please note that this study was not conducted on vegans and was only one week in duration. B12 intakes that low (.4 µg) or lower cannot be expected to keep HCY levels in check for long periods of time.

## Appendix J. Homocysteine & Kidney Disease

Homocysteine can be greatly elevated for people on dialysis or with a kidney transplant (typical average amounts range from 20-60 µmol/l).<sup>146</sup> Thus far, there have been no studies examining whether reducing homocysteine in these patients can reduce cardiovascular problems. Shemin et al.<sup>146</sup> report that, for this reason, the American Heart Association and the American Society of Nephrology consider screening and treatment of elevated HCY to be inappropriate. However, if I had kidney disease, the mere lack of direct evidence would not be comforting.

Unfortunately, to date, very high doses of folic acid (1,000-60,000 µg/day), B6 (50-110 mg/day), and B12 (12-1,000 µg/day) have not succeeded in normalizing homocysteine levels to 12 µmol/l in kidney disease patients. However, they have often succeeded in reducing homocysteine to 16-20 µmol/l which could be worthwhile. Note: vitamin B6 is sometimes toxic at very high doses for long periods of time.

Because people with kidney disease can have impaired cyanide metabolism or clearance, a non-cyanocobalamin form of B12 is preferable. Anyone with kidney disease who is concerned about homocysteine can suggest the review by Shemin et al.<sup>146</sup> to their dietitian and/or doctor.

## Appendix K. Ways to Get B12 Deficiency

(Reference: Herbert<sup>48</sup> (1994) where not otherwise noted.)

1. Inadequate dietary intake.
2. Inadequate absorption:
  - Loss of intrinsic factor: Genetically predetermined and age-dependent (sometimes as early as 45 yrs). The most common cause of B12 deficiency in nonvegetarians.
  - Autoimmunity to intrinsic factor: Circulating antibodies to intrinsic factor indicate eventual pernicious anemia if not treated. A chronic B12 deficiency damages immune function and the antibodies may disappear as B12 deficiency progresses.
  - Loss of gastric acid and/or protein digesting enzymes which break the protein-B12 bonds in food. This can be caused by stomach surgery, atrophy or inflammation of the stomach, medications that suppress acid secretion, or a stomach infection by *H. pylori* or anaerobic bacteria (which can be due to low stomach acid).<sup>49</sup>
  - Pancreatic disease reduces free calcium in the ileum (calcium is needed for B12 absorption). Can be improved with calcium and/or bicarbonate.
  - Unhealthy ileum.
  - Drugs decreasing absorption include cimetidine, metformin (ties up free calcium in intestines), potassium chloride, and cholestyramine.<sup>98</sup>
  - Infection by tapeworms,<sup>35</sup> *Diphyllobothrium latum*,<sup>117</sup> or *Giardia lamblia*.<sup>98</sup>
3. Defects in B12 enzymes, transport proteins, or storage proteins.
  - Defects in B12 enzymes that result in high MMA levels are rare. One researcher reported that they occur in 1 out of 28,000 people.<sup>179</sup> Ciani et al.<sup>179</sup> provide an informative diagram showing which enzymes are involved in the various B12 metabolic pathway defects.
4. Increased requirement during pregnancy or hyperthyroidism.
5. Increased excretion caused by alcoholism.
6. Nitrous oxide anesthesia in people with low sB12 (nitrous oxide can change the cobalt atom of B12).<sup>35</sup>
7. Hypothyroidism (possibly autoimmune).<sup>53</sup>
8. AIDS can cause B12 deficiency as shown through macrocytic anemia and neurological problems without elevated homocysteine levels.

### Insert K.1 Short-term Nitrous Oxide Causes Neurological Problems in a Vegetarian<sup>221</sup>

A 50 y.o. vegetarian woman eating mostly apples, nuts, and raw vegetables underwent nitrous oxide anesthesia for a hip fracture after injuring herself ice-skating. She was ventilated with nitrous oxide for 2 hours. Four weeks later, she developed rapidly increasing sensory impairment of the legs. Six weeks later, she could not walk. She was diagnosed with neurological degeneration secondary to B12 deficiency. After 5 months of treatment with cyanocobalamin injections, she could walk on crutches. At one year later, she had continued to improve. Vegetarians should make sure their B12 status is healthy before undergoing nitrous oxide anesthesia.

## Appendix L. Formulation of Recommendations

### L.1 Step 1.

Step 1 states:

If you have not had a regular source of B12 for some time, buy a bottle of sublingual B12. Place 2,000 µg under your tongue until the tablet(s) has dissolved, once a day, for 2 weeks. Then follow the advice under Step #2. (Note: you can break the remaining tablets in half or quarters for Step #2. It's okay to take more than recommended.)

These recommendations about how to replenish one's B12 stores are based on the success that people with B12 malabsorption problems have had with oral B12 supplements.

#### L.1.1 Oral B12 for People with Malabsorption

Intramuscular injections (IMI) of B12 are the typical way to treat B12 deficiency. The injections can be painful and expensive. Norberg<sup>75</sup> (1999, Sweden) points out that investigations in the 1950s and 60s showed that oral B12 is absorbed by an alternative pathway not dependent on intrinsic factor or an intact ileum. Approximately 1% of an oral dose in the range of 200-2000 µg/day was absorbed by the alternative pathway in those investigations. Based on this research, oral treatment, rather than IMI, has been in use for the majority of B12 deficiency cases in Sweden since the early 70s.

In a literature review encouraging the use of oral B12 therapy over injections for patients with pernicious anemia, Lederle<sup>58</sup> (1991, USA) reported that Swedish investigators recommend 2000 µg of oral B12 twice a day or injections to replenish B12 stores. After initial therapy, doses of 1000 µg/day appear to be enough.<sup>58</sup>

Kuzminski et al.<sup>56</sup> (1998, USA) studied 33 newly diagnosed B12-deficient patients (almost all had malabsorption) who received cyanocobalamin as either 1 mg intramuscularly on days 1, 3, 7, 10, 14, 21, 30, 60, and 90; or 2000 µg orally on a daily basis for 120 days (4 months). Results are in Table L.1.1. Kuzminski et al. conclude that 2000 µg/day of oral cyanocobalamin was as effective as 1000 µg injected intramuscularly each month, and may be superior.

Delpre & Stark<sup>28</sup> (1999, Israel) studied patients with B12 deficiency to see if B12 can be absorbed by holding a tablet under the tongue, known as sublingual. The theory behind sublingual is that the mucous membranes under the tongue are efficient at absorbing certain molecules, particularly if combined with something fat soluble such as a cyclodextrin (see: <http://www.nutrition.cornell.edu/nutriquest/sublingu.html>). 5 patients had pernicious anemia, 7 were vegetarians, and 2 had Crohn's disease (which can prevent the absorption of B12 in the ileum). The patients held two 1000 µg B12 tablets (equaling 2,000 µg/day), made by Solgar, under their tongues for 30 minutes until completely dissolved. This was done for 7 to 12 days. Average sB12 levels went from 127.9 ± 42.6 to 515.7 ± 235. All patients' sB12 normalized. There were no side effects and all patients preferred this to injections. Unfortunately, Delpre & Stark did not include a control group who chewed the B12 tablets, so there is no way to know if taking the tablets sublingually was more effective than chewing and swallowing them. On the basis of Kuzminski et al. above, swallowing seems to be as effective if done for 3 months.

Please note that the large doses mentioned in this section are for people with B12 malabsorption (or vegans who have neglected their B12 intake for a few months). People without malabsorption problems or current B12 deficiency do not need such large doses; hence Step 2 of the recommendations.

### L.2 Step 2 Recommendations.

#### L.2.1 Absorption Rates of B12

Russell et al.<sup>130</sup> (2001, Boston, MA) measured B12 absorption rates from milk and bread fortified with .1 µg B12, in healthy people who were over age 60 and had normal stomach acid (results are in Table L.2.1).

**Table L.1.1 Results of Kuzminski et al.**

	sB12	serum MMA µmol/l	HCY µmol/l
Pretreatment			
oral	93	3.850	37.2
injection	95	3.630	40.0
After 4 months			
oral 2000 µg/day	1005 <sup>A</sup>	.169 <sup>B</sup>	10.6
injection	325 <sup>A</sup>	.265 <sup>B</sup>	12.2

<sup>A,B</sup>Statistically significant difference between groups with same letters.

**Table L.2.1 B12 Absorption Rates in Various Foods**

Food / Oral Dose	% absorbed	µg absorbed (avg) <sup>C</sup>
fortified milk	65% <sup>A</sup>	
fortified bread	55%	
mutton <sup>130</sup>	65%	
chicken <sup>130</sup>	60%	
trout <sup>130</sup>	39%	
eggs <sup>130</sup>	24-36%	
liver <sup>206</sup>	11%	
.1 µg <sup>208</sup>	77%	.08
.25 µg <sup>208</sup>	75%	.19
.5 µg <sup>208,209</sup>	71-90%	.35-.45 (.4)
.6 µg <sup>208</sup>	63%	.38
1 µg <sup>206,208,209,211</sup>	50-80%	.5-.8 (.6)
2 <sup>208,209,211</sup>	40-50%	.8-1 (.9)
5 µg <sup>206,208,209,210,211</sup>	20-55%	1-2.8 (1.7)
10 <sup>208,211</sup>	15-16%	1.5-1.6
25 µg <sup>206</sup>	5%	1.25
50 <sup>209,211</sup>	3%	1.5 (1.5)
1,000 µg <sup>206,209</sup>	1-1.5% <sup>B</sup>	10-15 (12.5)

<sup>A</sup>Number represents only the fortified portion of B12 in the milk. <sup>B</sup>Even without intrinsic factor. <sup>C</sup>Average of all measurements (i.e., not the mid-range).



Absorption rates of different doses of cyanocobalamin have also been measured and appear in Table L.2.1. The second of two doses given 4-6 hours apart is absorbed as well as the first.<sup>206</sup>

Absorption of non-protein-bound B12 does not appear to decrease with age.<sup>206</sup>

If the circulating B12 exceeds the binding capacity of the blood, the excess is excreted in the urine. This normally happens only after a B12 injection.<sup>206</sup>

Various studies have indicated that .1-.2% of the body's B12 pool is lost per day; the .2% loss occurs in those with pernicious anemia.<sup>206</sup>

A 5 µg supplemental dose of cyanocobalamin appears to saturate the intrinsic factor absorption mechanism. Thus, B12 absorption drops to 1-1.5% for any additional B12 ingested above 5 µg.

There appears to be no published research comparing sublingual B12 absorption rates to oral (swallowing). It is possible that B12 is better absorbed when taken sublingually, but until research is published, we should not assume this. Thus, the recommendations given in this article are based on absorption rates of B12 that is swallowed.

### L.2.2 What Is a Healthy B12 Level?

Assuming that absorption is good, meeting the RDA of 2.4 µg for adults should keep macrocytic anemia from occurring. In 4 Indian vegetarians with B12-deficient anemia, .1-.25 µg of B12 (through the diet) was not enough to correct anemia, while .3-.65 was enough.<sup>212</sup> Not all patients, however, require so little. Studies have shown that 1.5 µg/day of injected B12 covers the needs of almost all patients with pernicious anemia.<sup>206</sup> In fact, the RDA for B12 intake for adults is based on this number.<sup>206</sup> However, I'm not convinced that meeting the RDA for B12 is enough to keep everyone's homocysteine (HCY) and methylmalonic acid levels in check, and to minimize DNA damage and nerve problems. Consider these findings:

- Selhub et al.<sup>145</sup> analyzed data from 8083 people, including white, blacks, and Hispanics, and found that elevated homocysteine levels (> 11.4 for men, > 10.4 µmol/l for women) were associated with sB12 levels less than 338 pg/ml.
- Fenech<sup>187</sup> studied folate and B12 levels and intake in respect to DNA damage in white blood cells (lymphocytes) which, according to Fenech, has been shown to be a good marker for future cancer. They found that sB12 > 405 pg/ml and a supplemental intake of 7 µg of B12/day were optimal for reducing DNA damage. Fenech says that folate and B12 intakes 3.5 times higher than the U.S. RDA minimize DNA damage. However, Fenech did not measure B12 intake from food, so not enough information was provided to determine the amount of B12 absorbed. It can be assumed that about 1.5 µg was absorbed from the 7 µg supplement; thus, more than 1.5 µg must have been absorbed per day (given that the subjects also had a normal dietary intake of B12) in order to minimize DNA damage.

- Lindenbaum et al.<sup>184</sup> studied MMA and HCY levels in 548 elderly people from the Framingham Heart Study (Table L.2.2). The authors chose a sB12 level of 350 pg/ml as the lower limit for suspecting a B12 deficiency because so many subjects with levels below this limit had elevated levels of sMMA.

Note: In light of recent evidence, a lower limit of .60 µmol/l for sMMA may be more descriptive of a deficiency, especially in elderly people (see Appendix B). Regardless, a sB12 of < 350 could indicate problems are starting to occur, especially if intake is zero.

Based on the above research, sB12 levels 338-405 seem necessary to limit damage.

### L.2.3 What Intake is Needed to Achieve a Healthy Level?

Tucker et al.<sup>98</sup> (2000, USA) examined the B12 status of 2999 subjects in the Framingham Offspring Study. sB12 was associated with B12 intake. Based on Figure 1 in Tucker et al.,<sup>98</sup> 2 µg from foods (at a 50% absorption rate) should keep one's sB12 levels at about 350 pg/ml. An intake of about 5 µg from foods (at a 50% absorption rate) keeps sB12 levels at about 430 pg/ml, a level at which people rarely, if ever, have B12-related problems (and which covers Fenech's findings on DNA damage). This translates into 2.5 µg/day being absorbed.

**Table L.2.2 Results of Lindenbaum et al.**

	#	# w/ MMA > .376 µmol/l	MMA > .376 µmol/l and HCY > 21.3 µmol/l	% taking B12 supplement <sup>B</sup> (reporting)
sB12 < 350	222	62 (28%)	21 (9%)	12% (160)
sB12 > 350	326	20 (6%) <sup>A</sup>	4 (1%)	40% (241)

<sup>A</sup>At least 10 subjects had elevated MMA due to poor kidney function, putting the number closer to 10 (3%). <sup>B</sup>Median amount of B12 in supplements was 6 µg.

**Table L.2.4.1 sB12 Levels from B12 (µg) provided from food/supplements.**

sB12 pg/ml	intake/day <sup>F</sup>	absorbed per day	Amount needed from:			
			fortified foods	1 daily SUP	1 SUP, 2x daily <sup>A</sup>	1 weekly SUP <sup>C,D</sup>
~ 350	2	1	2	2-5 <sup>E</sup>	2	700
~ 430	5	2.5	5	100	4-5 <sup>E</sup>	1700
Other <sup>B</sup>			3	10		2000

<sup>A</sup>Spaced 6 hours apart. <sup>B</sup>Minimum recommended amounts by vegan health professionals in *What Every Vegan Should Know about Vitamin B12*. <sup>C</sup>Chewed or sublingual. <sup>D</sup>Based on 1% absorption rate. <sup>E</sup>Absorption rates in doses of between 2 and 5 µg are not known. SUP - cyanocobalamin supplement <sup>F</sup>Extrapolated from Tucker et al.<sup>98</sup>

## L.2.4 Recommendations

Based on absorption rates listed in Table L.2.1, Table L.2.4.1 lists the amounts of B12 needed from fortified foods and supplements to match the intakes extrapolated from Tucker et al.<sup>98</sup>

A weekly supplement is the least tested of these methods. For this reason, I am more in favor of the fortified foods and daily supplement options. 2 daily supplements of 5 µg, spaced at least 6 hours apart, should result in an absorption of at least 2.5 µg per day which should keep sB12 levels above 400 pg/ml.

In *What Every Vegan Should Know about Vitamin B12* (<http://veganoutreach.org/health/b12letter.html>), minimum recommendations are given for vegans. As can be seen from Table L.2.4.1, these recommendations should put sB12 levels between 350 and 430 pg/ml. Thus, for consistency with other vegan health professionals, I have used them as minimum amounts.

Some people may wonder what levels are ideal, rather than minimal (assuming one does not suffer from intrinsic-factor-related problems). Thus I have suggested a range above which should provide no further benefit and below which there is evidence of at least modest adverse effects. My final recommendations to keep sB12 levels between 350-430 pg/ml are:

From fortified foods:	3-5 µg
From 1 daily supplement:	10-100 µg
From 2 daily supplements spaced at least 6 hours apart:	5 µg

## Appendix M. People Who Should Not Take the Cyanocobalamin Form of B12

### M.1 Cyanide Metabolism Defects

Cobalamin has a strong attraction to cyanide. While being a natural chemical produced in the body, cyanide is toxic, and the body turns it into thiocyanate in order to excrete it. If this pathway is defective or overwhelmed through ingestion of too much cyanide (such as in smokers, or people in Nigeria who eat large amounts of cassava which is high in cyanide), the body may detoxify the cyanide by attaching it to cobalamin and then excreting the cyanocobalamin. Leber's optic atrophy, tobacco-alcohol amblyopia, and other eye diseases can sometimes respond to high doses of hydroxocobalamin which serve to detoxify the cyanide. In these cases, there may be too much cyanide in the tissues (preventing conversion of cyanocobalamin to methylcobalamin or adenosylcobalamin) for cyanocobalamin supplements to be effective in maintaining B12 status.<sup>52</sup> In such cases, a different form of B12 should be given (speak to your health professional).<sup>206</sup>

### M.2 Chronic Kidney Failure

People with chronic kidney failure do not detoxify cyanide as well as people with healthy kidneys. It is thought that this may lead to nerve problems, especially in smokers.<sup>196</sup> For this reason it is better for kidney patients to take a form of B12 other than cyanocobalamin. Koyama et al.<sup>196</sup> suggest that patients on hemodialysis receive 500 µg of methylcobalamin intravenously after each dialysis. Vegans with kidney disease, whether or not they are on dialysis, should take a non-cyanocobalamin form of B12. These patients should talk to their health professionals about how much should be taken. 1,000 µg (1 mg) of methylcobalamin or adenosylcobalamin per day might be adequate.

## Appendix N. Non-cyanocobalamin B12 Supplements

### N.1 Methylcobalamin in a Small Sample of Vegans

Some researchers question whether these supplements are stable in their oral form. For this reason, much larger amounts are typically used with the hope being that at least some are absorbed intact. One study suggests that once absorbed, methylcobalamin may be retained in the body better than cyanocobalamin.<sup>30</sup>

Donaldson<sup>121</sup> (2000, USA) studied 3 vegans with elevated uMMA levels who were treated with 1/2 to 1 sublingual methylcobalamin tablet (from Enzymatic Therapy, Green Bay, WI. <http://www.enzy.com/products/display.asp?id=261&cpmid=293>), 2x/day for 3 weeks. Correspondence with the author (March 21, 2002) verified that these tablets contain 1,000 µg methylcobalamin each. Two of the subjects' uMMA normalized while the remaining subject's uMMA stayed slightly elevated at 4.1 µg/mg creatinine (normal: < 4.0 µg/mg creatinine). Thus, at a rate of 1-2,000 µg/day, methylcobalamin appears to be absorbed at a high enough rate to improve B12 status in some vegans. Additionally, this indicates that the methylcobalamin was able to improve the MMA pathway which requires adenosylcobalamin.

### Table L.2.4.2 Absorption rate extrapolations.

At a 5 µg supplement, about 1.5 µg is absorbed.

By adding 1.5 µg absorbed from the first 5 µg of a cyanocobalamin supplement, plus .1 µg (.01 \* 10 µg; absorption rate of 1%) for each additional 10 µg increment, we can compile enough B12 to equal 2.5 µg absorbed:

To obtain 2.5 µg absorbed from 1 supplement:

$$2.5 \mu\text{g} - 1.5 \mu\text{g} = 1.0 \mu\text{g}$$

$$1.0 \mu\text{g} / .1 \mu\text{g} = 10$$

$$10 * 10 \mu\text{g} = 100 \mu\text{g}$$

$$100 \mu\text{g} + 5 \mu\text{g} = 105 \mu\text{g}$$

Because 100 µg translates into 2.4 µg absorbed, which is almost the same as 2.5 µg, 105 µg can be rounded down to 100 µg.

## N.2 Therapeutic Use of Coenzyme Forms of B12

The coenzyme forms of B12 appear to be more effective in treating certain conditions than is cyanocobalamin (though they are often injected rather than taken orally). Kelly<sup>150</sup> (1997) reviewed the research on supplementing with the coenzyme forms of B12 (methylcobalamin and adenosylcobalamin). Results of studies performed on humans are listed in Table N.2.

### N.3 Hydroxocobalamin

Hydroxocobalamin is the form of B12 typically found in food. There are not many oral forms for people to take; it is normally injected. One study suggests that after injections, it is retained in the body better than cyanocobalamin.<sup>72</sup>

### N.4 SAME

Figure 3 shows that S-adenosylmethionine (SAM, aka SAME) is in the homocysteine/methionine pathway. SAM has been used in the treatment of liver disease, neurological disorders, rheumatoid arthritis,<sup>192</sup> and fibromyalgia.<sup>193,194</sup> Houston et al.<sup>169</sup> noted that SAM is depleted in B12 deficiency. Loehrer et al.<sup>192</sup> reported that SAM levels are low in a significant proportion of coronary artery disease patients. Kelly<sup>150</sup> suggests that SAM is involved in converting cyanocobalamin to methylcobalamin.

Loehrer et al.<sup>192</sup> showed that 400 mg of SAM (in the form of S-adenosylcobalamin bis(sulfate)-*p*-toluenesulfonate) taken orally after an overnight fast (with the fast continuing for 6 more hours), caused serum SAM levels to increase substantially, indicating that SAM is absorbed when taken orally. It was well tolerated by all 14 volunteers. Not enough research has been done to determine whether vegans who have depleted their stores of B12 can benefit from supplementing with SAM in addition to B12. Looking at the biochemical pathways, it would appear that if homocysteine levels are high (which would be expected in B12 deficiency) and folate is adequate, supplementing with B12 would be enough to replenish SAM stores. However, if supplementing with B12 does not resolve symptoms, someone might consider discussing SAM supplementation with their physician.

## Appendix O. Smokers and Cyanocobalamin

In one study, smokers were found to excrete 35% more B12 than nonsmokers.<sup>206</sup> In another study, smokers' sB12 did not differ from nonsmokers', and the Institute of Medicine concluded that "The effect of smoking on the B12 requirement thus appears to be negligible."<sup>206</sup>

Hydroxocobalamin injections decreased blood cyanide levels by 59% in smokers (1.5-3 packs/day); cyanide was eliminated in the urine as cyanocobalamin.<sup>215</sup> This indicates that cyanocobalamin may be actively excreted rather than used in people with elevated cyanide levels. Most smokers have an intake of hydroxocobalamin, and other non-cyanocobalamin forms of B12, through animal foods, which can counteract their bodies excretion of cyanocobalamin. However, unless vegans take a non-cyanocobalamin supplement, they do not have a non-cyanocobalamin source of B12. Thus, I am concerned that vegan smokers may not receive much benefit from cyanocobalamin supplementation.

In contrast, I know two vegan smokers whose B12 source has only been cyanocobalamin, and who have not developed overt B12 deficiency in over ten years on the diet. There are probably others. Unfortunately, I could find no studies looking at cyanocobalamin supplementation in smokers, much less in vegan smokers.

To be cautious, I am suggesting that vegan smokers supplement with a non-cyanocobalamin form of B12. The amounts will have to be somewhat arbitrary because of the lack of information on the absorption rates and detoxification action of the various forms of B12 in smokers. I have not seen evidence of oral adenosylcobalamin's effectiveness in counteracting B12 deficiency. Donaldson<sup>121</sup> (see Appendix N) had success with oral methylcobalamin. I would, therefore, tentatively recommend 500 µg/day, sublingually, of methylcobalamin for vegan smokers. Most tablets are 1,000 µg, so they will need to be broken in half to get 500 µg. At that rate, a month's supply of methylcobalamin should cost about \$4.

Please note: The case may be that the recommendations for smokers need not be any different than those for nonsmokers. At this time, I do not feel that there is enough evidence one way or the other.

Also note: There is no evidence that cyanocobalamin is harmful to vegan smokers; including a modest source of cyanocobalamin (e.g., 3-5 µg/day), in addition to methylcobalamin, could serve as insurance.

**Table N.2 Conditions Reported to Improve by Coenzyme Forms of B12**

	Therapeutic dose recommended by Kelly <sup>150</sup>	Conditions reported to improve from treatment <sup>150,A</sup>
methylcobalamin	1500-6000 µg/day	Diabetic neuropathy Infertility Hyperhomocysteinemia in diabetes Sleep disorders <sup>B</sup> Bell's Palsy
adenosylcobalamin, aka: 5'-deoxyadenosylcobalamin, dibencozide, coenzyme B12, cobamamide, and cobinamide	1000-6000 µg/day	Neurological problems secondary to anorexia Hepatitis A Viral hepatitis <sup>B</sup>

<sup>A</sup>In many cases, treatment was not compared to cyanocobalamin.

<sup>B</sup>More effective than cyanocobalamin.

Kelly's full paper can be found at <http://www.thorne.com/altmedrev/fulltext/b122-6.html>

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## Table of Contents

### 1. Introduction

### 2. Vitamin B12: A Pesky Molecule

#### 2.1 The B12 Molecule

##### 2.2.1 Digestion & Absorption of Protein-bound B12

##### 2.2.2 Digestion & Absorption of Unbound B12

#### 2.3 Enterohepatic Circulation

#### 2.4 Transport in the Blood

#### 2.5 Pernicious Anemia

### 3. Coenzyme Functions of Vitamin B12

#### 3.1 Homocysteine Clearance

#### 3.2 Anemia, DNA, and Folate

##### 3.2.1 Macrocytic Anemia: Not an Adequate Measure of B12 Status

#### 3.3 Methylmalonic Acid (MMA)

### 4. Serum B12 Level: Not a Reliable Measure of B12 Adequacy

#### 4.1 Seaweeds Can Falsely Inflate sB12 Levels

#### 4.2 Measuring Transcobalamin II

#### 4.3 B12-Deficient Nerve Damage with Normal sB12 and No Macrocytosis

### 5. Symptoms of B12 Deficiency

#### 5.1 Neurological Symptoms

#### 5.2 Early, Noticeable Symptoms of B12 deficiency

#### 5.3 Other symptoms of B12 deficiency

#### 5.4 When is it Time to Call a Doctor?

#### 5.5 Ways to Get B12 Deficiency

### 6. Unusually High B12

### 7. Sources of B12 for Vegans

#### 7.1 Myth about How Often Someone Needs B12

#### 7.2 Small Amounts of Animal Products.

#### 7.3 Fortified Foods

##### 7.3.1 Brewer's and Nutritional Yeasts

##### 7.3.2 Cooking Foods

#### 7.4 Multivitamins

#### 7.5 Supplements

##### 7.5.1 Safety

##### 7.5.2 Chew or Dissolve Supplements Under the Tongue

##### 7.5.3 Light

##### 7.5.4 Twinlab B12 Dots

##### 7.5.5 Non-cyanocobalamin Supplements

#### 7.6 Oral B12 for People with Malabsorption

### 8. The B12 Status of Vegans

#### 8.1. Infants



- 8.1.1 Infants of Vegan Mothers Who Do Not Use B12 Supplements
  - 8.1.2 Correction of B12 Deficiency in Infants
  - 8.1.3 Black Hebrews
  - 8.1.4 Vegan Infants Taking B12 Supplements
  - 8.2 Children & Teenagers
    - 8.2.1 Vegan Children & Teenagers Not Supplementing with B12
    - 8.2.2 Vegan Children & Teenagers Supplementing with B12
  - 8.3 Adult Vegans
    - 8.3.1 Studies on Adult Vegans Not Supplementing with B12
    - 8.3.2 Vegans Taking B12 Supplements
  - 8.4 Elderly Vegetarians & Vegans
  - 8.5 Raw Foodist Vegans
  - 9. Lacto-ovo Vegetarians (LOV)**
  - 10. Macrobiotics**
  - 11. Vegans, B12, Homocysteine & Disease**
    - 11.1 Background on Homocysteine
    - 11.2 Studies on Homocysteine in Vegans and Vegetarians not Supplementing with B12
    - 11.3 Conclusion
  - 12. B12 & Cancer**
    - 12.1 B12 & Breast Cancer
  - 13. Measuring B12 in Plant Foods: Why the Confusion?**
    - 13.1 Microbiological Assay
    - 13.2 Radioassay
    - 13.3 *Ochromonas malhamensis* Fares Better than an Intrinsic Factor Assay
    - 13.4 Paper Chromatography
    - 13.5 Methylmalonic Acid Reduction: The Gold Standard
  - 14. Are Intestinal Bacteria a Source of B12?**
    - 14.1 Bacteria in the Large Intestine
    - 14.2 Bacteria in the Small Intestine
    - 14.3 Conclusion
  - 15. Organic Produce & Soil as a B12 Source for Vegans**
    - 15.1 B12 Analogue-Producing Bacteria in the Soil
    - 15.2 Organic Produce Using Human Manure as Fertilizer
    - 15.3 Organic Produce Using Cow Manure as Fertilizer
      - 15.3.1 Soybean Plants Absorb B12 when Added to the Soil
      - 15.3.2 Plants Absorb B12 Analogue when Added to the Soil via Cow Dung
  - 16. B12 in Non-Human Animals**
  - 17. Immerman: The Exception**
  - 18. Conclusion**
    - 18.1 Can a *Natural* Diet Require Supplements?
    - 18.2 The Medical Community: Future of Research on Vegans
    - 18.3 Encourage New Vegans to Concern Themselves with B12 Supplements
    - 18.4 Daily or Weekly Supplementation?
- Appendix A. B12 Analogue in Tempeh, Seaweeds, and Other Plant Foods**
    - A.1 Foods with No Detectable Amount of B12 Analogue
    - A.2 Foods with Detectable B12 Analogue in Some Studies
    - A.3 Tempeh
    - A.4 Seaweeds
      - A.4.1 Various Seaweeds: Dulce Warrants Further Study
      - A.4.2 *Aphanizomenon flos-aquae*
      - A.4.3 *Chlorella*
      - A.4.4 *Spirulina*
      - A.4.5 *Nori*
      - A.4.6 *Coccolithophorid* Algae
  - A.5 Plant Sources of B12 Analogues Studied in Humans
  - A.6 Conclusion
  - Appendix B. Slightly Elevated MMA Levels**
  - Appendix C. Vegan Mothers' Infants & Toddlers with Serious B12 Deficiency**
  - Appendix D. B12 Levels in Lacto-ovo Vegetarians**
  - Appendix E. Vegan or Vegetarian Children with Serious B12 Deficiency**
  - Appendix F. B12 Levels in Macrobiotics**
  - Appendix G. Note about Twinlab B12 Dots**
  - Appendix H. Inactive Analogues: Worse than Useless**
  - Appendix I. Homocysteine & Disease**
    - I.1 Background
      - I.1.1 Measuring Homocysteine
      - I.1.2 Vitamin Supplementation Can Confound Study Results
    - I.2 Homocysteine & Cardiovascular Disease
      - I.2.1 Recent Reviews of the Studies on Elevated Homocysteine and CVD
      - I.2.2 Prospective Studies on Homocysteine and Cardiovascular Disease
      - I.2.3 Treatment of High Homocysteine with Folate, B12, & B6 in Nonvegetarians
        - I.2.3.1 Reduced Plaque in Carotid Arteries
        - I.2.3.2 Improved Exercise Test, Ankle-brachial Pressure, and Arterial Stenosis
        - I.2.3.3 Improved Endothelial Function
      - I.2.4 Elevated sB12 as an Increased Risk Factor for Disease
    - I.3 B12, Homocysteine, & Alzheimer's Disease
    - I.4 B12, Folate, Homocysteine, & Age-Related Hearing Loss
    - I.5 B12, Folate, Homocysteine, & Birth Defects
    - I.6 Homocysteine and Recurrent Early Pregnancy Loss
    - I.7 Homocysteine and Mortality
    - I.8 Homocysteine & Kidney Disease
    - I.9 Low Methionine Intakes May Exacerbate High Homocysteine Levels in B12 Depleted States
    - I.10 One Week of Vegan Diet (with B12) & Other Lifestyle Changes Lowers Homocysteine
  - Appendix J. Homocysteine & Kidney Disease**
  - Appendix K. Ways to Get B12 Deficiency**
  - Appendix L. Formulation of Recommendations**
    - L.1 Step 1.
      - L.1.1 Oral B12 for People with Malabsorption
    - L.2 Step 2 Recommendations.
      - L.2.1 Absorption Rates of B12
      - L.2.2 What is a healthy B12 Level?
      - L.2.3 What Intake is Needed to Achieve a Healthy Level?
      - L.2.4 Recommendations
  - Appendix M. People Who Should Not Take the Cyanocobalamin Form of B12**
    - M.1 Cyanide Metabolism Defects
    - M.2 Chronic Kidney Failure
  - Appendix N. Non-cyanocobalamin B12 Supplements**
    - N.1 Methylcobalamin in a Small Sample of Vegans
    - N.2 Therapeutic Use of Coenzyme form of B12
    - N.3 Hydroxocobalamin
    - N.4 SAME
  - Appendix O. Smokers and Cyanocobalamin**