Three B vitamins are involved in what is known as 1-carbon metabolism. This is the movement of 1 carbon units, generally methyl groups (CH₃). It is similar to the movement of the amino group that occurs in transamination. As shown in the figure below, folate, vitamin B₁₂, and vitamin B₆ are the B vitamins involved in 1-carbon metabolism.

Vitamin B₆ has been covered already in the previous chapter, so this chapter is going to focus on folate and vitamin B₁₂. We will examine this figure in pieces, so that hopefully by the time this chapter is completed, you will understand the role of all these vitamins in 1-carbon metabolism.

Sections:

11.1 Folate & Folic Acid
11.2 Vitamin B₁₂
11.3 B Vitamins, Homocysteine, & Cardiovascular Disease
11.1 Folate & Folic Acid

Folate is a B vitamin that exists in either its reduced form (folate) or oxidized form (folic acid). When folate is used in this section, we are referring to the reduced form, not the vitamin itself. Another key distinction between the 2 terms is that folic acid refers to the synthetic form, while folate refers to the natural form. Folic acid is only found in certain foods because they have been fortified with it, not because they produce it. The structure of folic acid is shown below.

![Figure 11.11 Structure of Folic Acid](image)

Table 11.11 summarizes the key differences between folate and folic acid.

<table>
<thead>
<tr>
<th><strong>Folate</strong></th>
<th><strong>Folic Acid</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Form</td>
<td>Oxidized Form</td>
</tr>
<tr>
<td>Natural</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Polyglutamate</td>
<td>Monoglutamate</td>
</tr>
<tr>
<td></td>
<td>More Stable</td>
</tr>
</tbody>
</table>

The bioavailability of folate was believed to be much lower than folic acid. To account for these differences, the DRI committee created dietary folate equivalents (DFEs) to set the DRIs. DFEs are defined as follows:

1 DFE = 1 ug food folate = 0.6 ug food folic acid = 0.5 ug folic acid on an empty stomach
DFE = ug food folate + (ug folic acid X 1.7)

The 1.7 came from research suggesting that folic acid from food was 85% bioavailable, compared to 50% for folate (85%/50% = 1.7)⁴. This was established in 1998 by the DRI committee, and it is likely that these conversions & the requirements will change based on the newer evidence suggesting folate's bioavailability from food is higher (80% of folic acid) than previously believed³. With this data, the new conversion factor for folic acid would be 1.25 (100%/80%). This conversion factor means that food folate levels are probably contributing more towards our dietary needs than currently being estimated by the DFE, but the DRI for folate/folic acid has not been updated.

Before folate (polyglutamates) can be taken up into the enterocyte, the extra glutamates must be cleaved prior to uptake into the enterocyte by the reduced folate transporter (RFT, aka reduced folate carrier)⁵-⁷. Folic acid, because it is a monoglutamate, requires no cleavage for uptake before it is taken up through the RFT. Once inside the enterocyte, the monoglutamate form is methylated and transported into circulation through an unresolved carrier⁵. This series of events is depicted in the figure below.

Thus, the methylated monoglutamate form is the circulating form. This is transported to the liver where it is converted back to the polyglutamate form for storage. Folate is excreted in both the urine and feces⁵.

Subsections:
11.11 Folate Functions
11.12 Folate Deficiency & Toxicity

References & Links
11.11 Folate Functions

The major function of folate is that it participates in 1-carbon metabolism. As described earlier, this is the transfer of 1-carbon units from 1 compound to another. The cofactor form of folate is tetrahydrofolate (THF). As is shown in the figure below, in order for THF to be formed, a methyl group is transferred to cobalamin (vitamin B\textsubscript{12}) from 5-methyl THF (THF plus a methyl group), forming methyl-cobalamin. You can see this on the left side of the figure below.

![One-carbon metabolism diagram](image)

There are 2 major cofactor functions of THF:\(^1\):

1. **DNA Synthesis**

   THF is required for the synthesis of DNA bases (purines and pyrimidines)\(^3\). As shown in the link below, N\textsuperscript{10}-formyl-THF (a form of THF) is needed in 2 reactions (3 and 9) in purine synthesis.

   [Web Link](Purine Synthesis)

2. **Amino Acid Metabolism**

   THF is a cofactor for enzymes that metabolize histidine, serine, glycine, and methionine\(^1\). The following link shows that THF is a cofactor for serine hydroxymethyltransferase, the enzyme that converts serine to glycine.
References & Links

Links
Serine to Glycine - http://themedicalbiochemistrypage.org/images/glycine-synthesis.jpg
11.12 Folate Deficiency & Toxicity

Folate deficiency is a vitamin deficiency that affects some Americans. The hallmark symptom of folate deficiency is megaloblastic (aka macrocytic) anemia. Megaloblastic anemia, as the name suggests, is characterized by large, nucleated (most red blood cells do not have a nucleus), immature red blood cells. This occurs because folate is needed for DNA synthesis; without it red blood cells are not able to divide properly\(^1\). As a result, fewer and poorer functioning red blood cells are produced that cannot carry oxygen as efficiently as normal red blood cells\(^2\).

A maternal folate deficiency can lead to neural tube defects in infants. The exact cause of neural tube defects is unknown, but folate supplementation has been shown to decrease the incidence of neural tube defects\(^3\). The most common of these neural tube defects is spina bifida (1 out of 2500 babies born in the United States), which is a failure of the neural tube to close and the spinal cord and its fluid protrude out the infant's back, as shown below\(^4,5\).

![Spina Bifida (Open Defect)](image)

Figure 11.121 Spina bifida\(^6\)

The neural tube closes 21-28 days after conception\(^1\), and with 50% of pregnancies estimated to be unplanned, many women aren't aware they are pregnant during this period\(^1,2\). Thus, it is recommended that women of childbearing age consume 400 ug of folic acid daily\(^1\). In addition, in 1998 the FDA mandated that all refined cereals and grains be fortified with 140 ug folic acid /100 grams of product\(^7\). As you can see below, spina bifida prevalence rates declined during the
optional fortification years and declined further once fortification became mandatory in the United States.

However, more recent research has found that folic acid supplementation begun before conception reduced the occurrence and severity of neural tube defects. The following link is an interesting account of the history that led up to the folic acid fortification. It is debatable whether folic acid fortification was fully responsible for the decrease in spina bifida rates shown above, but the rates are lower than they were pre-fortification. The second link is to the announcement that in 2016 the FDA approved the fortification of corn masa flour.

Web Link
Folic Acid Fortification: Fact and Folly
FDA Approves Folic Acid Fortification of Corn Masa Flour

Folate/Folic acid is not toxic, but it can mask a vitamin B$_{12}$ deficiency and prevent its diagnosis. This effect will be discussed further in the vitamin B$_{12}$ deficiency section.

References & Links
8. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6401a2.htm

**Link**

Folic Acid Fortification: Fact and Folly - http://www.fda.gov/AboutFDA/WhatWeDo/History/ProductRegulation/SelectionsFromFDLIUpdateseriesonFDAHistory/ucm091883.htm

FDA Approves Folic Acid Fortification of Corn Masa Flour - https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm496104.htm
11.2 Vitamin B\textsubscript{12} 

Vitamin B\textsubscript{12} is unique among vitamins in that it contains an element (cobalt) and is found almost exclusively in animal products. Neither plants nor animals can synthesize vitamin B\textsubscript{12}. Instead, vitamin B\textsubscript{12} in animal products is produced by microorganisms within the animal that the products came from. Animals consume the microorganisms in soil or bacteria in ruminant animals that produce vitamin B\textsubscript{12}\textsuperscript{1}. Some plant products, such as fermented soy products (tempeh, miso) and the sea algae supplement, spirulina, are advertised as being good sources of B\textsubscript{12}. However, fermented soy products are not a reliable vitamin B\textsubscript{12} source\textsuperscript{2} and spirulina contains a pseudovitamin B\textsubscript{12} compound that is not bioavailable\textsuperscript{3}. For vegans, supplements, nutritional yeast, and fortified products like fortified soy milk can help them meet their vitamin B\textsubscript{12} needs\textsuperscript{4}.

Vitamin B\textsubscript{12}’s scientific name is cobalamin, which makes sense when you consider it contains cobalt and many amine groups, as shown in the figure below.

Figure 11.21 Structure of vitamin B\textsubscript{12} (cobalamin)\textsuperscript{5}

The other feature that is important in cobalamin is the circled R group. This is what differs between the different cobalamins, whose names and R groups are shown in the following table.

Table 11.21 Different cobalamin forms
The 2 cofactor forms are adenosylcobalamin and methylcobalamin. We can convert most cobalamins into these 2 cofactor forms. Most foods contain adenosylcobalamin, hydroxocobalamin, or methylcobalamin. The most common form found in supplements is cyanocobalamin, with some also using methylcobalamin. Cyanocobalamin is a synthetic form of the vitamin B₁₂.

The uptake, absorption, and transport of vitamin B₁₂ is a complex process. The following descriptions explain, and figures illustrate, this process.

Vitamin B₁₂ is normally bound to protein in food. Salivary glands in the mouth produce haptocorrin (formerly known as R protein), which travels with the food into the stomach. In the stomach, acid converts pepsinogen into pepsin, and the protein intrinsic factor is released from the parietal cells.
As pepsin frees $B_{12}$ from protein, haptocorrin binds to the newly freed vitamin $B_{12}$ (haptocorrin + $B_{12}$). Intrinsic factor escapes digestion and, along with haptocorrin + $B_{12}$, exits the stomach and enters the duodenum$^{1,8}$.

In the duodenum, pancreatic proteases break down haptocorrin, and again vitamin $B_{12}$ is freed. Intrinsic factor then binds vitamin $B_{12}$ (intrinsic factor + $B_{12}$); intrinsic factor + $B_{12}$ continues into the ileum to prepare for absorption$^{1,8}$. 
In the ileum, intrinsic factor + B\textsubscript{12} is believed to be endocytosed by intrinsic factor binding to cubulin (aka intrinsic factor receptor), forming an endosome inside the enterocyte. Intrinsic factor is broken down in the enterocyte, freeing vitamin B\textsubscript{12}. The free vitamin B\textsubscript{12} is then bound to transcobalamin II (TC II + B\textsubscript{12}); TC II + B\textsubscript{12} moves into circulation\textsuperscript{8}.

The liver is the primary storage site for vitamin B\textsubscript{12}. Unlike most other water-soluble vitamins, the liver is able to maintain significant stores of vitamin B\textsubscript{12}. Uptake into the liver occurs...
through the binding of TC II + B₁₂ to the TC II Receptor and the endocytosis of both the compound and the receptor. Vitamin B₁₂ is once again freed after degradation of TC II. Vitamin B₁₂ is primarily stored in the liver as adenosylcobalamin.

The overall bioavailability of vitamin B₁₂ is believed to be approximately 50%, with the different cobalamin forms having similar bioavailabilities. Sublingual supplements of vitamin B₁₂ have been found to be equally efficacious as oral supplements. Excretion occurs mostly through bile, with little loss in urine.

Figure 11.26 Hepatic uptake and storage of vitamin B₁₂

Subsections:

11.21 Vitamin B₁₂ Functions
11.22 Vitamin B₁₂ Deficiency & Toxicity

References & Links
11.21 Vitamin B\textsubscript{12} Functions

Vitamin B\textsubscript{12} is a cofactor for 2 enzymes:

1. Methionine synthase
2. Methylmalonyl mutase

**Methionine Synthase**

Methionine synthase is an important enzyme in 1-carbon metabolism that uses methylcobalamin as its cofactor and converts homocysteine to methionine by adding a methyl group. Methionine then is converted to other compounds that serve as methyl donors, as shown below\textsuperscript{1}.

![Figure 11.211 One-carbon metabolism](image)

These methyl donors can donate methyl groups for methylating DNA, an epigenetic modification\textsuperscript{1}.

**Methymalonyl mutase**

This enzyme uses adenosylcobalamin as its cofactor, and is important in the breakdown of odd chain fatty acids (5 carbons etc.). As you know, odd chain fatty acids are less common than even chain fatty acids, but this enzyme is required to properly handle these less common fatty acids\textsuperscript{1}.
Demyelination

In addition to its role as a cofactor for enzymes, vitamin B\textsubscript{12} is also important for preventing degradation of the myelin sheath that surrounds neurons, as shown below.

![Myelin Sheath](https://en.wikipedia.org/wiki/Myelin#/media/File:Neuron_Hand-tuned.svg)

Figure 11.212 Vitamin B\textsubscript{12} is needed to maintain the myelin sheath that surrounds neurons\textsuperscript{2}

The mechanism through which vitamin B\textsubscript{12} prevents demyelination is not known\textsuperscript{3}.

**References & Links**
11.22 Vitamin B\textsubscript{12} Deficiency & Toxicity

There are 2 primary symptoms of vitamin B\textsubscript{12} deficiency:

Megaloblastic (Macrocytic) Anemia
Neurological Abnormalities

**Megaloblastic (Macrocytic) Anemia**

This is the same type of anemia that occurs in folate deficiency that is characterized by fewer, enlarged, immature red blood cells. In vitamin B\textsubscript{12} deficiency, this can occur because there is not enough cobalamin to convert 5-methyl THF to THF (illustrated in Figure 11.211). Thus, THF is not available for normal DNA synthesis and the red blood cells do not divide correctly.

**Neurological Abnormalities**

Vitamin B\textsubscript{12} deficiency also results in nerve degeneration and abnormalities that can often precede the development of anemia. These include a decline in mental function and burning, tingling, and numbness of legs. These symptoms can continue to worsen and deficiency can be fatal\textsuperscript{1}.

The most common cause of vitamin B\textsubscript{12} deficiency is pernicious anemia, a condition of inadequate intrinsic factor production that causes poor vitamin B\textsubscript{12} absorption. This condition is common in people over the age of 50 because they have the condition atrophic gastritis\textsuperscript{2}. Atrophic gastritis is a chronic inflammatory condition that leads to the loss of glands in the stomach, as shown in the figure in the following link.

[Web Link]
[Atrophic Gastritis]

The loss of glands leads to decreased intrinsic factor production. It is estimated that ~6% of those age 60 and over are vitamin B\textsubscript{12} deficient, with 20% having marginal status\textsuperscript{3}. In addition to the elderly, vegans are also at risk for vitamin B\textsubscript{12} deficiency because they do not consume animal products. However, the deficiency may take years to develop in adults because of stores and recycling of vitamin B\textsubscript{12}\textsuperscript{2}. Deficiency has the potential to occur much quicker in infants or young children on vegan diets because they do not have stores that adults do\textsuperscript{4}. 
Folate/Folic Acid masking vitamin B$_{12}$ deficiency

As mentioned above, folate and vitamin B$_{12}$ lead to the same megaloblastic (macrocytic) anemia. If high levels of folate or folic acid (most of the concern is with folic acid since it is fortified in foods and commonly taken in supplements) is given during vitamin B$_{12}$ deficiency, it can correct this anemia. This is referred to as masking because it does not rectify the deficiency, but it "cures" this symptom. This is problematic because it does not correct the more serious neurological problems that can result from vitamin B$_{12}$ deficiency. There are some people who are concerned about the fortification of cereals and grains with folic acid because people who are B$_{12}$ deficient might not develop macrocytic anemia, which makes a vitamin B$_{12}$ deficiency harder to diagnose$^2$.

No toxicity of vitamin B$_{12}$ has been reported.

References & Links

Links
11.3 B Vitamins, Homocysteine & Cardiovascular Disease

Homocysteine is a sulfur containing, non-proteinogenic (not used for making proteins) amino acid whose structure is shown in the figure below.

![Figure 11.31 Structure of homocysteine](http://en.wikipedia.org/wiki/File:Homocysteine_racemic.png)

Elevated circulating homocysteine levels have been found in people with cardiovascular disease. Folate, vitamin B₆, and vitamin B₁₂ contribute to the conversion of homocysteine to methionine by providing methyl groups, thereby decreasing homocysteine levels, as illustrated in the figure below. Thus, based on these facts, it was hypothesized that intake of these B vitamins may decrease the risk of cardiovascular disease.

![Figure 11.32 One-carbon metabolism](http://en.wikipedia.org/wiki/File:Homocysteine_racemic.png)

Research has found that intake of these B vitamins does decrease circulating homocysteine levels. However, most studies have not found that it results in improved cardiovascular disease outcomes²⁴. It is debated why B vitamin intake has not resulted in improved outcomes. Some think it is because the studies have not focused on individuals with elevated homocysteine levels², while others believe that homocysteine is a biomarker or indicator of cardiovascular disease, not a causative or contributing factor to cardiovascular disease development³.

References & Links