UNIT 4

Water-Soluble Vitamins

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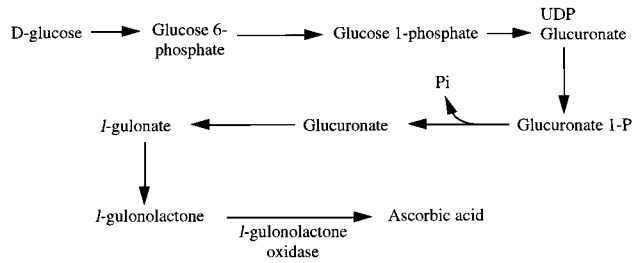


Figure 1 Biosynthesis of ascorbic acid.

I. ASCORBIC ACID

A. Overview

Ascorbic acid, or vitamin C, has been recognized as a needed nutrient for centuries. Although its chemical identity was unknown, foods rich in this micronutrient have long been used as treatment for the symptoms of scurvy. Ancient Egyptians, Greeks, and Romans referred to scurvy as a plague which interfered with victory in military campaigns. Accounts of the scourge of scurvy have appeared in writings of the sixteenth, seventeenth, and eighteenth centuries.

Perhaps the best known treatise on scurvy is that written by a Scottish surgeon, James Lind. Dr. Lind demonstrated that the inclusion of limes, oranges, and lemons in sailors' diets would successfully prevent the development of scurvy. Other reports similar to Lind's were published, but his remains the classic work in this field. Even though the value of citrus fruits was amply demonstrated by Lind, it took 30 years for his recommendations to be adopted. Most nutrition historians credit Captain James Cook, discoverer of the Hawaiian Islands, as the first sea captain to include citrus fruits as part of his ship's stores and part of the sailors' diets. He thus was able to demonstrate that long ocean voyages need not result in scurvy for the crew.

Despite the knowledge that scurvy could be avoided, many sailors and explorers continued to die from scurvy. Even as recently as 1912, Captain Scott and his team died of scurvy as they explored the polar regions of the Southern Hemisphere.

While the knowledge that certain foods prevented or cured scurvy was available in the eighteenth century, the isolation and synthesis of the antisorbutic factor did not occur until early in the twentieth century. The isolation of ascorbic acid was accomplished by two independent groups of chemists in 1928. Szent-Gyorgy isolated a compound which he called hexuronic acid from orange juice, cabbage, and adrenal glands. King likewise isolated the vitamin from lemon juice and showed that it was identical to the compound isolated by Szent-Gyorgy. The structure of the vitamin was accomplished by Haworth and its synthesis by Reichstein. While this exciting chemistry was being pursued an interesting fact became known, and that was that few species required ascorbic acid in their diets. The guinea pig, the primates (including humans), the fruit bat, and some fishes and birds were identified as being unable to synthesize the vitamin *in vivo*. All other species examined were found to be able to synthesize it from glucose (Figure 1). The reason why ascorbic acid-dependent species can not synthesize ascorbic acid is because they lack the enzyme L-gulonolactone oxidase.

B. Structure, Physical and Chemical Properties

Ascorbic acid and dehydroascorbic acid (the oxidized form) are the trivial names for vitamin C. The chemical name is 2,3-didehydro-L-threo-hexano-1,4-lactone. The compound can readily donate

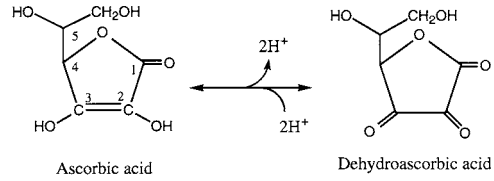


Figure 2 Structures of ascorbic acid and dehydroascorbic acid.

or accept hydrogen ions and thus exists in either state, as shown in Figure 2. In order for the compound to have vitamin activity it must have a 2,3-endiol structure and be a 6-carbon lactone.

L-Ascorbic acid (Figure 2) is a rather simple compound chemically related to the monosaccharide, glucose, with an empiric formula of $C_6H_8O_6$. It is a white crystalline solid with a molecular weight of 176 Da. It is soluble in water, glycerol, and ethanol, but insoluble in fat solvents such as chloroform and ether. It exists in both D and L forms but the L form is the biologically active form. This is in contrast to its related monosaccharide, glucose, which is biologically active as the D form. The vitamin is stable in the dry form but once dissolved in water it is easily oxidized. It is relatively stable in solutions with a pH below 4.0; as the pH rises, the vitamin becomes less stable. Ascorbic acid is easily oxidized by metals such as iron or copper. While ascorbic acid is readily oxidized, it is less perishable in food although it is still oxidized in alkaline environments, especially when heated, exposed to air, or in contact with iron or copper salts. Fortunately, those foods rich in ascorbic acid are relatively acidic and lack iron and copper. If the food is not cooked quickly with a minimum of water, significant losses will occur which will decrease the value of the food as a source of the vitamin.

Ascorbic acid is easily and reversibly oxidized to form dehydroascorbate (Figure 2). The ease with which this interconversion occurs is the basis for its biological function as an acceptor or donor of reducing equivalents. Further oxidation results in the formation of diketogulonic acid, which is biologically inactive. The conversion of ascorbate to dehydroascorbate is aided by sulfhydryl compounds such as glutathione. The strong reducing power of ascorbate can be used to good advantage in its assay. Ascorbate will react with a variety of cyclic compounds to form a color which can be measured spectrophotometrically. Dyes such as dichlorophenolindophenol and 2,4-dinitrophenylhydrazine are the most commonly used compounds in the assay for vitamin C. Chromatographic techniques are also available. An excellent enzymatic assay has been designed using the enzyme ascorbate oxidase. This assay is both sensitive and specific. However, because the need for ascorbic acid and the fact that is prevalent in relatively large amounts in those foods containing the vitamin, the spectrophotometric methods are usually satisfactory. For assays of the vitamin content of tissues such as liver or blood cells, the more sensitive HPLC and thin-layer chromatographic techniques are preferred. In animal and plant tissues vitamin C is present in milligram amounts. Human plasma, for example, contains about 1 mg/dl.

C. Sources

Ascorbic acid is provided mostly by citrus fruits, strawberries, and melons. Some of the vitamin can be found in raw cabbage and related vegetables.

D. Absorption, Metabolism

The metabolic fate of ascorbic acid depends on a number of factors including animal species, route of ingestion, quantity of material, and nutritional status. In species requiring dietary ascorbate, the ascorbate is absorbed in the small intestine, primarily the ileum, by an active transport system which is both sodium dependent and energy dependent. Studies *in vitro* have demonstrated clearly

Table 1 Distribution of Ascorbate in Humans and Rats

Tissue	Human (mg/100 g tissue, wet weight)	Rat (mg/100 g tissue, wet weight)
Adrenals	30–40	280–400
Pituitary	40–50	100–130
Liver	10–16	25–40
Spleen	10–15	40–50
Lungs	7	20–40
Kidneys	5–15	15–20
Testes	3	25–30
Thyroid	2	22
Heart	5–15	5–10
Plasma	0.4–1.0	1.6

that the vitamin moves from the mucosal to the serosal sides of the lumen against a concentration gradient. The influx of the vitamin at the brush border follows saturation kinetics and is specific for the L isomer. Influx can be inhibited by D-isoascorbate, a naturally occurring analog. If sodium is absent, influx does not occur.

Absorption is a sodium-dependent energy-dependent process involving a carrier for ascorbate. The carrier translocates the ascorbate into the enterocyte with sodium, whereupon the sodium must then be pumped out. The sodium dissociates from the carrier mechanism at the inner side of the mucosal membrane and the vitamin moves into the cytosol. The carrier then resumes its original position in the membrane and is available to repeat the process.

Although this process is similar to that envisioned for glucose and alanine, these compounds do not compete with ascorbate for absorption via the above-described carrier. Those species able to synthesize sufficient ascorbic acid do not possess this active transport system and dietary ascorbate is absorbed via passive diffusion. These species differences in transport phenomena lend further evidence of a bifurcation in the evolutionary process which separates guinea pigs, primates, and other ascorbate-requiring species from those species which do not require this vitamin in their diet.

Once absorbed, there appears to be a central pathway for metabolism common to all species. Any excess vitamin consumed beyond need is excreted. There is a very efficient reabsorption mechanism in the kidneys which serves to conserve ascorbic acid in times of need. In the guinea pig, the excess is oxidized to CO_2. In humans very little, if any, oxidation of ascorbate to CO_2 occurs. Ascorbic acid and its metabolites are excreted in the urine. Over 50 metabolites of ascorbate have been detected. Most of these are excreted in minor amounts. The main metabolites in the urine are ascorbate-2-sulfate, oxalic acid, ascorbate, dehydroascorbate, and 2,3-diketogulonic acid. *In vivo*, there is some exchange of ascorbate and ascorbate sulfate in the monkey. The significance of this exchange remains to be elucidated.

E. Distribution

One of the earliest investigations of ascorbic acid function included studies of the distribution of the vitamin throughout the body. Table 1 presents some of these findings in the human and rat. Ascorbic acid is also found uniformly in the brain distributed where it serves as a coenzyme for an enzyme which converts dopamine to norepinephrine.

Ascorbic acid pool sizes and turnover have been estimated using isotopically labeled vitamin. In depleted humans consuming a vitamin C-free diet, about 3% of the total existing pool of ascorbic acid is degraded daily. When the depleted subjects were given doses of vitamin C, this vitamin did not appear in the urine until the body pool approached the size of about 1500 mg. Body pool sizes of more than 1500 mg have not been observed, even when megadoses of the vitamin are consumed.

Table 2 Enzymes Using Ascorbate as a Coenzyme

Cytochrome P_{450} oxidases (several)
Dopamine-β-monooxygenase
Peptidyl glycine α-amidating monooxygenase
Cholesterol 7-α-hydroxylase
4 Hydroxyphenylpyruvate oxidase
Homogentisate 1,2-dioxygenase
Proline hydroxylase
Procollagen-proline 2-oxoglutarate-3-dioxygenase
Lysine hydroxylase
γ-Butyrobetaine, 2-oxoglutarate-4-dioxygenase
Trimethyllysine-2-oxoglutarate dioxygenase

These observations indicate that mega intakes are not useful with respect to the body's vitamin C content. The first signs of scurvy were observed in humans having pool sizes of 300 to 400 mg, and these signs did not disappear until the pool size increased to 1000 mg. Turnover is estimated by measuring the intake rate, the excretion rate, and the total body pool size. This can be accomplished by giving a dose of radioactively labeled vitamin and measuring its distribution and excretion. Vitamin C turnover has been estimated to be 60 mg/day for normally nourished humans. Smokers, incidentally, have higher turnover rates and require more ascorbic acid to maintain their pool sizes.

In contrast to many vitamins, ascorbic acid does not need a carrier for its transport within the body. Like glucose, it is readily carried in the blood in its free form and likewise freely crosses the blood-brain barrier.

F. Function

Although we had an abundance of information about the chemistry of this vitamin, its metabolic function remained elusive for many years. Because it readily converts between the free and dehydro form, it functions in hydrogen ion transfer systems and aids in the regulation of redox states in the cells. Since it is a powerful water-soluble antioxidant it helps to protect other naturally occurring antioxidants which may or may not be water soluble. For example, polyunsaturated fatty acids and vitamin E are protected from peroxidation by ascorbic acid. Ascorbic acid protects certain proteins from oxidative damage and, in addition to its role as an antioxidant, it serves to maintain the unsaturation:saturation ratio of fatty acids. Ascorbic acid aids in the conversion of folic acid to folinic acid and facilitates the absorption of iron by maintaining it in the ferrous state. Ascorbic acid plays a role in the detoxification reactions in the microsomes by virtue of its role as a cofactor in hydroxylation reactions. Table 2 provides a list of those enzymes in which ascorbate is a coenzyme. Many of these are dioxygenases. Again, this is due to the ascorbate-dehydroascorbate interconversion. A number of these enzymes are involved in collagen synthesis. This explains the poor wound healing found in deficient subjects.

The hydroxylation of proline to hydroxyproline, an important amino acid in the synthesis of collagen, is one example of the function of ascorbic acid. The vitamin has been shown to be needed for the incorporation of iron into ferritin. Another function is the role this vitamin plays in maintaining both iron or copper in the reduced state so that the metal can perform as part of an hydroxylation reaction. The hydroxylation of tryptophan to 5-hydroxytryptophan and the conversion of 3,4-dehydroxyphenylethylamine (DOPA) to norepinephrine are further examples. These roles may well explain some of the features of scurvy (Table 3). Poor wound healing is related to the need to form collagen to seal the wound; anemia may be related to the inability of iron and copper to remain in the reduced state in hemoglobin. Ascorbic acid serves as an important cofactor in hydroxylation reactions. Although these reactions will occur in the absence of the vitamin, they occur at a very slow rate.

Table 3 Signs of Ascorbic Acid Deficiency

Hyperkeratosis
Congestion of follicles
Petechial and other skin hemorrhages
Conjunctival lesions
Sublingual hemorrhages
Gum swelling, congestion
Bleeding gums
Papillary swelling
Peripheral neuropathy with hemorrhages into nerve sheaths
Pain, bone endings are tender
Epiphyseal separations occur with subsequent bone (chest) deformities

G. Deficiency

As with other nutrients there are large differences among individuals in their needs for ascorbic acid. As well, there are large differences in the duration of time needed to develop scurvy when consuming a vitamin C-deficient diet. Hodges et al. fed volunteers a diet devoid of vitamin C and described their symptoms as they developed. They noted an increased fatigability, especially in the lower limbs, and a mild general malaise as the symptoms of scurvy became apparent. Mental and emotional changes occurred after 30 days on the diet, with symptoms of depression and suicidal tendencies developing. After 112 days on the ascorbic acid-free diet, some subjects complained of vertigo (feeling of faintness), inappropriate temperature sensing, and profuse sweating. After 26 days of depletion, small petechial hemorrhages were observed on the skin, and after 84 to 91 days small ocular hemorrhages were present. Gingival hemorrhages and swelling in various degrees appeared at different times (42 to 76 days) in the subjects. Hyperkeratosis was observed after two months of depletion. All of these symptoms were reversed when the subjects were repleted.

H. Toxicity

Vitamin C is a water-soluble vitamin and is not usually stored. Thus, there is little evidence of toxicity. As mentioned earlier, oxalate is an end product of ascorbate metabolism and is excreted. Although some investigators have suggested that megadoses of vitamin C may be a risk factor in renal oxalate stores, urinary oxalate levels do not change with increasing intakes of ascorbate. Megadoses of vitamin C have been advocated for the treatment of cancer. However, studies of cancer patients have revealed that such treatment was of little benefit. Massive doses of vitamin C have been shown to reduce serum vitamin B_{12} levels. In part, this may be due to an effect of ascorbic acid on vitamin B_{12} in food. Ascorbic acid destroys B_{12} in food. Ascorbic acid also inhibits the utilization of β-carotene.

I. Recommended Dietary Allowances

Over the years, different countries have had widely different RDAs for ascorbic acid intake. This was due primarily to the differing standards of adequate nutritional status. In Canada, the absence of scorbutic symptoms was used as the indication of adequate nutrient intake. In the U.S., adequate intake has been defined as the saturation of the white blood cell with ascorbic acid. For many years these different definitions have meant that there was a twofold difference in the two countries' recommendations. The U.S. RDA has recently (1989) been revised downward from 75 mg/day for adults to 60 mg/day. Lactating and pregnant women should consume more (+40 and +20 mg, respectively) and children less, depending on age. These recommendations are shown in Table 4.

Table 4 Recommended Dietary Allowances (RDA) for Ascorbic Acid

Group	Age	RDA (mg/day)
Infants	Birth–6 months	30
	7–12 months	35
Children	1–3	40
	4–6	45
	7–10	45
Males	11–14	50
	15–18	60
	19–24	60
	25–50	60
	51+	60
Females	11–14	50
	15–18	60
	19–24	60
	25–50	60
	51+	60
Pregnancy	—	70
Lactation	0–6 months	95
	7–12 months	90

Vitamin C requirements may be higher in stressed or traumatized persons or in persons with diabetes mellitus. In rats, administration of ACTH or cortisone has been shown to lower plasma and hepatic levels of the vitamin. In addition, women taking contraceptive steroids may absorb less of the vitamin or may metabolize it more quickly, and thus may require more than 60 mg/day. Requirements by these groups of people have not been established as yet.

II. THIAMIN

A. Overview

The discovery of the chemical structure and synthesis of thiamin marked the end of a difficult search, spanning continents, to identify the substance in rice polishings responsible for the cure of the disease, beriberi. One of the earliest recorded accounts of the disease was by Jacobus Bonitus, a Dutch physician. He wrote in 1630, "A certain troublesome affliction which attacks men is called by the inhabitants [of Java] beriberi. I believe those whom this disease attacks with their knees shaking and legs raised up, walk like sheep. It is a kind of paralysis or rather tremor: for it penetrates the motion and sensation of the hands and feet, indeed, sometimes the whole body…"

In 1894, Takaki, a surgeon in the Japanese navy, suggested that the disease was diet related. By adding milk and meat to the navy diet, he was able to decrease the incidence of the disease. He thought the problem was a lack of dietary protein. About the same time (1890) a Dutch physician named Eijkman observed a beriberi-like condition (polyneuritis) in chickens fed a polished rice diet. He was able to cure the condition by adding rice polishings. Eijkman suggested that polished rice contained a toxin which was neutralized by the rice polishings.

In 1901, another Dutch physician named Grijens gave the first correct explanation for the cure of beriberi by rice polishings. He theorized that natural foodstuffs contained an unknown factor, absent in polished rice, that prevented the development of the disease.

Jansen and Donath in 1906 and Funk in 1912 reported the isolation of a material from rice polishings which cured beriberi. Funk called the material vita amine or vitamine.

In 1926 Jansen and Donath isolated a crystalline material which cured polyneuritis in birds. Jansen gave the material the trivial name, aneurine. This name was used extensively in the European

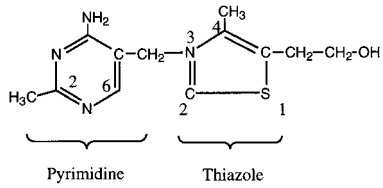


Figure 3 Structure of thiamin.

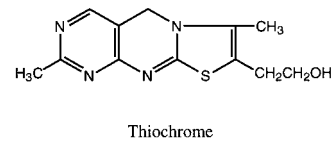


Figure 4 Structure of thiochrome.

literature. It is now considered an obsolete term, as are the terms vitamin B_1, oryzamin, torulin, polyneuramin, vitamin F, antineuritic vitamin, and antiberiberi vitamin. All these terms arose as early nutrition scientists identified diseases associated with thiamin deficiency which were reversed when the active principle, now known as thiamin, was provided.

In 1934 Williams et al. isolated enough of the material to make structure elucidation possible. In 1936 thiamin was synthesized by this same group. With synthesis demonstrated, the stage was set for the commercial preparation of thiamin followed by an outburst of publications on its function and metabolism.

B. Structure

Thiamin is a relatively simple compound of a pyrimidine and a thiazole ring (Figure 3). It exists in cells as thiamin pyrophosphate (TPP). TPP used to be called cocarboxylase. The name thiamin comes from the fact that the compound contains both a sulfur group (the thiol group) and nitrogen in its structure. Its biological function depends on the conjoined pyrimidine and thiazole rings, on the presence of an amino group on carbon 4 of the pyrimidine ring and on the presence of a quaternary nitrogen, an open carbon at position 2, and a phosphorylatable alkyl group at carbon 5 of the thiazole ring. In its free form it is unstable. For this reason, it is available commercially as either a hydrochloride or a mononitrate salt. The HCl form is a white crystalline material that is readily soluble (1 g in 1 ml) in water, fairly soluble in ethanol, but relatively insoluble in other solvents. The chemical name for the HCl form is 3-(4′-amino-2′-methyl-pyrimidine-5′-yl)-methyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride. It is stable to acids at up to 120°C but readily decomposes in alkaline solutions, especially when heated. It can be split by nitrite or sulfite at the bridge between the pyrimidine and thiazole rings. The mononitrate form is a white crystalline substance that is more stable to heat than is the hydrochloride form. This form is used more often for food processing than the HCl form. Other forms are also available. These include thiamin allyldisulfide, thiamin propyldisulfide, thiamin tetrahydrofurfuryldisulfide, and *o*-benzoyl thiamindisulfide. The molecular weight of the disulfide form is 562.7 Da, with a melting point of 177°C. While the hydrochloride form has a molecular weight of 337.3 Da the mononitrate form is 327.4 Da. The latter two are white crystalline powders whereas the disulfide form is a yellow crystal. Thiamin exhibits characteristic absorption maxima at 235 and 267 nm, corresponding to the pyrimidine and thiazole moieties, respectively.

When oxidized, the bridge is attacked and thiamin is converted to thiochrome. Thiochrome is biologically inactive. These structures are shown in Figures 3 and 4.

C. Thiamin Antagonists

Thiamin antagonists include pyrithiamin, a compound with the thiazole ring replaced by a pyridine, and oxythiamin, an analog having the C-4 amino group replaced by a hydroxyl group. It appears that thiamin activity is decreased when the number 2 position of the pyrimidine ring is changed.

Both molecules are potent thiamin antagonists but differ in their mechanisms of action. Oxythiamin is readily converted to the pyrophosphate and competes with thiamin for its place in the

TPP-enzyme systems. Pyrithiamin prevents the conversion of thiamin to TPP by interfering with the activity of thiamin kinase. Oxythiamin depresses appetite, growth, and weight gain and produces bradycardia, heart enlargement, and an increase in blood pyruvate, but it does not produce neurological symptoms. Pyrithiamin results in a loss of thiamin from tissues, bradycardia, and heart enlargement, but does not produce an increase in blood pyruvate.

A type of natural antagonist is a group of enzyme called thiaminases. The first antagonist was discovered by accident when raw fish was incorporated into a commercially available feed for foxes. Foxes fed this diet developed symptoms of thiamin deficiency. When heated, this enzyme is denatured and thus no longer is capable of destroying thiamin. The enzyme has several forms and has been found in fish, shellfish, ferns, betel nuts, and a variety of vegetables. Also found in tea and other plant foods are antithiamin substances that inactivate the vitamin by forming adducts. Tannic acid is one such substance; another is 3,4-dihydroxycinnamic acid (caffeic acid). Some of the flavinoids and some of the dihydroxy derivatives of tyrosine have antithiamin activity.

D. Assays for Thiamin

There are various chemical, microbiological, and animal assays available for thiamin. In animal tissues, thiamin occurs principally as phosphate esters, whereas in plants it appears in the free form. Both forms are protein bound.

The thiochrome method is the most widely used chemical assay for thiamin. It depends upon the alkaline oxidation of thiamin to thiochrome. Thiochrome, in turn, exhibits an intense blue fluorescence which can be measured fluorimetrically. Other chemical tests for thiamin are the formaldehyde-diazotized sulfanilic acid method, the diazotized *p*-aminoacetophenone method, and the bromothymol blue method. All of these assays must be preceded by extraction and removal of protein.

Lactobacillus viridescens is the microorganism most widely used to measure thiamin concentrations. It requires the intact thiamin molecule for growth. Other organisms are available but they are less useful.

Animal assays are used for determining the availability of thiamin in a food source. The rat is the preferred animal to use. The material being tested measures the curative effect of the food source on rats which have been made thiamin deficient and compares it to the curative effect of pure synthetic thiamin hydrochloride. The most sensitive assays are the chromatographic ones. Both HPLC (high performance liquid chromatography) and thin-layer chromatography yield excellent results. They have the advantages of sensitivity and reliability.

E. Sources

Thiamin is widely distributed in the food supply. Pork is the richest source, while highly refined foods have virtually no thiamin. Polished rice, fats, oils, refined sugar, and unenriched flours are in this group. Many products are made with enriched flour and so provide thiamin to the consumer. Enrichment means that the flour (or other food ingredient) has had thiamin added to it to the level that was there prior to processing. Peas and other legumes are good sources; the amount of thiamin increases with the maturity of the seed. Whole-grain cereal products contain nutritionally significant amounts of thiamin. Dried brewer's yeast and wheat germ are both rich in thiamin.

F. Absorption and Metabolism

Thiamin is absorbed by a specific active transport mechanism. In humans and rats, absorption is most rapid in the proximal small intestine. Studies *in vivo* on intact loops of rat small intestine revealed saturation kinetics for thiamin over the concentration range of 0.06 to 1.5 μ*M*. At higher

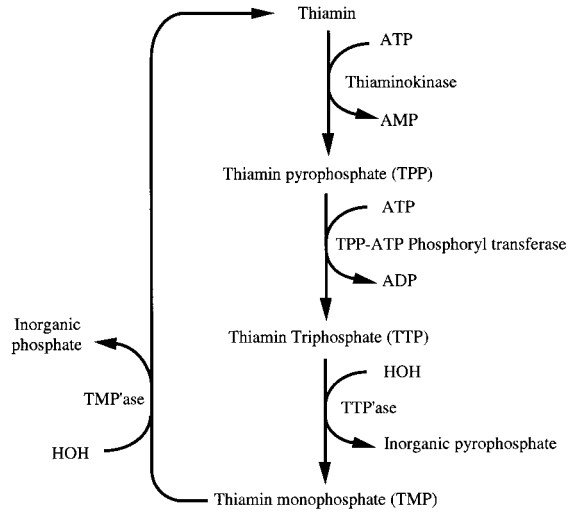
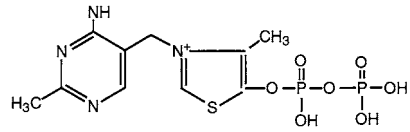


Figure 5 Formation of thiamin pyrophosphate through the phosphorylation of thiamin. About 80% of thiamin exists as TPP, 10% as TTP, and the remainder as TMP or free thiamin.



Thiamin pyrophosphate (TPP)

Figure 6 Structure of the coenzyme thiamin phyrophosphate.

concentrations (2 to 560 μ*M*) absorption was linearly related to the luminal thiamin concentration. *In vitro* studies using inverted jejunum sacs indicated an active transport mechanism, which is energy and Na^+ dependent and carrier mediated.

Thiamin undergoes phosphorylation either in the intestinal lumen or within the intestinal cells. This phosphorylation is closely related to uptake, indicating that the carrier may be the enzyme thiamin pyrophosphokinase. There is some argument about this, however. Figure 5 illustrates TPP synthesis.

While thiamin can accumulate in all cells of the body, there is no single storage site per se. The body does not store the vitamin and thus a daily supply is needed. Thiamin in excess of need is excreted in the urine. More than 20 metabolites have been identified in urine.

G. Biological Function

Thiamin is a part of the coenzyme thiamin pyrophosphate (TPP) (thiamin with two molecules of phosphate attached to it), also known as cocarboxylase, which is required in the metabolism of carbohydrates. Figure 6 illustrates this structure. The driving force for reactions with thiamin results because of the resonance possible in the thiazolium ring. The thiazolium ion, known as ylid, will form. Because of the formation of the ylid, the thiazole ring of TPP can serve as a transient carrier of a covalently bound "active" aldehyde group. Mg^{2+} is required as a cofactor for these reactions.

The metabolism of carbohydrates involves three stages in which the absence of thiamin as part of a coenzyme (TPP) leads to a slowing or complete blocking of the reactions. There are two

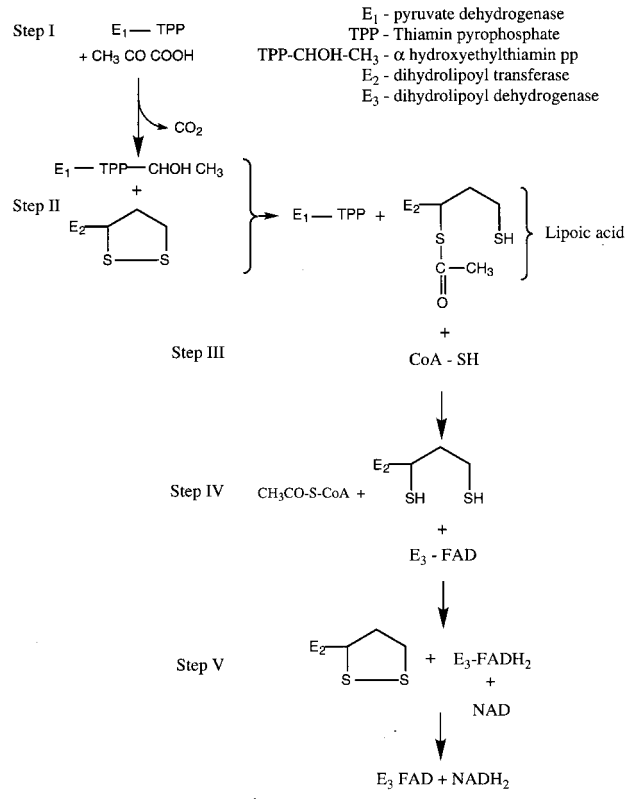


Figure 7 Oxidation of the pyruvate-mitochondria matrix.

oxidative decarboxylation reactions of α-ketoacids: the formation or degradation of α-ketols and the decarboxylation of pyruvic acid to acetyl CoA as it is about to enter the citric acid cycle. This reaction is catalyzed by the pyruvate dehydrogenase complex, an organized assembly of three kinds of enzymes. The mechanism of this action is quite complex. TPP, lipoamide, and FAD serve as catalytic cofactors; NAD and CoA serve as stoichiometric participants in the reaction.

As a consequence of the impairment of this reaction in thiamin deficiency, the level of pyruvate will rise. When thiamin is withheld from the diet, the ability of tissues to utilize pyruvate does not decline uniformly, indicating that there are tissue differences in the retention of TPP. Muscle retains more TPP than does brain. This role of thiamin arose from the discovery that thiamin alone promotes nonenzymatic decarboxylation of pyruvate to yield acetaldehyde and CO_2. Studies of this model revealed that the H at C-2 of the thiazole ring ionizes to yield a carbanion which reacts with the carbonyl atom of pyruvate to yield CO_2 and a hydroxyethyl (HE) derivative of the thiazole. The HE may then undergo hydrolysis to yield acetaldehyde or become oxidized to yield an acyl group. Figures 7 and 8 illustrate pyruvate metabolism and show where thiamin plays a role.

Thiamin is also active in the decarboxylation of α-ketoglutaric acid to succinyl CoA in the citric acid cycle. The mechanism of action is similar to that described above for pyruvate.

Step I. Similar to nonoxidative decarboxylation of pyruvate in alcohol fermentation.

Step II. The hydroxyethyl group is dehydrogenated and the resulting acetyl group is transferred to the sulfur atom at C-6 (or C-8) of lipoic acid, which constitutes the covalently bound prosthetic group of the second enzyme of the complex, lipoate acetyl transferase. The transfer of H^+ to the disulfide bond of lipoic acid converts the latter to its reduced or dithiol form, dihydrolipoic acid.

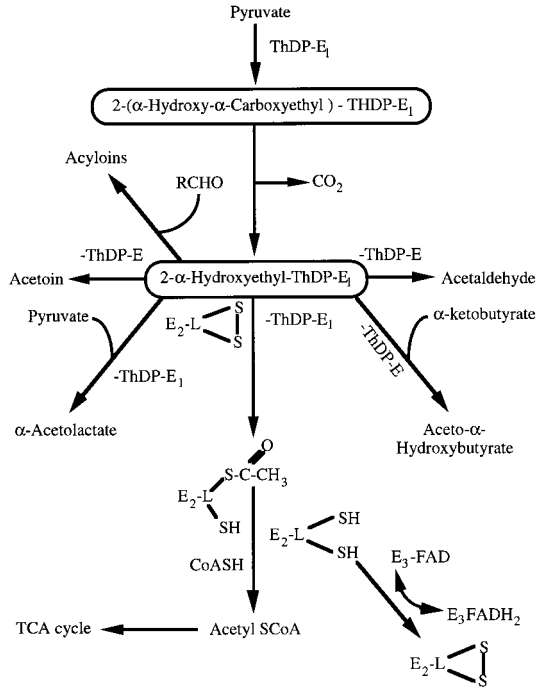


Figure 8 Summary of the metabolic pathways for pyruvate.

Step III. The acetyl group is enzymatically transferred to the thiol group of coenzyme A and a second H^+ is added to form the dihydrolipoyl transacetylase. The Ac CoA so formed leaves the enzyme complex in free form.

Step IV. The dithiol form of lipoic acid is reoxidized to its disulfide form by transfer of H^+ and associated electrons to the third enzyme of the complex, dihydrolipoyl (lipoamide) dihydrogenase, whose reducible prosthetic group is FAD. $FADH_2$, which remains bound to the enzyme, transfers its electron to NAD^+ to form NADH.

E_1 is regulated by PDH kinase and PDH phosphatase.

The oxidation of αKG to succinyl CoA is energetically irreversible and is carried out by the αKG DH complex:

$$\alpha KG + NAD^+ + CoA \Leftrightarrow \text{succinyl CoA} + CO_2 + NADH_2$$

$$\Delta G = 8.0 \text{ kcal mol}^{-1}$$

This reaction is analogous to the oxidation of pyruvate to acetyl-CoA and CO_2 and occurs by the same mechanism with TPP, lipoic acid, CoA, FAD, and NAD participating as coenzymes.

The metabolism of ethanol also requires thiamin. The same pyruvate dehydrogenase complex which converts pyruvate to acetyl-CoA will metabolize acetaldehyde (the first product in the metabolism of ethanol) to acetyl CoA. This system probably accounts for only a small part of ethanol degradation.

TPP participates in the transfer of a glycoaldehyde group from D-xylulose to D-ribose 5P to yield D-sedoheptulose 7P, an intermediate of the pentose phosphate pathway, and glyceraldehyde 3P, an intermediate of glycolysis. Transketolase contains tightly bound thiamin pyrophosphate. In this reaction the glycoaldehyde group (CH_2OH–CO) is first transferred from D-xylulose 5P to

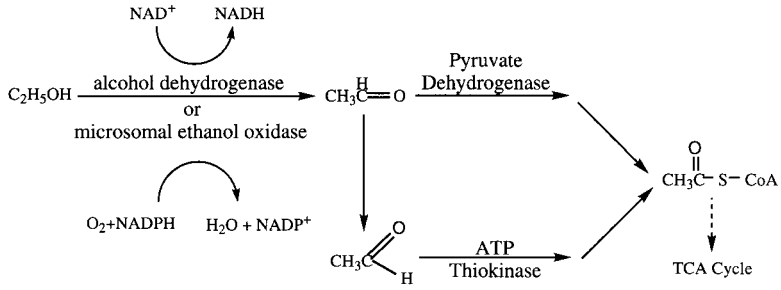


Figure 9 Metabolism of ethanol.

enzyme-bound TPP to form the α,β-dihydroxyethyl derivative of the latter, which is analogous to the α-hydroxyethyl derivative formed during the action of PDH. The TPP acts as an intermediate carrier of this glycoaldehyde group which is transferred to the acceptor molecule D-ribose 5P. This is a reaction in the hexose monophosphate shunt.

From the involvement of vitamin-containing coenzymes in the oxidation of alcohol (see Figure 9), it follows that vitamin deficiency could impair the rate of alcohol oxidation and thus increase the retention of alcohol in the blood of malnourished, chronic alcoholic subjects. Actually, there is little evidence for this assumption in animals or humans. The rate-limiting step appears not to be the level of vitamin concentration, but rather the amount of alcohol dehydrogenase present.

Large dietary intakes of carbohydrates will increase the need for thiamin. Ingestion of lipids, on the other hand, is considered to be thiamin sparing. This is a consequence of the fact that thiamin is required in the metabolism of lipids in fewer places than it is in the metabolism of carbohydrates.

In addition to its role as a coenzyme, it is speculated that thiamin has an independent role in neural tissue since it has been shown that stimulation of nerve fibers results in release of free thiamin and thiamin monophosphate. If a neurophysiologically active form of thiamin exists, it is as thiamin triphosphate. However, thiamin's role in the central nervous system is viewed at present as an intriguing enigma.

H. Deficiency

The major symptoms of thiamin deficiency (beriberi) are loss of appetite (anorexia), weight loss, convulsions, slowing of the heart rate (bradycardia), and lowering of the body temperature. Loss of muscle tone and lesions of the nervous system may also develop. Because the heart muscle can be weakened, there may be a cardiac failure resulting in peripheral edema and ascites in the extremities. The urine of rats with a thiamin deficit contains a higher pyruvate:lactate ratio than that of normal animals. Thiamin-deficient rats also exhibit a reduced erythrocyte transketolase activity. Administration of thiamin to rats brings about a remarkable reversal of deficiency symptoms in less than 24 hr.

Beriberi is classified into several types: acute-mixed, wet, or dry. The acute-mixed type is characterized by neural and cardiac symptoms producing neuritis and heart failure. In wet beriberi, the edema of heart failure is the most striking sign; digestive disorders and emaciation are additional symptoms. In dry beriberi, loss of function of the lower extremities or paralysis predominates; it is often called polyneuritis.

Thiamin deficiency is the most common vitamin deficiency seen in chronic alcoholics in the U.S. Clinical manifestations of the deficiency vary, depending upon the severity of the deprivation. However, all degrees of deficiency involve muscle and/or nerve tissue. The most serious form of thiamin deficiency in alcoholics is Wernicke's syndrome. It is characterized by ophthalmoplegia, 6th nerve palsy, nystagmus, ptosis, ataxia, confusion, and coma which may terminate in death.

Table 5 Clinical Features of Thiamin Deficiency

Wet and dry beriberi	Malaise Heaviness and weakness of legs Calf muscle tenderness "Pins and needles" and numbness in legs Anesthesia of skin, particularly at the tibia Increased pulse rate and palpitations
Wet beriberi	Edema of legs, face, trunk, and serous cavities Tense calf muscles Fast pulse Distended neck veins High blood pressure Decreased urine volume
Dry beriberi	Worsening of polyneuritis of early stage Difficulty walking Wernicke-Korsakoff syndrome; encephalopathy may occur: Disorientation Loss of immediate memory Nystagmus (jerky movements of the eyes) Ataxia (staggering gait)

Often the confusional state persists after treatment of the acute thiamin deficiency. This is known as Korsakoff's psychosis.

The next most serious level of thiamin deficiency is sometimes known as alcoholic beriberi. It is seen in those individuals who have had a minimal intake of thiamin. It is characterized by symmetrical foot and wrist drop associated with muscle tenderness. It may also affect cardiac muscle metabolism and may result in congestive heart failure.

The mildest and most common form of thiamin deficiency is the polyneuropathy affecting only the lower extremities of the chronic alcoholic. The signs and symptoms of thiamin deficiency are listed in Table 5. All the signs and symptoms of thiamin deficiency respond to thiamin treatment except the irreversible neurological lesions.

Red cell transketolase activity seems to be a sensitive index of thiamin nutritional status. Brin et al. have suggested an *in vitro* test using transketolase to differentiate between the enzymatic lesions caused by thiamin deficiency and those due to nonspecific causes. This test consists of stimulation of enzyme activity in the presence of saturating amounts of TPP. This so-called TPP effect (TPPE) is claimed to be a true measure of thiamin nutritional status.

In addition to the enzymatic test, a measure of urinary thiamin in relation to dietary intake has been the basis for balance studies to assess the adequacy of intake. When thiamin excretion is low a larger portion of the test dose is retained, indicating a tissue need for thiamin. A high excretion indicates tissue saturation. In the deficient state, excretion drops to zero.

I. Recommended Dietary Allowance

The thiamin needs of an individual are influenced by age, energy intake, carbohydrate intake, and body weight. Table 6 gives the RDAs for humans. The Food and Nutrition Board, on the basis of considerable evidence, recommends 0.5 mg/1000 kcal (4184 kJ). Because there is some evidence that older persons use thiamin less efficiently, it is recommended that they maintain an intake of 1 mg/day even if they consume less than 2000 kcal (8368 kJ) daily.

Since thiamin needs increase during pregnancy and lactation, an additional 0.4 mg/day is recommended during pregnancy and 0.5 mg/day during lactation.

Table 6 Recommended Dietary Allowances for Thiamin

Group	Age	RDA (mg/day)
Infants	Birth–6 months	0.3
	6–12 months	0.4
Children	1–3	0.7
	4–6	0.9
	7–10	1.0
Males	11–14	1.3
	15–18	1.5
	19–24	1.5
	25–50	1.5
	51+	1.2
Females	11–14	1.1
	15–18	1.1
	19–24	1.1
	25–50	1.1
	51+	1.0
Pregnancy	—	1.5
Lactation	—	1.6

Few studies have assessed the thiamin nutritional status of infants and children. The Food and Nutrition Board recommends 0.5 mg/1000 kcal (4184 kJ).

J. Toxicity

Although thiamin produces a variety of pharmacological effects when administered in large doses, the dose required is thousands of times greater than those required for optimal nutrition. Generally, toxic effects are reported with subcutaneous, intramuscular, intraspinal, or intravenous injections, but not with oral administration. In rare cases, thiamin excess has caused reactions in humans resembling anaphylactic shock. These usually develop only in individuals who have been given several large intravenous injections and may be related to the development of hypersensitivity to thiamin.

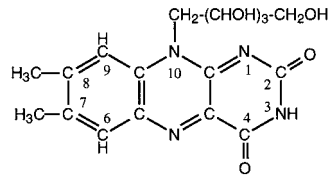
III. RIBOFLAVIN

A. Overview

While thiamin and niacin were being recognized as the causative factors in beriberi and pellagra, respectively, riboflavin, another B vitamin was ignored. A food fraction isolation by McCollum and Kennedy in 1916 was shown by Emmett and Luros, and later by Goldberger and Lillie, to have several functions: one as beriberi curative, another as a pellagra curative, and a third whose function was not known. These fractions were all water soluble but differed in their stability towards heat and light. In 1932 Warburg and Christian isolated and described a flavoprotein which was recognized by others as having vitamin properties. This became the vitamin, riboflavin. Since it was first isolated with thiamin and niacin it was given a letter designation in England as vitamin B_2 and in the U.S. as vitamin G. As our knowledge of vitamins increased, this nomenclature was dropped in favor of the name riboflavin.

B. Structure, Chemical and Physical Properties

Riboflavin (Figure 10) is a highly colored crystalline substance frequently associated with flavoproteins. As a solid, it is red-orange in color. In solution the color changes to a greenish yellow.



7,8-dimethyl-10-(1′ D-ribityl)-isoalloxazine
or
6,7-dimethyl-9-(D-1′ ribityl)-isolloxazine
if only the carbons are numbered
as in the older literature.

Figure 10 Structure of riboflavin.

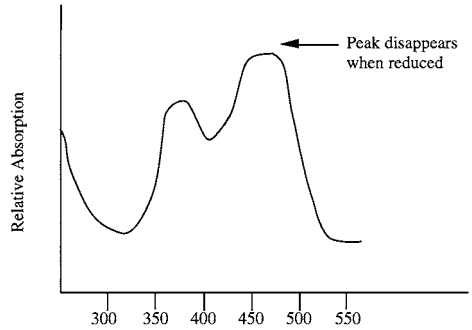


Figure 11 Absorption spectra of riboflavin.

It was first synthesized by Kuhn and also by Karrer et al. in 1935 as needle-like crystals with limited solubility in pure water or in acid solutions. Solubility increases as the pH of the solvent increases; however, as the pH of the solution rises, riboflavin's stability to heat and light decreases. Milk loses 33% of its riboflavin activity in 1 hr of sunlight. In solution, riboflavin is easily destroyed by light and must be protected at all times from exposure. Biochemists working with riboflavin take such precautions as using deep-red glassware and darkened work areas to ensure maximal recovery or assessment of vitamin activity. Because riboflavin has several absorbance maxima and fluoresces due to a shifting of bonds in the isoalloxazine ring, its presence can be quantified by spectrophotometric or photofluorometric techniques. Fluorescence can be measured before and after reduction by such compounds as sodium hydrosulfite. The reduced (hydroquinones) flavins do not fluoresce whereas oxidized flavins do. Fluorescence is pH dependent and is best measured between pH 4 to 8; maximal fluorescence occurs at 556 nm.

The oxidized forms of different flavoenzymes are intensely colored. They are characteristically yellow, red, or green due to strong absorption bands in the visible range. Upon reduction, they undergo bleaching with a characteristic change in the absorption spectrum. Figure 11 shows the change in absorption with changes in wavelength.

In order to have vitamin activity, positions 8 and 7 must be substituted with more than just a hydrogen and the imine group in position 3 must be unsubstituted. There must be a ribityl group on position 10. If the ribityl group is lost then vitamin activity is lost, as depicted in Figure 12 where photodecomposition is shown. There are some antivitamins that interfere with riboflavin's usefulness. These compounds compete for the prosthetic groups or competitively inhibit its phosphorylation and adenylation to form the coenzymes FMN (flavin mononucleotide) or FAD (flavin adenine dinucleotide), respectively. The structures of these coenzymes are shown in Figure 13.

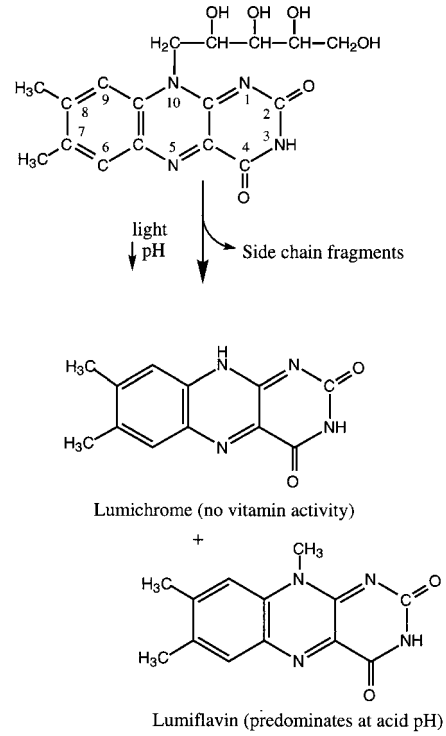


Figure 12 Degradation of riboflavin by light and acid or acidic conditions.

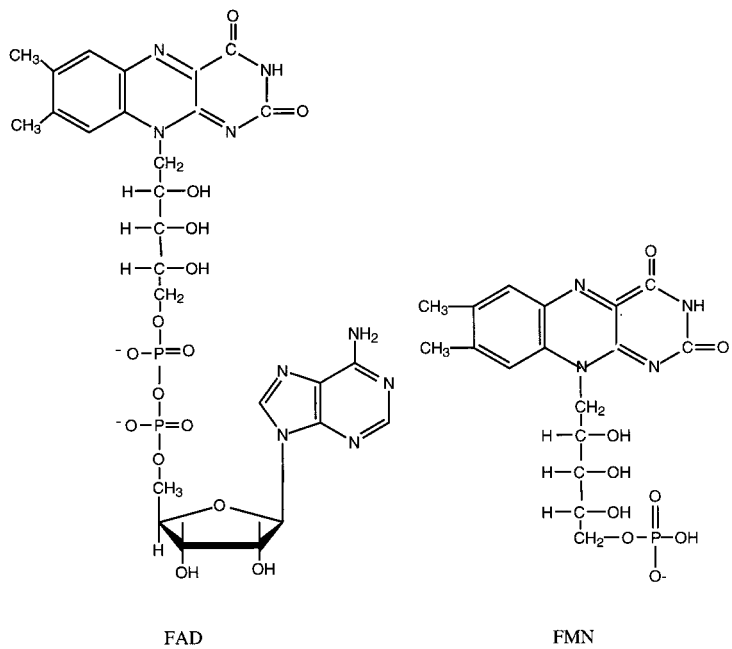


Figure 13 Structures of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

C. Sources

The best sources are foods of animal origin: milk, meat, and eggs. Wheat germ is also a good source.

D. Assay

The most sensitive and selective procedure for the determination of riboflavin is that which uses fluorescence detection coupled with HPLC. The vitamin and coenzymes must be protected from light and acid. The coenzyme forms can be separated from the free form by differential solubility. The free form is soluble in benzyl alcohol while the coenzymes are not.

E. Absorption, Metabolism

Absorption occurs by way of an active carrier and is energy and sodium dependent. Maximum absorption occurs in the proximal segment (the jejunum) of the small intestine, with significant uptake by the duodenum and ileum. After a load dose, peak values in the plasma appear within 2 hr. The phosphorylated forms (coenzyme forms) are dephosphorylated prior to absorption through the action of nonspecific hydrolases from the brush border membrane of the duodenum and jejunum. There is a pyrophosphatase which cleaves FAD and an alkaline phosphatase which liberates the vitamin from FMN. Bile salts appear to facilitate uptake, and a small amount of the vitamin circulates via the enterohepatic system. Prior to entry into the portal blood, some of the vitamin is rephosphorylated to re-form FAD and FMN. After absorption, the vitamin circulates in the blood bound to plasma proteins, notably albumin and certain gamma globulins.

Specific riboflavin-binding proteins have been isolated and identified in several species. These proteins are of hepatic origin. There is facilitated, mediated uptake of the free vitamin by all of the vital organs. For example, isolated liver cells will accumulate up to five times the amount of the vitamin in the fluids which surround them. Although cells will accumulate the vitamin against a concentration gradient, these cells also use the vitamin quite rapidly, so there is little net storage.

The usual blood levels of riboflavin are in the range of 20 to 50 μg/dl while 500 to 900 μg/day are excreted in the urine. Excretion products include 7- and 8-hydroxymethylriboflavin, 8-α-sulfonylriboflavin, riboflavin peptide esters, 10-hydroxyethylflavin, lumiflavin, 10-formylmethylflavin, 10-carboxymethylflavin, lumichrome, and free riboflavin. Very small amounts of riboflavin and its metabolites can be found in the feces.

Upon entry into the cell, riboflavin is reconverted to FMN and FAD, as shown in Figure 14. The initial phosphorylation reaction is zinc dependent. FMN and FAD synthesis is responsive to thyroid status. Hyperthyroidism is associated with increased synthesis whereas hypothyroidism is associated with decreased synthesis. FAD is linked to a variety of proteins via hydrogen bonding, and also to the purine portion of FAD, the phenolic ring of tyrosyl residues, and indolic-tryptophanyl residues in flavoproteins. Covalent bonding with certain enzymes also occurs, and involves the riboflavin 8-methyl group which forms a methylene bridge to the peptide histidyl-1 or 3-imidazole functions or to the thioether function of a former cysteinyl residue. When bound to these proteins, these coenzymes are somewhat protected from degradation although the proteins themselves are eventually degraded. However, flavins in excess of that which are protein bound are more rapidly degraded and excreted in the urine.

Degradation involves the oxidation of the ribityl chain and hydroxylation at positions 7 and 8 of the isoalloxazine ring by hepatic microsomal cytochrome P450 enzymes. The methyl groups at these positions are removed and the compound loses its activity as a vitamin. Because degradation and excretion occur at a fairly rapid rate, the rate of riboflavin degradation determines the requirement for the vitamin rather than the need for the vitamin in its function as a coenzyme, that is, the rate of FMN and FAD synthesis.

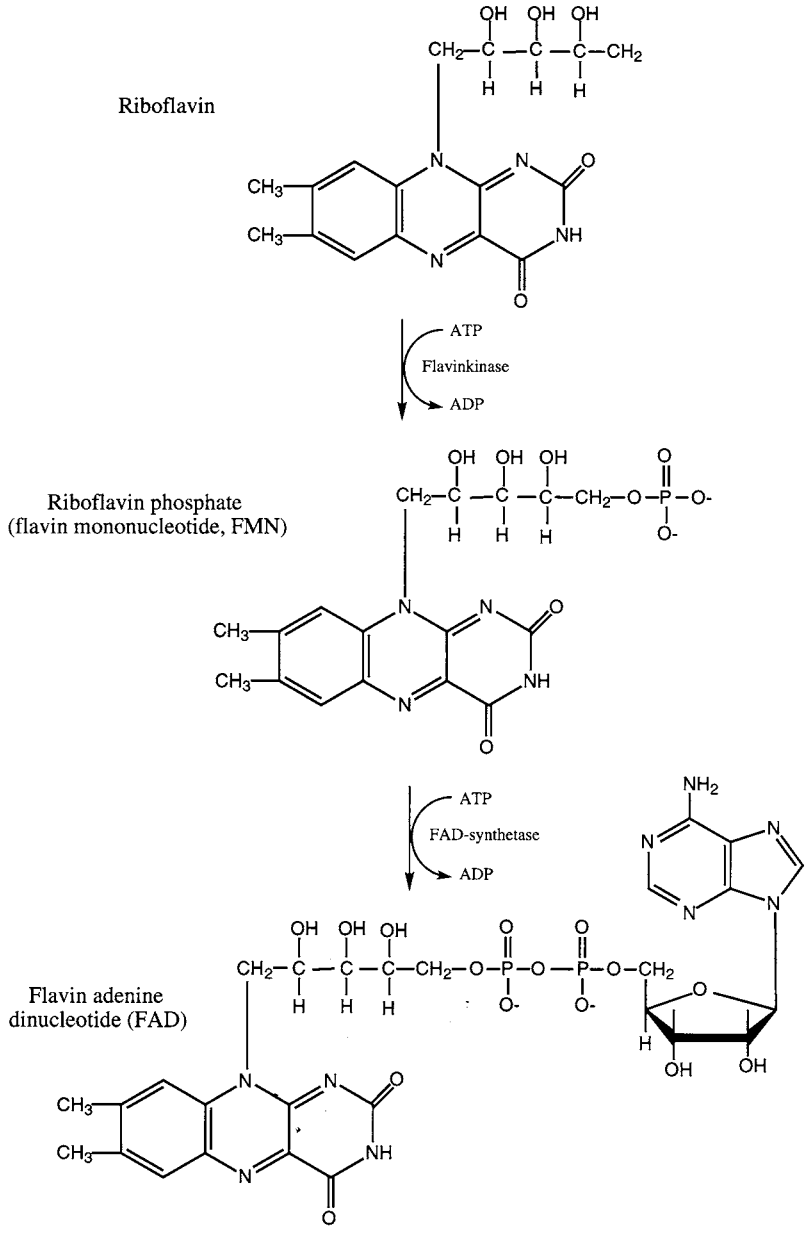


Figure 14 Synthesis of FMN and FAD.

F. Functions

FAD and its precursor FMN are coenzymes for reactions that involve oxidation-reduction. Thus, riboflavin is an important component of intermediary metabolism. The respiratory chain in the mitochondria and reactions in numerous pathways that utilize either FAD or FMN as coenzymes require riboflavin. Shown in Table 7 is a list of some of these enzymes. They include reactions where reducing equivalents are transferred between cellular compartments as part of a shuttle arrangement, as well as reactions that are in a mitochondrial or cytosolic sequence.

Table 7 Reactions Using FAD or FMN

FAD-Linked Enzymes	
Ubiquinone reductase	Xanthine oxidase
Monoamine oxidase	Cytochrome reductase
NADH-cytochrome P 450 reductase	Succinate dehydrogenase
D-Amino acid oxidase	α-Glycerophosphate dehydrogenase
Acyl CoA dehydrogenase	Electron transport respiratory chain
Dihydrolipoyl dehydrogenase (component of PDH and α-KGDH)	Glutathione reductase
FMN-Linked Enzymes	
NADH dehydrogenase (respiratory chain)	Lactate dehydrogenase
L-Amino acid oxidase	Pyridoxine (pyridoxamine)5′-phosphate oxidase

In most flavoenzymes, the flavin nucleotide is tightly but noncovalently bound to the protein; exceptions given in Table 7 include succinate dehydrogenase and monoamine oxidase, in which the flavin nucleotide, FAD, is covalently bound to a histidine residue of the polypeptide chain in the former case and a cysteinyl residue in the latter. The metalloflavoproteins contain one or more metals as additional cofactors. Flavin nucleotides undergo reversible reduction of the isoalloxazine ring in the catalytic cycle of flavoproteins to yield the reduced nucleotides $FMNH_2$ and $FADH_2$. The enzymes that have a riboflavin-containing coenzyme are of three general types:

1. Enzymes whose substrate is a reduced pyridine nucleotide and the acceptor is either a member of the cytochromes or another acceptor.
2. Enzymes that accept electrons directly from the substrate and can pass them to one of the cytochromes or directly to oxygen.
3. Enzymes that accept electrons from substrate and pass them directly to oxygen (true oxidases).

A simplified mechanism of action is shown in Figure 15.

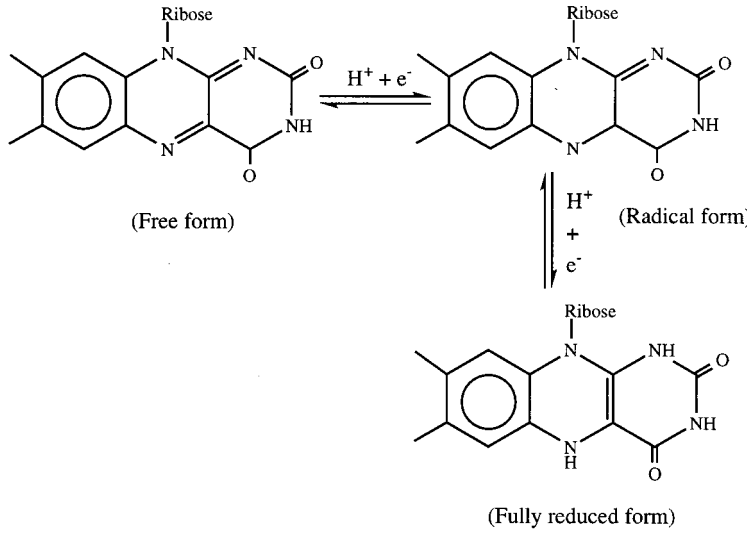


Figure 15 Mechanism of action of the riboflavin portion of the coenzyme.

Each of the steps in this sequence is reversible to the extent limited by the flavoprotein's capacity to accept or donate reducing equivalents which, in turn, can be joined to oxygen. Many of the flavoproteins also contain a metal such as iron, molybdenum, or zinc, and the combination of these metals and the flavin structure allows for its easy and rapid transition between single- and double-electron donors.

Note in Table 7 that a number of enzymes are members of the oxidase family of enzymes. The oxidases transfer hydrogen directly to oxygen to form hydrogen peroxide. Xanthine oxidase uses a variety of purines as its substrate, converting hypoxanthine to xanthine which is then converted to uric acid. Xanthine oxidase also catalyzes the conversion of retinal to retinoic acid (see Unit 3).

Among the important enzymes shown in Table 7 are those that are essential to mitochondrial respiration and ATP synthesis as well as to the mitochondrial citric acid cycle. Succinate dehydrogenase is one of these and its activity has been used as a biomarker of riboflavin intake sufficiency. The acyl CoA dehydrogenases catalyze another of the essential pathways, fatty acid oxidation. These are FAD linked. Fatty acid synthesis requires the presence of FMN-linked enzymes. The FMN-dependent pyridoxine (pyridoxamine) 5′-phosphate oxidase is essential for conversion of the two forms of vitamin B_6 to its functional coenzyme, pyridoxal-5′-phosphate. This is another example of vitamin-vitamin interaction. While the list of enzymes shown in Table 7 is by no means complete, it gives evidence of the intimate and essential need for riboflavin in the regulation of metabolism. In its absence, profound impairments can be expected and death should follow in a short time once all of the FAD and FMN are used up. In humans, clinical signs of deficiency appear in less than 6 weeks on intakes of less than 0.6 mg/day.

G. Deficiency

Despite our knowledge about riboflavin's function as a coenzyme, there are few symptoms that are specific to riboflavin deficiency. Poor growth, poor appetite, and certain skin lesions (cracks at the corners of the mouth, dermatitis on the scrotum) have been observed. However, some of these symptoms can occur for reasons apart from inadequate riboflavin intake, as in vitamin B_6 deficiency. As mentioned in the preceding section, the oxidase needed to convert B_6 to its functional form requires riboflavin as FMN. This lack of direct correlation of symptoms to intake is also due to the almost universal need for FAD and FMN as coenzymes in intermediary metabolism. Thus, it is impossible to pinpoint a specific deficiency symptom. Nutrition assessment of adequate riboflavin intake relies upon a few reactions in readily available cells, i.e., blood cells, that can predict intake adequacy. Erythrocyte FAD-linked glutathione reductase is one of these. Low enzyme activity is associated with inadequate intakes. Succinate dehydrogenase is another enzyme frequently used in nutrition assessment.

H. Recommended Dietary Allowance

As mentioned, there is almost no riboflavin reserve. Thus, a daily intake of riboflavin is essential. The RDAs for humans are shown in Table 8.

IV. NIACIN

A. Overview

Few vitamins have as tortuous a history of discovery as niacin, otherwise known as vitamin B_3, or nicotinic acid, or niacinamide. The synthesis of nicotinic acid was accomplished long before it

Table 8 Recommended Dietary Riboflavin Allowances for Humans

	Age	Riboflavin (mg/day)
Infants	Birth to 6 months	0.4
	7 months to 1 year	0.5
Children	1–3	0.8
	4–6	1.1
	7–10	1.2
Males	11–14	1.5
	15–18	1.8
	19–24	1.7
	25–50	1.7
	51+	1.4
Females	11–14	1.3
	15–18	1.3
	19–24	1.3
	25–50	1.3
	51+	1.2
Pregnant	—	+1.6
Lactating	1st 6 months	+1.8
	2nd 6 months	1.7

was discovered to be a vitamin. Some 50 years elapsed before it was connected to the disease pellagra. Pellagra was described in the mid-1800s in Italy and Spain and called "mal de la rosa" in the latter country. Its development was associated with the consumption of low-protein high-corn diets. The disease was more prevalent in very poor populations and associated with the consumption of corn. At one time it was thought to be due to a toxin found in corn; however, as descriptions of pellagra arose in the literature from populations that did not consume corn, this idea was discarded. Some years later, Goldberger demonstrated that pellagra was a nutrient deficiency disease and that the nutrient in question was niacin. The term niacin is a generic term which includes both the acid and amide forms.

Nicotinic Acid (Niacin) Nicotinamide

Figure 16 Structures of nicotinic acid (niacin) and nicotinamide.

B. Structure, Physical and Chemical Properties

Niacin occurs in two forms (as an acid or as an amide) as shown in Figure 16. The vitamin is widely distributed in nature. Nicotinamide is the primary constituent of the coenzymes NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide dinucleotide phosphate). The synthesis of these pyridine nucleotides is shown in Figure 17.

The molecular weight of nicotinic acid is 123.1 Da and that of nicotinamide is 122.1 Da. Nicotinamide is far more soluble in water than is nicotinic acid. Both are white crystals with an absorption maxima of 263 nm. The melting point of the acid form is 237°C while that of the amide is 128 to 131°C. In order to have vitamin activity there must be a pyridine ring substituted with a β-carboxylic acid or corresponding amide and there must be open sites at pyridine carbons 2 through 6.

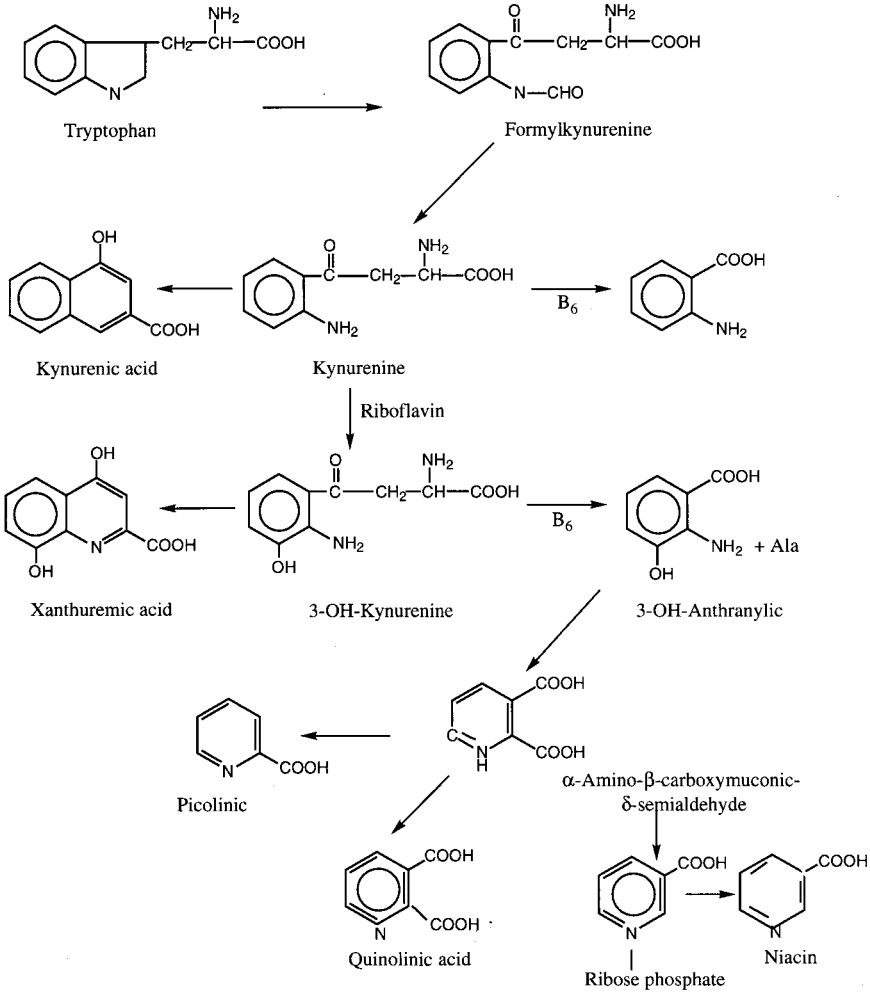


Figure 17 Synthesis of niacin from tryptophan.

Nicotinic acid is amphoteric and forms salts with acids and bases. Its carboxyl group can form esters and anhydrides and can be reduced. Both the acid and amide forms are very stable in the dry form, but when the amide form is in solution it is readily hydrolyzed to the acid form.

Several substituted pyridines can antagonize the biological activity of niacin. These include pyridine-3-sulfonic acid, 3-acetylpyridine, isonicotinic acid hydrazide, and 6-aminonicotinamide. HPLC is the analytical method of choice for this vitamin which does not occur in large amounts as the free form. Most often, it occurs as the coenzyme NAD^+ or $NADP^+$. Chemical analysis using the Koenig reaction, which opens up the pyridine ring with cyanogen bromide, followed by reaction with an aromatic amine to form a colored product, is one technique that is used. The most widely used method employs a chromophore-generating base, *p*-methylaminophenol sulfate, sulfanilic acid, or barbituric acid. The color intensity so developed is dependent on the concentration of the vitamin.

C. Sources

This vitamin is widely distributed in the human food supply. Especially good sources are whole-grain cereals and breads, milk, eggs, meats, and vegetables that are richly colored.

D. Absorption, Metabolism

Both nicotinic acid and nicotinamide cross the intestinal cell by way of simple diffusion and facilitated diffusion. There are species differences in the mechanism of absorption. In the bullfrog, absorption is via active transport. In the rat there is evidence of a transporter that is saturable and sodium dependent. This suggests facilitated diffusion. After absorption the vitamin circulates in the blood in its free form, as shown. That which is not converted to NAD^+ or $NADP^+$ is metabolized further and excreted in the urine. The excretory metabolites are N′-methylnicotinamide, nicotinuric acid, nicotinamide-N′-oxide, N′-methylnicotinamide-N′-oxide, N′-methylnicotinamide-N′-oxide, N′-methyl-4-pyridone-3-carboxamide, and N′-methyl-2-pyridone-5-carboxamide. Niacin can be synthesized from tryptophan in a ratio of 60 molecules of tryptophan to 1 of nicotinic acid. The pathway for conversion is shown in Figure 17. Note the involvement of thiamin, vitamin B_6, and riboflavin in this conversion.

E. Function

The main function of this vitamin is that of the coenzymes NAD^+ and $NADP^+$. Both function in the maintenance of the redox state of the cell. These coenzymes are bound to the protein (apoenzyme) portions of dehydrogenases relatively loosely during the catalytic cycle and therefore serve more as substrates than as prosthetic groups. They act as hydride ion acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom of the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidized NAD^+ or $NADP^+$ forms of these coenzymes to yield the reduced forms. The other hydrogen ion exchanges with water. Thus, the reduced coenzyme is represented as $NADH^+H^+$ or $NADPH^+H^+$. Most enzymes are specific for NAD or NADP and these enzymes are members of the oxidoreductase family of enzymes. Some will use either, e.g., glutamate dehydrogenase.

Most of the NAD- or NADP-linked enzymes are involved in catabolic pathways, e.g., glycolysis or the pentose phosphate shunt. NAD turns over quite rapidly in the cell. Its degradation is shown in Figure 18.

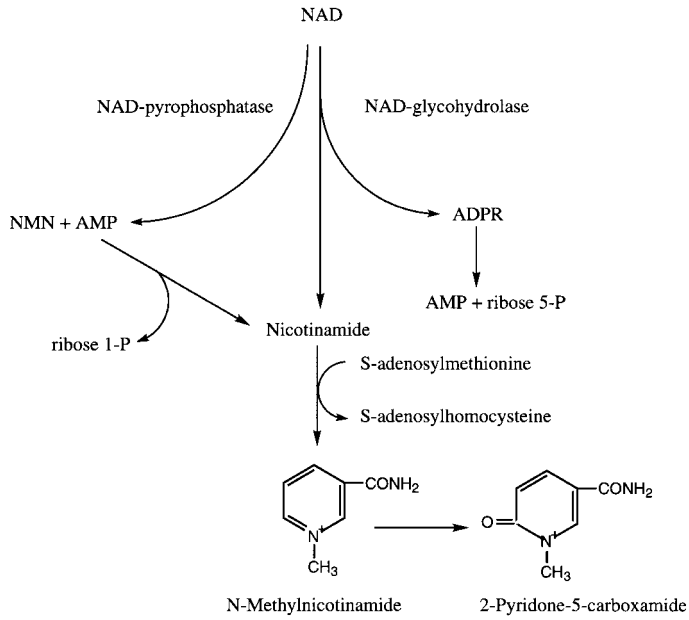


Figure 18 Degradation of NAD.

Beyond its use in biological systems as a precursor of NAD^+ or $NADP^+$, nicotinic acid has a pharmacological use. Nicotinic acid, the drug, is used as a lipid-lowering agent. Large intakes (1 g/day) lower serum cholesterol. However, large doses also result in flushing due to its effect on vascular tone. Nicotinic acid elicits a fibrinolytic activation of very short duration. Both nicotinic acid and nicotinamide can be toxic if administered at levels greater than 10 μmol/kg. Chronic administration of 3 g/day to humans results in a variety of symptoms including headache, heartburn, nausea, hives, fatigue, sore throat, dry hair, inability to focus the eyes, and skin tautness. In experimental animals, nicotinic acid supplements result in a reduction in adipocyte free fatty acid release by streptozotocin-diabetic rats, an inhibition of adipocyte adenylate cyclase activity in normal hamsters, and degenerative changes in the heart muscle of normal rats. All of these responses are those that characterize a defense against a toxic exposure to nicotinic acid rather than a response to a normal intake level.

F. Deficiency

Pellagra has been well described as the niacin deficiency disease. It is characterized by skin lesions that are blackened and rough, especially in areas exposed to sunlight and abraded by clothing. The typical skin lesions of pellagra are accompanied by insomnia, loss of appetite, weight loss, soreness of the mouth and tongue, indigestion, diarrhea, abdominal pain, burning sensations in various parts of the body, vertigo, headache, numbness, nervousness, apprehension, mental confusion, and forgetfulness. Many of these symptoms can be related to niacin deficiency-induced deficits in the metabolism of the central nervous system. This system has glucose as its choice metabolic fuel. Glycolysis, with its attendant need for NAD^+ as a coenzyme, is appreciably less active. As the deficient state progresses, numbness occurs, followed by a paralysis of the extremities. The more advanced cases are characterized by tremor and a spastic or ataxic movement that is associated with peripheral nerve inflammation. Death from pellagra ensues if the patient remains untreated.

More subtle biochemical changes have also been reported in experimental niacin deficiency. It is now well known that NAD^+ is the substrate for poly (ADP-ribose) polymerase, an enzyme associated with DNA repair. In the deficient state this repair does not occur readily, and one of the characteristics of niacin deficiency is an increase in DNA strand breaks. If niacin deficiency accompanies conditions known to increase oxidative damage via free-radical attack on DNA, then the two conditions are additive with respect to cell damage and subsequent tissue pathology. Such has been proposed as a mechanism for the induction of cancer in susceptible cells.

Early indications of niacin deficiency include reductions in the levels of urinary niacin metabolites, especially those that are methylated (N′-methyl-nicotinamide and N′-methyl-2-pyridone-5-carboxamide). Since the discovery of the curative power of nicotinic acid and nicotinamide, pellagra is very rare, except in the alcoholic population. This population frequently substitutes alcoholic beverages for food and thereby is at risk for multiple nutrient deficiencies including pellagra. The metabolism of ethanol is NAD^+ dependent. This dependency drives up the need for niacin in the face of inadequate intake, setting the stage for alcoholic pellagra. In part, the CNS symptoms of alcoholism are those of pellagra as described above.

There is another very small population at risk for developing niacin deficiency. This group carries a mutation in the gene for tryptophan transport. This results in a condition called Hartnup's disease. Its symptoms, apart from tryptophan inadequacy effects on protein synthesis, are very similar to those of niacin deficiency. This is because of the use of tryptophan as a precursor of nicotinic acid. If niacin supplements are given to people with Hartnup's disease, these pellagra-like symptoms disappear.

G. Recommended Dietary Allowance

Because tryptophan can be converted to nicotinic acid, the RDA is stated in terms of niacin equivalents. A niacin equivalent is equal to 1 mg niacin or 60 mg of tryptophan. The need is related

to energy intake as well, particularly the carbohydrate intake. However, the RDA takes into account varying diet composition as well as individual differences in nutrient need. Age and gender also influence need and these factors are considered in Table 9.

Table 9 Recommended Dietary Allowances for Niacin Equivalents (NE)

Group	Age	NE (mg/day)
Infants	Birth to 6 months	5
	7–12 months	6
Children	1–3	9
	4–6	12
	7–10	13
Males	11–14	17
	15–18	20
	19–24	19
	25–50	19
	51+	15
Females	11–14	15
	15–18	15
	19–22	15
	23–50	15
	51+	13
Pregnancy	—	17
Lactation	—	20

V. VITAMIN B_6

A. Overview

Of all the B vitamins whose nomenclatures have been changed to trivial names, one vitamin remains known by its letter designation: vitamin B_6. The vitamin was first defined by Gyorgy in 1934 as "that part of the vitamin B complex responsible for the cure of a specific dermatitis developed by rats on a vitamin-free diet supplemented with thiamin and riboflavin." The dermatitis is unlike that seen with other deficiencies of the B complex. There is a characteristic scaliness about the paws and mouth of rats in addition to a loss of hair from the body. The dermatitis is called rat acrodynia.

The vitamin was crystallized in 1938 by three different groups of researchers and was subsequently characterized and synthesized. Even though the vitamin was identified, crystallized, and synthesized in the late 1930s, it was not realized until 1945 that there were three distinct forms of the vitamin. Pyridoxine was isolated primarily from plant sources while pyridoxal and pyridoxamine were isolated from animal tissues. The latter two are more potent growth factors for bacteria and are more potent precursors for the coenzymes pyridoxal phosphate and pyridoxamine phosphate. When commercially prepared (synthesized) the vitamin is commonly available as pyridoxine hydrochloride.

B. Structure, Physical and Chemical Properties

Vitamin B_6 occurs in nature in three different forms which are interconvertible. It can be an aldehyde (pyridoxal), an alcohol (pyridoxine), or an amine (pyridoxamine). These three forms are shown in Figure 19. Vitamin B_6 is the generic descriptor for all 2-methyl-3-hydroxy-5-hydroxymethyl pyridine derivatives. To have vitamin activity it must be a pyridine derivative, be phosphorylatable at the 5-hydroxymethyl group, and the substituent at carbon 4 must be convertible to the aldehyde form.

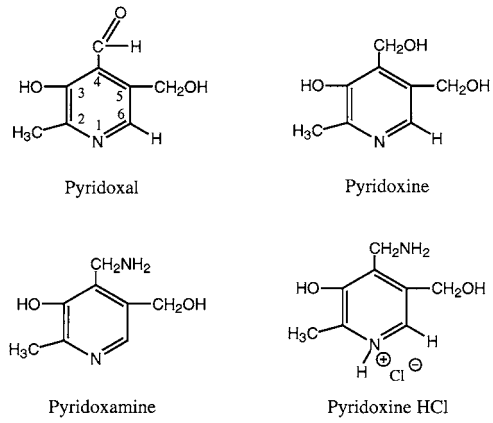


Figure 19 Structures of naturally occurring vitamin B_6.

Pyridoxine hydrochloride is the commercially available form of the vitamin and is shown in Figure 19. The molecular weight of pyridoxal is 167.2 Da and pyridoxine HCl has a molecular weight of 205.6 Da. Both occur as white crystals that are readily soluble in water. Pyridoxine is stable to light and heat in acid solutions. In neutral or alkaline solutions it is unstable to light and heat. The aldehyde form (pyridoxal) is much less stable. Its instability to heat is a major concern in food processing since foods that are rich in the vitamin are neutral to slightly alkaline. When heat treated, as is necessary to kill food-borne pathogens and prevent spoilage, vitamin activity may be lost. This is particularly true for foods that are autoclaved (i.e., infant formulas).

Pyridoxine, pyridoxal, and pyridoxamine can be assayed in a variety of techniques. Pyridoxal has an absorption maxima of 293 nm while pyridoxine HCl has absorption maxima of 255 and 326 nm. Microbiological, colorimetric/spectrophotometric, and chromatographic techniques are available. The method of choice is HPLC (high performance liquid chromatography).

C. Sources

Pyridoxine, pyridoxal, and pyridoxamine are widely distributed throughout the food supply. They are present in both plant and animal foods. Meats, cereals, legumes, lentils, nuts, fruits, and vegetables all contain the vitamin. Thus, persons consuming a diet containing a variety of raw and cooked foods likely will not develop a deficiency of the vitamin.

D. Absorption and Metabolism

In vivo and *in vitro* work with the rat and hamster small intestine provided no evidence of an active transport mechanism for the vitamin, however, more recent work suggests that facilitated diffusion may exist. Uptake into everted sacs of rat jejunum over a concentration range of 0.01 to 10 m*M* of pyridoxine was not inhibited by anoxia, DNP, lack of sodium, ouabain, or the presence of a structural analog 4-deoxypyridoxine. Thus, pyridoxine uptake is by passive and facilitated diffusion rather than by active transport (Figure 20). Once absorbed, it is carried by the erythrocytes to all cells in the body. Significant amounts of the vitamin may be found in liver, brain, spleen, kidney, and heart but, like the other water-soluble vitamins, there is no appreciable storage and this vitamin must be present in the daily diet. It is carried in the blood tightly bound to proteins, primarily hemoglobin and albumin. The vitamin binds via the amino group of the N-terminal valine residue of the hemoglobin α chain and this binding has twice the strength of its binding to albumin.

The B_6 vitamers are converted via a saturable two-step process to pyridoxal phosphate (PPS). The reactions, shown in Figure 21, are catalyzed by a B_6 vitamer kinase — an enzyme present in

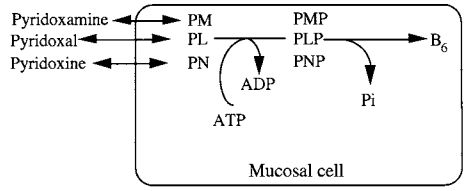


Figure 20 Absorption of the B_6 vitamins.

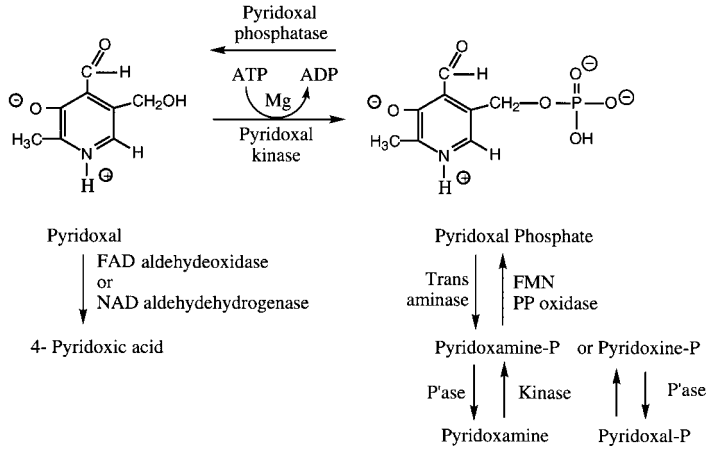


Figure 21 Phosphorylation of pyridoxal.

the cytoplasm of the mucosal cell. When the vitamers are phosphorylated, transmural absorption decreases whereas uptake is unaffected. Phosphorylation thus serves as a means of control of the cellular PMP, PLP, and PNP levels. This is called "metabolic trapping". Pyridoxine phosphate (PNP) and pyridoxamine phosphate (PMP) are oxidized by an FMN-dependent oxidase to form pyridoxal phosphate (PLP). At physiologic pH, zwitterionic structures pyridoxal and pyridoxal phosphate exist. These are shown in Figure 21. Pyridoxal phosphate can be converted (as shown) to either pyridoxine phosphate or pyridoxamine phosphate.

A major metabolite is 4-pyridoxic acid. It accounts for 50% of B_6 excreted in the urine. The reaction sequence is shown in Figure 21. Other metabolites have been found in the urine in addition to the three forms of the vitamin. Amphetamines, chlorpromazine, oral contraceptives, and reserpine all increase B_6 loss. Oral contraceptives had been found to increase tryptophan use and thus increase B_6 use. However, the current use of the minipill with its far smaller dose of hormones may not have this effect. The original observations were made in females using the larger dose of hormones.

E. Function

Pyridoxal phosphate serves as a coenzyme in reactions whose substrates contain nitrogen. Well over 100 reactions are known which involve pyridoxal phosphate. Many of these are transaminase reactions. Reactions such as transamination, racemization, decarboxylation, cleavage, synthesis, dehydration, and desulfhydration have been shown to be dependent on pyridoxal phosphate. In transamination, the α-amino group of amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, isoleucine, lysine, phenylalanine, tryptophan, tyrosine, and valine is removed and transferred to a carbon chain such as α-ketoglutarate, which in turn can transfer the amino group to the urea cycle for urea synthesis. Pyridoxal phosphate functions in transaminations in a Schiff

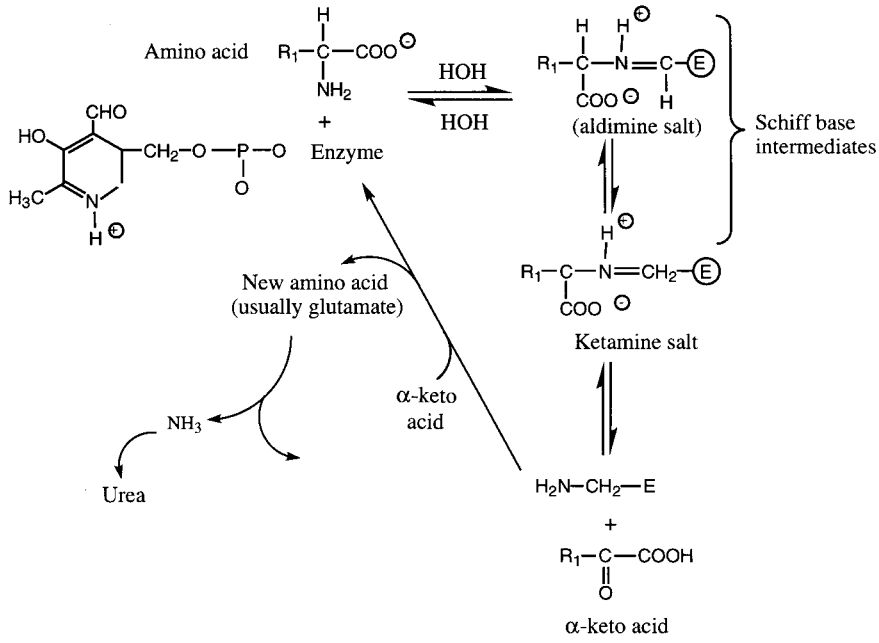


Figure 22 Schiff base mechanism for pyridoxal phosphate. Symbols used are R_1, amino acid; Ⓔ, apoenzyme; +, enzyme.

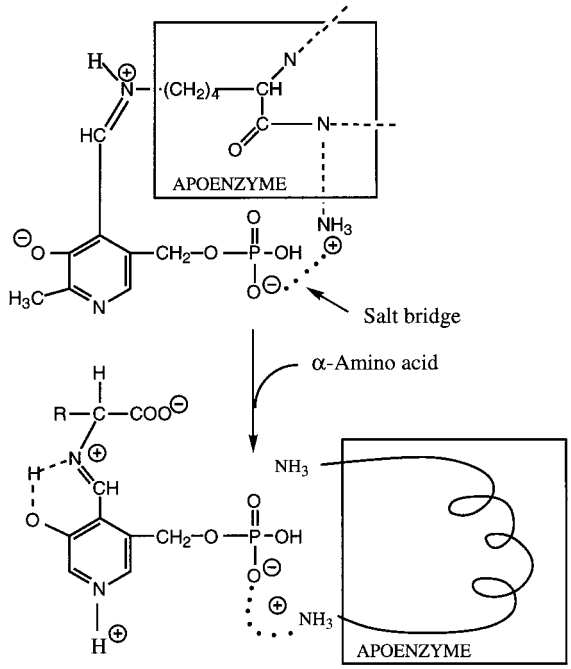


Figure 23 Binding of pyridoxal phosphate to its apoenzyme. When an α-amino acid enters, it displaces the ε-amino group of the lysyl residue of the apoenzyme.

base mechanism as shown in Figure 22. The binding of pyridoxal phosphate to its apoenzyme is shown in Figure 23. The active coenzyme forms of the vitamin B_6 are pyridoxal phosphate and pyridoxamine phosphate.

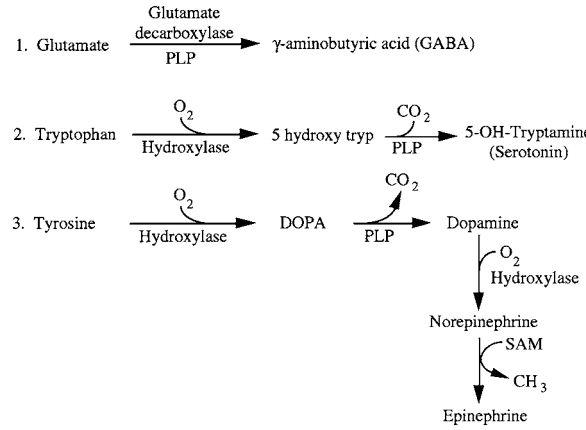


Figure 24 B_6 and synthesis of neurotransmitters.

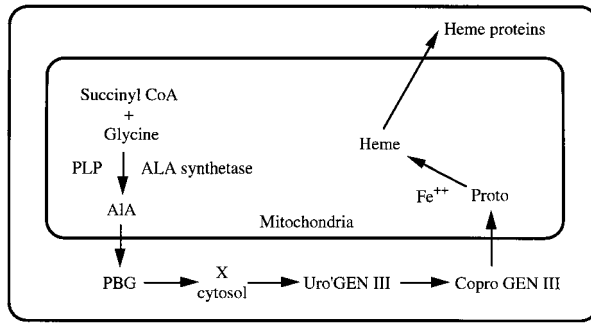
Our present understanding of the role of these coenzymes, in part, is from the early work of Snell et al., who found that pyridoxal will react nonenzymatically at 100°C with glutamic acid to yield pyridoxamine and α-ketoglutarate. This led to the proposal that pyridoxal phosphate functions as a coenzyme by virtue of the ability of its aldehyde group to react with the α-amino group to yield a Schiff's base between the enzyme-bound pyridoxal phosphate and the amino acid, converting it to the α-keto acid. The resulting bound pyridoxamine phosphate enzyme then reacts with another α-keto acid, called an amino acid acceptor, in a reverse reaction to yield a new amino acid and pyridoxal phosphate. The linkage of pyridoxal phosphate to the enzyme is a noncovalent bonding through the charged ring containing the nitrogen atom as well as ionic interaction from the 5′-phosphate moiety to counterionic residues of the transaminase enzyme proteins. In transamination, the unprotonated amino group of the amino donor is covalently bound to the carbon atom of the aldehyde group of enzyme-bound pyridoxal phosphate and, with the elimination of water, forms an aldimine which tautomerizes to the corresponding ketamine. X-ray crystallography has confirmed the covalent aldiminium and ketaminium forms. The step involves the movement of an electron pair from the amino acid to the pyridine ring of the prosthetic group, followed by tautomerization to the ketamine.

Addition of water leads to the formation of a free α-keto acid and Enz-PLP complex. By oscillating between the aldehyde and amino groups, the PLP and PMP act as an amino acid carrier. Thus, the transamination reaction is an example of a double displacement reaction.

Pyridoxal phosphate also acts with cystathionine lyase to catalyze the cleavage of cystathionine and yield free enzyme and free cysteine, with α-ketobutyrate and NH_3 as other products. Pyridoxal phosphate is important to the synthesis of the neurotransmitters γ-aminobutyric acid (GABA), serotonin, dopamine, norepinephrine, and epinephrine. These reactions are outlined in Figure 24.

This role of vitamin B_6 explains the CNS symptoms associated with the deficient state. Convulsions are a common symptom, together with other derangements in metabolism, as is anemia. The symptom of anemia arises from the role of pyridoxal phosphate in hemoglobin synthesis as is outlined in Figure 25.

More recently, we have come to understand a role of vitamin B_6 in steroid hormone-induced protein synthesis. New studies have shown that B_6 has an important role as a physiological mediator of steroid hormone function. In this role, B_6 binds to a nuclear steroid hormone receptor and, in so doing, inhibits the binding of the steroid hormone-receptor complex to specific DNA sites. In this way, B_6 acts as a negative control of steroid hormone action. Progesterone, glucocorticoids, estrogen, and testosterone effects on RNA polymerase II and RNA transcription have been shown to be inhibited by the presence of pyridoxal phosphate. In each of these instances, the pyridoxal phosphate binds to the receptor protein and, in so doing, has a negative effect on hormone-receptor



Abbreviations used:
ALA — aminolevulinic acid
PBG — porphobilinogen
Uro GEN III — uroporphyrinogen III
Copro GEN III — coproporphyrinogen III
Proto — protoporphyrin IX
X — intermediates
Enzymes catalyzing heme biosynthesis are omitted, except ALA synthetase

Figure 25 Role of pyridoxal-Ⓟ (PLP) in heme biosynthesis.

binding to DNA. This role for B_6 is in addition to its role as a coenzyme in a wide variety of enzymes involved in cell growth and cell division. One of these is ornithine decarboxylase, an enzyme that plays an important role in cell division. In rapidly growing tumor cells, B_6 levels are much lower than in normal cells and some of the major chemotherapies for cancer are based on the need for B_6 by these cells. Antivitamin B_6 compounds are important chemotherapeutic agents in this setting.

F. Deficiency

In laboratory animals, dermatitis (acrodynia) is the chief symptom. Lesions occur on paws, ears, nose, chin, head, and upper thorax. This skin disorder resembles EFA deficiency. A high-fat diet protects somewhat against a B_6 deficiency. Other symptoms include poor growth; muscular weakness; fatty livers; convulsive seizures; anemia; reproductive impairment; edema; nerve degeneration; enlarged adrenal glands; increased excretion of xanthurenic acid, urea, and oxalate; decreased transaminase activity; impaired synthesis of ribosomal RNA, mRNA, and DNA; and impaired immune response. High protein intakes accelerate the development of the deficiency.

In humans, the deficiency syndrome is ill defined. It is characterized by weakness, irritability and nervousness, insomnia, and difficulty in walking. Cheilosis (cracks at the corners of the mouth) appears but is not responsive to biotin or riboflavin. Infants consuming B_6-deficient milk formula have convulsive seizures which can be corrected almost immediately with intravenously administered vitamin. There is a deranged tryptophan metabolism and evidence of increased excretion of xanthurenic acid. In B_6 deficiency, the conversion of tryptophan to niacin is impaired and thus skin lesions develop which resemble those of pellagra and riboflavin deficiency. Behavioral changes have been described and these include depression and irritability.

Hypochromic, sideroblastic anemia is a common finding and is due to the role B_6 plays in hemoglobin synthesis. While B_6 is found in a wide variety of foods, B_6 deficiency can be observed when antivitamin drugs are used. For example, isoniazid, a drug used in the treatment of tuberculosis, results in excessive B_6 loss. Penicillamine, a drug used in the treatment of Wilson's disease, has antivitamin activity. Lastly, higher than normal doses of B_6 have been prescribed for the

Table 10 Recommended Dietary Allowances for B_6

Group	Age	Recommended Daily Allowance (mg/day)
Infants	Birth to 6 months	0.3
	7–12 months	0.6
Children	1–3	1.0
	4–6	1.1
	7–10	1.4
Males	11–14	1.7
	15–18	2.0
	19–22	2.0
	23–50	2.0
	51+	2.0
Females	11–14	1.4
	15–18	1.5
	19–22	1.6
	23–50	1.6
	51+	1.6
Pregnancy	—	2.1
Lactation	—	2.1

treatment of skin disease and for neuromuscular and neurological diseases. Whether this prescription has a positive effect on the pathophysiology of these diseases remains under discussion.

There are several congenital diseases of importance to B_6 status. Homocysteinuria, due to a defect in the enzyme cystathione-β-synthase, is characterized by dislocation of the lenses in the eyes, malformation of skeletal and connective tissue, and mental retardation. Pyridoxal phosphate is a coenzyme for this synthase. Another genetic disease, cystathionuria, due to a defect in cystathione-γ-lyase and characterized by mental retardation, also drives up the need for B_6. A third genetic disorder, GABA deficiency due to mutation in glutamate decarboxylase, is manifested by a variety of neuropathies. Lastly, sideroblastic anemia, due to a mutation in δ-aminolevulinate synthetase, is characterized by anemia, cystathionuria, and xanthurenic aciduria. All of these genetic disorders can be ameliorated somewhat by massive doses of the vitamin. Why this works is not known for all cases, but patients with these disorders do not have any symptoms of B_6 deficiency since the defects are not in the absorption or metabolism of B_6 per se, but in the inadequate function (often due to poor binding of PLP) of specific enzymes involved in amino acid metabolism.

G. Recommended Dietary Allowance

The need for B_6 depends on the composition of the diet and on the age and gender of the individual. The B_6 RDAs are shown in Table 10.

VI. PANTOTHENIC ACID

A. Overview

Pantothenic acid was isolated and synthesized in the late 1940s and recognized as an essential growth factor for yeast. Its essentiality for mammalian species did not become known until it was shown to prevent or cure chick dermatitis. It was subsequently recognized as essential for the rat, mouse, monkey, pig, dog, fox, turkey, fish, hamster and human. Pantothenic acid is synthesized by plant tissues but not by mammalian tissues. It is found in a variety of tissues in the bound form. In 1946, it was discovered to be an essential part of coenzyme A.

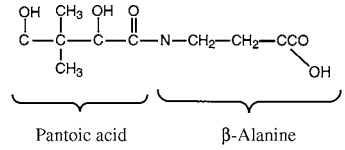


Figure 26 Structure of pantothenic acid.

B. Structure, Chemical and Physical Properties

Pantothenic acid is the trivial name for the compound dihydroxy-β,β-dimethylbutyryl-β-alanine. It has two metabolically active forms: as part of coenzyme A (CoA) and the acyl carrier protein. Pantothenic acid exists as the free acid (molecular weight 219.2 Da) or as a calcium salt (molecular weight 476.5 Da). It is the condensation product of β-alanine and a hydroxyl- and methyl-substituted butyric acid, pantoic acid. Its structure is shown in Figure 26. It is an unstable pale yellow oil, commercially available as a white, stable, crystalline, calcium, or sodium salt. When dry, the salt is stable to air and light but is hygroscopic. The salt is soluble in water and glacial acetic acid. The vitamin is stable in neutral solution but is readily destroyed by heat and either alkaline or acid pH. When heated in aqueous solution, there is hydrolytic cleavage of the molecule yielding β-alanine and 2,4-dihydroxy-3,3-dimethylbutyrate.

Pantothenic acid may be assayed colorimetrically following reaction with 1,2-naphthaquinone-4-sulphonate or ninhydrin. Radioimmunoassay also is used, as are microbiological methods. The method of choice is HPLC (high performance liquid chromatography).

C. Sources

Pantothenic acid is widely distributed in nature. Excellent food sources are organ meats, mushrooms, avocados, broccoli, and whole grains.

D. Absorption and Metabolism

Absorption occurs via facilitated diffusion and travels in the blood within the erythrocytes as well as in the plasma. Large doses of pantothenic acid are rapidly excreted in the urine, indicating no storage (except for that within the red blood cells and the fat cells) and little metabolism/degradation.

E. Function

Pantothenic acid functions in fatty acid metabolism as a component of coenzyme A. Unlike most vitamin coenzymes, pantothenic acid does not comprise the functional unit of CoA; instead, it provides the backbone for its derivative, pantotheine, whose SH group forms the reactive site. The structure of CoA is shown in Figure 27 and its synthesis is shown in Figure 28.

The function of CoA is to serve as a carrier of acyl groups in enzymatic reactions involving fatty acid oxidation, fatty acid synthesis, pyruvate oxidation, and biologic acetylations. It can not cross the cell membrane, and must therefore be synthesized in cells. Acetyl CoA (active acetate) is formed during the oxidation of pyruvate or fatty acids. It may also be generated from free acetate in the presence of the enzyme acetyl CoA synthetase. Acetyl CoA may then react with an acyl group acceptor such as choline to yield acetylcholine or oxaloacetate for citrate (Figure 29).

The sulfhydryl group of the β-mercaptoethylamine is the site at which acyl groups are linked for transport by the coenzyme. The ability of the CoASH to form thioesters with carboxylic acids is responsible for the vital role of the coenzyme in numerous metabolic processes.

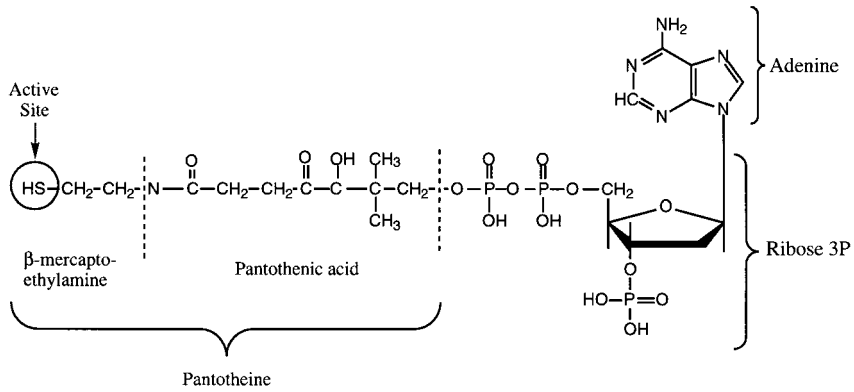


Figure 27 Structure of coenzyme A.

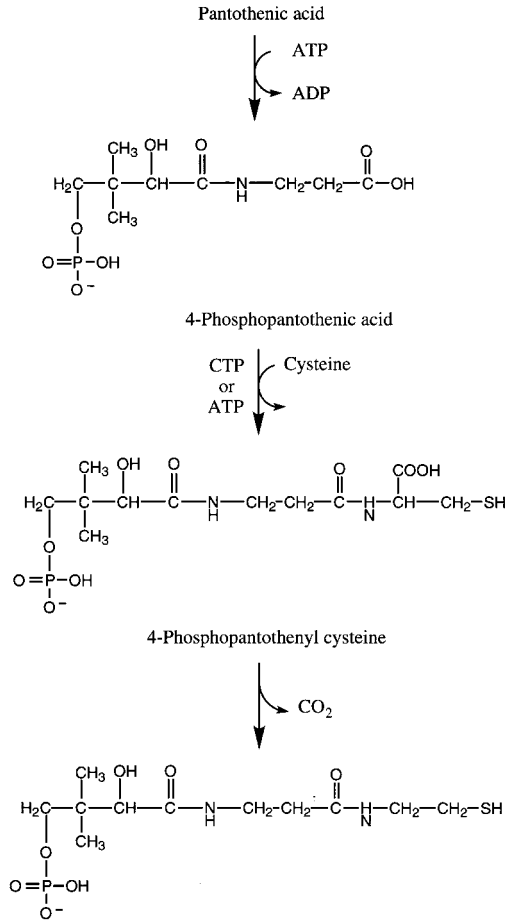


Figure 28 The intracellular synthesis of coenzyme A from pantothenic acid.

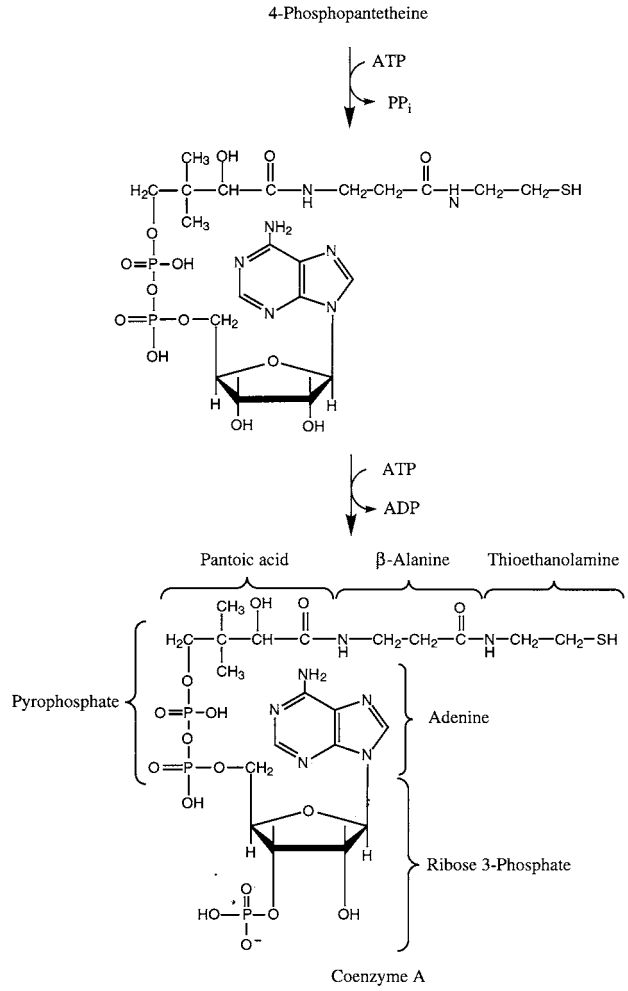


Figure 28 (continued)

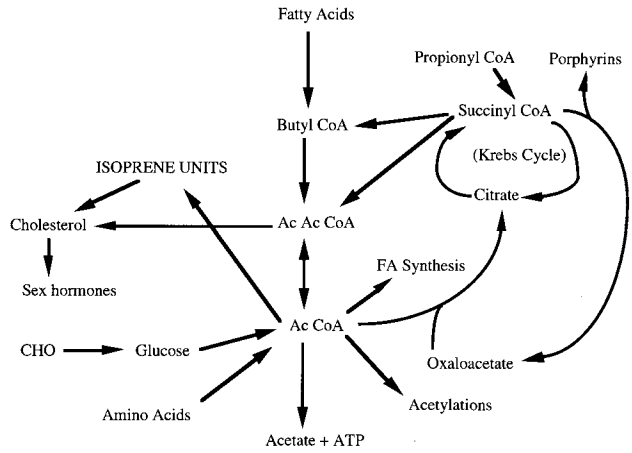


Figure 29 The central role of acetyl CoA in intermediary metabolism.

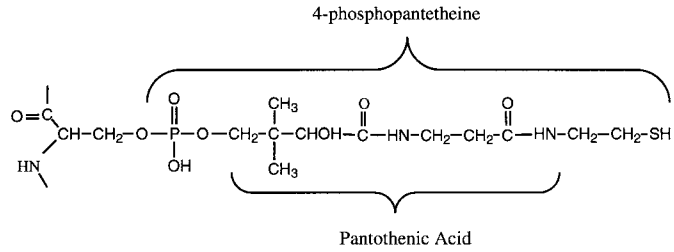


Figure 30 Structure of acyl carrier protein.

All known acyl derivatives of CoA are thiol esters. These acyl derivatives of CoA may participate in a number of metabolic reactions: condensation, addition, acyl group interchanges, and nucleophilic attack.

These reactions fall into three general categories:

1. Acetylation of choline and certain aromatic amines such as sulfonamides.
2. Oxidation of fatty acids, pyruvate, α-ketoglutarate, and acetaldehyde.
3. Synthesis of fatty acids, cholesterol, sphingosine, citrate, acetoacetate, porphyrin, and sterols.

Thus, CoA serves not only as an acetyl donor/acceptor but also as an acyl donor/acceptor and thus CoA serves as a central integrator of intermediary metabolism.

Fatty acid synthesis in the cytoplasm involves an additional role of pantothenic acid in the form of a cofactor, 4′-phosphopantetheine. This factor is bound to a protein commonly called acyl carrier protein (ACP). ACP plus 4′-phosphopantetheine appears to be involved in all fatty acid syntheses. Its structure attached to a seryl residue is shown in Figure 30. The acyl intermediates formed during fatty acid synthesis are esterified to the SH group. Phosphopantetheine is a cofactor bound to the GTP-dependent acyl CoA synthetase. Thus, 4′-phosphopantotheine serves in a capacity analogous to CoA during fatty acid oxidation.

Carnitine reacts with fatty acyl CoA esters to form carnitine esters capable of crossing the mitochondrial membrane. CoA does not travel across membranes and thus must be synthesized within each cell as the need for it arises.

F. Deficiency Symptoms

Deficiency symptoms are species specific (see Table 11). Pantothenic acid deficiency has not been described in humans as a single entity. If it occurs, it is accompanied by other deficiency disorders as well. The exception to this is in patients treated with the pantothenic acid antagonist, ω-methylpantothenic acid. In these patients, neurological symptoms (paresthesia of toes and feet), depression, fatigue, insomnia, vomiting, and muscle weakness have been reported. Changes in glucose tolerance, increased sensitivity to insulin, and decreased antibody production have also been noted.

G. Recommended Dietary Allowance

An RDA for pantothenic acid has not been determined, however, a provisional range for intake of 4 to 7 mg/day was suggested in 1980.

VII. BIOTIN

A. Overview

At the end of the nineteenth century, it was discovered that yeast needed a factor for growth that was not any of the already discovered essential nutrients. This factor was called “bios”. Later,

Table 11 Pantothenic Acid Deficiency Symptoms in Rats, Dogs, and Pigs

Rat
Dermatitis
Achromotrichia (graying)
Adrenal necrosis
Hemorrhage
Spectacle eye
Spastic gait
Anemia
Leukopenia
Impaired antibody formation
Gonadal atrophy
Infertility
Dog
Appetite
Hair loss
Runny nose
Fatty liver
Irritability
Hypoglycemia
Pig
Spastic gait
Hair loss
As above

scientists realized that bios was a mixture of inositol and biotin and that bios could overcome "egg white injury". At this point it was named vitamin H or factor H and was found to be needed for cellular respiration. Because of its essentiality for respiration, it was named coenzyme R. In the late 1930s Gyorgy finally integrated all these bits and pieces, and together with Kögl, du Vigneaud, and Harris realized the essential nature of a material they had isolated and synthesized. Biotin was chosen as its name.

B. Structure, Physical and Chemical Properties

Biotin is the trivial name for the compound *cis*-hexahydro-2-oxo-1H-thieno-(3,4-d) imidazole-4-pentanoic acid. Its structure is shown in Figure 31. In order to have vitamin activity the structure must contain a conjoined ureido and tetrahydrothiophene ring with the ureido 3′N sterically hindered, preventing substitution. The ureido 1′N is a poor nucleophile.

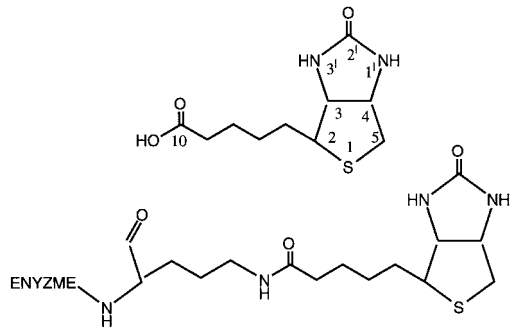


Figure 31 Structure of biotin and enzyme-bound biotin.

Biotin occurs in eight isomeric forms but only D-biotin has vitamin activity. Several biotin analogs have been synthesized or isolated from natural sources. Among these are oxybiotin or biotinol, biocytin, dithiobiotin, and biotin sulfoxide. The latter two are inactive as vitamins whereas the first two have some vitamin activity, albeit less than that of D-biotin.

Biotin is a white crystalline substance that, in its dry form, is stable to air, heat, and light. Its molecular weight is 244.3 Da and melting point is 167°C. It decomposes at 230 to 232°C. It has a limited solubility in water (22 mg/ml HOH) and is more soluble in ethanol. When in solution it is unstable to oxygen and strong acid or alkaline conditions and will be gradually destroyed by ultraviolet light. The analytical method of choice is HPLC; however, microbiological methods are also available. These methods use *Lactobacillus casei*, *Lactobacillus plantarum*, *Neurospora crassa*, *Ochromonas danica,* or *Saccharomyces cerevisiae*. These microorganisms require biotin for growth and are sensitive to varying quantities of biotin in the growth media. Using avidin, a protein found in egg white which binds biotin at the ureido group, an isotope dilution assay has been developed and is sensitive in the range of 4 to 41 pmol. Colorimetric assays based on the reaction of biotin with *p*-(dimethylamino)-cinnamaldehyde or on the absorbance of iodine formed during the oxidation of biotin to its sulfone with potassium iodide have been developed. The colorimetric assays are not as sensitive as HPLC or the avidin binding assays.

C. Sources

There are numerous food sources for biotin. Biotin is found in every living cell in minute amounts where it exists either in its enzyme-bound form or as a biotin ester or amide. Rich sources include organ meats, egg yolk, brewer's yeast, and royal jelly. Soy flour or soybean, rice polishings, various ocean fish, and whole grains are good sources of the vitamin.

D. Absorption, Metabolism

Biotin in food exists in the free and enzyme-bound form. The protein-bound form can be digested, which in turn yields biocytin, a combination of biotin and lysine. Biocytin is hydrolyzed via the action of biotinidase to its component parts. The resultant biotin is then available for absorption. Biotin is absorbed via facilitated diffusion. The jejunum is the major site for this absorption. Once absorbed, it circulates as free biotin. There may be some species differences in absorptive mechanisms. In addition, there is synthesis of the biotin by the gut flora.

Biotinidase is present in plasma as well, where it has a similar function. This enzyme plays a major role in biotin recycling. It acts as a hydrolase by cleaving biocytin and biotinyl peptides, thereby liberating biotin for reuse. Biotinidase, if mutated, results in an autosomal recessive disorder that causes a secondary biotin deficiency that can be overcome with biotin supplements. The clinical symptoms of this genetic disorder are the same as those of the biotin-deficient state and relate to the function of biotin as a coenzyme in intermediary metabolism, especially the carboxylase reactions.

Biotinidase has been cloned and sequenced and its distribution throughout the body has been determined. Although active in the intestinal tract, its activity is not sufficient to catalyze all of the bound biotin found in food. It has been estimated that less than 50% of the bound biotin found in foods of plant origin is hydrolyzed to provide the free form. The availability of biotin in food depends on the percent that is bound. In general, bound biotin found in foods of animal origin is more available than that of foods from plant origin. Biotin can be rendered unavailable by avidin, a protein found in raw egg white. Once the egg is cooked, the avidin is denatured and no longer binds the biotin. This binding is the explanation of the disorder "egg white injury". Other proteins, particularly membrane and transport proteins, bind biotin and are responsible for its entry into all cells that use the vitamin.

E. Function

Biotin serves as a mobile carboxyl carrier as it is attached to enzymes that catalyze carboxy group transfer. The formation of this biotin-enzyme complex is shown in Figure 32. A number of enzymes require biotin as a coenzyme for their function. These are listed in Table 12.

F. Deficiency

In humans the symptoms of severe deficiency include dermatitis, skin rash, hair loss (alopecia), developmental delay, seizures, conjunctivitis, visual and auditory loss, metabolic ketolactic acidosis, hyperammonemia, and organic acidemia. These symptoms have been reported in persons lacking normal biotinidase activity through a genetic error. In a genetically normal human population, a true biotin deficiency in the absence of other nutrient deficiencies is extremely rare. Only a few instances have been reported. In one instance, the deficient state was caused by the chronic consumption of 30 raw eggs per day for several months. In this individual, the symptoms were primarily related to the skin. Biotin deficiency may, however, be a secondary consequence of severe protein-energy malnutrition. Studies of severely malnourished children have shown improvement in biotin status with biotin supplements.

G. Recommended Dietary Intake

At present there is no RDA for biotin. Because the vitamin is present in a wide variety of foods, and because it can be synthesized by the intestinal flora, a fixed intake figure has been difficult to determine. However, the National Academy of Sciences Food and Nutrition Board has published a safe and adequate dietary intake for this vitamin. These suggested intakes are shown in Table 13.

VIII. FOLIC ACID

A. Overview

More than 50 years ago, folate was discovered to be a necessary constituent of every living cell of every organism, whether plant or animal. It took years of meticulous work to separate its function from that of vitamin B_{12}, for both are involved in the one-carbon transfers so important in the synthesis of the purines and pyrimidines that are constituents of DNA and RNA. Actually, until the genetic material and its function in the cell was worked out, there was no progress in understanding the role of the folates in nucleic acid synthesis. With the advent of our knowledge of how genes are made and how they work, we finally have come to understand the importance of folic acid in cellular function.

B. Structure, Chemical and Physical Properties

Folic acid, folate, or folacin are the generic terms for pteroylmonoglutamic acid and its related biologically active compounds. A number of derivatives (Table 14) have vitamin activity. The basic structure of pteroylglutamic acid is shown in Figure 33. The derivatives include the addition of hydrogen at N-5 and N-8 and to C-6 and C-7, with only one glutamate attached to *p*-aminobenzoic acid. This derivative is called tetrahydrofolic acid (THF). Other derivatives can have a methenyl group attached at N-5, a methenyl bridge between N-5 and N-10 or a methylene bridge at this position, or an aldehyde group at either N-5 or N-10, or an imino group at N-5. All of these derivatives have vitamin activity because vitamin activity is dependent on the presence of a pterin structure with variable hydrogenation or a methyl addition at N-5 or N-10 and the presence of at

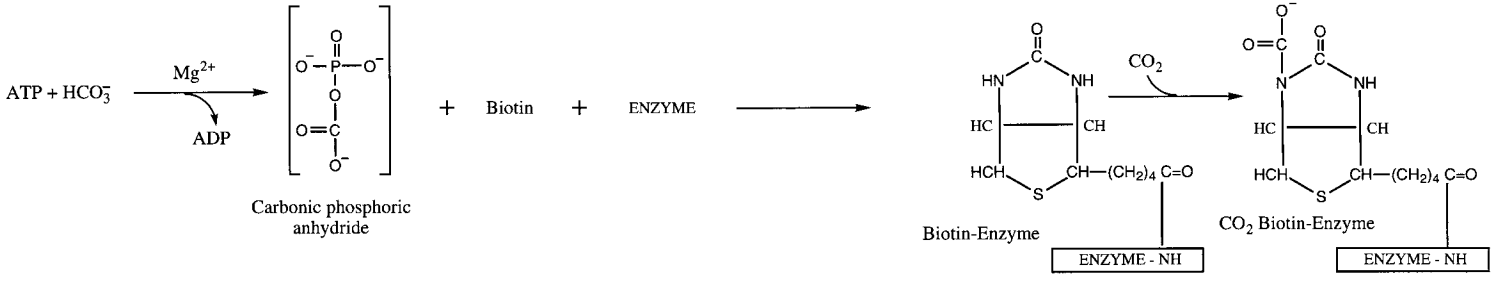


Figure 32 Formation of the CO_2-biotin enzyme complex.

Table 12 Biotin-Dependent Enzymes in Animals

Enzyme	Role	Location
Pyruvate carboxylase	First reaction in pathway that converts 3-carbon precursors to glucose (gluconeogenesis). Replenishes oxaloacetate for citric acid cycle.	Mitochondria (rate-limiting step in gluconeogenesis).
Acetyl-CoA carboxylase	Commits acetate units to fatty acid synthesis by forming malonyl-CoA.	Cytosol (rate-limiting step in fatty acid synthesis).
Propionyl-CoA carboxylase	Converts propionate to methylmalonyl-CoA which can be converted to succinyl CoA, an intermediate of the citric acid cycle.	Mitochondria
β-Methylcrotonyl-CoA carboxylase	Catabolism of leucine and certain isoprenoid compounds.	Mitochondria

Table 13 Safe and Adequate Dietary Intake of Biotin (μg/day)

Infants	0–6 months	35
	7–12 months	50
Children	1–3 years	65
	4–6 years	85
	7–10 years	120
Adolescents, Adults		100–200

Table 14 Derivatives of Folic Acid

Derivatives	N-5	N-10
Tetrahydrofolic acid	–H	–H
5-Methylfolic acid	$-CH_3$	–H
5,10-Methanylfolic acid	–CH=	–CH=
5,10-Methylenefolic acid	$-CH_2-$	$-CH_2=$
5-Formylfolic acid	–HCO	H
10-Formylfolic acid	–H	–HCO
5-Formiminfolic acid	–HCNH	H

least one glutamyl residue linked via peptide bonds to *p*-aminobenzoic acid. Methotrexate (4-amino-N^{10}-methyl folic acid, an antineoplastic agent) and aminopterin (4-amino folic acid, a rodenticide) are folate antagonists and as such are useful pharmaceutical agents against cell growth. Other drugs, sulfasalazine and diphenylhydantoin, for example, interfere with folacin uptake and use.

The pteroylmonoglutamate form has a molecular weight of 441.4 Da and is moderately soluble in water (0.0016 mg/ml water). It has absorption maxima at 256, 283, and 368 nm. It is an orange-yellow crystal with a melting point of 250°C. It is unstable to ultraviolet light, heat, oxygen, acidic conditions, and divalent metal ions such as iron and copper. As mentioned, it is present in all living cells in small amounts, so until the advent of sensitive HPLC techniques its determination in food and animal tissues relied on microbiological techniques. In addition to the HPLC methodology there is also a radioimmunological technique which involves the competitive binding of the vitamin to a protein, followed by quantification.

C. Sources

Folate is found in a wide variety of foods of both animal and plant origin. However, because it is so unstable, reliable food composition data have been difficult to obtain and it is altogether

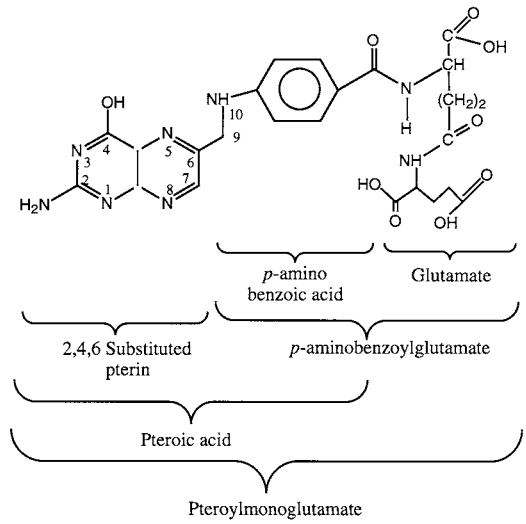


Figure 33 Structure of folic acid.

possible that food sources may be insufficient to meet dietary need. Good sources include meats, fruits, vegetables (especially asparagus), dry beans, peas and nuts, and whole-grain cereal products.

D. Absorption, Metabolism

Folate transport in the intestine is a carrier-mediated, pH-dependent process with maximum transport occurring after deconjugation to the monoglutamate form in the jejunum. There are specific folate-binding proteins that function in the absorption process. One is a low-affinity folate-binding protein found in the brush border membrane of the absorptive cell. There is another high-affinity folate-binding protein that is localized to the jejunal brush border cells. Affinity is optimized at pH 5.5 to 6.0. The high-affinity binding protein is similar to one found in the kidney.

A number of drugs inhibit or compete with folate for transport. These include ethacrynic acid, sulfinpyrazone, phenylbutazone, sulfasalazine, and furosemide. All of these are amphipathic substances. That is, they are compounds with a polar-apolar character. Absorption is also inhibited by cyanide and 2,4-dinitrophenol — drugs that poison oxidative phosphorylation and thus reduce the ATP supply. ATP is necessary for the active transport process to work. Absorption can also occur by passive diffusion but this is a secondary means for folate uptake. Very little folate appears in the feces.

After folate is absorbed it circulates in the plasma as pteroylmonoglutamate. That which is not used by the cells is excreted in the urine as pteroylglutamic acid, 5-methyl-pteroylglutamic acid, 10-formyltetrahydrofolate, or acetamidobenzoylglutamate. Uptake by cells is mediated by a highly specific folate binding protein. This protein has been isolated from the membranes of a variety of cells and a cDNA probe has been prepared. Folate appears to stimulate the transcription of the mRNA for this protein. In this role folate binds to a specific DNA binding protein (a folate receptor) which, in turn, serves as a transcription factor enhancing the transcription of the mRNA for the highly specific folate-binding protein.

Some of the food folate exists as 5,10-methylenetetrahydrofolate, which must be converted to 5-methyltetrahydrofolate, the circulatory folate form. This conversion requires the enzyme methylenetetrahydrofolate reductase. The gene for this enzyme has been mapped in the mouse to chromosome 4, and a common mutation that results in a substitution of valine for alanine at position 299

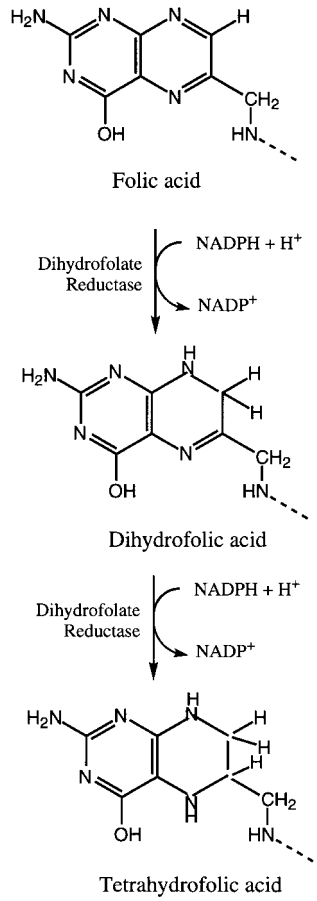


Figure 34 Activation of folic acid.

has been linked to neural tube defects. This mutation requires low folate status in the plasma for the development of mild hyperhomocysteinemia which in turn has been linked to neural tube defects.

E. Function

Folate's main function is as a coenzyme in one-carbon transfer. However, before it can do this it must be activated. Activation consists of the reduction of folic acid to dihydrofolic acid and thence to tetrahydrofolic acid as shown in Figure 34.

A number of folate derivatives have vitamin activity and these derivatives are interconvertible, as shown in Figure 35. Methyl-group transfer also involves vitamin B_{12}, as illustrated in Figure 36.

The regulation of methyl-group transfer is complex and involves a number of enzymes and substrates. Enzymes requiring folate as a coenzyme are listed in Table 15. While serine is a good source for the methyl group, methyl groups arise from other substrates as well. The major source of single methyl groups involves a cycle of reactions catalyzed by serine hydroxymethyltransferase, 5,10-methylene-FH_4 reductase, and methionine synthetase. The last of these reactions is rate limiting for the cycle, whereas the second is inhibited by a *S*-adenosylmethionine (SAM) as well as 5-methyl-FH_4. As described in Section IX on vitamin B_{12}, methionine synthesis depends on the transfer of labile methyl groups from 5-methyl-folate to B_{12} which, as methyl-B_{12}, donates this methyl group to homocysteine, making methionine.

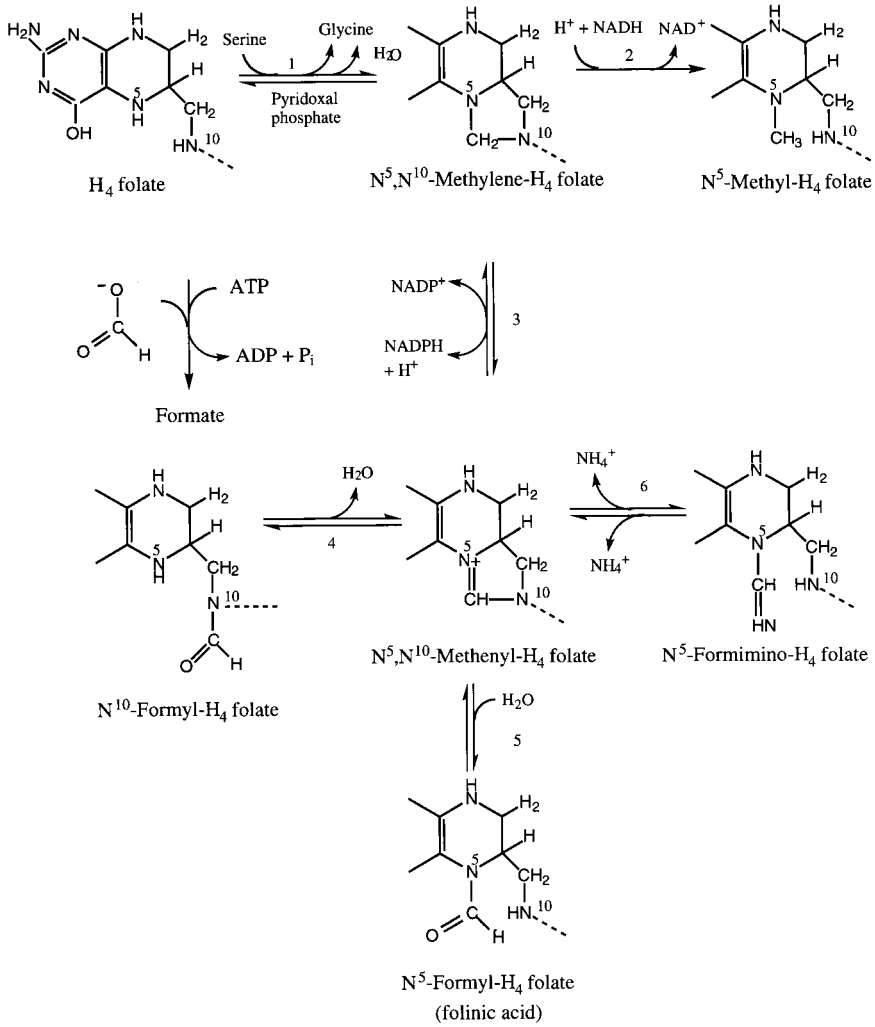


Figure 35 The interconversions of one-carbon moieties attached to tetrahydrofolate.

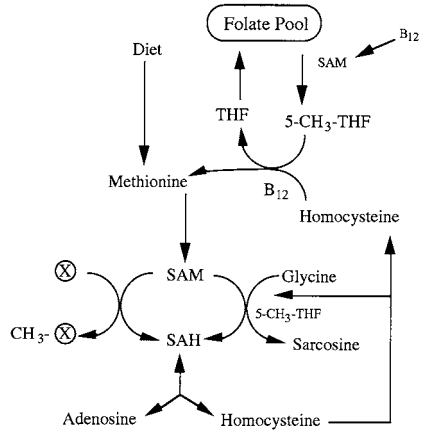


Figure 36 Involvement of B_{12} in methyl group transfer via S-adenosylation (SAM).

Table 15 Metabolic Reactions in Which Folate Plays a Role as a Coenzyme

Enzyme	Role
Thymidylate synthetase	Transfers formaldehyde to C-5 of dUMP to form a dTMP in pyrimidine synthesis (see Figure 5).
Glycinamide ribonucleotide transformylase	Donates formate in purine synthesis (see Unit 2).
5-Amino-4-imidazolecarboxamide transformylase	Donates formate in purine synthesis (see Unit 2).
Serine hydroxymethyl transferase	Accepts formaldehyde in serine catabolism.
10-Formyl-FH_4 synthetase	Accepts formaldehyde from tryptophan catabolism.
10-Formyl-FH_4 dehydrogenase	Transfers formate for oxidation to CO_2 in histidine catabolism.
Methionine synthase	Donates methyl group to homocysteine to form methionine.
Formiminotransferase	Accepts formimino group from histidine.

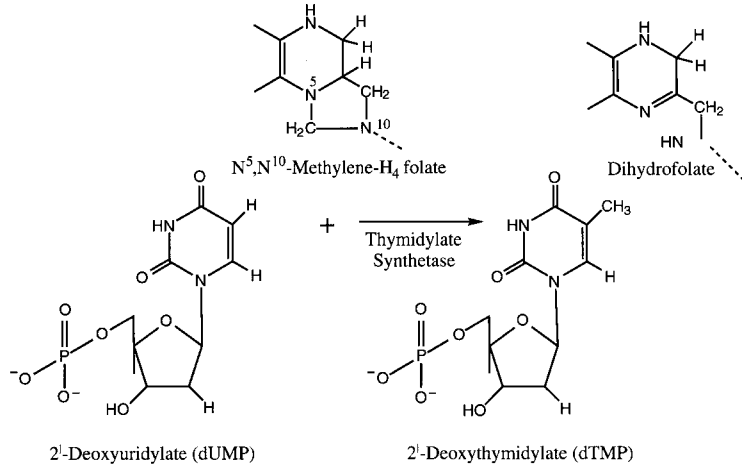


Figure 37 Addition of methyl groups to deoxyuridylate using the methyl-group transfer function of folate.

One-carbon transfer is particularly important in purine and pyrimidine synthesis. The mechanism of this transfer that involves folate (and also B_{12}) is illustrated in Figure 37. The whole reaction sequence for purine and pyrimidine synthesis is presented in Unit 2. In this sequence, methyl transfer via thymidylate synthetase converts 2′-deoxyuridylate to 2′-deoxythymidylate.

From this reaction (Figure 37) it is immediately apparent why folate and vitamin B_{12} are so important to gene expression. While nuclear DNA, once made, merely reproduces itself within the cell cycle, new messenger RNA is made every minute as new proteins are needed by the cell. While some of the purine and pyrimidine bases can be salvaged and reused, this recycling is not 100% efficient. Messenger RNA has a *very* short half-life (seconds to hours) compared to the other nucleic acid species in the cell. Thus, newly synthesized purines and pyrimidines must be available. If not available, mRNA synthesis, *de novo* protein synthesis, and, of course, cell renewal will be adversely affected.

F. Deficiency

While anemia, dermatitis, and impaired growth are the chief symptoms of folate deficiency in the human, scientists are now beginning to recognize the importance of adequate folate intake in early embryonic development. Inadequate intake by the mother prior to and/or during the early stages of development can have teratogenic effects on the embryo. Embryonic development, particularly closure of the neural tube, is impaired in folate deficiency. As a result, infants are born with spina bifida and other neural tube defects. It is estimated that about 2500 infants per year are

Table 16 1989 Recommended Dietary Allowances (RDA) for Folate

Group	Age	RDA (μg/day)
Infants	0–6 months	25
	7–12 months	35
Children	1–3	50
	4–6	75
	7–10	100
Males	11–14	150
	15–18	200
	19–24	200
	25–50	200
	51+	200
Females	11–14	150
	15–18	180
	19–24	180
	25–20	180
	51+	180
Pregnancy		400
Lactation		280

born with these defects, but not all of these infant defects are attributable to inadequate folate nutrients. Available evidence indicates that women contemplating pregnancy should consume 400 μg/day as a prophylactic measure. Low folate intake has been suggested as a factor in the development of colon cancer as well as in the bronchial squamous metaplasia (premalignant lesions) of smokers, and cervical dysplasia (another premalignant lesion) in women. Folate deficiency in rats has been shown to induce DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Whether this occurs in humans and can explain the link between folate status and cancer development remains to be explored. Other symptoms of deficiency are leukopenia (low white-cell count), general weakness, depression, and polyneuropathy. The latter sign is probably related to the folate-B_{12} interaction.

G. Recommended Dietary Allowance

Because of the concern over the folate deficiency effect on embryonic development, the RDA for folacin is under revision. The 1989 RDAs are given in Table 16. However, as mentioned in the preceding section, women contemplating pregnancy should at least double their intake (from an RDA of 180 to 400 μg/day). Since folate is not toxic this is probably a good idea, with the caveat that B_{12} status is normal. If this is not the case, excess folate could mask a B_{12} deficiency somewhat until the irreversible neurologic features of B_{12} deficiency appear. To avoid this consequence of masking, an evaluation of B_{12} status should be conducted. Alternatively, supplementation of B_{12} and folacin could be considered assuming that there is no deficit in intrinsic factor (see Section IX).

IX. VITAMIN B_{12}

A. Overview

Vitamin B_{12} is one of the most recently discovered micronutrients and is the most potent. Very little of the vitamin is required to prevent the symptoms of pernicious anemia and subsequent neurological change. It was isolated in 1948 and shortly thereafter was shown to be the required substance needed to prevent pernicious anemia.

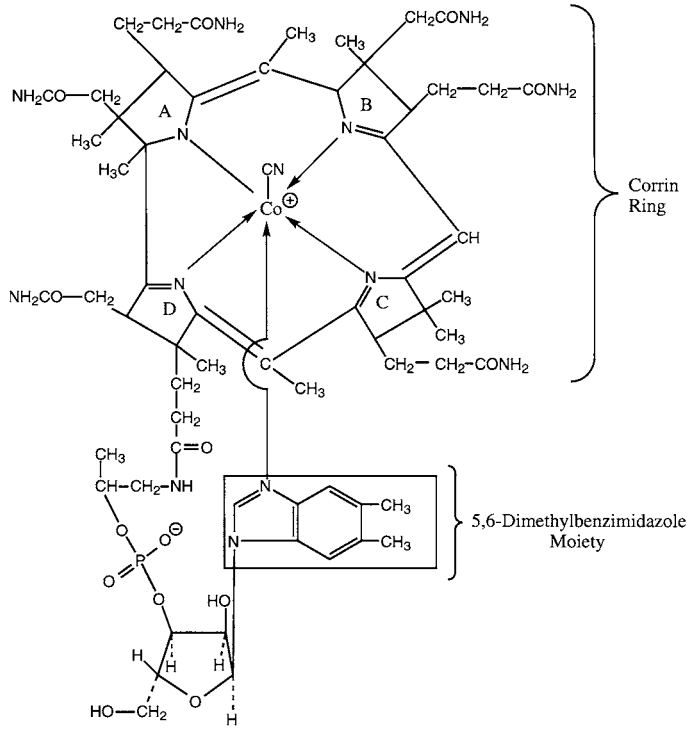


Figure 38 Cyanocobalamin; vitamin B_{12} ($C_{63}H_{88}N_{14}PCo$).

B. Structure, Chemical and Physical Properties

Vitamin B_{12} is a very complex structure as shown in Figure 38. The term B_{12} is the generic descriptor for all corrinoids — those compounds having a corrin ring. Cyanocobalamin is the trivial designation for this compound. In order to have vitamin activity it must contain a cobalt-centered corrin ring. Below the ring it may have a heterocyclic nitrogen side chain or may have nothing attached here. Above the ring it may have a hydroxo, aquo, methyl, 5-deoxyadenosyl, CN, Cl^-, Br^-, nitro, sulfito, or sulfato group. There are a number of structural analogs that have vitamin activity. Regardless of the substituents present above or below the cobalt-centered corrin ring, unless the ring is present there will be no vitamin function. The ring consists of four reduced pyrrole rings linked by three methylene bridges and one direct bond. The cobalt atom is in the 3^+ state and can form up to 6 coordinate bonds. It is tightly bound to the four pyrrole N atoms and can also bond a nucleotide and a small ligand below and above the ring, respectively. Commercially viable synthesis of this compound is very difficult although one such process has been developed using 2 mol of 5-aminolevulinate to form porphobilinogen, a pyrrole ring with an aminomethyl group on C-2, an acetate group on C-3, and a proprionate on C-4. Four of these are linked together and cyclized to form hydroxymethylcobalamin to which cobalt is attached. Vitamin B_{12} has a molecular weight of 1355.4 Da and is moderately soluble in water (12.5 mg/ml). It is insoluble in fat solvents. Its absorption maxima occur at 278, 361, and 550 nm. It is a heat-stable red crystal but will decompose at temperatures above 210°C. The crystal will melt at temperatures above 300°C. It is unstable to ultraviolet light, acid conditions, and the presence of metals such as iron and copper. Vitamin B_{12} is very difficult to assay. It is primarily the product of microbial synthesis and thus is not usually present in large amounts in most foods. Organ meats are good sources of B_{12}. It is synthesized in the gastrointestinal system by the resident flora. One assay system uses B_{12}-dependent

microorganisms. The most responsive and specific of these is *Ochromonas malhamensis*. Although sensitive to small amounts of the vitamin, these procedures are tedious. Spectrophotometric assays that can detect as little as 25 μg/ml are also available but not practical because the sensitivity is so limited. HPLC is the best system for detection and quantitation.

C. Absorption, Metabolism

Most mammals depend on a very complex system to extract vitamin B_{12} and absorb it. The process of absorption begins in the stomach, where preformed B_{12} is bound to a carrier protein called intrinsic factor. As B_{12} is made by the gut flora, it, too, is bound to a carrier protein. Whether this carrier is identical to that available in the stomach is not known. It probably is. Likely, future research will show that the synthesis of this carrier is directed by the vitamin in a manner analogous to that of retinol and the retinol receptor protein (see Unit 3).

Actually, there are four structurally distinct B_{12} carrier proteins. Intrinsic factor (IF) is one of these, and another, called R binder, is found in the proximal part of the alimentary tract. R binder is degraded by the pancreatic peptidases and proteases, while the intrinsic factor-B_{12} complex proceeds intact to the distal portion of the ileum where, in the presence of calcium and neutral pH, the complex binds to IF on the surface of the luminal epithelial cell. Subsequent to binding to IF, the vitamin appears in the portal blood bound to another protein called transcobalamin II or TCII. The blood contains an additional R-type protein called transcobalamin I (TCI) which assists in the transport of the vitamin to its target cells.

Although absorption occurs mainly in the distal ileum, it also occurs in the large intestine. This takes advantage of the fact that B_{12} is synthesized by the flora in this part of the intestinal tract. The mechanism of absorption by the ileum is mediated by a carrier, the intrinsic factor; however, the details of this mechanism are unknown except for the need for the carrier as described above. Passive diffusion also occurs when large B_{12} doses are given. Once absorbed, the transport of B_{12} within the enteral cell also involves a carrier and divalent ions. Calcium, in particular, is needed for the attachment of the intrinsic factor-B_{12} complex to its cognate receptor on the enteral cell plasma membrane. Likely, other factors are also involved; persons with a variety of diseases such as pancreatitis, tropical sprue, fluoroacetate poisoning, and pancreatic insufficiency do not absorb B_{12} efficiently and often show signs of pernicious anemia until provided with oral B_{12} supplements or injected with B_{12}. Absorption by the large intestine likely occurs via passive diffusion.

Once absorbed, B_{12} is transported in the blood bound to one of three transport proteins: transcobalamin I, II, or III. Small amounts are stored (as methylcobalamin) in the liver, kidney, heart, spleen, and brain. Thus, if an individual lacks intrinsic factor due perhaps to a genetic disease or surgical loss of the stomach (gastrectomy) or, as described above, has one or more diseases that affect B_{12} absorption, an injection of B_{12} can be given once a month (rather than daily) and this will correct the problem of inadequate supply.

D. Function

Vitamin B_{12} functions in one of two ways: (1) it participates as a coenzyme in reactions that utilize 5′-deoxyadenosine linked covalently to the cobalt atom (adenosylcobalamin), and (2) it participates as a substrate in reactions that utilize the attachment of a methyl group to the central cobalt atom (methylcobalamin). The conversion of cobalamin to methylcobalamin is catalyzed by the enzyme, B_{12} coenzyme synthetase. It catalyzes the reduction of the molecule then catalyzes the reaction with deoxyadenosyl derived from ATP. In addition to ATP, this reaction needs a diol or a dithiol group, a reduced flavin, or a reduced ferredoxin as the biological alkylating agent. The enzymes requiring B_{12} as a coenzyme are listed in Table 17. The first of these is required for L-methionine synthesis. This reaction removes a methyl group from methyl folate via methyl-B_{12} and delivers it to homocysteine. This allows recycling of the folate coenzymes required for purine

Table 17 Enzymes Requiring B_{12} as a Coenzyme

N^5-Methyltetrahydrofolate homocysteine methyltransferase
Acetate synthetase
Glutamate mutase
Methylmalonyl-CoA mutase
α-Methyleneglutarate mutase
Dioldehydrase a
Dioldehydrase b
Glyceroldehydrase
Ethanolamine ammonia-lyase
L-β-Lysine mutase
D-α-Lysine mutase
Ornithine mutase
L-β-Leucine aminomutase

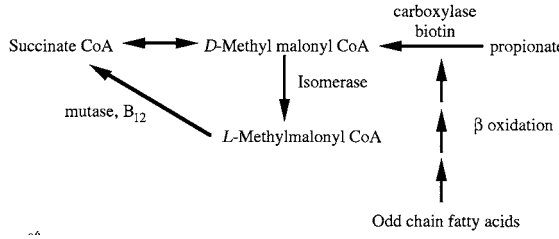


Figure 39 Overall reaction in the conversion of proprionate to succinate using biotin and vitamin B_{12} as coenzymes.

and pyrimidine biosynthesis. Thus, in B_{12} deficiency nucleic acid synthesis is arrested because it is indirectly dependent on adequate B_{12} intake. This process is directly dependent on folacin. B_{12}-deficient animals show decreased methylation of tetrahydrofolate and decreased cellular folate levels despite dietary folate sufficiency. One of the characteristics of the B_{12} deficient state is an anemia characterized by few mature red cells. Immature nucleated cells (megaloblasts) can be found, but not mature ones. Among the other enzymes listed in Table 17 is methylmalonyl CoA mutase, which participates in propionate metabolism. The overall reaction using B_{12} as a coenzyme in proprionate metabolism is shown in Figure 39.

Propionate metabolism, although a minor pathway in monogastric animals, is of some importance in neural tissue. Loss of this metabolic activity may explain the peripheral neural loss that characterizes long-term B_{12} deficiency.

Methylmalonic aciduria characterizes the B_{12}-deficient individual. Studies with rats made B_{12} deficient show an increase in odd-numbered fatty acids in the serum and in the neural and hepatic lipids and low hepatic methylmalonyl-CoA mutase activity. Replacement of B_{12} in the diet corrects both these responses. The effect of B_{12} on mutase activity is such that it is likely that the vitamin serves not only as the coenzyme in the reaction but also serves a role in the synthesis of that enzyme. Several reports of B_{12} activity vis à vis protein synthesis have appeared in the literature in addition to the one concerning the synthesis of the mutase. Whether this relates to the indirect role of B_{12} in DNA and RNA synthesis, i.e., the synthesis of pyrimidines and purines, or whether a B_{12}-protein complex acts as a *cis*- or *trans*-acting factor in the pathway for the expression of the specific genes for these enzymes is unknown.

E. Deficiency

As described in the preceding section, the deficiency state has as its main characteristic, megalobastic anemia. While inadequate B_{12} intake can result in this anemia, this is a rather unusual

nutritional state because most foods of animal origin contain B_{12} and so little is needed. More common as a cause of vitamin B_{12} deficiency is a genetically determined deficiency in the synthesis of intrinsic factor. This trait is inherited as an autosomal dominant trait and occurs in about 1 person in 1000. It can be treated with monthly B_{12} injections (~60 to 100 μg per dose). In the absence of this trait, the people most at risk for B_{12} deficiency are those who abstain from eating foods of animal origin. In addition to these are those who have had one of the illnesses described earlier that impair absorption. Humans that have had a gastrectomy or some disease of the gastric mucosa or some disease resulting in malabsorption are in this category.

Following the development of megaloblastic anemia, which is reversible, is the irreversible loss of peripheral sensation. This is due to the degenerative changes in the peripheral nerve tracts that include demyelination or loss of the lipid protective coat that surrounds the nerve tracts. Once the myelin is lost the nerve dies. Neural loss begins in the feet and hands and progresses upward to the major nerve trunks such that a progressive neuropathy can be followed. Sometimes this pattern of loss is not followed; instead the patient may have problems with balance or coordination of limb motions as in walking or picking up objects. Because both folate and B_{12} are interactively involved in DNA and RNA synthesis it used to be difficult to segregate one deficiency anemia from the other. Folacin supplements might mask the symptoms of B_{12} deficiency. However, measuring the presence of methylmalonic acid in urine and blood will allow for the differential analysis in the cause of the anemia. In addition to folacin and B_{12}, deficient intakes of ascorbic acid, vitamin B_6, niacin, iron, copper, and zinc can also explain anemia (see Units 1 and 6) and deficiencies of these nutrients must be ruled out.

F. Recommended Dietary Allowance

The daily dietary requirements for B_{12} are very small. The normal turnover rate is about 2.5 μg/day thus the recommendation for adults is close to this turnover rate or 2 μg/day. Table 18 gives the RDA for humans of different ages. The need for B_{12} is also related to the intake of ascorbic acid, thiamin, carnitine, and fermentable fiber. Each of these nutrients affects the production of propionate, and in their absence or relative deficiency, propionate production is increased and this, in turn, drives up the need for B_{12}. As already mentioned, the needs for B_{12} and folate are related.

Table 18 Recommended Dietary Allowances for Vitamin B_{12}

Group	Age	RDA (μg/day)
Infants	Birth to 6 months	0.3
	7–12 months	0.5
Children	1–3	0.7
	4–6	1.0
	7–10	1.4
Males	11–14	2.0
	15–18	2.0
	19–24	2.0
	25–50	2.0
	50 +	2.0
Females	11–14	2.0
	15–18	2.0
	19–24	2.0
	25–50	2.0
	51 +	2.2
Pregnant	—	2.6
Lactation	—	2.6

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