VITAMINS

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1. INTRODUCTION

Vitamins are enjoying renewed popularity with the lay public that in some ways mimics that seen in the early part of the twentieth century when they were first being discovered. Paralleling the interest by the general public is increased focus on the biochemical role of vitamins at the molecular level. Some vitamins can be considered prototypes for pharmacological agents used to treat diseases that do not appear directly related to the patient's vitamin status. Although all animals require vitamins, the discussion in this chapter will focus on humans and human biochemical pathways.

Vitamins are complex biochemically and functionally. There are many ways to classify vitamins. This chapter will examine two approaches: the traditional solubility model and a functional classification. Unfortunately, the loose regulation of nutritional products in the United States has led to misleading promotion of these items to the lay public such that individuals are confused as to what can be caused by a vitamin. General criteria for deciding if a substance truly is a vitamin will be presented. Another problem is to decide if a patient actually has a vitamin deficiency. As will be noted in the next section, this can be a complex question. Finally, deciding how much of a particular vitamin should be consumed has moved from the familiar Recommended Dietary Allowance (RDA) to four different ways to evaluate vitamin requirements and consumption. Unfortunately, today's vitamin product labels use older Food and Drug Administration (FDA) criteria for adult daily requirements.

1.1. What is Vitamin?

In general, a vitamin must be a naturally occurring organic molecule that is a normal constituent of the diet. It should be essential and only required in minute amounts. Finally, it is required to maintain normal cellular biochemistry and tissue integrity. These criteria need to be examined in more detail.

1.1.1. Vitamins are Naturally Occurring This criterion frequently is misinterpreted. While the vitamin is a natural product, there is no difference in efficacy between ingesting the all natural or a synthetic product as long as the natural and synthetic products are chemically identical. This includes stereochemistry. L-ascorbic acid is twice as active as the racemic D,L-ascorbic acid. A similar statement can be made for D-pantothenic acid and *S*-biotin relative to their racemic mixtures. The situation for α-tocopherol (vitamin E) with three asymmetric centers is more complex and will be discussed in more detail later in this chapter.

1.1.2. Vitamins are Essential Because They Are Not Produced by Human Biochemical Pathways With two exceptions, this requirement is obvious. If a compound is required for a biochemical process and our pathways cannot produce it, it must be obtained from exogenous sources. The evolutionary history of the human species is such that we sought out sources in our environment that contained these essential substances, and these substances became our food.

One of the substances commonly treated as a vitamin is niacin, which is synthesized from the essential amino acid tryptophan. In humans, the ratio currently is considered to requiring approximately 60 mg of tryptophan to produce 1 mg niacin [1]. This has led to niacin requirements being expressed as niacin equivalents (NE) based on the amount of tryptophan in the diet. It must be kept in mind that tryptophan is essential and is the precursor to the neurotransmitter serotonin plus being a part of protein structure. Therefore, niacin can be thought of as tryptophan sparing. The 60:1 ratio has been questioned based on examining the diets of patients diagnosed with pellagra, a disease caused by a niacin deficiency. The diets of these individuals contained adequate amounts of tryptophan [2,3].

The other exception is vitamin D_3 or cholecalciferol. Assuming adequate sunlight,

Burger's Medicinal Chemistry, Drug Discovery, and Development, Seventh Edition,
edited by Donald J. Abraham and David P. Rotella

cholecalciferol is the photochemical product from ultraviolet irradiation of 7-dehydrocholesterol found in our skin. A significant proportion of the world's population produces all of the cholecalciferol it needs from this photochemical reaction. It is true that a photochemical reaction is not a biochemical pathway, but exposure to adequate sunlight does fulfill the requirement for cholecalciferol.

1.1.3. Vitamins Are Organic Chemicals Trace elements are not properly called vitamins. Therefore, iron, zinc, magnesium, manganese, chromium, selenium, and the other trace elements that, obviously, are obtained from food sources are essential, but they are not vitamins. Conversely and by tradition, many of the essential organic substances in human diets are not vitamins. These would include essential fatty acids, essential amino acids, antioxidants, and fiber.

1.1.4. Vitamins Are Normal Constituents of the Human Diet With the exception of cholecalciferol, all of the other compounds that we treat as vitamins are normal constituents of our diets with most found in more than one food group. The situation with cholecalciferol is such that, except for the small part of the world's population that obtains its protein from marine species, neither cholecalciferol nor ergocalciferol from irradiation of the plant sterol ergosterol is a normal component of foods. Its presence in dairy products is caused by food processors added to it during pasteurization–homogenization of milk.

1.1.5. Vitamins Are Required in Minute Amounts This is arbitrary but ranges from 2.0 μg (10^{-6} gm) for cyanocobalamin to 90 mg for ascorbic acid. Other essential nutrients including the essential amino acids and fatty acids generally are required in larger amounts. For example, the National Research Council's recommended daily intake of amino acids range from a low of 245 mg for tryptophan to 980 mg for phenylalanine and tyrosine (combined) and leucine [4]. Note that the value for tryptophan will only provide about 4 mg of niacin assuming that all 245 mg of tryptophan is converted to niacin.

1.1.6. Vitamins Are Required to Maintain Normal Biochemical Functions of the Tissues Most vitamins function either as a hormone/chemical messenger (cholecalciferol) structural component in some metabolic process (pantothenic acid), or as a coenzyme (phytonadione, thiamine, riboflavin, niacin, pyridoxine, biotin, folic acid, cyanocobalamin). At least one vitamin has more than one biochemical role. Vitamin A as an aldehyde (retinal) is a structural component of the visual pigment rhodopsin and in its acid form (retinoic acid) a regulator of cell differentiation. In addition to a general antioxidant role, the precise biochemical functions of ascorbic acid and α-tocopherol are still not well defined.

1.2. Causes of Vitamin Deficiencies

There are a variety of reasons why a person might be experiencing a vitamin deficiency ranging from economics to genetics to diseased intestinal tract to a variety of medical conditions to lifestyle to drug interactions. Table 1 contains a classification scheme of possible causes of vitamin deficiencies. These include inadequate ingestion of food, inadequate absorption of nutrients from the gastrointestinal tract, inadequate utilization of the nutrient once absorbed systemically, and increased nutritional requirements usually due to changes in lifestyle.

2. DIETARY REFERENCE INTAKES AND DAILY VALUES

It has been a challenge to determine and present vitamin dosing to health professionals and the general public. Until 1989, the common dosage for a vitamin was expressed as the RDA and Estimated Safe and Adequate. The latter was used for those vitamins for which there was inadequate information. One could think of it as a best guess estimate. Even earlier, there were minimum daily requirements (MDR). Beginning in the 1990s, panels were assembled to develop a new reference system, Dietary Reference Intakes (DRIs), for nutrients that include vitamins, minerals, fiber, essential amino acids, essential fatty acids, and other nutrients. The DRIs are published by the Food and Nutrition Board of the

Table 1. Causes of Nutrient Deficiencies

Cause	Mechanism/Reason	Remarks
Inadequate ingestion usually from a poor diet	Economic deprivation	Inability to purchase adequate amounts and a variety of food
	Self-imposed reducing diets	A 1200 calorie diet professionally selected from the four major food groups (dairy, fruits and vegetables, grains, and meat) containing neither fried food nor added sugar has been considered the least amount of food not requiring a vitamin supplement
	Disease	This is usually due to loss of appetite from such conditions as cancer chemotherapy, depression, and eating disorders
Inadequate absorption	Diseased intestinal tract	Examples include chronic inflammatory conditions such as Crohn's disease and parasites
	Mineral oil laxatives that may dissolve the oil-soluble vitamins	This could include retinol, cholecalciferol/ergocalciferol, α-tocopherol, and vitamin K from food
	Ion exchange resins (colestipol, cholestyramine, colestid) that complex with the bile salts and can interfere with the absorption of the oil-soluble vitamins	A vitamin supplement can be taken 1 h before or 2 h after taking the resin
	Aluminum antacids can complex with some of the vitamins and, when used chronically, most definitely can cause hypophosphatemia	Aluminum antacids are no longer commonly used
	Chronic alcohol intake	Ethyl alcohol interferes with the uptake of some vitamins (folic acid, thiamine) or, because of liver disease, proper processing and/or storage of vitamins
	Cystic fibrosis that can cause fat malabsorption (steatorrhea) due to inadequate production of pancreatic lipases	This could include retinol, cholecalciferol/ergocalciferol, α-tocopherol, vitamin K from food, all of which are more readily absorbed when they can be part of normal mixed micelle process that occurs with lipid digestion and absorption
Inadequate utilization	Genetic diseases	Examples include variants of maple syrup urine disease (MSUD) that will respond to thiamine (vitamin B_1) supplements and homocystinuria that will respond to pyridoxine (vitamin B_6). The mutation lies with apoenzyme such that the equilibrium between the apoenzyme and the coenzyme lies to the left. To push the reaction to the right requires additional coenzyme.
	Drug–vitamin interactions	These can interfere with vitamin processing in the intestinal tract, tie up the vitamin preventing it from being used, or possibly promote elimination of the vitamin. Examples include isoniazid–pyridoxine, phenobarbital–cholecalciferol, methotrexate–folic acid, and phenytoin–folic acid

(*continued*)

Table (*Continued*)

Cause	Mechanism/Reason	Remarks
Increased requirements above the recommended daily allowances (RDA)	Reference dietary indices are based on an average population performing average duties	Individuals performing more strenuous activities (e.g., competitive athletics, construction in the arctic) requiring additional intake of calories also will require more nutrients including vitamins. Patients recovering from a debilitating illness, severe burn injuries, and cancer also have increased nutritional needs. Nutritional assessments are becoming a more common part of medical treatment

National Academy of Sciences, National Research Council (see Bibliography).

2.1. How Do the DRIs Differ from Earlier RDAs?

There will be one set of reference values for both Canada and the United States, and there will be clear documentation on how reference values are selected. A goal is the promotion of nutrient function and biologic–physical well-being. Evidence concerning the prevention of disease and developmental disorders will be examined in addition to the tradition of how much nutrient is needed to prevent a deficiency symptom. Data supporting food components that, up to this time, have not been considered essential nutrient will be examined. Finally, there will be recommendations for future research.

2.2. Uses of Dietary Reference Intakes

The DRIs consist of four components. Each type of reference value is calculated from daily intakes averaged over time (usually one or more weeks). The surveys include, but not limited to, random selection of healthy individuals and asking them to either report what they have eaten or maintain food diaries, monitoring overall food production and consumption, and correlating a defined population's health status with the group's food intake. The four components are estimated average requirement (EAR), RDA, adequate intake (AI) and tolerable upper intake level (UL). Table 2 describes each DRI in more detail.

2.3. Daily Values

The FDA has a set of daily values (DV) that are used on the labels of multivitamin products and the nutrition labels required on many food products [5]. Many times the label will indicate the percentage of the daily value (%DV) of the nutrient. In general, the daily values do not have the age and gender differentiation seen with the DRIs. Table 3 shows a comparison of the FDA's daily values with the National Academy of Sciences adult RDA/AI values.

2.4. Determination of a Vitamin Dose

This is not easy. Ideally, a dose–response curve could be constructed that would provide defined endpoints such as an ED_{50} or ED_{90} and to avoid serious toxicities, the LD_{50}. The problem is defining a biological or pharmacological endpoint. Not all vitamin deficiencies have defined syndromes. Little is known of the pharmacokinetics of individual vitamins. In contrast with most drugs, the body stores vitamins, sometimes several months' supply. This should not be surprising when one examines human history. Until fairly recent times, humans regularly experienced food plenty and food deprivation (famine) as crops failed and animals moved away from settlements. Causes ranged from weather to war. To survive, the evolutionary development of humans included ways to store and reuse vitamins. Assuming a good diet, humans have a supply of 6–9 months of vitamin A. Humans efficiently recycle cobalamin by enterohepatic circulation. The intestinal flora produces a precursor to vitamin K.

Table 2. Methods for Estimating Vitamin Requirements

Vitamin	Determination Methodology	Remarks
Retinol (vitamin A)	Dark adaptation test	Considered reasonably sensitive
	Pupillary response test	Data do not exit relating pupillary threshold sensitivity to retinol intake
	Plasma retinol concentration	Insensitive to liver stores
	Relative dose response	An initial plasma concentration is determined followed by a second concentration in a defined time after administration of a small dose of retinol. The ratio is proportional to the liver stores of retinol.
Calciferol (vitamins D_2 and D_3)	Serum 25(OH)D_2 or D_3	This is considered a more accurate indicator of vitamins D status compared to serum vitamins D or 1,25$(OH)_2$D.
α-Tocopherol (vitamin E)	Lipid peroxidation markers	They are not specific to vitamin E
	Plasma α-tocopherol concentration	It does not seem to correlate with daily intake, but there is a linear relationship seen in tocopherol-depleted subjects
Vitamin K	Prothrombin time	It is used to assess patients on coumadin anticoagulant therapy, but does not appear to be a reliable measure of vitamin K status in otherwise healthy subjects
	Factor VII	Even though factor VII only has a 6 h half-life, it does not appear to be reliable
	Plasma or serum phylloquinone concentration	This does respond to changes in dietary intake within 24 h
	Urinary γ-carboxyglutamyl residues	This is considered promising
Thiamine (vitamin B_1)	Erythrocyte transketolase activity	This is considered the most accurate
	Urinary thiamine excretion	This method is also considered good
	Erythrocyte thiamine concentration	It is not as widely used
Riboflavin (vitamin B_2)	Erythrocyte glutathione reductase activity	It is the most common assay
	Erythrocyte flavin	Some question the sensitivity of this test because the difference between adequacy and inadequacy is small
	Urinary riboflavin excretion	Care must be exercised with the size of the dose and method of administration
Niacin/niacinamide	Urinary excretion	N^1-methylnicotinamide and N^1-methyl-2-pyridone-5-carboxamide are the two metabolites that are considered sensitive measures of niacin status
	Plasma concentration	N^1-methyl-2-pyridone-5-carboxamide plasma levels provide an indication of low niacin intake by dropping below detection limits
	Erythrocyte pyridine nucleotides	Erythrocyte NAD levels may replace measuring metabolites found in the urine
Vitamin B_6 family	Plasma pyridoxal phosphate	It changes slowly in response to changes in vitamin intake
	Erythrocyte and total blood pyridoxal phosphate	There may be racial differences because of lower kinase activity
	Urinary pyridoxic acid	It tends to reflect recent vitamin intake rather than general vitamin status

(*continued*)

Table (*Continued*)

Vitamin	Determination Methodology	Remarks
	Various erythrocyte transaminases	Aspartate and alanine transaminases have been studied most extensively. There is a lack of consensus regarding their usefulness as indicators of vitamin B_6 status
	Tryptophan catabolites	While useful, some of the reactions are affected changes in steroid hormone status
	Plasma homocysteine	See discussion in the vitamin B_6 section
Pantothenic acid	Urinary excretion	It is strongly dependent on intake
	Blood levels	There is poor correlation with urinary excretion values
Biotin	Urinary excretion	There is a correlation with induced deficiencies caused by eating raw egg white
Folic acid	Erythrocyte folate	Only the developing erythrocyte takes up folate. Therefore, this test is an indicator of long-term status and not a measure of immediate changes in folate status
	Plasma homocysteine	There is no question that elevated homocysteine decreases with folate administration. There is not enough information to support its use for determining DRIs
	Serum folate	While a measure of dietary folate intake, it is not considered a reliable measure of folate status
	Urinary folate	This test may underestimate folate needs
	Hematological status	Characteristic megaloblastic anemia develops late in folic acid deficiency
Vitamin B_{12} (Cobalamin)	Hematological response	These are the typical hemoglobin, hematocrit, and erythrocyte counts. Partial responses are of limited values
	Serum or plasma Vitamin B_{12}	A problem is that serum values may be maintained at the expense of tissue stores
	Methylmalonic acid	Studies need to be done to see if it will correlate with vitamin B_{12} status
	Homocysteine	This can be elevated in folate and vitamin B_6 deficiencies
	Holotranscobalamin II	This protein is responsible for receptor-mediated uptake of B_{12} into cells. Further work needs to be done before it is adapted for routine clinical use
Ascorbic acid (vitamin C)	Antioxidant functions	A variety of markers have been evaluated including LDL, VLDL, malondialdehyde, hydroxynonenal, and reduced glutathione
	Cellular DNA damage	These have not proven useful for estimating ascorbate requirements
	Urinary markers	Oxidized DNA is nonspecific

Table 3. Comparison of Adult Daily Values (DV) with Adult Recommended Dietary Allowances (RDA) or Adequate Intakes (AI)

Vitamin	Daily Value	RDA or AI*
Retinol (A)	1500 μg	700-900 μg
Ascorbic Acid (C)	60 mg	25-90 mg
Cholecalciferol (D_3)	400 IU	*200-600 IU
α-Tocopherol (E)	30 mg	7-15 mg
Phylloquinone (K_1)	80 μg	*55-120 μg
Thiamin (B_1)	1.5 mg	0.9-1.2 mg
Riboflavin (B_2)	1.7 mg	0.9-1.3 mg
Niacin	20 mg	12-16 mg
Pyridoxine (B_6)	2.0 mg	1-1.7 mg
Folate	400 μg	200-400 μg
Cyanocobalamin (B_{12})	2.0 μg	1.8-2.4 μg
Biotin	30 μg	*20-30 μg
Pantothenic Acid	10 mg	*20 mg

*Adequate Intake for these vitamins.

2.5. Methods to Determine a Valid Dose of a Vitamin

Each of the methods outlined in the following sections have significant problems in determining a dose–response curve for a vitamin. These include

2.5.1. Extrapolate from Animal Studies This method will be dependent on the species. The vitamin requirements differ among the common laboratory animals. The classic example is ascorbic acid, which is not a vitamin in most animals. α-Tocopherol, biotin, and pantothenic acid cause definite deficiency syndromes not seen in humans.

2.5.2. Metabolic Balance Studies in Humans This usually involves a specified length of time on a defined diet in which the urine and feces are monitored. The problem is that corrections have to be made for the vitamins that are stored. The pharmacokinetics of most vitamins has not been carefully determined.

2.5.3. Compare Nutrient Intake in Areas with and Without the Deficiency Disease First, not all vitamin deficiencies lead to a defined deficiency state. Second, rarely does one find an area deficient in only one nutrient. Most common deficiencies are due to inadequate diet, which means several nutrient deficiencies will result.

2.5.4. Saturation of Biochemical Function A reliable biochemical indicator is required. For niacin, which NAD^+ or $NADP^+$ containing enzyme should be selected? Which transaminase will be the indicator for pyridoxine? Which function should be selected for vitamin A: vision in the rods or cell differentiation?

2.5.5. Serum Levels This probably is the most reliable, but it does require very sensitive assay methods for those vitamins required in microgram (10^{-6}) amounts. It also requires knowledge as to how the vitamin is transported: free or bound to a plasma protein or a specific transport protein. There are specific transport proteins for vitamin A and vitamin D_3. The tocopherols will be found in the lipoproteins (VLDL, LDL, etc.).

2.6. Do Vitamins Really Help Beyond Treating Their Deficiency Diseases?

Vitamins are essential nutrients, and their deficiencies cause diseases, some with definitive symptoms. It is also know that excess doses of some vitamins produce identifiable adverse reactions. The latter is the reason some vitamins have tolerable upper intake levels. But what about ingesting vitamins at doses between the RDA/AI and the UL? This is not an easy question to answer. Studies are expensive, usually are retrospective, and require years to complete. Many times the results are equivocal [6–9]. Nevertheless, there is evidence that doses of vitamins in excess of their RDA/AI, but less than their UL (for those vitamins with an UL), may, at a minimum, not be effective and possibly exacerbate certain diseases [10,11].

2.7. Antioxidant Supplement Controversy

Two vitamins, oil-soluble tocopherol family (vitamin E) and water-soluble ascorbic acid (vitamin C) plus the β-carotene, are considered biological antioxidants along with specific vitamin functions or, in the case of β-carotene, precursor to the vitamin A family. In addition to these three, there are other antioxidants in the foods we eat including lycopene. There have been several studies trying to answer the question if antioxidants can prevent or even treat cardiovascular dis-

ease and malignancies. In general, the results have been equivocal [9]. The one exception is β-carotene that may exacerbate lung cancer in smokers [12,13].

3. VITAMINS

The order of vitamins in this chapter will follow the classical classification-based solubility: fat soluble and water soluble. This classification originated before there was any understanding of the structural chemistry of the vitamins. There were fat-soluble A and water-soluble B. Thiamine was the first vitamin (B_1) whose structure was elucidated. It is an amine leading to the term "vital amine" and finally vitamin (without the "e"). The letter designations, for the most part, are being relegated to history. Many of the vitamins are actually groups of structures and, therefore, using a single letter can be misleading. Also, the structures occurring naturally in food and commercial preparations may actually be provitamins that have to be converted to the biochemically active form. Unless specified differently, the information in the following summaries will be found in the "Dietary Reference Intakes" published by the Institute of Medicine listed in the general bibliography at the end of this chapter.

3.1. Retinol (Vitamin A) Family

Early work on this vitamin was confusing because similar outcomes were seen with ingestion of "yellow vegetables" and colorless fish liver oils. It was finally shown that carotene (the yellow pigment) extracts from vegetables were converted to colorless retinal.

3.1.1. Chemistry The commercial form of vitamin A is all-*trans* retinol, usually formulated as the acetate or palmitate ester. The active forms are the two oxidation products (Fig. 1): retinal that is a structural component of the visual pigment, rhodopsin, and the two retinoic acids (all-*trans* and 9-*cis*) that are required for cell differentiation. There are specific nuclear receptors for retinoic acid, RAR and RXR. While the vitamin is marketed in the all *trans* form, both retinal and retinoic acid will be present in *cis* forms. There are also commercial forms related to the retinoic acid structure that have *cis* stereochemistry.

Although promoted commercially, carotenes are sold for their antioxidant activity rather than a source of the vitamin A group. Nevertheless, the carotenes are the source of the vitamin in yellow vegetables. There are many carotenes of which three are shown in Fig. 2. Only β-carotene is symmetrical (note the dashed line) and theoretically will produce two equivalents of retinal following the enzyme catalyzed oxidative cleavage. In reality, more recent studies indicate that vitamin A activity is six times the vitamin A activity derived from β-carotene. The problem is one of capacity in the intestinal mucosa cell to cleave the carotenes. Furthermore, there appears to be regulation of the cleavage of β-carotene. As the stores in the liver reach capacity, there is less conversion of β-carotene being oxidized to retinal. This is one of the reasons that β-carotene nutritional supplements enter the body intact. Furthermore, the bioavailability of β-carotene is significantly lower than that of retinol [14].

3.1.2. Uptake and Metabolism (Fig. 3) Both vitamin A esters, whether from animal tissues or a vitamin supplement, and β-carotene must be incorporated into mixed micelles along with other lipid material. The vitamin A esters are hydrolyzed by intestinal esterases. Both retinol and β-carotene are then absorbed into the mucosa cell where β-carotene is oxidatively cleaved to retinal and then reduced to retinol. The retinol, independent of the source, follows the same steps as seen with 2-monoglyceride from triglyceride digestion. The retinol is re-esterified, usually with palmitic acid, and incorporated into the chylomicrons along with the other dietary lipids. The chylomicrons first enter the lymph and then move to the circulatory system. The triglycerides are removed from the chylomicrons and deposited in the adipose and skeletal muscle cells leaving chylomicron remnants that are transported to the liver where they are stored [15]. Transportation from the liver to tissues where required is done on specific retinol binding proteins. Humans consuming a balanced diet store several months of retinol esters in their livers. It must be pointed out that the retinol binding pro-

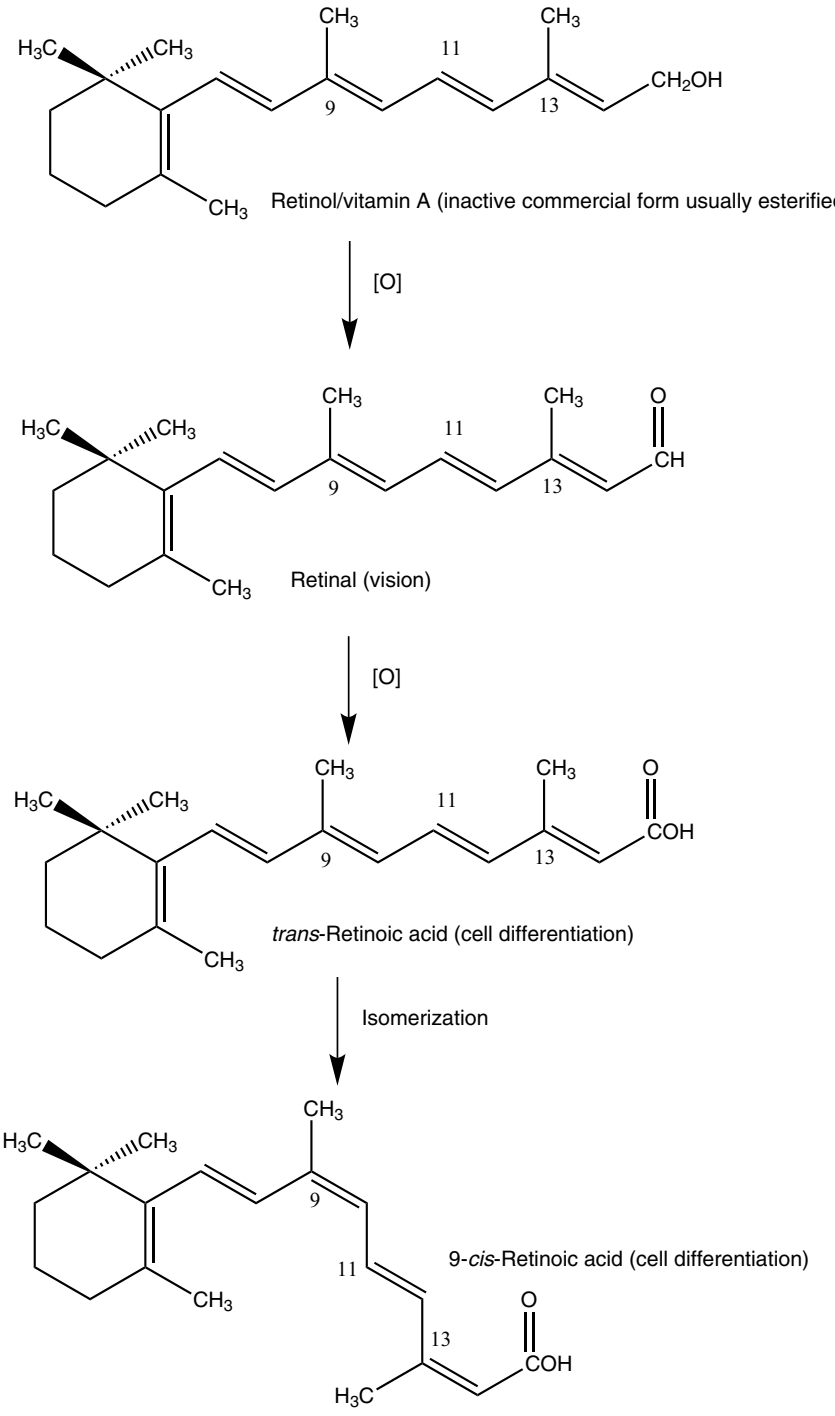


Figure 1. Retinol (vitamin A) conversions.

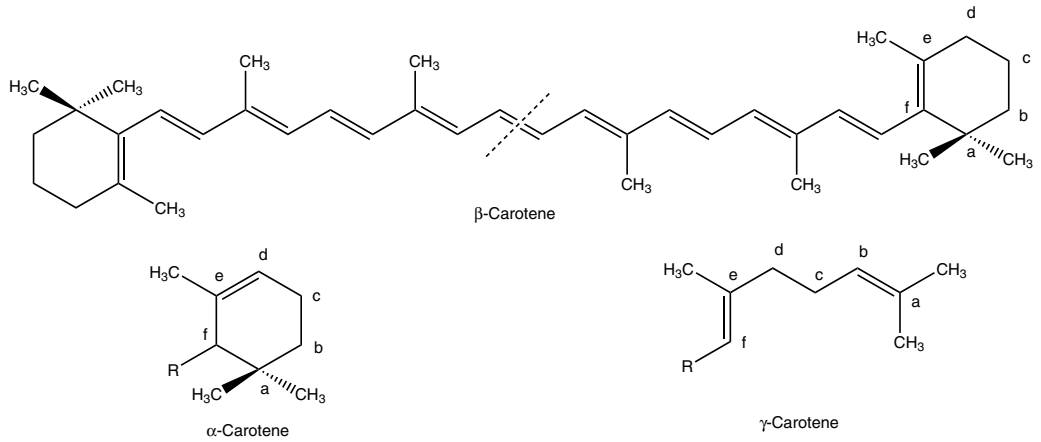


Figure 2. Carotenes.

teins have functions beyond that of transporting retinol esters. Retinol binding protein 4 (RBP4) is produced by adipocytes, and in obese subjects there is increased amounts of this protein. RBP4 may cause insulin resistance in skeletal muscle and increased gluconeogenesis in the liver. The combination of these two responses may contribute to the hyperglycemia seen in type 2 diabetes [16,17].

3.1.3. Biochemical Functions and Deficiency Three retinoids appear to have most of the biochemical functions attributed to vitamin A. The retinoic acids are required for cell differentiation and are the ligands for two families of nuclear receptors: *trans*-retinoic acid for $RAR_{\alpha,\beta,\gamma}$ and 9-*cis*-retinoic acid for $RXR_{\alpha,\beta,\gamma}$. These receptors are part of a family of super receptors that include the steroid hormones and cholecalciferol. Vitamin A deficiency can lead to a variety of symptoms depending on the age of the deficient person. The most serious syndrome is keratomalacia that results in desiccation, ulceration, and xerophthalmia of the cornea and conjunctiva. It is one of the leading causes of blindness in infants and children.

Retinoic acid is required for the development of goblet mucous cells. A deficiency results in proliferation of basal cells with increased keratinization of the epithelial structures. Mucous is one of the essential physical barriers that prevents pathogens from entering the body. Therefore, a vitamin A deficiency increases the risk of infection.

The aldehyde form, retinal, is an essential component of the visual pigment found in the rods of the eye. A very brief outline of the

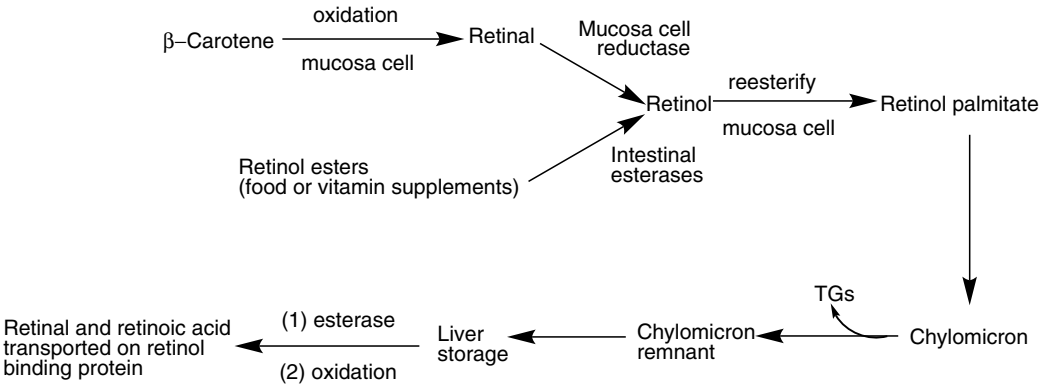


Figure 3. Uptake of dietary retinol and carotene.

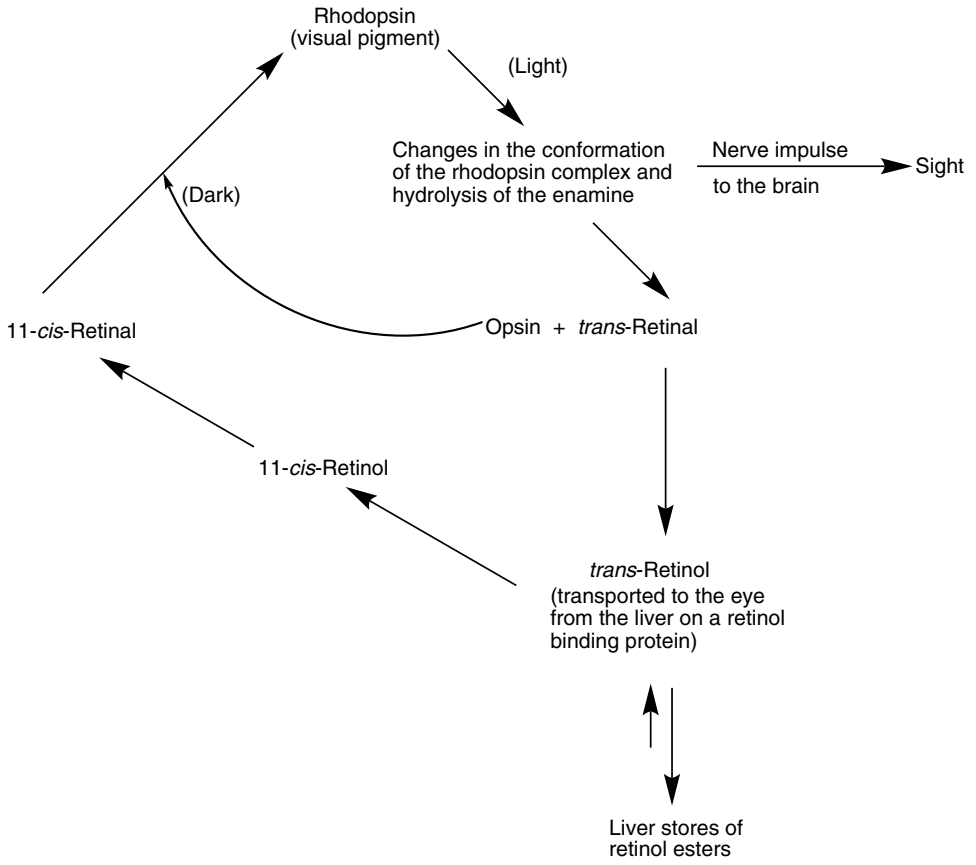


Figure 4. Outline of rhodopsin cycle.

rhodopsin cycle is shown in Fig. 4. Retinol is transported from the liver to the eye where it is converted to 11-*cis*-retinal. In the rod, the aldehyde forms an enamine with a lysine on opsin forming rhodopsin. In the presence of light, *trans*-retinal forms with cleavage of the enamine sending a nerve impulse to the brain along the optic nerve. In the dark, 11-*cis*-retinol re-forms followed by oxidation to 11-*cis*-retinal and the cycle repeats.

A deficiency causes night blindness, which is considered an early symptom of vitamin A deficiency. Night blindness refers to decreased ability to see in very dim light because there is an adequate amount of retinal in the eye to fully "stock" the rods with functional rhodopsin. There is some evidence that as retinol levels in the liver decrease, the equilibrium favors the movement of retinol from the eye back to the liver.

3.1.4. Dosage Forms Vitamin A is unstable. It easily dehydrates (Fig. 5) forming a resonance-stabilized carbonium ion. Therefore, the two most common forms for both oral and parenteral administration are the acetate or palmitate esters. With its extensive conjugation, vitamin A is light sensitive and subject to oxidation. Therefore, vitamin formulators must protect the product from light and air.

3.1.5. Hypervitaminosis A In high doses, vitamin A can be toxic. Acute poisoning is rare and dependent on the dosage form. Nausea and vomiting are the most common symptoms. Most rapidly absorbed are the "clear" emulsions (usually formulated with a Tween or other surfactant). Next in order are the standard emulsions, usually produced from fish liver oils. The most slowly absorbed are the dry tablet formulation or an oil solution in a

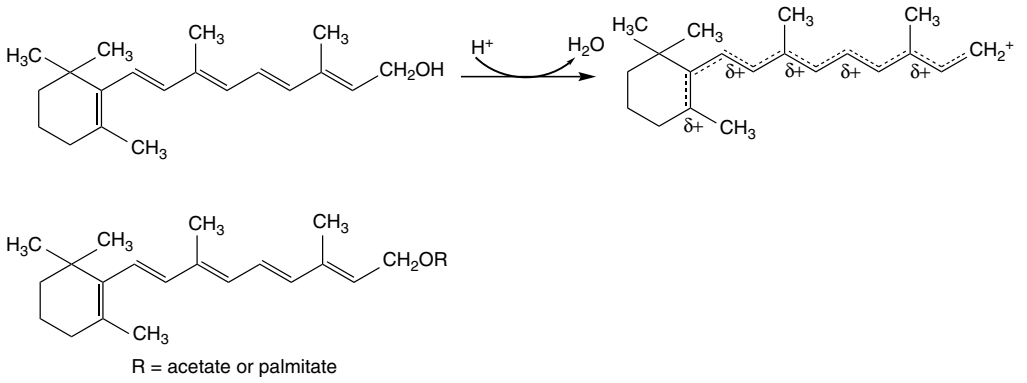


Figure 5. Vitamin A's commercial forms.

capsule. Chronic hypervitaminosis is more common and is more commonly seen when people consume fish liver oil concentrates. The symptoms are nondescript and include fatigue, malaise, lethargy, abdominal discomfort, bone/joint pain, severe and throbbing headache, insomnia, restlessness, dry and scaly skin, loss of body hair, brittle nails, constipation, and irregular menses, symptoms that might make a users conclude that they are vitamin A deficient. Depending on the health of person's liver, there is risk of developing cirrhosis. Multiple vitamins containing vitamin A contain a warning that excessive intake of the vitamin may increase the risk of osteoporosis in postmenopausal women [18]. There is a daily tolerable upper intake level (UL) of 3000 μg for this vitamin. The UL to RDA ratio is narrow, 3–5, relative to most vitamins.

3.1.6. Hypercarotenosis This occurs from massive doses of carotene, which exceed the capacity of the mucosa cells to cleave the molecule to retinal derivatives. The excess carotene becomes deposited in the body tissues. Except for the *yellow or bronze-orange* skin, there seem to be no other symptoms. The skin coloration will slowly disappear when carotene intake stops and the absorbed carotene is slowly eliminated from the body. A commercial form of carotene is indicated for the photosensitivity seen in erythropoietic porphyria. Carotene capsules also are sold with claim that a person can have a tanned appearance without the need of UV radiation. Patients who drink large amounts of carrot juice sometimes show signs of hypercarotenosis.

3.1.7. Dietary Reference Intakes

Vitamin A's DRIs are expressed at retinol activity equivalents (RAE)	
1 μg RAE—1 μg retinol, 12 μg β-carotene, and 24 μg α-carotene	
AI (infants 1–12 months)	400–500 μg/day
EAR	
Children (1–8 years)	210–275 μg/day
Boys (9–18 years)	445–630 μg/day
Girls (9–18 years)	420–485 μg/day
Men (19–70+ years)	625 μg/day
Women (19–70+)	500 μg/day
Lactating	880–900 μg/day
RDA	
Children (1–8 years)	300–400 μg/day
Boys (9–18 years)	600–900 μg/day
Girls (9–18 years)	600–700 μg/day
Men	900 μg/day
Women	700 μg/day
Pregnant	750–770 μg/day
Lactating	1200–1300 μg/day
UL	(Applies only to preformed vitamin A in foods, fortified foods, and supplements. It does not apply to vitamin A derived from carotenoids) 3000 μg/day for all adults including pregnant women. There is some concern of teratogenic effects based on the experience of the retinoids used in therapy

3.1.8. Pharmacologically Active Retinoids Because vitamin A deficiency shows in the

keratinization of epithelial tissue, vitamin A was, at one time, recommended for skin conditions including acne. There is no clinical evidence that vitamin A is effective for this type of skin condition. Now that it is realized that the active form is retinoic acid, the focus has been on developing pharmacologically active compounds based on this structure. These are divided into three groups: treatment of (1) acne, (2) the autoimmune disease, psoriasis, and (3) malignancies.

Retinoid and Retinoid-Like Drugs Used in the Treatment of Acne (Fig. 6) The first product introduced was tretinoin, which is topical all-*trans*-retinoic acid. Its effectiveness probably may not be related to any direct retinoid activity, but by its producing a complex response related to increasing the turnover of follicular epithelial cells. The result is decreased cohesiveness of follicular epithelial cells.

Tretinoin also is used as an antiwrinkle cream. There is disagreement on the mechanism and there may be more than one. Some improvement may be due to the irritation the drug causes. There is an increase in epidermal cell turnover shedding older cells and thickening the skin. Also, the drug may combine with epidermal retinoic acid receptors decreasing keratin production. Keratin can contribute to skin wrinkling [19].

Isotretinoin, 13-*cis*-retinoic acid, is very effective in treating severe forms of acne. It also is very teratogenic. There are elaborate procedures involving the prescribing physician, dispensing pharmacist before a female patient can receive the drug. There is also some concern that the sperm of men using the drug might be affected [20].

Although used topically, the nonretinoid, adapalene, does bind to the retinoic acid nuclear receptor and does affect cell differentiation, keratinization, and inflammatory responses.

Drug Based on the Retinoid Structure Used to Treat Psoriasis (Fig. 7) Etrinate is the ethyl ester of acitretin and is active after hydrolysis to the acidic drug. The terminal half-life after 6 months of etrinate therapy is 120 days. In contrast, the terminal half-life of acitretin is only 33–96 h. Both drugs are teratogenic and require elaborate warnings before being prescribed and dispensed. The third drug, tazarotene gel, is administered topically and is indicated for both acne and psoriasis. While there appears to be minimal absorption if used over limited skin area, there is some absorption with retention by the body for up to 3 months. It can cause fetal damage and cannot be used by pregnant women or women who may become pregnant.

Retinoids Used in the Treatment of Malignancies [21] (Fig. 8) Retinoic acid has been evaluated as a possible treatment for malignancies. This is based on the fact that it is required for proper cell differentiation, and products based on the retinoid structure are teratogenic. All-*trans*-retinoic acid is used in combination with other chemotherapeutic agents for the treatment of acute promyelocytic leukemia [22]. It does not kill the malignant cell. It seems to facilitate its differentiation into a nonproliferating myelocyte. Alitretinoin, 9-*cis*-retinoic acid, indicated for Kaposi's sarcoma is administered topically. It binds to all six retinoic acid receptors.

Bexarotene is most selective for the three $RXR_{\alpha,\beta,\gamma}$ receptors. It also binds several other nuclear receptors including the RAR, vitamin D, thyroid, and peroxisome proliferator activator receptors, which probably explains its numerous adverse reactions. The drug is indicated for cutaneous T-cell lymphoma and is available as capsules and a topical gel.

3.2. Vitamin D Family

Rickets was first reported in the mid-17th century. It could be lethal, but bone deformation was more common. Rickets, in the United States, continued to be a problem until the 1930s when vitamin D fortified milk became common. Older adults show the bow-legged characteristic of childhood rickets. There were many attempts at giving calcium and/or phosphorous supplements. Finally, it was realized that rickets was not found in sunny climates, and populations whose main source of protein was fish did not have rickets. In 1924, Professor Henry Steenbock, University of Wisconsin, demonstrated that irradiation of foods, including milk, produced food that was antirachitic [23,24].

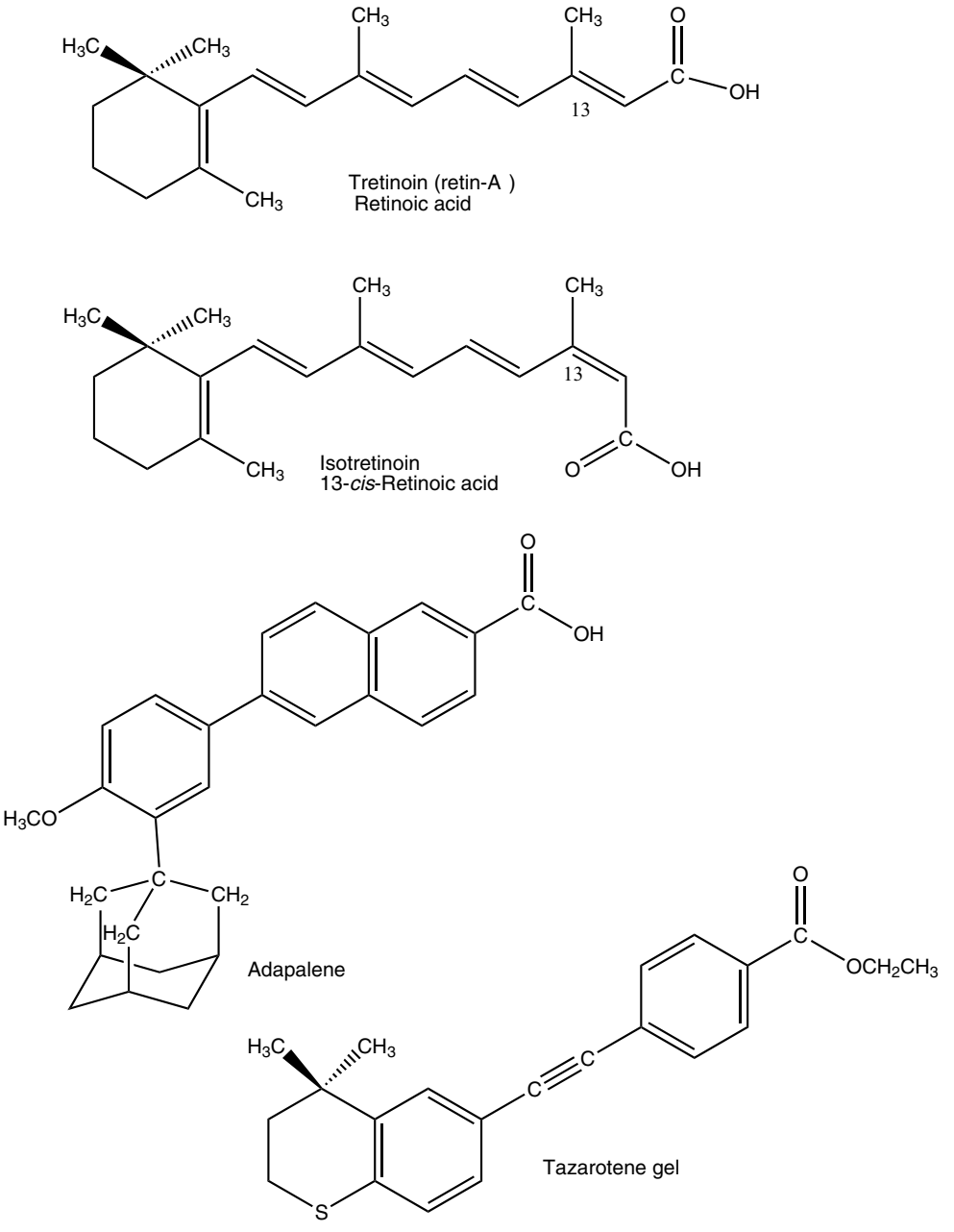


Figure 6. Retinoid and retinoid-like drugs indicated for acne.

When there is adequate sunlight, no dietary source of the vitamin is required. Indeed, an argument can be made that the vitamin D is not a normal component of the diet. In the United States, it is added to milk, other dairy products, and dairy substitutes. Fish is about the only natural food source. Cholecalciferol (vitamin D_3) is produced in the body from endogenously synthesized 7-dehydrocholecalciferol (Fig. 9). Consistent with a hormone

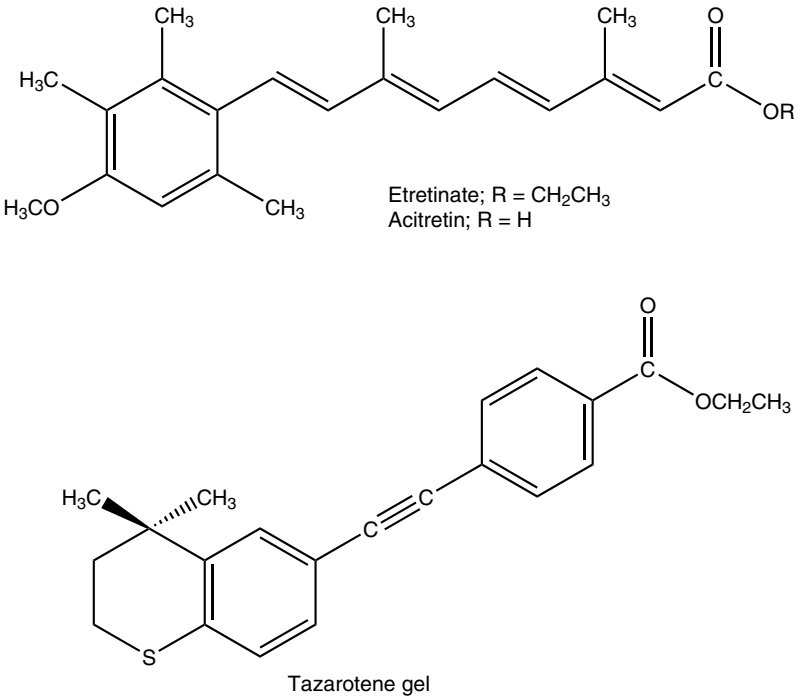


Figure 7. Drugs based on the retinoid structure indicated for psoriasis.

model, excess amounts of cholecalciferol can result in excess calcium uptake from the intestinal track leading to calcification of soft tissues.

3.2.1. Chemistry There are two forms of vitamin D, and originally both were considered biologically equivalent. Irradiation of the major plant sterol, ergosterol, produces ergocalciferol, also known as vitamin D_2 (Fig. 10). However, kinetic studies show significant differences between 25-hydroxy D_3 and 25-hydroxy D_2. Whereas both showed similar initial rises in serum concentration for the first 3 days, 25-hydroxy D_3 continued to increase over 14 days, but 25-hydroxy D_2 returned to baseline. The area under the curve (AUC) was significantly greater for 25-hydroxy D_3 [25,26].

Being photochemical reactions, in contrast to enzyme catalyzed biochemical reactions, the formation of cholecalciferol is not clean. Exposure of human skin to sunlight of 295–300 nm converts 7-dehydrocholesterol to previtamin D_3. The isomerization to cholecalciferol is heat catalyzed. Continuous exposure to ultraviolet radiation from the sun results in the reversible formation of lumisterol and tachysterol [27,28]. Once the B ring of the two steroids has been cleaved, the products should no longer be referred to as steroids.

3.2.2. Vitamin Uptake and Metabolism This is very complicated and is dependent upon the source. From a biochemical viewpoint, it is a hormone because in the presence of adequate sunlight, cholecalciferol is produced from 7-dehydrocholesterol in the skin. In this context vitamin D, when administered in supplements, could be considered replacement therapy. When photochemically synthesized in the skin, it is transported to the liver by a specific binding protein formed in the skin where the cytochrome enzyme system (CYP P450) hydroxylates it to 25-OH-cholecalciferol (25(OH) D_3; Fig. 11). This pulls the reversible reactions shown in Fig. 9 toward the desired cholecalciferol. Dependency on the binding protein for transport from the skin also provides a control to prevent overproduction of the hormone and possible hypercalcemia.

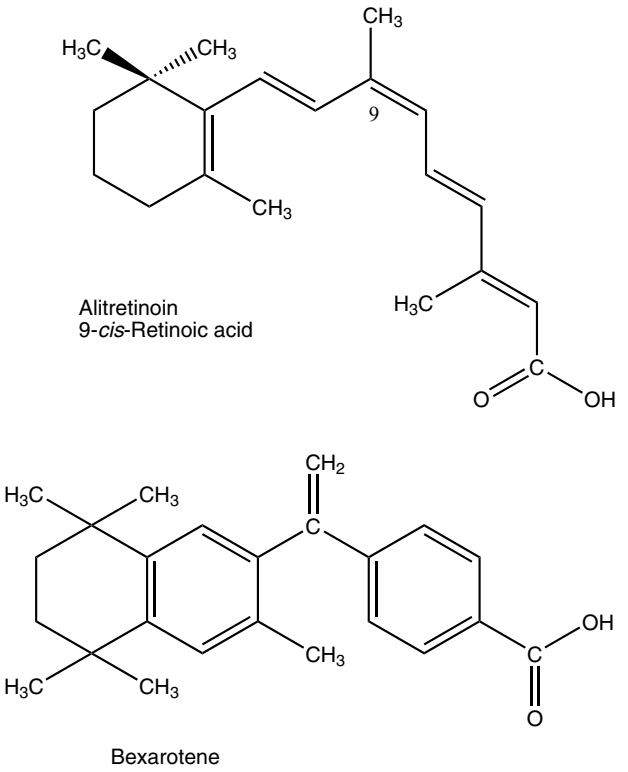


Figure 8. Retinoids indicated for malignancies.

When taken orally, it follows the same route as other dietary lipids. It is part of the mixed micelles and ends up on the chylomicrons formed in the intestinal mucosa cells. It is then transported to the liver where, like the endogenously produced cholecalciferol from sunlight, it is hydroxylated to $25(OH)D_3$.

The 25-hydroxylated product is then transported to the kidney where it is hydroxylated a second time by 25(OH)D-1α-hydroxylase (kidney CYP P450) forming the active 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). The latter is transported to the intestinal tract where it attaches to a nuclear receptor that signals the mucosa cell to synthesize a calcium transport protein. The final $1,25(OH)_2D_3$ product can be considered a kidney hormone that regulates calcium intake. Finally, $1,25(OH)_2D_3$ is oxidized to inactive calcitroic acid that is excreted through the kidney. The $24,24(OH)_2D_3$ metabolite may be part of the degradation process for $25(OH)D_3$ that is not transported to the kidney, but it also can elevate serum calcium levels [16]. Patients with kidney failure can experience vitamin D-resistant rickets. Because they cannot carry out the final hydroxylation step, $1,25(OH)_2D_3$ (calcitriol) is prescribed for their hormone replacement therapy.

3.2.3. Biochemical Function Vitamin D function is complex and, with the exception of calcium transport from the intestinal tract, is poorly understood. Specific vitamin D receptors (VDR) are found in 30 different tissues including bone, intestine, prostate, hematopoietic cells, and skin [29]. Like the retinoic acid receptors, the vitamin D receptor is a nuclear receptor belonging to the steroid hormone superfamily of nuclear receptors that includes receptors for estrogen, glucocorticoids, thyroid hormones, and retinoic acid. There are at least 50 genes that respond to hydroxylated calciferols regulating calcium release and uptake and cell division.

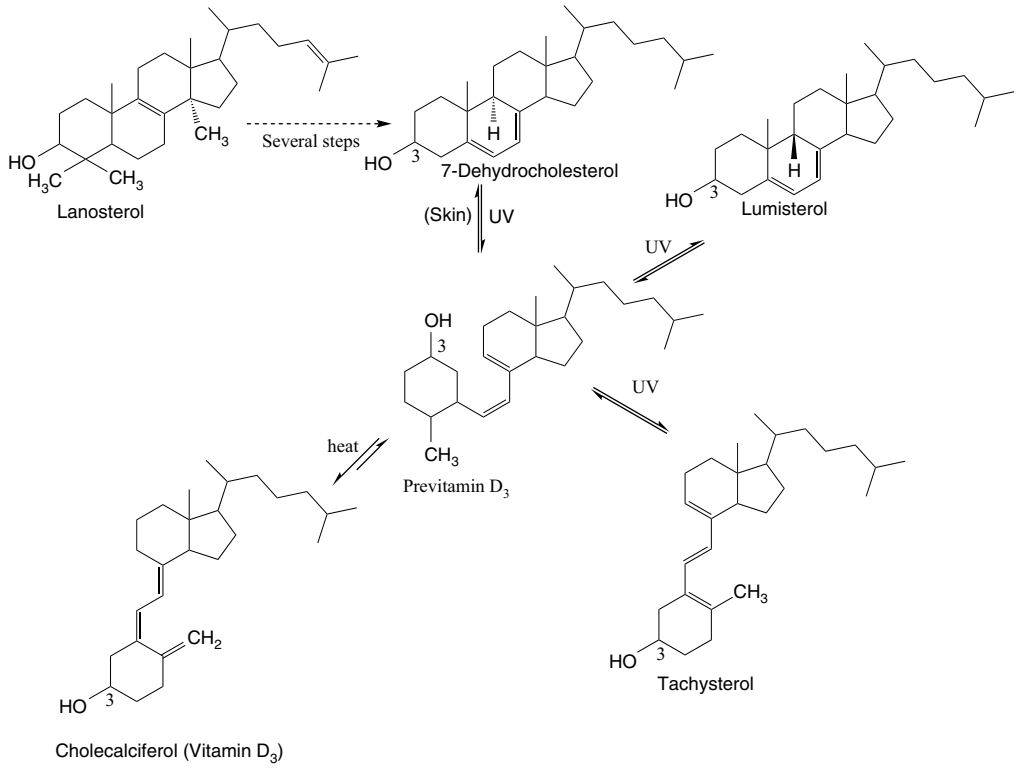


Figure 9. Photochemical formation of vitamin D_3 (cholecalciferol).

Calcium Regulation There are at least three, possibly four, hormones that regulate calcium metabolism, parathyroid (PTH), calcitonin, $1,25(OH)_2$, and gut-produced serotonin. Bone is the principal calcium reservoir, and it is a dynamic tissue with calcium being released and deposited. New calcium comes from our diet, and calcium is excreted through the kidneys. In response to low serum calcium levels, PTH stimulates the hydroxylation of 25(OH) D_3, leading to formation of calcium transport protein and osteoclast cells required to release calcium from bone. PTH also inhibits calcium excretion by the kidney. In contrast, calcitonin (produced in the thyroid gland) acts when serum calcium levels are high. It promotes the deposition of calcium into bone and excretion of calcium by the kidney. Using knockout mice, evidence has been published that serotonin produced in the gut may be the "master"

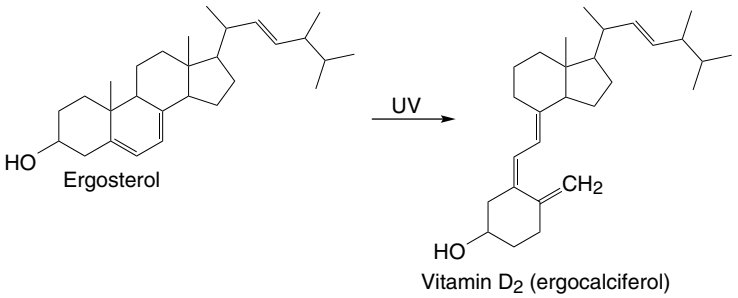


Figure 10. Photochemical formation of vitamin D_2 (ergocalciferol).

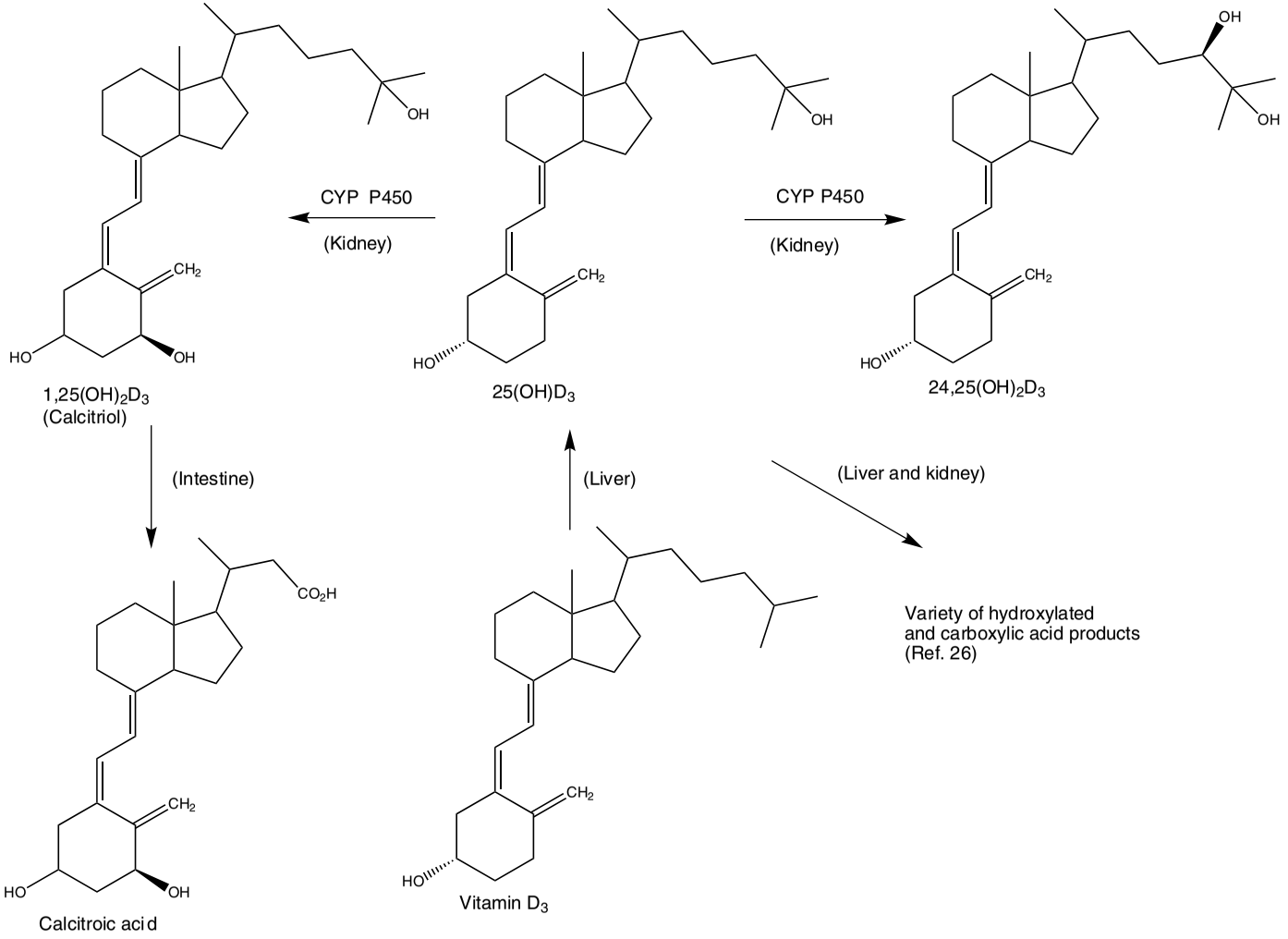


Figure 11. Vitamin D metabolism.

regulator of calcium flux to and from bone. This serotonin must act in the periphery because it does not pass the blood–brain barrier. In the knockout mice, the more the serotonin is released, the greater the calcium loss from bone. The process is reversed with low production of serotonin [30].

Rickets in infants and children and osteomalacia in adults are caused by a deficiency of vitamin D. Osteoporosis is complex involving many variables including vitamin D status and lack of calcium in the diet. Today most vitamin D deficiencies are caused by a lack of sunlight or restricted diet. The lack of sunlight may be caused by living in northern latitudes. The latter also can be affected by the amount of skin pigmentation [31–33]. A strict vegetarian diet lacking vitamin D fortified dairy products or fatty fish, particularly in children, may also result in rachitic lesions in the bone [34,35]. Mechanistically, rickets and osteomalacia are similar in that both are being characterized by bone softening. Normal bone growth and maintenance require that the osteoblast cells lay calcium hydroxyapatite onto a cartilage matrix. A deficiency of vitamin D results in a lack of mixed calcium salt available to the osteoblast cells. In infants and children, the cartilage continues to grow. Cartilage, being soft, cannot support the child's weight, leading to the typical bowlegs seen in a rachitic child. An adult also will have bone deformations, particularly in the pelvic area, because the bones cannot support the upper torso. Osteoporosis is a different disease. It can be thought of as osteoclast cells removing calcium more quickly such that osteoblast cells can lay calcium down. The result is porous, brittle bones that break easily. Incidence of hip fractures correlates with $25(OH)D_3$ status [36]. A patient's vitamin D status can be monitored by measuring the patient's $25(OH)D_3$. At one time calcitonin sometimes was prescribed to decrease the release of calcium from bone by osteoclast cells. Calcium with vitamin D is currently recommended to replace calcium being released from bone and excreted through the kidney.

Vitamin D Analogs Used in Chronic Renal Failure Because $1,25(OH)_2D_3$ is produced in the kidney, renal failure leads to a deficiency of this hormone and vitamin D-resistant rickets. The solution was prescribing synthetic $1,25(OH)_2D_3$ (generic name calcitriol). Nevertheless, these patients can experience hyperparathyroidism caused by the parathyroid gland attempting to compensate for the lack of $1,25(OH)_2D_3$. The result is increased osteoclast activity leading to a loss of calcium from the patient's bones, leading to hypercalcemia and either osteomalacia or osteoporosis. To overcome these complications, there are two synthetic ergocalciferol analogs (Fig. 12) indicated for secondary hyperparathyroidism associated with chronic renal failure. Note that both compounds contain the hydroxy group at position 1, the position where the kidney carries out its hydroxylation to produce the 1,3,25-trihydroxy product. Doxercalciferol is 1,3-dihydroxy-ergocalciferol and, in the liver, is hydroxylated to active $1,25(OH)_2D_2$ (the ergocalciferol analog of calcitriol). It is not clear why doxercalciferol is more selective than calcitriol. In contrast, paricalcitol is the 19-normethyl analog of $1,3,25(OH)_3D_2$ produced from ergocalciferol and requires no further hydroxylation reactions.

Other Activities Attributed to Vitamin D in Its Active Forms The status of vitamin D today is where vitamins C and E were years ago. Because of the wide distribution of the vitamin D receptors, vitamin D's role is cell biochemistry and maintenance of health is being widely investigated. A search in Medline will produce papers in highly regarded refereed journals showing that vitamin D deficiency is associated with cardiovascular disease, autoimmune diseases, increased bone fractures, metabolic syndrome, and diabetes. With the exception of bone strength, there is little evidence that intervention with vitamin D supplements will treat these diseases with the possible exception that a person definitely is vitamin D deficient.

The role of $1,25(OH)_2$ vitamin D in regulating cell division is under active investigation. Populations living in areas with higher exposure to sunlight have lower incidence of prostate, breast, and colon cancers [37]. On the other hand, there is some evidence that excessive vitamin D might exacerbate prostate cancer [38]. It is beyond the scope of this chapter to provide an in-depth, referenced review. An extensive review of vitamin D and

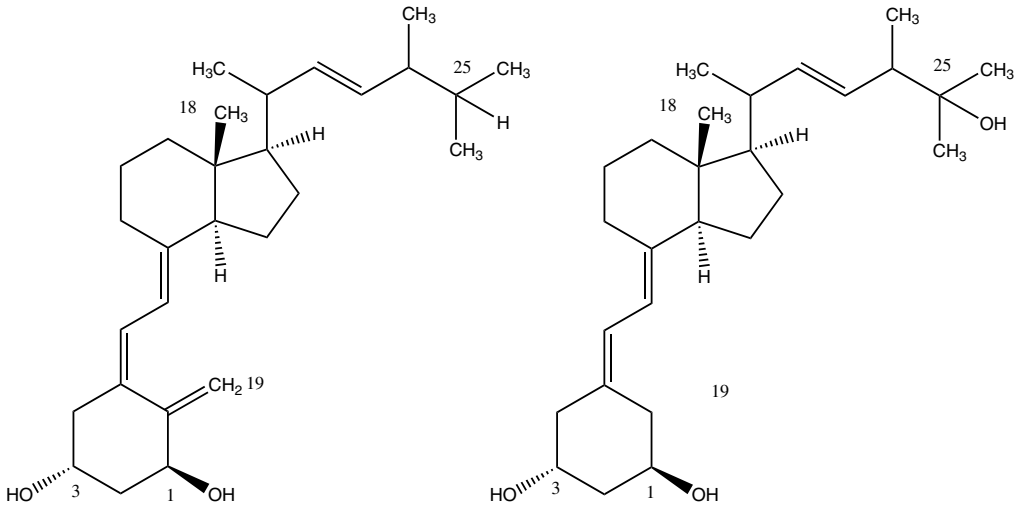


Figure 12. Ergocalciferol analogs indicated for chronic renal failure.

cancer is available from the International Agency for Research on Cancer of the World Health Organization [39].

There are concerns that taking megadoses of vitamin D causes hypercalcemia leading to possible calcium deposits in the soft tissues and possibly blood vessel walls. In contrast with vitamins C and E, vitamin D can be considered a hormone with the cautions associated with dosing any hormone therapy. Therefore, the challenge is to develop compounds that are selective for receptors involved with controlling cell division but will not affect calcium transport leading to hypercalcemia. The initial analog on the market is calcipotriene (Fig. 13), which is indicated for psoriasis, a nonmalignant proliferation of cells. Its use is limited to topical application. When administered internally, hypercalcemia can result. Attempts have been made at pulse dosing of $1,25(OH)_2D_3$ to maximize inhibition of cell division with minimal calcemic activity [40].

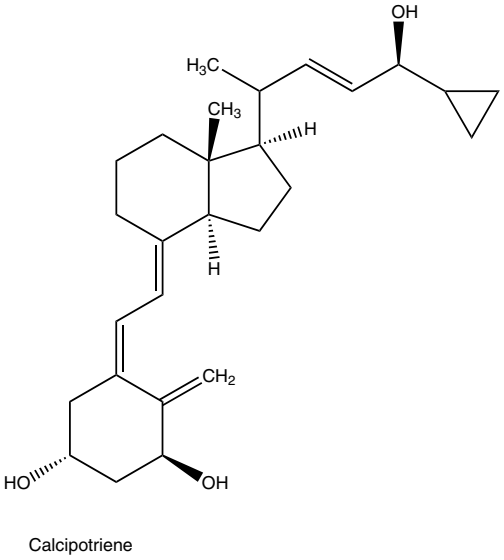
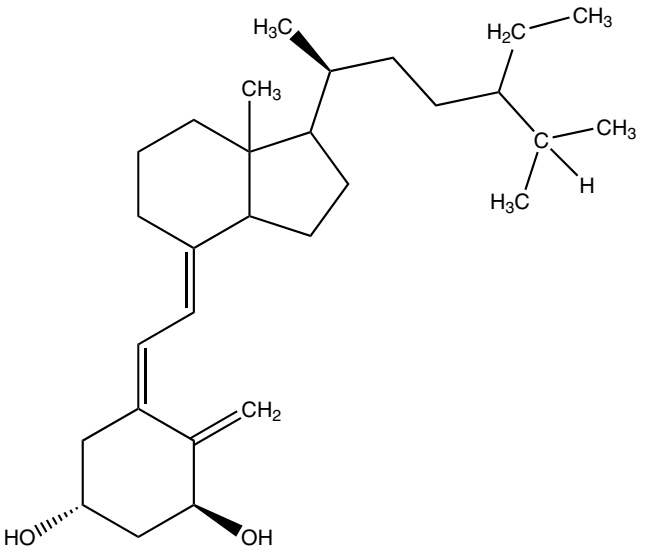


Figure 13. Calciferol analog indicated for psoriasis.

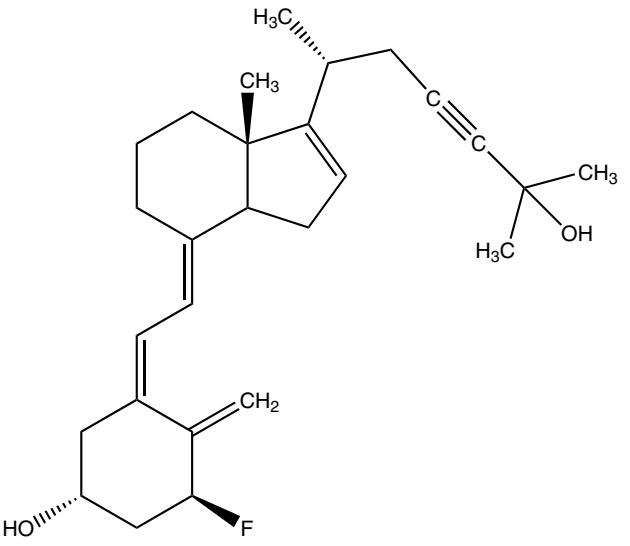
Most of the calciferol analogs are based on modifications of the 17-alkyl side chain. Modifications of the A ring include 19-nor methylene and 3-nor hydroxy analogs with and without the 1-hydroxy moiety. The 1α-hydroxy-24-ethyl cholecalciferol analog (Fig. 14) was less calcemic in mice and inhibited the development of preneoplastic lesions in mammary glands [41]. Unsaturation at position 16 (Fig. 15) provides modest antiproliferative effects on prostate cancer cells with little hypercalcemia [42]. A series of 20-cyclopropyl-cholecalciferol analogs



1-(OH)-24-ethyl-cholecalciferol

Figure 14. 1α-Hydroxyvitamin D_3.

(Figs 16 and 17) showed good activity against human prostate, breast, and myeloid leukemia cell lines [43]. The most potent in this series were the 19-nor methylene analogs independent of whether the side chain had ethylene or acetylene unsaturation. Modification of the side chain with a 25-keto or oxime (R_1 = O or NOH, respectively) with or without an additional hydrogen or methyl (R_2 = H or CH_3, respectively) produced analogs as antiproliferative *in vitro* as 1,25$(OH)_2D_3$ (Fig. 18). The oximes were less calcemic [44].



1,25-$(OH)_2$-16-ene

Figure 15. 16-Ene-25-yne D_3 analogs.

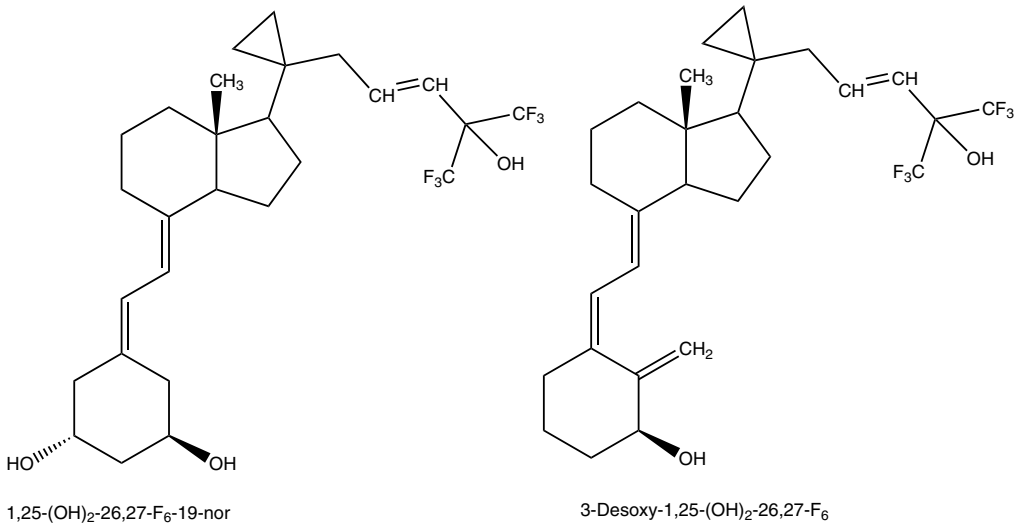


Figure 16. 23-*E*-Ene-20-cyclopropyl D_3 analogs.

3.2.4. Dosage Forms The commercial products are produced by irradiation of 7-dehydrocholesterol or ergosterol under controlled conditions. The final yield is about 50%. Although more stable than vitamin A, vitamin D is sensitive to oxygen and tend to isomerize into inactive isomers in the presence of trace metals, which can cause problems in formulating a vitamin mineral supplement. It is stabilized with antioxidants and protective coatings.

3.2.5. Hypervitaminosis D Like vitamin A, the calciferols are one of the few vitamins that have tolerable upper intake levels (UL) with the UL to RDA ratio of 5–10, which is similar to vitamin A. Hypervitaminosis D causes increased absorption of calcium and phosphorous (P follows Ca), leading to calcification of the tissues, vomiting, kidney damage, and so on. It can be the most serious of the hypervitaminoses. The reports that vitamin D inhibit proliferation of tissue and may protect against certain cancers could cause the public to overdose with this vitamin. Nevertheless, some medical specialties are recommending that adults take in 600–1000 IU (15–25 μg) per day divided between morning and evening. Because of vitamin D's low concentration in breast milk, pediatric vitamin D is being recommended.

3.2.6. Dietary Reference Intakes There is no RDA for this vitamin. It is difficult to derive a RDA for a population because a significant percentage will receive adequate amounts of the vitamin from sunlight because of the area of the country where they live. On the other hand, the diverse population of the United States means the people of color may need more of the vitamin from fortified milk relative to those whose ancestors came from Northern Europe.

AI	
Infants (0–12 months)	5 μg (200 IU)/day
Children (1–8 years)	5 μg (200 IU)/day
Boys (9–18 years)	5 μg (200 IU)/day
Girls (9–18 years)	5 μg (200 IU)/day
Men (19–50 years)	5 μg (200 IU)/day
Women (19–50 years)	5 μg (200 IU)/day
Men (51–70 years)	10 μg (400 IU)/day
Women (51–70 years)	10 μg (400 IU)/day
Men (70+ years)	15 μg (600 IU)/day
Women (70+ years)	15 μg (600 IU)/day
Pregnancy	5 μg (200 IU)/day
Lactation	5 μg (200 IU)/day
UL	
Infants	25 μg (1000 IU)/day
Children (1–18 years)	50 μg (2000 IU)/day
Adults (over 19 years)	50 μg (2000 IU)/day
Pregnancy	50 μg (2000 IU)/day
Lactation	50 μg (2000 IU)/day

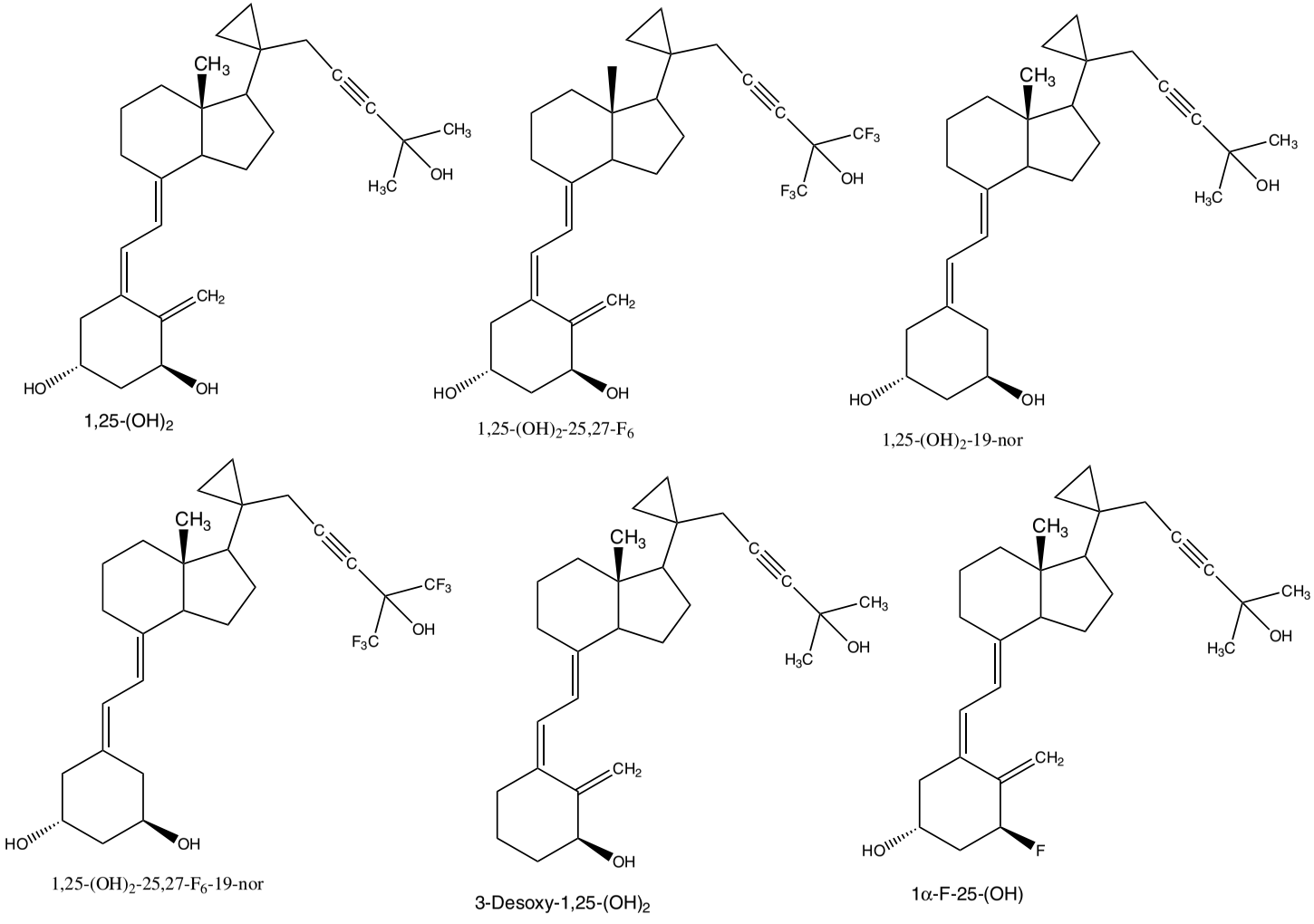


Figure 17. 23-Yne-20-cyclopropyl D_3 analogs.

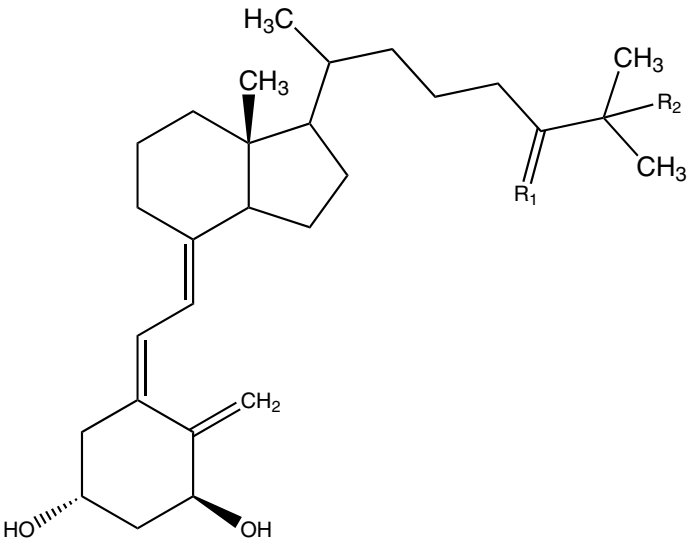


Figure 18. Keto and oxime vitamin D_3 analogs.

3.2.7. Drug Interactions Phenobarbital and possibly other anticonvulsants used in epilepsy induce liver hydroxylation leading to subsequent formation of the inactive end products. As long as the epileptic child receives a normal amount of fortified milk, there is no problem with this interaction.

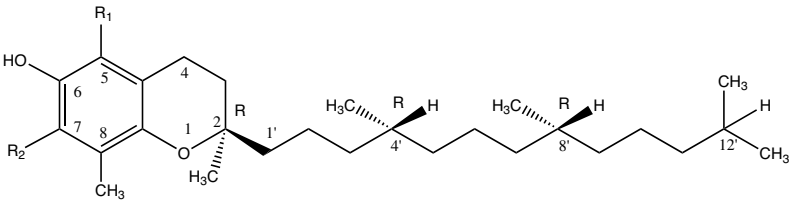
3.3. Vitamin E Family (Tocopherols and Tocotrienols)

This vitamin group was discovered in rat feeding experiments resulting in these animals unable to produce offspring. These same rats seemed normal in all other respects including physical growth [45]. The condition could be corrected by addition of lettuce, whole wheat, and cereal grains, with the best source being wheat germ followed by vegetable oils. Think of the tocopherols and tocotrienols (Table 4) as nature's antioxidants. Most of the defined vitamin activity in terms of rat fertility assays is found in α-tocopherol. "Tocopherol" means childbearing alcohol.

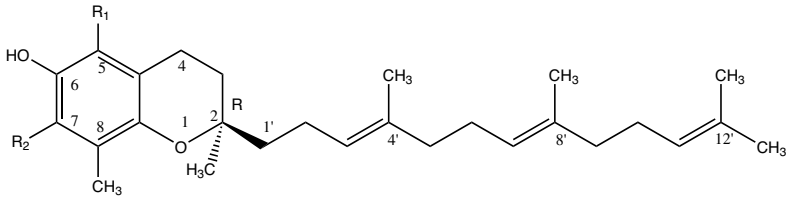
3.3.1. Chemistry The vitamin E family consists of tocopherols and tocotrienols. There has been considerable debate as to whether the *RRR* isomer is the only biologically active form of the vitamin. The vitamin of commerce is α-tocopherol either as a racemic synthetic mixture or the natural *RRR*-α-tocopherol. All of the tocopherols found in food have the *R*-configuration at position 2. The synthetic form of the vitamin is a mixture of all eight stereoisomers (now referred to as *rac*-α-tocopherol rather than D,L-α-tocopherol). Because there is both general and stereospecific antioxidant activity, the RDA tables state that the activity ratio of *RRR*-α-tocopherol to *rac*-α-tocopherol is 1:1.36 Table 5 [46]. All isomers are antioxidants and probably do provide general antioxidant protection internally or *in vivo*. On the other hand, evidence points to the *RRR* isomer as being specific for a variety of reductase systems, involving selenium (Se) and glutathione (GSH/GSSG). The "relative biological activity" in Table 4 is based on a rat resorption–gestation assay that probably has no parallel in terms of vitamin E status in human health.

3.3.2. Uptake and Metabolism Like the vitamin A esters, tocopherols, both esterified and nonesterified, require bile salts to become part of the mixed micelles containing the dietary lipids. They are absorbed into the mucosal cell by passive diffusion. The tocopherols follow the dietary lipids onto the chylomicrons. Because the latter first enter the lymphatic system before the circulatory system, the

Table 4. Tocopherols and Tocotrienols Relative Biological Activities[1]



Tocopherols	R_1	R_2	Relative Biological Activity[a]
α-Tocopherol	CH_3	CH_3	1
β-Tocopherol	CH_3	H	0.5
γ-Tocopherol	H	CH_3	0.1
δ-Tocopherol	H	H	0.03



Tocotrienols	R_1	R_2	Relative Biological Activity[a]
α-Tocotrienol	CH_3	CH_3	0.3
β-Tocotrienol	CH_3	H	0.05
γ-Tocotrienol	H	CH_3	Inactive
δ-Tocotrienol	H	H	Inactive

[1] *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, Food and Nutrition Board, Institute of Medicine, National Academy Press, p. 193, 2000.
[a] Rat fetal reabsorption assay.

Table 5. Factors for Converting International Units of Vitamin E to α-Tocopherol (mg) to Meet Recommended Intake[1]

	USP Conversion Factors[c]		Molar Conversion Factors	α-Tocopherol Conversion Factors
	IU/mg	mg/IU	μmol/IU	mg/IU
Synthetic Vitamin E and Esters[a]				
d,l-α-Tocopherol Acetate	1.00	1.00	2.12	0.45
d,l-α-Tocopherol Succinate	0.89	1.12	2.12	0.45
d,l-α-Tocopherol	1.10	0.91	2.12	0.45
Natural Vitamin E and Esters[b]				
d-α-Tocopherol Acetate	1.36	0.74	1.56	0.67
d-α-Tocopherol Succinate	1.21	0.83	1.56	0.67
d-α-Tocopherol	1.49	0.67	1.56	0.67

[1] *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, Food and Nutrition Board, Institute of Medicine, National Academy Press, p 192, 2000.
[a] Synthetic vitamin E supplements labeled as *d,l*-α-tocopherol can consist of all eight possible isomers (RRR-, RSR-, RRS-, RSS-, SSS-, SRS,-, SRR-, and SSR-).
[b] *d*-α-Tocopherol refers to the RRR-isomer, the only one found naturally in foods, and the other 2 R stereoisomers (RRS-, RSR-, and RSS).
[c] The United States Pharmacopeia (USP) has defined one IU as 1 mg of all racemic α-tocopherol acetate based on a 1940s rat fetal resorption assay.

tocopherols do not go directly to the liver. Instead, the chylomicrons are distributed throughout the body, and some of the tocopherols enter the adipose tissue with the fatty acids that also were being transported on the chylomicrons. The chylomicron remnants finally reach the liver. The remaining tocopherols leave the liver on the very low-density lipoproteins (VLDL) that become the low-density lipoproteins (LDL). In other words, tocopherols will be found wherever there is a significant amount of lipid material including the high-density lipoproteins (HDL). There is no specific organ where the tocopherols are stored. Consistent with antioxidant model, the RDA is based on the polyunsaturated fatty acid (PUFA) consumption. The implication is that if increased PUFA intake is recommended, the RDA for tocopherol should be increased.

There is increasing evidence that the *RRR*-stereoisomer is preferentially transferred in the liver onto the lipid transport proteins, but it is not absolute. This could explain why *RRR*-α-tocopherol is more active *in vivo*, but the other isomers are not inactive. Complicating the study of vitamin E in humans is the fact that the most common isomer in the human diet is γ-tocopherol, and it has anti-inflammatory properties. Excess α-tocopherol from vitamin supplements and fortified food can displace γ-tocopherol [47]. This raises the question if the conflicting report on the efficacy of vitamin E (as α-tocopherol) is related to its effects on γ-tocopherol and mix of stereoisomers.

3.3.3. Biochemical Function The best way to describe tocopherol's role is that of a lipid-soluble antioxidant. It protects unsaturated lipids from oxygen-induced peroxide formation. There is evidence for both free-radical one-electron chemistry (Fig. 19) and two-electron quinone–hydroquinone (Fig. 20) [48]. The oxidized–reduced glutathione system may be part of the system that regenerates reduced α-tocopherol. At one time it was thought that the preference for the 2*R* stereoisomers indicated that the vitamin was part of a biochemical oxidation–reduction system, possibly as a coenzyme. So far that role for α-tocopherol has not been confirmed. The current evidence points to the hepatic tocopherol transfer protein's preference for the *RRR*-stereoisomer as the explanation for the partial steric preference.

It is well known that oxygen-generated free radicals are tightly controlled by a variety of antioxidants, some generated *in vivo* and others dietary. A part of the current model for several diseases, including cardiovascular, is based on inflammatory process of which oxidative process is a part. This has led to extensive investigations on the effectiveness of α-tocopherol and ascorbic acid (vitamin C) in preventing and/or treating these diseases. Initially, the results looked promising. More recent studies show a lack of effectiveness and possibly exacerbating the patient's condition [49–53].

3.3.4. Deficiencies The current model for the cause of vitamin E deficiencies points to malabsorption of lipids. Thus, there may be malabsorption of other lipid-soluble vitamins. Little is know regarding the pharmacokinetics of the tocopherols. Part of the reason for this may be caused by there not being a specific storage organ for the vitamin.

Correlating human medical conditions with the biochemical role of the tocopherols is difficult because of the lack of correlations between deficiency diseases seen in animals relative to what is seen in humans. Deficiency diseases seen in animals include reversible reproductive failure in female rats; irreversible degeneration of rat testicular tissue leading to mail sterility; nutritional muscular dystrophies in monkeys, rabbits, guinea pigs, lambs, calves, turkeys, and chicks; and an anemia in monkeys. Vitamin E does not treat human muscular dystrophy, nor various causes preventing a couple from conceiving a child or inability of pregnant women to go to full term. What is seen in humans is partially reversible set of neurological problems and hemolytic anemia in premature infants. Because of poor placental transfer, newborn have little of the vitamin. Human milk contains 2–5 mg/L. Cow's milk contains less.

3.3.5. Hypervitaminosis E This is a relatively safe vitamin. Toxicities have been reported involving chronic administration of 300–1200 mg per day. The symptoms can be very serious and

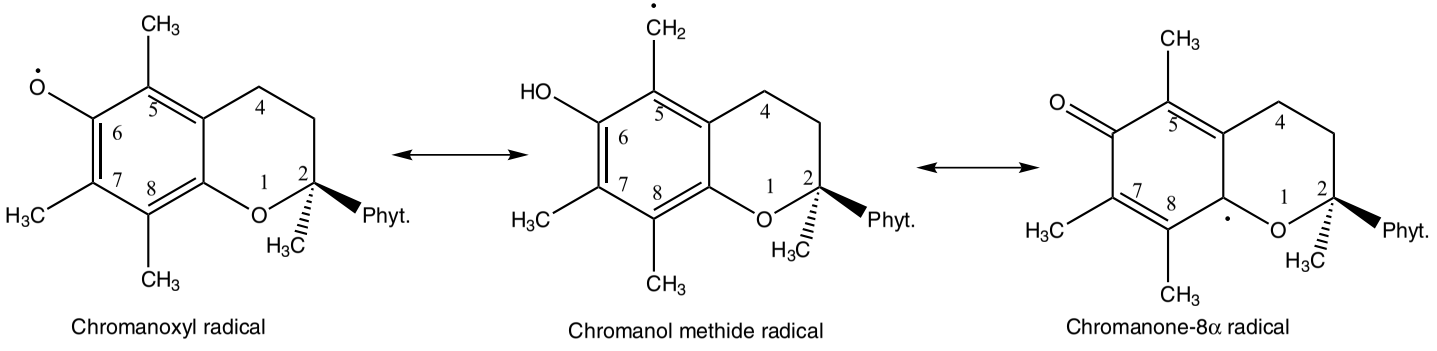


Figure 19. Resonance stabilized tocopherol radicals.

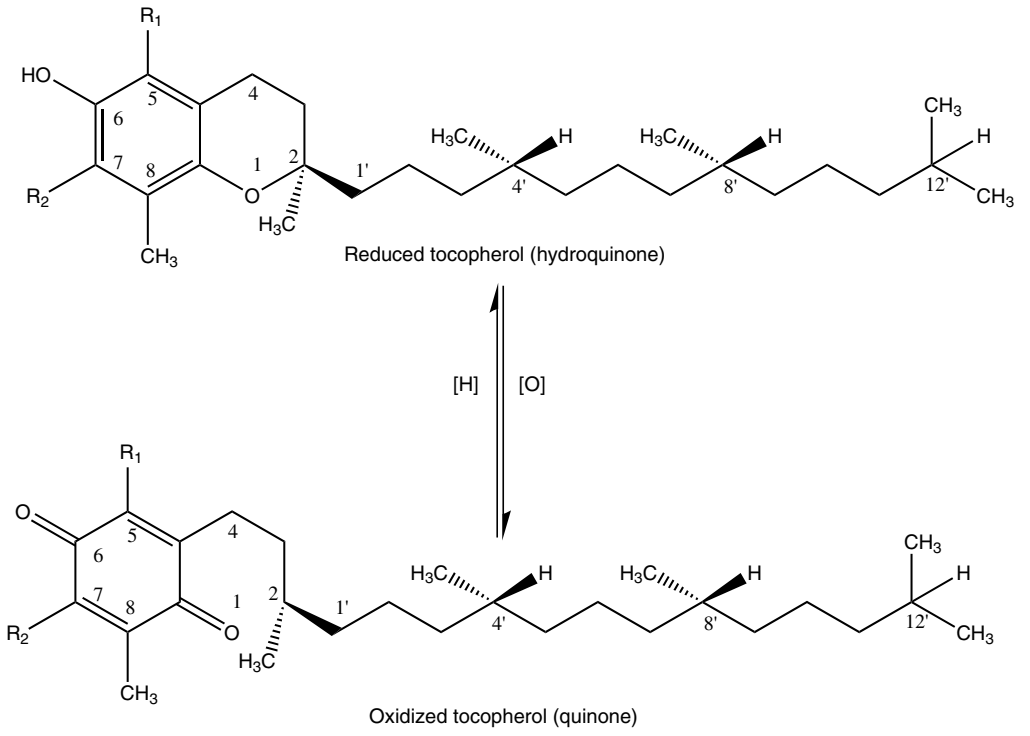


Figure 20. Tocopherol oxidation–reduction.

include thrombophlebitis, pulmonary embolism, hypertension, breast development in men and children, severe fatigue, and nonmalignant breast tumors. Nevertheless, the UL to RDA ratio is about 66 to 1 for adults, making it a very safe vitamin.

3.3.6. Dosage Forms The tocopherols, being antioxidants, are very sensitive to oxygen. Sensitive to UV light is another problem. There are two provitamin esters that are used commercially (Fig. 21), the oil-soluble acetate and water-soluble hemisuccinate. The latter is commonly found in dry dosage forms requiring a free flowing powder. Oxidation to the quinone form is blocked by esterifying the free phenolic hydroquinone.

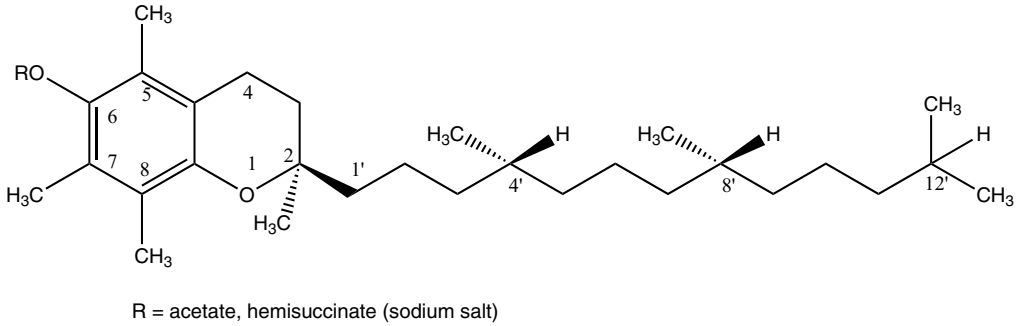


Figure 21. α-Tocopherol dosage forms.

3.3.7. Dietary Reference Intakes (Based on D-α-Tocopherol)

AI	
Infants (0–12 months)	0.6 mg/kg/day
EAR	
Children (1–8 years)	5–6 mg/day
Boys (9–18 years)	9–12 mg/day
Girls (9–18 years)	9–12 mg/day
Men (19–50 years)	12 mg/day
Women (19–50 years)	12 mg/day
Men (51–70+ years)	12 mg/day
Women (51–70+ years)	12 mg/day
Pregnancy	12 mg/day
Lactation	16 mg/day
RDA	
Children (1–8 years)	6–7 mg/day
Boys (9–18 years)	11–15 mg/day
Girls (9–19 years)	11–15 mg/day
Men (19–50 years)	15 mg/day
Women (19–50 years)	15 mg/day
Men (51–70+ years)	15 mg/day
Women (51–70+ years)	15 mg/day
Pregnancy	15 mg/day
Lactation	19 mg/day
UL	
Infants	Not established (do not give supplements; use only food and formula for sources)
Children	200 mg/day (1–3 years) up to 600 mg/day (9–13 years)
Adolescents	800 mg/day
Adults (19+ years)	1000 mg/day
Pregnancy	800–1000 mg/day
Lactation	800–1000 mg/day

3.4. Vitamin K Family

Vitamin K was discovered by accident by Danish scientists who, using a special fat-free diet designed to determine whether chickens synthesize cholesterol, observed that the animals developed a hemorrhagic condition characterized by a prolonged clotting time. The condition could be cured by an organic factor found in fresh cabbage, ether extract of alfalfa, putrefied fish meal, cereals, or hog livers. It was named *Vitamin K* for coagulation vitamin. This may be the only vitamin that humans receive in significant amounts from their intestinal bacterial, and there is some question regarding this commonly held assumption. Because of this source, it has been very difficult to establish a recommended daily allowance. An estimated safe intake was established for this vitamin for the first time with the recent 1989. With the release of the Dietary Reference Intakes, there is an adequate intake, but no RDA.

3.4.1. Chemistry There are two series for this vitamin (Fig. 22). The vitamin K_1 series is mostly obtained from green plants, whereas the K_2 series is the product of bacteria. The active vitamin is in the K_2 series. Menadione has sometimes been referred to as vitamin K_3. The common commercial form is called phytonadione in the United States Pharmacopeia and phylloquinone in Chemical Abstracts.

3.4.2. Vitamin K Uptake and Metabolism Dietary vitamin K_1 and the pharmaceutical form, phytonadione or vitamin $K_{1(20)}$, must be converted to the K_2 series known as menaquinones. The most common of these is menquinone-4 or $K_{2(20)}$. This conversion to the K_2 series occurs in the liver and possibly the intestinal flora. It involves removing the phytyl chain producing the intermediate menadione. Menadione sometimes is prescribed when there is impaired uptake of lipids from the intestine. There is little storage reserve in the liver, and a deficiency can result when dietary intake of vitamin K is restricted or absorption is impaired.

Dietary vitamin K and supplements are processed similarly as with the other fat-soluble vitamins. Bile salts are required for emulsification and formation of mixed micelles. They travel to the liver on chylomicrons along with vitamins A and E.

3.4.3. Vitamin K Biochemistry and Deficiency Deficiencies of this vitamin lead to serious hemorrhaging. The vitamin is required for formation of proteins that complex calcium. This is done by functioning as a coenzyme in the γ-carboxylation of glutamic acid (Fig. 23). Vitamin K_2 is reduced to the hydroquinone. After several steps, a complex oxidation occurs resulting in the "vitamin K base" that is integral part of the carboxylation step. In a key step, the vitamin K oxide is reduced to the

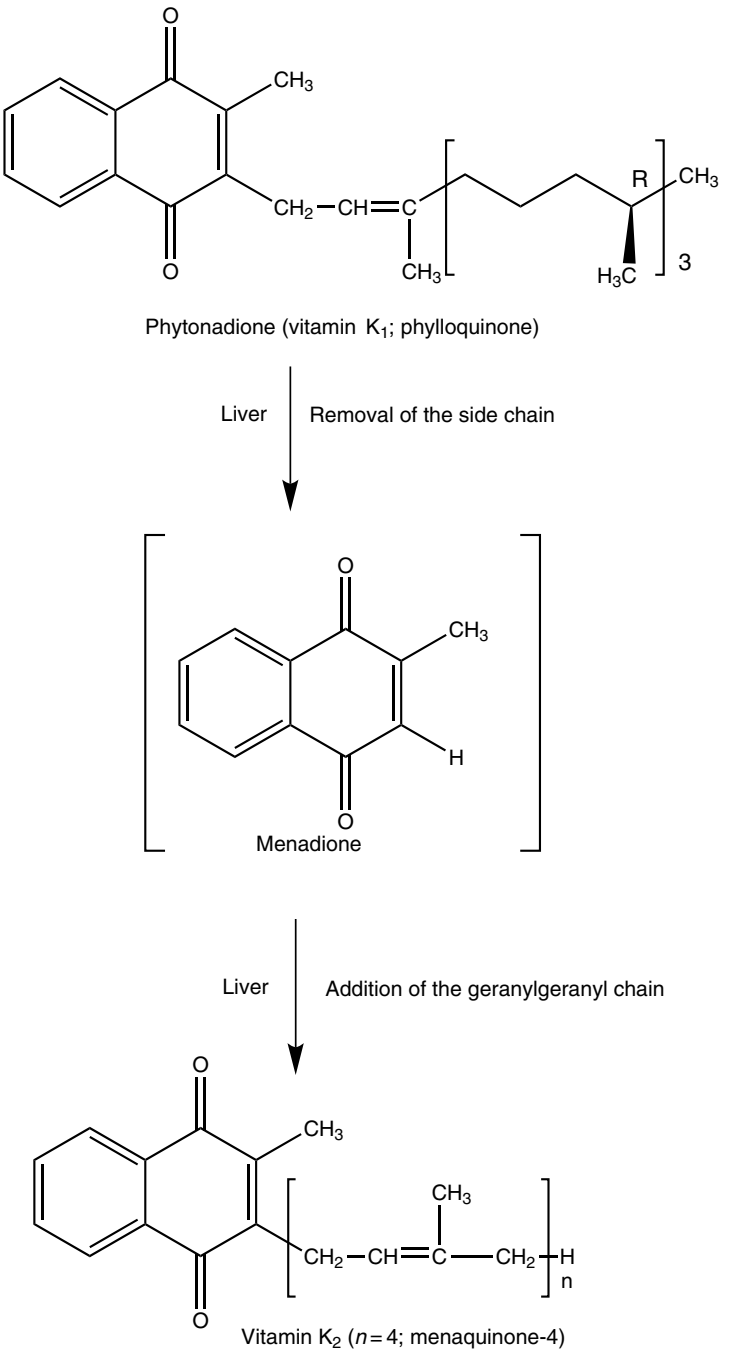


Figure 22. Formation of vitamin K_2.

original vitamin K_2. It is this final reduction that is inhibited by the coumadin anticoagulants widely used by patients susceptible to stroke, pulmonary embolism, phlebitis, and coronary thrombosis. This interaction between the coumadin anticoagulants and the regeneration of vitamin K_2 is the reason why patients on coumadin must monitor their vitamin

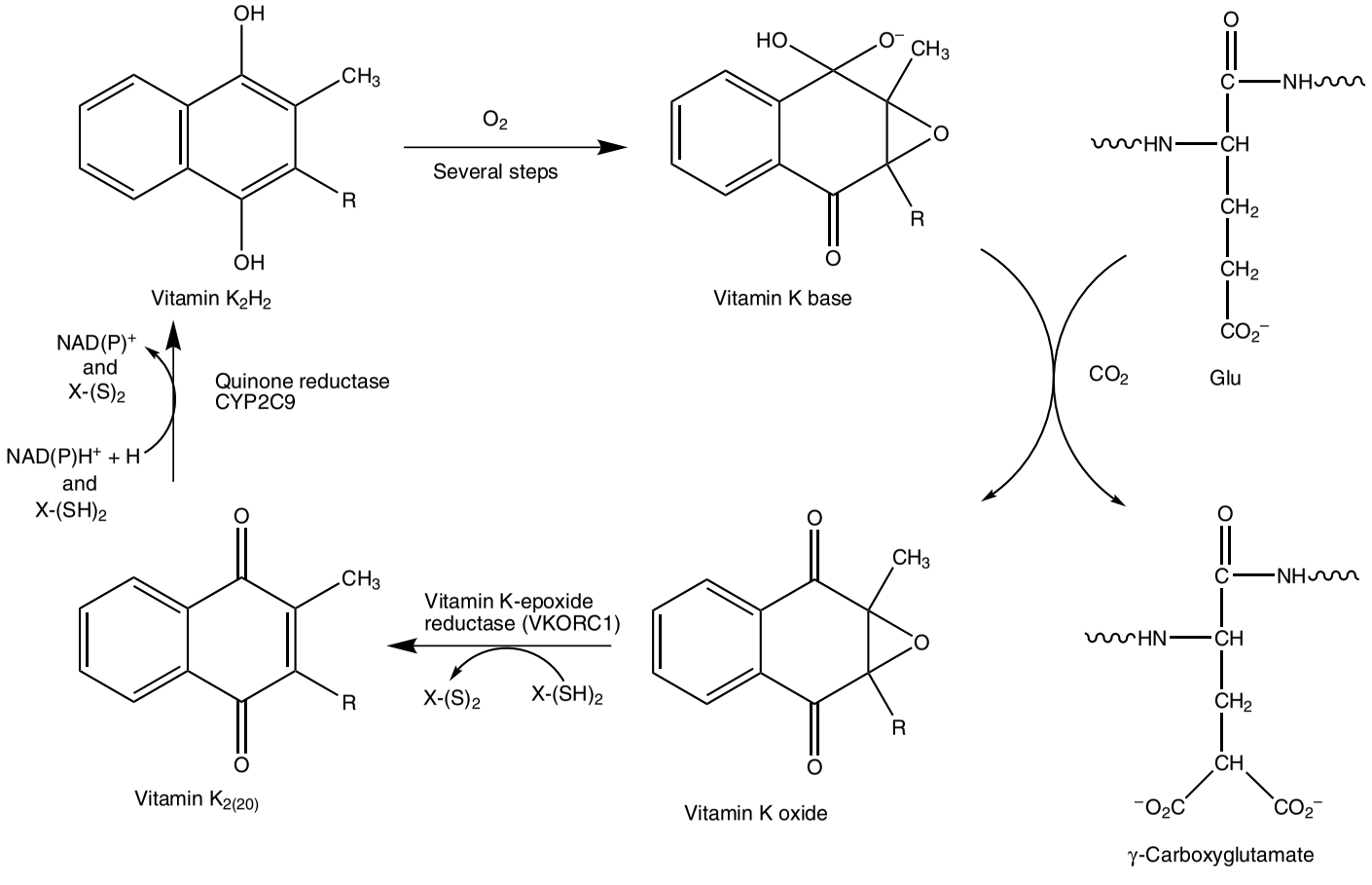


Figure 23. Outline of vitamin K carboxylation of glutamic acid.

K intake either by vitamin supplements or diet. This usually is done by regularly scheduled determinations of prothrombin time.

The carboxylation reaction is required for production of several clotting proteins including prothrombin, protein C, protein S, and factors VII, IX, and X. It is also required for γ-carboxylation of osteocalcin, an important calcium-binding protein found in the matrix of bone and required for proper deposition of calcium onto bone. This latter finding has led to several studies to determine if patients prescribed coumadin anticoagulants are at increased risk for osteoporosis and fractures [54–57]. Originally, it was postulated that vitamin K might help prevent osteoporosis. If this were correct, it would appear that patients on anticoagulant therapy would be at increased risk. Although there is some evidence that vitamin K_1 supplementation can increase bone mass density, there is little evidence that supplements reduce the incidence of fracture. Complicating the latter is an indication that vitamin K_1 supplement's ability to increase bone mass density may be gender specific with benefits seen in women and not men [58]. Nevertheless, some calcium supplements have both vitamin D and K added to their formulation.

3.4.4. Causes of Vitamin K Deficiency Rarely is a vitamin K deficiency caused by insufficient diet. It more likely is due to a medical condition. At one time, multivitamin supplements rarely contained vitamin K. It is now routinely found in these products. Causes of a vitamin K deficiency include obstructive jaundice (now uncommon), loss of intestinal flora in preparation for intestinal surgery, and hemorrhagic disease of the newborn.

Deficiencies caused by obstructive jaundice were caused by blockage of the bile duct, usually from cholelithiasis, preventing the release of bile salts into the intestinal tract for emulsifying the lipid contents. At one time, surgical removal of gallstones was delayed to see if the problem would correct itself. Menadione would be prescribed because it does not require micelle formation in that it is directly absorbed through the mucosa into the portal vein and flows to the liver. Alternatively, intramuscular injections of vitamin K_1 were given.

In nonemergency surgery of the intestinal tract, the patient would undergo 1–2 weeks of antibiotic therapy to reduce the level of intestinal bacteria. Usually the patient did not eat well because of the intestinal problem. The combination of reduced dietary vitamin K and vitamin from the intestinal bacteria could result in a vitamin K deficiency. Determination of prothrombin time is ordered by the surgeon to determine whether menadione or phytonadione is indicated.

The third cause is hemorrhagic disease of the newborn. Infants are born with a sterile intestinal tract. Until the flora is established, the infants have to get along with the vitamin K they received from their mothers. In the past, an infant might die from hemorrhaging. Most states requires that each newborn receive an injection of vitamin $K_{1(20)}$. Menadione injection should not be given because it can cause a hemolytic anemia in the newborn.

3.4.5. Hypervitaminosis K While it is possible to overdose with this vitamin, the fact that it is only available over the counter in small doses in multivitamin preparations has resulted in little knowledge of any toxicities. Toxicities do not appear in animals administered large doses. It is known that excess intake of the vitamin does not promote clot formation. There is no tolerable upper intake level.

3.4.6. Dietary Reference Intakes

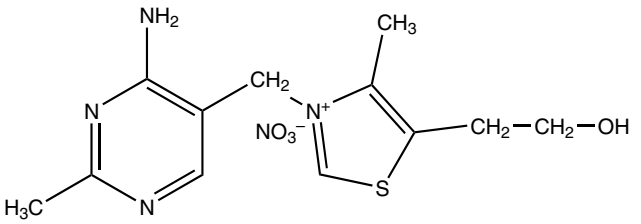
AI	
Infants	2–2.5 μg/day
Children (1–8 years)	30–55 μg/day
Boys and girls (9–18 years)	60–75 μg/day
Men	120 μg/day
Women	90 μg/day
Pregnancy	75–90 μg/day
Lactation	75–90 μg/day

3.5. Thiamine (Vitamin B_1)

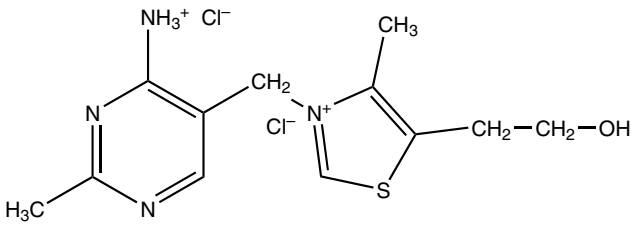
After 26 years of constant research, the vitamin preventative of the disease beri-beri has been isolated, its chemical constitution determined and the vitamin itself synthesized at a cost far lower than that of recovering it from bran.(Scientific American, February 1938; reprinted in **258**, 12, Feb. 1988)

The vitamin B complex, of which thiamine is considered the first one, generally includes the group of water-soluble vitamins found in rice polishings, bean extracts, yeast, and liver. There are no chemical relationships in the B complex. The nomenclature is very confusing. The common name originally implied something about the chemical nature of the vitamin. Even the concept of water soluble is somewhat misleading as some of the vitamins in this group would be considered poorly soluble by most pharmaceutical standards. The one thing the B complex vitamins have in common is that nearly all of them function either as a coenzyme or a structural component of a coenzyme.

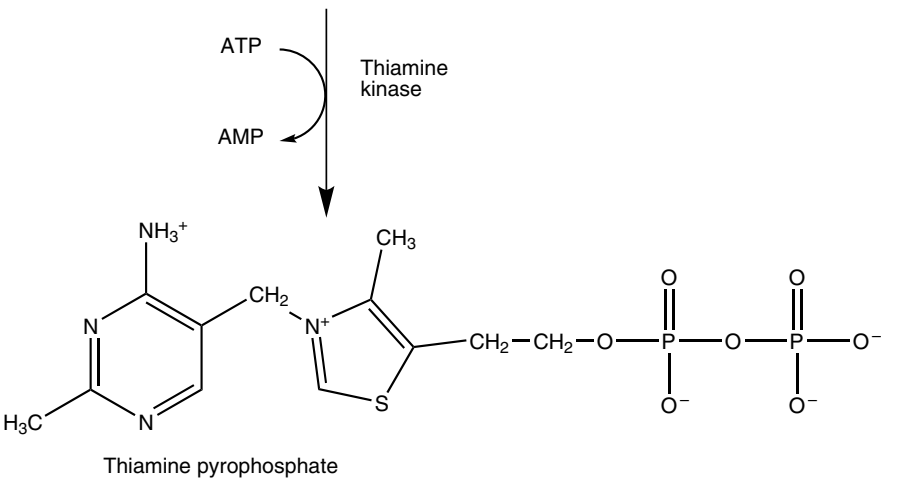
3.5.1. Chemistry (Fig. 24) Thiamine consists of a pyrimidine joined to a thiazole ring by a methylene bridge. The thiazole nitrogen is a quaternary with a permanent positive charge.



Thiamine mononitrate



Thiamine HCl
(thiamine chloride HCl)



Thiamine pyrophosphate

Figure 24. Commercial forms of thiamine and formation of thiamine pyrophosphate.

There are two commercial salts. Thiamine hydrochloride is, in reality, *thiamine chloride hydrochloride*. It is a double salt consisting of an amine hydrochloride on the pyrimidine amine and a chloride on the thiazole quaternary nitrogen. Thiamine nitrate is correctly named in that the nitrate anion is found on the quaternary nitrogen, and the pyrimidine amine is not protonated. Once the vitamin enters the acidic stomach, it will exist as the chloride hydrochloride salt.

Thiamine hydrochloride is very water soluble (1 gm/1 ml). It also is very hygroscopic, making it difficult to use in dry formulations. This salt is commonly used in liquid and injectable formulations. Thiamine nitrate is sufficiently water soluble (1 g/35 mL) that it can be used in liquid formulations since the RDAs are less than 2 mg per day. Because it is nonhygroscopic, it is commonly used in dry dosage forms.

3.5.2. Uptake and Metabolism An active transport system in the jejunum provides efficient uptake of the vitamin into the intestinal mucosa cell. A thiamine kinase in the intestinal mucosa cell transfers a pyrophosphate from the ATP to the propyl alcohol at position 5 of the thiazole ring forming thiamine pyrophosphate (TPP) (Fig. 24). The latter product is the coenzyme form of the vitamin. There is some evidence that this phosphorylation is the rate-limiting step and controls the absorption of the vitamin. The coenzyme is transported to the tissues where needed.

Thiamine pyrophosphate has two important coenzyme roles, both focusing mostly on carbohydrate metabolism (Figs 25 and 26). The active portion of the coenzyme is the thiazole ring. The first step in the oxidative decarboxylation of α-keto acids requires TPP. The two most common examples are pyruvate and α-ketoglutarate oxidative decarboxyated to acetyl CoA and succinyl CoA, respectively. The same reaction is found in the metabolism of the branched-chain amino acids valine, isoleucine, and leucine and also methionine. In all cases, TPP is a coenzyme in a mitochondrial multienzyme complex consisting of TPP, lipoic acid, coenzyme A, FAD, and NAD.

TPP is also the coenzyme in the transketolase reaction (Fig. 26) found in the pentose phosphate pathway that interconverts hexoses, pentoses, tetroses, and trioses. This reaction removes carbons 1 and 2 of a ketose and transfers them to an acceptor aldose. Examples include TPP transferring carbons 1 and 2 of xylulose-5-P to ribose-5-P producing glyceraldehyde-3-P (five carbons minus two carbons) and sedoheptulose-7-P (five carbons plus two carbons). This reaction is reversible. A second reversible reaction has TPP transferring carbons 1 and 2 of xylulose-5-P to erythrose-4-P producing fructose-6-P (four carbons plus two carbons) and glyceraldehyde-3-P (five carbons minus two carbons).

The dietary reference intakes for thiamine are based on carbohydrate consumption. This is because most pyruvate substrate comes from aerobic glycolysis of glucose, much of the α-ketoglutarate substrate originates from carbohydrate sources, and the transketolase reactions are part of carbohydrate metabolism.

3.5.3. Thiamine Deficiencies Thiamine deficiencies were reported in the very early medical literature and were called *beriberi*, a Japanese term. Sailors in the Japanese navy experienced thiamine deficiencies when fed rice in which the polishings had been removed to prevent mold growth. This is somewhat analogous to removing the germ from wheat in order to prolong the shelf life of flour containing foods. There are two forms of beriberi, wet and dry. Wet beriberi is characterized by edema and enlarged heart. Dry beriberi is more neurological and can include muscle wasting. A beriberi patient can experience both.

Assuming a reasonably balanced diet, most thiamine deficiencies today are caused by chronic alcoholism. This form of thiamine deficiency is called Wernicke–Korsakoff syndrome. It is common for emergency medical personal to add thiamine to the i.v. being administered to a comatose patient suspected of experiencing substance abuse.

3.5.4. Hypervitaminosis Thiamine The vitamin is considered very safe. There are no tolerable upper intake levels. Possibly the rate-limiting phosphorylation step in the intestinal mucosa reduces the risk of toxicity. The percentage of thiamine absorbed decreases as the dose increases.

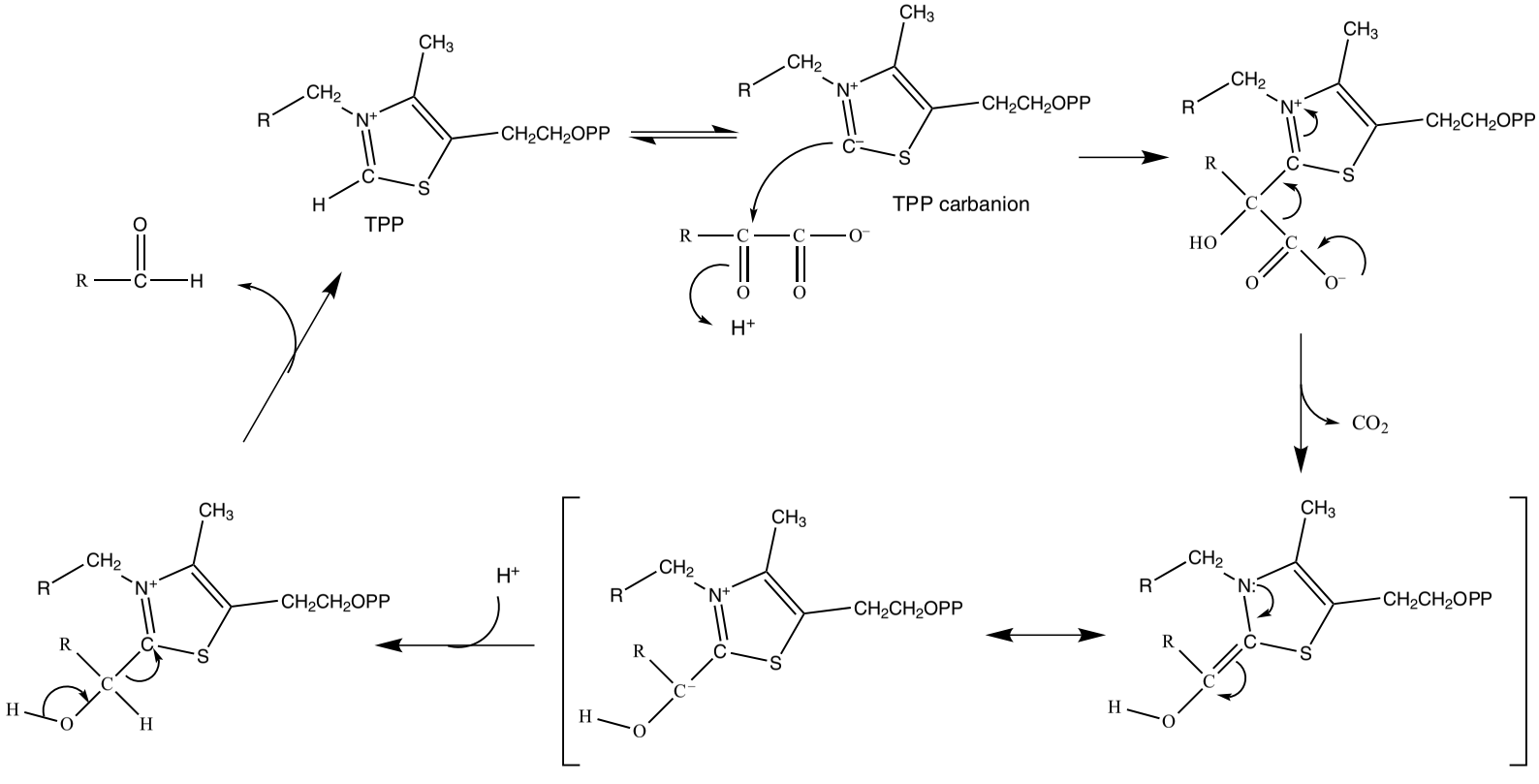


Figure 25. Thiamine pyrophosphate decarboxylatian of α-keto acids.

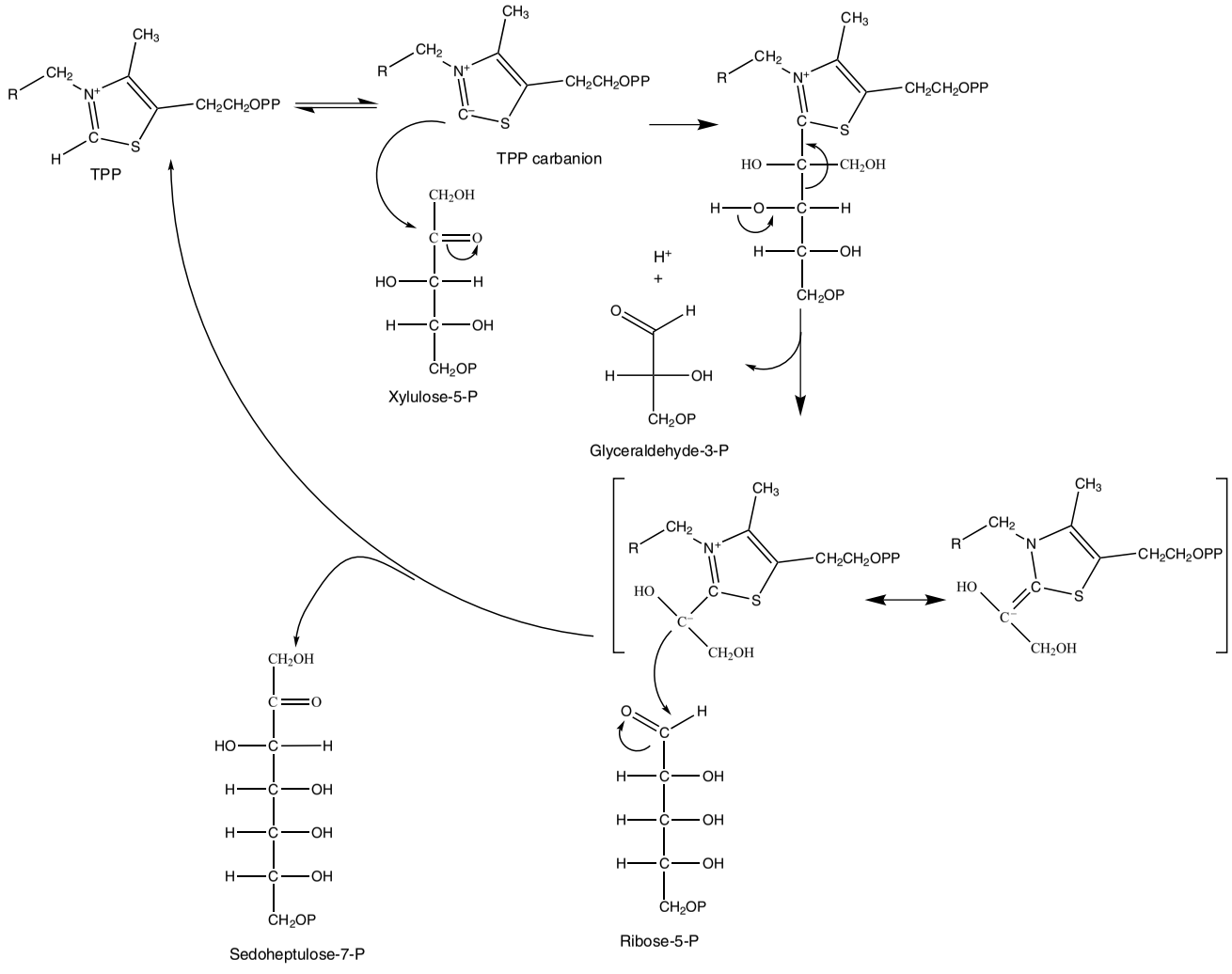


Figure 26. Thiamine pyrophosphate catalyzed transketolase reaction.

3.5.5. Dietary Reference Intake

AI	
Infants	0.2–0.3 mg/day
EAR	
Children (1–13 years)	0.4–0.7 mg/day
Males (14–18 years)	1.0 mg/day
Females (14–18 years)	0.9 mg/day
Men (19–50 + years)	1.0 mg/day
Women (19–50 + years)	0.9 mg/day
Pregnancy	1.2 mg/day
Lactation	1.2 mg/day
RDA	
Children (1–13 years)	0.5–0.9 mg/day
Males (14–18 years)	1.2 mg/day
Females (14–18 years)	1.0 mg/day
Men (19–50 + years)	1.2 mg/day
Women (19–50 + years)	1.1 mg/day
Pregnancy	1.4 mg/day
Lactation	1.5 mg/day
UL	
None reported	

3.6. Riboflavin (Vitamin B_2)

Shortly after the discovery of thiamine from yeast concentrates, the presence of a second nutritional factor in such materials was suggested. This second factor was also reported to have a pellagra-preventative activity since it alleviated a deficiency-induced dermatitis in rats. It was called vitamin B_2 in England and vitamin G in the United States.

3.6.1. Chemistry (Fig. 27) Riboflavin has a characteristic flavin ring system that gives it a unique spectroscopic and instability properties. There are two commercial forms. Riboflavin, itself, is poorly water soluble (1 g/10,000 mL) and is limited to oral dry dosage forms. Riboflavin phosphate, as the sodium salt, is very water soluble at 100 mg/mL. It is widely used in dry and liquid dosage forms.

3.6.2. Riboflavin Uptake and Chemistry (Fig. 27) Most dietary riboflavin is eaten as the FAD or FMN coenzymes. Intestinal pyrophosphatases and phosphatases produce free riboflavin that is actively transported through the proximal area of the small intestine into systemic circulation. Because of its poor water solubility, it is transported on albumin and immunoglobulin proteins. Conversion to the coenzyme forms occurs inside the cells that need these coenzymes.

3.6.3. Metabolic Role Riboflavin coenzymes are required for most oxidations of carbon–carbon bonds (Fig. 28). Examples include the oxidation of succinyl CoA to fumarate in the Krebs cycle and introduction of α,β-unsaturation in β-oxidation of fatty acids. Riboflavin is also required for the metabolism of other vitamins including the formation of niacin coenzymes from tryptophan (Fig. 29), reduction of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate (Fig. 45), and interconversion of pyridoxine-pyridoxal phosphate-pyridoxamine (Fig. 32). Because oxidation–reductions using FAD or FMN as the coenzyme is a two-step process, some flavin coenzyme systems contain more than one FAD or FMN.

3.6.4. Riboflavin Deficiency With riboflavin's central role in energy metabolism and conversion of folic acid and pyridoxine to their active forms, it is surprising that riboflavin deficiency does not produce a characteristic set of symptoms. One of the reasons may be that it is rare to see a patient who is solely deficient in riboflavin.

3.6.5. Hypervitaminosis Riboflavin The combination of regulated active transport and conversion to the coenzyme forms prevents hypervitaminosis problems with this vitamin. Toxicities from the water-soluble riboflavin phosphate have not been reported. There are no tolerable upper intake levels.

3.6.6. Dietary Reference Intakes

AI	
Infants	0.3–0.4 mg/day
EAR	
Children (1–13 years)	0.4–0.8 mg/day
Males (14–19 years)	1.1 mg/day
Females (14–19 years)	0.9 mg/day
Men (19–70 + years)	1.1 mg/day
Women (19–70 + years)	0.9 mg/day
Pregnancy	1.2 mg/day
Lactation	1.3 mg/day
RDA	
Children (1–13 years)	0.5–0.9 mg/day
Males (14–19 years)	1.3 mg/day
Females (14–19 years)	1.0 mg/day
Men (19–70 + years)	1.3 mg/day
Women (19–70 + years)	1.1 mg/day
Pregnancy	1.4 mg/day
Lactation	1.6 mg/day
UL	
None reported	

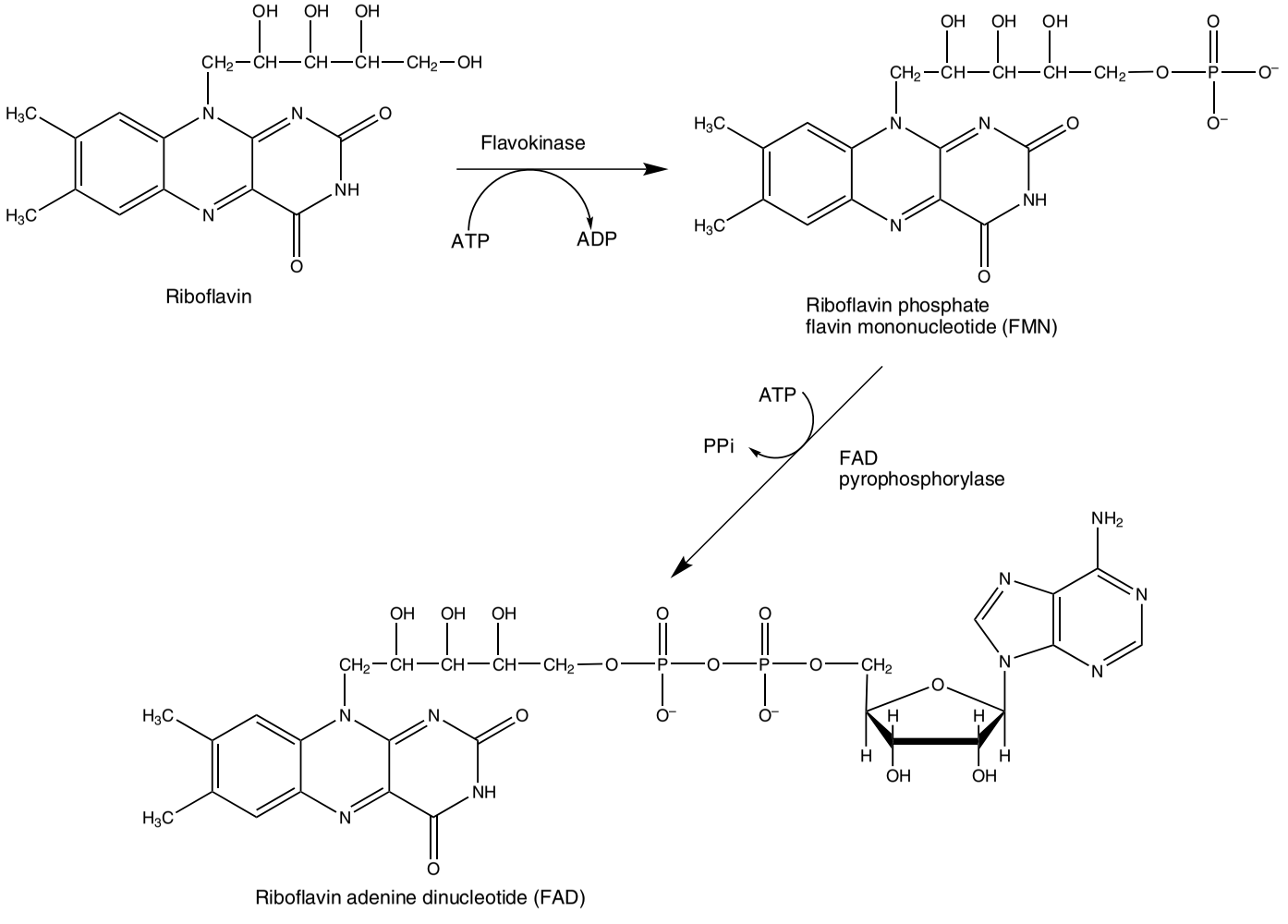


Figure 27. Riboflavin dosage and coenzyme forms.

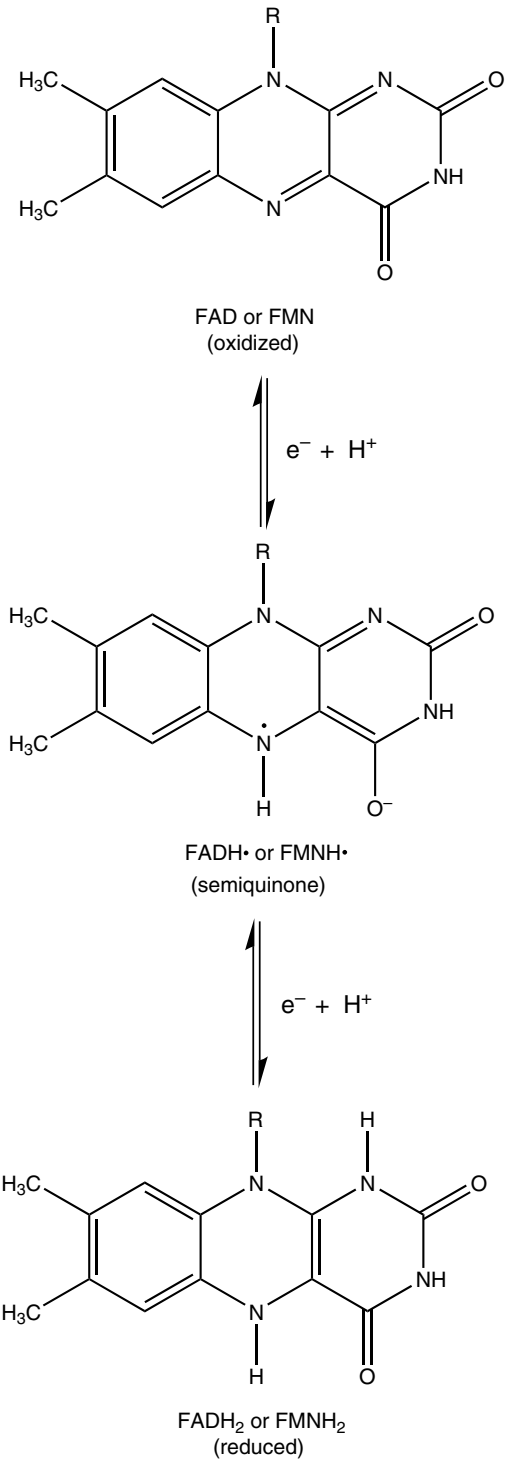


Figure 28. FAD/$FADH_2$–FMN/$FMNH_2$ oxidation–reduction.

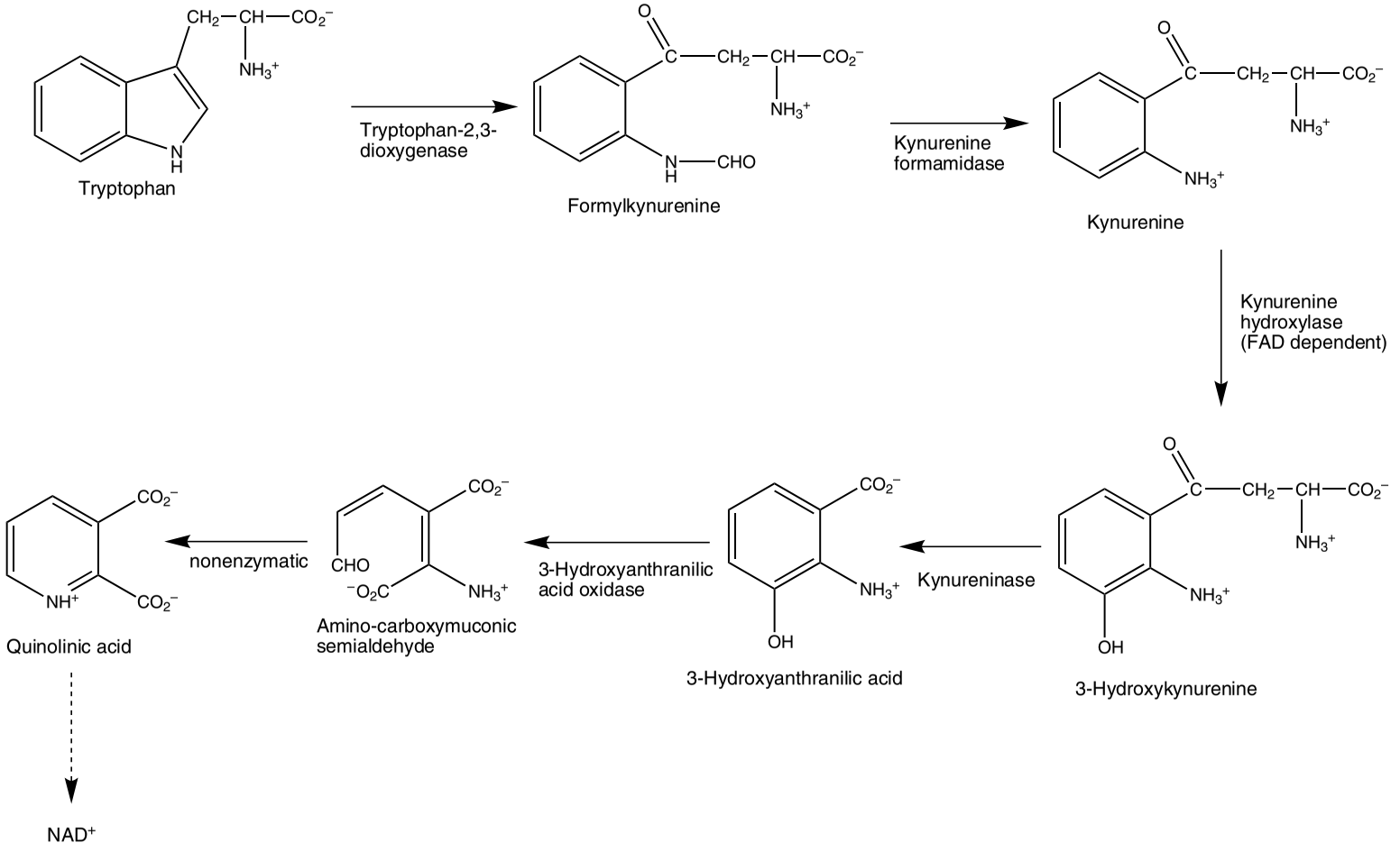


Figure 29. Biosynthesis of NAD^+ from tryptophan.

3.7. Niacin (Nicotinic Acid)—Niacinamide (Nicotinamide)

The history of niacin revolves around trying to find a way to prevent and cure pellagra, the late-stage deficiency disease caused by a niacin deficiency. Pellagra has been a serious nutritional disorder in the United States, mostly in the southeast. Two thousand deaths from pellagra were reported in 1941. This is ironic because nicotinic acid, later known as niacin, was first reported in during the structure elucidation of the alkaloid nicotine in the nineteenth century.

Like some of the other deficiency diseases, there was disagreement between those who thought pellagra was caused by poor sanitation versus those who concluded it was a nutritional disorder. Niacin deficiency, even today, is found in economically poor areas. Even when niacin was first isolated from foods, it was ignored because it did not cure beriberi.

3.7.1. Niacin/Niacinamide Chemistry, Uptake, and Metabolism Niacin is the simplest of the vitamins but has some of the most complex biochemistry of the vitamins. Structurally, it is pyridine-3-carboxylic acid. Strictly speaking, it is nonessential because the essential amino acid tryptophan is a source (Fig. 29). The biosynthetic route does not produce "free" niacin by decarboxylation of quinolinic acid. In a complex reaction, quinolinic acid loses the 2-carboxyl group and adds 5′-phosphoribose to form nicotinate mononucleotide (Fig. 30) [59,60]. Niacin and niacinamide in pharmaceutical dosage forms undergo a similar ribosylation reaction. Most vitamin products contain niacinamide because niacin can cause a distracting vasodilation leading to flushing.

Niacin and niacinamide are rapidly absorbed from both stomach and intestine. As the dose increases, absorption decreases. It is not clear if there is a feedback mechanism operating. Conversion to the coenzyme forms occurs in the cells where NAD and NADP are needed.

NAD primarily is the coenzyme required for oxidation–reduction of carbon–oxygen bonds and is required for oxidative catabolism (glycolysis, β-oxidation, Krebs cycle). NADP is the coenzyme in biosynthetic routes (fatty acid and cholesterol synthesis) and will be part of oxidation–reduction reactions involving both carbon–oxygen and carbon–carbon bonds.

The active part of the coenzyme is the pyridine ring (Fig. 31). When the substrate is labeled with deuterium, it has been shown that NAD systems can be categorized by the deuterium ending up on the A or B face of the pyridine ring. Examples of NAD dehydrogenases where the hydride anion attaches to the A face are isocitrate dehydrogenase, malate dehydrogenase, lactate dehydrogenase, and alcohol dehydrogenase. Dehydrogenases involving the B face include α-ketoglutarate dehydrogenase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase.

3.7.2. Niacin Deficiency Niacin deficiency, manifested as pellagra, is characterized by the four Ds: dermatitis, diarrhea, depression, and death. The dermatitis is characterized by a pigmented rash that develops on skin exposed to heat. Changes to the gastrointestinal tract can lead to vomiting, constipation, or diarrhea. Depression is one of the neurological symptoms that can also include apathy, headache, fatigue, and memory loss.

Deficiencies were common in populations whose main source of calorie was corn. Zein is the main protein found in corn and it is very low in both niacin and tryptophan. When corn is ground with lime water, the small amount of niacin and tryptophan becomes more bioavailable. Populations who consume corn meal by this process have a smaller incidence of pellagra. At the same time, it must be remembered that pellagra is the extreme form of a niacin deficiency. The dietary reference intakes for this vitamin use clinical chemistry assays listed in Table 2 to determine DRIs rather than the first appearance of pellagra symptoms.

3.7.3. Hypervitaminosis Niacin Niacin is considered nontoxic, but there are tolerable upper intake levels based on its use as a vitamin. These refer only to niacin and niacinamide, but not tryptophan and niacin equivalents (see next section). Large doses of niacin and niacinamide do have adverse reactions. These are sometime seen with patients prescribed niacin in doses up to 2 g daily for hyperlipidemia including both hypercholesterolemia and hypertriglyceridemia. For the former, there is

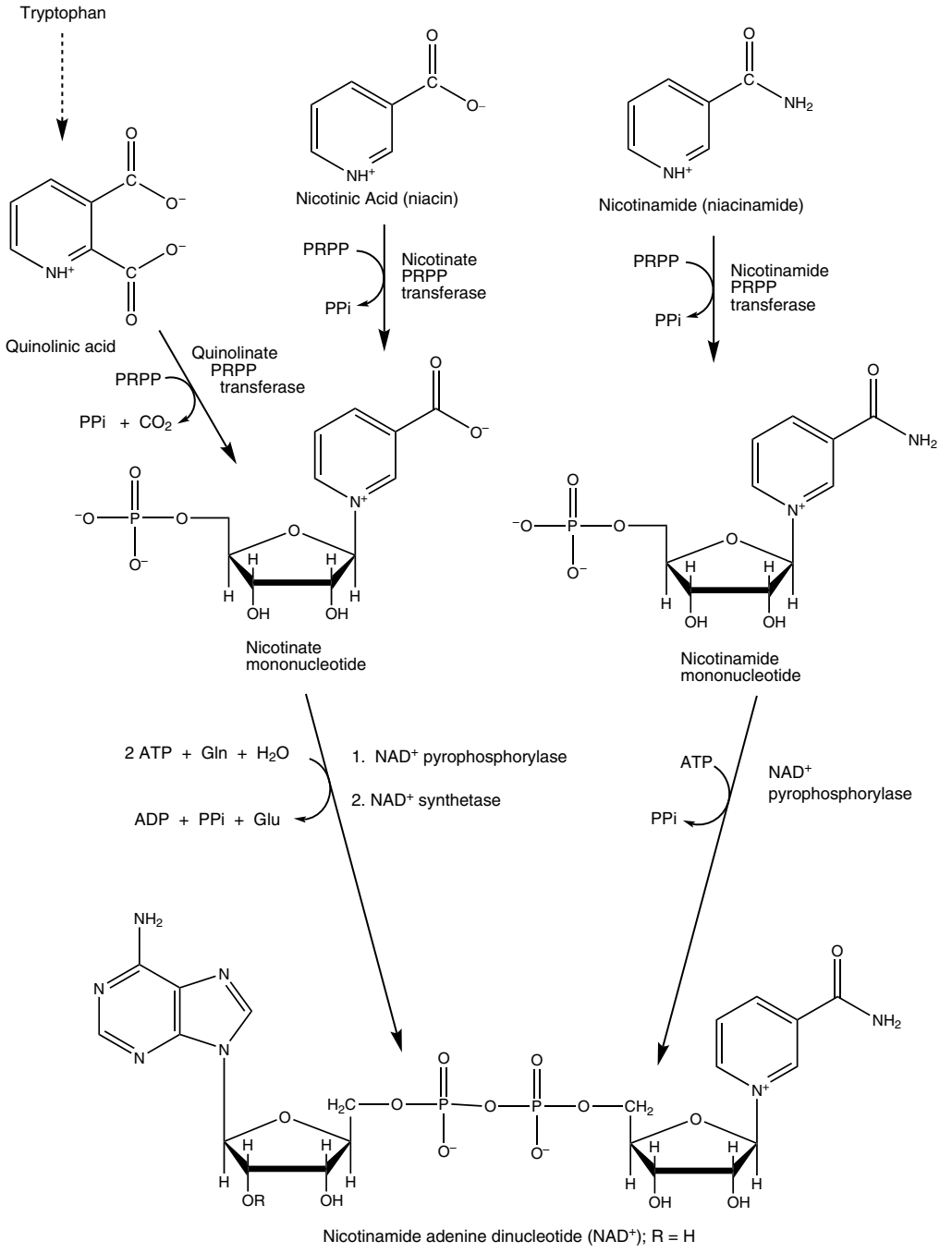


Figure 30. Biosynthesis of NAD^+ from niacin and niacinamide.

decreased LDL and increased HDL. There is the vasodilation from niacin, particularly in the head area, caused by increased intercranial blood flow. Niacin had been used for Raynaud's syndrome to treat the vasoconstriction seen with this disease, but it has largely been replaced by more specific vasodilators. Liver toxicities have been experienced by patients prescribed sustained release niacin products for hyperlipidemia.

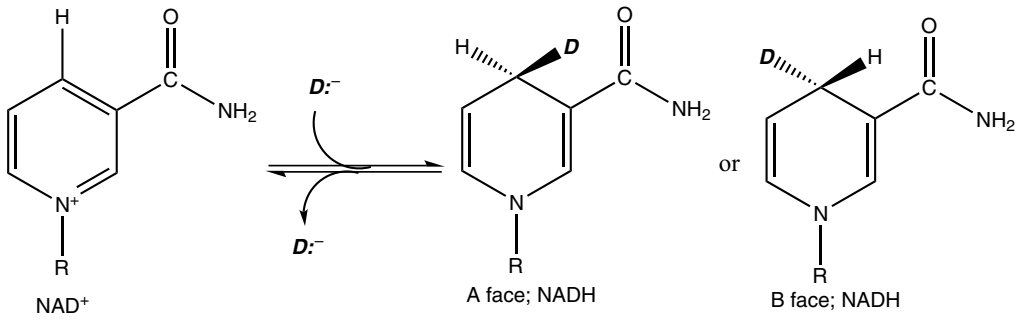


Figure 31. Stereochemistry of NAD^+ reduction.

3.7.4. Dietary Reference Intakes Niacin/niacinamide DRI units are expressed as milligram niacin equivalents (mg NE). These units assume that approximately 60 mg tryptophan produce 1 mg of niacin. For adult males, the RDA assumes a diet containing a mixture of tryptophan and niacin equivalent to 960 mg of tryptophan or 16 mg of niacin.

AI	
Infants (0–5 months)	2 mg/day of preformed niacin
Infants (6–11 months)	4 mg NE/day
EAR	
Children (1–13 years)	5–9 mg NE/day
Boys (14–18 years)	12 mg NE/day
Girls (14–18 years)	11 mg NE/day
Men (19–70+ years)	12 mg NE/day
Women (19–70+ years)	11 mg NE/day
Pregnancy	14 mg NE/day
Lactation	13 mg NE/day
RDA	
Children (1–13 years)	6–12 mg NE/day
Girls (14–19 years)	1.3 mg NE/day
Boys (14–19 years)	16 mg NE/day
Men (19–70+ years)	16 mg NE/day
Women (19–70+ years)	14 mg NE/day
Pregnancy	18 mg NE/day
Lactation	17 mg NE/day
UL	
Infants (0–12 months)	Source of intake should be formula and food only
Children (1–13 years)	10–20 mg/day of niacin
Adolescents (14–18 years)	30 mg/day of niacin
Pregnancy (14–18 years)	30 mg/day of niacin
Pregnancy (19 years older)	35 mg/day of niacin
Lactation (14–18 years)	30 mg/day of niacin
Lactation (19 years older)	35 mg/day of niacin

3.8. Vitamin B_6 Family

This group was discovered in the 1930s while conducting feeding experiments on rats. Its importance was realized tragically when the heat process for an infant formula reduced the bioavailability of the vitamin. Children developed convulsive disorders. Before realizing that the problem was caused by an induced pyridoxine deficiency, it was thought a contaminant had been introduced.

3.8.1. Uptake and Metabolism The vitamin B_6 family consists of pyridoxine, pyridoxal, pyridoxamine, pyridoxine phosphate, pyridoxal phosphate (PLP), and pyridoxamine phosphate (Fig. 32). Pyridoxal phosphate is the coenzyme form. Pyridoxal phosphate and pyridoxamine phosphate are from animal tissues. Pyridoxine is from plant tissues. All phosphorylated forms are hydrolyzed in the intestinal tract by phosphatases before being absorbed passively. Conversion to the phosphorylated forms occurs in the liver. Notice that niacin (NAD) and riboflavin (FMN, FAD) are required for interconversion among the vitamin B_6 family. The phosphorylated forms are transported to the cells where needed. The major excretory product is 4-pyridoxic acid.

3.8.2. Pyridoxal Phosphate Biochemistry Pyridoxal phosphate is required for amino metabolism and reactions involving amino acids. PLP is covalently bound to the apoenzyme through an enamine linkage between an ε-amino group of lysine and the aldehyde moiety of PLP (Fig. 33). The most common of the PLP catalyzed reactions are transaminations

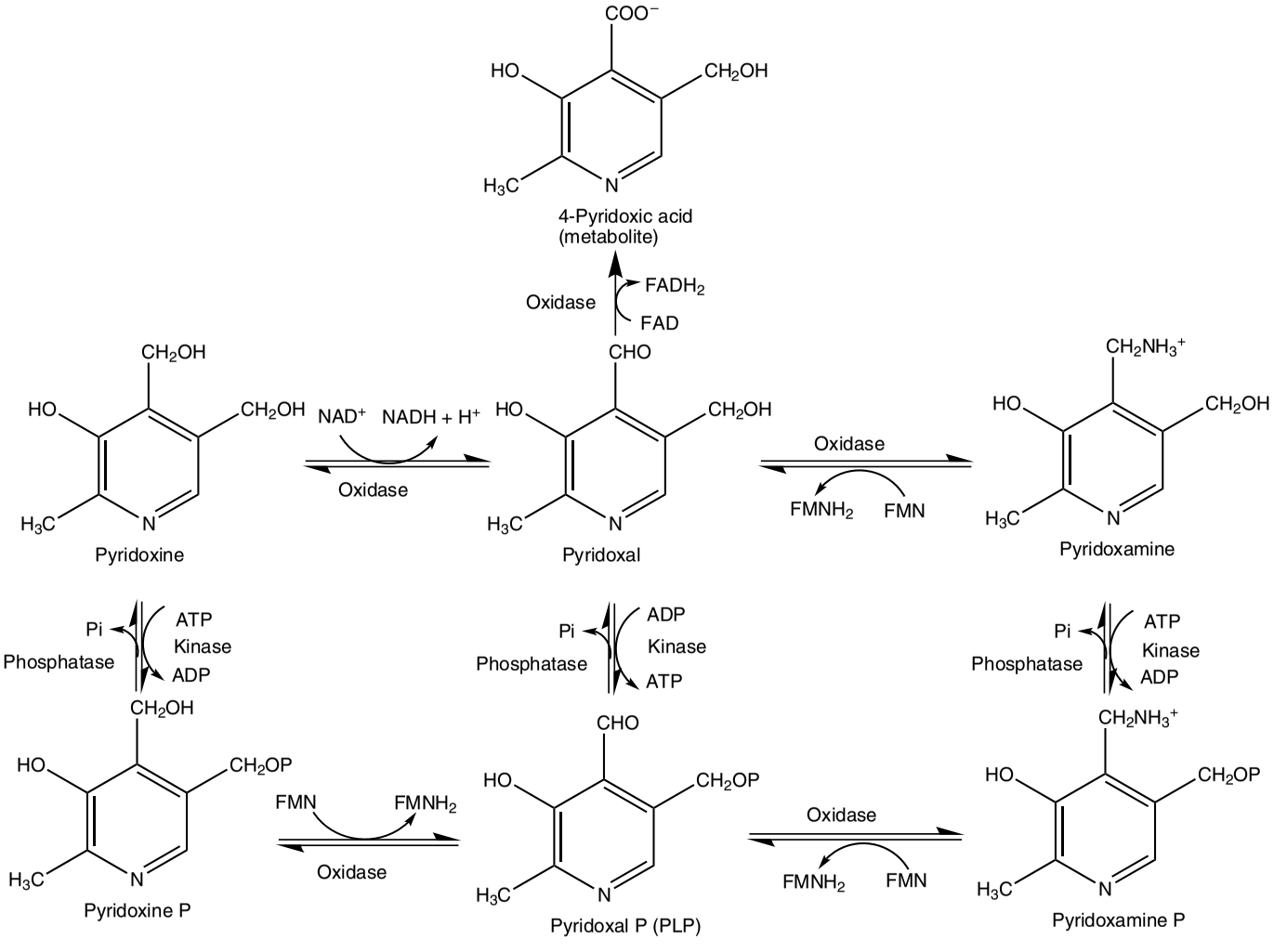
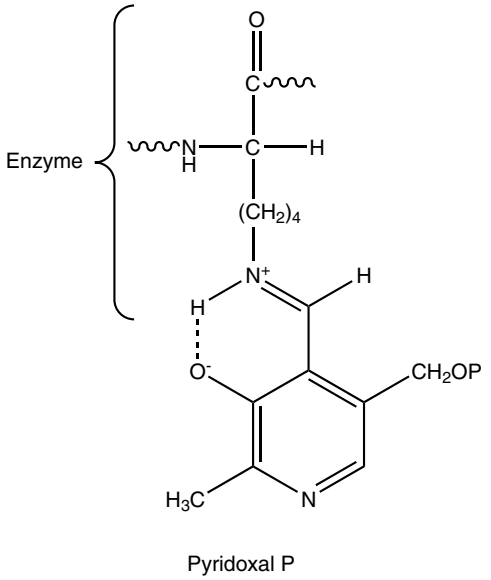


Figure 32. Interconversions among the vitamin B_6 family.



Pyridoxal P

Figure 33. Pyridoxal phosphate bound to the enzyme.

(Fig. 34). One-half of all transamination reactions involves

$$\alpha\text{-amino acid} + \alpha\text{-ketoglutarate} \rightarrow \alpha\text{-keto acid} + \text{glutamic acid}$$

α-ketoglutarate as the acceptor of the amine group forming glutamic acid. Alternatively, glutamic acid donates the amine group and an α-keto acid is the acceptor. Another PLP catalyzed reaction is decarboxylation of amino acids (Fig. 34). These are part of the biosynthesis of neurotransmitters including histamine, serotonin, dopamine, and γ-aminobutyric acid. Although not well understood, PLP is also required for the phosphorylase catalyzed glycogenolysis producing glucose-1-phosphate.

3.8.3. Vitamin B_6 Deficiency There is no distinct deficiency syndrome, but a deficiency can be serious. Seborrheic dermatitis, microcytic anemia, and eleptiform convulsions are observed. The anemia may be caused by decreased heme biosynthesis and the convulsions by imbalances in neurotransmitter biosynthesis.

At one time there was a hypothesis that vitamin B_6 deficiency might contribute to arteriosclerosis or possibly could be used with folic acid and vitamin B_{12} to reduce the risk of cardiovascular disease. This was based on an observation that elevated homocysteine correlated with increased incidence of arteriosclerosis, and homocysteine might contribute to the pathology of the vessel walls. Vitamins B_6, B_{12}, and folic acid are required for the metabolism of homocysteine (Fig. 35). The first two are cofactors in the "remethylation" back to methionine, and the latter for the initial step in the glycogenic catabolism of the amino acid. Note in Fig. 35 that biotin and a coenzyme form of vitamin B_{12} are also required for the catabolic reactions. While vitamins B_6, B_{12}, and folic acid will reduce homocysteine, there is little evidence that reduced homocysteine levels parallel a reduced risk of cardiovascular disease [61,62]. A better indicator is C-reactive protein (CRP). There is further discussion of vitamin B_6 supplementation for improving cognitive levels in the folic acid discussion.

3.8.4. Vitamin–Drug Interactions [63] The two most clinically significant interactions are pyridoxal phosphate with L-Dopa or isoniazid. Examine Fig. 34 and note that dopa decarboxylase requires PLP. This enzyme is found both centrally and peripherally. The latter includes the intestinal mucosa. The precursor to dopamine, L-Dopa is indicated for the treatment of Parkinson's disease. L-Dopa is prescribed because little dopamine crosses the blood–brain barrier relative to its precursor L-Dopa. A patient with Parkinson's disease prescribed L-Dopa and who takes a vitamin supplement with amounts of pyridoxine greater than the vitamin's RDA can experience an increase in Parkinsonian tremors. This is because L-Dopa will undergo decarboxylation in the intestinal mucosa and never reach the locations in the brain where it is converted to the needed dopamine.

Isoniazid is widely prescribed for tuberculosis. It can chemically react with pyridoxal and pyridoxal phosphate, significantly reducing the availability of this coenzyme (Fig. 36) [64]. Pyridoxine supplements commonly are recommended to prevent isoniazid-caused peripheral neuropathy, but they do not reduce the effectiveness of isoniazid.

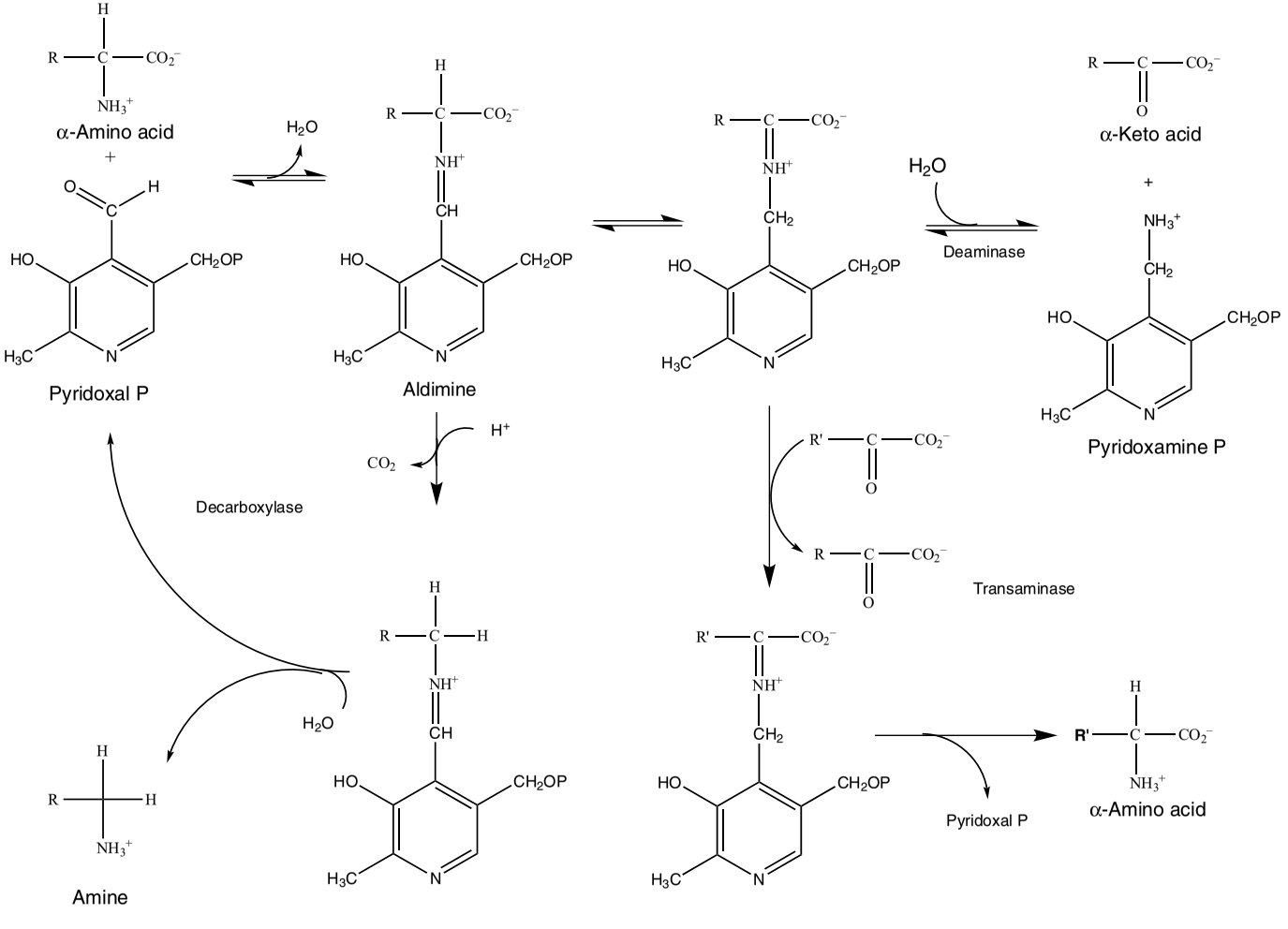


Figure 34. Pyridoxal phosphate catalyzed deaminations, transaminations, and decarboxylations.

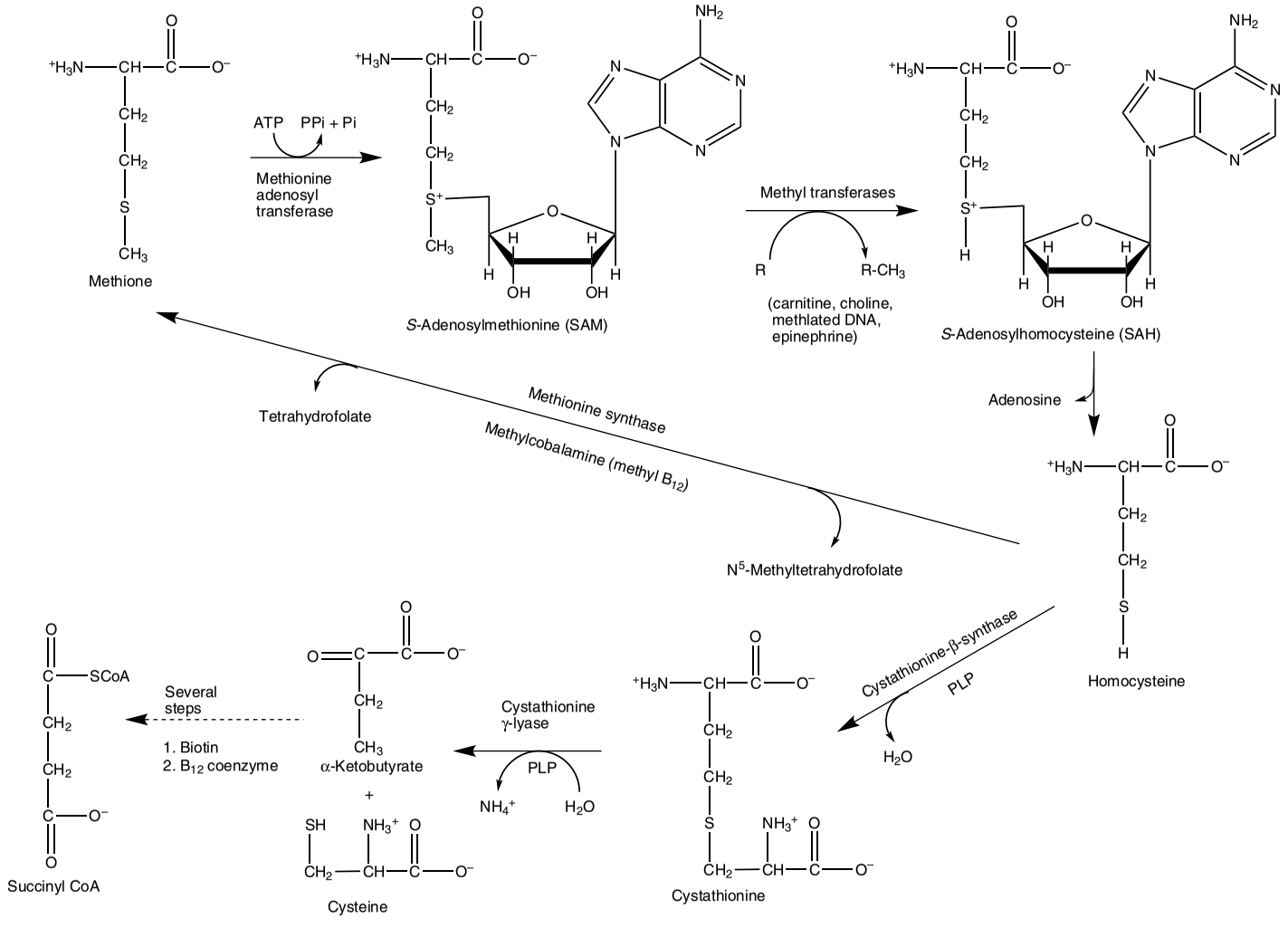


Figure 35. Methionine metabolism.

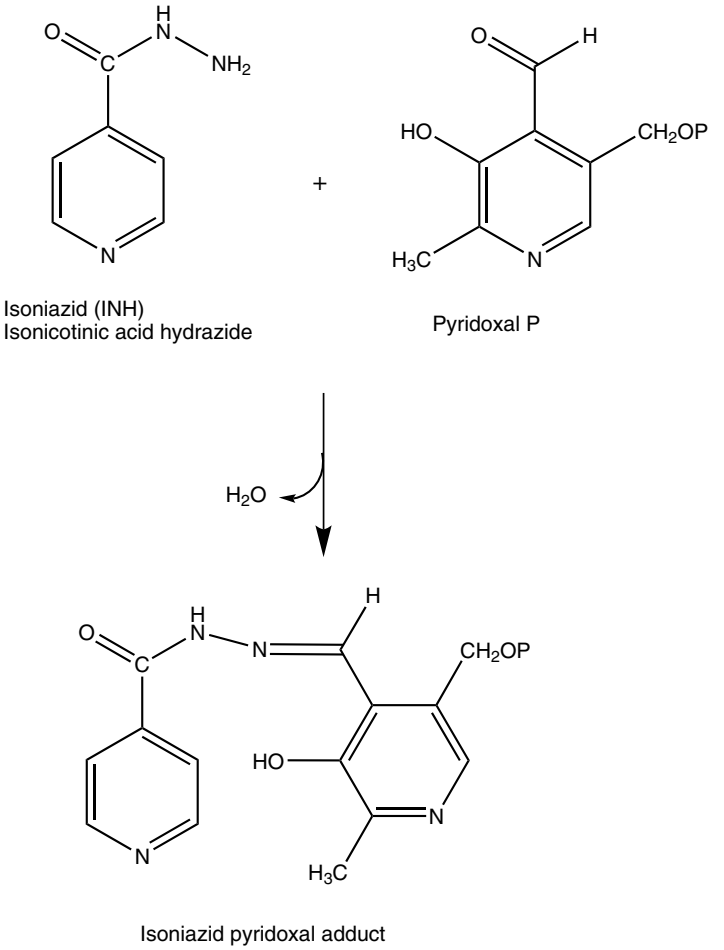


Figure 36. Pyridoxal phosphate–isoniazid interaction.

3.8.5. Hypervitaminosis Pyridoxine A certain mystique has built up around this vitamin resulting in individuals overdosing themselves with commercial vitamin supplements. Because of its role in glycogenolysis, some athletes will take large doses in an attempt to increase blood glucose levels. Serious neurological problems have been seen in doses of 2–6 gm/day for 2–40 months [65–67]. Megadosing below 2 gm/day seems safe, but all of this information is based mostly on anecdotal reports. There is a tolerable upper intake level, but the UL to RDA ratio is a comfortable 50–60.

3.8.6. Dietary Reference Intake

AI (any form of vitamin B_6)	
Infants	0.1–0.3 mg/day
EAR (any form of vitamin B_6)	
Children (1–13 years)	0.4–0.8 mg/day
Males (14–19 years)	1.1 mg/day
Females (14–19 years)	1.0 mg/day
Men (19–50 years)	1.1 mg/day
Men (51+ years)	1.4 mg/day
Women (19–50 years)	1.1 mg/day
Women (51+ years)	1.3 mg/day
Pregnancy	1.6 mg/day
Lactation	1.7 mg/day

RDA (any form of vitamin B_6)	
Children (1–13 years)	0.5–1.0 mg/day
Males (14–19 years)	1.3 mg/day
Females (14–19 years)	1.2 mg/day
Men (19–50 years)	1.3 mg/day
Men (51 + years)	1.7 mg/day
Women (19–50 years)	1.3 mg/day
Women (51 + years)	1.5 mg/day
Pregnancy	1.9 mg/day
Lactation	2.0 mg/day
UL (as pyridoxine)	
Children (1–13 years)	30–60 mg/day
Adolescents (14–18 years)	80 mg/day
Adults (19 + years)	100 mg/day
Pregnancy (14–18 years)	80 mg/day
Pregnancy (19 + years)	100 mg/day
Lactation (14–18 years)	80 mg/day
Lactation (19 + years)	100 mg/day

3.9. Pantothenic Acid [68]

Pantothenic acid is essential and is a normal component of our diet. There has been little research done on this vitamin, and therefore, it has adequate intakes and no RDAs.

3.9.1. Chemistry, Uptake, and Metabolic Role This vitamin, which can be considered a derivative of β-alanine, is asymmetric (Fig. 37). The natural form has the D(+) configuration. The L(−) stereoisomer is inactive. The reduced alcohol form, pantothenol, is considered as equally active as the parent acid. Many of the multiple vitamin products use a synthetic, racemic mixture. This means that double the amount of synthetic vitamin must be used to obtain equivalent active vitamin.

Dietary pantothenic acid is consumed as coenzyme A and the intermediates from coenzyme A's biosynthesis (Fig. 38). These are hydrolyzed to free pantothenic acid. Absorption is by saturable, active transport.

Calcium pantothenate is commonly used in dry dosage forms. It is moderately hygroscopic with a solubility of 1 g/2.8 mL and is unstable for autoclaving. Neither the parent pantothenic acid nor the sodium salt is commonly used in dosage forms.

Pantothenol (panthenol) is reasonably stable and freely soluble and is used both in injectable and oral dosage forms. Although widely used in cosmetics including skin creams and shampoos, there is no evidence that this vitamin is effective as a vitamin topically. It apparently has good emollient properties, but these have nothing to do with its systemic role.

Pantothenic acid is a structural component, but not the active site, of coenzyme A. The acyl thiol esters form on the mercaptan moiety that originates from a cysteine (Fig. 38). The biosynthesis of coenzyme A occurs in the tissues requiring it. Because coenzyme A is required for nearly all acyl transfers, biosynthesis takes place in nearly all cells.

3.9.2. Hypervitaminosis Pantothenic Acid There have been no reports of toxicity and no tolerable upper intake levels. Because its active transport is saturable, excessive uptake is doubtful. Also, this vitamin does not have the mystique that would prompt marketing "high potency" formulations.

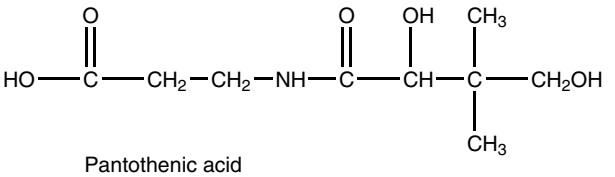
3.9.3. Dietary Reference Intakes There are too few studies to provide sufficient information to estimate EAR or RDA.

AI	
Infants	1.7–1.8 mg/day
Children (1–13 years)	2–4 mg/day
Everyone else	5 mg/day
Pregnancy	6 mg/day
Lactation	7 mg/day
EAR	
None reported	
RDA	
None reported	
UL	
None reported	

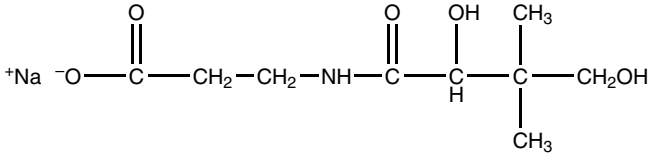
3.10. Biotin [69]

Biotin (Fig. 39) is essential, a normal constituent of the diet, and required for four biotin-dependent carboxylation reactions. Eating raw egg white can induce a deficiency.

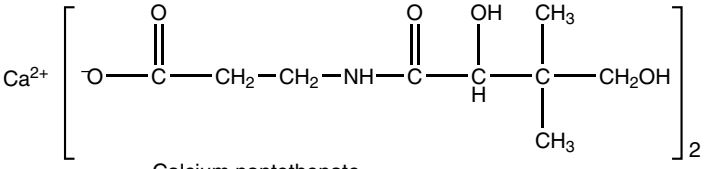
3.10.1. Uptake In foods, most biotin is covalently bound (Fig. 40) to the apoenzyme where it is the coenzyme for carboxylation reactions. Intestinal enzymes hydrolyze the amide link-



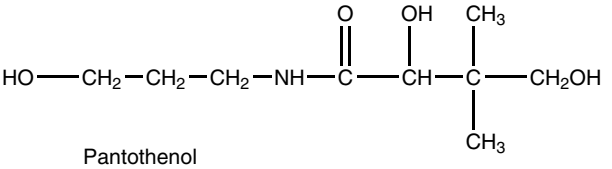
Pantothenic acid



Sodium pantothenate



Calcium pantothenate



Pantothenol

Figure 37. Forms of pantothenic acid.

age to produce free biotin. Biotin is actively transported through the intestinal flora into the portal vein and to the liver where it may be stored. It appears that adults may store several months of biotin. From the liver, it is transported to tissues where it is needed.

3.10.2. Chemistry Biotin consists of two five-membered rings *cis* fused to each other that can be drawn either as the keto (urea) or enolic form (Fig. 39). The enolic D-isomer is the active stereoisomer, but many times commercial multivitamin products contain the synthetic racemic D,L mixture. There is no activity with the L-stereoisomer.

3.10.3. Metabolic Role Biotin picks up carbon dioxide that has been activated by combining with an ATP donated phosphate producing the mixed anhydride of phosphoric and carbonic acids (Fig. 41). The biotin enolate receives the carbon dioxide producing the keto carbon dioxide releasing coenzyme.

There are four biotin-dependent carboxylation reactions (Fig. 42), three of which are in the mitochondria. They are

(a) Pyruvate carboxylase This reaction, which converts pyruvate to oxaloacetate, is in the mitochondria and has two functions. First, it is the initial reaction

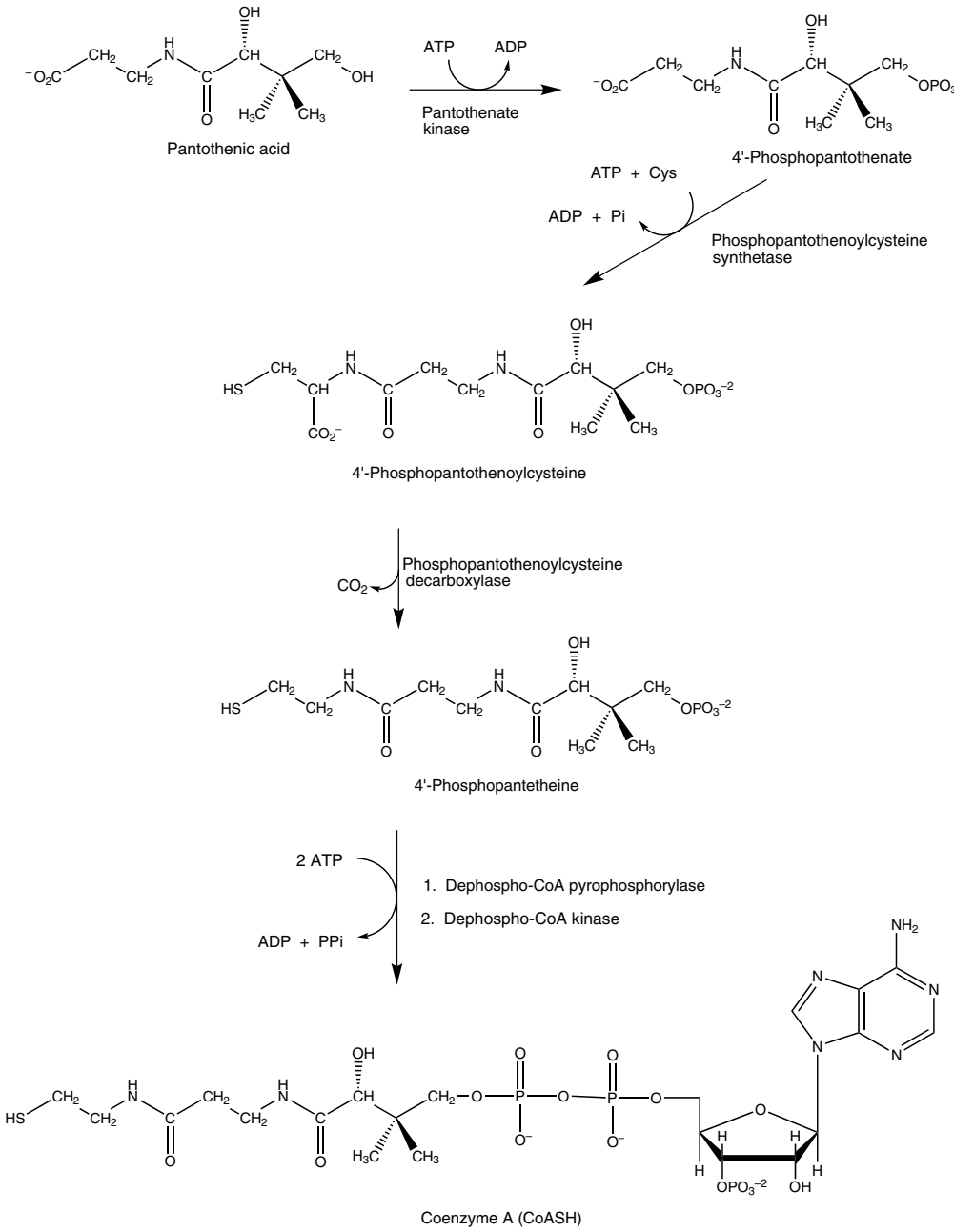


Figure 38. Formation of coenzyme A from pantothenic acid.

in gluconeogenesis to overcome the 14 kcal energy barrier to form phosphoenolpyruvate. Second, this same reaction, sometimes referred to as an anapleurotic reaction, insures that there is adequate oxalocetate when there is large amount of acetyl CoA entering the Krebs cycle.

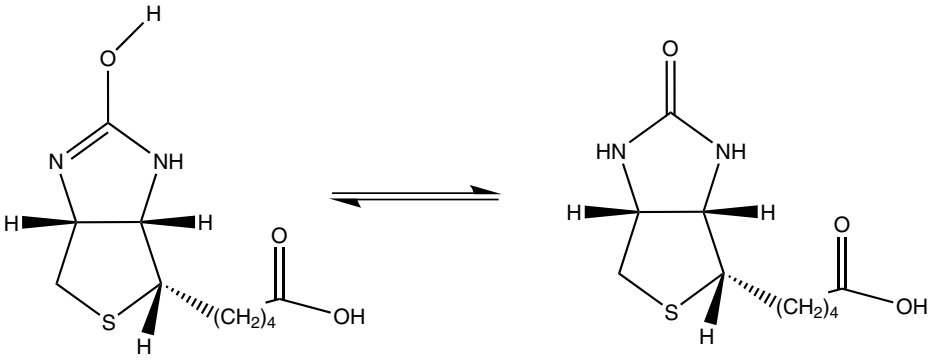


Figure 39. Biotin.

(b) Acetyl CoA carboxylase This reaction, found mostly in the cytosol, is the committed step in the synthesis of fatty acids.

(c) Propionyl CoA carboxylase Propionyl CoA is the product from the catabolism of valine, isoleucine, methionine, and odd numbered fatty acids. The carboxylation reaction, found in the mitochondria, produces methylmalonyl CoA. The latter undergoes a cobalamin (vitamin B_{12}) catalyzed rearrangement forming succinyl CoA, which is further metabolized in the Krebs cycle.

(d) β-Methylcrotonyl CoA Carboxylase This mitochondrial reaction permits the final steps in the catabolism of the branched-chain amino acid, leucine. The final products, acetoacetate and acetyl CoA, either are oxidative metabolized to carbon dioxide and water or enter other reactions in lipid metabolism.

3.10.4. Biotin Deficiency Relative to many of the vitamins, it is easy to induce a biotin deficiency by feeding volunteers raw egg white. Avidin, a basic protein found in egg white, forms salt linkages with acidic biotin and prevents its transport across the intestinal barrier. Cooked egg white is not a pro-

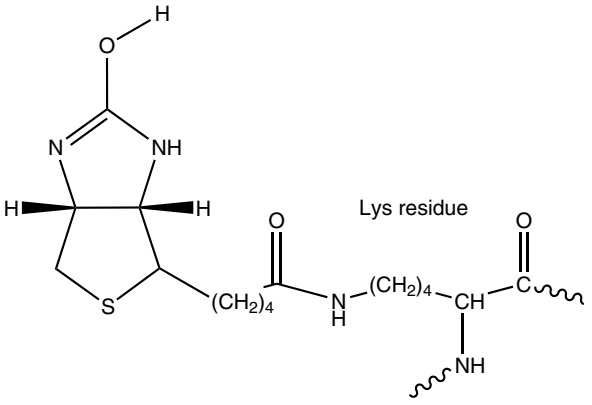


Figure 40. Coenzyme form of biotin.

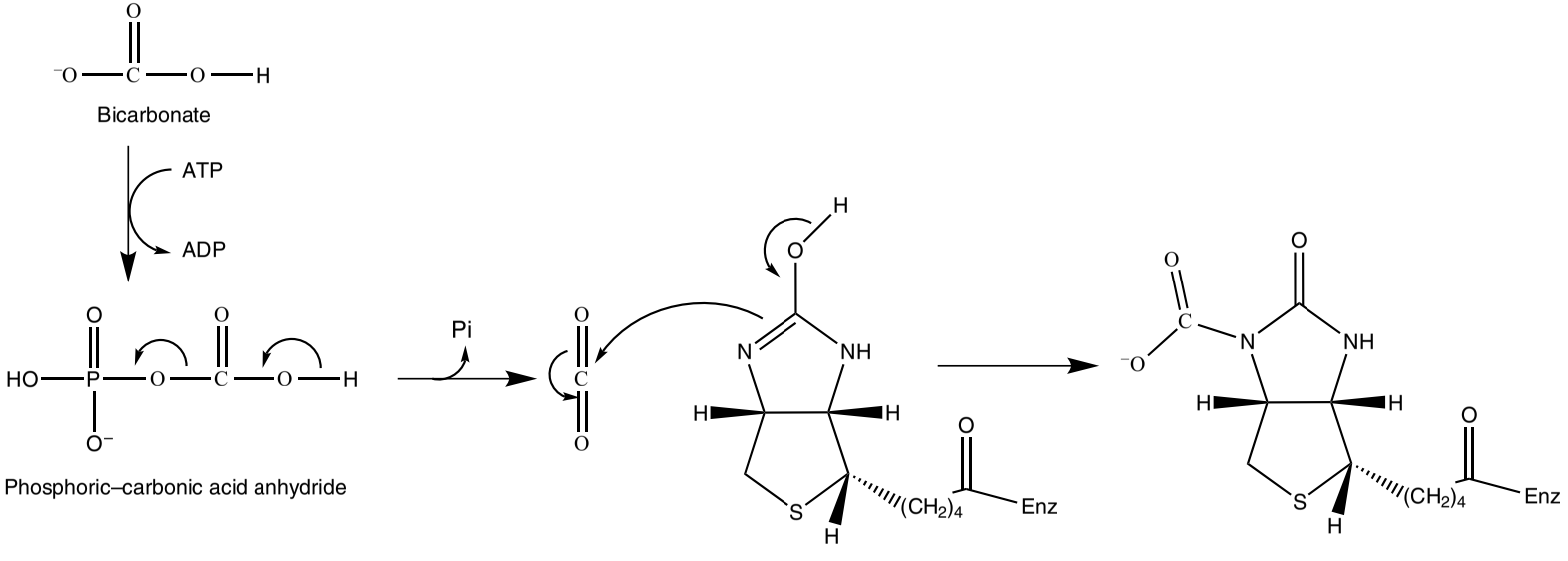


Figure 41. Biotin binding carbon dioxide.

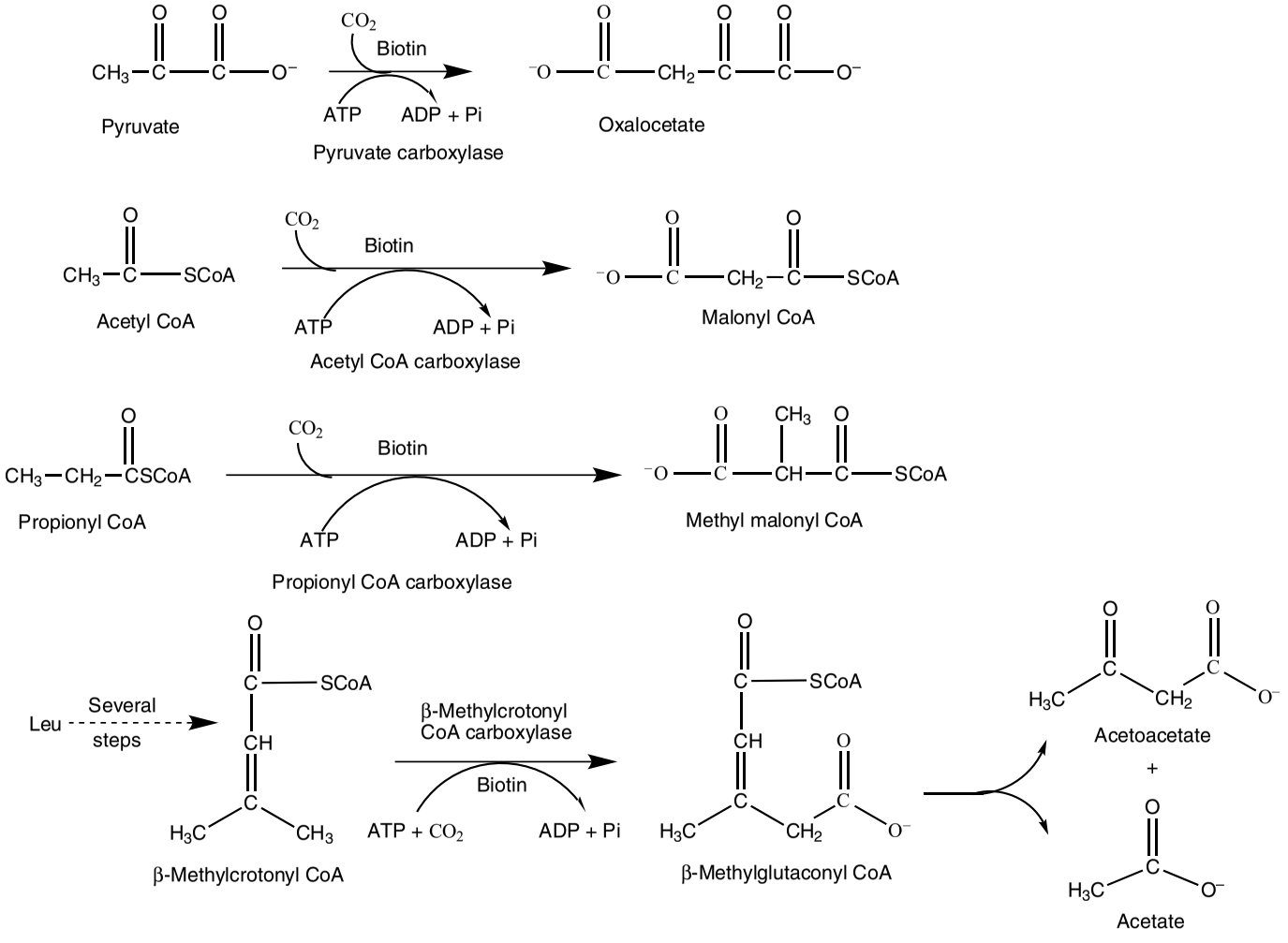


Figure 42. Biotin catalyzed carboxylations.

blem. Because biotin is found in the yolk, eating whole raw egg will not induce a deficiency. Deficiencies were also caused in patients on total parenteral nutrition (TPN) because biotin was not included in the early formulations. Symptoms include dermatitis, loss of hair color, and central neurological effects.

3.10.5. Hypervitaminosis Biotin None has been reported in humans and there are no tolerable upper intake levels.

3.10.6. Dietary Reference Intakes These have been difficult to determine. There has been some speculation that humans might obtain part of their biotin requirements from the intestinal flora in the colon. The question that has not been adequately answered is whether there is significant absorption of bacterial produced biotin from the colon.

AI	
Infants	5–6 μg/day
Children (1–13 years)	8–20 μg/day
Adolescents (14–18 years)	25 μg/day
Adults	30 μg/day
Pregnancy	30 μg/day
Lactation	35 μg/day
EAR	
None reported	
RDA	
None reported	
UL	
None reported	

3.11. Folic Acid [70]

With all vitamins being essential, it is difficult to state that one vitamin is more important than another. Nevertheless, folic acid, with its coenzyme role in purine biosynthesis, can be considered crucial for some of the cells most fundamental biochemistry, cell division. This vitamin is intimately tied to vitamin B_{12} (cobalamin), which has made estimating its DRIs difficult. Also, conditions that can cause a folic acid deficiency can also result in a vitamin B_{12} deficiency.

3.11.1. Chemistry The commercial form of the vitamin is folic acid (Fig. 43). It consists of a pteridine ring attached to a *p*-aminobenzoic acid that is attached to the α-amine of glutamic acid. Two biosynthetic changes must occur before it is active. First, it must be reduced to tetrahydrofolate by dihydrofolate reductase in a two-step reduction (Fig. 44). Notice that niacin is required for this reduction. Second a polyglutamate chain must be attached to the γ-carboxyl of the parent glutamic acid (Fig. 43). The remaining linkages of the polyglutamate chain are traditional α-amino-α-carboxyl peptides.

The natural vitamin is made up of a family of polyglutamates all connected to the initial glutamic acid at the previously described γ-carboxyl group. The length of this polyglutamate chain varies with the source of the vitamin, but lengths of three, five, and seven amino acids are seen. The most common polyglutamate found in food is 5-methyltetrahydrofolate polyglutamate (Fig. 43).

3.11.2. Uptake The dietary polyglutamates are cleaved to the monoglutamate vitamin by a γ-L-glutamylcarboxy peptidase commonly called conjugase. Folic acid is absorbed as the monoglutamate. Conjugase is found in the brush border of the intestine. Therefore, chronic inflammatory conditions in the intestine lead to low conjugase activity, which can result in significant decreased folic acid absorption.

The absorbed vitamin must be converted to the coenzyme form. This requires adding back a five to seven member glutamate chain and commercial folic acid also must undergo the two-step reduction to tetrahydrofolic acid. There are two common abbreviations for the reduced form, FH_4 and THF. These reactions apparently occur in a wide variety of tissues. The liver contains about 3–6 months supply of the vitamin, presumably in the polyglutamate form.

3.11.3. Metabolic Roles There are five forms of tetrahydrofolate polyglutamate, all but one of which are coenzymes (Fig. 45). The most highly oxidized is 10-formyl tetrahydrofolate polyglutamate produced by the addition of formic acid to tetrahydroformate polygluta-

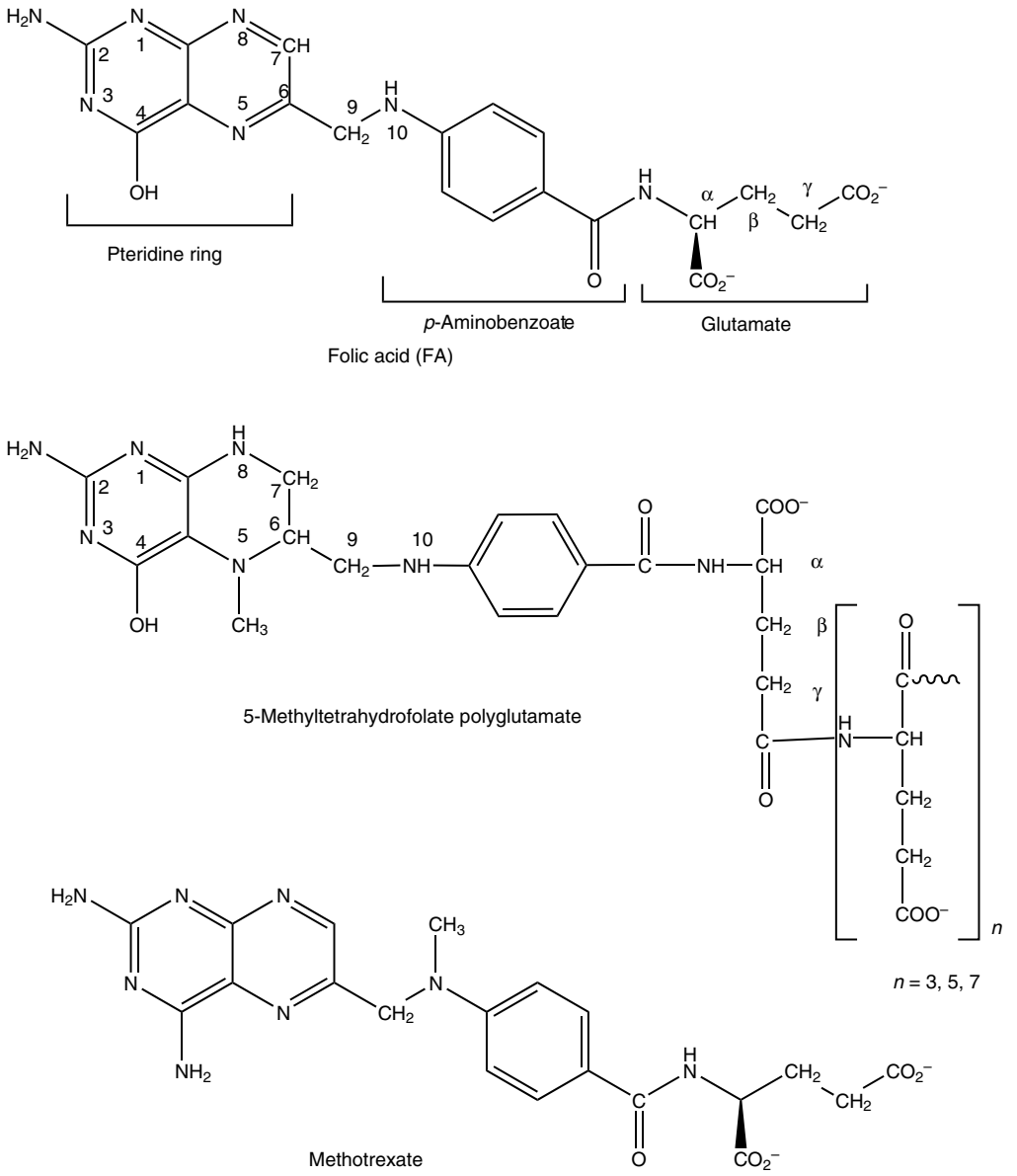


Figure 43. Folates and the antifolate, methotrexate.

mate. It is the coenzyme for two reactions in purine biosynthesis: (1) the synthesis of formylglycine ribotide (FGAR) from glycine amide ribotide (GAR) and (2) the formation of aminoimidazole carboxamide ribotide (AICAR) to formamidoimidazole carboxamide ribotide (FAICAR) (Fig. 46).

5,10-Methenyl tetrahydroformate polyglutamate is the cyclic enamime formed from 10-formyl tetrahydroformate polyglutamate. It also is formed from the catabolism of histidine and can be considered an intermediate between the 10-formyl compound and, upon

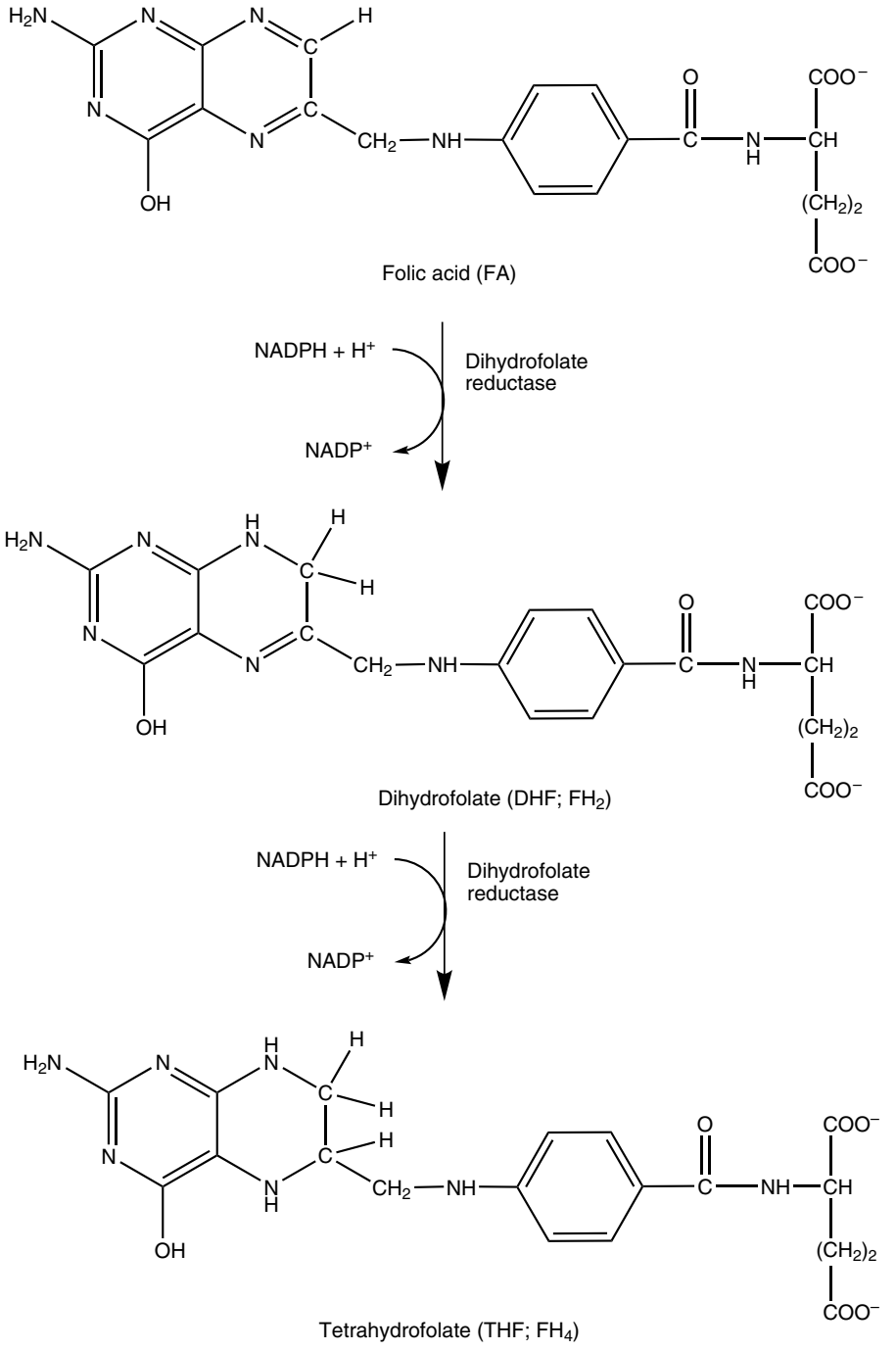


Figure 44. Reduction of folic acid to tetrahydrofolate.

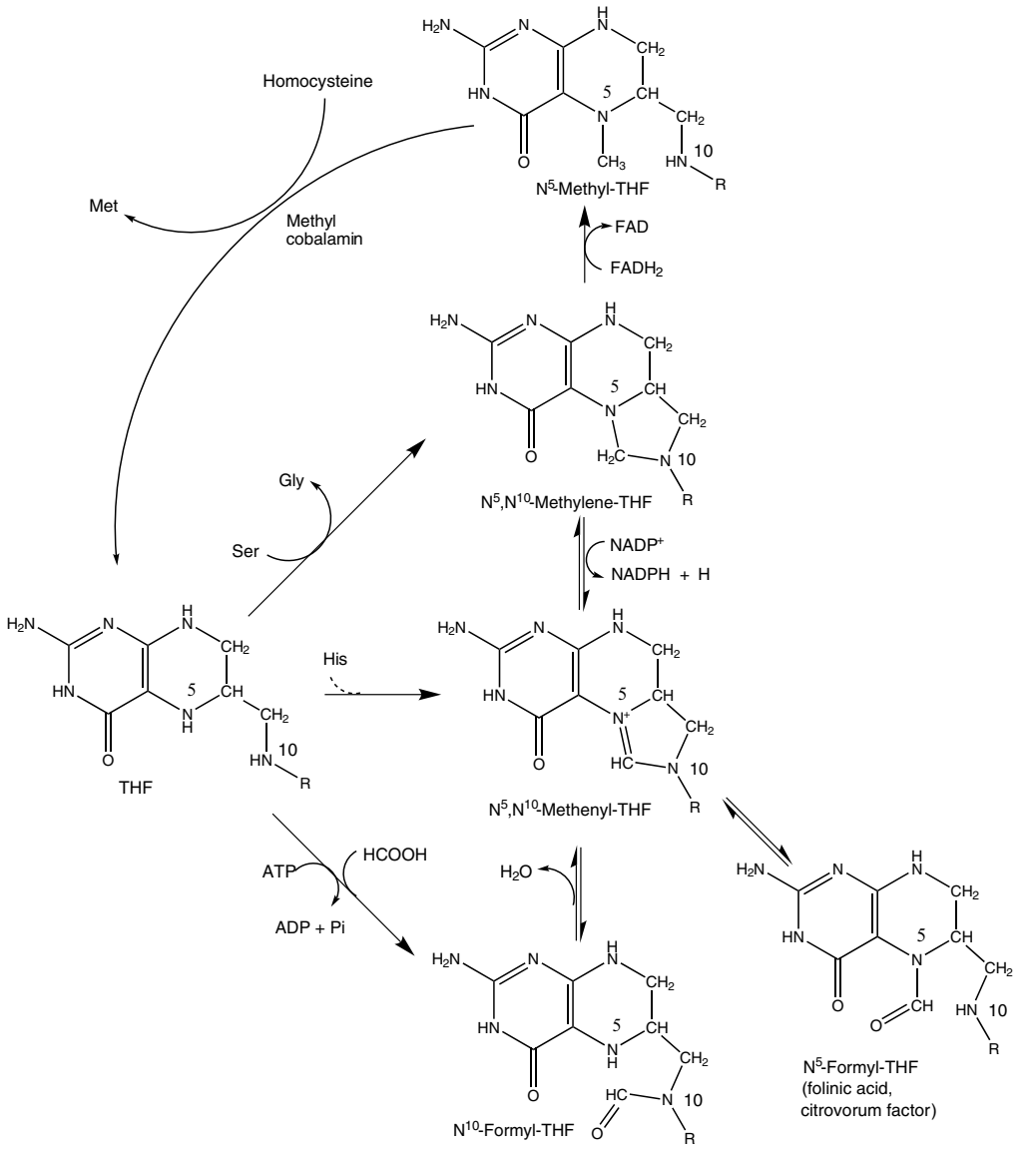


Figure 45. Formation of tetrahydrofolate coenzymes polyglutamates.

reduction, 5,10-methylene tetrahydroformate polyglutamate. The latter is the coenzyme required for the interconversion of serine and glycine and the methylation of deoxyuridylic acid forming deoxythymidylic acid (Figs 45 and 47). Most of the one-carbon units carried on position 5 or 10 or 5,10 bridge come from serine.

The most reduced coenzyme is 5-methyl tetrahydrofolate polyglutamate. It is the source of the methyl group added to homocysteine regenerating methionine and tetrahydrofolate, the latter capable of accepting a one-carbon unit from formate or serine. This last reaction is where folic acid and vitamin B_{12} come together (Figs 45 and 49). The implications of this reaction and how folic acid can mask pernicious anemia is discussed in the section on vitamin B_{12} (cyanocobalamin). Note that the formation of 5-methyl-THF

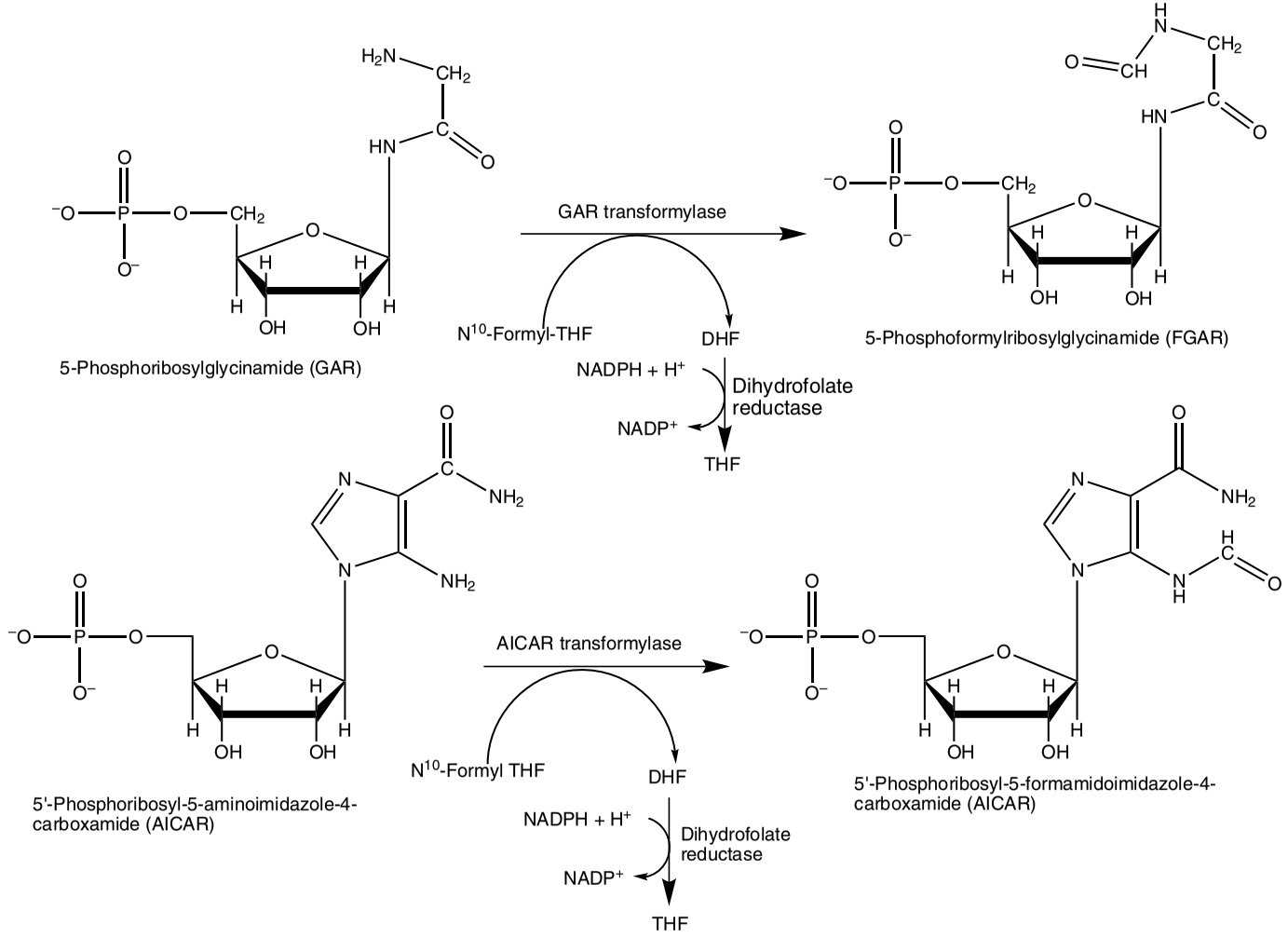


Figure 46. 10-Formyl THF coenzyme roles.

normally is not reversible. Tetrahydrofolate can only be regenerated if there is adequate methyl cobalamin coenzyme.

The fifth tetrahydrofolate compound is 5-formyl THF (folinic acid, citrovorum factor). This compound is not a coenzyme, but it can be converted to any of the active coenzyme forms. It is administered following treatment with the dihydrofolate reductase inhibitor, methotrexate (Fig. 43), as a form of rescue therapy. Because it already is in the reduced tetrahydrofolate form, it does not need dihydrofolate reductase to become an active coenzyme.

3.11.4. Folic Acid Deficiency It is obvious that folic acid is a very important vitamin for biosynthetic reactions, particularly those required for the biosynthesis of purines, methylation of deoxyuridylic acid, and regeneration of methionine from homocysteine. The main deficiency is a characteristic megaloblastic anemia due to a shortage of nucleotides required for the production of erythrocyte precursor cells.

Another clinical sign of folic acid deficiency is neural tube defects including spina bifida and anencephaly. Neural tube defects are one of the main reasons that Federal regulations mandate supplementing cereal grain-based foods with folic acid along with thiamine, riboflavin, and niacin [71–73]. While prenatal multiple vitamins contain adequate amounts of folic acid, pregnant women may not start taking these products until the second or third month of the their pregnancies, and this may be too late.

A third indication of inadequate folic acid is elevated blood homocysteine levels. As described in the vitamin B_6 section, at one time there was a hypothesis that elevated homocysteine correlated with increased risk of cardiovascular disease. This hypothesis was based on an observation that individuals with increased blood vessel plaque build-up also show increased levels of homocysteine. The elevated homocysteine can be corrected, at least partially, with folate supplements. Figure 35 illustrates why the three vitamins, pyridoxine, folic acid, and vitamin B_{12}, are indicated for elevated levels of homocysteine. Pyridoxal phosphate and cobalamin (B_{12}) are required for the catabolism of homocysteine to succinyl CoA. Methyl cobalamin (methyl B_{12}) is required for the conversion of homocysteine back to methionine.

There are reports that plasma homocysteine concentrations are inversely related to cognitive function in older people. While supplements of folic acid, vitamin B_{12}, and vitamin B_6 did lower homocysteine, there were no significant differences between the vitamin and placebo groups in the scores on tests of cognition [74].

There are many causes of folic acid deficiencies. Inadequate nutrition during periods of increased requirements is one of the main causes of megaloblastic anemia of pregnancy and neural tube defects. Alcoholism is considered the leading cause of folic acid deficiency in the United States [75,76]. There are two ways that excessive alcohol consumption can interfere with folic acid activity: impairment of folic acid reduction to the active THF forms and interference with folic acid storage and release from the liver. A third cause of folic acid deficiency is chronic inflammation of the intestinal mucosa. Inflammation can reduce production of the required conjugase enzyme, which removes the polyglutamate chain, and/or an inflamed mucosa inhibits folate transport. Finally, anticonvulsants such as phenytoin somehow interfere with folic acid uptake or utilization.

The dihydrofolate reductase inhibitor, methotrexate (Fig. 43), was developed as an anticancer drug whose inhibition of formation of folic acid coenzymes would block purine synthesis. In other words, it was designed to induce a folic acid deficiency. Notice in Figs 46 and 47 that formation of dTMP, FGAR, and AICAR also causes the oxidation of tetrahydrofolate to dihydrofolate. The latter must be reduced by dihydrofolate reductase to tetrahydrofolate before active coenzyme can form again. Thus, not only does methotrexate inhibit the initial formation of the tetrahydrofolate moiety from folic acid supplements, but it also blocks regeneration of the coenzyme form.

3.11.5. Hypervitaminosis Folic Acid With important exception of masking pernicious anemia characteristic of a vitamin B_{12} deficiency, excessive intake of folic acid has not been considered a problem. Transport across the

intestinal mucosa may be regulated by a feedback mechanism or the rate of hydrolysis of the polyglutamate chain or a combination of both. What is very important is that taking the vitamin in doses above 400 μg (800 μg in pregnant and lactating women) can mask the macrocytic anemia seen with pernicious anemia. The tolerable upper limit is based on trying to avoid this masking. Therefore, the UL to RDA ratio is low, 2–2.5, in adults.

There have been many trials to determine if folic acid supplements can ameliorate certain medical condition. These have not been promising and possibly might exacerbate the patient's disease, particularly if it is a malignancy [77–81].

3.11.6. Dietary Reference Intakes

AI	
Infants	65–80 μg/day
EAR	
Children (1–8 years)	120–160 μg/day
Children (9–13 years)	250 μg/day
Adolescents (14–18 years)	330 μg/day
Adults (19–50 + years)	320 μg/day
Pregnancy	520 μg/day
Lactation	450 μg/day
RDA	
Children (1–8 years)	150–200 μg/day
Children (9–13 years)	300 μg/day
Adolescents (14–18 years)	400 μg/day
Adults (15–50 + years)	400 μg/day
Pregnancy	600 μg/day
Lactation	500 μg/day
UL (from fortified foods or supplements)	
Children (1–3 years)	300 μg/day
Children (4–8 years)	400 μg/day
Children (9–13 years	600 μg/day
Adolescents (14–18 years)	800 μg/day
Adults (19 + years)	1,000 μg/day
Pregnancy (14–18 years)	800 μg/day
Pregnancy (19 + years)	1,000 μg/day
Lactation (14–18 years)	800 μg/day
Lactation (19 + years)	1,000 μg/day

3.12. Vitamin B_{12} (Cobalamin) [82]

This chemically very complex vitamin is required for two reactions, the methylation of homocysteine to methionine and rearrangement of methylmalonyl CoA to succinyl CoA. A deficiency leads to pernicious anemia, at one time a disease whose prognosis was death. Because folic acid can mask the blood picture of a cobalamin deficiency, it has become important for physicians to order tests specific for the vitamin, particularly in older patients who begin to show symptoms.

3.12.1. Chemistry The cobalamin family consists of a corrin ring (Fig. 48). It is similar to that of the porphyrin ring system except that there is no methylene or methine bridge between pyrrole rings A and D, and it contains cobalt rather than iron. The commercial form sold in the United States is cyanocobalamin. The hydroxy dosage form has also been used. The two coenzyme forms are methylcobalamin (cytoplasm) and adenosylcobalamin (mitochondria). The commercial vitamin is produced from bacterial fermentation. The dimethylbenzimidazole ring comes from rings A and B of the reduced form of riboflavin (Fig. 28) [83].

3.12.2. Uptake Uptake of the vitamin from food and vitamin products is complex. Indeed, most deficiencies are not from inadequate diet, but result from defects in the uptake process. Dietary cobalamin requires a fully functioning stomach and ileum of the small intestine [84]. Parietal cells in the stomach produce hydrochloric acid to free the vitamin from the food, R-factor that complex the dietary cobalamins and "intrinsic factor," a glycoprotein. The R-factor–cobalamin complex dissociates in the alkaline intestine, and the now free cobalamin combines with the intrinsic factor that also was produced in the stomach. In the presence of calcium supplied by the pancreas, specific receptors in the ileum in the mucosa take up the intrinsic factor–cobalamin complex. Without intrinsic factor, only about 1% of cobalamins are absorbed. Eventually the vitamin passes into systemic circulation and is transported by a series of plasma binding proteins, the transcobalamins. About 50% of the absorbed vitamin reaches the liver with the remaining transported to other tissues. Note that dietary cobalamins require both R-factor and intrinsic factor. Vitamin B_{12} supplements only require intrinsic factor.

Humans are efficient recyclers of vitamin B_{12} because of enterohepatic circulation. The vitamin is secreted in the bile. Upon

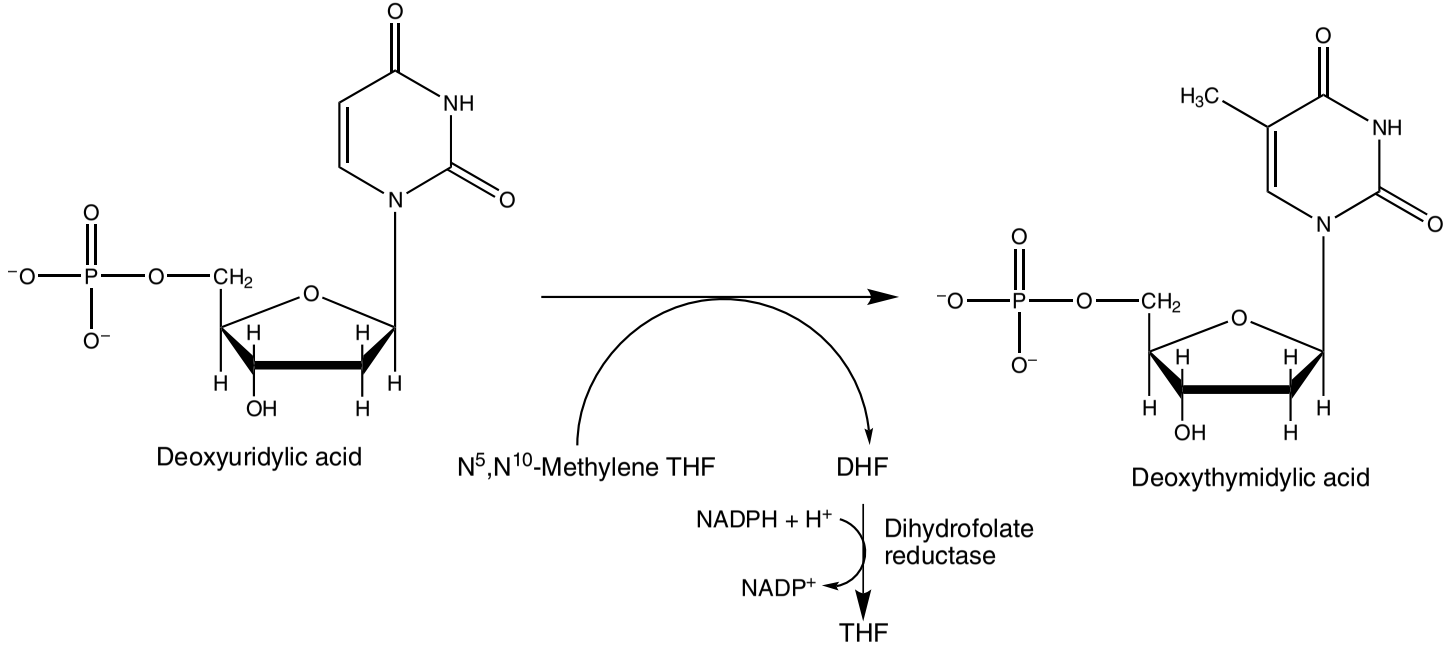


Figure 47. Methylation of deoxyuridylic acid forming deoxythymidylic acid.

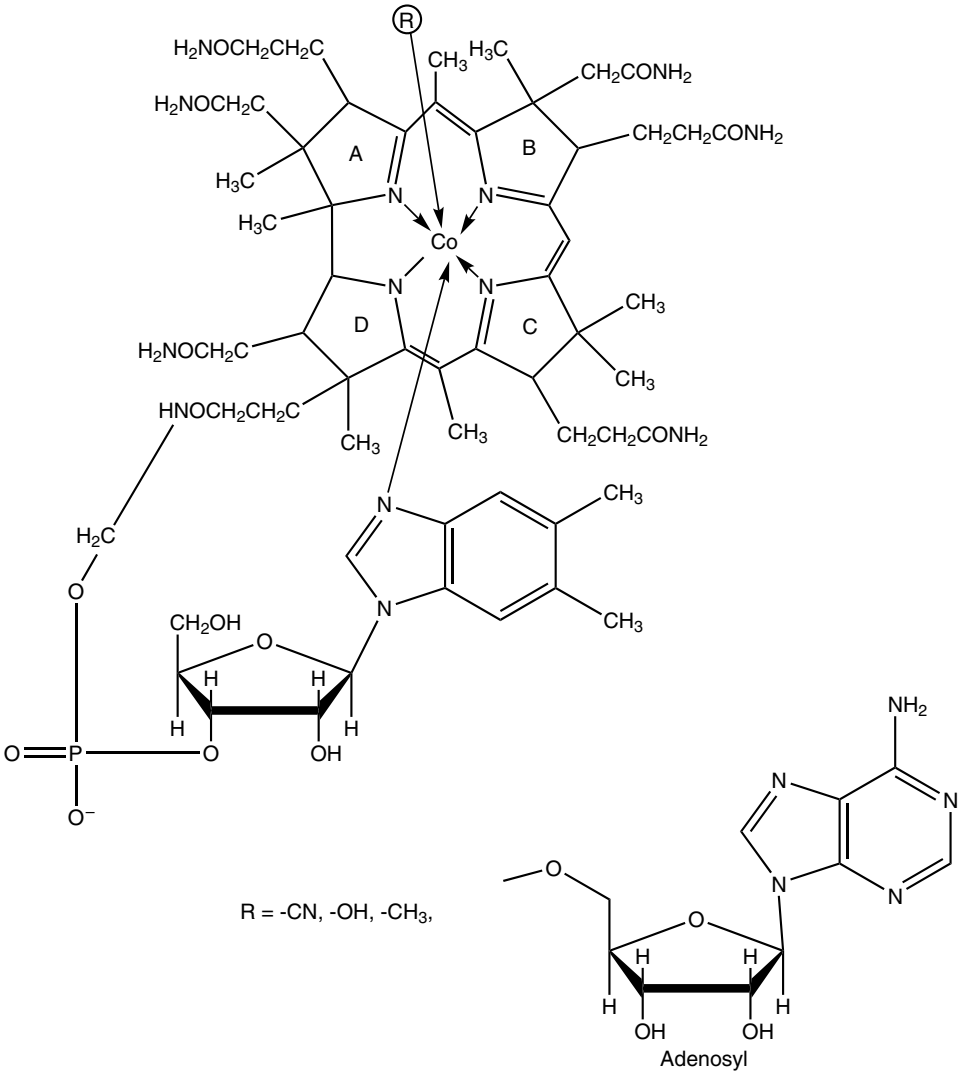


Figure 48. Vitamin B_{12} and coenzyme forms.

combining with intrinsic factor, the absorption process is repeated. This recirculation probably explains why dietary deficiencies are uncommon and why the inability to produce intrinsic factor results in vitamin B_{12} deficiency even though there may be adequate dietary intake.

3.12.3. Biochemical Role Cobalamin is a coenzyme in only two reactions, but they are basic to the health of the individual. Methyl cobalamin is required for the regeneration of methionine from homocysteine (Fig. 35). 5-Methyltetrahydrofolate polyglutamate is also required (Figs 45 and 49). The second reaction is the rearrangement of methylmalonyl CoA to succinyl CoA with adenosylcobalamin as the coenzyme (Fig. 50). Odd numbered fatty acids and the amino acids valine, isoleucine, and methionine produce propionyl CoA as part of their catabolism. Biotin catalyzed carboxylation (Fig. 42) yields D-methylmalonyl CoA, which must be epimerized to the L-stereoisomer. The latter undergoes a rearrangement to succinyl CoA, which enters the Krebs cycle for final degradation. There has been

considerable debate as to the mechanism of this rearrangement, but the general consensus is that there are free radical intermediates [85–87].

3.12.4. Cobalmin Deficiency Pernicious anemia is the disease associated with vitamin B_{12} deficiency. It is usually caused by the inability to produce intrinsic factor. Deficiencies can also result from total gastrectomy and sometimes resection of the ileum [88]. Indeed, many times the vitamin must be administered by injection although oral megadoses, and up to 1000 μg are usually tried initially. The blood picture, a megaloblastic anemia, is indistinguishable from that caused by folic acid deficiency. Indeed folic acid supplements can mask the blood picture. This is illustrated in Fig. 49. Removal of adenosylcobalamin eliminates the regeneration of tetrahydrofolate during the methylation of homocysteine to methionine. Folic acid supplements provide a fresh source of tetrahydrofolate coenzymes. DNA synthesis can continue and new erythrocytes form. Excess folic acid also may compete for the available vitamin, further exacerbating vitamin B_{12} deficiency.

Pernicious anemia can be lethal if not treated because of nerve damage. There are two explanations for the cause of this damage, both involving an excess of methylmalonyl CoA. Methylmalonyl CoA is a competitive inhibitor of malonyl CoA during fatty acid synthesis. This may impede repair of the myelin sheath surrounding nerves. Alternatively, methylmalonyl CoA replaces malonyl CoA as a substrate in fatty acid synthesis producing fatty acids with methyl substituents. These are incorporated into the lipids components of the myelin sheath producing a nonfunctioning myelin sheath.

3.12.5. Hypervitaminosis B_{12} The vitamin is considered nontoxic. There has been some concern that the presence of the CN^- anion in the commercial vitamin might cause problems with megadoses. However, 1000 μg of cyanocobalamin contains only 0.02 mg of CN. There are no tolerable upper intake levels.

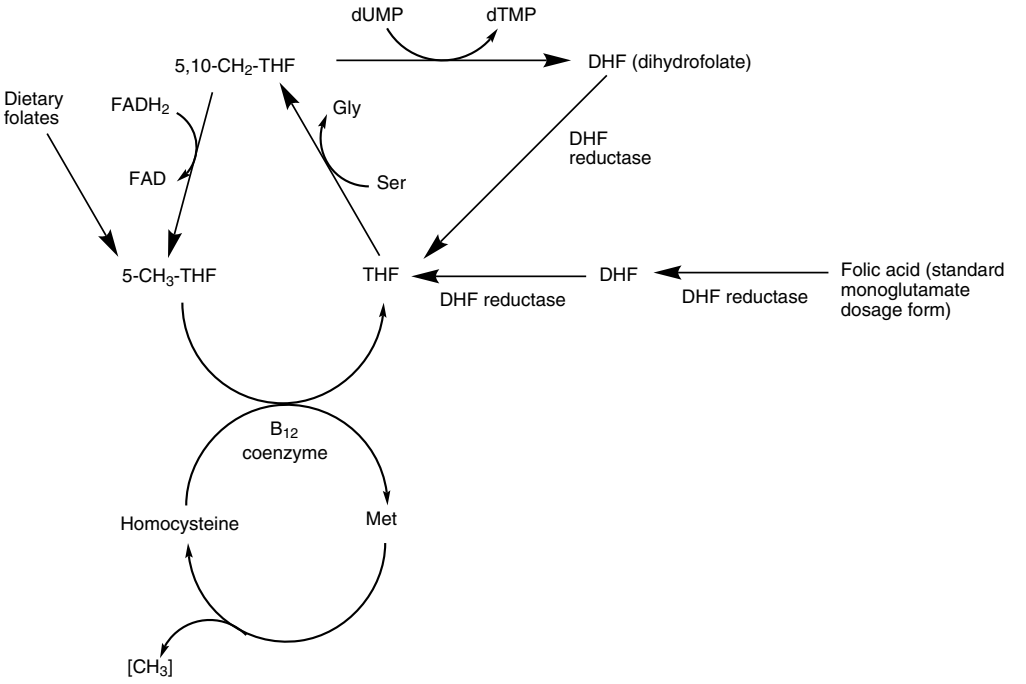


Figure 49. Methyl trap hypothesis.

3.12.6. Dietary Reference Intake Levels

AI	
Infants	0.4–0.5 μg/day
EAR	
Children (1–13 years)	0.7–1.5 μg/day
Adolescents (14–18 years)	2.0 μg/day
Men and women (19–50 + years)	2 μg/day
Pregnancy	2.2 μg/day
Lactation	2.4 μg/day
RDA	
Children (1–13 years)	0.9–1.8 μg/day
Adolescents (14–18 years)	2.4 μg/day
Men and women (19–50 + years)	2.4 μg/day
Pregnancy	2.6 μg/day
Lactation	2.8 μg/day
UL	
None reported	

3.13. Vitamin C (Ascorbic Acid) [89]

Deficiencies of this vitamin have been associated with the early sailors who lacked fresh fruit and vegetables. However, it was a problem on land and was seen in the Irish potato famine, the California gold miners, and territorial prisons. There long has been a mystique surrounding this vitamin with interest sharply increasing when Linus Pauling published his book "*Vitamin C and the Common Cold*" forcing the medical, nutritional, and biochemical professions to reexamine carefully the role of this essential nutrient in human health. A significant problem with studying this vitamin is the fact that ascorbic acid is not a vitamin in most animals. It was not until the discovery that guinea pigs also require ascorbate that animal experiments could be conducted.

3.13.1. Chemistry Ascorbic acid is derived from the aldonic acid form of L-gulose (Figs 51 and 52). There are two enolic proton donor groups with the one at position 3 being the most acidic with a pK_a 4.1. Ascorbic acid is easily oxidized to the dehydro form without loss of vitamin activity, but the lactone ring now hydrolyzes easily producing inactive open chain product. The triketo representation has been the common way to represent dehydroascorbic acid (DHA), but in the aqueous *in vivo* environment of the cell, it most likely is a bicyclic hemiacetal [90].

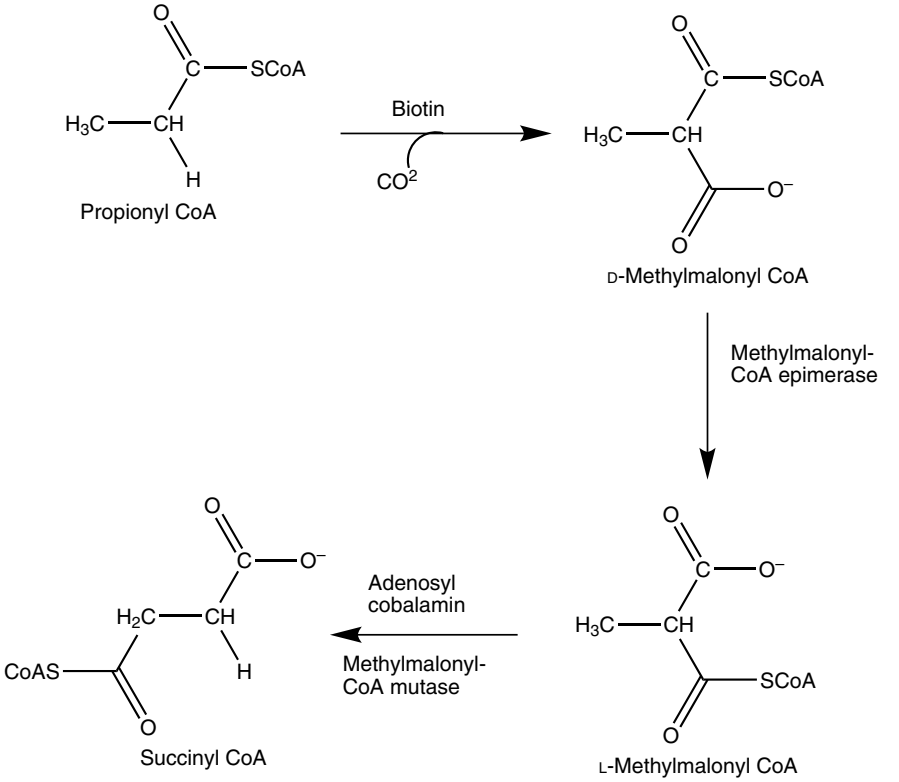


Figure 50. Vitamin B_{12} catalyzed mutase reaction.

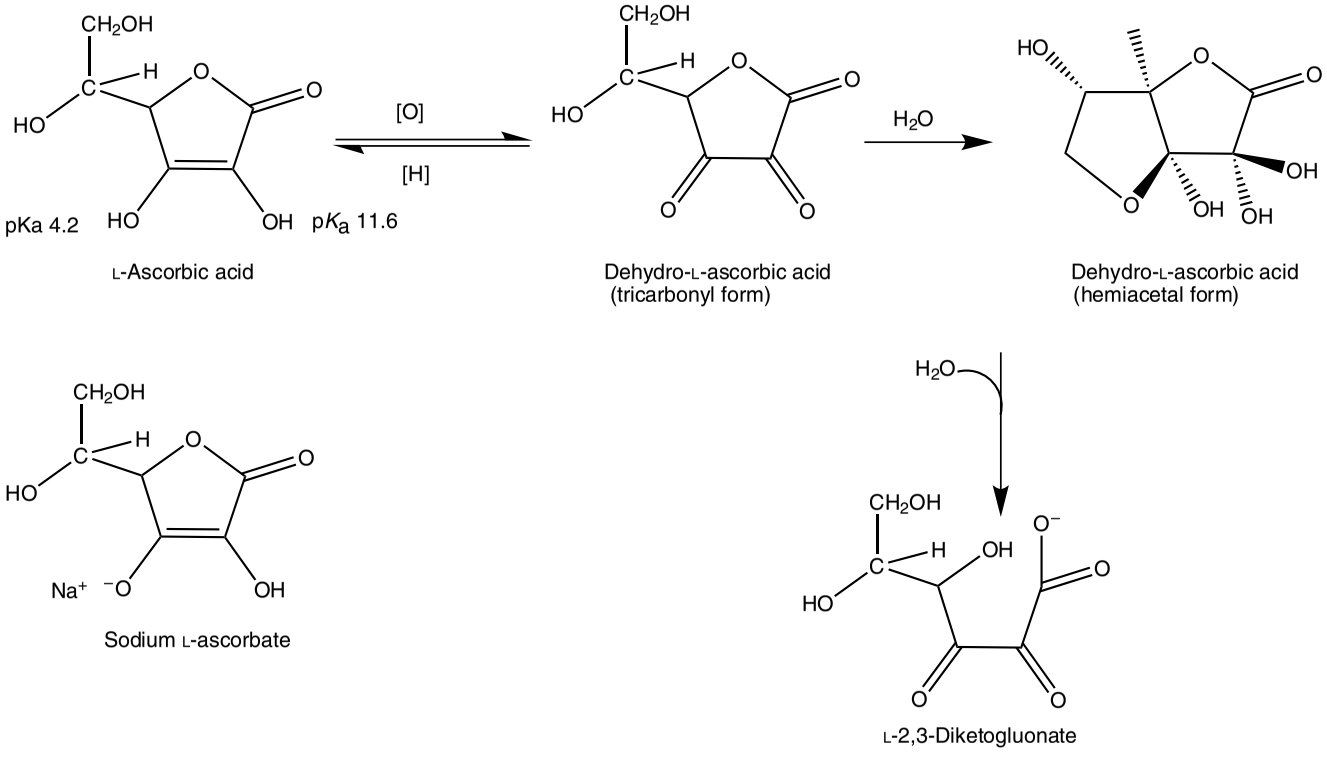


Figure 51. Ascorbic acid (vitamin C) chemistry.

Most animals, except for primates and guinea pigs, produce their own ascorbic acid from glucose (Fig. 52). The pathway follows the standard route to glucuronic acid. The aldose carbon is reduced to an alcohol and, following normal carbohydrate naming convention, the former carbon 6 of D-glucuronic acid becomes carbon 1 of L-gulonic acid. Cyclic L-gulonolactone forms and is oxidized to L-ascorbic acid. Humans and primates lack gulonolactone oxidase.

Because humans do not produce ascorbic acid, we have developed an efficient method of recycling it. The glucose transporter on human erythrocytes is altered by another protein, stomatin, such that dehydroascorbate, instead of glucose, is transported into the erythrocyte where it is reduced to ascorbic acid that can return to cells where needed [91].

3.13.2. Uptake Ascorbic acid is absorbed from the intestine by a sodium-dependent active transport system that is saturable. As the concentration of vitamin C increases in the intestinal tract, the absorption changes to passive diffusion. Once in systemic circulation, there are specific transporters based on cell types.

3.13.3. Metabolic Roles Ascorbic acid is an electron donor required for a variety of oxidative processes. It is readily regenerated by glutathione, NAD, and NADP and, therefore, has a long biological half-life. Currently, there are eight known human enzymes that require ascorbic acid. They are listed in Table 6. The precise metabolic roles have not been completely elucidated, but it appears that in the metalloenzymes, ascorbate reduces the active metal site. In addition to these specific enzymes, ascorbic acid seems to function as a free radical scavenger in the aqueous phase of plasma and cells.

3.13.4. Ascorbic Acid Deficiency Scurvy is the classical disease associated with ascorbate deficiency. It is a disease of the connective tissue and probably is caused by inadequate cross-linking because there is a lack of hydroxylated proline and lysine. Many consider scurvy to be an advanced stage of ascorbate deficiency. Chronic deficiencies may also (1) increase risk for malignancies as evidenced by oxidized DNA markers and increased concentrations of reactive oxygen species, (2) decrease immune function as evidenced by less vitamin in neutrophils and lymphocytes, (3) cardiovascular disease caused by the inflammatory response on the blood vessel walls, and (4) cataract formation caused by decreased concentrations of ascorbate in the ocular tissues. Nevertheless, see the discussion in the vitamin E section questioning the effectiveness of large doses of antioxidants.

3.13.5. Hypervitaminosis C The vitamin is considered very safe. At one time, many of the over-the-counter products contained significant amounts of sodium ascorbate, which would be contraindicated in people on low sodium diets. Today's products are virtually

Table 6. Metabolic Roles of Ascorbic Acid (Vitamin C)

Enzyme	Reaction	Contribution
Dopamine-β-hydroxylase	Hydroxylate dopamine phenethyl chain	Synthesis of norephrine
Peptidyl-glycine Monooxygenase	Amidate carboxyl end of peptide hormones	Biosynthesis of peptide hormones
4-Hydroxyphenylpyruvatedioxygenase	Hydroxylate phenylalanine	Synthesis of tyrosine
Proline hydroxylase	Post-translational Hydroxylation of proline	Cross linking of collagen
Lysine hydroxylase	Post-translational hydroxylation of lysine	Cross linking of collagen
Trimethyl Lysine Hydroxylase	Hydroxylation of trimethyl lysine	Carnitine synthesis
4-Butyrobetaine Hydroxylase	Oxidation of 4-butyrobetaine aldehyde	Carnitine synthesis
Cytochrome P450 Isozymes	Oxidation of steroids	Corticosteroid biosynthsis

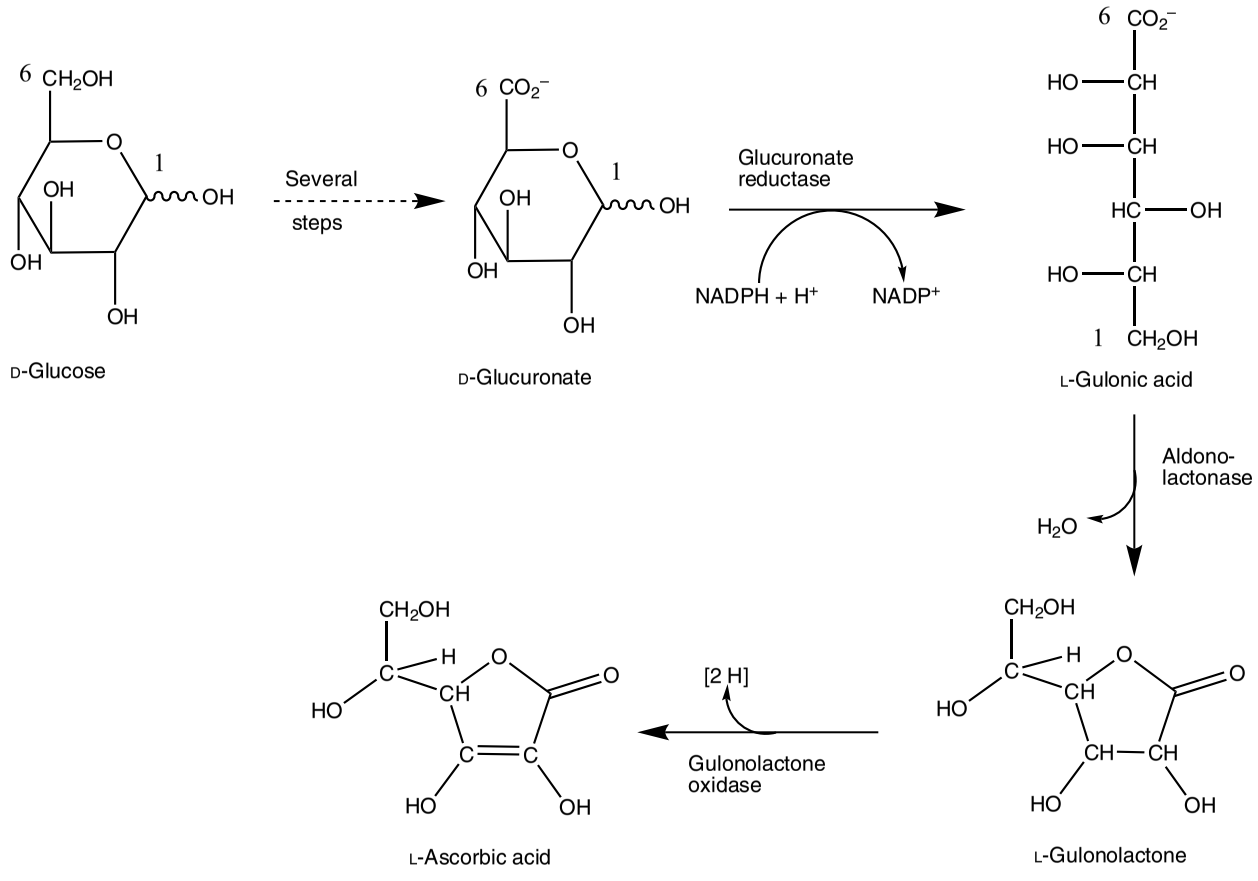


Figure 52. Outline of ascorbic acid biosynthesis.

sodium free unless labeled otherwise. Nevertheless, there are intermittent reports of adverse reactions associated with high doses. Therefore, there are tolerable upper intake levels, but these are very high relative to the RDAs. The UL to RDA ratio averages about 20.

3.13.6. Dietary Reference Intakes

AI	
Infants	40–50 mg/day
EAR	
Children (1–8 years)	13–22 mg/day
Boys (9–18 years)	39–63 mg/day
Girls (9–18 years)	39–56 mg/day
Men (19–70 + years)	75 mg/day
Women (19–70 +)	60 mg/day
Pregnancy	66–70 mg/day
Lactation	96–100 mg/day
RDA	
Children (1–8 years)	15–25 mg/day
Boys (9–18 years)	45–75 mg/day
Girls (9–18 years)	45–65 mg/day
Men (19–70 + years)	90 mg/day
Women (19–70 +)	75 mg/day
Pregnancy	80–85 mg/day
Lactation	115–120 mg/day
UL	
Infants	Not established; use formula and food only
Children (1–8 years)	400–650 mg/day
Boys and girls (9–13 years)	1200 mg/day
Adolescents (14–18 years)	1800 mg/day
Adults (19 + years)	2000 mg
Pregnancy	1800–2000 mg/day
Lactation	1800–2000 mg/day

BIBLIOGRAPHY

Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Food and Nutrition Board, Institute of Medicine, National Academy Press, 1997.

Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. Food and Nutrition Board, Institute of Medicine, National Academy Press, 1998.

Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. Food and Nutrition Board, Institute of Medicine, National Academy Press, 2000.

Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Food and Nutrition Board, Institute of Medicine, National Academy Press, 2001.

Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements. Institute of Medicine, National Academy Press, 2006.

REFERENCES

1. Horwit MK, Harper AE, Henderson LM. Niacin-tryptophan relationships for evaluating niacin equivalents. Am J Clin Nutr 1981;34 (3):423–427.
2. Jacob, R, Swenseid, M. Niacin. In: Ziegler EE, Filer LJ, editors. Present Knowledge in Nutrition. 7th ed. Washington DC: ILSI Press; 1996; p. 185–190.
3. Fu CS, Swendseid ME, Jacob RA, McKee RW. Biochemical markers for assessment of niacin status in young men: levels of erythrocyte niacin coenzymes and plasma tryptophan. J Nutr 1989;119(12):1949–1955.
4. The Role of Protein and Amino Acids in Sustaining and Enhancing Performance, Institute of Medicine, National Academy of Sciences, p. 171, 1999.
5. 'Daily Values' Encourage Healthy Diet. http://www.fda.gov/FDAC/special/foodlabel/dvs.html.
6. >National Institutes of Health State-of-the-Science Conference Statement: Multivitamin/mineral supplements and chronic disease prevention. Ann Intern Med 2006;145 (5):364–371.
7. Huang H-Y, Caballero B, Chang S, Alberg AJ, Semba RD, Schneyer CR, Wilson RF, Cheng M-Y, Vassy J, Prokopowicz G, Barnes GJ, Bass EB. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. Ann Intern Med 2006;145 (5):372–385.
8. State-of-the-Science Conference: Multivitamin/mineral supplements and chronic disease prevention. Am J Clin Nutr 2007;85 (Suppl).
9. Cook NR, Albert CM, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, Buring JE, Manson JE. A randomized factorial trial of vitamins C and E and beta carotene in the secondary pre-

vention of cardiovascular events in women. Arch Intern Med 2007;167(15):1610–1618.

10. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. JAMA 2007;297(8): 842–857.
11. Brzozowska A, Kaluza J, Knoops KTB, de Groot LCPGM. Supplement use and mortality: the SENECA study. Eur J Nutr 2008;47 (3):131–137.
12. Albanes D, Henonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barett MJ, Pietinen P, Malila N, Tala E, Liippo K, Sakinaam E-R, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. α-Tocopherol and β-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J Natl Cancer Inst 1996;88 (21):1560–1570.
13. Beta Carotene Cancer Prevention Study Group the Alpha-Tocopherol. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Eng J Med 1994;330(15):1029–1035.
14. West CE. Meeting requirements for vitamin A. Nutr Rev 2000;58(11):341–345.
15. Blomhoff R, Green MH. Transport and storage of vitamin A. Science 1990;250(4979):399–404.
16. Graham TE, Yang Q, Bluher M. Retinol-binding protein 4 and insulin resistance in lean and obese subjects and subjects with type 2 diabetes. N Engl J Med 2006;3554(24):2552–2563.
17. Polonsky KS. Retinol-binding protein 4, insulin resistance, and type 2 diabetes. N Engl J Med 2006;3554(24):2596–2598.
18. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. J Am Med Assoc 2002;287 (1):47–54.
19. Roberts L. Questions raised about anti-wrinkle cream. Science 1988;239(4840):564.
20. Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Curry CJ, Fernhoff PM, Grix AWJR, Lott IT, Richard JM, Sun SC. Retinoic acid embryopathy. N Engl J Med 1985;313 (14):837–841.
21. Liby KT, Yore MM, Sportn MB. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat Rev Cancer 2007;7357–369.
22. Tallman MS, Andersen JW, Schiffer CA, Applebaum FR, Feuser JH, Ogden A, Shepherd L, Willman C, Bloomfield CD, Rowe JM, Wiernik PH. All-*trans*-retinoic acid in acute promyelocytic leukemia. New Engl J Med 1997;337 (22):1021–1028.
23. Loomis WF. Rickets. Sci Am 1970;223(6):76–82.
24. Weick MT. A history of rickets in the United States. Am J Clin Nutr 1967;20(11):1234–1241.
25. Armas LAG, Hollis BW, Heaney RP. Vitamin D_2 is much less effective than vitamin D_3 in humans. J Clin Endocrinol Metab 2004;89 (11):5387–5391.
26. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D_2) as a vitamin supplement. Am J Clin Nutr 2006;84:694–697.
27. MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D_3 and its photoisomers in human skin. Science 1982;2161001–1003.
28. Koshy KT. Vitamin D: an update. J Pharm Sci 1982;71(2):137–153.
29. Minghetti PP, Norman AW. 1,25$(OH)_2$-vitamin D_3 receptors: gene regulation and genetic circuitry. FASEB J 1988;2(15):3043–3053.
30. Yadav VK, Ryu J-J, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, Karsenty G. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 2008;135(5):825–837.
31. Fitzpatrick S, Sheard N. Vitamin D-deficient rickets: a multifactorial disease. Nutr Rev 2000;58(7):218–222.
32. Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Isichei CO, Reading JC, Chan GM. A comparison of calcium, vitamin D, or both for nutritional rickets in Nigerian children. New Engl J Med 1999;341(8):563–568.
33. Stephens WP, Berry JL, Klimiuk PS, Mawer EB. Annual high-dose vitamin D prophylaxis in Asian immigrants. Lancet 1981;320(8309): 1199–1201.
34. Dwyer JT, Dietz WH, Jr, Hass G, Suskind R. Risk of nutritional rickets among vegetarian children. Am J Dis Child 1979;133(2):134–140.
35. Finberg L. Human choice, vegetable deficiencies, and vegetarian rickets. Am J Dis Child 1979;133(2):129.
36. Cauley JA, LaCroix AZ, Wu LL, Horwitz M, Danielson ME, Bauer DC, Lee JS, Jackson RD, Robbins JA, Wu C, Stanczyk FZ, LeBoff MS, Wactawski-Wende J, Sarto G, Ockene J, Cummings S. Ann Intern Med 2008;149(4):242–250.

37. Guyton KZ, Kensler TW, Posner GH. Cancer chemoprevention using natural vitamin D and synthetic analogs. Annu Rev Pharmacol Toxicol 2001;41:421–442.

38. Ahn J, Peters U, Albanes D, Purdue MP, Abnet CC, Chatterjee N, Horst RL, Hollis BW, Huang W-Y, Shikany JM, Hayes RB. Serum vitamin D concentration and prostate cancer risk: A nested case-control study. J Natl Cancer Inst 2008;100(11):796–804.

39. International Agency for Research on Cancer. Vitamin D and cancer. IARC Working Groups Report. 5. WHO Press, 2008. (ISBN 978 92 832 2446 4), http://www.iarc.fr/en/Media-Centre/IARC-News/Vitamin-D-and-Cancer.

40. Beer TM, Munar M, Henner WD. A phase I trial of pulse calcitriol in patients with refractory malignancies: pulse dosing permits substantial dose escalation. Cancer 2001;91(12): 2431–2439.

41. Mehta RG, Moriaty RM, Mehta RR, Penmasta R, Lazzaro G, Constantinou A, Guo L. Prevention of preneoplastic mammary lesion development by a novel vitamin D analogue, 1 alpha-vitamin D_5. J Natl Cancer Inst 1997;89 (3):212–218.

42. Schwartz GG, Hill CC, Oeler TA, Becich MJ, Bahnson RR. 1,25-Dihydroxy-16-ene-23-yne-vitamin D_3 and prostate cancer cell proliferation *in vivo*. Urology 1995;46(3):365–369.

43. Koike M, Koshizuka K, Kawabata H, Yang R, Taub HE, Said J, Uskokovic M, Tsuruoka N, Koeffler HP. 20-Cyclopropyl-cholecalciferol vitamin D_3 analogs: a unique class of potent inhibitors of proliferation of human prostate, breast and myeloid leukemia cell lines. Anticancer Res 1999;19(3A):1689–1698.

44. Posner GH, Halford BA, Peleg S, Dolan P, Kensler TW. Conceptually new low-calcemic oxime analogs of the hormone 1 alpha,25-dihydroxyvitamin D(3): synthesis and biological testing. J Med Chem 2002;45(8):1723–1730.

45. Evans HM, Bishop KS. On the existence of hitherto unrecognized dietary factor essential for reproduction. Science 1922;56(1433): 649–651.

46. The normal situation seen with vitamins that have two or more stereoisomers is only one isomer is active. Thus a racemic (usually represented as *d,l*) mixture contains 50% of the activity compared with an equi-molar amount of the active isomer. Examples where only one isomer is active includes *d*-pantothenic acid, D-biotin and D-ascorbic acid.

47. Wolf, G. How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. Nutr Rev 2006;64 (6):295–299.

48. Kamal-Eldin A, Appelqvist LA. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 1996;31(7):671–701.

49. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS. Vitamins C and E and the risks of preeclampsia and perinatal complications. N Engl J Med 2006;354(17):1796–1806.

50. Gaziano JM, Glynn RJ, Christen WG, Kurth T, Belanger C, MacFadyen J, Bubes V, Manson JE, Sesso HD, Buring JE. Vitamins E and C in the prevention of prostate and total cancer in men. The Physicians' Health Study II Randomized Controlled Trial. J Am Med Assoc 2009; published online December 9, 2008. DOI:101001/jama2008862.

51. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD, III, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook E, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL, Jr, Baker LH, Coltman CA, Jr. Effect of selenium and vitamin E on risk of prostate cancer and other cancers. The Selenium and Vitamin E Cancer Prevention Trial (SELECT). J Am Med Assoc 2009; published online December 9, 2008. DOI:101001/jama2008864.

52. Miller ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 2005;142(1):37–46.

53. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst 2008;100(11):773–783.

54. Kaneki M, Hosoi T, Ouchi Y, Orimo H. Pleiotropic actions of vitamin K: protector of bone health and beyond? Nutrition 2006;22:845–852.

55. Iwamoto J, Takeda T, Sato Y. Menatetrenone (vitamin K_2) and bone quality in the treatment of postmenopausal osteoporosis. Nutr Rev 2006;64(12):509–517.

56. Kruger MC, Booth CL, Coad J, Schollum LM, Kuhn-Sherlock B, Shearer MJ. Effect of calcium fortified milk supplementation with or without vitamin K on biochemical markers of bone turn-

over in premenopausal women. Nutrition 2006;221120–1128.

57. Volpe SL, Leung MM, Giordano H. Vitamin K supplementation does not significantly impact bone mineral density and biochemical markers of bone in pre- and perimenopausal women. Nutr Res 2008;28577–582.
58. Booth SL, Broe KE, Gagnon DR, Tucker KL, Hannan MT, McLean RR, Dawson-Hughes B, Wilson PWF, Cupples LA, Kiel DP. Vitamin K intake and bone mineral density in women and men. Am J Clin Nutr 2003;77:512–516.
59. Stone TW. Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 1993;45 (3):309–379.
60. Stone TW. Quinolinic Acid and the Kynurenines. Boca Raton, FL: CRC Press; 1989.
61. The Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering in folic acid and B vitamins in vascular disease. N Engl J Med 2006;354(15):1567–1577.
62. Bønaa KH, Njølstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;354 (15):1578–1588.
63. Bhagavan H. Interaction between vitamin B-6 and drugs. In: Reynolds RD, Leklem JE, editors. Vitamin B_6: Its Role in Health and Disease. New York: Alan R. Liss; 1985. p 410–415.
64. Byrd RB, Horn BR, Solomon DA, Griggs GA. Toxic effects of isoniazid in tuberculosis chemoprophylaxis. Role of biochemical monitoring in 1,000 patients. J Am Med Assoc 1979;241 (12):1239–1241.
65. Anonymous. Vitamin B_6: A new megavitamin syndrome. Nutr Rev 1984;42(2):44–46.
66. Anonymous. Sensory neuropathy from megadoses of pyridoxine. Nutr Rev 1984;42(2):49–51.
67. Schaumburg H, Kaplan J, Windebank A, Vick N, Rasmus S, Pleasure D, Brown MJ. Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. N Engl J Med 1983;309(9):445–448.
68. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. Food and Nutrition Board, Institute of Medicine, National Academy Press, 1998; pp. 357- 373.
69. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. Food and Nutrition Board, Institute of Medicine, National Academy Press, 1998; pp. 374- 389.
70. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. Food and Nutrition Board, Institute of Medicine, National Academy Press, 1998; pp 196- 305.
71. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. J Am Med Assoc 2001;285 (23):2981–2986.
72. Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, Gluckman RA, Block PC, Upson BM. Reduction of plasma homocyst(e) ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. New Engl J Med 1998;338(15):1009–1015.
73. Bar-Oz B, Koren G, Nguyen P, Kapur BM. Folate fortification and supplementation—are we there yet? Reprod Toxicol 2008;25 (4):408–412.
74. McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine lowering and cognitive performance. N Engl J Med 2006;354(26):2764–2772.
75. Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. Annu Rev Med 1982;33:345–354.
76. Halsted CH. Folate deficiency in alcoholism. Am J Clin Nutr 1980;33(12):2736–2740.
77. Kim Y-I. Does a high folate intake increase the risk of breast cancer? Nutr Rev 2006;64 (10):468–475.
78. Kim Y-I. Folic acid fortification and supplementation—good for some but not so good for others. Nutr Rev 2007;65(11):504–511.
79. Ulrich CM. Folate and cancer prevention: a closer look at a complex picture. Am J Clin Nutr 2007;86:271–273.
80. Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G, Rosenberg IH. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev 2007;16(7):1325–1329.
81. Solomons NW. Food fortification with folic acid: has the other shoe dropped? Nutr Rev 2007;65 (11):512–515.
82. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. Food and

Nutrition Board, Institute of Medicine, National Academy Press, 1998; pp 306- 356.

83. Taga ME, Larsen NA, Howard-Jones AR, Walsh CT, Walker GC. BluB cannibalizes flavin to form the lower ligand of vitamin B_{12}. Nature 2007;446(7134):449–453.
84. Kapadia CR, Donaldson RM. Disorders of cobalamin (vitamin B_{12}) absorption and transport. Annu Rev Med 1985;36:93–110.
85. Smith DM, Golding BT, Radom L. Understanding the mechanism of B_{12}-dependent methymalonyl-CoA mutase: partial proton transfer in action. J Am Chem Soc 1999;121(40): 9388–9399.
86. Grate JH, Grate JW, Gerhard GN. Studies on vitamin B_{12} and related compounds. 53. Synthesis and reactions of organocobalamins relevant to the mechanism of the methylmalonyl-CoA-succinyl-CoA mutase enzyme. J Am Chem Soc 1982;104:1588–1594.
87. Halpern J. Mechanisms of coenzyme B_{12}. Science 1985;227:869–875.
88. Duerksen DR, Fallow G, Bernstein CN. Vitamin B_{12} malabsorption in patients with limited ileal resection. Nutrition 2006;22:1210–1213.
89. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. Food and Nutrition Board, Institute of Medicine, National Academy Press, 2000; pp 95- 185.
90. Kerber RC. "As Simple as Possible, but not Simpler"—the case of dehydroascorbic acid. J Chem Educ 2008;85(9):1237–1242.
91. Montel-Hagen A, Kinet S, Manel N, Mongellaz C, Prohaska R, Battini J-L, Delaunay J, Sitbon M, Taylor N. Erythrocyte Glut1 triggers dehydroascorbic acid uptake in mammals unable to synthesize vitamin C. Cell 2008; 132.139–1048.