The **B-complex vitamins** are central to the metabolism of all mammalian cells since they act as coenzymes in specific reactions in glycolysis, the hexose monophosphate shunt (HMS), the tricarboxylic acid (TCA) cycle, and in lipid metabolism. They are generally associated with their respective enzymes through covalent bond formation near the active site.

### Thiamin (Vitamin B₁)

Thiamin is produced by some microbes, it is present in most plant and animal tissues, and it is involved in several reactions involving thiamin diphosphate (pyrophosphate) as coenzyme. An ATP-dependent thiamin diphosphotransferase present in brain and liver tissue is responsible for conversion of this vitamin to its active form. Although some thiamin triphosphate is also formed, and may play a separate role in brain cell viability, approximately 80% of thiamin in the body is found in the diphosphate form. This vitamin is not stored in the body (to any great extent), except in the sense of being attached to some enzymes. It is heat labile, and therefore easily destroyed. Thiamin is absorbed by both passive and active processes from the digestive tract, and is excreted from the body as multiple metabolites in urine.

There are two general types of reactions in mammals that utilize the activated coenzyme form of this vitamin: 1) **oxidative decarboxylation**, and 2) **transketolase reactions** (Fig. 40-1). Examples of common oxidative decarboxylation reactions are conversion of pyruvate to acetyl-CoA in glycolysis (see Chapter 27), conversion of α-ketoglutarate (α-KG) to succinyl-CoA in the TCA cycle (see Chapter 34), and decarboxylation of α-ketocarboxylic acid derivatives of the branched-chain amino acids (BCAAs; leucine, isoleucine, and valine;...
Figure 40-1

Oxidative decarboxylation and transketolase reactions utilize the activated form of thiamin as coenzyme. A deficiency of this vitamin, which can be judged by measuring erythrocytic transketolase activity, results in the accumulation of substrates for these reactions (i.e., pyruvate, α-KG, α-keto-carboxylate derivatives of the BCAAs, and pentose sugars of the HMS).
see Chapter 8), particularly in brain and muscle tissue.

Transketolase is an important enzyme in the HMS (see Chapter 28), and also in the “dark reactions” of plant photosynthesis, where CO$_2$ is converted to carbohydrate. Since erythrocytes depend heavily on HMS activity (and therefore thiamin availability; see Chapters 30 and 31), a deficiency of this vitamin can be judged by measuring erythrocytic transketolase activity. Given the above discussion, thiamin-deficient animals would be expected to exhibit accumulation of substrates involved in the above reactions (e.g., pentose sugars, pyruvate, $\alpha$-KG, and the $\alpha$-ketocarboxylate derivatives of the BCAAs).

Several foods have been found to exhibit thiaminase (or thiamin antagonist) activity (e.g., raw fish (e.g., tuna, salmon, and shellfish), rice bran, tannins (coffee and tea), bracken fern ($Pteridium aquilinum$), and horsetail ($Equisetum arvense$)). If cats, for example, are fed excessive amounts of raw fish in home-prepared diets, they may develop signs of thiamin deficiency. Additionally, some symbiotic microbes found in the digestive tract also produce thiaminase. Thiamin deficiency is associated with “beriberi” in primates, “polioencephalomalacia” in ruminant animals and horses, and “Chestak’s paralysis” in the fox, mink, and cat. Generalized symptoms of deficiency include peripheral neuropathy (most marked in the extremities), weakness, tenderness and atrophy of muscles, fatigue, and decreased attention span. Affects on the CNS are largely a result of its importance to aerobic metabolism, and when thiamin is deficient there is conversion to anaerobic glycolysis, local production of lactic acid, and neuronal dysfunction. Affected patients may exhibit vestibular “seizures,” fixed and dilated pupils, loss of physiologic nystagmus, plus stupor or coma. The condition may become fatal if left untreated. However, the response to therapy is reported as being dramatic, and the treatment innocuous, so that clinicians have been encouraged to treat whenever the diagnosis is suspected. Routine laboratory tests may be normal, although serum can be tested for vitamin B$_1$ metabolites, or erythrocytes can be tested for transketolase activity. Within the human population, thiamin deficiency is most often associated with alcoholism.

Riboflavin (Vitamin B$_2$)

Riboflavin was first isolated from milk in 1933, and functions as part of two coenzymes, flavin adenine dinucleotide (FAD), and riboflavin 5’-monophosphate (flavin mononucleotide, FMN; Fig. 40-2). Riboflavin 5’-monophosphate is formed by ATP-dependent phosphorylation of riboflavin, whereas FAD is synthesized by a further reaction with ATP in which the AMP moiety of ATP is transferred to FMN. Flavin
adenine dinucleotide and FMN are usually tightly bound to their respective apoenzyme protein, and contain one or more trace elements as essential cofactors (usually iron or molybdenum; see Chapter 48). Metalloflavoproteins are capable of reversible reduction, thus yielding FADH₂ and FMNH₂.

Flavins are useful in physiologic systems in that they are stronger oxidizing agents than NAD⁺, thus fitting in further along the electron transport chain (see Chapter 36). They participate in one or two electron processes (and thus reactions with free radicals or metal ions), and in reduced form react directly with O₂ (as in hydroxylation reactions). Riboflavin is thus an enzyme cofactor, or coenzyme, fundamental to many areas of metabolism, and is intimately involved in processes by which the oxidation of glucose and fatty acids are utilized for adenosine triphosphate (ATP) formation, and thus the support of metabolic processes. Riboflavin coenzyme formation (and thus trapping within cells), is initiated through phosphorylation by a flavokinase, which is positively regulated by the most active form of thyroid hormone (i.e., triiodothyronine, T₃; see Chapter 52).

The metalloflavoproteins are widespread in mammalian organisms, and participate in various oxidation/reduction reactions, exemplified as follows:

• **Ferredoxin reductase** (participates in renal vitamin D activation (see Chapter 45).

• **Succinate dehydrogenase** (which links the mitochondrial TCA cycle to oxidative phosphorylation; see Chapters 34-36).

• **NADH-CoQ reductase** and **succinyl-CoQ reductase** (complexes I and II of the mitochondrial ETC; see Chapter 36).

• **Mitochondrial glycerol 3-phosphate dehydrogenase** in transporting reducing equivalents from the cytosol into mitochondria (i.e., the glycerol 3-phosphate shuttle; see Chapters 35 and 36).

• **Xanthine oxidase** in purine degradation (see Chapter 17).

• **Acyl-CoA dehydrogenase** in mitochondrial fatty acid β-oxidation (see Chapter 55).

• **Glutathione reductase** in erythrocytes for the reduction of oxidized glutathione (see Chapter 30).

• **α-Amino acid oxidase** in hepatic and renal amino acid deamination (see Chapter 9).

All of these enzyme systems are impaired in riboflavin deficiency. Additionally, **glucose oxidase**, a non-mammalian FAD-specific enzyme prepared from fungi, is of interest because it is sometimes used in vitro for determination of blood glucose concentrations.

Riboflavin is a colored, fluorescent pigment that is synthesized by plants and microbes, but not mammals. In addition to vegetables, yeast, liver, and kidney are usually good sources of riboflavin. In contrast to thiamin, it is relatively heat stable, but decomposes in the presence of light. Therefore it is generally stored in dark bottles. Riboflavin absorption occurs primarily in the upper part of the small intestine, and is thought to occur via a carrier-mediated process.

Conversion of riboflavin to its coenzyme derivatives (FAD and FMN) occurs to a great extent in the liver. These compounds are thus stored there, and small amounts that enter bile generally return to the liver via the portal circulation following intestinal reabsorption. Thus, riboflavin, like bile acids, exhibits an enterohepatic circulation (EHC; see Chapter 62). It is ultimately degraded by hepatic microsomal mixed function oxidases, with degradative products being excreted in bile and some also entering blood. Degradative products entering blood are generally filtered by the kidneys and appear in urine largely in the form of 7- and 8-hydroxymethyl derivatives.

Riboflavin deficiency causes several non-specific signs and symptoms in animals, including mucus membrane inflammation,
Thiamin (B₁) and Riboflavin (B₂)

alopecia, dermatitis, anemia, photophobia, corneal vascularization, and cataracts. Unlike thiamin, however, deficiency of this vitamin does not usually lead to life-threatening conditions. **Erythrocytic glutathione reductase** activity has been used to assess riboflavin deficiency.

**OBJECTIVES**

- Recognize the two general types of reactions that utilize the activated coenzyme form of thiamin.
- Explain why erythrocytes are used to assess thiamin deficiency.
- Understand why cats fed excessive amounts of raw fish may develop thiamin deficiency.
- Associate various animal species with either beriberi, polioencephalomalacia or Chestak’s paralysis, and discuss the symptoms and causes of these conditions.
- Show how FMN and FAD can be formed from riboflavin, and explain what the metalloflavoproteins are.
- Show how the flavins (FAD and FMN) participate in oxidative phosphorylation (see Chapter 36).
- Identify and describe eight different biochemical reactions that would be impaired in riboflavin deficiency, and predict the consequences.
- Identify important dietary sources of riboflavin, and explain why it appears in the enterohepatic circulation (EHC).
- Explain why erythrocytic glutathione reductase activity can be used to assess riboflavin deficiency (see Chapter 30).

**QUESTIONS**

1. **Which one of the following glycolytic reactions involves thiamin?**
   a. Phosphoenolpyruvate —> Pyruvate
   b. Glucose —> Glucose 6-phosphate
   c. 3-Phosphoglycerate —> 2 Phosphoglycerate
   d. Fructose 6-phosphate —> Fructose 1,6-bisphosphate
   e. Pyruvate —> Acetyl-CoA

2. **Which of the following, if fed to cats in excessive amounts, could cause thiamin deficiency?**
   a. Raw fish (e.g., tuna or salmon)
   b. Fresh vegetables (e.g., squash, corn, or beans)
   c. Raw meat (e.g., beef)
   d. Milk
   e. Poultry (e.g., chicken)

3. **A thiamin deficiency can be judged by measuring the activity of which one of the following enzymes in mature erythrocytes?**
   a. Glutathione reductase
   b. Pyruvate carboxylase
   c. Transketolase
   d. Pyruvate dehydrogenase
   e. α-Ketoglutarate dehydrogenase

4. **Riboflavin 5’-monophosphate (FMN):**
   a. Is formed by ATP-dependent phosphorylation of riboflavin.
   b. Is formed from FAD by an ATP-dependent phosphorylation.
   c. Formation is initiated extracellularly through the activity of riboflavin dehydrogenase.
   d. Formation is inhibited in most cells by triiodothyronine (T₃).
   e. Activity is inhibited by molybdenum.

5. **Which one of the following is an FAD-containing enzyme?**
   a. Glycogen synthase
   b. Glutathione reductase
   c. Pyruvate carboxylase
   d. Glucose 6-phosphatase
   e. Glycogen phosphorylase

6. **Conversion of riboflavin to its coenzyme derivatives occurs to the greatest extent in which organ?**
   a. Brain
   b. Liver
   c. Kidney
   d. Lung
   e. Heart

7. **Alcoholics have been known to develop beriberi, steatosis and metabolic acidosis (see Chapter 24):**
   a. True
   b. False