Biotin and Pyridoxine (B$_6$)

Overview

- Biotin participates in carboxylation reactions.
- Biotin, pantothenate (B$_5$), and cobalamin (B$_{12}$) are needed by herbivores to move propionate into hepatic gluconeogenesis.
- Biotin-supplemented diets are sometimes fed to young, growing animals.
- Raw egg white reduces intestinal biotin absorption.
- Vitamin B$_6$ (pyridoxine) is used in muscle glycogenolysis, and in erythrocytes it is bound to hemoglobin.
- Pyridoxal phosphate is used in transamination reactions.
- Although rare in animals, a vitamin B$_6$ deficiency can result in increased amounts of amino acid metabolites appearing in urine, and it can reduce conversion of Trp to NAD$^+$.

Biotin

This water-soluble B-complex vitamin is a widely distributed **imidazole derivative** found in plants in the free form, but in animals it is linked to protein. Animal requirements for biotin are generally met through the diet, or through microbial synthesis within the digestive tract.

During digestion, biotin is initially released from protein as a lysine-adduct (**biocytin**), and either further digested to free biotin, or absorbed as such and hydrolyzed within intestinal mucosal cells. The enzyme involved in this process (sometimes referred to as **biotinidase**), may also be the **biotin carrier protein (BCP)** associated with this vitamin both intracellularly and within serum (**Fig. 42-1**).

Intestinal absorption of this vitamin in the jejunum is thought to occur by facilitated diffusion at low concentrations, and by simple diffusion at high concentrations. Although biotin deficiencies are rare, they can be induced through use of broad-spectrum oral antibiotics over a long period of time (which reduces microbial biosynthesis), or through excessive consumption of raw eggs. Raw egg whites contain the active form of **avidin**, a protein that binds tightly with biotin, thus making it unavailable for absorption from the digestive tract. Therefore, raw eggs should not be fed to young animals who need biotin for proper growth and development. Since heat denatures avidin, cooked egg consumption is not associated with biotin deficiency.

The function of biotin is to participate in **carboxylation** reactions. First, a **carbonic phosphoric anhydride** is formed through combination of a high energy phosphate from **ATP**, **CO$_2$** derived from HCO$_3^-$, magnesium (**Mg^{++}**), and potassium (**K$^+$**). Next, **biotin carboxylase** (sometimes referred to as **holoenzyme synthetase**), and manganese (**Mn^{++}**) help to facilitate movement of the activated carboxyl group of carbonic

phosphoric anhydride to biotin, thus forming **carboxybiotin**. This compound now becomes available to various carboxylases (or tran-scarboxylases) for **one carbon transfer**.

There are **four** important biotin-driven "**CO$_2$-fixing**" reactions that occur in animal cells (**Fig. 42-1**), and they are all catalyzed by substrate-specific carboxylases.

1) Conversion of pyruvate to oxaloace-tate (OAA, via **pyruvate carboxylase**; see Chapter 27 and 37).

2) Conversion of propionyl-CoA to methylmal-onyl-CoA (via **propionyl-CoA carboxylase**; see Chapter 37).

3) Formation of acetoacetate from leucine (via **β-methylcrotonyl-CoA carboxylase**; see Chapter 8).
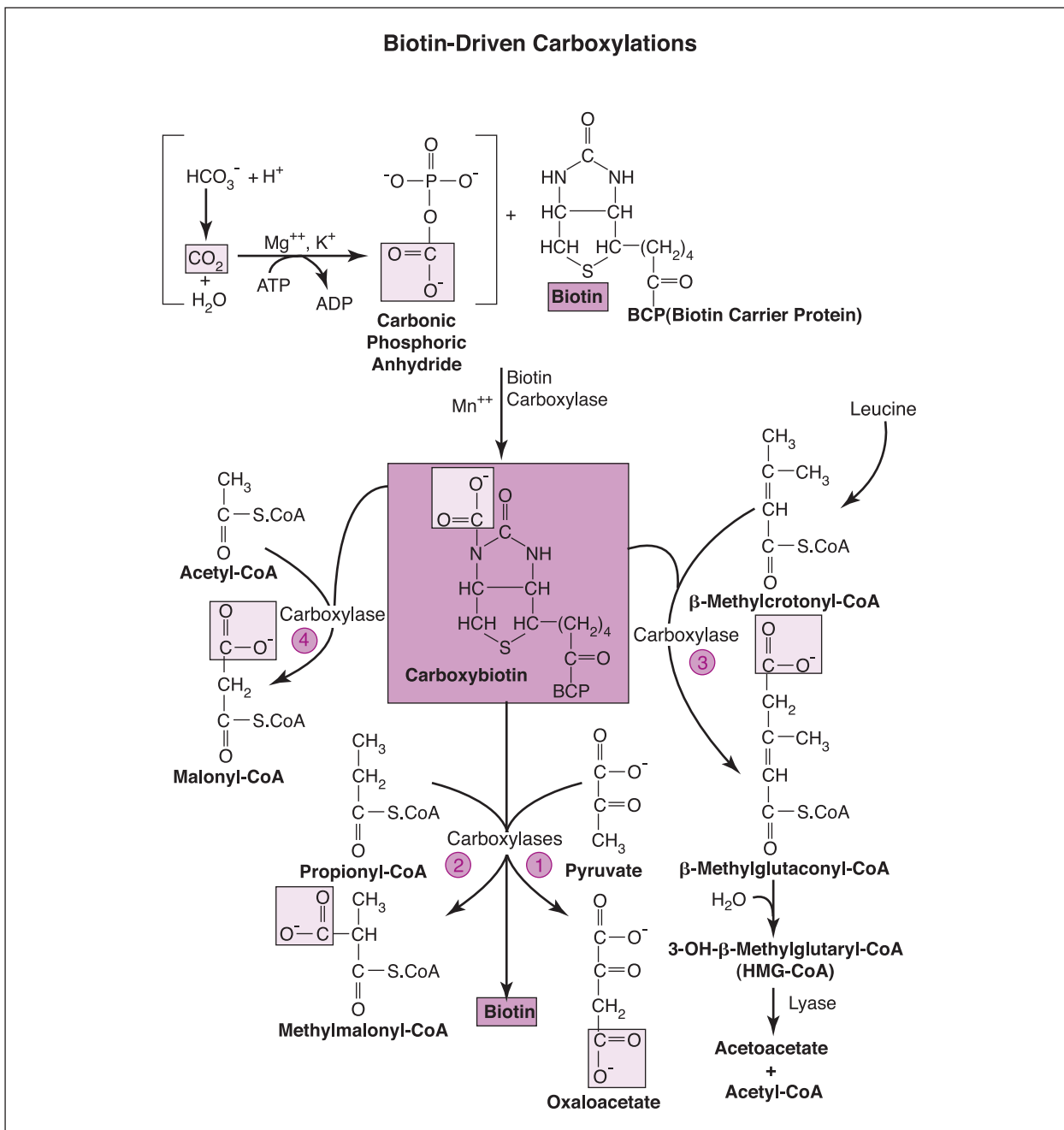
Figure 42-1

4) Formation of malonyl-CoA from acetyl-CoA in fatty acid biosynthesis (via **acetyl-CoA carboxylase**; see Chapter 56).
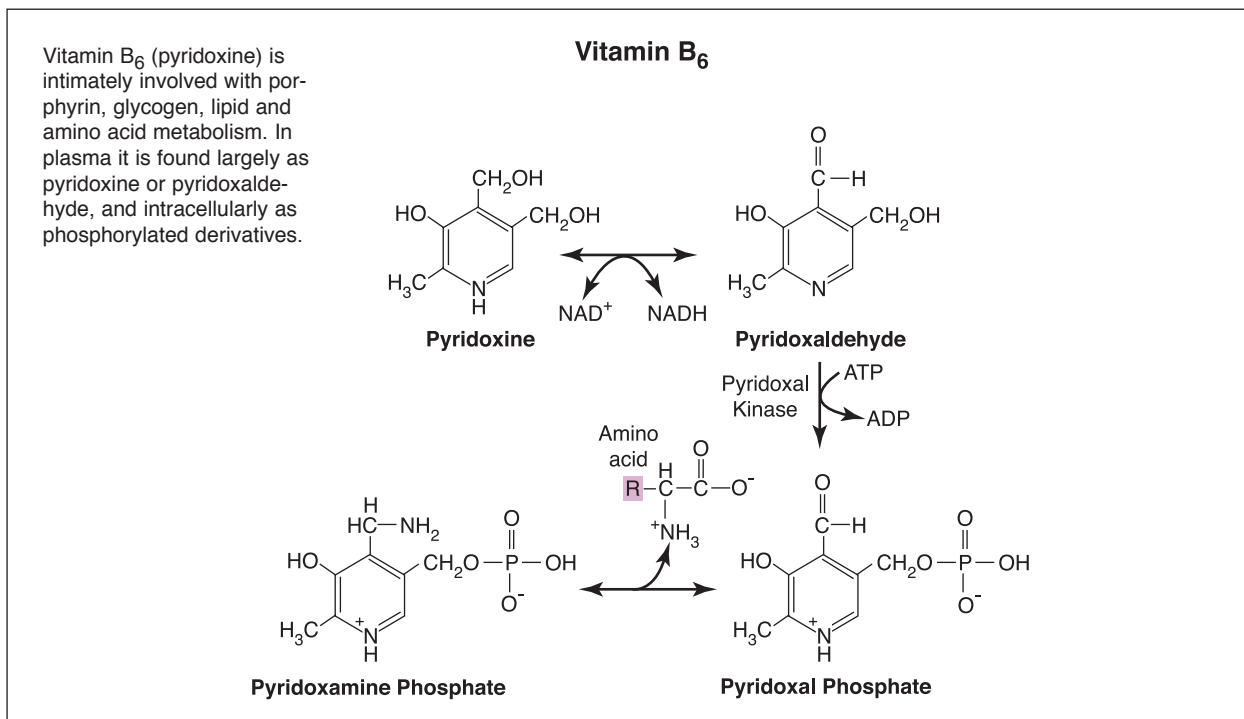
Hepatic **OAA formation** from pyruvate is allosterically enhanced by acetyl-CoA, a product of mitochondrial fatty acid β-oxidation. This enzyme is particularly important to liver and kidney tissue during gluconeogenesis, and to muscle tissue during exercise.

Propionyl-CoA is formed from the short-chain volatile fatty acid, propionate, from several glucogenic amino acids (i.e., threonine, methionine, isoleucine, and valine), from terminal three carbon segments of odd-chain fatty acids undergoing mitochondrial β-oxidation, and from β-aminoisobutyrate during thymine degradation. Propionate, generated from microbial fermentation of cellulose, is a significant source of carbon atoms for hepatic gluconeogenesis in herbivores, and the glucogenic amino acids listed above can be a significant source of carbon atoms for gluconeogenesis in carnivores, and in starved animals. Since most mammals store few odd-chain fatty acids, their terminal three carbon segments become an insignificant source of propionyl-CoA for gluconeogenesis. Since only modest amounts of β-aminoisobutyrate become available to the liver through pyrimidine degradation, this compound is also considered to be an insignificant gluconeogenic substrate. Following carboxylation of propionyl-CoA to methymalonyl-CoA, further conversion to succinyl-CoA requires cobalamin (vitamin B_{12}; see Chapter 43).

Hepatic catabolism of Leu to **ketone bodies** requires carboxybiotin, and forms β-methylglutaconyl-CoA as intermediate. Leucine and Lys are the only amino acids that are strictly ketogenic (see Chapter 8).

Biotin deficiency is not usually caused by simple dietary deficiency, but by defects in utilization. Symptoms can include dermatitis, alopecia, and muscle weakness. Biotin, like other B-complex vitamins, is not considered to be toxic, and supplementation can reverse these symptoms. In young animals, extra demands on biotin, a vitamin needed for proper

Vitamin B₆

Vitamin B₆ (pyridoxine) is intimately involved with porphyrin, glycogen, lipid and amino acid metabolism. In plasma it is found largely as pyridoxine or pyridoxaldehyde, and intracellularly as phosphorylated derivatives.

Figure 42-2

growth and development, are sometimes met by feeding biotin-supplemented diets.

Pyridoxine (B$_6$)

Vitamin B$_6$ is the collective term for **pyridoxine**, the form most prominent in plants, and for the phosphorylated coenzyme derivatives, **pyridoxal** and **pyridoxamine phosphate**, common forms found in animal tissues (**Fig. 42-2**). **Pyradoxine, pyridoxamine and pyridoxaldehyde** are major transport forms available to cells, and account for most of the vitamin in plasma. Once inside cells they become phosphorylated. In erythrocytes, for example, this vitamin becomes concentrated about four- to five-fold, where it is bound mainly to **hemoglobin** (it may enhance O$_2$ binding).

Vitamin B$_6$ coenzymes participate in over 100 different enzyme-catalyzed reactions, most of which occur in all living cells, and some of which are only present in liver and kidney cells (main sites of gluconeogenesis). They are involved with muscle **glycogenolysis**, and with **amino acid metabolism**, where they function in numerous transamination, decarboxylation, dehydratase, and side-chain cleavage reactions. Pyridoxal phosphate is an important coenzyme which aids the action of muscle **glycogen phosphorylase**, the enzyme mediating glycogen breakdown (see Chapter 23). **Muscle phosphorylase may account for as much as 70-80% of total body vitamin B$_6$ in mammals**.

Transamination reactions involving vitamin B$_6$ include those concerned with the synthesis of nonessential amino acids, and also those concerned with the first step in amino acid catabolism (see Chapter 9). Transaminases are rate-limiting for the catabolism of specific amino acids in the liver. **Decarboxylation** reactions involve the synthesis of several important substances, including neuroactive amines (e.g., serotonin, histamine, and γ-amino butyric acid (GABA)), Δ-aminolevulinic acid

(the first step in heme biosynthesis; see Chapter 32), and intermediates in the synthesis of sphingomyelin, phosphatidyl-choline (lecithin), carnitine, and taurine (important as a bile acid conjugate, and also in brain and eye function). **Dehydratase** reactions include conversion of serine and threonine to their α-keto acids through oxidative removal of the amino group as ammonia. **Side chain cleavage** reactions are exemplified by the formation of glycine from serine, and the splitting of cystathionine in methionine degradation. Thus, vitamin B$_6$ is intimately involved with **amino acid metabolism**, and also plays an important role in **porphyrin**, **glycogen**, and **lipid metabolism**. In addition to these actions, vitamin B$_6$ may be involved with growth hormone and insulin release (since deficiency of this vitamin in rats depresses uptake of amino acids by muscle cells). Some studies indicate that it may also be involved in the return (and thus inactivation) of steroid receptor complexes to the cytosol from the nucleus after they have promoted transcription of certain genes (which indicates that it plays a regulatory role in steroid hormone action).

Vitamin B$_6$ is thought to be absorbed from the digestive tract mainly as **pyridoxine**, and excreted in urine as **pyridoxic acid**. **Deficiencies** due to lack of pyridoxine are rare in animals, and when they occur they are usually part of a general B-complex vitamin deficiency. Due to its importance in amino acid catabolism, a deficiency of this vitamin can result in urinary excretion of increased levels of some **amino acid metabolites** which are normally degraded further (specifically metabolites of tryptophan, methionine, and glycine). Urinary **urea** excretion may also be enhanced, because of a decreased capacity to synthesize nonessential amino acids, resulting in decreased reutilization of NH$_3$ and amino nitrogen. As indicated in the previous chapter, a lack of B$_6$ can also **reduce NAD$^+$ formation**

from tryptophan. General deficiency symptoms include hyperirritability and convulsive seizures in infants, and inflammation of the oral cavity and oily dermatitis in adults.

In summary, biotin participates in four important "CO_2-fixing" reactions, and pyridoxine (B_6) is intimately associated with porphyrin, glycogen, lipid and amino acid metabolism. The majority of B_6 is complexed with muscle glycogen phosphorylase. Biotin deficiency is generally associated with defects in biotin utilization, while pyridoxine deficiency is usually associated with a broader B-complex vitamin deficiency. Neither vitamin is considered toxic.

OBJECTIVES

- Outline the protein-binding characteristics and requirements of biotin, and indicate how raw egg white consumption might affect biotin availability.

- Identify the substrate, energy, enzyme and cofactor requirements for carboxybiotin formation.

- Contrast and compare the ways in which folic acid, biotin, methylcobalamin and SAM participate in one carbon transfer (see Chapters 16 & 57).

- Identify and summarize the importance of the four key biotin-driven cellular carboxylation reactions.

- Explain the causes of biotin deficiency.

- Identify common extra- and intracellular forms of vitamin B_6.

- Explain the involvement of pyridoxine in glycogen metabolism.

- Recognize why a pyridoxine deficiency could result in an increased BUN concentration.

- Explain why phosphorylated forms of vitamin B_6 are not normally present in plasma.

- Discuss the involvement of vitamin B_6 in nonessential amino acid biosynthesis (see Chapter 9).

- Recognize how (and where) vitamin B_6 participates in heme biosynthesis.

QUESTIONS

1. **Which one of the following compounds, contained in egg white, binds strongly with biotin in the digestive tract, thus preventing its absorption?**
   a. Carnitine
   b. Avidin
   c. Pyridoxine
   d. Ascorbate
   e. Sodium

2. **Biotin is a coenzyme for certain:**
   a. Transamination reactions.
   b. Dehydratase reactions.
   c. Side-chain cleavage reactions.
   d. Carboxylation reactions.
   e. Decarboxylation reactions.

3. **Which one of the following mitochondrial reactions involves biotin?**
   a. Pyruvate —> Acetyl-CoA
   b. α-Ketoglutarate —> Succinyl-CoA
   c. Succinyl-CoA —> Succinate
   d. Fumarate —> Malate
   e. Propionyl-CoA —> Methylmalonyl-CoA

4. **Biotin is involved in ketone body production from which one of the following amino acids?**
   a. Leucine
   b. Glutamine
   c. Alanine
   d. Aspartate
   e. Proline

5. **Approximately 70-80% of vitamin B_6 in mammalian organisms is associated with which enzyme?**
   a. Hepatic glycogen synthase
   b. Renal glucose 6-phosphatase
   c. Muscle glycogen phosphorylase
   d. Adipolytic triglyceride lipase
   e. Cyclooxygenase (COX-1)

6. **Pyridoxine deficiencies are best associated with increased urinary excretion of:**
   a. Glucose metabolites (e.g., lactate).
   b. Ketone bodies.
   c. Amino acid metabolites.
   d. Cholesterol.
   e. Bilirubin diglucuronide.

6. c
5. c
4. a
3. e
2. d
1. b

ANSWERS