Chapter 45

Vitamin D

Overview

- Vitamin D can be synthesized in the skin, and it helps to facilitate intestinal Ca^{++} absorption.
- Ergosterol, which occurs in plants, is a vitamin D precursor.
- The active form of vitamin D ($1,25(OH)_2D$) is a steroid hormone.
- Renal activation of vitamin D is regulated by several endocrine factors.
- Liver and/or kidney disease can result in a $1,25(OH)_2D$ deficiency.
- Parathyroid hormone deficiency can be treated through vitamin D supplementation.
- Cholecalciferol rodenticide poisoning can cause symptoms of vitamin D toxicity.
- Glucocorticoids and calcitonin can be used to reverse symptoms of vitamin D toxicity.

Canine **rickets** was induced nearly 100 years ago through dietary manipulation, which could be cured with cod liver oil. The factor responsible was not vitamin A, but **vitamin D**.

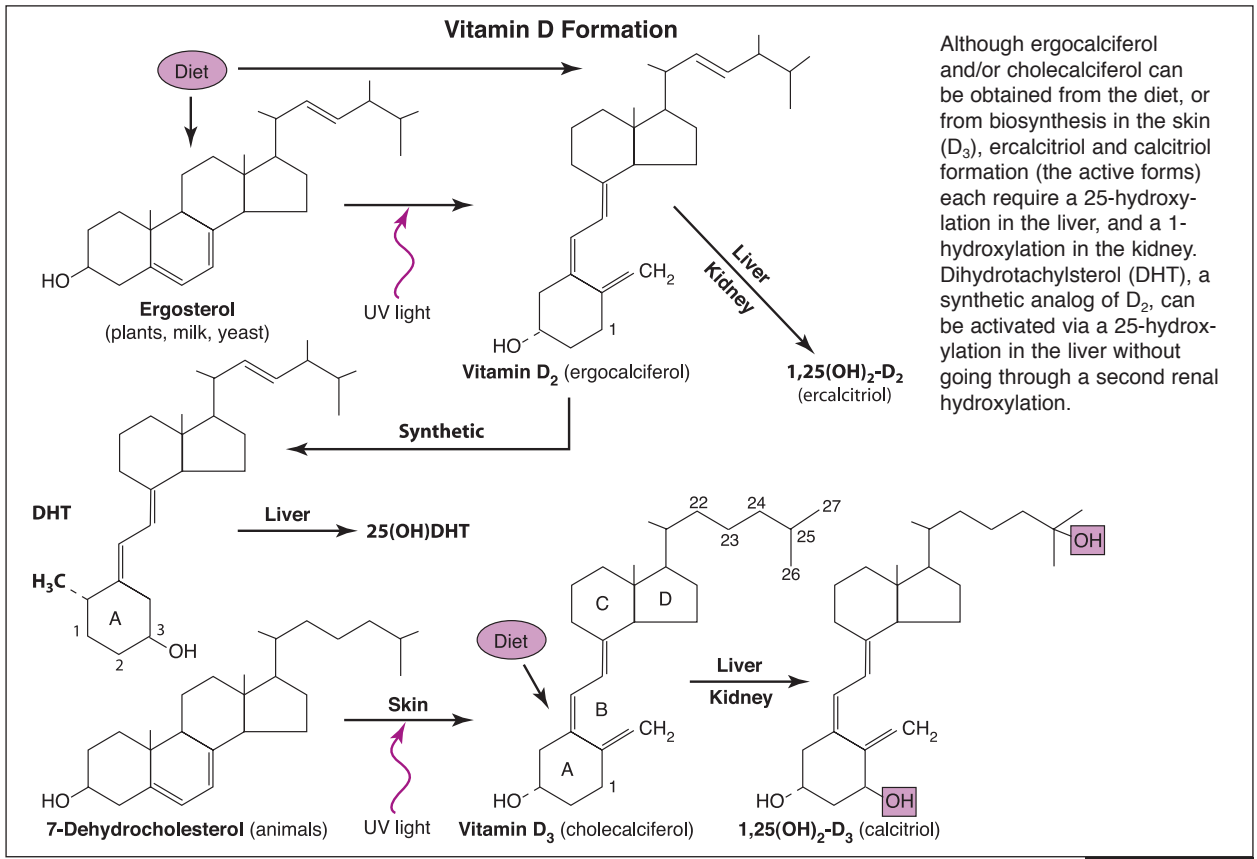
Unlike other fat-soluble vitamins, vitamin D can be synthesized by mammalian tissues (i.e., the malpighian layer of the epidermis in herbivores and omnivores); however, this source of the vitamin is not sufficient to meet body needs in dogs and strict carnivores, therefore they depend upon dietary intake. It is generally not produced by microbes, and its mechanism of action is primarily that of a steroid hormone.

Vitamin D_3 (**cholecalciferol**) is produced in the skin from **7-dehydrocholesterol** by a non-enzymatic process, catalyzed by **ultraviolet** (**UV**) **light** (**Fig. 45-1**). Although sunlight-activated vitamin D_3 formation may be an important source for many animals, this form of the vitamin can also be obtained via the diet. Ultraviolet light is partially filtered out by hair coat, pigmented layers of the skin, and window glass; however, the nose of hairy mammals may be a particularly important location for D_3 formation.

Ergosterol occurs in plants, milk and yeast, and differs from 7-dehydrocholesterol at its side chain, which contains an extra methyl group at C-24. Ultraviolet (UV) radiation cleaves the B ring of both ergosterol and 7-dehydrocholesterol yielding **ergocalciferol** and **cholecalciferol**, respectively. Irradiation of milk and yeast is a commercial means of producing D_2 from ergosterol, and **dihydrotachysterol (DHT)** is a synthetic analog of D_2. Although the antirachitic potencies of D_2 and D_3 (once properly hydroxylated) may differ between animal species, the following discussion will use vitamin D as a collective term for the two vitamers.

Vitamin D is slowly released from skin into plasma, where it is normally bound by **vitamin D binding protein** (**DBP**; a liver-derived α-globulin known as **transcalciferin**), and subsequently cleared from the circulation by the liver (**Fig. 45-2**).

Absorption of vitamin D in the small intestine



Although ergocalciferol and/or cholecalciferol can be obtained from the diet, or from biosynthesis in the skin (D_3), ercalcitriol and calcitriol formation (the active forms) each require a 25-hydroxylation in the liver, and a 1-hydroxylation in the kidney. Dihydrotachylsterol (DHT), a synthetic analog of D_2, can be activated via a 25-hydroxylation in the liver without going through a second renal hydroxylation.

Figure 45-1

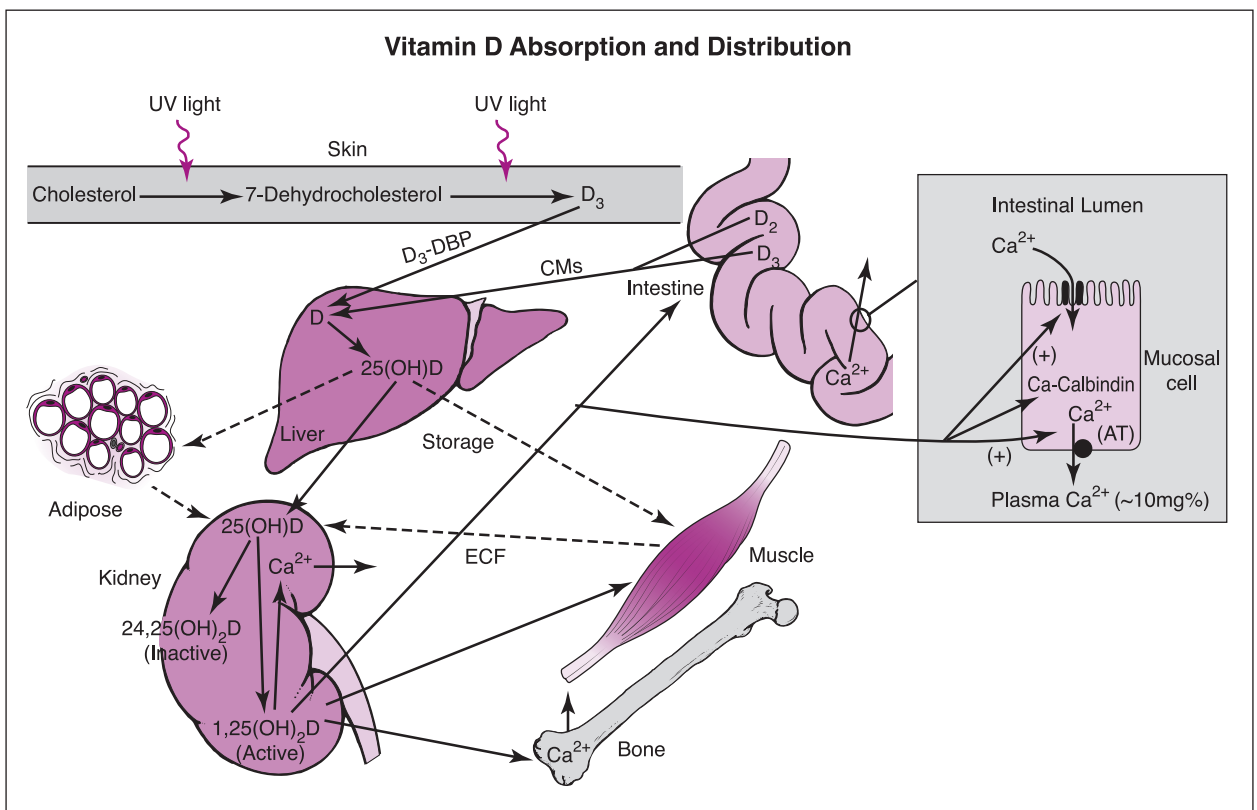


Figure 45-2

requires the presence of **bile acid micelles** (see Chapter 62). Like other fat-soluble vitamins, it is incorporated into **chylomicrons** (**CMs**) in mucosal cells of the small intestine, and then transferred to lymph (see Chapter 64). Chylomicron remnants containing fat-soluble vitamins are removed by the liver (like the D_3-DBP complex from skin), where D is hydroxylated (in a nonregulated fashion) by a **25-hydroxylase**. This reaction also occurs (at low efficiency) in the intestine. Calcifediol and/or ercalcifediol (25(OH)D) are normally the major forms of this vitamin in blood, and the major storage forms in liver, adipose, and skeletal muscle tissue.

In proximal renal tubular epithelial cells of the **kidney**, (and to a lesser extent in the placenta and in bone), **25(OH)D** is **activated** through **hydroxylation** at **position 1 of the A ring** by another mitochondrial enzyme, **1α-hydroxylase** (**Fig. 45-3**). This conversion involves a complex monooxygenase reaction system requiring **NADPH**, **Mg^{++}**, molecular **oxygen** (**O_2**), and at least three additional components: **1**) a **flavoprotein**, renal ferredoxin reductase (see Chapter 40) ; **2**) an **iron-sulfur protein**, renal ferredoxin; and **3**) **cytochrome P_{450}**. The products of this three-component reaction sequence produce the most potent form of vitamin D, **1,25-dihydroxycholecalciferol** (**$1,25(OH)_2D_3$** or **1,25-DHC**; **calcitriol**; and **ercalcitriol** (**$1,25(OH)_2D_2$**); **Table 45-1**).

The activity of **1α-hydroxylase** is positively regulated by several factors, including **low serum ionized Ca^{++}** and **$PO_4^{\equiv}$** concentrations, **parathyroid hormone** (**PTH**; needed to increase the serum ionized Ca^{++} concentration), **growth hormone** (**GH**; Ca^{++} needed for body growth), **placental lactogen** (**PL**; Ca^{++} needed for fetal

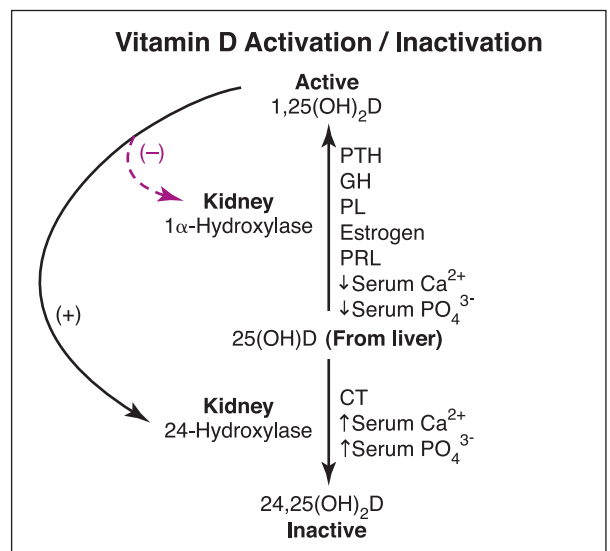


Figure 45-3

Table 45-1

Various Forms of Vitamin D

Active hormone* and precursors	Generic Name
D_1	(Impure preparation of D_2)
D_2 (Plants)	Ergocalciferol (Calciferol)
$25(OH)D_2$	Ercalcifediol
$*1,25(OH)_2D_2$	Ercalcitriol
Reduced D_2 (DHT)	Dihydrotachysterol
*25(OH)DHT	25-Hydroxydihydrotachysterol
D_3	Cholecalciferol
$25(OH)D_3$	Calcifediol
$*1,25(OH)_2D_3$ (1,25-DHC)	Calcitriol (1,25-Dihydroxycholecalciferol)
Inactive: $24,25(OH)_2D$ (major); $25,26(OH)_2D$ (minor); $1,25,26(OH)_3D$ (minor); Others	

growth), **estrogen** (Ca^{++} needed during pregnancy), and **prolactin** (**PRL**; Ca^{++} needed during lactation). In addition to estrogen, progestins and androgens cause marked increases in the renal 1α-hydroxylase activity of **ovulating birds**.

In general, 1,25$(OH)_2D$ serves to enhance serum Ca^{++} levels through facilitating intestinal Ca^{++} absorption, and through promoting the action of PTH on bone and the kidney. With the exception that PTH decreases while 1,25$(OH)_2D$ increases renal $PO_4^{\equiv}$ retention, PTH and 1,25$(OH)_2D$ exert similar actions, and are generally synergistic with each other. Therefore, this lipid-soluble steroid, which can be absorbed from the intestinal tract, is used to treat patients with PTH deficiency. Since PTH is a protein, if given orally it is destroyed in the digestive tract.

Calcitriol is also an important regulator of its own production. High levels of **1,25$(OH)_2D$ inhibit renal 1α-hydroxylase** in a negative-feedback fashion, yet stimulate formation and activity of a renal **24-hydroxylase** (that leads to formation of **24,25-$(OH)_2D$**, an **inactive** by-product of vitamin D). Other factors known to stimulate activity of 24-hydroxylase include **high** circulating levels of serum ionized **Ca^{++}** and **$PO_4^{\equiv}$**, as well as **thyrocalcitonin** (**CT**). Additional hydroxylations of this molecule are known to occur at positions 23 and 26. Although over 20 metabolites have been found, only **1,25$(OH)_2D$** has been shown to exhibit physiologic activity.

The effects of **1,25$(OH)_2D$** on the transfer of **Ca^{++}** (and possibly **$PO_4^{\equiv}$** across the **intestinal mucosa** requires special consideration, for it is this location where vitamin D is thought to exert its major control. **It is the only hormone known to effectively promote translocation of Ca^{++} against its concentration gradient in the intestine.**

Although **30-80% of ingested Ca^{++} can be absorbed from the digestive tract**, active absorption occurs primarily in the upper part of the small intestine by a multi-step mechanism (see **Fig. 45-2**). Calcium is not ionized at neutral pH, thus **gastric acid** (HCl) helps to solubilize calcium salts and free Ca^{++} from bound protein, thus permitting intestinal absorption. **1,25$(OH)_2D$** plays a critical role in duodenal Ca^{++} absorption by opening specific **Ca^{++} channels** in mucosal cell membranes, and stimulating transcription of certain proteins, including **calbindin** and **Ca^{++}-ATPase**. Through these actions 1,25$(OH)_2D$ can increase the efficiency of intestinal Ca^{++} influx from the lumen. This phenomenon, known as **transcaltachia**, occurs over a period of seconds to minutes, whereas the effects on transcription take hours. The gradient for Ca^{++} influx is secondarily maintained by the buffer action of calbindin, which as an intracellular Ca^{++}-binding protein exhibits significant homology with calmodulin, and mysoin light chain. It is important for the function of these cells that the concentration of free ionized Ca^{++} in the cytoplasm be maintained at a low concentration of about 10^{-6}M, thus the buffer action of calbindin fulfills this role. The active transport (AT) of Ca^{++} out of mucosal cells is the result of Ca^{++}-ATPase activity, which is also stimulated by **1,25$(OH)_2D$**.

Actions of **1,25$(OH)_2D$** on bone also deserve mention since both PTH and **1,25$(OH)_2D$** have receptors on (PTH) or in (**1,25$(OH)_2D$**) mature **osteoblasts**, but not on/in **osteoclasts**. These two hormones stimulate osteoblasts to produce **cytokines** that accelerate maturation of osteoclasts in a paracrine fashion. Local release of lysosomal enzymes from osteoclasts and end products of glycolysis then create a local environment that favors **bone dissolution**. This system appears to be synergistic, for alone PTH cannot account for its overall action of increasing the serum ionized Ca^{++} concentration. **1,25$(OH)_2D$** also promotes release of a **Gla protein, osteocalcin**, from osteoblasts (see Chapter 47). This protein binds to hydroxyapatite, preventing further mineralization.

Vitamin D and its metabolites are largely excreted into **bile**, with only about 3% normally appearing in urine. Since these are steroids, many are reabsorbed (and thus conserved) by the intestine.

Vitamin D Toxicity

As with vitamin A, vitamin D excess can result in symptoms of **toxicity**. Hypercalcemia and hyperphosphatemia, deposition of Ca^{++} in soft tissues (especially the kidney, heart, lung, and vasculature), hypercalciuria, and kidney stones have been described. Reptile pets seem particularly susceptible. Vitamin D toxicity can also occur, for example, when **cholecalciferol rodenticides** are consumed by dogs and cats, or when too much vitamin D is administered to animals with hypoparathyroidism. Glucocorticoids and CT are sometimes administered to reverse symptoms of toxicity. **Glucocorticoids** interfere with the mechanism of 1,25-DHC action on the intestine and kidney, and **CT** generally exerts opposite actions to both PTH and 1,25-DHC, including activation of renal 24-hydroxylase.

Vitamin D Deficiency

Vitamin D deficiency symptoms include abnormal bone mineralization and deformities (i.e., **rickets** in young animals, and **osteomalacia** in adults), hypocalcemia, and high circulating titers of PTH. Additionally, since vitamin D receptors are present on hair follicles, vitamin D deficiency can promote hair loss. In addition to **hereditary**, **dietary**, and **behavioral causes** (e.g., lack of UV light), **liver** and/or **kidney disease** may also result in a $1,25(OH)_2D$ deficiency. Also, **lead** (**Pb**) appears to block intestinal $1,25(OH)_2D$-stimulated Ca^{++} absorption, yet vitamin D supplementation enhances Pb uptake. As with vitamin A, normal hepatic stores of vitamin D are generally thought to be capable of supporting animals on vitamin-deficient diets for several months. Therefore, deficiency symptoms usually manifest themselves slowly.

25(OH)DHT, the synthetic analog of D_2, appears to be active in both the intestine and bone of nephrectomized rats. Comparison of the structures of DHT and $1,25(OH)_2D$ (**Fig 45-1**) shows that ring A of DHT is rotated so as to place its 3-hydroxyl group in approximately the same geometrical position as the 1-hydroxyl group of $1,25(OH)_2D$. It seems, therefore, that 25(OH)DHT interacts with receptor sites of $1,25(OH)_2D$ without undergoing 1-hydroxylation, thus bypassing renal mechanisms of metabolic control.

In summary, vitamin D conforms to the definition of both a **hormone** and a **vitamin,** and it plays an important role in control of the Ca^{++} concentration of the extracellular fluid compartment. Whether as a hormone produced in the skin, or as a vitamin provided in the diet, vitamin D has to be chemically modified by two hydroxylations, which occur sequentially in the liver and the kidneys, before it can play an active role in control of Ca^{++} distribution. Discovery of the necessity for hepatic and renal hydroxylations of cholecalciferol led to explanations for a number of clinical problems and their more satisfactory treatments.

Ergocalciferol (D_2) and cholecalciferol (D_3) are seco-steroids in which their B rings have been broken by fission of a carbon-carbon bond. In this case fission caused by UV light on ergosterol of plants producing D_2, and that on 7-dehydrocholesterol in the epidermis of the skin producing D_3.

Once D_2 and/or D_3 reach the liver, the first step in sequential activation occurs, namely hydroxylation by a monooxygenase (hydroxylase) to produce $25(OH)D_2$ and/or $25(OH)D_3$. These compounds, which can be stored in the liver and also in muscle and adipose tissue, are next transported to the kidneys where further hydroxylation takes place in the 1-position of each to produce the active hormones ($1,25(OH)_2D_2$ and/or $1,25(OH)_2D_3$), or in the 24-position of each to produce the inactive hormones ($24,25(OH)_2D_2$ and/or $24,25(OH)_2D_3$). In normal animals the relative plasma concen-

trations of the three hydroxylated forms (**25-hydroxy-, 24,25-dihydroxy-, and 1,25-dihydroxy-**) are about **100:10:1.**

The active form of vitamin D serves to enhance serum Ca^{++} levels through **facilitating intestinal Ca^{++} absorption,** and through **promoting the action of PTH on bone and the kidneys.** Since most of the actions of $1,25(OH)_2D$ and PTH are similar, vitamin D is frequently used to treat patients with PTH deficiency.

OBJECTIVES

- Identify normal sources of vitamin D_2, vitamin D_3 and DHT for animals, and discuss the hepatic metabolism of each.
- Discuss the intestinal absorption and plasma transport of vitamin D (see Chapters 62 & 64).
- Outline and explain the reasoning behind the renal endocrine control of vitamin D hydroxylation.
- Identify and discuss steps involved in vitamin D-stimulated intestinal Ca^{2+} absorption.
- Distinguish between the various active and inactive forms of vitamin D.
- Identify and describe primary physiologic roles for vitamin D in bone and in the kidneys.
- Explain why vitamin D is used to treat patients with PTH deficiency.
- Identify the signs and symptoms of vitamin D toxicity, and explain why CT and glucocorticoids can be used to treat this condition.
- Explain the signs and symptoms of vitamin D deficiency, and also explain why 25(OH)DHT is active in the bone and intestine of nephrectomized rats.

QUESTIONS

1. Ergosterol:
   a. Is a plant steroid.
   b. Differs from 7-dehydrocholesterol only in the orientation of its ring structure.
   c. Is a source of vitamin A in animals.
   d. Is vitamin D_3.
   e. Is the most active form of vitamin D in animals.

2. Calcifediol:
   a. Is usually the major form of vitamin D found in the blood of animals.
   b. Is $1,25(OH)_2D_3$, the most active form of vitamin D.
   c. Is normally hydroxylated in the kidney through the action of a 25-hydroxylase.
   d. Formation in the liver is closely regulated via the action of several different hormones.
   e. Is a polypeptide.

3. Renal 1α-hydroxylase, the activity of which is needed in the activation of Vitamin D, is thought to be stimulated by all of the following, EXCEPT:
   a. Parathyroid hormone.
   b. Prolactin.
   c. Hypocalcemia.
   d. Calcitonin.
   e. Hypophosphatemia.

4. Vitamin D can be used effectively to treat patients with which one of the following hormone deficiencies?
   a. Insulin
   b. Calcitonin
   c. Cortisol
   d. Thyroid Hormone
   e. Parathyroid Hormone

5. The active form of vitamin D is the only hormone known to "effectively" promote translocation of Ca^{++}:
   a. Against its concentration gradient in the renal tubules.
   b. Against its concentration gradient in the intestine.
   c. Against its concentration gradient, into bone.
   d. Into smooth muscle tissue.
   e. Out of bone.

6. Which one of the following elements is associated with a decline in 1,25(OH)2D-stimulated intestinal Ca^{++} absorption?
   a. Iron
   b. Copper
   c. Lead
   d. Magnesium
   e. Potassium

7. 25(OH)DHT could be used to effectively treat vitamin D-deficient animals with renal dysfunction:
   a. True
   b. False

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