Chapter 47

Vitamin K

Overview

- Vitamin K, which is needed for blood clotting, can be derived from plants, bacteria, and animal tissues.
- A water-soluble form of vitamin K can be converted to the active derivative, hydroquinone.
- The biologic half-life of vitamin K is shorter than the other fat-soluble vitamins.
- The liver is the main repository of vitamin K.
- Vitamin K is required for the hepatic postsynthetic transformation of several protein clotting factors.
- Vitamin K helps to facilitate the γ-carboxylation of glutamate residues, which in turn chelate Ca^{++}.
- A vitamin K cycle exists in the endoplasmic reticulum of liver cells.
- An important therapeutic use of vitamin K is as an antidote in poisoning by dicoumarol or warfarin.
- Vitamin K plays a role in bone metabolism, as well as in the renal reabsorption of Ca^{++}.
- Tissue distributions of this vitamin indicate that its actions are diversified.

Vitamin K ("**koagulation**"), a fat-soluble vitamin required for blood clotting, was discovered in Germany in 1929. The pure **vitamin** (K_1) was obtained from alfalfa in 1939, and later it was realized that bacteria synthesized a second form (K_2), with a more unsaturated side chain (**Fig. 47-1**). The **menaquinones** are a series of polyprenoid unsaturated forms of **vitamin** K_2 found in animal tissues, and synthesized by bacteria (actinomycete microbes) inhabiting the digestive tract. **Vitamin** K_3 (**menadione**) is a water-soluble form synthesized commercially, and also capable of being converted to the active form (hydroquinone; HQ).

Vitamins K_1 and K_2 are naturally occurring polyisoprenoid-substituted **napthoquinones**, that are absorbed from the small intestine with variable efficiency (10-80%). The amount of fat in the diet, as well as bile acid availability through the liver, will influence absorptive efficiency. Vitamins K_1 and K_2 are distributed much like the other fat-soluble vitamins, initially in chylomicrons (CMs), which enter the bloodstream through the lymphatics, then later partially in VLDL and LDL (see Chapters 64 and 65). Vitamin K_3, being water-soluble, can be absorbed in the absence of bile acids, passing directly from intestinal mucosal cells into the hepatic portal circulation.

The **liver** is the main repository of these vitamins, although there appears to be a rapid turnover; consequently, **body pools are thought to be small**. This high rate of turnover, compared to the other fat-soluble vitamins, supports the

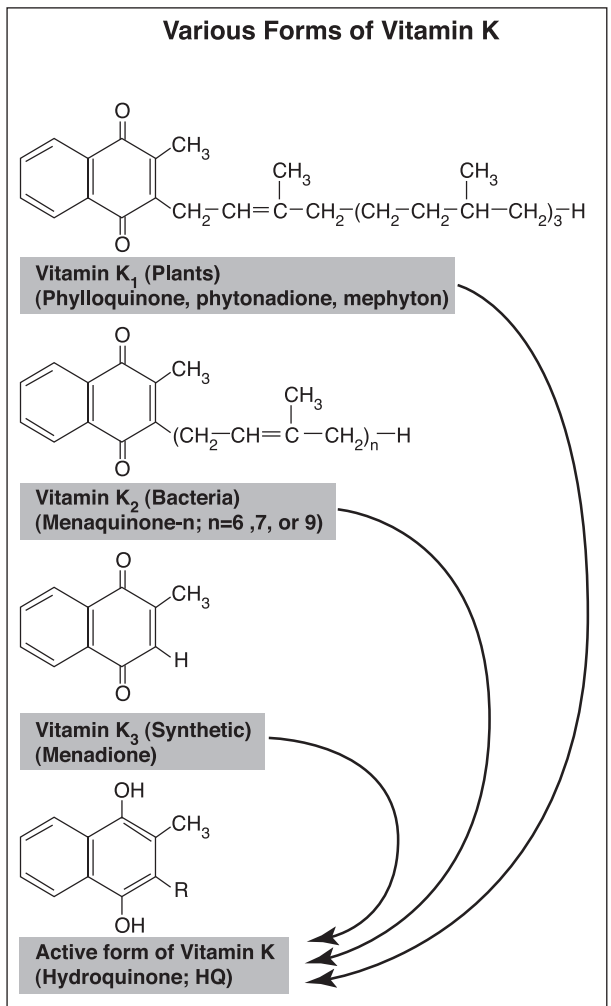

Figure 47-1

need for a continual supply through the diet (**K_1** and/or **K_2**), or from intestinal bacteria (**K_2**).

Although the liver form of this vitamin is **phylloquinone** (**K_1**) in horses, liver forms in dogs are usually half as **K_1**, and half as the **menaquinones** (**K_2**). Serum, however, contains mostly phylloquinones, indicating that the menaquinones may undergo side-chain saturation in the liver before being released into blood. Vitamin K and its oxidized metabolites are lost from the mammalian organism in both bile and urine.

Vitamin K is required for the **hepatic post-synthetic transformation of clotting factors II** (prothrombin), **VII** (proconvertin), **IX** (plasma thromboplastic component, Christmas factor, or antihemophilic factor B), and **X** (Stuart-Prower factor); as well as anti-coagulation **proteins C**, and **S** (which facilitates the inactivation of factors V_a and $VIII_a$ by facilitating the action of activated protein C). All are initially synthesized in the liver as inactive precursor proteins. In vitamin K deficiency, the liver continues its production of these coagulation proteins, however, they are nonfunctional.

The normal action of these factors in the clotting cascade is dependent upon the **carboxylation** of their terminal **glutamate** (**Glu**) residues to **γ-carboxyglutamate** (**Gla**; **Fig. 47-2**). Factor II contains ten of these residues, whose negativity allows **chelation of calcium** (**Ca^{++}**) in a specific protein-Ca^{++}-phospholipid interaction essential to their biologic role. Following injury, the binding of Ca^{++} by prothrombin anchors it to phospholipid membranes derived from blood **platelets**. This brings prothrombin

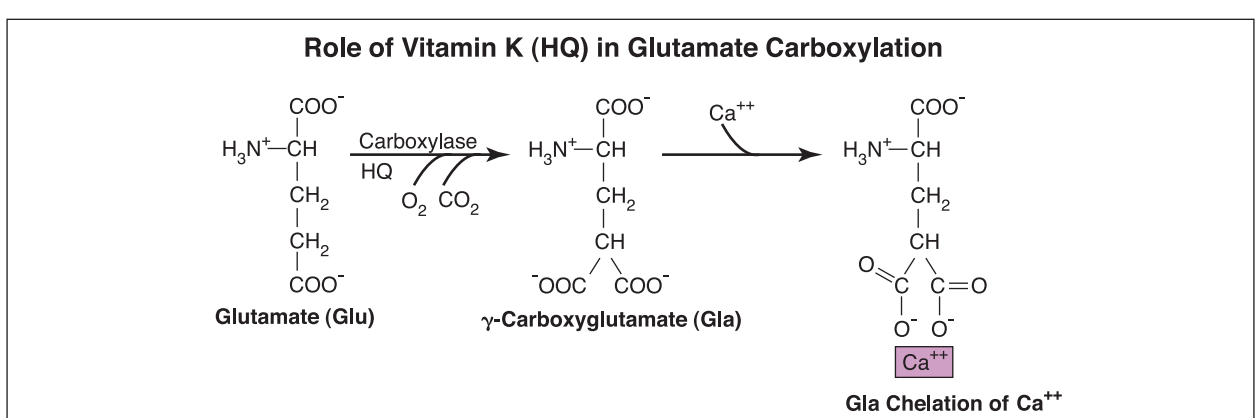

Figure 47-2

into close proximity with two proteins that mediate its conversion into **thrombin** -- factor X_a (a serine protease), and factor V (proaccelerin, a stimulatory globulin). The amino-terminal fragment of prothrombin, which contains the Ca^{++}-binding sites, is released in this activation step. Thrombin, which becomes freed in this way from the phospholipid surface, now becomes available to activate serum **fibrinogen**.

The involvement of vitamin K in the γ-carboxylation of several other proteins has also been demonstrated. These proteins are involved in **bone metabolism** (hydroxyapatite dissolution), and in **connective tissue** and **kidney function**. In all cases the vitamin appears to be required for the γ-carboxylation of specific Glu residues, which in turn allow chelation of Ca^{++}.

A small protein in bone (**osteocalcin** or "**bone Gla protein**"; **BGP**), which contains three Gla residues, is involved in the action of **vitamin D** in bone. The active form of vitamin D, **1,25(OH)$_2$D** (see Chapter 45), acts on osteoblasts to increase synthesis and secretion of osteocalcin, which then binds to hydroxyapatite, thus preventing further mineralization. Some osteocalcin also escapes into blood, especially if vitamin K status is compromised. Conditions associated with **hyperparathyroidism**, characterized by enhanced bone mineral turnover, thus show increases in blood osteocalcin levels.

A Gla protein found in the **kidney** is thought to be involved in the reabsorption of Ca^{++} by renal tubular epithelial cells (a function also shared by PTH and vitamin D). In addition, Gla proteins have been found in calcium-containing kidney stones.

Tissue distributions of vitamin K and its metabolites tend to indicate that the action of this vitamin is diversified, and not confined to a few tissues. Uptake by the spleen reportedly equals that of the kidney, at least in rats, and is exceeded by that of the lungs, skin, and muscle. Thus, it is likely that we are only beginning to understand the full functional significance of the phyllo- and menaquinones.

A **vitamin K cycle** exists in the endoplasmic reticulum of liver cells, where the **2,3-epoxide** product of the HQ carboxylation reaction is converted by **2,3-epoxide reductase** to the **quinone** form of the vitamin (using an as yet unidentified dithio reductant; **Fig. 47-3**). This reaction is inhibited by the anticoagulants, dicoumarol (**4-hydroxydicoumarin**) and **warfarin** (**Fig. 47-4**), with accumulation of the 2,3-epoxide resulting in the formation of an abnormal prothrombin that does not appropriately bind Ca^{++}. Normal reduction of the **quinone** to the **hydroquinone** form of the vitamin (by **NADPH**) completes the cycle for regenerating the active form. An important therapeutic use of vitamin K is as an antidote to poisoning by dicoumarol or warfarin. The quinone and water-soluble dione forms of the vitamin will bypass the inhibited 2,3-epoxide reductase step, thus providing an important source of the active form.

Dicoumarol is found in **spoiled sweet clover**, which contains a mold that produces this metabolite and can cause fatal hemorrhagic episodes in animals that graze on hay containing this compound. This coumarin derivative is used clinically as an anticoagulant to prevent thromboses in patients prone to clot formation. Both **dicoumarol** and **warfarin**, a structurally related 2,3-epoxide reductase inhibitor, are the active ingredients in rat poisons. Horses, swine, dogs, and cats that consume rodenticides containing these anticoagulants generally require vitamin K treatment. The duration of antagonism depends on the amount consumed, as well as on the physiologic half-life of these compounds (which can extend from days to weeks).

Vitamin K Deficiency

Vitamin K deficiency states are somewhat difficult to establish since the requirement for this vitamin in many species is met by intes-

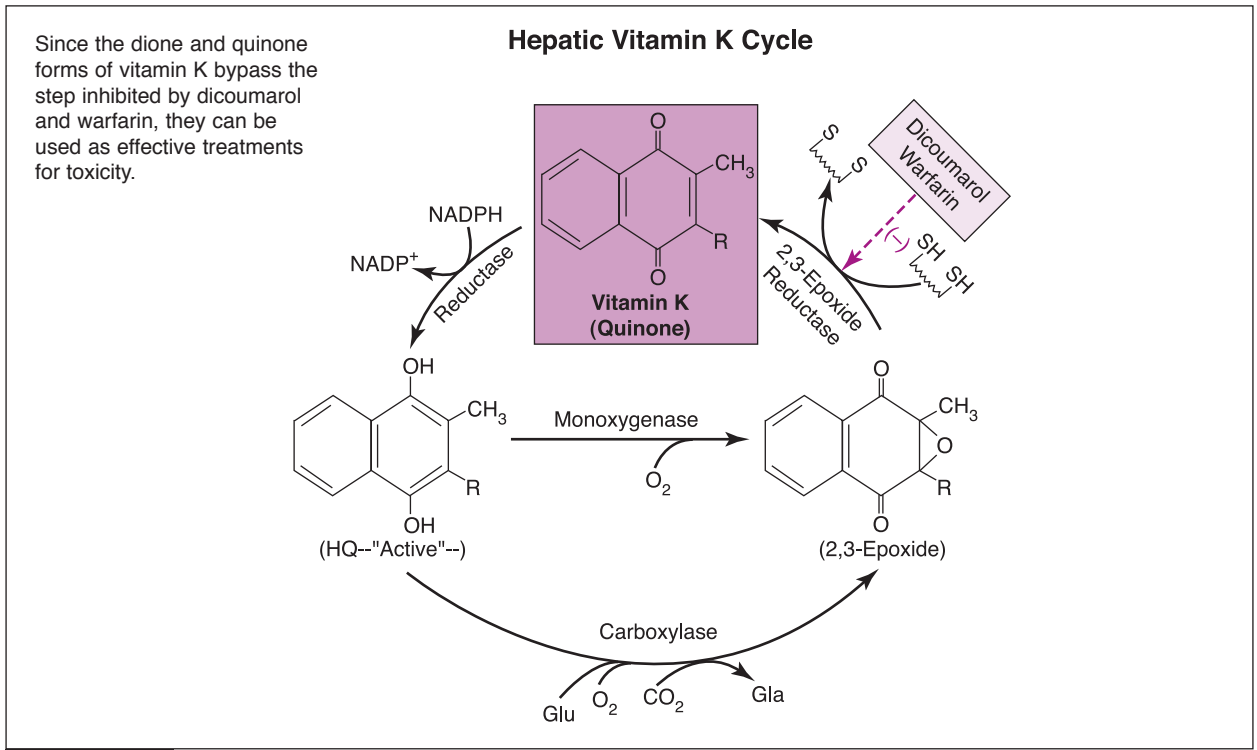

Figure 47-3

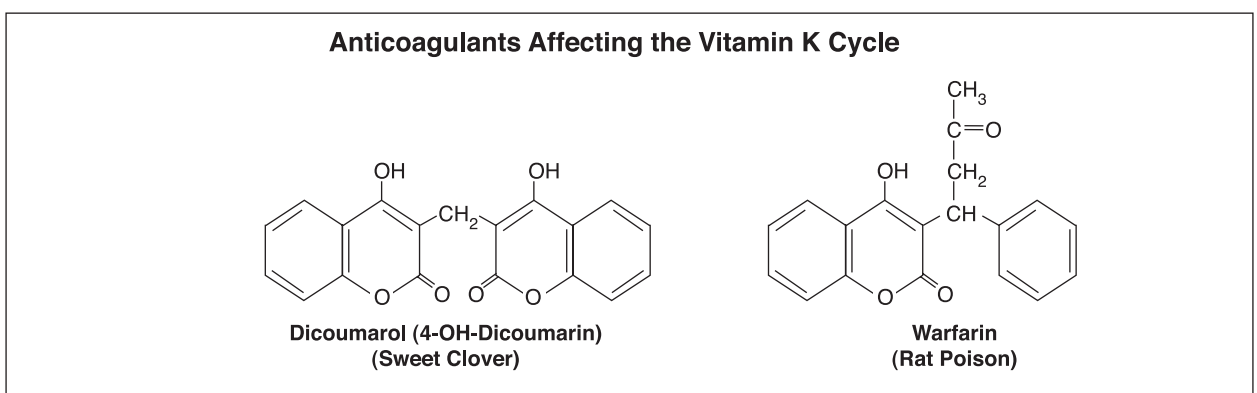

Figure 47-4

tinal microbial synthesis. Ruminant animals generally meet their vitamin K requirements through rumen **microbial biosynthesis** (with subsequent absorption in the small intestine), and the **coprophagy** practiced by rodents, rabbits, and other species provides vitamin K, produced in the large bowel. Horses generally receive sufficient vitamin K from pasture, hay, and intestinal bacteria to meet their needs. Dogs receive both $\mathbf{K_1}$ and $\mathbf{K_2}$ in their diets, and cats derive their quinones from eating meat.

Vitamin K deficiency can be caused by fat malabsorption, which may be associated with severe liver disease and/or biliary obstruction, pancreatic dysfunction, atrophy of the intestinal mucosa, or any cause of steatorrhea. In addition, sterilization of the large bowel by antibiotics can result in deficiency when dietary intake is limited. Vitamin K deficiency can present itself before other fat-soluble vitamin deficiencies, since the turnover of this vitamin is normally rather high. In newborn puppies,

a transient form of vitamin K deficiency may occur secondary to malnutrition of the bitch during gestation, or the marginal inadequacy of fetal hepatic protein synthesis.

Vitamin K Toxicity

Although few toxicity symptoms have been reported in animals, vitamin **K_3** (menadione), the synthetic water-soluble form of the vitamin used clinically, can apparently react with sulfhydryl groups on proteins, and therefore become toxic. Gastrointestinal disturbances and anemia have been associated with vitamin K excess.

OBJECTIVES

- Identify dietary and structural differences between the phylloquinones and menaquinone, and discuss differences in hepatic forms of vitamin K between dogs and horses.
- Describe how most ruminants, horses, rodents, rabbits, dogs and cats satisfy their vitamin K requirements.
- Explain why orally administered K_3 may appear at higher concentrations in the hepatic portal circulation than orally administered K_1 or K_2.
- Recognize why the liver can continue its production of clotting factors in vitamin K deficiency, yet those factors remain dysfunctional.
- Indicate how hepatic γ-carboxyglutamate is formed, where it is found, and how it functions.
- Discuss relationships that exist between bone Gla protein, $1,25(OH)_2D$, retinoic acid, PTH and Ca^{++} homeostasis.
- Understand why vitamin K is sometimes used as a therapeutic antidote to sweet clover and rat poisoning.
- Explain why vitamin K deficiency can present itself before other fat-soluble vitamin deficiencies, and identify vitamin K deficiency symptoms.
- Understand why newborn puppies might exhibit signs of vitamin K deficiency.

QUESTIONS

1. The water-soluble form of vitamin K is:
a. K_1.
b. K_2.
c. K_3.
d. K_4.
e. K_5.

2. Which one of the fat-soluble vitamins normally has the highest turnover rate?
a. A
b. D
c. E
d. K

3. In vitamin K deficiency:
a. The liver stops its production of prothrombin.
b. The liver stops its production of albumin.
c. The liver continues its production of prothrombin, however, it is nonfunctional.
d. Dicoumarol can be effectively used as a supplement.
e. Warfarin administration would have no effect.

4. Which one of the following enzymes is vitamin K-dependent?
a. Glutamate carboxylase
b. Vitamin K reductase
c. 2,3-Epoxide reductase
d. Purine nucleoside phosphorylase
e. PEP Carboxykinase

5. Which fat-soluble vitamins are best associated with osteocalcin of bone?
a. K and E
b. D and A
c. K and A
d. D and E
e. D and K

6. Dicoumarol is best associated with spoiled:
a. Milk.
b. Sweet clover.
c. Fish.
d. Meat.
e. Bread.

7. Biliary obstruction could cause vitamin K deficiency:
a. True
b. False

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