Chapter 6

Micronutrients: Minerals and Vitamins

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"Maybe my variety is due to bad absorption of vitamins."
Stephen Hawking

MINERALS

Introduction

Definition

The term "mineral" is generally used to denote all inorganic elements in a food. These inorganic elements constitute the majority of ash that remains after combustion of all organic matter. Ash analysis is of little value either for expressing mineral requirements or for indicating the useful mineral content of foods for two basic reasons: 1) body requirements are specific for certain inorganic elements (e.g., calcium, zinc, etc.) and 2) ash may not be a measure of total inorganic matter present, because some organic carbon may be bound as carbonate, and some inorganic elements (e.g., sulfur, selenium, iodine, fluorine and even sodium) may be lost during combustion.

The most important reason to determine total ash is to calculate the nitrogen-free extract by difference, as is required in the proximate analysis of foodstuffs. Specific minerals of interest can then be assayed (if not volatilized) from the ash component.

More than 18 mineral elements are believed to be essential for mammals (McDowell, 1992). By definition, macrominerals are required by the animal in the diet in percentage amounts, whereas microminerals or "trace" minerals are required at the mg/kg or parts per million (ppm). There are seven macrominerals: calcium, phosphorus, sodium, magnesium, potassium, chloride and sulfur. There are at least 11 trace elements or micronutrient minerals: iron, zinc, copper, iodine, selenium, manganese, cobalt, molybdenum, fluorine, boron and chromium. The last six are assumed to be essential for dogs and cats by analogy with other species. Calcium, phosphorus, magnesium, potassium, sodium and chloride are discussed below. Neither the Association of American Feed Control Officials (AAFCO) nor the National Research Council (NRC) lists a sulfur requirement for dogs or cats (AAFCO, 2007; NRC, 2006). Generally, there isn't a dietary need for sulfur per se, if a food is formulated to meet the sulfur-containing amino acid requirements of animals with simple stomachs.

Of the microminerals, only iron, zinc, copper, manganese, iodine and selenium will be discussed here. These trace minerals have been deemed essential for dogs and cats (although clinical cases of manganese deficiency have never been reported to occur in dogs or cats) (AAFCO, 2007). Cobalt and molybdenum are clearly important minerals in ruminant nutrition, but are not considered essential in monogastric species. Information about chromium and boron, two ultra-trace minerals, is included because of the potential importance these nutrients may have in companion animal nutrition. Other new trace elements discovered since 1970 include arsenic, lead, lithium, nickel, silicon, tin and vanadium. The essentiality of these minerals has not been elucidated in all species and under practical conditions may not be essential in the diet.

Function

Minerals are fundamental as: 1) structural components of body organs and tissues, such as calcium, phosphorus and magne-

Table 6-1. Mineral functions and effects of deficiencies and excesses.

Mineral	Function	Deficiency	Excess
Calcium	Constituent of bone and teeth, blood clotting, muscle function, nerve transmission, membrane permeability	Decreased growth, decreased appetite, decreased bone mineralization, lameness, spontaneous fractures, loose teeth, tetany, convulsions, rickets (osteomalacia in adults)	Decreased feed efficiency and feed intake, nephrosis, lameness, enlarged costochondral junctions. Increased calcium intake is a risk factor for calcium-containing urinary precipitates; however, moderate- to high-calcium levels may be protective against calcium oxalate precipitates. Calcium in meals may bind with oxalate in the gut decreasing the risk.
Phosphorus	Constituent of bone and teeth, muscle formation, fat, carbohydrate and protein metabolism, phospholipids and energy production, reproduction	Depraved appetite, pica, decreased feed efficiency, decreased growth, dull coat, decreased fertility, spontaneous fractures, rickets	Bone loss, uroliths, decreased weight gain, decreased feed intake, calcification of soft tissues, secondary hyperparathyroidism
Potassium	Muscle contraction, transmission of nerve impulses, acid-base balance, osmotic balance, enzyme cofactor (energy transfer)	Anorexia, decreased growth, lethargy, locomotive problems, hypokalemia, heart and kidney lesions, emaciation	Rare. Paresis, bradycardia
Sodium and chloride	Osmotic pressure, acid-base balance, transmission of nerve impulses, nutrient uptake, waste excretion, water metabolism	Inability to maintain water balance, decreased growth, anorexia, fatigue, exhaustion, hair loss	Occurs only if there is inadequate good-quality water available. Thirst, pruritus, constipation, seizures and death
Magnesium	Component of bone and intracellular fluids, neuromuscular transmission, active component of several enzymes, carbohydrate and lipid metabolism	Muscle weakness, hyperirritability, convulsions, anorexia, vomiting, decreased mineralization of bone, decreased body weight, calcification of aorta	Uroliths, flaccid paralysis
Iron	Enzyme constituent, activation of O_2 (oxidases and oxygenases), oxygen transport (hemoglobin, myoglobin)	Anemia, rough coat, listlessness, decreased growth	Anorexia, weight loss, decreased serum albumin concentrations, hepatic dysfunction, hemosiderosis
Zinc	Constituent or activator of 200 known enzymes (nucleic acid metabolism, protein synthesis, carbohydrate metabolism), skin and wound healing, immune response, fetal development, growth rate	Anorexia, decreased growth, alopecia, parakeratosis, impaired reproduction, vomiting, hair depigmentation, conjunctivitis	Relatively nontoxic. Reported cases of zinc toxicity from consumption of die-cast zinc nuts or pennies
Copper	Component of several enzymes (oxidases), catalyst in hemoglobin formation, cardiac function, cellular respiration, connective tissue development, pigmentation, bone formation, myelin formation, immune function	Anemia, decreased growth, hair depigmentation, bone lesions, neuromuscular disorders, reproductive failure	Hepatitis, increased liver enzyme activity
Manganese	Component and activator of enzymes (glycosyl transferases), lipid and carbohydrate metabolism, bone development (organic matrix), reproduction, cell membrane integrity (mitochondria)	Impaired reproduction, fatty liver, crooked legs, decreased growth	Relatively nontoxic
Selenium	Constituent of glutathione peroxidase and iodothyronine 5'-deiodinase, immune function, reproduction	Muscular dystrophy, reproductive failure, decreased feed intake, subcutaneous edema, renal mineralization	Vomiting, spasms, staggered gait, salivation, decreased appetite, dyspnea, oral malodor, nail loss
Iodine	Constituent of thyroxine and triiodothyronine	Goiter, fetal resorption, rough coat, enlarged thyroid glands, alopecia, apathy, myxedema, lethargy	Similar to those caused by deficiency. Decreased appetite, listlessness, rough coat, decreased immunity, decreased weight gain, goiter, fever
Boron	Regulates parathyroid hormone, influences metabolism of calcium, phosphorus, magnesium and cholecalciferol	Decreased growth, decreased hematocrit, hemoglobin and alkaline phosphatase values	Similar to those caused by deficiency
Chromium	Potentiates insulin action, therefore improves glucose tolerance	Impaired glucose tolerance, increased serum triglyceride and cholesterol concentrations	Trivalent form less toxic than hexavalent. Dermatitis, respiratory irritation, lung cancer

sium in bones and teeth, 2) constituents of body fluids and tissues such as electrolytes concerned with the maintenance of osmotic pressure, acid-base balance, muscle contraction, membrane permeability and tissue irritability (e.g., sodium, potassium, chloride, calcium and magnesium in blood, cerebrospinal fluid and gastric juice) and 3) catalysts/cofactors in enzyme and hormone systems, as integral and specific components of the structure of metalloenzymes, or as less specific activators within those systems. **Table 6-1** lists specific functions of each mineral.

Homeostasis

Specific concentrations and functional forms of minerals must be maintained within certain limits for optimal growth, health and fertility. Higher organisms possess homeostatic mechanisms that attempt to maintain concentrations of minerals at their active sites within narrow physiologic limits despite over- or under-ingestion. Such mechanisms include control of intestinal absorption or excretion, the availability of specific stores for individual elements and the use of "chemical sinks" such as metallothionein that can bind potentially toxic amounts of elements in an innocuous form (Underwood and Mertz, 1987).

The degree of homeostatic control varies from one element to another. Continued ingestion of diets or exposure to environments that are severely deficient, imbalanced or excessively high in a particular trace element, or in an interfering substance such as phytate or certain fibers, can induce changes in functioning forms, activities or concentrations of that element in body tissues and fluids so that they fall below or rise above the desired limits. Altered metabolism develops in these circumstances, which may affect physiologic function. Structural disorders may also arise in ways that differ with various elements, with the degree and duration of the dietary deficiency or toxicity and with the age, gender and species of the animal involved.

Deficiency/Adequacy/Toxicity

Traditionally, minerals were classified as "essential" or "toxic," but as more information was gathered, elements shifted from the latter to the former category (e.g., selenium). However, toxicity may occur with all elements. A "biologic dose-response curve" exists for each element (Underwood and Mertz, 1987). This curve (Figure 5-2) identifies a range of concentrations that spans three primary areas: 1) at low concentrations, physiologic function is consistently and reproducibly impaired (defined as deficiency), 2) at optimal concentrations, the nutrient is provided at levels necessary to meet the requirements of the animal and 3) at excessive concentrations, pharmaco-toxicologic effects occur. The intakes or dose levels at which these phases become evident and the width of the optimal plateau vary widely among minerals and can be markedly affected by the extent to which various other elements and compounds are present in the animal's body and in the food consumed (Box 6-1). Table 6-1 lists specific signs of mineral deficiencies and toxicities.

Several nutrients have specific therapeutic uses at high dosages (e.g., zinc is fed at growth-promoting levels in swine to prevent diarrhea). However, high doses may result in detrimental side effects after prolonged use. The pharmacologic actions of nutrients differ in several ways from their physiologic functions: 1) doses greatly exceeding the amount of a nutrient present in foods are usually needed to obtain a therapeutic response, 2) the specificity of the pharmacologic action is often different from the physiologic function and 3) chemical analogues of the nutrient that are often most effective pharmacologically may have little or no nutritional activity (RDA, 1989) (Box 5-7).

Claims of nutritional adequacy of pet foods are based on the current AAFCO nutrient allowances ("profiles"). These levels are neither minimal requirements nor necessarily optimal intake levels. It isn't possible to establish optimal levels without additional information about nutrient requirements for all lifestages and information concerning the availability of nutrients from pet food ingredients and complete diets. In some cases, insufficient margins of safety have been given to account for population variation, product diversity, processing effects and potentially low nutrient availabilities of certain pet food ingredients. In the case of trace minerals, the ratio between dietary allowance and absolute requirement can be as large as 100:1 (e.g., chromium) because of incomplete absorption, or can approach unity (e.g., iodine) when absorption is high (Underwood and Mertz, 1987). The nature of typical diets consumed strongly influences dietary allowances because numerous interactions among dietary components and different

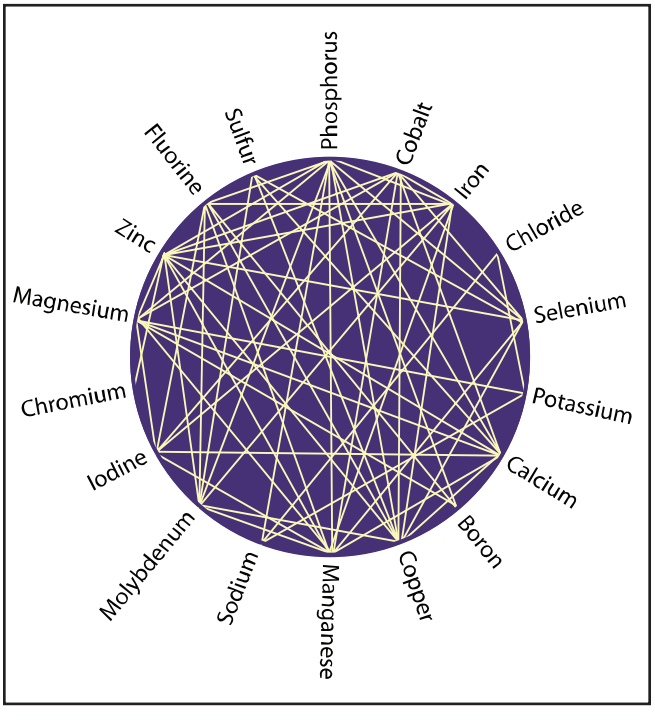

Figure 6-1. Mineral interrelationships. Minerals connected by a line clinically or experimentally interact with the other mineral. This interaction may be bidirectional (each mineral affects the use of the other) or unidirectional (one mineral affects the use of the second mineral but not vice versa). (Adapted from Puls R. Mineral Levels in Animal Health. Clearbrook, British Columbia: Diagnostic Data Sherpa International, 1990; 19.)

Box 6-1. Mineral Balance Studies.

The requirements for most nutrients are derived from experimental and clinical evidence of deficiency and the amount of nutrient needed to prevent signs of deficiency. When balance studies are used to estimate requirements, the requirement is defined as the intake at which zero balance is attained, or when intake is equal to excretion in urine and feces. However, the zero balance point will underestimate the requirement, if the measurement does not account for endogenous losses and losses in sweat.

Mineral balance studies have been criticized as inadequate and erroneous measures of body requirements. Small percentage errors in determining intakes and excretion can result in significant differences in balance calculations. One of the biggest problems in conducting balance studies is separating feces into time intervals that can be related to intake. Fecal markers aid in separation, but peristaltic reflux may still confound results. In addition, one animal's rate of passage can vary markedly. Adaptation to a different intake level may occur in a few days or weeks. Some adaptations, however, take several months or even years to occur. Thus, the adaptation period and collection period need to be sufficiently long to take into account animal adjustments to new foods, rates of passage and homeostatic adaptation.

Balance studies are probably more reliable when the mineral is excreted in the urine, rather than in the feces. This finding is true for sodium, potassium and selenium. When absorption of a nutrient is low, as is the case for a number of minerals, the amount of fecal mineral is large compared with the amount absorbed (e.g., the mineral concentration in feces is attributed to unabsorbed mineral and endogenous secretion). Failure to measure endogenous secretion may markedly underestimate the true amount of absorbed mineral. However, if radioisotopes or stable isotopes are used in conjunction with a balance study, the endogenous secretions can be distinguished from unabsorbed mineral and a measure of true absorption attained (as opposed to "apparent" absorption, which does not account for endogenous losses). Balance studies without the use of isotopes are fraught with inaccuracy and variability. Few analytical methods give a coefficient of variation as good as 5%, especially in complex matrices such as food, urine and feces. Thus, balance studies will not detect nutritionally important mineral differences when absorption efficiencies are low and radioisotopes or stable isotopes are not used.

Probably the biggest criticism of balance studies is that balance studies better reflect habitual intake than a requirement or zero balance. For example, an intake of 1 µg of selenium/kg body weight maintains a zero balance in Americans. Approximately one-tenth of that intake maintains a balance in people living in China; China is an area in which the risk of selenium deficiency is high and prevalence of Keshan disease is significant. Thus, zero balance does not necessarily indicate absence of disease. In New Zealand, selenium balance is maintained on an intake of one-third the amount required by Americans.

Analogous situations also exist worldwide for calcium balance. Widely different intakes of calcium result in zero balances in different countries. Thus, the previous dietary intake exerts a significant effect on the nutrient level that results in a zero balance and is more a reflection of the intake required to maintain an existing mineral pool size.

Balance studies should be evaluated with caution when attempting to determine requirements. A summary of their limitations follows:

- Prior long-term habitual intake influences whether positive, negative or equilibrium balance occurs at a particular intake.
- The duration of the study may not be long enough to allow for homeostatic adaptation.
- Cumulative errors occur from environmental contamination, individual variability and analytical methods. Thus, balance studies that demonstrate no treatment differences, may in fact be a result of the insensitivity and imprecision of the balance method.

The Bibliography for **Box 6-1** can be found at www.markmorris.org

chemical forms in individual foods determine biologic availability. Thus, it is important to understand the limitations of AAFCO nutrient profiles.

Mineral Interactions

A tremendous number of mineral-mineral interactions exist (**Figure 6-1**). In general, these interactions can be antagonistic (the presence of one mineral reduces the transport or biologic efficacy of the other) or synergistic (the two minerals act in a complementary fashion either by sparing or substituting for the other mineral or the two together enhance a biologic function). Most mineral interactions are antagonistic and can occur via a number of different mechanisms that include interactions (Solomons, 1988): 1) in the food during processing, before consumption, 2) in the digestive tract, where there is competition for uptake sites or intracellular-level mechanisms, 3) at the tissue level, either at storage sites or inhibition of enzyme activity, 4) at the time of transport and 5) in the excretory pathway.

The rigors of processing can affect the availability of minerals either positively or negatively via changes in solubility, pH, reduction potential and charge density and creation of complexes (Clydesdale, 1988). Charge density refers to the valence state and size of a metal. For example, the cations in the periodic table in groups 6B to 2B, with a relatively high ionic charge ($^{+}2$, $^{+}3$) and small size, form a large number of stable complex ions, whereas the large alkali metal cations, such as Na^{+} and K^{+}, with a small charge are much less likely to form complexes with proteins or carbohydrate moieties via ionic, coordinate or covalent bonds. Furthermore, among the transition metals, which may form more than one cation, complexes formed by the $^{+}3$ valence state (e.g., Cr^{+3}, Co^{+3}) are more numerous and more stable than complexes formed by their respective $^{+}2$ ions. Charge is also involved with cell permeability and ion solubility before ions enter cells. Solubility varies tremendously depending on ion size and degree of polarity or charge.

Solubility is of obvious importance; a mineral must come in contact with the intestinal mucosa if it is to be absorbed. Charge density is less obvious but important for its effect on complex formation and membrane permeability. Solubility as it refers to mineral availability includes the solubility of an ion, salt, hydrate or complex, and to the type and strength of chemical bonds within these molecules. Inhibition of mineral absorption by a food can be overcome by the use of mineral enhancers, such as ascorbate, meat, citric acid and other ligands (e.g., ascorbate enhances iron absorption but negatively affects copper uptake; both effects are brought about by a change in pH and reduction in valence state).

Mineral-mineral interactions that occur in the digestive tract result from chemically similar minerals sharing "channels" for absorption. In this situation, simultaneous ingestion of two or more such minerals will result in competition for absorption (Solomons, 1988). In other words, when the dietary supply of a nutrient and/or the body reserves of a mineral are low, the intestine adapts to improve the efficiency of uptake and transfer. When the adaptation is nonspecific, other similar minerals have enhanced absorption. In iron deficiency, an up-regulation of iron also increases uptake of lead (Solomons, 1988). Other examples of interactions occurring in the digestive tract include the formation of insoluble mineral complexes (e.g., foods containing phytate and excessive calcium will form an insoluble calcium/phytate/zinc complex that reduces zinc availability).

Mineral-mineral interactions can also occur at the tissue storage level. High levels of dietary iron, for example, reduce hepatic copper stores. In studies, when ratios of iron to copper exceeded 20:1, hepatic copper levels were reduced to less than 50% of control values (Solomons, 1988). Likewise, trace minerals such as zinc can be mobilized when calcium is deficient because co-mobilization of both minerals takes place from the skeleton, making both available for use.

Mineral-mineral interactions can also occur at the time of transport. Transferrin is a serum transport protein for iron. Transferrin is generally less than 50% saturated with iron in its transit from site to site (Solomons, 1988). Transferrin can also transport chromium and manganese; therefore, these minerals may compete for binding sites contained in transferrin.

Finally, mineral-mineral interactions can also occur within pathways of excretion. For example, levels of circulating ionized calcium govern the release of parathyroid hormone (PTH) from the parathyroid gland. PTH status, in turn, influences renal tubular handling of filtered phosphate. Evidence also points to an interaction between calcium and magnesium at the level of renal excretion (Solomons, 1988).

Availability

Evaluation of feeds as sources of minerals depends not only on what the feed contains (i.e., the analyzed nutrient content), but also on how much of the mineral can be used by the animal. The adequacy of a food, as determined by its analytical mineral concentration, can be misleading because a number of factors can influence mineral availability. These include: 1) the chemical form (which influences solubility), 2) the amounts and proportions of other dietary components with which it interacts metabolically, 3) the age, gender and species of the animal, 4) intake of the mineral and the need (body stores) and 5) environmental factors (Underwood and Mertz, 1987) (**Box 6-2**).

Few studies have been completed in dogs and cats to evaluate the availability of minerals in foodstuffs used in commercial pet foods. Thus, there are many unknowns about the availability of nutrients in pet foods and whether a given food is truly adequate for a given lifestage. The availability of different forms of a mineral can vary widely even among inorganic mineral supplements. In general, different forms of trace minerals (iron, zinc, manganese and copper) differ in availability as follows: sulfate and chloride forms >carbonates >oxides (Aoyagi and Baker, 1993; Wedekind and Baker, 1990; McDowell, 1992a; Henry et al, 1986). The oxides of iron and copper are poorly available and should not be used as mineral supplements in pet food (McDowell, 1992a; Morris and Rogers, 1994).

In general, meat-derived foodstuffs are considered a more available source of certain minerals than plant-derived foodstuffs. The organic forms of minerals found in meats are often more available or as available as those from inorganic mineral supplements, whereas the minerals in plants are often less available (Aoyagi et al, 1993; Hortin et al, 1993). This finding applies more for iron, zinc and copper than for selenium.

Although the mechanism has not been fully delineated, one theory has been suggested to explain why organic forms of minerals are better used than inorganic forms. This theory postulates that chelates or complexes provide the mineral in a protected form (Kratzer and Vohra, 1986), analogous to the iron contained in heme, wherein the iron is complexed to a protoporphyrin ring. Because the metal is complexed or bound, it is protected from being sequestered by other dietary components (e.g., phytate, fiber and sugars) and is less likely to compete with mineral excesses.

Regardless of whether the molecular species is plant- or animal-derived, the complex must be able to be absorbed by mucosal cells or be cleaved to release the mineral in a soluble form or have stability constants that allow the mineral to be transferred to mucosal or serosal acceptors for availability (Clydesdale, 1988). Other explanations for why animal products are generally more available forms of certain minerals than plants include the "meat-factor" effect, wherein meat provides an available form of the mineral and enhances the absorption of the mineral supplied by the rest of the food (Kapsokefalou and Miller, 1993; Turnlund et al, 1983). In addition, meats, unlike plants, do not contain anti-nutritional factors, such as phytate, oxalate, goitrogens and fiber, which reduce mineral availability.

Not all fiber sources, however, negatively affect mineral availability. Research in chicks (Wedekind et al, 1995, 1996) and puppies (Wedekind et al, 1996a) indicated marked differences about how fiber sources affect mineral availability (Table 5-7). In these studies, beet pulp consistently reduced the availability of minerals (zinc, calcium, phosphorus and iron); however, cellulose, corn bran and sunflower hulls had negligible effects. Pea fiber, peanut hulls and soy hulls inhibited availability of some

Box 6-2. Organic vs. Inorganic Minerals.

There is some debate about whether organic forms of trace minerals are more available than inorganic forms. The answer depends on the specific mineral, the dietary conditions and the physiologic state of the animal. Clearly, the organic forms of certain minerals (e.g., selenium, chromium, iron) are better used than inorganic forms. (See the selenium, chromium and iron sections of this chapter.) Which form is better used is less clear for other minerals (e.g., zinc, copper). For example, there are as many studies that have failed to show increased availability with zinc/copper organic forms as there are studies demonstrating improved availability.

A number of factors influence the outcome of availability studies, including: 1) the presence of non-nutritional factors (e.g., phytate, fiber, goitrogens), 2) nutrient interactions (e.g., excesses of other minerals) and 3) physiologic state (e.g., demand for certain minerals increases with reproduction and growth compared to that of maintenance, thus in these situations, the differences in availability are magnified between organic and inorganic sources).

Results of studies in puppies showed that as calcium levels increased from 1.0 to 1.5%, zinc usage (as measured by changes in plasma zinc concentrations) decreased, irrespective of whether the source was organic (zinc propionate) or inorganic (zinc oxide). Zinc from zinc propionate was approximately 1.8 to 2 times more available than from zinc oxide.

Other investigators likewise noted increased zinc retention (as measured by zinc deposition in hair and fecal zinc excretion) for adult dogs fed a zinc-amino acid chelate compared with the same dogs fed zinc polysaccharide or zinc oxide. Increasing calcium from 1.2 to 3.2% reduced zinc retention when dogs were fed zinc polysaccharide or zinc oxide, but not the zinc-amino acid chelate.

Similarly, researchers have demonstrated in livestock and fish that growth rate and calcium and phytate levels are factors that significantly affect zinc use. Thus, these factors determine whether the use of organic zinc sources is beneficial. Together, these data suggest little or no benefit to using organic zinc in foods low in phytate and calcium (e.g., low calcium is defined as calcium levels approximating NRC recommendations for respective species). However, as phytate and/or calcium levels increase, or demand for zinc increases (e.g., rapid growth rate), there is greater zinc use from organic zinc sources (parameters used to assess zinc availability included bone zinc, immune response and/or growth rate). Furthermore, the more rapidly the animal grows, the greater the benefit demonstrated for organic zinc (e.g., fish >chicks [broiler breeds >leghorn-type] >puppies >pigs). The efficacy decreased as the animal matured, suggesting organic zinc sources in foods may be less beneficial for adult animals.

In summary, organic forms of minerals may be beneficial when dietary or physiologic conditions limit mineral availability. These conditions include: 1) mineral antagonisms caused by phytate, fiber and imbalances/excesses of other minerals and 2) increased metabolic demand such as rapid growth rate, reproduction and immune challenge.

The Bibliography for **Box 6-2** can be found at www.markmorris.org.

but not all of the minerals evaluated. Additional mineral supplementation is warranted for foods known to have reduced mineral availability.

Macrominerals

Calcium

Calcium serves two important functions: 1) as a structural component in bones and teeth and 2) as an intracellular second messenger that enables cells to respond to stimuli such as hormones and neurotransmitters. Calcium's two major physiologic functions in bone are to serve as a structural material and as an ion reservoir. When calcium in bone acts as an ion reservoir, it is in equilibrium with serum ionized calcium and under tight homeostatic control.

The mechanism of calcium homeostasis in blood is complex and involves several organs. Blood concentrations of ionized (or free) calcium are the major initiator of calcium regulatory mechanisms in the body. Calcium in blood is in equilibrium between a free or ionized state (~50%), a protein-bound state (~40 to 45%) and a complexed or chelated state (~5 to 10%). The effects of changing ionized calcium concentration in blood are highlighted below (**Figure 6-2**) (Nap and Hazewinkel, 1994). Low concentrations of ionized calcium:

- Stimulate PTH secretion, which stimulates conversion of 25-hydroxycholecalciferol (25-OH-D_3) to the biologically more potent 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$-D_3) in the kidneys
- 1,25-$(OH)_2$-D_3 stimulates calcium uptake in the gut via receptor-mediated mechanisms
- 1,25-$(OH)_2$-D_3, in conjunction with PTH, stimulates bone resorption
- PTH induces phosphaturia.

High or normal concentrations of ionized calcium:

- Stimulate calcitonin secretion, which does not stimulate 1,25-$(OH)_2$-D_3 production
- 24,25-dihydroxycholecalciferol (24,25-$(OH)_2$-D_3) is now produced in the kidneys, which is considered biologically less active
- No stimulation of gut absorption or bone resorption occurs
- Increased renal calcium excretion results
- Calcitonin decreases osteoclastic activity.

PTH, calcitonin and 1,25-$(OH)_2$-D_3 act together to maintain calcium homeostasis in the face of variable dietary intakes and changing calcium requirements during growth, pregnancy and lactation.

The amount of true calcium absorption may range from 25 to 90%, depending upon calcium status, calcium form or intake (Nap and Hazewinkel, 1994). This exchangeable pool consists of the small amount of calcium in blood, lymph and other body fluids, and accounts for 1% of the total body calcium. The remaining 99% is located in bones and teeth. There are three routes of calcium absorption in the intestine. One is an active, saturable, transcellular process that occurs primarily in the duodenum and proximal jejunum. The process is regulated by vitamin D and involves a vitamin D-dependent, calcium-binding protein (CaBP or cal-bindin).

Active calcium absorption is affected by the physiology of the host (i.e., calcium and vitamin D status, age, pregnancy and lactation).

The other pathways of calcium absorption are facilitated and passive absorption, which are important in the distal gastrointestinal (GI) tract. Passive absorption is a nonsaturable, paracellular route that is independent of vitamin D regulation. The amount of calcium absorbed in this way depends primarily on quantity and availability of calcium in the food. No matter where absorption takes place, vitamin D is the most important regulator of calcium absorption (Birge and Avioli, 1990). Renal handling of calcium is also modulated by PTH and calcitonin but not as much by vitamin D.

Deficiencies and excesses of calcium, as well as calcium-phosphorus imbalances, should be avoided in dogs and cats (**Box 6-3**). A food grossly deficient in calcium, but adequate in phosphorus can cause secondary hyperparathyroidism. An all-meat diet devoid of bones, for example, is a very poor source of calcium. Inadequate calcium intake produces hypocalcemia, which stimulates release of PTH, which in turn stimulates production of 1,25-$(OH)_2$-D_3, resulting in a higher fractional absorption of calcium and phosphate, and lower calcium but higher phosphate concentration in urine. PTH acts with vitamin D to promote bone resorption and turnover, which may lead to pathologic fractures. Hypocalcemia is a common problem in diseased states (chronic or acute renal failure, pancreatitis, eclampsia, etc.), and parenteral supplementation of calcium and/or calcitriol (1,25-$(OH)_2$-D_3) is sometimes warranted (Chew and Carothers, 1995). Calcium excess is probably more detrimental in rapidly growing animals than in adults, especially large- and giant-breed puppies (Chapter 33). **Table 6-1** describes signs of calcium deficiency and excess.

Research suggests that the dietary requirement of calcium for growing puppies (especially large breeds) is higher at 1.2% dry matter (DM) (Hazewinkel et al, 1991; Schoenmakers et al, 1999; Nap et al, 1993) than the previous recommendation of 0.59% (NRC, 1985). The NRC (2006) recommended allowance for adult dogs and growing puppies after weaning is 0.40 and 0.59% DM calcium (both based on foods containing 4,000 kcal/kg), and for large- and giant-breed puppies at risk for developmental orthopedic disease, the recommendation is 0.7 to 1.2% DM calcium (based on foods containing 3,800 kcal/kg) (Chapter 33).

A balance study was conducted to determine the calcium requirement of adult cats (Pastoor et al, 1994). Four levels of calcium ($CaCO_3$) ranging from 0.27 to 1.62% DM were evaluated. The minimum level evaluated (0.27% calcium) resulted in positive mineral balance with no adverse effect on serum phosphorus, calcium, magnesium and alkaline phosphatase concentrations. This level is less than half that of current AAFCO (2007) recommendations (i.e., 0.6% calcium). Likewise, two groups of investigators conducting studies in kittens demonstrated lower calcium and phosphorus requirements than those currently recommended by AAFCO for growth (i.e., requirements for calcium and phosphorus were 0.5 and 0.63% [Morris and Earle, 1996] and 0.36 and 0.28%, respectively [Pastoor, 1993]). These investigators concluded that the 1% DM calcium recommended by AAFCO (2007) for kittens is excessive and that the NRC recommendation (2006) of 0.8% DM calcium was a more defensible allowance for kittens fed typical moist foods. The current AAFCO (2007) canine and feline recommendations for calcium are 1.0% for growth/reproduction and 0.6% for adult maintenance (DM for both values). For dogs, this calcium requirement is based on an energy density of 3.5 kcal/g metabolizable energy (ME), whereas an energy density of 4.0 kcal/g ME is assumed for cats (AAFCO, 2007). Foods with increased energy densities should have a proportionally increased amount of calcium to account for decreased food consumption.

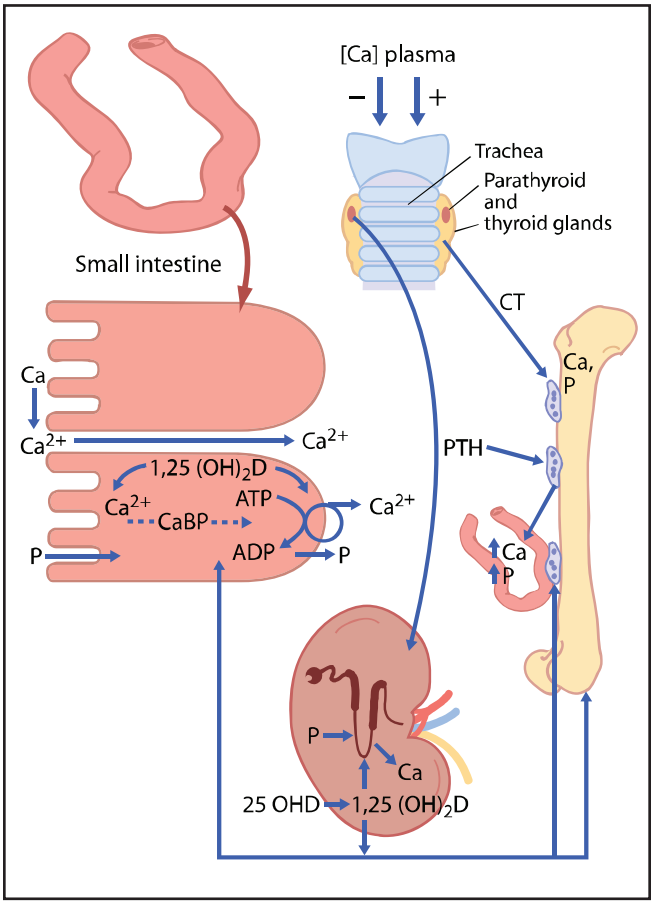

Figure 6-2. Calcium absorption by the intestine and bone resorption and reabsorption in the kidney are closely regulated by calcium-regulating hormones: parathyroid hormone, calcitonin and 1,25-$(OH)_2$-D_3. See text for details.

These meat meals are rich sources of calcium because of their bone content: poultry by-product meal, lamb meal and fish meal. Grains (corn, rice, etc.) are generally poor sources of calcium. Soybean meal and flaxseed have calcium contents between those of meat meals and grains. Meats without bone are poor sources of calcium. The most common calcium supplements used in pet foods are limestone (calcium carbonate), calcium sulfate, calcium chloride, calcium phosphate and bone meal, ranging in calcium from 16 to 39% (**Table 6-2**).

Box 6-3. Calcium-Phosphorus Ratios.

The "ideal" calcium-phosphorus ratio recommended for animals with simple stomachs is generally considered to be between 1:1 and 2:1. A number of factors, however, influence the importance of this ratio. Increasing levels of vitamin D reduce the significance of adverse calcium-phosphorus ratios. Furthermore, the ratio can differ markedly with the form and availability of the calcium and phosphorus supplied in the diet. For example, animals eating foods high in phytate phosphorus require greater phosphorus intake to meet their needs. Thus, the ideal calcium-phosphorus ratio would be lower when foods with these dietary characteristics are fed vs. foods composed of mostly meat ingredients.

Investigators sometimes debate whether the calcium-phosphorus ratio is more important than absolute calcium and phosphorus levels (**Table 1**). For all practical purposes, however, if a food were formulated to meet or slightly exceed an animal's requirement for calcium and phosphorus, it would by default provide an optimal calcium-phosphorus ratio. The more rapid the growth rate (e.g., large- and giant-breed puppies >small-breed puppies >adult dogs), the more critical it is to optimize calcium and phosphorus levels (Chapter 33). Increasing energy density increases the calcium and phosphorus requirement; the younger the animal the more critical it is that calcium and phosphorus be optimal.

Calcium-phosphorus ratios less than one have been evaluated in cats. Kealy et al compared the effects of feeding two foods with different calcium-phosphorus ratios to adult cats for 52 weeks. (The foods had 1:1 vs. 0.6:1 ratios; dry matter calcium and phosphorus levels were 1.27% calcium, 1.29% phosphorus and 0.75% calcium and 1.24% phosphorus, respectively.) Serum concentrations of total calcium, ionized calcium, phosphorus, PTH, alkaline phosphatase and vitamin D analogs did not differ between cats fed the two different foods at any sampling time and no signs of orthopedic diseases or bone loss developed during the study.

Likewise, Morris and Earle evaluated the effects of feeding foods containing calcium-phosphorus ratios as low as 0.65:1 to kittens and found no adverse effects, provided dry matter calcium and phosphorus levels exceeded 0.5 and 0.63%, respectively. Morris and Earle determined these calcium and phosphorus levels to be the calcium and phosphorus requirements for kittens. Results showed that this ratio (0.65:1) was well tolerated by kittens. Feed consumption, body weight gain, hematologic parameters and concentrations of plasma total and ionized calcium, total phosphorus, alkaline phosphatase, PTH, creatine phosphokinase, total plasma protein and albumin and plasma 25-OH-D did not differ from values in kittens fed foods with higher calcium-phosphorus ratios. Investigators noted significant changes in ionizable calcium concentrations; however, at 18 weeks in kittens fed foods with a calcium-phosphorus ratio of 0.38. These studies indicate that cats may tolerate wider dietary calcium-phosphorus ratios than the previously recommended ratios between 1:1 and 2:1.

The Bibliography for **Box 6-3** can be found at www.markmorris.org

Table 1. Examples of calcium-phosphorus percentages and ratios.

Examples	% calcium/% phosphorus (calcium-phosphorus ratio)
AAFCO adult allowance for calcium and phosphorus in dogs	0.6/0.5 (1.2:1)
AAFCO growth allowance for calcium and phosphorus in dogs	1.0/0.8 (1.25:1)
Example of why tuna is a poor source of calcium and has a poor calcium-phosphorus ratio	0.157/1.28 (0.12:1)
Example of a correct ratio, but excessive calcium and phosphorus levels	2/1.6 (1.2:1)

Phosphorus

Phosphorus is a vital participant in a number of tissues and functions. After calcium, phosphorus is the second largest constituent of bone and teeth. Phosphorus is a structural component of RNA and DNA, high-energy phosphate compounds such as ATP and cell membranes composed largely of phospholipids. As a component of nucleic acids, high-energy phosphate compounds and cell membranes, phosphorus is essential in cell growth and differentiation, energy use and transfer, fatty acid transport and amino acid and protein formation.

About 60 to 70% of phosphorus is absorbed from a typical diet (Allen and Wood, 1994). In general, phosphorus availability is greater from animal-based ingredients than from plant-based ingredients. Phosphorus in meat is found mainly in the organic form, whereas in plants, phosphorus is in the form of phytic acid. Phytate phosphorus is only about one-third available to monogastric animals but availability from different grains can vary markedly (McDowell, 1992). Intestinal phosphorus absorption represents the sum of a saturable, carrier-mediated component and a nonsaturable, concentration-dependent component. Regulation of total body phosphorus requires the coordinated efforts of the kidneys and intestine. Under conditions of low dietary phosphorus intake, the intestine increases its absorptive efficiency to maximize phosphorus absorption and the kidneys increase renal phosphorus transport or minimize urinary phosphorus losses. Hormonally, these adaptations result from changes in plasma levels of 1,25-$(OH)_2$-D_3 and PTH. Conversely, under conditions of dietary excess, the kidneys increase excretion of minerals. **Table 6-1** describes effects of deficiency and excess. Avoiding excess dietary phosphorus slows progression of kidney disease (Chapter 37).

There are few data to make a phosphorus recommendation; however, the most recent NRC (2006) suggests 1.0 and 0.30% DM for puppies and adult dogs, respectively.

Similar to the study design for calcium, investigators evaluated the minimum phosphorus requirement for adult cats (Pastoor et al, 1995). Four levels of phosphorus (provided as $NaH_2PO_4 \cdot 2H_2O$) ranging from 0.3 to 1.8% DM were evaluated. The minimum level evaluated (0.3% DM phosphorus) resulted in positive mineral balance with no adverse effect on serum phosphorus, calcium, magnesium and alkaline phos-

Table 6-2. Common mineral sources.*

Mineral	Source	Chemical formula	Mineral content (%)**		
Calcium	Calcium carbonate	$CaCo_4$	39 Ca	0.02 Na	
	Limestone	$CaCO_3$	38 Ca	0.05 Na	0.01 F
	Calcium citrate		24 Ca		
	Calcium sulfate	$CaSO_4$	23 Ca		
	Calcium chloride	$CaCl_2$	35 Ca	64 Cl	
Calcium and phosphorus	Bone meal		24 Ca	12.6 P	0.37 Na 0.05 F
	Phosphate, curacao		36 Ca	14 P	0.3 Na 0.54 F
	Defluorinated		30-34 Ca	18 P	5.7 Na 0.16 F
	Dicalcium		18-24 Ca	18.5 P	0.6 Na 0.14 F
	Mono and dicalcium		16-19 Ca	21 P	0.6 Na 0.20 F
	Soft rock		17 Ca	9 P	0.1 Na 1.2 F
	Sodium tripolyphosphate		0 Ca	25 P	31 Na 0.03 F
Phosphorus	Phosphoric acid	H_3PO_4	0 Ca	23 P	
	Tricalcium phosphate	Ca_3PO_4	31-34 Ca	18 P	
Magnesium	Magnesium oxide	MgO	54 Mg		
	Magnesium sulfate	$MgSO_4$	9 Mg		
Potassium	Potassium citrate		36 K		
	Potassium chloride	KCl	50 K		
	Potassium sulfate	K_2SO_4	42 K		
Sodium and chloride	Sodium chloride	NaCl	39 Na	61 Cl	
	Sodium acetate		28 Na		
	Sodium tripolyphosphate		32 Na	25 P	
Iron	Ferrous sulfate	$FeSO_4•H_2O$	33 Fe		
	Ferrous sulfate	$FeSO_4•7H_2O$	20 Fe		
	Ferric ammonium citrate		16.5-18.5 Fe		
	Ferrous fumarate	$FeC_4•H_2O_4$	32.9 Fe		
	Ferric chloride	$FeCl_3•6H_2O$	20.7 Fe		
	Ferrous carbonate	$FeCO_3$	48.2 Fe		
	Ferric oxide	Fe_2O_3	69.9 Fe		
	Ferrous oxide	FeO	77.8 Fe		
Copper	Cupric carbonate	$CuCO_3•Cu(OH)_2$	57.5 Cu		
	Cupric chloride	$CuCl_2•2H_2O$	37.3 Cu		
	Cupric hydroxide	$Cu(OH)_2$	65.1 Cu		
	Cupric oxide	CuO	79.9 Cu		
	Cupric sulfate	$CuSO_4•5H_2O$	25.4 Cu		
Manganese	Manganese carbonate	$MnCO_3$	47.8 Mn		
	Manganous chloride	$MnCl_2•4H_2O$	27.8 Mn		
	Manganous oxide	MnO	77.4 Mn		
	Manganese sulfate	$MnSO_4•5H_2O$	22.7 Mn		
	Manganous sulfate	$MnSO_4•H_2O$	32.5 Mn		
Zinc	Zinc carbonate	$5ZnO•2CO_3•4H_2O$	56.0 Zn		
	Zinc chloride	$ZnCl_2$	48.0 Zn		
	Zinc oxide	ZnO	72.0 Zn		
	Zinc sulfate	$ZnSO_4•7H_2O$	22.7 Zn		
	Zinc sulfate	$ZnSO_4•H_2O$	36.4 Zn		
Iodine	Calcium iodate	$Ca(IO_3)_2$	65.1 I		
	Potassium iodide	KI	76.4 I		
	Cuprous iodide	CuI	66.6 I		
	Iodized salt		48.2 mg/kg I		
Selenium	Sodium selenite	Na_2SeO_3	45.6 Se	26.6 Na	
	Sodium selenate	Na_2SeO_4	41.8 Se	24.3 Na	

*Adapted from National Research Council. Nutrient Requirements of Cats. Washington, DC: National Academy of Sciences, 1986.
**Actual mineral levels in technical grade sources may vary.

phatase concentrations. Again, this is lower than the 0.5% DM phosphorus level currently recommended by AAFCO (2007). Levels of phosphorus exceeding 0.6% DM were associated with lower plasma phosphorus concentrations, reduced creatinine clearance and decreased magnesium absorption. Thus, the authors concluded continued feeding of high levels of dietary phosphorus might be detrimental to renal function. The AAFCO (2007) recommendation for phosphorus, for both dogs and cats, is 0.8% for growth and reproduction and 0.5% for adult maintenance (DM). These values (0.72 and 0.26% [DM] for kittens and adult cats, respectively) are higher than those listed in the current NRC (2006).

In general, meat tissue (poultry, lamb, fish, beef) is high in phosphorus. Eggs and milk products are also relatively rich in phosphorus. Oilseeds, protein supplements and grains likewise contribute significant amounts of phosphorus to pet foods, due more to their high inclusion rate than to high-phosphorus concentrations. A number of phosphorus supplements (**Table 6-2**) are used in pet foods, including calcium phosphate (monocalcium, dicalcium and tricalcium phosphate, defluorinated rock phosphate), sodium phosphates and phosphoric acid.

Magnesium

Magnesium is the third largest mineral constituent of bone, after calcium and phosphorus. Magnesium is involved in the metabolism of carbohydrates and lipids and acts as a catalyst for a wide array of enzymes. It is required for cellular oxidation (e.g., ATP production), it catalyzes most phosphate transfers (e.g., alkaline phosphatase, hexokinase and deoxyribonuclease) and it exerts a potent influence on neuromuscular activity. In light of these functions, it is not surprising that magnesium deficiency in animals is manifested clinically in a wide range of disorders, which include retarded growth, hyperirritability and tetany, peripheral vasodilatation, anorexia, muscle incoordination and convulsions. Other metabolic aberrations that may occur in magnesium-deficient animals include calcification of the kidneys and liver, decreased blood pressure and body temperature and decreased thiamin concentrations in tissues (Underwood and Mertz, 1987).

From 20 to 70% of dietary magnesium is absorbed (Brody, 1994). Intestinal magnesium absorption represents the sum of both a carrier-mediated system at low intraluminal concentrations, and simple diffusion at higher concentrations. A number of dietary and physiologic factors negatively influence magnesium absorption, including high levels of dietary phosphorus, calcium, potassium, fat and protein.

The kidneys play a critical role in magnesium homeostasis. Approximately 70% of serum magnesium is filtered by glomeruli; healthy kidneys reabsorb about 95% of the filtered magnesium (Shils, 1996). Several physiologic and metabolic factors, drugs and disease states influence magnesium reabsorption in nephrons. Certain drugs, such as diuretics, aminoglycosides, cisplatin, cyclosporin, amphotericin and methotrexate, can cause increased renal wasting of magnesium (Freeman, 1995).

Avoiding excess dietary magnesium is recommended for the prevention of struvite urinary precipitates in cats and dogs; however, magnesium deficiency is reported to increase the risk of calcium oxalate urolithiasis in rats (Driessens and Verbeeck, 1990). Furthermore, magnesium supplementation has been advocated to prevent calcium oxalate urolithiasis in people. However, this practice is very controversial because clinical trials have demonstrated mixed efficacy. The relationship of magnesium to feline and canine urinary calcium oxalate precipitates is unknown; however, ensuring magnesium concentrations above the minimum requirement is considered safe (Chapters 40, 43 and 46).

Conversely, increased magnesium supplementation may be warranted under certain clinical conditions in which magnesium stores are depleted. The GI tract and kidneys are the primary potential routes for magnesium excretion. Magnesium deficiencies may also result from renal losses secondary to renal tubular acidosis, hypercalcemia, hyperthyroidism, hypoparathyroidism and use of diuretics. Additionally, epidemiologic data and rat studies suggest that low urinary magnesium to calcium ratios may increase the risk for calcium oxalate formation (Driessens and Verbeeck, 1990). **Table 6-1** describes signs of deficiency and excess.

The minimum requirement for magnesium in adult cats has been evaluated (Pastoor, 1993). Four levels of magnesium ($MgCO_3$) were compared. Positive mineral balance was observed even at the lowest magnesium level (0.02% DM) and no adverse effects were noted in serum magnesium and alkaline phosphatase concentrations. This magnesium level is half of the current NRC (2006) and AAFCO recommendation (2007). Extrapolation of these results, which were obtained by feeding semi-purified diets, to commercial foods should be made cautiously because of the differences in ingredients used and the greater potential for mineral antagonisms and decreased availability that may occur in practical diets. AAFCO (2007) recommends 0.08% DM magnesium for growth and reproduction and 0.04% DM magnesium for adult maintenance for cats (AAFCO, 2007). The AAFCO (2007) magnesium recommendation for dogs is 0.04% DM for both lifestages, whereas the current NRC (2006) suggests 0.04% DM for puppies and 0.06% DM for adult dogs.

Ingredients containing bone (bone meal, lamb meal), oilseed/protein supplements (flaxseed, soybean meal and other legumes such as pea protein) and unrefined grains and fiber sources (wheat bran, oat bran, beet pulp, soymill run) are rich in magnesium. Common magnesium supplements include magnesium oxide and magnesium sulfate.

Potassium

Potassium is the most abundant intracellular cation and the third most abundant mineral in the body. Potassium is involved in a number of functions, including: 1) maintaining acid-base balance, 2) maintaining osmotic balance, 3) transmitting nerve impulses, 4) facilitating muscle contractility and 5) serving as a cofactor in several enzyme systems (energy transfer and use, protein synthesis and carbohydrate metabolism).

Potassium is absorbed primarily by simple diffusion from the upper small intestine, although some absorption also occurs in the lower small intestine and large intestine. Potassium availability is relatively high (95% or higher) for most foodstuffs (McDowell, 1992). Yet, in contrast to most minerals, potassium is not readily stored and must be supplied daily in the diet. Thus, it is important that foods for dogs and cats contain adequate potassium. Increased intake of potassium is unlikely to cause sustained hyperkalemia unless renal excretion of potassium is impaired. Administration of certain drugs predisposes patients to hyperkalemia (e.g., nonspecific β-adrenergic blockers and angiotensin-converting enzyme inhibitors).

Table 6-1 describes signs of deficiency and excess. Increasing protein, energy density or chloride, and other factors such as stress (heat, exercise, vomiting and diarrhea) and milk production increase the requirement for potassium. AAFCO (2007) recommends 0.6% DM potassium for both dogs and cats for all lifestages. These levels are higher than the current NRC (2006) DM potassium recommendation of 0.44% for puppies, 0.40% for adult dogs, 0.40% for kittens and 0.52% for adult cats.

Rich sources of potassium include soybean meal, unrefined grains and fiber sources (soymill run, sunflower hulls, rice bran, wheat bran) and yeast. Potassium supplements commonly

added to pet foods include potassium citrate, potassium chloride and potassium sulfate.

Sodium and Chloride

Sodium and chloride, in addition to potassium, are important for maintaining osmotic pressure, regulating acid-base equilibrium and transmitting nerve impulses and muscle contractions via Na-K-ATPase (sodium pump). In addition, sodium and chloride control the passage of nutrients into cells. Sodium ions must be present in the lumen of the small intestine for absorption of sugars and amino acids. Insufficient sodium concentrations decrease the use of digested protein and energy. Sodium also influences calcium absorption and mobilization and may affect absorption of several water-soluble vitamins (e.g., riboflavin, thiamin and ascorbic acid) that are sodium coupled (McDowell, 1992).

Sodium and chloride are readily absorbed, principally from the upper small intestine, and excreted predominantly in the urine with smaller amounts in feces and perspiration. Marked losses of salt can occur through perspiration in some species, secretion in milk, vomiting and diarrhea. When sodium intake is inadequate, the body has a remarkable capacity for conserving sodium by excreting extremely low levels in the urine. Chloride metabolism is controlled in relation to sodium. For example, excess urinary excretion of sodium is accompanied by urinary excretion of chloride.

Hormones acting to maintain a constant sodium-potassium ratio in extracellular fluid regulate sodium concentrations in the body. Aldosterone, secreted from the adrenal cortex, regulates reabsorption of sodium from the renal tubules. Antidiuretic hormone from the posterior pituitary responds to osmotic pressure changes in the extracellular fluid. Both hormones maintain a constant sodium-potassium ratio.

A number of factors influence the sodium requirement. The requirement is increased during reproduction, lactation, rapid growth and heat stress and with high dietary potassium levels. In people, the average sodium intake exceeds the recommended requirement by 15-fold (Stamler, 1995). Likewise, the sodium content of certain pet foods exceeds the recommended level by four- to 15-fold (Chapter 36). Investigators determined the sodium requirement of kittens to be 0.16% (DM or 0.30 mg Na/kcal ME) based on aldosterone concentration in plasma (Yu and Morris, 1997). The same investigators determined the requirement for adult cats was 0.08% DM sodium or 0.15 mg Na/kcal ME (Yu and Morris, 1999). The AAFCO (2007) recommendation for sodium in cats is 0.2% DM for both lifestages, whereas in dogs, the recommendation is 0.3% DM for growth and reproduction and 0.06% DM for adult maintenance. The current NRC (2006) sodium recommendation is 0.14% DM for kittens and 0.068% DM for adult cats. The NRC (2006) suggests a 0.22% minimum DM sodium requirement for puppies and 0.08% DM for adult dogs.

High sodium intake has long been reported to increase the risk of hypertension in people and animals (Stamler, 1995). Pet populations with increased risk of hypertension include senior dogs and cats and those with renal disease, cardiac disease, hyperthyroid disease or obesity. Pet food manufacturers sometimes use dietary salt supplementation to increase water intake in cats with lower urinary tract disease. High sodium intake in the short term effectively increases water intake, urine output and urine dilution, thus lowering the risk of urolithiasis, but may have detrimental effects in the long term. Kirk (2002) showed that high-sodium intake (1.1% DM) over a three-month period, increased serum urea nitrogen, phosphorus and creatinine concentrations in cats with preexisting renal disease. In addition, the high-sodium food increased cardiac left ventricular fractional shortening and lowered plasma aldosterone levels, evidence suggesting that this sodium chloride load required both the heart and kidney to work harder. Similarly, both the NRC Mineral Tolerance of Animals (2005) and the NRC Nutrient Requirements of Dogs and Cats (2006) recommend a safe upper limit for sodium at 1.5% DM for adult dogs, and 1.0 and 1.5% DM for kittens and adult cats, respectively. At higher levels of sodium intake (≥2%), studies showed reduced food intake, negative potassium balance and vomiting.

In the absence of studies establishing chloride requirements for dogs or cats, the DM recommendation for chloride is 1.5 times that of sodium. This value is comparable to the Na:Cl requirement ratio for other species. **Table 6-1** describes signs of deficiency and excess (**Case 6-1**).

The effect of dietary sodium chloride on blood pressure has generally been attributed to the sodium ion. However, it is clear from a number of studies that both sodium and chloride are necessary to inhibit renin production (Kotchen et al, 1978; Kurtz et al, 1987). Salts such as sodium chloride, potassium chloride, lysine hydrochloride (but not lysine glutamate, sodium bicarbonate, potassium bicarbonate) inhibited renin production in sodium chloride-deprived rats and people.

Fish, eggs, dried whey, poultry by-product meal and soy isolate are ingredients high in sodium and chloride. Sodium and/or chloride supplements typically added to pet foods include salt, sodium phosphates, calcium chloride, choline chloride, potassium chloride and sodium acetate.

Microminerals

Iron

Iron is present in several enzymes and other proteins responsible for oxygen activation (oxidases and oxygenases), for electron transport (cytochromes) and for oxygen transport (hemoglobin, myoglobin). Because of the limited capacity of the body to excrete iron, iron homeostasis is maintained primarily by adjusting iron absorption. Iron in foods exists in two forms: 1) heme iron present in hemoglobin and myoglobin and 2) nonheme iron present in grains and plant sources.

Heme iron absorption is not greatly affected by iron status or other dietary factors. (Two exceptions are meat, which enhances heme iron absorption, and calcium, which inhibits heme and nonheme iron absorption.) In contrast to absorption of heme iron, absorption of nonheme iron is markedly influenced by iron status and by several dietary factors such as phytate, tannins and excesses of calcium, phosphorus, manganese, zinc, copper and ascorbic acid (Hallberg and Rossander-

Hulthen, 1993).

The amount of iron absorbed from food is thus determined by three factors: 1) iron status of the body, 2) availability of dietary iron (as affected by other ingredients and nutrients) and 3) amounts of heme and nonheme iron in food (Hallberg and Rossander-Hulthen, 1993).

Iron is transported by plasma and is taken up by the bone marrow for hemoglobin synthesis. Although a small amount of hemoglobin circulates in plasma, by far the greatest amount of plasma iron is complexed to the specific iron-binding β_1-globulin transferrin. The degree of saturation of transferrin affects deposition of iron in liver stores and the supply of iron to red blood cell precursors. At saturation levels above 60%, much of the iron is deposited in the liver. Under normal conditions, only 30 to 40% of the transferrin is saturated; the remaining 60 to 70% represents an unbound or latent reserve (Morris, 1987).

Iron is stored predominantly as ferritin and hemosiderin in liver, bone marrow and spleen. Normally, iron is stored primarily as ferritin. As tissue iron concentrations increase, however, the concentration of hemosiderin increases more than that of ferritin. Excretion of iron is limited. Only negligible amounts of iron appear in urine; the iron appearing in feces is predominantly unabsorbed iron. Iron is continuously lost in sweat, hair and nails.

Investigators determined that the iron requirement of kittens and puppies fed a phytate-free purified diet is 80 mg iron/kg of food (DM) (Chausow and Czarnecki-Maulden, 1987). This requirement is the AAFCO (2007) recommendation for iron for dogs and cats, for both growth/reproduction and adult maintenance lifestages. The new NRC (2006) recommends a minimum of 88 mg/kg DM iron for growth and 30 mg/kg DM iron for adult dogs. Similar to AAFCO allowances (2007), the minimum NRC (2006) iron recommendation for cats is 80 mg/kg DM for growth and adult lifestages. Most pet foods are high in iron because of the high iron concentrations found in meat ingredients, especially organ meats. Furthermore, studies have shown the availability of iron to be relatively high from liver, muscle and animal by-products (Elvehjem et al, 1933; Conrad et al, 1980). Consequently, iron deficiency is not of practical concern with most pet foods.

Although iron levels may be high in pet foods (levels sometimes exceed the requirement by 15-fold without supplementation), AAFCO has set a maximum level of 3,000 mg iron/kg of food for dogs (no maximum is established for cats), which clearly exceeds dietary concentrations of iron in most typical pet foods. Iron excesses should be avoided because of potential antagonism with other minerals (e.g., zinc and copper). **Table 6-1** lists signs of deficiency and excess.

Chronic blood loss eventually depletes iron reserves and causes a microcytic, hypochromic anemia. The most common chronic blood loss in dogs and cats occurs with blood-sucking intestinal (hookworms) and external (fleas, ticks) parasites. Young puppies and kittens are especially vulnerable because of the low-iron content of milk.

Iron concentrations are high in most meat ingredients, especially organ meats such as liver, spleen and lungs. Other ingredients rich in iron include dicalcium phosphate and fiber sources such as beet pulp, soymill run and peanut hulls. In fact, poultry studies have shown that the iron contained in dicalcium phosphate alone in a corn-soybean meal diet can meet a chick's requirement for iron (Deming and Czarnecki-Maulden, 1989).

Typical iron sources include ferrous sulfate, ferric chloride, ferrous fumarate, ferrous carbonate and iron oxide. The iron in iron oxide, however, is not biologically available. Iron oxide is often added to pet foods to impart a "meaty red" color. A relatively high level of iron oxide is added (up to 0.04%) when iron oxide is used as a pigment in pet foods. Analytically, a pet food containing iron oxide will appear to be high in iron, but may not be high in available iron. Thus, the contribution of iron from iron oxide should be considered when evaluating the iron adequacy of foods containing iron oxide (e.g., 0.04% DM iron oxide in a moist food contributes 933 mg iron/kg of food).

Zinc

Zinc is a constituent or activator of more than 200 enzymes, so it is involved in a number of diverse physiologic functions. Some of zinc's primary functions include: 1) nucleic acid metabolism, 2) protein synthesis, 3) carbohydrate metabolism, 4) immunocompetence, 5) skin and wound healing, 6) cell replication and differentiation, 7) growth and 8) reproduction. Zinc also interacts with hormone production, most notably testosterone, adrenal corticosteroids and insulin. Zinc homeostasis is controlled through absorption and excretion.

The mechanism and control of zinc absorption are still not fully understood. Zinc absorption occurs primarily in the duodenum, jejunum and ileum. Only small amounts are absorbed from the stomach. Zinc absorption is markedly affected by other dietary components. Phytate, for example, decreases zinc absorption, whereas low molecular weight binding ligands such as citrate, picolinate, ethylenediaminetetraacetic acid (EDTA) and amino acids such as histidine and glutamate enhance zinc absorption (Hambidge et al, 1986). The liver is the primary organ involved in zinc metabolism. When hepatic zinc content is increased above normal levels, additional zinc is associated with metallothionein, a metal-binding protein thought to have a role in storage and detoxification of zinc, copper, cadmium and other metals.

Zinc in plasma is bound to protein in two forms: 1) firmly bound zinc that appears to bind to globulin (approximately 33% of total plasma zinc) and 2) loosely bound zinc complexed with albumin (66% of total plasma zinc) (McDowell, 1992). Storage of zinc is limited except in bone; stores increase only slightly as dietary zinc increases. Zinc concentration in bone has been used as a measure of zinc absorption and/or zinc status in young growing animals, whereas plasma zinc is only a reliable index under controlled experimental conditions.

Zinc is excreted primarily in the feces as unabsorbed and endogenous zinc (pancreatic juice, bile, other digestive secretions). Excretion of endogenous zinc in feces varies according to the balance between true absorption and metabolic needs. Variable excretion is one of the primary mechanisms used to

maintain zinc homeostasis. Thus, both absorption and excretion are important in regulating zinc balance.

Zinc deficiency is probably more of a practical concern with pet foods than is toxicity, because: 1) zinc is relatively nontoxic and 2) its availability is decreased by a number of factors (phytate, high dietary levels of calcium, phosphate, copper, iron, cadmium and chromium). The antagonistic effects of calcium are greatest when phytate is also present, resulting in the formation of a highly insoluble complex of calcium, phytate and zinc. Signs of zinc deficiency have been reported to occur in dogs fed cereal-based dry foods (e.g., grains may contain significant concentrations of phytate), even when the zinc content of the food exceeded NRC minimum requirements (NRC, 2006; Morris and Rogers, 1994).

AAFCO recommends 120 mg/kg DM zinc for dogs and 75 mg/kg DM zinc for cats (2007). For trace minerals, AAFCO makes the same recommendations for adult maintenance and growth/reproduction foods. In livestock, however, the requirement for zinc is greatly increased during growth and reproduction. NRC (2006) recommends a minimum of 100 mg/kg DM zinc for growth vs. 60 mg/kg DM zinc for adult dogs; NRC makes similar zinc recommendations for cats (i.e., 75 and 74 mg/kg DM zinc in foods for growth and adults, respectively).

Signs of zinc deficiency include anorexia, decreased growth rate, alopecia, parakeratosis, impaired reproduction, depressed immune function and growth disorders of the skeleton (Chapter 33). Naturally occurring zinc-responsive dermatoses have been described (Chapter 32).

Although relatively nontoxic, excess dietary zinc can interfere with other minerals (iron and copper), thus excesses should be avoided. The only reported cases of zinc toxicosis in dogs or cats have been due to dietary indiscretion (e.g., consumption of die-case nuts from animal carriers or pennies) (**Case 6-2**). **Table 6-1** describes effects of zinc deficiency and excess. Ingredients naturally high in zinc include most meats, fiber sources and dicalcium phosphate. Zinc supplements most commonly used in pet foods are zinc oxide, zinc sulfate, zinc chloride and zinc carbonate.

Copper

Of the many copper-containing proteins, four enzyme systems may play key roles in the clinical signs associated with copper deficiency: 1) the ferroxidase activity of ceruloplasmin explains in part the disturbances of hematopoiesis in copper deficiency, 2) the monoamine oxidase enzymes may account for the role of copper in pigmentation and control of neurotransmitters and neuropeptides, 3) lysyl oxidase is essential for maintaining the integrity of connective tissue, a function that explains disturbances in lungs, bones and the cardiovascular system and 4) the copper enzymes cytochrome C oxidase and superoxide dismutase (SOD) play a central role in the terminal steps of oxidative metabolism and the defense against superoxide radicals, respectively. These functions have been postulated to account for the disturbances of the nervous system as seen in neonatal ataxia in several animal species with copper deficiency (Davis and Mertz, 1987).

In most species, copper can be absorbed in all segments of the GI tract; however, the small intestine is the major site of absorption (Davis and Mertz, 1987). Although the biochemical mechanisms are not fully understood, there is good evidence that intestinal absorption of copper is regulated by the need of the animal and that metallothionein (a metal-binding protein) plays a key role in regulation. Copper appears to be absorbed by two mechanisms, one saturable, suggesting active transport at low dietary copper concentrations and the other unsaturable, suggesting simple diffusion at high dietary copper levels.

Most copper in plasma is bound to ceruloplasmin, a copper-binding protein. Newly absorbed copper, however, may be transported from the intestine loosely bound to albumin or certain amino acids. In this form, the element is readily available to the liver and other tissues, in contrast to the much more tightly regulated distribution of ceruloplasmin-bound copper. This difference in availability may explain the tissue damage caused by copper accumulation in hepatotoxicosis seen in Bedlington terriers and people with Wilson's disease, in which the ceruloplasmin transport protein is lacking.

The liver is the central organ for copper metabolism. Hepatic concentrations reflect an animal's intake and copper status. Copper is excreted primarily through the feces. Most fecal copper is unabsorbed, but active excretion also occurs via the bile. Copper homeostasis is maintained primarily through absorption.

Dietary copper deficiency has been reported to occur in dogs and cats and thus is of practical concern (Morris and Rogers, 1994) (**Case 6-3**) Availability of copper from different foods and supplements can vary greatly, so the requirement for copper is difficult to define. The requirement can vary several-fold depending on the source of copper in the food and the level of other ingredients/nutrients/non-nutrients (e.g., interactions with phytate, calcium, zinc and iron). The AAFCO (2007) recommendation for copper in dogs is 7.3 mg/kg DM.

Separate copper requirements are recommended for extruded cat foods (15 mg/kg DM) vs. moist cat foods (5 mg/kg DM) during growth/reproduction. The recommended AAFCO (2007) copper level for maintenance of adult cats is 5 mg/kg DM, regardless of the food form. The rationale for separate copper requirements for cats (extruded vs. canned) is unclear. Investigators demonstrated increased needs for copper during reproduction in queens fed extruded foods.[a] Separate requirements for extruded and canned foods were recommended in the absence of reproduction data for cats fed moist foods.

Researchers studied chicks to evaluate the availability of copper from feed ingredients typically used in pet foods (Aoyagi et al, 1993). Results showed that copper availability was essentially zero from copper oxide and pork liver. Beef, sheep and turkey liver, however, were highly available sources of copper. AAFCO (2007) has recommended that pet food companies discontinue the use of copper oxide as a copper source based on studies of swine, poultry, dogs and cats in which researchers have demonstrated the poor availability of copper from copper oxide (Aoyagi and Baker, 1993; Cromwell et al, 1989; Czarnecki-

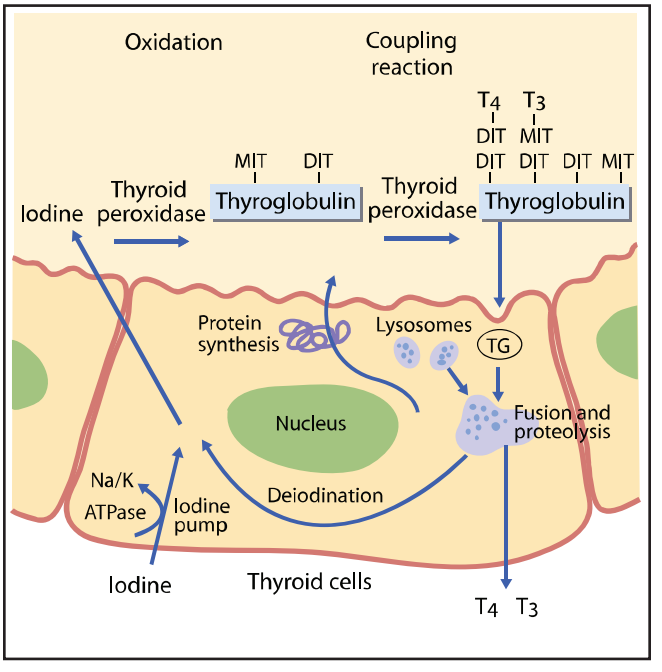

Figure 6-3. Diagram showing pathways of thyroid-hormone synthesis from iodine within the thyroid gland. (Adapted with permission from Hetzel BS, Maberly GF. Iodine. In: Mertz W, ed. Trace Elements in Human and Animal Nutrition, 5th ed. San Diego, CA: Academic Press Inc, 1986; 147.) See text for details.

Mauldin et al, 1993; Fascetti et al, 1998, 2000).

Signs of copper deficiency in cats include poor reproductive performance, early fetal loss, fetal deformities, cannibalism, coat hypopigmentation, kinked tails and inverted carpi.[a] Clinical signs in dogs include hair depigmentation and hyperextension of the distal forelimbs (Zentek and Meyer, 1991). **Table 6-1** lists signs of deficiency and excess.

Copper excess in dogs and cats with normal metabolism is of much less practical concern than copper deficiency, but can interfere with iron and zinc use. Bedlington, West Highland white and Skye terriers, however, are predisposed to hereditary autosomal recessive disease resulting in copper hepatoxicosis (Chapter 68). Anti-copper therapies such as zinc supplementation and orally administered tetrathiomolybdate have been used to treat dogs with this genetic disorder (NRC, 2006). AAFCO (2007) has set a safe upper limit of 250 mg/kg DM copper for dogs, but no safe upper limit for cats. NRC (2006) lists no safe upper limit of copper for either dogs or cats.

Most meat ingredients, especially organ meats, are rich in copper. Ruminant livers are extremely high in copper; concentrations are five to 10 times higher than in monogastric livers.[b] Typical copper supplements include cupric sulfate, cupric carbonate and cupric chloride.

Manganese

Manganese deficiency is of little practical relevance in dogs and cats, but is of practical relevance in birds. AAFCO (2007) recommends 5 mg/kg DM manganese for dogs and 7.5 mg/kg DM for cats, which is similar to NRC (2006) recommendations. The manganese requirement for birds is 10 to 12 times higher than that for people, pigs, dogs and cats (McDowell, 1992). Manganese functions as an enzyme activator or as a constituent of metalloenzymes. Although there are only a few manganese-containing metalloenzymes (e.g., arginase, pyruvate carboxylase and manganese-superoxide dismutase), many enzymes are activated by manganese, including hydrolases, kinases, decarboxylases and transferases. Other cations (especially magnesium), however, can partially substitute for manganese with little or no loss in enzymatic activity, thus manganese deficiency may not adversely affect physiologic or metabolic function (McDowell, 1992).

Manganese is also essential in bone and cartilage development because it activates glycosyltransferases (i.e., enzymes important for polysaccharide and glycoprotein synthesis). In addition, manganese is involved in reproduction and lipid metabolism (e.g., manganese is involved in the biosynthesis of choline and cholesterol).

Manganese homeostasis is maintained through regulation of absorption and excretion. Manganese is absorbed throughout the small intestine in a rapidly saturable process. Low molecular weight ligands, such as L-histidine and citrate, enhance absorption, whereas excessive concentrations of phosphorus, iron and cobalt can reduce absorption. Manganese is excreted via several routes that combine to provide an efficient homeostatic mechanism to regulate manganese levels in tissues. Bile flow is the primary route of excretion, but manganese is also excreted in pancreatic juice and in the small intestine.

Table 6-1 lists signs of manganese deficiency and excess. Ingredients rich in manganese include fiber sources, menhaden fish meal and dicalcium phosphate. Manganese supplements include manganese oxide, manganese sulfate, manganous chloride and manganese carbonate.

Iodine

Iodine is a constituent of the thyroid hormones 3,5,3',5'-tetraiodothyronine (thyroxine, T_4) and 3,5,3'-triiodothyronine (T_3). Thyroid hormones have an active role in thermoregulation, intermediary metabolism, reproduction, growth and development, circulation and muscle function. Thyroid hormones also: 1) influence physical and mental growth and differentiation and maturation of tissues, 2) affect other endocrine glands, especially the hypophysis and the gonads, 3) influence neuromuscular functioning and 4) have an effect on the integument, hair and fur (McDowell, 1992).

The thyroid glands actively trap iodine daily to ensure an adequate supply of thyroid hormone. This trapping mechanism regulates a more or less constant iodine supply to the thyroid glands over a wide range of plasma iodide levels. **Figure 6-3** outlines the steps of thyroid-hormone biosynthesis (Hetzel and Maberly, 1986). Iodine trapping is an active transport mechanism linked to Na^+/K^+-ATPase activity. Thyroid-stimulating hormone, which is released from the pituitary gland to regulate thyroid activity, also regulates this mechanism. A thyroid-peroxidase enzyme oxidizes iodide, which is released from thyroid

cells into the colloid space. Iodine then combines with tyrosine residues associated with thyroglobulin protein to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). The oxidation process proceeds further under the influence of the thyroid-peroxidase enzyme to couple MIT and DIT to form various iodothyronines (e.g., T_3 and T_4). Finally, iodinated thyroglobulin and thyroid hormones are reabsorbed into the thyroid cells and exposed to proteolytic enzymes. Much of the protein and iodinated tyrosines are lysed and returned as substrates to repeat the process. At the same time, some thyroid hormones are released into the circulation. Regulating the action of thyroid hormones is a complex process involving interaction among neurotransmitters, hormones and enzymes in the central nervous system, the pituitary gland, the thyroid glands, the circulation and peripheral tissues.

Investigators have estimated the iodine requirement for adult dogs to be 0.56 mg/kg DM (Belshaw et al, 1975). AAFCO (2007) recommends an iodine level of 1.5 mg/kg DM for dogs. This margin of safety is prudent in practical foods to overcome potential effects of goitrogens and negative mineral antagonisms.

A recent study (Wedekind et al, In press) estimated the iodine requirement for adult cats to be 0.46 mg/kg DM. This estimate was based upon three measurements of iodine status: regression of Tc 99m thyroid:salivary ratio (scintigraphy), iodine balance and urinary iodine excretion after iodine intake. These estimates agreed closely with the iodine requirement determined for dogs (Belshaw et al, 1975) and people (DRI, 2001). This estimate is higher than current AAFCO (2007) minimum iodine recommendations for adult cats (i.e., 0.35 mg/kg DM iodine), but is much lower than the NRC (2006) recommended iodine allowance (1.4 mg iodine/kg diet). Note that the NRC (2006) recommendation does not agree closely with current AAFCO recommendations and was based upon data derived from two studies (Scott et al, 1961; Ranz et al, 2002). Unfortunately, these studies should not have been used as a basis for establishing the iodine requirement of cats. The Scott (1961) study used a nutritionally incomplete or imbalanced diet (i.e., the diet used was an all meat diet [beef hearts], which was grossly deficient in calcium). The Ranz (2002) study was of short duration (i.e., 54 days total with only a seven-day period for each iodine level). In this study, the minimum iodine level evaluated, approximately 4.1 mg/kg iodine, was not low enough to yield a valid iodine requirement estimate. Interestingly, the previous NRC (1986) did not cite the Scott (1961) reference.

The iodine requirement is influenced by physiologic state and diet. Lactating animals require more dietary iodine because about 10% of the iodine intake is normally excreted in milk (McDowell, 1992). Likewise, the presence of goitrogenic substances and nutrient excesses of certain minerals (e.g., arsenic, bromide, fluoride, cobalt, manganese, calcium and potassium) may increase the need for iodine (NRC, 2005). Potential sources of goitrogens in pet foods include peas, peanuts, soybeans and flaxseed. Fish, eggs and iodized salt are rich sources of iodine, whereas most animal proteins are moderate sources of iodine. Iodine supplements typically used in pet foods include calcium iodate, potassium iodide and cuprous iodide.

Since the late 1970s, feline hyperthyroidism has become a more frequently diagnosed condition. However, much remains to be learned about this endocrinopathy (e.g., prevalence and cause). Hypothyroidism is a much more prevalent thyroid disorder in dogs. Both iodine excess and deficiency may result in subclinical or overt thyroid dysfunction.

Current AAFCO (2007) guidelines set a maximum safe level for iodine for dogs at 50 mg/kg, whereas NRC (2006) recommends 4 mg/kg as a safe upper limit. Neither NRC nor AAFCO sets a safe upper limit for iodine for cats. However, estimates for establishing safe upper limits and/or lowest observable adverse effect level for cats were determined in a recent study (Wedekind et al, In press). In people, guidelines have been established to define deficiency, adequacy and iodine excess (Laurberg et al, 2001) based upon urinary iodine concentrations. When these guidelines are applied to cats (corrected for metabolic equivalent basis; $BW^{0.67}$), dietary intakes between 0.46 and 3.5 mg/kg were considered optimal, whereas dietary intakes exceeding 3.5 mg/kg were defined as excessive. In addition, cats fed the highest dietary iodine intake (8.8 to 9.2 mg/kg iodine) for one year had significantly reduced FT_4 and numerically lowered TT_4 and TT_3 at 12 months. Thus, these findings suggest 3.5 mg/kg as a no observable adverse effect level or safe upper level and intakes of 8.8 mg/kg as a lowest observable adverse effect level. **Table 6-1** lists signs of iodine deficiency and excess. See Chapter 29 for more information about iodine.

Selenium

Selenium is an essential constituent of glutathione peroxidase, which helps protect cellular and subcellular membranes from oxidative damage. Glutathione peroxidase and vitamin E work synergistically to reduce the destructive effects of peroxidative reactions on living cells. Selenium spares vitamin E in at least three ways: 1) preserves the integrity of the pancreas, which allows normal fat digestion, and thus normal vitamin E absorption, 2) reduces the amount of vitamin E required to maintain integrity of lipid membranes via glutathione peroxidase and 3) aids retention of vitamin E in the blood plasma in some unknown way.

Vitamin E reduces the selenium requirement in at least two ways: 1) maintains body selenium in an active form, or prevents losses from the body and 2) prevents destruction of lipids within membranes, thereby inhibiting production of hydroperoxides and reducing the amount of the selenium-dependent enzyme needed to destroy peroxides formed in cells (Scott et al, 1982). Selenium also has a vital role in maintaining normal thyroid hormone and iodine metabolism, particularly through the control of deiodinase enzymes that regulate conversion of T_4 to T_3 (Arthur, 1993).

The duodenum is the main site of selenium absorption. There is no homeostatic control of selenium absorption regardless of the dietary selenium concentration. Likewise, selenium status also appears to have little effect on selenium uptake. Excretion of selenium, however, is homeostatically regulated. Urinary

excretion of selenium is closely related to dietary intake in rats and people (Levander, 1986). A dietary threshold is reached at low-selenium intakes, wherein excretion is shut down, thus conserving selenium. Urinary selenium increases proportionally at higher selenium intakes. Fecal excretion, on the other hand, remains constant over a wide range of selenium intakes.

Although selenium deficiency has been observed experimentally in dogs (Van Vleet, 1975), the incidence of selenium deficiency has not been reported for dogs and cats. Likewise, selenium toxicity has not been noted in dogs and cats, despite high concentrations (>4 mg selenium/kg food) in seafood and fish-containing cat foods. AAFCO (2007) selenium recommendations are 0.11 mg/kg of food DM for dogs and 0.1 mg/kg of food DM for cats. The NRC (2006) recommended allowance is 0.35 and 0.30 mg DM selenium/kg diet for dogs and cats, respectively (growth and adult recommendations were not different). This increase in minimum recommendations, relative to previous NRC publications, takes into account potentially low availability of selenium in pet food ingredients.

Selenium availability is highly influenced by the chemical form of selenium (supplied as a supplement or from foodstuffs). Furthermore, the requirement for selenium can be partially replaced by vitamin E. Selenium in animal feeds is highly variable primarily due to the variable selenium status of soils. Studies with pigs demonstrated that inorganic selenium (sodium selenite) and organic selenium (selenium yeast) were equally effective in supporting glutathione peroxidase activity, but that selenium stores in tissues (liver and muscle) were greater when organic selenium was fed (Mahan, 1995). Selenium content in milk is also higher when selenium is supplied in the organic form.

Fish products are rich in selenium (1 to 6 mg selenium/kg), but selenium availability in these ingredients is low (Wedekind et al, 1997; Wedekind et al, 1998; Combs and Combs, 1986). Selenium levels exceeding 2 mg selenium/kg of food DM have not been reported to be toxic for cats. Cats may be able to tolerate higher selenium levels because the high-protein foods typically fed to cats are protective against high-selenium levels (Levander, 1986).

Current AAFCO (2007) guidelines set a maximum safe selenium level for dogs at 2 mg/kg; however, NRC (2006) does not list a safe upper limit for cats. Neither NRC nor AAFCO has listed a safe upper limit for selenium for cats. Fish, eggs and liver are ingredients rich in selenium. **Table 6-1** lists signs of deficiency and excess (**Case 6-4**). Typical selenium supplements include sodium selenite and sodium selenite. See Chapter 29 for more information about selenium.

Ultra-Trace Minerals

The minimum dietary requirements for ultra-trace elements in dogs and cats have not been determined. Based on research in other species, it is probable that supplemental micronutrients, such as chromium and boron, may be beneficial under certain physiologic and dietary circumstances.

Chromium

In 1957, investigators identified a compound they called glucose tolerance factor (GTF) that restored impaired glucose tolerance in rats (Schwarz and Mertz, 1959). Chromium was identified as the active component. Chromium is ubiquitous in water, soil and living matter; however, tissue levels in animals are very low because of limited uptake by plants and absorption by animals. Furthermore, many forms of chromium are poorly available. Therefore, supplementation with an available form may be warranted. Chromium in an organically bound form (e.g., GTF) is absorbed better, has a different tissue distribution and is more available to fetuses than inorganic chromium (Mertz and Roginski, 1971).

Several studies in people and other animals have demonstrated beneficial effects of chromium supplementation (in chromium deficiency or diabetics) including: 1) improved glucose tolerance, 2) reversed hyperglycemia and glycosuria, 3) decreased circulating insulin concentrations, 4) decreased plasma lipid concentrations, 5) decreased body fat, 6) increased protein accretion, 7) improved immune response and 8) reduced cortisol production in response to heat and transport stress (Anderson, 1987; Page et al, 1993; Moonsie-Shageer and Mowat, 1993). Not all studies have shown improvements in these variables. This lack of consistent response may be accounted for by an adequate chromium nutriture for some individuals or some factor other than chromium deficiency that may have compromised the variable (impaired glucose tolerance, etc.). Few tests are available to specifically diagnose chromium status. The glucose tolerance test has been most commonly used to evaluate chromium deficiency. **Table 6-1** lists signs of deficiency and excess.

Boron

Boron indirectly influences PTH activity, thus it influences calcium, phosphorus, magnesium and cholecalciferol (vitamin D_3) metabolism. Investigators demonstrated that boron acts by at least three different mechanisms (Hunt et al, 1994): 1) it compensates for perturbations in energy substrate use induced by vitamin D deficiency, 2) it enhances macromineral content in normal bone and 3) it enhances some indices of growth and cartilage maturation, independent of vitamin D. Boron has a role in the control of urolithiasis. It decreases oxalate production in women fed magnesium-deficient diets (Hunt et al, 1994a). Boron also decreases calcium loss and bone demineralization in postmenopausal women (Nielsen et al, 1987). **Table 6-1** lists signs of deficiency and excess.

VITAMINS

Definition

The term "vitamine" was coined by Casmir Funk in 1912 when he described a class of nitrogen-containing compounds that were "vital-amines" (i.e., being vital to life). This term was later changed to vitamin when it was found that not all of these compounds contained nitrogen. The discovery, isolation and synthesis of vitamins have occurred in the last 100 years, although the effects of vitamin deficiency, specifically scurvy,

have been recorded since about 1150 B.C. (Combs, 1998).

A vitamin can be defined by its physical and physiologic characteristics. For a substance to be classified as a vitamin, it must have five basic characteristics: 1) it must be an organic compound different from fat, protein and carbohydrate, 2) it must be a component of the diet, 3) it must be essential in minute amounts for normal physiologic function, 4) its absence must cause a deficiency syndrome and 5) it must not be synthesized in quantities sufficient to support normal physiologic function.

These definitions are important to note because not all vitamins are essential for every species. For example, vitamin C is essential for primates, guinea pigs and some species of fish, but not for most other animal species. Lack of the enzyme L-gulonolactone oxidase prevents those species from synthesizing vitamin C from glucose, thereby, making vitamin C a required vitamin. Under certain conditions of disease or increased metabolism, however, vitamin C may be "conditionally essential" in those species that have de novo synthesis.

Two other terms warrant definition: vitamers and provitamins. A vitamer is chemically the same compound as a vitamin, but may exert varying physiologic effects because it is an isomer. Vitamin E is a good example of vitamers, because of its many forms. α-tocopherol is the most biologically active form, whereas γ-tocopherol has little biologic function, but acts as an in vitro antioxidant. A provitamin is a compound that requires an activation step before it becomes biologically active. β-carotene, for example, is cleaved by enzymatic processes to release two molecules of retinol (vitamin A).

The two main categories of vitamins are distinguished by their miscibility in either lipids (fat soluble) or water (water soluble). There are four fat-soluble vitamins (A, D, E and K) and nine generally recognized water-soluble vitamins (thiamin [B_1], riboflavin [B_2], niacin, pyridoxine [B_6], pantothenic acid, folic acid, cobalamin [B_{12}], biotin and vitamin C). Though not a true vitamin in the classic sense, choline is generally added to commercial dog and cat foods and treated as a vitamin in this chapter. The AAFCO dog food nutrient profiles list three fat-soluble and eight water-soluble vitamins including choline (vitamin K, biotin and vitamin C are not listed). The AAFCO cat food nutrient profiles list four fat-soluble and nine water-soluble vitamins including choline (vitamin C is not listed) (2007). There are also a number of compounds that are classified as "vitamin-like compounds" or "quasi-vitamins," which will also be discussed later in this section.

Function

Vitamins have incredibly diverse physiologic functions. Vitamins act as potentiators or cofactors in enzymatic reactions (**Figure 6-4**). They also play a significant role in DNA synthesis, energy release from nutrient substrates, bone development, calcium homeostasis, normal eye function, cell membrane integrity, blood clotting, free radical scavenging, amino acid and protein metabolism and nerve impulse transduction (**Table 6-3**).

Because of the differences between fat and water solubility and in chemical structure, vitamins are absorbed in the body through a variety of means. Fat-soluble vitamins require bile salts and fat to form micelles for absorption. They are then passively absorbed, usually in the duodenum and ileum, and transported in conjunction with chylomicrons to the liver via the lymphatic system. In contrast, most of the water-soluble vitamins are absorbed via active transport. Some vitamins (e.g., cobalamin) require a carrier protein called "intrinsic factor" whereas others need a sodium-dependent, carrier-mediated absorption pump.

Deficiency/Adequacy/Toxicity

Similar to other essential trace or micronutrients, differences in ingestion levels of vitamins create deficiency, adequacy or toxicity. The biologic dose-response curve (Figure 5-2) is appropriate for vitamins (Box 5-5). A deficiency is a lack of the vitamin in quantities required for normal physiologic function. In general, fat-soluble vitamins are stored in the lipid depots of all tissues, making them more resistant to deficiency, but also more likely to cause toxicity. Conversely, water-soluble vitamins are depleted at a faster rate because of limited storage; therefore, they are less likely to cause toxicity and more likely to be acutely deficient.

Within the range of adequate intake, requirements are met for each lifestage and tissue stores are maximized. Consuming more vitamins than is required to maximize stores can, in many cases, lead to clinical signs of toxicity if the ingestion period is prolonged and the body cannot excrete excesses. It is, therefore, prudent to provide vitamins in the appropriate balance for each lifestage to meet requirements and build tissue stores, but not to over-supplement in the pharmaco-toxicologic range.

Factors Affecting Requirements

Different lifestages of animals affect vitamin requirements. Growing and reproducing animals accrete tissues and therefore require higher levels of vitamins, minerals, protein and energy for optimal performance. Over-supplementation, however, is still contraindicated because these animals are also more susceptible to toxicity. As animals age, metabolic and physiologic changes may increase the requirement for some vitamins.

Various disease conditions also affect vitamin status. Prolonged anorexia deprives animals of vitamins and other nutrients and depletes vitamin stores. Polyuric diseases such as diabetes mellitus and kidney disease may increase excretion of water-soluble vitamins. Kidney disease can also lead to a secondary vitamin D deficiency by reducing the final hydroxylation step converting 25-OH-D_3 to 1,25-$(OH)_2$-D_3, which occurs in the proximal tubules of the kidneys.

In addition, certain drugs (e.g., antibiotics) may decrease the intestinal microflora responsible for vitamin K synthesis. Also, diuretic therapy may increase excretion of water-soluble vitamins. Some vitamin requirements depend on other nutrient levels. The amount of cobalamin required is related to the amount of folic acid, choline and methionine present because these nutrients interact metabolically and are dependent on each other. In addition, the amount of tryptophan influences

Table 6-3. Summary of names, functions and clinical syndromes associated with deficiency and toxicity of vitamins.

Vitamin	Function	Deficiency	Toxicity
Vitamin A	Component of visual proteins, (rhodopsin, iodopsin), differentiation of epithelial cells, spermatogenesis, immune function, bone resorption	Anorexia, retarded growth, poor coat, weakness, xerophthalmia, nyctalopia, increased CSF pressure, aspermatogenesis, fetal resorption	Cervical spondylosis (cats), tooth loss (cats), retarded growth, anorexia, erythema, long-bone fractures
Vitamin D	Calcium and phosphorus homeostasis, bone mineralization, bone resorption, insulin synthesis, immune function	Rickets, enlarged costochondral junctions, osteomalacia, osteoporosis	Hypercalcemia, calcinosis, anorexia, lameness
Vitamin E	Biologic antioxidant, membrane integrity through free radical scavenging	Sterility (males), steatitis, dermatosis, immunodeficiency, anorexia, myopathy	Minimally toxic. Fat-soluble vitamin antagonism, increased clotting time (reversed with vitamin K)
Vitamin K	Carboxylation of clotting proteins II (prothrombin), VII, IX, X and other proteins, cofactor of the bone protein osteocalcin	Prolonged clotting time, hypoprothrombinemia, hemorrhage	Minimally toxic. Anemia (dogs)
Thiamin (B_1)	Component of thiamin pyrophosphate (TPP), cofactor in decarboxylase enzyme reactions in the TCA cycle, nervous system	Anorexia, weight loss, ataxia, polyneuritis, ventriflexion (cats), paresis (dogs), cardiac hypertrophy (dogs), bradycardia	Decreased blood pressure, bradycardia, respiratory arrhythmia
Riboflavin (B_2)	Component of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) coenzymes, electron transport in oxidase and dehydrogenase enzymes	Retarded growth, ataxia, collapse syndrome (dogs), dermatitis, purulent ocular discharge, vomition, conjunctivitis, coma, corneal vascularization, bradycardia, fatty liver (cats)	Minimally toxic
Niacin	Component of nicotinamide-adenine dinucleotide (NAD) and adenine dinucleotide phosphate (NADP) coenzymes, hydrogen donor/ acceptor in energy-releasing dehydrogenase reactions	Anorexia, diarrhea, retarded growth, ulceration of soft palate and buccal mucosa, necrosis of the tongue (dogs), reddened ulcerated tongue (cats), cheilosis, uncontrolled drooling	Low toxicity. Bloody feces, convulsions
Pyridoxine (B_6)	Coenzyme in amino acid reactions (transaminases and decarboxylases), neurotransmitter synthesis, niacin synthesis from tryptophan, heme synthesis, taurine synthesis, carnitine synthesis	Anorexia, retarded growth, weight loss, microcytic hypochromic anemia, convulsions, renal tubular atrophy, calcium oxalate crystalluria	Low toxicity. Anorexia, ataxia (dogs)
Pantothenic acid	Precursor to coenzyme A (CoA), protein, fat and carbohydrate metabolism in the TCA cycle, cholesterol synthesis, triglyceride synthesis	Emaciation, fatty liver, depressed growth, decreased serum cholesterol and total lipids, tachycardia, coma, lowered antibody response	No toxicity established in dogs and cats
Folic acid (folate)	Methionine synthesis from homocysteine (vitamin B_{12} dependent), purine synthesis, DNA synthesis	Anorexia, weight loss, glossitis, leukopenia, hypochromic anemia, increased clotting time, elevated plasma iron, megaloblastic anemia (cats), sulfa drugs interfere with gut synthesis, cancer drugs (methotrexate) are antagonistic	Nontoxic
Biotin	Component of four carboxylase enzymes: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase and 3-methylcrotonyl CoA carboxylase	Hyperkeratosis, alopecia (cats), dry secretions around eyes, nose and mouth (cats), hypersalivation, anorexia, bloody diarrhea	No toxicity established in dogs and cats
Cobalamin (B_{12})	Coenzyme functions in propionate metabolism, aids tetrahydrofolate-containing enzymes in methionine synthesis, leucine synthesis/degradation	Cessation of growth (cats), methylmalonic aciduria, anemia	Altered reflexes (reduction in vascular conditioned reflexes and an exaggeration of unconditioned reflexes)
Vitamin C	Cofactor in hydroxylase enzyme reactions, synthesis of collagen proteins, synthesis of L-carnitine, enhances iron absorption, free radical scavenging, antioxidant/pro-oxidant functionality	Liver synthesis precludes dietary requirement, no signs of deficiency have been described in normal cats and dogs	No toxicity established in dogs and cats
Choline	Component of phosphatidylcholine found in membranes, neurotransmitter acetylcholine, methyl group donor	Fatty liver (puppies), increased blood prothrombin times, thymic atrophy, decreased growth rate, anorexia, perilobular infiltration of the liver (cats)	None described for cats and dogs
L-carnitine*	Transport long-chain fatty acids into the mitochondria for use in β-oxidation	Hyperlipidemia, cardiomyopathy, muscle asthenia	None described for cats and dogs

*L-carnitine is a vitamin-like substance.

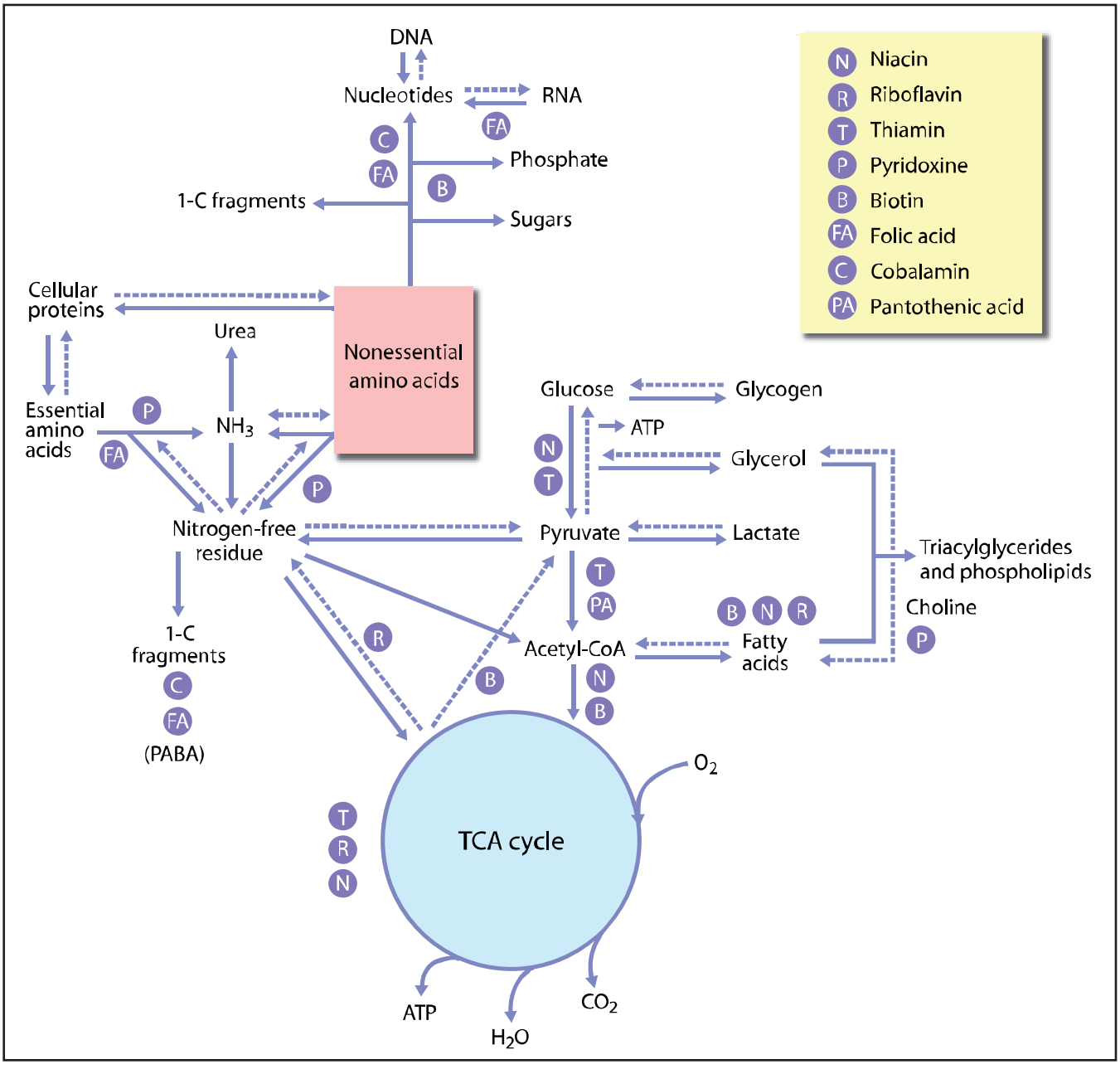

Figure 6-4. The role of B vitamins in intermediary metabolism.

niacin requirements because tryptophan is the precursor for that vitamin.

Finally, certain foods may contain antivitamin activity, for example, thiaminases in tissues of some fresh water fish may deactivate thiamin.

Feeding scientifically based formulas that specifically target the lifestage, nutrient interaction and disease condition will address changing vitamin requirements.

Vitamin Interactions

Much of the experimental work with vitamin deficiency disease has focused on the deficiency of a single vitamin. However, multiple deficiencies occur more frequently than a single-vitamin deficiency in patients. Pellagra is a classic example, in which deficiencies of niacin and tryptophan are usually accompanied by deficiencies of vitamin B_6 and riboflavin.

Many critical pathways require the concerted action of several B-complex vitamins (**Figure 6-4**). For example, one of the key reactions of metabolism is the conversion of pyruvate to acetyl-CoA. It is a key reaction because it occurs at the intersection of glycolysis and the TCA cycle. This pathway point is critical in the production of energy and the synthesis of fat and protein. Four different vitamins (niacin, thiamin, pantothenic acid and biotin) act as coenzymes in this one enzymatic conversion. Thus, a deficiency of any one of these four vitamins compromises the efficiency of the other three.

Table 6-4. Examples of vitamin-vitamin interactions.*

One vitamin needed for optimal absorption of another
Vitamin B_6 for vitamin B_{12}
Folate for thiamin
A high level of one vitamin may interfere with absorption or metabolism of another
Vitamin E interferes with vitamin K
Vitamin B_6 interferes with niacin
Thiamin interferes with riboflavin
One vitamin needed for metabolism of another
Riboflavin needed for vitamin B_6 and niacin
Vitamin B_6 needed for niacin
One vitamin protects against excess catabolism or urinary losses of another
Vitamin C spares vitamin B_6
One vitamin protects against oxidative destruction of another
Vitamin E spares vitamin A
Vitamin C spares vitamin E
A high level of one vitamin can obscure the diagnosis of deficiency of another
Folate deficiency obscures vitamin B_{12} deficiency

*Adapted from Machlin LJ, Langseth L. Vitamin-vitamin interactions. In: Bodwell LE, Erdman JW Jr, eds. Nutrient Interactions. New York, NY: Marcel Dekker Inc, 1988; 287-306.

The interactions between vitamins may involve the processes of absorption, metabolism, catabolism and excretion. Some vitamins may spare the requirements of others, whereas others may have potentially adverse effects. Even the marginal deficiency of one vitamin can exacerbate a deficiency or increase the requirement of another vitamin. Some examples of vitamin-vitamin interactions appear in **Table 6-4**.

Availability

Estimating the vitamin content of foods and foodstuffs and ultimately the adequacy of a given food is difficult at best because of cumulative errors made in estimating vitamin content and availability. These errors include: 1) analytical errors in sampling and determination of the vitamin, 2) variation in the actual amount of the vitamin (e.g., lot-to-lot variation, seasonal effects, demographics, different cultivars), 3) the presence of vitamins in bound forms in many foodstuffs and foods, 4) storage losses and 5) processing losses. All of these factors make it difficult to define what vitamin level is optimal for a given lifestage and food. To account for potential errors, references recommend that analytical values in databases be discounted by 10 to 25% (Combs, 1998).

Supplementation

Nearly all commercial pet foods contain added vitamins. Formulating a ration to meet vitamin requirements entirely from ingredient sources is extremely difficult and poses risks for the animal. The body uses synthetic and naturally formed vitamins in the same way, although they may have different availabilities. The effects of processing on vitamin stability and the availability in conjunction with disputed requirement levels in complex foods make fortification necessary (Chapter 8).

Commercial pet foods, therefore, are fortified to meet an animal's vitamin requirement for a given lifestage, overcome processing and storage losses and avoid toxicity. Because commercial pet foods are fortified with vitamins, it is usually unnecessary, and perhaps contraindicated, to concurrently give multipurpose vitamin-mineral supplements. Supplementation may be warranted in the management of diseases that affect vitamin metabolism, but should be monitored if long-term treatment is planned.

Fat-Soluble Vitamins

Vitamin A

Vitamin A is a general term describing a group of compounds with the biologic activity of retinol. Carotenoids are provitamin A. There are about 600 carotenoids known in nature, but only about 10% (e.g., α-carotene, β-carotene and β-cryptoxanthin) are provitamin A (Yeum and Russell, 2002). The international unit (IU) is still used by the pet food industry to measure the biologic activity of vitamin A. One IU of vitamin A activity can be provided by 0.3 μg of all-trans-retinol. In people, 12 μg β-carotene, 24 μg α-carotene or 24 μg β-cryptoxanthin is equal to one retinol activity equivalent (1 μg retinol) (DRI, 2001). Dogs, but not cats, can use provitamin A as a source of vitamin A. The retinol equivalent of β-carotene in dogs has not been defined. Most of the preformed vitamin A in food is in the form of retinyl esters, whereas the source of vitamin A from plants is in the form of provitamin A carotenoids.

FUNCTION

Vitamin A is essential for a number of distinct biologic functions. It is necessary for normal vision, growth, reproduction, immune function and maintenance of healthy epithelial tissue. Vitamin A is also involved in the expression and regulation of many genes (McClintick et al, 2005).

METABOLISM

Retinyl esters in food are hydrolyzed by hydrolases from the pancreas and the mucosal brush border to yield retinol. Retinyl esters and carotenoids are hydrophobic, thus their dispersion into the aqueous environment of the small intestinal lumen requires bile salts for micellar solubilization. This process allows access of hydrolytic enzymes to retinyl esters and exposes retinol to the mucosal surface, allowing free retinol and intact β-carotene to diffuse passively into mucosal epithelial cells. Absorption of vitamin A esters appears to be high (80 to 90%), but absorption may vary depending on the level and type of dietary fat and protein, which exert surfactant effects (Combs, 1998).

Dietary carotenoids are only absorbed half as well as preformed dietary vitamin A. As the amount of carotenoids in the food increases, however, the absorption efficiency decreases. Intestinal absorption of carotenoids is much more critically dependent on the presence of bile salts than is absorption of vitamin A. Cats can absorb β-carotene but are unable to convert it to retinol (Schweigert el al, 2002).

In the body, enzymes convert provitamin A carotenoids to retinols. β,β-carotene 15,15'-monooxygenase is a central cleav-

age enzyme that cleaves β,β-carotene into two molecules of retinol at the 15,15' double bond. The gene encoding for the enzyme has been cloned from several species including people, mice and chicks (Wyss, 2004). This central cleavage enzyme is found in the intestinal mucosa, liver, lungs, kidney, testes and brain. Recently, a second type of cleavage enzyme, β,β-carotene 9',10'-dioxygenase, was identified and cloned in mice (Kiefer et al, 2001). This enzyme exclusively catalyzes the asymmetric oxidative cleavage of β-carotene at the 9',10' double bond, resulting in the formation of β-apo-10'-carotenal and β-ionone.

Vitamin A is absorbed almost exclusively as the free alcohol retinol. Within mucosal cells, retinol is re-esterified mostly to palmitate and incorporated into the chylomicrons of the mucosa. Afterwards, it diffuses into lymph. A small amount of retinol may be oxidized first to retinal and then to retinoic acid, which may form a compound (glucuronide) that passes into the portal blood. Vitamin A is transported through the lymphatic system with low-density lipoprotein (LDL) to the liver, where it is deposited mainly in hepatocytes and stellate and parenchymal cells.

Some vitamin A derivatives are re-excreted into the intestinal lumen via the bile. This is true for much of retinoic acid and some retinol. The major vitamin A components of bile are vitamin A glucuronides, many of which are reabsorbed. Thus, enterohepatic circulation may provide an important means of conserving vitamin A. Although dogs and cats excrete vitamin A in urine, cats excrete a lesser amount.

When vitamin A is mobilized from the liver, stored vitamin A ester is hydrolyzed before it is released into the bloodstream. Vitamin A retinol is transported to tissues in the bloodstream by a specific transport protein called retinol-binding protein (RBP). RBP is synthesized and secreted by hepatic parenchymal cells.

In contrast to most other species, dogs and cats have a unique way of metabolizing vitamin A. Cats require preformed vitamin A because they lack the oxygenase enzyme necessary for β-carotene cleavage. In addition, studies have shown that cats and dogs do not depend on RBP to transport vitamin A in plasma (Schweigert, 1988; Wilson et al, 1987; Schweigert et al, 1990; Schweigert et al, 1990a). Cats and dogs transport vitamin A as retinyl esters (mostly retinyl stearate) bound to LDL and very low-density lipoprotein in amounts 10 to 50 times those of other mammals (Schweigert, 1988). This is of interest because free circulating retinyl esters are a sign of hypervitaminosis A in almost all other animal species, including people.

REQUIREMENTS

The AAFCO (2007) recommended allowance for vitamin A is 5,000 IU/kg DM for dogs for all lifestages (growth, reproduction and maintenance) and 9,000 IU/kg DM for cats for growth and reproduction and 5,000 IU/kg DM for maintenance. NRC (2006) recommends a vitamin A allowance of 1,515 RE (retinal equivalent) (5,050 IU)/kg DM for dogs for all lifestages, 1,000 RE (3,333 IU)/kg DM for cats for growth and maintenance and 2,000 RE (6,667 IU)/kg DM for cats during gestation and lactation. Unlike dogs, cats cannot meet their vitamin A requirement from carotenoids.

DEFICIENCY AND TOXICITY

The appreciable stores of vitamin A in the body are mobilized as needed to mitigate against the effects of low dietary intakes of the vitamin. The only unequivocal signs of vitamin A deficiency are the ocular lesions nyctalopia (night blindness) and xerophthalmia (extreme dryness of the conjunctiva). Other signs include anorexia, weight loss, ataxia, skin lesions, increased susceptibility to infection, retinal degeneration, poor coat, weakness, increased cerebrospinal fluid pressure, nephritis, skeletal defects (periosteal overgrowth and narrowing of foramina) and impaired reproduction (NRC, 2006).

Vitamin A toxicities have been encountered in numerous species. The most characteristic signs of hypervitaminosis A are skeletal malformation, spontaneous fractures and internal hemorrhage (**Case 6-5**). Other signs include anorexia, slow growth, weight loss, skin thickening, suppressed keratinization, increased blood clotting time, reduced erythrocyte count, enteritis, congenital abnormalities, conjunctivitis, fatty infiltration of the liver and reduced function of liver and kidneys. Queens fed a diet with 606,000 RE/kg food had an increased number of kittens born with defects such as cleft palate, cranioschisis, foreshortened mandible, stenotic colon, enlarged heart and agenesis of the spinal cord and small intestine (Freytag et al, 2003). Dogs seem less sensitive to excess dietary vitamin A than some other mammals (Cline et al, 1997).

The dietary maximum of vitamin A in the AAFCO (2007) dog and cat nutrient profiles is 250,000 IU/kg DM for dogs and 750,000 IU/kg DM for cats. NRC (2006) proposed a safe upper limit of 15,000 RE (50,000 IU)/kg DM for growing puppies and gestating and lactating bitches, and 64,000 RE (213,333 IU)/kg DM for adult dogs. The safe upper limit of vitamin A for cats is 80,000 RE (266,667 IU)/kg DM for growth and 100,000 RE (333,333 IU)/kg DM for maintenance, gestation and lactation.

SOURCES

Naturally rich sources of vitamin A include fish oil, liver, egg and dairy products. The most common vitamin A supplements used in pet foods include vitamin A esters (all trans retinyl palmitate, acetate or propionate) or vitamin A provided as fish oils. Because of stability issues, vitamin A sources are often coated, beaded, prilled or spray dried with antioxidants and emulsifying agents.

Concentrations of carotenoids in plants vary widely according to geographic location, maturity, method of harvest, amount and type of processing, length and conditions of storage and exposure to high temperature, sunlight and air. As a result, vitamin A is among the most variable nutrients in the diet. The vitamin A content in animal tissues can also be variable; concentrations can be very high in certain tissues such as liver. Levels of vitamin A in animal tissue vary depending on either the level of vitamin A or carotenoid present in the dog's or cat's diet.

Vitamin D

Two important forms of vitamin D are cholecalciferol (vitamin D_3), which occurs in animals and ergocalciferol (vitamin D_2), which occurs predominantly in plants. In pet food, vitamin D activity is typically expressed in IU. One IU of vitamin D can be provided by 0.025 µg of cholecalciferol or vitamin D_3. The skin of most mammals can produce cholecalciferol from the provitamin 7-dehydrocholesterol via activation with ultraviolet-B light. However, this photosynthesis pathway is inefficient in dogs (Hazewinkel et al, 1987) and cats (Morris, 1999) because of the higher activity of the enzyme 7-dehydrocholesterol- Δ^7 reductase that converts 7-dehydrocholesterol to cholesterol. Therefore, dogs and cats need dietary vitamin D.

FUNCTION

The primary function of vitamin D is to enhance intestinal absorption and mobilization, as well as retention and bone deposition of calcium and phosphorus. This function is manifested through its active form of 1,25-$(OH)_2$-D_3 as a hormone that binds to the nuclear 1,25-$(OH)_2$-D_3 receptor (VDR) in many types of cells. The active vitamin D_3 also has a direct effect on Ca^{2+} channels located on the plasma membrane (Norman et al, 1992).

METABOLISM

Vitamin D is absorbed from the small intestine by nonsaturable, passive diffusion, which depends on bile salts. Vitamin D then enters the lymphatic circulation primarily (~90%) in association with chylomicrons; the remainder of vitamin D is associated with an α-globulin fraction (Combs, 1998). Like other steroids, vitamin D is transported in association with proteins. In most species, the binding protein is vitamin D-binding protein (DBP) or "transcalciferin." The concentration of DBP greatly exceeds the concentration of vitamin D metabolites in blood. This concentration difference, in conjunction with the binding affinity, results in less than 5% of the available binding sites being occupied by vitamin D compounds. The distribution between bound and free vitamin D compounds greatly favors the bound form. In this fashion, DBP facilitates peripheral distribution of vitamin D from dietary origin and mobilizes endogenously produced vitamin D from the skin.

Vitamin D is distributed relatively evenly among the various tissues where it resides in lipid depots. Vitamin D can be found in adipose, kidneys, liver, lungs, aorta and heart. The primary circulating form of vitamin D is the parent vitamin D (~50%), with the next most abundant form (i.e., 25-OH-D_3 [also called calcidiol]) accounting for approximately 20% of the total (Combs, 1998).

In mammals, both vitamin D_2 and D_3 are not the active form of vitamin D. They are activated in the body by hydroxylation to 25-OH-D_3 first in the liver and again to 1,25-$(OH)_2$-D_3 (also called calcitriol) in the kidneys. Vitamin D_2 is less efficiently used than vitamin D_3 in cats (Morris, 2002). At normal plasma concentrations, only small amounts of 25-OH-D_3 are released from this pool to enter tissues. Thus, circulating levels of 1,25-$(OH)_2$-D_3 are a good indicator of vitamin D status.

Several factors tightly regulate the vitamin D endocrine system: 1,25-$(OH)_2$-D_3, PTH, calcitonin, several other hormones and circulating levels of calcium and phosphate. The vitamin D-dependent homeostatic system responds to perturbations in calcium concentration. For example, when serum calcium falls below a given level, PTH is secreted by the parathyroid glands, which function to detect hypocalcemia. The kidney responds to PTH, resulting in phosphate diuresis and stimulation of 25-OH-D_3 1-hydroxylase. The latter effect increases production of 1,25-$(OH)_2$-D_3, which acts to increase enteric absorption of calcium and phosphate. In addition, 1,25-$(OH)_2$-D_3 acts jointly with PTH in bone to promote mobilization of calcium and phosphate. The combined result of these responses is to increase plasma concentration of calcium and phosphate. Calcitonin is secreted by the thyroid gland ("C" cells) when circulating concentrations of calcium are increased. Calcitonin suppresses bone mobilization and may increase the renal excretion of calcium and phosphate. In that situation, 25-OH-D_3 1-hydroxylase may be inhibited by 1,25-$(OH)_2$-D_3, and may actually be converted to 24,25-$(OH)_2$-D_3, which may down-regulate the absorption of calcium in dogs (Tryfonidou et al, 2002).

These events tightly regulate hydroxylase activity and maintain nearly constant plasma concentrations of 1,25-$(OH)_2$-D_3, calcium and phosphorus. Once formed, 1,25-$(OH)_2$-D_3 binds to specific receptors on the enterocyte nucleus and initiates events that stimulate calcium and phosphorus absorption. In addition, 1,25-$(OH)_2$-D_3, acting with PTH, mediates resorption of bone with the release of calcium and phosphorus.

Many metabolites of vitamin D circulate in plasma other than 25-OH-D_3 and 1,25-$(OH)_2$-D_3. One metabolite, 24,25-$(OH)_2$-D_3, may also be biologically active, whereas other metabolites are generally considered physiologically inactive excretory forms (Combs, 1998).

REQUIREMENTS

The AAFCO (2007) dietary allowance for vitamin D is 500 IU/kg DM for dogs for all lifestages. For cats, the AAFCO (2007) allowance is 750 IU/kg DM for growth and reproduction and 500 IU/kg DM for maintenance. The vitamin D allowance recommended by NRC (2006) is 13.8 µg cholecalciferol (552 IU)/kg DM for dogs regardless of lifestage. The NRC recommended vitamin D allowance for cats is 5.6 µg cholecalciferol (250 IU)/kg DM for growth and 7 µg cholecalciferol (280 IU)/kg DM for maintenance and reproduction.

DEFICIENCY AND TOXICITY

Signs of vitamin D deficiency are frequently confounded by a simultaneous deficiency or imbalance of calcium and phosphorus. Clinical signs generally include rickets (young animals), enlarged costochondral junctions, osteomalacia (adult animals), osteoporosis (adult animals) and decreased serum calcium and inorganic phosphorus concentrations. Experimental vitamin D deficiency has been produced in cats, resulting in neurologic abnormalities associated with degeneration of the

cervical spinal cord (Morris, 1996). Other signs included hypocalcemia, elevated PTH concentrations, posterior paralysis, ataxia and eventual quadriparesis.

Excessive intake of vitamin D is associated with increases in 25-OH-D_3, with the D_3 form being more toxic than the D_2 form. When circulating at very high concentrations, 25-OH-D_3 can compete effectively with 1,25-$(OH)_2$-D_3 for receptors in the intestine and bone. Therefore, during vitamin D toxicosis, 25-OH-D_3 can induce actions usually attributed to 1,25-$(OH)_2$-D_3. Thus, 25-OH-D_3 is believed to be the critical factor in vitamin D intoxication (NRC, 1987). Excessive vitamin D_3 supplementation below the toxic level decreases bone remodeling and causes focal enlargement of the growth plate in growing puppies (Tryfonidou et al, 2003). Excessive vitamin D concentrations may result in hypercalcemia, soft-tissue calcification and ultimately death (Morita et al, 1995; Nakamura et al, 2004) (**Case 6-6**).

The vitamin D maximum in the AAFCO (2007) dog and cat nutrient profiles is 5,000 IU/kg DM for dogs and 10,000 IU/kg DM for cats. The NRC (2006) proposed a safe upper limit of 80 µg cholecalciferol (3,200 IU)/kg DM for dogs and 750 µg cholecalciferol (30,000 IU)/kg DM for cats regardless of lifestages.

SOURCES

Marine fish and fish oils are the richest natural sources of vitamin D in foodstuffs but they may pose a risk for toxicity. One group of investigators found that moist foods generally contained higher levels of vitamin D than extruded foods and that some moist foods exceeded the AAFCO maximal recommended allowance of 10,000 IU/kg for cats (Morris, 1996). Other sources of vitamin D include fresh water fish and eggs (especially yolks). Beef, liver and dairy products contain smaller amounts of vitamin D. The most common synthetic sources of vitamin D in pet foods include cholecalciferol (D-activated animal sterol), vitamin D_3 supplement, ergocalciferol (D-activated plant sterol) and vitamin D_2 supplement.

Vitamin E

Vitamin E is a term for a group of compounds with the biologic activity of α-tocopherol. In nature, there are eight isomeric forms of vitamin E, four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ). Naturally occurring α-tocopherol (*d*-α-tocopherol) is now designated as RRR-α-tocopherol based on RS or CIP system of chiral configuration. Synthetic α-tocopherol (*dl*-α-tocopherol), a mixture of eight stereoisomers of α-tocopherol, is designated as all-*rac* (racemic)-α-tocopherol. Vitamin E activity in pet food is generally expressed in international units. One IU of vitamin E equals 1 mg of all-*rac*-α-tocopheryl acetate or 0.91 mg of all-*rac*-α-tocopherol. The most biopotent form of vitamin E is α-tocopherol. The relative biopotencies of vitamin E isomers are as follows: α >β >δ >γ (McDowell, 1989). Also, tocopherols are generally more available than tocotrienols (Combs, 1998). Because some forms of vitamin E have little biologic activity, total vitamin E analysis is not a reliable means of determining vitamin E activity.

FUNCTION

Vitamin E functions as an antioxidant in the body and in food. Of the vitamin E isomers, α-tocopherol is the most active biologic form in the body, whereas the γ-isomer is the most active form in food. Mixed tocopherols, including γ-tocopherols, are widely used to prevent lipid oxidation in pet food products (Chapter 8).

Vitamin E works in conjunction with glutathione peroxidase to protect cells against the adverse effects of reactive oxygen and other free radicals that initiate the oxidation of polyunsaturated membrane phospholipids. Vitamin E in cellular and subcellular membranes is the first line of defense against peroxidation of vital phospholipids. However, some peroxides are formed even when adequate levels of vitamin E are present.

Selenium, as part of the enzyme glutathione peroxidase, is a second line of defense that destroys peroxides before they damage membranes. Therefore, selenium, vitamin E and sulfur-containing amino acids, through different biochemical mechanisms, are capable of preventing some of the same nutritional diseases (McDowell, 1992). Vitamin E prevents fatty acid hydroperoxide formation, sulfur-containing amino acids are precursors of glutathione peroxidase and selenium is a component of glutathione peroxidase. In addition, vitamin E is important for normal reproduction and is involved in modulating cellular signaling, regulating gene transcription, modulating immune function and inducing apoptosis (Brigelius-Flohe et al, 2002).

METABOLISM

Vitamin E is absorbed from the small intestine by nonsaturable, passive diffusion, which depends on micellar solubilization. Whether presented as free alcohol or as esters, most vitamin E is absorbed as the alcohol. Esters are largely hydrolyzed in the gut wall before absorption, probably by a duodenal mucosal esterase. The free alcohol enters the intestinal lacteals and is transported via lymph to the general circulation.

The efficiency of vitamin E absorption is low and variable (35 to 50%); the absorption efficiency is much lower than that of vitamin A (Combs, 1998). Absorption of vitamin E is enhanced by the simultaneous digestion and absorption of dietary lipids. Transfer of vitamin E across epithelial cells may require several stages, most of them poorly understood. In mammals, vitamin E is transported from the intestine to lymphatic capillaries in association with chylomicrons. Conversely, in birds, tocopherol is transported via the portal vein directly to the liver. Unlike cholesterol or vitamin A, α-tocopherol is not re-esterified during the absorption process.

Vitamin E circulates in the lymph and blood bound to all of the lipoproteins. There is a very high correlation between tocopherol levels and the total lipid or cholesterol concentration in serum.

All tissues show linear increases in tocopherol concentrations with increases in tocopherol intake. This relationship differs from that of most other vitamins, which usually have distinct deposition thresholds in tissues other than the liver, and may provide an explanation for the pharmacologic effects of vitamin

E. Vitamin E levels in tissues vary markedly with no consistent relationship to lipid parameters. The vitamin is most concentrated in membrane-rich cell fractions such as mitochondria and microsomes.

Vitamin E undergoes very little metabolism. Usually less than 1% of orally ingested vitamin E is excreted in urine (Combs, 1998). The major route of excretion is fecal elimination.

The need for vitamin E in the diet is markedly influenced by dietary composition. The requirement increases with increasing levels of polyunsaturated fatty acids (PUFA), oxidizing agents, vitamin A, carotenoids and trace minerals and decreases with increasing levels of fat-soluble antioxidants, sulfur-containing amino acids and selenium. Various researchers have recommended up to 60 mg of α-tocopherol per g of PUFA; however, there is no consensus among experts about the quantitation of this relationship (Combs, 1998).

REQUIREMENTS

The AAFCO (2007) recommended allowance for vitamin E is 50 IU/kg DM for dogs and 30 IU/kg DM for cats, irrespective of the lifestage. For cat foods containing fish oils, AAFCO recommends an additional 10 IU vitamin E/g of fish oil/kg of food above the allowance. The vitamin E allowance recommended by NRC (2006) is 30 mg α-tocopherol (33 IU)/kg DM for dogs for all lifestages. For cats, the vitamin E allowance is 38 mg α-tocopherol (42 IU)/kg DM for growth and maintenance and 31 mg α-tocopherol (34 IU)/kg DM for gestation and lactation.

DEFICIENCY AND TOXICITY

The clinical manifestations of vitamin E deficiency vary markedly between species. In general, however, the neuromuscular, vascular and reproductive systems are affected most commonly. Signs of vitamin E deficiency are mostly attributed to membrane dysfunction as a result of the oxidative degradation of polyunsaturated membrane phospholipids and disruption of other critical cellular processes. Clinical findings of vitamin E deficiency in dogs include degenerative skeletal muscle disease associated with muscle weakness, degeneration of testicular germinal epithelium and impaired spermatogenesis, failure of gestation, brown pigmentation (lipofuscinosis) of intestinal smooth muscle and decreased plasma tocopherol concentrations. In cats, deficiency signs include steatitis, focal interstitial myocarditis, focal myositis of skeletal muscle and periportal mononuclear infiltration in the liver (**Case 6-7**).

Vitamin E is one of the least toxic fat-soluble vitamins. Animals and people apparently tolerate high levels without adverse effects. However, at very high doses, antagonism with other fat-soluble vitamins may occur, resulting in impaired bone mineralization, reduced hepatic storage of vitamin A and coagulopathies as a result of decreasing absorption of vitamins D, A and K, respectively. A maximum of 1,000 IU/kg DM was recommended by AAFCO for dogs; however, AAFCO (2007) set no maximum for cats. There is no evidence of vitamin E toxicity in dogs and very limited information when vitamin E is given orally to cats. In certain conditions, higher dietary vitamin E levels may be beneficial (Morris and Rogers, 1994). A number of inflammatory dermatoses in animals have been treated with oral vitamin (Chapter 32). People have been given much higher dietary concentrations of vitamin E without adverse clinical signs (Combs, 1998).

SOURCES

Only plants synthesize vitamin E. The richest sources of vitamin E are vegetable oils and, to a lesser extent, seeds and cereal grains. Tocopherol concentrations are highest in green leaves. Tocotrienols are not found in green leaves, but instead are found in the bran and germ fractions of certain plants. Animal tissues tend to be low in vitamin E, with the highest levels occurring in fatty tissues. Common vitamin E supplements used in pet foods include α-tocopherol and α-tocopherol acetate.

Vitamin K

Like other fat-soluble vitamins, vitamin K is a generic descriptor for a group of compounds exhibiting the antihemorrhagic activity of phylloquinone. Phylloquinone (vitamin K_1) and menaquinone (vitamin K_2) are the two major naturally occurring forms of vitamin K. Green leafy vegetables are the primary sources of vitamin K_1 whereas vitamin K_2 is produced from actinomycete bacteria found in normal intestinal microflora. Vitamin K_2 is now called menaquinone-7 (MK-7) in recognition of the seven-isoprenoid units. Menadione or vitamin K_3 (2-methyl-1,4-napthoquinone) is the parent compound of the vitamin K series. When used in pet food, it is usually added in a complex form such as menadione sodium bisulfate complex (MSBC) or menadione dimethylpyrimidinol bisulfate (MPB), as the bioactive form of vitamin K.

FUNCTION

Vitamin K plays a major role in the carboxylation of proteins (factors II, VII, IX, X and proteins C and S) to convert prothrombin to thrombin for normal blood clotting. Vitamin K is also involved in the synthesis of osteocalcin, a protein that regulates the incorporation of calcium phosphates in growing bone (Combs, 1998).

METABOLISM

The absorption of natural vitamin K in food is between 40 and 70% (Combs, 1998). Ingested phylloquinone is absorbed from the proximal small intestine into the lymphatic system by an energy-dependent process. Menaquinone is absorbed from the small intestine by a passive noncarrier-mediated process. Conditions that impair lipid absorption also adversely affect vitamin K absorption. Upon absorption, vitamin K is transported to the liver in chylomicrons. The vitamin is rapidly concentrated in the liver, but in contrast to other fat-soluble vitamins, vitamin K has a very rapid turnover in this organ. No specific carriers have been identified for any of the K vitamers.

Although phylloquinones and menaquinones are ingested, much of the vitamin K in tissues is from bacterial origin. Menadione is rapidly excreted in urine as the phosphate, sulfate or glucuronide form of menadiol. However, catabolism of phyl-

loquinones and menaquinones is much slower than that of menadione and they are primarily excreted in feces as a glucuronide conjugate.

Because microbially synthesized K_2 is readily absorbed by passive diffusion in the colon in most mammalian species, dietary supplementation is unnecessary for most cats and dogs.

REQUIREMENTS

AAFCO (2007) does not have a recommended allowance for vitamin K for dogs, but recommends 0.1 mg/kg DM for cats when cat foods contain more than 25% fish. This recommendation is warranted because vitamin K deficiency has been observed in cats fed certain commercial foods containing high levels of salmon or tuna (**Case 6-8**). NRC (2006) recommends that the vitamin K allowance is 1 mg/kg DM for cats for all lifestages. For dogs, the recommended allowance of vitamin K is 1.64 mg/kg DM for growth, 1.63 mg/kg DM for maintenance and 1.6 mg/kg DM for gestation and lactation.

DEFICIENCY AND TOXICITY

Prolonged clotting times and excessive bleeding have been reported in vitamin K deficiency in cats and dogs under various conditions. Vitamin K deficiency usually occurs secondary to other conditions such as malabsorptive diseases (inflammatory bowel disease), ingestion of coagulant antagonists (coumarin, indanedione), destruction of gut microflora by antibiotic therapy (sulfonamides and broad-spectrum antibiotics) and congenital defects (γ-glutamyl carboxylase defect in Devon Rex breed of cats). Vitamin K_3 (menadione) has lower lipid solubility and is the most effective form of vitamin K for cases of malabsorption. Vitamin K_1 is the only form of vitamin K effective in anticoagulant antagonism (Edwards and Russell, 1987).

Phylloquinone produces no adverse effects when administered to animals in massive doses by any route (NRC, 1987). The menaquinones are similarly thought to have negligible toxicity. Menadione, however, can produce fatal anemia, hyperbilirubinemia and severe jaundice. The intoxicating doses appear to be at least three orders of magnitude above those levels required for normal physiologic function (Combs, 1998). Neither AAFCO nor NRC has set maximum or safe upper limits for dogs and cats.

SOURCES

Data for vitamin K content of foods are limited by the lack of good analytical methods. Nevertheless, because dietary needs for vitamin K are low, most foods contribute significantly to those needs. Rich sources of vitamin K include alfalfa meal, oilseed meals, liver and fish meals. Menadione sodium bisulfite complex and menadione dimethypyrimidinol bisulfate are commonly used as vitamin K sources in pet food because of their stability during manufacturing and storage.

Water-Soluble Vitamins

Deficiency of B vitamins occurs in veterinary medicine but may be difficult to specifically diagnose because analytical tests are not readily available. Therefore, diagnosis relies almost entirely upon clinical signs and nutrient intake history.

B vitamins are relatively nontoxic and may be supplied to veterinarians in individual or combination forms. Because many of the B-vitamin deficiencies present with overlapping clinical signs, it may be prudent to treat deficiency with vitamin-B complex. If signs are specific for a particular B-vitamin deficiency, and if the single preparation form of the vitamin is available, individual targeted treatment may be initiated. However, individual preparations of B vitamins are often more expensive, and the relative nontoxic levels of B vitamins warrant treatment with the combination form.

Thiamin

Thiamin or vitamin B_1 consists of one pyrimidine ring and one thiazole ring linked via a methylene group. Thiamin may exist as free thiamin or in the mono-, di-(pyro), or triphosphate configuration. Thiamin pyrophosphate (80%) is the major form found in tissues; the other three forms are found in lesser amounts (Rindi, 1996; Brody, 1994a). Thiamin is very labile, especially in wet foods, being susceptible to neutral and alkaline conditions, heat, oxidation and ionizing radiation.

FUNCTION

Thiamin pyrophosphate (TPP) is the major coenzymatic form of thiamin and is required for only a small number of enzymatic reactions. TPP is involved in the following general scheme of reactions: 1) nonoxidative decarboxylation of α-ketoacids, 2) oxidative decarboxylation of α-ketoacids and 3) transketolation reactions. Thiamin may also have a function unrelated to coenzyme activity. TPP is concentrated in neuronal cells and may affect chloride permeability by controlling the number of functional channels, possibly by phosphorylation.

METABOLISM

Dietary thiamin may be present in any of the four forms mentioned above or may be of synthetic origin. Whatever the form, thiamin is hydrolyzed to free thiamin by intestinal phosphatases before absorption by enterocytes. Absorption takes place primarily in the jejunum by an active, carrier-mediated transport that also phosphorylates the vitamin. Passive diffusion becomes an important mode of absorption when dietary thiamin intake is high.

Absorbed thiamin is transported in erythrocytes, which contain free thiamin and its phosphorylated forms, and in plasma, which only contains free thiamin and its monophosphate form. Tissues take up thiamin and may interconvert it between any of its four forms. The liver, heart and kidneys have the highest concentration of thiamin.

REQUIREMENTS

The AAFCO (2007) recommended allowance for thiamin is 1 mg/kg DM for dogs and 5 mg/kg DM for cats, irrespective of the lifestage. NRC (2006) recommends a thiamin allowance for dogs of 1.38 mg/kg DM for growth and 2.25 mg/kg DM for maintenance and reproduction. For cats, the NRC (2006)

Table 6-5. Blood levels, allowances and tests for B-complex vitamins in cats and dogs.

Cats

Vitamin	Blood level	AAFCO allowance*	NRC allowance*	Best test
Thiamin	20-90 ng/ml (WB)	5 mg/kg	5.6 mg/kg	Erythrocyte transketolase activity
Riboflavin	196-660 ng/ml (WB)	4 mg/kg	4 mg/kg	Erythrocyte glutathione reductase**
				Urine riboflavin
Niacin	1.8-5.8 μg/ml (WB)	60 mg/kg	40 mg/kg	Urine methyl nicotinamide or methyl-pyridones**
Pantothenic acid	104-270 ng/ml (WB)	5.0 mg/kg	5.75 mg/kg	Urinary excretion of pantothenate
Pyridoxine	86-350 ng/ml (P)	4.0 mg/kg	2.5 mg/kg	Blood levels of pyridoxine
				Urinary metabolites of pathway intermediates
Folic acid	3.2-34 ng/ml (P)	0.8 mg/kg	0.75 μg/kg	Serum folate
Vitamin B_{12}	120-1,200 pg/ml (WB)	20 μg/kg	22.5 μg/kg	Blood levels of cobalamin
				Serum and urine methylmalonic acid
Biotin	1,000-3,000 pg/ml (WB)	70 μg/kg	75 μg/kg	Urinary biotin
				Urinary organic acids
Choline	180-490 μg/ml (P)	2,400 mg/kg	2,550 mg/kg	Plasma choline and phosphatidylcholine

Dogs

Vitamin	Blood level	AAFCO allowance*	NRC allowance*	Best test
Thiamin	46-112 ng/ml (WB)	1.0 mg/kg	2.25 mg/kg	Erythrocyte transketolase activity
Riboflavin	185-420 ng/ml (WB)	2.2 mg/kg***	5.25 mg/kg	Erythrocyte glutathione reductase**
				Urine riboflavin
Niacin	2.7-12 μg/ml (WB)	11.4 mg/kg	17 mg/kg	Urine methyl nicotinamide or methyl-pyridones**
Pantothenic acid	120-380 ng/ml (WB)	10 mg/kg	15 mg/kg	Urinary excretion of pantothenate
Pyridoxine	40-270 ng/ml (P)	1 mg/kg	1.5 mg/kg	Blood levels of pyridoxine
				Urinary metabolites of pathway intermediates
Folic acid	4-26 ng/ml (P)	0.18 mg/kg	0.27 mg/kg	Serum folate
Vitamin B_{12}	135-950 pg/ml (WB)	22 μg/kg	35 μg/kg	Holotranscobalamin II**
Biotin	530-5,000 pg/ml (WB)	None established	0†	Urinary biotin
				Urinary organic acids
Choline	235-800 μg/ml (P)	1,200 mg/kg	1,700 mg/kg	Plasma choline and phosphatidylcholine

Key: WB = whole blood, P = plasma, AAFCO = Association of American Feed Control Officials, NRC = National Research Council.
*AAFCO allowances are similar for growth and adult maintenance and are expressed on dry matter basis (AAFCO Official Publication, 2007). NRC allowances are "recommended allowances" for adult maintenance and are also expressed on a dry matter basis (NRC. Nutrient Requirements of Dogs and Cats. Washington, DC: National Academies Press, 2006.
**Not currently available in veterinary medicine.
***Investigators have shown a riboflavin requirement approximately 20 to 33% higher than the AAFCO allowance listed here. (Cline JL, Odle J, Easter RA. The riboflavin requirement of adult dogs at maintenance is greater than previous estimates. Journal of Nutrition 1996; 126: 984-988.)
†For normal foods not containing raw egg whites, adequate biotin is probably provided by intestinal microbial synthesis (assuming the patient is not receiving antimicrobial therapy).

recommended allowance for thiamin is 5.5 mg/kg DM for growth, 5.6 mg/kg DM for maintenance and 6.3 mg/kg DM for gestation and lactation. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Clinical thiamin deficiency is rarely observed in dogs and cats because most commercial pet foods have adequate supplementation. Signs of thiamin deficiency are often related to the nervous system and heart. They include anorexia, failure to grow, muscle weakness, paraparesis, convulsions, seizures, ventriflexion of the head, ataxia and cardiac hypertrophy (Read and Harrington, 1981; Jubb et al, 1956; Everett, 1944).

Thiamin deficiency may result from inadequate intake of thiamin, attributable to foods with low-thiamin content or processing losses, or high intake of thiamin antagonists. The processing conditions used to prepare commercial pet foods are destructive to thiamin. However, this anticipated loss is overcome by adding synthetic thiamin before processing (**Case 6-9**).

Thiamin antagonists may be synthetic or natural compounds that modify the thiamin structure rendering it inactive. The natural antagonists include thiaminases (enzymes that degrade thiamin), and polyhydroxyphenols (caffeic acid, chlorogenic acid, tannins), which inactivate thiamin by an oxyreductive process. Thiaminases are found in high concentrations in raw fish, shellfish, bacteria, yeast and fungi (**Table 6-6**). Cooking destroys thiaminases.

Thiamin deficiency may be diagnosed by measuring erythrocyte transketolase activity or thiamin metabolites in blood directly. **Table 6-5** lists concentrations of thiamin in blood for cats and dogs (Baker et al, 1986). Activity of erythrocyte transketolase is an excellent indicator of thiamin status, if determined in a laboratory familiar with the analysis. Thiamin toxicosis via the oral route is very rare.

SOURCES

Thiamin occurs in animal tissues almost entirely in phospho-

rylated forms, whereas it occurs mostly as free thiamin in plants. Thiamin is widely distributed in foods, but is mostly present at low concentrations. The richest sources are whole grains, yeast and liver (especially pork liver). Meat products may also supply significant amounts of thiamin. Up to 90% of thiamin in natural ingredients may be lost as a result of processing (Morris and Rogers, 1994). Therefore, thiamin supplementation is common in pet foods. Thiamin hydrochloride and thiamin mononitrate are the most commonly used supplements in commercial foods for dogs and cats.

Table 6-6. Thiaminase activity in fish products.*

Food	Thiaminase activity**
Marlin	0
Yellowfin tuna	265
Red snapper	265
Skipjack tuna	1,000
Dolphin (mahi mahi)	120
Ladyfish	35
Clam	2,640

*Adapted from Hilker DM, Peter OF. Anti-thiamin activity in Hawaii fish. Journal of Nutrition 1966; 89: 419-421.
**mg thiamin destroyed/100 g fish/hour.

Riboflavin

Riboflavin, or vitamin B_2 belongs to the class of isoalloxazines. Riboflavin has a planar structure and has limited solubility in water. This property has clinical significance because it is difficult to deliver massive doses of the vitamin via intravenous solutions. Riboflavin is heat stabile, but sensitive to light, and acidic and alkaline conditions.

FUNCTION

Riboflavin is the precursor to a group of enzymatic cofactors called flavins. Flavins linked to protein are called flavoproteins. The two major coenzymes derived from riboflavin are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Flavins are used as coenzymes in about 50 enzymes in mammals. Flavins participate in intermediary energy metabolism and function mainly in oxidoreductase types of reactions (**Figure 6-4**).

METABOLISM

Most riboflavin found in food sources is in the form of free coenzyme derivatives that are not readily absorbed unless hydrolyzed, and covalently bound riboflavin that is not well used. The free flavin compounds are hydrolyzed before they are absorbed in the upper GI tract. A specialized transport system that is saturable and sodium dependent is necessary for absorption of flavins. After absorption, about 50% of the riboflavin in blood is bound to albumin and the other half to globulins (Brody, 1994a; Rivlin, 1996). Tissues requiring riboflavin convert it to FMN by phosphorylation catalyzed by flavokinase and subsequently to FAD catalyzed by FAD synthase. Excess riboflavin in the body is eliminated largely as riboflavin via the kidneys.

REQUIREMENTS

The AAFCO (2007) recomended allowance for riboflavin is 2.2 mg/kg DM for dogs and 4 mg/kg DM for cats for all lifestages. The NRC (2006) recommended allowance for riboflavin for dogs is 5.25 mg/kg DM for growth and maintenance and 5.3 mg/kg DM for gestation and lactation. For cats, the NRC (2006) recommended allowance for riboflavin is 4.0 mg/kg DM for all lifestages. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Deficiency of riboflavin in dogs and cats is uncommon but may manifest as dermatitis, erythema, weight loss, cataracts, impaired reproduction, neurologic changes and anorexia (NRC, 1985; Street and Cowgill, 1939; Street et al, 1941). Measurement of erythrocyte glutathione reductase activity is commonly used to evaluate riboflavin status in people and animals. **Table 6-5** lists riboflavin blood values for dogs and cats. Most commercial pet foods are supplemented with synthetic riboflavin. Toxicity has not been reported to occur in dogs and cats.

SOURCES

Riboflavin is widely distributed in foods, primarily bound to proteins as FMN and FAD. Rich sources include dairy products, organ meats (e.g., liver, heart, kidney), muscle meats, eggs, green plants and yeast. Cereal grains are poor sources of vitamin B_2. The supplemental form for addition to foods is usually riboflavin.

Niacin

Niacin is the generic term used to describe compounds that exhibit biologic activity of nicotinamide. Two major forms of niacin are nicotinic acid and nicotinamide.

FUNCTION

Nicotinic acid and nicotinamide are substituted pyridine ring structures (pyridine 3-carboxylic acid and nicotinic acid amide). Niacin must be converted to either nicotinamide-adenine dinucleotide (NADH) or nicotinamide-adenine dinucleotide phosphate (NADPH) to participate in enzymatic reactions or protein modification.

Niacin, in its cofactor form, is essential to several physiologic reactions: 1) oxidoreductive reactions, 2) nonredox reactions, 3) cleavage of β-N-glycosidic bonds with transfer of ADP-ribose to proteins (post-translational modification) and 4) formation of cyclic ADP-ribose (mobilizes intracellular calcium).

Oxidoreductive reactions are the primary function, but the others are significant in proper cell function. Generally, NAD/NADH is involved in catabolic reactions and transfers the reducing power (electrons) acquired from intermediary metabolites to the electron transport chain to ultimately produce adenosine triphosphate. Alternatively, NADP/NADPH is generally involved in biosynthetic reactions that transfer reducing power (electrons) to macromolecules such as fat, protein and carbohydrate.

METABOLISM

Niacin in foods is found mainly as NADH and NADPH, which may be free or bound to other macromolecules. After ingestion, NADH and NADPH undergo hydrolysis by the intestinal mucosa to release free nicotinamide, which is readily absorbed (Brody, 1994a). Dietary niacin (nicotinic acid and nicotinamide) is absorbed readily through the gastric and small intestinal mucosa. Both free nicotinic acid and nicotinamide are found in blood. Tissues readily take up these compounds to synthesize required cofactors, which also trap the compound in cells. Excess niacin is methylated and excreted in urine.

Niacin may also be synthesized from tryptophan via the kynurenin pathway, which results in formation of nicotinic acid ribonucleotide. Some enzymes in this pathway require vitamin B_6 and iron as cofactors. In most mammals, foods high in tryptophan can alleviate signs of niacin deficiency. However, cats cannot efficiently use tryptophan to synthesize niacin because they have a very high enzymatic activity of picolinic carboxylase that decisively leads the metabolism of tryptophan to acetyl-CoA and CO_2 instead of NAD (Sudadolnik et al, 1957; Baker and Czarnecki-Maulden, 1991). Thus, cats have a strict dietary requirement for preformed niacin.

REQUIREMENTS

The AAFCO (2007) recommended allowance for niacin is 11.4 mg/kg DM for dogs and 60 mg/kg DM for cats for all lifestages. The NRC (2006) recommended allowance for niacin is 17 mg/kg DM for dogs and 40 mg/kg DM for cats for all lifestages. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Deficiency of niacin results in pellagra with its classic 4D signs: dermatitis, diarrhea, dementia and death. Clinical deficiency is uncommon in dogs because most commercial pet foods are supplemented with niacin. Cats, however, are more likely to develop signs of deficiency because of their strict requirement for niacin. Niacin is a fairly stable vitamin. Processing conditions may release some bound niacin, which increases availability. Niacin deficiency may occur when foods with low quantities of niacin and tryptophan are ingested.

Measurement of methylated nicotinamide levels in urine best substantiates niacin deficiency. Niacin metabolites in whole blood have been reported for dogs and cats (**Table 6-5**), but these values generally have not been useful markers of deficiency in other species (Baker et al, 1986; Jacob and Swendseid, 1996). No niacin toxicity information in cats is available. However, excessive ingestion of nicotinic acid causes bloody stool, convulsions and even death (Chen et al, 1938).

SOURCES

Niacin is a very stable vitamin found in a variety of foodstuffs. The greatest amounts of niacin are found in yeast, animal/fish by-products, cereals, legumes and oilseeds. Niacin occurs in animal tissues as NAD and NADP and in plants mostly as protein-bound forms. Niacin is generally added to most pet foods as nicotinic acid or nicotinamide.

Pyridoxine

Pyridoxine is also generally called vitamin B_6. However, vitamin B_6 is a generic descriptor for all 3-hydroxy-2-methylpyridine derivatives exhibiting the biologic activity of pyridoxine. The three naturally occurring forms of vitamin B_6 are pyridoxal, pyridoxine and pyridoxamine.

FUNCTION

The biologically active forms of vitamin B_6 are the coenzymes pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP). PLP is involved in most reactions of amino acid metabolism, including transamination, decarboxylation, desulfhydration and nonoxidative deamination. PLP is also involved in the catabolism of glycogen and metabolism of lipids. As a coenzyme for decarboxylase enzymes, PLP functions in the synthesis of serotonin, epinephrine, norepinephrine and γ-aminobutyric acid (GABA). Pyridoxine is involved in vasodilatation through the production of histamine and is required in the pathway where niacin is produced from tryptophan. Pyridoxine helps catalyze the synthesis of taurine from cysteine and participates with ascorbic acid and NAD in the synthesis of carnitine from the amino acid lysine. Pyridoxine is also involved with the synthesis of the heme precursor porphyrin (as a coenzyme for δ-aminolevulinate synthase).

METABOLISM

The various forms of vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine, PLP, PMP) are freely absorbed via passive diffusion in the small intestine. The glucuronide form is not absorbed.

The predominant form of vitamin B_6 in blood is PLP, which is tightly bound to proteins. Pyridoxal crosses cell membranes more readily than PLP does. After uptake by cells, the vitamin is again phosphorylated by pyridoxal kinase to yield the predominant tissue form, PLP, which is considered to be the most active vitamin B_6 form.

The vitamin B_6 forms are readily interconverted metabolically by reactions involving phosphorylation/dephosphorylation, oxidation/reduction and amination/deamination. Phosphorylation appears to be an important means of retaining the vitamin intracellularly. Only small quantities of vitamin B_6 are stored in the body. The products of vitamin B_6 metabolism are excreted in the urine; pyridoxic acid is the predominant metabolic product. Different from other species, cats excrete little pyridoxic acid in urine even after a large oral dose of pyridoxine hydrochloride (Coburn and Mahuren, 1987). The main metabolites of vitamin B_6 in cat urine are pyridoxine 3-sulfate, pyridoxal 3-sulfate and N-methylpyridoxine.

REQUIREMENTS

The AAFCO (2007) recommended allowance for pyridoxine is 1 mg/kg DM for dogs and 4 mg/kg DM for cats for all lifestages. The NRC (2006) recommended allowance for

pyridoxine is 1.5 mg/kg DM for dogs and 2.5 mg/kg DM for cats for all lifestages. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Signs of vitamin B_6 deficiency include anorexia, reduced growth, muscle weakness, neurologic signs, (e.g., hyperirritability and seizures), anemia, and irreversible kidney lesions. Oxalate crystalluria is also a notable sign in pyridoxine-deficient cats (NRC, 2006). **Table 6-5** lists normal plasma levels of pyridoxine for cats and dogs (Baker et al, 1986).

Because pyridoxic acid is not detected in the urine of vitamin B_6-deficient subjects, this metabolite is useful in the clinical assessment of vitamin B_6 status. Measurement of xanthurenic acid excretion after a tryptophan load, however, is a more sensitive indicator of vitamin B_6 status. When vitamin B6 is deficient, the conversion of tryptophan to niacin is impaired, resulting in increased production of xanthurenic acid. Other indices of vitamin B_6 status are plasma concentrations of PLP and erythrocyte transaminase.

The prevalence of vitamin B_6 toxicity appears to be low. Earliest detectable signs include ataxia and loss of small motor control. Many of the signs of toxicity resemble those of vitamin B_6 deficiency: ataxia, muscle weakness and loss of balance. Histologic examination of tissues from dogs fed more than 200 mg pyridoxine hydrochloride/kg body weight/day revealed bilateral loss of myelin and axons in the dorsal funiculi and loss of myelin in the dorsal nerve roots (Phillips et al, 1978). There is no information regarding vitamin B_6 toxicity in cats.

SOURCES

Vitamin B_6 is widely distributed in foods, occurring in greatest concentrations in meats, whole-grain products, vegetables and nuts. The chemical forms of vitamin B_6 tend to vary among foods of plant and animal origin; plant tissues contain mostly pyridoxine, whereas animal tissues contain mostly pyridoxal and pyridoxamine. Pyridoxine is far more stable than either pyridoxal or pyridoxamine, thus processing losses are greatest in foods containing animal products. Losses can be as high as 70% (average losses from 0 to 40%) (McDowell, 1989). Pyridoxine hydrochloride is most often used for supplementation because it is relatively stable.

Pantothenic Acid

Pantothenic acid is the trivial designation for dihydroxy-β, β-dimethylbutyryl-β-alanine. Only the dextrorotatory form of pantothenic acid has biologic activity. It occurs mainly in bound form, (i.e., coenzyme A [CoA] and acyl-carrier protein), in most foods and feedstuffs. Pantothenic acid in foods is fairly stable at cooking temperatures and during storage. However, appreciable losses (up to 50%) have been reported during canning and storage of some foods at pH values greater than 7 and less than 5 (Combs, 1998).

FUNCTION

CoA is found in all tissues and is one of the most important coenzymes for metabolism. CoA plays a critical role in the tricarboxylic acid cycle for production of ATP from fat (glycerol and fatty acids), glucose and amino acids. CoA is also involved in the synthesis of fatty acids, steroid hormones and cholesterol. CoA is necessary for oxidation of fatty acids, pyruvate and ketoglutarate (Machlin, 1991).

METABOLISM

CoA and acyl-carrier protein are the predominant forms of pantothenic acid in foods and foodstuffs. Thus, hydrolytic digestion of these protein complexes is the first step in metabolism of this vitamin. Both forms are degraded to pantothenic acid in the lumen of the intestine in a series of steps. Absorption occurs via a saturable, sodium-dependent, energy-requiring process. At high concentrations, simple diffusion occurs throughout the small intestine. In dogs, more than 80% of free pantothenate is absorbed from the gut (Taylor et al, 1974). Urinary β-glucuronide is the major form of excretion (Taylor et al, 1972). Pantothenic acid is transported in the free acid form in plasma. Erythrocytes contain predominantly acetyl-CoA.

REQUIREMENTS

The AAFCO (2007) recommended allowance for pantothenic acid is 10 mg/kg DM for dogs and 5 mg/kg DM for cats for all lifestages. Less pantothenic acid is apparently required to optimize growth when high-protein foods are fed, whereas high-fat diets may increase the requirement for pantothenic acid (McDowell, 1989). The NRC (2006) recommendation for pantothenic acid allowance is 15 mg/kg DM for dogs regardless of lifestage. For cats, the NRC (2006) allowance for pantothenic acid is 5.7 mg/kg DM for growth, and 5.75 mg/kg DM for maintenance and reproduction. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Naturally occurring deficiency of pantothenic acid is rare. Dogs with pantothenic acid deficiency have erratic appetites, depressed growth, fatty livers, decreased antibody response, hypocholesterolemia and coma, in later stages. Pantothenic acid-deficient cats developed fatty livers and became emaciated (NRC, 2006). Normal whole blood concentrations of pantothenic acid for dogs and cats are listed in **Table 6-5** (Baker et al, 1986).

Pantothenic acid is generally regarded as nontoxic. No adverse reactions or clinical signs other than gastric upset have been observed in any species following ingestion of large doses.

SOURCES

"Pantothenic acid" is derived from the Greek word "pantos" meaning "found everywhere." Although this vitamin is found in practically all foodstuffs, the quantity present is generally insufficient for most monogastric species. The most important sources are meats (especially liver and heart), rice and wheat bran, alfalfa, peanut meal, yeast and fish solubles. Calcium pantothenate is the predominant form added to pet foods.

Folic Acid

Folic acid was first discovered in 1943 and was classified as vitamins B_{10} and B_{11}. The structure of folic acid may be subdivided into three functional components: the middle group is para-aminobenzoic acid (PABA), flanked on one side by a pteridine ring, and on the other side by a polyglutamic acid chain. Folate is the name commonly used to designate a family of compounds with the biologic activity of folic acid (Brody, 1994a; Selhub and Rosenberg, 1996).

FUNCTION

Folic acid functions as a one-carbon (methylene, methenyl, methyl) donor and acceptor molecule in intermediary metabolism. Specific pathways include nucleotide biosynthesis, phospholipid synthesis, amino acid metabolism, neurotransmitter production and creatinine formation. In addition, vitamin B_{12} is closely paired with folate in the production of methionine from homocysteine, which will be discussed later in this section.

METABOLISM

Natural sources of folic acid undergo hydrolysis by the intestinal enzyme γ-glutamyl hydrolase to form folylmonoglutamate, which is subsequently absorbed by enterocytes. Thus, the major form of folic acid in blood is the monoglutamate form. After target cells absorb folylmonoglutamate, additional glutamates are added to the tail, which trap the molecule within cells.

Folic acid must be in the reduced form (i.e., dihydro or tetrahydro) to participate in one-carbon metabolic reactions. The enzyme dihydrofolate reductase (DHFR) interconverts dihydrofolates to tetrahydrofolates. Inhibition of this enzyme interferes with intermediary pathways that require reduced folates for coenzymes. C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene also affects folate metabolism and requirement (Golbahar et al, 2005).

REQUIREMENTS

The AAFCO (2007) recommended allowance for folic acid is 0.18 mg/kg DM for dogs and 0.8 mg/kg DM for cats for all lifestages, respectively. The NRC (2006) recommended allowance for folic acid is 270 µg/kg DM for dogs and 750 µg/kg DM for cats regardless of lifestage. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Folate deficiency is characterized by poor weight gain, megaloblastic anemia, anorexia, leukopenia, glossitis and decreased immune function. In cats, folate deficiency is associated with hyperhomocysteinemia and greatly augmented urinary excretion of formiminoglutamic acid (Yu and Morris, 1998). Folate deficiency has been linked to the risk of neural tube defects in people (Mitchell, 2005). Folate levels in blood may be measured to confirm a deficiency suggested by clinical signs. **Table 6-5** lists plasma levels for healthy cats and dogs (Baker et al, 1986).

There have been no reported cases of folate toxicity. Neither NRC nor AAFCO has proposed a dietary maximum concentration for folic acid.

SOURCES

Folate is found in several foods, but is unstable in a variety of conditions. Liver, egg yolks and green vegetables are good sources of folate. The vitamin is destroyed by heating, prolonged freezing and during storage in water. Commercial pet foods are supplemented with folate to overcome the effects of processing and storage.

Biotin

Biotin consists of an imidazole ring fused to a tetrahydrothiophene ring with a valeric acid side chain. It has eight possible stereoisomers in nature but only d-(+)-biotin is physiologically active (NRC, 2006). Biotin is unstable to oxidation and heat.

FUNCTION

Biotin is an essential cofactor for four different carboxylase reactions in mammals. These carboxylases have important functions in the metabolism of lipids, glucose, some amino acids and energy.

METABOLISM

The majority of biotin in food sources is thought to be covalently bound to proteins. After ingestion, biotin must be hydrolyzed from protein by the enzyme biotinidase in pancreatic juice before absorption in the intestine (Brody, 1994a; Mock, 1996). After hydrolysis, free biotin is actively absorbed through a biotin transporter that requires both an intact ureide group and a free carboxyl group on valeric acid (NRC, 2006). Avidin in raw egg white can tightly bind biotin and is resistant to intestinal proteolysis and heat treatment, making biotin unavailable for absorption. After absorption from the intestine, biotin is transported in the free form in the plasma to the required tissues where it is linked to its target apoenzyme by the enzyme holocarboxylase synthetase. The kidneys eliminate excess biotin. Increasing urinary excretion of 3-hydroxyisovaleric acid, an indicator of reduced activity of the biotin-dependent enzyme methylcrotonyl-CoA carboxylase, and decreasing biotin in urine are early and sensitive indicators of biotin deficiency (Mock et al, 2002).

REQUIREMENTS

Neither AAFCO (2007) nor NRC (2006) has a recommendation for biotin for dogs. However, diets containing raw egg white and/or antibiotics may need biotin supplementation. The AAFCO (2007) biotin recommendation for cats is 0.07 mg/kg DM for all lifestages. The NRC (2006) recommended allowance for biotin for cats is 75 µg/kg DM regardless of lifestage. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Naturally occurring biotin deficiency is very rare in dogs and cats (NRC, 2006). Feeding raw egg whites and administering oral antimicrobials are probably the two most common causes of biotin deficiency. Raw egg whites contain the glycoprotein avidin, which binds biotin rendering it unavailable for absorp-

tion. Feeding avidin to cats may result in signs of biotin deficiency that include dermatitis, alopecia and a dull coat (Pastoor et al, 1993). Because gut microbial synthesis may meet half the biotin requirement, antimicrobials that decrease the population of the intestinal microflora may also result in signs of biotin deficiency. Clinical signs include poor growth, dermatitis, lethargy and neurologic abnormalities (**Case 6-10**). **Table 6-5** lists biotin blood values for dogs and cats (Baker et al, 1986).

Biotin toxicity has not been reported. Neither AAFCO (2007) nor NRC (2006) has proposed a dietary maximum concentration for biotin.

SOURCES

Mammalian tissues are incapable of synthesizing biotin. The biotin requirement is probably met by two sources: diet and microbes (Brody, 1994a; Mock, 1996). Biotin is widely distributed in foods, but mostly in very low, highly variable concentrations. Oilseeds, egg yolks, alfalfa meal, liver and yeast are the most important natural sources of biotin. Marked losses of biotin may occur as a result of oxidation, canning, heat and solvent extraction of foodstuffs. Less than one-half of the biotin in various foodstuffs is biologically available (McDowell, 1989). Most commercial pet foods are supplemented with synthetic biotin.

Vitamin B_{12}

Vitamin B_{12} or cobalamin is the generic descriptor for all corrinoids exhibiting the biologic activity of cyanocobalamin. Vitamin B_{12} is the largest and most complex B vitamin and the only one to contain a metal ion, cobalt. The structure consists of four pyrrole rings linked to form a macrocyclic ring designated as corrin, which is similar to hemoglobin. Substitutions on the corrin ring account for the different recognized forms of vitamin B_{12}. The active forms of B_{12}, 5'deoxyadenosylcobalamin and methylcobalamin, are very unstable (Brody, 1994a; Herbert, 1996). Substituted forms of vitamin B_{12} are much more stable and may be used as pharmaceutical supplements (cyanocobalamin, hydroxocobalamin, nitritocobalamin).

FUNCTION

Vitamin B_{12} is important in one-carbon metabolism. In dogs and cats, methylcobalamin, which contains cobalt in the 1^+ state, is a coenzyme for methionine synthase and 5'deoxyadenosylmethionine, which contains cobalt in the 2^+ state is a coenzyme for methylmalonyl-CoA mutase. Vitamin B_{12} is required by the enzyme methionine synthase that removes a methyl group from methyl tetrahydrofolate (THF) to regenerate THF, which is needed for pyrimidine biosynthesis. This intimate relationship with folate may result in folate trapping in B_{12} deficiency and the resultant megaloblastic anemia of folate deficiency.

METABOLISM

Dietary vitamin B_{12} is freed from food peptides and proteins by hydrolysis (gastric acidification and pancreatic enzymes). Free vitamin B_{12} binds to intrinsic factor (IF), a glycoprotein secreted from gastric parietal cells. IF is essential for vitamin B_{12} absorption in people. In dogs, the pancreas is the major and the stomach a lesser source of IF. In cats, the pancreas appears to be the sole source of IF (NRC, 2006). The stable vitamin B_{12}-IF complex is absorbed in the ileum via cell surface specific receptors. Vitamin B_{12} may also be absorbed in the jejunum of dogs and cats (Gazet and McColl, 1967). After absorption, vitamin B_{12} is transported in blood by transcobalamin I and II. Transcobalamin I (haptocorrin) is a glycoprotein that carries almost all vitamin B_{12} in the blood of people. Transcobalamin II, a protein without a carbohydrate moiety, carries about 75% of vitamin B_{12} in the blood of dogs and cats. Cat and dog plasma do not contain transcobalamin I, but have another transport protein, transcobalamin O, which carries about 10 to 15% of vitamin B_{12} (Linnel et al, 1979). All DNA-synthesizing cells take up vitamin B_{12} from the blood via cell surface specific receptors.

REQUIREMENTS

The AAFCO (2007) recommended allowance for vitamin B_{12} is 0.022 mg/kg DM for dogs and 0.020 mg/kg cats for all lifestages. The NRC (2006) recommended allowance for vitamin B_{12} is 35 µg cobalamin/kg DM for dogs and 22.5 µg/kg DM for cats regardless of lifestages. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Vitamin B_{12} deficiency is very rare but may result in poor growth and neuropathies in dogs (**Case 6-11**). Because vitamin B_{12} is only made by microbes and found in animal tissue, long-term feeding of vegetarian diets may lead to vitamin B_{12} deficiency.

Vitamin B_{12} may be directly assessed by determination of serum vitamin B_{12} levels or indirectly by determination of serum or urine methylmalonic acid (MMA) (Brody, 1994a). MMA levels in serum and urine increase with vitamin B_{12} deficiency. A newer test, serum holotranscobalamin II, may prove useful in the future to detect early vitamin B_{12} deficiency (Herbert, 1996). Whole blood levels of cobalamin for dogs and cats are listed in **Table 6-5** (Baker et al, 1986).

Oral toxicity of vitamin B_{12} has not been reported in dogs and cats. Neither AAFCO (2007) nor NRC (2006) has proposed a dietary maximum concentration for vitamin B_{12}.

SOURCES

Only certain microorganisms synthesize cobalamin. Microbes and yeast can make vitamin B_{12} for absorption by animals. Plants generally contain very small amounts of vitamin B_{12}. Meat and, to some degree, milk products are good sources of vitamin B_{12}. Most commercial pet foods are supplemented with stable vitamin B_{12}.

Choline

Choline is traditionally classified as one of the B-complex vitamins although it does not entirely satisfy the strict definition of a vitamin; many animals can synthesize choline in the liver. In

addition, choline is required in the body in substantially greater amounts (>1,000 mg/kg) than the other B vitamins (<100 mg/kg). Furthermore, choline does not function as a coenzyme or cofactor as do most other B vitamins.

Choline, 2-hydroxy-N, N, N-trimethylethanaminium, has three methyl groups that enable choline to serve as a methyl donor in the body. It is an integral component of lecithin (phosphatidylcholine). Choline is a strong base and decomposes in alkaline solution.

FUNCTION

Choline plays several important roles in the body. It is an integrated component of phosphatidylcholine, a structural element of biologic membranes. Phosphatidylcholine also promotes lipid transport. Diminished synthesis of phosphatidylcholine in the liver due to choline deficiency results in accumulation of lipids in the liver. Choline, as acetylcholine, is a neurotransmitter. Choline, as a component of platelet-activating factor (1-O-alkyl-2acetyl-sm-glycero-3-phophocholine), is important in clotting and inflammation. After oxidation to betaine, choline is a source of labile methyl groups for transmethylation reactions (e.g., the formation of methionine from homocysteine and creatine from guanidoacetic acid).

METABOLISM

Choline is present in food predominantly as phosphatidylcholine; less than 10% is present as either the free base or sphingomyelin (Combs, 1998). Choline is released from phosphatidylcholine and sphingomyelin by digestive enzymes and absorbed from the jejunum and ileum mainly by a carrier-mediated process. Intestinal microorganisms metabolize most free choline ingested to trimethylamine, which is absorbed and excreted in urine. Phosphatidylcholine is not subject to such extensive microbial metabolism; therefore, metabolism of phosphatidylcholine results in less urinary trimethylamine. Once absorbed, choline is transported in the lymphatic circulation primarily in the form of phosphatidylcholine bound to chylomicrons.

Most species can synthesize choline, as phosphatidylcholine, by the sequential methylation of phosphatidylethanolamine. The activity is greatest in the liver, but is also found in many other tissues.

REQUIREMENTS

The requirement for choline is affected greatly by dietary factors such as methionine, betaine, myoinositol, folate and vitamin B_{12}, as well as the combination of different levels and composition of fat, carbohydrate and protein in the diet. In addition, age, gender, caloric intake and growth rate influence the lipotrophic action of choline and thereby its requirement.

Choline and methionine are the two principal methyl donors in transmethylation. Therefore, dietary adequacy of methionine and choline directly affects the requirement of the other. Methionine can completely replace choline as a methyl donor. For example, in cat foods, if dietary methionine exceeds 0.62% DM, 3.75 parts of methionine can be substituted for 1 part choline (AAFCO, 2007).

Vitamin B_{12} and folate are required for the synthesis of methyl groups and metabolism of the one-carbon unit. Biosynthesis of labile methyl from a formate carbon requires folate, whereas vitamin B_{12} plays a role in regulated transfer of the methyl group to tetrahydrofolic acid. Therefore, a deficiency of one or both of these vitamins increases the requirement for choline.

Excess dietary protein and/or high-fat foods increase the choline requirement. In most species, the choline requirement is greater for younger animals than for adults. Some adult species may not require choline.

The AAFCO (2007) recommended allowance for choline is 1,200 mg/kg DM for dogs and 2,400 mg/kg DM for cats for all lifestages. The NRC (2006) recommended allowance for choline is 1,700 mg/kg DM for dogs and 2,550 mg/kg DM for cats regardless of lifestage. **Table 6-5** lists AAFCO and NRC allowances for dogs and cats.

DEFICIENCY AND TOXICITY

Choline deficiency in most animal species is characterized by depressed growth, hepatic steatosis and hemorrhagic renal degeneration (Combs, 1998). Additional signs of choline deficiency in dogs include thymic atrophy and elevated plasma phosphatase values and increased blood prothrombin time. **Table 6-5** lists normal plasma levels of choline for cats and dogs (Baker et al, 1986). Choline and phosphatidylcholine levels in blood may be measured to confirm deficiency suggested by clinical signs.

Studies with dogs suggested a low tolerance to lecithin (phosphatidylcholine). Reduced erythrocytes resulted from daily oral administration of lecithin (equivalent to 150 mg of choline) (NRC, 1987). However, neither AAFCO (2007) nor NRC (2006) has recommended a maximum or safe upper limit for dietary choline for dogs and cats.

SOURCES

All natural fats contain some choline; therefore, choline is widely distributed in foods and foodstuffs. Lecithin has also been shown to be an effective emulsifying agent in foods and is the form of choline ingested in most foods. Egg yolks, glandular meals and fish are the richest animal sources and cereal germs, legumes and oilseed meals are the best plant sources. Choline is added to most pet foods as choline chloride and is added separately from the vitamin premix because of its hygroscopic nature and propensity to reduce the stability of other vitamins if added in the premix.

Vitamin C

Because of de novo synthesis, vitamin C is not technically a vitamin for healthy dogs and cats. (See vitamin definition.) However, it is included here because of its biochemical functions, including in vivo and in vitro antioxidant properties (Chapter 7).

FUNCTION

Vitamin C, ascorbic acid or specifically L-ascorbic acid, is a very labile compound that is readily oxidized to dehy-

droascorbic acid. It requires a reducing enzyme (dehydroascorbic acid reductase) to transform it back to the active form. Vitamin C primarily functions in the body as an antioxidant and free radical scavenger. Ascorbic acid is best known for its role in collagen synthesis, where it is involved in hydroxylation of prolyl and lysyl residues of procollagen (Combs, 1998). It is also involved in drug, steroid and tyrosine metabolism (McDowell, 1989) and electron transport. Ascorbic acid is also necessary for synthesis of L-carnitine, an important carrier of acyl groups across mitochondrial membranes. Normal circulating plasma levels are 4 µg/ml in dogs and 3 µg/ml in cats (Baker et al, 1986).

More recently, research into the role of ascorbic acid has shifted from prevention of deficiency to the treatment and prevention of disease. Because ascorbic acid protects against free-radical damage induced by the "oxidative burst" of neutrophils (Combs, 1998; Levine et al, 1994), and stimulates the phagocytic effect of leukocytes, it plays a role in immune function (McDowell, 1989). Larger doses may play a protective role against carcinogenesis. Ascorbic acid acts as a nitrate scavenger, thereby reducing nitrosamine-induced carcinogenesis. Vitamin C has been associated with a reduced risk for gastric cancer, oral cancer and perhaps lung cancer, but had no effect on cancer of the pancreas, colon and prostate gland (Sauberlich, 1991).

Vitamin C may even play a role in the prevention of gingival and periodontal disease. Studies with people have shown that 600 mg/day (10x the recommended dietary allowance) significantly reduced gingival bleeding upon probing (Leggott et al, 1986). Whether this effect can be demonstrated in species that synthesize their own ascorbate (i.e., cats and dogs) remains to be seen.

Ascorbic acid may have some benefit in exercise stress recovery (Kronfeld, 1983). However, megadose supplementation to prevent hip dysplasia has not proved effective (Richardson, 1992).

METABOLISM

Most higher animals can synthesize vitamin C from glucose via the glucuronic acid pathway. People and some animals such as guinea pigs, fish, fruit-eating bats, insects and some birds cannot synthesize vitamin C because they lack the key enzyme L-gulonolactone oxidase. In these species, vitamin C is absorbed by a saturable, carrier-mediated, active-transport mechanism that is sodium dependent. Species that can synthesize ascorbic acid absorb it strictly by passive diffusion. In either case, absorption efficiency of physiologic doses is more than 80% (Combs, 1998).

Vitamin C is transported in the plasma in association with albumin, mostly in a reduced form. Under physiologic conditions, vitamin C exists as ascorbate, which cannot cross most membranes readily. Cellular uptake of vitamin C involves dehydroascorbic acid in erythrocytes, lymphocytes and neutrophils. Once inside the cell, dehydroascorbic acid is quickly reduced to ascorbic acid by an intracellular enzyme (dehydroascorbic acid reductase), which uses reduced glutathione (GSH) as the source of reducing equivalents. Ascorbic acid is widely distributed throughout tissues, both in animals capable of synthesizing ascorbic acid and those that depend on dietary vitamin C. The pituitary and adrenal glands have the highest concentrations of vitamin C; high levels are also found in the liver, spleen, brain and pancreas. Ascorbic acid is excreted in urine, sweat and feces. Losses in feces and sweat are usually minimal.

Because vitamin C is not an essential nutrient for dogs and cats, neither AAFCO nor NRC lists recommendations.

DEFICIENCY AND TOXICITY

Acute vitamin C deficiency results in scurvy (in animals unable to synthesize the vitamin). In general, high intake of vitamin C is considered to be of low toxicity.

SOURCES

Fruits, vegetables and organ meats are generally the best sources of vitamin C. The vitamin C content of most foods decreases dramatically during storage and processing. Polyphosphorylated forms of vitamin C are available that can survive processing conditions.

Vitamin-Like Substances

Vitamin-like substances are substances that exhibit properties similar to those of vitamins, but do not fit the strict definition of a vitamin. They have physiologic functionality, but questionable essentiality. These compounds can be "conditionally essential" depending on the metabolic capacity of the animal.

L-carnitine

L-carnitine is one of the most well known vitamin-like substances. L-carnitine is a natural component of all animal cells (Bremer, 1983; Rebouche and Paulson, 1986). Its primary function is to transport long-chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix for β-oxidation (Bremer, 1983; Fritz, 1958). Skeletal and cardiac muscle contain 95 to 98% of the L-carnitine in the body and are significant storage sites (Rebouche and Engel, 1983).

The biosynthesis of L-carnitine requires five enzymatic steps that occur in many cells in the body (Bremer, 1983). The final step in which butyrobetaine is converted to L-carnitine is rate limiting and occurs primarily in the liver (Bremer, 1983). Lysine, methionine, ascorbic acid, ferrous ions, vitamin B_6 and niacin are important in L-carnitine metabolism; these nutrients are required substrates and cofactors for the enzymes involved in L-carnitine biosynthesis (Borum, 1986).

Clinical signs of L-carnitine deficiency include chronic muscle weakness, fasting hypoglycemia, cardiomyopathy, hepatomegaly and dicarboxylic aciduria (Stanley, 1987). In many cases of L-carnitine deficiency, no clinical signs are apparent (Borum, 1986).

Carotenoids

Carotenoids are a class of lipophilic natural pigments that are widely distributed throughout the plant and animal kingdom. Only plants, bacteria, fungi and algae synthesize carotenoids; however, animals can accumulate carotenoids in their tissues

after oral ingestion. In plants, carotenoids play essential light-harvesting roles during photosynthetic events and protect membranes against photo-oxidative damage. More than 600 different compounds are classified as carotenoids, but fewer than 10% can be metabolized into vitamin A. In contrast to many other mammals, cats are unable to convert β-carotene to vitamin A; therefore, cats must rely solely on preformed vitamin A in their diet (Schweigert et al, 2002). The carotenoids found in greatest abundance in a variety of foodstuffs are β-carotene, α-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin, canthaxanthin and astaxanthin. A primary characteristic of the carotenoids is their conjugated polyene structure.

ABSORPTION AND TRANSPORT

Because carotenoids are lipophilic compounds, concurrent ingestion of fat facilitates intestinal carotenoid absorption. Bile salts are necessary for absorption of ingested fat and carotenoids. The aggregation of bile salts into micelles, and the formation of mixed micelles with the products of lipid digestion and other lipid-soluble food constituents are essential in facilitating absorption of lipophilic compounds from the intestine. At the brush border, micelles interact with enterocytes where the lipophilic contents of micelles diffuse out of the micelles and across the cell membrane. It is believed that the uptake of carotenoids by enterocytes occurs passively and is not carrier-mediated. Enterocytes package carotenoids into chylomicrons, which migrate to the basal-lateral cell membrane where they are exocytosed into the intracellular space for passage to the lymphatic system. After transportation in chylomicrons via the lymphatic system, carotenoids are carried by lipoproteins and transported in the bloodstream.

FUNCTION

Although carotenoids do not strictly fit the definition of a vitamin for mammalian species, they have biologic activity beyond their provitamin A role. Carotenoids with nine or more double bonds function as antioxidants by quenching singlet oxygen and other reactive oxygen species such as hydroxyl radicals, superoxide anion radicals and hydrogen peroxide, which are produced in normal metabolism (Chew, 1995; Bendich, 1989). Carotenoids sacrifice highly reactive multiple double bonds to free radicals via hydrogen donation, thereby stabilizing reactive products. Carotenoids also protect cell membranes by stabilizing the oxygen radicals produced when phagocytic granulocytes undergo respiratory bursts that destroy intracellular pathogens (Bendich, 1989).

The immune-modulating properties of carotenoids have been studied in dogs and cats. Supplementation with β-carotene increases the CD4 T cell population in older dogs to levels found in young dogs and improves T-cell proliferation (Massimino et al, 2003). Supplementation with β-carotene or lutein, an oxycarotenoid found in corn and other vegetables, stimulates cell-mediated and humoral immune responses in dogs and cats (Chew et al, 2000; Kim et al, 2000; Kim et al, 2000a).

SOURCES

Carotenoids are responsible for the striking colors of many yellow, orange and red fruits and vegetables, plant leaves, as well as the colors in some species of fish, crustaceans and plumage of some birds.

Bioflavonoids

The flavonoids are a group of red, blue, yellow and colorless compounds that have vitamin-like activity. This class of compounds was originally mistaken for vitamin C because crude extracts of lemon juice and yellow peppers had antiscorbutic effects. Originally called citrin (mixture of eriodictyol and hesperidin), vitamin P or vitamin C_2, these compounds were reclassified as flavonoids in 1950 (Combs, 1998; Machlin, 1991; Harborne, 1994). More than 5,000 flavonoids have been identified (Harborne and Baxter, 1999). Flavonoids are classified in major and minor groups. Classes include flavonols, flavanols, flavones, isoflavones and anthocyanins. Flavonols, (e.g., kaempferol, quercetin and myricetin, are present in tea, apples and onions. Flavanols (also called catechins) are found in tea, apples and red wine. Isoflavones such as genistein and daidzein are constituents of soybeans. Anthocyanins provide the deep red color to fruits such as berries.

ABSORPTION AND TRANSPORT

The availability varies widely among flavonoids depending on the food source and the forms of flavonoids they contain. Flavonoids are usually found naturally as glycosides linked to sugars, except for catechins. The type of sugar moiety of the glycoside affects availability, (e.g., quercetin glucosides are more efficiently absorbed than quercetin rutinosides) (Hollman et al, 1999). Mammalian enzymatic systems are unable to hydrolyze flavonoid glycosides, but the necessary glycosidases are present in the gut microflora. After hydrolysis and absorption in the small intestine, flavonoids are bound in the liver as glucuronides or sulfate conjugates (Machlin, 1991). Recent studies with flavanols have shown that glycosides can be absorbed without previous hydrolysis by microorganisms (Hollman et al, 1995). Most of the flavonoids are further metabolized into phenolic compounds and rapidly excreted, usually within 24 hours.

FUNCTION

Although many different flavonoids exist with many different physiologic effects, this class of compounds shares some similar functions. The most notable is the sparing effect that flavonoids have on vitamin C. Flavonoids have the ability to perform similarly to vitamin C: reduce capillary fragility and permeability and chelate the divalent metal ions copper and iron (Combs, 1998). Flavonoids can act as antioxidants because they are very effective scavengers of free radicals. In fact, flavonoid assays in vitro often exhibit stronger antioxidant activity than vitamins E and C. Other non-antioxidant related beneficial effects include prevention of angiogenesis (Cao and Cao, 1999) and inhibition of cyclooxygenase and lipoxygenase (Laughton et al, 1991). Catechins, found in abundance in tea, have been shown to modulate signal transduction pathways,

have antiinflammatory activities and decrease cell proliferation (Dong et al, 1997; McCarty, 1998). Data from studies using animal models have shown that green and black tea consistently decrease cancers of the skin, lung, stomach, liver, mammary gland and colon (Chung et al, 2003). Isoflavones present in soybeans have been associated with reduced risk of cardiovascular disease, certain cancers and other degenerative diseases.

SOURCES

Flavonoids are ubiquitous in the plant kingdom. Significant variation in the flavonoids present in leaf, petal, root, fruit and seed can occur within the same plant. Flavonoid concentration can vary within a given plant organ (i.e. in apples), flavonoids tend to concentrate in the skin (Harborne, 2000).

Other Vitamin-Like Substances

Some other substances with vitamin-like activity include lipoic acid, ubiquinones, orotic acid, inositol and p-aminobenzoic acid. Animals synthesize most of these compounds, which are important metabolic intermediates. They function: 1) in the metabolism of fatty acids, 2) in the electron transport chain, 3) as antioxidants and 4) as growth factors. Continued research in "conditionally essential" nutrients may lead to vitamin classification for many of these compounds.

ENDNOTES

a. Kirk CA. Hill's Science and Technology Center, Topeka, KS, USA. Personal communication, 1997.
b. Wedekind KJ. Hill's Science and Technology Center, Topeka, KS, USA. Unpublished data, 1997.

REFERENCES

The References for **Chapter 6** can be found at www.markmorris.org.

CASE 6-1

Seizures in an Airedale Terrier

Patient Assessment

A 20-kg, eight-year-old, neutered male Airedale terrier was admitted to an emergency clinic after a 45-minute episode of continuous seizure activity. The dog was moribund at presentation. Thirty-six hours before the onset of seizures, the dog had ingested a salt-flour figurine, weighing approximately 100 g. The dog vomited a clear fluid three times within 12 hours after ingesting the figurine and became progressively more polydipsic and polyuric. The dog then consumed an unknown additional volume of uncooked salt-flour dough. Within an hour after ingesting this mixture, the dog developed generalized, fine-muscular fasciculations, which rapidly progressed to clonic-tonic motor activity.

The moribund dog was unresponsive to painful stimuli, pyrectic (41.6°C [106.9°F]), tachypneic and had an irregular heart rhythm. A generalized seizure occurred during the examination. Serum electrolyte and blood gas analysis revealed severe hypernatremia (serum sodium 211 mEq/l, normal 145 to 158), hyperchloremia (serum chloride 180 mEq/l, normal 105 to 122) and metabolic acidosis (serum pH 7.135, normal 7.32 to 7.38).

Treatment Plan

Treatment was initiated with intravenous fluids (5% dextrose in water), sodium bicarbonate, diazepam, phenobarbital and furosemide. The dog was also cooled with ice-water wraps and electric fans. The dog suffered cardiopulmonary arrest five hours later and died.

Further Assessment

At postmortem examination, one liter of putty-like, grayish-white material and clear, watery fluid were found in the stomach. Hemorrhage was noted throughout the stomach and the proximal two-thirds of the small intestine. Acute renal and hepatic necrosis was found histopathologically. Sodium and chloride levels in tissues were higher than normal. The brain sodium level was 108 mEq/l (80 mEq/l is considered indicative of sodium salt toxicosis). Analysis of the liquid portion of the gastric contents showed that a minimum of 20 g of sodium chloride remained in the stomach.

Bibliography

Khanna C, Boermans HJ, Wilcock B. Fatal hypernatremia in a dog from salt ingestion. Journal of the American Animal Hospital Association 1997; 33: 113-117.

CASE 6-2

Vomiting and Diarrhea in a Yorkshire Terrier

Patient Assessment

A one-year-old, intact female Yorkshire terrier weighing 2.7 kg had a sudden onset of lethargy, watery diarrhea, vomiting, icterus and red-colored urine. Abnormal laboratory findings included hemolyzed plasma, anemia, azotemia, leukocytosis, hemoglobinuria and an elevated total bilirubin concentration. Abdominal radiographs revealed a metal object at the pylorus. The object was recognized by the owner as a nut that had been missing for two weeks from an airfreight kennel used to house the dog. Serum zinc concentration was 32 mg/kg, compared with 1.1 mg/kg in serum obtained from a clinically normal dog at the same time.

Assess the Food and Feeding Method

No dietary history was available. The manufacturer of the kennel indicated that the nut was made of pure zinc.

Treatment Plan

The nut was removed from the stomach using a fiberoptic endoscope. Additional therapy included intravenous fluids and a blood transfusion.

Reassessment

The dog stopped vomiting but remained depressed and continued to have profuse watery diarrhea. Semi-solid feces were passed on Day 5 after removal of the nut. The dog's appetite returned on Day 6 and the dog steadily improved until discharge seven days later. Serum zinc concentrations on Days 11 and 21 were 8.5 mg/kg and 1.0 mg/kg respectively (values for a clinically normal dog were 0.7 mg/kg). The owner reported that the dog seemed completely normal three months after discharge.

On analysis, the nut contained 97% zinc, 2% aluminum and other elements. The nut removed from the stomach was highly corroded and when its weight was compared with that of a new nut of the same design, it appeared that the dog received a total dose of 703 mg zinc/kg body weight.

Bibliography

Torrance AG, Fulton RB. Zinc-induced hemolytic anemia in a dog. Journal of the American Veterinary Medical Association 1987; 191: 443-444.

CASE 6-3

Reproductive Problems in a Group of Cats

Patient Assessment

A group of breeding domestic shorthair cats, ranging in age from two to five years and weighing from 3 to 4.5 kg, was presented for poor reproductive performance, including failure to conceive, fetal resorption, small weak kittens and cannibalism. Neonatal kittens from these queens had graying of hair, dry and curled coat texture and skeletal abnormalities including inverted carpi and metatarsi, "kinked" tails and fused digits.

Physical examinations of the unbred queens were unremarkable. Some pregnant queens appeared slightly underweight for their date of gestation but were otherwise normal when examined. Newborn litters contained several small kittens, weighing less than 70 g or kittens with gray-to-whitish coats over the caudal one-half to three-fourths of the body. The coat color over the head and feet was unaffected. The coat texture of kittens less than three days old was somewhat dry and had a slightly curled appearance. Several newborn kittens had kinked tails and inverted carpi. Kittens older than three weeks had normal coats and improvements in carpal and tarsal malformations, but normal function or structure did not return in many kittens. Kittens with kinked tails and fused toes did not improve with age.

Reproductive problems included a decline in conception rate from 100% to between 0 to 50% over an eight-month period. In utero monitoring of pregnant queens through biweekly ultrasound examinations showed that the fetal loss rate was 67% and occurred between 25 to 30 days of gestation. Food intake was only about two-thirds of that expected for the queens.

The initial evaluation included complete blood counts and serum biochemistry profiles for many of the queens and serum trace mineral analyses and heavy metal toxicity screens for queens and affected kittens. The hemogram results included normal hematocrit and hemoglobin values with low mean corpuscular hemoglobin concentrations (hypochromasia) in four of six queens evalu-

ated. Heavy metal toxicity screens of affected kittens were unremarkable with the exception of high-hepatic zinc level in one kitten and a single low-hepatic iron value. Serum copper concentrations for queens were normal, but hepatic copper values were not determined because queens were in active reproduction. In one- to two-week-old affected kittens, hepatic copper concentrations ranged from 26.6 to 35.7 mg/kg and serum copper values from 0.3 to 0.4 mg/kg, which were deemed borderline low based on literature values.

Assess the Food and Feeding Method

A commercial dry cat food that had passed an AAFCO feeding trial for feline growth and maintenance had been fed for approximately eight months to cats in this colony before any abnormalities were noted. The food was plant-based; the first four ingredients were corn, corn gluten meal, soybean meal and poultry by-product meal. In addition to containing typical vitamin and mineral supplements, the food also included copper oxide as a copper source and iron oxide as a colorant.

Analysis of the food disclosed no deficiencies when compared with recommended levels established for growing kittens. However, high levels of zinc and iron were noted in the food. High levels of dietary phytates, which can reduce mineral absorption, were expected to be in the food because of the plant ingredients it contained.

Feeding Plan

A dietary copper deficiency was considered the most likely cause of the reproductive failure noted in these queens. Although food analysis revealed that dietary copper levels were more than adequate, the copper oxide used in the food is a completely unavailable copper source for animals. Additionally, factors that impair copper absorption by chelation (phytates in plants) or transport competition (zinc and iron) were found in high concentrations in the food and would further impair absorption of available copper.

The cat food was supplemented with 15 mg/kg copper from an available source (i.e., copper sulfate).

Reassessment

After the food was supplemented with copper sulfate, the conception rate increased to 80% of breedings and in utero fetal death rates decreased to 12.5%. Food intake increased to expected levels. Three months after feeding the supplemented food, coat pigmentation abnormalities and limb and tail deformities again became evident in newborn kittens. Serum samples were again collected from pregnant queens for copper analysis. Copper values were low in four of nine queens, indicating continuing copper deficiency. An additional 10 mg/kg of dietary copper as copper sulfate were added for a total of 25 mg/kg supplemental copper. No abnormal kittens were born during the next five months.

Some less severe clinical signs of copper deficiency in kittens not consuming copper-supplemented food (i.e., queen's milk only) were reversible. Pigmentation and coat texture returned to normal and improved carpal flexion was observed with skeletal maturation.

Bibliography

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CASE 6-4

Sudden Death in a Chihuahua

Patient Assessment

A three-year-old female Chihuahua was found dead one hour after being given 1.5 ml of a vitamin E preparation by intramuscular injection. The owner routinely administered the vitamin preparation twice yearly to all dogs of breeding age in his kennel. One week earlier, a similar incident occurred with a two-year-old female Yorkshire terrier. The owner had purchased the vitamin E product from the same veterinarian for several years. The Chihuahua and the vitamin E preparation were delivered to a diagnostic laboratory for examination.

At necropsy, the lungs were wet, glistening and mottled pink. White foam was found in the trachea and bronchi. All other internal organs appeared normal. Histopathologic examination of the lungs showed congested capillaries, perivascular edema and abundant proteinaceous fluid in the alveolar lumina. Liver and kidney specimens from the dog contained 12.9 and 12.1 mg selenium/kg, respectively (normal values <3 mg/kg).

Assess the Food and Feeding Method

No food was available for evaluation. When contacted, the veterinarian suggested that the bottle of vitamin preparation might also

contain selenium. Selenium had been added to a bottle of vitamin E intended for use in calves, but the mixture had not been dispensed. The veterinarian was concerned that the bottle might have been sold inadvertently to the owner of the dog.

Two liquid phases, one oily and the other watery, were visible in the vitamin preparation bottle. The water-base liquid from the bottle contained 5,317 mg selenium/l. Subcutaneous tissue at the injection site contained 129 mg of selenium/kg. The calculated dose of selenium that had been administered to the dog was 2.5 mg/kg. The minimal lethal dose of selenium administered by intramuscular injection in dogs is 2.0 mg/kg.

Comments

Selenium toxicosis in cattle, sheep, horses, swine and poultry has been documented and usually develops as a subacute to chronic disease resulting from ingestion of seleniferous plants or feeds that contain high concentrations of selenium because of errors in ration formulation. Lesions of subacute to chronic selenium toxicosis have also been produced in dogs by long-term parenteral selenium administration. Acute selenium toxicosis causes increased vascular permeability, which is manifested as hemorrhages and edema in many tissues.

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CASE 6-5

Cervical Rigidity in a Cat

Patient Assessment

A 10-year-old, castrated domestic shorthair cat weighing 7 kg was examined for lethargy, decreased appetite and weight loss of several months' duration. Weight loss of 2 kg over the preceding 12 months was evident from the medical record.

The cat appeared depressed, had a matted, unkempt coat and extended its cervical region and held its head low and directly in front of its body. The cat was afebrile, obese and dehydrated. On palpation, the cervical region was rigidly extended with tense musculature. A hard mass was palpable in the midcervical region. The rigidly extended neck was the only neurologic abnormality.

Evaluation included a complete blood count (mild leukocytosis with mature neutrophilia), serum biochemistry analysis (mild hyperglycemia and hypercholesterolemia), feline leukemia virus antigen test (negative) and cervical and thoracic radiographs. Radiography revealed a bone-dense, cervical mass ventral to the C_1-C_2 intervertebral space. Much of the normal vertebral architecture appeared to be obliterated and the trachea and soft tissues were deviated ventrally and laterally. Thoracic radiography revealed ventral, bony proliferations extending from thoracic vertebrae T_2 through T_7. Marked bony proliferation was evident along the sternum and several of the costal cartilages.

The cat's serum vitamin A concentration was markedly high (315 μg/dl, normal 20 to 80 μg/dl).

Assess the Food and Feeding Method

The cat was fed a commercial dry cat food ad libitum supplemented with fresh beef liver daily.

Treatment and Feeding Plan

A tentative diagnosis of hypervitaminosis A was made based on the dietary history, clinical signs and radiographic lesions. The daily liver supplementation was considered the source of the excess dietary vitamin A. The cat was given a single intramuscular injection of dexamethasone and an oral analgesic was prescribed. The owner was advised to discontinue feeding beef liver and to feed only a balanced commercial cat food. The owner was further advised to encourage the cat to eat with hand feeding.

Reassessment

Six months later, the cat was euthanatized for reasons unrelated to the hypervitaminosis A. The cat had been eating fairly well, although its stiff-necked posture remained.

Bibliography

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CASE 6-6

Vomiting and Anorexia in a German Shepherd Mixed-Breed Dog

Patient Assessment

A five-year-old, 10.6-kg, neutered female German shepherd mix was examined after three days of vomiting, anorexia and lethargy. The owners reported the dog was allowed free access to the neighborhood, which included a radiator machine shop where cholecalciferol-based rodenticides were used. The dog appeared depressed and moderately dehydrated.

Abnormal laboratory findings included moderate hypercalcemia, mild azotemia, proteinuria and isosthenuria. These results suggested vitamin D_3 toxicosis.

Assess the Food and Feeding Method

No dietary history was available.

Treatment and Feeding Plan

Treatment consisted of intravenous 0.9% saline solution, diuretics, salmon calcitonin and corticosteroids. Hypercalcemia persisted throughout hospitalization. Further diagnostic testing did not identify a cause for persistent hypercalcemia. After seven days of hospitalization, the dog improved markedly and was discharged to the owners' care. Oral prednisone (at tapering dosages) and a veterinary therapeutic food formulated for renal patients were given at home.

Reassessment

The dog was evaluated several times during the next four weeks and appeared normal despite persistent hypercalcemia. The dog became normocalcemic five weeks after discharge from the hospital and remained normocalcemic when examined at two and three months.

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CASE 6-7

Subcutaneous Nodules in a Young Cat

Patient Assessment

A five-month-old female domestic shorthair cat was examined for depression, anorexia, firm nodular subcutaneous fat in the groin region and abdominal hyperesthesia of one week's duration. The cat was normally docile and tractable but began to resist being handled and petted. Body condition was normal (3/5).

Hematologic abnormalities included a neutrophilic leukocytosis and a normocytic, normochromic, nonregenerative anemia. Urinalysis and fecal examination results were normal. Biopsy specimens were obtained from the affected subcutaneous tissue. The biopsy specimens were firm, nodular and brownish-orange when examined grossly. Serosanguineous fluid oozed from the biopsy sites. Histopathologic examination revealed pyogranulomatous panniculitis, ceroid pigment and multifocal areas of fat necrosis and mineralization.

Assess the Food and Feeding Method

Since weaning, the cat had only been fed sardines, anchovies and mackerel free choice.

Treatment and Feeding Plan

A diagnosis of pansteatitis was made based on the dietary history and histopathologic lesions. Treatment included α-tocopherol (50 mg/kg body weight) once daily per os for two months and prednisolone for 15 days in a decreasing dosage schedule. A fish-free, complete and balanced moist cat food was offered. Because the cat was anorectic and unaccustomed to commercial cat food, it was initially force-fed.

Reassessment
Marked clinical improvement occurred within one week and the cat appeared clinically normal within one month.

Comments
Vitamin E protects cells against lipid peroxidation. α-tocopherol appears to localize within cell membranes to prevent or inhibit initiation of lipid peroxidation. Animals fed oily fish and fish oils containing high levels of unsaturated fat require greater amounts of vitamin E to limit fat oxidation.

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CASE 6-8

Hemorrhagic Diathesis in a Group of Kittens

Patient Assessment
A group of adult intact female cats and their kittens were involved in an AAFCO feeding trial to establish nutritional adequacy for gestation, lactation and growth. Necropsy of four kittens that died during the feeding trial revealed hepatic or GI hemorrhages. Fourteen of the surviving kittens were divided into two groups. Blood samples were taken on Days 1, 3, 4 and 6. After the Day 3 blood samples were taken, seven of the kittens were injected subcutaneously with a vitamin K preparation (200 mg K_1), and the other seven were left untreated. Clotting times were determined for each sample.

The mean clotting time for kittens not receiving vitamin K treatment was 50 ± 9 seconds (values for normal kittens 22 ± 0.1 seconds). Mean clotting times for kittens receiving treatment decreased significantly from 59 ± 10 seconds for Days 1 and 3 to 22 ± 0.4 seconds for Days 4 and 6.

Assess the Food and Feeding Method
Queens and kittens were fed a commercial feline food formulated primarily from tuna, free choice. Individual food intake measurements were not available for the kittens because they were group housed for the AAFCO feeding protocol.

Feeding Plan
Further studies using purified diets did not identify the specific cause of vitamin K deficiency in kittens eating this fish-based food. These studies led to a recommendation that pet food companies include a supplemental source of vitamin K in moist fish-based foods for cats.

Bibliography
Strieker MJ, Morris JG, Feldman BF, et al. Vitamin K deficiency in cats fed commercial fish-based diets. Journal of Small Animal Practice 1996; 37: 322-326.

CASE 6-9

Weight Loss in a Group of Cats

Patient Assessment
Twenty-eight cats in a humane shelter in England developed lethargy, a mild decrease in food consumption and weight loss. Analysis of blood samples taken from three of the cats revealed a normocytic, normochromic anemia.

Three days after the onset of clinical signs, 13 of the cats rapidly lost body condition and developed an uncoordinated gait. Within eight to 12 hours, these cats developed ventriflexion of the head and had fully dilated pupils with no light reflex. Five of the cats subsequently developed seizures and died despite treatment with anticonvulsant drugs. A diagnosis of thiamin deficiency was made based on necropsy results.

Assess the Food and Feeding Method

The cats were fed a commercial moist cat food for six months. The food was not a complete and balanced product but was designed as a "complementary" food to be mixed with other complete dry foods. Two different lots of the moist food contained 0.56 and 0.04 mg thiamin/kg food. Assuming the food contained 75% water and had a metabolizable energy content of 1.25 kcal/g as fed, the food should contain at least 1.25 mg/kg food of thiamin for kittens and 0.5 mg/kg food of thiamin for adult cats.

Treatment and Feeding Plan

The other severely affected cats were treated with intravenous fluids and intramuscular injections of vitamin B complex for five days. These cats responded to therapy within 12 hours and were clinically normal five days later. No other cases have occurred since the humane shelter switched to a complete and balanced moist cat food.

Bibliography

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Finke MD. Alpo Viewpoints in Veterinary Medicine 1993; 3(1).

CASE 6-10

Skin and Hair Disorders in a Group of Kittens

Patient Assessment

Twenty female kittens were involved in a feeding trial to evaluate dietary phosphorus requirements. The kittens were eight weeks old at the beginning of the trial. After eating the experimental food for 11 weeks, most kittens developed dried secretions around the eyes, mouth, nose and feet, focal dermatitis of the lips near the canine teeth, alopecia along the back, neck and tail, achromotrichia, dull fur and a brownish appearance of the skin. Growth of the kittens was not impaired. Results of hemograms and urinalyses were normal.

Assess the Food and Feeding Method

The food was a purified diet that contained dried egg whites, fish meal, beef tallow, corn oil, glucose, cooked starch, cellulose, taurine, vitamins and minerals. Food and demineralized water were provided free choice.

Feeding Plan

A tentative diagnosis of biotin deficiency was made based on the dietary history and clinical signs. The biotin content of the food was increased from 0.066 mg/kg to 3.0 mg/kg of food.

Reassessment

The kittens were markedly improved after eating the biotin-supplemented food for 10 weeks. Serum biotin concentrations of kittens fed unsupplemented food was about one-fifth of that of adult female cats fed a commercial complete and balanced dry cat food. Serum biotin concentrations responded to increased biotin intake.

Comments

Biotin deficiency induced by avidin in raw egg whites is a classic example of vitamin deficiency in experimental nutrition. Avidin is a glycoprotein that irreversibly binds biotin and renders it unavailable. Biotin deficiency was an unwanted side effect in this group of research cats due to egg whites in the formulation. The researchers ordered ovalbumin expecting to receive a purified fraction of egg protein. However, they received dried total egg whites, which contained avidin.

Bibliography

Pastoor FJH, Van Herck H, Van't Klooster ATh, et al. Biotin deficiency in cats as induced by feeding a purified diet containing egg white (expanded abstract). Journal of Nutrition 1991; 121: S73-S74.

CASE 6-11

Cachexia in a Young Giant Schnauzer

Patient Assessment

A five-month-old female giant schnauzer was admitted for lethargy, depression and cachexia (body condition score 1/5). The dog weighed 7.8 kg and was 47 cm high at the shoulder. It had gained no weight in the previous eight weeks. The dog's four normal female littermates weighed 20.5 to 22.5 kg and were 48 to 52 cm high at the shoulder.

Hematologic abnormalities included chronic nonregenerative anemia and neutropenia. Peripheral blood smears revealed marked erythrocyte anisocytosis and poikilocytosis, occasional hypersegmented neutrophils and large platelets. Analysis of bone marrow aspirates revealed decreased to normal cellularity with adequate iron stores. Serum iron and total iron binding capacity were normal. Serum biochemistry analyses were within normal limits for age-matched controls.

Intestinal maldigestion and malabsorption were ruled out based on normal GI contrast radiography, normal absorption of starch and fat and normal serum trypsin-like immunoreactivity. Normal hepatic function was documented by ammonia tolerance and BSP retention tests.

A urine sample was submitted for metabolic screening. Analysis revealed methylmalonic aciduria, which is a sign of vitamin B_{12} deficiency. Two serum samples had vitamin B_{12} concentrations of 21 and 36 pg/ml (values for normal dogs 209 to 483 pg/ml). Results of a test to measure intestinal absorption of an orally administered dose of vitamin B_{12} were suboptimal.

Assess the Food and Feeding Method

The puppy was fed a variety of homemade and commercial dog foods free choice, supplemented with an oral liquid hematinic.

Treatment and Feeding Plan

Vitamin B_{12} (1 mg) was administered intramuscularly once daily for seven days. A complete and balanced commercial dry growth dog food was offered free choice.

Reassessment

Within 12 hours of the vitamin B_{12} injection, the puppy became bright and alert and developed a voracious appetite. Two weeks after treatment, the puppy had gained 7 kg; six weeks after treatment the puppy weighed 25 kg. Reticulocytosis occurred five days after parenteral vitamin B_{12} therapy was started. Neutrophil counts increased within 10 days and all hematologic abnormalities resolved within two months. The dog remained clinically normal when given 1 mg vitamin B_{12} intramuscularly every four to five months.

Subsequent testing of this puppy's mother documented an inborn error of vitamin B_{12} metabolism leading to selective vitamin B_{12} malabsorption. Inherited selective malabsorption of vitamin B_{12} has been described in other giant schnauzer puppies and in a cat.

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Fyfe JC, Jezyk PF, Giger U, et al. Inherited selective malabsorption of vitamin B_{12} in giant schnauzers. Journal of the American Animal Hospital Association 1989; 25: 533-539.

Vaden SL, Wood PA, Ledley FD, et al. Cobalamin deficiency associated with methylmalonic acidemia in a cat. Journal of the American Veterinary Medical Association 1992; 200: 1101-1103.