Chapter 7

Antioxidants

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"Scientists now believe that free radicals are causal factors in nearly every known disease, from heart disease to arthritis to cancer to cataracts. In fact, radicals are a major culprit in the aging process itself."
Lester Packer

INTRODUCTION

Oxidation is characterized by the loss of electrons, which results in an increase in positive or a decrease in negative charges on an atom. Usually, in biologic systems this occurs by the loss of one or two electrons by transfer to another atom, which accepts the electron(s) into its orbit, resulting in a more stable state. Conversely, reduction of an atom is the gain of electrons. A substance that donates electrons (i.e., becomes oxidized) to another substance is a reducing agent and one that accepts electrons (i.e., becomes reduced) is an oxidizing agent. Oxidizing agents are always reduced in a reaction, whereas reducing agents are always oxidized. Redox reactions occur when oxidation and reduction take place in the same chemical equation between two substances. In general, the balance of this potential energy equation is a measure of the ease with which a molecule gives up an electron compared to its willingness to accept an electron in relation to the hydrogen half-cell equation developed by Nernst.

An antioxidant is any substance, that when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (i.e., it prevents oxidation) (Halliwell, 2002). Thus, antioxidants may preserve the structural integrity and function of biologic molecules in cells. However, this concept may be too simplistic because some cellular signaling pathways appear to depend on redox chemistry to manifest "normal" biologic function.

Free radicals are unstable atoms (e.g., oxygen, nitrogen) with at least one unpaired electron in the outermost shell. Oxygen free radicals (also called reactive oxygen species or ROS) will be used as the prototypical molecule for this chapter (**Figure 7-1**).

An unpaired electron creates a thermodynamically unstable situation; therefore, the molecule will either attempt to gain (reduction) or lose (oxidation) an electron to achieve thermodynamic stability. Thus, a free radical may act as either an oxidizing or reducing agent depending on its thermodynamic propensity for stability. For example, superoxide (O_2^-) is a normal byproduct of cellular respiration. Thermodynamically, superoxide attempts to lose an electron to become oxygen and eventually water by a hydrogen peroxide intermediate. Alternatively, the hydroxyl radical (OH) strongly prefers to gain an electron (i.e., oxidize other molecules) to achieve its OH^- configuration. The chemistry of free radical reactions depends not only on which free radical species is generated in vivo but also where the molecule is generated within the cell. For example, a highly reactive free radical produced in mitochondria is unlikely to diffuse into the cytoplasm. A less reactive species, however, such as hydrogen peroxide may diffuse into the cytoplasm before it engages chemically in a redox reaction.

Redox and free radical chemistry reactions may occur directly or be catalyzed by other molecules, metals or proteins acting

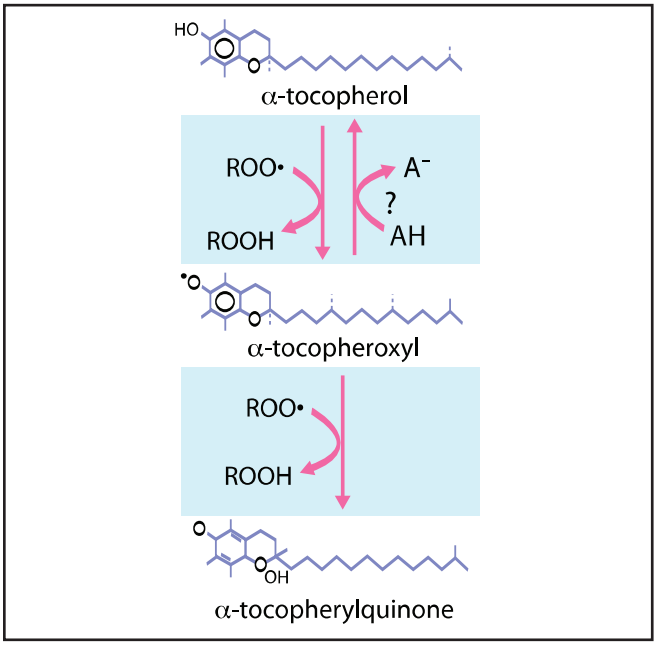

Figure 7-1. Oxidation of tocopherols by reaction with peroxyl radicals.

Reaction 1	$2O_2^{-\cdot} + 2H^+ \xrightarrow{\text{superoxide dismutase}}$	$H_2O_2 + O_2$
Reaction 2	$2\,H_2O_2 \xrightarrow{\text{catalase}}$	$2H_2O + O_2$
Reaction 3	$H_2O_2 + Fe^{2+}$	$OH + OH^- + Fe^{3+}$
Reaction 4	$O_2^- + Fe^{3+}$	$O_2 + Fe^{2+}$

Figure 7-2. An example of detoxifying free radicals.

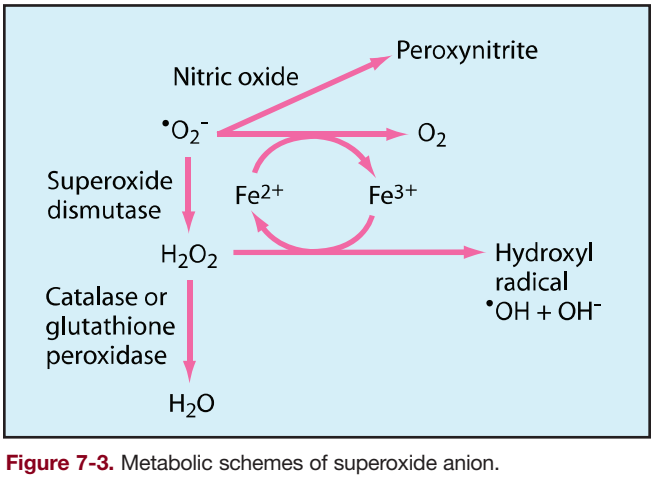

Figure 7-3. Metabolic schemes of superoxide anion.

as enzymes. Also, these systems may work in networks that depend on the proximity and species of redox coupling required. For example, mitochondria produce superoxide as a normal byproduct of cellular respiration. Normally, electrons "leak" from the electron transport chain, converting 1 to 3% of oxygen molecules into superoxide.

Cells can detoxify free radicals by several mechanisms; in the case of superoxide, cells use a two-step enzymatic method.

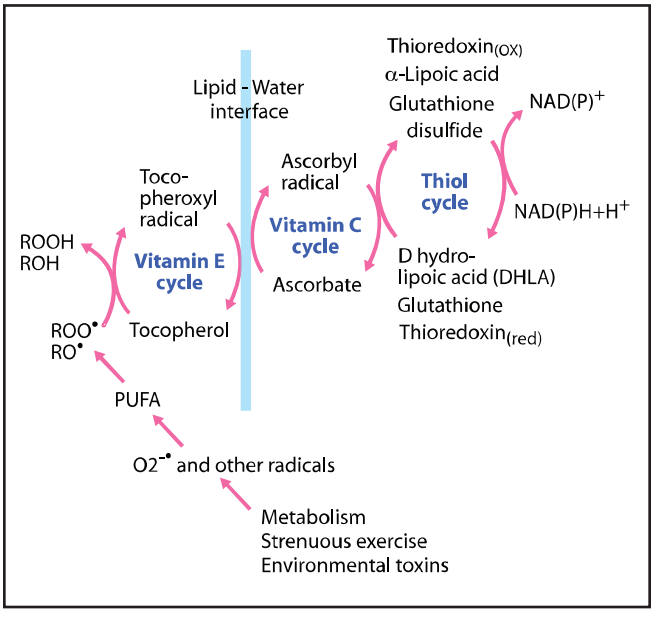

Figure 7-4. An antioxidant network to detoxify free radicals.

First, the superoxide free radical is simultaneously reduced and oxidized (dismutated) by superoxide dismutase to form hydrogen peroxide and oxygen (reaction 1) (**Figure 7-2**). Although hydrogen peroxide is a ROS, it is much less reactive than superoxide. As mentioned above, it may diffuse out of the mitochondria before reacting with another molecule. In the second step, catalase enzymes convert hydrogen peroxide into water and oxygen (reaction 2). Ironically, the hydroxyl (OH) free radical, the most mutagenic of the ROS, is generated when superoxide is converted to hydrogen peroxide. Peroxide readily reacts with ferrous iron (Fe^{2+}) or other transition metal ions (Fenton reaction) to produce hydroxyl radicals (reaction 3). Ferric iron (Fe^{3+}) can accept an electron from superoxide and cycle it back to the ferrous state where it is available to react with another peroxide molecule (reaction 4). Trace amounts of ionic iron can potentially catalyze formation of large quantities of hydroxyl free radicals.

A more dynamic metabolic picture of potential pathways emerges when these individual reactions are linked together in a biologic system (**Figure 7-3**). Thus, free radical production depends on multiple pathways and the availability of detoxification mechanisms vs. reactive materials. Overproduction of oxidative/reactive materials vs. detoxification mechanisms is called oxidative stress.

Hydroxyl radicals are highly reactive and oxidize most organic compounds at almost diffusion controlled rates (K >10/molar/sec.) (Dorfman and Adams, 1973). Due to their high reactivity, hydroxyl radicals are indiscriminate, reacting with the first substrate available. Therefore, hydroxyl radicals are highly destructive and have mutagenic potential. Mitochondrial membranes and DNA are particularly susceptible because ROS are formed in close proximity.

Redox reactions are complicated and involve multiple reactions for completion. As mentioned above, antioxidants may require several steps, cellular components or both to successful-

ly detoxify oxidizing agents. **Figure 7-4** demonstrates the production and the stepwise detoxification of an oxidant.

NUTRITIONALLY EFFECTIVE ANTIOXIDANTS

Theoretically, adding antioxidants to a biologic system should positively affect the aging process (**Box 7-1**). However, many interventional studies designed to prove this hypothesis have produced limited or contradictory results.

Distribution and availability of antioxidants are important determinants of biologic outcome. For example, several plant flavonoids and other polyphenols have limited solubility and absorption in the gut compared to other water- or fat-soluble compounds (Carbonaro and Grant, 2005). Physiologic factors such as food intake and composition may markedly influence the effects of antioxidants considered to be easily absorbed and distributed (Hacquebard and Carpentier, 2005; Leonard et al, 2004). One study showed that vitamin E absorption was least effective from gel capsules given without a meal and variably effective when given with a meal. However, vitamin E adsorbed onto a cereal provided consistently higher rates of availability.

Metabolic transformation may alter biologic activity and distribution of orally administered antioxidants between species. Cats lack β-carotene 15,15'-dioxygenase that cleaves β-carotene (provitamin A) into two retinal molecules, whereas herbivores have relatively high activity of this enzyme (Combs, 1998). Thus, cats (and possibly other carnivores) are more likely to absorb carotenoids intact, whereas carotenoids serve relatively more of a pre-vitamin A function for herbivores.

Cats metabolize and eliminate α-lipoic acid at a much slower rate than other species (Hill et al, 2004). Age is another functional consideration. Although vitamin C is not considered essential for rats, as rats age the metabolic enzymes responsible for recycling and transporting vitamin C in hepatocytes become impaired which, if severe, may impart a conditionally essential status for vitamin C to older rats (Lykkesfeldt et al, 1998; Michels et al, 2003).

NON-CLASSIC ANTIOXIDANT MECHANISMS

Many "antioxidant" molecules have other important physiologic functions, including regulating second messengers, cell cycle signaling and controlling gene expression. These cellular redox functions are well regulated and coordinated and are probably inherent rather than random.

Resveratrol, a polyphenol from red grapes, activates sirtuin 2, a member of the sirtuin family of NAD^+ dependent de-acetylases, which mimics the effects of caloric restriction and prolongs cell life (Howitz et al, 2003). Hydrogen production mimics insulin signaling and is now recognized as a component of insulin signaling physiology (Goldstein et al, 2005). Nuclear factor kappaB (NF-κβ) signaling of apoptosis is activated by an alternative pathway via hydrogen. Furthermore, antioxidants that specifically target mitochondria alter this signaling pathway (Hughes et al, 2005; Kutuk and Basaga, 2003; Haddad, 2002). NF-κβ is not the only redox-sensitive transcription factor; several other factors have been characterized with these properties over the past several years (Azzi et al, 2004; Haddad, 2002). Antioxidant molecules are far reaching and go beyond the understanding of classic chemistry.

Box 7-1. Free Radical Theory of Aging.

Denham Harman first proposed the free radical theory of aging in 1956. His theory postulates that reactive oxygen species (ROS) damage cells with resulting age-dependent pathology. Today, it is generally accepted that mitochondria, through aerobic respiration byproducts, are the primary source of ROS in mammals. Accordingly, slowing or reversing the effects of ROS may slow aging.

The free radical theory of aging hypothesis has produced many strategies to mitigate the effects of ROS. One highly touted strategy is to suppress ROS effects with antioxidants or antioxidant defense mechanisms through nutritional supplementation. The effectiveness of this strategy depends on a wide range of different biologic factors.

The Bibliography for **Box 7-1** can be found at www.markmorris.org

MEASURING OUTCOMES OF ANTIOXIDANT STUDIES

Interpreting the vast number of studies involving antioxidant supplements is challenging. The biologic effects of antioxidants may occur by multiple divergent or convergent pathways, thus making interpretation difficult. The effects of ROS are considered insidious and temporally delayed, thus, predicting long-term outcomes from short-term experiments is another challenge to interpretation. Finally, determining the outcome event is also problematic because of the variety of endpoints that have been developed to measure the effects of antioxidants. Some outcomes discussed below highlight potential pitfalls of current methodologies.

Antioxidant Concentrations in Foods, Supplements and Tissues

Oral antioxidant administration as a supplement or in combination with food does not ensure absorption and distribution into tissues. Some antioxidants are more readily absorbed than others. Species differences may further affect absorption. Vitamin E is usually easier to absorb than water-insoluble plant phenols; however, variable absorption and distribution may occur depending on several factors. Vitamin E was more efficiently absorbed when administered with a meal (Leonard et al, 2004). Vitamin E depletion and repletion also appears to have different kinetic parameters depending on tissue type (Pillai et al, 1993, 1993a). Absorption and distribution of oral antioxidants must be relevant to the target tissue and the intended bio-

Table 7-1. Examples of biomolecules and specific markers.

Molecules	Markers
DNA	8-oxodeoxyguanosine
Lipids	Alkenals, malondialdehyde, thiobarbituric acid reaction substances
Prostaglandins	Isoprostanes
Protein	Nitrotyrosine, protein carbonyls
Advanced glycation end products	—

Table 7-2. Blood concentrations (μmol/l at seven days) of cats and dogs supplemented with β-carotene for at least seven days.

Species	Dose (mg/day)	Body weight (kg)	Peak plasma concentration
Cat	10	3 to 3.5 kg	0.95
Dog	25	7 to 9 kg	Approx. 0.02

logic outcome. Variabilities in bioavailability and distribution have not limited the number of studies attempting to link either increased ingestion or increased serum values of antioxidants to a variety of health outcomes in target tissues. If absorption and distribution fail to prove causality, what measurements are available for developing arguments about biologic efficacy?

Decreased Markers of ROS Damage

ROS are short-lived and difficult to measure as their native species. Several laboratory methods have been developed to measure biologically stable molecules as markers of ROS production in a biologic system. Presumably, if levels of these markers increase in serum or tissue, then more ROS are being produced and more damage results. If marker levels decrease, production of ROS has presumably also decreased. These markers are specific for different biomolecules (**Table 7-1**). The utility of these measurements has been debated because they indirectly measure presumed ROS reactions, sometimes in distant tissues. As such, they are responses to oxidative events, but do not provide direct mechanistic effects of antioxidant action in target tissues.

The next investigative modality is to look directly at target tissue effects of orally administered antioxidants. These studies can provide biochemical information about tissue mechanisms compared to indirect measures. Several interesting results have emerged with a variety of antioxidants. For example, aged rats, a vitamin C independent mammal, have decreased ability to recycle vitamin C in their hepatocytes, which may be restored by administration of lipoic acid and acetyl-carnitine (Lykkesfeldt et al, 1998). As mentioned above, aged rats had increased oxidative damage to hepatic proteins, which decreased enzymatic activity and increased susceptibility to protein degradation (Starke-Reed and Oliver, 1989). Finally, oxidative damage increases in the brains of aging beagles and rats; the damage was correlated with memory loss in rats (Head et al, 2002; Liu et al, 2002). Intervention with acetyl-carnitine and lipoic acid partially reversed the memory loss in older rats (Liu et al, 2002).

Biologic Outcomes of Antioxidant Interventions

Intervention studies are much more difficult to perform because of their greater expense, length of time required for intervention and the inability to control dietary intake of individuals. However, animals that have shorter lifespans are useful for developing strategies that may benefit people and other animals with longer lifespans. Models with shorter lifespans and/or accelerated aging, attributable to more rapid ROS damage, may; therefore, be more translucent to interventions and assessed more quickly for efficacy (Magwere et al, 2006). In addition, specific genetic models such as the senescent accelerated mouse, which overproduces free radicals, and transgenic models are becoming more available. These models may provide insight into efficacy and modes of action of supplemental dietary antioxidant regimens.

VETERINARY APPLICATIONS

The science of nutritional antioxidants has advanced over the past several years. Numerous studies have revealed biologic benefits to supplementing foods or dietary regimens with oral antioxidants in a variety of species. A review of mainstream antioxidants and their application to canine and feline nutrition follows.

Vitamin E

Dogs

Requirements for vitamin E in dogs and cats were suggested as early as 1939 and modified based on selenium and polyunsaturated fatty acid (PUFA) content of foods in the 1960s (Anderson et al, 1988; Harris and Embree, 1963; Hayes et al, 1969). From published research, the National Research Council (NRC) recommends that dogs receive 22 IU vitamin E per kg/food dry matter (DM) (based on a food containing 0.1 ppm selenium, not more than 1% linoleic acid and 3,670 kcal metabolizable energy/kg DM). This results in a range roughly equivalent to 0.4 to 1.4 IU/kg body weight for maintenance (lower number) up to pregnant/lactating dogs (upper number) (2006).

Effects of vitamin E on other biologic outcomes have been tested in dogs. Investigators found that levels higher than the requirement may confer targeted biologic benefits. Increasing dietary intake of vitamin E up to 2,010 mg/kg DM in geriatric beagles improved immune function (Hall et al, 2003; Meydani, 1998). Increased intake of vitamin E in food is related directly to increased vitamin E content of skin, which may provide health benefits for dermatologic disease processes (Jewell et al, 2002). Vitamin E concentrations in blood decrease with exercise, whereas higher levels have been associated with improved performance (Piercy et al, 2001; Scott et al, 2001). Finally, vitamin E protects from ischemic damage in a variety of tissues (Jorge et al, 1996; Sebbeg et al, 1994; Fujimoto et al, 1984).

There are no published toxicity data for vitamin E in dogs; however, concentrations exceeding 2,000 IU/kg DM of food have been fed for 17 weeks without observable negative reactions (Hall et al, 2003). Although an upper limit of toxicity has not been documented, a level of 1,000 IU/kg DM of food, or 45 IU/ kg of body weight, has been suggested (NRC, 2006).

Cats

Cat foods are often higher in fat and PUFAs than dog foods, which may provide a different matrix for determining requirements. Nonetheless, several studies have shown that the amount of vitamin E needed to support growth and reproduction in cats is approximately in the same general range as that for dogs, when accounting for adequate selenium and excessive PUFAs. Thus, a range of 0.5 to 1.7 mg of vitamin E/kg body weight has been suggested by NRC (2006) for maintenance and pregnancy/lactation, respectively.

Food supplemented with vitamin E at 272 and 552 IU/kg DM food improved immune function in aged cats (Hayek et al, 2000). Supplementation with 1,000 IU D-α-tocopherol enhanced neurologic recovery in a spinal cord compression model (Anderson et al, 1988). Vitamin E supplementation at 800 IU/day via gel caps did not protect better than placebo for preventing onion powder or propylene glycol induced Heinz body anemia in cats (Hill et al, 2001). Food supplemented with vitamin E and cysteine (2,200 IU vitamin E + 9.5 g cysteine/kg food DM) protected against acetaminophen-induced oxidative production of methemoglobinemia (Hill et al, 2005). Also, pretreatment of cats with vitamin E and selenium (200 IU vitamin E + 50 μg selenium) for five days delayed motor nerve degeneration in a model of axonal degeneration (Hall, 1987). A presumed safe upper level for oral administration has not been established; however, administration of vitamin E parenterally at 100 mg/kg body weight to kittens resulted in significant mortality (Phelps, 1981).

Vitamin C

Dog and Cats

Dogs and cats are capable of synthesizing required amounts of vitamin C by de novo mechanisms (Innes, 1931; Naismith, 1958). One group of investigators showed that hepatic in vitro synthesis of vitamin C in dogs and cats was much less (i.e., 10 to 25%) than in other mammals leading to speculation that ability to synthesize vitamin C may be limited in these species; however, no followup work has been performed (Chatterjee et al, 1975). In dogs, both ascorbic acid and ester-C are rapidly absorbed, possibly by use of an active transport mechanism in the gastrointestinal tract (Wang et al, 2001).

The subchronic intravenous toxicity (i.e., LD_{50}) dose for vitamin C has been reported to be greater than 500 mg/kg/day and 2,000 mg/kg/day for cats and dogs, respectively (Körner and Weber, 1972). Supplementation of vitamin C (0, 200, 400 or 1,000 mg/day) to cats resulted in a small progressive reduction in urinary pH (Kienzle and Maiwald, 1998). In people, intake of ascorbate at the upper recommended limit of 2,000 mg/day increased urine oxalate excretion and risk of kidney stone formation (Massey et al, 2005). However, moderate supplementation of vitamin C in healthy cats (i.e., up to 193 mg/kg DM food, approximately 2 mg/kg body weight), did not appear to increase the risk of oxalate stone formation (Yu and Gross, 2005). Supplementing rats with vitamin C at 1,500 mg/kg DM food may decrease erythrocyte fragility when vitamin E is near the requirement level in food (Chen, 1981). Additionally, oral vitamin C supplementation at 1 g/day may slow racing times in greyhounds (Chapter 18) (Marshall et al, 2002).

β-Carotene and Other Carotenoids

The carotenoids, predominately β-carotene, have been subjected to preliminary studies in canine and feline nutrition. β-carotene can serve as a precursor to vitamin A in dogs, but not cats. Although carotenoids possess antioxidant properties, most of the research in dogs and cats has focused on immunomodulatory benefits.

β-carotene supplementation increases concentrations of β-carotene in canine and feline plasma and white blood cells (Chew et al, 2000, 2000a). However, the concentrations reached in feline plasma are approximately 50-fold higher than those in canine plasma at the same approximate time and dose rate, indicating that most of the β-carotene administered to dogs is probably converted to vitamin A rather than absorbed directly as b-carotene. People convert approximately 60 to 75% of β-carotene into vitamin A and absorb approximately 15% intact. From this, the mean concentration of serum β-carotene in people is approximately 0.3 μmol/l, which is approximately 10-fold greater than concentrations found in dogs receiving supplements. Nevertheless, supplementation with β-carotene reportedly improves immune function in young and aged dogs (Kearns et al, 2000; Chew et al, 2000b).

Supplementation with the carotenoid lutein increases plasma and leukocyte concentrations in dogs and cats. Food supplemented with lutein improves immune function in both species (Kim et al, 2000, 2000a). A novel form of astaxanthin provides cardioprotection from vascular occlusion in dogs (Gross and Lockwood, 2005).

β-carotene has been evaluated in beagles at very high doses (i.e., 50 to 250 mg/kg/day as an oral dose in beadlets) (Heywood et al, 1985). Although coat discoloration and liver vacuolization were noted at all dose levels, no consistent findings of toxicity were found. Carotenoid safety is not well evaluated in cats; however, it may be presumed to be very safe based on wide margins of safety in other mammals and lack of conversion to vitamin A in this species. Canthaxanthine supplementation in cats for six months induced retinal pigment epithelial changes that included some vacuolization but no functional electroretinogram changes (Scallon et al, 1988).

Selenium

Selenium was first recognized as an essential nutrient in 1957 based on its ability to spare vitamin E in exudative diathesis in chicks (Schwarz et al, 1957). The metabolic basis for selenium's nutritional function remained unclear until it was discovered in

1973 (Rotruck et al, 1973) that selenium was a component of glutathione peroxidase. Subsequently, investigators discovered several selenium-dependent glutathione peroxidase isoforms (phospholipid, cytosolic, plasma and gastrointestinal). In addition, other selenoproteins were discovered including (three iodothyronine 5'-deiodinases [types I, II and III]); two thioredoxin reductases and four other selenoproteins (in plasma [P], muscle [W], liver and prostate) (Combs, 2001).

Glutathione peroxidase primarily defends against oxidative stress by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides, which react with the selenol group of the active center of selenocysteine. As a constituent of 5'-deiodinases, types I to III, selenium combats oxidative stress by deactivating large amounts of hydrogen peroxide produced by the thyroid gland, which is used for iodination of thyronine residues. The activity of phospholipid and cytosolic glutathione peroxidase protects the thyroid gland from oxidative damage.

Glutathione peroxidase and thioredoxin reductase activities are involved in a variety of key enzymes, transcription factors and receptors. Thioredoxin reductase's involvement in the modulation of redox-regulated signaling including ribonucleotide reductase, prostaglandin and leukotriene synthesis, receptor-mediated phosphorylation cascades (i.e., activation of NF-κβ) and in apoptosis is of great interest (McKenzie et al, 1998; Neve, 2002).

The selenium requirement of most animals is similar and based on maximization of glutathione peroxidase in plasma and red blood cells. The estimated selenium requirement for kittens and adult cats is 0.15 and 0.13 mg selenium/kg food, respectively (Wedekind et al, 2003, 2003a) and 0.10 mg selenium/kg food for adult dogs (Wedekind et al, 2002). Recommended allowances of selenium in pet foods, which account for bioavailability, for dogs and cats are 0.35 and 0.30 mg selenium/kg food, respectively (NRC, 2006).

Animal studies and clinical intervention trials involving people have shown selenium to be anticarcinogenic at intakes 5- to 10-fold greater than recommended daily allowances or minimum requirements (Combs, 2001; Neve, 2002). Several mechanisms have been proposed to account for selenium's anticancer effects: 1) antioxidant activity through glutathione peroxidase and thioredoxin reductase, 2) enhanced immune function, 3) altered carcinogen metabolism, 4) inhibited tumor proliferation and enhanced apoptosis and 5) inhibited angiogenesis (Neve, 2002). Studies indicate antioxidant protective ranges for selenium would be approximately 0.50 to 1.3 mg selenium/kg food DM for dogs and cats. Interestingly, the complementary nature of antioxidants such as vitamins C and E and selenium suggests that one "spares" the need for the others in protecting against lipid peroxidation. In the case of all of these antioxidants, effective levels necessary to reduce disease risk are much higher than levels needed to merely prevent nutritional deficiency.

Safe upper limits for selenium for most species are similar (Koller and Exon, 1986), approximately 2 mg selenium/kg food, although neither the Association of American Feed Control Officials (AAFCO, 2007) nor NRC (2006) suggests a safe upper limit for cats (Wedekind et al, 2003). Cats have approximately fivefold higher serum selenium concentrations compared to other species (Foster et al, 2001). This is probably attributed to the fact that selenium intakes are higher for cats than for most other species. For example, fish and other seafood, which are highly concentrated sources of selenium, are fed much more widely to cats than dogs. However, studies show that even when dogs and cats are fed foods containing the same selenium concentration, serum selenium concentrations are 40 to 60% higher in cats. Cats have significantly higher selenium concentrations in blood even when fed similar dietary selenium intakes compared to most other species including dogs. It is unclear whether cats have a higher tolerance for selenium; however, the literature suggests that diets containing similar sources and levels of selenium were more toxic for swine (Kim and Mahan, 2001) than for cats (Wedekind et al, 2003). AAFCO (2007) suggests a safe upper limit of 2 mg selenium/kg diet for dogs (Wedekind et al, 2002).

Thiols: S-Adenosyl-L-Methionine, α-Lipoic Acid, N-Acetylcysteine

Thiol metabolism has gained research momentum as redox chemistry has matured. Thiols are capable of redox reactions similar to those of oxygen and have many metabolic correlates within cells. Glutathione, S-adenosyl-L-methionine (SAMe), thioredoxin and other sulfur-containing molecules have important roles in metabolism and antioxidant defenses.

SAMe has been used to successfully treat acetaminophen toxicity in cats and dogs (Wallace et al, 2002; Webb et al, 2003). Administration of SAMe to clinically healthy cats improved indices of redox status as indicated by decreased RBC thiobarbituric acid reaction substances and increased hepatic glutathione (Center et al, 2005).

α-Lipoic acid is another thiol that may influence reduced glutathione content of cells. As a food additive, α-lipoic acid resulted in increased ratios of reduced white blood cells to oxidized forms (GSH:GSSG) in dogs (Zicker et al, 2002). Administration to cats prolongs elimination of α-lipoic acid compared to that of other species; therefore, administration rates should be adjusted accordingly (Hill et al, 2004).

N-acetylcysteine increases reduced glutathione in cats challenged orally by onion powder compared to values in controls (Hill et al, 2001). N-acetylcysteine combined with ascorbic acid inhibits virus replication in cell lines infected with feline immunodeficiency virus (Mortola et al, 1998). Cysteine in combination with vitamin E also protects cats from acetaminophen-induced oxidative damage (Hill et al, 2005).

Fruits and Vegetables

Fruits and vegetables are often rich in flavonoid, polyphenol and anthocyanidin ingredients that may possess antioxidant properties. Exhaustive research of the effects of these ingredients in dogs and cats is unavailable; however, a few studies have tried to evaluate some potential benefits of adding fruits and vegetables to dietary regimens. Oral administration of a bioflavonoid complex reduced the extent of Heinz body anemia caused by acetaminophen administration to cats (Allison et al,

2000). A combination of fruits and vegetables in a supplemented food increased selected flavonoids in the blood of aged dogs (Zicker, 2005). Although effective doses and safety of fruits and vegetables are not well evaluated, administration of onion powder to cats can result in Heinz body anemia, perhaps through increased oxidation, although it has purported antioxidant benefits in some species (Robertson et al, 1998).

Combination Therapies

Because many antioxidants work in networks, several studies looked at complex mixtures of these compounds. Physiologic outcomes are variable, but generally, effects on immune function have been positive (Devlin et al, 2001) and markers of antioxidant status or damage from oxidative stress have been reduced (Baskin et al, 2000; Jewell et al, 2000; Piercy et al, 2000; Wedekind et al, 2002a; Yu and Paetau-Robinson, 2006). Long-term supplementation with a complex mixture of antioxidants slowed cognitive decline in aged dogs and resulted in improved behavioral correlates in an in-home study (Roudebush et al, 2005). The contribution to the final results of each individual compound is unknown in any of these studies; thus, this remains an area of future research.

REFERENCES

The references for **Chapter 7** can be found at www.markmorris.org.