Chapter 9
Metabolic Constituents: Water, Minerals and Vitamins

Water is the reactive medium for metabolic pathways in plants and animals. Although water loss is potentially lethal to cells, some organisms (e.g., bacterial and fungal spores, plant seeds and brine shrimp eggs) undergo arrested metabolism and tolerate desiccation. The availability of water affects primary production in terrestrial habitats; rainfall stimulates the growth and reproduction of plants and animals in hot deserts, and snow melt provides water for annual production in boreal forests (Chapter 1).

Water holds a wide range of solutes, from simple mineral ions to sugars, fatty acids, amino acids and complex proteins and vitamins. Enzymes are affected by mineral ions in the same way that hydrogen ion concentration (pH) affects protein structure and digestion (Chapter 8). High concentrations of sodium (Na) and chlorine (Cl) ions cause proteins to unfold and denature in a phenomenon called 'salting out'. Interactions with ions also facilitate enzyme activity; muscle contraction and blood clotting both involve activation by calcium (Ca) ions. Moreover, mineral ions form the catalytic core or active site of several enzymes. For example, zinc (Zn) is essential for protein digestion by carboxypeptidase A, and iron (Fe) in hemoglobin is required to bind oxygen. Complexes of minerals and carbon (C) comprise a wide variety of structures found in vitamins, which then participate in the transfer of electrons or functional groups. For example, cobalt (Co) is the catalytic center of vitamin B_{12}, which participates in the transfer of methyl groups ($—CH_3$) during the formation of fatty acids and nucleic acids in the production of blood cells. The metabolism of animals therefore depends on the reactive mix of ions and trace nutrients in body fluids.

9.1 Water and Electrolytes

Water availability influences the distribution of wildlife because all animals require water to maintain the volume and composition of their body fluids. Although animals can use a variety of mechanisms to conserve the water in their bodies, impaired regulation of water and solutes results in rapid death. Oriental-white backed vultures die quickly after consuming carcasses of livestock that were treated with the anti-inflammatory drug diclofenac. Vultures die from impaired regulation of body fluids

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because the drug is highly toxic to the kidney of this bird (Meteyer et al. 2005). Water functions as a solvent for salts, ions and gasses, and as a transport medium for metabolic products in the body. It is important for digestion, excretion and temperature regulation, as well as lubrication of joints and as the medium to conduct sound and light.

Water is a small molecule that readily diffuses across membranes in the body. A single oral dose of water labeled with isotopes of hydrogen (^2H_2O; ^3H_2O) or oxygen ($H_2{}^{18}O$) diffuses from the digestive tract and into the blood to equilibrate with tissues throughout the body within 90 minutes in ducks and within 180 minutes in larger animals such as reindeer and seals. The dilution space of labeled water is used to measure the total body water pool and is related to the amount of lean tissue and lipid in the animal (see Chapter 10). Most water in the body is distributed in the intracellular space, which is closely regulated to maintain the activity of metabolic pathways within cells (Fig. 9.1). The extracellular space includes interstitial fluids between cells in the blood (plasma or serum), connective tissues between joints and muscles, and peritoneal fluids between organs. Extracellular spaces are more variable in volume and composition than the intracellular space because they are a buffer between the cells of the animal and its environment. The most variable of these extracellular spaces are those in the digestive tract and urinary bladder (Fig. 9.1). Fluids in the rumen of desert goats and the urinary bladder of desert tortoises vary in composition and volume with feeding and excretion; those volumes may be more than 10% of body mass and serve as stores of water between drinking bouts (Shkolnik et al. 1980; Nagy and Medica 1986). Dehydrated goats can drink a volume of water equivalent to 30% of their body mass; the excess water dilutes the ruminal fluid by 90% but only

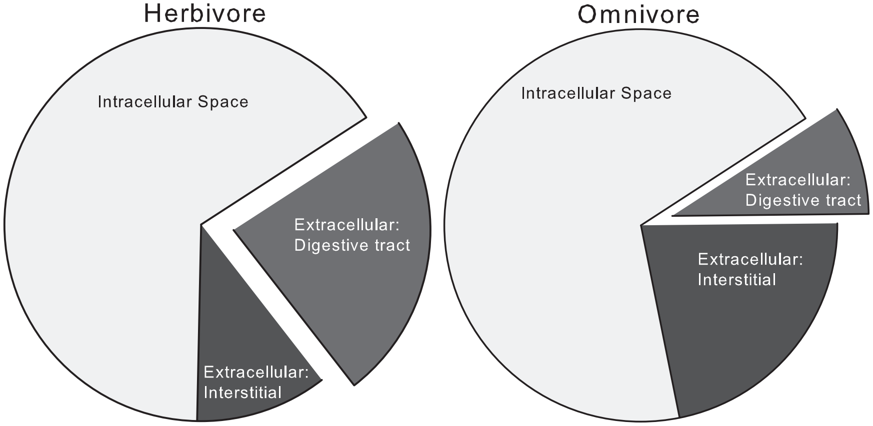

Fig. 9.1 Proportions of total body water within cells (intracellular) and outside cells (extracellular) in connective tissues (interstitial) and fluid compartments such as the digestive tract. Intracellular volume (space) and solute concentration are more closely regulated than those of the extracellular spaces. Fluids in the digestive tract are a larger proportion of the extracellular space in herbivores such as white-tailed deer (National Research Council 2007b) than in omnivores such as black ducks (Barboza and Jorde, unpublished data)

dilutes the concentration of the blood by 10% because water is only slowly absorbed from the digestive tract (Brosh et al. 1983; Choshniak et al. 1984).

A molecule is dissolved in water when the solute is surrounded by a solvation shell of water molecules. The solute and its solvation shell are usually too large to move across membranes without specific channels or transporters. Unbound water molecules that are not associated with solvation shells can continue to diffuse through membranes by osmosis. Water moves down concentration gradients from high to low concentrations of unbound water, that is, from low to high concentrations of solutes. Osmotic potential of a fluid increases with the concentration of solutes, which is measured as osmolarity (milliosmols per liter, mOsmol•L^{-1}); sea water is 1,000 mOsmol•L^{-1} whereas pure water is free of solutes and is 0 mOsmol•L^{-1} (Fig. 9.2). The concentration of charged solutes such as mineral ions may also be measured as the electrical conductivity or resistivity of the solution. Electro-fishing uses the electrical conductivity of body fluids to temporarily immobilize fish for capture by disrupting nerve and muscle function; captures require less voltage in fresh water than sea water because the fluids of the fish are more conductive than the surrounding fresh water. Solutes are also measured as total dissolved solids (TDS); TDS includes large uncharged molecules as well as the smaller charged ions. Water sources that are below 1,000 ppm TDS are suitable for consumption by most animals. Some animals can ingest solute concentrations similar to that of sea

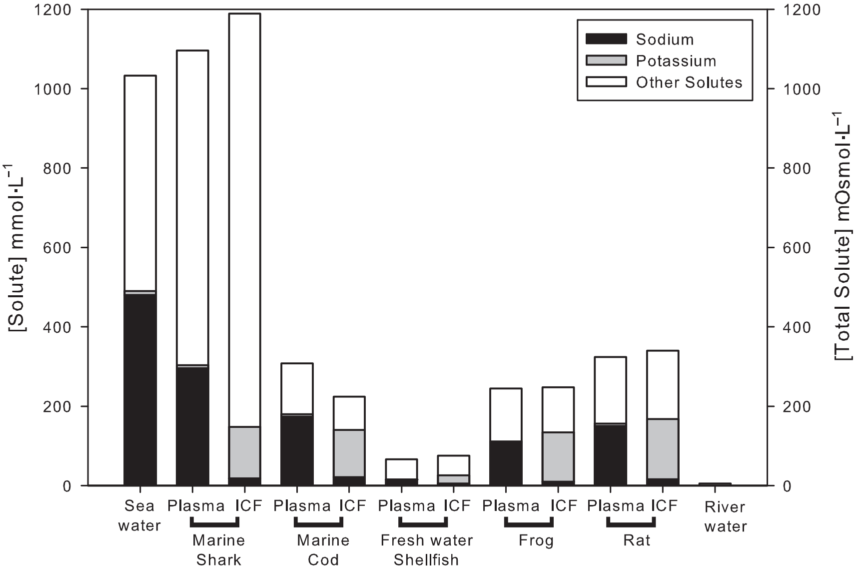

Fig. 9.2 Concentrations of sodium, potassium and other solutes (mmol•L^{-1}, mOsmol•L^{-1}) in sea water, fresh water from a river, and the body fluids of animals (Schmidt-Nielsen 1997). Solute concentrations are also compared between extracellular (ECF; blood plasma) and intracellular (ICF; muscle) fluids of dogfish shark, marine cod, freshwater shellfish, edible frogs and brown rats (Kirschner 1991)

water (approximately 3,000 ppm TDS), but more concentrated solutions draw water from the body into the digestive tract, causing osmotic diarrhea in a manner similar to the malabsorption of lactose (Chapter 6) (National Research Council 1974; National Research Council 2005).

Most of the osmotic potential of sea water is attributed to Na^+ and Cl^-, with smaller contributions from magnesium (Mg^{2+}), sulfate (SO_4^{2-}) and Ca^{2+} (Fig. 9.2). Blood plasma and interstitial fluids are also high in Na^+ and Cl^- but are low in potassium (K^+) ions. Conversely, intracellular osmotic potential is mainly attributed to K^+, phosphate (PO_4^{3-}) and proteins, with smaller contributions from Mg^{2+} and SO_4^{2-} (Linder 1991). The differences in solute concentrations produce gradients that are maintained in the animal by hydrolysis of ATP, which powers membrane transporters or pumps. The imbalance in Na^+ and K^+ is produced by active transport through the Na/K pump in the cell membrane. This gradient also produces an imbalance of charge between the cell contents and the plasma that is used for other cellular exchanges and for the action of nerves and muscles. The activity of the Na/K pump accounts for 31% of the energy expended by domestic animals at rest, which contributes to the energy required for maintenance of the whole animal (Chapter 10) (Underwood and Suttle 2001).

9.1.1 Transport Mechanisms

Exchangeable membranes control the composition of an animal’s blood plasma and the extracellular space. Water is channeled while solutes are filtered and pumped across gills, salt glands, rectal glands, kidneys, urinary bladders and the digestive tract. Water channels allow frogs and tortoises to resorb water from urine stored in the bladder. Such water exchanges across membranes are controlled by combining the osmotic potential of solutes with transcellular channels called aquaporins (Agre et al. 2002). The permeability of a membrane to water and solutes depends on its lipid composition and structure (Chapter 7). Thick shells and membranes that are invested with cholesterol and waxes are relatively impermeable and provide a strong barrier to water exchange at the surface of desert animals (Withers 1992). Conversely, lungs and gills have thin membranes that are permeable to respiratory gases (O_2 and CO_2) as well as to water and some solutes.

In the glomerulus of the kidney, thin membranes with gap junctions between cells allow both paracellular and transcellular exchanges for filtration of blood. Ultrafiltration in the kidney separates cells and proteins from small charged and uncharged sugars, amino acids and mineral ions. Glomerular filtration rates (GFR) are increased by changing the pressure and flow of blood across the filter when an excess of water or solutes is cleared from the extracellular space. GFRs of terrestrial animals are typically greater for species from water-abundant (mesic) areas than those from arid (xeric) areas (Yokota et al. 1985). Selective pumps return glucose and amino acids to the blood, but leave mineral ions (e.g., Na, Cl) and nitrogenous wastes in the urine for excretion (Chapter 8). Fish, amphibians, reptiles and birds produce dilute urines with an osmotic pressure similar to their blood. These animals can easily clear water loads through the kidneys but risk losing solutes in the large volume of filtrate. Palestine sunbirds, for example, consume up to six times their body mass as watery nectar each day and excrete most of that water by increasing GFR to produce dilute urine. The ability of their kidneys to recover 98% of the glucose from the nectar may be partly due to a reduction in water absorption from the digestive tract, which reduces the load of water on the kidneys (McWhorter et al. 2004). Conversely, dehydrated desert goats reduce GFR and urine excretion after drinking a large volume of water, which regulates the loss of Na^+ and Cl^- as water is slowly absorbed from the digestive tract (Choshniak et al. 1984).

In mammals, the kidney can produce urine with a higher concentration of solutes than the blood by combining solute pumps with a counter-current flow that can return more water to the blood. Kidneys with long concentrating tubules have proportionately large central medullary sections relative to the volume of glomerular tissue in the peripheral cortex. The relative medullary thicknesses (RMT) of desert mammals are greater than those from mesic areas among both placental mammals and marsupials (Calder and Braun 1983; Brooker and Withers 1994). RMT correlates with urine concentrating ability in rodents (Al-kahtani et al. 2004); for example, the large RMT of the hopping mouse is associated with a maximum urine concentration of $9{,}400\,\mathrm{mOsmol{\bullet}L^{-1}}$, which is 9.4 times the concentration of sea water and 25 times the concentration of blood plasma (Schmidt-Nielsen 1997). In marsupials, the urine concentrating ability of young animals is low at birth but increases with the development of the kidney medulla and with increases in RMT (Wilkes and Janssens 1988). Kidney mass and GFR may vary among adults within a population as water and solute intakes change during the year, especially in species with large seasonal changes in activity and reproduction (McAllan et al. 1996, 1998).

The removal of salt loads from the blood at chloride cells in the gills of fish and salt glands of birds, reptiles and sharks is accomplished via specific transporters such as the Na/K pump. The ability to secrete a fluid with high concentrations of Na and Cl allows gulls and waterfowl to gain water from drinking in brackish estuaries or the ocean because the solute excretion requires little water (Fig. 9.3) (Schmidt-Nielsen 1997). Secretions from salt glands account for 40% of Na excretion and only 11% of the total water loss of mallard ducks drinking brackish water (300 mM NaCl, 67% sea water) at the maximum concentrating ability of their salt gland (Bennett et al. 2003). The combined effect of Na/K pumps and water resorption in the mammalian kidney also allows harp seals and desert wallabies to drink sea water (Kinnear et al. 1968; How and Nordoy 2007).

9.1.2 Aquatic Exchanges of Water

Fish readily exchange water with their surroundings, especially at the thin vascularized surfaces of the gills. The net loss or net gain of water at the gills depends on

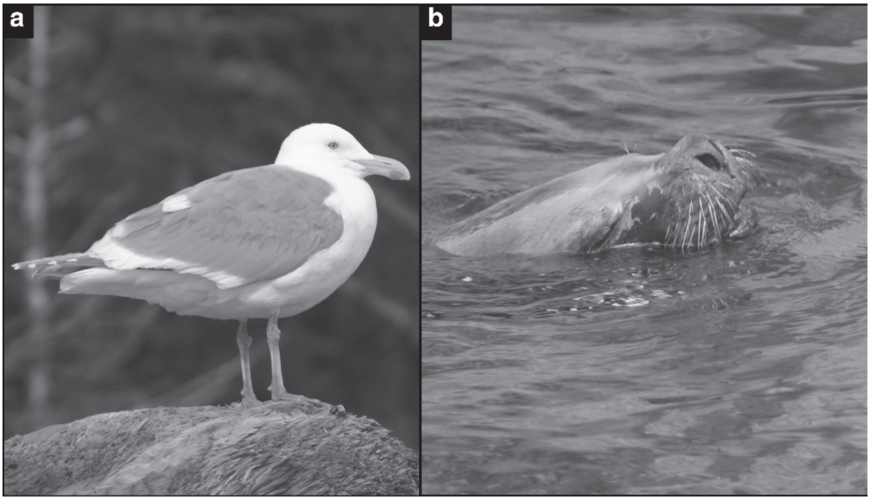

Fig. 9.3 Water for some mammals and birds can be gained by drinking sea water if Na^+ and Cl^- ions can be removed and secreted at a higher concentration than the blood. **a** Seabirds such as the herring gull eliminate excess salt in secretions from the nasal salt glands. **b** Marine mammals such as the South American sea lion eliminate excess salt in urine from the kidney

the osmotic gradient between the blood and the medium. Salty surroundings may be osmotically opposed by maintaining a lower osmolarity (e.g., bony fishes, Family Teleostei) or complemented with alternative solutes to Na^+ and Cl^-. Many bacteria and invertebrates use sugars, amino acids and their derivatives to maintain an osmotic balance with marine and hyper-saline environments such as salt lakes (Hochachka and Somero 2002). Some of those solutes also are used for cellular exchanges within vertebrate tissues such as the kidneys (Yancey 1988). The elasmobranch fishes (e.g., sharks and rays) use urea as an uncharged internal solute to maintain plasma osmolarity near that of the surrounding ocean by retaining urea at the kidney and at the gills (Fig. 9.2; Chapter 8) (Moyle and Cech 2004). Plasma osmolarities of bony fish are lower than sea water and greater than fresh water (Figs 9.2 and 9.4), which results in a net efflux of water in the ocean and a net influx of water in a freshwater stream. Water is replaced by drinking in the ocean; the excess Na^+ and Cl^- are excreted by α-chloride cells in the gills whereas loads of Mg^{2+} and SO_4^{2-} are excreted by the kidneys (Moyle and Cech 2004). Freshwater teleosts do not drink but urinate to excrete the continuous influx of water (Fig. 9.4), resulting in higher GFRs for fresh water than for marine species of fish (Yokota et al. 1985). Solutes lost in the urine of freshwater fish are replaced by solutes in the food and by absorbing Na^+ and Cl^- at the gills by β-chloride cells. Fish that move between the ocean and rivers can adjust to the changing demands for ion excretion by changing the number and activity of the α-chloride and β-chloride cells to favor salt excretion and absorption respectively. Survival of young Atlantic salmon is related

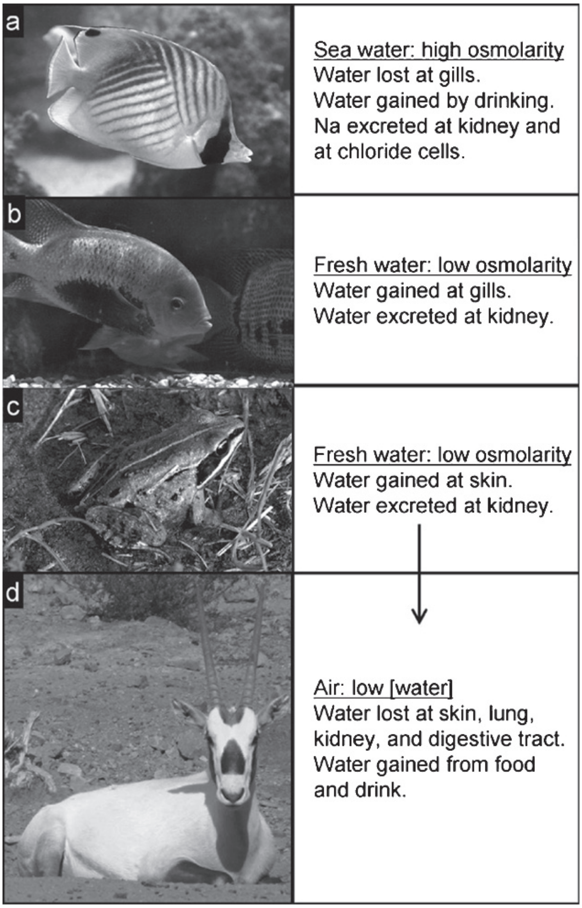

Fig. 9.4 Exchanges of water depend on the difference in the concentration of solutes (osmolarity) and unbound water between the animal and its environment. **a** Marine teleosts such as butterfly fish lose water at the gills because the medium has a higher osmolarity than the body. Salt gained from feeding and drinking is excreted at the kidneys and at the salt glands in the gills. **b** Freshwater teleosts such as spotted tilapia gain water at the gills because the concentration of solutes in the medium is lower than the body; excess water is excreted at the kidneys. **c** Terrestrial animals continuously lose water to air at the skin and lungs. Wood frogs can gain water through the skin in summer, but minimize evaporative losses by hibernating in the leaf-litter on the forest floor during winter. **d** Desert animals such as the Arabian oryx minimize excretory losses of water by producing dry feces and concentrated urine

to the activity of chloride cells in the gills as they move from their natal streams to the ocean (McCormick et al. 1999). The energy required to regulate the water and solute balance in fish is less than 5% of the energy used at rest, but that cost may increase and detract from growth as the osmotic difference between the animal and the medium increases and diverges from the optimal range for the species (Withers 1992; Moyle and Cech 2004).

9.1.3 Terrestrial Exchanges of Water

Fish can always replace their water losses from the medium as long as they can resolve imbalances in solutes. Terrestrial vertebrates must resolve imbalances of both water and solutes between their body and the environment (Fig. 9.4). Water evaporates from animals when their surfaces are exposed to air. Vertebrates cannot gain water from air, but small invertebrates such as ticks and mites can use specialized structures with high osmolarities to condense water vapor (Withers 1992), which allows the parasites to remain hydrated while waiting for their next vertebrate host.

Evaporation increases with air temperature as more energy is available for liquid water to enter the gaseous phase. Water vapor equilibrates with the liquid phase in still air; moving air disrupts the equilibrium between phases and increases evaporative loss. Bird eggs typically lose 10–12% of their laid mass as water is lost at warm temperatures during incubation even though air flow in the protected nest is low. Pores in the shell conduct gases between the air and the growing embryo. Gas conductance is a trade-off between O_2 supply and the loss of water from the egg. Birds that nest at low elevation lay eggs with low conductances that minimize water loss, whereas higher conductances ensure the diffusion of O_2 to eggs laid by birds that nest at high altitudes (Vleck and Bucher 1998).

Respiratory tracts are most vulnerable to evaporation because their surfaces are moistened to facilitate the dissolution of O_2 and CO_2 and because ventilation removes moist air from the animal each time it exhales. Long air passages and counter-current exchanges with the blood reduce the temperature of the exhaled air in ostriches and camels; this reduces the water-holding capacity of the air and thus reduces the amount of water lost through respiration (Withers 1992). Flying increases the rate at which birds breathe and thus the rate at which they lose water during respiration (Adams et al. 1997). Respiratory water loss may limit the flight range of migratory shorebirds; predicted flight ranges are longest at altitudes where cool temperatures minimize evaporative losses (Klaassen 1995; Landys et al. 2000).

Skin surfaces are most resistant to evaporation because water permeabilities are reduced by multiple layers of skin cells and waxy coats. However, amphibians such as frogs that can absorb O_2 and water through the skin are also vulnerable to losing water by evaporation when they emerge from ponds (Fig. 9.4). Frogs that estivate during droughts or hibernate during winter reduce evaporative losses by secreting layers of skin or mucus and by using burrows or leaf-litter where the air is humid and still (Fig. 9.4). Surface evaporation also may be used for thermoregulation; the energy used by water molecules to enter the gaseous phase removes heat from the surface, and this produces evaporative cooling. Heat transfer is most efficient when the evaporative surface is close to an area that produces heat (e.g., muscle) or can conduct the heat (e.g., blood vessels). Most animals can use high-frequency breathing or panting to evaporate air from the tongue and mouth where the thin skin covers a dense network of blood vessels. Although large ungulates such as elk and eland commonly rely on cutaneous evaporation and the smaller ungulates such as mule deer and hartebeest use respiratory evaporation for thermoregulation in summer (Parker and Robbins 1984), moose take advantage of free water in their environment for evaporative cooling. Saliva also can be used to transfer water to the body surface. For instance, red kangaroos lick their forelimbs to cool the blood flowing through a capillary bed close to the skin (Dawson 1983), and heat-stressed wood storks and black vultures urinate on their legs (Withers 1992). In horses and humans, sweating secretes water from glands on the skin surface, especially near the major muscle groups at the shoulder and rump where most heat is generated during running.

Animals including small ground squirrels and African gazelles that need to conserve water at high temperatures may reduce evaporation by allowing body temperature to rise; this reduces the difference in temperature between the animal and the air (Withers 1992; Maloney and Dawson 1998). Birds that are routinely exposed to high temperatures may reduce evaporative water loss further by changing the ultrastructure of their skin and by reducing their metabolic rate and heat production (McKechnie and Wolf 2004). The effects of temperature on energy metabolism are further discussed in Chapter 10.

9.1.4 Water Turnover and Balance

The water requirements of terrestrial animals are determined by the rate at which water enters (influx) or leaves (efflux) the body water pool (Fig. 9.5). Water efflux is the combined loss of water in the excreta (urine and feces) and the evaporative or insensible losses of water from the skin (cutaneous) and respiratory tract. Intracellular and extracellular spaces of water are maintained when rates of influx and efflux of water are balanced (Fig. 9.5). The body water pool can be maintained over a wide range of complementary influxes and effluxes. Red knots balance water influx and efflux at daily rates equal to eight times the body water space when the shorebirds feed on clams; daily water efflux declines to 0.3 times the body water pool when the same birds stop feeding (Visser et al. 2000). Water turnover (also referred to as water flux) generally increases with energy requirements because animals consume more food, excrete more solutes and lose more water to respiration as activity increases (Macfarlane and Howard 1972). Conversely, low water turnovers are associated with low energy requirements in spinifex pigeons from deserts and in sloths from tropical rainforests (Nagy and Montgomery 1980; Williams et al. 1995). Water requirements can be estimated on the basis of energy expenditures for domestic dogs and cats at 2.93–4.18 g $H_2O{\bullet}kJ^{-1}$ (National Research Council 2006).

Water turnovers increase with the energy demands of lactation and with the secretion of water in milk, especially for species that produce large volumes of dilute milk such as horses (Maltz and Shkolnik 1984; National Research Council 2007a). Water requirements may be increased further by enhanced insensible losses at high ambient temperatures (Fig. 9.5). Water turnovers of desert tortoises, kanga-

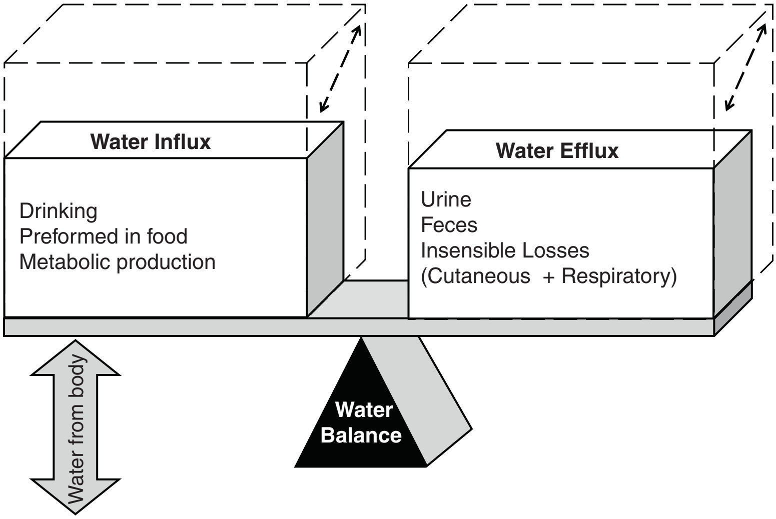

Fig. 9.5 Water enters and leaves the body water pool by several routes in terrestrial animals. Water also is produced within the body during metabolism and when water is absorbed from the digestive tract and urinary bladder. The balance between total influx and total efflux of water is zero when animals maintain total body water. Dehydration reduces the body water pool and results in negative water balance. Accumulation of water in extracellular spaces results in positive water balance. Water turnover or flux (ml•d^{-1}) is the rate at which water enters the body water pool. The turnover changes with both influx and efflux of water (*broken lines*) when food intakes, solute loads and respiration rates are altered

roos and koalas increase with activity, air temperatures and radiant heat loads from winter to summer (Ellis et al. 1995; Henen et al. 1998; Bradshaw 2003). Water turnovers and insensible water losses of nestling birds and young mammals are usually high because growing animals have high energy demands and proportionately large surface areas in relation to their small body masses (Chapter 1). Young red kangaroos are more susceptible to the heat and water restrictions of droughts than adults because their insensible water losses are as high as those of adult females three times their body mass (Munn and Dawson 2001).

Birds and mammals may rely on sources of surface water for drinking in hot, dry habitats such as deserts and savannahs. The distribution of watering holes influences the foraging areas used by animals; in semi-arid rangelands, domestic cattle and sheep heavily graze the areas around watering holes (Landsberg et al. 1999). In Australia, water from wells allows feral cats and goats to persist in dry habitats often to the detriment of native marsupials such as bilbies that do not require surface water for drinking (Hume 1987; Hume et al. 2004). Drinking behaviors may be influenced by other factors such as the presence of predators (e.g., crocodiles) and the quality of the water. Ephemeral ponds and seasonal wetlands become increasingly salty as the water evaporates and they become contaminated by decomposing plants and animals. In cold climates, drinking water and eating snow incur an added energy cost because ingested water must be warmed to body temperature; the energy required to warm ingested water is equivalent to 3–14% of the digestible energy intake of muskoxen and 3–30% of the total energy expended by smaller mammals (Berteaux 2000; Crater and Barboza 2007).

Many animals rely on water in food because drinking water may be scarce and drinking may incur other risks to survival such as predation, thermal or solute loads and microbial pathogens. Preformed water in foods varies from less than 30% of the mass of seeds and winter twigs to more than 80% of the mass of algae and fruit (Fig. 1.2) or lush green forage in early spring. The water content of animal foods is 73% for lean tissues such as muscle, but declines with increasing concentrations of fat. However, the dry portion of foods also can yield metabolic water when reducing equivalents (e.g., NADH and $FADH_2$) are oxidized by the following reaction (Fig. 6.1):

$$O_2 + 4H^+ \rightarrow 2H_2O$$

Metabolic water production is therefore related to the fuel value of each substrate: oxidation produces 1.07g $H_2O \bullet g^{-1}$ of fat but only 0.56 g $H_2O \bullet g^{-1}$ of carbohydrate. Protein oxidation yields a similar amount of water to that of carbohydrate, but some metabolic water must be used to excrete the amino-N. Protein oxidation therefore produces 0.50 g $H_2O \bullet g^{-1}$ when N is excreted as uric acid and only 0.40 g $H_2O \bullet g^{-1}$ when N is excreted as urea (Chapter 8). Carnivores such as desert kit foxes may derive most of their water requirements from their prey because lean tissues are rich in preformed water, and fat provides oxidative water. Omnivores such as bilbies and Merriam's kangaroo rats survive in deserts without drinking water by using preformed and metabolic water from plants and insects (Tracy and Walsberg 2002). Metabolic water also is produced by oxidation of the animal's own tissues; oxidation of fat provides most of the water required by fasting northern elephant seal pups, which maintain the osmolarity of their blood plasma after they are weaned by their mothers (Adams and Costa 1993; Ortiz et al. 2002). During hibernation, bears, ground squirrels and prairie dogs also rely on water produced from tissue breakdown. Birds that catabolize lean body tissues during long migratory flights produce metabolic water from oxidation of protein and also release intracellular and extracellular water from cells and connective tissues (Lindström and Piersma 1993).

Protein catabolism has the potential to increase urinary water loss because urea and uric acid are solutes that must be removed from the extracellular space. High N intakes therefore may increase urinary volumes when animals can increase their water intakes from food or drink (National Research Council 2007a). Restrictions on water influx can alter N excretion as animals reduce urinary water losses; recycling of N-catabolites decreases excretory N loads. The proportion of urea-N recycled increases when drinking water is restricted for desert sheep, goats, camels, kangaroos and wombats (Mousa et al. 1983; Barboza 1993b; Freudenberger and Hume 1993). In birds, reflux of urine from the cloaca to the hindgut allows recycling of urate-N by microbes and resorption of water and ions in the ceca (Hughes et al. 1999; Laverty and Skadhauge 1999). Although high solute loads can increase urinary water losses, the concentrating abilities of the kidney and/or salt glands may minimize changes in water flux. Urine flow rates of emus were similar among diets with different concentrations of NaCl because kidney function changed with solute load and water availability (Dawson et al. 1991). Similarly, water fluxes only increased by 2–3% in mallard ducks when fresh drinking water was replaced with brackish water (Bennett et al. 2003).

The amount of water lost in feces varies with the digestive function of the animal and the structure of the diet. Coarse residues from fibrous diets of leaves and stems result in more feces with a greater water-holding capacity than the dense residues from more digestible items such as seeds or animal prey. Desert carnivores such as cats and foxes lose little water in the feces because their diet is highly digestible and the feces are mainly comprised of the skin and bones of prey. Water is removed from the digesta by muscular contractions that extrude fluid from the digesta toward the intestinal wall and by osmotic exchanges across the wall between intestinal contents and blood. Fecal water losses by desert species are usually lower than those of closely related species from mesic regions, even on the same diet. For example, common wombats from mesic regions produce feces with large particles that are similar to feces produced by horses, whereas hairy-nosed wombats from the desert produce feces with fine particles in small, dry pellets (Barboza 1993a, 1993b). Particles that are fine and dense have large surface areas and small volumes that facilitate water resorption across the intestinal mucosa. Also, the longer distal colon of hairy-nosed wombats increases the time and surface area for water resorption from feces as they form pellets (Barboza and Hume 1992). Several ruminants such as caribou, white-tailed deer, sheep and goats can produce small fecal pellets from a long distal colon that is arranged in concentric loops called the spiral colon (Hofmann 1989). Hindgut morphology may reflect evolutionary selection for water conservation in some groups of ungulates; the desert-adapted dromedary camel also has a spiral colon whereas the aquatic hippo has only a short hindgut (Stevens and Hume 1995).

Food intake is dependent on hydration, and mammals and birds often reduce their intake of DM when water availability declines (Langhans et al. 1995; Forbes 2007). Consequently, water deficits may cause secondary deficits of energy and nutrients; white-tailed deer decreased food intake and lost body mass when drinking water was restricted to 33% of ad libitum levels (Lautier et al. 1988). The effects of limited water availability on animals can be measured by comparing food intakes and balances between periods when drinking water is available ad libitum and when water is either restricted (Table 9.1) or completely withdrawn. Kangaroos and goats responded to moderate restrictions of drinking water (43–68% of ad libitum) by reducing water turnover without changing body mass or total body water (Freudenberger and Hume 1993).

Water turnovers decline with reductions in influxes of drinking, preformed and metabolic water, and with commensurate reductions in both fecal and urinary effluxes of water (Table 9.1). Fecal water losses are minimized by decreases in the moisture content of feces whereas urinary losses are minimized by increases in urine osmolarity. Water resorption at the distal colon and the kidney is facilitated

Table 9.1 Water balance of a ruminant when drinking water that is either freely available (ad libitum) or restricted to 50% of ad libitum. Values are derived from responses of white-tailed deer and goats to water restriction (Lautier et al. 1988; Freudenberger and Hume 1993; National Research Council 2007b)

Parameter	Units	Calculation	Ad libitum	Restricted	Change (%)[1]
Body mass	kg	A	60	60	0
Drinking water	$g \bullet d^{-1}$	B	1,200	600	–50
Food intake	$g \bullet d^{-1}$	C	2,000	1,600	–20
Food moisture	$g \bullet g^{-1}$	D	0.48	0.48	0
Preformed water	$g \bullet d^{-1}$	$E = C \times D$	960	768	–20
DM intake	$g \bullet d^{-1}$	$F = C - E$	1,040	832	–20
Metabolic water content	$g \bullet g^{-1}$	G	0.35	0.35	0
Metabolic water gain	$g \bullet d^{-1}$	$H = F \times G$	364	291	–20
Total water influx (turnover)	$g \bullet d^{-1}$	$I = B + E + H$	2,524	1,659	–34
Fecal output	$g \bullet d^{-1}$	J	1,100	704	–36
Fecal moisture	$g \bullet g^{-1}$	K	0.6	0.5	–17
Fecal water loss	$g \bullet d^{-1}$	$L = J \times K$	660	352	–47
Fecal DM loss	$g \bullet d^{-1}$	$M = J - L$	440	352	–20
DM digestibility	$g \bullet g^{-1}$	$N = (F - M)/F$	0.58	0.58	0
Urinary water loss	$g \bullet d^{-1}$	O	947	574	–39
Excretory water loss	$g \bullet d^{-1}$	$P = L + O$	1,607	926	–42
Insensible water loss	$g \bullet d^{-1}$	$Q = I - P$	917	733	–20

[1] 100 × (Restricted – Ad libitum) ÷ Ad libitum.

by the anti-diuretic hormones (ADH) arginine vasotocin (AVT) and arginine vasopressin (AVP). AVP is found in mammals whereas AVT is found in fish, reptiles and birds (Norris 1997). These hormones open water channels in the mucosa of the colon and in the renal tubules to return water to the blood, and secondarily act on the brain to stimulate drinking behavior. Responses to plasma ADH and water restriction are more pronounced in desert animals than similar species from mesic habitats (Macfarlane and Howard 1972). Concentrations of plasma ADH were inversely related to water turnovers of sympatric wallabies during a drought; the most mesic bettongs had water turnovers that were 2.8 times higher than those of an arid-zone species of hare-wallaby, but plasma ADH concentrations of the bettong were only 2% of those measured in the hare-wallaby (Bradshaw 2003).

Loss of body mass during water restriction may be associated with changes in both extracellular and intracellular water spaces (Fig. 9.1). Most of the reduction in the extracellular water space is often in the digestive tract, especially in large herbivores such as sheep, kangaroos and donkeys (Macfarlane et al. 1961; Maloiy et al. 1978). Although increases in rumen osmolarity may serve as a post-ingestive feedback that reduces food intake, desert ruminants are able to maintain fermentation and digestion during bouts of dehydration and rehydration (Brosh et al. 1983; Langhans et al. 1995). Prolonged periods of low availability of both food and water during drought may be associated with a loss of body tissue, which reduces the intracellular water space but may also reduce energy demand. Sand gazelles lost dry mass from the digestive tract, kidney, heart and muscle during 4 months of food and water restriction, which resulted in reductions in the rates of energy metabolism and insensible water loss (Ostrowski et al. 2006).

Animals that survive hot dry environments and droughts use both physiological and behavioral mechanisms to minimize water losses and maximize water gains. Antelope ground squirrels tolerate elevated body temperature but also evade radiant heat loads in burrows, where higher humidity also increases the water content of stored seeds (Robbins 1993; Schmidt-Nielsen 1997). Springbok antelope feed at night on moist flowers and leaves to maximize water intake at lower air temperatures and avoid high radiant heat loads (Nagy 1994). Populations of some desert animals may depend on refuges where supplies of water and food are adequate for a minimal viable number of animals to survive drought. Populations of black-tailed jack rabbits fluctuate widely because the species is vulnerable to drought, but high fecundity restores the population from a few individuals that survive in refugia during severe drought (Nagy 1994). The energy and nutrient requirements of animals for survival and reproduction are further discussed in Chapter 10.

9.2 Minerals

Water conveys dissolved minerals from rocks and soils to plants and up the trophic chain of animals. Minerals released from underwater hydrothermal vents sustain unique communities of invertebrates and fish in the immediate vicinity while currents distribute those minerals throughout the ocean. Minerals are conveyed by lava flows and volcanic eruptions to the surface of the earth and are weathered into water courses that flow below and above ground through riparian systems. Geology and hydrology affect the availability of minerals for plants and animals over large geographic areas. Sodium (Na) and iodine (I) are relatively abundant in marine systems and are thus more available to plants and animals in coastal habitats than at the center of continents (Underwood and Suttle 2001). Conversely, selenium (Se) concentrations of plants are low on the Pacific and Atlantic coasts of the United States of America in high rainfall areas where leaching occurs, but some plants (e.g., milk vetch) can accumulate Se to toxic levels in the center of the country. Soil minerals therefore may limit animal populations in some regions; low Se in soils is associated with poor reproduction of black-tailed deer in northern California (Flueck 1994). The diversity among plants and foraging areas, however, can ameliorate some of these local affects on mineral supplies. Herbivores can generally avoid Se toxicity from milk vetch by consuming other plants as long as forage abundance is high (Van Soest 1994). Similarly, inland populations of herbivores may supplement their diet with salts of Na and Mg from natural deposits and springs at mineral licks (Tankersley and Gasaway 1983; Ayotte et al. 2006).

The metabolizability of minerals depends on their form. Complexes and compounds that contain minerals are indigestible if the element cannot dissolve into an absorbable form in the digestive tract. Iron (Fe) is abundant in soils as rust or ferric oxide (Fe_2O_3), but the compound is insoluble and therefore unavailable to animals. Conversely, ferrous sulfate ($FeSO_4$) can dissolve to form Fe^{2+} ions that can be absorbed. Organic complexes of minerals are often more metabolizable than inorganic forms because the mineral is either absorbed with the complex or more easily released; Fe in blood and muscle is readily absorbed as heme and retained in hemoglobin and myoglobin. Dietary minerals are likely to be more available for carnivores than herbivores because the tissues of prey contain minerals in readily digestible forms. Minerals in plants may be contained within indigestible cell walls or complexed with a wide diversity of PSMs and other minerals that can interfere with absorption and retention.

Mineral concentrations vary widely among the tissues of plants and animals because each element can serve several functions. Silicon (Si) is deposited as silica in the cell walls of grasses that are readily consumed by bison, and in the spicules of sponges that are consumed by hawksbill turtles. Silicon has only a minor biochemical role in the tissues of vertebrates and is therefore present in low concentrations in bison and turtles despite the abundance of the element in their diet. Elements that are required for structural compounds or osmotic balance are accumulated to high concentrations in the organism and are considered macrominerals (typical units are grams per hundred grams or grams per kilogram, $g \bullet 100\,g^{-1}$ or $g \bullet kg^{-1}$). The common macrominerals of vertebrate animals include Na, Cl, K, Ca, phosphorus (P), magnesium (Mg) and sulfur (S) (Table 9.2). Each macromineral can serve more than one function in the same organism, as in birds that use Ca for bones, enzyme activation cascades and egg shells (Table 9.2). Trace minerals usually serve catalytic roles in enzymes or hormones that may be involved in many biochemical pathways and tissues, but nonetheless only require small amounts of the element (typical units are miligrams per kilogram or micrograms per kilogram, $mg \bullet kg^{-1}$ or $\mu g \bullet kg^{-1}$). Trace minerals commonly required by vertebrate animals include manganese (Mn), Fe, copper (Cu), Zn, I and Se (Table 9.3).

Mineral dynamics are similar to those of water (Fig. 9.5) because influxes and effluxes are equal when the body pool is maintained. Positive balances indicate the accumulation of mineral in tissues whereas negative balances indicate depletion of body pools. Body pools of macrominerals are large and widespread in contrast to pools of trace minerals that are small and distributed among intracellular spaces. Bone is a large pool of Ca, whereas Zn is associated with numerous enzymes in both the cytosol and nucleus of cells in several organs. Mineral fluxes reflect the turnover of their principal pools. Na turnovers are high and commensurate with the turnover of the extracellular space, whereas Ca turnovers may be slow, especially in adults with their low rates of bone deposition. Mineral status is usually evaluated as the amount of mineral in an indicator tissue or the activity of an associated enzyme in the body. For example, net changes in Ca flux are often reflected in mass, density and Ca content of bone, and the plasma concentration of vitamin D (a hormone that stimulates Ca absorption). Homeostatic

Table 9.2 Common forms and functions of macrominerals in animals

Name	Chemical form	Biologically active forms	Chemical function	Body function
Sodium	Na^+	Free ion outside cells	Gradient	Nerves, muscles, body fluid volumes
Potassium	K^+	Free ion within cells	Gradient	Nerves, muscles, acid-base balance
Chlorine	Cl^-	Free ion	Gradient	Gastric acidity, acid-base balance
Calcium	Ca^{2+}	Free ion	Activation cascades	Nerves, muscles
	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite	Structural rigidity	Bone, teeth
	$CaCO_3$	Calcium carbonate	Structural rigidity	Egg shell
Phosphorus	HPO_4 esters	Phospholipids	Linkage	Membranes
		ATP, ADP, AMP	Energy store	Metabolism, RNA, DNA
	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite	Structural rigidity	Bone, teeth
Magnesium	Mg^{2+}	Free ion	Activation cascades	Energy metabolism
Sulfur	SO_4^{2-}, —SH	Methionine Glutathione Vitamins B_1 and B_8	Proteins, electron (e^-) transfer	Feathers, hair Oxidative defense, TCA cycle

Table 9.3 Common forms and functions of selected trace minerals in animals

Name	Chemical form	Biologically active forms	Chemical function	Body function
Manganese	Mn^{2+}	Superoxide dismutase	e^- transfer	Oxidative defense
Iron	Fe^{2+}	Hemoglobin, transferrin	e^- transfer	O_2 transport, respiration
Copper	Cu^{2+}	Superoxide dismutase, ceruloplasmin	e^- transfer	Oxidative defense, immunity
Zinc	Zn^{2+}	Metallothionein, superoxide dismutase	e^- transfer	Gene expression, oxidation
Iodine	I^-	Thyroid hormones	Signal transduction	Energy metabolism
Selenium	SeO_3^{2-} Se-Cysteine Se-Methionine	Glutathione peroxidase	e^- transfer	Oxidative defense, thyroid function
Cobalt	Multi-ring complex with Co at center	Cobalamines, vitamin B_{12}	C transfer	Synthesis of amino acids, bases and SCFA

controls regulate the availability of minerals to tissues by modulating excretion from the kidney, absorption from the digestive tract and release from tissues. Consequently, the concentrations of mineral ions in blood may not indicate body stores. Plasma concentrations of Ca^{2+} are maintained by the large store of Ca in bone and do not decline until the exchangeable pool of Ca is depleted. Apparent digestibilities of minerals reflect both the demand for a mineral and the availability of that mineral in a diet. An animal that is replete in Ca will not absorb as much Ca as an animal with depleted bones and high concentrations of vitamin D, even though both animals consume the same diet.

Minerals in plants and animals are measured by removing organic material and by dissolving the residue in acid. Combustion in a furnace at 450–600°C removes all the C, N, H and O to leave a residue of minerals or ash, which is dissolved in acid for further analysis. Unfortunately, some minerals are volatile (e.g., Se and I) and escape from the ash during combustion. 'Wet digestion' in a boiling mixture of strong oxidants such as nitric (HNO_3), hydrochloric (HCl), perchloric ($HClO_4$) and sulfuric (H_2SO_4) acids retains the volatile elements but removes the organic material. Some macrominerals can be assayed by electrochemistry (e.g., Na^+, Cl^-) or by reactions that produce a compound that absorbs wavelengths of light for spectrophotometry (e.g., P). Spectral methods are also used for direct analysis of the minerals in atomic absorption spectrophotometers or plasma spectrometers (e.g., inductively coupled plasma, or direct current plasma spectrometers). These devices measure the absorption or emission of light when the minerals enter a flame or an electrical arc. Mineral analysis is often expensive because the equipment is costly and the procedures use specialized gases and generate hazardous wastes. Samples that are intended for analysis of trace minerals should be handled carefully because they are vulnerable to contamination with dust from other samples and soils. The quality of analysis should be monitored by including certified standards in each batch of samples and by comparing results between laboratories.

9.2.1 Sodium, Chlorine and Potassium

Concentrations of Na^+, Cl^- and K^+ contribute largely to acid–base balance in the animal; they account for most of the balance in osmotic pressure and charge between intracellular and extracellular fluids (Fig. 9.2). Exchanges of these ions allow nerves to transmit impulses, muscles to contract, glands to secrete acids and membranes to transport solutes. Large changes in transcellular gradients are potentially fatal because they disrupt the ratio of Na^+:K^+. A rapid rise in plasma K^+ can cause heart failure, whereas rapid rises in plasma Na^+ cause osmotic changes in the spinal cord and brain that produce convulsions (National Research Council 2005). Na, K and Cl homeostasis is mainly controlled by excretion at the kidney. The hormone aldosterone from the adrenal gland stimulates resorption of Na at the kidney as plasma Na^+ declines. Concentrations of aldosterone in plasma of common wombats and gray kangaroos are low on the coast and high in the mountains, reflecting the high concentrations of Na in coastal environments and low concentrations of Na in plants and soils of montane habitats (Hume 1999).

Excretion of excess K^+ also is stimulated by aldosterone because it restores the high Na^+: K^+ ratio in plasma and other extracellular fluids such as saliva (Underwood and Suttle 2001; Wang 2004). Increases in plasma Na^+ cause the release of atrial naturetic hormone (ANH) from the heart, which opens Na channels in the kidney and allows excess Na^+ to be removed in the urine. Concentrations of plasma Na and K are affected by water balance and dietary loads of these electrolytes. Ornate dragons are lizards that maintain transcellular gradients during a drought by allowing concentrations of both Na and K to rise in plasma and also tissue. These lizards continue to acquire Na from their diet of ants, but allow Na to accumulate in the extracellular space until rain provides sufficient drinking water for excretion (Bradshaw 1992).

Marine fish mainly absorb Na and Cl at the gills from the surrounding water in the sea but rely on dietary sources of K (Fig. 9.2) (Kaushik 2001). Terrestrial animals and freshwater fish rely on their diet for all three electrolytes. Dissolved Na^+ and K^+ are absorbed across the mucosa of the digestive tract by a variety of exchanges with H^+, Cl^-, Mg^{2+} and other solutes that are linked to ATP and the Na/K pump. Chlorine is an abundant mineral in both plant and animal tissues and is therefore rarely limiting for wildlife (Underwood and Suttle 2001). Some plants in deserts and island habitats contain potentially toxic concentrations of Na, but animals such as kangaroos and rodents usually avoid the Na loads that would accompany consumption of halophytic shrubs such as salt bush and blue bush (Hume 1999). Concentrations of Na in plants consumed by animals are usually lower than those of animal tissues (Fig. 9.6). Conversely, the leaves of legumes, grasses and forbs are rich in K (Fig. 9.6), which may impose large excesses of K on herbivores. Desert reptiles can eliminate K loads with a minimum loss of water by using salt glands and by storing K as stable urate salts in the urinary bladder (Minnich 1972; Bradshaw 1992). High intakes of K, however, may impair net absorption of low concentrations of Na in leafy plants. Ratios of Na^+:K^+ in plasma and saliva decline from winter to spring as reindeer switch from lichens that are low in K to forbs and browse that are 5–10 times richer in K but contain the same levels of Na (Staaland et al. 1980). Mineral licks are an important source of Na for ungulates such as elk, moose, mountain goats, muskoxen and Stone's sheep in habitats far from the sea (Risenhoover and Peterson 1986; Klein and Thing 1989; Ayotte et al. 2006) (Fig. 9.7). Tree bark also may provide a source of Na for rodents such as red-backed voles and porcupines, which can result in considerable damage to trees planted for commercial forestry (Roze 1989; Hansson 1991).

Demands for Na and K increase with the growth of body tissues and thus gains in both intracellular and extracellular spaces. Requirements for these electrolytes therefore increase with water turnover and energy expenditure in ungulates such as white-tailed deer (Hellgren and Pitts 1997). Incremental demands for Na during reproduction are more likely to limit herbivores, especially fecund species with rapid growth rates such as California voles because of low Na concentrations in plants compared to prey tissues (Batzli 1986). Because Na is not accumulated in high concentrations in plants, deficiencies in herbivores are most likely to occur in

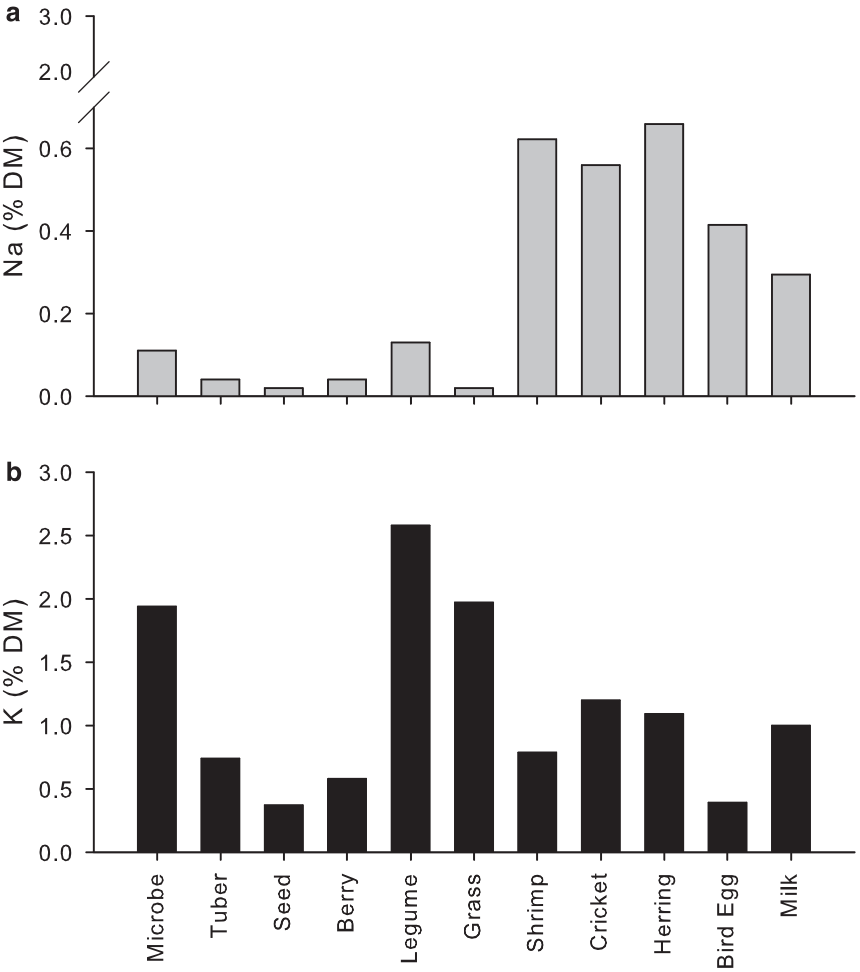

Fig. 9.6 Concentrations of two electrolytes in the dry tissues of selected microbes, plants and animals. **a** Sodium (Na). **b** Potassium (K). Microbes: yeast (National Research Council 2003). Plants: tubers of sweet potato, seeds of corn, blueberries, leafy alfalfa (National Research Council 2003) and hay from mixed species of temperate grasses (National Research Council 2007a). Animals: muscle from mixed species of shrimp (National Research Council 2006); whole cricket and herring (National Research Council 2003); chicken eggs with shells (Johnson 2000; National Research Council 2003); average of milks from domestic cattle, sheep, goats and water buffalos (Agricultural Research Service 2007)

areas where alpine and mountain soils are leached by rain and snow. Increases in activity also may increase the Na demands of animals that secrete high concentrations of Na in sweat, such as horses (National Research Council 2007a).

Fig. 9.7 Inland populations of herbivores such as moose may be limited by Na, especially during summer when high forage intakes for body mass gains, antler growth and reproduction result in the ingestion of large amounts of K

9.2.2 Calcium and Phosphorus

Ca and P influence skeletal formation, and contribute to nerve impulse transmission, muscle contractions and metabolism. The predominant biological form of P is phosphate (PO_4^{3-}), which links with other phosphates in ATP (Fig. 9.8), glycerol in phospholipids (Chapter 7; Fig. 7.5) and sugars in DNA (Chapter 8; Fig. 8.7). The biologically active forms of Ca are ionic; Ca^{2+} activates enzymes by binding to carboxyl groups ($\text{—}COO^{2-}$) on proteins and to both carbonate (CO_3^{2-}) and phosphate (Fig. 9.8). Calcium carbonate is the mineral component of a protein matrix that forms the shells of bird eggs and mollusks. Bone is a matrix of proteins called collagens that are integrated with a complex of Ca and phosphate called hydroxyapatite (Fig. 9.8). The matrix of bone is not static but continuously exchanging Ca and P with the blood (Fig. 9.9) as bone cells mineralize and demineralize the protein matrix in response to both blood Ca^{2+} and the physical stimuli of gravity and activity. Bears can maintain the concentrations of Ca and P in serum as well as the mineral content of their bones through the long inactivity of their winter dormancy (Floyd et al. 1990). The exchangeable nature of the bone matrix allows the gradual incorporation of other ions. The trace mineral fluoride (F^-) substitutes for hydroxyl groups in teeth to produce a less porous matrix; excess exposure to F^- from ground waters may alter bone structure and tooth hardness, especially in young animals (National Research Council 2005), and is associated with tooth breakage and reduced life span of elk wintering near hot springs in Yellowstone National Park (Garrott et al. 2002).

Homeostatic controls of Ca and P in vertebrates are cued by changes in plasma Ca^{2+} and the large reserve of both Ca and P in bone. Declines in blood Ca^{2+} are opposed by the action of parathyroid hormone (PTH), which stimulates release of Ca from bone, resorption of Ca at the kidney and absorption of Ca from the digestive tract mediated by activated vitamin D. Bone mobilization releases both Ca and P, but the relative excess of P usually is cleared at the kidney with the stimulus of PTH. Rises in blood Ca are opposed by calcitonin, which suppresses Ca release from bone. Calcitonin suppresses the effect of PTH on the kidney to allow P to be retained in blood and Ca to be lost in urine. Calcium homeostasis in fish differs

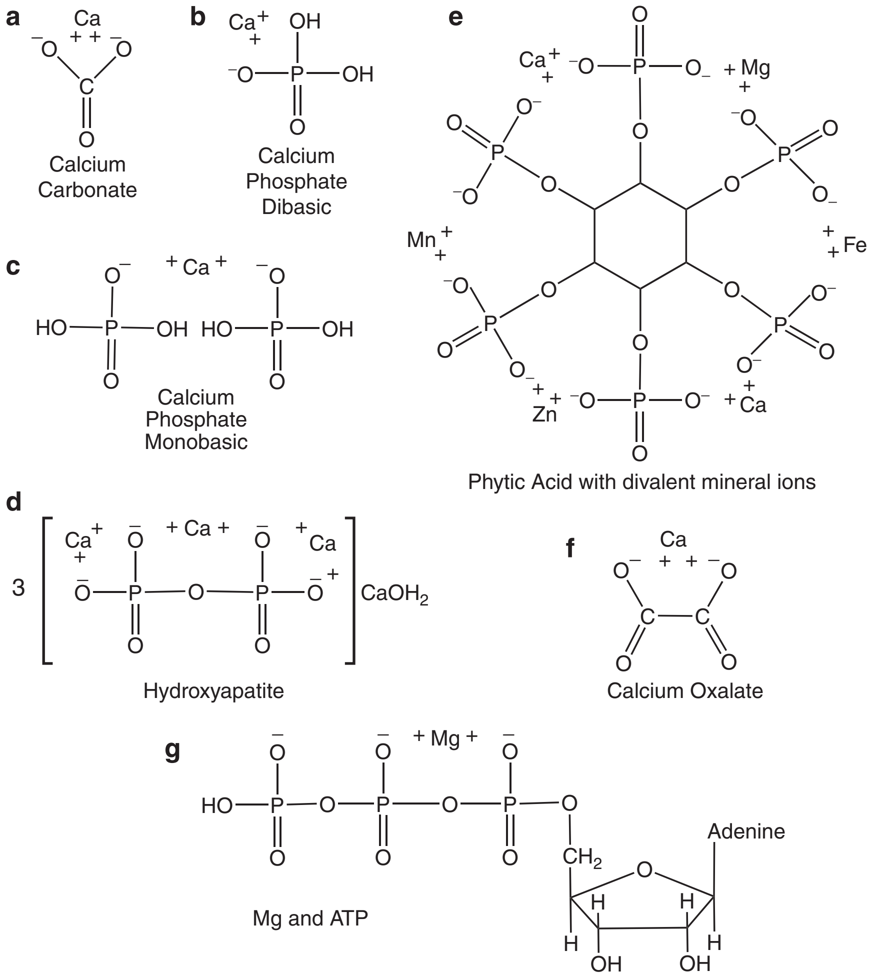

Fig. 9.8 Chemical forms of Ca and P and their interactions with other divalent mineral ions. **a** Calcium carbonate from shells of birds and molllucs. **b** and **c** Calcium phosphates found in foods. **d** Hydroxyapatite from bone. **e** Phytic acid from seeds with associated mineral ions. **f** Calcium bound to oxalic acid from browse. **g** Phosphate linkages of ATP interact with magnesium

Fig. 9.9 Bones are dynamic systems of cells and channels that continuously exchange Ca, P and protein. Minerals and protein are deposited in rings as bones enlarge; the pattern of growth is evident in the scutes on the carapace of the Galapagos tortoise, which continues to grow for several decades

from other vertebrates because vitamin D and hormones from the pituitary gland substitute for PTH when blood Ca declines. Fish can also use Ca from water and from their integument; Pacific salmon can absorb Ca at the gills and mobilize Ca from scales during migration (Lall 2002).

Bone may be more important as a store of P for fish because concentrations of plasma phosphate are lower in cartilaginous fish (e.g., sharks) than in bony fish (e.g., flounder) (Lall 2002). Mammals and birds can release phosphate from glucose-phosphate in bone by the action of the enzyme alkaline phosphatase (AP); AP in plasma increases with bone mineralization in growing birds and during antler growth in white-tailed deer (Grasman and Hellgren 1993; Tilgar et al. 2008). Similarly, the bone protein osteocalcin is used as a serum indicator of bone growth and antlerogenesis. Osteocalcin and AP indicate bone deposition whereas PTH indicates the adequacy of Ca and P supplies. During mineralization of antlers in fallow deer, activities of AP increase and the concentration of PTH remains unchanged if the dietary supply of Ca is adequate (Eiben et al. 1984; Grasman and Hellgren 1993).

Tissues vary widely in their contents of Ca and P and in their availability for digestion (Fig. 9.10). Milk contains Ca and P in high concentrations and in forms that are readily absorbed by infant mammals; Ca availability is enhanced by interactions between lactose and Ca^{2+}, while milk phospholipids provide a ready source of phosphate (Linder 1991). Phosphate is also available in high concentrations from the membranes of microbes, muscles and egg yolks (Fig. 9.10). Acid digestion releases soluble Ca and phosphate from within cells and protein complexes. Insoluble Ca in bones and shell of whole prey and eggs, however, may require prolonged retention in the stomach, which would limit the digestion of other components of the diet and the supply of energy and protein. Consequently, a large proportion of ingested Ca and P is lost in the feces or regurgitated as pellets by carnivores such as owls that ingest whole prey. Grinding bone into small particles increases the surface area for digestion of both the protein and its minerals. Hyenas, Tasmanian devils and timber wolves chew bones and leave only small fragments and the hardest parts of the jaws and claws. Cape Griffon vultures scavenge small fragments of bone left by large predators to feed their chicks; the eradication of large predators in some areas of Africa lead to bone anomalies in young vultures (Richardson et al. 1986). Herbivores such as voles and porcupines also obtain Ca and P from bones by gnawing on antlers dropped from moose and elk.

Concentrations of Ca and P differ markedly in plants because the two minerals are not always linked by function or structure as they are in the bone matrix of animals. For example, P varies seasonally and is highest in growing plants. High concentrations of Ca^{2+}, however, can bind phosphate and reduce the availability of dietary P. The optimum ratio of dietary Ca: P is between 1:1 and 2:1 for growing bones in most animals. Adults may tolerate much higher ratios (up to 7:1) of Ca: P as long as the diet provides enough P because Ca absorption is regulated in relation to blood Ca^{2+}. Birds may require high ratios of Ca:P (up to 4:1) during egg laying because Ca is needed for calcium carbonate in egg shell, although both Ca and P are required for the yolk and albumen (Fig. 9.10). Although not common, low Ca:P ratios result in an excess of P in the body pool because there are few places to shunt excess P, in contrast to Ca that can be moved to bone or eggs. The resulting condition is known as nutritional secondary hyperparathyroidism (NSH); levels of PTH increase in response to low plasma Ca^{2+} and cause mobilization of Ca from bones, which can deform growing animals (National Research Council 2006, 2007a).

The availability of Ca and P is reduced by interactions with charged elements of the plant fiber matrix and by PSMs such as oxalic acid in leaves and phytic acid in seeds (Fig. 9.8). Microbial phytases in the digestive tract can release P and its associated mineral from phytates in herbivores such as ruminants. Fecal contamination of water near dense aggregations of animals such as cattle feedlots, piggeries and poultry farms provides phosphates and N for rapid growth of algae that may reduce stream flow and water quality for fish and other wildlife.

Inadequate dietary supplies of Ca and P usually result in the net loss of mineral from bone, and in elevated concentrations of PTH or vitamin D metabolites in plasma. Bone demineralization reduces the ability to resist the physical stresses of gravity and activity, which may result in enlarged joints and bent limbs or rib cages in growing animals (rickets) and a high incidence of fractures during capture and handling. Malformation of bones may be more easily discerned in growing animals with long limbs such as cranes (Olsen and Langenberg 1996). Skeletal bones are also a temporary store of Ca and P for production of milk, antlers and eggs. Red deer and mule deer accumulate bone Ca and P from the diet through spring and

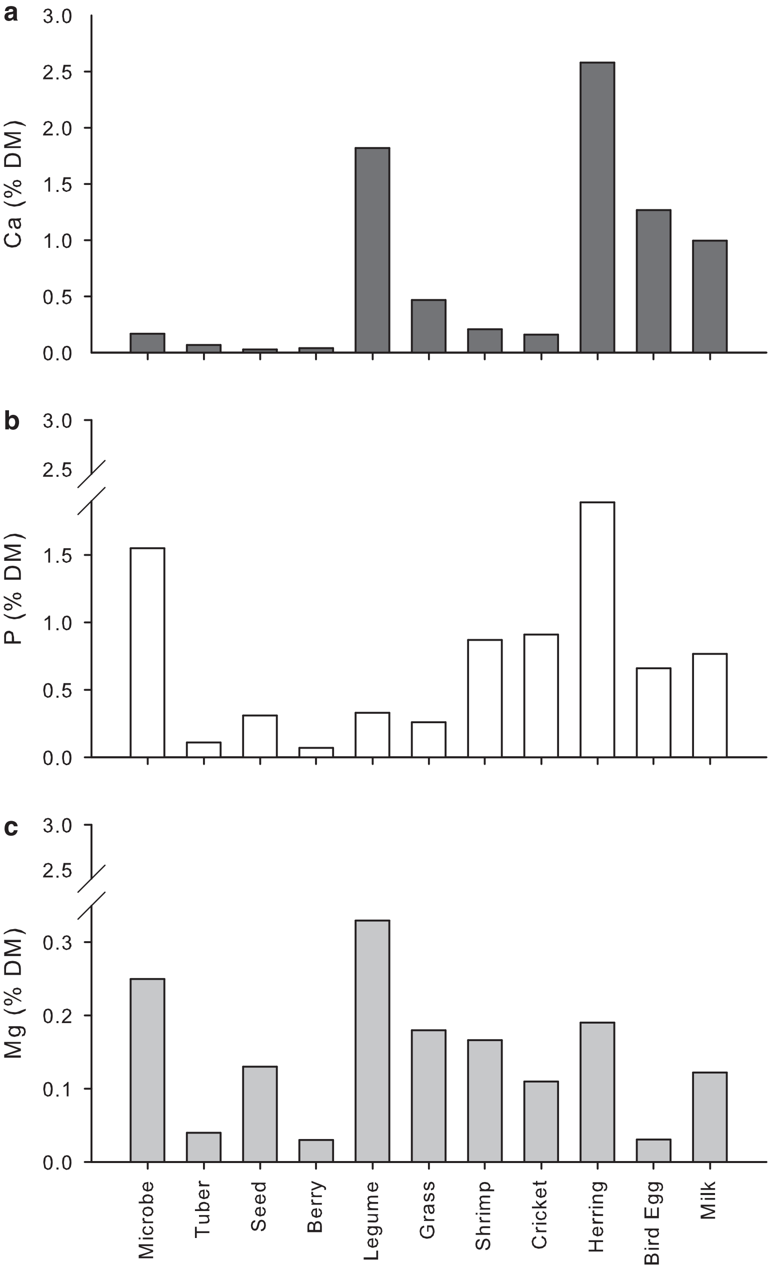

Fig. 9.10 Concentrations of three macrominerals in the dry tissues of selected microbes, plants and animals. **a** Calcium (Ca). **b** Phosphorus (P). **c** Magnesium (Mg). See Fig. 9.6 for details of the selected organisms

summer and translocate those stores to the hardening antler in the last phase of antlerogenesis (Hillman et al. 1973; Muir et al. 1987).

The development of the avian egg shell requires a steady delivery of Ca to the oviduct, which is provided by medullary bone at the core of leg and pelvic bones (Klasing 1998). Vitamin D stimulates absorption of Ca and P from the diet, both of which are deposited in medullary bone. The protein calbindin carries Ca from bone to the oviduct where bicarbonate is produced to combine with Ca as $CaCO_3$ in the egg shell (Fig. 9.8). Net loss of bone is minimal during egg formation in chickens, even though Ca deposited in the egg is equivalent to more than five times the amount of exchangeable Ca^{2+} in the body (Clunies et al. 1993). Granivorous songbirds rely on dietary Ca in insects and snails to support egg formation (Graveland 1996). Pollutants that alter the abundance of invertebrate prey therefore may alter Ca availability and egg production in songbirds such as pied flycatchers (Eeva and Lehikoinen 2004). In tree swallows, Ca availability affects the onset of laying, the size of eggs and the growth of nestlings (Bidwell and Dawson 2005; Dawson and Bidwell 2005). Severe depletion of Ca, P, Na and other minerals may cause pica (the consumption of items that are not usually considered food, such as wood, bone and hair) or soil (geophagia). These unusual materials may increase tooth wear, physical damage to the soft tissues of the mouth and digestive tract and increase the risk of infection (Underwood and Suttle 2001).

9.2.3 Magnesium and Sulfur

Magnesium and sulfur are widely distributed in animals because they form linkages with minerals and amino acids in bone, connective tissue and the enzyme complexes within cells. The largest amount of Mg in animals is associated with bone, where Mg^{2+} binds to hydroxyapatite. Mg^{2+} plays a wide variety of catalytic roles as a cation in the cells of both plants and animals. For example, Mg^{2+} ions stabilize the high-energy phosphate bonds in ATP and interact with enzymes to control the use of this energy store (Fig. 9.8) (Linder 1991). Plants use Mg as a central component of chlorophyll in their photosynthetic systems. Consequently, Mg concentrations are high in leaves and typically lower in plant storage organs such as tubers (Fig. 9.10). The absorption of Mg from plants may be reduced by binding to phosphates in phytic acid or by high concentrations of K (Figs 9.6, 9.8 and 9.10). Grass tetany is a condition found in high-producing livestock when high demands for lactation coincide with a switch to fertilized pastures where plants are high in K and N (Underwood and Suttle 2001). Low Mg availability apparently limits the supply of ATP for muscular contraction and animals quickly become immobilized, with rigid limbs, staggered gait, uncoordinated movements, convulsions and often death. This condition is uncommon among wild ruminants, even though lactating cervids often shift to highly digestible forbs or browse soon after birth. Mg tetany may be rare because those foods are high in Mg or because susceptible females would be quickly predated.

Animal tissue provides Mg in a readily available form for carnivores (Fig. 9.8). Excess Mg is excreted in urine with phosphate and other solutes. Urinary minerals can crystallize into 'stones' or uroliths that are large enough to obstruct the renal system, especially the narrow urethral duct of male mammals. Struvite is a urolith composed of Mg, ammonium and phosphate that is often found in domestic cats. The formation of struvite and other uroliths depends on the concentrations of the component ions, urinary pH and urinary flow rate. Urinary pH is influenced by the dietary load of ions and by the endogenous production of acid for digestion. After large meals, bicarbonate and ammonium ions titrate the acid in an alkaline tide in the urine. Water restriction or dehydration increases the concentration of solutes and reduces urinary flow, which favors the formation of uroliths. Captive cats and dogs may be predisposed to formation of uroliths because their low energy expenditures result in low water turnovers and urinary volumes, and because their food may be consumed in large, regular meals that result in concomitantly large fluctuations in urinary pH. The incidence of struvite uroliths in domestic cats has been reduced by formulating diets that are low in Mg and result in an acid urine (National Research Council 2006). Uroliths also may result from chronic metabolic conditions in wild animals. Uroliths of the sulfur amino acid cysteine (Fig. 8.1) are prevalent in both captive and wild maned wolves because cysteine is insufficiently resorbed at the kidney (Boveé et al. 1981). The genetic defect persists because uroliths mainly shorten the lives of adult males, but do not prevent the animals from reproducing. Captive breeding programs maintain maned wolves with diets that minimize excessive intakes of sulfur amino acids (Barboza et al. 1994; Childs-Sanford and Angel 2006).

Sulfur is found in all proteins, especially the keratins in skin, horns, claws, nails, hair and feathers. Animals therefore ingest most S as the amino acids cysteine and methionine in proteins from both plants and animals (Figs. 8.1 and 8.2). Microbes in the foregut of ruminants and kangaroos may use sulfate and non-protein N (e.g., urea) to synthesize S amino acids for microbial proteins that are subsequently absorbed by the animal (Chapter 8). Cysteine forms the sulfhydryl cross links (—S—S—) between proteins in hair, feathers and connective tissues whereas taurine is used as a charged sulphite group (—SO_3^-) in bile salts (Fig. 8.1). S compounds are used for numerous reactions of the vitamins biotin, thiamine and pantothenic acid, and in the antioxidant glutathione. Oxidation of S amino acids produces sulfate ions that contribute to the cation balance within cells. Sulfate excretion at the kidney contributes to decreases in urinary pH that counterbalance the alkaline tide following a large meal of protein.

Aberrations in S metabolism are primarily due to inadequate intakes of methionine and cysteine that can result in malformation of hair, feathers and connective tissue, especially in growing animals. Increases in requirements for S are usually accompanied by much greater increases in the requirements for N and energy to support tissue synthesis. For example, only 3% of the energy expended by molting birds is deposited in feathers (Lindström et al. 1993). Signs of limited S availability such as poor fur and feathers in wildlife therefore may arise from primary limitations on energy and protein, which are discussed further in Chapter 10.

9.2.4 Trace Metals

A prominent group of trace nutrients consists of trace metals such as Mn, Zn, Cu and Fe. Imbalances of Mn and Zn are not likely in free-ranging populations of wildlife, but cases of both Cu deficiency and Cu toxicity sometimes are reported. A deficiency of Cu results most commonly in wildlife that consumes low-Cu foods or when other trace minerals in excess inhibit Cu uptake from the gut. Cu toxicity may occur when animals have access to polluted water bodies and settling ponds. In the case of Fe, a deficiency usually occurs when animal health is already compromised, such as by blood-sucking ticks on moose, but Fe toxicity in wildlife is rare.

Ionic forms of metals such as Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} are highly reactive and are present in low concentrations both in extracellular fluids and within cells. These trace metals are usually bound to proteins that serve as transport molecules or enzymes. Trace metals are used to catalyze an enormous number of reactions that involve the loss (oxidation) or gain (reduction) of electrons (Table 9.3). Consequently, imbalances in trace metals result in numerous symptoms that range from impaired O_2 uptake for physical activity (Fe) to infertility (Zn) and increased susceptibility to infection (Cu). Reactions that transfer small functional groups include Mn-containing enzymes involved in the production of urea from arginine (arginase), and the coordinated oxidation of C from carbohydrate (pyruvate carboxylase) and lipid in the TCA cycle (Fig. 7.9) (Brody 1999). Interactions between trace metals and macromolecules include those between Zn and nucleotides that stabilize the DNA helix during transcription of RNA. Reversible electron-transfer reactions are used for gas exchange and respiration; the red heme complex at the center of the hemoglobin and myoglobin proteins contains Fe, which forms a readily reversible bond with O_2 and allows the gas to be transported in blood and stored in muscle (Fig. 9.11). A similar complex with Cu in hemocyanin carries O_2 in the blood of mollusks, albeit with a change in color to blue (Mathews and Van Holde 1996).

Protein complexes involving both Fe and S form parts of the electron transport chain for respiration in mitochondria and for photosynthesis in plant chloroplasts (Mathews and Van Holde 1996). Enzymes with trace metals catalyze reactions that defend against toxic products of respiration. The respiratory chain uses O_2 and H^+ to produce water (H_2O), but these reactions also can release toxic superoxide ions (O_2^-) that randomly destroy macromolecules such as lipids. Superoxide dismutase is a family of enzymes that contain one of the trace metals (Mn, Cu, Zn or Fe; Table 9.3) and convert superoxide to the less toxic hydrogen peroxide by the reaction $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. Peroxides are rapidly degraded to harmless water and oxygen by the enzyme catalase, which involves Fe in heme for the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$. Trace metals such as Cu are also involved in production of peroxides

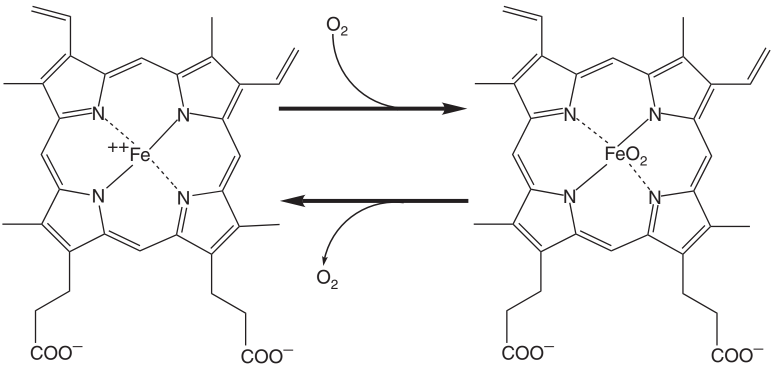

Fig. 9.11 Iron (Fe) readily interacts with oxygen (O_2) to form oxides that are too robust to release O_2 under cellular conditions. However, when combined with heme, an organic complex, Fe binds with O_2 in a controlled and reversible fashion that makes possible the transport of O_2 between lungs and tissues by hemoglobin, and storage of O_2 in muscle by myoglobin (Mathews and Van Holde 1996)

and superoxides for immune defense (Weiss and Spears 2006). White blood cells such as neutrophils, macrophages and lymphocytes use a rapid burst of O_2 consumption to produce toxic superoxides and hydrogen peroxide that destroy invading bacteria (Bonham et al. 2002).

Mucosal cells of the intestine absorb trace metals in the ionic state; Fe is also absorbed as heme but degraded within the mucosal cells or enterocytes (Brody 1999). Metal ions are transferred to intracellular proteins such as ferritin for Fe storage within the mucosal cells. Carrier proteins such as plasma albumen, ceruloplasmin (Cu) and transferrin (Fe) mediate the transfer of nutrients from mucosa to blood and target tissues such as liver, muscle or bone marrow. Blood samples are used to assess Fe status by measuring the total iron-binding capacity of plasma and serum ferritin. These parameters of Fe status have been used to assess the development of O_2 storage and diving ability in harbor seals, which fast on shore after they are weaned (Burns et al. 2004). Ceruloplasmin activities in blood samples are used to assess Cu status; activities of ceruloplasmin reflect concentrations of Cu in the liver of moose, muskoxen and caribou (Barboza and Blake 2001). Enzymes and proteins that indicate Cu, Zn and Fe status should be used to complement other measures because many of the enzymes are affected by development and stressors. For example, ceruloplasmin activities do not reflect liver Cu in muskox calves less than 90 days of age or liver Cu in male reindeer during rut (Barboza and Blake 2001; Rombach et al. 2002b). The liver mediates the uptake and storage of metals in proteins such as hemosiderin (Fe) and metalothionein (Cu and Zn). Concentrations of metals in the liver are therefore the most common method for assessing the status of Cu, Zn and other metals in fish and wildlife. Apparent digestibilities of trace metals are usually low, and some trace metals such as Mn can be used as a digestibility marker (Chapter 4). Low digestibilities of metals reflect both unavailable forms and fecal losses of absorbed metals in sloughed intestinal mucosal cells, and unresorbed secretions from the liver and pancreas.

Animals contain Fe, Cu and Zn in readily available forms that reach their highest concentrations in internal organs such as the liver, kidney and gonads (Fig. 9.12). Milk contains highly available forms of trace metals that are highest in the colostrum, which is produced soon after birth, but concentrations of these minerals are low for the rest of lactation (Underwood and Suttle 2001). Mothers transfer Fe, Cu and Zn to their young during fetal development in utero; suckling muskoxen and caribou rely on stores of Fe, Cu and Zn in the liver until they begin consuming forages (Rombach et al. 2002a; Knott et al. 2004). In marsupials, for which milk is used to support most of the development of the offspring in the pouch, milks are more concentrated in Fe, Cu and Zn than those of placental mammals (Green and Merchant 1988; Krockenberger 2006). Plants usually contain lower concentrations of trace metals than animals. High metal concentrations in plants are often associated with less available forms that are complexed with plant fiber or with PSMs such as phytate (Fig. 9.12). Steady consumption of soil may increase the uptake of trace metals. Drought is associated with increases in soil ingestion by hairy-nosed wombats, which leads to increases in liver Fe, Cu and Zn (Gaughwin et al. 1984). Lack of access to soils, as with Fe-deficient diets, by captive animals may result in a variety of deficiencies, including the Fe-deficient depigmentation of underfur known as cotton-fur that decreases the value of mink from fur farms (Robbins 1993).

Chronic exposure to trace metals can slowly increase their concentrations in liver, kidney and even hair as the metal ions bind to weakly negative groups such as the sulfhydryl group (—SH) on cysteine. Metalothionein is a S amino acid-rich storage protein which binds Cu, Zn and toxic heavy metals such as Cd (Bremner and Beattie 1990). High concentrations of Cd from invertebrate prey accumulate on metalothionein in the kidney of beluga whales (Dehn et al. 2006). Similarly, moose accumulate Cu in the liver from plants growing in acidic soils in Finland (Hyvarinen and Nygren 1993). Metals become toxic when large amounts of the ion are released; Cu is released to the blood when the storage capacity of the liver is exceeded, resulting in the oxidation and lysis of blood cells in sheep and hairy-nosed wombats on high-Cu diets (Barboza and Vanselow 1990; Underwood and Suttle 2001). Tolerances to metals vary with the ability to control absorption, safely store the metal in protein and excrete excesses. Trout can tolerate a diet of 665 ppm Cu whereas domestic sheep may exhibit signs of Cu toxicity on diets as low as 10 ppm Cu (Lall 2002; National Research Council 2005). Metal tolerances and requirements sometimes differ among closely related species and among subpopulations of the same species of domestic animals (Oldham 1999). Browsing rhinos such as the Sumatran rhino and black rhino are susceptible to Fe overloads and hemolytic crises in captivity, whereas captive African white rhinos and Indian rhinos are grazers that tolerate diets based on agricultural forages and products (Paglia et al. 2001).

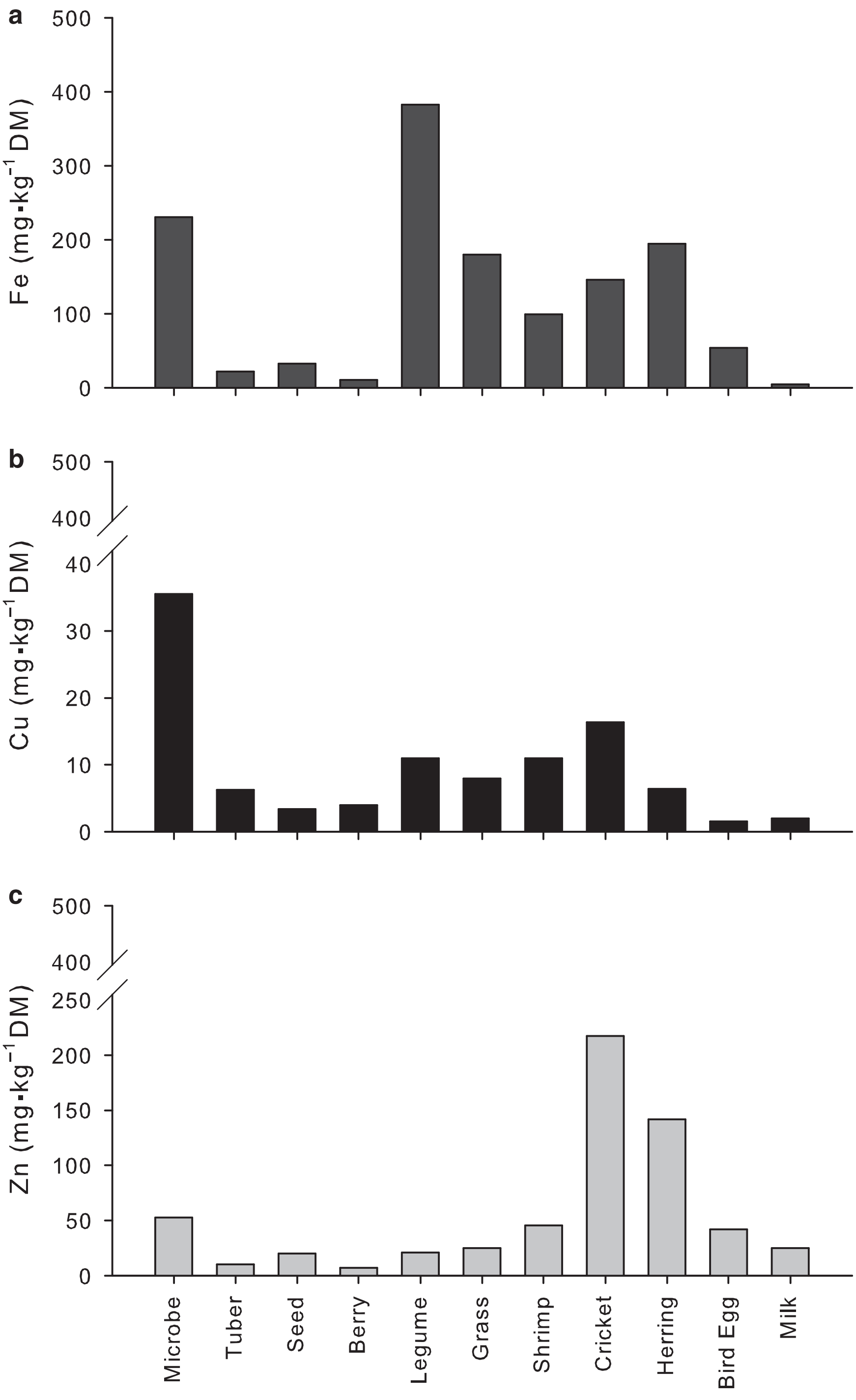

Fig. 9.12 Concentrations of three trace minerals in the dry tissues of selected microbes, plants and animals. **a** Iron (Fe). **b** Copper (Cu). **c** Zinc (Zn). See Fig. 9.6 for details of the selected microbes, plants and animals

Regional deficiencies of trace metals are probably more common than toxicities in populations of wild animals. Soils with low Cu availability for plants result in low Cu concentrations in the livers of Californian elk and Canadian muskoxen, and in the hair and hooves of Alaskan moose (Flynn et al. 1977; Gogan et al. 1989; Barboza et al. 2003). Alkaline soils or mine spoil areas with high concentrations of molybdenum (Mo) and sulfate reduce the availability of Cu by forming an insoluble copper thiomolybdate ($CuMoS_4$) in the forestomach of ruminants and kangaroos. Deficiencies are exemplified by defective keratinization of hooves in ungulates, which decreases mobility and increases the risk of predation. In ruminants such as moose, Cu deficiency can be induced on diets containing normal levels of Cu but relatively high levels of Mo and inorganic sulfate (Suttle 1991; Frank et al. 1994, 2000). Thiomolybdates are also associated with apparent Cu deficiency in the quokka, a small wallaby living on the limestone soils of Rottnest Island off the coast of Western Australia. Rottnest Island quokkas exhibit seasonal anemias whereas the same species living on better soils on the adjacent mainland show no signs of Cu deficiency (Barker 1961a,b; Hume 1999). Regional differences in Cu availability may become more apparent as populations grow. High densities of muskoxen increase the exposure of calves to intestinal infection, which increases their use of liver Cu and impairs their growth (Swor 2002). Intestinal infections debilitate and eventually kill calves by reducing food intake and increasing water loss through diarrhea. Trace metal imbalances therefore may intensify the adverse effects of disease and weather on young animals and thus reduce the rate of population growth (Barboza and Reynolds 2004) (Chapter 2).

9.2.5 *Iodine and Selenium*

The trace elements iodine and selenium serve critical roles in biochemical pathways and development. They also interact to affect the animal's metabolism and thermoregulation. In free-ranging wildlife, iodine toxicities are rare; deficiencies occur in iodine-deficient areas and in response to some plants and pesticides. Se toxicities result from the consumption of seleniferous plants and Se deficiencies are most common in regions leached by precipitation.

I is readily available as the iodide ion (I^-) in salts of Na or K, especially in the tissues of marine organisms such as seaweeds and sponges. The primary role of I in the body is in the function of the thyroid gland. Iodide is absorbed by the thyroid and attached to a derivative of the amino acid tyrosine to form the hormone thyroxine (T_4; Fig. 9.13). The thyroid gland stores T_4 as the protein thyroglobulin, which is hydrolyzed to form the hormone secreted into the blood. Removal of iodide from T_4 produces a more active form of the hormone, tri-iodo-thyronine (T_3). Both T_4 and T_3 are absorbed into target cells and bind to receptors in the nucleus and mitochondria.

Thyroid hormones stimulate transcription of nuclear genes, as well as protein synthesis and respiration in mitochondria (Norris 1997). Increasing concentrations

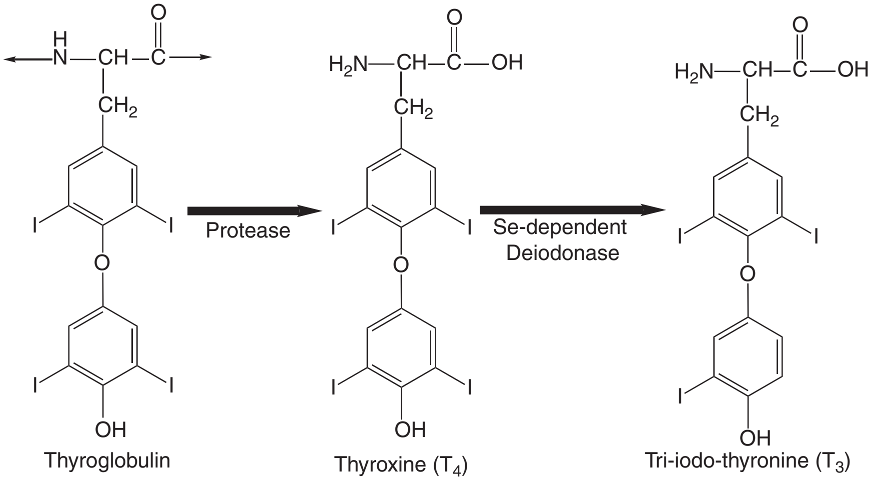

Fig. 9.13 Iodine (I) modifies the amino acid tyrosine to form a hormone (thyroxine; T_4) that regulates energy metabolism. The hormone is stored in the thyroid gland as a polymer (thyroglobulin) and released as T_4 when the peptide bonds are broken (Groff et al. 1995). Hormonal activity is further increased by removing one I to convert T_4 to tri-iodo-thyronine (T_3) with an enzyme that uses Se. Inadequate Se intakes therefore cause secondary symptoms of I deficiency

of plasma T_4 and T_3 therefore are associated with increases in resting energy expenditure and the production of metabolic heat for thermoregulation (Chapter 10). High concentrations of thyroid hormones in newborn muskoxen and caribou reflect high rates of heat production used to combat low ambient temperatures of late winter and early spring in the Arctic (Knott et al. 2005). Plasma T_4 increases with seasonal changes in daily food intake and mass gain of red deer and reindeer during summer (Ryg and Langvatn 1982; Ryg and Jacobsen 1983; Barboza et al. 2004) and with the increased activity of migration in Pacific salmon (Norris 1997). Thyroid hormones activate genes that cause the asymmetry of the eyes in flounder and the resorption of fins and tails in frogs during metamorphosis (Norris 1997). Similarly, T4 is also associated with muscular development in birds such as barnacle geese (Deaton et al. 1997). The suppression of thyroid hormones in I deficiency produces many symptoms that include impaired development of the brain (e.g., cretinism in humans), skin anomalies and suppressed rates of metabolism that vary with the role of the T_4 and T_3 in each species and life stage.

Thyroid secretions are stimulated by hormones released from the central nervous system at the hypothalamus and the pituitary gland; these hormones integrate thyroid function with seasonal changes in growth and reproduction. Plasma concentrations of T_3 and T_4 are influenced by the sensitivity of receptors and the clearance rate of the hormones from the blood, both of which may change with season (Tomasi and Mitchell 1994). Increasing concentrations of thyroid hormones in blood suppress the secretion of the thyroid gland by negative feedback. Iodine deficiency does not allow enough thyroid hormone to be released for negative feedback. Consequently, the thyroid continues to produce protein and enlarges into a tumor or goiter. Goiters also are produced by PSMs called goitrogens. Plants in the Family Brassicacea, which include turnips and cabbages, contain a PSM called progoitrin that is converted to goitrin when the plant is damaged; goitrin suppresses iodination and thus production of T_4 by the animal (Norris 1997). Goiters can be caused by cyanogenic glycosides from a variety of plants, including clover and cassava; the release of thiocyanate (CNS^-) ions from the hydrolysis of the PSM blocks iodide absorption by the thyroid (Chapter 3) (Harborne 1993). The pesticide DDT causes enlargement of thyroid glands in ring doves, which is associated with thinning of egg shells and low rates of survival in chicks. The widespread effects of DDT on egg shell thickness in peregrine falcons and other birds may be due to both a direct effect on Ca deposition in the oviduct and a secondary effect of thyroid hormones on this energetically demanding process (Moriarty 1999).

Deficiencies of Se impair thyroid function because the conversion of T_4 to T_3 requires a Se-dependent iodinase (Fig. 9.13). Se deficiency therefore is accompanied by some symptoms of I deficiency such as low T_3 and impaired thermogenesis in newborn ruminants exposed to the cold (Underwood and Suttle 2001). Se is incorporated into enzymes and other proteins as the modified S amino acids Se-methionine and Se-cysteine (Table 9.3). These amino acids are produced by substitution of S with Se immediately before translation of mRNA to protein (Brody 1999). Animals absorb the Se-amino acids with the same transporters for methionine and cysteine in the small intestine (Chapter 8). Inorganic Se is passively absorbed as selenite (SeO_3^{2-}) and actively absorbed as selenate (SeO_4^{2-}) in a carrier shared with sulfate. Plants that accumulate Se can discriminate between sulfate and selenate and can store Se-methionine and Se-cysteine in vacuoles, where the modified amino acids cannot interfere with protein synthesis (Harborne 1993). In animals, Se toxicity is associated with sloughing of hooves and nails and with rough hair coats because sulfhydryl cross links (—S—S—) between keratin proteins are lost when cysteine is replaced with Se-cysteine. In ungulates, the chronic form of Se toxicity is known as alkali disease; acute Se toxicity leading to respiratory failure is called blind staggers. Seabirds may be exposed to high levels of Se from sediments and invertebrate prey. Emperor geese, common eiders and spectacled eiders that winter in the seas off Alaska have high levels of blood Se when they arrive at the spring breeding grounds but those levels decline through the summer. Eiders and emperor geese may be more tolerant of Se than other waterfowl because blood Se concentrations of these waterfowl were 10 times greater than those of freshwater birds such as mallards at the start of spring (Franson et al. 2002; Grand et al. 2002).

The cells of the liver, heart and blood are rich in Se and its associated proteins. Liver Se and the activity of the Se-dependent enzyme glutathione peroxidase are used to assess Se status (Underwood and Suttle 2001). Glutathione is a derivative of cysteine that serves as a substrate for detoxification of peroxides of hydrogen (H_2O_2) and lipids (—ROOH) in the cytosol and mitochondria of cells throughout the body (Fig. 9.14). Peroxides are highly reactive byproducts of O_2 utilization that can damage proteins and membranes; oxidative defenses such as glutathione peroxidase are therefore crucial to maintaining tissues with intense O_2 consumptions such as exercising muscles, liver and kidney (Hulbert et al. 2007). Glutathione and glutathione peroxidase may serve as stores of S and Se respectively during feather synthesis in birds such as white-crowned sparrows when demands for S-amino acids and energy for protein synthesis are high (Murphy and King 1990; Underwood and Suttle 2001). Se deficiency is associated with fragile red cells and muscle damage or myopathy in ruminants and birds, especially after the intense exercise of capture and handling (Spraker 1993). White-muscle disease, characterized by dystrophy and white streaks in muscle tissue, results from low levels of Se and vitamin E, which are augmented by capture stress, in species such as mountain goats.

Demands for Se are affected by complementary systems of oxidative defense such as superoxide dismutase and vitamin E; inadequate supplies of trace metals and vitamin E may intensify the role of Se in oxidative defense and constrain the supply of Se for thyroid function. Signs of Se deficiency therefore may vary with environmental conditions and with the supply of trace metals and vitamin E (Underwood and Suttle 2001). Populations of wild animals often can tolerate chronically low supplies of Se because other nutrients ameliorate the need for Se and because stores of Se in the liver can satisfy the low demands for Se in adults. The constraints of low intakes of Se and other trace minerals become apparent when high demands for growth and reproduction coincide with high environmental demands. These problems are more readily discerned in livestock that are confined to small managed areas than in wild animals with large home ranges. However, the subtle effects of trace nutrients can be revealed by experimental approaches such as supplementation. For example, Se supplementation of wild mule deer increased the survival of fawns even though mothers did not exhibit any symptoms of Se deficiency (Flueck 1994). Trace mineral status can contribute to high mortality and low recruitment in a population when home range and age structure are altered by changes in the environment (Chapter 2).

9.3 Vitamins

Vitamins are comprised of structures that cannot be synthesized by animals. Many vitamins contain N, which led to the original name of ‘vital amines’ for this group of essential nutrients (Linder 1991). Some vitamins also contain essential elements such as cobalt (Co) in cyanocobalamin (vitamin B_{12}) and S in biotin (vitamin B_8) and thiamine (vitamin B_1; Table 9.4). The structures of vitamins include C rings and C chains. Rings are used to carry electrons on niacin and riboflavin (vitamin B_2) for respiration, and to absorb light on retinal for vision (vitamin A; Table 9.5). Chains are used to incorporate tocopherol (vitamin E) into membranes and to transfer functional groups between larger molecules in the TCA cycle with thiamine, transaminate amino acids with pyridoxine (vitamin B_6), and synthesize amino acids and bases with folic acid (vitamin B_9).

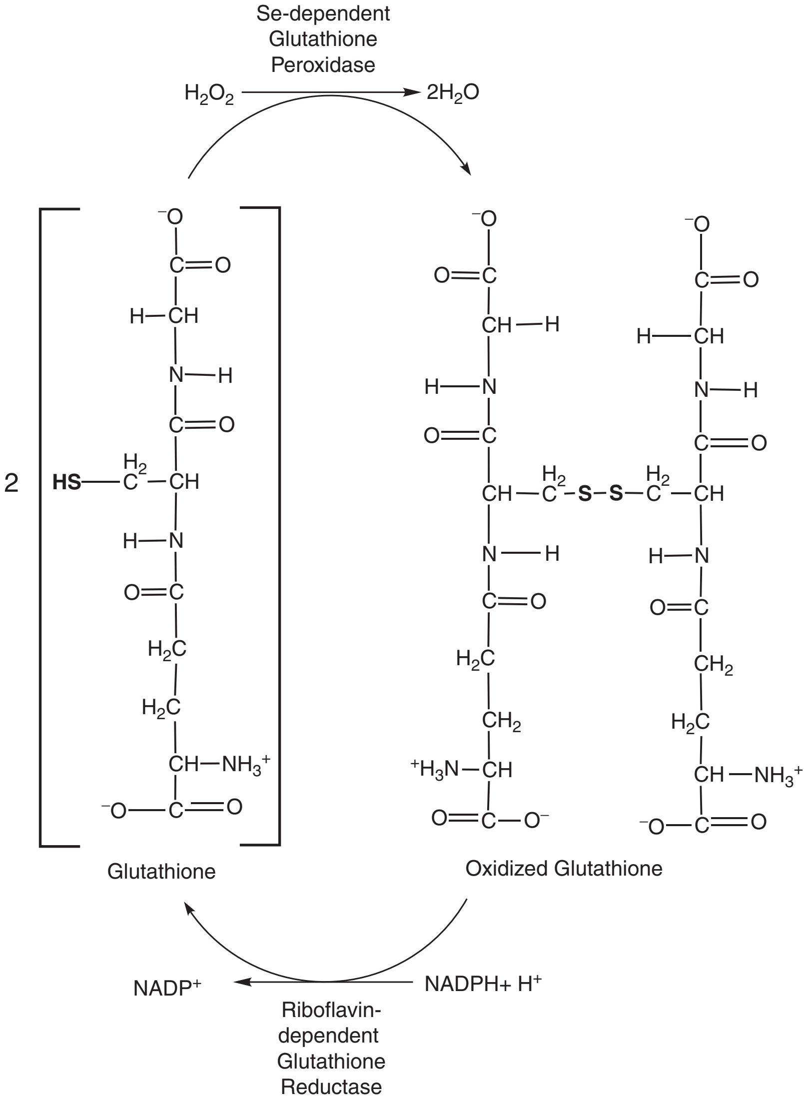

Fig. 9.14 The amino acids cysteine, glycine and glutamate are combined in glutathione, which serves as a substrate for reactions with peroxides of hydrogen (H_2O_2) and lipids (—ROOH) in the cytsol and mitochondrial matrix of cells (Underwood and Suttle 2001). Glutathione peroxidase is a Se-dependent enzyme that destroys peroxides of hydrogen and free fatty acids. Glutathione reductase is an enzyme that depends on riboflavin to recycle glutathione by using electrons from NADPH (Brody 1999)

Vitamin deficiencies are difficult to identify in populations of wild animals because symptoms may be subtle and require repeated testing that is often only feasible in captivity. Deficiencies are also slow to develop because these metabolites are conserved and recycled within the body. Seasonal or short-term deficits

Table 9.4 Common forms and functions of selected water-soluble vitamins in animals

Name	Chemical structures	Biologically active forms	Chemical function	Body function
Vitamin C	Sugar	Ascorbic acid	e^- transfer, reduction	Collagen synthesis, oxidative defense
Thiamine, Vitamin B_1	Pyrimidine and side chain with S and N	Thiamine-pyrophosphate	C oxidation	TCA cycle
Riboflavin, vitamin B_2	Triple C ring containing N	Flavin nucleotides (e.g., FMN, FAD)	e^- transfer	Energy metabolism
Niacin, nicotinic acid	Single ring containing N	Nicotinamide adenine dinucleotide (e.g. NADH)	e^- transfer	Energy metabolism
Pantothenic acid, vitamin B_5	C chain with N	Coenzyme A (e.g., acetyl-CoA)	C transfer	TCA cycle
Pyridoxine, vitamin B_6	Single C ring containing N	Pyridoxal-phosphate	C transfer	Transamination of amino acids
Biotin, vitamin B_8	Double C ring containing S and N	Biotin-AMP	C transfer	TCA cycle
Folic acid, vitamin B_9	Two C ring complexes containing N with C side-chain	Tetrahydrofolates	C transfer	Synthesis of amino acids and bases
Cyanocobalamin, vitamin B_{12}	Multi-ring complex with Co at center	Cobalamines	C transfer	Synthesis of amino acids, bases and SCFA

Table 9.5 Common forms and functions of fat-soluble vitamins in animals

Name	Chemical structures	Biologically active forms	Chemical function	Body function
Vitamin A	Single C ring and chain	Retinoids	Light transduction	Vision
			Signal transduction	Cell division
Vitamin D	Sterols	Ergocalciferol (D_2 from plants), cholecalciferol (D_3 from animals)	Signal transduction	Ca absorption
Vitamin E	Double C ring and chain	Tocopherols	e^- transfer	Membrane integrity
Vitamin K	Double C ring and chain	Phylloquinone (K_1 from plants), menaquinone (K_2 from bacteria)	C transfer	Activation of blood clotting enzymes

in vitamin intake are also offset by stores in tissues such as the liver. Consequently, symptoms of vitamin deficiency may only appear briefly in a portion of the population. For example, folic acid deficiencies produce specific anemias that are associated with impaired development, which is most likely to appear among neonatal animals when the demands for cell synthesis are highest. Anomalies of vitamin nutrition are most frequently reported in animals that are restricted to a small area with a relatively small number of dietary choices for long periods of time, such as populations that are isolated on islands by water, or in fenced reserves. Animals are less vulnerable to these problems if they are able to consume a diversity of foods that compensate for the low concentration or availability of vitamins among food items.

Vitamins are categorized on the basis of their extraction from tissues in animals and plants. Water-soluble vitamins include the B vitamins and ascorbic acid (vitamin C) that are mainly associated with the aqueous contents of cells (Table 9.4). The fat-soluble vitamins A, D, E and K are found in lipids and membranes (Table 9.5). Fat-soluble vitamins can be absorbed and transported with dietary lipids, whereas water-soluble vitamins are usually absorbed by specific carriers at the intestinal mucosa. Water-soluble vitamins are deposited in cells throughout the body, whereas fat-soluble vitamins can be accumulated in body lipids and may lead to toxic effects. Fat-soluble vitamins often are excreted in bile from the liver, whereas water-soluble vitamins are usually excreted in urine from the kidney. High doses of B vitamins in captive animals are readily discerned by urinary excretion of fluorescent cyclic compounds. The specific analysis of vitamins and their associated metabolites is often complex. Samples are usually frozen immediately in liquid N_2 or dry ice (solid CO_2), stored in ultra-low freezers (−80°C), and shielded from light in opaque containers to minimize degradation. Extractions of samples may involve several steps to remove extraneous compounds (e.g., cell proteins) before the specific metabolites can be isolated and quantified by one of the many chromatographic methods available. The activity of vitamins in foods is measured with bioassays on cell cultures or laboratory animal models (e.g., chickens, mice or rats). As with mineral analyses, the quality of vitamin analyses should be monitored closely by including certified standards and controls in each batch of samples and by comparing results between laboratories.

9.3.1 Water-Soluble Vitamins

9.3.1.1 B Vitamins

Vitamins in the B group are involved in several pathways of intermediary metabolism (Table 9.4) that interact with the functions of other trace nutrients. For example, riboflavin is required for maintaining glutathione reductase, the enzyme that recycles glutathione for oxidative defense with Se-dependent peroxidase (Fig. 9.14) (Brody 1999). Inadequate supplies of B vitamins can therefore impair the production and maintenance of many tissues and are most evident as anomalies of the skin, muscle and blood cells. Symptoms vary between species and life stages with the relative demands for each vitamin.

Plant storage organs such as tubers and the endosperm of seeds are poor sources of B vitamins and trace minerals, but the synthetic portions of the plant such as the photosynthetic leaves and the germ of seeds are rich sources of these trace nutrients. Many B vitamins are synthesized by microbes. Microbial fermentation provides most of the requirements of foregut fermenters (e.g., ruminants and kangaroos) for these vitamins. The cecotrophs ingested by rabbits, rats and ringtail possums are important sources of these microbial vitamins for these small hindgut fermenters (Stevens and Hume 1995). The presence of mucosal transporters in the colon indicates that some microbial vitamins also may be absorbed directly from the hindgut (Said 2004). Prolonged antibiotic treatments of captive animals can reduce the supply of these endogenous sources of vitamins by altering the microbial fermentation. Muscle and organs of animal prey are rich sources of vitamins for carnivores. However, plant and animal tissues begin to oxidize B vitamins soon after injury. These vitamins continue to be oxidized with exposure to air, light and increasing temperature. The loss of vitamins in stored food is therefore a major concern for the nutrition of captive wildlife in zoos and aquaria.

Some foods contain compounds that destroy vitamins or impair their absorption. Thiamin is degraded by enzymes called thiaminases in fish and some plants (e.g., horsetail, nardoo and bracken fern). Similarly, much of the biotin in eggs is bound to the protein avidin (Brody 1999). Conversely, vitamins such as niacin are partly produced from other dietary components. Niacin can be synthesized from tryptophan in a series of reactions that require mineral ions (Cu^{2+}, Fe^{2+}, Mg^{2+}) and another B vitamin (pyridoxine); this source of niacin may be only important when intakes of protein are high, because only 3% of the tryptophan from dietary protein is converted to niacin by this pathway (van Eys 1991). Obligate carnivores such as cats receive both tryptophan as well as niacin from NAD and NADP in the muscles of their prey (Morris 2002). Dietary changes also may affect requirements for B vitamins; requirements for pyridoxine may increase with protein intake and carnivory because the demands for transamination increase as amino-acid C is used for energy (Leklem 1991). Similarly, changes in the fermentation of dietary carbohydrate may increase the demand for vitamin B_{12} when propionate production is increased; high starch diets and low B_{12} have been associated with chronic wasting and inflammatory bowel disease in captive moose (Shochat et al. 1997). Supplements of cobalt, which is essential to the formation of B_{12}, and increased offerings of dietary browse are used to partially remedy these problems.

9.3.1.2 Vitamin C

Unlike the other water-soluble vitamins, vitamin C (ascorbic acid) is a carbohydrate and does not contain N (Table 9.4; Fig. 9.15). Ascorbic acid can be synthesized from glucose in a pathway that includes the enzyme glucono-lactone oxidase (GLO). Sturgeons express the gene for GLO, but most other fish do not

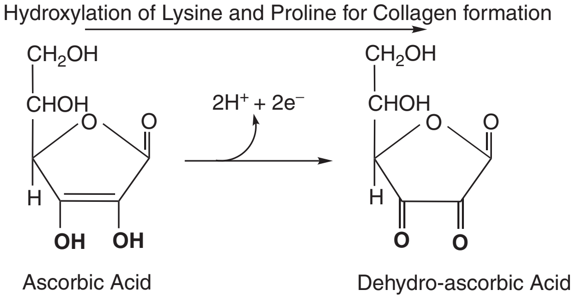

Fig. 9.15 Ascorbic acid (vitamin C) donates electrons for modifying amino-acid side chains that are involved in the cross-linking of collagen in connective tissues (Linder 1991)

express the enzyme and therefore require dietary sources of vitamin C (Halver 2002). The expression of GLO follows a phylogenetic pattern that progresses from kidney to liver. In amphibians, reptiles and monotremes (echidna, platypus), the enzyme is only expressed in the kidney. GLO is expressed in both the kidney and liver in marsupial bandicoots, but only in the liver of other marsupials and placental mammals (Hume 1999). A similar progression of GLO expression from the kidney to the liver is also reported in passerine birds. Ascorbic acid is required by some songbirds, some rodents (e.g., guinea pigs) and by most primates and bats. Fruits and flowers provide the highest concentrations of ascorbic acid for herbivores, whereas organs such as the liver may provide a rich source of the vitamin for carnivores.

Vitamin C deficiency causes weak connective tissues because ascorbic acid is used to cross-link collagen. Vitamin C deficiency causes skeletal abnormalities in growing fish (Halver 2002) and weak membranes in the mouth and blood vessels of other vertebrates. Symptoms of vitamin C deficiency include poor wound healing, increased rates of oral infection and tooth loss, hemorrhages and bruising. Vitamin C deficiency in humans is called ‘scurvy’, a condition frequently incurred by explorers when supplies of citrus fruits or preserved vegetables were unavailable. Requirements for ascorbic acid may increase with infection, energy use and exposure to toxins, as the vitamin is part of the oxidative defenses of the body (Moser and Bendich 1991; Halver 2002). Ascorbic acid may be used to protect the nervous system of hibernators from oxidative damage during the intense metabolic activity of arousal from torpor. In Arctic ground squirrels, the concentration of ascorbic acid in the blood plasma increases during torpor when body temperatures are low, but declines quickly as the animal increases O_2 consumption during rewarming (Drew et al. 1999). Glutathione and its Se-dependent peroxidase also are used to protect the fragile mucosa of the intestine from oxidative damage during rewarming of thirteen-lined ground squirrels (Carey 2005).

9.3.2 Fat-Soluble Vitamins

9.3.2.1 Vitamin A

Compounds with vitamin A activity are involved with vision, bone remodeling of young, reproductive output of adults, and epithelial tissue and coloration. Both deficiencies and toxicities of vitamin A have been reported in wildlife. Lipid-soluble retinoids of plant origin are the source of vitamin A for animals (Table 9.4; Fig. 9.16). Retinoids are transported with lipoproteins to lipid stores in the liver and adipose tissue. The liver exports retinol on retinol-binding protein, which is recognized by the light-sensitive cells on the retina at the back of the eye. The retinal cells convert retinol (an alcohol) to retinal (an aldehyde), which is bound to the protein opsin to form rhodopsin. Rhodopsin is degraded by light in a reaction that releases retinol, but also transfers the energy to a Na pump in the cell membrane, allowing the transmission of a nerve impulse that is perceived as vision (Linder 1991). Small amounts of retinol are lost from this system, but most of the retinol is recycled to retinal and returned to

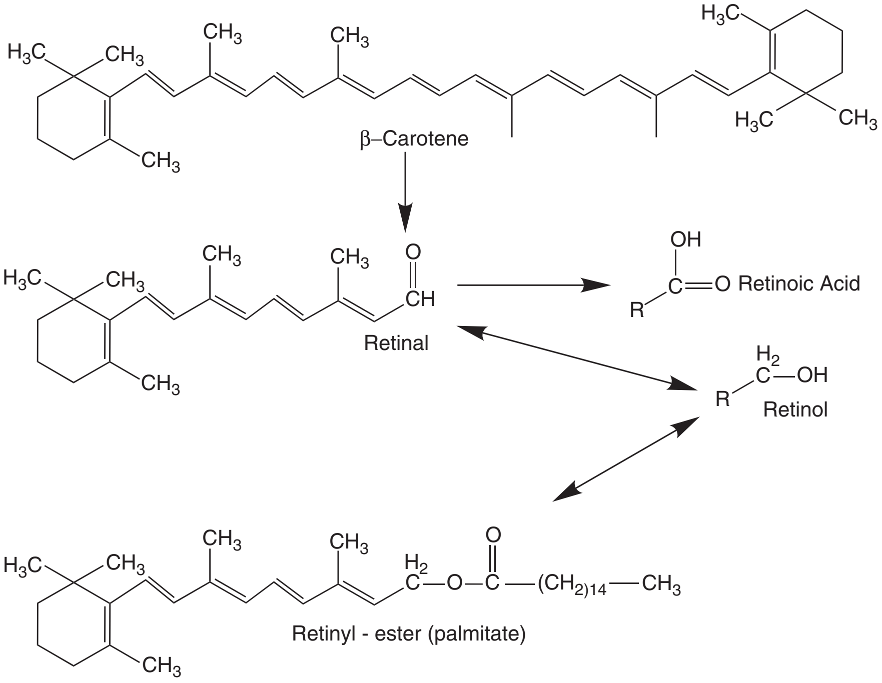

Fig. 9.16 Carotenoids in plants are converted to retinoids that have vitamin A activity in animals (Groff et al. 1995). Reversible reactions convert retinal to retinol and then to retinyl-esters for storage and mobilization from liver and body fat. Retinal is used at the eye for vision when it is bound to the protein opsin to form the light transducer rhodopsin. Retinal is also converted to retinoic acid, which serves as a hormone for cell differentiation

rhodopsin. Although the supply of retinol is maintained by small stores of retinyl-ester in the retina, depletion of that store in vitamin A deficiency impairs vision, especially in low light. Night-blindness is the earliest sign of vitamin A deficiency in humans, but this sign may only be apparent as subtle changes in behavior of animals such as domestic pigs and horses (Olson 1991).

Retinol is also absorbed at other tissues and converted to retinoic acid, which is used to control differentiation and division of cells (Fig. 9.16). Vitamin A deficiency affects cells in the outer layer (cornea) of the eye and the associated mucus glands; dry eyes accumulate keratin which eventually causes inflammation of the eye and blindness. Insufficient retinoic acid impairs growth and reduces reproductive output of adults by impairing development of both sperm and the fetus (Olson 1991). Cell division is also impaired by vitamin A toxicity; cartilage and bone overgrow and fuse in fish and mammals (Halver 2002). Carnivorous mammals tolerate higher concentrations of retinyl-esters in their blood than omnivorous and herbivorous mammals (Schweigert et al. 1990) and safely accumulate these retinyl-esters in specialized cells within the liver (Leighton et al. 1988). Toxicities of vitamin A may occur in people consuming liver from marine carnivores such as cod, whales, seals and polar bears. High concentrations of vitamin A in the liver of whales and polar bears are an example of biomagnification, that is, the accumulation of a compound in each successive level of the food chain. The concentration of vitamin A or any other potential toxin (e.g., organochlorines, heavy metals) in an organ depends on the rates of uptake and elimination by each prey and predator in the trophic chain, and by the population dynamics of each species (Moriarty 1999). Long-lived apex predators such as whales that consume large quantities of prey with short lives are more likely to accumulate lipid-soluble compounds such as vitamin A because they undergo several years of lipid storage and depletion during their lifetime.

Herbivores derive most vitamin A from carotenoids in the diet (Fig. 9.16). Carotenoids are converted to retinal by carotenoid-dehydrogenases in the intestinal mucosa and to a lesser extent in the liver. This conversion varies widely with the structure of the carotenoid, the lipid content of the diet and the digestive function of the species. Carotenoids are converted to retinal in domestic dogs but not in domestic cats, which require retinoids in the diet (Morris 2002). Obligate carnivores therefore rely on prey for vitamin A as well as amino acids (Chapter 8). Vitamin A activity of carotenoids is expressed as the equivalent activity of the same mass of retinol. In humans and domestic herbivores, β carotene has a retinol equivalent of 1/6 (167 μg retinol = 1,000 μg β carotene), whereas mixed carotenoids are equal to only 1/12 the same mass of retinol (Olson 1991; National Research Council 2007a). These conversions vary widely across the 50 carotenoids known to provide vitamin A activity; consequently the carotenoid content of the diet may be poorly related to the vitamin A activity derived by the animal.

Carotenoids also provide the functions of light absorption and oxidative defense in organisms. The large planar structure of carotenoids (Fig. 9.16) reinforces membranes in bacteria and fungi. In plants the linear structure and cyclic ends of carotenoids trap light on membranes during photosynthesis. These same attributes produce the colors of fruits and flowers that attract animals, in which the same compounds produce colors in skins and feathers. Vertebrate animals cannot synthesize carotenoids de novo, but they can modify carotenoids in the liver, kidney and at the skin to change the colors they produce. Enzymes for modification may be expressed differently as plumage coloration changes from chicks to adults and between adult males and females. Female scarlet tanagers use dietary carotenoids with little modification to produce yellow plumage, whereas males modify the same carotenoids extensively to produce red plumage (McGraw 2006a).

Animals require a dietary source of carotenoids to produce colors that range from red to yellow, depending on the mixture of carotenoids. Common dietary carotenoids are α and β carotene, lutein, zeaxanthin and canthaxanthin (McGraw 2006a) (Fig. 9.17a). The orange feet of mallards and greylag geese are a combination of β carotene, lutein, zeaxanthin, astaxanythin and canthaxanthin. Flamingos, canaries, goldfish and rainbow trout may lose their colors in captivity unless the diet includes appropriate carotenoids (Choubert 2001; Hill 2006) because carotenoid coloration depends on a continuous supply of carotenoids in the blood serum to the skin during seasonal production of new skin, scales or feathers. Dietary carotenoids therefore may influence the intensity of the red–yellow colorations of plumage on some birds such as American goldfinches (McGraw 2006a). Consequently, some of the variation in carotenoid coloration of wild songbirds and fish is associated with the foods selected in their foraging area or territory (Hill 2006). Carotenoids in tissues and serum reflect diet, but they also reflect selective uptake and retention of compounds as well as their conversion (McGraw 2006a). Carotenoids could serve as dietary markers for some animals, as indigestible markers similar to the alkanes

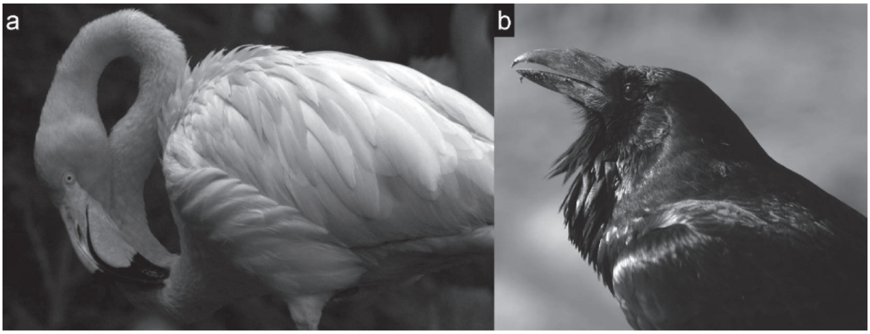

Fig. 9.17 Carotenoids and melanins are the two most common color compounds in birds. Carotenoid coloration is directly influenced by diet; dietary carotenoids are incorporated into the lipid of fat depots, skin and feathers. Carotenoids produce red, orange and yellow hues in feathers. **a** The pink pigmentation of flamingos is derived from the carotenoids in invertebrates that obtained carotenoids from algae. Feather coloration signals mate quality in greater flamingos and many other birds. **b** Eumelanins produce black and blue colors in the feathers of common ravens. Phaeomelanins produce brown and rust colors in the feathers of ptarmigan and mallards. Melanins are produced endogenously from the amino acids tyrosine and cysteine

in plant cuticle, or as assimilable markers such as the essential fatty acids in prey items (Chapter 4).

Carotenoids may indicate the condition of an animal when these compounds are used for oxidation reactions in immune responses. Infections affect the concentrations of carotenoids in liver and bursal (immunogenic) tissue of growing chickens, and supplementation with carotenoids enhances the immune response of zebra finches (McGraw 2006a). Carotenoid coloration of feathers declines with the intensity of bacterial, protozoal and ectoparasitic infections in several songbirds (Hill 2006). These colors therefore may indicate the quality of mates for some birds because they integrate characteristics of foraging ability as well as disease resistance.

Not all colors are based on carotenoids; melanins are also an important group of pigments in animals. Eumelanins produce black and blue colors, whereas phaeomelanins produce brown to rust colors. Melanins combine with structural proteins such as keratin and collagen in feathers and skin. Ultrastructural properties of pigmented feathers produce the iridescent feathers on the heads of peacocks and male mallards (McGraw 2006b). Melanins are synthesized de novo from the amino acids tyrosine and cysteine that are derived from the essential amino acids phenylalanine and methionine (Fig. 9.17b, Chapter 8). Melanophores synthesize melanin in the skin and control its deposition in feathers; melanophores can produce bands of pigment in mottled feathers that provide camouflage for ground birds and waterfowl. Although the amino acids in melanin are conditionally essential, diet does not affect melanization of feathers until dietary protein is so severely restricted that it limits feather synthesis (Hill 2006). In cats, the enzymes that synthesize eumelanin have a greater affinity for tyrosine than those that route tyrosine to other lean tissue; imbalanced diets that restrict the supply of tyrosine and phenylalanine therefore may affect coat color in cats (Morris 2002). The structural colors of melanized feathers are decreased by intestinal infections with coccidia in turkeys during molt (Hill et al. 2005). Coat color of mammals and plumage quality of birds may be adversely affected by diet and disease that limit supplies of energy, amino acids and carotenoids to the skin during molt.

9.3.2.2 Vitamin D

The principal function of vitamin D is the homeostatic control of Ca and P (Table 9.5); imbalances in vitamin D result in deficiencies or toxicities in wildlife. Light also plays a role in the metabolism of vitamin D; ultraviolet (UV) wavelengths (290—315 nm) provide the energy to break specific bonds in sterols produced by both plants and animals (Fig. 9.18). Vitamin D from animals is called cholecalciferol (D_3) whereas ergocalciferol (D_2) is produced in terrestrial plants and phytoplankton (Table 9.5). Light exposure has the greatest effect on concentrations of D_2 when plants senesce because less energy is captured by other light-sensitive compounds such as carotenoids (Collins and Norman 1991). Skin pigments (e.g., melanin), feathers and fur all reduce the UV light that is available for synthesis of D_3.

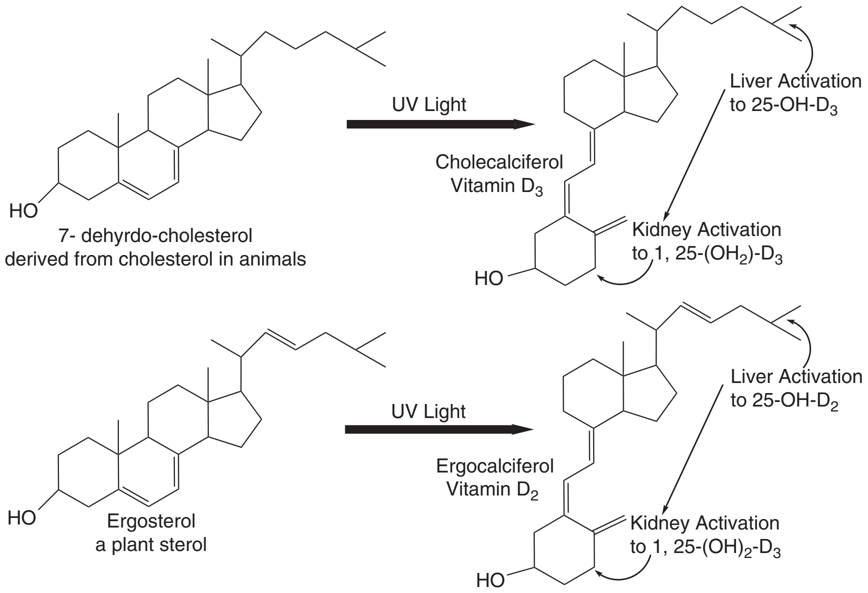

Fig. 9.18 The sterols of plants and animals can be converted to compounds with vitamin D activity in reactions that use ultraviolet (UV) radiation from sunlight at the surface of the leaf or the skin (Linder 1991; Groff et al. 1995). Hydroxyls are added at the *curved arrows* to activate vitamin D to 25-OH-D, which circulates in the blood, and to 1,25-$(OH)_2$-D, which stimulates Ca uptake from the intestine and release of Ca from bone

Full sunlight for only a few minutes each day, however, is often sufficient to produce the daily requirement of D_3 from a small portion of the skin surface at the face, legs and forelimbs of many animals, including humans. Consequently, dietary sources of vitamin D are not essential for ruminants and horses that are active in full sunlight during the day (National Research Council 2007a,b).

The light reaction for synthesis of D_3 can vary in its sensitivity to UV among animals with different diurnal behaviors; a comparison of sympatric species of lizards from the genus *Anolis* indicates that a shade-loving species produces more D_3 than a basking species when both animals are given the same UV exposure (Ferguson et al. 2005). Burrowing animals with crepuscular or nocturnal patterns of activity may still be able to use brief periods of UV exposure to produce D_3 if they bask at the entrances of their burrows to thermoregulate. Production of D_3 at the skin is also dependent upon the supply of the steroid precursor. Basking cats do not produce D_3 because the precursor 7-dehydro-cholesterol is catabolized before it can be used for D_3 synthesis (Morris 1999). Similarly, UV exposure is not a significant source of vitamin D_3 synthesis in fish because water blocks the radiation and because the concentration of the precursor is low (Holick 1989). Transfer of D_3 from skin to blood is regulated by vitamin D binding protein (DBP) from the liver; D_3 is catabolized by further UV exposure at the skin if the vitamin is not absorbed into the blood. Consequently, UV exposure cannot cause toxicity of vitamin D because the accumulated vitamin is degraded; UV protection may have been the principal role of these steroids in the evolution of plants and animals (Holick 1989).

Dietary vitamin D is absorbed at the small intestine and transported with either lipoproteins or DBP. The lipoproteins deliver vitamin D to stores in fat depots and DBP delivers the vitamin to the liver where it is converted to 25-OH-D_3 and 25-OH-D_2 and returned to the blood with DBP (Fig. 9.18). Serum 25-OH-D therefore is used as an indicator of vitamin D stores (Collins and Norman 1991; Klasing 1998). Vitamin D is activated further to 1,25-$(OH)_2$-D_3 and 1,25-$(OH)_2$-D_2 by the cells of the proximal tubules in the kidney (Collins and Norman 1991). More 1,25-$(OH)_2$-D_3 enters the circulation than 1,25-$(OH)_2$-D_2 in fish, cats, dogs, primates and birds because D_2 may be less efficiently absorbed and activated than D_3, and excreted more rapidly than D_3 when activated to 1,25-$(OH)_2$-D (Klasing 1998; Halver 2002; National Research Council 2003, 2006). Consequently, vitamin D_2 from plants may provide less than 10% of the activity of the same mass of vitamin D_3 (Klasing 1998).

Production of 1,25-$(OH)_2$-D is controlled by PTH when serum Ca is low. Absorption of Ca from the diet is increased by the action of 1,25-$(OH)_2$-D on mucosal cells in the small intestine, which increase production of Ca transporters. Blood Ca is elevated by the complementary effects of 1,25-$(OH)_2$-D and PTH on bone and kidney; bone releases both Ca and phosphate while the kidney retains Ca and excretes phosphate to increase blood Ca (Groff et al. 1995). In birds, growth hormone also increases production of 1,25-$(OH)_2$-D when blood P is low. Blood phosphate is elevated by the complementary effects of 1,25-$(OH)_2$-D and growth hormone on bone and kidney; bone releases both Ca and P, while the kidney excretes Ca and retains phosphate to elevate blood phosphate (Klasing 1998). Serum concentrations of 1,25-$(OH)_2$-D increase during mineralization of antlers, eggs and the growing skeleton (Eiben et al. 1984; Klasing 1998). Serum concentrations of 1,25-$(OH)_2$-D probably depend on the kinetics of clearing the hormone as well as the sensitivity of target tissues to the hormone. New World primates such as common marmosets from the Family Callitrichidae have serum concentrations of 1,25-$(OH)_2$-D that are five times higher than other primates such as rhesus monkeys (National Research Council 2003). The apparently low sensitivity of New World moneys to 1,25-$(OH)_2$-D may reduce their susceptibility to a plant analog of the hormone that is produced as a glycoside or PSM by some plants in the Family Solanaceae. These calcigenic plants cause vitamin D toxicity in ruminants, which includes malformations of bone and mineralization and malfunction of vital organs such as the heart, lungs and kidney (Mello 2003).

Deficiency of vitamin D results in the same symptoms as inadequate Ca and P, that is, weak bones and egg shells. The seeds, fruits and leaves of most plants are poor sources of vitamin D, as are milk and the lean tissues of animals (Collins and Norman 1991). Animals therefore must derive vitamin D from UV exposure or from stores of vitamin D in fat. Maternal vitamin D is transferred to the fetus of mammals and to the yolk of birds for development. Fat depots also provide most of the vitamin D required by terrestrial animals in winter because fat gained in the bright light of summer is mobilized in the dark of winter when D_3 production is low or absent. Dietary vitamin D for terrestrial predators varies with the fat content, light exposure and diet of their prey. For example, the vitamin D content of wild rodents available to cats varies from 0.06–0.33 IU•kg^{-1} DM (Morris 1999). The livers of terrestrial prey are low in vitamin D (< 4 IU•kg^{-1}), but the livers of marine fish such as cod, tuna and shark are rich in the vitamin (> 600 IU•kg^{-1}) because the liver is also a principal fat depot in these fish (Halver 2002). Vitamin D is usually not limiting for marine predators, but may be limiting for terrestrial predators with a low diversity or availability of prey. Captive carnivores such as lions that are fed muscle (meat) which is low in Ca and vitamin D are vulnerable to bone fractures, especially during growth and lactation (Allen et al. 1996).

The need for vitamin D in Ca absorption may be obviated in some animals. Fish can absorb Ca across the gills; signs of vitamin D deficiency and inadequate absorption of dietary Ca are only evident in fish kept in water with low concentrations of Ca (Halver 2002). Some animals can absorb Ca by transport systems that are not dependent on vitamin D, especially during growth and lactation, but also during normal maintenance of the body. Mole rats (Family Bathyergidae) are completely subterranean animals that feed on tubers; these animals do not produce D_3 from UV exposure or receive D_2 from their diet of tubers (Skinner et al. 1991) even though they are able to activate D_3 in a similar fashion to other mammals (Buffenstein et al. 1993). Fortunately, their favored diet of gemsbok cucumbers is high in Ca. Damara mole rats passively absorb Ca independently of vitamin D (Pitcher et al. 1992).

9.3.2.3 Vitamin E

Vitamin E is critical to the maintenance of all cell membranes. Although it is found in plant and animal fats, deficiencies are most likely in carnivores with access to only young prey without large fat reserves and in herbivores consuming forages with low vitamin E activity. Toxicities of vitamin E are unlikely in free-ranging animals, but could have severe consequences associated with bleeding because they antagonize the absorption of vitamin K.

Vitamin E is part of the oxidative defence of cells that is provided by other trace nutrients including glutathione (S) and its Se-dependent peroxidase, ascorbic acid (vitamin C), superoxide dismutases (Mn, Cu, Zn) and catalase (Fe; Tables 9.2–9.5). Superoxides (O_2^-), peroxides (H_2O_2) and free radicals ($OH^\bullet$) are reactive species of oxygen that randomly damage membranes. Antioxidant systems prevent damage to fatty acids and can even reverse some of the changes to lipids. Antioxidant systems that resist and repair oxidative damage to cell membranes are associated with the prolonged life spans of some mammals including naked mole rats and humans (Hulbert et al. 2007; Buffenstein 2008). Unlike the other antioxidant systems, vitamin E provides an oxidative defense within membranes (Table 9.5). The role of vitamin E therefore increases in importance with the risk of producing strong oxidants and with the vulnerability of membrane lipids to oxidative damage. The most vulnerable membranes are those that contain high concentrations of polyunsaturated fatty acids (PUFA), including the essential fatty acids (Chapter 7). All reactions that involve O_2 present some risk of producing highly reactive free radicals ($OH^{\bullet}$), which lead to spontaneous degradation of double bonds (Fig. 9.19). Membranes with high risk of oxidative damage during respiration include the mitochondrial membranes of muscles and the plasma membranes of red blood cells and lungs. Immune cells such as neutrophils and lymphocytes also incur oxidative risks when they produce peroxides to destroy invading pathogens.

The tocopherols are a group of eight compounds in plants that have vitamin E activity (Machlin 1991). The most active is α-tcopherol, which is the reference for all other natural and synthetic forms of the vitamin (Fig. 9.19). All forms of the vitamin have a hydrocarbon tail that dissolves in the lipid bilayer of membranes. The reactive part of the molecules is the chromanol ring. Vitamin E is a sacrificial substrate because it reacts with a free radical to form a stable radical on the chromanol ring that does not damage the surrounding lipids (Fig. 9.19). Subsequent reactions with glutathione and ascorbate, however, can regenerate the vitamin. The concentration of vitamin E in membranes is similar to that of the essential fatty acids (Chapter 7); the minimum concentration is defined by the function of the

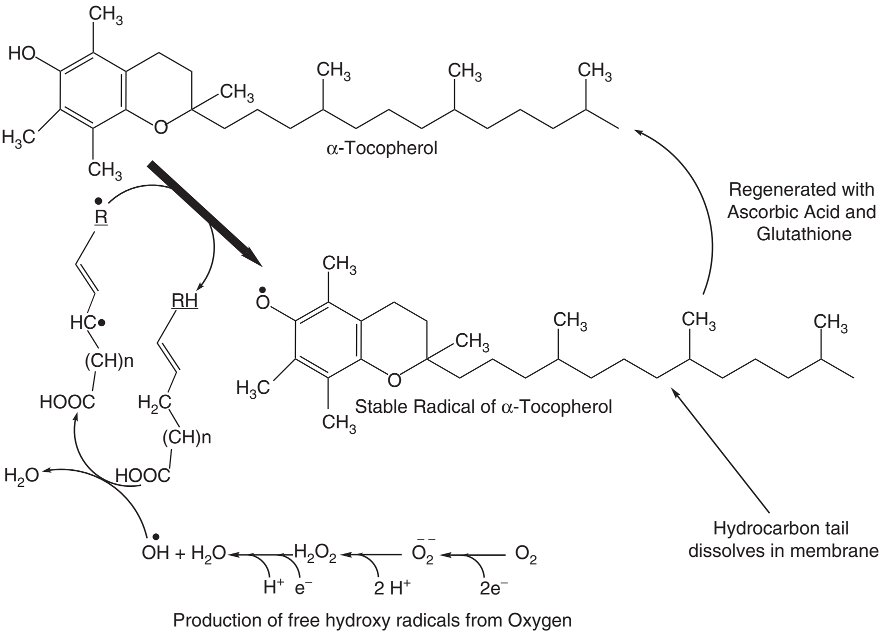

Fig. 9.19 The use of O_2 as an electron acceptor for respiration spontaneously forms superoxides (O_2^-), peroxides (H_2O_2) and free radicals ($OH^{\bullet}$). These compounds can start chain reactions in unsaturated fatty acids by forming fatty acid radicals ($R^{\bullet}$) that randomly exchange H and lose double bonds. Tocopherols (vitamin E) discharge free radicals and protect membrane lipids from disruption by returning the fatty acid to the stable form (RH) (Linder 1991). The oxidized tocopherol can be recycled with ascorbic acid (vitamin C) and glutathione

membrane, but additional vitamin E and PUFA may be incorporated when available from the diet. Tocopherol concentration of lungs and muscle increases with vitamin E consumption in rats, chickens and sheep (Machlin 1991; National Research Council 2007b). Similarly, tocopherol concentrations in plasma and red cells increase with vitamin E intake as the vitamin accumulates in adipose tissue, liver and muscle (Machlin 1991).

In plants, concentrations of tocopherols also follow those of PUFA, both being highest in the germ of seeds and lowest in tubers. Animal lipids with high PUFA content such as fish are also good sources of tocopherol for carnivores. Tocopherol contents of foods may vary by a factor of 10 as plants and prey change seasonally in lipid content and membrane composition. Vitamin E content of foods can also decline quickly, especially as PUFA are oxidized. Stored diets for captive wildlife are often fortified with vitamin E to offset the loss of tocopherol during storage.

Deficiencies of vitamin E are associated with muscle degradation or myopathy in fish, birds and mammals. These lesions are most prevalent when wild animals struggle intensely during capture and restraint (Spraker 1993). Myopathies associated with vitamin E have been reported in wallabies, white-tailed deer and domestic ruminants (Tramontin et al. 1983; Hume 2006; National Research Council 2007b). Low vitamin E concentrations also are associated with vascular problems such as leakage of fluids (exudative diathesis) and hemolysis (Machlin 1991). Signs of vitamin E deficiency include more general problems with growth and reproduction that have been associated with low concentrations of tocopherol in the blood of mammals and birds in captivity. Captive conditions may increase requirements for vitamin E with exposure to disease and other stressors; supplementation of vitamin E in the diets of captive wildlife can alleviate some of these problems (Robbins 1993; Dierenfeld 1994; Hume 1999). Similarly, supplementation of domestic cattle with vitamin E and Se increases the production of superoxides and the ability to kill pathogens by neutrophils (Weiss and Spears 2006). The requirement for vitamin E therefore varies with the availability of other antioxidants and the type of disease or oxidative stress on the animal. Changes in population density and movements of animals alter their exposure to disease and other stressors, which may in turn alter vitamin E requirements.

9.3.2.4 Vitamin K

Vitamin K was named by its Danish discoverer for 'koagulation' of blood because it serves as a cofactor for activating 4 of the 13 steps in the clotting cascade. Blood clots are formed from soluble proteins and small cells called platelets. Activation of the soluble proteins and the platelets requires conversion of specific glutamate residues to γ-glutamate that can bind Ca^{2+} (Fig. 9.20). The clotting cascade is similar to the serial activation of zymogen enzymes in protein digestion (Chapter 8). Each step of the clotting cascade activates the next level, which results in rapid precipitation of the soluble proteins in a matrix with platelets; the resultant clot blocks further blood loss from a damaged vessel. Vitamin K also is involved in similar reactions with glutamate residues in osteocalcin, which facilitates the release of Ca^{2+} from bone (Linder 1991).

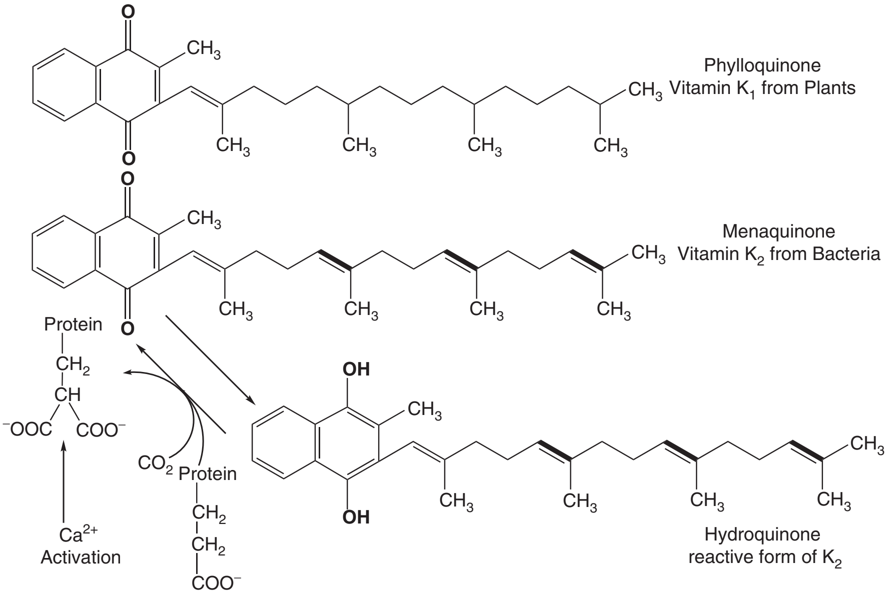

Fig. 9.20 Quinones with vitamin K activity are produced by plants and bacteria. Vitamin K is used to add a carboxyl group (—COO^-) to the end of glutamate residues in proteins (Groff et al. 1995). The divalent negative charge on γ-glutamate allows the protein to interact with Ca^{2+}, which activates a cascade of proteins in the formation of a blood clot. Dicoumarol and Warfarin impair blood clotting by blocking the reactions that convert vitamin K to active hydroquinone and back to quinone

Vitamin K activity is derived from quinones that are involved in electron transfer reactions in photosynthesis and oxidation in plants and bacteria (Fig. 9.20). Plant leaves are a rich source of phylloquinone (vitamin K_1), and intestinal bacteria provide menaquinone (vitamin K_2). Phylloquinone is absorbed actively from the small intestine and transported on lipoproteins, whereas bacterial menaquinone is absorbed passively from the colon (Groff et al. 1995). Both forms of vitamin K accumulate in the liver. Excessive bleeding and slow clotting times are the principal signs of vitamin K deficiency. Neonatal mammals may be vulnerable to vitamin K deficiency because they have meager stores of the vitamin and because they are born without the intestinal bacteria that produce menaquinone (Linder 1991). Poor clotting reactions are also the result of impaired cycling of vitamin K between the reactive form of hydroquinone and the original quinone. Fungal damage and spoilage of sweet clover releases the anticoagulant dicoumarol, which blocks the vitamin K cycle in grazing ruminants (Harborne 1993; Van Soest 1994). Animals that are exposed to rodenticides such as Warfarin may show signs of poor clotting because the poison also blocks the vitamin K cycle (Groff et al. 1995). Supplemental intakes of vitamin K can offset these toxins. The effects of stressors such as toxins on nutrient requirements and body condition are discussed further in Chapter 11.

9.4 Summary: Metabolic Constituents

Animals require water for metabolic reactions. Water is channeled while solutes are filtered and pumped across gills, salt glands, rectal glands, kidneys, urinary bladders and the digestive tract. The body water pool can be maintained by a wide range of complementary influxes (free water, preformed water in food, metabolic water produced from chemical reactions) and effluxes (excreta, respiratory and cutaneous water losses). Water requirements vary with age, body size and environmental conditions including temperature and drought. High water turnovers are associated with high energy demands during growth and reproduction. High energy demands increase respiratory water loss as well as intakes of food and solutes that enhance fecal and urinary water losses. The availability of minerals for animals depends on the geology and hydrology of their habitat. Mineral concentrations vary widely among the tissues of plants and animals, and each element can serve several functions. Body pools of macrominerals (Na, K, Ca, P, Mg, S) are large and widespread whereas pools of trace minerals (Mn, Cu, Fe, Zn, I, Se) are small and distributed among intracellular spaces. Trace minerals are mainly used as catalytic centers in enzymes and transport proteins. Both trace minerals and vitamins are required for metabolism of energy and for protecting cells against oxidative damage. Water-soluble vitamins include the B vitamins and ascorbic acid (vitamin C), which mainly are associated with the aqueous contents of cells distributed throughout the body. The fat-soluble vitamins (A, D, E and K) are found in lipids and membranes; accumulations in body lipids may be at high enough concentrations to have toxic effects. Requirements for trace minerals and vitamins are affected by interactions among nutrients and by stressors (such as disease), which may change with population density and animal movements.