Edward D. Harris

MINERALS IN FOOD

Nutrition, Metabolism, Bioactivity

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To Dr. Boyd L. O'Dell,
my mentor and close friend
and a pioneer in mineral nutrition

Contents

Preface

THIS book is written for students and professionals who seek meaningful insight into biominerals in foods, or more precisely, into metals and non-metals that function as nutrients. Over the past fifty years, scientists have greatly expanded our understanding of the physiological absorption and transport of minerals, as well as their assimilation into target cells. Much has been learned about the physiological functions of specific minerals, which has provided a rationale for identifying and preferring certain minerals as nutrients.

Food scientists and engineers have developed procedures to minimize mineral loss during food processing, and such techniques have ensured that food products meet the nutritional requirements of consumers.

At a more theoretical level, chemists investigated the structure and biochemistry of minerals, which has led to a much greater appreciation of their roles in biological processes. With this expanded knowledge, we can now explain at the molecular level why, for example, iron is part of hemoglobin and copper is selected as a co-factor for enzymes using oxygen.

The present book assembles and connects such insights into a cohesive description of the multiple functions of dietary minerals in animals and humans. The book should help readers grasp why twenty chemical elements, over one sixth of the periodic table, are biologically necessary. However, this understanding is only the beginning, as this volume contains a comprehensive description of minerals as dietary nutrients. The reader will see the scope of *in vivo* reactions carried out or cata-

lyzed by minerals, the ways the status of minerals are assessed, the consequences of dietary mineral deficiencies or excesses, and the unique differences between macrominerals and microminerals in all of these phenomena. The book is a systematic introduction to the functions of ingested inorganic chemicals in living systems, and thus forms an important complement to treatments focused mainly on organics.

The layout of the text follows a pattern, beginning first with principles that apply to all minerals, which is followed by an in-depth explanation of specific examples. Since minerals have unique roles in the brain, an entire chapter is devoted to mineral chemistry and the brain. Similarly, a chapter is devoted to mineral-mineral interactions, given that no single element acts in isolation. The focus however remains on the chemistry, biochemistry and nutritional properties with the goal being to illustrate the necessity of minerals in physiological functioning. Building on this, the book discusses ways to evaluate the mineral status of individuals and populations, and the strengths and weaknesses of procedures in place to perform such assessments. A discussion of microminerals demonstrates why this class of minerals is akin to vitamins in terms of their quantity in the diet and their roles in the biological system. Like vitamins, minerals are minute fractions of foods that have a profound impact on the status quo of an organism. Indeed, one is not wrong in saying biominerals are vitamins in the guise of inorganic elements.

E. D. Harris
College Station, Texas
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Acknowledgements

THIS book could not have been written without the input and advice of colleagues who have devoted their scientific careers to the study of biominerals. Indeed in putting together a comprehensive book on biominerals, I have drawn exclusively on their published research findings to let the reader see the science behind current understandings. I would like to express my appreciation to those colleagues in particular who have lent their expertise in evaluating the accuracy of the information. To that end I acknowledge the advice of Dr. Christopher Fredrickson, University of Texas, James Vincent, University of Alabama, Stephen Talcott, Texas A&M University, Suresh Pillai, Texas A&M University, Todd See, North Carolina State, Karen Kabena, Texas A&M University, Robert Cousins, University of Florida, Julian Spallholtz, Texas Tech University, and Alice Villalobos, Texas A&M University. The biominerals have always been regarded as a hidden nutrition whose impact on human and animal health is secondary to vitamins and other organic nutrients. It is hoped that the book can justify the importance of biominerals in many aspects of health and why they merit a stand-alone role in educational programs in food science and nutrition. Finally, I would be remiss if I did not acknowledge DEStech Publications who have lent support and encouragement throughout the preparation. I owe a special debt of gratitude to Dr. Joseph Eckenrode, Publisher, who had a vision of a book on biominerals that would greatly serve the fields of food science and nutrition not only for students but professionals who have daily contact with biominerals in their work. I am also indebted to Steven Spangler, Production Director who put the final touches on the book's assembly. Their patients and encouragement is duly noted.

CHAPTER 1

Introduction to the Minerals

MINERALS are omnipresent components of the living environment and a major class of nutrients in foods. A brief look at their history, how they are categorized, their properties and functions, and the various forms in which they occur, will be discussed here in a summary way with more detailed information in chapters that follow. Our goal is to understand how minerals fit into the framework of life, keying on the properties that underlie their uniqueness and selection for functions. Specific objectives are:

1. To provide a brief historical perspective
2. To define a mineral in a biological setting
3. To learn the role of minerals in biology
4. To introduce mineral properties that play a role in function
5. To predict a mineral's function by knowledge of its structure

1.1. DEFINITION

The word mineral comes from "mine", or more specifically, a substance in the earth's crust that is obtained by "mining". From a biological perspective, minerals are essential inorganic components in foods that play multiple functional and structural roles in cells—functions that their organic counterparts cannot replicate. Minerals thus represent a special class of food nutrients with no parallels.

1.2. A BRIEF HISTORY OF BIOLOGICAL MINERALS

James Lind's investigation (1747) into the cause of scurvy in British sailors is generally acknowledged as the first scientific experiment in nutrition. Antoine Lavoisier's discovery that equated body heat with oxygen utilization put an emphasis on the energy components in foods. By the 1800s, foods were generally thought to be composed of four elements: carbon, hydrogen, nitrogen and oxygen. The technology to investigate these factors took a leap forward when Justus von Liebig turned his interest in plant growth to understanding how food molecules become assimilated into body matter. Minerals were basically ignored at the time because conventional wisdom regarded minerals as contaminants taken into the body by accident or from an imperfect food supply. This did not stop early investigators, driven by curiosity, to learn if minerals performed useful bodily functions. Early interest in the nutrition of minerals was spurred by studies with animals, not humans, however. The concern was to identify factors in the soil that promoted optimal growth of livestock. In Australia, where probes into the biology of microminerals began in earnest, there was an urgent need to optimize the quality of the wool sheared from sheep. Linking mineral deficiencies with disease states erased any doubt of their essentiality and when scientists showed it was possible to control the growth and health of animals by modulating the minerals in the diet, the stage was set for learning precisely how minerals functioned in living systems. Table 1.1 chronicles some of the discoveries that made the study of biological minerals a worthy scientific endeavor.

From these early studies came a greater appreciation of the value of minerals to living organisms; specifically, how they compared to vitamins in being essential to growth and health. Still, minerals could not be entirely divorced from environmental hazards. In the 1880s, citizens in New York City organized an effort to oversee the condition of animals in slaughter houses and extended the concern to the way animals were housed on farms. Pigs wallowing in the mud were an attractive target. With much badgering from groups that demanded hygienic environments, farmers replaced the mud pits with concrete patios, but with disastrous consequences. Unbeknown at the time was that pigs in a rapid state of growth have a special need for copper. Insufficient amounts in the diet caused major artery rupture, similar to the aortic rupture observed in chicks and a symptom of severe copper deficiency. Apparently a mud bath, despite its obvious lack of cleanliness, supplied the animals with sufficient copper to prevent the pathology.

TABLE 1.1. Historical Events in Mineral Nutrition.

130–200 A.D.	Galen, a Greek physician and student of Hippocrates, suggested that most foods acted as drugs. The Hippocratic school also held that foods were a source of "ailment" and regarded most foods as differing in content of poisons, drugs and other active ingredients unrelated to beneficial nutriment. On the other hand, Paracelsus believed that food contained both nourishment and poison. The idea that foods contained a single ailment persisted as late as 1833 in the writings of William Beaumont, famous for his work on digestion.
1534	Jacques Cartier's sailors were saved from death through scurvy in what is now Quebec by drinking infusions of evergreen needles.
1828	Friedrich Wohler, a German organic chemist, synthesized urea by heating ammonium cyanate. The experiment showed that a so-called body chemical could be made in a laboratory flask.
1834	William Prout showed that hydrochloric acid was the factor that made stomach juice acidic.
1840	Jean Baptiste Boussigault, a French chemist, and Justus von Liebig, a German organic chemist, collaborated in recognizing the importance of minerals. Wild animals would walk many miles to salt licks. Bone was found to be composed of calcium and phosphorus.
1850	Boussingault later demonstrated that iodine was the active component in salt deposits shown to be effective against goiter.
1873	After studying the chemical makeup of body components and their derivation from foods for more than 30 years, Liebig postulated that the essential nutrients consisted of "albuminous substances (proteins), fuel substances (carbohydrates and fats), and mineral salts (calcium and phosphorus)".
1928	Conrad Elvehjem and colleagues at the University of Wisconsin showed that rats made anemic by low iron in the diet required copper as well as iron to fully restore blood hemoglobin, one of the earliest demonstrations of mineral synergism.
1934	Todd and Elvehjem showed that zinc was essential for the optimal growth of animals.
1953	Prasad and coworkers, while studying malnutrition in remote areas of Egypt, equated a type of human dwarfism with the low zinc in the diet. The cause of the low zinc was apparently due to phytate-rich unleavened bread, a main staple in the native diet.
1957	Schwarz and Foltz showed that selenium in the diet prevented liver necrosis in rats.
1959	Schwarz and Mertz discovered that rats fed yeast diets lacking chromium were impaired in utilizing blood glucose.
1961.	O'Dell and coworkers at the University of Missouri discovered that a diet deficient in copper caused aneurysms and ruptured chick aortas. Coulson and coworkers at the University of Utah made a similar discovery with baby pigs.
1962	Holmberg and Laurell discovered two globulins in serum. One, which they named "transferrin", appeared to function in the transport of iron between cells. The other, a copper protein, they called "ceruloplasmin" because of its heavenly blue color.
1972	Carlisle and later Schwarz and Milne showed that silicon was an essential element in animal nutrition.

1.3. MINERALS AND THE BUILDING BLOCKS OF LIFE

Referring to minerals as "inorganic" distinguishes them from the more familiar "organic" components in foods, such as, proteins, carbohydrates, and fats. As the word implies, inorganic compounds stand apart from the larger, more complex molecules that have carbon skeletons at their foundation. In reconstructing the biochemical elements of life, carbon, hydrogen and oxygen provided the fundamental building atoms for carbohydrates and fats. Adding nitrogen and sulfur brought proteins into the mix, but not nucleic acids. Finally, phosphorus completed the assembly (Figure 1.1). Together, these six elements embody 97 percent of the body weight, with oxygen alone making up more than 60 percent.

We may ask, however: will six elements suffice to give all the features of life? More specifically, will six elements allow hemoglobin to bind oxygen or enzymes to have catalytic prowess? Will the six provide an internal supporting skeleton, stimulate muscles to contract, propagate nerve impulses, control cell division, or turn genes on and off? Evidently, the six elements denoted as essential to life fall short of making life happen. In essence, the six can only give us the components of life, not life itself.

So, the list of elements essential to life must extend beyond six in the chemist's Periodic Table of Elements. Put in a biological perspective, more than six are needed to allow movement, growth and development, turnover, energy production and utilization and maintenance of internal homeostasis. What elements in food are essential for these other critical functions? The remaining elements not accounted for—some 21 in all—comprise the class of substances called minerals. In the final analysis, anywhere from 26–32 elements are needed for life (Table 1.2). Some still remain questionable. Therefore, we are justified in conclud-

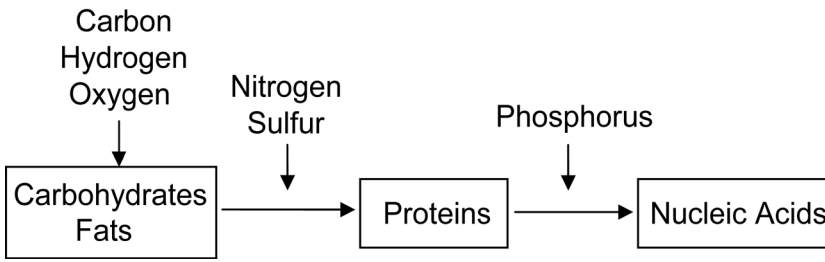

FIGURE 1.1. Basic Elemental Requirement for Biological Molecules.

TABLE 1.2. Chemical Elements Essential for Life.

Macro-elements			Micro-elements		
Basic	**Metals**	**Non-metals**	**Metals**	**Non-Metals**	**Questionable**
Carbon	Sodium	Chlorine[1]	Chromium	Selenium[2]	Tin
Hydrogen	Potassium		Manganese	Iodine[1]	Arsenic[2]
Nitrogen	Calcium		Iron	Fluorine[1]	Lithium
Oxygen	Magnesium		Cobalt	Silicon[2]	Aluminum
Sulfur			Nickel	Boron[2]	Tungsten
Phosphorus			Copper	Bromine[1]	Beryllium
			Zinc		
			Molybdenum		
			Vanadium		

[1]Halide
[2]Metalloid

ing that minerals acting alone or in combination, although contributing less than 3 percent of the body weight, make up nearly 80 percent of the total elements in living matter. Of the 92 natural elements in the Periodic Table, about one third are found in living systems and most of these are classified as nutrients.

1.3.1. The Biominerals

1.3.1.1. Macro- , Micro- (Trace) and Toxic Minerals

Minerals in living systems are referred to as biominerals. As a group, the biominerals make up the inorganic complement of tissues and organs. Depending on abundance in a food source or in the system, biominerals are further sub-classified as either macro- or microminerals. As seen in Table 1.2, minerals in both subclasses are represented by metals, non-metals, or metalloids (non-metals with properties of metals). Non-metals are present as halides, metalloids, or multi-atom complexes such as NH_4^+, $HPO_4^=$, and $SO_4^=$. Macrominerals have the advantage of abundance and thus can derive a critical mass for performing functions. As the name denotes, "micro" implies a scarcity in foods and the system and the need for only small amounts in the diet. The term "trace element" is also used for this category, denoting a period in their history of discovery when instrumentation was insufficient to quantify their exact amount in tissues and fluids. Their scarcity predicts func-

tions other than giving mass or structure to a system; serving as activators, regulators or cofactors is more likely for this subclass. A substantial group of minerals comprise the transition metals (Chapter 2) and includes chromium (Cr), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), molybdenum (Mo) and cobalt (Co). Also included are non-metallic trace minerals such as selenium (Se), iodine (I), fluorine (F), bromine (Br) and boron (B). A further subset, the "ultratrace", designates a group of minerals, some of questionable significance, that fall below micro in quantity. This rather narrow but important class includes tin (Sn), lithium (Li), beryllium (Be), and arsenic (As). The final category, the toxic minerals, imply a danger to normal function, even at very low amounts. Included are cadmium (Cd), mercury (Hg), lead (Pb), beryllium (Be), arsenic (As) and aluminum (Al). Given excessive amounts or prolonged exposure as extenuating factors, it is important to note that any mineral in a food source or at the site of action in a cell is toxic.

1.3.1.2. Mineral Complexes

As noted above, some minerals are not singular elements but rather complexes of two or more elements. Complexes built around nitrogen, for example, give rise to ammonia (NH_3) or ammonium ion (NH_4^+), as well as nitrates (NO_3^-) and nitrites (NO_2^-). Sulfur is commonly found as sulfate (SO_4^{2-}) or sulfite (SO_3^{2-}) and vanadium and boron as vanadate (VO_3^-) and borate ($B(OH)_4^-$), respectively. Biological phosphorus as phosphate makes its appearance as a free ion or bound to organic molecules. Bones and teeth are composed of a crystallized hydroxyapatite, a complex of calcium and phosphate. Heme, a complex of iron within a porphyrin ring, binds to the protein globin to form hemoglobin that transports dioxygen. Heme is also the iron component of proteins that transport electrons. Binding a mineral to an organic molecule is a common structural motif in biological systems and in some cases is required for the organic component to be recognized by an enzyme. Examples are phosphate-bound sugars, fatty acids and nucleic acids that make up major constituents within the cell or are part of the cell's biochemical architecture.

Forming complexes with proteins is the most common—and could arguably be considered the more typical—state of minerals *in vivo*. Acting as large molecular ligands (molecules that bind metal ions), proteins with metal-binding properties engage small ions in complexes that vary

in stability. Macrominerals such as Ca^{2+} and Mg^{2+} favor electrostatic or ionic interactions with proteins, whereas microminerals (Zn^{2+}, Cu^{2+}, and Mn^{2+}) are more apt to use coordinate covalent bonds (Chapter 2). The binding of a metal ion to a protein can be a signal for a biological event to occur or cease. As an example, when Ca^{2+} binds to the actin-myosin complex in muscle, the muscle is stimulated to contract. When magnesium replaces the calcium, the muscle is given the signal to relax. Proteins with metal binding properties have amino acids selected to tether the metal ions to their structure. A case is *metallothionein*, a protein that binds Cu^{2+}, Zn^{2+}, and Cd^{2+}. About one third of the amino acids in metallothionein are sulfur amino acids ideally suited for binding heavy metals.

1.4. PROPERTIES OF MINERALS RELATED TO FUNCTION

1.4.1. Chemical Properties

1.4.1.1. Metal Ions and Valence

Metal ions are positively charged cations with valence states determined by the number of electrons lost on ionization. A movement toward the electronic configuration of a noble gas is the driving force for ionization. Whereas macro metals such as Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} have only one stable valence state, microminerals exist mostly as ions with multiple valencies. Only zinc with a single +2 valence (Zn^{2+}) is an exception (Table 1.3). The ability of a metal to give rise to stable multivalence ions can be a deciding factor in a metals ion's selection for a

TABLE 1.3. Major Stable Valence States of Macro- and Microminerals.

Macro		Micro	
Sodium	Na^{+}	Iron	Fe^{2+}, Fe^{3+}
Potassium	K^{+}	Zinc	Zn^{2+}
Magnesium	Mg^{2+}	Copper	Cu^{+}, Cu^{2+}
Calcium	Ca^{2+}	Manganese	Mn^{2+}, Mn^{4+}, Mn^{5+}
Chloride	Cl^{-}	Cobalt	Co^{+}, Co^{2+}
		Nickel	Ni^{+}, Ni^{2+}
		Molybdenum	Mo^{4+}, Mo^{5+}, Mo^{6+}
		Iodine	I^{-}

biological process. By giving or receiving electrons, multiple valence metal ions have the capacity to move between states. This very important property is found in "redox metals" as explained below.

1.4.1.2. Solubility

Ionization imparts both charge and polarity to metal ions, two properties that contribute to solubility in water and attraction to opposite charge. The smaller the ion, the more intense the charge. The polar features of many mineral salts (NaCl, $MgCl_2$, K_2HPO_4) lets these metal ions coexist freely with water molecules. Microminerals, however, are just the opposite. Despite the presence of strong positive charges, ferric iron (Fe^{3+}) and cuprous copper (Cu^{+}) and Zn^{2+} are sparingly soluble in water as noted by the magnitude of their solubility constants in aqueous medium (Table 1.4). Insolubility is due to the capacity to form insoluble metal hydroxides, which limits the number of ions that exist freely in solution.

As an example, both ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron in aqueous solution at neutral pH form insoluble complexes. Fe^{3+} iron [Equation (1)] is more active than Fe^{2+} iron [Equation (2)] in the reaction, however. Thus, when compared to ferric, a greater fraction of ferrous iron will be soluble. The figure also shows that an acidic pH shifts the equilibrium toward the free ion. While ferric iron may have greater solubility in the stomach, it is practically insoluble in the alkaline environment of the duodenum.

$$Fe^{3+}(\text{aqueous ferric}) + 3H_2O \rightleftarrows Fe(OH)_3\ (\text{solid}) + 3H^{+} \qquad (1)$$

$$Fe^{2+}(\text{aqueous ferrous}) + 2H_2O \rightleftarrows Fe(OH)_2\ (\text{solid}) + 2H^{+} \qquad (2)$$

Two points are brought out by this observation. First, to be absorbed by intestinal cells, Fe^{3+} must (1) form a complex with some factor that renders it soluble, or (2) engage reducing agents (such as vitamin C) to convert the less soluble Fe^{3+} to the more soluble Fe^{2+}. Both of these events have been shown to enhance the absorption of iron in the gut (Chapter 13). $Ca_3(PO_4)_2$ is weakly ionized in aqueous solution and is poorly absorbed in the intestinal tract. Insolubility is due to a strong association between the calcium ions and phosphate, an attraction that can only partially be broken by water molecules. Insolubility in an aqueous medium impedes a metal ion's ability to exist freely in the aqueous phase, and hence figures in a major way in the mineral's absorption and bioavailability.

TABLE 1.4. Relative Solubility Constants for Macro- and Microminerals.

Mineral	Ion/Complex
Na^+, K^+	10^{-1} M
Mg^{2+}, Ca^{2+}	10^{-3} M
Zn^{2+}	10^{-12} M
Cu^+	10^{-13} M
Fe^{3+}	10^{-17} M

1.4.1.3. Oxidation-Reduction

Table 1.5 lists elements, mostly metal ions, that have a well defined oxidation-reduction (redox) activity. To be so designated, a redox agent must be able to exist in multiple electronic forms and partake in chemical reactions that donate or acquire electrons. This basically determines the mineral's "redox potential", which is a measure of the ion's affinity for the electron. To gain further insight into their behavior in the cell, Equation (3) below shows the oxidation of iron by copper. Reading from left to right, Fe^{2+} (ferrous) is oxidized to Fe^{3+} (ferric) concomitant with Cu^{2+} (cupric) reduced to Cu^+ (cuprous). The change in valence signifies that

$$Cu^{2+} + Fe^{2+} \rightarrow Cu^{+} + Fe^{3+} \qquad (3)$$

a single electron has been transferred from the Fe^{2+} to the Cu^{2+}, reducing the Cu^{2+} to Cu^+ and oxidizing the Fe^{2+} to Fe^{3+}. If the equation is read from right to left, Fe^{3+} would be considered the electron acceptor (oxidant) and Cu^+ the electron donor (reductant). The direction of electrons' flow when all components are at the same concentration is always towards the metal ion with the stronger affinity for the electron. Redox metals in biological systems have the potential to donate and receive

TABLE 1.5. Redox Elements in Biological Systems (Adopted from da Silva and Williams, 1991).

Redox Metals	Redox Non-metals
Chromium	Hydrogen
Manganese	Carbon
Iron	Nitrogen
Cobalt	Oxygen
Nickel	Sulfur
Copper	Selenium
Molybdenum	

electrons and in so doing have the capacity to behave as oxidants or antioxidants in a biological systems.

1.4.1.4. Electron Configuration

Electronic charge is not the only factor that determines the metal ion's binding affinity to proteins, nucleic acids, etc. Geometry is another deciding factor. Whether a metal will bind strongly or weakly is determined by how well it "fits" into the structure of the host molecule, which in turn must be compatible with its geometric shape. As an example, consider that in its ionized state, iron exists primarily in an octahedral arrangement with 6 binding ligands. The six are arranged in space so as to allow minimal interaction with one another. This same arrangement, however, is only weakly duplicated by copper. Instead, copper generally has four ligands arranged in a square planar configuration or in a tetrahedral arrangement. (Figure 1.2) Zinc follows a similar binding pattern. Because they can only form weak octahedral complexes, copper and zinc cannot engage in an octahedral site on a protein or enter into complexes with porphyrin, as iron does to form heme. Specificity in electronic configuration in its most selective form allows one metal ion to engage a site on a protein or perform a biological function. Likeness in complex formation also forms the basis for metal-metal interactions which can be both antagonistic and synergistic.

1.4.2. Biochemical Properties

Life at the biochemical level is a series of well-regulated integrated pathways designed to keep the status quo of the system. Living systems strive to maintain a balance between energy production and energy utilization. Although minerals are not sources of energy, they can and do assist cells in extracting and storing energy from compounds in foods.

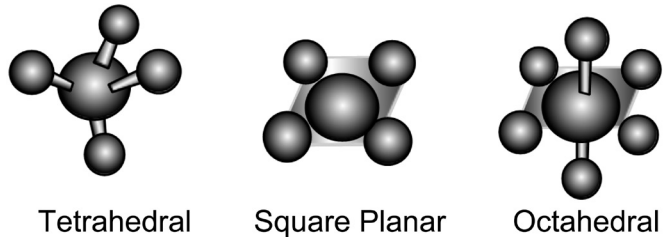

FIGURE 1.2. *Common Geometric Complexes of Minerals.*

As discussed in Chapter 3, attaining internal metabolic change relies strongly on the properties of minerals. Most are manifested through their cofactor role for enzymes. Literally one-third of all enzymes require a metal ion as a cofactor. Oxidation reactions make up a large core of energy yielding reactions in cells. Removing electrons from one molecule during the course of a reaction places a burden on the component that receives the electron. If the receiving component is an amino acid in the enzyme, the amino acid is destroyed and the enzyme may cease to function. Enzymes for that reason have coenzymes that take up the electrons and prevent damaging the enzyme's structure. Vitamin-derived cofactors such as NAD^+ and FAD from niacin and riboflavin, respectively, are examples of organic cofactors that bind electrons safely and dispose of them in subsequent metabolic steps. By far, the bulk of the protection for enzymes resides with metal ions attached to their surface in what may be the "active site". The fact that metal ions receive and donate electrons without permanently changing their structure makes them ideal factors to protect the enzyme while performing a vital function. All of this points to biometals being more suited to rigors of life at the chemical level.

1.4.3. Nutritional Properties

The sciences of nutrition and food science are devoted to knowing the elements in food that are essential to life. To be categorized as essential, a mineral (or any nutrient) must meet certain criteria. These criteria tend to be unequivocal evidence for necessity. To establish essentiality, a mineral must meet the following:

1. It must be present in all tissues at nearly uniform concentrations where it exerts biological effect;
2. Withdrawal of the mineral from the diet produces deficiency symptoms reflecting an impairment or abnormality in physiological functioning;
3. Returning the candidate mineral removes the impairment and restores the health of the animal.

In the nutritionist's list of requirements for essentiality, the concern is to identify gross symptoms that are readily observed or can be measured when a subject experiences a mineral deficiency. A more refined biochemical approach is to probe into causes at the molecular level. Some answers that are sought are the following:

1. What protein, enzyme, cell component specifically requires the mineral for function?
2. Can another mineral substitute for the mineral in question or do circumstances show a strict requirement for only one mineral?
3. What specific biochemical processes are impaired when the mineral is absent or present in substandard amounts in the diet? Are these events reversible?

These criteria provide prima fasciae evidence for concluding a given mineral is essential. Symptoms could reflect deficiencies induced *in utero* or early in the postnatal period. Semi-purified diets that lack the unknown are the instrumental tools in these studies. Perhaps the staunchest criterion for classifying a mineral as essential is to show a reversal of symptoms after a deficiency has been induced. This depletion/repletion approach is designed to rigorously test the suspected mineral not only as the cause but the sole component that can reverse the impairment. Identifying the biochemical locus pinpoints the location within the system where the depletion is most critical—generally a pathway or, more specifically, an enzyme in that pathway. As we continue to explore the role of minerals in the system, we must be cognizant that in many instances only the one element performing the function is capable of performing that function. Herein may be the essence of essentiality and the reason there is a need for many minerals to carry out the functions of a living system.

1.4.4. Structure-Function Relationships

Throughout this book the emphasis will be placed on chemical properties of minerals as a rationale for determining their behavior and selectivity for biological functions. Because minerals differ in abundance, size, solubility, ionization potential, complex stability, and redox properties, the structure-function principle is the guide to learning the basis of their selection for a particular biological task. The diversity is not only functional but also becomes instrumental in identifying how minerals are handled in a living system, their metabolism, and the manner in which they are absorbed, transported and assimilated into biological molecules. Mineral deficiencies can be devastating, life-threatening disorders that affect the organism much the same way as a vitamin or essential amino acid deficiency. As with vitamins, mineral deficiency symptoms provide valuable clues to their underlying biological site of action as well as confirm their necessity for sustaining the status quo of the system.

1.5. SUMMARY

Most of the nutrients in foods are minerals. Although by quantity they may be regarded as minor, by sheer variety of types they supersede organic components in functions and presence. Other than calcium and phosphorous, which appear as major components of the skeleton, minerals tend to be more in the background and represent a type of "hidden nutrition". Based on quantity in the system and food source, minerals are classified as macro if they occur in great amounts and micro or trace if their quantity is below the threshold for a critical mass. While their presence may be subtle, the functions they perform are extremely important to the processes of life. When dealing with minerals, however, it is important to take note of the food sources of the minerals. How they appear in the matrix of the food will determine the ease with which they are extracted by digestive processes or lost during a food processing step. Solubility, ionization, binding interactions—all must be taken into account when questioning a mineral's function and bioavailability. Minerals follow a unique set of rules when working within the confines of a living system. A goal of nutrition and food sciences is to bring these rules to light.

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1.7. PROBLEMS

1. For each of the following minerals, determine if it belongs to the class of Macro- or Microminerals.

a. sodium	e. silicon	i. selenium
b. phosphorus	f. calcium	j. chromium
c. manganese	g. cobalt	k. nickel
d. magnesium	h. potassium	l. molybdenum

2. Draw the structure of three biomolecules that have phosphate in their structure. The three should represent carbohydrates, fats, and nucleic acids.

3. Which member of the pair below is more soluble in water and why?
   a. sodium chloride vs ferric chloride
   b. potassium phosphate vs manganese phosphate
   c. ferric chloride vs ferrous chloride
   d. calcium phosphate vs sodium iodide
   e. silver chloride vs potassium chloride

4. Answer the following:
   a. if iron is reduced by copper, which of the two acts as an oxidant? As a reductant?
   b. draw an equation showing the oxidation of manganese (Mn^{2+}) by ferric iron (Fe^{3+}).
   c. what would be the product of reaction of Ca^{2+} with Fe^{3+}?

5. How does a metal ion protect an enzyme from destruction? Suppose the reaction catalyzed is an oxidation (electrons are removed); would any metal ion suffice to fill the role as a protectorate? Explain.

6. Iron is classified as a micromineral. Yet, the body has about 4–5 grams of iron. How does one justify calling iron "micro" when there is so much in the system? (*Hint:* you may want to read chapter 13 for the answer.)

7. Can you name a micro-metal ion that exists in only one valence state? A macro-metal ion that exists in more than one valence state? (*Hint:* Chapter 2 has the answers to these observations).

8. Starting with BIOMINERAL and ending with ULTRA-TRACE METAL, construct in outline form all of the sub-categories that occur in between.

CHAPTER 2

Chemical Properties of Minerals

BIOMINERALS are unique elements selected to perform distinct biological functions. In principle, however, the function performed by the selected mineral must ultimately conform to the chemical properties of that mineral. This same principle applies to absorption, transport, and utilization by biological systems. In this chapter we will see that the configuration of electrons around the nucleus of a metal, aptly described by the principles of quantum chemistry, provide insight into a mineral's properties and determine its functional limitations. Although a detailed examination of quantum principles is beyond this book, the chapter will introduce students to the chemistry behind a mineral's properties. Specific objectives are:

1. To relate the scope of minerals' functions to its electronic structure
2. To associate electronic configurations with the geometry of mineral complexes
3. To learn the basis of configurations and valence states of minerals

2.1. BASIC QUANTUM THEORY APPLIED TO MINERALS

Quantum theory gives us a rationale for understanding the electronic structure of atoms. To appreciate the impact of the theory on minerals, first consider that a mineral's functional applications and limitations will depend largely on its electronic structure. The position of electrons

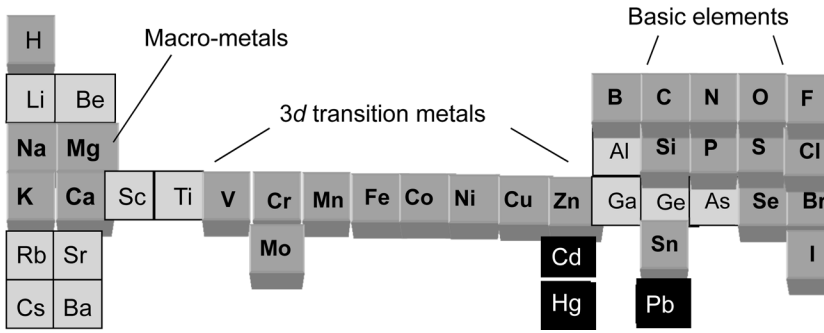

FIGURE 2.1. The Periodic Table of Elements Highlighting Biologically Important Minerals. Essential elements are shown in projection. Toxic elements with no redeeming nutritional value are in black.

around the central nucleus of the atom allows minerals not only to form specific geometric complexes with other molecules, but also provides oxidation/reduction properties as well as a basis for antagonism with other minerals. Quantum numbers become a primary consideration in predicting all of these properties.

2.1.1. Periodic Table Applied to Biological Minerals

Most biological elements fall in the upper third of the Periodic Table. Figure 2.1 highlights the chemical elements of life—all told, some 27–29—that extend from hydrogen (the lightest) to iodine (the heaviest) in atomic weight. In quantum theory, each horizontal row or period matches up to filling a quantum shell of electrons. Those in greater abundance, i.e., the macrominerals Na, K, Mg, and Ca, are in the first and second columns. The basic elements carbon, nitrogen, and oxygen are in the second row and have the quantum number $n = 2$ while for sodium and magnesium in the third row $n = 3$. Most of the microminerals dominate the fourth row and are designated 3*d* transition metals. Those minerals grouped by principle quantum number are likely to have similar electronic configurations, which permits one to predict similarities and differences in chemical properties. To understand how this translates into biological structures and properties, one must first focus on the electron orbitals ensconced within each energy level.

2.1.2. Energy States and Positions of Electrons Around a Nucleus

Starting with hydrogen, an increase in atomic number (Z) is matched

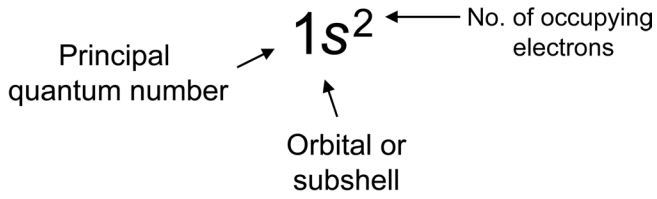

FIGURE 2.2. *Designating the Electronic Configuration of Helium.*

by an increase in electron density. As Z grows, so do the values for quantum number that describe the energy state of the electrons. Higher numbers penetrate into the 2nd, 3rd, and 4th quantum energy levels corresponding to *s*, *p*, *d*, and *f* orbitals. The simple way to designate quanta values for helium is shown in Figure 2.2. Helium and hydrogen occupy the first row of the periodic table and thus both have the principle quantum number $n = 1$. The two differ, however, in the number of electrons occupying the single s orbital.

2.1.3. Quantum Numbers Predict Orbital Numbers and Shapes

The principle quantum number also determines the number of allowable orbitals for that energy level. For example, when $n = 1$, only one orbital (the *s* orbital) is allowed; for $n = 2$, *s* and *p* orbitals are permitted, and for $n = 3$, *s*, *p*, and *d*. Regardless of the energy level, *s* orbitals will always be spherically shaped with a capacity for 2 electrons. The *p* orbitals will be cylindrically symmetric along the *x*, *y* and *z* axis labeled *px*, *py* and *pz*, respectively, each with 2 electrons, or 6 when all 3 are filled (Figure 2.3). *d* orbitals occur first at the $n = 3$ energy level and re-

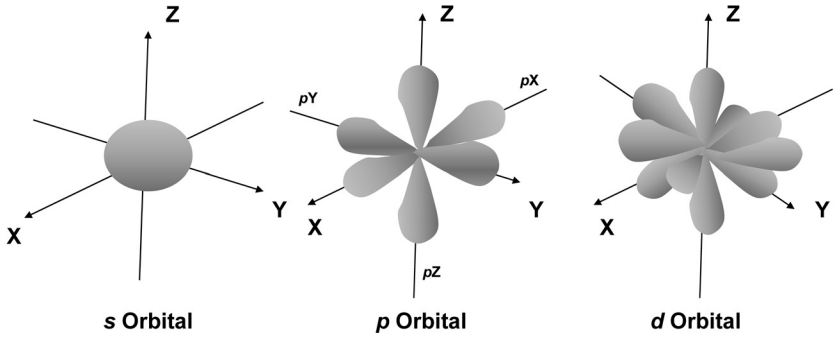

FIGURE 2.3. *Shapes of Orbitals. The s orbital is sphere-shaped whereas p orbitals are sausage-shaped extensions along each long axis out from the nucleus. The d orbital is the most complex, consisting of a composite of 5 suborbitals. The overall shape allows minimum electron-electron interactions.*

late to 5 atomic suborbitals of equal energy (degenerate). The *f* orbitals further out from the *d* can accommodate 14 electrons. Because f orbitals play only a minor role in biological minerals, they will not be discussed further. Knowing the position of electrons in the outer reaches of the atom gives a rationale for the type and shapes of metal ion-centered complexes that can be formed.

2.1.4. Transition Elements: 3*d* Orbitals

Biological metal ions must be given special attention. This applies more directly to the 3*d* first transition metals. As seen in Figure 2.4, the 3*d* shell is comprised of 5 subatomic orbitals that can accommodate a total of 10 electrons. The combined configuration overall is shown in Figure 2.3. Biologically important metals with a 3*d* configuration include vanadium, chromium, manganese, iron, nickel, copper, and zinc, all known to be important in living organisms (Table 2.1). Elements with 3*d* orbitals make their appearance in the fourth row of the periodic

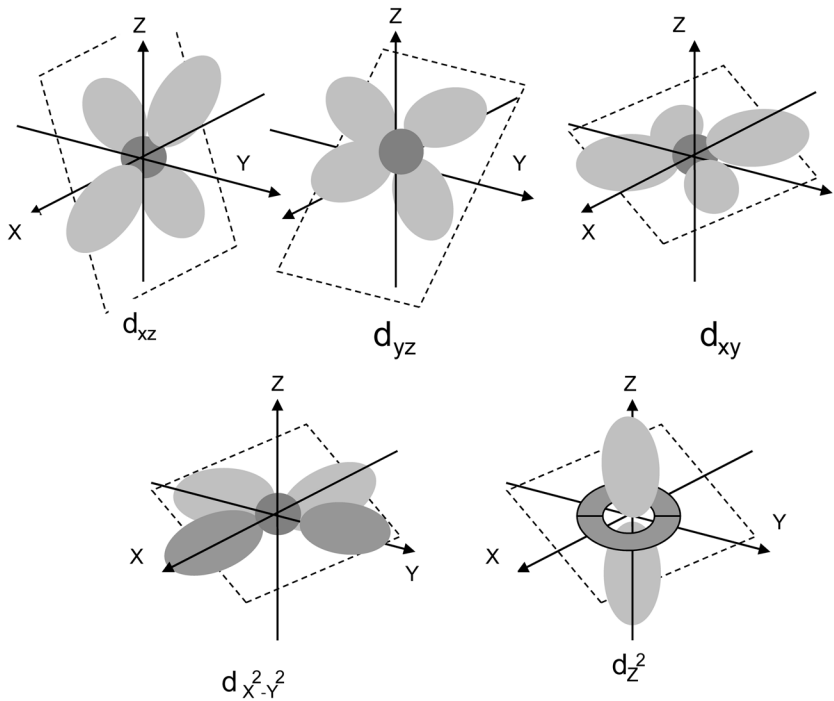

FIGURE 2.4. *Shape and Orientation of 5, 3d orbitals. See Figure 2.3 for a composite picture.*

TABLE 2.1. Elements in the Third Quantum Series.

Sodium, potassium, magnesium and calcium have no electrons in the 3*d* orbital. 3*d* orbital electrons make their first biological appearance in vanadium and are part of the first transition series elements. [Ne] is the abbreviation for the electronic configuration of neon, [Ar] is for the configuration of Argon.

Element (At. No.)	Electron Configuration	Abbreviated Form
Sodium (11)	$1s^2 2s^2 2p^6 3s^1$	[Ne]$3s^1$
Magnesium (12)	$1s^2 2s^2 2p^6 3s^2$	[Ne]$3s^2$
Silicon (14)	$1s^2 2s^2 2p^6 3s^1 3p^2$	[Ne]$3s^2 3p^2$
Phosphorus (15)	$1s^2 2s^2 2p^6 3s^2 3p^3$	[Ne]$3s^2 3p^3$
Sulfur (16)	$1s^2 2s^2 2p^6 3s^2 3p4$	[Ne]$3s^2 3p^4$
Chlorine (17)	$1s^2 2s^2 2p^6 3s^2 3p5$	[Ne]$3s^2 3p^5$
Potassium (19)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^1$	[Ar]$4s^1$
Calcium (20)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2$	[Ar]$4s^2$
Vanadium (23)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^3$	[Ar]$4s^2 3d^3$
Chromium (24)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^1 3d^5$	[Ar]$4s^1 3d^5$
Manganese (25)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^5$	[Ar]$4s^2 3d^5$
Iron (26)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^6$	[Ar]$4s^2 3d^6$
Cobalt (27)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^7$	[Ar]$4s^2 3d^7$
Nickel (28)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^8$	[Ar]$4s^2 3d^8$
Copper (29)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^1 3d^{10}$	[Ar]$4s^1 3d^{10}$
Zinc (30)	$1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^{10}$	[Ar]$4s^2 3d^{10}$

table beginning with potassium ($Z = 19$) and calcium ($Z = 20$). Because 4*s* orbitals are at a lower energy state than 3*d*, the 4*s* orbital is filled before the 3*d* and upon ionization, electrons are lost from the 4*s* first. For potassium and calcium, the loss of the 4*s* electrons gives rise to a highly stable Argon configuration, making further loss of electrons extremely energy demanding. Consequently, potassium exists as a monovalent (K^+) and calcium as a divalent ion (Ca^{2+}), respectively. The term "transition element" is used to designate elements that fill 4*s* orbitals before completing the 3*d*. Only chromium (Cr) and copper (Cu) are exceptions to this rule in that both have only a single 4*s* electron before filling the 3*d* (Table 2.1).

2.2. THE FIRST TRANSITION SERIES ELEMENTS

The 10 elements scandium (Sc) through zinc (Zn) represent the first transition series of elements in the periodic table. Within the series are

8 nutritionally essential metals (Table 2.1). The complexes that form with transition elements tend to be highly colorful. For example, iron complexes are red, copper takes on a blue tinge, and chromium and nickel have green and blue complexes, respectively. These colors arise by light-induced electronic transitions between partially filled 3d suborbitals. Zinc (Zn^{2+}) with all 10, 3*d* electrons in place is prohibited from undergoing electronic transitions between orbitals in response to external light energy. Consequently Zn^{2+} cannot absorb light and its complexes are colorless and proteins with zinc in their structure have no zinc-related spectral properties.

2.2.1. 3*d* Orbital Geometry

The 5 suborbitals in a 3*d* orbital occupy the total space around the nucleus. From their orientation there may be many as 6 binding sites for other atoms (ligands). As noted in Figure 2.5, octahedral complexes

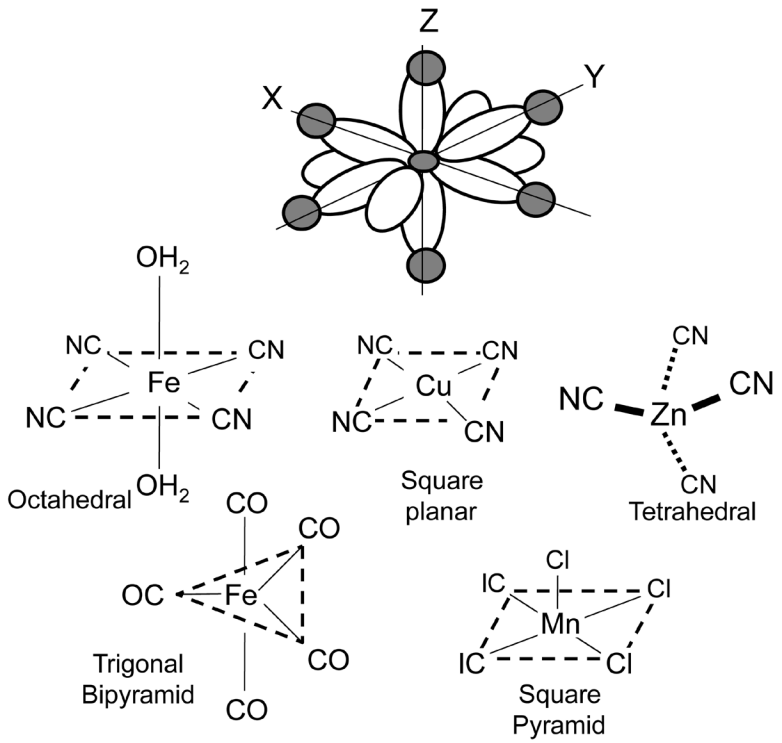

FIGURE 2.5. Complexes that form from interaction with 3d orbitals. Note how shape of complex conforms to shape of orbitals.

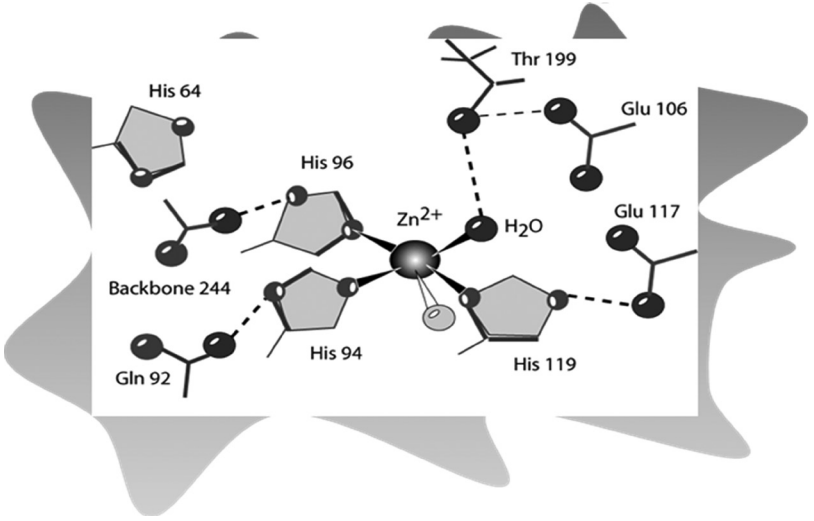

FIGURE 2.6. *Geometry of Zn^{2+} at the active site of the enzyme carbonic anhydrase.*

form when 6 ligands approach the central metal ion, 4 in a plane and 2 above and below the plane. Tetrahedral complexes feature 4 ligands spaced to minimize inter-ligand interference. The same is seen in a square planar arrangement in which all 4 ligands exist in the same plane with the metal ion in the center. Octahedral, square planar, and tetrahedral complexes are quite common. Zinc is capable of a 5-coordinate bonding as seen in the enzyme carbonic anhydrase (Figure 2.6). These basic arrangements tend to carry over when biological complexes of the metal are formed and basically translate into restraints that are put on proteins or nucleic acids to fit the metal ion into a compatible pocket that distorts neither the distances nor the direction of the orbitals.

2.3. PREDICTING PROPERTIES BASED ON CHEMICAL STRUCTURE SIMILARITY

Sharing electronic configuration has its drawbacks as well as advantages. Metal ions are known to compete with other metal ions for binding sites on proteins and entry portals in cell membranes. The strength of the competition is weighed by the structural similarity between the competing ions. Other than overlapping electronic structures and valences, a judgment of similarity also takes into account coordination number, which refers to the number of ligands that can be bound when

TABLE 2.2. Structural Similarity Based on Valence, Coordination Number and 3d Orbital Geometry.

Metal	Ion	Orbital	Configuration	Coordination No.
Copper	Cu^{+}	d^{10}	tetrahedral (dsp^3)	4
Zinc	Zn^{2+}	d^{10}	tetrahedral (dsp^3)	4
Cadmium	Cd^{2+}	d^{10}	tetrahedral (dsp^3)	4
Mercury	Hg^{2+}	d^{10}	linear (dsp)	2
Copper	Cu^{2+}	d^9	sq. planar (dsp^2)	4
Silver	Ag^{2+}	d^9	sq. planar (dsp^2)	4
Iron	Fe^{2+}	d^5	octahedral (d^2sp^2)	6

the metal forms a complex. Table 2.2 illustrates this principle for transition metals. Cu^{+}, Zn^{2+}, Cd^{2+} and Hg^{2+} all have closed shell (filled with 10 electrons) d orbital configurations. Based on the table, one is able to predict potential antagonism in the behavior of copper, zinc and cadmium. On the other hand, mercury should be unable to compete with any of these metal ions because mercury only favors a linear complex with two ligands. This observation is verified by observing that copper, zinc and cadmium all bind to the protein metallothionein (Chapter 3); mercury, however, shows no reaction. Also, zinc in the diet is very effective in blocking the absorption of copper (Chapter 15).

2.3.1. Ionization Properties

The driving force for atoms to gain or lose electrons is to achieve a stable electronic configuration. The target configuration is that of a noble gas in the same row of the Periodic Table. Metals have a propensity to lose the outer, higher energy electrons first, giving rise to ions deficient in one of more electrons. In viewing the Periodic Table, it is clear that first and second column elements lose 2*s* and 3*s* electrons, respectively, to obtain a stable "noble gas" core. Cations formed this way include Na^{+}, K^{+}, Mg^{2+} and Ca^{2+}—basically the major minerals in the macromineral class. Because the core is highly stable (referred to as a closed shell), these ions cannot form coordinate-covalent bonds with ligands, but instead engage other ions and organic molecules primarily through electrostatic (charged) interactions. They are further precluded from emulating the geometrical shape of transition element complexes. A closed shell also prohibits electron transitions between orbitals, and hence the ions and complexes of macrominerals (regardless of the ligand) tend to be colorless and are void of redox properties.

2.3.2. Oxidant and Antioxidant Properties of 3*d* Metal Ions

Having partially-filled 3*d* orbitals allows a metal ion to accept or donate electrons concomitant with a change in the valence state. In Equation (1), a single 3*d* electron from Fe^{2+}, when transferred to hydrogen peroxide, forms a hydroxyl radical, the most dangerous free radical in a biological system. In contrast, a single electron from the radical superoxide anion, when transferred to Cu^{2+} in the enzyme *superoxide dismutase*, reduces and gives rise to Cu^{+} without harming the enzyme. The electron borne on Cu^{+} is then transferred to a second superoxide anion, giving rise to hydrogen peroxide and molecular oxygen, destroying two radicals in the overall process [Equation (2)].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH^{\bullet} \quad (1)$$

$$Cu^{2+} + O_2^{-} \rightarrow Cu^{+} + O_2 \quad (2)$$
$$Cu^{+} + O_2^{-} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2$$

2.4. SUMMARY

Quantum theory of atoms helps us correlate electronic states with mineral properties. For our purposes, it gives us insight into the scope and limitations of these properties. Monovalency and divalency of important minerals can be rationalized by knowing the ease with which one or two electrons, respectively, are lost upon ionization. The importance of electron orbitals and valence is made even more relevant when considering transition metal ions that comprise most of the micromicrominerals such as iron, copper, zinc, manganese, etc. Well-defined geometrical complexes with proteins and other ligands are the result. Multiple valences allow these minerals to accept and donate electrons. For a protein to accommodate a metal ion will depend on whether that protein has a binding pocket that is in synchrony with geometric constraints of the metal ion, a key determinant of selection. Gaining and losing electrons is a major property of redox minerals. Insights into electronic structure shows us why macrominerals cannot be used as redox metals and are more fitting as stable ions. The electronic state of a mineral helps us understand what a mineral can and cannot do in a living system. It also gives us insight into the potential

for toxicity incurred by non-biological metals having the same electronic structure, and therefore able to thwart the biological metals at the functional site.

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2.6. PROBLEMS

1. Draw the electron configuration of sodium and explain why Na^+ is the only ionized form.

2. What electrons are lost when calcium ionizes?

3. Name all atoms with the principle quantum number $n = 2$

4. Iron exists as 2^+ and 3^+. Explain how this happens.

5. What is the minimum principle quantum number for an atom with *p* orbitals? *d* orbitals?

6. Can an octahedral complex be formed with a metal that has no *d* orbitals?

7. Of the two, Zn^{2+} and Cu^{2+}, which has diamagnetic properties? Explain why.

8. Which of the following would be considered "closed shell" ions: Ca^{2+}, Mg^{2+}, K^+, Zn^{2+}?

9. Identify the elements that correspond to the following electron configurations:
   a. $1s^2 2s^2 2p^2$ _____
   b. $1s^2 2s^2 2p^6$ _____
   c. $1s^2 2s^2 2p^6 3s^2 3d^5$ _____
   d. $[Ar]4s^2 3d^{10}$ _____
   e. $[Ne]2p^6 3s^2$ _____

10. Predict the valence and identify the element with the following configuration:
    a. $1s^2 2s^2 2p^6 3s^2$ _____
    b. $1s^2 2s^2 2p^6 3s^2 3p^6 4s^2$ _____
11. What is the electronic configuration of the following ions? (Hint: ions will not have the same configuration as atoms. You want the ion's configuration.)
    a. H^+ _____
    b. Mg^{2+} _____
    c. Cu^{2+} _____
    d. Cu^+ _____
    e. Fe^{3+} _____
    f. Co^{3+} _____

CHAPTER 3

Biochemical Insights into Minerals

BIOCHEMISTRY allows one to view minerals in a natural setting. These insights are needed for several reasons. First, to show that most minerals in the system are bound to proteins and other ligands. Second, to see how minerals fit into the general scheme of biochemical events. Third, to realize that selection of a particular mineral must be compatible with the biochemical function it performs. Basically, if a mineral is to be designated essential, its biochemical function(s) must be known. Later chapters will discuss the biochemical properties of individual minerals. In this chapter, we key on some important mineral complexes and see how form is inseparable from function. Specific objectives are:

1. To learn the natural state of biominerals as they exist within the organism
2. To identify complexes of minerals with proteins and other macromolecules
3. To see how distinguishing a metal-activated from a metalloenzyme depends on the mineral cofactor
4. To relate a mineral's function to its structure, ionic state, and cellular location.

3.1. THE FUNDAMENTALS

Minerals are everywhere . . . in organs, tissues, in every cell and fluid

of the body. Serum is a rich source of sodium and chloride ions, the cytosol is filled with potassium ions, bone is composed of crystalline calcium and phosphate, red blood cells are rich in heme iron, and the nucleus has many zinc-bound proteins. This brief scenario captures the omnipresence of minerals in living systems. One must pause to consider the potential chaos that such a picture presents. With minerals everywhere, what determines order, specificity or function? Are minerals unique or can the function of one be duplicated by others? The answer to these questions gives us insight into the statement: "no two minerals are alike because no two can have the same biochemical properties".

3.2. BIOCHEMICAL PROPERTIES OF MINERALS

The view that minerals are abundant components of all living systems is reflected by the multitude of biochemical processes that cannot function without minerals and the variety of forms in which they occur. Table 3.1 gives an overview of mineral requirement in a living system. Three points become apparent from the table: (1) many minerals per-

TABLE 3.1. Biochemical Forms and Functions of Minerals.

Mineral	Major Forms	Functions
	Macrominerals	
Na^+, K^+, Cl^-	Free ion	Ion currents, Osmotic balance
Calcium	Crystalline complex	Bones and teeth, Muscle contraction, blood clotting
Phosphorus	Phosphate	Sugar phosphates, phospholipids, ATP, RNA, DNA
Magnesium	Complex with ATP	Enzyme cofactor
	Microminerals	
Iron	Heme and non-heme	Oxygen transport, Electron transport
Zinc	Protein-bound	Metalloenzymes, Cell signaling
Copper	Protein-bound	Cu-metalloenzymes
Manganese	Protein-bound	Mn-metalloenzymes
Molybdenum	Protein-bound	Mo-metalloenzymes
Cobalt	Vitamin B12	B12-enzyme
Nickel	Protein-bound	Ni-metalloenzymes
Chromium	Unknown	Insulin activator
Selenium	Selenocysteine	Peroxidation reactions
Iodine	Thyroxine	Thyroid hormone

TABLE 3.2. Mineral Cofactors for Enzymes.

Inorganic Cofactor	Function	Enzyme Class
	Macrominerals	
Magnesium	ATP activation, substrate binding	Transferases
Calcium	substrate activation	Hydrolases
Potassium	substrate binding	Transferases
	Microminerals	
Iron	oxygen binding, electron transport	Oxidoreductases
Zinc	substrate binding structural stability	Lyases, ligases
Copper	dioxygen activation	Oxidoreductases
Manganese	oxygen radical dismutation	Oxidoreductases
Cobalt	intramolecular shifting	Isomerase (with B12)
Selenium	peroxide destruction	Oxidoreductases

form more than one biochemical function; (2) complexes with organic molecules are the predominate form of the minerals; and (3) functions performed by macrominerals for the most part are not duplicated by microminerals and vice versa. As an example of (1), note that calcium is a component of bone but it is also needed in a protein-bound form to activate muscle contractions and cause blood to clot. Iodine and selenium, respectively, give thyroid hormones and peroxidase enzymes their function. Enzymes are perhaps the most dominant category of mineral-dependent functional molecules. Table 3.2 shows that minerals, mostly metal ions, appear as cofactors for all the classes of enzymes. Indeed, it has been estimated that about a third of all enzymes have minerals as cofactors.

3.3. BIOCHEMICAL FUNCTIONS OF MINERALS

3.3.1. Distinguishing Macro- from Micromineral Dependent Functions

As noted in Table 3.1, there is little duplication of functions performed by macrominerals and microminerals. By exploiting a greater abundance in the system, macrominerals are structural components (bones and teeth), or control osmotic fluid balance and create energy gradients across cell membranes. In contrast, microminerals (due to their scarcity) function as coenzymes and regulatory factors, thus gen-

erating energy indirectly. These, of course, are not hard fast rules, but they do explain the difference in the function of these two families of minerals in a living system.

3.3.2. Function of Minerals in Metabolism

Life is sustained by a series of well-regulated biochemical reactions designed to maintain the status quo in energy and cellular constituents. A large part of the maintenance is based on channeling energy to and from cells. Nutrition goes one step further by labeling molecules in foods as energy-rich (or poor) or nutrient-rich (or poor). As to being energy rich, the principles of chemistry state that to yield energy, a food molecule must be oxidized or have its structure altered chemically. Moreover, part of the energy released by the oxidation must be set aside for future use. Although minerals cannot be considered energy sources, they can and do assist cells in extracting and preserving energy from foods. *Metabolism* refers to the series of internal reactions that lead to chemical change, or more specifically *catabolism* when the focus is on the destructive processes that derive energy as opposed to *anabolism* which use energy. To appreciate the role of and scope of minerals in these reactions will be a valuable lesson in learning the role of minerals in the overall process of metabolism.

3.3.3. Minerals Required to Metabolize Glucose

As an example of the importance of minerals in metabolism, consider the energy obtained by postprandial aerobic oxidation of glucose. The total energy yield conforms to Equation (1):

$$C_6H_{12}O_6 + 6O_2 \rightleftarrows 6CO_2 + 6H_2O \quad (1)$$

Arriving at the final products requires a series of enzyme-catalyzed reactions that take place both within the cytosol and the mitochondria. Figure 3.1 shows where minerals take an active part, beginning with absorption across the intestine and ending with the release of CO_2 and H_2O. Absorption of glucose into intestinal cells strongly depends on a gradient of sodium ions to drive glucose into the cells and potassium ions to restore the gradient (Chapter 10). Upon entering the blood stream the glucose is transported to the liver, where it is catabolized

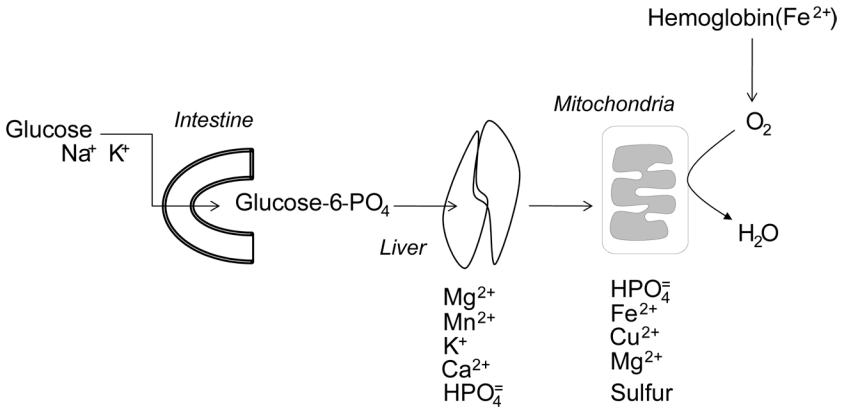

FIGURE 3.1. *Minerals Required for the Metabolism of Glucose.*

by a series of enzymes that require Mg^{2+}, K^+ and Mn^{2+} as cofactors. Inorganic phosphate from ATP is transferred to the glucose molecule from a Mg-ATP complex. Binding the phosphate group prevents free diffusion of the glucose from the cell and primes the glucose molecule for enzymes that recognize phosphate-bound sugars as metabolites destined for chemical change.

The products of the first phase are channeled into the mitochondria where the final stages of energy extraction takes place. Enzymes in the Krebs cycle owe their activity to Fe^{2+}, Mg^{2+} and phosphate. Hemoglobin with iron entrapped as heme brings oxygen into the cell to drive the electrons down the transport chain. In the final stage, Fe and Cu mediate the transfer of electrons to oxygen, forming water as a product. A byproduct of all of these steps is ATP, which is formed by condensing phosphate with ADP which preserves most of the energy from the extraction steps. Adding up all the steps, it can be seen that at least eight different minerals, free or as complexes, take an active part in the aerobic metabolism of glucose to CO_2 and H_2O.

3.3.4. Minerals as Cofactors for Enzymes

The bulk of the sight-unseen minerals alluded to in Figure 3.1 are associated with enzymes. As noted, one-third of all enzymes require a metal ion as a cofactor. Enzymes catalyze chemical changes in cells. The indispensability of its cofactor can be traced to the number of functions the cofactor is capable of performing. These include (1) stabiliz-

ing the structure of the enzyme, (2) assisting the substrate in binding to the active site of the enzyme, and (3) preventing electrons removed from a substrate from contacting the delicate structure of the enzyme. The large category of enzymes that require metal ions as cofactors can further be subdivided into those in which the metal ion is in equilibrium with the enzyme and those where it is firmly attached to the structure. The former are referred to as "metal-activated" and the latter as "metalloenzymes". Table 3.3 compares their properties.

3.3.4.1. Metal-Activated Enzymes

By definition, a metal-activated enzyme does not form a tight chemical bond with its metal ion cofactor. Rather, the metal ion exists in a state of equilibrium with the enzyme. Upon removal of the metal from the vicinity of the enzyme, the enzyme loses activity. Such occurs when the enzyme is isolated from the tissues. Adding back the metal ion is necessary to restore activity. Being in an equilibrium state, the addition of more metal ion into the medium will have a positive effect on the catalytic efficacy of the enzyme.

3.3.4.2. Metalloenzymes

Metalloenzymes, in contrast, bind the metal strongly to the surface of the enzyme protein. The metal is basically an integral part of the enzyme's structure. For many metalloenzymes, the metal ion is at the enzyme's active site. Because the binding is tight, there is no equilibrium. Instead, there is an integral number of metal ions per enzyme molecule. Adding more metal ion will not improve the catalytic efficiency. Isolated metalloenzymes retain their function despite being removed from the biological milieu, which speaks of the strong metal-protein bond. Table 3.4 shows some metalloenzymes and their cofactors.

3.3.4.3. Selection of the Metal Ion Cofactor

One of the most intriguing aspects of metal-activated versus metalloenzymes is in the nature of the metal ion cofactor. For metal-activated enzymes, the cofactor exists partly or wholly as a free ion. Macrominerals such as K^+, Mg^{2+} and Ca^{2+} fit this property. In contrast, metalloenzymes accept metal ions of the first transition elements. Included are Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}. Indeed, sequencing the human genome has

TABLE 3.3. Comparison of Properties of Metal-Activated vs Metalloenzymes.

Metal Activated Enzymes	Metalloenzymes
Metal ion is in equilibrium	Metal ion is firmly bound
No stochiometry	Fixed number of metals/protein
Metal ion lost on isolation	Metal ion retained on enzyme
Macro-metals are main factors	Micro-metals are main factors

revealed that as many as 900 proteins have zinc binding sites, and of this number more than 300 are enzymes. Ca^{2+} as a metal ion cofactor is unique because it straddles the boundary between metal-activated and metalloenzymes. Some hydrolase enzymes (those that break bonds by adding water across the bond) require Ca^{2+} in the medium for maximum function, whereas Ca^{2+} in the enzyme thermolysin is bound strongly to the enzyme's surface.

3.3.5. Metalloproteins

By definition, metalloproteins are a broader class of proteins that bind metals. Most have no perceived catalytic activity but instead are used mainly to store or transport metal ions (Table 3.5). Their role in storage is linked to detoxification, which basically prevents or limits

TABLE 3.4. Examples of Metalloenzymes and Their Cofactors.

Iron	Calcium
Ribonucleotide reductase	Thermolysin
Cytochrome oxidase	Manganese
Zinc	Arginase
Dehydrogenases	Water splitting enzyme
Aminopeptidase	Pyruvate carboxylase
Carboxypeptidase	Molybdenum
Carbonic anhydrase	Nitrogenase
RNA, DNA polymerase	Xanthine oxidase
Copper	Cobalt (Vitamin B12)
Superoxide dismutase	Homocysteine transmethylase
Tyrosinase	Methylmalonyl CoA mutase
Lysyl oxidase	Nickel
Peptide-α-amine monooxidase	Urease
Cytochrome oxidase	
Dopamine β-monooxygenase	

TABLE 3.5. Examples of Metalloproteins.

Protein	Function
Calmodulin	Ca binding, regulator
Calbindin	Ca transport
Metallothionein	Cu, Zn, Cd storage
α_2-macroglobulin	Zn transport in plasma
Serum albumin	All purpose metal ion transport
Selenium proteins P and W	Selenium transport in plasma and muscle
Transferrin	Fe, Mn transport protein
Ferritin	Fe storage protein

heavy metal toxicity when metal ions amass inside cells. For example, the protein ferritin, an iron-binding protein, is capable of binding up to 5,000 atoms of iron. The protein is generally considered a storage form of iron inside cells. Metallothionein binds zinc, copper and cadmium, utilizing the –SH group on the amino acid cysteine to bind the metal ions. Up to a third of the amino acids in metallothionine are cysteine. Transferrin in plasma binds and transports iron. Each transferrin molecule can hold two iron atoms for transit and delivery to tissues. Albumin in the plasma also binds and transports metals, mainly zinc and copper. Thus, metalloproteins have a varied but highly important role in maintaining cells in a healthy state and keeping physiological systems functional.

3.4. BIOMINERALIZATION

The combination of calcium with phosphorus (phosphate) and calcium with carbon (carbonate) are two examples of minerals being deposited en masse for the purpose of forming major crystalline structures. Familiar examples include bone, tooth enamel and the shell of an egg. These unique happenings should not go unnoticed. Bone is brought about by layering units of hydroxyapatite, a calcium phosphate complex, over the organic matter (collagen) to form a hard crystalline surface. This process occurs spontaneously and requires osteoblasts and osteoclasts, two special bone forming and remodeling cells (Figure 3.2). Egg formation requires a special organ called the shell gland. Mineralization begins with the deposit of calcium carbonate around a fibrous collagen protein layer that encompasses the yolk and ovalbumin

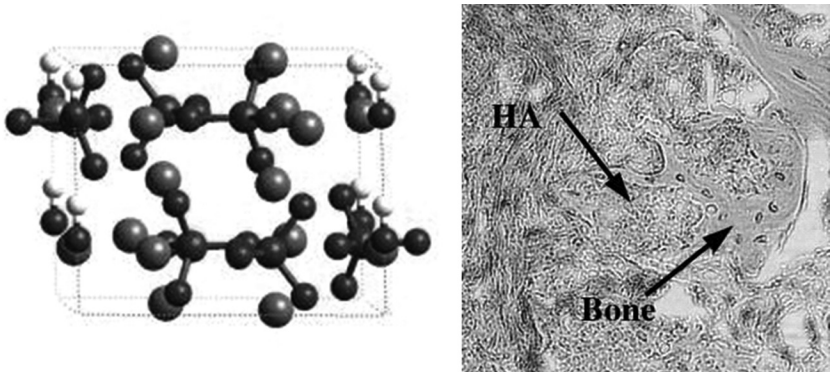

FIGURE 3.2. *Biochemical Structure of Bone.*

as it passes down a tube. Putting on a crystalline coat takes place in the final stage and involves adding the same quantity of calcium and carbonate committed to the shell, regardless of the size of the yolk and ovalbumin core. Large diameter cores, therefore, could result in thin-shell or even no-shell eggs. For the consumer, this manifests as large and extra-large size eggs caused by a mineralization process that cannot adjust to egg size.

3.5. SUMMARY

When we view a mineral in its biological setting, it becomes apparent that minerals are present in a multitude of biochemical forms. Only a few exist entirely as free ions. One reason is that ions in a free state have limited biochemical capabilities. The biochemical form reflects the active form of the mineral and in many instances applies to only one mineral. Na^+, K^+ and Cl^- are only present as free ions in the system and therefore can have only one biochemical form. In contrast, iron has many. For instance, iron is one of the few metal ions that exists in a heme-like structure with porphyrin. Heme is the active form of iron in many proteins. Only cobalt can activate vitamin B12. It is not unusual for one mineral to have more than one function. Calcium in the bone is the same calcium, but a different form, of the calcium that modulates muscle contraction and elicits responses in cell signaling. A large group of minerals is bound to proteins and most of these are cofactors for enzymes. Two categories of mineral-dependent enzymes, “metal-activated” and “metalloenzymes”, differ in the strength

of metal binding—whether the metal is in equilibrium or firmly fixed to the structure. Metal ions with redox properties tend to be bound to enzymes that remove or add electrons to substrates. Iron is unique in being able to reduce O_2 to water yet transport O_2 to tissues intact. Iron is also found in enzymes that use O_2 as a substrate. These three unrelated functions of iron are consistent with distinctly different biochemical forms. Zinc is important in enzyme cofactors and as a regulator of genetic expression. Minerals amass to the greatest extent in the calcium-phosphate matrix of bones and teeth and the calcium carbonate coat of egg shells. The biochemical properties of minerals reveal the multitude of ways minerals function in biological systems. On closer inspection, it becomes apparent that appearance dictates the function a mineral is designed to perform. Some of these, nonetheless, still remain a mystery, such as why iodine must be bound to thyroid hormones in order for the hormone to be active.

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3.7. PROBLEMS

1. Explain the difference between a metalloprotein and a metalloenzyme. Give two examples of each.

2. Why do some enzymes lose catalytic activity when isolated in a pure state?

3. Obtain a biochemical textbook to answer the following.
   a. What role do chloride ions play in the absorption of glucose?
   b. A kinase enzyme uses ATP as a substrate. What mineral is needed for the enzyme to function? What function does the mineral perform?
   c. The enzyme pyruvate carboxylase uses a mineral as a cofactor. Name it.
   d. Where is iron found in the electron transport system in mitochondria? How many different forms of iron are present? Where is copper found in the mitochondria?
   e. What is the biochemical form of Na^+ and K^+ in living systems? How many different forms of these two ions are present?

4. Where do you find hydroxyapatite in a biological system? What is hydroxyapatite?

5. Calcium carbonate also occurs in crystalline form. Where would you find this mineral?

CHAPTER 4

Bioavailability of Minerals in Foods

FOODS are composed of proteins, fats, carbohydrates, minerals and water—the so-called "foundation biochemicals". No two foods provide the same quantity and quality of nutrients; this also applies to minerals. Dairy products rich in calcium are nonetheless poor sources of iron, zinc and copper. With an increased emphasis on plant-based foods in the diet, concern has been raised as to the bioavailability of plant minerals as compared to minerals from animal sources. Nutrition and Food Science come together in closely examining the potential threat of mineral deficiencies and oversupply to the health-promoting properties of foods. Technical advances in food processing have focused on preserving mineral elements during processing, storage, and cooking and lowering levels of minerals such as sodium that can have adverse effects. This chapter takes a closer look at the steps food science is addressing to assure that food sources meet the standards of a healthy diet. Specific objectives are:

1. To examine the quantity and quality of biominerals in foods
2. To identify steps in food processing that affect minerals
3. To investigate if food processing procedures influence mineral bioavailability
4. To view minerals in foods from the standpoint of food safety.

4.1. HISTORICAL PERSPECTIVE

Government initiatives in food processing were initially intended to

address issues of food safety. The conditions of slaughterhouses, when brought to light, threatened American food exports. During Theodore Roosevelt's administration, Congress passed legislation under the auspices of the Pure Food and Drug Act to address issues of food safety and in 1927 passed legislation giving rise to the Food, Drug, and Insecticide Administration [shortened to the Food and Drug Administration (FDA)] to enforce the Pure Food and Drug Act. Among the many charges garnered by the new agency was the power to regulate food supplements and insecticide levels in foods. In these early beginnings, the minerals in food were not an important issue. This all changed when studies reported that sheep and cattle foraging on plants or plant products grown in soils deplete or overly unbalanced in one or more essential minerals showed impaired growth, health and gave rise to substandard commodity products (wool, meat, etc.). Periodic changes in the grazing area effectively resolved the problem and reversed the deficiency signs. Elements in the soil thus became a focus. In time it was found that a cobalt- or selenium-deficient soil could be linked to specific symptoms. The swayback in lambs, the white muscle disease in sheep, and fallings disease in cattle were all shown to have common ground in mineral deficiencies. Seminal to humans was the discovery that higher incidences of goiter could be traced to lower levels of iodine in the soil. Such findings promoted a strong awareness of the value of minerals to growth and development and stimulated interest in mineral-dependent functions that could explain the deficiency symptoms. More importantly, they shifted the emphasis to the minerals in foods.

4.2. THE FUNDAMENTALS

The minerals in foods contribute to flavor, texture, and when digested provide the cofactors for enzymes that assist digestion. Their content in any one food source will depend on climatic changes, agricultural practices and genetics. Minerals are also responsible for food spoilage while in storage and must be viewed in the context of shelf life and food safety. Nutritional issues address the ease with which minerals are rendered bioavailable to the organism. A major concern is the losses incurred during processing and cooking that render the processed food item less mineral-rich than the raw product. The minerals retained are in the digestate presented to the intestine for absorption. Digestion, however, has limitations. Undigested fibers in food can engage iron,

zinc, copper, calcium and magnesium ions and limit their bioavailability to the organism. Plant foods, particularly legumes, are rich in organophosphate compounds that limit absorption of certain key minerals. To confront these problems and as a means to restore intended levels, food manufacturers and processors use supplementation and biofortification to enhance the content of important minerals in foods.

4.3. BIOAVAILABILITY OF FOOD MINERALS: EFFECTS OF PROCESSING

The nutritional term "bioavailable" refers to a fraction of the total amount of a nutrient taken into the system that becomes functional to the organism. In food processing, the term further infers the amount remaining in a processed food source up to the time of consumption (Figure 4.1). That fraction then is referred to as "chemically available". Chemically available further denotes the highest level that can be "biologically available" to the organism.

Chemically available minerals are determined by measuring specific minerals in the ash after the organic matter has been destroyed by calcinations or combustion. Most take the form of metal oxides, phosphates, sulfates, nitrates, and halides that can be quantified by various colorimetric and spectrophotometric procedures—procedures that can be sensitive to the nanomolar (10^{-9}M) or picomolar (10^{-12}M) level of the nutrient.

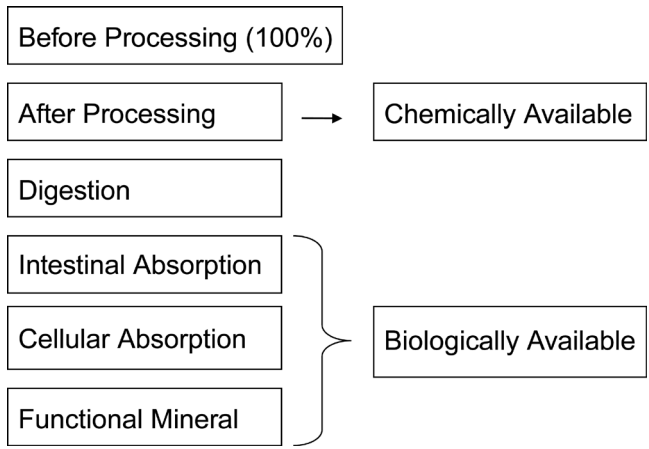

FIGURE 4.1. *Steps in Food Processing that can Impact on Mineral Content.*

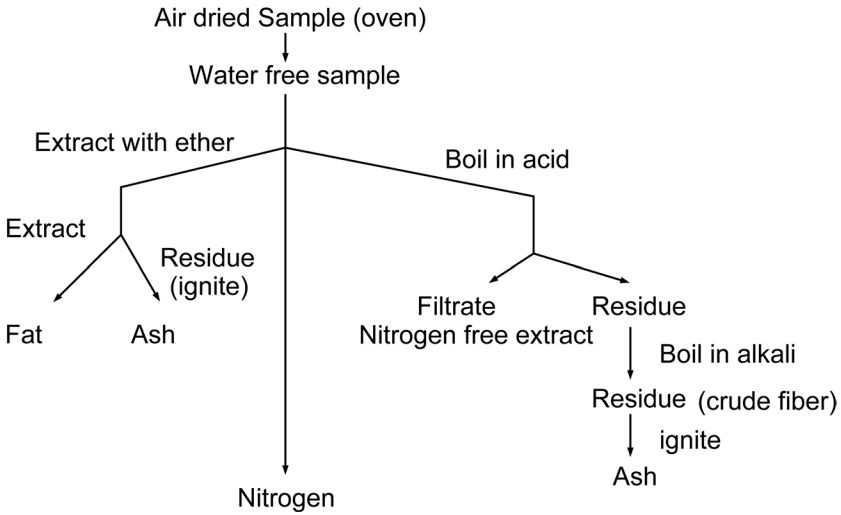

FIGURE 4.2. *Basic Scheme of Proximate Analysis. Sample to be assessed is first oven dried and treated successively with acid and alkali. The residue from the alkali is the crude fiber fraction. Ash from ether extract and the residue from the crude fiber are used to assess the mineral content.*

4.3.1. Determining Chemically Available Minerals in Forage

4.3.1.1. Proximate Analysis

Proximate analysis was first performed in 1865 at the Weende Agriculture Experiment Station in Germany. The analysis was designed to learn why certain feeds fed to cattle were more advantageous than others for growth. By dissecting the feed into its composite parts and emulating digestive processes that occurred in the rumen (exposure to acid and alkaline solutions), the investigators hoped to learn the biochemical basis for feed superiority. The basic scheme is shown in Figure 4.2. A sample to be analyzed was first oven dried then treated successively with boiling acid and alkali. The fraction that resisted digestion was carefully weighed at each stage. Fat content was determined separately by extracting an untreated sample with an organic solvent such as ether. The mineral content was estimated by weighing the ash from each fraction after combustion. The outcome of the experiments was to show that the feed consisted of a series of major component ingredients. Nondigestible material was referred to as "crude fiber" and because it resisted digestion was considered to have little nutritive value. Because the outcome was based solely on weight, these gross analyses were unable

to discern potential differences in micronutrient content, which today are known to have a major impact on growth. Today, the food industry still uses a modified proximate analysis scheme with basically the same goal in mind: to equate superior food quality with the ingredients in the food.

4.4. FOOD PROCESSING STRATEGIES AND MINERAL BIOAVAILABILITY

Food processing strategies are designed to make foods healthier, taste better, and increase shelf life. Processing also increases accessibility of micronutrients and decreases anti-nutrients. Because processing of plant foods uses procedures such as heating, grinding, soaking, fermentation, germination/malting, etc. to achieve these goals, processing can have a telling effect on mineral bioavailability. Mineral losses during processing, particularly microminerals, are irretrievable.

Depending on the particular plant or mineral, processing can also have a beneficial effect on mineral nutrition. Saying this is beneficial relates to rendering minerals in a food's matrix more diffusible and absorbable. For example, blanching and cooking of spinach leaves has been shown to improve the extractability of iron, calcium and zinc (Yadav and Sehgal, 1995; 2002). Table 4.1 illustrates the more negative aspects of processing. Minerals such as calcium, iron, and zinc are lost by milling and heat treatments; the higher the degree of milling or the longer the exposure time, the greater the proportion of minerals lost. Likewise, packaging can alter food composition and thus influ-

TABLE 4.1. Processing Procedures that Affect the Mineral Content of Food.

Thermal Threats		Mechanical Threats	
Detrimental	**Beneficial**	**Detrimental**	**Beneficial**
Sterilization	Baking	Milling	Canning
Pasteurization	Blanching	Extrusion	Fermentation
Boiling		Soaking	
Steaming		Drying	
Frying		Freezing	
Blanching		Storage	
Baking		Packaging	

ence mineral bioavailability (Johnson, 1991). Although baking removes phytic acid—a major anti-nutrient (see below)—baking also destroys vitamin C, an important stimulator of iron absorption (Hallberg, 1981). These dual effects that impinge on mineral bioavailability illustrate the scope of problems one encounters when attempting to assure food products retain acceptable bioavailable levels of minerals.

Perhaps the greatest loss of minerals from foods occurs in the kitchen. Minerals leaching into hot cooking fluids are of particular concern. Other losses occur by forming heat-induced chemical complexes that bind minerals and deter their absorption. Reactions between reducing sugars and amino acids or proteins—part of the so-called browning reactions—are examples of the latter. Browning reaction products are more resistant to digestion and hence capable of retaining their mineral-binding properties intact in the lumen.

4.4.1. Anti-Nutrients in Plant Foods

4.4.1.1. Phytic Acid and Phytases

Key divalent metals such as Ca^{2+}, Fe^{2+}, and Zn^{2+} have the capacity to form complexes with phytic acid (phytate), fiber, and tanning and lectin compounds. The phytates in particular are common to cereal grains and because they target divalent metal ions, phytates compete with absorbing cells for Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+}. The outcome is a deficit of these essential minerals to the organism. Consequently, negating the action of phytates has received considerable attention in food processing procedures. Treating the grains of cereals with phytases (enzymes that destroy the phytates), a process known as dephytinization, is one approach that has been used with success. When applied to infant cereals, dephytinization increased the uptake, transport and retention efficiency of iron and zinc (Frontela *et al.*, 2009). Calcium uptake is also enhanced by this treatment.

4.4.1.2. Oxalic Acid and Oxalates

Partially or completely ionized salts of oxalic acid (oxalates) form water soluble complexes with Na^+, K^+ and (NH_4^+), but insoluble complexes with Ca^{2+}, Fe^{2+} and Mg^{2+}. With the exception of Zn^{2+}, which is relatively unaffected, oxalates are on a par with phytates as anti-nutrients that target divalent cations. At a pH of 2, oxalates exist as mon-

ovalent ions primarily in association with potassium as the counter ion. Above pH 6, the potassium salt is completely dissolved and the oxalate anion is free to complex with Ca^{2+} and Mg^{2+}. Thus, acid sensitivity is an important consideration for stability of the complex. Soaking or cooking foodstuff removes oxalates from certain foods such as tea leaves, spinach or cocoa—foods that may contain as high as 300 to 2000 mg of oxalic acid (Noonan *et al.*, 1999).

4.5. REVERSING MINERALS LOSS BY BIOFORTIFICATION

Biofortification refers to ways to enhance the nutrients value of a food source. The term is generally applied to plants. Fertilization, plant breeding, and biotechnology are the most commonly used practices to achieve a biofortified product. Losses of minerals incurred during processing can be replenished by enriching the raw or unprocessed food product with the minerals. This can be accomplished either by direct addition or by environmental or genetic manipulation. As an example of the former, enriching rice with a powdered mineral mix followed by heating to coat the rice with an edible film is a good strategy for restoring minerals lost from rice hulls during milling. Akin to this is parboiling, a process that drives minerals from the hull into the endosperm of the rice grain before removing the hull or coat by milling.

4.6. MINERAL BIOTECHNOLOGY

In one of the earliest applications of biotechnology to plant minerals, rice seeds were iron-fortified by overexpressing a soybean ferritin gene (Goto *et al.*, 1999). The amount of iron the treated rice retained in the endosperm was as much as three-fold over non-treated rice. In a related application, enriching the calcium content of carrots was achieved by over expressing the CAX1 gene that codes for a vacuolar Ca/H^+ antiporter. Increasing the antiporter resulted in higher amounts of calcium in the edible portion of carrots. When consumed, the carrots provided twice the amount of calcium into the bones of experimental animals and over 40 percent more calcium to human subjects. A similar enrichment of calcium in tomatoes and potato tubes was obtained when CAX1 and CAX2 genes from Arabidopsis was over-expressed in these plants (Park *et al.*, 2005; Kim *et al.*, 2006). Calcium enrichment did

not harm the tomato; on the contrary, it gave the tomato a longer shelf life. Because the Ca/H^+ anti-porter mediates passage of other minerals besides calcium, however, manipulating the CAX1 gene has the potential to increase the bioavailability of other minerals in the tomato and carrot. More recent studies, however, seem to suggest that only calcium was enriched. An important concern, nonetheless, is that when grown, the genetically altered plants may show indiscriminant uptake and accumulation of toxic metals such as cadmium and lead, in addition to calcium. Such unwanted accumulation can be minimized, however, by genetically modifying critical residues at the metal binding site, tailoring them to bind only calcium (Shigaki *et al.*, 2005).

4.7. ISSUES OF FOOD SAFETY AND MINERALS

4.7.1. Toxic Mineral Absorption by Plants

Because soil-grown plants are unable to screen harmful minerals from beneficial ones, toxic minerals in the soil can be a serious health threat, particularly if the toxic minerals are absorbed into edible parts of the plant. The same concern applies indirectly to meat products obtained from animals grazing on plants grown in soils exposed to toxic metals. For them, there is also the risk of essential mineral insufficiency, which can have the same impact as a toxicity. The source of toxic minerals could be deposits of sludge, spillage, or mining operations conducted in the area or even the fertilizer applied to the field. Minerals seemingly of greatest concern are arsenic, cadmium, mercury, lead, and selenium (McLaughlin *et al.*, 1997). Cadmium in particular tends to build up in body tissue over time and is not readily removed from the system. Arsenic poses a primary threat to drinking water. Arsenic as well as chromium are known to be carcinogens, which places a special concern for arsenic-contaminated rice.

4.7.2. Redox-Active Metal Ions

A mineral liberated from its food confines is not necessarily a safe mineral. This is especially true for transition metal ions such as Fe^{2+} and Cu^{2+} that have the capacity to act as pro-oxidants and catalyze formation of free radicals that destroy organic molecules in membranes and cell nuclei. In their free state, redox-active metal ions can lead to

spoilage and shorter shelf lives. Fatty acids and fat soluble vitamins are particularly vulnerable. Despite this apparent hazard, supplementing food products with iron is still a common practice for enhancing food quality. To minimize exposure, the iron is generally added at a late stage in the processing procedure. Because it can release a single electron, ferrous sulfate is particularly dangerous, with the potential to form reactive oxygen species (see Chapter 3); iron compounds less prone to food spoilage tend to have lower bioavailability (Hurrell, 1989). A second concern of redox-active metals is their effects on food storage and shelf life. In addition to destroying essential vitamins and fat, redox-active minerals can catalyze peroxidations that can lead to rancid food products.

4.7.3. High Sodium Content

Ever since common table salt was linked to hypertension and risk of heart disease, the food industries have responded by taking steps to lower the amount of sodium in a processed food source. Lowering sodium, however, comes with consequences. Foods with reduced sodium can show differences in texture and palatability and have a lower shelf life. To the consumer, a low salt food could mean modifying the cooking procedures to fit the low salt. Changes in microwave heating patterns and cooking time may be required. There is also a requirement to eliminate the risk of a wide range of microbes in the food—microbes that could cause food-borne illnesses. Optimizing cooking strategies may require a different type of cooking procedure. One such technique still in the experimental stages is the use of high hydrostatic pressure. This procedure not only stops microbial growth, but also restores the quality of the low salt food.

4.8. A COMPARISON OF MINERALS IN FOODS FROM ANIMALS AND PLANTS

Although most of the emphasis of food minerals has been on plants, there remains to be decided whether plants or animals are the better source of essential minerals. Table 4.2 shows that foods from plants tend to be richer in calcium, potassium and phosphorus, whereas dairy products exceed plants as sources of calcium. It's important to note that quantity alone does not always translate into better bioavailability. Be-

TABLE 4.2. Comparison of Calcium, Potassium and Phosphorus Content of Foods from Plants and Animals.

	Calcium	Potassium	Phosphorus
Plant			
Brown Rice	10	250	310
Peanuts	16	183	100
Spaghetti	15	146	110
Lentils	15	287	40
Peas	21	330	118
Broccoli	89	588	138
Potato	23	119	190
Animal			
Hard Cheese	202	27	143
Beef	3	85	134
Egg White	4	94	8
Milk	252	306	209

low is a list of advantages foods from animals have over plant foods as sources of dietary minerals:

1. Foods from animals such as meats, eggs, and dairy products undergo very little processing before being submitted to consumers, which basically means that bioavailability is determined after, not before, consumption.
2. Animal products regardless of tissue source are void of phytates and oxalates, thereby avoiding two major anti-nutrients that interfere with mineral absorption. In food from plants, phytate is the major biochemical source of phosphorus in the plant. In animal foods, phosphorus is broadly distributed among the major biochemical categories of nutrients—proteins, fats and carbohydrates.
3. The iron in animal proteins, muscle protein in particular, is a richer source of both heme and non-heme iron, the former generally considered to be a more absorbable and bioavailable form of iron.

Weighting the two can lead to no hard and fast conclusions as to which is better, although the lack of processing and the known losses that occur in processing tend to put animal products ahead of plants as preferred sources of minerals.

4.9. SUMMARY

There is ample evidence that certain procedures used by the food industry have the potential to alter the mineral content of foods and in so doing can be both detrimental and beneficial to mineral bioavailability. As a benefit, processing can increase absorptivity by enhancing the diffusability of food-locked minerals. The negative factors, however, are a greater concern to food science. The losses that occur through milling, extrusion cooking, thermal treatments, etc. can result in a food product that lacks essential minerals or falls below levels to retain health-promoting properties. Plant phytates and oxalates must also be weighed as confounding factors. Losses incumbent on processing can be addressed by enriching a food source with the minerals prior to processing. Another approach is biofortification, which employs biotechnology to modify the genetics of plants as a means to enrich the mineral content while not harming the plant. Unfortunately, this technique to date has only been applied to a few important minerals and may not be applicable to all in many cases. Lingering on the dark side of minerals is the need to be constantly aware of the potential toxic minerals that are manifested in crops grown in contaminated fields as well as in the vicinity of a processing plant. It is still undecided if plant minerals are less bioavailable than minerals from animals sources, although present understanding leans favorably towards animal products as the better source for some.

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4.11. PROBLEMS

1. Of the various procedures, determine which would be detrimental or have a minimum effect on the mineral content:
   a. The amount of Na^+ and K^+ in corn after boiling in water for 6 minutes
   b. The amount of calcium in a meat source after heating in the oven at 375 degrees
   c. The amount of iron in rice after boiling for 3 minutes in water
   d. The amount of zinc in lima beans after boiling in a water bath for 6 minutes
   e. The amount of selenium in broccoli after boiling in water for 6 minutes
   f. The amount of iron in spinach after cooking in vegetable oil

2. What is meant by the term anti-nutrient? Give examples and explain why these are classified as "anti". When using the term is it important to specify the mineral involved? For example, is there an anti-nutrient for Na^+ or Cl^-?

3. Explain how "packaging" affects the mineral content of foods.

4. There is the concern that soil contaminated with heavy metals risks having these metal ions transferred to plants that are grown in that soil. What does this tell you about the ability of food plants to discriminate between essential and non-essential minerals? Also, what does this tell you about the ability of plants to overcome deleterious effects of toxic minerals?

5. What could be the cause of a zinc deficiency in an isolated population of people whose main staple in their diet is unleavened bread? Explain your answer.

6. Why is eliminating or lowering the salt content from a food source not a good idea?

7. Give examples of processed and non-processed animal products. Repeat this for plant products.

8. Explain the theory behind overexpressing genes to confer greater density of minerals in a food source. How are the genes to be manipulated identified? Explain the rationale for using a ferritin gene as opposed to a transferrin gene; the Ca/H^+ anti-porter gene as opposed to a calbindin gene.

9. "Mary, Mary quite contrary, how does your garden grow?" List 3 minerals that Mary would not like to have in her garden? Explain why, for Mary's sake, these minerals are undesirable. Remember how the nursery rhyme ends before you answer the question.

CHAPTER 5

Nutritional Approaches to Minerals

EVALUATING mineral status allows one to make recommendations for tolerable levels of minerals for an at-risk population. From balanced diets and biomarkers of adequacy to signs of deficiency and toxicity, the recommended dietary allowance (RDA), dietary reference index (DRI), lowest observed adverse effect level (LOAEL), etc. hold tangible meaning only when the values reported are backed up by sound scientific evidence. How that evidence is obtained and why it must be critically evaluated will be the focus of this chapter. Specifically, the chapter will address:

1. How mineral adequacy is determined.
2. What are biomarkers and how do they fit in to the evaluations?
3. How are risks of inadequate and overly adequate intake determined?
4. What precautions must be taken when evaluating scientific evidence?
5. How does mineral status connect with the health of the individual or the population?

5.1. DIETARY REFERENCE INDEXES AS GUIDELINES

Setting criteria for adequacy is a goal of nutrition. The Dietary Reference Index (DRI) represents numbers gleaned from nutritional sci-

ence reports that in their broadest interpretation represent probabilities for meeting standards of adequacy in the population as a whole. Such guidelines are expressed as the Adequate Intake (AI), the Recommended Dietary Allowance of the United States (RDA), the Recommended Nutrient Intake of Canada (RNI) and the Estimated Average Requirement (EAR). Also included are the Tolerable Upper Intake Level (UL) and the Acceptable Macronutrient Distribution Ranges (AMDR), which address issues of toxic exposure. Although these values are subject to change, each represents a current norm for the general public to follow in order to minimize risk of a deficiency or toxicity. In setting such standards, one must be alert to the dynamics of living systems. Minerals are constantly being taken in from the diet, stored temporarily and eventually released from the system as part of a dynamic process referred to as turnover. Meeting mineral needs, therefore, is akin to finding a level of intake that maintains functional status quo in the face of these dynamic changes. Because the system is considered to be open to its environment, this is not an equilibrium, but rather a steady state that forms the basis for such guidelines.

5.2. ASSESSING MINERAL STATUS

An ultimate goal of Nutrition and Food Science is to link nutrients in the diet with the health status of the individual or consumer. The objective is to know the quantity of any one nutrient that meets optimal growth, maintains a steady-state balance between input and excretion, or prevents deficiencies or toxicities. Such information depends on reliable quantifiable tests that specifically relate to the mineral in question. How this information is obtained will be discussed here. When applied to the general population, the data from such tests sets standards based on probability for lowering the risk of adequacies and inadequacies. When applied to the individual, the data assist health care professionals to determine if a disease or adverse symptoms has improper mineral nutrition as the underlying cause.

5.2.1. Using Balance to Assess Status

The philosophy behind balance studies is to determine a level of mineral that matches input with excretion—a level that sets a homeostasis condition. A simplistic representation of balance is shown in Figure 5.1.

When $K_{in} = K_{out}$, the system is in balance. If K_{in} exceeds K_{out}, more is retained than excreted and system is in positive balance. When K_{in} is less than K_{out} the system is in negative balance. A system in positive balance is tantamount to one in growth; a negative balance symbolizes wasting. In the figure, B and C represent internal variables denoting temporary retention and storage or functional retention, respectively. C is away from the excretory path. The dynamics of the temporary storage or retention pools must likewise be viewed as competing rates. When $K_1 = K_{-1}$, B and C remain at the same level. If K_1 is greater than K_{-1}, more of B is being put to functional use. A greater K_{-1} would indicate a favored movement towards breakdown and removal from the system. Basically when the two occur at the same rate, which can also be interpreted as adequacy being reached and maintained, the system has achieved homeostasis internally. A negative balance impinges on the body's stores and functional pools of the mineral, channeling the mineral towards excretion, the blueprint for a mineral deficiency. A positive balance reflects enhancing the stores or functional pools, a blueprint for adequate nutrition or growth.

The importance of proper balance can be illustrated by the Sky Lab program (Figure 5.2). In the eighty four-day mission, three astronauts were tested to determine if the effects of prolonged weightlessness altered calcium or protein homeostasis. A second goal was to determine if the diet fed to the astronauts was adequate to sustain health in the weightless environment. Within the first 30 days, urinary calcium excretion was twice the amount observed at preflight and remained high throughout the flight. Fecal calcium was also increased but at a slower rate and continued through the flight, but never returned to post-flight levels. Urinary nitrogen was raised 20–30 percent over preflight but returned to baseline during post-flight monitoring. Throughout the mission, calcium losses averaged about 200 mg per day and nitrogen losses

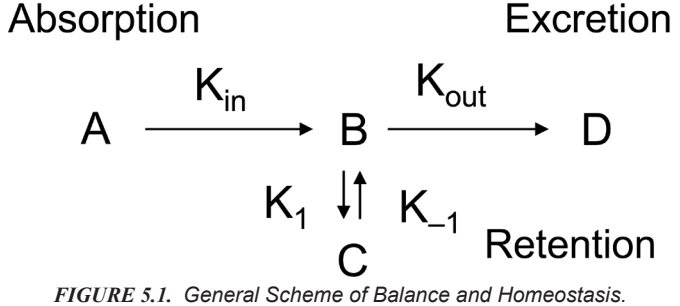

FIGURE 5.1. *General Scheme of Balance and Homeostasis.*

FIGURE 5.2. *Sky Lab in Orbit Around the Earth.*

averaged 590 mg per day. Based on these data, it was clear that the amount of calcium and nitrogen in an astronaut's diet must be increased substantially to offset losses brought on by a weightless environment.

5.2.1.1. Problems with a Balance Study to Determine Mineral Adequacy

Although the procedure seems straight forward, there are concerns with data derived from balance studies. Four major ones are listed below:

1. The endpoint is in doubt
2. Excretion is episodic, not continuous
3. Multiple connecting pools can lead to internal shifting
4. System adaptation

Time to achieve balance may be days or even weeks, and often is not known or can only be estimated. This makes the end point uncertain. To assume that intake and excretion occur at uniform rates in a given

period of time may be more hypothetical than real. For one, excretion is under episodic, not continuous control. Another concern is that the mineral in question may be multifunctional and one of the functions could be compromised by internal shifting between mineral-dependent factors, which sometimes become apparent when the results of a balance study are not in agreement with results of other analyses (see below). But, by far the greatest weakness to a balance approach is system adaptation. A low intake can force an adjustment of the system to absorb more or excrete less of a mineral and a high intake can do the opposite. Hence, the system may appear to be in balance when it may actually be shifting internally to accommodate sub- or super-optimal levels of intake. These factors, when combined, make balance studies less reliable for assessing mineral requirements.

5.2.2. Clinical Approaches

Clinical approaches rely mostly on observations performed in a clinical setting. The term clinical carries the connotation that the results pertain to live subjects as opposed to model systems. A suboptimal level of a mineral could cause deficiency symptoms to appear, symptoms such as anemia, diarrhea, hair loss, scaly skin, bone demineralization, etc. These are just a few of the clinical signs of a poorly managed system that could be deficient in minerals. In clinical assessments it is important to link the physical symptoms to an underlying biochemical flaw, a defective enzyme in a pathway, for example. Only then is it possible to rationally link the overt sign with some specific mineral-dependent component in the system.

5.2.2.1. Problems with a Clinical Approach

A problem encountered with the clinical approaches is that the deficiency symptoms for the different minerals tend to overlap. For example, diarrhea is a common occurrence in young children experiencing a deficiency in zinc and could also appear in a magnesium deficient or copper deficient child as well. Frequently, more than one deficiency sign (stunted growth, loss of hair, skin rash, etc.) can have numerous initiating progenitors. Thus, nutritionists are constantly challenged with finding symptoms that are linked unequivocally with the mineral in question. Often, this means performing numerous tests to pin down the exact cause.

5.2.3. Biochemical Approaches to Assessing Mineral Adequacy

Having viewed procedures for evaluating minerals' status, the focus now turns to the more common methods in practice today. These are:

1. Observing or measuring body stores
2. Response to low or high intake
3. Functional assays
4. Reversal of deficiency symptoms

Each index while providing useful information also has limitations. Seldom are all four used for any one mineral. Body stores, for example, refer to the total amount of a particular mineral present in the system. Obviously, this is not subject to direct or even rational assessment and must be obtained indirectly. For example, a person suspected of low iron stores can be diagnosed for signs of anemia or low blood iron or by keying on some measurable component related to iron storage and known to change with intake. Reversing symptoms of a deficiency will assess and possibly confirm if the mineral suspected as causing the problem is connected to the symptoms observed, which further points to the current level of that mineral as being sufficient or insufficient.

5.2.3.1. Body Stores

Vertebrates have a limited capacity to store minerals. This is particularly true for the microminerals. Storage capacity nonetheless can be determined by simple tests. Iron status, for example, equates with iron bound to ferritin (Chapter 13). A direct test of plasma levels of ferritin is easy to perform. A second approach is measuring total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC). Both are an index of the amount of iron bound to transferrin, and a direct assessment of potentially functional iron level in the plasma. While transferrin is not a major storage site for iron, the number of iron atoms bound to each transferrin molecule tends to correlate with the body stores of iron. Thus, both serum ferritin and serum transferrin can be used to make judgments as to whether a person is deficient or adequate in iron.

While this may work for iron, a simple blood test for microminerals is risky and the data are generally not reliable. This is because the level of microminerals in blood can be infinitesimally small and any change at the micromolar level challenges the most sophisticated and

sensitive instrumentation. Zinc in the serum accounts for only 0.1% of the total zinc in the system (body load). Consequently, serum zinc under-represents the body load of zinc, and small fluctuations in plasma zinc when zinc intake is suboptimal (as opposed to severely deficient) are difficult to interpret with surety. Using body stores to assess macromineral status, however, can be accomplished with greater assurance of validity. Macrominerals are plentiful in body fluids and tend to be widely distributed without amassing in any one location. Thus blood levels of sodium, potassium, phosphate, calcium, etc. are a clear indication of mineral status for these minerals and the values obtained from the measurements can be interpreted as representing the body load.

5.2.3.2. Overt Response to Mineral Intake

Measuring the growth rate of an experimental animal as a function of the mineral intake is a common procedure in nutrition research. One obtains insight into the "conditional" amount of the mineral that gives optimal growth. Referring to this as "conditional" implies the level so observed may depend on other factors in the diet and the values obtained could reflect these other influences. This happens when the status of one mineral depends on another mineral (Chapter 8).

Microminerals can also be judged by overt responses, but valid quantitative judgments are not always possible. A deficiency in copper, zinc or iron can stunt growth. Finding the amount that gives steady-state growth is not always a direct procedure. This is because zinc, copper and iron are antagonistic and could interfere with each other's bioavailability. Thus, a value that is set for copper must pay heed to the level of zinc and iron in the diet. Pre- and post-absorption interactions make it hard to quantify precisely the level of a mineral needed to achieve optimal or even adequate status. Small quantities of minerals added to test diets are sometimes just above the level of contaminants. This slight difference can reflect a disparity between adequate and suboptimal levels. More often than not, status of a micronutrient is best determined by functional assays.

5.2.4. Functional Assays

Functional assays base conclusions on quantifiable mineral-dependent functions, not on the amount in the food source. A functional assay, therefore, puts the focus on what the mineral is doing internally, not

simply that it is there—which bypasses digestion and absorption influences in the assessment. Often a functional assay is a simple biochemical test of a metal-dependent enzyme in a tissue or fluid. Because other minerals also elicit nearly identical responses or could be confounding factors in the response, function assays can also be misleading. This could happen if two minerals are required to fulfill a particular function or if the biomarker is influenced by non-mineral factors. Because copper is required to mobilize iron, overt signs of anemia could reflect either a deficiency in iron or copper. As observed in a classical study by Hart *et al.* (1928), both were shown to play a role in building hemoglobin to correct an anemic condition in rats. Ceruloplasmin levels in serum, measured by oxidase activity towards ferrous iron, is used to assess copper status in humans. Because ceruloplasmin is an acute phase protein, its level in serum is influenced by stress hormones and inflammatory factors. Linking the ceruloplasmin's oxidase activity to copper status alone is tenuous unless there are assurances that test results are not influenced by non-mineral factors.

5.2.5. Reversing Deficiencies

Sub-adequate intake of minerals can be pathogenic. In a more refined application, a severe deficiency in zinc is known to cause rashes on the skin in humans and hair loss, most noticeably around the orbits of the eye, in rats and mice. Children suffering from acrodermatitis enteropathica (AE), a genetic impairment in zinc absorption, develop a rash over most of the body (Chapter 14). Treating the infant with zinc supplements clears the rash and returns the skin to normal appearance, implicating low zinc as the likely cause of the condition. In a similar way, cobalt as vitamin B12 can reverse the symptoms of pernicious anemia. The organic component of B12 without the cobalt and cobalt alone have no effect, clearly showing the cobalt must be present in the correct form to correct the anemia. In both examples, the mineral—or the proper form of the mineral—was connected to a reversal of the symptoms, which gave credibility to the identity of the causative factor. Furthermore, linking that mineral to the impairment also revealed an important function performed by the mineral in the system.

5.2.6. Employing Ideal Standards

One could argue that assessing the optimal quantity for any essential

nutrient could be based on measurement obtained from an ideal food source. Can this apply to minerals? Milk is often spoken of as nature's perfect food. Hence, quantifying the minerals in milk can set a standard with which to judge all other food sources for mineral quality. When using milk as a standard for minerals, however, milk is not nature's perfect food and is far from being put in that category. Among the concerns are the very low iron, zinc and copper content of milk (Table 5.1) . One liter of human milk meets one-third the daily requirement for these microminerals, but less than 10 percent of the requirement for iron. There is also concern that whey protein in milk interferes with calcium absorption and that levels of sodium and potassium are unusually high. All this leads one to conclude that milk strays quite a bit from ideal and should not be used as a food paradigm for setting a mineral nutritional food standard.

5.2.7. Biomarkers: Advantages and Limitations

By definition, a biomarker is an internal or external signal elicited in

TABLE 5.1. Average Mineral Content of Human and Bovine Milk.

Macrominerals	**Human (milligrams/Liter)**	**Bovine (milligrams/Liter)**
Calcium	259 ± 59	1180
Sodium	207 ± 94	580
Potassium	543 ± 78	1400
Magnesium	31 ± 6	120
Phosphorus	142 ± 25	930
Chloride	453 ± 53	1040
Microminerals		
Iron	0.4–0.8	0.2–0.6
Copper	0.2–0.4	0.05–0.2
Zinc	1–3	4
Manganese	3–6	21
	(micrograms/Liter)	**(micrograms/Liter)**
Selenium	15–20	10
Iodine	12–178	70–219
Molybdenum	1–2	22
Chromium	0.2–0.4	5–15
Nickel	0.5–2	4–40
Fluorine	4–15	19

response to a nutrient. The strength of the signal is directly related to the amount of nutrient at the eliciting site. The importance of biomarkers in mineral nutrition is crucial and cannot be overstated. As pointed out by O'Dell, an ideal biomarker is some reliable internal factor that responds directly, specifically and quantitatively to changes in a mineral's homeostasis. Its purpose is to signal a disturbance in the functional stores of a minerals. The most common signal elicited is a depression in blood or tissue levels of the mineral. Biomarkers, in effect, allow rapid assessments of a developing condition before symptoms of the condition appear. If, for example, one wishes to know the amount of iodine that prevents a goiter from forming, one can wait for the goiter to develop and then by adding iodine systematically, seek the level that delays the response. This could take weeks or months. A better ploy is to measure some internal factor whose function depends on iodine. An example is thyroid hormone, which is derived from thyroglobulin in the thyroid gland (Chapter 18). Either thyroglobulin or thyroxine could serve as biomarkers of iodine and a pending risk of developing a goiter. Thus, confronting the problem with a valid biomarker allows a preclinical assessment of iodine status of the individual before the pathology develops.

A change in a biomarker could be the first clinical sign of abnormal mineral status. It is comforting to know that a decision from a biomarker is not simply based on what was in the food source, which basically ignores the organism's role entirely. Rather, biomarkers more accurately reflect what is happening in the internal milieu, thus obviating concerns for absorption and transport in the assessment. Biomarkers can relate to biochemical impairments that lead to pathologies. For example, a selenium-dependent enzyme is required to synthesize the thyroid hormone triiodothyronine (T3) from thyroxine (T4). Either the enzyme or T3 in the plasma could be a biomarker for a selenium requirement and a window to assess whether the recommended amount is adequate or below.

Table 5.2 lists biomarkers for many minerals. Sodium, potassium, magnesium and calcium, which together account for 99% of the minerals in the body, can usually be quantified by a simple blood or urine analysis employing automated instruments. Calcium, however, because of its close association with bone density, can be judged by an additional analysis (see below). In contrast, micronutrients, which include most of the minerals listed in the table, are present in too low of quantity for a direct chemical analysis of the blood; more sophisticated instrumentation is required. Thus, as noted above, micromineral status is

TABLE 5.2. Examples of Biomarkers to Assess Mineral Status.

Mineral	Biomarker Measured	Physiological System
Calcium	bone density	bone resorption
Magnesium	blood and urine levels	bone structure, energy
Sodium	blood and urine levels	electrolyte balance
Potassium	blood and urine levels	electrolyte balance
Phosphorous	blood and urine levels	bone structure
Chloride	blood and urine levels	electrolyte balance
Iron	blood hemoglobin, transferrin saturation, ferritin	oxygen transport
Copper	plasma ceruloplasmin	energy, pigment, antioxidant
Zinc	plasma zinc, zinc enzymes	growth and development
Manganese	manganese enzymes	antioxidant
Selenium	selenium enzymes	antioxidant
Vanadium	none	uncertain
Molybdenum	molybdenum enzymes	purine metabolism
Chromium	chromodulin	glucose tolerance
Iodine	T3, T4	thyroid hormone

generally best achieved by a functional analysis that exploits cofactors for enzymes. Enzymes can be biomarkers of adequacy or inadequacy when a nutritional deficiency is suspected, which is common in a clinical setting. Invariably, the cofactor itself is specific for the enzyme and not subject to substitution. In experimental nutrition, the ebb and flow of enzyme activity is a window into the effectiveness of the diet to meet a level of metabolic stasis and avoid a nutritional deficiency.

5.3. ASSESSING RISK OF TOXICITY

With the above tools in hand, it is now possible to define a range of intake for a certain mineral that meets the daily requirement. There are two concerns in the assessment: risk of deficit and risk of excess. First, a biomarker must be selected that will set the criteria for the decision. Next, the "estimated average requirement", or EAR, must be determined. The EAR can be defined as the intake level that meets the needs of 50% of an average "healthy" population in a particular age (or gender) group of individuals. Once an EAR is set, the recommended dietary allowance (RDA) can be determined. In essence, the 50th percentile must be defined to allow the 97–98th percentile to be calculated.

RDA (or RNI, recommended nutrient intake, the Canadian equivalent of RDA) is the daily intake represented by two standard deviations from the EAR, which in a normal Gaussian distribution is the level that satisfies the needs of 97.5% of the population. This means that, at most, an RDA or RNI (when followed) puts only about 2.5% of the population at risk of a deficiency. In addition, one must always be mindful of the "upper limit", or UL, which looks at the opposite extreme—i.e., the limit of safe consumption before crossing into the danger (toxic) zone and observing adverse signs.

5.3.1. Risk of Excess

Risk of excess is another concern that must be addressed if one is to recommend a level of intake. There are different terms and acronyms associated with the excess, however. In each instance, one is concerned with bad signs—more specifically, the upper limit (UL) of intake—that is attained without showing adverse symptoms. Tantamount to UL are the NOAEL and LOAEL, which represent the "no observed adverse effect level" and "lowest observed adverse effect level", respectively. The latter refers to the point when adverse signs are first noticed. Most of these terms are illustrated in Figure 5.3, which is taken from the Food and Nutrition Board. The uniqueness of the UL is sometimes overlooked. UL defines the point where the risk of inadequacy or excess

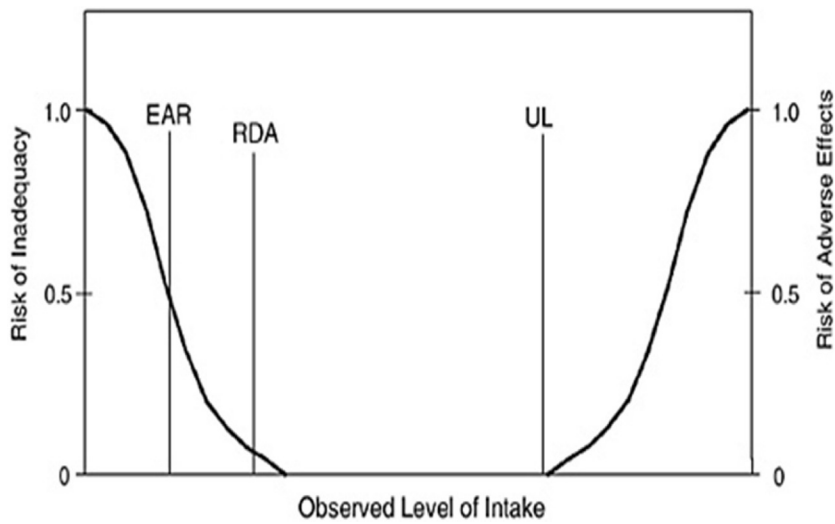

FIGURE 5.3. Assessments of Risks as a Function of Dietary Intake.

is practically zero. That is comforting to know for an individual, but when the concerned party is a manufacturer who is adding a nutritional supplement to thousands of cartons of a product, putting in more than is needed could be highly undesirable and very expensive.

5.4. ASSESSING BIOAVAILABILITY

Bioavailability represents the fraction (or percentage) of a nutrient in the diet that is put to a functional use. One could even say that bioavailability is a means of determining, quantitatively, the first law of nutrition which states, "no nutrient is absorbed and utilized to the full extent that it is fed". By acknowledging that only a fraction of the nutrients taken in the diet is put to some useful purpose, the amount of mineral in the food source that ultimately becomes functional must be determined to complete the assessment of health benefit. This definition presupposes losses that occur along the way towards becoming functional. Using Equation (1) as defined by O'Dell, it is possible to determine bioavailability by knowing the percentage of the total that is absorbed and the percentage assimilated (rendered functional) as follows:

$$\%\ \text{Bioavailable} = \%\ \text{Absorbed} \times \%\ \text{Assimilated} \times 10^{-2} \qquad (1)$$

The fraction assimilated takes into account losses that occur in post-absorption transport to the cells, passage across the membrane and exchange with factors in the cell's milieu. As shown in Figure 5.4, a mineral must pass through a number of barriers en route to its functional target. Each step represents potential losses either by an active excretion through the kidneys, incomplete transfer across membranes, or misdirection once inside the cell. All of these steps combined impact negatively on a mineral's bioavailability.

By itself, bioavailability is more of a reporting factor than a defining factor. Bioavailability measurements give insight into a system's internal components that limit utilization. Competing metals, sequestering proteins, hormonal and genetic regulation all impact on numerical values for bioavailability. Knowing that a nutrient's bioavailability is low is a signal that regulatory factors may be controlling access to the biomarker. For example, a bioavailability value based on an enzyme assay could be influenced by the presence of the enzyme protein and the need to coordinate its synthesis with the arrival of the metal ion cofac-

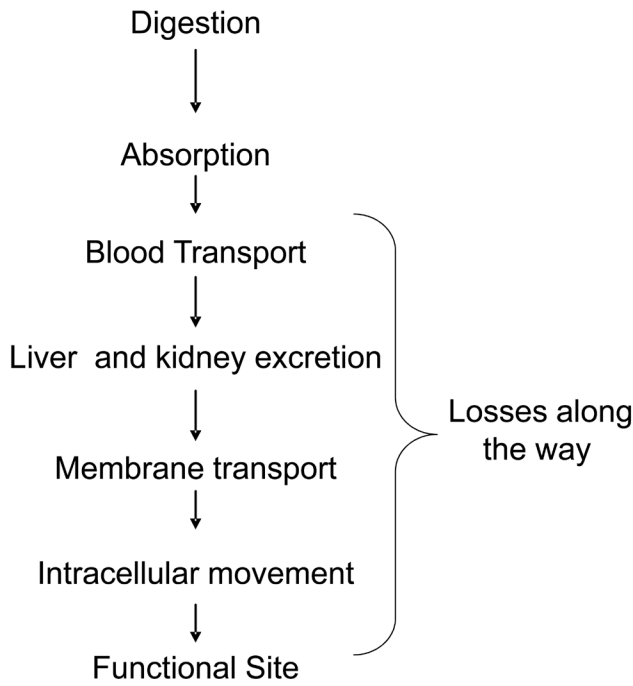

FIGURE 5.4. *Overview of a Mineral's Movement Related to its Bioavailability.*

tor. Indeed, genetic regulation, which basically controls the expression of proteins in cells, may be a deciding factor in a mineral's functional appearance.

A nutrient not needed is a nutrient shunned. This simple principle reiterates the common sense observations that bioavailability is highest when the need is greatest and lowest when it is not. There will always be a certain amount of mineral held in reserve. The reserve pool is designed to feed the cell in the time of need. Generally, this dynamic pool is small and tends to be depleted quickly. Body pools are not static, but are maintained in a dynamic state typical of all biological molecules. Minerals tend to maintain a steady state of utilization or loss from the system. In the case of iron, this could mean incorporation into ferritin concomitant with breaking down the ferritin to release the iron. Although one can interpret this as a futile effort, it fits the needs of the cell by being able to shift rapidly between synthesis and degradative processes, depending on need. The simple principle, however, remains: when the need is greatest, so too is the bioavailability of the needed mineral.

5.5. SUMMARY

Assessment of mineral status relies on a bevy of important tests and procedures. Some of these make use of simple observations that are readily performed in a clinical setting. Others require more sophisticated procedures and obtain answers indirectly. The latter make use of biomarkers of adequacy. Biomarkers operate on the premise that an internal factor related to the mineral's presence responds directly, specifically, and quantitatively to changes in a mineral's level in the system. There are, however, caveats in their application. To be valid and reliable, a biomarker must be both sensitive and specific for the eliciting mineral. Measurements must be repeatable and not subject to change with time of day, age and gender or health issues afflicting the individual. Moreover, any observed differences must correlate strictly with changes in the mineral's level in the system. Data obtained from biomarkers have been used to formulate tables that provide values for adequate intake and lower risk. Another concern is overly strong intake of a mineral and the potential for toxicity. Judgments of this phase of mineral nutrition rely on the appearance of adverse signs that relate to pathogenic changes that are taking place and signal a dangerous situation developing. The concept of bioavailability recognizes that any set standard may be subject to factors that affect absorption and assimilation. For this reason, standards cannot depend on the amount of mineral in the food source, but rather must take into account factors that affect the mineral's metabolism and adjustments made accordingly by the system.

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5.7. PROBLEMS

1. Explain the difference between the following nutritional terms:
   a. EAR vs RDA
   b. RDA vs RNI
   c. RDA vs AI
   d. UL vs NOAEL

2. What minerals are likely to be involved in the following clinical symptoms:
   a. A hemoglobin level that is below normal-condition 1
   b. A hemoglobin level that is below normal-condition 2
   c. A hemoglobin level that is below normal-condition 3

3. If you suspect a deficiency in copper could be one of the conditions, other than measuring serum levels, what test would you run to confirm the diagnosis?

4. Suppose another condition in problem 2 could be pernicious anemia. How would you confirm this diagnosis? Read Chapter 21 to see if you are correct.

5. Copper/Zinc superoxide dismutase is an important antioxidant enzyme. If you used this enzyme as a biomarker and found the level low, what can you conclude as to the mineral status of the patient . . . i.e., which mineral is responsible for the suppressed level of activity?

CHAPTER 6

Intestinal Absorption of Minerals

IN this chapter, we focus on general principles governing the absorption of minerals across the intestine. Solubility, valence, and geometric configuration discussed in Chapter 1 all come into play when discussing mineral absorption. Here, we explore the general principles that apply to all minerals as they enter the system. Later chapters will discuss transport to functional sites. For information about a specific mineral, you are urged to read the appropriate chapter for that mineral. The specific objectives of the chapter are:

1. To seek common principles in the intestinal absorption of minerals.
2. To comprehend the role of carrier proteins in the entrance and transcellular movement.
3. To compare and contrast macro- with micromineral absorption.

6.1. OVERVIEW

In Chapter 5, it was pointed out that whatever enters the lumen of the intestine is not necessarily what is absorbed or rendered functional. This fundamental principle of nutrition recognizes that nutrients incur irretrievable losses along the path from intake to function. It is fair to say that most of the losses in minerals occur at the intestinal absorption phase of metabolism. Any nutrient is judged outside the body until it passes through the wall of the intestine and enters capillaries on the serosal side. The intestinal epithelium, therefore, is a barrier of cells

separating the internal from the external environment. To enter means penetrating a phospholipid bilayer that is basically impermeable to free ions. Integral membrane proteins distributed along the surface provide entry channels which largely overcome this impediment. Moreover, high concentrations in the lumen and low concentrations in the cytosol establish a gradient across the membrane that provides the driving force for the mineral to enter. In contrast, microminerals whose critical mass is below what is needed to drive diffusion must rely on mobile proteins to execute the movement within the absorbing cell's confines. Cells in the intestine are in a constant flux, first formed as stem cells in the crypts and then migrating to the surface as they mature and differentiate into absorbing epithelial cells. Mature epithelial cells are basically secretory in nature and give the lumen its largely aqueous environment. The life of an intestinal cell is short, sometimes no longer than three days, at which time the cell undergoes death through apoptosis and becomes detached from the underlying basement membrane. It is in that three day span that a mineral is either taken into the system or stays trapped within the cytosol of cast off cells.

6.2. DIGESTION AND ABSORPTION OF MINERALS

6.2.1. Digestion

Viewed broadly, digestion is the process that prepares nutrients for absorption. Minerals in a food source are locked within a matrix composed primarily of proteins, complex carbohydrates and fats. The purpose of digestion is to render large composite molecules into smaller manageable units, liberating minerals as free ions in the process. Hydrolytic enzymes (those that break bonds by inserting the elements of water across the bond) comprise the greatest number of enzymes for digestive purposes (Figure 6.1). These enzymes reduce the size without total destruction (metabolism) of the liberated components.

6.2.2. Absorption

Intestinal absorption of minerals is the most critical determinant of a mineral's bioavailability and ultimate value to the organism. One can view the absorptive process as occurring in three stages. Stage I involves transfer of newly liberated minerals from the lumen into the enterocyte,

	Enzyme	Location	Target	Action
I	Pepsin	gastric juice	proteins	breaks peptide bonds
	Trypsin and chymotrypsin	duodenum	proteins	breaks peptide bonds
	Amylases	saliva and duodenum	starch and glycogen	breaks glycosidic bonds
	Lipases	duodenum	complex lipids	breaks ester bonds
II	Glycosidases	microvilli	di- and tri-saccharides	breaks glycosidic bonds
	Peptidases	microvilli	small peptides	breaks peptide bonds

FIGURE 6.1. *Enzymes involved in digestion of food minerals. Stage I consists primarily of enzymes in salivary, gastric and pancreatic secretions. Stage II represents enzymes on the surface of absorbing cells.*

the absorbing cell. This stage involves passage through a lipid membrane bilayer at the apical surface. In Stage II, the mineral, upon entrance into the enterocyte, must traverse the distance from the apical surface to the basolateral surface in order to reach an exiting portal on the serosal side. Depending on the type of mineral, this stage is accomplished by either free movement in the cytosol (as is the case for the macrominerals) or while tethered to a protein or entrapped within a vesicle (which is typical of microminerals). Stage III involves exiting on the serosal side of the cell. As with Stage I, in Stage III the mineral must again pass through a lipid bilayer, only this time using membrane proteins as exit portals. Minerals similar in size and geometric shape have the capacity to compete for the same entrance or exit portal. An excess of one mineral, therefore, can potentially block or delay the absorption of another. Having identical electronic and geometric configurations is the rationale for this type of mineral antagonism. Below is a list of key factors that have application to the absorption of minerals as determined by careful studies of the absorption process relative to particular minerals.

1. Absorption tends to be selective for the form and type of mineral.
2. Absorption is greater when there is a nutritional need for that mineral and less when the mineral is adequate or in excess.
3. Mineral antagonism can arise when two chemically look-alike minerals compete for common membrane entry and exit portals or binding sites on mobile proteins.

4. Vitamins and hormones have the capacity to facilitate the passage of specific minerals (calcium, phosphate, magnesium, iron, etc.) by influencing their solubility or ease of movement across the intestine.

6.2.2.1. Transcellular vs Paracellular Transport

Together, three stages constitute transmural (*L. mural,* "wall") passage. An alternative mechanism, paracellular transport, is characterized by movement in the spaces between cells. Unlike transmural passage, paracellular transport is not regulated and its contribution to the overall absorption depends on the mineral's concentration in the lumen.

6.2.2.2. Regulating Factors

Studies of transcellular movement have led to the discovery of specific proteins in epithelial cell membranes that transport minerals. The level of activity for some is under vitamin, hormonal or genetic control, brought about through signaling mechanisms that sense the quantity of minerals impinging on the cell or from signals within the system. In the short term, however, the action of transport proteins is modulated by phosphorylating kinase enzymes that either activate or suppress a carrier's action.

6.2.3. Mechanism of Absorption

6.2.3.1. Cytosolic Trapping Proteins

A sudden surge of a heavy metal such as zinc, copper or iron can induce the synthesis of a metal-binding protein within the cytosol of the absorbing cells, trapping and effectively limiting the amount of mineral that passes into the system. This response protects against high concentrations of potentially toxic metals entering the system en masse.

6.2.3.2. Vesicles and Absorption Efficiency

A second mechanism involves receptor-laden vesicles that function as mobile compartments (Figure 6.2). These vesicles move between the cell surface and an internal membrane site. Ensconcing a mineral in a vesicle occurs at the membrane surface. Because they cycle between the cytosol and the inner surface, vesicles are subject to down regu-

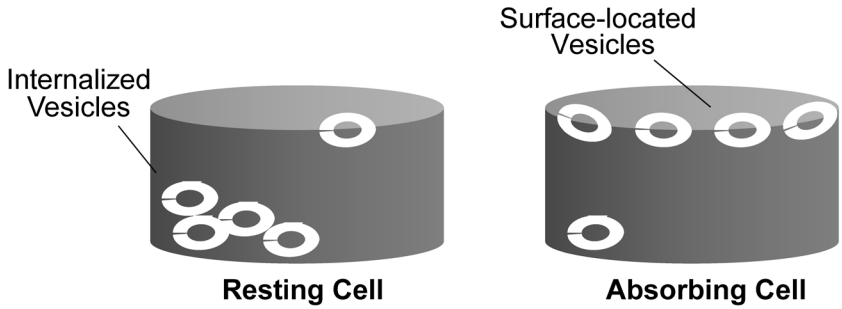


FIGURE 6.2. *Mobile vesicles involved in intestinal absorption. Typically, a resting cell has fewer surface-exposed vesicles to trap and move minerals inward. The opposite is true of a cell actively absorbing minerals and passing them into the cell.*

lation when the lumen content of the mineral is high and up regulation when the mineral in the lumen is low. Down regulation decreases absorption efficiency by lowering the surface density of a particular transport protein. In chronic oversupply, fewer carriers appear on the surface because of translocation to internal storage sites or removal by an ubiquitin-based destruction process when they are no longer in needed. The opposite occurs when a metal ion is deficient. Such conditions will increase the number of absorbing proteins on the surface. For some minerals, a deficiency will activate specific genes that code for the transporters. The effect is to raise the density of absorbing proteins, thus raising the probability for more of the mineral being absorbed. Justifiably, absorption efficiency (the percent absorbed as a function of the amount present) increases when a mineral is present in low supply.

6.2.3.3. Solute-linked Carriers (SLC)

Solute link carriers represent a family of membrane proteins that conduct passage of molecules into cells. Many use the energy of ATP or a gradient of ions to provide the driving force (Table 6.1). The carriers are basically integral membrane proteins (passing through the entire membrane lipid bilayer) and in most instances recognize and are specific for only one factor. Typically, the carrier proteins have tandem domains in their structure, one for the ion that provides the energy and the other for the component being co-transported. A large family of sodium-dependent SLCs co-transports glucose, amino acids, phosphate and other organics into intestinal and peripheral cells (Chapter 10).

TABLE 6.1. Solute-Linked Carriers Responsible for the Passage of Minerals into the Intestinal Cells.

Mineral	SLC	Trivial Name, function
Copper		CTR1, DMT-1, Nramp2 (divalent ion transporter)
Zinc	SLC39A4	Zip4 (acrodermatitis enteropathica factor)
Sodium	SLCA5	Family of sodium driven carriers
Iodine	SLC5A11	Sodium driven transport of iodide ion into the thyroid gland
Phosphorus	NPT2	Intestine
Chloride/bicarbonate	SLCA26A6	Chloride/bicarbonate antiporter

6.2.4. Absorption of Macrominerals

Because of their abundance in the diet and within the system, macrominerals are able to use diffusion-controlled mechanisms combined with specific channel proteins to enter the system. High quantities of macrominerals in the lumen also allow paracellular transport mechanisms to operate.

6.2.4.1. Sodium, Chloride and Potassium

Like most macrominerals, sodium, potassium and chloride exist basically as free ions in the lumen of the intestine and cytosol of cells. As such, these minerals are unaffected by the pH of their environment. They also have little propensity to form stable complexes with other components in the digestate. As noted, sodium ions en masse create high energy gradients across membranes. There is, therefore, more concern for too much sodium entering the system rather than for a deficiency of this mineral. Maintaining the sodium gradient is a function of potassium ions (Chapter 10). Neither ion, however, is regulated at the level of the intestine, but instead uses excretion through the kidney and to a lesser extent biliary secretions and sweat glands to control internal levels (Chapter 10).

6.2.4.2. Calcium, Magnesium and Phosphorus

Compared to monovalent ions, divalent cations show a stronger tendency to interact with factors in the diet—factors that could deter absorption. Consequently, Ca^{2+} and Mg^{2+} do not share the same high

absorption efficiency as Na^+ and K^+. Using an everted sac technique in which intestinal loops are turned inside out exposing their absorbing surfaces to the medium, Bonner was able to show that Ca^{2+} absorption depended largely on ATP generated aerobically by the enterocytes. Repeating the procedure at different concentrations of Ca^{2+} gave evidence for both carrier-mediated and paracellular transport mechanisms. These tests have also defined the region along the intestine that most likely contains the membrane carriers for bringing calcium into the enterocyte [Figure 6.3(a)].

The everted sac experimental approach has also been used to study the effects of vitamin D on calcium absorption. As shown in Figure 6.3(b), without the vitamin, paracellular, non-regulated transport seems to dominate the uptake of calcium into the sac. This non-regulated diffusion only occurs when the lumen calcium is high, which is not the typical scenario for dietary calcium. In the presence of the vitamin, however, there is evidence for a more sensitive saturable mechanism, indicative of carrier intervention in the absorption. The data, therefore, suggest that one function of the vitamin is to increase the amount of carrier that regulates the rate of calcium infusing into the cells.

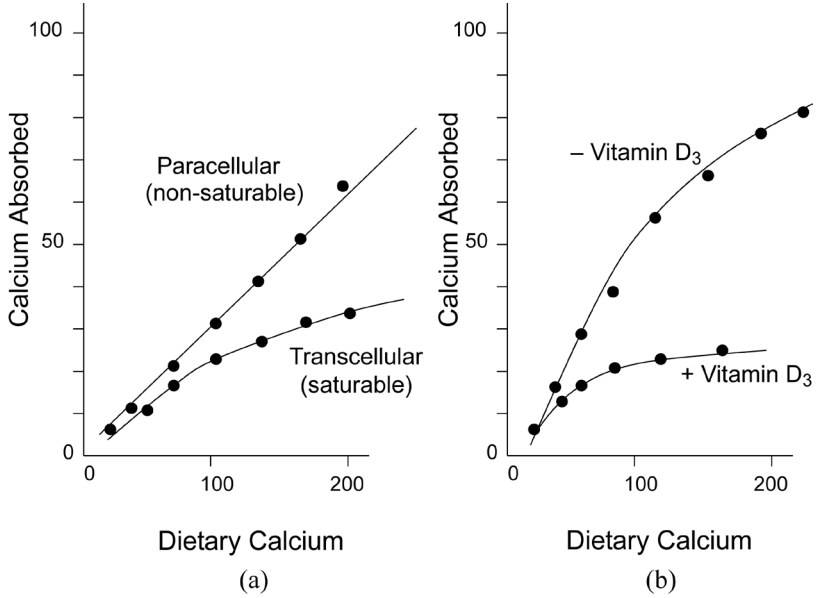


FIGURE 6.3. *Intestinal calcium absorption using everted intestinal loops. (a) Effusion to distinguish paracellular from transcellular. (b) Data showing the addition of vitamin D_3 restores regulated (transcellular) calcium absorption.*

Like calcium, magnesium also employs both energy-driven and diffusion-driven transport systems. Thus, the absorption of calcium and magnesium, in contrast to sodium and potassium, is clearly subject to regulation at the absorption stage. The absorption of phosphorus, as phosphate, also requires a saturable carrier whose synthesis is stimulated by vitamin D (Chapter 11).

6.2.5. Absorption of Microminerals

6.2.5.1. Overview

Solubility and concentration are major concerns for absorbing microminerals. Lacking a sufficient mass to force inward diffusion, microminerals must rely on high affinity metal-binding proteins to mediate the entrance into intestinal cells and move across the intestinal epithelium generally as a complex with the carrier protein. The proteins work in conjunction with internal metal binding factors. Vesicle transport is also more common for microminerals. Solubility in aqueous medium and stereo-specific restrictions of membrane and cytosolic transporters also must be taken into account. Some transporters recognize more than one mineral or are selective for only one valence state. Internal movement of iron, zinc and copper, for example, is primarily via vesicles using membrane proteins to move the metal ion into the vesicle (see below). Not only does this solve the problem of limited concentration, but provides a vector for moving the mineral to its functional site.

6.2.5.2. Solubility and pH

Solubility for many microminerals is limited and is strongly influenced by the pH of the immediate environment. As seen in Figure 6.4, the solubility of Fe^{3+} and Zn^{2+} falls off rapidly as the pH rises. As a consequence, when the two minerals are in the stomach, an acidic environment allows them to attain their highest solubility. But, when these ions move into the duodenum, the slightly alkaline environment (pH ~8) renders both insoluble and unable to enter cells in the aqueous phase.

6.2.5.3. Importance of Mucins in Solubility

Ferrous iron (Fe^{2+}) is more soluble than ferric iron (Fe^{3+}). In an oxygen-rich aqueous environment, however, membrane-bound enzymes

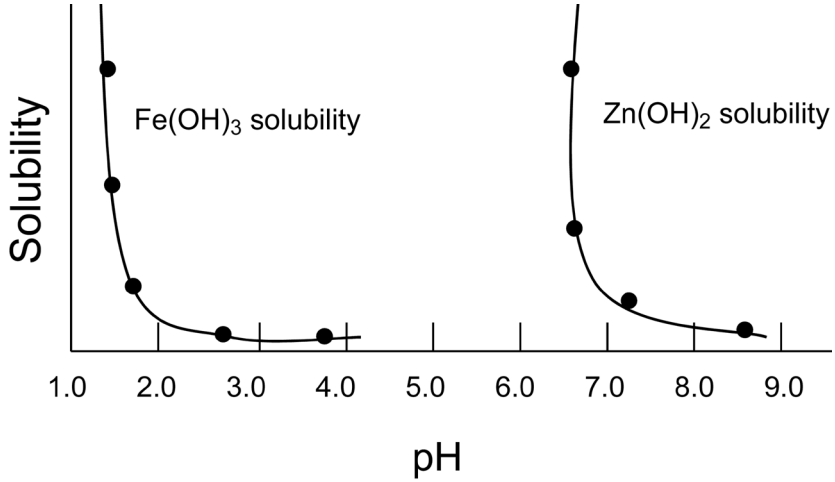


FIGURE 6.4. *Solubility of ferric hydroxide and zinc hydroxide as a function of pH.*

oxidize Fe^{2+} to Fe^{3+}, rapidly converting ferrous iron to its less soluble ferric ion. To overcome ferric iron insolubility, gastric and intestinal cells secrete heteropolysaccharides called mucins that complex the metal ions, rendering them more soluble. Mucins literally coat the surface of the stomach and mucosal lining of the intestine, allowing rapid and fascile interactions. Mucins, therefore, are able to counter the alkaline-induced polymerization of metal ions (primarily those in Category II metals) and make the metal ion more accessible for ease of absorption at the apical surface of the enterocyte. Gastroferrin is perhaps the best recognized mucin, its name eponymous with the iron-binding protein secreted by cells lining the stomach.

6.2.5.4. Importance of Valence State and Electronic Configuration

A change in valence could determine if minerals are absorbed or shunned by a transporter. This principle is illustrated most vividly with iron. Ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}) have their own transporters (Chapter 13). Copper absorption favors the cuprous form (Cu^{+}), primarily because the membrane transporter for copper only recognizes Cu^{+} (Chapter 15). Zinc, whose ion can only exist as Zn^{2+}, has its own transporter. More importantly, because zinc has only one ionic form, the geometry of zinc complexes become a deciding factor. As noted in Chapter 1, both zinc ions and copper ions favor tetrahedral and square planar arrangements with four binding ligands, which allow Zn^{2+} to be

a formidable antagonist of Cu^{+}. Non-heme iron favors the octahedral configuration and thus is only weakly able to compete with either zinc or copper. These factors must be taken into account when considering the mechanism for iron, zinc and copper penetration across the intestine.

6.2.6. Internal Transport

The cytosol of the absorbing cells represents a huge expanse laden with membranes, proteins, metabolites and organelles, blocking the movement to the exit portal on the opposite side of the cell. Moreover, the apical and basolateral surfaces of polarized cells are spatially far apart and made up of distinctly different biochemical factors. Polarized cells also employ vesicles as mobile compartments to guide the transit from one surface to the other. Given this scenario, it is no surprise that vesicle transport is a major modus operandi for transporting microminerals through enterocytes. Vectorial movement is critical at this stage of intestinal absorption because the path through the cytosol is filled with molecules that have the potential to engage, block, or reroute movement. Table 6.2 shows some of the known factors that bind to minerals during passage in the cytosol of the enterocyte. Their function is to assure timely and orderly passage of minerals from the lumen to the serosal capillaries. For some minerals, such prosthetic factors have yet to be identified, which suggests all minerals might not depend on mediating factors. Those that have been identified have come through studies of known genetic disease where the main phenotype is a disruption in mineral transport.

TABLE 6.2. Intracellular Factors in the Intestinal Absorption of Minerals.

Mineral	Transport Factor	Vesicles
Sodium	None	No
Potassium	None	No
Chloride	None	No
Iron	Mobilferrin	Yes
Zinc	Zip4	Yes
Copper	ATOX1, ATP7A	Yes
Magnesium	Unknown	?
Calcium	Calbindin	?
Manganese	Unknown	?

6.2.6.1. Cytosolic Transport Proteins

A series of metal-binding proteins called "metallochaperones" work in concert with vesicles to move metal ions between the two surfaces. Most metallochaperones have been identified by studying the movement of copper ions though the cytosol of intestinal cells (Chapter 15). A series of 4 chaperones are known for copper. Each one is specific for a particular target site in the cell. In essence, the chaperone to which it is bound will determine the ultimate location of the transiting copper. Internal transport of many of the other minerals is unknown and can be assumed to operate in a manner similar to what has been described for the well-studied minerals, i.e., a coordinated and systematic protein-vesicle interaction with vectorial movement from surface to surface.

6.2.7. Release at the Serosal Side

In the final phase of intestinal absorption, metal ions either bound to transit proteins or harbored within vesicles move to the opposite membrane where they are discharged from the cell. Vesicles fuse with the cell membrane which allows them to open up and discharge their cargo. Protein-bound metals, however, employ a protein-metal-protein exchange at the site of the discharging protein. Escape from the cell for some metals is valence dependent, meaning the discharging protein will not discharge the metal ion if it is presented in the wrong valence. This implies that some microminerals need to engage redox-active factors in order to be primed for release from the cells. The releasing mechanism also appears to be mineral-specific, with little overlap between minerals. More on the release of minerals from the intestinal cells will be discussed in the sections dealing with specific mineral elements.

6.3. SUMMARY

Bringing minerals into the system is tantamount to passing a formidable barrier designed to allow only the most essential factors to pass. Selection rests with a series of proteins that recognize, bind and take an active part in the transmembrane movement. Alternatively, minerals can gain access by passing between cells in a type of non-regulated diffusion. Because mineral absorption for many of the minerals is in tune with body needs and/or concern for oversupply, mineral uptake

as a process is highly regulated. A case in point is the role of vitamin D, which appears to regulate passage of both calcium and phosphorus (Chapter 11). As can be predicted, macrominerals operate mainly by diffusion mechanisms, whereas microminerals lean more toward proteins and internal vesicles to execute passage. An important part of the absorption process is the release from the absorbing cells at the basolateral surface. Like input from the lumen, effusion from the enterocyte is carefully regulated and requires specific components to execute.

6.4. PROBLEMS

1. What major factors determine the absorption efficiency of macrominerals? Do the same factors come into play for microminerals? Explain points of similarity and divergence.

2. What is the advantage of using membrane-derived vesicles to transport metal ions through intestinal cells? What minerals are apt to use vesicles in their transport from the lumen to the serosal side?

3. Would you expect a reducing agent such as vitamin C to influence the absorption of magnesium or calcium in the intestinal cells? Explain why or why not.

4. Based on the data in Table 6.1, predict the consequences of having insufficient amounts of sodium in the diet. Do the same for excess sodium.

5. Explain how too much sodium in the diet can contribute to obesity.

6. The importance of regulating the flow of minerals into the systems cannot be overstated. What is the difference between transcellular and paracellular transport mechanisms of absorption with regard to the following:
   a. source of energy
   b. effect of concentration
   c. type of mineral taking part
   d. role of the enterocyte
   e. regulation capability

7. What is the function of gastroferrin? Why is this important?

8. Define the meaning of "metallochaperone". What is its function in mineral absorption and transport?

9. What protects the cell (and organism) from a sudden rush of heavy metal ions into the system? For example, beer made from the Teff grain has a very high iron content, yet consumers of this beverage show little signs of iron toxicity.

10. The cytosol of intestinal cells has redox-active factors capable of changing the valence state of metal ions. What metal ions would be most likely to engage such factors? Explain why this is important.

CHAPTER 7

Post-absorption Metabolism of Minerals

THE ultimate goal of absorption is to assimilate minerals into their biological targets in outlying cells. In this chapter, the focus is on the three phases of post-absorption processing: (1) transport in the blood, (2) movement across membranes, and (3) intracellular relocation. Each phase is influenced by the solubility and abundance of the mineral in question. Previously, it was noted that heavier metals such as Zn^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+}, because of their insolubility as free ions, are more apt to be bound to proteins when they relocate to their targets. This same ploy applies to intracellular transport, where again the more soluble macrominerals move freely in the cytosol, whereas the weakly soluble microelements require specific ligands. The bioavailability of a mineral ultimately depends on the efficacy of its post-absorption movement. Through these studies we hope to learn:

1. The processes of moving minerals from the intestine to peripheral cells
2. Important plasma proteins that transport minerals
3. Mechanisms for penetrating the membrane of target cells
4. The handling of minerals within the cell
5. Diseases that can arise by improper handling of minerals.

7.1. PLASMA MINERALS

As their name implies, macrominerals comprise the bulk of the ions

TABLE 7.1. Status of Macrominerals in Plasma.

Mineral	Total	Bound	Fraction Free
Sodium (mm/L)	135–145	minor	~100%
Potassium (mm/L)	3.5–5	minor	~100%
Chloride (mm/L)	98–108	minor	~100%
Magnesium (mm/L)	0.44–0.66	0.1–0.3	68%
Calcium (mm/L)	2.5	1.0–1.5	54%
Phosphate (mm/L)	0.7–1.4	0.5–1.0	30% (non-phospholipids)

in plasma. In addition to bulk, macrominerals are more soluble than microminerals in aqueous medium. As shown in Table 7.1, sodium, potassium, and chloride exist almost totally as free ions in plasma, whereas magnesium and calcium have a lower percentage of free ions and a stronger tendency to be bound. There are several important points that come to light by these data. First, because they bind weakly to proteins, macrominerals exist in a quasi-equilibrium state with bound ions, which allows them to readily dissociate from the carrier when the surrounding environment of the mineral is low. Second, as free ions, sodium and potassium can form concentration gradients across cell membranes and use the energy of diffusion to force movement inward. In contrast to macrominerals, microminerals are more apt to bind tightly to proteins, thus making the protein a determinant of their movement to a functional site. Moreover, microminerals cannot use the energy of diffusion to penetrate cell membranes, but instead must rely on membrane receptors and cellular-derived energy to force entrance. A requirement for energy also holds true for macrominerals that move against a concentration gradient. The latter is exemplified by ATP-driven expulsion of sodium ions and calcium ions from the interior of cells to maintain a low intracellular concentration of these ions.

7.2. DELIVERY OF MINERALS TO PERIPHERAL CELLS

Transport proteins not only carry minerals, but must also execute their delivery to cells. To accomplish the delivery phase, the receiving cell expresses receptors on the membrane surface. The receptor lets transport proteins dock and discharge their cargo while still exterior to the cell. Alternatively, for some the binding triggers an inward movement (invagination) of the membrane, which brings the mineral into

the cell interior while remaining attached to its transporter. Once in the interior, the membrane forms a vesicle and the mineral is discharged. As will be discussed below, the latter is the means by which iron is brought into a cell while remaining attached to the protein *transferrin*. Receptors for mineral-bound proteins are well regulated and have a high affinity (capable of engaging at very low levels) for the transport protein, thus increasing the probability that the mineral in question will be available to the cell when needed.

7.2.1. Plasma Transport Proteins

Proteins known to take part in the transport of minerals in plasma are listed in Table 7.2. Because of their unique chemical properties, a particular micromineral may have a preferred carrier. This does not preclude sharing carriers, however. For example, it is evident from the table that calcium, magnesium, manganese, zinc and copper all use albumin as a transport carrier but transferrin is the sole carrier for iron. Manganese, however, may also use transferrin. Thyroglobulin presents another interesting dimension to mineral transport. Iodide ion not only binds to tyrosine residues in the protein, but once excised from the protein iodinated tyrosine residues become the skeleton of thyroid hormone (Chapter 18). Perhaps the most enigmatic of all is selenoprotein P, a plasma protein that is believed to serve as a cellular source of selenocysteine. To obtain the selenocysteine requires that the protein be disas-

TABLE 7.2. Mineral Transport Proteins in Serum.

Mineral	Protein	Comment
Calcium	Albumin	50% is protein-bound
Magnesium	Albumin	32% is protein-bound
Iron (non-heme)	Transferrin	2 iron atoms per protein
Zinc	α_2-macroglobulin, albumin	Moderately attached
Copper	Ceruloplasmin, albumin	firmly bound to both proteins
Manganese	Transferrin, albumin	firmly bound
Selenium	Selenoprotein P	as selenocysteine
Iodine	Thyroglobulin	covalently bound
Phosphorus	Lipoproteins	as phosphate associated with lipids
Chloride	none	exist as free ions
Sodium	none	exist as free ions
Potassium	none	a small fraction is albumin bound

sembled at the membrane site or within the cell. Additional information on the role of specific transport proteins will be discussed in chapters that deal with specific minerals.

7.2.1.1. Properties of Plasma Transport Proteins

Recognizing the many functions they perform, proteins that transport metal ions to cells must have the following properties:

1. freely soluble in plasma
2. high affinity metal binding sites
3. receptor recognition groups on the surface

Proteins that transport metal ions to outlying cells must be able to travel freely in the plasma and exist in a freely soluble form. Their metal ion cargo must be ensconced in specific sites in the protein. These sites must be stable to transport but labile to delivery. Binding for the metal ion must occur at levels in the micro- or nanomolar range, implying these sites must have a high affinity for the metal ion. At the delivery site, the protein must have groups that allow it to anchor to the membrane of target cells in order to discharge its cargo.

Proteins that transport minerals in the plasma have the general features of transferrin, as shown in the act of delivering iron to a cell (Figure 7.1). To accommodate the iron, the protein must have configured iron binding sites that allow the iron to fit snugly into the protein. The protein also has a carbohydrate appendage that serves as a recognition site for its receptor on the surface of the target cells. These carbohydrate appendages interact with complementary groups on the membrane receptor and allow the iron-laden transferrin to bind to the cell membrane. Iron is released when the internal compartment (endosome) fuses with the lysosome, leaving the iron-free transferrin free to recycle out of the cell.

7.2.2. Rules Governing Mineral Delivery

In evaluating transport/delivery of minerals to cells, a series of 5 rules that defines important facets of the mechanism must be noted. These five rules state the case for transport proteins and their high affinity receptors and recognize a critical need for microminerals to exist as protein-bound entities in plasma.

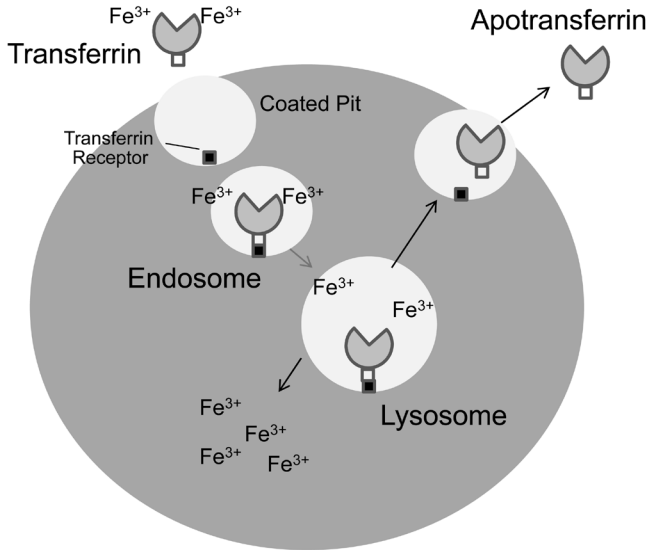


FIGURE 7.1. *Transferrin as a transport and delivery protein for iron.*

Rule 1: Whereas macrominerals (Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-} etc.) travel in the blood and access cells primarily as free ions, the micronutrients (Cu^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}) rely on proteins and other ligands for delivery.

Rule 2: Targeting microminerals to select organs and locations within cells is a function of membrane receptors that work in concert with transport proteins, providing the docking and binding sites for the delivery.

Rule 3: Common to both macro- and microminerals are specific portals through which the mineral must pass to access the cell's interior, or conversely effuse from the interior.

Rule 4: The input and outflow channels may not be the same, which allows them to be regulated separately.

Rule 5: In general, because of their bulk, macrominerals use the energy of diffusion and electrochemical potential across the membrane to gain access to the cytosol from the exterior. Microminerals, in contrast, exploit energy systems and components derived from the cell's metabolism.

7.2.3. Membrane Penetration

As the major interface between the cytosol and the extracellular en-

vironment, the plasma membrane is designed to protect the cell from wanton uptake of plasma components. The lipid bilayer resists free movement inward (and outward) of water, charged particles and polar molecules. Passage, therefore, requires holes in the bilayer, which take the form of specific protein-lined channels that open and close in response to internal or external signals. The above scenario must also include the energy factors that drive the movement. When considering membrane passage, there are four mechanistic avenues by which minerals pass into cells: (1) simple diffusion, (2) facilitated diffusion, (3) active transport, and (4) receptor-mediated endocytosis. All require energy for the transfer but differ in the source of that energy.

7.2.3.1. Simple Diffusion

Diffusion refers to the spontaneous movement of freely mobile substances from an area of high concentration to one of low concentration. The energy for the movement is provided by a concentration gradient established across a semipermeable barrier that separates the two. The rate of diffusion can be calculated by the below Equation (1) known as Fick's law, where A is the area of cross section, L is the length of the diffusion path and $C_2 - C_1$ represents the gradient across the membrane expressed as the difference between interior and exterior concentrations of the mineral being transported:

$$F = \frac{AD}{L}(C_2 - C_1) \tag{1}$$

The diffusion coefficient (D) is in reference to an aqueous medium. High concentrations of minerals in the plasma (as compared to the cytosol) allow minerals to diffuse into the cell through openings in the membrane. Movement will continue until the interior and exterior concentration of the mineral is the same ($C_2 - C_1 = 0$), at which point the rate of movement outward matches the inward flow and the two compartments are in equilibrium.

7.2.4. Facilitated Diffusion

When movement through a membrane barrier is aided by another component—a ligand or a carrier in the membrane, for example—the movement is referred to as "facilitated". Facilitated diffusion uses the

energy of the gradient as the driving force but uses a small molecule such as an amino acid or a nucleotide to form a complex with the mineral to facilitate its passage into the cell.

7.2.4.1. Active Transport

Active implies the requirement for ATP as an energy source. ATP is singled out because most active transport systems for minerals are enzymes classified as P-type ATPases, owing to their property of activating the transport mechanism using ATP to auto-phosphorylate a serine residue in the protein structure. Phosphorylation puts the protein into an energy-rich, high affinity conformation favorable to bind the mineral and relocate it across the membrane. P-type ATPases, also referred to as "pumps", tend to be effective in one direction. Examples of ATPases that move minerals across membranes are shown in Table 7.3.

7.2.4.2. Receptor-mediated Endocytosis

A dramatic departure from the other three mechanisms, receptor-mediated endocytosis is an internal invagination of the plasma membrane which entraps the bound protein-mineral complex. The part of the membrane taken in becomes a closed clathrin-coated vesicle inside the cell. Upon losing the supporting clathrin, these vesicles (referred to as endosomes) fuse with other internal organelles such as lysosomes and through that interaction the protein carrier is destroyed and the metal ion is released. In the case of transferrin, the protein remains affixed to its receptor and the acidic environment of the vesicles dissociates the iron from the protein (Figure 7.1). Receptor-mediated endocytosis is not used by all plasma protein carriers. Albumin, for example, which binds Ca^{2+}, Cu^{2+} and Mg^{2+}, tends to affix to the cell surface, but discharges its bound ions from the protein at the external surface.

TABLE 7.3. Membrane-bound P-type ATPase Enzymes.

ATPase	Minerals	Role	Function
Na^+/K^+ ATPase	Na^+, K^+	Exchanges K^+ for Na^+	Maintains Na+ gradient
Calcium ATPase	Ca^{2+}	Expels Ca^{2+} from cytosol	Restores low cytosolic Ca^{2+}
Cu-ATPase	Cu^{2+}	Expels Cu^{2+} from cells	Intestinal Cu^{2+} absorption

7.2.5. Membrane Channel Proteins for Microminerals

In addition to intestinal transport, valence is an important concern for post-absorption transport of microminerals. One reason is that the carriers in the membranes themselves are valence-state specific. One such carrier is the "divalent cation transporter" DCT1 (or divalent metal ion transporter, DMT1) that potentially transports all divalent cations including Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, etc. Discriminating between macro- and micro metal ions does not seem to matter to this carrier. With numerous minerals vying for the same site on the carrier, a competing ion in excess could cause a deficiency of other ions—that is, assuming the affinity of each metal ion for the carrier is the same, which it is not. Nonetheless, metal ion antagonism can still occur. Another example of a valance-specific carrier is the membrane transporter CTR1, which will only transport Cu^{+}. While giving +1 copper a clear pass for uptake, the carrier is also a "keep out" signal for all non-univalent metal ions.

7.3. INTRACELLULAR TRANSPORT

As in intestinal transport, when a mineral penetrates the membrane of a peripheral cell, it is challenged to find a direction to go. Instead of leaving movement to chance, cytosolic minerals (like their plasma counterparts) rely on proteins within the cytosol to provide directional support. Moreover, such proteins have structural signals built in that allow entrapment at specific locations. Thus, calcium ions bound to calbindin tend to locate within the endoplasmic reticulum, whereas parvalbumin-bound calcium is directed to the actin-myosin complex of muscle. Copper, zinc, and iron, for the most part, are vesicle-bound and tend to move into all the organelles, including the nucleus and mitochondria. As noted previously, chaperones for copper assist in directing the mineral to an internal organelle, enzyme or secretory vesicle; whichever chaperone is holding the copper is the direction the copper goes.

7.4. MINERAL TRANSPORT AND DISEASES

A failure to transport minerals to target cells as part of post-absorption movement could lead to mineral deficiencies. Of the proteins listed

in Table 7.2, some are specific and represent the only carrier protein that can handle the mineral. A genetic defect in transferrin, for example, can render the system incapable of delivering iron to cells, which no doubt would be fatal. In terms of the disruptions to metabolism, a defect in a transport protein is tantamount to subsisting on an iron deficient diet. Ceruloplasmin has been suspected of being a key protein for delivering copper to tissues. Genetic defects in the structure of ceruloplasmin give rise to aceruloplasmanemia. The loss of ceruloplasmin, however, does not impede the delivery of copper to cells, an observation that has challenged ceruloplasmin's essential role as a transport delivery protein for copper. Structural problems with membrane receptors likewise represent a potential locus for cellular mineral deficiency if such defects result in a failure to dock a transport protein for delivery. Not being able to bind transferrin can stifle iron uptake into cells that need iron, which is every cell in the system.

7.5. SUMMARY

Proteins play an important role in post-absorption nutrition and metabolism of minerals. Specific proteins act as ligands that bind minerals for transport in the blood and entrance into cells. Protein transporters permit minerals to transcend barriers and to locate targets within cells. As a complex, proteins heighten the metal's solubility and membrane penetrating properties under conditions where diffusion-driven events are unworkable. Most are part of a protein/receptor system with power to select what mineral is taken in by the cell. This puts sensitivity on a par with the protein's binding affinity for the mineral as well as receptor on the cell surface. The latter statement heeds the notion that ligands can be forceful determinants of the selectivity and sensitivity of the transport process in general. By binding to their surface, the fate of the metal depends on what happens to the protein. If the protein enters the cell, the metal ion goes with it. There are both similarities and differences in movement mechanics between the two classes of minerals. Similarities can be found in the requirement for specific passageway components that have the power of discriminating among the different minerals. Differences lie in the source of energy and the need for a piggy-back factor to carry the mineral through the lipid bilayer. Each mineral seems to have its own set of principles for bringing the mineral to its site of activity. The best way to fully appreciate the importance of

protein carriers is to consider that a protein-mineral complex acting as a unit makes the mineral functional.

7.6. PROBLEMS

1. Trace the path of iron from the liver into hemoglobin in bone marrow cells. In this path, identify components that are valence-state specific. This question is best answered by drawing an outline and consulting Chapter 13 for more information.

2. What is the concentration of Na^+ and Cl^- in plasma? What would be the consequence if the level of these two ions in plasma were twice their normal physiological level? The question has relevance in explaining why one set adrift in an ocean is cautioned against drinking ocean water to quench thirst.

3. A misinformed person is concerned about having too little iron in the blood. The person wants to take iron tablets, but a friend suggests that a better way is to inject iron salts directly into the blood to get the full benefit. Is this good or bad advice? Explain.

4. Explain what would happen if the calcium in blood was bound tightly to a protein such as albumin and could not diffuse by normal means. What effect would this have on bone structure?

5. According to Fick's law, what two factors determine the rate of diffusion across the membrane? If the gradient across the membrane was doubled, what changes would need to be made in order to re-establish the same diffusion rate?

CHAPTER 8

Mineral-Mineral Interactions

No one mineral within a living system acts with the total abandonment of other minerals. This is tantamount to saying that mineral-mineral interactions are a common occurrence in living systems and can affect absorption, transport, catalysis, etc. Indeed, interactions with other mineral can be both beneficial and detrimental. Thus, we see interdependence between minerals or interlinking two or more to execute a function. Such chaos is not without boundaries, however. As noted earlier, when called upon to perform a function, some minerals are locked into one specific carrier protein or one specific enzyme. In this chapter, the focus will be on specific mineral-mineral interactions, their consequences to the organism, and why they occur. Through these studies we hope to learn:

1. The more common mineral-mineral interactions occurring in humans.
2. Studies that have supported their occurrence.
3. Mineral reality that characterizes minerals in living systems.

8.1. NATURE OF THE INTERACTION

Mineral-mineral interactions have major health implications and hence have been the focus of many nutritional studies. As pointed out by O'Dell (1963), a serious interference can occur when the principal mineral in the list in Table 8.1 is in excess and its major competitor is

TABLE 8.1. Some Metal-Metal Interactions in Living Systems.

Principal Mineral	Major Competitor
Sodium	Potassium
Calcium	Magnesium
Manganese	Iron
Iron	Copper
Zinc	Copper

at the lower limit of requirement. Lönnerdal has further pointed out that imbalances between ratios of trace elements such as iron/zinc, zinc/copper and iron/manganese may cause one to rethink values for upper limits for trace metals in formulae and parenteral solutions. Oversupply of a look-alike mineral can also have positive health effects as well. Modulating sodium-induced hypertension with potassium ions and inducing muscle relaxation by replacing calcium with magnesium are specific examples where interactions benefit the organism and serve a useful biological purpose.

8.2. INTERACTIONS BETWEEN MACROMINERALS

Figure 8.1 shows interactions between 5 macrominerals. The two-way arrows indicate reversible interactions; no arrow signifies that there is no direct connection. As the figure shows, the need for calcium in the system is influenced by phosphorus, magnesium, potassium and sodium. Likewise, calcium can influence the requirement for these four as well. Sodium and potassium, while isolated from phosphorus and magnesium, tend to be antagonistic towards one another, whereas sodium shows no influence on magnesium or phosphorus, but plays a major role in controlling calcium. The health implications for these interactions are given below.

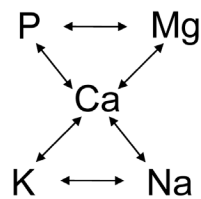


FIGURE 8.1. *Interactions Between Macrominerals. (Adopted from O'Dell, 1999).*

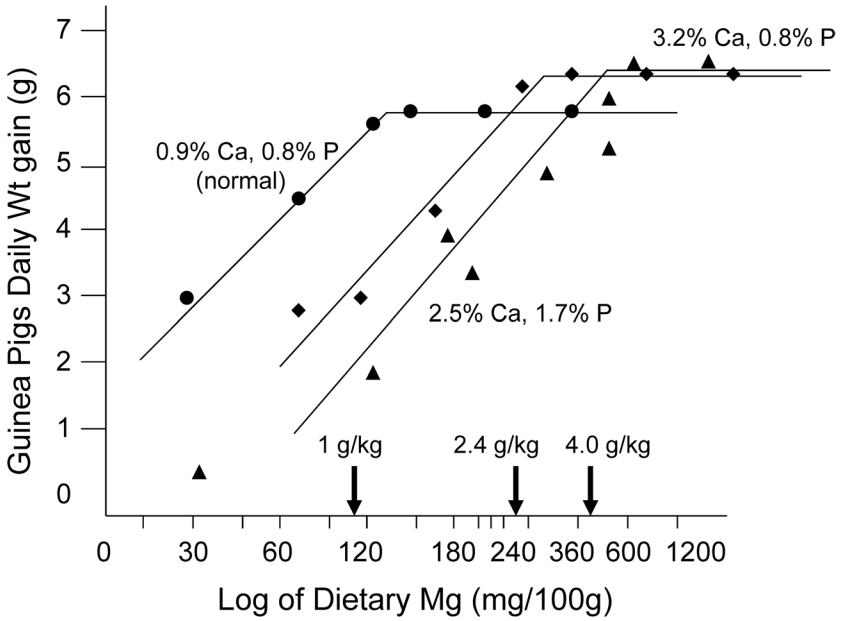
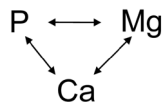


FIGURE 8.2. *Assessing Calcium, Magnesium and Phosphorus Levels for Optimal Growth. The y- axis shows daily weight gain (not to be confused with cumulative weight gain) for Guinea pigs. The x-axis shows the adjustments in magnesium needed to meet the changes in calcium and phosphorus. Plateau values indicate constant daily weight gain. (Adopted from O'Dell and Morris, 1963).*

8.2.1. Calcium/Phosphorus/Magnesium



Calcium, magnesium, and phosphorus are known to play a pivotal role in the growth rate of guinea pigs. All three show an interdependence in meeting standards of optimal growth, as illustrated in Figure 8.2. Note that tripling the intake of calcium in a standard diet (0.9% to 3.2%) while holding phosphorus steady nearly doubles the requirement for magnesium. If phosphorus and calcium are both doubled, the magnesium requirement is nearly 4 times greater. Failure to adjust magnesium levels to accommodate changes in calcium and phosphorus could result in a magnesium deficiency. Moreover, each mineral taken individually can influence the other two. Thus prevailing levels of all three, not just one, must be taken into consideration when setting standards for optimal growth rate. One can surmise from these data that

high calcium can block magnesium, most likely at the level of intestinal absorption. Elevating the level of magnesium overcomes the deleterious effects of the high calcium, which can be further be exacerbated by raising the phosphorus in the diet and thus limit both calcium and magnesium absorption.

8.2.2. Sodium/Potassium

$$Na \longleftrightarrow K$$

Sodium and potassium are mutually antagonistic towards one another. Many of the health implications that arise from high sodium/potassium ratios in the modern diet, such as hypertension, osteoporosis, and cardiovascular problems, are still without mechanistic insights. Owing to a preference for processed foods and a lower intake of potassium-rich fruits and vegetables, Morris et al. (2006) have argued that current trends in the human diet are leading to undesirable effects on health. One theory recognizes the diet of early humans as consisting mostly of plants, which led to a high intake of potassium. The potassium taxed the excretory function of the kidney to achieve potassium homeostasis, which eventually led to the kidney becoming more efficient at excreting potassium. Sodium was not considered a serious health threat in the early human diet because intake was low and the high potassium further moderated whatever sodium effects were manifested, which brings about the conundrum: is high sodium or low potassium to blame for hypertension and other maladies associated with the modern diet? The uncertainty is further amplified by recognizing that the resting postprandial blood level of sodium and potassium does not change despite the undercurrent of pathogenic changes that may be occurring. Since potassium modulates sodium's hypertensive effects, both ions must be taken into account when deciphering a cause/effect relationship for nutritionally induced hypertension.

8.2.2.1. Sodium/Potassium Interactions in the Kidney

A severe dehydration or blood loss stimulates sodium retention and potassium excretion by the kidney. The underlying mechanism is still unclear, but appears to involve the renin-angiotensin-aldosterone system impinging on the kidney (Figure 8.3). The initial reaction is the release of renin from the kidney. Renin, a proteolytic enzyme, converts angiotensinogen to angiotensin I, which through the action of the an-

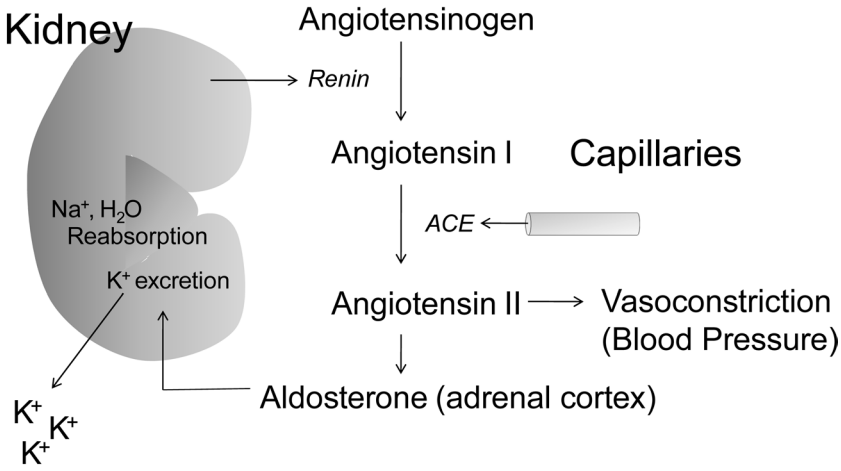


FIGURE 8.3. *Regulation of Sodium and Potassium Excretion by the Kidney.*

giotensin I converting enzymes (ACE) in the capillaries gives rise to angiotensin II, a major vasoconstrictor. The overall effect is to raise blood pressure and to stimulate aldosterone release from the adrenal cortex, which stimulates the kidney to excrete potassium while at the same time increasing sodium retention.

8.2.3. Sodium/Calcium Interactions

Na ⟷ Ca

The kidney is also the site of sodium/calcium interactions. A high sodium diet is conducive to causing excessive calcium excretion. In this instance, losing calcium through desorption over time could lead to osteoporosis and other low calcium-related problems. The actual mechanism by which sodium stimulates calcium excretion is quite complex but overall relates to the capacity of sodium to limit reabsorption of calcium ions filtered through the kidney tubules (Chapter 11). Stimulating calcium excretion suppresses blood levels of the calcium, which stimulates bone resorption. It has been estimated that a 2.3 g increase in sodium can results in 24–40 mg loss of calcium through the kidney. Not only is the risk of kidney stones elevated, but in the long run the risk of heart disease is also increased.

8.2.4. Calcium/Magnesium Interaction

Ca ⟷ Mg

Magnesium ions in excess impedes calcium, more specifically the events ensuing after calcium has been absorbed into cells in the post-absorption phase. Magnesium is present in low amounts extracellullarly but in rich supply in the cytosol. In contrast, calcium is rich in plasma but almost undetectable in the cytosol. The two ions thus emulate the high potassium, low sodium condition prevailing in cells. As a consequence, magnesium is positioned to modulate the effects incurred when calcium enters the cell. One of the more important of these is muscle contraction initiated by calcium. Whereas calcium ions cause the actin/myosin complex to contract, magnesium exerts a muscle-relaxing effect (Chapter 12). No more is the role of the two ions more important than in the rhythmic contractions of the heart. Here, contraction/relaxation is translated into calcium initiating the heart beat and magnesium regulating the heart beat. Interrupting the flow of the two ions internally can seriously stress cardiac functions and lead to cardiac arrest. A similar interaction occurs in nerve cells where calcium promotes the transmission of nerve impulses and magnesium moderates impulse conduction.

8.2.5. Calcium/Phosphorus Interactions

$$P \longleftrightarrow Ca$$

Calcium and phosphorus (as phosphate) make up the crystalline structure of bone and tooth enamel. This fact alone alerts one to consider that when both ions are present within the same confines, a biochemically unstable situation is likely to occur, rendering both ions to precipitate from solution. Such is likely to occur when one is in excess. Serum levels of calcium (8.4–10.2 mg/dL) and phosphate (2.5–4.5 mg/dL) are generally held steady at a ratio of about 3:1. Because the two ions are separated by a membrane barrier, there is little propensity to interact. But, if the serum concentration of either ion rises without a corresponding fall in the other, the stability of the free ions is challenged and precipitation can occur. For example, bone resorption releases calcium and phosphate into the serum, whereas raising dietary phosphate without a concomitant increase in calcium lowers the Ca/P ratio in serum. Two hormones, parathryroid hormone (PTH) and calcitonin, counter the effects of a calcium/phosphate imbalance. PTH stimulates phosphate excretion in the kidney while at the same time maximizing the reabsorption of calcium ions (Chapter 11). PTH also stimulates 1,25- dihydroxy D_3 synthesis, which in turn promotes intestinal calcium and phosphate absorption. Calcitonin, a hormone produced in the thyroid, responds to

elevation in the serum calcium (hypercalcemia) by suppressing the reabsorption of calcium in kidney tubules and the release of calcium from bone. Overall, the effect is to restore or maintain the calcium/phosphate ratio in the serum.

8.3. INTERACTIONS BETWEEN MICROMINERALS

Microminerals in general have a narrower window for avoiding conditions that lead to abnormal functioning. A slight excess or deficiency of one could play a more decisive role in creating an imbalance among other minerals, which could lead to pathogenic changes. If, for example, 0.9 mg of copper per day is considered adequate, a diet containing 0.5 mg could result in signs of a copper deficiency. In a similar situation, the 0.9 mg intake could be *inadequate* if the zinc in the diet is higher than its requirement. Such high zinc could suppress copper absorption. Because suppression will only occur at levels approaching toxicity, the ability of copper to suppress zinc absorption is not feasible. It seems clear that chemical similarities in structure between participating ions could be at the root of micromineral interactions. Examples of interlinking in the micromineral category is one redox metal ion changing the valence state another in order for the second to engage a valance-specific membrane or plasma transport protein.

8.3.1. Iron/Zinc/Copper

Interactions between iron, zinc and copper occur mostly at the absorption phase of metabolism. Studies with growing animals have confirmed that such reactions can stunt the growth of young animals and dramatically affect the requirement for adequacy. Figure 8.4 shows that increasing iron intake impacts negatively on zinc absorption in humans. In contrast, the same amount of iron had little or no effect on copper absorption.

That zinc has the potential to antagonize copper is seen in Figure 8.5. Raising zinc levels from 28 mg/kg to 84 mg/kg brought about a sharp drop in cumulative body weight gain. The drop, however, could be overcome by supplementing the diet with copper, confirming that zinc in excess has the capacity to deter system access to the copper.

The interlinking of copper with iron does not always result in antagonism. Sometimes the two metals are interlinked in performing a func-

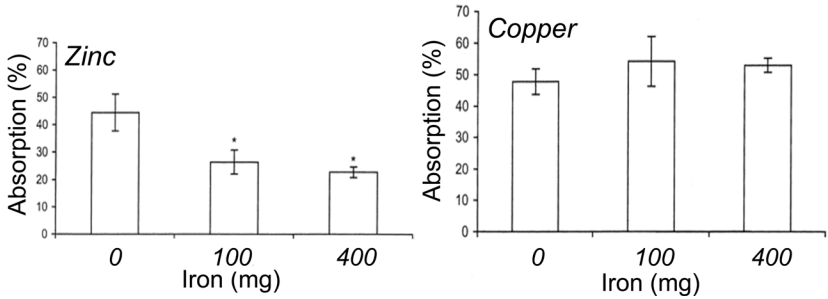


FIGURE 8.4. Iron Effects on the Absorption of Zinc and Copper.

tion or executing movement or transport. Table 8.2 shows that rats fed diets deficient in iron or copper for 35 days experience the expected low serum and liver levels of these minerals. Iron deficiency caused a 90% drop in serum iron and about an 80% drop in liver iron. What had not been expected, however, was the strong rise in liver iron and lowering of serum iron when the animals were deficient in copper. In contrast, liver copper showed only a modest increase when the diet was deficient in iron. The rise in liver iron with low copper in the diet shows that copper is needed to mobilize iron from its storage place in the liver to the peripheral cells. In contrast, liver copper was only slightly elevated by removing iron, suggesting that copper homeostasis in liver is only weakly dependent on the iron status of the animal.

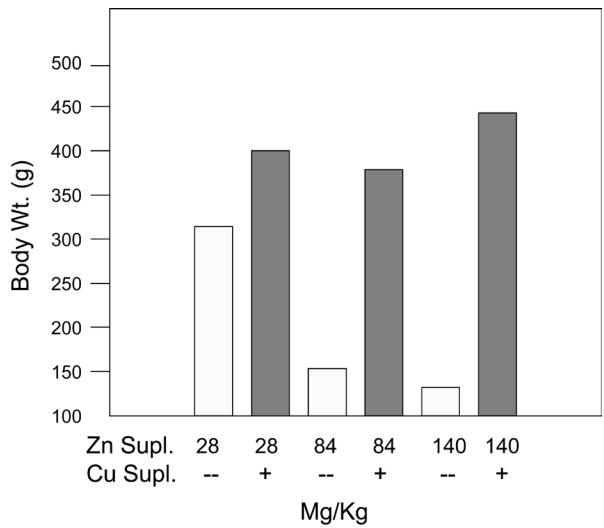


FIGURE 8.5. Zinc/Copper Antagonism in Growing Chicks.

TABLE 8.2. Copper/Iron Interactions in vivo.

Diet	Serum Fe (µg/dL)	Serum Cu (µg/dL)	Liver Fe (µg/gm)	Liver Cu (µg/gm)
Control	331.7 ± 10	0.48 ± 0.3	376.6 ± 66	10.7 ± 2.1
Fe deficient	33.6 ± 7.3	0.65 ± 0.1	63.3 ± 7.4	15.3 ± 3.1
Cu deficient	165.0 ± 0.03	0.03 ± 0.02	578.0 ± 60.4	3.56 ± 1.8

8.3.2. Copper/Iron Interactions in Humans

Rarely will a copper deficiency, mild or severe, cause iron to accumulate in the liver of humans. This is because ceruloplasmin activity, which copper controls, never reaches the point of zero activity and is thus able to maintain liver iron homeostasis. In a landmark study reported by Logan (1996), however, two brothers initially diagnosed with dementia and diabetes were shown to have elevated liver iron. Both brothers had virtually undetectable levels of ceruloplasmin in the blood. A further analysis using ^{59}Fe iron to trace iron flow resulted in the data shown in Figure 8.6.

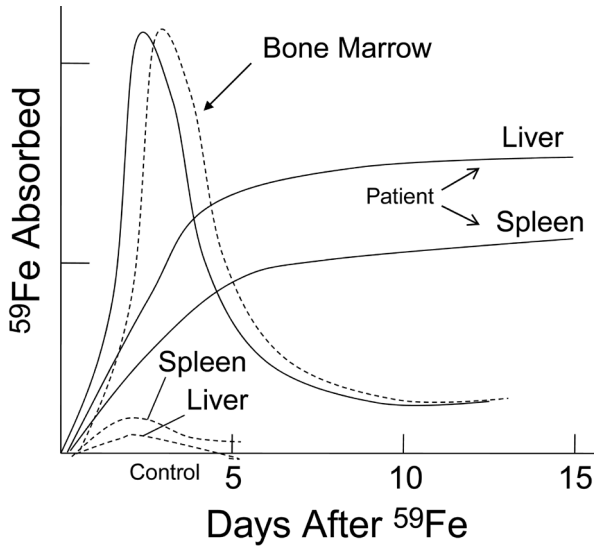


FIGURE 8.6. *Abnormal Iron Metabolism in Tissues of a Patient with a Genetic Defect in Ceruloplasmin. These data show that radioactive iron given to a person who is unable to synthesize ceruloplasmin results in steady accumulation of iron in the liver and spleen. Note that the initial surge of iron into the bone marrow is unaffected, implying that the movement of iron to bone marrow cells does not depend on ceruloplasmin (Adopted from Logan, 1996).*

Both the patient and a non-affected control, upon being injected with the iron, showed a rapid rise of ^{59}Fe in the bone marrow. Turnover from the bone marrow for both occurred at about the same rate. In contrast, a strong retention of radioactive iron in liver and spleen occurred in the patient with no ceruloplasmin. The data suggest that without ceruloplasmin, iron can be taken up by the liver and spleen but not released. Subsequently, it was shown that an injection of ceruloplasmin into the affected patient raised serum iron two-fold, which is consistent with other studies that have implicated ceruloplasmin as being essential to modulating the rate of iron flow from liver to plasma and, via transferrin, to other organs. This study allowed investigators to observe for the first time the consequences humans experience when there is no functional ceruloplasmin.

8.3.3. Copper/Iron Interactions in Yeast

Linking copper with iron metabolism is more vividly portrayed in yeast. When grown in a medium lacking copper, yeast cells are unable to absorb iron. Adding copper back to the medium, however, enables iron to enter. A diagram of the yeast cell shows why this happens (Figure 8.7).

Copper entering the cell reacts with Atox1, a copper chaperone that takes the copper to the Fet3p-permease assembly site in the Golgi. Through the action of Cccp, a copper ATPase, the copper is transferred from Atox1 to apo-Fet3p, rendering Fet3p active as an oxidase. The active complex moves to the plasma membrane, where it is positioned to govern the movement of Fe^{2+} entering into the cell by first oxidizing Fe^{2+} to Fe^{3+}, the form that is recognized and transported by the permease. Fet3p thus catalyzes the oxidation in tandem with the movement of Fe^{3+} through the permease. Without copper, the complex is inactive and no iron can be transported into the cell.

8.3.4. Copper/Molybdenum/Sulfur

When copper/molybdenum and sulfur come together, the outcome is to form a complex that suppresses copper absorption in the intestine and its uptake by peripheral cells.

Such was the conclusion that was made when it was discovered that a molybdenum-sulfur complex, referred to as tetrathiomolybdate, trapped copper ions and seriously limited their absorption and metabo-

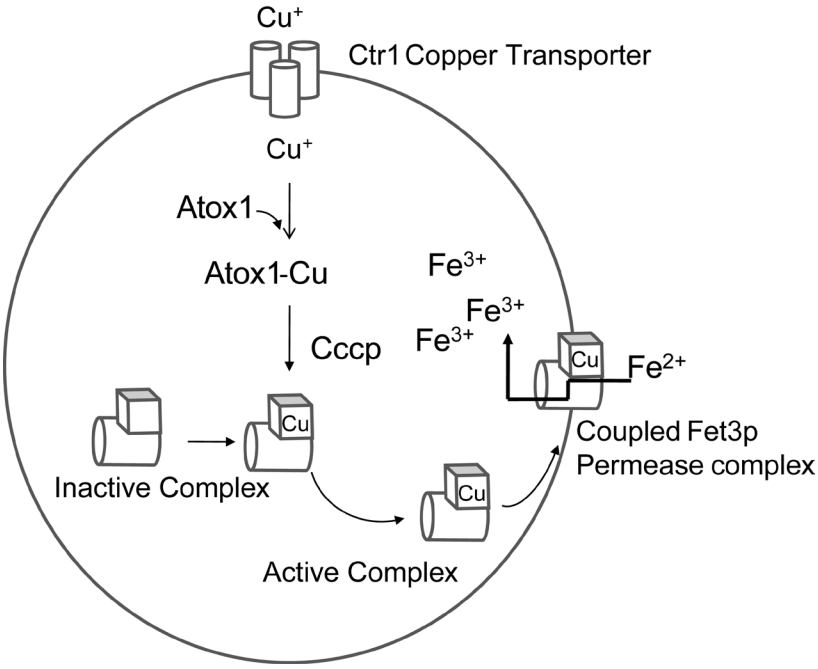


FIGURE 8.7. *Role of Copper in the Uptake of Iron into Yeast Cells.*

lism (Figure 8.8). Suppressing copper absorption in the rumen of animals grazing in molybdenum-rich soils had a major effect on cattle that required copper as an essential nutrient. More recently, thiomolybdates have been used as anticancer agents in human medicine. Cancerous tumors depend on copper ions to form the capillaries that provide blood for the tumor to grow, what is referred to as "angiogenesis". Because of the formidable risks, a patient with an inoperable tumor cannot rely on surgical removal. Such patients now have the option of using thiomolybdate as a medicine to limit tumor growth by shutting down the tumor's access to copper.

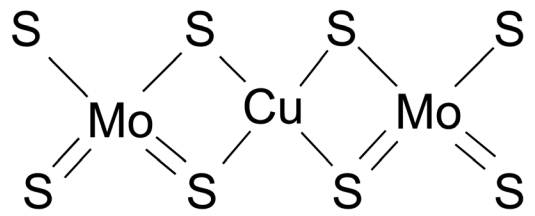


FIGURE 8.8. *A Tetrathiomolybdate Complex with Copper.*

8.3.5. Iron/Manganese

Manganese is a major cofactor for the mitochondrial enzyme mangano superoxide dismutase (SOD-2). Iron and manganese as Fe^{3+} and Mn^{2+}, respectively, have identical $3d^5$ electronic configurations, which makes the enzyme capable of binding iron at the manganese site. To lessen the possibility of interference, cytosolic Fe^{3+} is sequestered and kept at very low amounts in the cytosol. When sequestering is prevented by a genetic defect in iron storage, the Fe^{3+} will compete with Mn^{2+} for binding sites on metal-free apo-SOD-2, resulting in an inactive enzyme and thus making mitochondria vulnerable to free radical damage.

8.3.6. Selenium/Sulfur

No two dissimilar elements match the closeness of selenate and sulfate in their structures and dimensions. As seen in Table 8.3, the similarity is borne out by each complex having nearly identical ionic and covalent radii. The covalent radii will be more of a factor in determining the overall dimensions of the complex. Because their radii are nearly identical, Na_2SO_4 and Na_2SeO_4 compete with one another in correcting White Muscle Disease in lambs. The sulfate salt, however, has no biological activity and will not interfere when selenocysteine or selenomethionine is applied as the therapeutic compounds.

8.3.7. Iodine/Selenium

A striking interlinking between two non-metals is exemplified by iodine and selenium. The synthesis of an active thyroid hormone depends on the incorporation of iodine into the precursor protein thyroglobulin (Chapter 18). The resulting hormones, referred to as T4 and T3, are formed by a series of events in follicle cells of the thyroid gland. Of the two, T3 is considered the more active form of the hormone. The synthesis of T3 occurs by a deiodination at the 5′ position (Figure 8.9). That reac-

TABLE 8.3. Similarity in Ionic and Covalent Radii of Selenium and Sulfur.

Ionic Radii	Covalent Radii
Se = 1.9 nm	Se = 1.03 nm
S = 2.0 nm	S = 1.07 nm

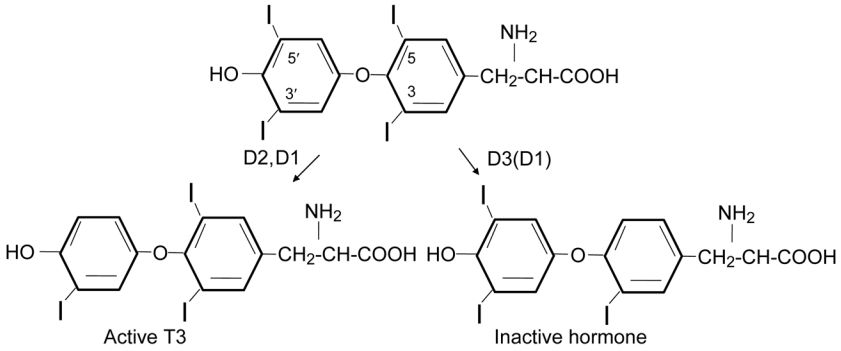


FIGURE 8.9. *Scheme of Thyroid Hormone Synthesis and Activation. D1, D2, and D3 refer to selenium iododiodinase enzymes that remove one iodine from the T4 rings. Numbers refer to positions on the rings. D2 and D3 in combination remove the iodine at position 5', giving rise to an active T3 hormone. D3 and D1 remove the iodine at the 5 position and hence give rise to an inactive hormone.*

tion is catalyzed by isomers of selenium enzyme, 5′ iododiodinase, D2 and D1. Each deiodinase enzyme contains a selenocysteine that takes part in removing the iodine. The combination of isomers D3(D1) removes iodine from the 5 position on the ring, resulting in an inactive hormone. The importance of selenium in thyroid hormone synthesis is noted by a number of studies with humans (mainly children) that have shown the interlinking of the two minerals in preventing goiters and cretinism.

8.4. SUMMARY

Mineral-mineral interactions occur for both the macro and micro families of minerals. The connection between minerals is through both interlinking and interdependency of their properties, which in turn depends to a large extent on their structural similarity. The reactions can be both beneficial and antagonistic. The conclusion one can draw from these observations is that many of the functions performed by a mineral in the system is influenced by or depends on other minerals in the system. In the macromineral family, calcium is at the hub of a series of interactions that governs growth and sets standards for other minerals. One cannot ignore the chemical properties when attempting to describe existing mineral interactions and predicting others. Of those noted, the confluence of copper and iron is of special significance. A defect in the gene for ceruloplasmin in humans and a copper-deficient diet in animals both result in a strong retention of iron in the liver, an indication of a

failure to mobilize iron into the blood. Yeast as a model eukaryotic cell has provided insight into the requirement for copper in iron uptake into cells. Fet3p in yeast has analogies to ceruloplasmin in being a copper-dependent ferrous iron oxidase. These observations gain significance when it is realized that iron deficiency is pandemic and in some cases resistant to iron salts alone as the correcting factor. Thyroid hormone metabolism appears to be a function of both iodine and selenium. This raises the possibility that a selenium deficiency could exacerbate cretinism and goiters, two disorders associated mainly with dietary iodine.

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8.6. PROBLEMS

1. What two conditions can elevate the serum calcium and phosphate level of the blood?

2. What hormones respond to rises in serum calcium and phosphate (hypercalcemia)? To a fall in serum calcium (hypocalcemia)? Responding to elevations in serum calcium and phosphate are two hormones. Name them and describe their function in restoring calcium and phosphate homeostasis.

3. What mineral is capable of relaxing muscle contraction? How does this work?

4. What mineral reestablishes sodium gradients across membranes?

5. What minerals, alone or in a complex, are able to limit the absorption of copper?

6. Is selenium needed to synthesize thyroxine (T4)? Explain your answer.

7. Where would sulfur have the greatest impact on selenium: when selenium is used a cofactor for an enzyme or when selenate is added to soils to prevent a disease?

8. Why is iron capable of interfering with the antioxidant activity in the mitochondria?

9. As a nutritionist, what advice would you give to someone who believes that increasing the intake of calcium is the best way to lower the risk of osteoporosis?

CHAPTER 9

Minerals in the Brain

IT'S difficult to single out a particular tissue or organ to call attention to a specific mineral's function. Basically, minerals perform the same functions in all organs. An exception, however, is the brain. In the brain, the minerals are the same but the functions they perform take on new meaning. Perhaps the most multifunctional mineral in the brain is zinc. At least 10 percent of brain zinc is located in presynaptic vesicles that modulate the action of glutamatergic neuron receptors. This deviates quite strongly from zinc's role in other organs. Heavy metals in the brain do not come without risk. As pointed out by Knight, "our brains are full of metals that are absolutely vital, but handled the wrong way can be devastating". This statement foresees brain minerals as factors that lead to neurotoxicities or propagate disorders such as Parkinson's and Alzheimer's diseases. The key is "if handled the wrong way". In this chapter, we will focus on four key metals in the brain and explain their essentiality to brain functions. The outcome of these lessons is to understand the following:

1. The functions of minerals in brain tissue aside from their function in other organs.
2. Transport and uptake of brain minerals.
3. Consequences of mineral deficiencies in the brain.
4. The dark side of minerals in the brain—their role as pathogens in brain disorders.

TABLE 9.1. Functions of Minerals in the Brain.

Mineral	Brain Function
Zinc	Glutamatergic neuron receptor modulator
Iron	Myelin synthesis and neurotransmitter release
Calcium	Neurotransmitter release
Cu, Zn and Mn	Cofactors for antioxidant enzymes
Copper	Dopamine, norepinephrine synthesis, activation and release of neuropeptide hormones

9.1. SUMMARY OF FUNCTIONS

As stated by Ashley Bush, "the brain concentrates minerals better than any other organ"; and, as pointed out by Bourre, "the brain is an organ elaborating and functioning from substances present in the diet". A brief summary of some of the functions of minerals in the brain is shown in Table 9.1. The functions range from for stimulating and modulating impulses across synapses to the synthesis and activation of at least 18 neuropeptide hormones. Some of these mineral targets are found only in brain tissue. The metabolism of brain tissue emphasizes a unique set of reactions that occur in specific regions of the brain such as the pituitary, hippocampus, and hypothalamus. These are literally life and death functions that only the inorganic elements in the diet can perform.

9.2. ZINC

9.2.1. Function in the Brain

Zinc is sometimes referred to as the most abundant trace mineral in the brain. Unlike other tissues, free zinc ions amass exclusively in the presynaptic vesicles of the glutamatergic neurons in the forebrain of the cerebral cortex. The highest concentrations are found in neurons in the hippocampus region which controls learning and memory. They are not found in the cerebellar cortex or thalamus. Zinc-laden vesicles release zinc as a free ion in response to calcium. Figure 9.1 shows a zinc stained section through the midbrain and cortex of a rat. Zinc-containing vesicles appear as dense particles in an electron micrograph [Figure 9.2(a)].

Zinc uptake into vesicles is a function of ZnT_3 zinc transporter found

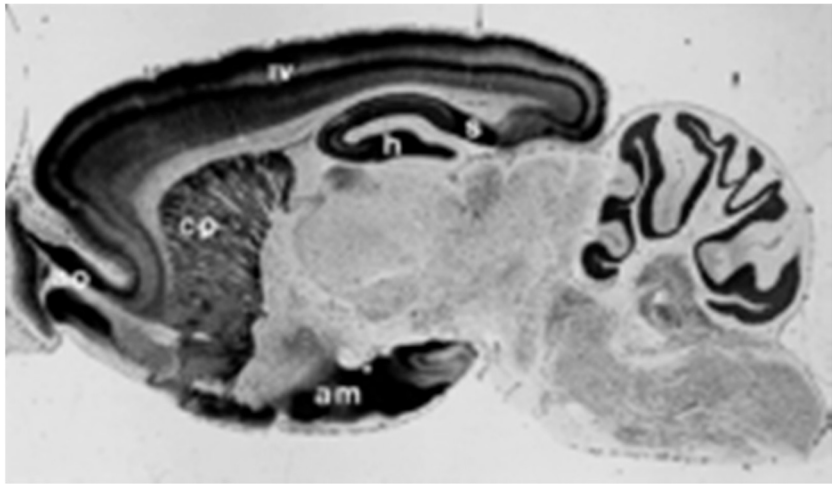
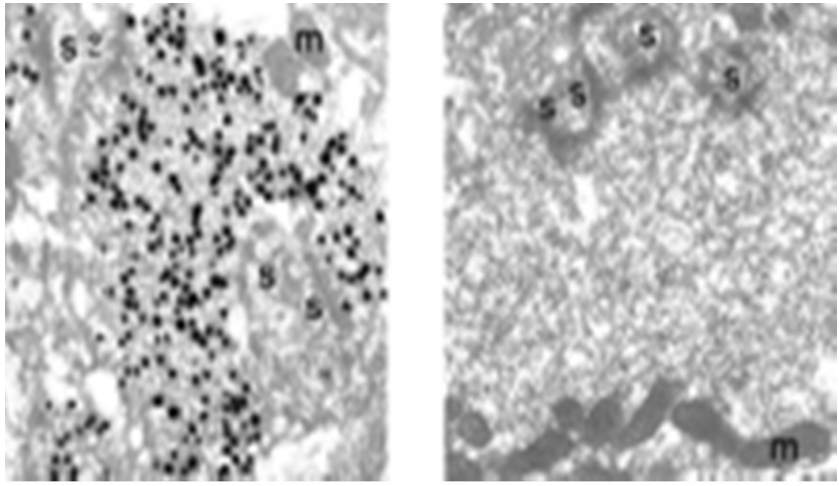


FIGURE 9.1. *Cross Sections of a Rat Brain Stained for Zinc. Zinc-containing vesicles are in neurons in the hippocampus region but missing from the cerebellar cortex and thalamus.*

chiefly in neural tissue. As shown in Figure 9.2(b), zinc cannot localize in vesicles if ZnT_3 is absent or defective. Figure 9.3 shows zinc and glutamate vesicles in the afferent neuron.

When stimulated by the action potential, both zinc and glutamate are released into the synaptic junction between the two neurons and



(a) (b)

FIGURE 9.2. *Zinc-laden Vesicles in Brains. (a), control; (b) ZnT_3 knockout mice. (Adopted from Frederickson et al., 2004)*

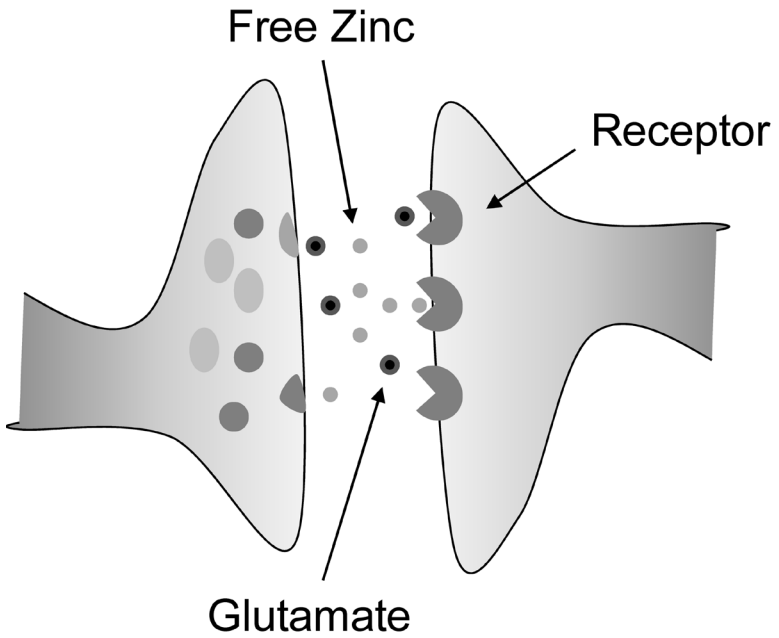


FIGURE 9.3. Function of Zinc as a Transmitter Modulator. The diagram shows the synaptic junction between two adjoining neurons. Free Zn and glutamate compete for the receptor on the distal side of the synapse (Adopted from Frederickson et al., 2004).

compete for the glutamate receptor on the other side of the junction. In this way, zinc is able to control the intensity of the action potential of the distal neuron. Zinc that has reached the other side of the synapse is quickly sequestered by metallothionein 3, one of the family of metal binding metallothioneins, but like ZnT_3 is found mainly in the brain.

9.2.2. Brain Zinc Homeostasis

The brain receives zinc from both the plasma (across the blood brain barrier) and cerebral fluid (across the blood cerebral spinal fluid barrier). Once in the brain, the zinc is slow to turnover, but is still sensitive to nutritional deprivation and therefore capable of causing brain dysfunctions. In general, the greater the neuronal activity, the greater will be the need for zinc to exert a modulating action.

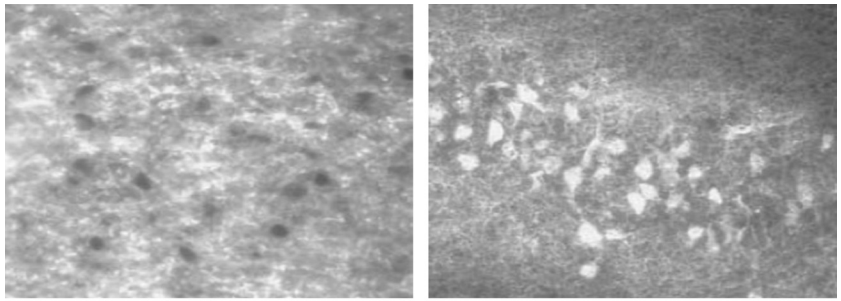
9.2.3. Zinc Toxicity

Sequestering zinc into vesicles precludes any free zinc from exerting

toxic effects. There are, however, instances, pathogenic in nature, where large amounts of free zinc can be released prematurely from their vesicular confines. As an example, the level of free non-sequestered zinc in the brain is estimated to be around 3 nanomolar. By depriving the brain of oxygen or interrupting the blood supply to neurons (ischemia), the level of free zinc can be raised more than 10,000 times. All this excess free zinc overwhelms metallothionein sequestering and is now poised to induce apoptosis (programmed cell death) of brain neurons. Figure 9.4(a) shows a normal distribution pattern for zinc in the hylus of the dentate gyrus of a rabbit. Healthy neurons appear as dark areas amid Zn-positive axonal plexes. Figure 9.4(b) shows the hilar region 24 hours after a 7 minute ischemia. Zn-positive areas are shown in white. Note that darker areas indicative of live neurons are strongly diminished. Free zinc that is liberated after seizures, stroke and brain trauma can be toxic to neurons and contribute to excitotoxic brain injury.

9.2.4. Zinc and Antioxidant Activity

The rich oxygen environment within brain cells is conducive to oxidation reactions that give rise to free radicals as by-products of metabolism. Because antioxidant enzymes are in lower levels in the brain than in other tissues, it is imperative that metal ions cofactors for these enzymes remain at steady-state levels. As one of the cofactors for superoxide dismutase (SOD1), zinc is in a position to quench free radicals before they harm brain cells. Copper, the other cofactor in the enzyme, works with zinc in this reaction. Together the two metals protect neurons from oxidative reactions that have the potential to cause neurological diseases.



(a) (b)

FIGURE 9.4. *Ischemia-induced Changes in Zinc Distribution in the Hylus of the Dentate Gyrus.*

9.2.5. Zinc and Cognitive Functions

Is there evidence that zinc in the brain controls behavioral and learning parameters? Studies from the USDA Human Nutrition Center in Grand Forks, North Dakota have shown zinc to have an impact on behavior and cognitive reasoning. In one study, Penland and coworkers (2000) supplemented the diet of adolescent seventh graders with 20 mg of zinc, 5 days a week for 20 days. Subjects given the zinc were more attentive and responded more quickly and accurately to questions. Earlier Penland, in attempting to establish a critical level, modified zinc diets of adults and tested for cognitive and psychomotor skills. In the critical period, adults were given low zinc (ranging from 1–4 mg/day) for 35 days then switched to 10 mg per day. Testing performance in the zinc restriction period gave clear evidence of a deterioration in skill level in most of the tests performed.

9.3. COPPER

9.3.1. Functions in the Brain

9.3.1.1. Dopamine β-monooxygenase (DMO)

The copper-dependent enzyme dopamine-β-monooxygenase (DMO) catalyzes the conversion of dopamine to norepinephrine, the major neurotransmitter of the sympathetic nervous system. Like PAM (below), DMO requires ascorbic acid as an electron donor. The soluble form of

TABLE 9.3. Pituitary Hormones Activated by Peptidyl-α-amidating Monooxygenase.

Hormone	Location or Action
Galanin	Monoaminergic neurons
Gastrin	Gastric acid
Gonadotropin releasing hormone	Sex hormone
Neuropeptide Y	Control of hunger and obesity
Pancreastatin	Insulin control
Substance P	Emotions
Thyrotropin releasing hormone	Thyroid hormone synthesis
Vasopressin	Water homeostasis

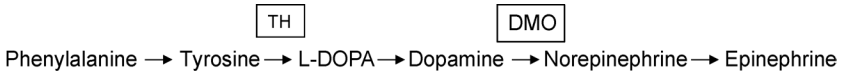


FIGURE 9.5. *Pathway for the Biosynthesis of Neurotransmitters in Brain and Nervous Tissue.*

the enzyme is localized in chromaffin granules of the adrenal medulla, which is rich in ascorbic acid. Tyrosine hydroxylase (TH), an iron-dependent enzyme, also partakes in regulating dopamine and epinephrine synthesis by controlling the formation of L-DOPA, the third intermediate in the pathway (Figure 9.5).

9.3.1.2. Peptidyl-α-amidating monooxygenase (PAM)

At least eight pituitary hormones are activated (not synthesized) by PAM (Table 9.3). Ascorbate serves as an electron donor in this two-step reaction that effectively adds an amide group to the C-terminus of the pituitary hormones (Figure 9.6). Without an amide group, the hormones are functionless. PAM's distribution is widespread and includes the adrenal medulla and pancreas, where its functions are still unclear. Sequence data provides evidence that PAM and DOM show some structural similarity, possibly meaning that both have binding sites for ascorbic acid on the protein surface.

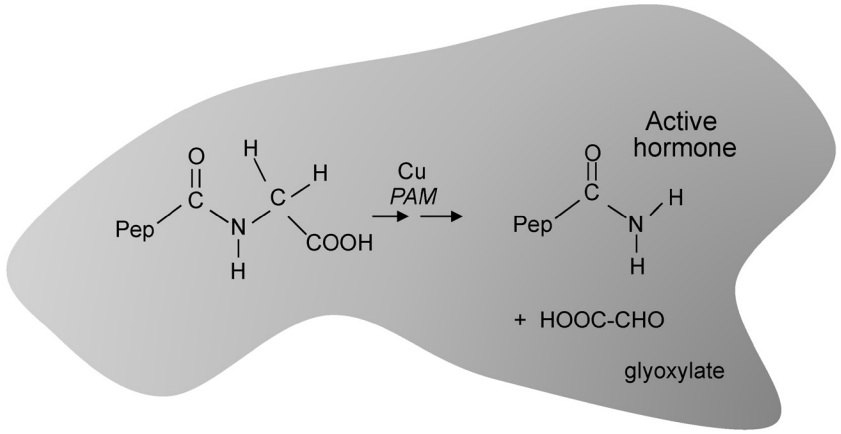


FIGURE 9.6. *Basic Reaction Catalyzed by Peptidyl-α-amidating monooxygenase (PAM).*

9.3.1.3. Cu/Zn Superoxide Dismutase (SOD_1)

This ubiquitous copper/zinc enzyme is a major defense against oxygen toxicity. The need for the enzyme in the brain is critical, considering that the brain is one of the highest oxygen-requiring organs in the body. A rich oxygen environment increases the propensity to form oxygen radicals, which makes the enzyme a key component in the defense against oxygen toxicity.

9.4. IRON

9.4.1. Overview of Iron Homeostasis and Functions in the Brain

Iron enters the brain by two mechanisms: transport across the blood brain barrier and transport through the blood choroid plexus. Transferrin is the iron carrier in both pathways. This scenario paints a picture of how iron must pass through two key barriers in order to engage neurons and glial cells prepared to receive the iron. It also gives insight into the mechanism of iron homeostasis in the brain. Although limited, ferritin in the brain has the capacity to store iron. As noted previously, tyrosine hydroxylase (TH) is an iron-dependent enzyme that determines the rate of dopamine and epinephrine synthesis (Figure 9.5). Iron's other, more prominent role is in the mitochondria as a cofactor for iron-dependent enzymes and a component of heme in the cytochromes that transfer electrons to oxygen to generate ATP.

9.4.2. Consequences of Iron Deficiency in the Brain

Of its many important functions in the brain, none surpasses the brain's need for iron in the process of myelination. Myelination begins late in gestation and extends into the third year postnatally. Iron's role in myelin synthesis in the developing brain is in the series of enzyme reactions involved in membrane lipid biosynthesis, specifically fatty acids, phospholipids and cholesterol, from which is formed the substance of myelin sheaths that surround the axons of neurons. A deficiency in iron intake can cause a widespread myelin deficiency in critical brain regions such as the hippocampus which, in the developing brain, is the foundation for learning and memory.

9.5. MANGANESE

9.5.1. Overview of Functions in the Brain

Manganese, like iron, accesses brain cells by crossing the blood brain barrier and choroid plexes. Transferrin is believed to be the carrier in the transmembrane movement. The brain contains a large number of manganese-requiring enzymes; two of the more prominent are manganese superoxide dismutase in the mitochondria and glutamine synthetase in astrocytes. It has been speculated that free manganese, in a reaction emulating zinc, modulates nervous impulses in glutamatergic neurons. At the same time, manganese is regarded as a potent neurotoxin capable of causing damage to the brain cells and eliciting behavioral disorders.

9.5.2. Brain Areas Susceptible to Manganese

Manganism (manganese poisoning, also called "welder's disease") results from excessive exposure to manganese oxide. Workers so exposed over time have been observed to exhibit unusual behavior bordering on psychosis. It is now known that the psychotic behavior was caused by Mn accumulating within the basal ganglia, especially the caudate putamen, globus pallidus, substantia nigra, and subthalamic nuclei. These areas of the brain are known to control voluntary movement, active learning, and certain character traits. Symptoms of manganism at early stages are compulsive or violent behavior, emotional instability, hallucinations, fatigue, headache, muscle cramps, and loss of appetite. Other descriptions tend to show an overlap between manganism and Parkinson's disease with patients showing dystonia, hypokinesia, rigidity and muscle tremors.

The mechanism leading to the development of manganism in the brain is still in doubt. Neuronal damage, however, appears to be central to development. It is suspected that when Mn accumulates in the basal ganglia, there is a potential decrease of gamma-amino-butyric acid (GABA) innervations into subthalamic nuclei, which further deregulates glutamate release into the substantia nigra. Both would ultimately increase dopamine released into the striatum. Because Mn can oxidize dopamine, brain areas dependent on dopamine could be seriously impaired. Zwingmann *et al.* proposed that glucose metabolism in the neu-

rons could also be impaired due to Mn-mediated oxidative stress. The stress can render neuronal mitochondrial dysfunctional which eventually leads to a general energy failure with less ATP being synthesized and neuronal death.

9.5.3. Mineral Links to Brain Pathologies

A question yet to be resolved is whether heavy metals in the brain are primary or secondary to the development of brain pathologies. Two of the most detrimental with aging are iron and copper. Supporting a primary role is evidence that shows that some metal ion concentrations tend to change as a person ages. Second, the changes occur in specific brain regions that are consistent with the symptoms displayed. Third, in some cases the biochemical factors linked to abnormalities have been shown to bind and store heavy metals.

9.5.4. Age as a Factor

Figures 9.7 and 9.8 show changes in iron and copper, respectively, with aging in the human brain. Typically, the changes are confined to

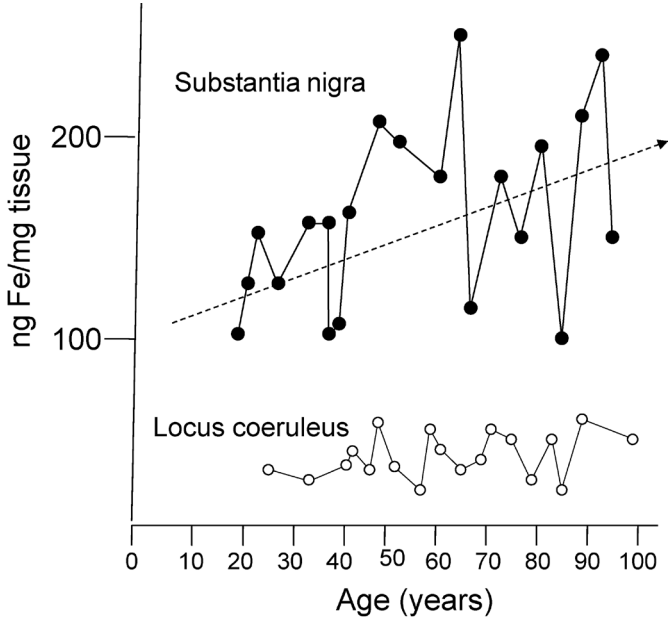


FIGURE 9.7. Changes in Iron Concentration in the Human Brain with Aging.

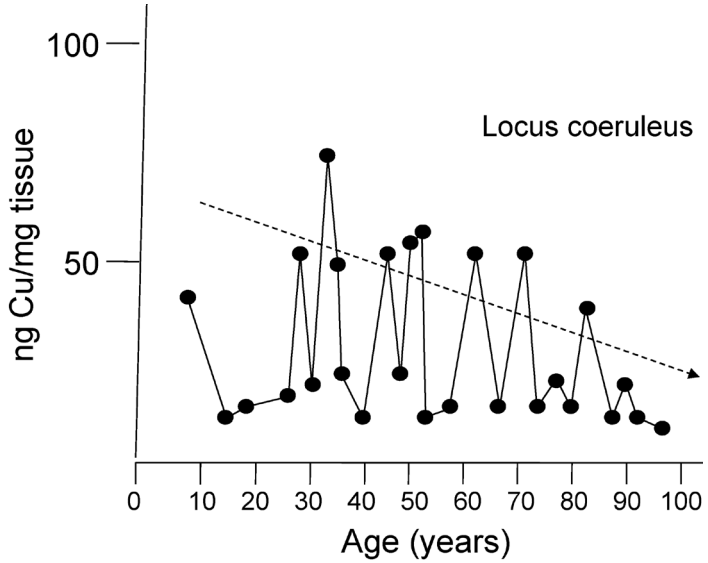


FIGURE 9.8. *Changes in Copper Concentration in the Locus Coeruleus of Human Brains with Aging.*

specific brain regions. For example, over time, iron builds up slowly in the substantia nigra (SN) but not in the locus coeruleus (LC). In some cases, the prevailing neurological disorder is accompanied by higher transferrin saturation in the blood, allowing more iron to enter the brain, which could be a cause of the gradual iron buildup. In contrast, copper concentration steadily declines in the locus coeruleus, increasing the likelihood that dopamine and epinephrine concentrations will be affected.

9.6. SPECIFIC DISEASES WITH A MINERAL CONNECTION

Three diseases highlight evidence for mineral involvement in either the cause or progression of a neurological disease. Metal ions have destructive properties when in excess or when brain metabolism is altered. Also, pathologies arise when the mineral in question is deficient. The following diseases illustrate both possibilities.

9.6.1. Restless Leg Syndrome (RLS)

RLS has been connected to an iron deficiency, more specifically an impairment of transferrin to cross the blood brain barrier. The disease

is more apt to be evident in adults. Pregnancy, for example, can be associated with an iron deficiency if the mother does not take into account the need for iron by the fetus. The connection with iron is strengthened by observing symptoms of RLS improve or disappear with iron supplements.

9.6.2. Alzheimer's Disease (AD)

Alzheimer's disease affects mainly the elderly. Appearance of symptoms before the fifth decade of life is rare. AD is characterized by short term memory loss and severe disorientation. The role of metal ions as primary or secondary in the onset of AD is yet to be resolved. A hallmark of AD is the presence of plaques composed of amyloid beta (Aβ) protein that deposits on the neurons as fibular tangles. The insoluble fibrils entrap metal ions and have toxic properties, possibly exacerbated by the metal ions they bear. Studies have provided evidence that Aβ has binding sites for copper with the capacity to reduce the copper to its cuprous (Cu^{+}) form. In addition, iron and zinc accumulate within the neutrophil and are highly concentrated in the plaques. There is also evidence that copper and zinc trigger plaques to form fibrils and thus become a major factor in the slow build up of Aβ protein deposits. The stability of the metal induced fibrils is weakened by metal chelators, suggesting that metals not only have a role in amyloid plaque and fiber formation but provide resistance to metabolic turnover once they form. Another concern is that zinc released into the synapse as the free ion can react with Aβ to cause precipitation around blood vessels, which may explain why plaque formation is more prominent in zinc-rich regions of the brain.

9.6.3. Parkinson's Disease (PD)

PD is a movement disorder that is both chronic and progressive. Like AD, PD is also characterized by a buildup of amyloid plaques that surround the neurons. In this case, plaques are referred to as alpha synuclein or Lewy bodies in honor of the investigator who first correlated their presence with the disease. The incipient death of dopaminergic neurons has also been connected with PD onset and epidemiological studies have connected Fe, Zn and Cu with the pathogenesis. Parkinsonian patients display a buildup of copper in neurons that secrete dopamine. If copper is a factor, copper homeostasis at the

cellular level is disrupted and cells are apparently unable to export copper from dopamine neurons, ultimately causing neuronal death through a copper toxicity.

9.7. SUMMARY

Zn, Cu, Fe, and Mn play indispensible roles in normal brain functions. Both a deficiency and an excess of these metals has telling effects on the brain. Brain neurons function as a signaling network that conveys electrical impulses. In a deficiency, the absence of the metal or its presence in low amounts could seriously hinder synthesis of neurotransmitters or deregulate neuronal activity. Zinc and manganese ions in a free form modulate the action potential. Low levels of zinc and copper make the brain tissue more susceptible to free radical damage. Low levels of iron seriously hinder myelin formation in the developing brain. The dark side of metals in the brain is their toxic effects at high concentrations and when liberated from vesicles or binding proteins. Under such conditions, key brain functions are compromised by metal toxicity. Whether toxic actions of untethered metal ions are behind the onset of Alzheimer's or Parkinson's disease is still unsettled. Clearly, metal ions associated with amyloid fibrils characterize these disease states and as such can be considered factors in the destructive action that ensues.

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9.9. PROBLEMS

1. Compare and contrast the mechanism by which zinc, copper, iron and manganese gain access to the brain. What regulates the inward flow of these metals?

2. Free ions have the potential to be pathogenic. How does the brain protect against the toxic action of zinc, copper and iron? Is there a common mechanism for the three ions?

3. Chapter 15 discusses Wilson's disease. What causes this disease and how do the symptoms resemble a Parkinson-like toxicity?

4. You saw in Figure 9.7 that with aging, iron tends to accumulate in the Substantia Nigra region of the brain. Efforts to explain the increase have focused on serum transferrin levels. What else could explain the increase in iron as one ages?

5. Can sodium ions substitute for zinc's as a neuromodulator? What conditions would have to be met for this to occur?

6. Repeat question 5, only consider calcium ions as the potential displacer.

7. It is said that neurons in the brain, once lost, cannot be replaced. In essence, neurons are laid down in a critical window during development and do not turnover. How does this observation cause one to think of metal ion homeostasis in the brain? What factors must be met for homeostasis to occur?

8. How does adequate zinc in the diet link up with learning? What is the zinc doing that helps one learn faster?

CHAPTER 10

Sodium, Chloride and Potassium

THE three minerals in this chapter control fluid balance and membrane potential. These two functions, however, are only part of their action. Sodium gradients across membranes supply the energy that drives amino acids and sugars into cells and also stimulate nerve impulses that activate muscle contractions. Sodium ions are located almost exclusively *outside* of cells, whereas potassium ions amass *inside* cells; chloride is the counter anion for both. Because of their antagonism, compartmentalization must be maintained in order for the two to perform basically diametrically opposite functions. There is no RDA for sodium, chloride or potassium, but instead adequate intake (AI) is used to assess nutritional need. Even this figure can be misleading, however, since specifying an adequate amount for sodium does not take into account high sodium losses in the urine and body pores. Nutritionists are more concerned with adverse effects arising from too much sodium in the diet rather than a deficiency.

10.1. HISTORY AND EARLY INSIGHTS

Sir Humphrey Davy recognized sodium as a new chemical element in 1807. The chemical symbol Na is derived from the Latin *natrium* or arabic *natrum*, and the word sodium comes from the Latin "sodanum" meaning headache remedy. For early Egyptians sodium salts were used as cleansing agents that barred food from spoiling. Common salt (NaCl) was a highly valued food preservative whose locale (city, town, etc.)

was generally a target of armed conquest. On a more practical side, having sodium as a preservative eliminated dependence on seasonal foods. Nomads subsisting mainly on cereal, vegetables and boiled meats had to rely on salt supplements to stay healthy; this example is one of the earliest references in the literature regarding low sodium intake. Once regarded as a luxury for the rich, sodium salts have become a staple in the diet of humans and animals. Only within the last 30 years have nutritionists questioned possible deleterious effects arising from too much sodium in the diet. Concern was aroused by studies that showed incidences of hypertension and heart disease correlating with elevated sodium intake. Even today, the biological action of sodium is still actively studied. Thus, despite its omnipresence in nearly all foods and biological systems, we still know little about the nutritional need for sodium and its role in nutritionally associated disease. Potassium was isolated by Davy using electrolysis, the first element to be obtained in nearly pure form by this procedure. The name potassium refers to potash (KOH), an alkaline-forming residue left in the pot after burning.

10.2. CHEMICAL PROPERTIES

As group IA metal ions, sodium and potassium are colorless, water-soluble monovalent cations with little or no capacity to bind strongly to organic or inorganic components. Biological Na^+ and K^+, therefore, exist as non-tethered free ions in aqueous media, free to ooze through pores in cell membranes and to form ion gradients across cell membranes. Both have nearly identical electronic configurations, thus providing a rationale for antagonism between the two. As hydrated ions, however, Na^+ is larger than K^+, which opens the way for the more permeable K^+ to regulate the action potential of Na^+ gradients that drive nutrients into cells and excite neurons.

10.3. BIOCHEMICAL PROPERTIES

Sodium and chloride ions work in concert in controlling water distribution between cells, the interstitial fluid and plasma. In contrast, Na^+ and K^+ have the potential to counter each other's movements, which results in the two ions being in perpetual conflict within the system. In effect, there are no synergistically beneficial effects of Na^+ and K^+

in biological systems. Potassium can interrupt sodium flux into cells and cause contracted muscles to relax, which is one reason potassium salts or potassium-rich foods can overcome muscle cramps. Such conflict between the two becomes critical when it threatens the action of energy-rich sodium gradients across membranes. Overseeing the conflict, therefore, are membrane ion pumps that keep the two ions apart. Maintaining the energy of a sodium gradient is the function of Na^+/K^+ATPase, an enzyme that forcefully expels Na^+ from the cell's interior in exchange for K^+ (Figure 10.1). Referred to as a Na-K pump, the enzyme is found on the basolateral surface of cells and uses the energy of one ATP to displace 3 Na^+ ions from the interior in exchange for 2 K^+ ions entering from the exterior. One chloride ion is also expelled to maintain electro-neutrality.

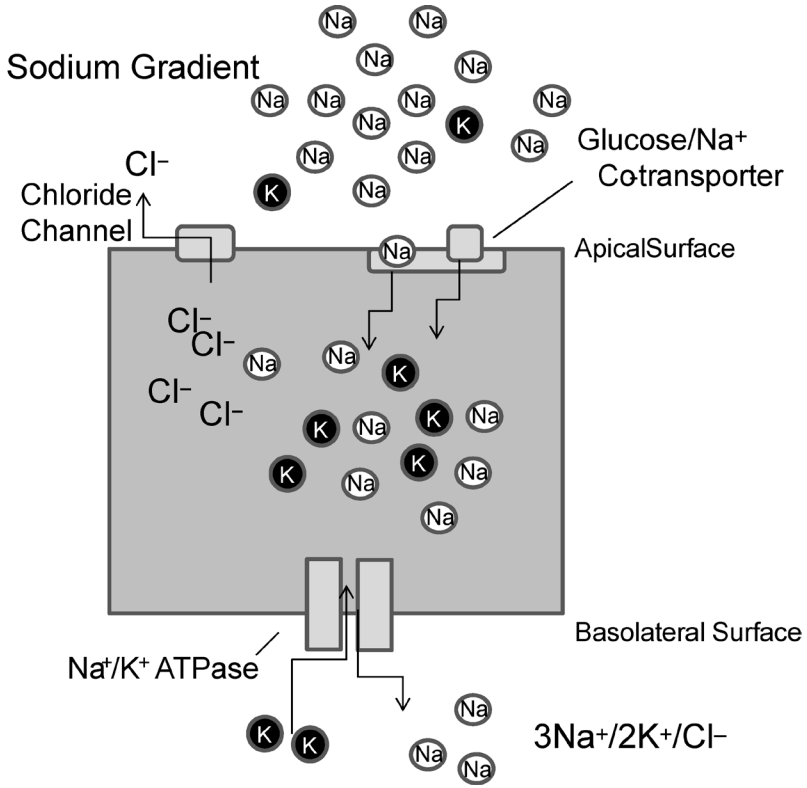

FIGURE 10.1. *Sodium-Potassium Homeostasis in Cells. Sodium ions en masse provide the driving energy for glucose and other sugars to enter the intestinal cells. Homeostasis is achieved by expelling the Na^+ from the cytosol with an energy-driven Na^+/K^+ ATPase on the basolateral surface.*

10.3.1. Sodium Dependent Transport Carriers

The role of solute linked carriers (SLC) for nutrient absorption across the intestine was introduced in Chapter 6. Suffice to say, sodium-dependent carriers with Na^+ ions providing the driving force extend beyond the intestine. The sodium/glucose co-transporter gene family in humans is one example. This family consists of 11 members, all coded by the SLC5 family of genes (Table 10.1). SLC5A1, for example, transports D-glucose and D-galactose across the brush border membrane of mature enterocytes in the small intestine. Two Na^+ ions are cotransported in sync with each sugar molecule and chloride ion is used as a counter ion. SLC5A1 also serves as a uni-transporter for Na^+. SLC5A5, a Na^+/iodide co-transporter (NIS) found principally in the follicle cells of the thyroid gland, accumulate iodide ions for thyroid hormone biosynthesis (Chapter 18).

TABLE 10.1. The SLC5A Family of Solute Carriers Associated with Sodium Transport Functions.

Human Gene	Protein	Function	Cellular Location
SLC5A1	SGLT1	Na+/glucose, galactose (C)[1]	Small intestine, kidney, heart, plasma membranes
SLC5A2	SGLT2	Na+/glucose (C)	Kidney cortex
SLC5A3	SMIT	Na+/myo-inositol (C)	Brain, heart, kidney, lung, plasma membranes
SLCA4	SGLT3	Glucose-activated Na^+/H^+ channel	Small intestine, muscle, kidney, uterus, plasma membranes
SLC5A5	NIS	Na^+/iodide (C)	Thyroid gland
SLC5A6	SMVT	Na^+/Biotin, lipoate	Brain, heart, kidney, lung, plasma membrane
SLC5A7	CHT	Na^+/Cl^-/choline (C)	Spinal cord, medulla vesicles
SLC5A8	SGLT4	Unknown	Small intestine, kidney, liver, lung and brain
SLC5A9	SGLT5	Unknown	Kidney
SLC5A10	SGLT6	C/Na^+	Small intestine, brain, kidney, liver, heart and lung
SLC5A11	AIT	Iodide	Thyroid; apical plasma membrane

[1](C)= co-transporter

10.3.2. Chloride Channels

Chloride channels are integral membrane proteins that provide a passageway for negative ions across the membrane. Both chloride and bicarbonate ions (HCO_3^-) use this path through the membrane. Ion channels regulate cell volume, stabilize membrane potential, and control signal transduction and transepithelial transport. Their structural classes, revealed through cloning, have been categorized either as ligand-gated or voltage-gated chloride channels. The selectivity of anions over cations minimizes Na^+ and K^+ interference. As part of a super family of membrane proteins regulated by ATP and cAMP, chloride channels rely on ATP, not diffusion, to provide the energy; this conclusion is based on levels of chloride ions in the cytosol that are higher than would be expected if diffusion alone was the driving force. Two of the more intensely studied chloride channel proteins are ClC and CFTR. ClC is the principle chloride channel protein in muscle; CFTR (cystic fibrosis transmembrane conductance regulator) is located in the apical membrane of cells in the intestine, pancreas, sweat glands and airway passages. CFTR is thus central in determining transepithelial fluid flow at these locations. The importance of chloride channel protein is dramatically illustrated by cystic fibrosis (CF), a disorder that arises from a dysfunction of CFTR. CF is manifested by excessive fluid and mucous accumulation in airway passages that can lead to airway disease. The dysfunction also causes pancreatic failure and elevated salt levels in sweat. A defective ClC can cause muscle myotonia, characterized by prolonged muscle contraction.

10.3.3. Potassium Channels

The size differential between hydrated sodium and potassium ions appears to be the basis for potassium and sodium ions not sharing the same ion channel. Knowing that there are specific channels through which only K^+ may pass gives insights into the biological necessity for potassium. As noted previously, the physiological function of sodium and potassium relies on segregating the two ions from one another, a process referred to as "compartmentalization". Failure to segregate the two cancels the energy of the co-transport systems. Four channels for potassium that occur in every cell have been identified as major. These are:

1. Na^+/K^+ ATPAse:

2. K^+–H^+ ATPase:
3. Na^+–Cl^-–K^+ transporter
4. K^+ conductance channel

In contrast to the Na^+/K^+ ATPases on the basolateral surface, K^+–H^+ATPase is situated on the apical surface, positioned favorably to conduct an ATP-driven inward flow of potassium ions in exchange for H+. In the gastric mucosal cells, this ATP-dependent exchanger generates and controls the acidity of stomach acid. In contrast, the Na^+–Cl^-–K^+ transporter has binding sites for all three ions and is driven by a sodium gradient. Two Cl^- ions are transported for each K^+ and Na^+ moved, thus retaining electric neutrality. A total of 6 proteins that are highly selective for K^+ exist in cells. Proteins that form the conductance channel for potassium are the more complicated. These use the electrochemical energy across the membrane (electronic attraction/repulsion) as a driving force. Of the six, one is activated by Ca^{2+} and at least 3 are controlled (gated) either by nucleotides, voltage, or specific ligands. These proteins have external influences triggering their action and therefore are important mediators of nutrient actions.

10.4. NUTRITIONAL PROPERTIES

Several unique factors must be considered in a discussion of the nutrition of sodium, chloride and potassium. First and foremost is to recognize that these ions have no biomarker to judge adequacy. This precludes determining an EAR (estimated average requirement) which further precludes determining an RDA (Chapter 5). Second, these ions are rapidly and nearly completely absorbed into the blood from the intestine and released into the urine to maintain plasma levels. Another major route of exit for sodium and chloride, however, is through the sweat pores in the skin. Thus, individuals who partake in high physical activity or who are exposed to heated environments are more prone to have a greater need for sodium in the diet. Only when skin loss is minor can one match intake with urinary excretion to assess sodium balance. Potassium, in contrast, is at its highest concentration inside cells. The major flux of potassium ion is therefore inward, not outward. Entrance of potassium into the system from the diet relies on conductance channels mentioned earlier, but selective for potassium ions. As with so-

TABLE 10.2. Dietary Reference Index for Sodium and Chloride[1].

	Sodium (grams/day)		Chloride (grams/day)		Potassium[2] (grams/day)
Age	**AI**	**UL**	**AI**	**UL**	**AI**
0–6 mo	0.12	ND	0.18	ND	0.4
7–12 mo	0.37	ND	0.57	ND	0.7
1–3 yr	1.0	1.5	1.5	2.3	3.0
4–8 yr	1.2	1.9	1.9	2.9	3.8
9–13 yr	1.5	2.2	2.3	3.4	4.5
14–50 yr	1.5	2.3	2.3	3.6	4.7
51–70	1.5	2.3	2.0	3.6	4.7
>70	1.2	2.3	1.8	3.6	4.7

[1]Values for groups apply to both males and females. Pregnancy and lactation have no influence on the values.
[2]An upper limit is not specified for potassium.

dium, potassium absorption is unregulated at the intestinal stage and requires the kidney to maintain homeostasis.

Table 10.2 shows the age dependency for sodium, chloride and potassium. Suffice to say that gram quantities equivalent to adults are recommended for all three minerals beginning at ages 1–3. UL values for sodium and chloride relate to intake levels, which if exceeded, have the potential to raise blood pressure. Upper limit values for up to one year of age cannot be quantified accurately due to the difficulty of determining adverse effects in this age group. For potassium, the AI's are in reference to the amount of potassium that counters a sodium-induced elevation in blood pressure or lowers the risk of recurrent development of kidney stones. There is no evidence that a high intake of potassium from a food source has an adverse effect.

10.4.1. Food Sources of Sodium and Chloride

Nearly 80% of the salt in the diet comes from processed foods; only about 12% comes from naturally occurring salt in food. The remaining 8% represents salt added during cooking or when partaking in a meal.

10.5. INTESTINAL ABSORPTION

Since Na^+ and Cl^- occur in propitious amounts in the postprandial

lumen, their passage into the system presents no major challenge. Both ions have membrane transporters stretching the length of the small intestine and colon. In addition to their role as co-transporters, these ions also maintain osmotic balance. As an example of the latter, consider that gradients of chloride ions drive H_2O secretions from the lumen into the cells, whereas sodium ions force a reverse fluid flow back into the lumen. Glucose- and amino acid cotransporters and the Na^+/H^+ transporter together account for about 70% of the Na^+ taken in through the intestine. Maintaining homeostasis within the system shifts to the kidney and to a lesser extent biliary secretions and sweat glands. The co-transport with glucose renders carbohydrate- rich diets competent to raise the sodium intake, which makes organic components in the food source a potentially important determinant of the amount of sodium taken in by the system. All told, maintaining energy gradients through ATP driven mechanisms accounts for about 40 percent of the resting energy expenditure.

10.6. SODIUM AND HYPERTENSION

Hypertension is defined as having a resting systolic/diastolic blood pressure in excess of 140/90 millimeters of mercury (Benos, 2010). A high salt intake has been linked to the condition. High, for most people in Westernized countries is around 8 to 10 grams per day, which translates into sodium intake of around 3–5 grams per day (Temple, 2011). Sodium ions play a major role in controlling blood pressure, which means sodium in excess in the diet can be a factor in hypertension etiology. Because hypertension can lead to heart failure and a sundry of other problems, hypertension is regarded as a major health problem on a global scale. Because the kidney has a major role in regulating the Na^+ content of the blood and thereby extracellular fluid volume, the kidney is a prime candidate for hypertension onset. More specifically, the kidney's ability to actively reabsorb sodium ions that have been filtered by the nephron tubules can raise the sodium load in the blood and increase blood volume; both are prime factors in causing hypertension. Evidence has further suggested that mutations in epithelial Na^+ channel proteins (ENaCs) could lead to a "gain in function", resulting in overly active reabsorption and retention of sodium ions. Having a high dietary sodium or low potassium intake could further exacerbate the condition. One key to controlling hypertension,

therefore, is to limit sodium intake. This measure, although effective, cannot overcome the condition if mutations in ENaC are the primary cause, however.

Despite a plethora of data linking hypertension to sodium intake, there are some doubts as to the validity of such a connection. How the upper limit of sodium intake has been determined has been subject to criticism. Without this knowledge, what constitutes "excessive" cannot be determined (Satin, 2011). The burden, therefore, shifts to the food industry to lower the content of salt in foods, given the fact that cold storage has replaced salting when storing or preserving foods. Other studies cited based on meta analysis have determined that the intake of salt in American diets has remained fairly steady over the years, yet the incidences of hypertension in the general populace has gone up in that same time period, which suggests that there may be factors other than sodium alone that play a role in controlling hypertension.

10.7. SUMMARY

Sodium, chloride and potassium epitomize electrolyte physiology of living systems. Their ubiquitous distribution in plants and animals gives assurance that no dietary source would be void of these nutrients. An important feature of all three is their synergism in establishing and maintaining sodium gradients across cell membranes. Extracellular potassium ions are needed in the process that drives Na^+ out of the cell, reestablishing the gradient, and chloride maintains the electrochemical potential. Through studies employing the tools of molecular genetics, it has been possible to identify a family of carriers to which sodium ions serve as a co-transporter. These carriers have weak binding sites for sodium ions and thus exploit the disproportionate location of sodium to execute uphill transmembrane movement into cells. In the final analysis, it becomes apparent that the free flow nature of sodium, chloride and potassium is suited to their major movement across membranes via channels and in the form of current flow. An excess of sodium in the diet can raise blood pressure and lead to more serious complications, such as heart failure and neurological problems. Although the exact reason for raising blood pressure remains to be determined, the kidney has been considered a major candidate. There are strong indications that the fault may lie in the sodium ion reabsorption at the level of the kidney.

10.8. REFERENCES

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10.9. PROBLEMS

1. Outline a transport system that uses sodium gradients as an energy source. How are extracellular potassium ions able to modulate the energy of a sodium gradient? What is the role of chloride ions?

2. Explain why potassium in excess can be lethal. If you say potassium stops muscles from contracting, would you be correct?

3. It is very difficult to find a biomarker of sodium or potassium adequacy or upper limit. How then is it possible for a physician to determine if a person is suffering from a deficiency in either of these ions?

4. Offer an explanation as to why a person who sweats profusely is in little danger of losing excessive amounts of potassium ions.

CHAPTER 11

Calcium and Phosphorus

CALCIUM and phosphorus appear most conspicuously in the crystalline matrix of bone. Calcium also plays a critical, if not unique role in stabilizing membranes, activating muscle contraction, promoting blood clotting and inducing cell signaling. Phosphorus is more commonly seen as phosphate in proteins, carbohydrates, fats and nucleic acids. Phosphorylation, or adding a phosphate group to a protein, has been seen as a major mechanism for regulating biological activity in cells.

11.1. CALCIUM

11.1.1. History and Early Insights

Calcium derives its name from the Latin word calx or calcis meaning "lime", alluding to an early usage of calcium oxide (lime) by the Romans. Elemental calcium was isolated and named by Sir Humphrey Davy in 1808, who used electrolysis to separate the calcium from a mixture of mercuric acid lime. Sydney Ringer's demonstration that withdrawing calcium from a bathing solution caused a frog's heart to cease beating was one of the first insights into a biological function other than bone structure.

11.1.2. Chemical Properties

Calcium is an alkaline earth metal with an atomic number 20 and an

atomic weight of 40. The divalent Ca^{2+} is the dominant ionic form, a property it shares with Mg^{2+} and Zn^{2+}. ^{45}Ca, a gamma emitting isotope, is commonly used in experimental studies. In nature, calcium's more familiar forms are limestone ($CaCO_3$), gypsum ($CaSO_4 \times 2H_2O$) and fluorite (CaF_2). With an ionic radius of 0.99 nm, Ca^{2+} is nearly one and a half times larger than Mg^{2+}. Typical of alkaline earth metals, the outer electrons in calcium ions occupy the *s* and *p* orbitals. A loss of its two 4*s* electrons gives rise to the highly stable, closed-shell configuration typical of Na^+ or K^+. With its closed shell and lack of 3*d* electrons, Ca^{2+} behaves more like a spherical ion, with less potential to enter into coordinate covalent bonding or form complexes with well-defined spatial geometry. The coordination number for calcium, however, can exceed 7, indicative of a large ion with the potential to engage multiple centers at once, thus allowing Ca^{2+} to serve as a bridging ion or cross-linking agent, properties that are suited for membrane rigidity, permeability and viscosity as well as cell signaling in response to hormones and other activators.

11.1.3. Biochemical Properties

Predictably, about 99% of the body load of calcium is in bones and teeth. Serum and cells account for 0.1 percent and 1 percent, respectively. Bone thus is a major repository of body calcium. Serum calcium homeostasis is maintained by dissolving calcium from the bone matrix (resorption) in response to low serum levels or affixing calcium into the matrix (reabsorption) when levels are high. Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], a calcium-phosphate complex, is the major building block of vertebrate bones and teeth and the most prominent calcium complex in the system. There is a 10^4-fold difference between the concentration of the free ion in the cell and extracellular fluid, 10^{-7} and 10^{-3} M, respectively, which clarifies a requirement for ATP to drive Ca^{2+} out of the cell. Buffering free Ca^{2+} is essential in order for calcium and phosphate to be present in proper proportions at the crystallization site. This delicate balance between crystalline and free calcium is maintained by calcium ion transporters, pumps, membrane channels, binding proteins, and calcitropic hormones. The latter include parathyroid hormone, vitamin D, and calcitonin (see below). An imbalance is characterized by bone loss, calcium deposits in arteries, gall bladder, kidney, or cell death through apoptosis. Giving support to the endoskeleton is not the only important function of calcium. As seen in Table 11.1,

TABLE 11.1. Non-structural Roles for Calcium in Biology.

Active Functions
Enzyme cofactor (lipases, hydrolases)
Blood clotting cascade
Relaxation and constriction of blood vessels
Cell aggregation and adhesion
Muscle protein contraction
Cellular protein turnover
Hormone secretion
Nervous impulse transmission
Intracellular trafficking
Cell signaling

numerous physiological processes are either controlled by or triggered by Ca^{2+}. The latter include cofactor roles for enzymes, modulators of their activity, and inducers of cell signaling.

11.1.3.1. Calcium's Role as an Enzyme Cofactor

Calcium is a cofactor for numerous enzymes that perform a variety of essential cell functions. Many of these are hydrolases, which are enzymes that break down (hydrolyze) biological molecules by inserting the elements of water across the bond (Table 11.2). Foods provide the Ca^{2+} for digestive enzymes that break down food molecules in the intestine. Examples of the latter are the lipases and phospholipases that have a specific need for Ca^{2+} in order to break down complex fats.

11.1.3.2. Calcium-Binding Proteins

Families of calcium-binding proteins that are designed specifically

TABLE 11.2. Enzymes Dependent on Calcium for Function.

Enzyme	Location	Function
Phospholipase A2	intestine	acylphospholipid hydrolysis
Lipase	intestine	triacylglycerol hydrolysis
Thermolysin	bacteria	protein hydrolysis
Trypsin	duodenum	protein digestion
Caspases	wide	protease activation
Protein Kinases	wide	cell signaling

TABLE 11.3. Biological Functions of Calcium-binding Proteins.

Protein	Function
Annexins	cell adhesion
CaT1	membrane calcium transport
Calbindin	cytosolic calcium transport
Calmodulin	enzyme regulator
Calpains	protease activators
GLA proteins	blood clotting
Calcium ATPase	cellular calcium efflux
Troponin C	muscle calcium action
Parvalbumin	calcium ion buffer, muscle activator
Calnexin	glycoprotein folding
Cadherins	epithelial cell adhesion
Calcitonin	blood calcium regulator

to transport, store, or take active part in calcium-dependent regulation are listed in Table 11.3. Some tend to serve as buffers between the free ion and calcium deposits in bone. A brief description of each is given below.

11.1.3.1.1. Annexins

Annexins are mainly intracellular proteins widely distributed in animals, plants and fungi but notably absent in bacteria. The annexins form complexes with phospholipids in the cell membrane, giving rise to a sturdier membrane structure and overall architecture. Annexins also take part in dynamic changes at the membrane surface such as formation of vesicles for import. Ca^{2+} is needed to connect the protein to the phospholipid bilayer, which is reciprocated by having the annexins make up the basic structure of calcium channels in the membrane.

11.1.3.1.2. Calcium Transporter-1 (CaT1)

CaT1 is a calcium channel protein located in the brush border of intestinal cells. Its main function is to bring calcium ions into the interior of the cell, thus taking the first step in the absorption of calcium ions from the lumen. Vitamin D3 and the intracellular concentration of Ca^{2+} are important regulators of CaT1 mRNA synthesis. Transcription activation is triggered when the intracellular concentration of Ca^{2+} is low; inactivation occurs when it is high.

11.1.3.1.3. Calbindin

Another target of Vitamin D3 action, calbindin is present in the cytosol of many cells. Transport of calcium ions through the cytosol, i.e., ferrying Ca^{2+} from one side to the other, occurs mainly while attached to calbindin. The quantity of calbindin, therefore, modulates the rate of calcium absorption across the intestine as it moves from the apical to basolateral surface. Calbindin mRNA synthesis, however, may be less of a telling target for vitamin D3-stimulated calcium absorption. Figure 11.1 compares calbindin mRNA activation with CaT1 mRNA in response to Vitamin D3, showing that CaT1 mRNA synthesis is the greater responder to vitamin D3 stimulation.

11.1.3.1.4. Calmodulin

Calcium ions have the capacity to stimulate biological processes by regulating the activity of enzymes. Calmodulin, a calcium-binding protein, proficiently alters the conformation of the enzyme proteins and acts as a trigger for a calcium-dependent response. Its many features include sensing and binding Ca^{2+} (as many as four ions when saturated) and, as a calcium-calmodulin complex, stimulating muscles to contract, immune cells to respond, nerve cells to grow, etc. Calcium-calmodulin

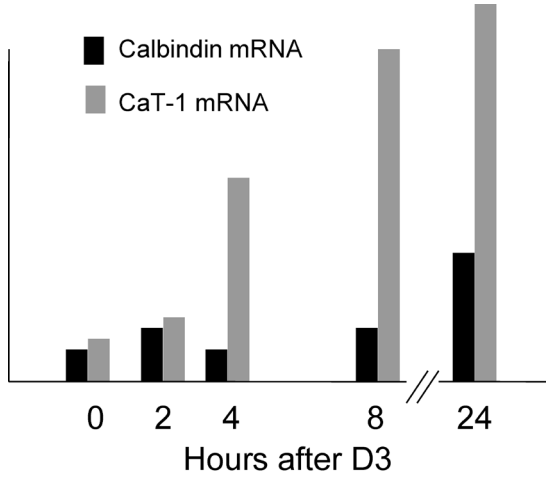

FIGURE 11.1. *Comparison of Vitamin D3 stimulation of mRNAs for Calbindin and CaT-1. The vitamin was administered at time zero. Readings of the respective mRNAs were taken as shown up to 24 hours after vitamin injection. (Adoped from Wood et al., 2001).*

in effect links calcium levels in a cell with the activity of calcium-dependent enzymes within the cell.

11.1.3.1.5. Calpains

Calpains are a family of enzymes referred to as "cysteine proteases". The name distinguishes this class of proteases by having a cysteine residue as part of the active site. Ca^{2+} is required for their activity. Familiar names include the "caspases" that regulate apoptosis. The moniker "modulator protease" is given to calpains to recognize that these proteins have limited proteolytic activity and what activity they manifest represents modulating rather than destroying the functional target. Calpain dysfunction for whatever reason could be lethal or lead to muscular dystrophy and other serious disorders.

11.1.3.1.6. GLA Proteins

Vitamin K is a coenzyme for a carboxylase enzyme that transfers CO_2 to the gamma carbon of about 10 glutamate residues in prothrombin, the blood clotting protein. The carboxylglutamates thus formed (abbreviated gla) with their tandem carboxyl groups are now able to bind Ca^{2+}, which when bound, anchors the prothrombin to the phospholipid bilayer of a cell membrane and initiates the blood clotting (thrombotic) sequence.

11.1.3.1.7. Calcium ATPase

As noted previously, the almost 10,000-fold difference in concentration between exterior and interior calcium mandates an energy-driven mechanism to release Ca^{2+} from the interior of cells into the blood. Calcium ATPases fit that role. These enzymes represent a large class of membrane-bound enzymes that serve as pumps for moving ions across cell membranes against concentration gradients. The ATPase for Ca^{2+} activity is required to restore low intracellular levels of Ca^{2+} following the influx of Ca^{2+} in response to an external signal from a cytokine or hormone. Lowering the calcium in the cell's interior effectively shuts down the response. Recall that, in a resting cell, intracellular Ca^{2+} must be maintained at extremely low levels. Failure to do so could cause overstimulation of calcium-dependent processes, including cell death through apoptosis.

11.1.3.1.8. Troponin C

Troponin (TN) is a complex of three proteins (TN-T, TN-I and TN-C) resting on the actin filaments in muscle. Together the three mediate muscle contraction. Troponin C (TN-C), a calmodulin-like protein, is the calcium-binding component of the three. In the absence of Ca^{2+}, muscle contraction is suppressed by a TM-TN complex that prevents myosin from sliding along the actin filament. With Ca^{2+} present, the suppression is relieved by a TN-C-Ca^{2+} complex. In skeletal muscle, this action occurs concomitantly with opening of myosin binding sites and engaging actin, which allows the myosin to slide and the muscle to contract.

11.1.3.1.9. Parvalbumin

This small calcium-binding protein is present in skeletal and heart muscles as well as in the brain. In the brain, the parvalbumin is mainly within neurons that respond to the neurotransmitter gamma-amino butyric acid (GABA). As an extension of the neuronal role, parvalbumin is also present in endocrine tissue. Because parvalbumin is more concentrated in fast contracting muscles, its function appears to be related to muscle contraction. This may be only one function of this multifunctional protein, however. Suffice to say parvalbumin may act as a nonspecific chaperone, sequestering and shuttling calcium ions internally to systems that require calcium.

11.1.3.1.10. Calnexins/Calreticulins

These calcium-binding protein are components in a dynamic cycle that is responsible for proper folding of glycoproteins synthesized in the endoplasmic reticulum. The reaction is a post-translational modification to the 3D structure of the protein. Calreticulin is a major Ca^{2+} storage protein in the cycle, whereas calnexin guides the folding and also serves as a Ca^{2+} chaperone to the spot where folding occurs.

11.1.3.1.11. Cadherins

Cadherins are a family of Ca^{2+} binding proteins that are responsible for holding epithelial sheets of polarized epithelial cells together. These proteins function in a variety of epithelial cells that line the surfaces

of kidney tubules, small intestines, pancreas, etc. About 30 are known. Similar to annexins, cadherins depend on Ca^{2+} to establish a firm cell-to-cell contact and will detach if the Ca^{2+} is removed from the bathing medium of cells in culture.

11.1.3.1.12. Calcitionin (Thyrocalcitonin)

Not a calcium binding protein per se, but a regulator of blood calcium, calcitonin opposes the action of parathyroid hormone (PTH) in response to lower serum calcium levels. In so doing, it suppresses Ca^{2+} absorption in the intestine, reabsorption of Ca^{2+} by the kidney, and release of more Ca^{2+} from bone by bone osteoclasts. Its action on the kidney also results in less phosphate being reabsorbed.

11.1.4. Nutritional Properties

An average adult human has about 1 kg (2.2 pounds) of calcium, making it the most abundant mineral in the body. Calcium's requirement in the diet predictably varies with age, gender, and physiological state. An adult human between the ages of 19 and 50 requires about 1 gram of calcium per day. Adults over 50 require 1.2 g per day. As noted in Table 11.4, calcium needs in younger adults increase with pregnancy and lactation. Part of the increase reflects the needs of the mother,

TABLE 11.4. RDA for Calcium.

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infant	0–6 months	210	210
Infant	7–12 months	270	270
Children	1–3 years	500	500
Children	4–8 years	800	800
Children	9–13 years	1,300	1,300
Adolescent	14–18 years	1,300	1,300
Adult	19–50 years	1,000	1,000
Adult	51+ years	1,200	,1200
Pregnant	18 years and younger	–	1,300
Pregnant	19+ years	–	1,000
Lactating	18 years and younger	–	1,300
Lactating	19+ years	–	1,000

TABLE 11.5. Food Sources of Calcium.

Richest: (600–900 milligrams/100 grams)
Cheese
Wheat-soy flour
Molasses
High: (120–350 milligrams/100 grams)
Dairy products: milk, yogurt, sour cream, ice cream
Low: (<100 milligrams/100 grams)

whose bone growth is still progressing, as well as calcium set aside for neonate consumption. The final stages of bone growth tend to reach a climax just beyond the second decade of life. Older adults, both male and female, also show an enhanced need for calcium, owing perhaps to alterations in bone metabolism with aging and the greater likelihood of bone loss in senior years.

11.1.5. Dietary Sources

Sources of dietary calcium are listed in Table 11.5. Those richest in calcium meet the daily requirement with about 200 grams of the food item. Included as richest sources are cheese and soy flour. The density of calcium in dairy products and fruits is one-third or less, implying a need for three times or more (600–1000 grams) to meet the daily requirement. Calcium that occurs in plants is bound mainly as calcium oxalate, which tends to make plants less favorable in supplying calcium in the diet. Its attraction towards phosphate also makes calcium in plants vulnerable to binding to phytic acid (phytate). These organophosphate complexes tend to diminish the absorption of calcium at the level of the intestine and within the system.

11.1.5.1. Dietary Supplements

Taking calcium supplements is common in the populace. The U.S. National Heath Survey in 1986 found that 14% of men, 25% of women, and 7.5% of children surveyed admitted taking calcium supplements. A follow up study in 1992 showed that supplements for men increased the calcium intake, but not significantly. For women, the increase was statistically significant, signifying a concern for preventing osteoporosis.

11.1.5.2. Upper Limit

Based on milk-alkali syndrome, which is a measure of renal insufficiency, the upper limit for calcium is 2,500 mg per day (about double the RDA). This represents calcium that is taken from food, water and supplements. This UL value is common across all life stages.

11.1.5.3. Calcium Deficiency

Of the many concerns of calcium deficiency, risk of osteoporosis is major. Given its role in mineralization, a calcium deficiency will affect bone structure and tensile strength. Sub-adequate intake will also compromise muscle contraction and other intracellular events, although such is not a common occurrence. A low system level can be related to calcium-binding factors in the diet or an impairment in intestinal absorption. Low vitamin D can be a factor in the latter. Calcium deficiency will impact on bone health and if left unchecked can result in a loss of bone density and osteoporosis. Annually, osteoporosis accounts for around 1.5 million bone fractures in the U.S.; in Canada, the incidence is around 76,000 fractures.

11.1.5.4. Excessive Intake

An increased propensity to develop kidney stones and renal failure (alkali disease) is a concern when calcium is in excess. Enriched intake interferes with the absorption, excretion and metabolism and is more likely to interrupt mineral balance in general. As an example, very high levels of calcium, nearly twice the RDA, can decrease iron and magnesium absorption; this effect is enhanced when combined with high sodium in the diet. Zinc absorption is also impaired by high calcium. The effect is more apparent when dietary zinc intake is marginal.

11.1.6. Digestion and Absorption

11.1.6.1. Digestion

Nearly all of the calcium taken in the diet is locked into a food matrix made up of protein, complex carbohydrates, and polyphosphate complexes. Salivary amylases combined with mastication assist in partial release of calcium from starches. A greater portion, however, is liber-

ated by amylases in the proximal intestine. Proteases in the stomach and duodenum effect a partial hydrolysis of protein molecules down to small peptides and in the process liberate calcium as free ions by disrupting the calcium binding sites in the protein. The moderately strong acidity of the gastric juice (pH 1.7) also helps dissociate the Ca^{2+} from the peptides and renders salts of calcium, such as calcium phosphate, more soluble.

11.1.6.2. Absorption

Only about one-third of dietary calcium is absorbed. Absorption efficiency is generally the same for all foods. This aspect of calcium metabolism has received much interest because absorption efficiency tends to wane with age. The overall scheme for Ca^{2+} absorption is shown in Figure 11.2. The calcium transport protein (CaT1) in enterocytes mediate transcellular Ca^{2+} movement; paracellular transport only becomes a factor when Ca^{2+} is high. CaT1 is distributed all along the surface of the small intestine and colon. More pertinent is a clear need for 1,25-dihydroxy vitamin D3 (calcitriol) when Ca^{2+} intake is low. The hormone is the primary homeostatic regulator of Ca^{2+} absorption. CaT1 is very specific for Ca^{2+} and because the Ca^{2+} concentration in the lumen (10^{-3}M) is greater than the cytosol, movement across the membrane is by a diffusion-driven electrochemical gradient. Effective transcellular movement requires calbindin, a cytosolic calcium-binding protein, to chaperone Ca^{2+} through the cell. Calbindin levels determine the rate of calcium passage. In the extrusion from the cell, the electrochemical

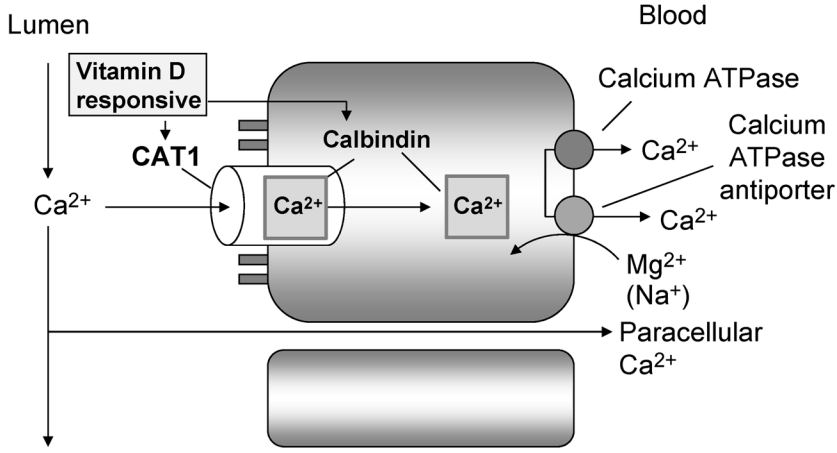

FIGURE 11.2. *Overview of Calcium Absorption in the Enterocyte.*

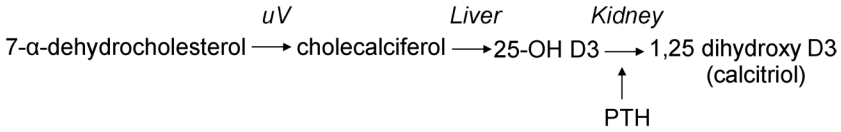

FIGURE 11.3. *Metabolic Steps in the Biosynthesis of 1,25 dihydroxy D3 (calcitriol).*

gradient that facilitated Ca^{2+} uptake works against its release, which mandates a need for a calcium ATPase to overcome the concentration and charge differential.

11.1.7. Calcium Homeostasis

11.1.7.1. Role of Calcitriol (1,25-dihydroxy D3) and Parathyroid Hormones

Calcitriol is the active form of vitamin D3 which, along with parathyroid hormone (PTH), comprises the major regulators of calcium absorption and homeostasis. Calcitriol is synthesized by a uv-dependent transformation of 7-α-dehydrocholesterol to choleciferol, vitamin D3 (Figure 11.3). The ensuing events in the activation involve two hydroxylation reactions, first in the liver and then in the kidney. Parathyroid hormone stimulates the hydroxylase enzyme that catalyzes the kidney reaction, thereby indirectly stimulating the uptake of calcium into the absorbing cells. As will be discussed below, calcitriol is also a major regulator of phosphorus absorption.

As noted previously, both CaT1 and calbindin mRNAs are raised in response to calcitriol, which translates into two sites for 1,25-dihydroxy D3 action on calcium absorption. In tandem, both control the rate of calcium passage into the system. Calcium uptake is sensitive to the calcium level in the cytosol and membrane storage sites. Activation of CaT1 and calbindin are needed to assure effective transport when the level of calcium in the intestine is low. Parathyroid hormone (PTH) is secreted in response to low serum calcium. Triggering PTH sets in motion a series of reactions designed to raise the serum calcium levels. These events are summarized in Figure 11.4. Elevation in PTH stimulates the formation of calcitriol from 25-OH D3, which in turn increases the inward flow of calcium from the intestinal lumen. Calcitriol in turn shuts down calcium excretion in the kidney by inducing a calbindin-like protein which, by promoting kidney calcium reabsorption, prevents calcium loss. PTH, however, will also promote the breakdown of bone

by stimulating osteoclasts that release calcium from the bone layer. This scenario shows a close interplay between the intestine, kidney, and parathyroid gland in an attempt to correct low Ca^{2+} in the serum. Not mentioned in Figure 11.4 but discussed earlier is the role of calcitonin, which is to counter all phases of PTH action, thereby moderating the effects of the hormone.

11.1.7.2. Interaction with Other Nutrients

Calcium absorption and its post-absorption metabolism is strongly influenced by magnesium, phosphorus and sodium in the diet. These minerals block Ca^{2+} movement, which in extreme cases can lead to bone loss and excessive urinary calcium output. Table 11.6 summarizes other dietary factors that can block or delay calcium absorption and disrupt calcium retention by the system.

A magnesium deficiency, not excess, will trigger bone resorption by lowering serum calcium levels (hypocalcemia). In some animal studies, a severe magnesium deficiency was needed to produce a depression in blood calcium, but surprisingly in humans a mild deficiency reportedly will give the same effect. Excessive intake of phosphorus is detrimental

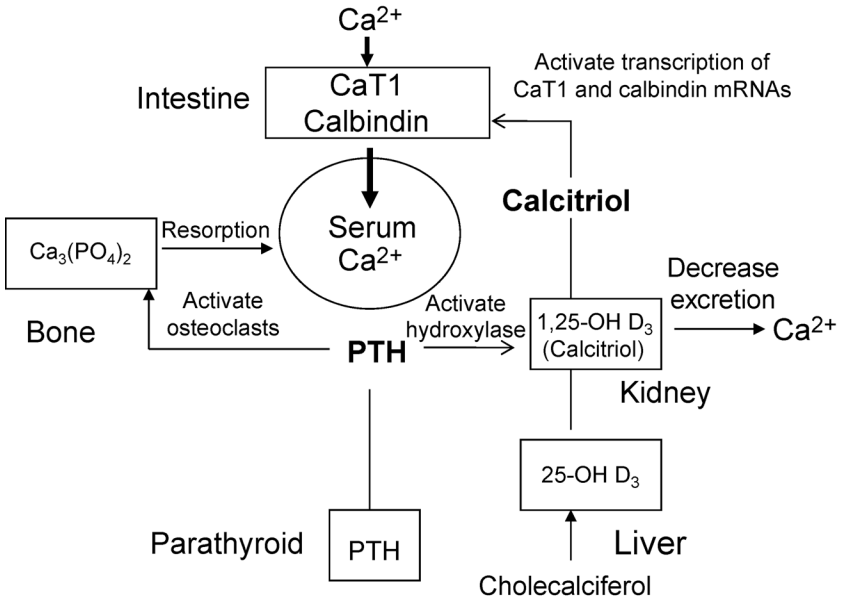

FIGURE 11.4. *Response of System to Low Serum Calcium: Hormonal Steps.*

TABLE 11.6. Dietary Factors with the Potential to Influence Calcium Homeostasis.

Factor	Effect on Calcium Homeostasis
Minerals	
Magnesium deficiency	hypocalcemia
Phosphorus excess	absorption
Sodium	excretion
Non-Minerals	
Protein	excretion
Oxalic acid	absorption
Phytic acid	absorption
Caffeine	absorption and excretion

to calcium absorption, betraying a close interaction between the two bone minerals. Foods rich in phosphorus such as dairy products, soft drinks and meats can exacerbate a mild calcium deficiency. Sodium ions in excess also affect calcium absorption, but a more detrimental effect is promoting calcium excretion by the kidney. High sodium has only a minimal effect on bone loss, however.

Non-mineral components in the diet also impinge on the absorption and excretion of calcium. Calcium chelators such as oxalic acid and phytic acid bind luminal Ca^{2+} and prohibit its transport across the intestinal membrane. While phytic acid is a potent Ca^{2+} binding ligand, this organophosphate complex is also the major phosphate structure in green plants, grains and nuts. Thus, vegetarians risk a greater calcium insufficiency, even with adequate calcium in their diets. A more detailed explanation of phytic acid is given below. Another concern is caffeine that is commonly found is teas, sodas and coffee. Caffeine operates mainly at the level of calcium excretion, resulting in excessive urinary loss and is especially detrimental when calcium intake is sub-adequate. It can also affect bone calcium desorption, although a direct link between caffeine and accelerated bone loss has not been established. Postmenopausal and lactating women appear to be more susceptible.

11.2. PHOSPHORUS

11.2.1. History and Early Insights

Phosphorus derives its name from two Greek words *phôs* (light)

and *phorus* (bearer). The term traces back to the discovery by Hennig Brand, a German alchemist, who in 1669, while attempting to make gold by heating urine, observed white substances in the mix. In the presence of air, the substance glowed in the dark. Unbeknown to Brand, the light was created by an oxidation reaction between elemental phosphorus and O_2, a spontaneous event tantamount to setting the phosphorus on fire. Robert Boyle, the great English chemist, was the first to combine phosphorus with sulfur-coated wooden sticks, the forerunner of wooden matches. The biological role of phosphorus came to light in 1769 when Jonathan Gann and Carl Scheele found the matrix of bone to be composed largely of calcium and phosphate. Phosphorus was also found in serum and urine and, in time, nucleic acids. Although the tendency in nutrition is to speak of phosphorus as a nutrient, in the biosphere phosphorus is almost always present as phosphate, the salt of phosphoric acid.

11.2.2. Chemical Properties

Phosphorus is a Group V non-metal configured as $[Ne]3s^2 3p^3$. Because it is highly reactive in air, elemental phosphorus is not found in a free state, but rather as oxy-anions, mainly phosphates ($H_2PO_4^-$, HPO_4^{2-}) and organic esters of phosphate such as sugar phosphates, phosphate bound lipids, and nucleic acids. Phosphates can also form polyphosphates by a heat-induced polymerization. Each bond between the phosphates is formed with the loss of one molecule of water, hence giving it the name phosphoanhydride. When present in biochemical pathways, phosphoanhydrides are sources of high energy and are used to drive energy-dependent reactions.

11.2.3. Biochemical Properties

Like calcium, phosphorus is found mainly in bones and teeth, amassing between 500 and 700 grams in an adult human. About 15 percent appears in phosphate-bound lipids and nucleic acids. Only about 0.1% is present in plasma, which, due to pH considerations, is mainly the dibasic HPO_4^{2-} salt of phosphoric acid. The phosphate ions in the blood and cells acts as buffers to stabilize pH. Phosphate-bound intermediates in metabolic pathways make up a large but highly unstable pool of

phosphate in the cell. Phosphate esters are extremely abundant in living systems, most notably sugar phosphates in metabolic pathways and diesters in DNA and RNA. Diesters of phosphate also make their appearance in acylglycerol phosphates that comprise the major lipids in cell membranes. Divalent cations such as Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} bind weakly to organophosphate complexes, but nonetheless compete with one another for phosphate groups on proteins. A phosphate on a sugar molecule such as glucose effectively traps the glucose in the cytosol of a cell and seals its fate for destruction via enzymes in the glycolytic or hexose monophosphate shunt pathways. Phosphate-bound sugars—and compounds in general—require specific carrier proteins to cross membranes. One of the most important non-structural roles for phosphate is that of a mediator of hormonal action and cell signaling. Signaling responses are controlled by a series of protein-kinase enzymes that use ATP as a phosphate source to activate (or inhibit) a biological responder, such as an enzyme or membrane protein. To be effective, the phosphate group forms ester bonds with serine, threonine and tyrosine residues within the target protein. This action either raises or lowers the catalytic efficacy of an enzyme or in the case of a membrane receptor, allows the receptor protein to engage other signaling molecules and propagate the signal internally.

11.2.4. Nutritional Properties

11.2.4.1. Recommended Intake

Data derived from the National Health Survey estimate that the average diet for both men and women contains about 62 mg/100 Kcal phosphorus. Its widespread appearance in foods makes it practically impossible in a balanced diet to create a phosphorus deficiency. Table 11.7 shows data taken from the Institute of Medicine Dietary Reference Index (DRI).

Phosphorus RDA reaches a peak between 9 and 18 years of age for both genders. For both, there is a substantial drop in the RDA as maturity approaches and rapid growth stabilizes. Surprisingly, pregnancy and lactation have no effect on the phosphorus requirement.

11.2.4.2. Food Sources

Table 11.8 illustrates the phosphorus content of a variety of foods.

TABLE 11.7. Dietary Reference Intakes for Phosphorus as a Function of Life Stage and Gender.

	DRI values (mg/day)	
	RDA	
Life Stage	**Males**	**Females**
Life Stage		
1–3 yr	460	460
4–8 yr	500	500
9–13 yr	1,250	1,250
14–18 yr	1,250	1,250
19–30 yr	700	700
31–50 yr	700	700
51–70 yr	700	700
Pregnancy		
<18 yr 1,250		1,250
19–50 yr		700
Lactation		
<18 yr 1,250		1,250
19–50 yr		700

The data are taken from the USDA Handbook No. 8 series. From the table, one may surmise that dairy products (as compared to other sources) provide the highest amounts of phosphorus. Vegetables are lower than meats and dairy products. Grains such as oats, wheat bran, beans and plant material in general are also very high, but this phosphate is represented primarily by their phytic acid content. Because the inositol ring structure of phytate holds up to six phosphates per molecule (Figure 11.5), phytate is the major component of phosphate in the diet of animals subsisting on grains.

11.2.4.3. Upper Limit

The upper limit of phosphorus intake in adults is roughly 8 times the RDA. The need to maintain a delicate balance between phosphorus and calcium presages a potential harm to a system with higher levels of intake of either mineral. The condition of hyperphosphatemia signals a serious adverse effect. This condition can lead to reduced calcium intake (more problematic when intake of calcium is sub-adequate) and calcification of soft tissues, particularly the kidneys.

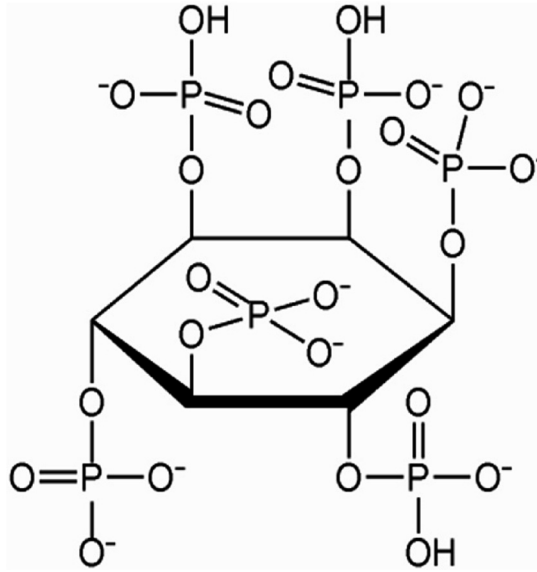

FIGURE 11.5. Structure of Phytic Acid (Phytate). The major source of phosphate in plants, phytate's abundant phosphate groups have a strong propensity to bind to calcium, zinc, and other divalent cations, inhibiting their absorption.

11.2.5. Digestion and Absorption

11.2.5.1. Digestion

As noted, the phosphorus in food sources is present mainly as esterified organic phosphates, which in cereals, seeds and grains is present as phytic acid. There are no enzymes in humans and animals capable of removing the phosphate from phytate. That reaction requires the enzyme phytase, which is present in plants and bacteria. Phytases, therefore, strongly influence the bioavailability of phosphate from grains. Ruminants have phytases abundantly available through bacteria in the rumen. Mammals also have bacterial phytases, but the enzyme is mainly in the colonic bacteria and thus beyond the phosphate absorption sites in the small intestine. Because the concentration of phytase in plants is not the same for all plants, it has been advantageous to supplement animal feed with microbial phytase to aid the digestibility and increase the amount of absorbable phosphate. Yeast are rich in phytase, which contributes to the higher bioavailability of phosphate from leavened bread products. However, once phytic acid has formed a complex with multiple calcium ions, it is able to resist phytase action.

11.2.5.2. Role of Hormones in Phosphorus Absorption and Homeostasis

Phosphate liberated from its organic components is free to enter the system. As with calcium, internal homeostasis of phosphate is regulated at the level of absorption and renal excretion and can be influenced by parathyroid hormone (PTH), calcitriol (1,25-dihydroxycholecalciferol) and calcitonin as follows. A decrease in plasma phosphorus (hypophosphatemia) stimulates renal production of calcitriol, enhancing the level of hormone in the circulation. Calcitriol's direct action is to raise serum phosphorous by stimulating Npt2 in the upper small intestines, thus bringing more phosphate into the system (Figure 11.6). The hormone also suppresses phosphorus excretion by the kidney. Calcitriol acts indirectly by mobilizing bone phosphorus (resorption), an action it shares with PTH (Figure 11.4). Calcitonin, however, counters the action of both hormones by promoting bone formation, which lowers serum phosphorus. Thus, three hormones that play an active role in controlling serum calcium levels are also instrumental in maintaining serum phosphorus.

TABLE 11.8. Phosphorus Content of Selected Foods.

Item	Amount	P content (mg)
Macaroni and cheese	1 cup	322
Milk (2% fat)	1 cup	248
Ham	3 oz.	210
Almonds	1/4 cup	184
Oatmeal	1 cup	178
Cheddar cheese	1 oz.	146
Broiled shrimp	1 oz.	137
Cooked ground beef	3 oz.	135
Tofu	1/2 cup	120
Baked potato	1	115
Egg	1	86
Whole wheat bread	1 slice	74
Peas	1/2 cup	72
Cola beverage	1 can	46
Potato chips	14	43
Dark chocolate	1 oz.	41
White bread	1 slice	30
Orange	1	18

Data taken from U.S. Department of Agriculture.

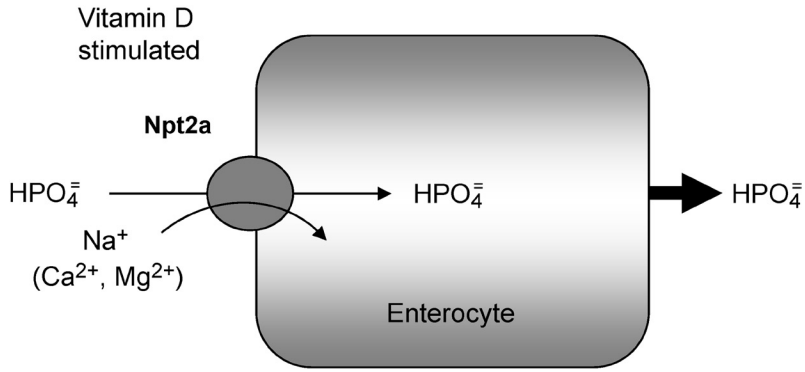

FIGURE 11.6. Intestinal Absorption of Phosphorus.

11.2.6. Calcium-Phosphorus Interactions

11.2.6.1. Biomineralization

Biomineralization is best represented by the deposit of calcium and phosphate over an organic layer of protein (collagen), giving rise to the crystalline structure of bones and tooth enamel. Mineralization, when applied to bone remodeling, regulates the levels of calcium and phosphorus in the serum—an illustration of the dynamic nature of crystallization at the liquid-solid interface. In forming bone structure, the underlying layer of collagen controls uniformity in crystal size, orientation and growth. Distortion in the organic foundation of collagen caused by disrupting intermolecular crosslinks between the collagen chains can weaken bone structure by disrupting uniform growth of the crystals. The resulting bone fractures more easily.

Because phosphate has a propensity to form insoluble aggregates with calcium, the recommended intake of phosphorus in the diet must pay heed to the calcium level of the diet to prevent wanton crystallization. Generally, a ratio of 1.3:1, Ca:P, is considered optimal. In quantitative terms, an adult human consuming 1,200 mg of calcium per day should have 900 mg of phosphorous to maintain the ratio. Indeed, a sharp deviation from this ratio can upset absorption efficiency and alter the homeostasis of either mineral. This in turn can trigger homeostatic changes internally that lead to pathologies, including bone loss. The basis for these undesirable reactions relate to the tendency of the two minerals to precipitate out of solution when either one is in excess. Pathologies become apparent when mineralization occurs in soft tissue,

such as small crystals of calcium-phosphate accumulating in the gall bladder and kidney.

11.3. SUMMARY

Calcium and phosphorous are best known for their role in hard tissues such as teeth and bone. By weight, the two are the major macrominerals in the system, found in every cell and fluid in the body. Of the two, calcium has the more varied non-structural roles, as illustrated by its requirement for cell signaling, membrane stability, muscle contraction, blood clotting and metabolic regulation in general. Phosphorus as phosphate, by contrast, is primarily a structural component in all classes of biological molecules and makes up a substantial fraction of nucleic acids, the phospholipids in membranes, ATP, and other compounds that generate or trap energy. Intestinal absorption of calcium and phosphorus (phosphate) must be carefully regulated because Ca^{2+} and phosphate together represents a potentially unstable chemistry. When either ion as a free ion is disproportionately high in the plasma, the propensity toward biomineralization is raised. Such events could lead to kidney or gall stones. CaT1 and calbindin control the rate of calcium absorption and Npt2 regulates uptake of phosphorus. All three are directly under the control of 1,25-dihydroxy D3 (calcitriol) and indirectly by parathyroid hormone. Exporting calcium requires an energy-driven transport protein, also controlled by calcitriol. What cannot be controlled by the system, however, are the effects of anti-nutrients phytic acid and oxalic acid on calcium absorption, which can be detrimental to calcium status.

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11.5. PROBLEMS

1. Name at least 5 non-structural roles of calcium in a biological system.

2. Of the 5 you named in problem 1, which are regulatory as opposed to catalytic?

3. Explain how a person who drinks a lot of high-phosphate sodas could be in danger of developing osteoporosis.

4. What would be the impact of a vitamin D deficiency other than the development of rickets in a young child?

5. Explain why the RDA for phosphate actually becomes less as a person grows older.

6. PTH has two sides in calcium metabolism and homeostasis. Explain why the hormone is both beneficial and a detriment to bone health.

7. Compare and contrast the actions of parathyroid hormone with calcitonin. Point out similarities as well as differences in the following:
   a. Effect on calcium levels in the blood
   b. Effect on phosphorus levels in the blood
   c. Calcium absorption from the intestinal lumen
   d. Calcium excretion from the kidney
   e. Activity of osteoclasts (bone destroying cells)
   f. Tissue of origin
   g. Effect on calcitriol biosynthesis

8. Besides transporting minerals, what else do calbindin, CaT1 and Npt2 have in common?

9. Nutritionists are concerned that the general populace as a whole tends to take in less vitamin D in their daily eating habits. They are also alarmed by the rise in hip fractures in the elderly. Are these two factors involved in a cause/effect relationship? Explain.

10. Why does calcium export from the basolateral surface of enterocytes into the blood require the energy of ATP?

Magnesium

MAGNESIUM is the fourth most abundant mineral in the body, and like calcium, has richer deposits in bone and muscle. Magnesium's multitude of functions tend to center on a cofactor role for enzymes that use ATP. Its recently discovered role in blood pressure and blood sugar modulation, as well as nerve and muscle functions tend to suggest magnesium could be a factor in hypertension, cardiovascular disease, diabetes and other nutritionally related disorders. This chapter gives insight into the multitude of biochemical reactions that depend on magnesium and why a magnesium deficiency could have health-challenging consequences.

12.1. HISTORY AND EARLY INSIGHTS

The word magnesium comes from Magnesia, a region in southeastern Greece where it was first discovered. This same region yielded magnetite and manganese. Magnesium's biological importance first took notice as the active component in Epson salt, at the time known for a remarkable healing property. Not finding any specific diseases connected with a magnesium deficiency delayed a more ambitious undertaking in human and animal health. Awareness of magnesium's importance in biology was reinforced when sea water was shown to have abundant amounts of magnesium and the chlorophyll molecule in plants had a magnesium ion at its central core. Later magnesium was shown to play key roles in energy metabolism, primarily reactions that utilized ATP. Another important insight was its structural role in DNA and RNA.

When calcium absorption and blood levels were shown to be influenced by magnesium in the diet, there was clear evidence for an interdependence of the two minerals and more importantly the potential for one to regulate the action of the other.

12.2. CHEMICAL PROPERTIES

Magnesium is an alkaline earth metal positioned just above calcium in the Periodic Table of Elements. Its most stable oxidation state is +2 brought about by the loss of 2, 3*s* electrons, a property magnesium shares with calcium and a foreteller of competition between the two ions. Magnesium's ionic radius, however, is two-thirds the size of Ca^{2+}, which works against competition based on size. In fact the smaller radius gives Mg^{2+} a more intense electric charge and an ability to displace Ca^{2+}, which in muscle is the basis for muscle relaxation after contraction. The lack of 3*d* electrons further predicts that Mg^{2+} behaves mainly as a spherical ion with less of a tendency to form covalent bonds. The +2 charge aids the solubility of Mg^{2+} in aqueous solutions, which makes Mg^{2+} a freely diffusible cation in the plasma and the cytosol of cells. One would also predict that missing 3*d* electrons precludes complexes of magnesium having a color. This is true with one exception. Magnesium coordinate—covalently bound to the tetrapyrrole ring of the chlorophyll molecule not only allows chlorophyll molecules to trap and channel photons of light, but also gives chlorophyll its unique green color.

12.3. BIOCHEMICAL PROPERTIES

Over 300 enzymes require Mg^{2+} as a cofactor. Many are kinase enzymes, which are defined as enzymes that transfer phosphate groups from a Mg-ATP complex to substrates. Indeed, nearly every biosynthetic pathway in a cell has a magnesium-requiring kinase enzyme. Singling out just one example, the synthesis of cholesterol uses Mg^{2+} as Mg-ATP not only to activate cholesterol precursors but eventually form the familiar ring structure of the cholesterol molecule. Magnesium is also present in the nucleus where giant polymers of DNA and RNA are assembled from nucleotide triphosphates. Their action is testament to varied roles of magnesium at the biochemical level of cell function.

12.4. NUTRITIONAL PROPERTIES

In 1997 the Food and Nutrition Board of the institute of Medicine raised the magnesium requirement for children and adults to values shown in Table 12.1. The need for change was based on balance studies that utilized more sensitive methods for measuring magnesium homeostasis. As shown in the table, an adult human requires between 350–500 mg of magnesium per day to stay in magnesium balance. Because the balance approach cannot be applied to children under the ages of six months, there is insufficient information to establish an RDA for infants. Consequently, the values in the table reflect the magnesium content of breast milk or formula for the period. For infants and toddlers between 6 months and three years, an RDA of under 100 mg/day is recommended (Table 12.1). Gender differences are not seen until early adulthood. Pregnancy and lactation brings about only a modest increase in the RDA for magnesium. By comparison, magnesium at its highest level is still less than one half the calcium requirements for individuals of the same age and gender (Chapter 11).

12.4.1. Food Sources

A summary of food sources for magnesium is shown in Table 12.2. Green leafy vegetables, legumes, whole grains, nuts and shell fish are the richest source, estimated to be around 500 mg per kilogram of fresh weight. Meats, starches and milk tend to be intermediate, generally

TABLE 12.1. Magnesium Requirement as a Function of Age and Gender.

Life Stage	Age	Males (mg/day)	Females (mg/day)
Children	1–3 years	80	80
Children	4–8 years	130	130
Children	9–13 years	240	240
Adolescent	14–18 years	410	380
Adult	19–30 years	400	310
Adult	31–50 years	420	320
Adult	>51 years	420	350
Pregnant	19–30 years	–	350
Pregnant	31–50 years	–	360
Lactating	19–30 years	–	310
Lactating	31–50 years	–	320

TABLE 12.2. Magnesium Requirement as a Function of Age and Gender.

Richest: (500 milligrams/100 grams)
Green leafy vegetables
Wheat-soy flower
Shell fish
Whole grains and nuts
Chocolate
Middle (150 milligrams/1000 grams)
Meats
Milk
Banana
Fruits
Lowest
White flour (refined)

amounting to less than 30 percent of the requirement per day. Refined or processed foods tend to have the lowest magnesium content. Hard water is particularly rich in magnesium and, as opposed to soft water, a good source of the mineral. The rise in refined foods entering the food chain gives a rationale to the statement that only about one-third of Americans meet the daily requirement for magnesium.

12.4.2. Diet and Bioavailability

Absorption efficiency for magnesium is very high, ranging between 50-90 percent for human milk and formula. Absorption of magnesium from solid foods is estimated to be about 50 percent of the amount taken in. Higher absorption percentages reflect magnesium's solubility in aqueous solution. A notable exception are plant foods rich in phytic acid that acts as a major deterrent to magnesium absorption. As noted earlier, phosphate salts and organic phosphate compounds have a strong attraction for divalent cations such as Ca^{2+} and Zn^{2+}. Magnesium (Mg^{2+}) does not escape this attraction.

12.4.3. Dietary Supplements

A typical adult supplement for magnesium is around 100 mg/day with women tending to take in more than men. For children, the number is around 25 mg/day. The order of occurrence in supplements is MgO > $MgCO_3$ > $Mg(OH)_2$ > Mg-citrate > Mg-lactate > $MgCl_2$ > $MgSO_4$.

12.4.4. Upper Limit

Using milk alkali syndrome as a measure of renal insufficiency, magnesium has been shown to have no upper limit when food or water is used as the source. When taken in the form of supplements, however, the upper limit for children 1–3 years is 65 mg/day; for 4–8 years, 100 mg/day and for adults 350 mg/day.

12.5. DIGESTION AND ABSORPTION

12.5.1. Digestion

With the exception of a chlorophyll-magnesium complex in green leafy vegetables, magnesium in foods is generally present as a weak complex with other food molecules. Digestion causes magnesium to dissociate from the digestate and enter the system as a soluble cation. Salivary amylases assist in the release of magnesium ions from glycogen and starch, although a greater portion is liberated by pancreatic amylases in the proximal intestine. Proteases in the stomach and duodenum also liberate magnesium, as does the acidic environment of the stomach.

12.5.2. Absorption

On first impression, one may consider the absorption mechanism for magnesium to follow in step-by-step fashion with the pathway for calcium uptake. Such may be only partly true, however. Magnesium's entrance into the system is by both diffusion and active transport. The first barrier confronted is the Transient Receptor Protein (TRPM6), a cation channel protein on the apical surface. The soluble, non-chaperoned Mg^{2+} than moves to the basolateral surface for discharge in a reaction driven by ATP (Figure 12.1). Like Ca^{2+}, Mg^{2+} absorption occurs all along the intestinal tract, with the ileum and colon being more active. Diffusion, however, only accounts for 7–10% of the magnesium taken in. Since TRPM6 operates solely by diffusion without co-transporters, absorption efficiency is a function of the ratio of luminal Mg^{2+} to cellular Mg^{2+}. Not only does TRPM6 function in the intestine, but this channel protein also occurs in the distal tubules of the kidney and is poised to control reabsorption of Ca^{2+} and Mg^{2+} in

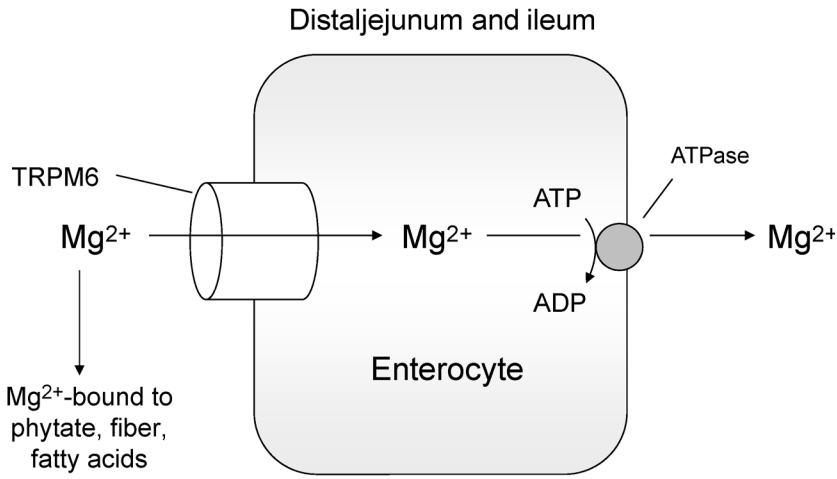

FIGURE 12.1. Magnesium Absorption in the Intestine.

the kidney. Because of a strong preference of Mg^{2+} over Ca^{2+}, however, it is more likely that TRPM6 functions as the major cation channel for Mg^{2+} excretion.

Unique to magnesium that was not seen with either sodium or potassium is a lowering of the fractional absorption with increasing amounts in the diet. In one report, raising dietary magnesium from 7 mg to 36 mg lowered the fractional absorption of magnesium from 65–75% to 11–14%. Although Mg^{2+} absorption is regulated, no data support a vitamin D3 dependence, even though the vitamin will cause a slight increase in the amount of magnesium absorbed. This dilemma for magnesium has not been resolved, but speculation is that other than TRPM6, the divalent transporter DCT1/DMT1 could also play a role in magnesium transport across the intestine.

12.6. MAGNESIUM/CALCIUM INTERACTIONS

12.6.1. Interactions in the Intestine

Even though the atomic radius of Ca^{2+} is 1.3 times bigger than Mg^{2+}, Ca^{2+} still has the potential to impede the movement of Mg^{2+} into absorbing enterocytes. That observation supports a common carrier or entry portal for the two cations. Long term studies, however, dismiss interference between the two, but in the short term the fractional uptake of

Mg^{2+} is clearly influenced by Ca^{2+}. Nonetheless, a calcium intake must exceed 2,600 mg/day—i.e., nearly two and one-half times its RDA—to disrupt magnesium balance. Phosphorus as phosphate likewise interferes with Mg^{2+} absorption. Alkaline earth metals in general are strongly attracted to phosphate either as the free ion or as an organophosphate complex. Conversely, magnesium inversely affects phosphate and, to a lesser extent, the absorption of calcium.

12.6.2. Ca^{2+}/Mg^{2+} Interactions in Muscle Contraction

The series of reactions that are part of the muscle contraction-relaxation cycle exemplify a biological synergism between Mg^{2+} and Ca^{2+} (Figure 12.2). When Ca^{2+}, through troponin C, triggers the actin-myosin complex in muscle to contract, Mg-ATP provides the energy. To restore muscle relaxation, a second Mg-ATP drives the Ca^{2+}-ATPase that pumps Ca^{2+} out of the cell. The most important function of Mg^{2+}

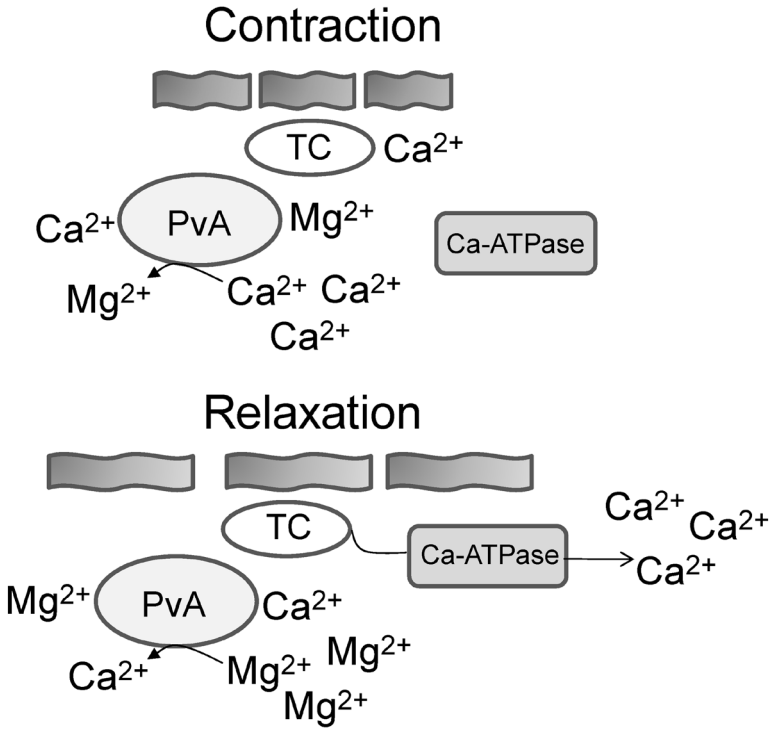

FIGURE 12.2. *Magnesium-Calcium Interactions in Muscle. TC = troponin C; PvA = parvalbumin.*

is in the relaxation phase of the cycle, where Mg^{2+} displaces Ca^{2+} from parvalbumin to initiate a relaxation. How rapidly the Ca^{2+}-Mg^{2+} exchange takes place basically controls the rapidity of muscle contraction. Rapidly contracting skeletal muscles are richer in parvalbumin for that reason. In a resting muscle, a Mg-parvalbumin complex is the more prevalent form of parvalbumin.

12.6.3. Bone Resorption

Bone is particularly sensitive to magnesium/calcium interactions. In a magnesium deficiency, bone density decreases. Lowering serum calcium stimulates bone resorption by osteoclasts, which could account for the lower bone density. As noted previously, however, the deficiency in magnesium must be rather severe for blood calcium levels to decline and bone density to suffer.

12.7. MAGNESIUM DEFICIENCY AND TOXICITY

Magnesium deficiency in well-fed infants and adults is a rare occurrence. Even so, there is concern that the diet of most adults is sub-adequate in magnesium. A focus of the concern is that dietary intake may not be sufficient to maintain magnesium stores in the body. In those instances where moderate to serve depletion has occurred, the symptoms include:

- hypocalcemia
- muscle cramps
- interference with vitamin D metabolism
- neuromuscular hyperexcitability
- tetany
- carpal spasm
- seizures

Less discernible, but implicated in a magnesium deficiency are:

- cardiovascular and neuromuscular diseases
- malabsorption syndrome
- diabetes mellitus
- renal wasting syndrome
- osteoporosis

A rationale to deficiency symptoms is based on the role of Mg^{2+} in calcium homeostasis and muscle biochemistry. Because Mg^{2+} is required for relaxation, a low Mg^{2+} level leads to retention of Ca^{2+} in the sarcoplastic reticulum. Too much Ca^{2+} can cause cramping and muscle spasms and, if left unchecked, give sustained contractions characteristic of tetany.

12.7.1. Excessive Intake

A high intake of magnesium from food sources will not cause adverse effects. Magnesium taken as a pharmacological dose in the form of a salt, however, has that potential. Large doses of magnesium-containing laxatives and antacids have been implicated in magnesium-induced kidney failure and symptoms of other conditions which include the following:

- diarrhea (the primary symptom)
- nausea
- abdominal cramps
- difficulty breathing
- metabolic alkalosis
- hypokalemia
- paralytic ileus
- low blood pressure

Although not life threatening, these conditions can have a telling effect on the quality of life and, of greater concern, can be mistaken for serious pathologies when the ultimate cause relates to excessive intake of magnesium.

12.8. SUMMARY

Magnesium performs a number of important functions in biological systems, many of which are life-sustaining in their consequences. An example is the role of Mg^{2+} in the chlorophyll molecule. Not only does magnesium form the basis for the green color of plants, but may be indispensable in the light-capturing mechanism. In animals, Mg^{2+} is generally accompanied by other ions at functional sites. An important companion is Ca^{2+}, with which Mg^{2+} shares many synergistic actions. Regulating muscle contraction, particularly the relaxation phase, criti-

cally depends on Mg^{2+} to restore resting muscle. Perhaps the most important role for magnesium is as a complex with ATP, which energizes the ATP in preparation for phosphate group transfer. Here, magnesium is unique and with the possible exception of Mn^{2+} has no other ion that can take its place. A deficiency in Mg^{2+} in the diet does not lead to a drastic system shutdown. Likewise, consumption of high magnesium foods does not give rise to toxic symptoms. A concern, however, lies with magnesium as a supplement on a steady basis, which could have an effect on calcium homeostasis and result in bone loss.

12.9. REFERENCES

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12.10. PROBLEMS

1. What chemical features allow Mg^{2+} to be soluble in water? To bind strongly to proteins?

2. What would be the consequences to the organism if the binding of Mg^{2+} to parvalbumin was covalent and not electrostatic?

3. Compare the electronic structure of Mg^{2+} with Ca^{2+}. How do the two differ? How are they similar? Which ion relies on 3*d* orbitals to explain its properties?

4. With reference to the conditions below, explain the likely outcome to the organism if magnesium in the diet was totally suppressed:
   a. The amount of calcium bound to calbindin
   b. The amount of calcium bound to parvalbumin
   c. The ability of the enzyme tyrosine kinase to respond to a hormone
   d. The stability of DNA and RNA
   e. The process of DNA replication

5. Chlorosis is a condition in plants in which the green pigment is bleached gradually to a yellowish pigment. The condition can be reversed by putting more iron into the soil. Offer another way to treat this condition.

6. Look up Epsom Salts and offer an explanation as to what magnesium may be doing to reverse the symptoms.

7. Justify the comment that magnesium may be considered an anti-calcium.

8. Give two functions of parvalbumin in muscle. Why is the off rate of magnesium from the protein an important factor? What function predominates when a state of relaxation is reached?

Iron

HUMANS have between 3 to 4 grams of iron, most of which is in blood cells. The daily intake ranges between 15 and 25 mg; 1 to 2 mg (5–10%) is absorbed by the gut. About 80 percent of the iron intake on a daily basis is used for hemoglobin synthesis. As noted by the multitude of biological systems that require iron, the metal is strongly linked to molecular oxygen. In addition to hemoglobin, the transporter of oxygen in the blood, iron is used by oxygen-requiring enzymes and proteins that transfer electrons to oxygen to generate cellular energy. The freedom to exist in stable multivalent forms is one key to understanding iron's functional versatility. With it comes caution. Its pro-oxidant activity mandates that iron be sequestered inside cells; consequently, there is very little free iron anywhere in the system. Food sources richest in iron are mainly animal products. A most intriguing aspect of human iron nutrition is the system's ability to regulate iron intake but not excretion. While designed to guard against a sudden loss of iron in the blood, this one feature of iron metabolism opens the way to serious iron-storage and iron-overload diseases that can be life threatening.

13.1. HISTORY AND EARLY INSIGHTS

The study of iron goes back to ancient Greece, when soldiers in battle were given iron to overcome muscle weakness. Bordering on the supernatural, Menghini showed that dried blood was attracted to a magnet. The biology of iron began to attract attention when low iron was linked

to an anemic condition referred to as chlorosis, a yellowing of leaf tissue due to a failure of a plant to absorb iron from the soil. In humans, however, chlorosis was diagnosed as an anemic condition characterized by fewer numbers of red blood cells. In 1873, Boussingault wrote the first essay on the essentiality of iron in living systems, and 20 year later Bunge showed infants on a strict milk diet were vulnerable to low iron intake. Later, infantile anemia was shown to be avoided by using iron-fortified formulas. These observations brought attention to the nutritional value of iron and some of the consequences of insufficient intake.

13.2. CHEMICAL PROPERTIES

Biological iron is a multivalent metal, with ferric (Fe^{3+}) and ferrous (Fe^{2+}) the two principle oxidation states. Both forms readily interconvert, making iron a reactive redox metal. The loss of two 4*s* electrons generates the quasi-soluble $3d^6$ ferrous ion. The ferric ion ($3d^5$) is formed when the two 4*s* and one 3*d* electron are removed. This form is highly insoluble in aqueous medium. Like all 3*d* metal ions, iron must conform to a specific geometric arrangement at the ligand binding sites. An octahedral arrangement as seen in heme proteins is the most common [Figure 13.1(a)]. A second arrangement is iron-sulfur centers in proteins [Figure 13.1(b)]. Proteins bearing iron-sulfur centers are at the proximal end of an electron transport chain. The pro-oxidant property of iron, however, is of greater concern. As shown below, a non-enzyme transfer of one electron from ferrous iron to hydrogen peroxide generates a hydroxyl radical, one of the most reactive oxygen-centered radicals in biological systems [Equation (1)]. Paradoxically, when exposed to catalase, an iron-dependent enzyme, hydrogen peroxide is reduced to H_2O and O_2 [Equation (2)], thereby protecting the cell against peroxidation. Recognizing that free iron is a hazard gives a justification for shielding iron from the immediate environment, thwarting iron's capacity to generate free radicals. Hemoglobin, which harbors iron within the confines of a porphyrin ring, is a good example of shielding the iron.

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\cdot} + Fe^{3+} \quad (1)$$

$$H_2O_2 + Fe^{2+} \rightarrow H_2O + \tfrac{1}{2}O_2 + Fe^{3+} \quad (2)$$

13.3. BIOCHEMICAL PROPERTIES

Nutritionists distinguish iron as either heme or non-heme iron. Heme refers to a porphyrin ring encompassing the iron in an octahedral complex [Figure 13.1(a)]. Non-heme designates either an iron sulfur center in a protein or low molecular weight iron complexes [Figure 13.1(b)]. Three heme proteins of note are hemoglobin and myoglobin, which function in the transport of O_2 in the blood and cytosol, respectively, and membrane-bound cytochromes that are part of the electron transport system that transfer electrons to O_2 in the mitochondria. Whereas hemoglobin and myoglobin iron exists primarily in a stable ferrous form, iron in cytochromes is free to exchange between Fe^{2+} and Fe^{3+}, allowing the protein to accept and donate electrons. Another important heme protein, cytochrome P450, plays a major role in lipid metabolism as well as detoxifying drugs and harmful organic molecules.

13.3.1. Distribution of Iron in Tissues and Cells

The multiple biochemical forms of iron are present in all organs and tissues, and about two-thirds of these contain iron as heme iron (Table 13.1). Ferritin and hemosiderin, two iron storage proteins, make up the second largest iron pool. As noted in the table, stored

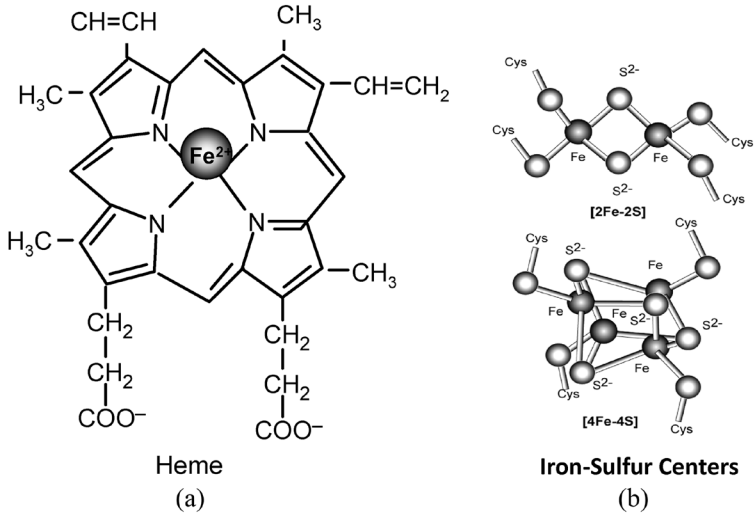

FIGURE 13.1. *Principal Biochemical Forms of Iron in Biological Molecules.*

TABLE 13.1. Distribution of Iron in Human Tissues.

Protein (mg/kg)	Male	Female
Functional Iron		
Erythrocyte hemoglobin	31	26
Myoglobin	4	3
Heme iron	1	1
Non-heme iron	1	1
Transferrin Iron	0.05	0.05
Total	**37 (74%)**	**33 (87%)**
Storage Iron		
Ferritin	9	4
Hemosiderin	4	1
Total	**13 (26%)**	**5 (13%)**

iron makes up about 15 percent of the total iron in females and 25 percent in males.

13.3.2. Iron-Dependent Enzymes

A list of the more familiar and better studied iron enzymes is shown in Table 13.2. Based on the list, one is justified in concluding that iron's main biochemical functions in living systems is to partake in physiological events requisite to:

1. Transport of O_2 to cells and within cells (hemoglobin, myoglobin)
2. Transport of electrons to O_2 to generate energy (cytochrome proteins)
3. Protection against oxidants and toxicants (catalase, peroxidases, cytochrome P450)
4. Fixation of atmospheric nitrogen (nitrogenase)
5. Synthesis of nucleic acids and lipids (ribonucleotide reductase, fatty acid desaturase).

13.4. NUTRITION

From a nutritional perspective, recycled iron from senescent blood cells makes up a large portion of the iron required to sustain the system on a day to day basis. This phenomena, unique to iron more so than any

TABLE 13.2. Iron-dependent Enzymes.

Enzyme	Biochemical Function
Aldehyde Oxidase	Oxidizes reactive aldehydes
Amino Acid Oxidase	Amino acid catabolism
Cytochrome P450	Antioxidant and antitoxicant
Fatty acid desaturase	Unsaturated fatty acid synthesis
Nitric oxide synthetase	Nitrogen cycle in plants
Nitrogenase	Nitrogen fixation
Peroxidases	Destroys peroxides
Phenylalanine hydroxylase	Tyrosine biosynthesis
Ribonucleotide reductase	Deoxyribose synthesis
Tryptophan 5-monooxygenase	Serotonin biosynthesis
Tyrosine hydroxylase	L-DOPA biosynthesis

other mineral, reflects the proclivity of the system to retain iron once it is absorbed. Daily losses in the stool, sweat, or by menstruation are matched by the amount of iron absorbed to maintain iron balance. Most iron taken into the system from the diet is transferred to bone marrow for hemoglobin biosynthesis. This could amount to 80 percent of the daily intake. There is reason to suspect that on a global scale the intake of iron in the average adult, particularly the elderly, is insufficient to achieve optimal health.

13.4.1. Food Sources

Table 13.3 lists some iron-rich foods in the diet. Because of their ease

TABLE 13.3. Richest Food Sources of Iron.

Red meat
Egg yolks
Seeds and nuts (sesame, sunflower, pecans)
Dark, leafy greens (spinach, collards)
Dried fruit (prunes, raisins)
Cereals and grains
Mollusks (oysters, clams, scallops)
Turkey or chicken giblets
Beans, lentils, chick peas and soybeans
Liver
Artichokes

of absorption and high bioavailability, foods rich in heme iron are preferred over other sources. Meat, especially dark meat, tops the list. Of lesser quality but still important are poultry, especially drumstick meat, which is superior to breast meat. Oily fish (oysters, mollusks and clams) are good sources due to their modest amounts of heme iron. Non-heme iron, in contrast, is found mainly in plant foods. Whole grains, barley and oats in particular, are a good source. Green leafy vegetables (spinach, kale, and water cress) have ample amounts of iron but are inferior to seeds and nuts. It is important to note that dairy products provide almost no iron in the diet.

13.4.2. Recommended Intake

Table 13.4 shows the RDA and UL for iron at various life stages. Gender differences do not becomes a factor until late in the adolescent period. The increased needs of females over males probably reflects gains and losses associated with menses. Upper limits for iron are generally in the range of 4–5 times the RDA for all ages and genders.

TABLE 13.4. Recommended Intake for Iron as a Function of Age and Gender.

Subject	RDA (mg/day)		UL (mg/day)
	Male	Female	
Infants (0–6 mo)	–	–	40
Children and adolescents			
7–12 mo	11	11	40
1–3 yr	7	7	40
4–8 yr	10	10	40
9–13 yr	8	8	40
14–18 yr	11	15	45
19–30 yr	8	18	45
Pregnancy			
<18 yr		27	45
19–50 yr		22	45
Lactation			
<18 yr		10	45
19–50 yr		9	45

TABLE 13.5. Food Components that Promote or Suppress Iron Absorption.

Promoters	Suppressors
Amino acids	Carbonates
Animal protein	Calcium
Ascorbic acid	Phosvitin in egg yolk
Organic acids	Fibers
Sugars	Oxalates
Mucins	Phosphates
	Phytates
	Plant polyphenols
	Soy proteins

13.5. DIGESTION AND ABSORPTION

13.5.1. Overview

Although about a third of the iron in the diet is heme, both heme and non-heme iron are bound to proteins and thus require protease enzymes synthesized in the gastric juice and pancreas to liberate (Chapter 6). While the majority of the heme iron is stable and remains intact, non-heme iron once released presents problems in solubility that have to be overcome for absorption to occur. Moreover, since non-heme iron can either be ferrous or ferric, absorption requires a multifaceted system of valence-specific transporters to recognize both forms, as well as one for heme.

13.5.2. Food Factors Influencing Absorption

A multitude of factors in the lumen play ancillary roles in the amount of iron that enters the system. Few, if any, influence heme iron. As seen in Table 13.5, factors that influence iron absorption can be divided into factors that either promote or hinder absorption. Included in the former are amino acids, sugars, and water soluble organics. The importance of mucins will be discussed below. Ascorbic acid (vitamin C) is especially important to note because the reduced form of the vitamin promotes iron absorption by reducing ferric iron to ferrous, the more soluble form. Suppressors of iron absorption are mainly compounds that deter its passage across the enterocyte membrane or metal ions that compete for the same entry site.

13.6. MECHANISM OF IRON ABSORPTION

Once released as a free ion, the iron is primed to be taken up by absorbing enterocytes that line the stomach and small intestines. An exception is heme iron, which stays bound to the porphyrin ring and is absorbed as an intact complex. As noted previously, the presence of three forms of iron in the lumen mandates that absorption at the apical surface requires three separate channels designed to recognize, trap and shuttle iron into the interior. A description of events taking place is shown in Figure 13.2.

13.6.1. The Heme Pathway

The heme group, with its ferrous bound iron, is fairly stable and can enter the duodenal cells as an intact complex. Passage into the enterocytes utilizes a heme carrier protein (HCP1). Unregulated uptake across the lipid bilayer also occurs. Once inside the cell, the enzyme *hemeoxygenase* liberates the iron from the porphyrin ring and transfers the iron as Fe^{3+} to the paraferritin complex. The porphyrin ring is subsequently catabolized to biliverdin and bilirubin and cast into the lumen as biosalts.

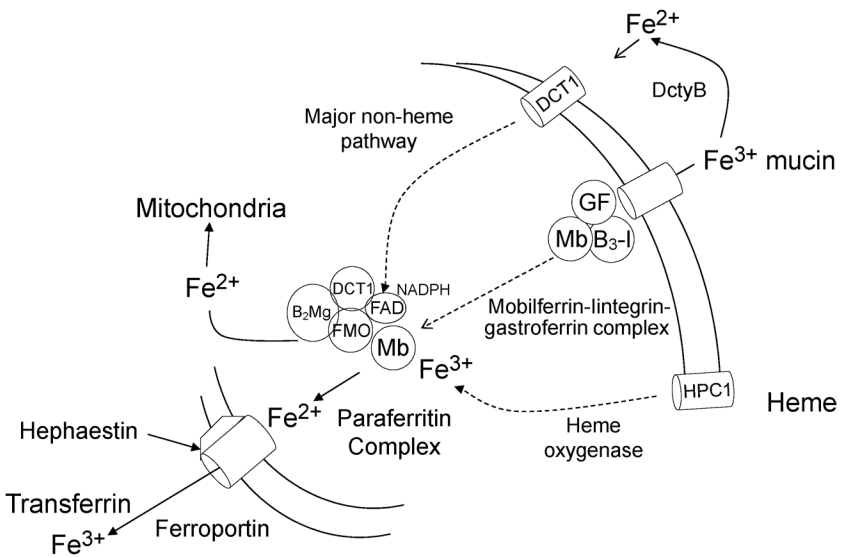

FIGURE 13.2. Pathways of Iron Uptake into Enterocytes. Three paths for iron entrance into the enterocyte: the Fe^{3+}-mucin pathways, the heme pathway, and the ferrous pathway all converge at paraferritin which redistributes the iron to different locations within the cell and for export.

13.6.2. Ferric Iron Pathway, Role of Mobilferrin and Paraferritin

As the name implies, the ferric pathways (also called the Fe^{3+}-mucin pathway) only recognize Fe^{3+}. Mobilferrin (Mb) is a mediator in this pathway. Located in vesicles near the apical surface of the microvilla, mobileferrin is believed to exist as a complex that is linked to β3-integrin and gastroferrin (GF). Gastroferrin binds the insoluble Fe^{3+}, forming a quasi-soluble Fe^{3+}-mucin complex that engages mobilferrin. The mobilferrin moves through the cytosol carrying the entrapped Fe^{3+}. One major destination is paraferritin, a protein complex which, although the name suggests otherwise, is not used to store the iron, but rather to mediate its passage to the exporting surface for discharge from the enterocyte (see below).

13.6.3. The Ferrous Iron Pathway

Ferrous iron (Fe^{2+}), the more soluble iron form, utilizes DCT1 (divalent cation transporter)—also called DMT1 (divalent metal ion transporter)—to enter the cell. Of the three pathways for iron entry, the DCT1 is the most dominant. As the name implies, DCT1 also transports divalent cations in general, such as Zn^{2+}, Cu^{2+}, Fe^{2+}, and Mn^{2+} and plays a minor role in the absorption of Ca^{2+} and Mg^{2+}. Mutations in human DCT1 have been linked with severe hypochromic microcytic anemia. Due to the ease with which Fe^{2+} is oxidized by O_2 in aqueous medium, a sizable amount of the iron in the duodenal lumen has the potential to be Fe^{3+}. Fe^{3+}, however, is subject to reduction to Fe^{2+} by ascorbate (Vitamin C) or through the action of duodenal cytochrome B reductase (DcytB) on the membrane surface. Either way, Fe^{3+}—now as Fe^{2+}—has a direct conduit to DCT1. The role of DCT1 as a membrane iron transporter is overshadowed by a lingering uncertainty as to how iron penetration utilizing this protein is achieved. An electrochemical gradient created by amassing protons on one side of the membrane has been postulated as a driving force. Once inside, however, the DCT1-Fe^{2+} complex is on the path to paraferritin and export of iron from the cell.

13.6.4. Export from the Cell, Role of Ferroportin (FPN)

Ferroportin-1 (IREG-1, MPT-1) provides an exit portal for iron to escape the enclosed membrane of the enterocyte. This could apply to

release from a vesicle within the cell or at the membrane surface. Ferroportin is located at the basolateral surface of the cell, which lets iron exit on the serosal side of the intestine. Because ferroportin only recognizes Fe^{2+}, the ferric iron in the paraferritin complex must be reduced to ferrous in order to exit. Levels of ferroportin are closely regulated by hepcidin, which promotes internalization and degradation of ferroportin. A failure to regulate ferroportin levels in the membrane could result in uncontrolled amounts of iron entering the system. Ferroportin also plays an important role in iron release from the liver Kupffer cells (macrophages) and the movement of iron from endosomal compartments. Iron release from these internal sources maintains a steady pool of iron for engaging transferrin on the serosal side.

13.6.5. Ferritin Intervention

A fraction of the iron absorbed by enterocytes is shuttled to ferritin for storage. How much is taken in by ferritin depends on the amount taken up by the cell. This is a regulatory step to assure that only graded amounts of iron actually pass through the cell and are absorbed into the system. Binding to ferritin thus protects the system from a sudden iron surge. Ferritin itself is controlled by a series of regulatory proteins that control ferritin mRNA translation (see below).

13.6.6. Role of Paraferritin

Paraferritin is a large complex that forms when mobilferrin fuses with β_3-integrin, flavin monooxygenase, and β_2-microglobulin. As noted in Figure 13.2, the importance of paraferritin is shown by its strategic positioning at the crossroads of the three iron entrance pathways. To be exported, the iron must first be reduced to ferrous iron and the paraferritin complex performs the reduction.

13.6.7. Role of Hephaestin

Hephaestin was identified as the factor responsible for a condition in mice known as sex-linked anemia (SLA). Inferring that the defect is sex-linked indicates the defective gene is located on the X-chromosome and therefore foremost in males. The hephaestin gene codes for a multicopper oxidase similar to ceruloplasmin. It has been speculated that the binding of newly released ferrous iron to transferrin uses hephaestin to

perform the oxidation to ferric iron. Failure of the enzyme to catalyze the conversion to ferric causes the anemic condition.

13.7. REGULATION OF IRON ABSORPTION

Because of its potential harm to the system, events in iron absorption are carefully regulated at all steps. Since vertebrates have no control over iron excretion, should an excess input occur, the system is vulnerable to iron overload. Insight into the regulation has been gained through studies of hemochromatosis, specifically the role of HFE and hepcidin in this human iron overload disorder.

13.7.1. HFE

The HFE gene has been implicated as a primary factor responsible for hereditary hemochromatosis. Located on chromosome 6, the gene codes for a 348 residue membrane protein whose structure is similar to MHC Class 1-type proteins that associate with β_2-microglobulin. Binding to β_2-microglobulin shields HFE from attack by proteases. Membrane-bound HFE engages a transferrin receptor and blocks the receptor from binding transferrin, thereby deterring iron uptake in peripheral cells, a protective measure. Alternatively, the HFE-transferrin complex can also enter the cell, but HFE prevents the release of iron into the endosome. HFE may also be associated with undifferentiated crypt cells of the intestine, which acts as a sensor of body iron status. By sensing iron associated with the transferrin uptake, HFE assists in determining if more or less iron needs to be absorbed. Mutations in the HFE gene prohibit HFE protein from engaging β_2-microglobulin and open the way to the destruction of HFE and unchecked uptake of transferrin-bound iron, which typically is what is seen in hemochromatotic subjects.

13.7.2. Hepcidin

Hepcidin, a small 25-residue protein made in the liver and released into the plasma, is considered the master regulator of iron metabolism. One of hepcidin's actions is to inhibit ferroportin by causing its destruction internally, thus shutting off iron flow from the intestine. A hepcidin mutation results in a steady continuous influx of iron regardless of the

iron status of the individual. Because the input is steady, ferritin synthesis is not triggered. Both HFE and HJV (hemojuvelin) control the synthesis of hepcidin at the level of transcription. Although these two proteins are membrane-bound, a hydrolysis reaction liberates a soluble form that activates hepcidin mRNA transcription. A serious iron overload in juveniles has been associated with a mutation in HJV gene. Newly emerging data seems to suggest hepcidin may also suppress the synthesis of DCT1, thus providing a direct connection with apical surface iron transport and the release of transferrin-bound iron from the endosome (see below).

13.7.3. Understanding the Causes of Hemochromatosis

Hemochromatosis is an iron overload disease that has revealed important insights into the regulation of iron export from the intestine and import into peripheral cells. Iron is a dangerous chemical that must be managed closely. Figure 13.3 summarizes the important factors that are responsible for regulating iron within the system. A defect in any one of these factors could lead to hemochromatosis.

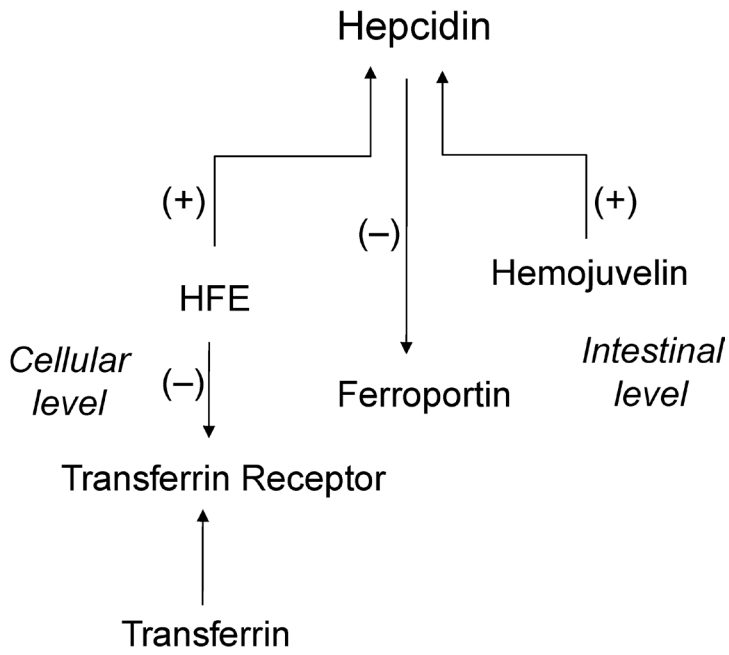

FIGURE 13.3. Overview of Iron Regulation in Hemochromatosis.

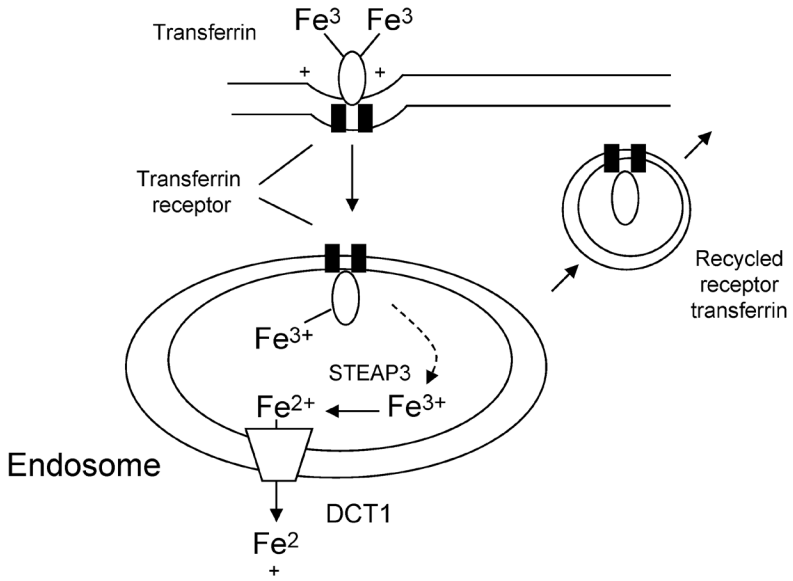

FIGURE 13.4. *Uptake of Iron from Transferrin.*

13.8. METABOLISM AND ASSIMILATION

13.8.1. Role of Transferrin in Iron Uptake

The iron transport protein transferrin is the major conveyor of iron to the tissues. Transferrin is also believed to play a role in regulating internal iron homeostasis. This 80 kDa monomeric glycoprotein has two binding sites for iron located on opposite ends of the protein chain. The C-terminal site and the N-terminal site can both bind iron but may differ in their efficiency in releasing iron to the tissues. Filling both sites results in a saturated molecule. The second component in the assimilation process is the receptor. To deliver iron to a cell requires transferrin to bind to its receptor on the cell surface. Binding to the receptor is followed by entrance into the cells through a receptor mediated endocytosis. The entrapped transferrin within the confines of a clathrin-coated pit will eventually merge with an endosome (Figure 13.4). The environment within the endosome is slightly acidic, causing the iron to dissociate from the transferrin. Exit from the endosome to the cytosol is believed to occur through the DCT1 transport protein. The ferric iron (Fe^{3+}) must first be reduced by STEAP3 in order to be recognized by DCT1 and exit the endosome. In the cytosol, the liberated Fe^{2+} is

free to bind to iron proteins or to ferritin for storage, and the iron-free transferrin still bound to the receptor cycles back to the surface. This recycling allows the cell to release intact transferrin protein and prepare the receptor for another transferrin molecule.

13.9. REGULATION OF IRON METABOLISM AT THE GENETIC LEVEL

13.9.1. Synthesis of Ferritin and Transferrin Receptor

The synthesis of ferritin and transferrin receptors has revealed mechanistic parallels between genes regulating iron-protein expression. By sequestering iron for storage, ferritin levels in the cell critically control the amount that passes across the intestine. Similarly, the transferrin receptor on the cell surface controls the amount of iron entering a peripheral cell. Both proteins, therefore, need to be tuned to system needs. How ferritin and transferrin receptors are modulated by iron has been a topic of considerable interest. As shown in Figure 13.5, both are controlled at the level of translation and require an important class of proteins known as iron-response proteins (IRPs). IRPs recognize a specific stem-loop structure on ferritin mRNAs and transferrin receptor mRNAs and in so doing can modulate the action of both mRNAs. The IRE on ferritin mRNA is located on the 5′ end, the segment that binds to the ribosome; the IRE on transferrin receptor is on the 3′ end. By binding to the IRE, the IRP prevents ferritin mRNA from engaging the ribosome, effectively canceling the reading of the mRNA and blocking ferritin synthesis. Elevated cellular iron, however, prevents the IRP from binding, whereas low iron allows binding; the first permits mRNA to be read, the second blocks the reading of the message.

The IRE for a transferrin receptor is located on the 3′ end of the message, the end that controls mRNA stability and turnover. Again, high cellular iron blocks the IRE from binding, but with no IRE bound, the mRNA is destabilized and open to degradation. Lowering the level of transferrin receptor by lowering the amount of its message is a predictable outcome, considering the cell has sufficient iron and does not need to take up more. In contrast, low cellular iron allows the IRP to bind, thus stabilizing the mRNA to turnover and allowing more mRNA for translation and more receptor protein to be made—exactly what would be expected if the cell is in need of iron.

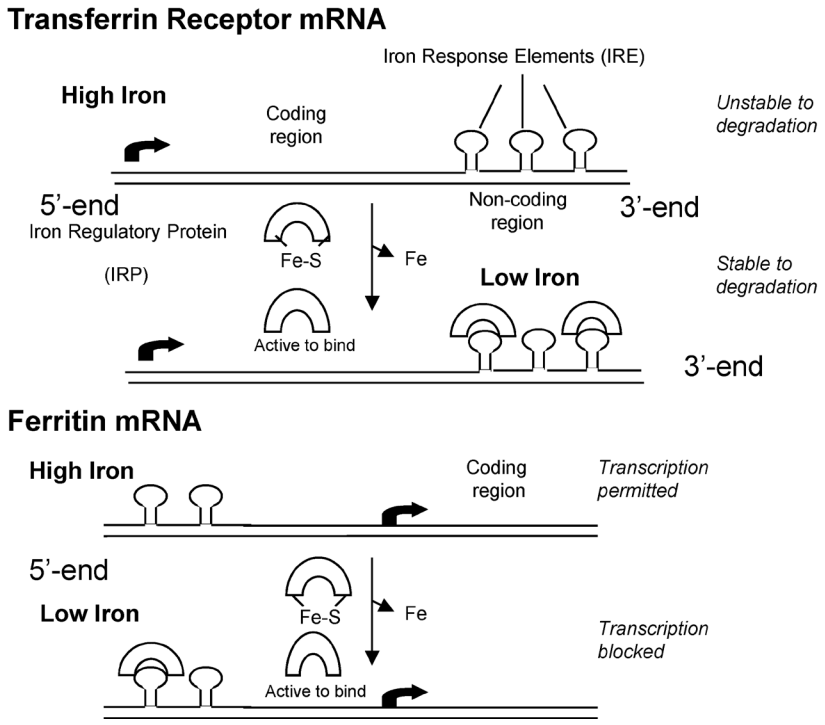

FIGURE 13.5. *Transcriptional Regulation of Ferritin and Transferrin Receptor Synthesis.*

13.10. SUMMARY

Although the 3–4 grams of iron in a human body may be considered out of the realm of a micronutrient, nearly one third of the iron is in hemoglobin and the rest is sparingly present inside non-erythropoetic cells. Iron's ability to exist in multiple valence states underlies a role for this metal in redox reactions and electron transport. Redox properties also translate into antioxidant and pro-oxidant reactions. Its ability to bind O_2 reversibly allows hemoglobin to carry O_2 to the tissues. Iron's strategic location in membranes as part of iron-sulfur complexes and cytochrome proteins has made iron suitable for transferring electrons that enable cells to generate energy for living processes. Due to its relative ease of absorption, foods richest in heme iron are the preferred source for the diet. Because foods have both heme and non-heme iron, systems that absorb iron have had to adapt to multiple forms of the metal. Intestinal iron absorption, therefore, utilizes membrane factors that recognize ferric, ferrous, and heme forms of iron and are adept at

absorbing all three. Regardless of how iron enters, there is a convergence of the three pathways within the enterocyte and mechanisms are in place for sorting out the movement to either storage or exit from the cell. A characteristic of systemic iron is that once absorbed, the iron cannot be excreted by an active process. This makes the input stage responsible for controlling iron levels in the system. Whereas low levels of iron can lead to anemia, failure to control iron absorption across the intestine or by peripheral cells can lead to iron overload diseases such as hemochromatosis. Both conditions represent problems that can occur when iron homeostasis is interrupted. Because only iron input is subject to regulation, carrier proteins such as DCT1 or ferroportin1 must be carefully tuned to the system's need for iron. This means that there must be a means of shutting off input when iron is sufficient. Hemochromatosis and other iron overload diseases have helped identify key factors that modulate release of iron from the intestine and uptake into cells.

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13.12. PROBLEMS

1. Outline the electronic configuration of iron and explain the transi-

tions that occur in order to form ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms. Of the two, which is more likely to be a pro-oxidant and therefore the more dangerous form? Explain your answer.

2. Which is the safer route for administering an iron supplement, oral or intravenous? Explain why you chose this answer.

3. A defect in which of the following components is likely to cause a severe anemic condition:
   a. mobilferrin
   b. DCT1
   c. paraferritin
   d. HCP1
   e. hepcidin
   f. ferritin
   g. ferroportin
   h. HFE

4. Repeat question 3 for the components whose defect could cause a serious iron overload.

5. Check all observation that would occur or be related to an increase in an IRP-Fe complex:
   a. The level of cellular iron is high
   b. The level of cellular iron is low
   c. Levels of transferrin receptor mRNA are elevated
   d. Levels of transferrin receptor mRNA are lowered
   e. More ferritin protein is synthesized
   f. More transferrin receptor protein is synthesized

6. A person who absorbed 1–2 mg of iron a day is likely to: (Check all correct answers)
   a. Be adequate in iron
   b. Be at risk for iron deficiency
   c. Be at risk for iron overload
   d. Develop anemia
   e. Be in iron homeostasis

7. Look at Figure 13.2. What component in the figure seems to be the point where all three pathways of iron's entrance into the cell converge?

8. What is gastroferrin and what role does gastroferrin play in iron absorption? Which form of iron is most likely to benefit from its presence? Explain.

Zinc

An adult human has about 1.5–2.5 grams of zinc, more than eighty percent of which is in bone and skeletal muscle. Food sources range from between 0.5–4 mg/100 grams with grains and cereals on the low end and meat, poultry and sea foods on the upper end of the scale. Zinc exists basically as a complex with proteins and nucleic acids in all cells. It functions as a cofactor for more than 300 enzymes and 200 transcription factors. Genetic-linked disease impinging on zinc absorption or diets low in zinc can lead to stunted growth and skin disorders. Its role as a brain neurotransmitter regulator is underappreciated, as is its role in cell signaling. Because of a multitude of known functions associated with zinc, this mineral must rightly be considered the most multipurpose mineral in the system, which challenges one to consider the properties that allows this to be.

14.1. HISTORY AND EARLY INSIGHTS

Although zinc was recognized as a chemical element as early as 1509, it took more than three hundred years to acknowledge its value to biological systems. Early studies reported zinc in plants, bodily fluids and organs of animals. Zinc's essentiality for life and health, however, was not accepted until early in the 20th century. Several decisive discoveries led the way. In 1955, Tucker and Salmon reported that zinc supplementation of a plant-based diet prevented a hitherto-unknown parakeratosis in pigs, a disease characterized by abnormal

hardening of the epidermal layer of the skin. At nearly the same time, Prasad and coworkers reported a type of human dwarfism characterized by stunted growth and repressed sexual development in people living in secluded areas of Egypt where unleavened bread was a dietary staple. The disease was hypothesized to be caused by high levels of phytate in the bread which suppressed zinc absorption. This was confirmed when enriching zinc intake reinstated growth in a dramatic reversal of the condition. Subsequent studies provided evidence that zinc was required to maintain vitamin A levels of the blood. Price and Vallee showed that *Euglena gracillis* was an ideal organism to study zinc utilization at the cellular level, focusing on biochemical factors that engaged the zinc or were affected by its presence or absence. A psychological approach later showed zinc to impact on cognitive development in animals and, in time, humans. All of these studies led to an increased awareness of zinc as an essential element and opened the way to learning the better-known functions of zinc that have since come to light.

14.2. CHEMICAL PROPERTIES

Zinc is last in the line of first transition series metals. As a consequence, the 3*d* and 4*s* orbitals are filled. Ionization occurs with the loss of the two 4*s* electrons, giving rise to Zn^{2+}, zinc's only allowable oxidation state. As an ion, Zn^{2+} behaves as a Lewis acid, which translates into a metal ion that is strongly attracted to electron pairs. Carbonyl, alcohol and imidazole groups are common structural features of ligand binding sites in zinc-dependent enzymes. Zinc's +2 charge confined within a small ionic radius (0.65 A) promotes electrostatic interactions that have a greater intensity than Ca^{2+}, Mg^{2+} or other divalent cations. Having only one oxidation state precludes zinc from propagating destructive free radicals, thereby allowing Zn^{2+} to coexist safely with organic molecules in cells. Although its closed $3d^{10}$ shell makes Zn^{2+} behave as a spherical ion, zinc's 3*d* electrons allow the metal to enter into strong coordinate covalent bonding. All zinc complexes have well-defined spatial geometries but lack color (Chapter 2). Finally, the disassociation of zinc complexes tends to be very rapid, which is a desirable feature for a cofactor that must swiftly discharge the product from the enzyme surface after it forms. The strong attraction of Zn^{2+} for electrons, however, weakens its solubility in aqueous medium. Zn^{2+} literally displaces

protons from water molecules and in the process forms insoluble zinc hydroxides as shown in Equation (1) below:

$$Zn^{2+} + 2H_2O \rightarrow Zn(OH)_2 + 2H^+ \quad (1)$$

Because the hydrolysis reaction occurs readily at physiological pH, zinc's solubility as a free ion in the blood and cells is about 20 μM and requires protein carriers to transport Zn^{2+} in fluids.

14.3. BIOCHEMICAL PROPERTIES

An element as adaptable as zinc can be predicted to have multiple biochemical roles. One observes, for example, that about 10 percent of the human genome is estimated to code for proteins with zinc-binding domains, which is consistent with zinc's widespread occurrence in biochemical pathways and structures. As is typical of most biominerals, zinc's biochemical functions can be divided into three categories: catalytic, structural, and regulatory. These in turn can be delegated to four major subcategories: (1) as a cofactor for all the major classes of enzymes; (2) as a regulator of genetic expression and cell signaling; (3) as a major stabilizer of proteins and nucleic acid structures; and (4) as a modulator of neurotransmissions in certain regions of the brain. Biochemical and physiological functions that depend on zinc are listed in Table 14.1.

14.3.1. Zinc as an Enzyme Cofactor

Having zinc-binding domains in ten percent of the proteins in hu-

TABLE 14.1. Biochemical Functions that have been Attributed to Zinc.

1. Enzyme Catalysis
2. Protein biosynthesis
3. Nucleic acid metabolism
4. Carbohydrate and Energy metabolism
5. Lipid metabolism
6. Epithelial tissue integrity
7. Cell division
8. Vitamin A and E transport
9. Immune function
10. Reproduction

TABLE 14.2. Examples of Zinc-dependent Enzymes (Adopted from Vallee and Auld, 1995)

Classification	Zinc Enzyme
Oxidoreductases	Alcohol dehydrogenase
	Dehydroquinate synthase
Transferase	Aspartate carbamoyltransferase
Hydrolase	Carboxypeptidase A
	Carboxypeptidase B
	DD carboxypeptidase
	Thermolysin
	Alkaline phosphatase
	Phospholipase C
	Beta Lactamase
	RNAase, DNAase
	5'-nucleotidase
Lyase	Carbonic anhydrase I
	Carbonic anhydrase II
Isomerase	Triose isomerase
Ligase	DNA polymerase
	RNA polymerase

mans translates into a potential for more than 900 different enzymes and proteins that are capable of binding zinc. As a cofactor, zinc stabilizes the overall structure of enzymes and also takes an active part in the catalytic event on the enzyme's surface. These functions require the zinc to be bound covalently to the amino acid side chains in the enzyme protein. Table 14.2 lists some of the more familiar zinc metalloenzymes. In search of a common principle for the selection, one notes that many zinc enzymes function with a water molecule bound to the zinc, which is consistent with the zinc having the capacity to displace a proton from the water to create a stronger nucleophile. In the case of alcohol dehydrogenase and dehydroquinate synthase, the zinc activates the alcoholic –OH by assisting in the removal of a hydride ion as shown in Equation (2):

$$\text{R-OH} + \text{Zn}^{2+} \rightarrow \text{R-O-Zn} + \text{H}^{-} \qquad (2)$$

Hydrolases (enzymes that break bonds by inserting water across the bond) make up a large number of zinc-dependent enzymes. Carbonic anhydrase, the enzyme that catalyzes a reversible release of CO_2 in

the lung, is one example of a zinc-dependent enzyme. In the reactions, the stable +2 charge on the zinc ion accepts an electron pair, which is paramount for the reaction to occur and is why zinc is selected (Figure 14.1).

14.3.2. Precision of the Zinc Binding Site

A bound zinc atom is shaped precisely to the specifications of the binding site on the protein. A small distortion, therefore, can alter the catalytic property of the enzyme. In carbonic anhydrase, zinc is present in a trigonal bipyramidal arrangement (Chapter 2), with three histidine groups engaging directly and one open valence for water. The fifth valence does not take part in the binding to either protein or substrate. In the reaction, zinc displaces a proton from the water molecule, giving rise to OH^-, which is a stronger nucleophile than water (Figure 14.1). Activating the water molecule by displacing a proton is a quintessential reaction for converting CO_2 to carbonic acid, and in the reverse, converting carbonic acid back to CO_2.

14.3.3. Non-Cofactor Functions of Zinc

14.3.3.1. Protein Biosynthesis and Nucleic Acid Metabolism

Integrated pathways of protein assembly and nucleic acid synthesis strongly depend on zinc. More specifically, polymerase enzymes that assemble mRNA on a DNA template or replicate and repair DNA use

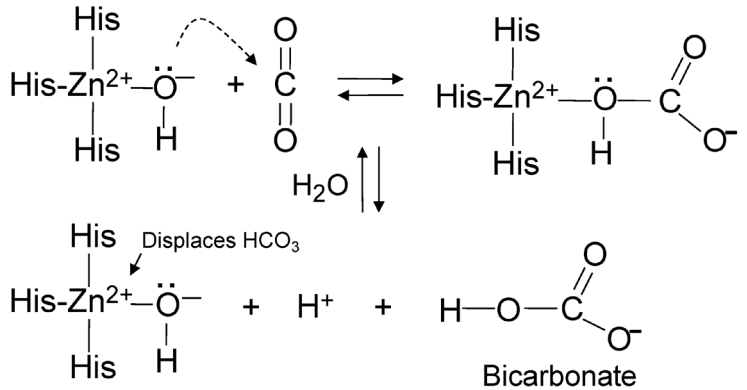


FIGURE 14.1. *Mechanism of Action of Zinc in Carbonic Anhydrase (Adopted from Voet and Voet, 1995).*

zinc as a cofactor. These would include DNA and RNA polymerases, DNA repair enzymes and RNA modifying enzymes.

14.3.3.2. Regulation of Genetic Expression

Zinc is a structural component of many transcription factors that bind to the promoter region of genes and initiate mRNA transcription. In this capacity, zinc forms a structural motif that engages the major grooves on the DNA. What have been called zinc-finger proteins are extremely prominent transcription regulators that provide a foundation for RNA polymerase to engage the DNA.

14.3.3.3. Cell Signaling

Cell signaling occurs when a hormone or cytokine binds to receptors on the membrane surface and activates a response within the cell. Zinc-dependent factors play an integral part in the transmission of external signals by mediating a cascade of events terminating in cell division or cell death or various sundry functions performed within the cell.

14.3.3.4. Carbohydrate and Lipid Metabolism

Zinc is a prominent player in the metabolism of fats and sugars and thus is essential for energy metabolism. An example is the requirement for a zinc-insulin complex to activate glucose uptake into the muscle and adipose tissue and subsequent reactions using zinc-dependent enzymes to digest food molecules and extract energy.

14.3.3.5. Neurotransmitter Modulator

Although zinc is classified as a micronutrient, Zn^{2+} is known to concentrate in certain areas of the brain where presynaptic glutamate-containing termini release vesicular Zn^{2+} to act as a free ion modulator of glutamate-stimulated impulses at the glutamate receptor. This less-appreciated role for zinc is discussed in more detail in Chapter 9.

14.4. NUTRITION

Most of the zinc in humans and animals is in skeletal muscle, bone,

TABLE 14.3. Distribution of Zinc in Human Organs and Tissues.

Organ or Tissue	Percent Zinc
Skeletal Muscle	47
Bone	29
Skin	6
Liver	5
Brain	1.5
Kidneys	0.7
Heart	0.4
Hair	0.1
Plasma	0.1

skin and liver, which combined accounts for nearly 97% of the total body load (Table 14.3). Less than 1% is in the kidneys and the heart. The brain is the fifth richest organ for zinc. Only about 0.1% of the body's zinc is in the plasma.

14.4.1. Food Sources of Zinc

Although shellfish are the richest food sources of zinc, plant-based foods, lean red meats and dairy products provide most of the zinc in a typical American diet. Three ounces of oysters may have as much as 2 mg of zinc. Poorer sources are grains (mostly bran), which incur large losses of the zinc (80%) by milling (Chapter 4). Grains are also rich in phytic acids (hexa- and pentaphosphates), which suppress zinc absorption (Chapter 6). In contrast, meats (skeletal muscle) offer a further advantage in that cysteine and methionine released upon digestion aid zinc absorption. Egg yolk, not egg white, is particularly rich in zinc, whereas milk tends to be middle range as a food source. One liter of milk, for example, contains only about 2 mg of zinc, which means that

TABLE 14.4. Food Sources of Zinc.

Oysters
Red meats
Eggs
Dairy products
Grains and cereals
Nuts
Legumes

a person must consume 5 quarts (5.68 liters) to meet the daily zinc requirement.

14.4.2. Recommended Intake

Table 14.5 shows the RDA and UL for zinc at various stages of life. Data from a number of studies have concluded that the average adult does not consume sufficient amounts of zinc to maintain health and that marginal zinc diets could be at the root of a series of physiological impairments. This is particularly true for the elderly. Gender differences do not become a factor until late in the adolescent period. The increase in zinc for males reflects not only body size and weight but the high zinc content of seminal fluid. In adult females, very little zinc is lost by menses. Upper limits for zinc generally are 3–4 times the RDA.

14.4.3. Zinc Homeostasis

About 1% of the body zinc is replenished daily by foods in the diet. Within the same time interval, an equal amount of zinc must be lost from the system to maintain status quo. Figure 14.2 outlines the overall homeostasis of zinc in an adult human, beginning with a daily in-

TABLE 14.5. Recommended Intake for Zinc.

	RDA (mg/day)			
Subject	**Male**	**Female**	**AI**	**UL (mg/day)**
Infants (0–6 mo)			2	4
Children and adolescents				
7–12 mo	3	3		5
1–3 yr	3	3		5
4–8 yr	5	5		12
9–13 yr	8	8		23
14–18 yr	11	9		34
19–30 yr	11	8		40
Pregnancy				
<18 yr		12		34
19–50 yr		11		40
Lactation				
<18 yr		13		34
19–50 yr		12		40

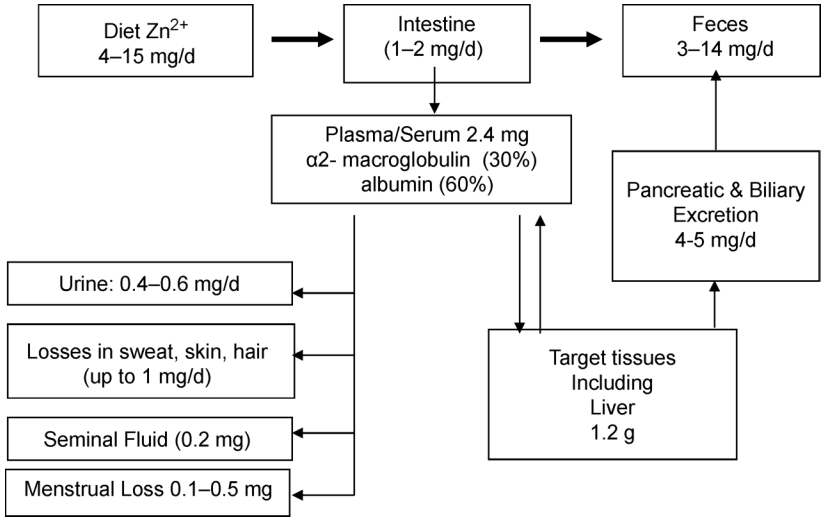


FIGURE 14.2. *Zinc Homeostasis and Distribution in Adult Humans.*

take of between 4–15 mg. Of that amount, only about 1–2 mg is absorbed and the remainder is excreted in the feces. Zinc taken into the system passes into the plasma and is distributed between albumin and α_2-macroglobulin, roughly 60 percent and 30 percent, respectively; about 10 percent is bound to other serum factors. Free Zn^{2+} in serum is estimated to be less than 0.1 micromolar. Plasma zinc is rerouted to the liver and other organs that have membrane portals for absorbing Zn^{2+} into the cell. Liver is particularly noted as an assembly site for many zinc enzymes as well as releasing zinc into the bile. The latter contributes to the pool of zinc excreted in the feces. Excretion through the kidneys into the urine appears to play only a minor role in regulating body zinc. A sizeable amount, however, is lost in sweat and hair follicles. Zinc-rich seminal fluid is an avenue for removing zinc in males. Because of the low levels in blood, very little zinc is lost in menses. Stress induced hormones tend to lower plasma zinc by increasing liver absorption and retention.

14.5. DIGESTION AND ABSORPTION

14.5.1. Digestion

Zinc in foods appears as either the sparingly-soluble free ion (mi-

nor) or as a complex with macromolecules or smaller organic chelates. Digestion liberates the zinc from its entrapped food environment, allowing Zn^{2+} to enter the system as a free ion. Liberating the zinc takes place initially in the stomach and later in the proximal intestine. In the stomach, digestive enzymes combined with an acidic environment produce mainly free zinc ions. Digestion in the proximal intestine occurs primarily from enzymes secreted from the pancreas into the duodenum. The alkaline environment of the duodenum, however, is unfavorable to the solubility of liberated zinc ions. Poor solubility is overcome by *gastroferrin*, the mucous-like protein released into the intestinal lumen by cells in the stomach and along the digestive tract. (Chapter 13).

14.5.2. Intestinal Absorption

Studies with inverted intestinal sacs have shown zinc absorption occurs all along the small intestine, but may be strongest in the jejunum. Very little zinc is absorbed in the stomach, cecum, or colon. An overall scheme of absorption is shown in Figure 14.3. Zinc entrance into the enterocyte is believed to be through Zip4, a member of the zinc family of transporters (See below). DCT-1 (divalent cation ion transporter) or DMT-1 (divalent metal transporter), discussed earlier, may also import zinc at the apical surface. This suggestion has not had strong experimental support, however. Once inside the enterocyte, zinc's transport to the basal surface can be rapid or delayed depending on the amount absorbed and the zinc status of the individual. A surge in zinc induces the synthesis of metallothionein, which sequesters the zinc while at the

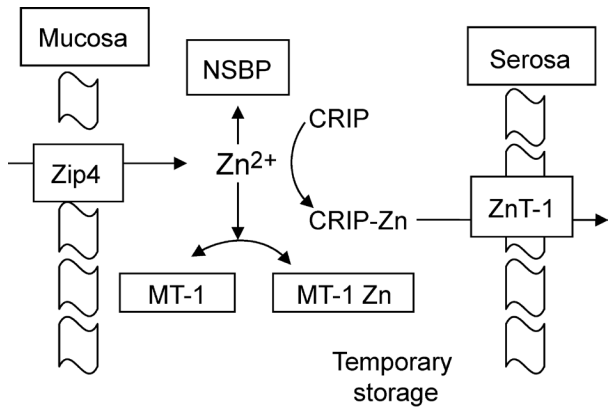


FIGURE 14.3. Intestinal Absorption of Zinc.

same time Zip4 is internalized away from the apical surface, further shutting down uptake. The combined effect is to suppress the amount of zinc both entering and passing through the enterocyte. In a low zinc state the opposite happens; more Zip4 is surface located and less metallothionein is synthesized, allowing absorbed zinc to pass with less impedance. CRIP, a cysteine-rich intracellular protein, may transport zinc to the basal surface for release via the transport protein ZnT-1. Other avenues of movement involve NSBP, a non-specific binding protein whose function in the regulation has not been clarified, but which has the capacity to sequester zinc temporarily out of the main stream.

14.5.3. Regulation of Zinc Absorption

Typically, one-third of the zinc ingested is absorbed. This figure may vary between 15 and 60 percent depending on a number of factors. The zinc status of the individual is a primary consideration. A zinc deficiency, subtle or severe, will increase the absorption of food zinc. Zinc's level in the plasma appears to have no influence on the amount of zinc that is absorbed, which singles out the enterocyte as the primary factor regulating the amount taken into the system. The poor solubility of the zinc in the alkaline environment of the duodenum is another factor to consider, as are zinc chelators and competing metal ions that affect absorption efficiency. By far the greatest impediment to zinc absorption lies with phytic acid and phytates in plant foods; of less importance are oxalates, which have only minimal effects on zinc absorption.

14.5.3.1. Competing Ions

Because Zn^{2+} and Cu^{+} both have a stable $3d^{10}$ configuration, there is reason to suspect the two metals will compete for entrance into the enterocyte and peripheral cells. Such has been shown by experiment to be correct. Due to its toxic effects at small levels, however, an intake of copper sufficient to lower plasma zinc and raise fecal zinc is unreasonable to consider. What is feasible is for the less toxic zinc to suppress copper. Acting in a therapeutic role, zinc has been used to suppress copper absorption in diseases such as Wilson disease, where excess copper intake is an extenuating factor. In fact, by adjusting the amount of zinc given to the patient it is possible to regulate the amount of copper entering the system. Such is needed to assure that the patient undergoing therapy does not suffer a copper deficiency. Finally, not to be excluded

is iron, which when taken as a supplement greater than 60 mg/day has the capacity to lower plasma zinc levels (Chapter 8). Of its three forms, ferrous iron is the most effective impediment; heme iron has no effect.

14.6. ZINC METABOLISM

Once zinc has been absorbed into the system, ensuing post-absorption events under the guise of zinc metabolism follow. These include: (1) transport in the plasma; (2) binding to target cells; (3) transmembrane transport into the cytosol; and ultimately, (4) delivery of zinc to zinc-dependent components within the cell. Failure of any of the steps through genetic mutations or other causes can lead to zinc deficiency symptoms.

14.6.1. Plasma Transport

In addition to albumin and α_2-macroglobulin, there is evidence that some zinc may be associated with transferrin and a histidine-rich glycoprotein (HRGP); only a small fraction is present as free zinc ions. All of these proteins are candidates for transporting zinc to cells. Albumin-bound zinc is believed to be part of an exchangeable zinc pool that makes zinc available for peripheral cell absorption. Albumin itself cannot enter cells, but will allow zinc ions to dissociate at the cell surface and enter a specific portal (such as Zip4) as a free ion. Such a concept implies that zinc uptake by peripheral cells may be multifaceted. In one case, it depends on its transport protein docking temporarily to the cell membrane and discharging the zinc. The other is for the zinc-binding protein to recognize a specific receptor at the membrane surface that, by endocytosis, transfers the protein-bound zinc into the cytosol. A receptor-mediated mechanism with transferrin and α_2-macroglobulin in the role of zinc delivery proteins would favor the latter. Conceptually, this may be correct, but definitive evidence is lacking. In fairness, therefore, it can be said that the identity of the transport factor(s) accountable for zinc uptake into cells has not been determined.

14.6.2. Zinc-Specific Membrane Transporters

Evidence that cells have membrane-bound transporters capable of translocating extracelluar zinc ions into the cytosol was first reported

by Palmiter and Findley in 1995. With the discovery came new insight into the absorption mechanism, but more importantly gave valuable clues as to how zinc uptake and homeostasis was regulated. Zinc transporters are part of two structurally dissimilar families of solute-linked carriers (SLC, Chapter 3); the Zip family (SLC30) and the ZnT family (SLC39A). Sequencing the human genome has led to the prediction that there could be at least 15 members of the Zip family and 9 members of the ZnT family in humans (Table 14.6). Family members for zinc transporters share sequence identity but differ in their overall size and intracellular location. As to why two families of transporters are needed to metabolize zinc, the answer appears to be that the two differ in their movement of zinc. Zip transporters move zinc ions into cells whereas ZnT transporters release zinc from the cell or sequester it in vesicles (Figure 14.4). Together, the two families maintain intracellular zinc homeostasis. Neither Zip nor ZnT transporters require ATP as an energy source, which contrasts with the ATP7a and ATP7b transporters for copper. Because ATP hydrolysis is not a factor, it has been postulated that that energy for transmembrane translocation is derived from anti-portal substitution of H^+ or K^+ for Zn^{2+} or from a Na^+/Zn^{2+} exchanger. Both provide the energy necessary to drive zinc into cells or release it from its cytosolic confines. Another transporter, ZnT-7 (not shown), directs cytosolic zinc to the Golgi site, where zinc-dependent enzymes are assembled. Of the Zip family, Zip4 has received much attention recently

TABLE 14.6. Summary of Membrane Zinc Transport Proteins.

ZnT Family	Cell, Location
ZnT-1	Plasma membrane
ZnT-2	Small intestine, vesicles, lysosomes
ZnT-3	Glutamatergic and GABAergic brain neurons
ZnT-4	Mammary gland, brain
ZnT-5	Pancreatic beta cells, insulin secretory vesicles
ZnT-6	Complexes with ZnT5
ZnT-7	Small intestine, Golgi
ZnT-8	Pancreatic beta cells, insulin secretory vesicles
Zip Family	
Zip-1	Small intestine, pancreas
Zip-2	Small intestine, liver, spleen, bone marrow
Zip-3	Bone marrow, spleen, intestine, liver
Zip-4	Small intestine, kidney

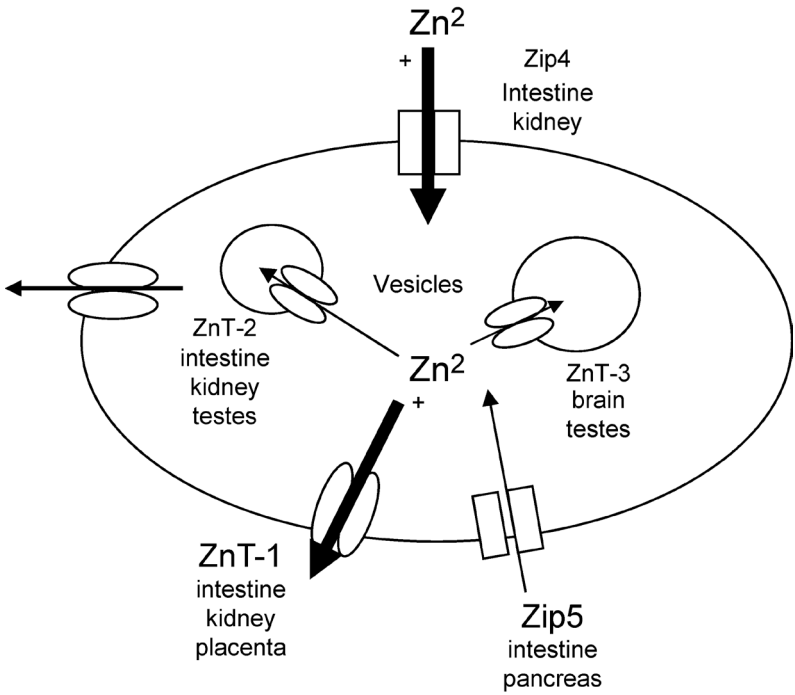


FIGURE 14.4. ZnT and Zip Family of Membrane Transporters. Note that Zip4 transports zinc ions into the cytosol, whereas ZnT-1 transports zinc out (Adopted from Cousins et al., 2006).

for its role in acrodermatitis enteropathica (AE), a well-characterized zinc malabsorption disorder (see below). Linking Zip4 with AE has confirmed its critical role in zinc movement.

14.6.3. Delivery of Zinc to Intracellular Targets

Cells that express a higher density of zinc receptors and transporters are in a more advantageous position to absorb zinc from plasma carriers. Movement into the cells follows the basic pattern of the intestinal enterocyte, requiring proteins and members of the Zip and ZnT family of transporters. The closest semblance of a storage site for zinc is metallothionein, whose biosynthesis is regulated by the level of zinc in the cell. Because the cytosol is slightly alkaline, free ion diffusion is not possible, which means transport within the cytosol relies on zinc bound to proteins or in vesicles. There is still much to be learned about that mechanism.

14.6.4. Zinc and Brain Functions

Brain functions of zinc stand apart from its more familiar role as a cofactor for enzymes. Nonetheless, zinc is the most abundant trace mineral in the brain. The important functions of zinc in the brain are discussed in Chapter 9.

14.7. ZINC DEFICIENCY

14.7.1. Assessing Zinc Deficiency

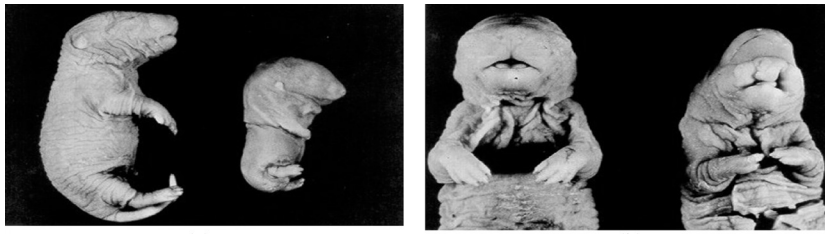
Subtle changes in zinc intake can go virtually unnoticed. More severe changes in human and animals give rise to the symptoms shown in Table 14.7. Many deficiency signs appear with slight impairments to internal metabolism. The multitude of protein factors that transport zinc or regulate its homeostasis are vulnerable to genetic mutations. Diabetics tend to excrete more zinc in the urine and the elderly suffer from both low intake and an altered physiology. As noted, a zinc deficiency or inadequacy can impair growth (particularly sexual maturation), cause hair loss, produce skin rashes, and predispose the sufferer to infections due to a weakened immune system.

14.7.1.1. Zinc Deficiency in Pregnancy

A more telling effect of zinc deficiency is seen with pregnant animals (Figure 14.5). Keen and colleagues have shown that zinc-deficient rats

TABLE 14.7. Symptoms of Severe Zinc Deficiency.

Growth retardation
Hypogonadism and hypospermia
Parakeratosis and acroorificial skin lesions
Diarrhea
Anorexia
Alopecia, glossitis and nail dystrophy
Impaired taste acuity
Immune system compromise
Delayed wound healing, burns, decubitus ulcers
Eye lesions, photophobia, dark adaptations
Behavioral changes



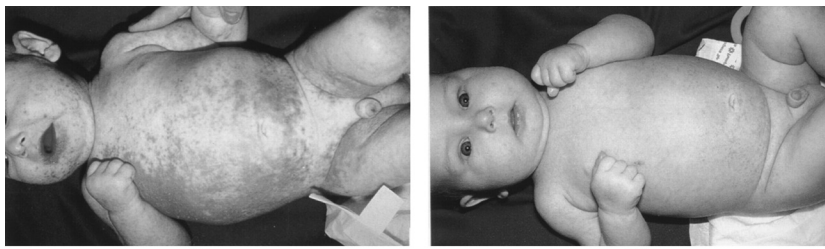
(a) (b)

FIGURE 14.5. *Zinc Deficiency during Gestation in the Rat.*

will have lower conception rates and their offspring will have a higher incidence of deformities of the brain, skull, limbs, eyes, hearts and lungs, most of which are attributed to post-conception events as early as the blastula stage (Uriu-Adams and Keen CL, 2010). Inducing a zinc deficiency in later stages of pregnancy produces less severe changes but nonetheless could lead to lower birth weight and result in a more difficult parturition.

14.7.1.2. Zinc Deficiency in Children

Acrodermatitis Enteropathica (AE) is a disorder noted primarily in children. Symptoms are a serious epidermal skin rash uniform over the body but more concentrated in the back and skin crevices (Figure 14.6). The rash and other symptoms of the disorder disappear within 4 days after giving the child supplements of zinc. Current research has shown that a mutation in the gene coding for Zip4 could be responsible for the disorder.



(a) (b)

FIGURE 14.6. *Clinical Signs of Acrodermatitis Enteropathica in a Four Week Old Infant. (a) Before zinc treatment; (b) after receiving zinc supplements for 4 days.*

TABLE 14.8. Means of Determining Zinc Status.

1. Zinc levels in the serum
2. Urinary zinc excretion (24 hour)
3. Fecal zinc excretion
4. Lymphocyte zinc content
5. Hair zinc
6. Liver zinc
7. Activity of zinc-dependent enzymes
8. Metallothionein levels of blood or metallothionein mRNA levels in monocytes
9. Body pools of zinc

14.7.1.3. Zinc Deficiency in Adults

The role of zinc in post-puberty growth was revealed by Prasad and colleagues while investigating the cause of nutritional dwarfism in isolated areas of Egypt. Growth and asexual development correlated with diets low in zinc. The low dietary zinc in turn was attributed to consuming unleavened bread that was rich in phytates. Feeding supplements of zinc to afflicted individuals resulted in an amazing growth spurt, showing the condition could be reversed with zinc.

14.7.2. Evaluation of Zinc Status

Physicians are often confronted with the task of determining if a patient's symptoms can be attributed to insufficient zinc intake. Ways to confirm a diagnosis of zinc deficiency become paramount under such circumstances. Table 14.8 lists various procedures that have been applied to determine the preclinical zinc status of an individual. It should be noted that each procedure listed in the table is itself subject to shortcomings and that the application of more than one procedure is desirable for an accurate assessment.

14.8. SUMMARY

The role of zinc in a biological system is multiple and varied. This seems somewhat unexpected for a simple non-redox cation with only one valence state. Much of zinc's versatility has to be attributed to its relative blandness, which allows zinc to react safely with many biologi-

cal ligands. It's absence of redox activity is also a factor in zinc's wide acceptance. Of greater importance may be its capability as a Lewis Acid to form strong bonds with a variety of proteins and nucleic acids. The biochemical requirement for zinc is met by a variety of different compounds that function as enzymes, signaling proteins, hormones, etc. In the brain, ionic zinc is both a neuromodulator and potent neurotoxin. Phytates in plants make vegetables less desirable as a source of zinc in the diet. Meats and sea food are a better choice. Considerable progress in understanding zinc metabolism has come through studies of the Zip and ZnT families of zinc transporters. The two families control cellular zinc homeostasis and perhaps act as protective factors against zinc toxicity. A variety of diseases have been documented as related to zinc deficiency or excess. Humans exposed to severe zinc deficiencies risk developing stunted growth, arrested sexual development, a compromised immune system, brain and cognitive disorders and serious disruptions to genetic level functions in general.

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14.10. PROBLEMS

1. Compare and contrast the electronic structure of Zn^{2+} with Ca^{2+}. Both are considered closed shell ions. Why then is Zn^{2+} but not Ca^{2+} more prone to form coordinate covalent bonds?

2. Although Cu^{+} and Zn^{2+} have identical electronic configurations, they don't participate in the same reactions. Give an example of a reaction that Cu^{+} can perform but not Zn^{2+}, and one that Zn^{2+} can perform but copper cannot.

3. Although plants contain zinc, plant foods in the diet are not al-

ways desirable to meet the zinc requirement. Explain why this is the case.

4. Describe how the presence of zinc allows hydrolase enzymes to function effectively.

5. What is a zinc-finger protein? In what part of a cell would you find zinc-finger proteins? What is the role of zinc in a zinc-finger protein?

6. If a person or animal is suspected of being deficient in zinc, what tests can be performed to confirm the diagnosis?

CHAPTER 15

Copper

An enrichment in atmospheric O_2 may have been a driving force for bringing copper into the biosphere. As evidenced by the many copper enzymes that use O_2 as a substrate, copper-endowed living systems with the means to cope with a potentially toxic gas and generate a greater energy yield from foods in the diet. Copper's role at the cellular level is primarily catalytic, not structural. At least two copper enzymes react directly with iron, showing a clear interplay in the metabolism of the two metals. Other copper enzymes play major roles in the synthesis of neurotransmitters and the establishment of a firm connective tissue protein network. The body load of copper in humans is estimated to be about 80–150 mg. Although deficiencies are rare, genetically impaired functions of copper have caused fatal diseases in humans and animals. Wilson disease and Menkes disease, in particular, result from mismanagement of copper transport and homeostasis. In addition to their medical implications, these diseases have given mechanistic insights into how living systems handle copper. Although recent years have seen a wealth of new insights into the absorption and cytosolic transport, there are still gaps in our understanding, particularly copper's role in chronic diseases and aging.

15.1. HISTORY AND EARLY INSIGHTS

Greeks on the island of Cyprus were the first to discover copper, a discovery that ushered in the bronze age. Its ecological necessity for

life, however, was not recognized until the 19th century, when chlorosis or "green sickness", an anemic condition afflicting mainly adolescent women, was characterized by both low serum iron and copper. The interdependence between the two metals was strengthened in 1928 with the discovery that both iron and copper—not iron alone—was required to fully restore blood hemoglobin levels in rats made severely anemic by iron deprivation. Later, workers found that feeding diets deficient in copper caused young pigs and chicks to suffer aneurysms and ruptures of major blood vessels. The finding resulted in pig and poultry farmers supplementing feed with copper salts, a precautionary practice still in use today. Pursuing the cause of the rupture led to the discovery of a copper-dependent enzyme responsible for cross-linking chains of collagen and elastin that both supported and gave elasticity to connective tissue. Wilson's disease, first described in 1906 by an English physician, was characterized by copious amounts of copper accumulating in the brain and liver. A second disorder, Menkes disease, was not recognized until the early 1960's, and copper (or lack thereof) as the factor that caused the disease became known a decade later. This X-linked disease, fatal to infants, was traced to a defect in an intestinal transport protein essential for copper absorption. Both Wilson and Menkes diseases provided important insights into the metabolism of copper and the pathologies that occur if copper intake or metabolism is disrupted.

15.2. CHEMICAL PROPERTIES

Copper, element number 29, is located between nickel and zinc in the Periodic Table of Elements. With a $3d^{10}4s^{1}$ electronic configuration, elemental copper readily loses one 4*s* electron to give rise to Cu^{+} ($3d^{10}4s^{0}$), an electronic configuration identical to Zn^{2+}. Cu^{2+}, the more common form of copper, is a stable, water-soluble, redox active, metal ion. Proteins engage Cu^{+} and Cu^{2+} ions primarily through nitrogen (histidine) and sulfur (cysteine, methionine) ligands. Copper ion bound to a porphyrin ring is a rare occurrence in biology. More importantly, changing valence states occur without major disruptions to the structure of the binding site on the protein. This allows protein-bound copper ions to readily donate or receive electrons while retaining a firm grip on the protein, a desirable property for a redox active center in a protein. One of the more familiar copper proteins is ceruloplasmin, whose prefix "cerulo" means "heavenly blue" (Chapter 3). As noted, the redox behavior

of copper is consistent with its most familiar property, that of an oxidant of ferrous iron.

15.3. BIOCHEMICAL PROPERTIES

15.3.1. Copper Enzymes

As is typical for microminerals in general, biological copper is bound to protein. Although its role is primarily catalytic, one rare incident worth noting is that mollusks and horseshoe crabs transport O_2 via a porphyrin ring-bound copper in the protein hemocyanin. Copper's primary use in higher animals and humans, however, is catalytic, as typified by copper oxidases and oxygenases that draw on O_2 as a substrate. The copper enzymes are akin to iron-oxidases and oxygenases (Chapter 13), but are more widely distributed in cells and tissues.

15.3.2. Mono- and Multicopper Oxidases

The prefixes mono- and multi- refer to the number of copper atoms bound to the enzyme. Multi-copper oxidases generally have 2 to 4 bound copper atoms and generate water as the reduced oxygen product. Mono-copper oxidase has one copper atom and produces hydrogen peroxide as the reduced product. A multi-copper oxidase with 4 copper atoms thus transfers 4 electrons to O_2, giving rise to two molecules of water as shown in Equation (1). In contrast, mono-copper oxidases with only one copper transfer 2 electrons in a catalytic cycle, resulting in hydrogen peroxide as the reduced oxygen product [Equation (2)].

$$2AH_2 + O_2 + 4e^- \rightarrow 2H_2O + 2Aox \quad (1)$$

$$AH_2 + O_2 + 2e^- \rightarrow H_2O_2 + Aox \quad (2)$$

Multi-copper oxidases are exemplified by ceruloplasmin, which has as many as 7 copper atoms bound to a single polypeptide chain. A notable feature is the presence of a "blue" copper center in the protein. The center gives ceruloplasmin a blue tint that is more obvious when the protein is in pure form. Other enzymes that require copper in order to function are shown in Table 15.1. A brief description of each and its importance to biological systems is given below.

TABLE 15.1. Human Enzymes Dependent on Copper in Order to Function.

Enzyme	Function of Copper
Ceruloplasmin	Oxidation of Fe^{2+} to Fe^{3+}
Hephaestin	Oxidation of Fe^{2+} to Fe^{3+}
Tyrosinase	Oxidation of tyrosine to L-DOPA
Lysyl oxidase	Oxidative deamination of lysyl residues
Cytochrome c oxidase	Transfer of electrons to oxygen
Dopamine β monooxygenase	Synthesis of L-DOPA from dopamine
Cu_2/Zn_2 Superoxide dismutase	Dismutation of O_2^- to H_2O_2
Peptidyl-α amine-monooxidase	Amidation of peptide hormones in the pituitary
Ascorbate oxidase	Vitamin C biosynthesis in plants

15.3.2.1. Ceruloplasmin (Ferroxidase I)

Most of the copper (estimate range up to 95 percent) in serum is bound to ceruloplasmin, a copper-laden glycoprotein. The remaining copper is distributed disproportionately among albumin, amino acids and transcuprein. Four of the 6–7 copper atoms in ceruloplasmin constitute the enzyme's active center and take part in oxidative functions. One such function is the oxidation Fe^{2+} to Fe^{3+} preparatory to binding to the iron-transport protein transferrin (Chapter 13). Because only 4 copper ions are needed to execute the action of a four-electron transfer, the remaining 2 or 3 have no specified function. The non-catalytic copper atoms, therefore, have been theorized to represent copper in transit to the tissues. This view that casts ceruloplasmin in the role of a copper delivery protein has been challenged and is still open for debate.

15.3.2.2. Superoxide Dismutase (SOD-1)

Since ionic copper can readily give and take electrons to and from substrates, another important role is that of an electron acceptor acting as an antioxidant. One of the most prominent antioxidant in cells is the enzyme Cu_2/Zn_2 superoxide dismutase (SOD-I). SOD-1 destroys superoxide anion (O_2^-), giving rise to O_2 and hydrogen peroxide as products. In this two-step reaction, Cu^{2+} first receives a single electron from the substrate O_2^- releasing O_2 as a product but retaining the electron in Cu^+ [Equation (3)]. The electron is then transferred to a second O_2^-, which together with $2H^+$, forms hydrogen peroxide (H_2O_2), the

second product in the reaction. The second reaction also restores Cu^{2+} in the enzyme [Equation (4)]. Equation (5) shows the net effect of the 2 reactions.

$$O_2^- + Cu^{2+} \rightarrow Cu^+ + O_2 \quad (3)$$

$$O_2^- + Cu^+ + 2H^+ \rightarrow H_2O_2 + Cu^{2+} \quad (4)$$

Net effect:

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (5)$$

15.3.2.3. Cytochrome c Oxidase

Arguably one of the most important enzyme in aerobic metabolism, the mitochondrial enzyme *cytochrome c oxidase* has both copper and iron in combination to reduce molecular oxygen to water. ATP is synthesized concomitantly to preserve the energy. The enzyme is present in the inner mitochondrial membrane and is the terminal complex in a chain of complexes designed to convey electrons from NADH to O_2. With the loss of 2 two electrons, the NADH is oxidized to NAD^+ and the electrons are transferred to the cytochrome c oxidase complex, which transfers them to O_2. Repeating this for 4 electrons gives rise to H_2O as the product.

15.3.2.4. Lysyl Oxidase

Lysyl oxidase was identified as the enzyme responsible for post-translational cross-linking of collagen and elastin. The enzyme is found primarily in soft connective tissues and catalyzes a reaction in which select lysine residues in soluble precursor proteins (tropoelastin or tropocollagen) are oxidized to aldehydes. The aldehydes then condense with other peptide-bound aldehydes (aldo condensation) or with unreacted lysines (Schiff base) to unite two chains in a covalent linkage (Figure 15.1). Modifications to the secondary structure of connective tissues, proteins, elastin and collagen are essential to form a sturdy supporting framework characteristic of ligaments and tendons. Moreover, elastin's resilient properties necessitate the protein to have periodic cross-links distributed along the coiled peptide chains to provide anchors for the stretchable chains. Lysyl oxidase is known to be very sensitive to dietary copper. This is especially true in a neonate where blood vessel synthesis is occurring at an accelerated rate.

A severe copper deficiency at this critical growth stage has the potential to cause malformed blood vessels that are prone to aneurysms or rupture, causing instant death.

15.3.2.5. Tyrosinase

Tyrosine metabolism gives rise to melanin, the characteristic pigment of the skin, hair or fur of animals, humans and other species. In forming melanin, tyrosine is first oxidized by the copper enzyme tyrosinase in the melanocytes, giving rise to L-DOPA. The L-DOPA is then converted to dopaquinone and eventually to the biopigments pheomelanin (reddish brown) and eumelanin (dark brown to black). Loss of tyrosinase activity in a copper deficiency results in the loss of a dark skin or hair pigment, characteristic of albinism. Such is noticeable in the dark brown or black fur of animals fed diets lacking copper. Under such conditions, the fur takes on a light brown or gray appearance (Figure 15.2).

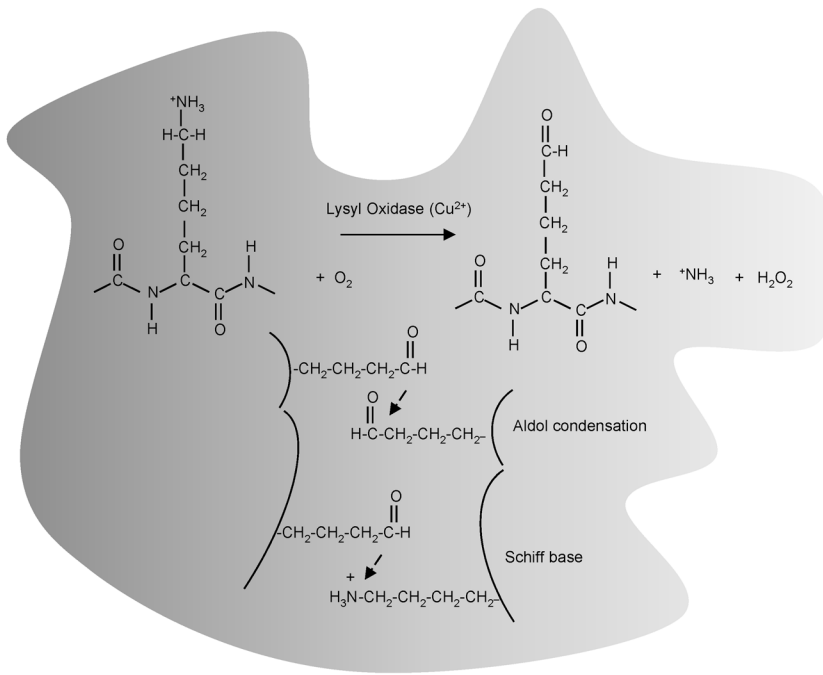

FIGURE 15.1. *Formation of Cross Links Catalyzed by Lysyl Oxidase.*

FIGURE 15.2. *Copper Deficiency in Rabbits: Effect on Pigment and Hair. The above rabbit was raised on a copper adequate diet, the one below on a diet deficient in copper. Achromatricia (loss of color) and alopecia (loss of hair) were both evident in the deficient rabbit. (Kindly donated by T. See, North Carolina State University)*

15.4. NUTRITIONAL PROPERTIES

Estimates of the adult human body load of copper go as high as 150 mg. Copper is distributed disproportionately to all organs with the highest concentration in the liver, kidney and heart muscle. Seafood such as oysters and other shellfish, as well as organ meats and muscle, are particularly rich sources of copper in the diet. So too are whole grain cereals, nuts, leafy green vegetables and white potatoes. Because of its slow turnover, the RDA for adults is set at 0.9 mg, indicating only about one percent of the body load is replaced per day. As noted in Table 15.2, the average intake (AI) for copper in adult humans in about 3–4 mg. This is well within the boundaries of safety since the element has an upper limit of around 10 mg. Infants and children up to the age of 8 years have an AI of about 1 mg per day.

TABLE 15.2. Requirement for Copper in Humans.

Subject	RDA (μg/day)		AI	UL (μg/day)
	Male	Female		
Infants				
0–6 mo			200	
7–12 mo			220	
Children and adolescents				
1–3 yr	340	340		1,000
4–8 yr	440	440		3,000
9–13 yr	700	700		5,000
14–18 yr	890	890		8,000
19–30 yr	900	900		10,000
31–50 yr	900	900		10,000
51–70 yr	900	900		10,000
>70 yr	900	900		10,000
Pregnancy				
<18 yr		1,000		8,000
19–50 yr		1,000		10,000
Lactation				
<18 yr		1,300		8,000
19–50 yr		1,300		10,000

15.4.1. Risk of Deficiency

Even though copper deficiency is a rare occurrence in adults, there are situations where the need to monitor the amount of copper in the diet is warranted. Examples include infants on a strict milk diet which is known to be low in copper (Chapter 5) or who experience prolonged diarrhea or malabsorption associated with celiac disease or short bowel syndrome. Nourishment through total parenteral nutrition or gastric bypass surgery are other concerns. In ruminants, copper deficiency can occur in grazing animals foraging on plants grown in copper poor soil or soils with high levels of molybdenum present as molybdenum-sulfate complexes that bind copper and render it non-absorbable in the intestine and within the system.

15.5. ABSORPTION AND METABOLISM

Copper transport at the cellular level is achieved by an integrated

series of active and passive mediators. Vesicles and soluble peptides assist in moving copper to copper-dependent sites in the cell and excreting or sequestering the copper to prevent build-up. This basic scenario for maintaining cellular copper homeostasis is repeated for all cells, but with some notable exceptions occurring in the liver. A general overview of components taking part in intracellular copper metabolism is shown in Table 15.3. An overview of intestinal copper absorption and metabolism is shown in Figure 15.3.

15.5.1. Intestinal Absorption of Copper

Copper ions utilize mobile copper transport proteins embedded in the cell membrane to enter the enterocyte. These proteins cycle between the outer membrane and internal storage sites. Two transporters, each recognizing a different valence form, take part in the movement. CTR1 recognizes the Cu^{+} form, while DCT1, the divalent transporter previously discussed for iron, may play a secondary role. Of the two, CTR1 carries the bulk of the permeable copper, which in turn necessitates an enzyme to reduce Cu^{2+} to Cu^{+} for timely transmembrane movement.

15.5.2. Intestinal Transport

To deliver copper to the serosal side of the intestine requires the chaperone ATOX1 and ATP7a, the latter a copper ATPase embedded in the surface of vesicles of the trans-Golgi network (TGN). Copper bound to ATOX1 is transferred to ATP7a which, by the energy of ATP hydrolysis, forces the copper ions into the interior of the vesicles. The vesicles themselves are part of the secretory pathway designed to discharge components from the cell's interior. In the act of secreting, the

TABLE 15.3. Biochemical Factors in the Absorption and Metabolism of Copper.

ATP7A	Copper transport into vesicles
ATP7B	Copper transport into vesicles, biliary excretion
CTR1-3	Plasma membrane transporters identified first in yeast membranes and shown to have human counterparts
DCT1/DMT1	Non-specific transporter for divalent Cu^{2+}
CCS	Intracellular chaperone for Cu/Zn superoxide dismutase
Cox17	Intracellular chaperone to mitochondrial cytochrome oxidase
ATOX1	Intracellular chaperone to Cu-ATPases for export

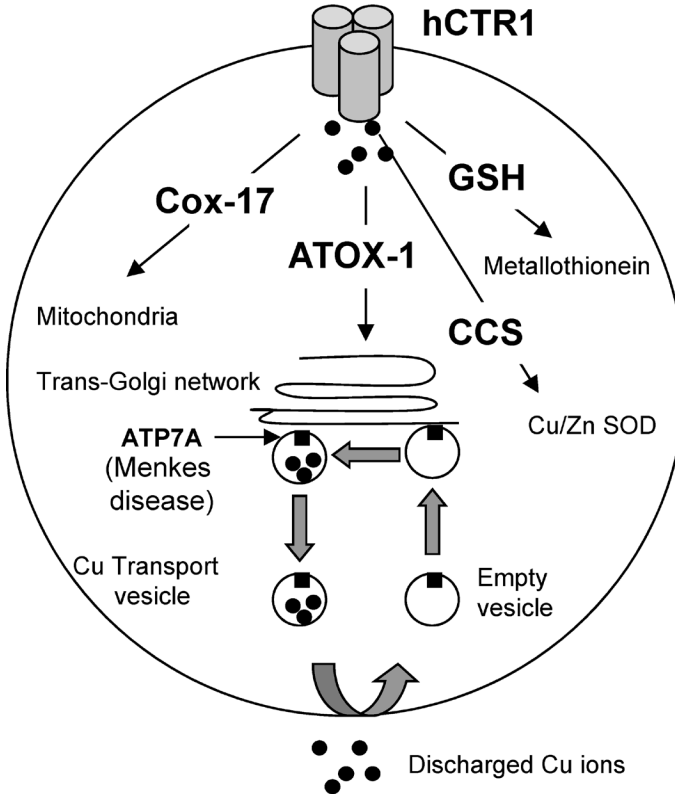

FIGURE 15.3. Intracellular Metabolism and Absorption of Copper in Humans. Shown is the basic mechanism for absorbing copper across the intestine. Cox-17, CCS, ATOX1 are copper chaperones that take the copper to specific locations in the cell. GSH delivers copper to metallothionein for storage. Only ATOX1 takes copper to ATP7a in the trans-Golgi network for discharge on the serosal side, the key event in copper absorption.

copper-laden TGN vesicles move to the basal surface on the serosal side of the intestine and effuse the copper (Figure 15.3). The ATP7a pathway is the only means by which intestinal copper ions can exit the enterocyte and enter the system. Irrefutable evidence for ATP7a's essential role comes from studies that have linked ATP7a mutations with Menkes disease, a disease characterized by a severe copper deficiency brought about by defective intestinal transport.

15.5.3. The Role of the Liver in Copper Metabolism

The liver is the main organ for controlling internal copper homeostasis. In achieving homeostasis the liver performs two major functions:

distribution of copper to the peripheral organs and excretion of copper into the bile. Both events maintain a steady-state level of copper not only in the liver but throughout the system. As a distribution center for copper, the liver releases ceruloplasmin (Cp) with its bound copper into the plasma or excretes copper into the bile which eventually is excreted in the feces. Once released into the bile, the copper enters the duodenum in a form that cannot be reabsorbed back into the system. Figure 15.4 shows how these events combine to help maintain safe levels of copper within the liver and the system in general. The copper chaperone ATOX1 and the ATPase ATP7B, a Cu-ATPase akin to ATP7A, perform the internal transfer to the bile. ATP7B also is accountable for incorporating copper into ceruloplasmin. The ATP7B is defective in Wilson disease, which is characterized by high levels of copper in the liver and brain and low levels of copper in ceruloplasmin. The strong buildup in the liver is testament to diminished copper excretion in the bile.

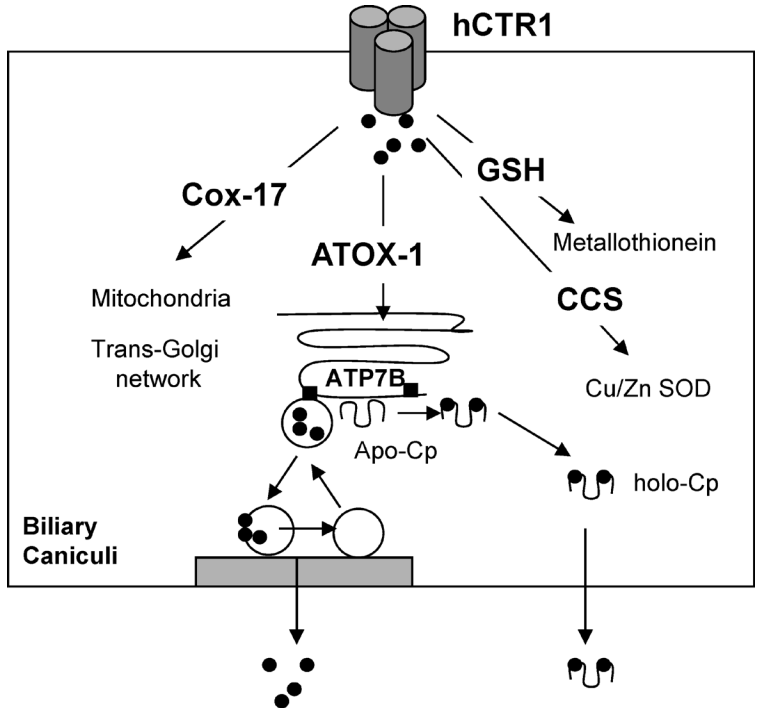

FIGURE 15.4. *Metabolism of Cu in the liver. The liver maintains safe levels of copper in the system by (1) transferring copper ions to vesicles for excretion in the bile, and (2) incorporating copper into apo-ceruloplasmin (Apo-Cp) for release into the blood. Both transfers require ATP7B, an enzyme defective in Wilson Disease.*

15.6. TRANSPORT AND DELIVERY TO CELLS

Copper-laden proteins released from the liver are positioned to transfer their copper load to cells. There is still considerable doubt as to how this is accomplished because the principle plasma transport protein for copper, if such exists, has not been identified. Evidence pointing to ceruloplasmin as a delivery protein cites the presence of receptors for ceruloplasmin on cell membranes; however, evidence connecting copper delivery with membrane receptors is lacking. Radioactive ^{67}Cu bound to ceruloplasmin, however, has been shown to exchange with Cu in the cell. Further studies have shown ceruloplasmin protein does not enter the cell, implying copper must be released at the membrane site. Ascorbate (vitamin C) has been shown to prime copper for release, implying converting Cu^{2+} to Cu^{+} may be requisite for its uptake into the cells via Ctr1. Cu^{+} is thus accessible to Ctr1 on the plasma membrane. The second candidate for a copper delivery protein is serum albumin. This protein has a binding site for the copper on the N-terminus of the protein. The firmness of the binding, however, works against a ready release of copper to cells. Finally, early studies noted that a select number of amino acids and small peptides in the plasma are likely to take part in assisting copper movement across cell membranes. Which of these three candidates is the transport/delivery form of copper is still to be determined.

15.7. INTRACELLULAR METABOLISM

Events occurring within the intestinal cell and the liver tend to be repeated in peripheral cells targeted for receipt of the copper. Once inside a cell, Cu^{+} has the option of moving to four different pathways (Figure 15.3). The decision of which pathway to take is met through chaperones that steer the copper to select sites in the cell. The chaperone Cox17 brings copper into the mitochondria where it engages the protein cytochrome c oxidase. Binding to the chaperone CCS (copper chaperone for superoxide dismutase) transfers copper to the enzyme superoxide dismutase. Movement to ATOX1 allows copper to enter the secretory pathway governed by ATP7a and ATP7b. As noted earlier, ATP7a is part of the pathway that discharges copper from intestinal cells and the only pathway that controls the level of copper entering the system. ATP7b appears to work primarily in the liver; ATP7a is absent in the liver. Finally, in mammalian cells, the tripeptide glutathione, which is known

to bind copper although it lacks the properties of a chaperone, has been shown to be a precursor of copper binding to metallothionein, implying the peptide delivers copper to the protein.

15.8. COPPER-IRON INTERACTIONS IN COPPER METABOLISM

Recall the two biological forms of iron are Fe^{2+} and Fe^{3+}. Cells are selective in recognizing only Fe^{3+} which is transported into the cell via transferrin (Chapter 13). Iron escaping from a cell must be Fe^{2+} if it is to be recognized by ferroportin. This shifting between valence states is the best way to visualize the manner in which copper regulates iron metabolism. Two copper proteins, ceruloplasmin and hephaestin, catalyze the oxidation of Fe^{2+} to Fe^{3+}. In the reaction, 4 electrons are transferred to O_2, resulting in the formation of water and 4 ferric ions [Equation (6)].

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (6)$$

As multi-copper oxidases, ceruloplasmin and hephaestin avoid the more dangerous H_2O_2 as a byproduct, but more importantly are requisite to form an iron-transferrin complex. Not allowing iron to bind to transferrin restricts iron transfer and incorporation into hemoglobin, which provides a rationale for why inadequate copper in the diet can mimic an iron deficiency.

15.9. ASSESSING COPPER ADEQUACY

15.9.1. Symptoms

Table 15.4 list some of the symptoms associated with a copper deficiency in humans and animals. Substandard functioning of a copper-dependent enzyme is at the root of many overt symptoms. Although anemia is listed, cases of anemia by dietary copper deficiency are rare. A postnatal ataxia (sway back) in newborn lambs has been traced to ewes foraging on copper-deficient plants. Ataxia is caused by a major disruption of the integrated circuits in the cerebellum that control movement and coordination. The myeloneuropathy reflects changes in peripheral nerves that can have repercussions throughout the system.

TABLE 15.4. Symptoms of Copper Deficiency.

Anemia
Spasticity
Ataxia
Myeloneuropathy
Leukopenia
Optic neuritis
Demyelination
Blood vessel aneurysms

Defects in connective tissue which can result in aneurysms and bone weakness represent connective tissue malformations that evince as a general loss of tensile strength in tendons and the elastic property of major blood vessels.

15.9.2. Biomarkers for Assessing Copper Status

Several copper-dependent proteins lose catalytic function when dietary copper is restricted. Measuring ceruloplasmin levels in the blood is the most common clinical procedure for humans. The ferroxidase activity of the protein is the basis for the analysis, and because ceruloplasmin makes up nearly all the copper in plasma, there is some assurance that the quantities obtained are a valid indicator of copper status. Experimental studies with animals have also used cytochrome c oxidase or superoxide dismutase activity as markers. These proteins draw favorable attention because, unlike ceruloplasmin, they are not subject to variations caused by stress or hormonal influences. Copper in hair follicles is used to gauge copper intake over long periods of time.

TABLE 15.5. Genetic Diseases in Humans Associated with Copper.

Marfan's Syndrome	Low lysyl oxidase?
Aortic aneurysm	Low lysyl oxidase
Down's Syndrome	Excessive Cu, Zn Superoxide dismutase production
Albinism	Lower tyrosinase activity
Mitochondria myopathy	Lower cytochrome c oxidase
Cutis Laxa	Lower lysyl oxidase?
Ehlers-Danlos Syndrome	Lower lysyl oxidase?
Wilson Disease	Defective copper transporter ATP7B
Menkes Disease	Defective copper transporter ATP7A

15.10. COPPER'S LINK TO GENETIC DISEASES

15.10.1. Marfan's Syndrome

Marfan's syndrome is a genetic disorder traceable to a defect in the gene that encodes fibrillin, a glycoprotein that is part of the extracellular connective tissue matrix. Its link to copper is through the copper enzyme lysyl oxidase. A key to this conclusion is based on a substantial decrease in the desmosine content (as much as 50%) in Marfan's patients (Perejda *et al.*, 1985). The tensile strength of the elastin relies on desmosine crosslinks, which in turn are derived from the oxidative deamination of the protein precursor (pro-elastin) through the action of lysyl oxidase. The defect thus appears to be related to elastinogenesis at a pre-crosslinking stage.

15.10.2. Aortic Aneurysm

In humans, it is estimated that about 15,000 new cases of aortic aneurysm are reported each year and nearly 50,000 people die from the condition. The disease is characterized by a weakening of the abdominal aorta in the region of the heart. The weakened structure causes a bulge to appear. Such blood vessel weakening is reminiscent of disorders observed in experimental animals fed diets deficient in copper, which shuts down lysyl oxidase. Although the link between the enzyme and blood vessel weakening is firmly established in experimental animals, the human condition and other types of aneurysms in a seemingly healthy person have yet to be connected to a dietary deficiency of copper.

15.10.3. Ehlers-Danlos Syndrome (EDS)

Two doctors, Edward Ehlers of Denmark and Henri-Alexandre Danlos of France, identified this disease at the turn of the 20th century. EDS is characterized by a weakening in the fibrous property of ligaments caused by a lower content of Type III collagen. Lacking the support, the tendons and ligaments are more prone to have hypermobility. Over the years it has become clear that EDS could have as many as six different types. In the past, copper was thought to be more directly related to the condition. Recent data, however, seems to rule out a copper-related deficiency, toxicity, or genetic defect as the cause.

15.10.4. X-linked Cutis Laxa

X-linked cutis laxa is a rare disorder characterized by a pronounced skin laxity with pulmonary and cardiovascular complications. The disease resembles Ehlers-Danlos Syndrome in having weak underlying support, but unlike EDS, the skin show no elastic rebound when stretched. Elastin biosynthesis is implicated in the symptoms possibly at the stage of mRNA synthesis. The genetic basis may have little involvement with copper.

15.10.5. Menkes Disease

Symptoms of Menkes disease are seen in neonatal males; the disease is X-linked and reaches a fatal course before the third year of life. First described by John Menkes in 1960 who referred to the disease as an X-linked neuropathy with a poor prognosis and kinky or steely hair as the most prominent feature. In 1971, David Danks in Australia showed that the angiograms from a Menkes patient had the same twisted tortuous blood vessels of rats fed copper-deficient diets. This diagnosis was confirmed when a child suspected of having the disease was unable to absorb copper. The kinky or steely hair is referred to as "pili torti" and results in a brittle, stiff hair shaft (Figure 15.5). The hair has also lost pigment and takes on a somewhat reddish appearance. All these symptoms reflect a severe copper deficiency. The cause of the disease is known to be a mutation in the ATP7a gene.

15.10.6. Wilson Disease

Whereas Menkes disease is primarily a copper deficiency, Wilson disease is a copper toxicity. The disease was first described as an autosomal recessive disorder characterized by a strong buildup of copper in the liver and brain. Because the excessive copper was toxic and could erode tissues, the disease was referred to as "hepatolenticular degeneration", reflecting the two major organs affected. Patients with the disease had no trouble absorbing copper; rather, the concern was absorbing too much and failing to manage copper homeostasis within the liver. Release of copper into the bile was blocked, as was the incorporation of copper into ceruloplasmin. As a result, the liver and brain accumulated massive amounts of copper, resulting in disruptions to nervous system and liver functions. Treatment of Wilson disease involves restricting

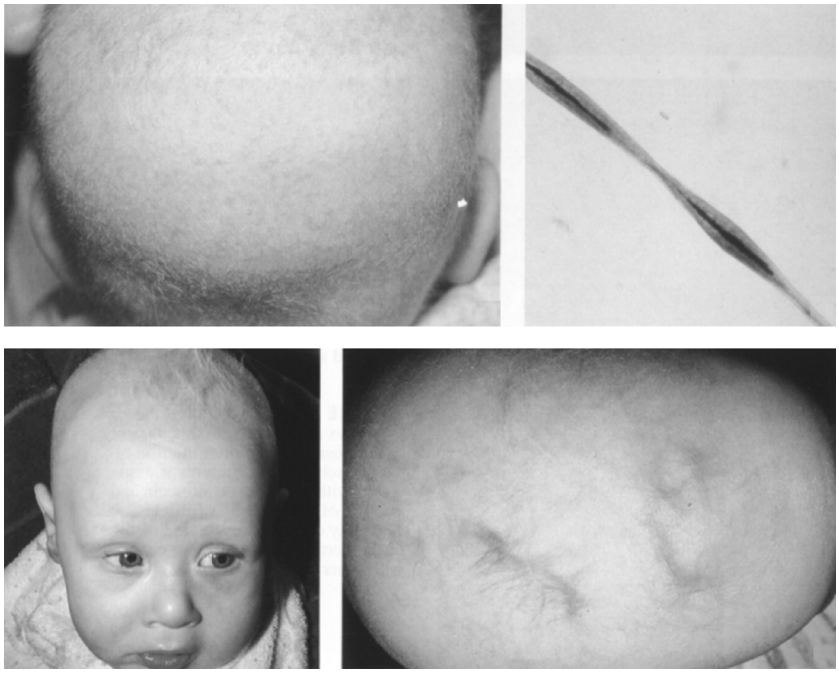

FIGURE 15.5. *Menkes Disease. Typical symptoms of the disease include a reddish brown color of hair, high cheek bones, a somewhat distorted cranium, and brittle hair fibers showing a twisted appearance (upper right).*

the amount of copper entering the system and redistributing the copper compartmentalized within the organs. High levels of zinc given as a twice-daily supplement tends to slow gut absorption. As noted previously, the cause of Wilson disease has been traced to a defect in ATP7b, the Cu-ATPase in the liver responsible for transferring copper to ceruloplasmin and releasing copper in the bile.

15.11. SUMMARY

The first hint of copper's essentiality in a biological system came when the metal was linked to the metabolism of iron. This may be secondary to copper's other purpose in biology, which is the safe utilization of oxygen. Enzymes that use oxygen as a substrate or insert oxygen into products of metabolism are testament to a need for copper. With a RDA bordering on less than a milligram per day, the small amount of copper in the diet challenges the systems to handle infinitesimally small

amounts and yet maintain copper homeostasis. Proteins that bind and transport copper are key factors in meeting its nutritional challenge. Fatal diseases such as Menkes disease and Wilson disease provide invaluable insights into what can go wrong if copper metabolism is impaired. A deficiency in copper can affect all organs of the body; a severe deficiency can have fatal consequences. Pronounced defects are noted in the cardiovascular system, where copper is needed to form the architecture of blood vessels—specifically, giving major arteries elasticity and resilience. In the brain, copper plays a major role in the synthesis of neurotransmitters and is responsible for the pigmentation of skin and fur. Of equal concern are diets subadequate in copper that can compromise connective tissue stability, antioxidant protection and pituitary functioning in animals and humans. Identifying the factors that are affected by deficiencies gives insight into how copper is handled, as well as clues as to its role in specific metabolic pathways.

15.12. REFERENCES

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15.13. PROBLEMS

1. Name two enzymes whose activity can be impaired by a low intake of copper. What symptoms will be manifested if these enzymes have diminished activity?

2. Why is it necessary to reduce copper to its cuprous form in order to be absorbed?

3. Ascorbic acid (vitamin C) is both a detriment and a benefactor of copper metabolism. Its beneficial effects occur at the level of in-

testinal absorption. Its detrimental effects occur within the system. Explain the rationale for this dual effect of ascorbate.

4. What is the hallmark of an oxidase enzyme? The question is asking what is unique about oxidases that is not found in other enzymes. Could Zn^{2+} be used as a catalytic cofactor for an oxidase enzyme? Explain and justify your answer.

5. The amount of copper in cow's milk is about 0.1 mg/liter (Chapter 5). For human milk, the level is about 0.3 mg/liter. Based on these figures, how much cow's milk must a one-year-old consume in order to meet the RDA value for copper? Repeat the calculation for human milk.

6. Suppose you are a sheep farmer in Western Australia who owns a herd of midnight black Murino sheep. In order to protect your sheep from dingos (wild dogs), you alternate between the east paddock and the west paddock two months at a time. This gives maximum grazing protection. When your sheep are ready to be sheared and the wool harvested, the sheep shearer tells you to come look at your sheep. He says "Aye mate, tis a zebra jumpbuck for sure" (jumpbuck is Australian slang for sheep). What did he mean and what caused the symptoms you observed?

CHAPTER 16

Manganese

ITS scarcity in living systems has made manganese one of the lesser known and poorly studied essential minerals. Indeed, manganese is better acknowledged as a neurotoxin than an essential nutrient. Manganese is known to be important for bone and connective tissue development and plays a catalytic role in carbohydrate, amino acid and lipid metabolism. Its role as a cofactor in the mitochondrial antioxidant enzyme *superoxide dismutase* and oxygen evolving *water splitting enzyme* in chloroplasts is testament to the variety of Mn-dependent functions in the biosphere. Quite simply, if it were not for manganese, atmospheric oxygen would not be possible. A manganese deficiency seldom occurs in humans, but elevated intake is always a concern. Excess exposure of Mn-containing particles in the environment can give rise to manganism, a disorder that disrupts normal brain functions. In this chapter, the emphasis with be on manganese's role in health as opposed to life-threatening disorders, although the latter will be alluded to in order to give the full scope of functions attributed to this intriguing mineral.

16.1. HISTORY AND EARLY INSIGHTS

The name manganese is derived from the Greek "manganesum or magnes", referring to black minerals from Magnesia that did not attract iron. Carl Scheele in 1774 first proposed manganese as a new chemical element, but Johan Gahn, a Swedish chemist, is generally acknowledged

with the discovery. It took a series of rather imaginative approaches by a number of investigators to demonstrate manganese's essentiality to life, however. Hart and Elvehjem at Wisconsin observed that a milk diet with adequate iron and copper did not correct infertility in rodents, but adding manganese with the other minerals did. Calcium and phosphorus were later shown to be of no value in treating perosis, a bone deformity in chickens, but manganese added with the other minerals prevented the deformity. In 1837, James Couper showed that humans exposed to high amounts of manganese oxide developed Parkinsonian-like symptoms. Later, manganese accumulating in the brain by repetitive inhaling of dust particles was shown to cause violent psychotic behavior in occupational workers. These observations were instrumental in establishing manganese as essential yet dangerous to health, which is basically how manganese is perceived today.

16.2. CHEMICAL PROPERTIES

Manganese is the twelfth most abundant element in the earth's crust and occupies the 25th position in the Periodic Table of Elements. Its electron configuration [Ar]$4s^2 3d^5$ is conducive to valence states between –3 to +7; Mn^{2+} and Mn^{3+}, however, are the more dominant biological forms of the metal. As noted by da Silva and Williams, the $3d^5$ shell in Mn^{2+} is half-filled, allowing Mn^{2+} to behave more like a spherical ion with coordination properties similar to Mg^{2+} and Ca^{2+}. Moreover, like calcium, manganese can form seven coordinate complexes. This makes it reasonable to suspect Mn^{2+} can substitute for or antagonize Ca^{2+} or Mg^{2+} in biological systems. Another potential interaction arises with iron. Both Fe^{3+} and Mn^{2+} have identical [Ar]$3d^5$ electronic configurations, which forecast a potential competition between both metals for binding sites on transferrin and major disruption of iron metabolism.

16.3. BIOCHEMICAL PROPERTIES

Although relatively few in number, enzymes that require manganese are responsible for a number of irreplaceable biochemical reactions in cells. As noted in Table 16.1, there appears to be no set pattern to the functionality of these enzymes. The multi-valence structure of the manganese ion is perhaps a key to this diversity. Two crucial enzymes to

TABLE 16.1. Manganometalloenzymes and their Reactions.

Enzyme	Function of Copper
Arginase	Urea and nitrous oxide synthesis
Glutamine synthetase	Nitrogen assimilation
Pyruvate carboxylase	Gluconeogenesis
Phosphoenolpyruvate decarboxylase	Carbohydrate metabolism
Manganese superoxide dismutase	Antioxidant enzyme in mitochondria
Glycosyl-xylosyltranferases	Proteoglycan synthesis and bone formation

note are glutamine synthetase, which is responsible for assimilating nitrogen into organic compounds, and manganese superoxide dismutase, an enzyme in the mitochondrial matrix that destroys the superoxide anion and protects mitochondria from free radical damage.

16.4. NUTRITION

16.4.1. Food and Environmental Sources of Manganese

Plant foods provide the richest sources of manganese in the average diet, mainly whole grains, green leafy vegetables, nuts and tea. According to the USDA Food Composition Database, plant foods have between 0.4–1.9 mg of manganese per serving. Drinking water can also be a significant source of manganese. Water sources, however, tend not be uniform in manganese content and depend on location. For example, large city reservoirs may contain anywhere between 1–100 micrograms/L whereas rural locales report around 10 micrograms/L. Air contamination has been another source of manganese intake. Inhaled fumes from aerosols or gasoline additives contribute an estimated 2 micrograms of manganese per day.

16.4.2. Intake and Adequacy

Replies to questionnaires suggest that an adult human averages less than 5 mg of manganese per day with a ranges of between 0.7–10.9 mg. Current FDA listings do not give an RDA for manganese. Instead, DRI values for Adequate Intake (AI) and Upper Limit (UL) are reported. Their dependence on developmental stage, gender and age is shown in Table 16.2.

16.4.3. Assessing Manganese Adequacy and Toxicity

Because there are no reliable biomarkers for manganese, the values reported in Table 16.2 are based on healthy individuals who have shown no overt clinical signs of deficiency or excess. Determining manganese adequacy in the general public has been a vexing problem and is of special concern for patients undergoing long term parenteral nutrition. Measurements of manganese concentration in whole blood and plasma has tended to be highly variable, and measuring Mn-superoxide dismutase activity in tissues, although more sensitive, is discouraged because of a high variability in the tissues examined (Figure 16.1).

Assessing the upper limit (UL) for manganese intake likewise faces challenges. Because Mn^{2+} is paramagnetic and tends to accumulate in the brain, MRIs have been used to diagnose manganism, a neurotoxicity caused by the accumulation of manganese in the basal ganglia. The test is especially valid if MRI intensity in several brain regions correlates with high blood levels of manganese. Like other minerals, the symptoms of manganese neurotoxicity are shared with other factors, which make preclinical diagnosis less than a reliable undertaking.

TABLE 16.2. Adequate Intake and Upper Limit Values for Manganese.

Age	Male (mg/day)	Female (mg/day)	Upper Limit
0–6 mo	0.003	0.003	Not determined
7–12 mo	0.6	0.6	Not determined
1–3 yr	1.2	1.2	2
4–8 yr	1.5	1.5	3
9–13 yr	1.9	1.6	6
14–18 yr	2.2	1.6	9
19–30 yr	2.3	1.8	11
31–50 yr	2.3	1.8	11
51–70 yr	2.3	1.8	11
>70 yr	2.3	1.8	11
Pregnancy			
<18 yr		2.0	9
19–50 yr		2.0	11
Lactation			
<18 yr		2.6	9
19–50 yr		2.6	11

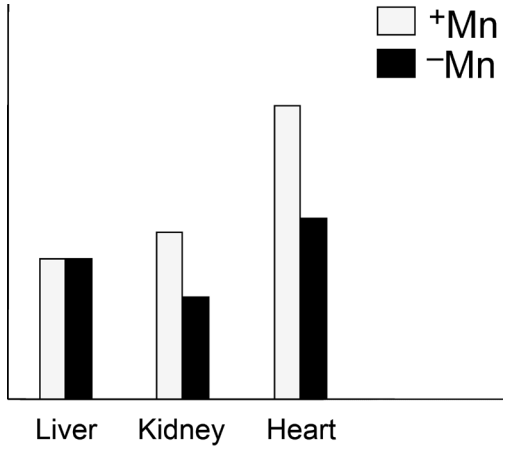

FIGURE 16.1. *Suppression of Mn-Superoxide Dismutase Activity by Feeding Rats a Diet Low in Manganese.*

16.5. DIGESTION AND ABSORPTION

Manganese absorption is generally less than 10% of the manganese in the food source. This will vary with age. Neonates, for example, are more efficient in absorbing manganese, possibly reflecting an enhanced requirement in early development. Women, for reasons yet not clear, have a higher absorption efficiency than men, which is the rationale in Table 16.2 for the AI for adult women being about one-quarter less than men. Compared to a gastric route, manganese intake by parenteral nutrition is 100% bioavailable. A high bioavailability heightens concern for a manganese neurotoxicity which is always a possibility when manganese enters the system by a non-oral route.

16.5.1. Absorption Efficiency

Many factors impinge negatively on the absorption efficiency of manganese. Perhaps foremost are competing divalent metal ions such as Ca^{2+}, Mg^{2+}, and Zn^{2+}. Competition is especially critical if manganese in the food source is marginal. As noted earlier, the identical [Ar]3d^5 electronic configurations for Mn^{2+} and Fe^{3+} is the rationale for a lower percentage of Mn^{2+} entering the system when ferric ion is high. In contrast, a low iron diet could increase the fraction of manganese absorbed and, by raising the level of manganese bound to transferrin, enter the brain and other tissues via a receptor mediated mechanism.

16.5.2. Absorption Mechanism

Details as to the mechanism for absorbing manganese across the intestine are not complete. Because divalent cations interfere with absorption, there is reason to suspect that DCT1, the divalent cation transporter, controls most of the Mn^{2+} that crosses the apical surface. Other possible candidates exploiting similarities in valence are voltage-gated channels such as the Na^{+}/Ca^{2+} exchanger, the Na^{+}/Mg^{2+} antiporter and a mitochondrial active Ca^{2+} uniporter. The zinc transporter Zip8 has also been postulated to play a role in Mn^{2+} uptake into cells. With all these possible membrane transporter candidates, there may not be a definitive transporter selective only for Mn^{2+} or such has yet to be identified.

16.6. POST-ABSORPTION TRANSPORT

16.6.1. Manganese Transport in the Blood

Nearly all the manganese in the blood is present as a complex. An estimated 40 percent is bound to transferrin and the remaining manganese is distributed among albumin, citrate and carbonate.

16.6.2. Tissue Turnover and Excretion

Manganese is distributed homogeneously throughout the body. Mitochondria and tissues with a higher pigmentation tend to have greater amounts. Figure 16.2 shows manganese distribution in rat organs tested two different times after being injected with ^{54}Mn, a radioactive isotope. Twice measuring the ^{54}Mn gives insight into manganese turnover, specifically how rapidly manganese tissue levels change. The liver assimilated the most ^{54}Mn but showed little change in the amount with time, indicative of a rapid uptake and slow turnover. In contrast, the kidney—although slower to accumulate—retained the greatest amount of manganese. The spleen showed a slow rate of accumulation and a turnover similar to the liver. Excretion of manganese occurs through the liver, which retains a small labile pool that conjugates with components in the bile. Manganese released into the bile enters the intestine and is excreted from the system through the feces. Failure to excrete manganese effectively, such as in patients with chronic liver disease, risks

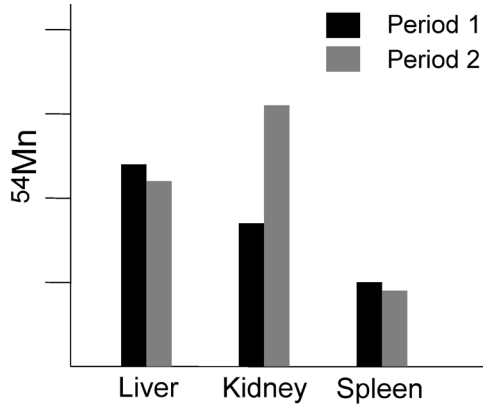

FIGURE 16.2. *Turnover of Manganese in Rat Tissues. Rats were tested at two different times for changes in manganese in tissues following injections of ^{54}Mn.*

raising blood manganese concentrations, which can lead to an incipient neurotoxicity developing in the brain.

16.7. MANGANESE DEFICIENCY

Depriving animals or humans of manganese for long periods puts them at risk of developing the symptoms shown in Table 16.3. From the list, one may surmise that a deficiency in manganese translates into disrupted glucose uptake and metabolism, altered cholesterol metabolism, and reproductive failure, as well as a compromised antioxidant system in the mitochondria. One of the more notable effects, however, would be to the effects manifested in skeleton and bone in general. Young livestock grazing on forage grown in Mn-deficient soil suffer major bone and skeletal defects (Figure 16.3). A similar type of bone disorder has been seen in Mn-deficient poultry.

TABLE 16.3. Symptoms of Manganese Deficiency in Animals.

Impaired growth
Skeletal defects
Reduced reproductive function
Altered lipid and carbohydrate metabolism
Reduced levels of HDL
Diabetic-like glucose tolerance curves
Suppressed levels of insulin receptors

Although a rare occurrence in humans, clinical cases of manganese deficiency have shown some surprising results. In one of the earliest reports, exposure of human subjects to a formulated diet that provided 0.34 mg of manganese per day—literally only 15 percent of the average intake—caused a reddening of the hair, scaly skin, lower cholesterol and weight loss in the subjects. In an earlier study reported by Friedman and coworkers, 5 out of 7 male subjects who consumed < 0.1 mg of manganese for 39 days developed a mild dermatitis but showed no significant changes in serum manganese levels (Friedman *et al.*, 1987) . This rather unexpected finding has several important implications. First, adult humans who are exposed to a severe manganese deficiency for a prolonged period can be expected to have major alterations in skin, hair, and internal organs, and second, that manganese intake has little effect on blood manganese levels. The latter challenges blood manganese as a valid biomarker of manganese status, but more importantly, provides evidence for a well-controlled homeostatic mechanism for retaining manganese in the system in the face of severe deprivation.

FIGURE 16.3. *Manganese Deficiency in a Calf. The skeletal defects shown result from reduced levels of glycosyltransferases or prolidase, two manganese-requiring enzymes that play major roles in connective tissue metabolism and prevention of bone malformation.*

16.8. NUTRITIONALLY RELEVANT MANGANESE TOXICITY

Toxic effects attributed to manganese overexposure extend beyond environmental health hazards. There are, however, more subtle areas where manganese exposure may be by accident or unsuspected when performing traditional nutritional procedures.

16.8.1. Parenteral Nutrition

Due to the 100 percent bioavailability and low excretion, the risk of developing manganism-like symptoms is elevated substantially in patients undergoing total parental nutrition (TPN). Data to support this hypothesis is firm but still incomplete, however. What is known is the symptoms of manganese neurotoxicity depend on the dose, age of the subject, and TPN duration; children and young adults are more susceptible than older adults.

16.8.2. Neonatal Nutrition

The amount of manganese in human milk is estimated to be between 1.8–27.5 μg/L while infant formulas may contain between 33–300 μg/L. Infants receiving formula, therefore, have greater exposure and since young children absorbed more manganese than adults, the risk of neurotoxicity is enhanced. Erikson *et al.* have opined that excessive levels of manganese in a neonate, if left uncorrected, would damage a different area of the brain than adult manganism and could lead to oxidative stress and other neural problems.

16.9. SUMMARY

Manganese is known for its nutritional essentiality as well as toxicity. A deficiency or a neurotoxicity of manganese seldom occurs under normal nutritional or physiological conditions. Manganese deficiencies have been linked with impairments, mainly at developmental stages. Deficiency-related bone defects have been seen in young livestock and poultry. A reddening of the hair, scaly skin, weight loss and dermatitis has been reported in humans who received marginal levels of manganese in experimental diets. In contrast, excessive environmental expo-

sure of manganese over time can induce psychotic behavior in humans. Nearly all functions of manganese in living systems exploit its multiple valency and redox properties. However, there are other factors to consider. A close resemblance electronically to the chemically stable Mg^{2+} and Ca^{2+} offers the potential for Mn^{2+} to substitute for these two macrominerals in biological compounds. Electronic similarity also forecasts Mn^{2+} and Fe^{3+} to be antagonistic towards one another. Substitution and antagonism may be manifested at levels of intestinal absorption, transport and uptake by cells. Biomarkers for diagnosing manganese status of humans and animals currently lack precision, specificity and reliability.

16.10. REFERENCES

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16.11. PROBLEMS

1. Manganese has been likened to iron. Does that mean manganese can form a heme-like structure with porphyrin?

2. Manganese ions (Mn^{2+}) resemble magnesium ions (Mg^{2+}). Predict the consequences to the environment as we know it if, instead of binding Mg^{2+}, chlorophyll bound Mn^{2+}. Now consider the consequences of substituting Mg^{2+} for Mn^{2+} in the water splitting enzyme of chloroplasts. Based on these important functions privy to each ion, do you think substituting Mg^{2+} for Mn^{2+} in biological systems will permit normal functions to continue?

3. Is a person with a liver disorder also vulnerable to develop a psychosis? Explain how this can happen.

4. What evidence justifies considering Mn^{2+} an antioxidant?

5. When making a judgment as to the importance of manganese in the

biosphere, there are some who believe that in its absence the level of oxygen in the air would gradually fall. What justifies making such a bold prediction?

6. A consumer who is easily confused by similar names accidentally puts tablets of manganese sulfate instead of Epsom Salt (Chapter 12) in a bath. Is this person is serious trouble? Explain.

7. As a practicing dietitian, you are approached by a friend who you suspect is suffering from a manganese deficiency. You find out later that your friend, on the advice of her mother, is regularly taking large supplements of iron tablets to correct her anemia. Answer the following:
   a. What symptoms caused you to suspect your friend was manganese deficient?
   b. When your friend assures you that she is eating foods rich in manganese, what is she eating and what is she overlooking and needs to know?
   c. What do you tell your friend if she thinks taking more manganese tablets will correct the problem?

CHAPTER 17

Selenium and Sulfur

As a biological entity, selenium is best acknowledged for its protective action against oxidative damage to tissues. A relatively low reducing potential has been seen as favoring destruction of metabolically generated peroxides that are known to harm sensitive organic molecules. Selenium's biological activity is expressed through a wide variety of chemical compounds, not all of which function as antioxidants. The enzyme glutathione peroxidase with selenium as selenocysteine best illustrates selenium as an antioxidant. While plants have no perceived selenium requirement, plants provide much of the selenium in the diet. An adequate intake of selenium is essential for reproductive, cognitive, immunologic and hormonal functions and can be life-threatening when deficient. Selenium in excess can have pro-oxidant properties that can devastate biological systems. In this chapter, our focus will be on beneficial effects of selenium and the way selenium from foods is incorporated into living molecules that are responsible for the spectra of catalytic functions associated with its actions.

17.1. HISTORY AND EARLY INSIGHTS

The Swedish chemist Berzelius (1817) named selenium for a nonmetallic element that co-mixed with sulfur compounds. The name typified the practice of naming elements after Greek gods and goddesses: helium (Helius) for the sun, tellurium (Tellurus) for earth and now selenium (Selene) for the moon. At first, selenium's presence in foods was

regarded as toxic. Its essentiality was slow to be realized until Schwarz and Foltz discovered that a yeast source practically void of selenium caused a severe liver necrosis in rats. Rotruck and colleagues later connected selenium to the antioxidant enzyme glutathione peroxidase and in time Ganther identified selenocysteine as the active site selenium factor in the enzyme. These observations led to the realization that biological selenium was not only essential, but its biochemistry was uniquely different from its sulfur analog. A broad base of chemists, biologists and nutritionists now recognize this element's exceptional properties and importance to life.

17.2. CHEMICAL PROPERTIES

Selenium is classified as a metalloid, i.e., a non-metallic element with the properties of a metal. Because its chemical properties closely emulate sulfur, selenium and sulfur chemistry and biochemistry are practically identical with one exception—only selenous enzymes function as antioxidants (peroxidases). Both are Group VI non-metals that share the same multiple valence states: selenate (sulfate), selenite (sulfite) and selenide (sulfide). Selenocysteine (cysteine), selenomethionine (methionine) and Se-methyl-selenocysteine (S-methyl-cysteine) are the major organic selenocompounds (Figure 17.1). The nearly identical atomic radii allow selenium to substitute for sulfur in amino acids and other complexes. As pointed out by Sunde, the difference in reduction potentials of selenous and selenic acid as compared to their sulfur analogs could explain why biological selenium behaves as an antioxidant and sulfur does not.

17.3. BIOCHEMICAL PROPERTIES

17.3.1. Selenium in Plant and Animal Systems

Biological selenocompounds can be both organic and inorganic. Only the organic, however, are active and function in an antioxidant capacity. As seen in Figure 17.2, cells have the capacity to convert selenate and selenite into the organic selenocysteine (SeCys) and incorporate the latter into a protein. Selenocysteine and selenomethionine in foods also have the capability of being incorporated into the newly assembled proteins.

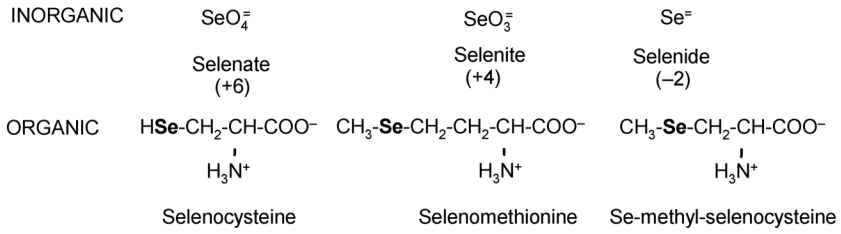

FIGURE 17.1. *Selenium Compounds Common to Biological Systems.*

Although both selenomethionine and selenocysteine are the dominant biological forms, their distribution in plants and animals is not the same (Table 17.1). Selenomethionine, for example, comprises about two thirds of the selenium content of wheat, corn, and rice, but only one third of the selenium in animal products. In contrast, one-half to two-thirds of the selenium in animal protein is selenocysteine. This inequality in distribution is due in part to (1) plants having a greater capacity to synthesize selenomethionine de novo, and (2) selenomethionine undergoing a rapid conversion to selenocysteine in animal systems (Whanger, 2002). Table 17.1 shows that 5 days after rats were injected with radioactive selenomethionine, nearly half of the radioactivity was recovered in selenocysteine. In plants, the same experiment yielded less than 20% of the dose in selenocyteine. Organ preference for selenium in terms of relative abundance is: kidney > liver > endocrine glands > pancreas > heart > spleen > brain.

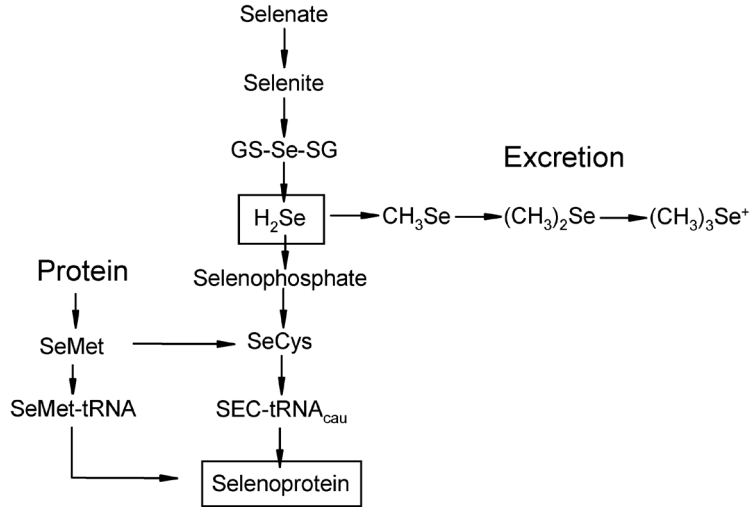

FIGURE 17.2. *Overview of Selenium Metabolism.*

TABLE 17.1. Comparison of Selenium Compounds in Plants and Animals (Adopted from Wanger, 2002).

	% Distribution		
	Selenomethionine	**Selenocysteine**	**Other**
Plants			
Corn	61–64	15–16	20–24
Rice	68–81	6–10	19–31
Wheat grain	58–83	4–12	4–26
Grassland legumes	51–70	19–39	10–13
Animals			
Rat[1]	6–10	64–70	20–34
Rat[2]	63	22	15
Rat[3]	14–25	46–57	18–40

[1]Injected with selenite before analysis.
[2]Injected with selenomethionine one day before analyzing for selenium.
[3]Injected with selenomethionine 5 days before analyzing for selenium.

17.3.2. Selenoproteins and Enzymes

Selenium's presence as selenocysteine in a select group of antioxidant enzymes underscores its most important biochemical role in animals and humans. Table 17.2 lists a series of major enzymes and proteins that require selenium for activity or to perform non-enzyme functions in selenium metabolism. Selenocysteine is the compound required for antioxidant activity and only the seleno amino acid—as opposed to the sulfur analog (cysteine)—can impart that activity.

17.3.3. Glutathione Peroxidases

The glutathione peroxidases are a family of antioxidant enzymes that destroy hydrogen-alkyl- and lipid peroxides. Sequence data have revealed at least four major forms, all with selenocysteine as the active site cofactor. Classical glutathione peroxidase (GSH-Px or GPX1) was identified as the factor that protected hemoglobin from oxidation by ascorbate or H_2O_2. The plasma enzyme counterpart (GPX3) gained interest as a potential biomarker of selenium status because its activity paralleled selenium levels in the plasma. Phospholipid hydroperoxide (GPX4), in contrast to the other forms, prefers lipid peroxides (cumene hydroperoxide) and excels in retaining activity in a selenium deficiency. Gastrointestinal glutathione peroxidase (GPX-GI or GPX2), the fourth glutathione peroxidase to be described but second to be sequenced, is

TABLE 17.2. Seleno-Enzyme and Proteins in Biological Systems (Adopted from Sunde, 1997).

Enzymes	
Classical glutathione peroxidase	GPX1
Gastrointestinal glutathione peroxidase	GPX2
Plasma glutathione peroxidase	GPX3
Phospholipid hydroperoxide	GPX4
Iodothyronine 5′-deiodinase-1	
Iodothyronine 5′- deiodinase-2	
Iodothyronine 5′- deiodinase-3	
Selenoproteins	
Selenoprotein P (plasma)	
Selenoprotein W (muscle)	

very prominent in gastrointestinal cells but notably absent in heart, lung and kidney cells (Sunde, 1999).

All the glutathione peroxidases are part of a dual enzyme system designed to rid the system of peroxides in a continuous cyclic manner. Reduced glutathione (GSH) is the key substrate that provides the electrons, and alcohol and water are the products of the reaction (Figure 17.3). Oxidized glutathione (GSSG) formed is reduced to 2GSH by glutathione reductase, using electrons provided by NADPH and FAD to mediate the transfer.

17.3.4. Thioredoxin Reductases (TrxRs)

As their name implies, the enzyme thioredoxin reductase catalyzes

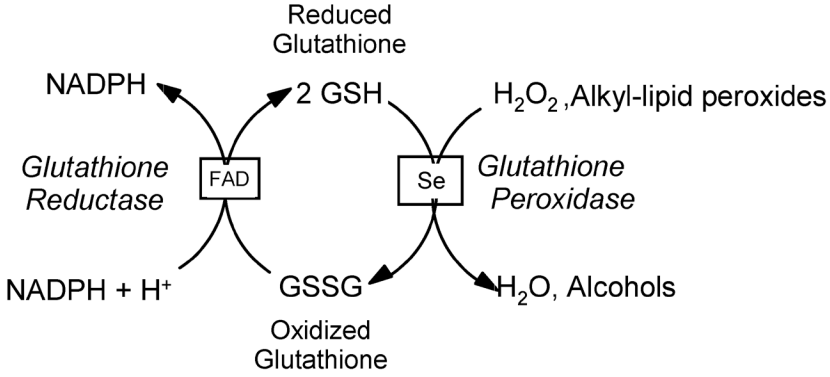

FIGURE 17.3. *Destruction of Reactive Oxygen Species by the Glutathione Peroxidase-Glutathione Reductase Cycle.*

electron flow to thioredoxin, a small disulfide protein and growth regulator in plants and animals. The source of the electrons is NADPH. In plants, TrxRs triggers a switch from starch synthesis during the daytime to energy-yielding starch utilization at night. TrxRs also function in DNA synthesis, specifically converting ribonucleotides into deoxyribonucleotides. Because of the free radical intermediate as a byproduct, a selenocysteine is requisite at the active site of the enzyme. Being a part of selenocysteine is not the only function of selenium in the enzyme. Selenium as selenate also regulates intracellular concentration of TrxRs and also operates separately as an antioxidant.

17.3.5. Iodothyronine Deiodinases

Iodothyronine deiodinases are selenoenzymes that play a significant role in thyroid hormone biosynthesis, specifically the conversion of T4 to T3. Selenocysteine is at the active site. Isomers of the enzyme differ as to the specific iodine removed. There are three types. Types 1 and 2 are selenoenzymes that convert T4 to T3, the more active form of thyroid hormone (Figure 17.4). Type 3 removes iodine from the 5-position on the ring, which inactivates the hormone (Chapter 8). The discovery of selenium in the enzyme provided a rationale for linking selenium deficiency with the development of goiters in humans.

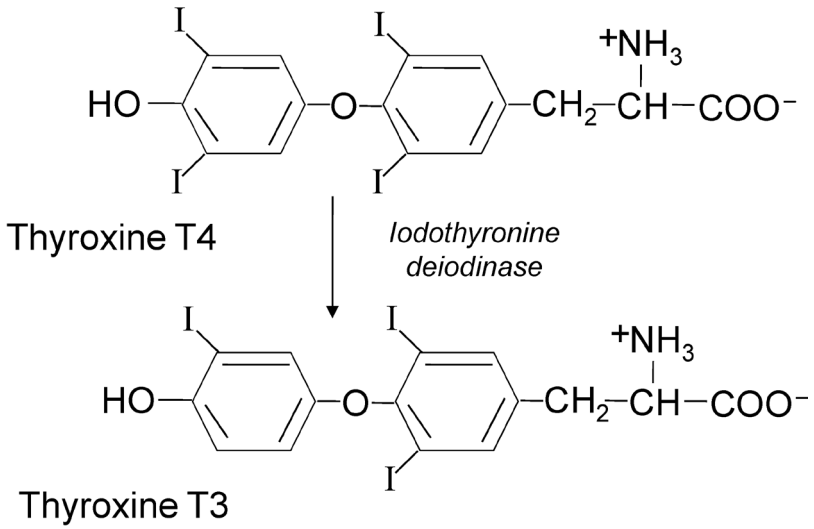

FIGURE 17.4. Synthesis of T3 from T4 by Iodothryonine deiodinase.

17.3.6. Selenoprotein P

Selenoprotein P (Sepp1) accounts for about two-thirds of the selenium in vertebrate plasma. Depending on the species, this small basic glycoprotein may contain between 10–17 selenocysteines in its structure. Two functions for Sepp1 have been postulated. One is to provide selenium for the biosynthesis of selenoproteins in tissues and, (2) to serve in a non-enzyme antioxidant capacity as a protector of endothelial cells from oxidative damage. Tissues and organs gain access to Sepp1 via specific receptors present in the plasma membrane. Mice with a mutation in the receptor molecule are blocked from absorbing Sepp1. As a consequence, these mice experience a sharp drop in selenium levels in the brain and testes, causing infertility and severe damage to neurons in certain brain areas.

17.3.7. Selenoprotein W

Interest in Selenoprotein W (Sepw1) is based on a possible link of the protein with white muscle disease in young growing sheep and goats. The disease, a type of myopathy, manifests in a number of tissues but is predominant in heart and skeletal muscle. Sepw1, however, is also found in parts of the brain (thalamus, cortex, hypothalamus) and spinal cord. The discovery that lambs diagnosed with white muscle disease have only rudimentary amounts of this 10 kDa selenoprotein in their semitendinosus muscles has linked selenoprotein W with risk of developing the disease. That this protein may have a protective role in normal development of brain, heart, and skeletal muscles has also been suggested. As explained below, white muscle disease is more prevalent in the young of larger, fast growing animals.

17.4. NUTRITIONAL PROPERTIES OF SELENIUM

17.4.1. Food Sources

Plants, animals, and sea foods are all good sources of selenium. Because selenoniferous soils vary in selenium content, the potential for highly variable levels of selenium in the same plant species is possible. In seafood, the preponderant selenium compounds are elemental selenium, dimethylselenide, and a complex of mercury and selenium. The

selenium content in some of the more common foods is shown in Table 17.3. A wide spectrum of foods provides selenium in the diet, ranging from 13 micrograms (mcg) in one hard-boiled egg to four milligrams in one cup of Brazil nuts (grown in selenium-rich soil). The values reported in Table 17.3 reflect total selenium and do not distinguish among the various biochemical forms. With the exception of freshwater fish, most aquatic species are a rich source. Wheat germ and seeds appear to be the richest plant sources. Surprisingly, skim milk is poor in selenium, estimated to be only 5 micrograms in one cup of milk. Drinking water alone is not a reliable source for supplying nutritionally significant quantities of selenium.

17.4.2. Assessing Selenium Status

A rise in blood levels of selenium either through selenium rich foods or supplements is accompanied by a sharp and nearly linear rise in plasma GSH-Px activity. This observation suggests GSH-Px is a valid biomarker of blood selenium levels and selenium status in general. Consequently, measuring plasma GSH-Px activity has become a standard in the nutritional assessments of the mineral. A drawback, however, are species differences in the amount of selenium associated with the en-

TABLE 17.3. Selenium (total) in Various Foods. (www. Fineberg.northwestern.edu/nutrition).

Food Source	Total Selenium (mcg)
Oysters (3.5 oz)	115
Steamed Clams (3.5 oz)	64
Sardines (3.5 oz)	46
Crab (3.5 oz)	40
Freshwater Fish (3.5 oz)	15
Chicken Liver (3.5 oz)	71
Beef Liver (3.5 oz)	57
Whole Wheat Pasta (1 cup)	36
White Pasta (1 cup)	30
Wheat Germ (1 cup)	112
Sunflower Seeds (1 cup)	94
Cooked Oat Meal (1 cup)	19
Soy Nuts (1 cup)	34

TABLE 17.4. Selenium Requirement in Males and Females Over the Life Span.

Age	Male (µg/day)	Female (µg/day)	Upper Limit
Infants			
0–6 mo	15 (AI)[1]	15 (AI)	45
7–12 mo	20 (AI)	20 (AI)	60
Children			
1–3 yr	20	20	90
4–8 yr	30	30	150
9–13 yr	40	40	280
Adolescents			
14–18 yr	55	55	400
Adults			
>19 yr	55	55	400
Pregnancy			
all ages	–	60	–
Lactation			
all ages	–	70	–

[1]AI = Adequate Intake.

zyme. Humans, for example, have less than 10% of the selenium in the erythrocyte enzyme, whereas rats and sheep have more than 70 percent. Such disparity between species questions the validity of GSH-Px as a universal assessment gauge and has challenged investigators to seek other biomarkers such as GSH-Px mRNA or selenoprotein P levels in the plasma.

17.4.3. Daily Requirement

Table 17.4 shows that the average intake and RDA for selenium changes with age but not gender. With the exception of infants, the values recorded were obtained by optimizing plasma glutathione peroxidase activity. Infants one year or less in age use the selenium content of human milk and adequate intake (AI) to specify quantity. Values for groups beyond 30 years are extrapolated from the values obtained from the 19–30 group (not shown in Table 17.4), and adolescents up to 18 yrs. of age draw on the values extrapolated from adults. An upper limit (UL) of 400 µg/day is set for adults, with tolerability for selenium developing quickly in infants as they mature.

17.4.4. Regional Differences in Selenium

On a global scale, selenium content in soils varies from high concentrations in certain regions of China, Venezuela and the USA to much lower levels in Finland and New Zealand. Figure 17.5 gives a graphic picture of selenium distribution in the USA. Richest levels are in the central part of the country encompassing the upper and lower Great Plains region, whereas low selenoniferous soils are confined mostly to the coastal states and Great Lakes region. It should be noted that food supplies from certain regions of the USA tend to be widely distributed throughout the country, making it less likely that people living in areas with low selenium in the soil will be exposed to food products grown in that area. Not having a country-wide food distribution system in place has had disastrous consequences in China, where people consume crops grown in an area where selenoniferous soils have low selenium. These people risk developing Keshan's disease, which is a type of cardiac myopathy (see below). Surprisingly, broccoli accumulates selenium far above the level in the soil (Finley, 2003) and has been recommended as a major crop for people living in the affected regions.

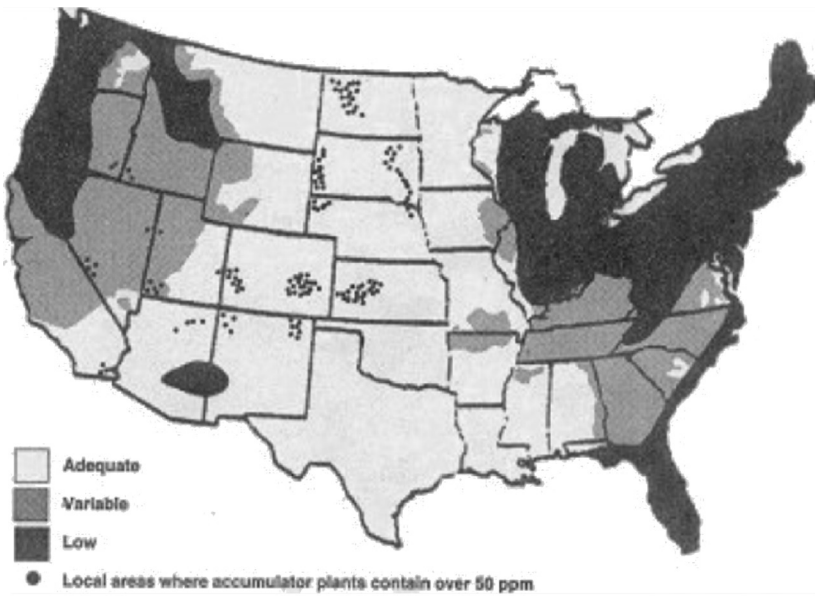

FIGURE 17.5. Geological Distribution of Selenium in Soils in the USA.

17.5. DIGESTION, ABSORPTION AND METABOLISM

17.5.1. Digestion and Absorption

Most chemical forms of selenium are absorbed rapidly. An exception is selenite in ruminants, where the high reducing environment of the rumen favors reduction to elemental selenium (van Ryssen *et al.*, 1987). Selenomethionine and selenocysteine are absorbed as rapidly as the sulfur amino acids, suggesting the seleno counterparts use the sulfur transport system to gain access. Also absorbed with high efficacy are selenate and selenite, which are likely to be present as dietary supplements or products of selenoamino acid catabolism. Rats, for example, absorb between 95-98% of the selenium when administered as selenomethionine and nearly that same amount when dosed with selenite.

17.5.2. The Incorporation of Selenium into Proteins

Most of selenium's biological functions are associated with selenocysteine and selenomethione, two amino acids that are incorporated into proteins. How these seleno analogs become part of the protein structure has been a subject of intense interest. Both inorganic and organic forms of selenium are incorporated into proteins by pathways that allow communication between selenium forms. The close similarity in structure between selenium and sulfur carries over to selenite being formed from selenate by the same enzyme systems that convert sulfate to sulfite. Animal systems further have the capability of converting selenomethionine to selenocysteine, thus making selenomethionine a precursor of protein-bound selenocysteine.

17.5.2.1. The Inorganic Pathway of Selenocysteine Biosynthesis

The formation of selenite from selenate is shown in Figure 17.6. In the first step, selenate reacts with ATP to form adenosine 5′-phosphoselenate (APSe). This step is followed by the addition of a second ATP to form 3′-phosphoadenosine-5′ phosphoselenate (PAPSe). A NADPH-linked reduction reaction releases selenite from the PAPSe complex. Selenite coming from the diet can enter the pathway at this point. In the terminal reactions in the pathway, selenite binds to two molecules of glutathione and is reduced to selenide (Se^{2-}). Elemental selenium (Se^0) is an intermediate in the reaction. To be incorporated into pro-

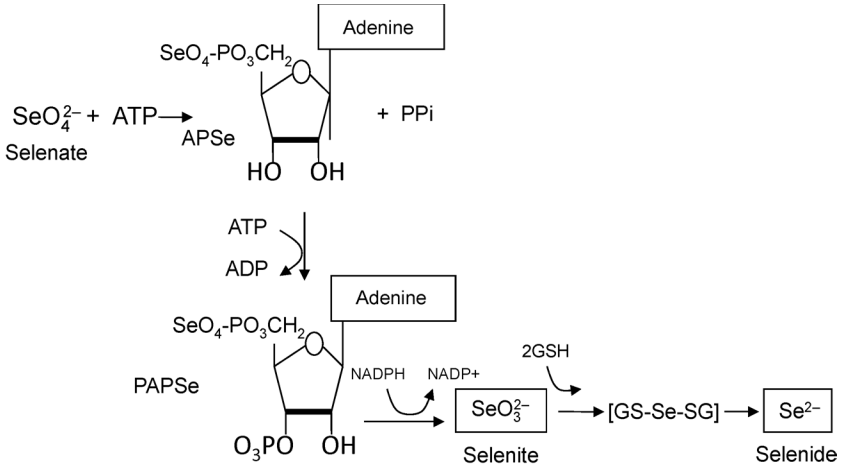

FIGURE 17.6. Pathway for the Reduction of Selenate to Selenide.

tein as selenocysteine, the selenide reacts with ATP to form the highly unstable intermediate, selenophosphate (Figure 17.7). Selenophosphate thus becomes the last intermediate in the inorganic pathway for incorporating selenium into proteins.

Identifying selenophosphate as the proximal selenium donor opened the way to learning details of the incorporation pathway. Tracing the origin and fate of selenophosphate in prokaryotes eventually led to the discovery of 4 genes, SelA, SelB, SelC, and SelD, that control 4 key steps in the incorporation pathway. SelD encodes selenophosphate syn-

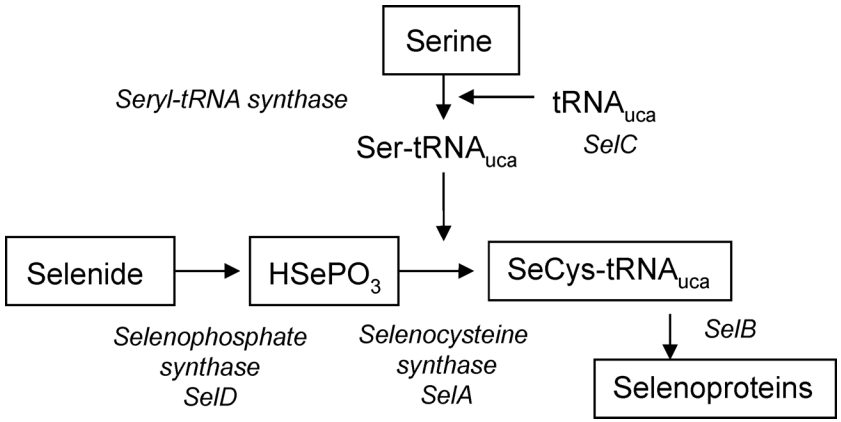

FIGURE 17.7. The Inorganic Pathway for Incorporating Selenium as Selenide into Selenoproteins.

thase, the enzyme that catalyzes the formation of selenophosphate from selenide, the first step in the pathway (Figure 17.7). Selenocysteine synthase, the product of SelA, catalyzes the condensation serine-$tRNA_{uca}$ with selenophosphate to form selenocyteine-$tRNA_{uca}$. SelB codes for an elongation factor that attaches selenocysteine-$tRNA_{cua}$ to the mRNA at the UGA codon. Mutations of any one of these four genes will lead to failure to form protein-bound selenocysteine.

17.5.2.2. The Organic Pathway of Selenocysteine Biosynthesis

The organic pathway leading to selenocysteine biosynthesis starts with selenomethionine in animals and O-acetylserine in plants. In plants, selenocysteine is formed from a direct reaction of O-acetylserine with selenium sulfide (H_2Se). In animals, selenocysteine arises by first forming selenocystathionine, a complex of serine with selenohomocysteine. A lyase enzyme frees the selenocysteine by effectively transferring the selenium to the serine residue of the complex, giving rise to selenocysteine as a split product (Figure 17.8). The selenocysteine is then transferred to a special selenocysteine $tRNA_{uca}$ for incorporation into protein.

17.5.2.3. Incorporation of Selenocysteine into Selenoproteins

Both the inorganic and organic pathways end with selenocysteine bound to a unique species of tRNA, selenocysteine tRNA (SEC-tRNA).

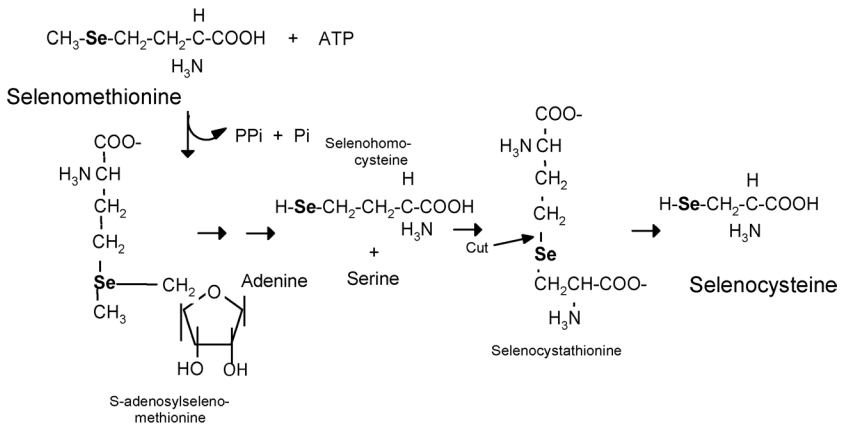

FIGURE 17.8. *Organic Pathway for the Synthesis of Selenocysteine from Selenomethionine.*

This tRNA is special because it has the anticodon CAU, which lets the tRNA bind to a UGA codon on the mRNA. UGA is normally a stop codon that terminates translation. Overcoming the suppression is a function of the product of the SelB gene. This highly unusual reaction requires a specific stem loop structure in the 3′ untranslated region (3′-UTR) of the mRNA, referred to as an elongation selenocysteine insertion sequence (eSECIS). Although the distance from the UGA on the message to the eSECIS in the 3′-UTR is known to vary for the different selenoproteins, eSECIS is absolutely essential to place selenium at the UGA site on the mRNA.

17.6. SELENIUM DEFICIENCY

Selenium deficiency puts a person or animal at risk of developing cancer, muscle degeneration, infertility, cardiovascular and immune system failure. Outward signs are most conspicuous in two diseases of skeletal muscle and heart. White muscle disease generally occurs in young growing animals; Keshan Disease, a cardiomyopathy, occurs in humans. Although rare in humans, a selenium deficiency can arise from genetic mutations and selenium malabsorption. Patients undergoing long term parenteral nutrition have been shown to develop symptoms of selenium deficiency. Deficiency symptoms appear to be associated with a depletion of selenium enzymes and proteins designed to protect against oxidative stress.

17.6.1. White Muscle Disease (WMD)

Gross muscle degeneration and loss of pigmentation typifies white muscle disease (Figure 17.9). Much of the early work to identify a cause focused on Selenoprotein W (SepW1), a small selenoprotein found mainly in muscle. Although SepW1 appeared to be low in animals that developed the disease, not having a firm connection between SepW1 and the disease handicapped its acceptance as a protective factor. Importantly, glutathione peroxidase in the affected muscle remained at normal levels in white muscle disease tissues, suggesting a more dominant antioxidant selenium-containing factor was at the core in preventing WMD. That observation led to the discovery of selenoprotein N (SEPN1) as a second candidate for a protective factor. Evidence in support of SEPN1 was based mainly on showing that mutations in the

FIGURE 17.9. *Lamb Skeletal Muscle Afflicted with White Muscle Disease. Control muscle is on the left. Loss of pigment and evidence of necrosis are typical symptoms of the disease.*

protein gave rise to a series of neuromuscular disorders referred to collectively as selenoprotein N-related myopathies, some of which closely resembled WMD. Thus, mutations in SEPN1 and omission of selenium from the diet were both apt to cause the same disorder. That SEPN1 mutations is inherited made the muscle-related disorders a genetic disease and SEPN1 protein a target of the disease, the first instance where a genetic disease has been linked to the expression of a selenoprotein. Consistent with the earlier observation, the expression of the SEPN1 gene is not modulated by selenium, which holds open the possibility that another selenium-responsive factor is yet to be identified.

17.6.2. Keshan Disease

This endemic disease, first reported in 1935, was named for a province in northeast China where it was first discovered. Sufferers of Keshan disease had poor blood circulation with an endocardium abnormality and myocardium necrosis. Once it was learned that the disease may be linked to selenium-deficient soil in the region, steps were taken to enrich the soil in the surrounding area with sodium selenite to prevent further cases. The treatment drastically lowered incidences of the dis-

ease. Other instances not related to selenoniferous soils have also been reported. An adult patient subsisting on TPN for 6 years who eventually succumbed to heart failure was shown in a postmortem diagnosis to have a cardiomyopathy and myocytolysis closely resembling Keshan disease. Low concentrations of glutathione peroxidase in blood, heart, liver and skeletal muscle were also noted.

17.6.3. Kashin-Beck Disease

Kashin-Beck disease is a chronic and degenerative osteoarthritis with skeletal deformations and dwarfism. First characterized in areas of China and Tibet, the disease has also been traced to low selenium soils. Sufferers show low blood iodine and thyroid hormone, making iodine deficiency a risk factor for developing the disease. Low selenium combined with fulvic acid (a mixture of water-soluble reactive organic products of microbial decomposition of plants) is believed to be the major cause. Figure 17.10 shows the dwarfism and irregular bone formations that are typical symptoms of the disease. In experimental animals, bone irregularities have been traced to a defect in the collagen component of bone, specifically the lysine residues which form stable crosslinks in the collagen protein (Chapter 15).

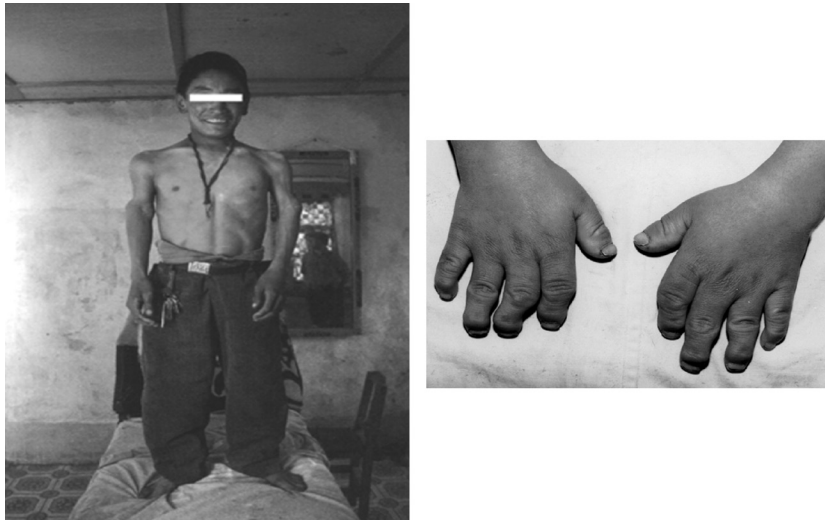

FIGURE 17.10. Symptoms of Kashin-Beck Disease.

17.6.4. Immune System Functions

Effective functioning of the immune system depends on selenium. A deficiency can impugn the protective functions of glutathione peroxidase which tends to protect neutrophils, T- and B-lymphocytes, Natural Killer (NK) cells and macrophages from oxidative reactions. This is especially important for NK cells and neutrophils that use superoxide anion and peroxides generated in situ to kill ingested foreign microbes. Basically, all components of the immune system that use cell-mediated or humoral responses depend on a selenium-related factor for protection.

17.6.5. Cancer

A link of selenium with cancer has been paradoxical since either too little or too much selenium in the diet can be carcinogenic. Because cancer is a neoplastic disease, dietary deficiencies, excesses, or imbalances can be part of the etiology. Lowering the protective antioxidant barrier can make the system vulnerable to neoplastic changes, which basically means that selenium can delay or block the onset of a rapid growth phase typical of cancerous cells. On the other hand, too much selenium in the diet can result in a toxicity characterized by a loss of control over cell division. According to the EPA, only one form of selenium, selenium sulfide, is a probable carcinogen for humans. Fortunately, selenium sulfide is not found in food. This is the paradox faced by nutritionists and food scientists in coming to an understanding of the role of selenium in cancer.

Results from geographic areas and intervention trials have fortified the belief that the risk of developing many cancers can be lowered by elevating selenium intake (Willet *et al.*, 1983). A landmark study that turned attention from causing cancer to protecting from developing cancer was the Nutritional Prevention of Cancer (NPC) study reported in 1996. Designed initially to study protective effects of selenium against skin cancer, the investigators followed 1,312 high-risk dermatology patients who consumed 200 µg/day of selenium as selenized yeast for an average of 4.5 years. Although no significant difference occurred in basal layer skin carcinoma, those subjects that consumed selenium as opposed to a placebo had a significant decrease in incidences and mortality of lung, prostate and colon cancers. In connecting the deficiency with the cancer, it has subsequently been shown that lowering selenium

intake causes DNA damage which can lead to mutations that cause cells to be transformed. One must also take into account that any component in the diet that interferes with the incorporation of selenocysteine into selenoproteins can negate the protective effects of selenium.

17.6.6. Male Infertility

The link between selenium deficiency and male infertility shows the importance of selenium to normal spermatogenesis. Low testicular levels of selenium can be a prelude to the destruction of mature germinal cells and seminiferous epithelium. Thus, maintaining fertility by retaining selenium appears to be a priority in testicular tissue; enhancing vitamin E intake does not provide protection. While the selenium factor has not been identified, it is known that impairment in selenoprotein P causes infertility in male rats and gives rise to spermatozoa with flagellar defects. The protein as a likely candidate for protection is bolstered by observing testes readily take up selenoprotein P from the plasma. Also to consider is GPx4; the hydroperoxidase that protects oxidation of phospholipids is located in the midpiece of the sperm. As the most dominant glutathione peroxidase in testes, GPx4 activity is also the most resistant to suppression by a moderate to severe dietary selenium deficiency.

17.7. INTERPLAY BETWEEN SELENIUM AND VITAMIN E

Vitamin E joins selenium as a major protectorate of cells and tissues from oxidative damage. The fat solubility property of the vitamin favors its action in the lipid environment of the cell membrane. The two do not merely mimic one another's action, but instead are unique in their target selection and site of action. For example, both prevent lipid peroxidation when rats are given oxidants that initiate destructive chain reactions. However, animals given only the vitamin accumulate lipid peroxides in the tissues, whereas those same products are not seen when the animals are given selenium. The data, therefore, suggest the internal target for the two is not the same. A possible explanation recognizes that although both give protection, vitamin E appears to act within the lipid membrane, neutralizing free radicals in the membrane before they are formed, whereas selenium destroys the peroxidation products after they have formed. Figure 17.11 shows the synergism in this action of the two antioxidants.

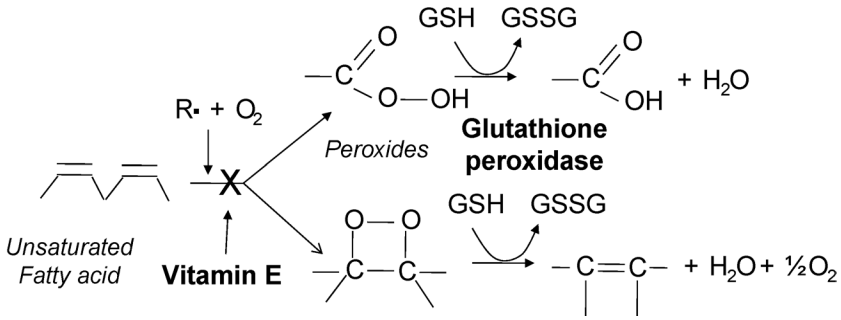

FIGURE 17.11. *Peroxidation of a Polyunsaturated Fatty acid: Differential Actions of Vitamin E and Glutathione Peroxidase. Seen is the abstraction by a free radical (R·) of a hydrogen from the –CH_2 group joining the double bonds of an unsaturated fatty acid. Oxygen reacts with the radical that is formed to initiate a chain reaction by abstracting a second hydrogen atom. Vitamin E effectively blocks formation of an alkylhydroperoxide from the fatty acid. Glutathione peroxidase destroys the peroxide by converting the peroxide group into a less reactive alcohol.*

17.8. SELENIUM EFFECTS ON GENETIC EXPRESSION

Regulating genetic expression implies that selenium, possibly through selenocysteine, has the capacity to regulate the synthesis or turnover of mRNAs that code for selenoproteins. Hypothetically, when selenium is in adequate supply, the levels of selenoprotein mRNAs are kept at a steady-state level. In a deficiency, however, some selenoproteins and enzymes fail. Moreover, the fall in activity could revert back to a drop in the mRNA. As an illustration, in a selenium deficiency, glutathione peroxidase (GSH-Px) and type-1 iodothyronine 5′-deiodinase (5′IDI) in rat liver, thyroid and heart undergo a progressive loss in enzyme activity concomitant with a drop in the enzyme's mRNA. The same is seen for selenoprotein P in liver. This is not seen in all tissues, however. For example, GPX4 activity in liver falls at a rate that is greater than the change in its mRNA. The same is true for thioredoxin reductase which shows only a modest change in its mRNA and five selenoproteins in pituitary and thyroid fail to respond to either a decrease or increase in selenium. This apparent selectivity and inconsistency counters the notion that a selenium deficiency works through decreases in mRNA levels for specific selenoproteins and enzymes and suggest a more complex, tissue-specific response.

17.9. SUMMARY

The importance of selenium to humans and animals lies in its cofactor role in selenoproteins and enzymes that protect the system from oxidants. Selenium's function is inseparable from Vitamin E, although selenium cannot replace the vitamin, nor can the vitamin replace selenium in the preventative action. As a cofactor for glutathione peroxidase, selenium as selenocysteine destroys peroxides and free radicals. What sets selenium apart from metal ion cofactors, however, is its presence in an amino acid (selenocysteine) within the protein structure as opposed to being attached as an ion to the protein structure. Although selenium has no recognized role in plants, plants nonetheless are a rich source of the mineral. Selenomethionine, the most common selenium compound in plants, is readily converted to selenocysteine in animals. Soils poor in selenium impact on plant selenium levels and ultimately on animals that feed on the plants. Its chemical resemblance to sulfur allows selenium to be metabolized by the same metabolic pathways and enzymes that recognize the sulfur analogs. A unique feature, however, is the incorporation of selenocysteine at the UGA codon site on the mRNA. No other amino acid-tRNA complexes share this property. With the multitude of important functions performed by selenium, a deficiency in selenium has wide-spread effects on animal and human health. Liver necrosis in rats, white muscle disease in lambs, and Keshan disease in humans are better known examples. Selenium deficiency risks malfunction to the immune system and spermatogenesis as well as malignant transformations that can lead to many types of cancers. Selenium must also be thought of in the context of a toxic element capable of causing severe disorders when in excess. Selenium intake exceeding 400 micrograms a day is not advisable. Most of the toxicity is attributed to the presence of selenide. The role of selenium as it relates to its fundamental actions in tissue is still an active area pursued by research.

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17.11. PROBLEMS

1. Examine the Periodic Table of Chemical Elements. Where does selenium appear relative to sulfur? Are the outer orbital electrons in selenium and sulfur the same? If so, how can you explain the unique biological properties of selenium?

2. What foods tend to provide the most selenium in the diet? Is it fair to say that plants rich in selenium can be toxic?

3. What enzyme class is more likely to have selenium as a cofactor?

4. What would be your reply to a friend who says that he doubles the amount of vitamin E in his diet so that he doesn't need to take any selenium?

5. How much broccoli (grams) must a 20 year old man consume to meet the RDA for selenium?

6. What is the normal function of the UGA codon on a mRNA? How is it possible for this codon to be a site for the incorporation of an amino acid? What component must be modified in order for this to happen?

7. What data support or negate Selenoprotein W as being the factor that prevents the development of white muscle disease? What data support or negate Selenoprotein N as being the factor?

8. Offer suggestions a medical doctor might consider to make a definitive diagnosis of selenium deficiency in his/her patient.

CHAPTER 18

Iodine

HISTORY advises that iodine may have been the first biological mineral with curative and preventative properties, a reference to iodized salts and oils to prevent or treat goiters. About 70–80 percent of the 20 mg of iodine in a human body is in the thyroid gland; the remainder is mostly in muscle. The biochemistry of iodine is limited primarily to a structural role, exclusively in T4 (thyroxine) and T3 (triiodothyronine), the two hormones produced by the thyroid gland. Thus, iodine has a dominant role in controlling the pace of metabolism, mental and physical growth, and preventing disease. Although many foods contain iodine, its variation in soils cannot assure that all foods will contain adequate amounts. Moreover, iodine in foods tends to be poorly absorbed and thus supplementing diets with iodized salts is a practice recommended for the populace in general. In this chapter, we will explore how iodine is rendered an essential nutrient as well its functions in maintaining health and normal metabolic functions.

18.1. HISTORY AND EARLY OBSERVATIONS

Although Courtois in 1811 was the first to report a purplish vapor that evolved from sea weed during the manufacturing of gun powder, it was Gay-Lussac and Humphrey who recognized iodine as a new chemical element. The name iodine has its origin from the Greek word "iodes", meaning purple or violet. The biological awakening occurred when Boussingault showed that iodine was the active component in salts used to cure

goiters. The discovery held no meaning until Baumann showed iodine to be present in the thyroid gland (1895), which was followed about 20 years later by Kelberg and Osterberg's report that iodine was in the structure of thyroxine, the most prominent thyroid hormone. The practical benefits of this information was provided by Kimball and Martin (1918), who used regular iodine supplements to prevent the occurrence of goiters in school aged children, a discovery that may have prompted the use of dietary supplements in general to prevent diseases. In time, iodized salts have been a mainstay in maintaining iodine levels in the system.

18.2. CHEMICAL PROPERTIES

With an atomic weight of 126.9, iodine has the distinction of being the heaviest (atomic weight-wise) mineral in humans and animals and second only to tungsten in the biosphere. Classified as a halogen, meaning "salt former", iodine exists in the free state as the gas, I_2. Upon reacting with alkali metals, iodine forms a white crystalline solid or halide. The chemical property more relevant to its biological function, however, is the electropositive charge brought about when iodide anion (I^-) is oxidized to I^+. This one reaction in a biological system permits iodine (as I^+) to bind covalently to the phenyl ring of tyrosine in a reaction that precedes formation of thyroid hormones.

18.3. BIOCHEMICAL PROPERTIES

The biochemistry of iodine basically takes the form of 3 biological molecules: thyroglobulin (TG), thyroxine (T_4), and triiodothryonine (T_3). Figure 18.1 shows the biochemical pathway that connects all three. Thyroglobulin is a precursor of both T4 and T3. The sequence of events leading to the synthesis of thyroxine (T4) and triiodothyronine (T3) begins with the iodination of tyrosine residues in thyroglobulin. Breaking peptide bonds in iodinated thyroglobulin releases T4 and T3 into the blood. T3 is also the product of post-cleavage deiodination of T4. Although the more abundant of the two, T4 has only one-third the activity of T3. In terms of iodine concentration, the thyroid gland is the richest, followed by saliva > stomach > mammary gland > pituitary and liver. Brain and muscle have the lowest per wet weight of tissue, but because of its mass, muscle is the second richest store of iodine.

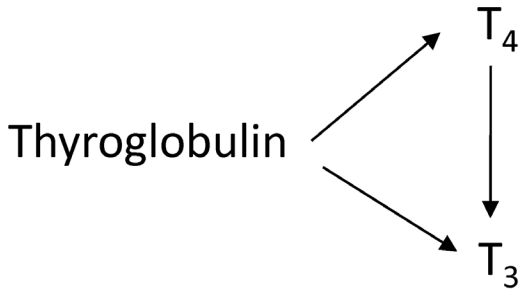

FIGURE 18.1. *Biosynthetic Scheme for the Production of Thyroid Hormones.*

18.4. NUTRITION

Food sources vary in iodine content. Similarly, the iodine content of any one crop or forage depends on the amount of iodine in the soil where it is grown, suggesting the immediate environment has a strong impact on the iodine density of foods. For example, as compared to fresh water varieties, ocean varieties of marine animals produce larger quantities of organoiodine compounds, which is perhaps why seafoods top the list of richest sources of iodine (Table 18.1). Moderate sources include eggs and leafy vegetables such as spinach, turnips, chard and asparagus. A surprising finding is the lower amounts of iodine in dairy products as well as most fruits and nuts such as almonds, cashews, and walnuts. It's important to note that meat is not considered a good source, perhaps reflecting the very low concentration of iodine in muscle.

TABLE 18.1. Dietary Sources of Iodine.

High (30–500 µgm/100 g)	Medium (10–50 µgm/100 g)	Low (1–10 µgm/100 g)
Sea food (ocean)	Fresh water fish	Nuts
Kelp	Eggs	Grains
Irish moss	Beef liver	Most fruits
Mushrooms	Peanuts	Vegetables
Sunflower seeds	Pineapple	Milk and cheese
	Leafy greens	Honey
	Cheddar cheese	
	Chocolate	
	Bone meal	
	Iodized salt	
	Mayonnaise	

18.4.1. Daily Requirement

Table 18.2 shows the RDA values for iodine as a function of age and gender. The figures are based on the Total Diet Study performed by the Food and Drug Administration. As with most nutrients, postnatal values up to one year of age represent adequate intake (AI) and are based on breast milk or formula content. An infant's average intake through 6 months of age is estimated by this means, whereas values for 7 to 12 months represent extrapolations from 0–6 month average intakes. The values for 1–8 years come mainly from balance studies and 9–18 years are extrapolated from adult EAR values. Values for 19–50 years are based on thyroid iodine accumulation and turnover. It should be noted that there are no gender differences in the RDA for each stage and group after 1 year. This does not hold for pregnancy and lactation, however, where a strong increase in RDA is observed. The upper limit for both genders increases progressively, reaching a maximum of about 1 mg per day at ages between 19 and 30 years. Although post puberty RDA values of 150 µg per day is recommended, the thyroid gland needs only about half this amount to sustain the requisite requirement for T4 and T3 synthesis.

TABLE 18.2. Dietary Reference Intakes for Iodine.[1]

Life Stage	RDA[2] Male (µg/day)	RDA[2] Female (µg/day)	AI	Upper Limit Male or Female
0–6 mo			110	Not determined
7–12 mo			130	Not determined
1–3 yr	90	90		200
4–8 yr	90	90		300
9–13 yr	120	120		600
14–18 yr	150	150		900
19–30 yr	150	150		1,100
31–50 yr	150	150		1,100
51–70 yr	150	150		1,100
>70 yr	150	150		1,100
Pregnancy				
<18 yr		220		900
19–50 yr		220		1,100
Lactation				
<18 yr		220		900
19–50 yr		220		1,100

[1]Values take from FDA Total Diet Study.
[2]RDA = Recommended Dietary Allowance.

18.5. DIGESTION AND ABSORPTION

Iodine in the diet is present mainly in an organic-bound form, i.e., covalently bound to carbon and not readily dissociated by gastric juice or digestive enzymes. This translates into less than 30% of the food iodine becoming absorbed into the system. Overall, the low bioavailability of iodine in humans and animals reflects an ineffective system for extracting iodine from foods. Digestion becomes an important consideration when natural foods low in iodine are a main dietary staple, which perhaps justifies the need to supplement the diet with iodized salts to meet the RDA and avoid deficiencies. In contrast, inorganic forms, which are basically salts of iodine, are water soluble and readily pass through the intestine and into the blood.

18.5.1. Goitrogens

As their name implies, goitrogens have the potential to cause goiters. They do this by blocking iodine absorption at the level of the gut or interfering with the production of thyroid hormones. Goitrogens in foods was first reported by Chesney in 1928. Examples include thiocyanates (SCN), which compete with iodine for the iodine membrane transporters and isoflavonoids, which inhibit the action of a thyroperoxidase required for the production of I^+, the oxidized form of iodine needed for thyroid hormone biosynthesis. Thiocyanates are present in foods such as brassica (rape seed), sorghum, and cruciferae (cabbage, broccoli, brussel sprouts, cassava, mustard and horse radish). Isoflavonoids such as genistine are found in soybean-related foods. Fortunately, most goitrogens are unstable in heat and moisture and are readily destroyed in cooking.

18.6. TRANSPORT AND UPTAKE OF IODINE

About 88 percent of the iodine absorbed into the system is taken up by the thyroid gland. Most of what is not taken in by the thyroid is excreted in the urine. Only a small percentage (5 percent) is removed through the liver via the bile. Highly efficient uptake of iodine by the thyroid is due to a large number of sodium/iodide symporters (NIS) on the basal surface of the follicle cells of the thyroid gland. These transporters screen the blood that is infusing into the gland and mediate

passage of iodide ions (I^-) into the cells. A sodium gradient across the membrane provides the energy (Chapter 10).

18.7. SYNTHESIS OF THYROID HORMONES

18.7.1. Overview

The synthesis and activation of thyroid hormones is the major and perhaps only biological requirement for iodine. How the thyroid hormones are synthesized, particularly at the stage of iodine incorporation, requires knowing not only the biochemistry but also the anatomy of the thyroid gland. As shown in Figure 18.2(a) and 18.2(b), the thyroid gland is composed of spherical sacs (follicles) ringed by a single layer of cuboidal epithelial cells (follicle cells) enclosing a matrix-like interior (colloid). Nearly all of the iodine entering follicle cells goes into thyroid hormone. Once iodine is internalized, the following events ensue: (1) transport of iodide ions to the apical membrane-colloid interface for iodination of select tyrosine residues in thyroglobulin, and (2) re-entrance of iodinated thyroglobulin into the follicle cells and release of T4 and T3 into the blood following proteolysis of the thyroglobulin. These events are pictured in Figure 18.3.

18.7.2. Transfer of Iodine to Thyroglobulin

The absorbed iodide ions are transported to the apical surface and

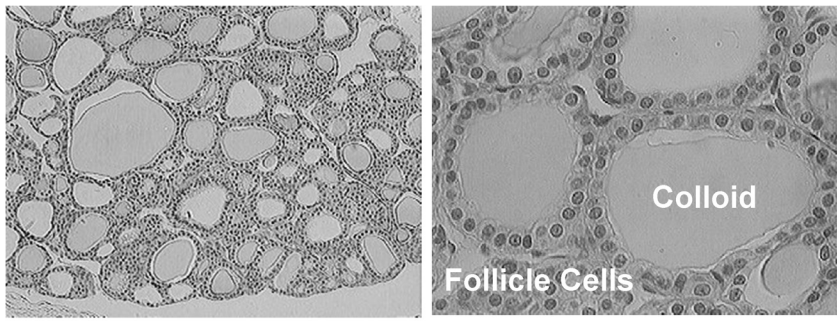

FIGURE 18.2. *Anatomy of the Thyroid Gland showing Follicle Cells and Colloid. A single layer of follicle cells encircle the colloid. Iodine is transferred to the apical surface of follicle cells and released into the colloid. Iodination occurs at the membrane-colloid interface. (a) x 100: (b) x 400.*

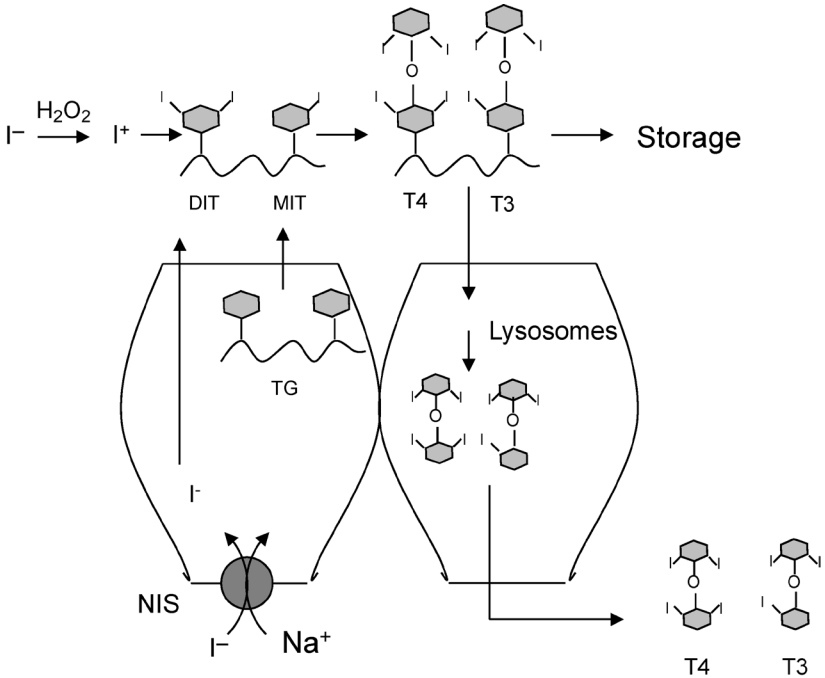

FIGURE 18.3. *Synthesis of Thyroid Hormones. Iodine ions are actively transported into the follicle cells by a sodium-iodide symporter (NIS) on the basal surface. Release at the apicial surface allows an interaction with thyroglobulin (TG). Freeing the active hormone requires re-entrance of iodinated TG into the follicle cells and a proteolysis catalyzed by a lysosomal protease.*

released into the colloid of the follicles. Thyroglobulin, a 666,000 kDa glycoprotein dimer, is synthesized in the follicle cells and released into the lumen at the colloid surface. Subsequent oxidation and binding of iodide ion to thyroglobulin is catalyzed by *thyroperoxidase*, an integral membrane protein on the apical surface; H_2O_2 is the oxidant in the reaction. Iodination gives rise to mono- and di-iodotyrosine residue while still bound within the protein. Each thyroglobulin chain has 4 tyrosine residues subject to iodination, 3 on the C-terminus and one on the N-terminus, a total of 8 potential sites for forming the ring structure of the thyroid hormone. The juxtapositioning of the iodinated tyrosines across from one another permits coupling of iodinated tyrosine with its iodinated cross neighbor (Figure 18.4). The tyrosines must be iodinated to take part in the coupling and ring transfer. T4 and T3 arise from the mono-iodinated or di-iodinated rings, respectively, generally at a ratio of 15:1 in the protein.

18.7.3. Release of Thyroid Hormones

The final steps in the processing involve the release of thyroid hormones (T4 and T3) from the protein bearing the bound precursors. Another option is to store the iodinated protein in the colloid for release at a later time. To release T4 and T3, the iodinated protein re-enters the follicle cell via endocytosis and is transferred to the lysosomes. A protease enzyme in the lysosomes breaks the peptide bond, liberating T4 and T3, which exit the cell at the basal surface and enter the blood. Liberated T4 and T3 engage thyroid binding proteins (TBPs) and are transferred to their target cells. Most T3 molecules arise from preformed T4 after release, using the selenoenzyme 5′ iodo-deiodinase (Chapter 17).

18.7.4. Role of Thyroid Stimulating Hormone

Thyroid Stimulating hormone (TSH) produced in the pituitary regulates the amount of T4 and T3 produced by the thyroid gland. It does this by controlling the activity of the Na^+/I^- symporter (NIS). The symporter has power over the amount of iodide ion taken in by the follicle cells. An iodine deficiency that lowers blood levels of T4 will stimulate production of TSH and activate the symporter; overproduction of T4 will restrict iodine uptake and suppress hormone synthesis. This feedback

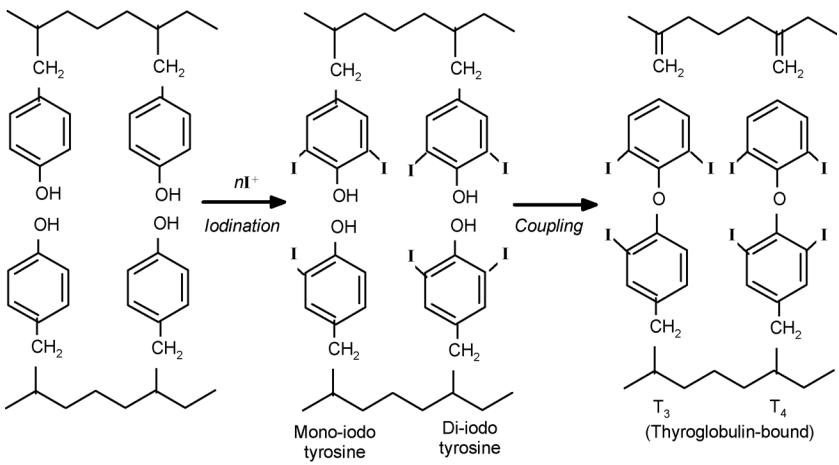

FIGURE 18.4. *Synthesis of Thyroid Hormones T4 and T3. Thyroperoxidase oxidizes I^- to I^+ in preparation for iodination. Mono- and di-iodo tyrosine residues juxtaposed couple through an ether linkage and detach from one of the chains. The final step releases the thyroid hormone from the peptide chain by a lysosomal enzyme in the colloid.*

mechanism assures that the thyroid hormone in the system remains at a steady-state level and is the reason TSH is used as a biomarker for iodine adequacy (see below).

18.8. TRANSPORT AND UPTAKE OF THYROID HORMONES

Over 99% of the T4 and T3 in plasma is bound to the thyroid binding proteins (TBPs), of which there are three types. *Thyroxine-binding globulin* (TBG), a 54 kDa protein with one binding site for T4, makes up about 70% of the T4 in plasma. TBG releases T4 slowly at the membrane site. *Transthyretin,* although 10 times richer than TBG in the plasma, accounts for only 10–15% of the bound T4. Transthyretin is a multifunctional protein that also binds and transports retinol and retinal, which competes with T4. Lastly, *serum albumin,* which makes up about half the protein in plasma, is believed to have 6 non-specific binding sites for thyroxine, but because of their non-specificity, these sites have a weak binding affinity for the hormones. Albumin, therefore, is not considered a major transport/delivery protein for thyroid hormones. Furthermore, a protein-bound form of the hormone is not active, implying that to be rendered functional, the hormone must completely disengage from its plasma protein carrier prior to entering the cell.

18.8.1. Membrane Penetration

Once free of its carrier, T4 and T3 enter peripheral cells, exploiting their lipophilic property to pass through the membrane. Energy to effect the movement from plasma to cytosol is not universal, however. For instance, liver cells take up T3 by an energy-driven mechanism and effluxes T3 by passive diffusion. Non-hepatic organs require energy for T3 to enter, but T4 enters by simple diffusion. These conflicting data suggests that the transport mechanism for the two thyroid hormones are separate and could involve structurally distinct transport mediators. The larger picture, therefore, is that both tissue type and hormone type must be considered in the transport of thyroid hormones into cells.

18.9. IODINE DEFICIENCY

A deficiency in iodine can threaten normal development at all stages

of the life cycle beginning in utero. Chronic deficiency can lead to the formation of goiters and cretinism. The World Health Organization estimates that there may be 1.6 billion people in the world who are at risk of developing iodine deficiency disorders. Included are 211 million people who are goiter prone and 5 million showing symptoms of cretinism. Clearly on a global scale, iodine deficiency is a major nutritional problem.

18.9.1. Geographical Considerations

Some countries are at greater risk than others for having an iodine deficient populace. Part of the reason can be related to low iodine levels in soils, which makes geographical considerations a priority. Listed below are locations that could be low iodine areas:

- High mountain regions typical of Switzerland and other Alpines as well as the Andes and Himalayas, where glaciated run off could be detrimental
- Flood plains typical of Asia (Nepal, India, China and Bangladesh)
- Old exposed soil where iodine was lowered by sun exposure that sublimates iodine.

Geographical areas that are identified as being at risk must execute ways to overcome the deficiency. Of those listed, increasing the content of iodized salts may be the single and most effective preventative.

18.10. ASSESSING IODINE DEFICIENCY

18.10.1. Measurement of T4 (free) in the Plasma

Only a small fraction of the T4 in plasma is unbound. This fraction, however, represents the active thyroid hormone. Thus, measuring free T4 (fT4) as opposed to total T4 is using a biomarker for the amount of hormone performing some useful function within the system.

18.10.2. Measurement of TSH levels in Plasma

A deficiency of T4 and T3 will cause the pituitary gland to synthesize and release more TSH into the blood. Therefore, high plasma

TSH values, at least above a resting average, would indicate unbound T4 and T3 are at low levels, which in turn could be due to a low iodine intake.

18.10.3. Urinary Iodine Excretion

The level of iodine in the urine of an adult free living subject is about 100–200 μg/L. A level below 20 μg/L would be considered severely deficient in iodine and greater than 200 μg/L would be consistent with excessive intake. Ranges in between are moderate (20–49 μg/L) and mild (50–99 μg/L).

18.10.4 Iodine Deficiency Disorders with Age

18.10.4.1 Fetus and Neonate

Miscarriages, stillbirths, congenital abnormalities and increased perinatal and infant mortality have been associated with iodine deficiency during pregnancy. Incidences of neonatal hypothyroidism and neonatal goiter have also been reported. As the child matures, there is an increased risk of neurological and hypothyroid cretinism, which is characterized by mental deficiency, deafness, and dwarfism.

18.10.4.2. Adults

In adults, the symptoms of iodine deficiency center on the goiter, with complications that include hypothyroidism and impaired mental function.

18.11. TOXICITY

The thyroid gland shows amazing resilience. Even at intake levels approaching 30 mg per day, the thyroid gland is functionally active. High dietary iodine, however, disrupts regulation, particularly the level of thyroid stimulating hormone (TSH). High blood iodine will shuts down the Na/I^- symporter and block the release of T4 and T3 from the thyroid. Nonetheless, a person who regularly takes in high iodine from food sources does increase the risk of developing Graves disease (hyperthyroidism) or Hashimoto's disease (hypothyroidism).

18.12. SUMMARY

All the functions of iodine center on its requirement for thyroid hormone biosynthesis. Proteins play an important role in the post-absorption transport and assimilation of iodine. Nearly all of the iodine absorbed is transferred to the thyroid gland exclusively for the production of thyroid hormones. The two hormones, T4 and T3 arise from the proteolytic cleavage of iodinated thyroglobulin. To access the protein the iodine is drawn into the follicle cells by a TSH-sensitive sodium/iodide symporter (NIS). Iodination occurs when the two components, iodine and the protein, merge at the apical surface. To release the hormone the iodinated protein is broken down by lysosomal proteases in the colloid. In the blood the thyroid hormones bind to a series of proteins that differ in their capacity to engage T4 and T3. An iodine deficiency in foods tends to reflect the level of iodine available to the plant or animal. Sea foods from a high salt aqueous environment are the richest source of iodine. Animal proteins tend to be low in iodine because muscle is not an iodine-rich tissue. Soil conditions can be improve by iodination treatment. The two most common symptoms of a severe deficiency are goiters and cretinism. Neonates up to adults can be susceptible to these deficiencies. Inherited conditions of hyperthyroidism (Graves disease) and hypothyroidism (Hashimotos disease) are testaments to overactive or underactive, respectfully, conditions of the thyroid gland.

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18.14. PROBLEMS

1. Based on the average values shown in Table 18.1, how much meat or cheese must be consumed to meet the RDA for iodine? Recall, a microgram is one-thousandth of a milligram and there are 2.2 pounds in a kilogram.

2. Repeat the same analysis as problem 1, assuming your meal this time is seafood and mushrooms.

3. Does the iodine that enters the follicle cells have the same chemical form as the iodine that becomes bound to the tyrosine residues in thyroglobulin? Explain your answer.

4. What is a goitrogen? What would you say to a friend who was told to eat more soybean foods to get more iodine in the diet?

5. Show how T4 and T3 differ structurally. Can T3 be used to make T4? Can T4 make T3? Explain. If you said yes to one or both, what enzyme is needed to carry out the reaction?

6. How does thyroid stimulating hormone, which is made in the pituitary, activate the biosynthesis of thyroid hormone? What controls the levels of TSH in the blood?

7. What source of energy drives iodine uptake into the follicle cells? Explain.

8. Suppose as a practicing dietitian you were challenged to determine if a patient was at risk of developing a goiter. What is the first thing you would want to know before recommending a dietary intervention? What information would allow you to make this assessment?

CHAPTER 19

Fluorine (fluoride)

FLUORIDE is an integral component of calcified tissues, which explains the interest of fluoride in foods. Treating fluoride as an essential mineral, however, is not without controversy. Lacking a serious disease state or physiological impairment attributable to its absence from the diet has led some to question if fluoride meets the criteria for essentiality. A counter argument cites tooth decay and dental caries as a disease whose impact is narrowed by fluoride. Fluoride also has toxic properties that have raised the alarm about too much fluoride from fluoridated food supplies and drinking water. Despite its limited arena of action, fluoride nutrition and metabolism continues to occupy the interests of a general public concerned with osteoporosis and hip fractures. Research has focused on fluoride-calcium interactions at the level of intestinal absorption and in calcified tissues. Because of its omnipresence in toothpaste and mouth rinse, fluoride deficiency seldom occurs, whereas excess exposure is a concern that has led some to question if fluoridation is a safe practice.

19.1. HISTORY AND EARLY DEVELOPMENTS

Fluoride's history of biological interest goes back to fluoridation as a means to prevent tooth decay. The auspicious beginnings, however, were delayed when Frederick S. McKay, a dentist, showed fluoride in drinking water from a natural well caused brown-stained, mottled tooth enamel. Rather than dismissing fluoride outright, H. Trendley Dean, a dental officer of the U.S. Public Health Service, provided evidence that fluoride

up to 1 ppm in water—about one-tenth the amount in the natural water—did not cause mottling of the enamel but instead significantly lessened the incidence of dental decay. In effect, preventing dental carries formed the basis for designating fluorine an essential element. Following Dean's observations, Gerald Cox, a biochemist and industrial scientist, recommended that fluoridation of drinking water should be made public policy in the U.S. These observations led to fluoridating city water reservoirs as a means to control a dental scourge, but at the same time raised the alarm that too much fluoride is a hazard that also needs to be addressed.

19.2. CHEMICAL PROPERTIES

Fluorine is a gas that does not occur naturally. Fluoride ion, its halide derivative, is a member of the halogen (salt formers) family of elements and like chlorine, iodine and bromine, is a highly reactive Group VII element. Its small atomic radii gives fluoride ion an immutable –1 charge, together with a strong electronegativity with the potential to form highly polar chemical bonds. The strong electronegativity also resists oxidation, which precludes a positive oxidation state. As related to strengthening teeth, fluoride ion, when applied to the surface of enamel, can readily displace the OH^- group of hydroxyapatite, thus making the enamel more resistant to dissociation by acids. Also pertinent is fluoride's attraction to calcium, which depending on amount can affect bone density positively and negatively. The strong attachment to calcium and magnesium also impedes intestinal absorption of fluoride and impacts negatively on the bioavailability of calcium and magnesium ions.

19.3. BIOCHEMICAL PROPERTIES

The biochemistry of fluorine rests mainly with fluoride's remineralization action on enamel and bone. In addition to hardening teeth by forming fluoroapatite [$Ca_5(PO_4)_3F$], fluoride also inhibits the action of enolase, an enzyme in the glycolysis pathway, limiting production of lactic acid in acid-producing bacteria.

19.3.1. Fluoride Action on Teeth

To fully appreciate fluoride's protective action on tooth enamel, it is

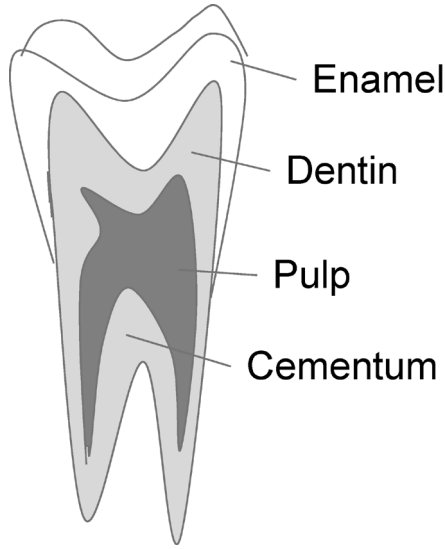

FIGURE 19.1. *Main components of a tooth. Enamel makes up the dense, outermost portion of the tooth crown, resting on a layer of porous dentine. Hydroxyapatite forms the basic crystalline substance of enamel and dentin. From this diagram, it is evident that fluoride treatment will be directed primarily at the enamel and dentin components of the tooth and have little effect on the pulp or cementum.*

necessary to first discuss the anatomy of the tooth. Basically, the four components that make up the tooth—the enamel, the dentin, the cementum and the pulp—differ in their composition, hardness and surface exposure. The enamel is the hardest mineralized component and is constantly bathed by the saliva in the oral cavity. Forming the outer covering of the tooth, enamel is slightly porous and rests on a foundation of dentin. Unlike enamel, dentin contains collagen, a connective tissue protein, which aids in supporting the enamel. Although dentin is more porous, the hydroxyapatite in dentin is shielded from fluid exposure and, like enamel, has no blood supply. Thus, exposing enamel or dentin to fluoride can only be by topical application and not systemically. The other two components, the cementum and pulp, make up the vascularized core of the tooth. The cementum is a deeper calcified layer that covers the root. The pulp, whose main component is collagen, makes up the core of tooth. These features are illustrated in Figure 19.1.

19.3.2. Fluoride Action on Bone

Although tooth decay is the concern, nearly all the fluoride in the

system (99%) has been estimated to be in bone. This finding correlates with the natural tendency of calcium to attract fluoride ions. Bone, like teeth, is richly endowed with crystalline hydroxyapatite which, as noted previously, produces fluoroapatite upon contact with fluoride ions. It seems reasonable to consider fluoride remineralization a means by which the crystalline density of bone is strengthened. This, however, is not always the case. Fluoride's impact on bone depends on the type of bone. For example, trabecular bone, which makes up the axial skeleton (pelvis, ribs, and spine)—as opposed to cortical bone in the appendicular skeleton (hips, legs and arms)—is more prone to react with fluoride. Testing fluoride exposure in bone can be performed by DXA (dual energy X-ray absorptiometry), an effective tool for diagnosing bone density (Figure 19.2). A DXA test gives a T-score which correlates directly with risk of fracture.

Although fluoride ions readily intercalate into bone structure, the bone strength may be unaltered and in some cases may even be weakened by too much fluoride. Using demographic data, Yi *et al.* (2001) found a U-shaped curve when fluoride in drinking water was correlated with the incidence of total bone fractures in six human populations in China (Figure 19.3). Water fluoride levels below or above 1.0 ppm made the bone more susceptible to fracture. This rather surprising discovery led to the conclusion that fluoride exposure has an optimal level of about 1.00–1.06 ppm in preventing bone fracture. Deviations on either side of this figure increases—rather than decreases—susceptibility to bone fractures in a populace as a whole.

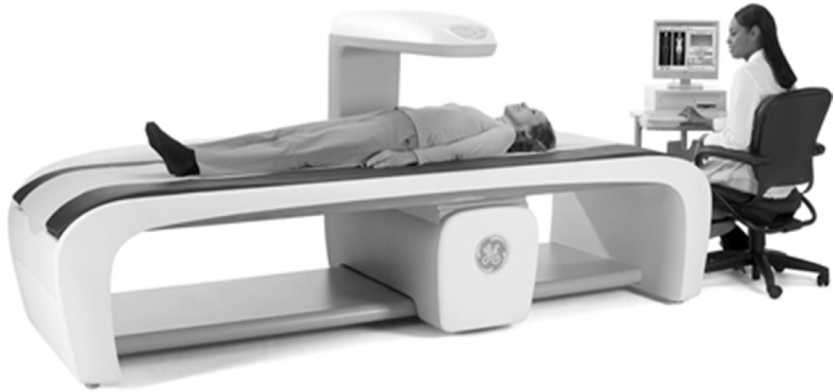

FIGURE 19.2. *Dual Energy X-ray Absorptiometry (DXA) of Subject Undergoing Bone Density Test.*

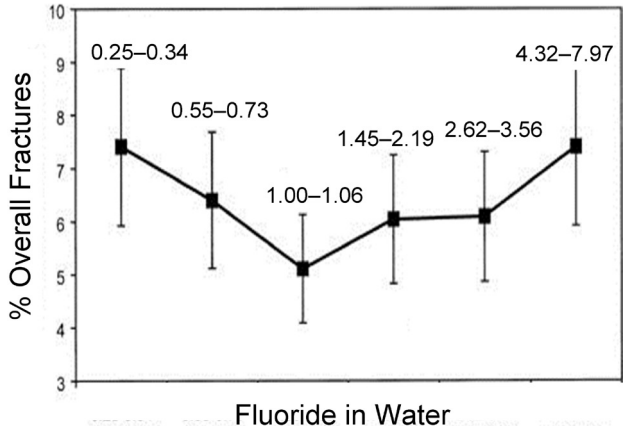

FIGURE 19.3. *Correlating the Incidences of Bone Fractures with Fluoride Concentration in Drinking Water: a Population Study (Adopted from Yi et al., 2001).*

19.4. NUTRITION

19.4.1. Food Sources

Suffice to say that most food contains fluoride, but in amounts less than 50 mg in 100 grams. The more common food source, therefore, is drinking water, where fluoride occurs naturally. The fluoride concentration of spring water may vary from 0.1 ppm (microgram/ml) on the low end of the scale to as much as 10 ppm. Plants with an ability to leech fluoride from soils and store the mineral in leaves are also good sources. Tea leaves is an example of the latter. Other sources include canned sardines (with bones) and fish and chicken.

19.4.2. Recommended Intake

Table 19.1 shows the age-dependence of adequate intake (AI) and upper limit (UL) on fluoride at different stages of life. AI values for subjects over 7 months of age reflect levels required to prevent dental caries; UL values reflect risk of signs of skeletal fluorosis. The periods of greatest concern is in infants and young adults when bone is continually being formed or enamel is being laid. At these times, fluoride exposure can have the greatest impact on the crystallization processes. In the adult, adjustments made between males and females reflect body

TABLE 19.1. Dietary Reference Intake for Fluoride for Different Age Groups.[1]

Age	AI		UL
	Male (mg/day)	Female (mg/day)	
0–6 mo	0.01	0.01	0.7
7–12 mo	0.5	0.5	0.9
1–3 yr	0.7	0.7	1.3
4–8 yr	1	1	2.2
9–13 yr	2	2	10
14–18 yr	3	3	10
19–30 yr	4	3	10
31–50 yr	4	3	10
51–70 yr	4	3	10
>70 yr	4	3	10
Pregnancy			
<18 yr		3	10
19–50 yr		3	10
Lactation			
<18 yr		3	10
19–50 yr		3	10

[1]Data are taken from the 2006 Dietary Reference Index-Institute of Medicine of the National Academy of Sciences.

size and bone structure. Pregnancy and lactation have little effect on the fluoride requirement.

19.5. DIGESTION AND ABSORPTION

Fluoride absorption, although mainly passive, is very efficient. Like other minerals, the chemical form and food environment have the greatest impact. In drinking water, fluoride is nearly 100 percent absorbable, whereas in foods it may be between 50–80 percent absorbable. Absorption occurs from the stomach and all along the upper intestine. Divalent cations are a major impediment to absorption, specifically calcium and magnesium, which can form insoluble complexes with fluoride ions and delay or block fluoride ion passage across the intestinal wall. Once in the blood, the fluoride is rapidly ensconced by bone. Some fluoride is also stored in soft connective tissue. Fluoride ions show little tendency to accumulate in the blood.

19.5.1. Fluoride Homeostasis

Balance studies have shown that adult humans excrete about 50–60% of the fluoride consumed each day. Fluoride not excreted is stored in tissues, primarily bone. As a consequence, fluoride accumulates over time and is released when bone undergoes resorption.

19.6. FLUORIDE TOXICITY

19.6.1. Fluorosis

Fluorosis is a cumulative condition resulting from fluoride overexposure. Enamel and bone in the process of forming are highly sensitive to fluoride, which places infants particularly at risk. Under normal conditions, tooth enamel has a smooth, white glossy appearance. Depending on exposure time and amount, dental fluorosis is seen as a yellowing of the teeth, and in advanced cases as mottled, pitted enamel with yellow-brown stains. According to an index provided by H. Trendley Dean, fluorosis first shows up as white spots on the enamel which gradually begins to cover the tooth surface (Figure 19.4). Under mild conditions, these opaque white areas cover less than 50 percent of the tooth. As the condition progresses, the entire surface is affected and accompanied by pitted areas with brown stains. These conditions are permanent and cannot be reversed by lower exposure or without dental intervention.

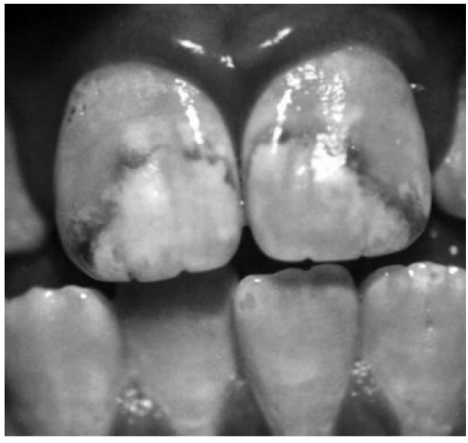

FIGURE 19.4. *Dental Fluorosis Affecting Tooth Enamel.*

19.6.2. Inhibition of Metabolism

Inhibiting the activity of the glycolytic enzyme enolase was one of the earliest negative actions attributed to fluoride. Suppressing the activity shut down production of lactic acid, a major end product of the anaerobic pathway. Inhibiting lactic acid in Streptococcus mutans, the bacteria most closely linked with tooth decay, is considered an alternative action in fluoride's protective effects against tooth decay. Its attraction to calcium ions also puts enzymes that utilize calcium as a cofactor subject to impairment by fluoride. These would include enzymes in the pathways of lipid metabolism as well as hormones and other factors that control gene expression and cell signaling (Chapter 11). It should be made clear that fluoride inhibition of these factors has been observed under controlled conditions. Their significance, therefore, is in showing a potential for disruption of metabolic processes, not an actual occurrence. Evidence does not support fluoride having deleterious effects when given at the level of fluoride in foods or fluoridated drinking water.

19.6.3. Fluoride Exposure in Neonates

Milk given to breast-fed infants has a fluoride content of less than 0.005 ppm (mg/liter). Some would argue that this level of fluoride is safe for neonates. In contrast, infants fed formula milk can be exposed to fluoride levels 200 times higher (1.0 ppm) if fluoridated water is used to reconstitute the formula. A further concern is that infants excrete only about 20% of the intake and store the rest in the system, primarily in bone.

19.7. SUMMARY

Combining fluoride's intrinsically high chemical reactivity with a small atomic radius gives a rationale as to why fluoride is able to displace hydroxide ion from hydroxyapatite and form fluoroapatite. Fluoride's action in a biological system thus appears to be one of remineralization of the hydroxyapatite foundation in bones and teeth, rendering the complex as a whole more resistant to acid. Lowering the incidences of dental carries may have been the main factor in designating fluoride an essential nutrient. It should be noted, however, that fluoride exposure cannot stop all teeth from decaying, nor is it capable of strengthening

bone despite modifications to these structures. According to the American Dental Association, a 50–60 percent reduction in dental caries has been seen since World War II, when fluoridation of communities' drinking water first began. The impression that fluoride can only benefit by such a step has been challenged by those who cite deleterious effects of fluoridation, particularly in the young. Since we know very little about fluoride's movement in the blood and uptake into cells, one has to be wary of not treating fluoride as just another halide anion.

19.8. REFERENCE

Li, Y., Liang, C., Slemenda, C.W., et al. (2001) Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. *J. Bone Miner. Res., 16*(5):932–939.

19.9. PROBLEMS

1. How would you determine the adequate intake (AI) for fluoride? Explain what you would measure. How would you quantify results to determine if there is a correlation of what you measured with fluoride intake? Based on your answers, for fluoride which parameter is more readily ascertained—adequate intake or upper limit (UL)? (Hint: it's not always teeth you should be thinking of)

2. Why is water-borne, as opposed to food-borne, fluoride a better choice for preventing tooth decay?

3. What is the approximate concentration of fluoride in drinking water for those cities that opt to fluoridate their water reservoirs? Based on this value, how much water must a person 20 years of age drink to meet his/her adequate intake level for fluoride?

4. Who is receiving a higher dose of fluoride, a coffee drinker or a tea drinker?

5. Suppose you wanted to do an epidemiological study on a global scale to see if fluoride in plants lowers the incidence of tooth decay. What country would you expect to surpass the U.S. in having lower dental caries? Explain why you chose that country.

6. Explain why fluoride does not contribute to the electrolyte pool in the blood.

7. What is known about fluoride's movement in the blood and uptake by tissues? Basically, is there any evidence for a protein-bound form of fluoride and if so, how strong would be the protein-fluoride bond?

8. Regarding the dangers of fluoridation, why are young children regarded as a high-risk group?

CHAPTER 20

Chromium

CHROMIUM is one of the scarcest minerals in foods and the human body. Despite its rareness in biological systems, some deem chromium the most important trace metal in higher animals. Chromium plays an important role in the management of carbohydrate and lipid metabolism, which in effect, underscores chromium's function in preventing or treating diabetes. There is still conflicting data as to the efficacy of chromium supplements in reversing symptoms of diabetes and glucose dysfunctions. The onset of Type 2 diabetes has been linked to lower levels of chromium in the blood and elevated levels in the urine. As one ages, chromium levels in the tissues and organs tends to decrease. Recent advances have shown that insulin action on cells may be potentiated by chromium. Whereas the observation has supported chromium's designation as nutritionally essential, it still leaves many questions unanswered.

20.1. HISTORY AND EARLY DEVELOPMENTS

Louis-Nicolas Vaquelin, a French chemist, is credited with the discovery of chromium. Vaquelin's 1897 discovery came when an unknown compound was isolated from crioite, a lead-chromium ore. Because of a resemblance to the ore chromite, the unknown was called chromium. The pathway to the discovery of chromium as a biological essential mineral was not as straightforward. About 50 years ago, Walter Mertz and Klaus Schwarz showed that feeding torula yeast to rats

affected the rate of glucose uptake (glucose tolerance) from the blood. Earlier, these investigators had shown a torula yeast diet caused liver necrosis in rats and identified selenium as the missing factor. Selenium added to the yeast, however, did not correct glucose tolerance. Contributing to the perplexity, Impaired tolerance was not always seen in the experiments. A baffling observation was when the glassware used in the study was washed in a dichromate-sulfuric acid solution, impaired glucose intolerance was never observed, leading the investigators to suspect chromium as a possible factor. Subsequent testing of a multitude of heavy metals eventually singled out chromium as the only mineral that restored normal tolerance. Suppressing diabetic-like symptoms in patients undergoing prolonged total parenteral nutrition has provided the strongest evidence for chromium's necessity in humans. Thus, ground work was in place for a multidisciplinary effort to learn more about a role for chromium in glucose uptake into cells, focusing in particular on events controlled by insulin.

20.1.1. Chemistry

Chromium sits between vanadium and manganese in first transition elements. Its electronic configuration $[Ar]4s^1 3d^5$ gives rise to compounds with a variety of valence states, although +3 and +6 are the most common in biological systems. Hexavalent chromium is almost always linked to oxygen and because if lacks all 6 outer shell electrons, is a powerful oxidizing agent. Such is exemplified by the dichromate anion $(Cr_2O_7^{2-})$, a powerful oxidant and active factor in some cleaning solutions. Humans and animals possess enzymes that reduce Cr(VI) to Cr(III) for safe usage, but not without generating free radicals as byproducts in the process, which is another way Cr(VI) toxicity is expressed.

20.1.2. Biochemistry

A search for chromium complexes that mediate chromium action has been at the forefront of chromium research. Fittingly, attempts to link chromium with insulin action has been the strategy adopted by most investigations. Schwarz and Mertz (1959) isolated a low molecular weight Cr(III) compound from brewer's yeast and, believing it to be the biologically active form, named it glucose tolerance factor (GTF). Although never in pure form, GTF was projected to be composed of

trivalent chromium bound as an octahedral complex to nicotinic acid and three amino acids known to be present in glutathione (glutamate, glycine and cysteine). Subsequently, Yamamoto *et al.* (1988) reported the isolation of a small, naturally-occurring, chromium-containing peptide, referred to as a low molecular weight chromium-binding substance (LMWCr), from bovine colostrum. LMWCr, since named *chromodulin*, is a carboxlylate-rich oligopeptide with a molecular weight of about 1,500, composed of glycine, cysteine, aspartate and glutamate and capable of binding four trivalent chromium ions (Vincent, 2004). Of its four chromium atoms, three are believed to exist in a trimetal array linked through oxygen with the carboxyl groups on the peptide providing the ligand-binding sites. Unlike GTF, recent studies have shown that LMWCr can engage an insulin receptor on the cell surface and could be a factor in insulin signaling (see below).

20.2. NUTRITION

Most foods in the human diet contain only trace amounts of chromium. All told, the body load of chromium is estimated to be about 4–6 mg. Calculations of chromium intake based on food database values cannot be relied upon to give an accurate assessment of chromium status. Moreover, food processing procedures diminish the amount of chromium in foods, making the computed amount lower than the actual amount. Lowering chromium by processing, however, can be countered by chromium gleaned from stainless steel utensils used in the processing procedures. Adult males consume 40–50 micrograms per day, whereas adult females take in 25–30 micrograms per day; both values suggest that the average daily intake meets the recommended level. The data speak strongly against a chromium deficiency in the population as a whole.

20.2.1. Food Sources

Of the different food categories, high bran cereals, broccoli, and grape juice tend to be the richest sources of Cr(III). Egg yolk, nuts, green beans, brewer's yeast, oysters, potatoes and red wine are also good sources. Dairy products tend to be on the low end, with meats and poultry in between and vegetables and fruits showing the greatest variation. It has been estimated that at best, food sources provide no more than 1–2 micrograms of chromium per serving.

TABLE 20.1. Symptoms of Low Chromium Intake.

High insulin resistance (hyperinsulinemia)
Low insulin sensitivity
Elevated blood glucose
Syndrome X (hypertension, elevated cholesterol, high triglycerides, low HDL)

20.2.2. Deficiency Symptoms

Insufficient intake of chromium is a rare occurrence. Patients undergoing prolonged total parenteral nutrition (TPN) , however, have been known to show signs symptomatic of a diabetic-like condition if chromium is not added to the TPN solution. Table 20.1 lists some of the preclinical symptoms resulting from insufficient chromium in the diet.

20.2.3. Recommended Intake

Table 20.2 shows the Adequate Intake (AI) values for chromium at

TABLE 20.2. Recommended Adequate Intake for Chromium with Age and Gender.

	AI	
	Male (µg/day)	**Female (µg/day)**
Children, adolescents and adults		
0–6 mo	0.2	0.2
7–12 mo	5.5	5.5
1–3 yr	11	11
4–8 yr	15	15
9–13 yr	25	21
14–18 yr	35	24
19–30 yr	35	25
31–50 yr	35	25
51–70 yr	30	20
>70 yr	30	20
Pregnancy		
<18 yr		29
19–50 yr		30
Lactation		
<18 yr		44
19–50 yr		45

various ages and physiological conditions. Data are from the Food and Nutrition Board at the Institute of Medicine. This same board concluded that chromium in foods or supplements does not represent a health hazard and therefore no tolerable upper intake level (UL) was set. Gender differences do not become a factor until late in the adolescent period. Since a quart of breast milk is estimated to contain about 0.2 micrograms of chromium, a child fed this amount of breast milk per day meets the AI value for a child less than six months of age.

20.3. DIGESTION, ABSORPTION AND METABOLISM

Obtaining chromium as a nutrient from a food source is a serious challenge to the system. Estimates range between 0.4% to 2.8% of the total in the diet is absorbed. The higher values reflects the absorption percentage when picolinate-chromium is the source of the chromium (Figure 20.1). High fiber, phytate, and carbohydrates tend to suppress absorption efficiency. On the other hand, oxalate, vitamin C and amino acids enhance chromium absorption.

20.3.1. Chromium Absorption in Diabetics

When given Cr(III) as a nicotinate or picolinate complex, subjects

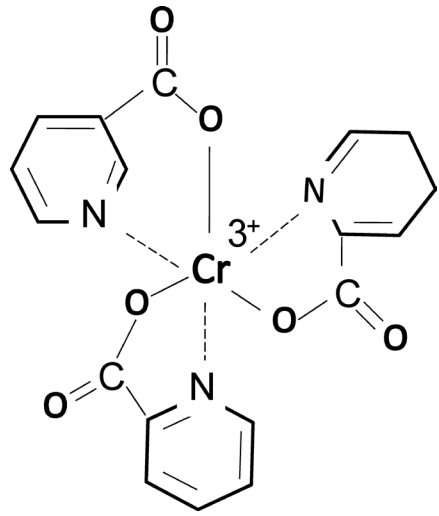

FIGURE 20.1. *Structure of Chromium (III) Picolinate.*

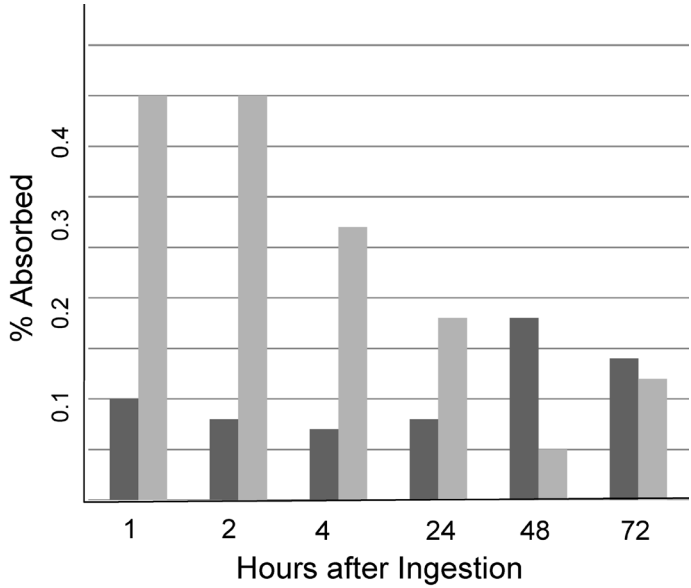

FIGURE 20.2. Comparison of Chromium Absorption Profiles Between Normal and Type 2 Diabetic Subjects. Dark bars represent the absorption percentage of chromium picolinate in normal subjects at various times after receiving a glucose load; light bars are for individuals diagnosed with Type 2 Diabetes.

diagnosed with Type 2 diabetes as compared to non-diabetic subjects have a higher absorption efficiency of chromium, especially in the first two hours after a glucose feeding (Figure 20.2). In contrast, non-diabetic subjects show a steady uptake over the first 24 hours, with only a small increment after 48 and 72 hours.

20.3.2. Post Absorption Metabolism

In a series of actions yet to be clarified, post-absorption chromium is distributed to the tissues as a complex with transferrin. Whether chromium binds to the iron binding sites on transferrin—suggesting the possibility of competition with the more prevalent iron—is unknown. Liver, spleen, kidneys and thyroid glands receive most of the chromium absorbed.

20.4. EVIDENCE FOR ESSENTIALITY

Many of the experiments that have tested the effects of chromium

TABLE 20.3. Results of Treating Type 2 Diabetic Patients with Chromium Picolinate.

Symptom
Improved glucose tolerance
Decreased insulin output
Decline in cholesterol and total lipids
Increased insulin sensitivity
Lower glycosylated hemoglobin levels
Lower fasting level of insulin
Lower fasting level of glucose

have used a picolinate complex of chromium. The complex does not occur naturally in foods nor is it a product of chromium metabolism. The use of an organocomplex, as opposed to a chromium salt, has some advantages, one of which is a higher absorption efficiency. Protocol to achieve a response with chromium-picolinate extends into months with daily doses of about 1,000 micrograms, or about 300 times the average intake. Table 20.3 lists a series of symptoms that result from administering chromium supplements to subjects with Type 2 diabetes. The effects observed were more pronounced in subjects that showed a heightened insulin resistance or poor insulin sensitivity, as well as impaired control of carbohydrate metabolism. End points for these experiments have generally focused on restoring serum glucose to normal levels. While clinical trials have shown persons afflicted derive the benefits shown in Table 20.3, chromium picolinate does not have the same beneficial impact for non-diabetic subjects. The data, therefore, are biased towards an abnormal state of insulin dependence and resistance.

20.5. CHROMIUM TOXICITY

The extremely low quantities of chromium in a food source speaks against the diet as being a factor in chromium toxicity. Rather, chromium supplements to a normal diet pose a greater risk. It has been estimated chromium picolinate supplements are almost as widespread and sought after as calcium supplements. This raises the question as to the relative safety, especially when chromium is presented in a form that is more absorbable and more readily taken up by peripheral cells. What seems clear from these report is chromium toxicity cannot be regarded as an

TABLE 20.4. Symptoms of Chromium Toxicity.

Symptom
Cultured Cells
Chromosomal Damage
Mutation in DNA
Mitochondrial damage
Induction of apoptosis
Free-radical cleavage of DNA
Animal Studies
Elevated levels of 8-hydroxydeoxyguanosine (DNA damage) in rats
Elevated levels of peroxidized lipids in rats
Lethal mutations in fruit flies
Sterility and arrested development in fruit flies

acute response, but rather one brought about by a prolonged exposure to the complex and at levels far in excess of recommended amounts.

Table 20.4 lists a series of symptoms ascribed to chromium toxicity, paying heed to the model used to generate the data. Cells suspended in growth medium and animals in a state of rapid growth have shown toxic effects when chromium picolinate is added to the culture medium or diet, respectively. In some instances, it has been possible to discern specific metabolic changes leading to the pathology. For example, chromium can act as a mutagen that causes chromosomal damage in culture cells. Mitochondria also become a target for the chromium. Chromium's presence in a cell could lead to an apoptotic response, resulting in cell death. In live animals there is evidence for chromosomal damage, lipid peroxidation, arrested development and sterility arisng from metabolites generated from a chromium-picolinate complex. Moreover, evidence supports a dissociation of chromium from the picolinate complex as a likely occurrence upon entering a cell. While there has been no direct connection to humans, symptoms observed in cell cultures or model animal systems have shown chromium has pathogenic potential that can lead to cellular and system dysfunctions. This concern has caused the FDA to weigh a decision as to whether usage of chromium picolinate should be regulated.

20.6. CHROMIUM AND INSULIN SIGNALING

Within the past decade, a strong effort has been made to identify the

mechanism of chromium's impact on insulin action. Because events appear to extend beyond promoting glucose uptake, the insulin signaling pathway has also been considered a target of chromium action. The working model is shown in Figure 20.3. First, insulin binding to the alpha subunit of its receptor triggers a conformation change in the beta subunit of the receptor, activating the beta subunit's tyrosine kinase enzyme, which autophosphorylates select tyrosine residues. Second, binding to the receptor also stimulates the metalation/activation of apochromodulin, resulting in chromium-bound chromodulin engaging the receptor. Three focal points to consider are: (1) that insulin alone can affect a signaling response; (2) filling the chromium binding sites renders chromodulin functionally capable of binding to the insulin receptor; and (3) chromodulin can only bind after the receptor has been activated by insulin. Internal signaling proteins are also targets for the receptor's tyrosine kinase activity. Referred to as insulin receptor substrates (IRS), these internal signaling proteins upon phosphorylation perpetuate the signal by activating other signaling proteins, one of which is PI(3)-K (phosphoinositol-3 kinase). IRS-1(P) binds to the regluatory subunit of PI(3)-K and as a complex with the latter initiates a series of glucose-related metabolic actions as shown in Figure 20.3. Glucose transport into the cell is stimulated by translocating GLUT4 to the membrane surface (Chapter 6). Countering insulin signaling (basically shutting off the re-

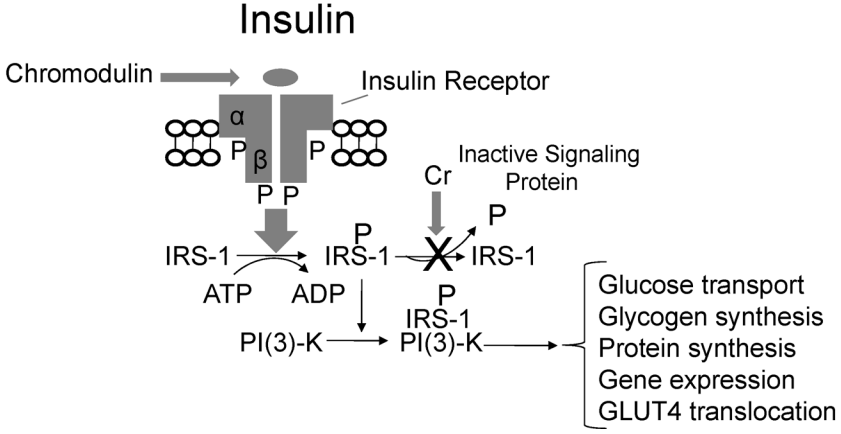

FIGURE 20.3. *Insulin Signaling Pathway in the Cell. Insulin binding initiates an autophosphorylation of the beta subunits of the insulin receptor by the receptor's tyrosine kinase. Chromium as chromodulin is postulated to potentiate the action of insulin after the receptor has been activated as well as suppress the phosphatase enzyme that inactivates IRS-1.*

the response) are cell phosphatase enzymes that remove the phosphate groups from IRS-1. Insulin-receptor substrates are now regarded as a family of proteins that control downstream events in insulin action. As many as 6 have been characterized. How chromium's presence or absence may affect other members of the IRS family is unknown.

20.7. SUMMARY

As a micromineral, chromium's presence in a food source is barely at the level of detection. Its scarcity in food is countered by the relatively low amounts needed to sustain health, which gives some assurance that chromium deficiency is a rare occurrence in humans. Like other microminerals in this category, valence-state related toxic properties must be considered when dealing with chromium metabolism. Chromium's transport, storage and excretion are hampered by a very low bioavailability at both intestinal absorption and when used internally. Although it may be valid to conclude that chromium is a key factor in cellular metabolism of carbohydrates and lipids in humans and therefore should be deemed nutritionally essential, chromium action eludes a factual insight and leaves a void in an understanding of mechanism. This void has been partially filled by the finding of a biochemical factor that requires chromium to mediate the action of insulin on a cell. While many studies have reported a positive results of chromium supplements in reversing symptoms of diabetes, the conclusions have been biased by the high pharmacological doses and long exposure times required to show an effect. It is still not known if a non-diabetic subject derives any benefit from chromium, perhaps acting in the capacity of a preventative to the development of Type 2 diabetes. More recent information on chromium as an insulin potentiator and mimetic of insulin signaling holds much promise in gaining more insight into the mechanism of chromium action at the molecular level. Such information will provide insight into how a mineral that is barely detectable in a food source and within the system has a profound effect on life-sustaining functions of the system.

20.8. REFERENCES

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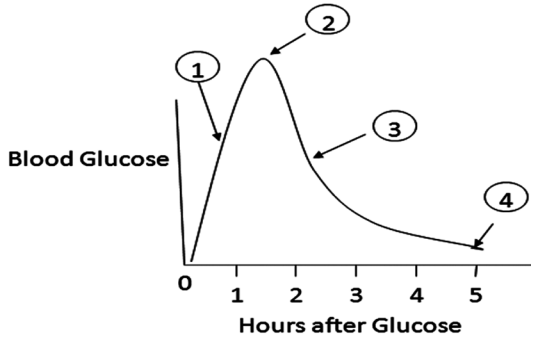
20.9. PROBLEMS

1. Offer an explanation as to why Cr(VI) is more toxic than Cr(III).

2. Below is a glucose tolerance curve. Based on the figure, which part of the curve would be expected to be altered when the diet is deficient in chromium? Select the number.



3. Which number in the figure provides the best view of insulin action?

4. Is it correct to assume that knowing all the functions of insulin will help us understand the nutritional essentiality of chromium? What research observations have tended to question the validity of that statement?

5. Safety issues aside, as a physician, what is your response to a patient who says "I take chromium picolinate because I don't want to get diabetes"?

6. As a nutritional consultant, would you ever recommend a high chromium food source to a patient who has a family history of Type II diabetes? Give the pros and cons.

7. What is the advantage of correcting a chromium deficiency with chromium picolinate as opposed to a chromium salt?

Cobalt and Molybdenum

THE two minerals discussed in this chapter have well-defined biochemical factors underscoring their presence in living systems. Cobalt as an inorganic ion is required by some species of bacteria and algae, but is not essential for plants. Animals and humans link cobalt inextricably with vitamin B12. Although molybdenum has an assigned RDA, the consequences of a dietary deficiency are less understood.

21.1. COBALT

21.1.1. History and Early Insights

Georg Brandt, a Swedish chemist, in the 18th century reported the discovery of a new element that had the properties of a semi-metal. Brandt was able to connect the new element with the blue color of glass, eliminating bismuth as the long suspected pigment. A biological connection came almost two hundred years later with the discovery of a factor in the soil where sheep grazed that stymied their growth and quality of wool. Considerations that this could be due to a toxin were dismissed when it was shown that poor growth could be corrected by enriching the soil with cobalt. In humans, the seminal discovery was the unexpected finding of cobalt in the corrin ring structure of vitamin B12. These studies all pointed to an essential mineral for animals and humans disguised as a vitamin. In time, it was realized that the vitamin expressed cobalt's requirement for essentiality in animals and humans.

21.1.2. Chemistry

Cobalt, atomic number 27, is between iron and nickel in the first transition series of elements. Its electronic configuration [Ar]$4s^2 3d^7$ gives rise to ions with oxidation states of –1 to +4 , although +2 and +3—which emulate the valence states of iron—are the most common in biology. Of the two, the +2 is the more stable. Like iron, cobalt's coordination chemistry is expressed through octahedral complexes referred to collectively as cobamides, which are cobalt-containing corronoids. Vitamin B12, cobalamin, is a corronoid with a methyl, cyano, or 5'-deoxyadenine group bound to the axial position. These give rise to methylcobalamin, cyanocobalmin, or 5′-deoxyadenosylcobalamin, the three main and functionally distinct structures of vitamin B12 (Figure 21.1). Some anaerobes that synthesize corronoids do not possess a similar ability to synthesize a porphyrin ring, implying from an evolutionary perspective that the corrin ring fixture for cobalt may have predated the porphyrin ring for iron.

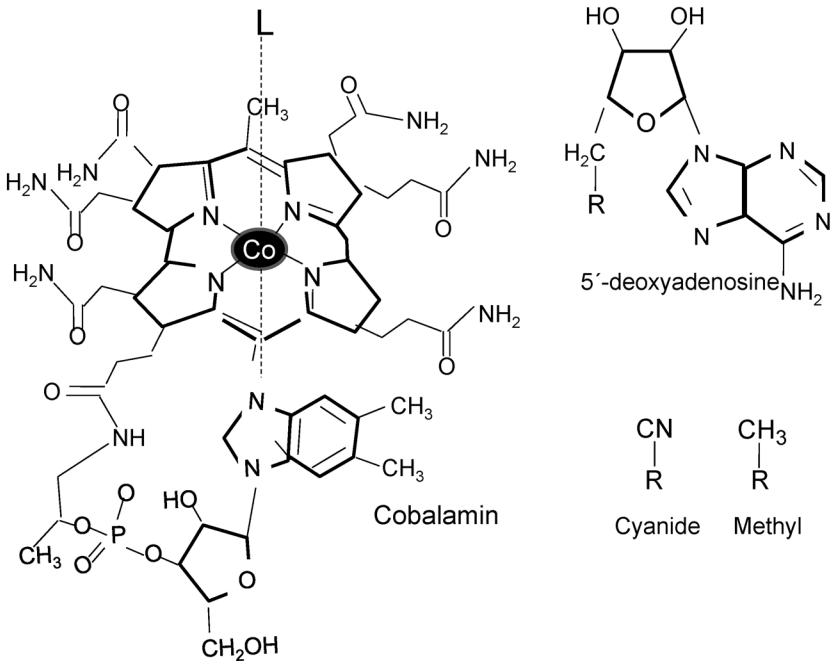


FIGURE 21.1. *Forms of Vitamin B12 in Animals and Humans. The vitamin in nature exists as an octahedral complex with a flat corrin ring and cobalt in the same plane. The axial position marked L signifies positions for binding 5'-deoxyadenosine, cyanide, or methyl groups. The group that binds must conform to the reaction catalyzed by the enzyme.*

21.1.3. Biochemistry

From the foregoing discussion of its chemistry, cobalt's essentiality is inseparable from vitamin B12, either as a cofactor for enzymes that require the vitamin or for microorganisms that synthesize the vitamin as a secondary metabolite. Ruminants require cobalt to build up the rumen population of methanogenic bacteria that synthesize vitamin B12. Not all bacteria species synthesize cobalamin, however, and like humans, must acquire it from the environment.

21.1.3.1. Cobalt (B12) Enzymes

Only a small number of enzymes in animals and humans are known to require vitamin B12 as a cofactor (Table 21.1). Methionine synthase requires methylcobalamin as a cofactor to transfer methyl groups, obtained initially from methyltetrahydrofolate, to form methionine from homocysteine. Using the folate derivative spares S-adenosylmethionine, the primary methyl donor in the cell. More importantly, methylating homocysteine removes an important oxidant in the blood and the folate in the methyltetrahydrofolate, a dead end cofactor, can be recycled back into the pool of active folate cofactors. 5′-Deoxyadenosyl cobalamin is the cofactor for methylmalonyl CoA mutase, the enzyme that synthesizes succinyl CoA from methylmalonyl CoA. This reaction plays a prominent role in lipid and amino acid metabolism, especially propionate, which the rumen digests when odd chain fatty acids are metabolized.

Of those enzymes listed in Table 21.1, methionine aminopeptidase requires special mention. In eukaryotes and prokaryotes, the enzyme cleaves the N-terminal methionine from a newly synthesized peptide chain. Uniquely, methionine aminopeptidase is only one of eight and the only eukaryotic enzyme with inorganic cobalt, not the vitamin, as its cofactor.

TABLE 21.1. Cobalt/Vitamin B12-containing Enzymes.

Enzyme	Cofactor	Reaction Catalyzed
Methionine synthetase	Methyl cobalamin	Methyl group transfer
Methylmalonyl CoA mutase	5'-Deoxyadenosyl cobalamin	Internal rearrangement
Methionine aminopeptidase	Cobalt	Removes N-terminal methionine from nascent peptides

21.1.3.2. Cobalt (B12) Binding Proteins

In addition to enzymes, a series of vitamin B12 binding proteins are known. Although this class of proteins displays no catalytic activity, they are prominent in protecting B12 from destruction and transporting the vitamin to absorption sites in the intestine and beyond. A brief description of some of the more important ones is given below.

21.1.3.2.1. Haptocorrin

Haptocorrin, otherwise known as transcobalamin 1 or R protein, is a glycoprotein secreted into the saliva that binds strongly to vitamin B12 and shields the acid-labile B12 from stomach acid.

21.1.3.2.2. Intrinsic Factor

Intrinsic factor, a glycoprotein produced by parietal cells lining the stomach, mediates intestinal absorption of vitamin B12 by recognizing a receptor on the apical surface of enterocytes. Intrinsic factor receptors line the apical surfaces of the ileum.

21.1.3.2.3. Transcobalamin II

Transcobalamin II (TCII) engages B12 in the cytosol of cells of the ileum and translocates the vitamin to the capillaries on the serosal side of the enterocyte. The protein thus acts as a shuttle for the vitamin. Its action, however, extends beyond the intestine, where transcobalamin II- B12 complex stays intact in the blood in its movement to target cells.

21.1.4. Nutrition

Excluding vitamin B12, humans are believed to take in about 40–50 micrograms of cobalt per day, more than half of which is excreted in the feces and urine. An estimated daily requirement for humans is about 7–15 micrograms, which means the amount of cobalt taken in the diet far exceeds the amount required for adequacy. Milk cows require about 7–20 mg of cobalt per day (1,000 times the human requirement). In understanding the importance of cobalt to the nutrition of animals and humans, it must first be realized that a cobalt deficiency will impinge strongly on the population of microorganisms in the gut and/or rumen. From the standpoint of absorption, however, the production site for B12 in the colon is beyond the absorption site in the ileum. Thus, for

humans to ward off possible deficiencies, the vitamin (as opposed to cobalt alone) provides the greater health benefit.

21.1.4.1. Tissue Levels

A typical 70 kg human has a body load of cobalt estimated to be less than 1.5 mg with the liver, heart and bone having the highest concentration. Such a low amount in toto makes it difficult to discern the specific amounts of cobalt in each tissue. Liver cobalt, for example, reflects stores of the vitamin when the animal has sufficient cobalt in the diet. If the intake is adequate, the tissue levels of the vitamin are steady from birth through aging. If the levels are not adequate, a dietary deficiency of cobalt causes a rapid loss of the vitamin from the tissues. A dietary deficiency of cobalt in sheep is known to affect the liver, kidneys, heart, and pancreas to the same extent. Liver and kidney concentrations of cobalt in sheep, however, are increased substantially by injections of cobalt, presumably by stimulating B12 biosynthesis in the rumen.

21.1.4.2. Food Sources

Seafoods such as shrimp, scallops, clams and oysters are good sources of inorganic cobalt and cobamides. Also included are leafy vegetables, red meat, liver, and milk. Ruminants obtain cobalt from forage crops that take up inorganic cobalt from the soil. Leguminous plants especially exceed other plants in storing cobalt. Although plants appear to show less of a need for cobalt to grow and do not produce cobamides, they nonetheless accumulate cobalt in the leaves and stems, which for some plants may be as high as 100 mg per kg of dry matter.

21.1.4.3. Deficiency Symptoms

There is little concern that a typical human diet will fail to meet health standards of cobalt. A cobalt deficiency, nonetheless, will lead to many disruptions to normal metabolism and physical symptoms of wellness. Table 21.2 categorizes psychological and physiological symptoms that have been observed when dietary cobalt is low. A caveat to consider is that many of the symptoms reflect a deficiency of vitamin B12 and thus impinge on cobalt indirectly. Since the functions of cobalt and the vitamin are basically inseparable, however, the overall interpretation is the same. Because plants neither synthesize nor show a need for B12,

TABLE 21.2. Symptoms of Cobalt (B12) Deficiency.

Physical and Psychological
Dizziness and loss of balance
Painful tingling sensation in hands and feet
Memory loss
Depression
Pathological
Megaloblastic anemia
Impairments in carbohydrate and lipid metabolism
Endemic disease (ruminants)
Impaired biosynthesis of erythropoietin
Neurological defects

individuals who adhere to a strictly vegetarian diet (vegans) could be at greater risk of a deficiency. One of the most lethal B12 deficiency symptoms is pernicious anemia (PA). PA can arise by either a lack of the vitamin in the diet or molecular defects in intrinsic factor protein. The latter include a destruction of the parietal cells in the stomach that synthesize IF or an autoimmune response that inactivates it. Finally, mutations in the gene coding for the factor will negate the effectiveness of IF to perform transport and uptake functions.

21.1.5. Absorption and Metabolism

21.1.5.1. Inorganic Cobalt versus Vitamin B12

The absorption efficiency for inorganic cobalt in the human diet is estimated to be around 25%; the absorption efficiency for vitamin B12, however, may be as high as 80%. There are several reasons for the discrepancy. Since inorganic cobalt represents only a modicum of the total, the critical mass of cobalt needed to perform energy driven transport is compromised. Because Fe^{2+} and Co^{3+} have identical electronic configurations, cobalt must compete with iron for the same entrance site on the apical surface; iron, being the more prevalent mineral, will outcompete cobalt for absorption.

In contrast to inorganic cobalt, vitamin B12 upon ingestion engages a series of proteins that bind and transport the vitamin to the absorption site in the intestine (Figure 21.2). In the pregastric phase, the vitamin binds to Haptocorrin (HT) prior to entering the stomach. The vitamin is labile in acid and by forming a complex with haptocorrin stays intact

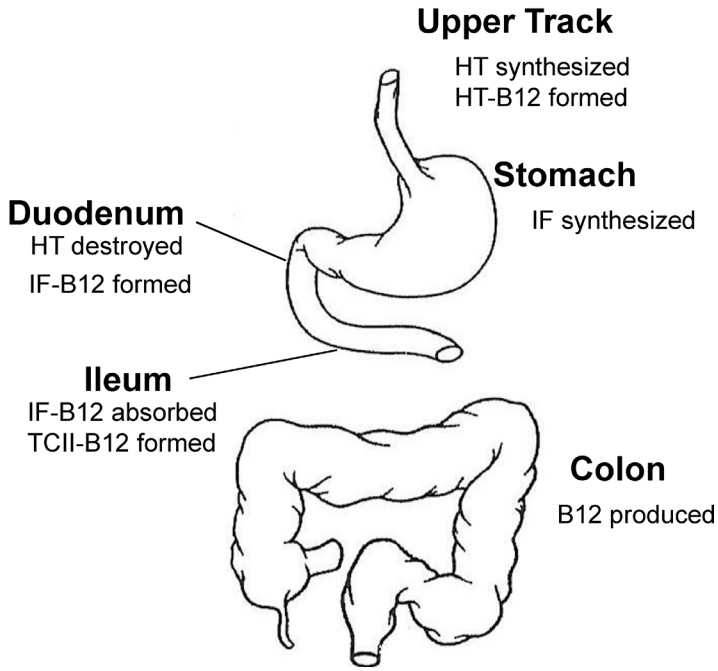


FIGURE 21.2. *Scheme Summarizing Factors in the Absorption of Vitamin B12. Three B12 binding proteins function at different locations in the absorption pathway. Haptocorrin (HT) in saliva gives safe passage of B12 through the stomach. Intrinsic factor (IF) engages B12 after HT is hydrolyzed and transports B12 to the ileum. B12 released from HT in the cytosol binds to transcobalamin II (TCII) for transport to the blood as a TCII-B12 complex.*

as it passes through the stomach into the duodenum. In the postgastric phase the HT- B12 complex enters the duodenum where protease enzymes secreted from the pancreas hydrolyze the HT, freeing the vitamin and making it available to bind to intrinsic factor (IF). Even though IF was in the gastric juice, its weaker affinity for B12 prevented displacing the vitamin from the HT- B12 complex. This rationalizes the need to destroy HT by proteolytic enzymes before B12 can course its way through the intestine as a IF- B12 complex. Such is necessary for the B12 to engage receptors on the absorbing surface of the ileum. Uptake into absorbing enterocytes in the ileum is by receptor-mediated endocytosis analogous to iron uptake via transferrin. Shortly after entering the cytosol, the B12 detaches from the IF- B12 complex and binds to transcobalamin II in the cytosol. Details as to what happen after entrance have not been clarified. What is known is that transcobalamin II (TCII), the third transport protein, binds the freed B12 moiety and transfers it

to the basolateral surface of the enterocyte for effusion into the blood. The vitamin stays firmly attached to the TCII throughout its transits through the cytosol and out of the cell into the blood capillaries, which by the portal circulation delivers the complex to the liver or distributes it to peripheral cells.

21.1.6. Cobalt Toxicity

Cobalt in the environment is considered a health hazard. Because foods contain very little cobalt, and most of what is present is in the form of cobamides, there is less concern that overindulgence in the diet can give rise to a cobalt toxicity. Table 21.3 lists a series of symptoms observed by industrial exposure of cobalt in solid or powdered form. Goiter reflects a reduced production of thyroid hormones (hypothyroidism) brought about by blocking iodine uptake into follicle cells (Chapter 18). Other symtoms reflect hazards to lungs from airborne particles or skin.

21.1.7. Summary

The essentiality of cobalt as a nutrient is not questioned. Different situations arise when one compares the need for cobalt in the human and ruminant diet. In grazing cattle or sheep, the evidence points to an increased need for cobalt in the soil primarily to stimulate the growth of microflora in the rumen that produce vitamin B12. Humans and other non-ruminants have less of a need for cobalt but instead rely on the vitamin. Plants, however, neither synthesize nor use vitamin B12 in their metabolism. Only a limited number of enzymes are known to require a vitamin B12 cofactor. Applying B12 as a cofactor requires either the methyl derivative or the deoxyadenosine derivative. Lipid metabolism uses 5′-deoxyadenosyl cobalamin to convert propionate to

TABLE 21.3. Consequence of Excessive Cobalt Exposure.

Symptom
Goiter
Elevated Hemoglobin
Contact dermatitis
Cardiomyopathy
Asthma
Contact dermatitis

succinyl CoA, whereas regenerating methionine from homocysteine requires methylated B12. These two reactions tend to underscore the functions of vitamin B12 and reveal cobalt's essentiality at the molecular level. The absorption of B12 requires three proteins whose function is to protect the acid-labile vitamin from destruction by gastric juice, chaperone the vitamin to the entrance portals in the ileum, and transfer the vitamin in the blood to the tissues. These proteins provide a scheme for uptake of vitamin B12 into the system and why eukaryotes are more programmed for uptake of the vitamin as opposed to cobalt.

21.2. MOLYBDENUM

21.2.1. History and Early Developments

Molybdenum comes from the Greek word, molybdos, meaning lead. Carl Scheele, a Swedish chemist, however, affirmed that molybdenum was neither lead nor graphite but a new element. The importance of molybdenum in biology did not come to light until the early 1900s. Plants flourished and animals grazing on these plants seemed to benefit when soils were enriched with molybdenum. Thirty years later, the nitrogen-fixing nitrogenase enzyme in azobacter bacteria was shown to have a molybdenum cofactor, which rekindled interest in molybdenum and gave a rationale to molybdenum's importance to plant growth. Its importance to animals and humans came in the form of three oxidase enzymes that required molybdenum for activity. Perhaps the one that received most of the attention was xanthine oxidase, which synthesized uric acid from xanthine in the purine catabolism pathway. Suppressing the enzyme by omitting molybdenum from the diet, however, had no effect on either the rate of growth or the production of uric acid in experimental animals. Suppressed activity did occur when tungsten, in copious quantities, was added to the diet. Thus molybdenum as a stand-alone cofactor for an enzyme was doubtful and its essentiality to humans questioned. Tungsten later was shown to be a strong antagonist of molybdenum, which explained the apparent tungsten effect and put the question of molybdenum essentiality to rest.

21.2.2. Chemistry

Molydenum with a $4d^5 5s^1$ configuration is the only mineral other

than iodine with quantum numbers extending into the fourth energy level. Hence, there are multiple stereochemistries and oxidation states of molybdenum prevalent in biological systems.

Molybdenum binding to molybdopterin gives rise to the molybdenum cofactor (Figure 21.3). As an enzyme-bound cofactor, molybdenum shuttles between +4, +5 and +6 oxidation states. Its most common inorganic form is molybdate (MoO_4^{2-}). The chemistry of molybdenum is similar to tungsten, which is known to compete with molybdenum for binding to molybdopterin.

21.2.3. Biochemistry

The list of important molybdenum-requiring enzymes in humans and animals is shown in Table 21.4. Based on similarities in stereo arrangement and oxidation state, two families of molybdenum enzymes have been identified: the xanthine oxidase family and the aldehyde and sulfite oxidase family. All three enzymes in the two families catalyzed oxidative hydroxylations consistent with the adaptable chemistry of molybdenum. According to da Silva and Williams, the enzymes are capable of a two-electron oxygen atom transfer that avoids generating a free radical, owing to the ease of which the M=O bond is broken and two electrons are in the leaving group. In addition to molybdenum, the three also contain iron-sulfur centers as cofactors (Table 21.4). Xanthine oxidase, also referred to as xanthine oxidoreductase, uses the molybdenum cofactor shown in Figure 21.3. A brief description of the reactions is given below.

21.2.3.1. Xanthine Oxidoreductase (XO) and Xanthine Dehydrogenase (XDH)

Two molybdenum enzymes catalyze the formation of uric acid from

TABLE 21.4. Molybdenum-Requiring Enzymes in Animals and Humans.

Enzyme	Cofactor
Xanthine Oxidoreductase	Fe/S molybopterin
Aldehyde Oxidase	Fe/S molybopterin
Sulfite Oxidase	Fe/S molybopterin
Xanthine dehydrogenase	NAD+, NADH

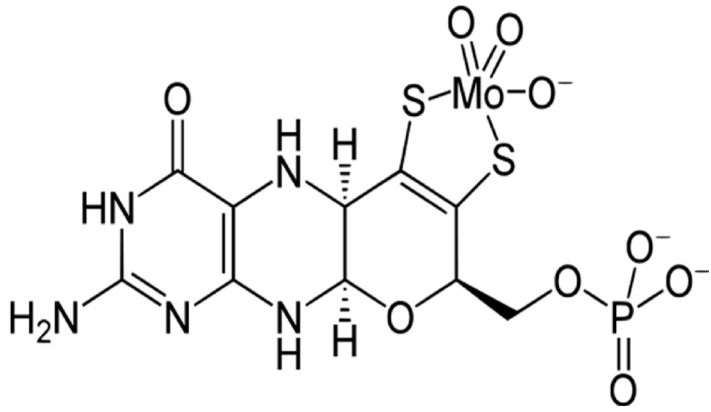


FIGURE 21.3. *Structure of Molybdopterin: One of the Cofactors in Molybdenum Enzymes.*

xanthine, a key reaction for maintaining nitrogen homeostasis in the system. XDH is the more dominant of the two. Two reactions convert XDH into XO—one by an oxidation (reversible), the other by the removal of a N-terminal peptide (irreversible). XO catalyzes a two-step conversion of hypoxanthine, first to xanthine and then to uric acid (Figure 21.4). The reaction requires molecular oxygen as the oxidizing agent and forms hydrogen peroxide as a product. XDH uses NAD+ as

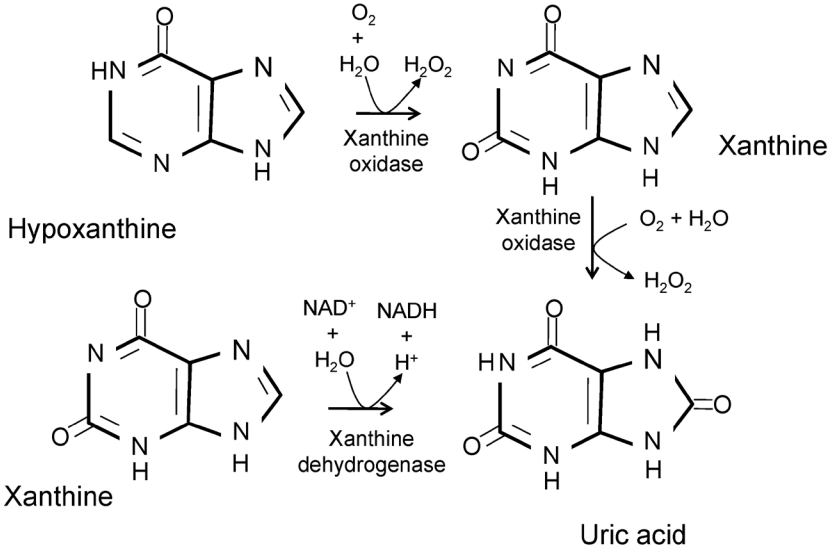


FIGURE 21.4. *Two Pathways for the Formation of Uric acid from Xanthine.*

the final product has not been determined. A defect in xanthine dehydrogenase has been postulated to result in excess xanthine in the urine (hyperuricemia) and other stress disorders, suggesting XDH is the major source of XO in the system.

21.2.4. Aldehyde Oxidase

Aldehyde oxidase (AO) function is to convert compounds with an aldehyde functional group into carboxylic acids using molecular oxygen as substrate [Equation (1)]. Like xanthine oxidase, AO has both a molybdenum cofactor and an iron-sulfur center on each subunit. The enzyme is more concentrated in the liver and kidneys, but reports of the enzyme in bone, intestine, and lung have also emerged.

$$\text{R-CHO} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{R-COO}^- + \text{H}_2\text{O}_2 + \text{H}^+ \qquad (1)$$

Although cells also express an aldehyde dehydrogenase (AD), the enzyme does not use a molybdenum cofactor.

21.2.4.1. Sulfite Oxidase (SO)

Sulfite oxidase occurs in the mitochondria of all eukaryotes. The enzyme catalyzes the oxidation of sulfite to sulfate, a major metabolite in the biosynthesis of heteropolysaccharides in connective tissue [Equation (2)]. SO is also believed to catalyze

$$\text{SO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+ + 2\text{e}^- \qquad (2)$$

the last stage in the oxidation of methionine and cysteine, two sulfur amino acids. In the reaction, two electrons are transferred to the cofactor, effectively reducing Mo(VI) to Mo(IV). Unlike the other two molybdenum oxidases, molybdenum in SO channels electrons to the electron transport system using cytochrome c as the entrance point. In the transfer, Mo(VI) is restored. Consequently, the oxidation is energy-yielding and results indirectly in the production of ATP. SO draws parallels to the other two molybdenum oxidases with two exceptions: O_2 is not a substrate for SO (despite referring to the enzyme as an oxidase) and iron in the enzyme is present as a heme group. Both of these features may be necessary in the transfer of electrons from sulfite into the electron transport chain.

21.2.5. Nutrition

The average body load of molybdenum in the adult is about 5 mg. Despite low molybdenum on a global scale, a molybdenum deficiency is a rare occurrence in humans. Prolonged total parenteral nutrition can be an extenuating factor in creating a deficiency. Table 21.5 shows the RDA and UL for molybdenum intake as a function of age. The figure 45 µg/day was determined by balance studies using stable isotopes. The average daily intake of molybdenum is estimated to be between 120 to 240 µg, well beyond the RDA. The variation no doubt reflects the different amounts of molybdenum in a food source. When compared to the RDA value for molybdenum (Table 21.5), this level of intake on a daily basis offers a rationale for the populace as a whole not suffering a molybdenum deficiency.

TABLE 21.5. Requirement for Molybdenum in Humans.[1]

	DRI values (µg/day)			
	RDA[2]			
Age	**Male**	**Female**	**AI**	**UL**
Infants				
0–6 mo			2	Not determined
7–12 mo			3	Not determined
Children and adolescents				
1–3 yr	17	17		300
4–8 yr	22	22		600
9–13 yr	34	34		1,100
14–18 yr	43	43		1,700
19–30 yr	45	45		2,000
31–50 yr	45	45		2,000
51–70 yr	45	45		2,000
>70 yr	45	45		2,000
Pregnancy				
<18 yr		50		1,700
19–50 yr		50		2,000
Lactation				
<18 yr		35		1,700
19–50 yr		36		2,000

[1]Data are from the Dietary Reference Index, Institute of Medicine of the National Academy of Sciences, published in 2006.
[2]RDA = Recommended Dietary Allowance.

21.2.5.1. Food Sources

Due to the very low stores of molybdenum in mammalian tissues and organs, foods derived from animals would be expected to be intrinsically low in molybdenum. An exception is sea food, owing to the very high amount of molybdenum in sea water, which is higher than any other micromineral. In addition, meats, milk and dairy products are all regarded as good sources of the mineral. Plants such as legumes, which include beans and peas, leafy vegetables and grains can be designated as good. The amount of molybdenum in plants will vary strongly and depend on the level of molybdenum in the soil. It's particularly important to note that molybdenum ores do not have a strong omnipresence in the geosphere, which translates into a low molybdenum content in soils in general.

21.2.5.2. Deficiency Symptoms

Overt signs of molybdenum deficiency reflect impairments in molybdenum enzymes. No one particular biomarker has been used to assess molybdenum adequacy, however. Because free-living subjects rarely suffer a molybdenum deficiency, information on the subject has come from patients undergoing prolonged total parenteral nutrition (TPN) without the aid of molybdenum supplements. Reportedly, a deficient TPN patient will have elevated sulfate and uric acid in the blood as well as an abnormal heart rhythm. There also could be signs of mental disturbances. Some symptoms result from high levels of sulfite or suppressed levels of sulfate. Reports in the literature cite low molybdenum soils in China as a preemptory cause to the development of esophageal and stomach cancers. Linking the disease with molybdenum soil content is tenuous, however, and has yet to single out low molybdenum as the only factor responsible for the condition.

21.2.6. Absorption and Metabolism

Little is known about molybdenum's absorption in higher animals. Algae are the only eukaryotes for which there is evidence of a high affinity transport protein for molybdenum. The protein may belong to the high sulfate carrier family and unexpectedly is localized on the internal membranous system of the mitochondria, not the plasma membrane. Within the system, molybdenum is absorbed into the blood and transported as the hexavalent molybdate anion (MoO_4^{2-}). Again, it is not

known if proteins are need for the transport and uptake by cells. The general homeostasis of molybdenum in the system is likely to be controlled by the kidneys. An important point to consider is that molybdenum metabolism is closely dependent on iron. One notes, for example, molybdenum enzymes depend on iron either as iron-sulfur centers or heme for intra-electron transfer.

21.2.7. Molybdenum Toxicity

What is known about molybdenum toxicity, referred to as molybdenosis, has come mainly from animal studies. The values for upper limits (Table 21.5) suggest that a daily intake in the range of 1–2 mg would be expected to produce toxic signs similar to those shown in Table 21.6. In addition to the amount fed, tolerable amounts will also depend on the particular molybdenum compound in the diet. One condition that requires further explanation is the effect of high molybdenum on copper intake. Recall from Chapter 8 that molybdenum in the presence of sulfur readily forms tetrathiomolybdates (TTM), a complex that has strongly attracts copper ions. TTM will prevent copper absorption and give rise to conditions reflective of low copper intake.

21.2.8. Summary

The very small amounts of molybdenum in the environment is evinced by its scarcity in animals and human tissues. There are only 5 known enzymes in humans and animals that use molybdenum as a cofactor. Basing molybenum's role in these life-sustaining enzymes gives credence to molybdenum's designation as essential. Diets deficient in molydenum when given to rats, chicks or sheep have no effect on the metabolism of purines or production of uric acid or allantoin. Tungsten, a known antagonist, when added to the diet will show symptoms

TABLE 21.6. Symptoms of Molydenum Toxicity.

Growth retardation in young
Diarrhea
Infertility in adults
Gout
Low birth weight in offspring
Low copper absorption

of dysfunction. These data show that the requirement for molybdenum internally can be met by very small amounts in the system, which was one of the early reasons for dismissing molybdenum as an essential mineral. There is still much to learn about the metabolism of molybdenum. Insights into how molybdenum as MoO_4^{2-} is taken into the system or transported to functional sites is still incomplete and in need of further research.

21.3. REFERENCES

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21.4. PROBLEMS

1. What plants are the best source of cobalt in the diet? How do you reconcile your answer with the statement that plants don't need cobalt to grow?

2. What is the difference between a cobamide and a corronoid? List two cobamides (corronoids) that are required for animal and human health. Why are two forms of this corronoid used in metabolism?

3. What does the designation "pernicious" in pernicious anemia mean?

4. Figure 21.1 shows the structure of Vitamin B12. The structure is composed of fixed and variable components. Which are fixed and which will vary?

5. What protein provides safe passage of vitamin B12 through the stomach? What is the acid-sensitive site in the vitamin?

6. Cobalt absorption in the intestine is subject to interference by iron. Based on what you learned in Chapter 13, which entry path through the enterocyte would cobalt be expected to take?

7. Explain why excessive amounts of cobalt in the diet risks developing a goiter.

8. When molybdenum serves as a cofactor, how is it bound to the enzyme?

9. It is generally agreed by nutritionists that a standard American diet has no risk of developing a molybdenum deficiency. What nutritionally related circumstance could lead to this risk?

10. Suggest a biomarker that could be used to assess molybdenum status.

11. Why is it not a good idea to add molybdenum to soils frequented by grazing animals? The question is asking what molybdenum compounds could be formed and what would be the consequences of their presence.

CHAPTER 22

Arsenic, Boron, Silicon, and Vanadium

THE four minerals discussed in this chapter lack strong evidence for essentiality to human health. Of the four, arsenic is the furthest from being deemed essential to life. Indeed, arsenic in any form or amount is toxic and potentially lethal. Boron has a high presence in plants but in animals and humans, nutritional and biochemical evidence supporting boron's need for survival is lacking. Silicon has a strong chemical presence in bones and connective tissues and, with properties akin to carbon, has been shown to have a structural role in fungi, bacteria, and viruses, but not humans. Vanadium, despite circumstantial evidence to the contrary, has yet to be connected to specific biochemical factors dependent on vanadium for function, which has made the assessment of its value to human health questionable.

22.1. ARSENIC

Arsenic is a poison with toxic properties that outweigh any semblance of benefit. Nutritionists and food scientists should know that arsenic in food is ubiquitous, fed into streams and soils mainly from a pervasive geological presence in the environment. Furthermore, humans and animals have the capacity to render inorganic arsenic into a non-toxic organic form. Although the list of suspected biological functions have grown, physiological and biochemical evidence for a life-essential role of arsenic in humans is fragmented and weakly supportive. In this chapter, we will look at arsenic and see where it may depart from a toxin to a potential benefactor in a living system.

22.1.1. History and Early Insights

Arsenic (As) derives its name from the Latin "arsenicum" or Greek "arsenikon", relating to something of strength and masculine character. Early history alludes to arsenic as a homicidal suicidal agent. The 14th century alchemist, Albertus Magnus, by extracting the gold-like pigment from an arsenic ore (As_2S_3), is generally credited with isolating arsenic in a near-pure form. A medicinal connection came later when the British physician Thomas Fowler concocted a regimen that contained 1% potassium arsenic to treat patients with fevers and headaches. Despite side effects such as vomiting and nausea, Fowler's solution, as it came to be known, quickly gained the support of medical professionals who used it to treat skin diseases, epilepsy and asthma. One such patient was Charles Darwin, who in his later years complained of headaches, eczema, nausea, and heart palpitations—all signs of arsenic poisoning. Stable trivalent organoarsenoxides were used to treat the spirochete bacterium that caused syphilis, a practice that stayed active until arsenic was replaced by penicillin in the 1940s and 1950s. Other beneficial avenues opened with the discovery that animals fed arsenic excreted non-toxic organoarsenals in their urine. Knowing that organoarsenics are not toxic stimulated efforts to learn the biochemistry behind the transformation of a toxin to a metabolite. A possible essential role was evidenced by a series of arsenic-deficient dietary studies that showed effects on growth rate and reproductive capabilities of animals. The momentum gained from these early studies has tended to wane, leaving many questions about arsenic's presumed essentiality unanswered.

22.1.2. Chemical Properties

Being a group V element, arsenic joins phosphorus as a metalloid with a considerable range in chemical behavior. Some estimates have been as high as 450 different mineral species with arsenic in their structure. Arsenic can exist as a gas (As_4O_6) or as the crystalline trioxide (AsO_3), the latter giving rise to arsenic acid in aqueous solution. The trivalent arsenic [As(III)] as the ortho-arsenite anion ($H_2AsO_3^-$), forms complexes with sulfhydryl groups juxtaposed to receive the complex (Figure 22.1). By targeting adjacent –SH groups, As(III) has the capacity to block critical enzymes in lipid and ATP synthesis pathways. This same interaction is not seen with the pentavalent form [As(V)]. Complexes with betaine and choline are examples of organoarsenics in biol-

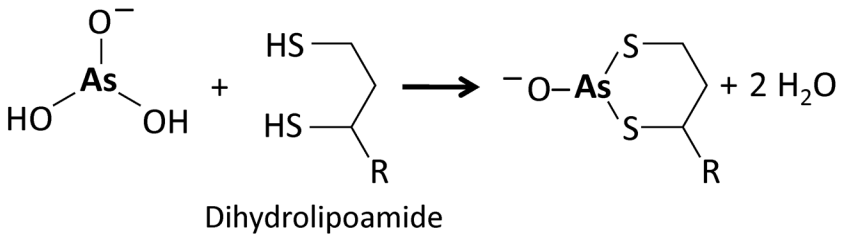

FIGURE 22.1. *Reaction of Arsenite with Dihydrolipoamides.*

ogy. Higher animals and humans tolerate organic forms; protozoans, however, tend to show a greater sensitivity.

22.1.2.1. Biochemical Properties

Two organoarsenic compounds have been identified as metabolites in human and animal systems, arsenobetaine and dimethyl arsenic acid. Arsenobetaine is also a major organoarsenal in fish and other marine animals. Indeed, Molin *et al.* (2012) reported high levels of arsenobetaine and dimethyl arsenic acid in human urine following a meal of blue mussels (Figure 22.2). Sugar derivatives were also detected. Once ingested, inorganic arsenic is readily converted to the organic form within the system, further implying animals and humans have evolved weak but effective biochemical means to lower arsenic toxicity. Considering the possibility that arsenic may be essential to life, conversion to a non-toxic form would be a necessary first step. As to why the trivalent form of inorganic arsenic is more toxic, it has been speculated that in solution at neutral pH, As(III) exists as an undissociated acid capable of engaging proteins covalently through relatively strong hydrogen bonds. In contrast, As(V) anions engage proteins through weaker electrostatic forces, which prohibits a strong toxic action.

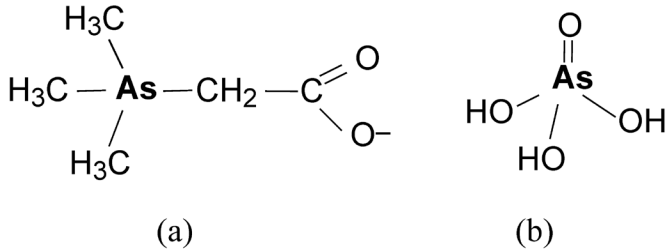

FIGURE 22.2. *Arsenobetaine (a) and arsenic acid (b), the major organic arsenic compounds in human urine.*

22.1.3. Nutrition

Impeding weight gain in young goats and causing lactating goats to succumb was the first report of a possible essential role for arsenic in animals. This discovery was further bolstered by observing rats raised on arsenic-deficient diets after weaning showed impaired methionine metabolism accompanied by increased bleeding time and central nervous system disorders. None of these studies, however, connected definitive arsenic compounds with the observations, thus weakening the soundness of the evidence.

22.1.3.1. Food Sources

Marine animals and plants grown in arsenic-rich soils represent the most plentiful sources of arsenic. Sea water contains about 0.002–0.005 micrograms per gram, whereas rivers and lakes—owing to runoff from alluvial soils and herbicides—can be as high as 0.8 micrograms per gram. Soils generally have 40 micrograms per gram or 50 times the content of rivers and lakes. Crops grown in such soils easily accumulate arsenic in their stems and leaves. Despite such omnipresence, it has been estimated that the typical diet exposes human and animals to less than 0.3 micrograms per gram. Table 22.1 shows the distribution. Foods are the major source of bioorganic arsenic complexes. One exception is blue mussel, where nearly half (48%) of the arsenic present is in inorganic complexes.

22.1.4. Absorption, Excretion and Storage

Both inorganic arsenate and arsenite are absorbed in toto into the system from the diet. Penetration across the intestinal barrier occurs via specific channel proteins and may use phosphate transporters. Once in the bloodstream, the arsenic is quickly transported to the liver, where conversion to arsenobetaine or dimethylarsenic complexes takes place. Controlling the level of these compounds is a function of the kidney, which excretes most of the arsenic absorbed. Storage of arsenic in tissues is very low. Studies using radioactive arsenic (^{74}As) have shown that two days after consumption, rats retain only about 0.3% of the arsenic administered. Arsenic in the blood is bound to hemoglobin in erythrocytes. Retention in tissues is weak, highly vari-

TABLE 22.1. Prevalence of Arsenic in Human and Animal Foods.

Land-Based Foods (µg/g)	
Forage Crops	0.1–1.0
Cereals	0.05–0.4
Vegetables	0.05–0.8
Fruits	0.03–1.0
Meat	0.005–0.1
Milk	0.01–0.05
Eggs	0.01–0.1
Marine-Based Foods (µg/g)	
Fish	2.0–80.0
Oysters	3.0–10.0
Blue mussels	1.5–4,900[1]

[1]Highest observed arsenic in marine animals. Data were obtained from the National Institute of Nutrition and Sea Food Research and represents arsenic found in blue mussels in fjords along the Norwegian coast.

able and species-dependent. There are no data available for human retention of arsenic.

22.1.5. Metabolism

For simplicity, one can consider the metabolism of arsenic centering on two main steps: reduction and oxidative methylation. Inorganic arsenate [As(V)] taken into the system is quickly reduced to arsenite [As(III)] by glutathione (GSH) and transferred to the liver [(Equation (1)]. This non-enzyme reaction occurs in the blood of most species. The same reaction in the cell is catalyzed by the enzyme *arsenic reductase*. Because of their toxicity, it is of utmost importance that trivalent arsenous compounds be eliminated quickly or risk cell death.

$$\underset{\text{(arsenate)}}{H_3AsO_4} + 2GSH \rightarrow \underset{\text{(arsenite)}}{HAsO_2} + 2H_2O + GSSG \qquad (1)$$

22.1.5.1. Detoxification of Arsenite

Figure 22.3 shows the series of reactions that form the crux of arsenite detoxification. In the liver, the arsenite is oxidatively methylated to monomethylarsonic acid [MMA(V)] and dimethylarsinic acid

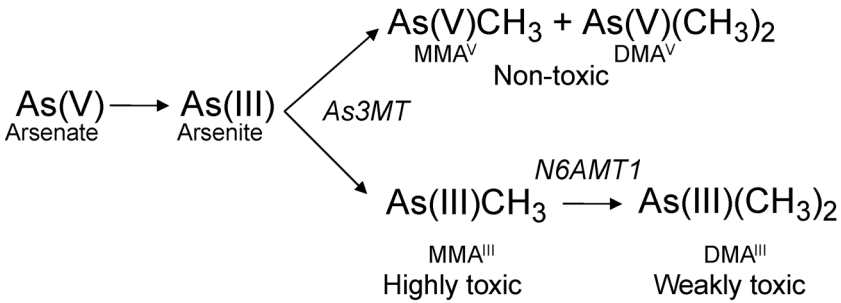

FIGURE 22.3. *General Scheme of Arsenic Metabolism that Renders Arsenic Safe.*

[DMA(V)], two pentavalent forms that are relatively non-toxic to the organism. The reaction is catalyzed by the enzyme *arsenic methyltransferase* (As_3MT) and uses S-adenosylmethione (SAM) or methylated Vitamin B12 as the methyl group donor. In addition to the pentamethylated complexes, there is evidence that trivalent monomethylarsonous acid [MMA(III)] also forms in the reaction. MMA(III) is highly toxic, perhaps more so than inorganic arsenite. Observing that animals lacking As_3MT can still survive a dose of arsenic has led to the discovery of *N-6 adenine-specific DNA methyltrasnferase* (N6AMT1), an enzyme that specifically converts MMA(III) to the weakly toxic dimethylarsonous acid [DMA(III)] (Figure 22.3). Forming the dimethyl derivative apparently prevents MMA(III) from binding to proteins and blocking biological functions. Failure to methylate MMA(III) leads to fatal consequences.

22.1.6. Arsenic Toxicity

22.1.6.1. Exposure

The Environmental Protection Agency (EPA) has set the arsenic standard in drinking water at 0.01 micrograms per mL (ppm). Although there is no upper limit for arsenic, this figure is the maximum concentration allowable for organic and inorganic forms, either alone or in combination. According to the EPA, about 90% of the industrial arsenic in the environment comes from compounds used as wood preservatives. Lesser amounts are found in paints, dyes, soaps and semiconductors. Non-industrial avenues of exposure include waste water, water runoff from arsenic-contaminated soils, and arsenic released into the environment as a gas.

22.1.6.2. Symptoms and Diseases

According to the National Research Council (NRC), amounts of inorganic arsenic greater than 10 mg/kg/day can lead to serious skin and pigment alterations referred to as arsenicism. Lower amounts over time can cause debilitating diseases. Table 22.2 lists a series of symptoms and diseases known to result from acute and chronic exposure to low, non-fatal doses of arsenic.

22.1.6.3. Safe Levels

With the potential to be pathogenic at nearly all levels, it's difficult to arrive at what may be deemed a safe level of exposure to arsenic. Typically, the intake over time for both men and women ranges between 1.7–2.9 micrograms per day. Unfortunately, this level has steadily increased due to increases in avenues of exposure. In 1997, it was estimated that the average amount of arsenic in drinking water was below 10 micrograms per liter for 98 percent of the human population in the U.S. The EPA has since revised the maximum contamination level (MCL) downward and as of 2000 was between 3–20 micrograms per liter with the likelihood of going lower. It's important to point out that a substantial number of people in the U.S. and North America in general are exposed to arsenic by inhaling arsenic in the air from smelters and chemical plants (airborne arsenic); exposure by this mean far exceeds EPA's MCL.

22.1.7. Summary

The deleterious effects of arsenic far outweigh its nutritional con-

TABLE 22.2. Diseases Arising from Acute and Chronic Arsenic Exposure.

Short term Exposure	Long Term Exposure	Diseases
Nausea	Muscle weakness	Skin, bladder, lung cancer
Vomiting	Hearing loss	Diabetes (Type 2)
Diarrhea	Headaches	Peripheral neuropathy
Abdominal Pain	Chronic pain	
Burning sensation in the mouth	Keratosis and skin changes	

tributions. To date, only fragmented evidence supports arsenic as essential. In the environment, arsenic occurs in two forms (inorganic and organic) and in two oxidation states [As(III) and As(V)]. Of the two, arsenite [As(III)] is more toxic. Organic arsenic appears to be a product of biological systems that possess the capacity to both oxidize and biomethylate arsenic. There seems to be very clear evidence that humans and animals have the capability of detoxifying inorganic arsenic taken into the system. Arsenic turnover in living systems is rapid, with little tendency for storage in the tissues or blood. Urinary excretion is the principal route for removing arsenic from the system and nearly all that is released is organic arsenic, of which arsenobetaine and dimethylarsenous acid are the two principal forms. The question of arsenic's essentiality to humans and higher animals in still in abeyance. Animal studies tend to suggest an arsenic-deficient diet has the potential to lower growth rate and interfere with reproductive efficiency. These findings, however, have not been shown to apply to humans. Attempts to identify specific biological systems that are impaired by a deficiency are a pressing matter in need of more rigorous research.

22.2. BORON

Best known as a component of the cell walls of plants, boron is an element of indisputable importance to vegetation but of questionable necessity for animals and humans. Current research is attempting to challenge the latter. A collected body of evidence has shown that many animal species require boron to conduct the metabolism and turnover of calcium, phosphorus and magnesium, possibly by regulating hormones that control these minerals. Other studies have shown that diets low in boron suppress serum levels of 17β-estradiol and calcium in postmenopausal women. Boron supplements counter a magnesium-depleted state by restoring magnesium balance and interaction with other minerals. The physiological function that impinges upon these observations has yet to be determined, however. In viewing the proposed requirement in humans, one must therefore recognize that functional boron comes to light under conditions of nutritional stress. Consequently, a system in status quo may show less need for boron. This is the dilemma faced by researchers who have used traditional dietary approaches to identify essential nutrients.

22.2.1. History and Early Insights

Its ominous presence in igneous rock and shale, fresh water and sea water make boron a familiar compound in the environment. Sir Humphrey Davy used electrolysis to separate the components of boricium, one of the more common boron ores, and succeeded in isolating a new element which he called boron in reference to its resemblance to carbon. Biological insights into boron came in 1923, when Warrington correlated growth and structural integrity of legumes with the soil's content of boron. Speculation favored boron playing a structural role, most likely giving stability to the plant's cell walls. Boron in humans and animals was never firmly established until it was shown that a boron deficiency, while not life-threatening, had the capacity to disrupt the metabolism and turnover of other minerals as well as to alter blood levels of the hormones that controlled their homeostasis. One important study showed that the impaired growth of vitamin D3-deficient chicks could be overcome with supplements of boron. The boron, however, had no effect on the growth of vitamin D3-adequate chicks. Although a lack of definitive biochemical and physiological evidence has precluded wide acceptance of boron's essentiality to humans, the evidence gathered thus far justify continuation of studies of essentiality of boron in humans.

22.2.1.1. Chemical Properties

Boron oxides, specifically boric acid, and borate anions, make up the majority of boron in plants and consequently are the main forms of boron in animal and human diets. Oxyboron compounds common to a biological form are either in the trigonal $B(OH)_3$ or tetrahedral $B(OH)_4$ configuration. In the cell, mononuclear $B(OH)_4^-$ anion and $B(OH)_3$ acid are dominant. Based on their chemistry, trigonal boric acid and tetrahedral borate anions form very stable complexes with cis-diol (–OH groups) groups on sugars. Moreover, tetrahedral borate has the capacity to form stable crosslinks across adjacent polysaccharide chains (Figure 22.4). A reactive hydroxyl group is considered a key to boron functions and has guided research efforts to isolate boron complexes from animals and human tissues.

22.2.2. Biochemical Properties

Figure 22.5 summarizes the physiological and biochemical param-

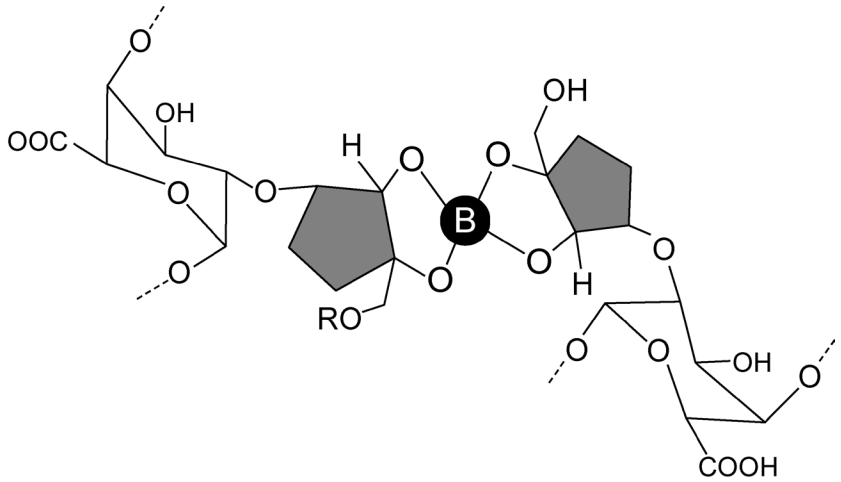

FIGURE 22.4. Reactions of Borates with Sugars. By binding to viscinal cis-OH groups in neighboring sugars, boron forms bridges across polysaccharide chains in pectin, giving a sturdier supporting structure overall. (Adopted from da Silva and Williams, 1991).

eters that are influenced when boron is taken as a supplement in the diet. Because no stable biochemical complex of boron has been isolated from a mammalian system, what is known of the biochemical properties of boron has come from studies that have focused mainly on metabolic pathways and regulating factors influenced by boron. Table 22.3 lists the factors that fit the category of "responsive to boron deficiency". Many of those listed are hormones with known effects on absorption, retention and excretion of other minerals. It should be realized at the

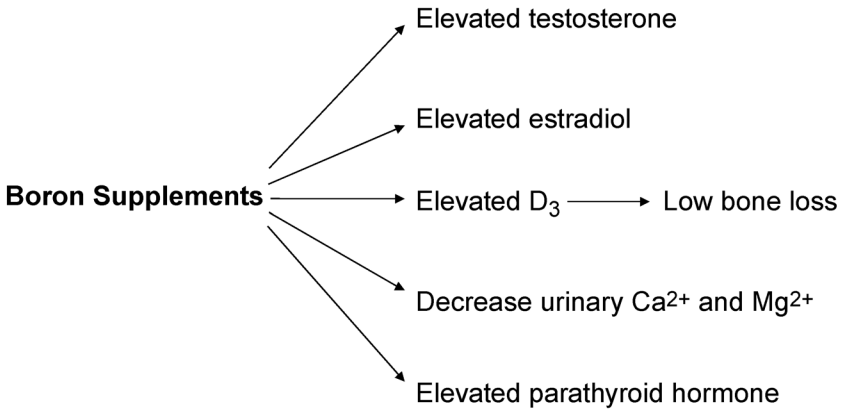

FIGURE 22.5. Physiological Parameters Altered by Boron Supplementation.

TABLE 22.3. Mineral-Metabolizing Hormones and Other Factors that Respond to Boron Deficiency.

Factor	Observed
17β-estradiol	lowered in boron deficiency
Testosterone	lowered in boron deficiency
Calcitonin	supplementation lowers loss of calcium
Osteocalcin	lowered in boron deficiency
Dihydroxyvitamin D3	no effect in boron deficiency
Parathyroid hormone	no effect in boron deficiency

onset that creating a boron deficiency is not easily accomplished, given the low amount of boron in the diet. Then, too, boron is classified in the literature as an ultra-trace mineral with extremely small amounts needed to keep body levels adequate.

22.2.2.1. Animal Studies

Much of our current understanding and support for the essentiality of boron comes from non-traditional animal studies. The zebra fish, for example, responds to both boron deficiency and supplementation. Deficiencies impinge on embryonic development which can be traced back to the sperm and ovum. Although sperm from a low boron male can successfully fertilize eggs from a boron deficient female, embryo survival is very low, with growth terminating at an early cleavage stage.

22.2.3. Nutrition

Because it is yet to be classified as essential, boron has no RDA. Based on literature values, the average adult human consumes about 3–5 mg of boron daily. The safe and adequate level, however, is 1–3 mg/day for adults.

22.2.3.1. Dietary Sources

Boron occurs in all plant foods, typically where pectin is found—i.e., part of the extracellular material that coats the outer surface. The pulp of citrus fruits such as apples, beets, and carrots are rich in pectin and hence are good sources of boron. The same applies to fibrous plants where boron acts as a stabilizer.

22.2.4. Absorption, Storage and Excretion

Turnover of boron, as measured by competitive rates of absorption and excretion, is very high, implying that under condition of normal intake, little boron is retained in the tissues. Estimates range between 30 and 90 percent of dietary boron is rapidly excreted in the urine; there is little set aside for storage. Although boron is found in all tissues, the highest amounts are in bone. An anomaly of interest is that plasma level of boron in human neonates is high at birth but quickly drops to half the level by the fifth postnatal day. Whether this reflects adaptation to an environment outside the womb is open to speculation.

22.2.5. Suspected functions of Boron

22.2.5.1. Bone Structure

The link between boron and osteocalcin (vitamin D3), and parathyroid hormone—both hormones affecting calcium absorption and homeostasis—supports the conclusion that boron is required for bone strength and composition. It is not clear if the effect is primary or secondary to the action of steroid hormones that control bone mineralization (Chapter 11). Supplementing postmenopausal women with boron resulted in a greater retention of calcium in the urine and raised the levels of estrogen and vitamin D3 in the plasma. While questions have been raised as to the validity of the data, the results have established a possible link between boron and osteoporosis and other disorders of calcium metabolism.

22.2.5.2. Calcium and Magnesium Metabolism

Symptoms of calcium and magnesium deficiency are less severe and calcium and magnesium homeostasis is partially restored when boron is added as a supplement to the diet. In an early experiment, it was shown that boron supplements eliminated signs of magnesium deficiency in chicks, suggesting an effect on magnesium homeostasis. Phosphorus deficiency was unaffected, however. A study with postmenopausal women given supplements of boron (3 mg/day) showed a marked decrease in urinary excretion of calcium and magnesium. Suppression was stronger if subjects were also deficient in magnesium. Plant cell walls are also known to have boron/calcium interactions. Plants grown

in low boron soil have less calcium in pectin and display defects in the assembly and mechanical properties of cell walls. An impaired plant is less able to endocytose external cell wall pectin, which must be continuously remodeled for the plant to grow and thrive. At present, no evidence supports human plasma membranes undergoing a similar boron-dependent remodeling.

22.2.5.3. Stamina

A number of studies have supported the concept that boron taken daily as a supplement improves body stamina and athletic performance. These findings suggest boron may function to assist muscle contraction, but the data are too preliminary to make definitive conclusions.

22.2.6. Boron Toxicity

What constitutes a toxic dose of boron for humans is still an open question. One study suggested toxic symptoms appear at an intake of about 0.1 grams boric acid. Another study suggests an intake of at least 4 grams/day can be tolerated, but increasing the dose or the frequency of the dose can have toxic consequences. For example, a single dose of 20 grams of boric acid can be given without toxic signs but repeated intakes of as little as 0.5 grams per day for 50 days can cause digestive problems (diarrhea, nausea) consistent with toxicity. Toxicity also arises when the kidney falters, which can lead to the accumulation of boron in vital organs such as the brain, lungs, heart and kidneys. For adults, a lethal dose can be 15–20 grams; for children, 3–6 grams. Recognizing the propensity of boron to cause serious consequences to health on a global scale, the World Health Organization has banned boric acid as a food additive and preservative.

22.2.7. Future Directions for Research

It is quite clear that much of boron nutrition has yet to be discovered. Recent studies in plants have questioned whether boron is strictly a cell wall factor or performs additional functions. One basis for the inquiry is the observation that plants, bacteria, and fungi—all lacking cell walls—nonetheless show a need for boron. Evidence that plasma membrane structure and turnover are controlled by boron and may operate at the level of genetic regulation is another finding supporting an expanded

role for boron. These findings have opened new ways of thinking about boron's importance to living organisms.

Recently, a sodium-driven boron transporter on the basolateral surface of mammalian cells and encoded by the SLC4A11 gene has been projected to be essential for boron to access cells. The transporter protein (NaBC1) functions as a channel for Na^+ and OH^- (H^+) in the absence of boron. With borate present, however, the channel converts to an electrogenic Na^+-coupled borate co-transporter. Because it fails to transport arsenic, the transporter is believed to be specific for boron and no other metalloid. Figure 22.6 shows how the transporter is believed to function. NaBC1 mRNA has been detected in jejunum, ileum and kidneys of pigs. Boron supplements added to the feed raised NaBC1 mRNA in jejunum but suppressed the gene in kidney. Thus, supplements of boron to a boron-deficient animal, working through NaBC1 expression, increased absorption but decreased excretion. The importance of the discovery of this transporter is two-fold: (1) it supports the concept of a multi-tissue specific factor designed to transport boron into cells, and (2) because dietary boron exerts opposite effects on transporter mRNA in the intestine and kidney, it would appear that NaBC1 plays a central role in controlling boron homeostasis. The fact that boron may control genetic expression further implies an essential role for this mineral as a transcriptional or cell signaling agent. Turning on and off genes that control transport, storage and excretion of minerals could

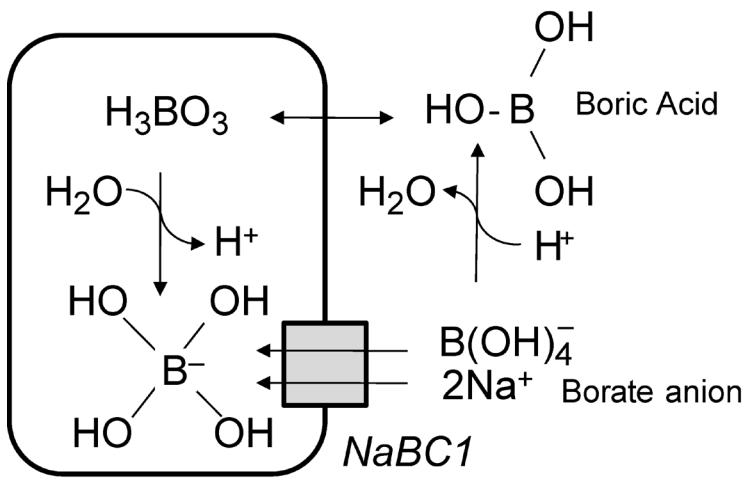

FIGURE 22.6. Transport of Boron into cells; Role of the NaBC1 Transporter (Adopted from Park et al., 2004).

be at the crux of boron functions yet to be clarified in animals, but having stronger evidence in plants.

22.2.8. Summary

In a practical sense, the case for boron essentiality is established for plants, but questionable for animals and humans. This does not dismiss boron as an essential nutrient for humans, but shows that the field of boron research has yet to meet all criteria for establishing boron as having a serious impact on the quality of life. Considering that boron is more effective when animals are under nutritional stress, the focus of boron may not be on prevention or treatment, but as a factor that can "rescue" or "reverse" a distressed system and restore homeostasis. That boron supplements respond to and correct low calcium, phosphorus and magnesium in the system suggests that in order to judge boron's effectiveness, a system must first be put in an off balance status. A positive step forward has been the discovery of a biochemical factor that moves boron across cell membranes. In the absence of boron, this sodium-driven transporter also serves as an entry portal for sodium and protons, which argues against the transporter controlling only the movement of boron. The potential for boron to regulate genetic expression has also come to light in plants and animals. It has not been determined, however, if controlling expression at the genetic level underpins many of the suspected functions of boron.

22.3. SILICON

As the second most abundant element in the earth's crust, silicon would be predicted to play a preeminent role in the evolution of life on earth. This expectation is realized in early life forms, but does not carry through to more advanced biosystems. Owing perhaps to its chemical properties or lack thereof, silicon and silicates play only minor roles in the nutrition of humans, animals, and plants. Much of the research on silicon in animals has focused on the bone and soft connective tissue formation and rigidity. A search for a silicon-dependent enzyme has not met with success. Therefore, silica—like the other minerals in this section—has no RDA and much of its evidence for essentiality is based on responses to dietary deficiencies or oversupply. Only a few of these studies have focused specifically on humans.

22.3.1. History and Early Developments

Early studies established the presence of silicon in viruses, bacteria and fungi. Its finding in primitive organisms and ability to form long carbon-like chains raise the questions as to whether a silicon-based architecture preceded a carbonaceous one in early life forms. Follow-up work showed that primitive marine organisms such as diatoms, sponges and algae have a special need for silicon and silicates for growth. Silicon deficiencies in the soil have had a telling effect on the growth rate of oats, rice, barley, and tomatoes. These early endeavors became blue prints for studying a possible essential role for silicon in animals and humans. Carlisle, Schwarz and Milne in the early to mid 70s used a deplete/replete design to show that a silicon deficiency affected the growth rate of rats and chicks, presumably by causing defects in bone structure and connective tissue. Such changes correlated positively with the amount of silicon fed. Acute or chronic decreases in bone mineral density (BMD) that typifies osteoporosis has since become a motivating factor for learning more about the action of silicon in bone structure and health.

22.3.2. Chemical Properties

Silicon occurs naturally in foods as silicon dioxide (SiO_2, also known as silica) and as silicates (salicic acid esters). Orthosilicic acid [$Si(OH)_4$] is the major silicon in H_2O and liquids in general, and is the main silicon species absorbed from the diet. As a group IV non-metal, silicon shares properties with carbon, the most biologically relevant of which is catenation, which can be pictured as forming an amorphous array of finite long or circular chains with oxygen atoms bridged between silicon atoms (Figure 22.7). The chains of silica, however, have strong anionic character with a capacity to bind heavy metals such as Mg^{2+}, Ca^{2+}, Fe^{2+} and Mn^{2+}. The strong ionic character may carry through to forming tight complexes with collagen, the protein in bone that serves as the foundation for bone mineralization.

22.3.3. Biochemistry

Insights into the biochemical properties of silicon in higher animals are limited. Although the body load has been estimated to be 1 to 2 grams, a precise biochemical compound that requires silicon has not

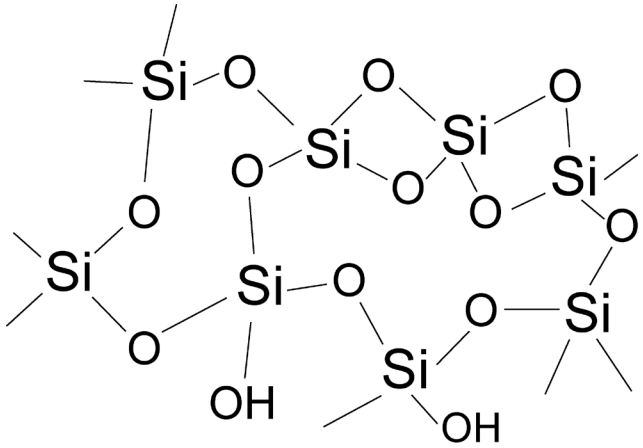

FIGURE 22.7. *Amorphous Array of Hydrated Silicic Acid (Adopted from da Silva and Williams, 1991).*

been identified. There is evidence that chains of $Si(OH)_4$ interact weakly with cis-diols typically found in catechols and are able to replace phosphorus as a central element in condensation reactions (da Silva and Williams, 1991). A silicon deficiency has been observed to lower the hydroxyproline content of collagen by lowering the activity of prolyl hydroxylase, a key enzyme in the post-translational modification of collagen and an essential factor for bone crystallization. Lower levels of alkaline and acid phosphatases, two other key enzymes in bone, have also been reported in a silicon deficiency.

22.3.4. Nutrition

Based on dietary studies, the intake of silicon from foods in the typical U.S. diet ranges from 40 mg per day for males to 19 mg/day for females. Wide variations in foods reflects soil conditions where the crops are grown for harvesting or used as forage for livestock. Geographical location on a global input scale must also be taken into account. China and India, for example, have plant-based foods as a main staple in the diet and have an estimated average intake of silicon as high as 200 mg per day (Jugdaohsingh, 2007). As to meeting nutritional needs, there is no RDA or Adequate Intake established for silicon. Most authoritative sources, however, recommend between 5 to 33 mg/day, basing this estimate on studies that have shown marked improvement in bone mending and skin health.

22.3.4.1. Food Sources

Plant based foods as opposed to meats and dairy products tend to have higher levels of silicon. The most available source is drinking water, and here again the variation in the silicon content is quite large. An estimated 55 percent of silicon is provided by liquid beverages such as beer, coffee, and water. Grains and grain products contribute about 14 percent and vegetables only 8 percent. Processed foods, particularly those receiving silicate additives as antifoaming and anticaking agents, can raise the intake.

22.3.4.2. Body Load

Major blood vessels, tendons, ligaments, cartilage and bone, all rich in connective tissue, have the highest deposits of silicon in the system. Blood levels, which consist almost entirely of unbound silicic acid, are low, averaging about 0.5 mg/liter.

22.3.4.3. Human Studies

Two cross-sectional studies have supported silicon importance in nutrition, specifically bone formation and bone density. Jugdaohsingh *et al.* (2004) reviewed the data from a Framingham Offspring cohort composed of 1,251 men and 1,596 women and concluded that a higher hip bone mass density (BMD) correlated with high Si intake in men and pre-menopausal women, but interestingly, not post-menopausal women. More recently, Macdonald *et al.* (2012), as part of an osteoporosis screening study of 3,199 premenopausal and early post-menopausal women, reported a positive increase in the BMD at the spine and femur in pre-menopausal and menopausal women treated with Si while on hormone replacement therapy.

22.3.5. Absorption, Storage and Excretion

Orthosilicic acid is readily absorbed into the system. How much is absorbed, however, depends on the food source and the pH of the internal environment. Plant silicates are composed mainly of water-insoluble polymeric chains that break down to smaller molecules of orthosalicic acid. The importance of this step is illustrated in Figure 22.8. Only 2% of the silicon in a banana, one of the highest food sources for silicon

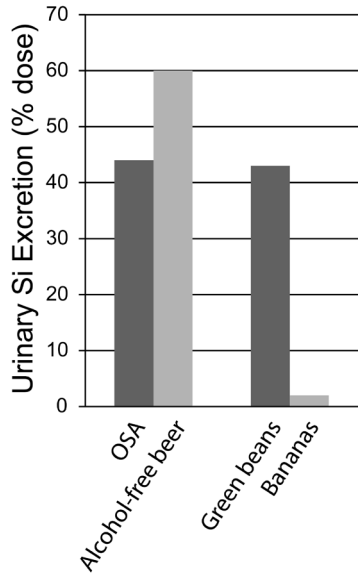

FIGURE 22.8. *Percentage of Silicon Dose Excreted in the Urine 6 hours after Ingestion of OSA, orthosalicic acid (21.4 mg); Alcohol-free beer (22.9 mg); Cooked green beans (6.1 mg); Bananas (13.6 mg). Values reflect estimated Si intake. (Adopted from Sripanyakorn et al., 2009).*

(5.5 mg/100 g), is absorbed, whereas green beans (2.5 mg/100 g) have an absorption efficiency of 60%. In the stomach (pH ~1) the orthosalicic acid is mainly non-ionic and water insoluble. In the proximal intestine (pH ~8) most of the orthosalicic acid is in a water soluble ionized form favorable for absorption. Permeation events that occur at the apical surface have not been clearly defined. The silicon that is absorbed is rapidly excreted. A rat study using the radioactive isotope of silicon found that 48 hours after ingestion, all the silicon had been eliminated from the system by excretion in the urine. An earlier study by Goldwater (1936) reported that human subjects excreted an average 10 mg of silicon per day and a follow up study by Kelsay and coworkers (1979) reported 12–16 mg/day in the urine following a high fiber diet. These studies did not comment on the storage of silicon within the system, which based on radioactive data appears to be minor.

22.3.6. Toxicity

Current understanding holds that silicon from a naturally occurring food source poses no risk of adverse effects. This does not hold for

silicon taken as an additive or supplement. A case in point is magnesium trisilicate (6.5 mg of silicon per tablet). Used as an antacid over a prolonged period (years) could lead to the development of urolithiasis, which manifests as kidney stones. This is a rare occurrence, but one of concern. Attempts to link silicon intake with carcinogenicity has been futile. For example, feeding rats and mice with silica (SiO_2) orally and daily for 2 years gave no evidence of inducing tumors.

22.3.7. Summary

Research on silicon essentiality in humans has reached an impasse. The data appear to support an important role in bone structure and possibly in the prevention of bone loss with aging. The latter, however, would suffice to meet one criteria set for essentiality. Although classified as an ultratrace mineral, silicon (like iron) has a heavy presence within the system. One must consider that most of this silicon performs a structural role, implying the silicon is permanently fixed in place with little opportunity for turnover. Studies are needed to give more insight into the dynamics of silicon within the system. Evidence supports a disparity in the absorption of silicon from different food sources. This, too, is in need of clarification. These findings tend to suggest that a failure to break down polymeric forms of silicon in foods could lead to less being absorbed. This is one instance where digestion takes precedence over absorption in determining bioavailability of a mineral. It seems clear that silicon taken in foods poses no harm to an individual. The same cannot be said of silicon-based medicine or supplements. Here, the disquieting effect is to prevent formation of silicon-induced kidney stones and less concern for other effects.

22.4. VANADIUM

Vanadium's health-promoting properties have been the subject of much debate. Favorable to an essential role are numerous studies reporting enhancements to physiological functions in response to vanadium supplements to the diet. The opposing position is that a time-dependent loss of function relative to some vanadium-specific biomarker has yet to be demonstrated. While vanadium is known to be at the active center of *vanadium bromoperoxidase* in algae, a human enzyme requiring vanadium as a cofactor has yet to be discovered.

This chapter can only bring attention to what is known from studies that have used traditional nutritional approaches to gain insight into possible targets of vanadium's action. Although no official recommendation for an RDA or AI has come from these studies, exposure to vanadium in a wide range of foods makes a deficiency of this mineral an unlikely occurrence.

22.4.1. History and Early Insights

Vanadium is named for Vanadis, the Norse Goddess of beauty; beauty in this instance refers to an array of colors associated with the different oxidation states of vanadate ions. The biological history of vanadium focused initially on a strain of algae that required a vanadium enzyme to grow and survive. Tunicates and toadstools also relied on the enzyme. Another discovery showed that the bacterium Azobacter used vanadium in place of molybdenum as a cofactor for nitrogenase, the nitrogen fixing enzyme. In one of the earliest studies to suggest an essential role in mammals, Schwarz and Milne reported reduced growth and reproductive capabilities in rats fed diets purged of vanadium. Other studies showed that chickens fed vanadium supplements, when compared to the controls, had a greater feather length and significantly lower blood cholesterol. Few if any studies reported a vanadium deficiency having adverse effects on humans. Present understanding, therefore, considers vanadium's role in human nutrition very limited but safe.

22.4.2. Chemical Properties

As number 23 in the Periodic Table of Elements, vanadium is a metal that exhibits multivalency and 3d coordination properties. Vanadium's electronic configuration [Ar]$4s^2 3d^3$ gives rise to oxyanions and oxycations with multiple valence states, ranging from –3 to +5; +2 to +5 is perhaps more relevant to biology. The analogous oxidation scheme with molybdenum (Figure 22.9) may help explain why molybdates and vanadates share cofactor functions in some enzymes. da Silva and Williams further point out that of the two, vanadate oxy

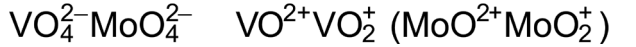

FIGURE 22.9. *Structural Similarity between Oxymolybdates and Oxyvanadates.The data offer a rationale for substituting vanadate for molybdate is enzyme catalysis.*

complexes are more powerful reducing agents than the corresponding molybdate complexes. Suffice to say that with such a wide range of oxidation states, vanadium has the potential to exist in many different forms and complexes, not all of which have equal efficacy in vanadium's action.

22.4.3. Biochemical Properties

Table 22.4 summarizes the biochemical properties that have been attributed to vanadium complexes in biology. The haloperoxidase with vanadate as a cofactor is essential to the survival of sea algae. This organism has a vanadate ion at the active site of bromoperoxidase and uses hydrogen peroxide to incorporate bromine into a wide variety of marine metabolites. The reaction is needed for growth, defense and structural integrity of the algae. The finding of an enzyme with a vanadate cofactor has encouraged studies to determine if vanadium in higher animals also functions as an enzyme cofactor. To date, however, there is no evidence for a haloperoxidase or any vanadate-requiring enzyme in human tissues. Biochemical functions of vanadate as an insulin mimetic has also been proposed. A number of cell culture studies have reported that cells from a variety of tissues acquire a greater sensitivity to insulin when grown in a medium supplemented with vanadium. Pharmacological doses approaching toxic levels and ranging from 10 to 100 times the amount in the diet are needed to show effects, however, which argues against the response having physiological significance.

TABLE 22.4. Suspected Functions of Vanadium.

Cofactor Role	Action
Haloperoxidases (bromoperoxidase)	Brominated compounds produced by sea weed
Nitrogenase	Replaces molybdenum in nitrogen fixing enzyme
Biochemical	
Lipid Metabolism	Cholesterol and triacylglyceride biosynthesis
Glycogen synthesis	Stimulates glycogen synthesis in liver
Physiological	
Insulin mimetic	Anti-diabetic agent
Growth stimulator in rats	Unknown
Muscle builder in rats	Unknown
Thyroid gland	Structure and thyroid hormone production

Another consideration that has received experimental support is to regard vanadium's presence as that of a natural inhibitor and thus a factor that regulates enzymes that metabolize phosphate. ATPases, protein kinases, phosphatases, and nucleases are subject to interference by vanadium salts.

22.4.4. Nutrition

Typical of microminerals, vanadium's presence in foods is measured in micrograms. The estimated body load is less than one mg. Americans consume about 30 to 50 micrograms of vanadium per kilogram of diet to sustain this load. Only about one percent of the vanadium ingested is absorbed.

22.4.4.1. Food Sources

Vanadium's presence in soils, water, air and ocean environments is conducive to providing adequate amounts of vanadium from foods in the diet. According to the U.S. Department of Agriculture, the best food sources are whole grains, mushrooms, shellfish, dill and black pepper. Those low in vanadium are dairy products, legumes and fresh fruits. Airborne vanadium may also be an important source. Food processing may contribute to the content of vanadium in a food source due to possible contact with the stainless steel equipment. Processed or refined foods may contain higher levels of vanadium than unprocessed foods, possibly because of contamination from stainless steel processing equipment.

22.4.4.2. Recommended Intake

Vanadium has neither an RDA nor a biomarker to assess its adequate level of function. A guide to the quantity needed is first to recognize that the body requires less than 1 mg to sustain the body load. Secondly, that absorption estimates suggest less than one percent of the amount ingested is absorbed into the system. For infants up to one year of age the suggested daily intake is 10–50 micrograms; one year through adulthood the estimate rises and steadies out at 50–100 micrograms/day.

22.4.5. Digestion, Absorption and Metabolism

A drawback to vanadium is its very poor absorption efficiency. Such

low absorption results in most of the vanadium taken in being excreted in the feces; very little is lost in the urine. The causes of poor absorption is unknown, although early studies suggested that vanadium enters the system through phosphate channels in the membrane, suggesting competition with an ion present in bulk quantities.

22.4.6. Toxicity

Toxicity is a concern for those who regularly take vanadium supplements such as vandyl sulfate. Among the symptoms that appear are a loss of appetite and gastrointestinal discomfort. More serious signs include nervous disorders and a disturbance in heart rhythms and bipolar disorders.

22.4.7. Summary

Although the biology for vanadium is extensive, vanadium itself has yet to be accepted as essential for humans. Oxidation/reduction potential may play a significant role in vanadium's biochemical actions, particularly since primitive organisms use vanadium as a cofactor for peroxidase enzymes. Because of a lack of a molecular understanding, knowing the specific targets of vanadium action or its bioactive form is more challenging. From its body load to its need in the diet, there is every indication that vanadium must be regarded as an ultra-trace mineral, yet studies to find a specific function have been compromised by the very large quantities needed to show an effect. Perhaps the poor absorbability of vanadium and vanadates is a barrier that must first be overcome.

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22.6. PROBLEMS

1. Offer an explanation as to why some forms of arsenic are very toxic and others are not. What in your opinion is responsible for the discrimination?

2. What enzymes are needed for a human to tolerate arsenic? Based on what you know about arsenic, how effective are such systems?

3. Based on its known action with adjacent -SH groups (Figure 22.1), predict a biochemical compound in the conversion of glucose to fatty acids that would be susceptible to arsenic action. (Hint: the compound is in the pyruvate dehydrogenase complex.)

4. In order to avoid taking steroids, and hence risk detection of high steroid levels in the blood, some athletes have resorted to taking supplements of boron instead. What is the scientific basis behind such a move?

5. Why has the discovery of the NaBC1 transporter bolstered the case for ruling boron an essential element?

6. What element in the Periodic Table appears directly above silicon? What does this tell you about silicon's propensity to form polymeric chains?

7. Name a suspected function of vanadium that benefits animals and humans.

8. What minerals in the diet are apt to lower the efficiency of vanadium absorption from the intestine?

Answers to End of Chapter Problems

Chapter 1

1. a, b, d, f, h are macrominerals; c, e, g, j, k, I, L are microminerals
2. Refer to a Biochemestry textbook for the structures
3. a. sodium chloride
   b. potassium phosphate
   c. ferrous chloride
   d. sodium iodide
   e. potassium chloride

   Solubility is determined by polarity and compatibility with water. Monovalent ions are more soluble than divalent ions; ferrous is less prone to form insoluble hydroxides with water.
4. a. Cu^{+} is a reductant (electron donor); Fe^{3+} is an oxidant (electron acceptor)
   b. $Mn^{2+} + Fe^{3+} \rightarrow Mn^{3+} + Fe^{2+}$
   c. no reaction
5. Metal ions prevent chemical damage to enzymes. To function they must be able to change valence states to accommodate electron changes. Calcium ions, for example, with only a +2 valence cannot donate or accept electrons.
6. Nearly a third of the iron in the system is hemoglobin iron. Very

little iron is excreted. Iron needed on a daily basis is less than 20 mg (Table 13.4), which is in the range for a micromineral.

7. Zinc. All macro biometal ions have only one valence.
8. Biomineral → macro or micromineral → metal ion or metalloid → trace metal → ultratrace metal.

Chapter 2

1. $1s^2 2s^2 2p^6 3s^1$ Upon ionization, loss of the $3s^1$ electron forms Na^+ and a stable Ne core.
2. Ca^{2+} arises by removing both $4s^2$ electrons.
3. $n = 2$ signifies atoms that have 1*s,* 2*s* and 2*p* shells inclusive for filling and no shells beyond 2*p*. Included in the list are all second row elements, Li, Be, B, C, N, O, F, Ne.
4. Elemental iron has the configuration $[Ar]4s^2 3d^6$. Ferrous iron is formed through the loss of the 2, 4s electrons $[Ar]3d^6$; ferric iron loses these two and one 3*d*.
5. $n = 2$; $n = 3$
6. No. Only atoms with *d* orbitals can fit the spacial requirement of an octahedron, i.e., 6 bound ligands, 4 in a square planar arrangement and 2 in the axial position.
7. Diamagnetic means no magnetic properties. The phenomenon occurs when all electrons in the orbitals are paired. Paramagnetic has magnetic properties due to an unpaired electron arising from an odd number, e.g., Cu^{2+} $[Ar]3d^9$. In contrast Zn^{+2} $[Ar]3d^{10}$ with no unpaired electrons is diamagnetic as is Cu^+ $[Ar]3d^{10}$.
8. Close shell means all available shells or subshells are filled with electrons. Such would happen to all of the ions listed (see Table 2.1).
9. a. carbon
   b. neon
   c. chloride
   d. zinc
   e. none

10. a. magnesium, Mg^{2+}
    b. calcium, Ca^{2+}

11. a. $1s^0$
    b. $1s^2 2s^2 2p^6$
    c. $[Ar]3d^9$
    d. $[Ar]3d^{10}$
    e. $[Ar]3d^5$
    f. $[Ar]3d^6$

Chapter 3

1. Metalloproteins do not function as catalysts. Their main function is metal ion storage and transport. For iron, examples include hemoglobin and transferrin. Metalloenzymes function as catalysts. Examples include zinc in carbonic anhydrase and copper in ceruloplasmin.
2. Most likely the loss of activity can be attributed to a lost cofactor. Metal-activated enzymes have loosely bound metal ions that readily dissociate from the enzyme protein surface.
3. a. Chloride ions are a counter ion to Na^+ to maintain charge neutrality (see chapter 10)

   b. Magnesium ions. Mg^{2+} energizes the terminal phosphate group of ATP preparatory to transferring the group to a substrate.

   c. Manganese as Mn^{2+}

   d. Iron is in three places: in the electron transport chain the most prominent forms of iron are heme iron in cytochrome proteins and as iron sulfur centers. Iron is also in the terminal cytochrome oxidase, an iron/copper enzyme, that transfers electrons to O_2.

   e. Both Na^+ and K^+ exist as free ions. That is there major if not only biological form.
4. Hydroxyapatite is a complex of calcium and phosphate that is the building block unit of crystalline bone (Figure 3.5).
5. Calcium carbonate makes up the shell of eggs in avians and reptiles.

Chapter 4

1. a. detrimental to both ions
   b. minimal
   c. detrimental
   d. detrimental
   e. minimal
   f. minimal

2. An anti-nutrient is any compound or factor that is capable of interfering with the bioavailability or function of a nutrient. Examples include phytate or phytic acid that blocks the absorption of zinc and other essential divalent metal ions. Na^+ and Cl^- exist primarily as free ions with little tendency to bind to ligands that could interfere with absorption.
3. By adjusting the water content (condensing), salt content, or physical properties of food, packaging has the potential to alter the mineral composition of a food product.
4. Plants apparently do not discriminate between potentially toxic minerals and minerals deemed essential for health of humans and animals. The danger is some plants are better able to cope with toxic minerals, which are then passed on to human and animals in the food supply.
5. Because of the high level of phytate in grains, unleavened bread suppresses zinc absorption. Low zinc intake on a steady basis could affect physical growth. Adding yeast, a source of the enzyme phytase, breaks down the phytate and overcomes a zinc deficiency.
6. Salt, primarily sodium, is a major essential nutrient that has the highest recommended intake level of any mineral (AI =1.5 grams/day for adults). Although too much sodium is a concern, severely restricting sodium intake will interfere with many key metabolic events.
7. Processed meats such as sausage, deli ham, and cottage cheese are animal products. Freshly cut meat from slaughtered animals is not. For plants, nearly all produce is either freshly cut or available in packaged form. Non-processed include fresh fruits such as apples and grapes. Canned tomatoes, packaged rice, whole grain cereals, etc. are processed foods.
8. Over expression of genes coding for storage or membrane trans-

port proteins increases the content of specific minerals in the plant. For example, when the gene for ferritin is enhanced, more iron is retained and as a food source the plant is richer in iron. Pumping more mineral into the plant by enhancing the gene for a membrane transporter or increasing the amount of a storage protein is a better strategy than enhancing intracellular or extracellular transport proteins.

9. Any heavy metal that is present in high amounts in the soil could make Mary unhappy. Cadmium, arsenic, or lead that have no nutritional value or can evince toxic properties at low levels are a special concern.

Chapter 5

1. a. Both terms set statistical standards for assessing risk of deficiency or oversupply. EAR (estimated average requirement) applies to the level that puts 50 percent of the population at risk of a deficiency (or 50 percent not at risk). EAR also allows one to calculate the RDA (recommended daily requirement), the intake level that puts a 2.5 percent at risk.

   b. RNI (recommended nutrient intake) is the Canadian equivalent of RDA.

   c. AI or adequate intake is a safe level that meet standards consistent with health. AI often replaces RDA when there is no quantifiably reliable biomarker to support a more definitive conclusion.

   d. Both terms address safe intake. UL (upper limit) refers to the tolerable upper intake level that theoretically puts no one at risk of deficiency or excess. LOAEL (lowest observed adverse effect level) basically denotes that level that can be reached without observing adverse effects. Although both terms may seem alike, LOAEL is more concerned with toxic exposure rather than a healthy intake.

2. All three reflect the overlap that occurs when 3 different minerals can give rise to the same symptoms, in this case iron deficiency. It is known that cobalt (as vitamin B12) when deficient can lead to pernicious anemia (Chapter 21). A deficiency in iron and copper can also lead to anemia.
3. Measure the level of ceruloplasmin in the plasma.

4. See chapter 21
5. Superoxide dismutase is more sensitive to copper deprivation rather than zinc. Zinc is primarily a structural element in the enzyme whereas copper is present at the catalytic center.

Chapter 6

1. When dealing with absorption, free ion solubility and quantity are paramount. Macrominerals have mass and hence can force entrance by diffusion. Microminerals form complexes with transport proteins residing in the membranes and cytosol of absorbing cells. Specific membrane transporters, however, are required for both.
2. Vesicles shield minerals from other binding factors that may interfere with movement in the cytosol. They also allow vectoral transport between input and output surfaces. Vesicles also regulate intake by appearing on the surface when needed and sequestered internally when not. Microminerals especially those with limited solubility are more apt to use vesicles to enter and leave cells.
3. No. Both ions cannot accept electrons and hence cannot change valence to accommodate movement.
4. The energy of a sodium gradient drives the uptake of sugars, amino acids and other nutrients across the membrane of intestinal cells. Failure to form sodium gradients can impair effective uptake. Alternatively, if the sodium is the rate-determining factor, oversupply can result in a greater uptake of these essential nutrients.
5. As note in answer 4 above, the uptake of glucose and other sugars depends on sodium gradients across membranes. This means the amount of sodium in the diet could be a factor in governing the amount of glucose that passes into the enterocyte and into the system. In essence high sodium could augment the amount of glucose absorbed which over time could lead to obesity.
6. a. transcellular uses energy generated within the cell; paracellular relies on diffusion

   b. concentration is critical for paracellular and less of for transcellular.

   c. not an important factor for either.

   d. factors on the surface membrane of absorbing cells are impor-

tant only for transcellular transport. Paracellular denotes entrance via the junctions between enterocytes.

e. Paracellular is regulated by amount, transcellular by the availability of transporters.

7. Because of their limited solubility in water at neutral pH, ferric ions and others engage gastroferrin (a mucin) and enter cell as a soluble metal ion-gastroferrin complex.
8. Metallochaperones take part in a non-vesicle type transport of metal ions through the cytosol. These are small metal binding proteins selected for the mineral being transported.
9. A sudden surge of iron inward will trigger the synthesis of ferritin, an intracellular iron storage protein. A surge of zinc will stimulate metallothionein. Both proteins buffer the amount of metal ion entering the system and protect the cells from toxicity.
10. Changing valence pertains only to microminerals. Some ions must be at a specific valence in order to be recognized by transport factors. These would include iron, copper, manganese, and others.

Chapter 7

1. Use chapter 13 to help identify components and valence changes.
2. The concentration of NaCl in sea water (500 mM) is about 3.5 time greater than plasma (135–145 mM) (Table 7.1). Raising plasma NaCl will draw water from the tissues and cells into the plasma; lowering the level will cause cells to absorb more water.
3. Bad advice. Injecting iron directly into the blood bypasses mechanism in the gut designed to modulate iron uptake and prevent overdosing.
4. As shown in Table 7.1 about half the calcium in serum is bound to proteins and other factors. To perform its functions calcium must be available as a free ion. A tightly bound calcium cannot be used in hydroxyapatite formation and hence is restricted from taking part in bone formation.
5. The diffusion rate (F) is directly proportional to the cross section area (A) and C2-C1; it is inversely proportional to the path length (L). Doubling C2-C1 will require A to be halved or L to be doubled to achieve the same diffusion rate.

Chapter 8

1. In the short term increasing dietary calcium and phosphorus intake will raise both ions in the blood. In the long term, bone resorption in response to low intake will raise serum calcium and phosphate levels. Both conditions are carefully regulated by hormones.
2. Only calcitonin produced by the thyroid gland responds to a rise in calcium and phosphorus in the blood. Calcitonin suppresses reabsorption of Ca^{2+} in the kidney and calcium and phosphorus release from bone. A fall in serum calcium and phosphorus evokes the action of calcitriol and PTH which stimulate intestinal absorption and suppress kidney excretion. PTH also stimulates osteoclasts to breakdown bone and release more calcium into the blood.
3. Magnesium and potassium. Magnesium displaces the calcium effectively blocking the trigger for muscle contraction. Potassium induces relaxation by modulating sodium effects on nervous impulses that stimulate contraction.
4. Potassium
5. Zinc can act alone; molybdenum with sulfur can act as a complex to bind copper.
6. No. T4 has the full complement of iodine on the molecule. Formation of T3 requires a selenium-dependent enzyme.
7. Selenate or basically a free selenium salt. As a cofactor selenium is locked within the structure of an amino acid (selenocysteine or selenomethionine) and cannot exchange with sulfur.
8. Iron can replace manganese and render the activity of superoxide dismutase-2, an antioxidant enzyme in the mitochondria, functionless.
9. To only increase calcium may work when blood levels are low, but maintaining a balance between calcium and phosphorus is the important goal.

Chapter 9

1. Iron and manganese are believed to access the brain via transferrin and hence require transferrin receptors. Copper and zinc access has not been clarified, although movement inward after first detaching from serum proteins is a strong possibility.

2. Little is known about heavy metal sequestering in brain. Zinc uptake into synaptic vesicles depends on ZnT3 a specific zinc transport factor in brain. Copper requires a copper-transporting ATPase enzyme to access vesicles (Chapter 15). Iron uses a combination of binding proteins and vesicles.
3. Wilson disease is characterized by copper loading in brain and liver. Unregulated uptake of copper and other metals in brain is believed to cause the formation of amyloid fibers characteristic of Alzheimer's and Parkinson's diseases. A cause-effect relationship, however, has not be established.
4. Accumulation implies a disruption in homeostasis favoring retention. That retention appears to be region dependent would suggest that areas where amyloid fibers appear in abundance could be regions that would favor iron sequestration since the fibers tend to trap iron, zinc and copper.
5. No. One could assume that for a metal ion to modulate the action of glutamate, it must be able to form stable complexes with the glutamate receptor.
6. Calcium could be a candidate, but calcium is the trigger that releases zinc ions into the synaptic junction. Also, calcium in the cytosol is generally kept at extremely low amounts.
7. The question raises the spectra that neurons are continually being synthesized throughout the life span of the individual. Iron accumulation in certain brain regions may be a testament to such an occurrence. Alternatively, neurons in place could be demonstrating a shift in iron homeostasis. The answer requires further research on neurobiological phenomenon involving minerals.
8. If learning is regarded as a regulated electrochemical event involving the synergistic action of neurons in select brain regions, then zinc effects may target neural transmissions. There is, however, no mechanistic answer to this question.

Chapter 10

1. See figure 10.1
2. Potassium has the potential to antagonize sodium at membrane surfaces. Practically speaking, such will occur only when the intake of potassium by whatever means (injection or diet) is very high. Po-

tassium interference with sodium at neuromuscular junctions could help explain potassium action on muscle contraction.

3. Because tests for assessing sodium or potassium status are not reliable, RDA for the two ions has been dropped in favor of an AI. If either ion is involve, the clinician may attempt to correct the symptoms by administering sodium or potassium. Severe muscle cramps, for example, can be eased by giving the patient potassium or recommending a food source rich in this mineral.
4. There is little free potassium in the blood and hence available to be excreted through the sweat glands.

Chapter 11

1. See Table 11.1
2. Regulatory implies the modulation of the action of an enzyme or signaling event; catalytic means the mineral is an intricate component of the catalytic property of an enzyme.
3. Calcium in soda is less than one percent of the RDA. If not accompanied by calcium, phosphate can lower blood levels of calcium. Nutritionist recommend a 1.3:1 C:P ratio for intake. Over a long period high phosphate intake without calcium will contribute to bone loss.
4. Vitamin D3, a precursor to calcitriol, regulates calcium and phosphorus absorption by controlling the level of their transporters. Rickets is only one concern. Low calcium could affect muscle contraction and compromise cell signaling.
5. The requirement for phosphorus is tuned to growth. The decline reflects stability at the end of a period of rapid growth.
6. Parathyroid hormone (PTH) controls blood levels of calcium (Figure 11.4). Detrimental effects are to stimulate bone resorption by activating bone osteoclasts. Positive effects increase the level of calcitriol to bring more calcium into the system.
7. a. PTH raises calcium blood levels; calcitonin lowers blood calcium

   b. PTH activates bone resorption to normalize serum phosphous; calcitonin lowers serum phosphorus by promoting bone formation

   c. PTH stimulates calcium absorption; calcitonin suppresses calcium absorption

d. PTH suppresses calcium excretion; calcitonin enhances calcium excretion

e. PTH activates osteoclasts; calcitonin prevents release of bone calcium

f. PTH is produced in the parathyroid gland; calcitonin is synthesized in the thyroid gland

g. PTH activates calcitriol biosynthesis; calcitonin has no direct effect on calcitriol although current understanding holds that this hormone counters all phases of calcitriol action.

8. Genetic expression of all three transporters is influenced by calcitriol
9. A correlation with hip fracture seems logical in that bone erosion over time can be attributed to low serum calcium which in turn can reflect calcitriol insufficiency.
10. Effusion of calcium from the enterocyte is against a strong external calcium concentration. Not only in enterocytes by all cells in general require an ATPase enzyme to release calcium because cytosolic calcium is only a fraction of the external concentration.

Chapter 12

1. Magnesium is a polar divalent cation that lacks the water hydrolyzing properties of minerals with 3*d* orbitals. Its binding to protein is primarily through electrostatic bonds.
2. Covalent binding prohibits ease of diffusion from the protein. Mg^{2+} bound to parvalbumin would be unable to displace Ca^{2+} from troponin C, the muscle protein. The result would be catastrophic in that muscle would be unable to relax after contraction.
3. Both are divalent cations with closed shell, nobel gas configurations. Magnesium $[Ne]3s^2$ as an ion is smaller than Calcium $[Ar]4s^2$. Both lose their s shell electrons to form ions. Both lack a 3*d* orbital.
4. a. minor if at all since calbindin is a specific Ca^{2+}-binding protein

   b. parvalbumin calcium would more apt to increase

   c. kinase enzymes in general would be rendered less functional

   d. impaired DNA and RNA structure

   e. would be shut down

5. Add more Mg^{2+} to the soil since Mg^{2+} give the chlorophyll molecule its green pigment
6. Epson salts, generally used in baths, are touted for their healing and relief of muscle pain. If such pains reflects muscle tension (also referred to as muscle knots), then one can see a possible scientific basis for magnesium as a factor that allows muscle relaxation.
7. Anti implies a countering action. Although the two ions are strikingly different in size there is evidence for antagonism between the two (see chapter 8).
8. Parvalbumin is a Ca^{2+}/Mg^{2+} protein that controls the muscle contraction/relaxation cycle. Controlling the cycle depends on the ease of displacement of one ion by the other. A rapid off rate of magnesium is characteristic of a rapidly contracting muscle. Relaxation is characterized by less Ca^{2+} (having been displaced by Mg^{2+}) bound to the parvalbumin.

Chapter 13

1. $Fe^{2+}[Ar]3s^6$ and $Fe^{3+}[Ar]3s^5$. Loss of the $4s^2$ electrons gives rise to Fe^{2+}; Fe^{3+} occurs though loss of both $4s^2$ and one $3d^6$ electrons. Because upon oxidation Fe^{2+} releases a single electron, Fe^{2+} is a stronger pro-oxidant.
2. Oral. Intravenous bypasses all factors in place for safe intake.
3. The question must pay heed to the multiple paths of entrance. Blocking either mobilferrin or DCT1 will affect non-heme iron absorption but have no effect on heme iron. Paraferritin blockage will affect both non-heme and heme and is the starting factor for releasing iron from the cell. A defective ferroportin will lock iron in the enterocyte. Defects in hepcidin or HFE will not lead to anemia. They along with ferritin are factors for preventing iron overload (question 4).
4. Iron overload begins at ferroportin. Factors that regulate ferroportin concentration at the exporting surface include HFE and hepcidin. HFE controls hepcidin. A defective hepcidin leads to more ferroportin and more iron entering. HFE also suppresses iron uptake from transferrin. A defect puts more iron into peripheral cells. The others play only minor roles.

5. Observe Figure 13.5 for the following
   a. high iron influx favors formation of IRP-Fe and elevates ferritin mRNA translation
   b. low iron influx favors IRP remaining free and suppresses ferritin mRNA translation
   c. transferrin receptor mRNA turnover is suppressed when its IRP is free of iron
   d. transferrin receptor mRNA turnover is elevated when IRP-Fe is present in quantity
   e. condition (a) above
   f. condition (c) above
6. Key word is "absorbed". About 10% dietary iron is absorbed. A male, 19–30 years of age has a RDA of about 8 mg/day with a UL of 45 mg/day (Table 13.4). The following deductions can be made. Based on the amount absorbed, the iron intake is between 10–20 mg. Intake, therefore, is about adequate and there is no risk of a deficiency. If the subject was a female in the same age bracket the RDA is 18 mg/day. The subject is taking 10–20 mg daily which is a safe level for the female.
7. All three pathways converge at paraferritin. Paraferritin is also responsible for reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in order to be exported by ferroportin.
8. Gastroferrin, a mucin, aids solubility of ferric iron.

Chapter 14

1. Zinc's 3*d* orbitals permit coordinate covalent binding; calcium has no electrons in 3*d* orbitals.
2. Copper is a redox metal which means it can accept and donate electrons. With only a +2 valence state, zinc cannot take part in redox reactions and Cu^{+}, which has a reductant, is unable to behave as an inert structural component in zinc-dependent enzymes.
3. Plants are also a rich source of phytate which chelates the zinc and prevents absorption.
4. Zn^{2+} can coordinate with electron pair donors such as histidine and cysteine as well as oxygen in a water molecule. The latter reaction favors formation of OH^{-} which is a stronger nucleophile than water.

5. Zinc finger proteins, so named because of a finger-like projections surrounding a zinc ion in the protein chain, are suited to bind to the major and minor grooves of DNA. Zinc-finger proteins are transcription factors in the nucleus of cells that control the biosynthesis of specific mRNAs.
6. Zinc deficiency is difficult to diagnose. Overt signs are listed in Table 14.6 and means of determining zinc status are shown in Table 14.7. Laboratories rely on zinc levels in the blood and the activity of zinc-dependent enzymes such as alkaline phosphatase in blood cells to determine zinc status.

Chapter 15

1. Of the many copper-dependent enzymes used to assess copper status in humans and animals, ceruloplasmin is a favored choice because of ease of obtaining a blood sample for measurement. Another favored choice is superoxide dismutase. Depressed levels of ceruloplasmin can affect iron metabolism; low serum and tissue copper can cancel the effectiveness of superoxide dismutase, an antioxidant enzyme in blood cells and tissues
2. Copper's main accesses to a cell's interior is via the Ctr1 transporter that will only recognizes cuprous copper.
3. Ascorbic acid (vitamin C) reduces Cu^{2+} to Cu^{+}, which for reasons not clear, renders the copper less absorbable by intestinal cells. Once absorbed, however, reducing Cu^{2+} to Cu^{+} is required for uptake by the Ctr1 transporter in peripheral cells.
4. Oxidase enzymes use molecular oxygen as an electron acceptor. Depending on the number of coppers in the enzyme, the oxygen is either reduced to H_2O_2 or to H_2O. Because it lacks redox properties, Zn^{2+} cannot be used in reactions where changes in the valence state of the metal ion cofactor is required.
5. The RDA for a one-year old infant is 340 µg per day. Human milk contains 200–400 µg per liter (Table 5.1). Thus an infant would have to consume upwards of one liter (1.06 quarts) of human milk per day to meet the RDA. An infant consuming cow's milk would require three times this amount.
6. The black-white pattern reflects on-off pigmentation controlled by the enzyme tyrosinase. Apparently the soil content of one of the paddocks is low in copper salts.

Chapter 16

1. Mn^{2+} has an electronic configuration ([Ar]3d^5) that resembles ferric ion, not ferrous, and, compared to iron, has a larger ionic radius. Mn^{2+} is also less reactive with electron pair donors, which makes binding to a porphyrin ring less likely
2. Both would lead to disastrous consequences. Mn^{2+} is a potent oxidant whose redox properties are suited for the water-splitting enzyme that forms O_2 from H_2O. In contrast Mg^{2+} has no redox properties.
3. The onset of a psychosis can be traced to mismanagement of manganese in the liver. The liver excretes Mn^{2+} into the bile and synthesizes transferrin to give safe passage of Mn^{2+} in the blood. Patients with liver disease are unable to excrete manganese in a timely manner. This could lead to greater levels of Mn^{2+} in the blood and eventually neurotoxicity affecting brain neurons.
4. Manganese is a cofactor for the mitochondrial antioxidant enzyme superoxide dismutase-2, the mangano form of superoxide dismutase.
5. As noted in problem 2, manganese is the key cofactor for the water splitting enzyme in plants. The enzyme literally sustains the dioxygen (O_2) level of the atmosphere
6. It would appear so for all reasons stated in the chapter.
7. 1) High levels of manganese salts taken as supplements can suppress iron absorption resulting in an anemic-like condition. If your friend is showing problems controlling blood sugar:

   2) She could be mistaking manganese for magnesium

   3) In her case manganese is the cause not the cure for her ailments.

Chapter 17

1. Selenium is just below sulfur in the VIB column of the Periodic Table of Elements. Both are metalloids that have nearly identical atomic radii (Table 8.3) and 4 electrons in an outer *p* orbital. There is no clear understanding of selenium's superior oxidant biological properties over sulfur, although differences in reduction potentials have been suggested.

2. Table 17.3. No. Although plants contain selenium, most is present as selenomethionine and hence locked within the structure of an amino acid.
3. Most, if not all selenium enzyme are categorized as oxidoreductases.
4. Your friend is not informed. Although both serve in the capacity of antioxidants, selenium action cannot be mimic by vitamin E and vice versa (Figure 17.11).
5. One serving of broccoli (one-half cup) provides about one microgram of selenium, which is 2 percent of the daily requirement for this age group.
6. UGA is a stop codon that causes a nascent peptide to abort and be release from the ribosome. To overcome UGA's action requires a special tRNA that contains an insertion sequence (eSECIS).
7. Correlating the disease onset with low levels of selenoprotein W was the first indication. Finding no change in glutathione peroxidase suggested a selenium deficiency may not be the only cause. Mutations in selenoprotein N that gave basically the same symptoms shifted the focus to this protein.
8. We know from Kashin-Beck and Keshan disease that chronic deficiencies in selenium can affect body stature and give rise to degenerative osteoarthritis and a myocardium necrosis. Shorter term deficiencies can cause a liver necrosis as seen in rodents. All symptoms lack a unique connection to selenium as the causative factor. A medical doctor would need further evidence to provide a firm diagnosis.

Chapter 18

1. Assuming the concentration of iodine in meat or cheese on average is about 30 μgm per 100 g, an adult would require 0.5 kilograms (about one-fourth pounds) per day to meet the requirement.
2. Sea foods and mushrooms are 3 times richer in iodine. Therefore one third less or 0.17 kilograms would meet the requirement.
3. No. The NIS transports I^-, an anion. To be incorporated in thyroglobulin the I^- must be oxidized to I^+ by the enzyme *thyroperoxidase.*

4. A goitrogen is any compound with the capacity to cause a goiter. Suppressing iodine absorption or limiting access of I^- to thyroglobulin is a key to its action. The isoflavonoid genistine in soybeans is an example of a known goitrogen.
5. Thyroglobulin, upon iodination and coupling contains both T4 and T3 at a ratio of 15:1. A fraction of T4 released is converted into T3 by the enzyme 5′ iodo-deiodinase. Because the reaction is irreversible, T4 cannot arise from T3.
6. Thyroid stimulating hormone (TSH) controls the action of NIS, the Na^+/I^- symporter. TSH, in turn, is controlled by the level of thyroxine in the blood.
7. By powering a sodium-iodide symporter on the basal surface of follicle cells, a sodium gradient provides the energy that drives iodide ions into cell.
8. You must first have a dietary record of all foods and their amounts. These data can be used to calculate the subjects iodine intake with special attention to goitrogens.

Chapter 19

1. Both AI and UL are used. Studies to determine adequate intake use risk of developing dental carries (generally applied to 7 months and older). Risk of excess employ symptoms of skeletal or dental fluorosis to determine the tolerable upper limit.
2. Water borne fluoride in the oral cavity as the free ion can immediately access tooth enamel; food borne fluoride locked into the food particles must be released by digestion.
3. About 1 ppm (mg per liter). Based on the values in Table 19.1, adequate daily intake for a male would be 4 liters, a female 3.
4. A tea drinker since fluoride in the soil tends to accumulate in plant leaves.
5. One option is to use countries such as China or Japan where tea drinking is more common. European countries have experienced a substantial reduction in tooth decay owing to fluoridation of water. Whether plants also played a role is uncertain.
6. Blood levels of fluoride are too low to be considered.

7. Very little. Because of its strong tendency to bind, protein-bound fluoride is not the active form nor the form transported in the plasma.
8. In young children enamel and bone are in various stages of formation and fluoride could interfere with these active building processes.

Chapter 20

1. Cr(VI) is more electron poor than Cr(III) and therefore less stable. To be stable the Cr(VI) must acquire electrons, which gives it strong oxidant properties.
2. Tolerance is measured by the steepness of the downward curve (position 3). A curve with a deep down slope is indicative of strong insulin action.
3. Position 3.
4. No. Symptoms have no value unless the underlying mechanism is revealed.
5. There is no evidence for chromium picolinate as protective factor against Type 2 diabetes.
6. High food sources, like supplements, offer no protection. They do, however represent a safe way to increase chromium intake.
7. The picolinate complex is more readily absorbed.

Chapter 21

1. Leguminous plants such as beans, peas and lentil are high in cobalt. Plants accumulate cobalt in leaves and stems but are unable to synthesize cobamides (octahedral complexes) including corronoids.
2. Cobamides is a general term for octahedral complexes of cobalt. Corronoids are cobamides with a corrin ring, a feature that is readily apparent in the multiple structures of vitamin B12.
3. “Deadly” or “exceedingly harmful”.
4. The variable components are localized to an axial position of the complex.
5. Haptocorrin. Cobalt binding to the corrin ring is acid sensitive.

6. Because Co^{2+} is the more stabile ion, the DCT1 transporter is the likely uptake site.
7. Industrial exposure to cobalt powder lowers the level of thyroid hormones which is conducive to developing goiters.
8. As molybdopterin.
9. Iron supplements to the diet or high iron intake in general can lead to a molybdenum deficiency.
10. Levels of molybdenum enzymes for a deficiency; high levels in the urine for excess.
11. Molybdenum combines with sulfate in soils to form thiomolybdates that are known to bind copper and result in a copper deficiency.

Chapter 22

1. Arsenate [Ar(V)] binds by weaker electrostatic bonds, whereas arsenite [Ar(III)] binds by hydrogen bonds and thus is more effective in canceling the activity of key enzymes.
2. Enzymes that oxidize and methylate arsenite to form a methlated derivatives of arsenate. It is assumed that detoxification, although conducive to modifying toxic properties, is still susceptible to arsenite in the diet.
3. The lipoic acid moiety in the pyruvate dehydrogenase complex.
4. Boron will elevate levels of testosterone in the blood and hence eschew taking supplements of steroid hormones.
5. A biochemical factor that shows specificity towards a mineral or mineral metabolite supports the mineral as being a necessary component of the system.
6. One observes that both silicon and carbon are capable of forming polymeric compounds for structural purposes.
7. As supported by rigorous scientific evidence, there is none. Not knowing the underlying biochemical or nutritional factor(s) in their action precludes making a definitive statement of necessity.
8. Phosphate and possibly iron.

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