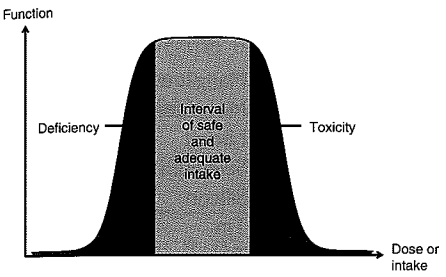

CHAPTER 6

BIOLOGICAL FUNCTIONS OF THE ELEMENTS

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I. Essentiality of Elements
II. Major, Minor, and Trace Elements in Biology
III. Brief Description of the Function of Major Elements
IV. Brief Description of the Functions of Minor Elements
V. The Functional Value of Trace Elements

I. ESSENTIALITY OF ELEMENTS

Much discussion has taken place with regard to how to define the essentiality of elements, particularly trace elements. The earliest definition was actually borrowed from protein chemistry. In this definition, an element is essential if:

- It is present in living tissues at a relatively constant concentration.
- It provokes similar structural and physiological anomalies in several species when removed from the organism.
- These anomalies are prevented or cured by supplementation of the element.

The current definition, suggested by an expert consultation of the World Health Organization/Food and Agricultural Organization/International Atomic Energy Agency (Mertz, 1998), states:

> *An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in the organism.*

The concept of essentiality has the practical consequence that it is necessary to supply an organism with adequate amounts of the concerned elements. An immediate question raised by this consequence is how much is adequate. For most elements, ranges of safe and adequate intakes have been defined. In some cases, however, there is considerable uncertainty regarding the limits of such ranges. Adequate intakes do vary substantially among elements, in both amount and width of the range. In very general terms, the range may be visualized as shown in Figure 1. For a detailed discussion of deficiencies and toxicities, the reader is referred to Chapter 8.

TABLE I. Abundance by Mass of Major and Minor Elements in the Human Body

Element	*Mass Percent*	*Element*	*Mass Percent*
Oxygen	65.0	Magnesium	0.50
Carbon	18.0	Potassium	0.34
Hydrogen	10.0	Sulfur	0.26
Nitrogen	3.0	Sodium	0.14
Calcium	1.4	Chlorine	0.14
Phosphorus	1.0	—	—

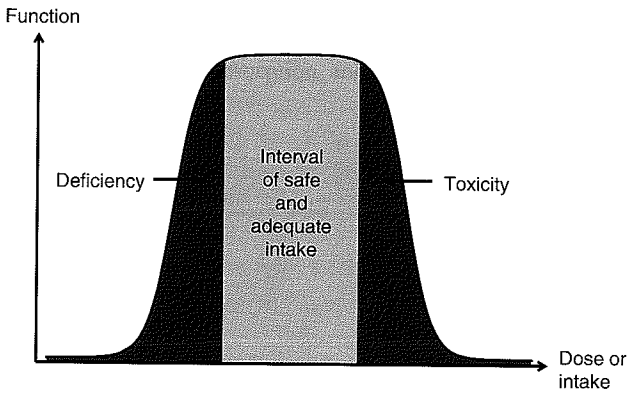

FIGURE 1 Dose–response of essential trace elements.

II. MAJOR, MINOR, AND TRACE ELEMENTS IN BIOLOGY

Eleven elements seem to be consistently abundant in biological systems: hydrogen, oxygen, carbon, nitrogen, sodium, potassium, calcium, magnesium, phosphorus, sulfur, and chlorine. In humans, these elements comprise 99.9% of the atoms. Usually, these elements are divided into two groups of major and minor elements. The major elements—hydrogen, oxygen, carbon, and nitrogen—make up 99% of the atoms, or just over 96% of the body mass. Sodium, potassium, calcium, magnesium, sulfur, and chlorine comprise 3.78% of the body mass (Table I).

The entire group of noble gases is excluded from consideration because their chemical properties make them unlikely to fulfill any biological function. The remaining elements are considered to be trace elements. There are 90 naturally occurring elements in the periodic table; thus, 73 are trace elements. Of these 73, 18 are considered to be essential or possibly essential trace elements: lithium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, tungsten, molybdenum silicon, selenium, fluorine, iodine, arsenic, bromine, and tin. Their abundance in the human body is reflected in Table II.

TABLE II. Abundance of Certain Trace Elements in the Human Body by Mass ($\mu g\ g^{-1}$)

Element	*Mass Fraction*	*Element*	*Mass Fraction*
Arsenic	0.26	Manganese	0.17
Bromine	2.9	Molybdenum	0.08[a]
Cobalt	0.021	Nickel	0.14
Chromium	0.094	Selenium	0.11
Copper	1	Silicon	260
Fluorine	37	Tin	0.24
Iron	60	Tungsten	0.008[a]
Iodine	0.19	Vanadium	0.11[a]
Lithium	0.009*	Zinc	33

[a]Estimated from Iyengar *et al.*, (1978) and Li (2000).

Several problems are associated with proving the essentiality of trace elements. Experiments are based upon the general acceptance that, if an essential trace element is completely withdrawn from the diet of experimental animals, signs and symptoms of a deficiency should occur, such as growth retardation and loss of hair. When a state of deficiency has been established, supplementation of the trace element should alleviate these symptoms and reverse the deficiency state. The first basic problem is that it is not possible to completely eliminate every bit of an element in food. Even if this were possible, the analytical techniques are inadequate due to their limits of detection. A second problem is that when essentiality is being evaluated, there is usually no well-grounded hypothesis for a possible biological function. Withdrawal of one essential trace element from the diet may result in altered uptake patterns for other trace elements, which makes results ambiguous. Most results have been obtained on plants and rodents. Veterinary medicine has contributed with information on the essentiality of elements in domestic animals. When it comes to humans, however, our knowledge of essential trace elements is less advanced for obvious reasons.

The essentiality of 12 of the trace elements in Table II is generally agreed upon, although perhaps not for all

biological species. Without exhausting the available data on vanadium biology, a conspicuous property of ascidians must be pointed out. The blood cells of some ascidians accumulate vanadium at a degree of 10^7 as compared to the sea concentration. *Ascidia gemmata* has been shown to have the highest vanadium concentration—350 m*M*—which corresponds to about 1.7% (Michibata *et al.*, 2002).

III. BRIEF DESCRIPTION OF THE FUNCTION OF MAJOR ELEMENTS

Because the human body is roughly 71% water, it is not surprising that the quantities of hydrogen and oxygen are so substantial. Water makes up more than two-thirds of the weight of the human body, and without water a human would die in just a few days. All the cell and organ functions depend on water for functioning. It serves as a lubricant and forms the base for saliva and the fluids that surround the joints. Water regulates body temperature, as cooling and heating occur through perspiration. Water helps to alleviate constipation by moving food through the intestinal tract, thereby eliminating waste. Water also contributes to the high contents of oxygen in the body.

A. A Few Important Points About Hydrogen

Hydrogen is a very special element in biology. It appears in three states, H^+ (cation), H— (a covalently bound state), and H^- (anion). It is a very strong acid as a proton (H^+). In the H— form, it takes part in stable non-metal bonds such as C—H and N—H. Even in the presence of dioxygen, these bonds are kinetically stable. Hydrogen can also be transferred from a non-metal, not just as H^+ or H— but also as H^- (Fraústo da Silva & Williams, 2001). This makes it possible for hydrogen atoms to take part in one- or two-electron processes. Many biological redox reactions are based upon this property.

B. Carbon: The Backbone of Organic Chemistry and Biochemistry

In the chemical sense, use of the term "organic" means that carbon is involved; thus, an organic compound contains carbon atoms, and organic chemistry is the chemistry of carbon compounds. There are a few exceptions, however, as oxides, carbonates, and cyanides are considered inorganic compounds. Biochemistry, the chemistry of life, is a special branch of organic chemistry. Slightly contradictory is the fact that biochemistry involves both organic and inorganic compounds. To be more precise, it is appreciated in this field of study that the large molecules found in cells all contain carbon but many of the small molecules may be inorganic. A carbon atom is capable of combining with up to four other atoms, but in some cases a carbon atom combines with fewer than that. These bonds are covalent. Not only can a carbon atom form covalent bonds with four other atoms, but it can also combine with other carbon atoms; thus, carbon atoms can form chains and rings onto which other atoms can be attached. The atomic number of carbon is six; it has two electrons in the K shell and four in the L shell. Carbon must gain or lose four electrons to be ionized, but this process is difficult so instead it shares electrons to fill its L shell. In summary, carbon turns out to be a very versatile atom. The organic compounds in biology are represented by carbohydrates, lipids, proteins, and nucleic acids.

1. Carbohydrates

Carbon, hydrogen, and oxygen comprise most of the carbohydrates. For each carbon and oxygen there are two hydrogen atoms (*i.e.*, CH_2O). Carbohydrates perform a series of important functions in biology, such as short-term energy storage (*e.g.*, monosaccharides), long-term energy storage (*e.g.*, starches and glycogen), and structural support (*e.g.*, cellulose found in all plant cell walls), as well as important components of DNA and RNA.

2. Lipids

Lipid molecules are insoluble in polar solvents such as water. They dissolve in nonpolar solvents, and they are nonpolar. Lipids work as energy storage molecules, as insulation and protection for internal organs, as lubricants, and as hormones. Additionally, phospholipids are the major structural elements of membranes that are composed of a bilayer of phospholipids.

3. Proteins

Amino acids can be combined to form peptides, in which case the order of the amino acids is significant. The amino acids consist of an amino (–NH) group and a carboxylic acid ($-CO_2H$) group bonded to a central

carbon atom. When the combination of amino acids exceeds more than about ten amino acids, the resulting peptide is referred to as a *polypeptide*. If the number of amino acids in a combination is more than 50, the molecule is referred to as a *protein*. The most important function of proteins is to maintain and drive the reactions of cells; however, additional properties include acting in supportive tissue like cartilage, and they are involved in muscle movement. Many proteins are enzymes, and their association with trace elements is described later in this chapter.

4. Nucleic Acids

Ribose, which is a monosaccharide, interacts with nitrogenous bases to form nucleosides such as adenosine. When nucleosides exist in cells mainly in the form of esters with phosphoric acid they are called *nucleotides*. If the sugar is ribose, the molecule is called *ribonucleotide*. The two nucleic acids, DNA and RNA, are polymers of nucleotides. In DNA, the bases are adenine, cytosine, guanine, and thymine; in RNA they are the same, except that uracil takes the place of thymine. The nucleotides in both DNA and RNA are joined by covalent bonds between the phosphate of one nucleotide and the sugar of the next one. A chain of many nucleotides is a polymer forming a nucleic acid.

C. Oxygen: The Savior and Reactionist

Without oxygen, humans and other mammals would not survive. Structures with a high degree of organization require specific transport mechanisms, and it is well known that in mammals oxygen is transported in the blood by hemoglobin; however, other oxygen-transporting molecules, such as hemerythrin, are used by some marine invertebrates, and hemocyanin is found, for example, in snails. In muscles, the diffusion of oxygen is facilitated by myoglobin. Oxygen is intimately involved in the production of energy-rich molecules (adenosine triphosphate, or ATP) which takes place in the mitochondrial membrane. The reduction of dioxygen to water requires the transfer of four electrons onto dioxygen at the same time:

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

Such a process, however, is not chemically possible; instead, on the way to becoming water, dioxygen passes through stages of aggressive power: the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\bullet$). All three intermediates are extremely reactive. Even hydrogen peroxide is very reactive, although it is not a radical. Electron- and proton-transfer reactions are extremely rapid, so the reactive intermediates are generally kept in the enzyme. In summary, the reduction of dioxygen to water involves four one-electron transfers:

$$O_2 \rightarrow O_2^{-\bullet} \rightarrow H_2O_2 \rightarrow OH^\bullet \rightarrow H_2O$$

Nature has evolved protective functions to take care of leaking intermediates. The superoxide anion is metabolized by superoxide dismutase to hydrogen peroxide, which is metabolized to water by catalase or glutathione peroxidase without releasing the extremely reactive hydroxyl radical. This balance, however, is delicate, and attacks from metals may result in the release of radicals. The plethora of reactions and functions that could be described for oxygen are too voluminous to present in this chapter, and the reader is referred to standard biochemistry textbooks.

D. Nitrogen Fixation

The supply of nitrogen to biological systems comes from gaseous N_2, which is very abundant in the atmosphere (80%). The bond between the nitrogen atoms is very strong; hence dinitrogen is chemically unreactive. Similar to dioxygen, dinitrogen has to be reduced in order to be biologically available. Reduction to ammonia requires a very specific and sophisticated enzyme in a process known as *nitrogen fixation*.

In the biosphere, most nitrogen fixation is carried out by a few species of bacteria that synthesize the enzyme nitrogenase. This enzyme is found in the species *Rhizobium*, living in symbiosis with root nodules of many leguminous plants, such as beans, peas, alfalfa, and clover. Nitrogen fixation also takes place in free-living soil bacteria such as *Azobacter*, *Klebsiella*, and *Clostridium* and by cyanobacteria found in aquatic environments.

Two proteins comprise nitrogenase. One of them contains a [4Fe–4S] cluster, and the other has two oxidation–reduction centers. Iron is involved in one of these centers, molybdenum in the other. The net reaction is

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

To obtain the reducing power and ATP required for this process, symbiotic nitrogen-fixing microorganisms rely on nutrients obtained through photosynthesis carried out by the plant with which they are associated.

IV. BRIEF DESCRIPTION OF THE FUNCTIONS OF MINOR ELEMENTS

The minor elements in biology are comprised of sodium, magnesium, phosphorus, sulfur, chlorine, potassium, and calcium. The biological functions of the minor elements are discussed in three groups: (1) sodium, potassium, and chlorine; (2) magnesium and phosphorus; and (3) calcium.

A. Sodium, Potassium, and Chlorine: Interactions and Ion Properties

Life evolved from water. Do concentrations of abundant elements in sea- or freshwater reflect this evolution? Table III makes a crude comparison between seawater and blood serum. It shows that intracellular concentrations are in most cases much lower than extracellular, the exception being potassium, which is an abundant intracellular ion. To function properly, organisms must actively pump sodium and chloride out of cells and actively take in potassium. It is not quite clear why life must reject the most abundant anion (Cl^-) and the most abundant cation (Na^+), although this is probably due to a need for the cellular stability that comes from maintaining an osmotic balance. Maintaining the intracellular and extracellular balance requires active transport processes that require considerable energy. For details regarding uptake and transport processes, the reader is referred to Chapter 5.

TABLE III. Concentrations of Free Cations and Anions of Calcium, Magnesium, Potassium, and Sodium in Seawater, Human Blood Serum, and Human Red Cells (mmol/L)

Ion	*Seawater*	*Serum*	*Red Cells*
Calcium	10.25	2.20–2.55	10^{-4}
Magnesium	53.60	0.76–1.10	2.5
Potassium	9.96	3.5–5.1	92
Sodium	471	136–146	11
Chloride	549	98–106	50
Bicarbonate	—	22–29	10?
Phosphate	2×10^{-3}	0.74–3.07	10?

1. Biological Functions of Sodium, Potassium, and Chloride

Circuits maintained by K^+, Na^+, and Cl^- generally control the following properties in all cells of all organisms:

- Osmotic pressure
- Membrane potentials
- Condensation of polyelectrolytes
- Required ionic strength for activity

The first two properties are quite easy to understand; however, condensation of polyelectrolytes may have to be clarified. Biopolymers are polyelectrolytes; DNA, for example, possesses a linear series of charges. Other polyelectrolytes such as fats show two-dimensional arrays, whereas proteins have curved surfaces not seldom almost spherical. The surfaces of polyelectrolytes are stabilized by the surrounding ionic environment mainly due to sodium, potassium, and chloride ions.

Absorption of glucose, or any molecule for that matter, entails transport from the intestinal lumen, across the epithelium and into blood. The transporter that carries glucose and galactose into the enterocyte is the sodium-dependent hexose transporter, known more formally as SGLUT-1. As the name indicates, this molecule transports both glucose and sodium into the cell and, in fact, will not transport either alone.

The essence of transport by the sodium-dependent hexose transporter involves a series of conformational changes caused by the binding and release of sodium and glucose. It can be summarized as follows:

- The transporter is initially oriented facing into the lumen, and at this point it is capable of binding sodium, but not glucose.
- Sodium binds, which induces a conformational change that opens the glucose-binding pocket.
- Glucose binds, and the transporter reorients in the membrane such that the pockets holding sodium and glucose are moved inside the cell.
- Sodium dissociates into the cytoplasm, which causes glucose binding to destabilize.
- Glucose dissociates into the cytoplasm, and the unloaded transporter reorients back to its original, outward-facing position.

Sodium is intimately involved in vitamin transport. For example, the uptake of biotin, a member of the vitamin B complex, is dependent on the sodium-dependent multivitamin transporter, or SMVT (Stanley *et al.*, 2002). There are also indications that the transport in brain

parenchyma of *N*-acetylaspartate (the second most abundant amino acid in the adult brain) is accomplished by a novel type of sodium-dependent carrier that is present only in glial cells (Sager *et al.*, 1999).

Sodium is also closely connected to the transport of vitamin C. It has been shown that the sodium-dependent vitamin C transporter (SVCT) is responsible for an age-dependent decline of ascorbic acid contents in tissues (Michels *et al.*, 2003). The transport of vitamin C into the brain relies heavily on the sodium-dependent ascorbic-acid transporter Slc23a1 (Sotiriou *et al.*, 2002). A conspicuous finding of Handy *et al.* (2002) was that copper uptake across epithelia seems to be sodium dependent.

Experiments have shown the presence of an ouabain-insensitive, potassium-dependent *p*-nitrophenylphosphatase in rat atrial myocytes. This enzyme is suggested to be an isoform of an H-transporting, potassium-dependent adenosine triphosphatase (Zinchuk *et al.*, 1997). The enzyme pyruvate kinase requires potassium for maximal activity (Kayne, 1971); however, it was recently shown that in the absence of potassium dimethylsulfoxide induces active conformation of the enzyme (Ramirez-Silva *et al.*, 2001).

There is an interesting coupling of sodium and chloride in the transport of the neurotransmitter gamma-aminobutyric acid (GABA). The removal of GABA from the extracellular space is performed by sodium- and chloride-dependent high-affinity plasma membrane transporters (Fletcher *et al.*, 2002). It has been suggested that chloride is involved in the regulation of proteolysis in the lysosome through cathepsin C. This enzyme is a tetrameric lysosomal dipeptidyl–peptide hydrolase that is activated by chloride ion (Cigic & Pain, 1999). Alpha-Amylases have also been shown to be chloride dependent (D'Amico *et al.*, 2000).

B. Magnesium and Phosphate: Close Connections

In contrast to many metal and non-metal ions, magnesium is rather homogeneously distributed in organisms with about the same intra- and extracellular concentration of 10^{-3} *M*. It is also unique among the biological cations due to its size, charge density, and structure in aqueous solution, as well as its aqueous chemistry. These properties make magnesium generally different from all other cations, monovalent or divalent. Mg^{2+} has the largest hydrated radius of any common cation; in contrast, its ionic radius (*i.e.*, minus waters of hydration) is among the smallest of any divalent cation (Kehres & Maguire, 2002). Magnesium is an essential element, a fact that was first demonstrated 77 years ago (Leroy, 1926); however, its general role in cellular function is poorly understood.

Although there does not seem to be any significant chemical gradient, the net electrochemical gradient for Mg^{2+} is markedly directed inward because of the negative membrane potential inside; consequently, mechanisms must be in place for maintaining a low intracellular free Mg^{2+} concentration and to regulate Mg^{2+} homeostasis. At least two transport processes have been discovered; one is sodium dependent and the other works even in the absence of sodium. The sodium-dependent exchanger operates with a $3Na^{+}_{in} : 1Mg^{2+}_{out}$ ratio (Romani & Maguire, 2002). Various monovalent and divalent cations may replace the Na^{+} when sodium is absent. In that case, the stoichiometry is 1 : 1 (Romani & Maguire, 2002). The physiological significance of this mechanism is, however, unclear as low extracellular concentrations of sodium are unlikely.

1. Hormonal Regulation of Magnesium Homeostasis

In physiological terms, it is reasonable to envision that, because hormones stimulate Mg^{2+} extrusion, other hormones or agents must also operate in eukaryotic organisms to promote Mg^{2+} accumulation and the maintenance of Mg^{2+} homeostasis (Romani & Maguire, 2002). The mechanism behind hormonal regulation seems to be hormones or agents acting on the production of cyclic AMP. Another possibility is that activating a protein kinase C pathway decreases cyclic AMP in many tissues. An exiting finding was identifying the protein family Mrs2p in mitochondria; this is the first molecularly identified metal ion channel protein in the inner mitochondrial membrane.

2. Magnesium Binding and Magnesium Enzymes: Magnesium and Phosphates

The best known example of the very strong binding of magnesium ions is the Mg^{2+} in chlorophyll. A few other cases are ATP-synthetases in thylakoids and mitochondria and in the ATPases of muscles. Nucleoside diphosphates and triphosphate, both in aqueous solution and at the active site of enzymes, usually are present as complexes with magnesium (or sometimes manganese) ions. These cations coordinate with oxygen atoms of the phosphate groups and form six-membered rings with ADP or ATP (Figure 2). A magnesium ion can form several different complexes with ATP. In solution, formation of the β,γ complex is favored.

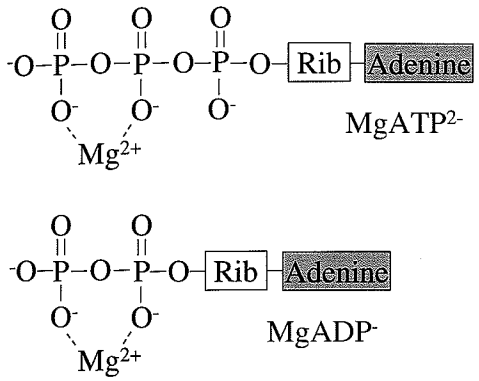

FIGURE 2 Mg^{2+} complexes with ADP and ATP.

Glycolysis is one of two important pathways in carbohydrate metabolism. The other is the pentose phosphate pathway. Both pathways provide energy to cells and both are also involved in the formation and degradation of other molecules such as amino acids and lipids. The glycolysis pathway has ten enzyme-catalyzed steps. In one of them, 2-phosphoglycerate is dehydrated to phosphoenolpyruvate in a reaction catalyzed by enolase. This enzyme requires Mg^{2+} for activity. Two magnesium ions participate in this reaction: A "conformational" ion binds to the hydroxyl group of the substrate and a "catalytic" ion participates in the dehydration reaction. Some other magnesium-dependent enzymes of the general metabolism are shown in Table IV together with their biological functions.

TABLE IV. Some Examples of Magnesium-Dependent Enzymes and Their Functions

Enzymes	*Function*
Kinases	G-transfer reactions
Adenylate cyclase	cAMP formation from ATP
ATPases	Hydrolysis of ATP
Alkaline phosphatase	Splitting off phosphorus
Isocitrate lyase	Formation of succinate and glyoxylate in the citric acid cycle
Methyl aspartase	Glutamate receptor
Ribulose bisphosphate carboxylase	Carboxylation and oxygenation of ribulose bisphosphate
Myosin ATPase	Hydrolysis of ATP in muscles
Nucleases	Hydrolysis of phosphodiesters in nucleic acids
GTP-dependent enzymes	Restriction enzymes cleaving DNA, for example

3. Magnesium and Nucleic Acid Biochemistry

Monovalent metal ions such as Na^+ and K^+ act more or less as bulk electrolytes to stabilize surface charge. Divalent magnesium, on the other hand, interacts with nucleic acids with higher affinity. The role of Mg^{2+} is to neutralize negative charges from phosphates, either electrostatically or by forming hydrogen bonding networks from waters of solvation. Mg^{2+} may lower the pK_a of coordinated water, thereby facilitating phosphate ester hydrolysis. It is becoming increasingly clear that many drug molecules interact with DNA in a specific and Mg^{2+}-dependent manner. An understanding of the latter may aid in the design of other novel DNA binding drugs (Sreedhara & Cowan, 2002).

In addition to the enzymes noted in Table IV, it is worth mentioning a newly discovered class of enzymes requiring magnesium. Several categories of RNA have been found to catalyze reactions autonomously without protein or with only secondary protein assistance. Ribozymes are RNA molecules adopting three-dimensional structures that allow them to catalyze a variety of chemically important reactions, including but not restricted to phosphate ester hydrolysis, amide bond formation, and ligation (Sreedhara & Cowan, 2002). Divalent magnesium seems to be involved in both the structure of RNA and the catalytic mechanism of ribozymes. Other metals such as manganese and calcium might take part in this structural and catalytic mechanism. Due to the intracellular abundance of magnesium, this would be the preferred metal ion. Cleavage of RNA is yet another exciting Mg^{2+}-dependent process of ribozymes.

4. Magnesium and Photosynthesis

As living organisms became abundant on the primitive Earth, their consumption of organic nutrients produced by geochemical processes outpaced production. Developing alternative sources of organic molecules that provided energy and the raw materials required for biosynthetic processes became critical for survival. The abundant CO_2 in the Earth's early atmosphere was a natural carbon source for organic synthesis; hence, photosynthesis became the pragmatic solution to the problem. Organisms capable of photosynthesis include certain bacteria, cyanobacteria (blue-green algae), algae, nonvascular plants, and vascular (higher) plants. Photosynthesis is also the source of the Earth's molecular oxygen. With the exception of anaerobic bacteria, all organisms capable of photosynthesis give off O_2 as an end product.

The net reaction of photosynthesis is:

$$\xrightarrow{\text{light}}$$
$$CO_2 + H_2O \rightarrow (CH_2O) + O_2$$

where (CH_2O) represents carbohydrate. The oxidation of water, a thermodynamically unfavorable reaction, is driven by solar energy. Electrons from this oxidation pass through electron-transport systems that resemble the mitochondrial electron-transport chain. Photosynthesis encompasses two major processes that can be described by two partial reactions:

light

$$H_2O + ADP + P_i + NADP^+ \rightarrow O_2 + ATP + NADPH + H^+$$

$$CO_2 + ATP + NADPH + H^+ \rightarrow (CH_2O) + ADP + P_i + NADP^+$$

light

$$\text{Sum: } CO_2 + H_2O \rightarrow (CH_2O) + O_2$$

Photosynthesis takes place in chloroplasts. Chlorophyll is the most abundant pigment involved in the light harvesting, and the tetrapyrrole ring of chlorophylls, called *chlorine*, is similar to heme but contains Mg^{2+} chelated to the nitrogen atoms of the ring (Figure 3). Chlorophylls a and b are the most abundant types. Magnesium ions are inserted into the chlorin ring through the action of a chelatase enzyme. Without the enzyme, magnesium could not combine with chlorins. Other metal ions, such as Zn^{2+}, Cu^{2+}, and Fe^{2+}, are more likely to combine with chlorins.

Mg^{2+} has an additional function in the chloroplast. When light shines on the thylakoids, pH drops to about 4.0. The protons force Mg^{2+} out of the thylakoid, and some chloride enters. The consequence is increased levels of Mg^{2+} in the stroma, where magnesium activates the carboxylase rubisco for incorporation of CO_2 into ribulose bisphosphate. This reaction is a step in the Calvin cycle of photosynthesis.

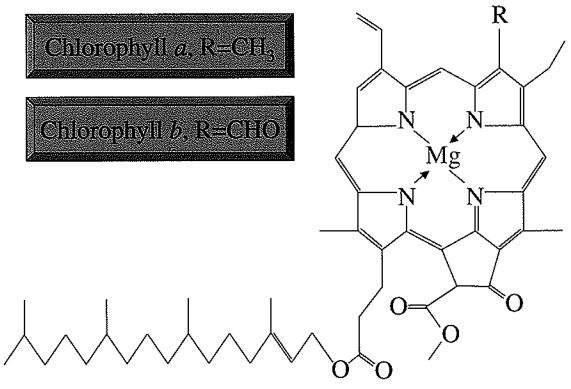

FIGURE 3 Magnesium in chlorophyll.

C. Calcium: Messenger and Support

Calcium has a plethora of functions in biology, and new roles are still being discovered. Calcium triggers new life at fertilization. It controls several developmental processes, and when cells have differentiated it functions to control such diverse cellular processes as metabolism, proliferation, secretion, contraction, learning, and memory (Jaiswal, 2001). The best known function of calcium is its being an integral component of bone and teeth phosphates. Of the approximately 2.14 kg of calcium in the entire body, the skeleton contains 1.1 kg and the soft tissues 13.8 g. The bulk of the calcium, therefore, is contained in the skeleton as more or less crystalline calcium phosphate (called *bone apatite*), and it promotes the stability and rigidity of bones. Although only a minor portion is contained in soft tissues, the role of calcium here should not be overlooked. Control over metabolic processes probably involves a calcium-dependent step (Table V).

TABLE V. Calcium-Controlled Events in Cells

Activity	*Controlled Events or Systems*
Photosynthesis	Dioxygen release
Oxidative phosphorylation	Dehydrogenases
Receptor responses	Nerve synapse
	IP3-linked reactions
Contractile devices	Muscle triggering (actomysin)
	Cell filament controls
Phosphorylation	Activation of kinases (e.g., in fertilization)
Metabolism	Numerous enzymes inside cells
Membrane/filament organization	Annexin-like proteins modulate tension
Cell division	S-100 proteins, immune system
Cell death (apoptosis)	Internal proteases
Hormone/transmitter release	Homeostasis
Binding to membranes	C-2 domains of enzymes
Cross-linking	Outside cells
Enzyme-activation	Outside cells; in membranes

Source: Adapted from Fraústo da Silva and Williams (2001).

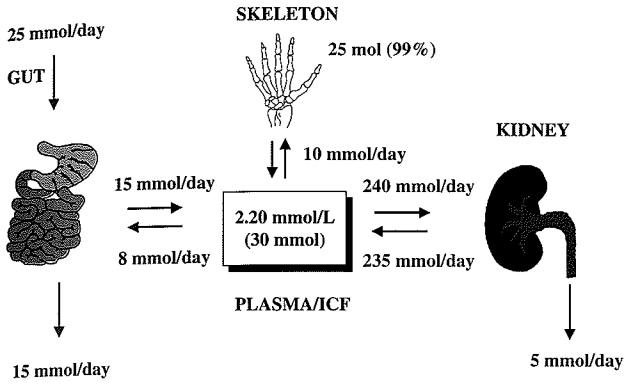

FIGURE 4 Normal daily calcium exchange.

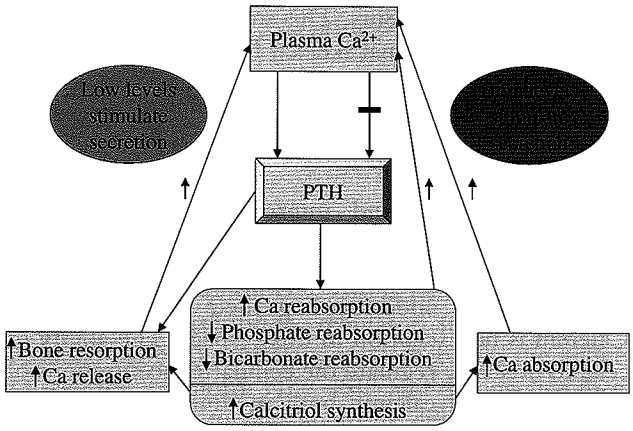

FIGURE 5 Hormonal control of calcium metabolism.

1. Calcium Homeostasis

Only a minor proportion of skeletal calcium (about 1%) is rapidly exchangeable with plasma, although remodeling of bone results in the turnover of nearly 20% of skeletal calcium each year. Approximately 1% of body calcium is present in the extracellular fluid, where functions include the regulation of neuromuscular excitability and acting as a cofactor for clotting enzymes. The gradient of extracellular to intracellular free calcium is around 10,000:1, although cellular calcium is also found as insoluble complexes. Within cells, free calcium ions (Ca^{2+}) regulate the activity of various enzymes directly and also exert second-messenger hormonal functions by interaction with calcium-binding proteins such as troponin C and calmodulin.

The normal daily calcium exchange is represented in Figure 4. Calcium in the gastrointestinal tract originates from the diet and also from secretions. Approximately half is absorbed, mainly in the upper small intestine (jejunum and duodenum), by active transport. Up to 250 mmol calcium is filtered daily by the kidney. With the majority reabsorbed in the proximal tubule and loop of Henle, urinary excretion is normally 2.5 to 7.5 mmol L^{-1}, depending on uptake. The small amounts lost in sweat are usually insignificant, unless profuse sweating occurs for a prolonged period.

Parathyroid hormone (PTH) is a key regulatory hormone of calcium metabolism whose secretion is stimulated by low plasma Ca^{2+} concentrations and by low plasma magnesium concentrations. The physiological importance of regulation by magnesium is unclear, although magnesium depletion can cause hypocalcemia. The secretion of PTH is inhibited by increased Ca^{2+} levels. Within the parathyroid glands, PTH is transcribed as an 115-amino-acid polypeptide and processed to form an 84-amino-acid polypeptide, in which form it is stored prior to secretion. The biological activity of PTH is contained within a 32- to 34-amino-acid fragment of the molecule located at the N-terminal end. Following secretion, PTH is cleaved, mainly in the liver, to produce two fragments, one of which is an inactive (C-terminal) fragment. PTH is usually measured in blood by assays that depend on immunoreactivity rather than biological activity (immunoassays). In renal failure, the rate of metabolism of the C-terminal fragment is reduced as the kidney normally removes this. If assays for measuring PTH are based on the immunoreactivity of the C-terminal end of the molecule, levels may appear higher than would be apparent if biological activity were determined. This is important, because renal failure causes secondary hyperparathyroidism.

The main effect of PTH is to raise plasma Ca^{2+} concentrations through actions on bones, the kidney, and, indirectly, the gastrointestinal tract (Figure 5). In the bones, PTH stimulates osteoclast activity, while in the kidney it increases the reabsorption of calcium and reduces the rate of transport of phosphate and bicarbonate. PTH also stimulates the hydroxylation of 25-hydroxycholecalciferol to form calcitriol, which then acts on the gut to increase calcium and phosphate absorption.

Vitamin D is converted to its biologically active form, 1,25-dihydroxycholecalciferol (calcitriol) by successive hydroxylations in the liver and kidney. Calcitriol stimulates intestinal absorption of calcium and phosphate by regulating the synthesis of a protein that transports calcium across the enterocyte. In addition, calcitriol is required for normal mineralization of bone, which is defective in deficiency states. Weakness of skeletal muscles also occurs in vitamin D deficiency, which

responds to supplements. This suggests that vitamin D is important for normal skeletal function, although the basis of this is not understood.

2. *Calcium Signaling*

The development and improvement of analytical methods to qualitatively and quantitatively determine the presence of specific elements are of profound importance for understanding their biological roles. Calcium is no exception in this respect. The ability of cells to precisely regulate the cellular concentrations of free and bound calcium both in time and space is a feature that adds to the versatility of the calcium ion. Calcium plays an important role in cellular signaling and therefore has been classified as one of the major second-messenger molecules. Calcium signals control a vast array of cellular functions, ranging from short-term responses such as contraction and secretion to longer term control of transcription, cell division, and cell death (Berridge *et al.*, 2000; Venkatchalam *et al.*, 2002). Eukaryotic cells have developed specialized internal Ca^{2+} stores that are localized in the sarco- or endoplasmic reticulum (Carafoli & Klee, 1999; Sorrentino & Rizzuto, 2001), at least for higher vertebrate cells. These stores represent a significant contribution to intracellular signaling, as the stores can release Ca^{2+} either in conjunction with or independently of the Ca^{2+} entry pathways localized on the plasma membrane after stimulation by agonists (Sorrentino & Rizzuto, 2001). Molecular studies have both identified the genes and characterized the proteins participating in intracellular Ca^{2+} homeostasis (Carafoli & Klee, 1999; Sorrentino & Rizzuto, 2001).

Intracellular Ca^{2+} stores have a specific molecular organization with regard to the way in which Ca^{2+} is released through specific intracellular Ca^{2+} release channels (ICRCs). Accumulation and storage, however, are mediated by dedicated Ca^{2+} pumps and Ca^{2+} binding proteins, respectively.

Ca^{2+} release from intracellular stores is mediated by channels encoded by a superfamily of genes, which includes three genes encoding channels capable of binding the vegetal ryanodine (RY) receptors and three genes encoding channels that bind inositol (1,4,5)-trisphosphate (IP3 receptors). These should be common for vertebrates (Sorrentino & Rizzuto, 2001). In most nonexcitable cells, the generation of receptor-induced cytosolic calcium signals is complex and involves two interdependent and closely coupled components: the rapid, transient release of calcium from stores in the endoplasmic reticulum and then the slow and sustained entry of extracellular calcium (Putney & McKay, 1999; Berridge *et al.*, 2000; Venkatchalam *et al.*, 2002). Through the activation of phospholipase C subtypes, G-protein-coupled receptors and tyrosine-kinase-coupled receptors generate the second messenger inositol (1,4,5)-trisphosphate and diacylglycerol. Doing so functions as a chemical message that diffuses rapidly within the cytosol and interacts with inositol-trisphosphate receptors located on the endoplasmic reticulum lumen and generates the initial calcium signal phase (Sorrentino & Rizzuto, 2001).

3. *Structural Role of Calcium*

Bone is not a static tissue, although it would be easy to think that when growth is finished bone does not change anymore; however, bone is a very dynamic tissue undergoing continuous destruction and remodeling. This process is important for the maintenance of bone volume and calcium homeostasis. The cells responsible for these dynamics are osteoblasts and osteoclasts, respectively. Osteoblasts produce bone matrix proteins, of which type I collagen is the most abundant extracellular bone protein. In addition, osteoblasts are critical to mineralization of the tissue (Aubin & Triffitt, 2002; Katagiri & Takahashi, 2002). Undifferentiated mesenchymal cells become osteoblasts, chondrocytes, myocytes, and adipocytes. Progenitor cells acquire specific phenotypes that are under the control of regulatory factors during the differentiation. Bone morphogenetic proteins (BMPs) play critical roles in the differentiation of the mesenchymal cells to osteoblasts (Katagiri & Takahashi, 2002).

Osteoclasts are multinucleated cells responsible for bone resorption. They are differentiated from hematopoietic cells of monocyte/macrophage lineage under the control of bone microenvironments. The presence of ruffled borders and a clear zone is characteristic for these cells (Väänänen & Zhao, 2001). Osteoblasts or bone marrow stromal cells have been shown to regulate osteoclast differentiation, thus providing a microenvironment similar to bone. The resorbing area under the ruffled border is acidified by vacuolar H^+–ATPase. A clear zone surrounds the ruffled border. This zone allows for the attachment of osteoclasts to the bone surface and maintains a microenvironment favorable for bone resorption. The recent discovery of the tumor necrosis factor (TNF) receptor–ligand family has clarified the molecular mechanism of osteoclast differentiation (Katagiri & Takahashi, 2002).

D. Sulfur Bioinorganic Chemistry

About 10 nonmetallic elements are essential for life. Of these, sulfur stands out due to its astounding chemical versatility. This versatility is reflected by several papers dealing with the biology of sulfur—for example, the chemistry of sulfane sulfur, disulfide as a transitory covalent bond and its significance in protein folding, the role of protein sulfenic acids in enzyme catalysis and redox regulation, and sulfur protonation in $[3Fe–4S]^{0,2-}$ clusters. In addition, it has been suggested that sulfur plays a role with regard to the μ_3 sulfide, where μ_3 indicates the number of metal ions connected to a multivalent ligand of Fe–S clusters in the catalysis of homolytic reactions, such as in pyruvate–formate lyase, anaerobic ribonucleotide reductase, 2,3-lysine amino mutase, biothin synthase, and the thioredoxin reductase (Beinert, 2000). A close neighbor in the periodic table, phosphorus, works in biological systems by ionic reactions in the form of its oxo-anions. In contrast, sulfur in its most oxidized form, sulfate, is of limited use to higher organisms except for sulfation and detoxification reactions; rather, it is the chemical versatility of the lower oxidation states of sulfur that is vital for anabolic reactions (Beinert, 2000).

1. Inorganic Systems

A conspicuous example of Fe–S clusters is cytoplasmic c-aconitase/iron regulatory protein (IRP). The aconitases lose one Fe readily on exposure to O_2, or even faster to O_2^-, with formation of the enzymatically inactive 3Fe form $[3Fe–4S]^+$, which can be reconstituted to form active $[4Fe–4S]^{2+}$ enzyme (Figure 6a). In addition, c-aconitase alternates as aconitase and, after complete loss of its Fe–S cluster, as an IRP (Figure 6b) that can bind to iron-responsive elements (IREs) in the untranslated regions of mRNAs and thereby regulate translation (Haile *et al.*, 1992; Beinert *et al.*, 1996; Beinert, 2000). There is some spontaneous breakdown of aconitase; however, this process is probably too slow to be used in regulation but for subtle adjustments.

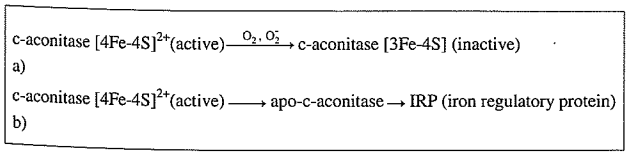

FIGURE 6 Possibilities for aconitases.

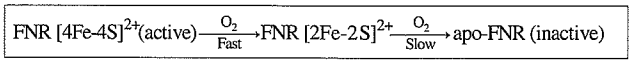

FIGURE 7 Transition from anaerobic to aerobic metabolism in *E. coli*.

Agents such as NO or H_2O_2 can speed up the process significantly. Transition of the organism from anaerobic to aerobic metabolism (*Escherichia coli*) is governed by the global transcription regulator fumarate nitrate reduction (FNR). FNR alternates between the active, O_2-sensing, holoform $[4Fe–4S]^{2+}$ and the inactive form $[2Fe–2S]^{2+}$ and, finally, apoform during O_2 sensing (Bates *et al.*, 2000) by means of its very O_2-sensitive Fe–S cluster (Figure 7). Obviously, in all these cases, there must be a continuous cycle of disassembly and reassembly or repair of clusters *in vivo*.

2. Organic Systems

If the processes in which Fe–S clusters are involved are considered primarily inorganic, it is interesting to look for sulfur processes without the involvement of these clusters. It appears that at least one process is devoid of Fe–S clusters—the biosynthesis of thiamine. This pathway is complicated and not yet fully understood (Begley *et al.*, 1999). Thiamine synthesis involves at least six peptides (ThiF, S, G, H, I, J). Cystein is the original source of the sulfur, and the immediate donor, which completes the heterocycle synthesis, is thiocarboxylate formed at the carboxyl terminus of ThiS in an ATP-demanding reaction.

V. THE FUNCTIONAL VALUE OF TRACE ELEMENTS

Trace elements are found in minute amounts in tissues and body fluids. Nevertheless, they play an extremely important role in biology. The paramount function is to be necessary for the structure and function of significant biomolecules, mainly enzymes. An intriguing property of many of the trace elements is that they are found among the transition elements, and most of them are also in the fourth row of the periodic table. Only boron, selenium, molybdenum, iodine, and tungsten are found at other sites.

A. Vanadium, Cobalt, and Nickel Are Not Used Extensively

These trace elements are treated more or less collectively without any specific chemical argument. They can be found in the transition metal area in the periodic table and are known to have or are suspected to have biological functions.

1. Vanadium

The rich aqueous chemistry of vanadium suggests that its metabolism is complex. Both the anion vanadate (VO_3^-) and the cation vanadyl (VO^{2+}) can complex with molecules of physiological significance. Vanadium species such as the oxyanion and oxycations VO_4^{2-}, VO^{2+}, and VO_2^+ are oxidizing agents capable of reacting by one-electron transfer or by two-electron steps when V acts as an O-atom donor. In addition, vanadium can form sulfur-containing anionic centers (*e.g.*, VS_4^{3-}) and sulfur-containing cationic centers such as VS^{2+} and $VSSH^+$ (Fraústo da Silva & Williams, 2001). Apart from the strikingly high concentrations of vanadium in some ascidians, for which there still is no clear biological function, vanadium concentrations in tissues and body fluids generally are low, as can be seen from Table VI.

Most of the vanadium in the vanadocytes of ascidians, which are known to be the signet ring cells, has been shown to be in the V(III) state, with a minor part occurring in the V(IV) state; consequently, reducing agents have to participate in the accumulation of vanadium in vanadocytes. Among the proposed agents are tunichromes. Earlier it was thought that the vanadium incorporated by ascidians was dissolved as ionic species or associated with low-molecular-weight substances rather than proteins. This is in contrast to other metals that generally bind to macromolecules such as proteins and are incorporated into the tissues of living organisms. Michibata *et al.* (2002), however, demonstrated the presence of a vanadium-associated protein in vanadocytes. This protein was estimated to associate with vanadium in an approximate ratio of 1:16. The protein was comprised of three peptides of estimated molecular weights of 12.5, 15, and 16 kDa; however, the physiological roles of vanadium still remain to be elucidated.

Other functions of vanadium are associated with various defense systems, such as peroxidase and catalase activity or haloperoxidases, as well as dinitrogen fixation. The peroxidase and catalase activity is exerted by the vanadium-containing substance amavadine (V(IV) *bis*-complex of *N*-hydroxyimino-di-a-propionate), the mechanism of which is illustrated in Figure 8 (Matoso *et al.*, 1998). Amavanadine is found in some *Amamita* toadstools. It seems that amavadine could be a kind of primitive protective substance able to use H_2O_2 for self-regeneration of damaged tissues or to defend against foreign pathogens and predators, decomposing it when not necessary (Fraústo da Silva & Williams, 2001).

An intriguing and exciting property of vanadium is that it mimics insulin both *in vitro* and *in vivo*. Even more fascinating is that vanadium as sodium vanadate ($NaVO_3$) was used to treat patients with diabetes mellitus as early as 1899 (Lyonnet *et al.*, 1899), before insulin was even discovered. The proposed mechanism by which vanadyl acts is on at least three sites (Sakurai, 2002), as illustrated in Figure 9. Vanadate behaves in a manner similar to that of phosphate; therefore, the effects of vanadium are thought to inhibit the production of protein phosphotyrosine phosphatase that follows stimulation of protein tyrosine phosphorylation. Vanadate also activates autophosphorylation of solubi-

TABLE VI. Concentrations of Vanadium in the Tissues of Several Ascidians Compared with Human Serum (mmol/L)

Species	*Tunic*	*Mantle*	*Branchial Basket*	*Serum*	*Blood Cells*
Ascidia gemmata	ND	ND	ND	ND	347.2
A. ahodori	2.4	11.2	12.9	1.0	59.9
A. sydneiensis	0.06	0.7	1.4	0.05	12.8
Phallusia mammillata	0.03	0.9	2.9	ND	19.3
Ciona intestinalis	0.003	0.7	0.7	0.008	0.6
Homo sapiens	—	—	—	0.000003–0.000018	—

lized insulin receptors in a way similar to the insulin activation. Vanadate also stimulates the tyrosine kinase activity of the insulin receptor β-subunit. Both vanadate and vanadyl are effective in stimulating glucose metabolism in adipocytes. Vanadate restores the expression of the insulin-sensitive glucose transporter and induces the recruitment of the GLUT4 glucose transporter to the plasma membrane. The 3′,5′-cyclic adenosine monophosphate (cAMP)-mediated protein phosphorylation cascade in adipocytes is activated during diabetes

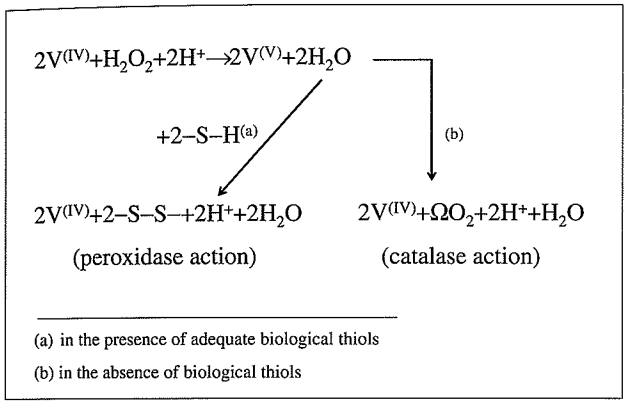

FIGURE 8 Peroxidase and catalase activity of amavadine. (Adapted from Matoso *et al.*, 1998.)

or in the presence of adrenalin. Both glucose and vanadyl, which are incorporated in the adipocytes in response to vanadyl treatment, lead to the restored regulation of this cascade. Free fatty acid (FFA) release is thought to be inhibited by vanadyl. Vanadyl, thus, acts on at least three cell sites—phosphatidyl inositol 3-kinase, glucose transporter, and phosphodiesterase—to normalize both glucose and FFA levels in diabetes.

It has been demonstrated that vanadium compounds can be used in the treatment of diabetes mellitus, and vanadium also seems to be able to prevent the onset of diabetes. A novel hypothesis has been proposed in which nitric oxide (NO) production can be attributed to macrophages (Mϕ). The possible mechanism is shown schematically in Figure 10 (Sakurai, 2002). In the macrophages of normal animals treated with $VOSO_4$, incorporation of vanadium and responses to enhanced NO production are low. In the prediabetic phase of mice treated with streptozotocin (STZ), activated Mϕ exudes through pancreatic islets, and the NO produced concomitantly by activated Mϕ destroys normal islet β cells. The onset of diabetes by STZ administration is proposed to be based on the enhancement of the generation of superoxide anions ($\cdot O_2^-$) in β cells. NO reacts with $\cdot O_2^-$ to produce peroxynitrite ($ONOO^-$). One of the degradation products of peroxynitrite is a hydroxyl radical (·OH). The radicals $\cdot O_2^-$ and ·OH destroy the β

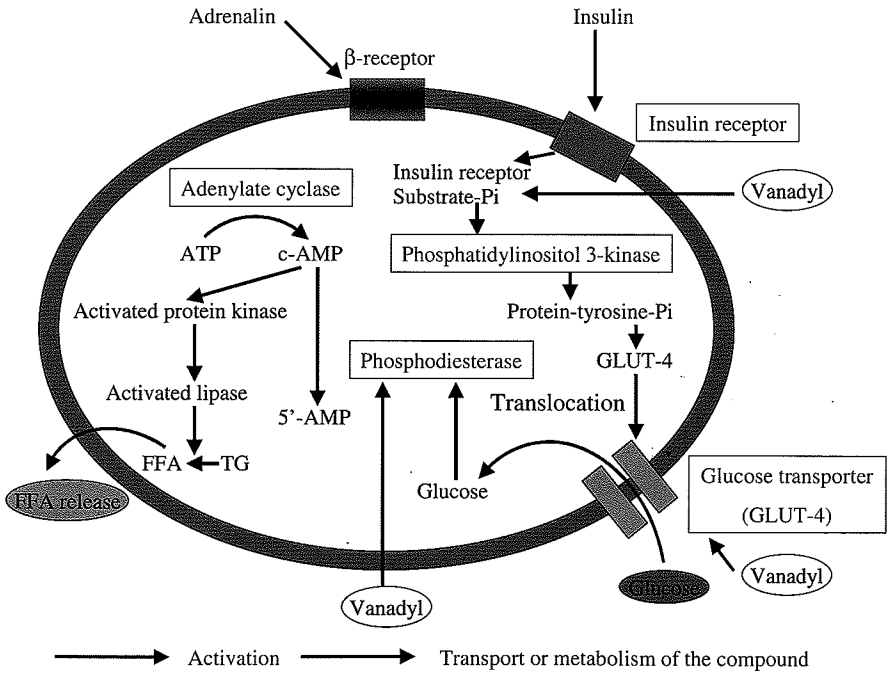

FIGURE 9 Proposed mechanisms of vanadyl action. (Adapted from Sakurai, 2002.)

cells. Treatment with $VOSO_4$ suppresses NO production and consequently formation of ·OH that damages the β cells.

2. *Cobalt*

A spontaneous association with cobalt biological functions is most likely with vitamin B_{12}, as cobalt is an integral cofactor in this vitamin. Although the biological functions of vitamins are slightly out of the scope of this book, a few comments are necessary here. Vitamin B_{12} is involved in four types of important reactions:

1. Reduction of ribose to deoxyribose
2. Rearrangement of diols and similar molecules
3. Rearrangement of malonyl to succinyl
4. Transfer of methyl groups

Worth mentioning is the enzyme ribonucleotide reductase that requires vitamin B_{12} for the reduction of ribonucleotides to the corresponding deoxyribonucleotides. Not only is this reaction dependent on the enzyme ribonucleotide reductase and vitamin B_{12}, but the reaction is also considered to proceed via the radical pathway. This constitutes an interesting example of how radicals can have important functions in biological processes that are not destructive in nature. In vitamin B_{12}, cobalt is associated with a corrin ring, a relative of porphyrin. Non-corrin cobalt is receiving increased interest, and ten non-corrin-cobalt-containing enzymes have been isolated (Kobayashi & Shimizu, 1999) and characterized (Table VII).

Metionine aminopeptidase cleaves the N-terminal methionine from many newly translated polypeptide chains in both prokaryotes and eukaryotes. It is an important catalyst for N-terminal modification involved in functional regulation, intracellular targeting, and protein turnover. The *E. coli* methionine aminopeptidase is a monomering protein of 29 kDa and consisting

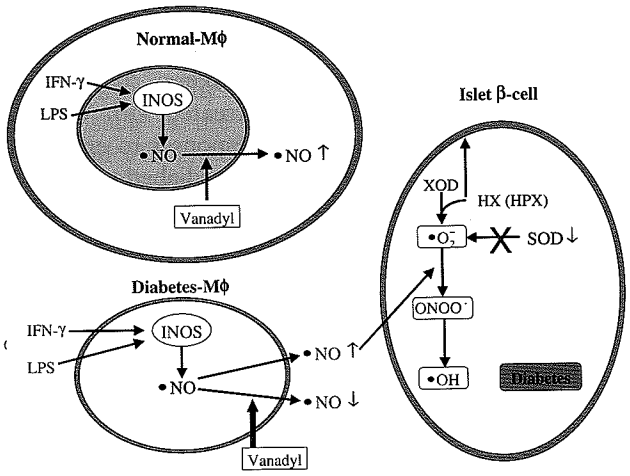

FIGURE 10 A possible mechanism of vanadium prevention of diabetes. (Adapted from Sakurai, 2002.)

TABLE VII. Cobalt-Containing Proteins

Enzyme or Protein	*Source*	*Cofactor content*	*Postulated role of cobalt*
Methionine aminopeptidase	Animals, yeast, bacteria	2 Co per subunit	Hydrolysis
Prolidase	Archaea	1–2 Co per subunit	Hydrolysis
Nitrile hydratase	Actinomycetes and bacteria	1 Co in each α-subunit	H_2O activation, CN-triple-bond hydration and protein folding
Glucose isomerase	Actinomycetes	1 Co per 4 subunits	Isomerization
Cobalt transporter	Actinomycetes and yeast	—	Cobalt uptake
Methylmalonyl-CoA carboxytransferase	Bacteria	1 Co, 1 Zn per subunit	Carboxytranserfation
Aldehyde decarbonylase	Algae	1 Co-porphyrin per αβ-subunit	Decarbonylation for aldehyde
Lysine-2,3-aminomutase[a]	Bacteria	0.5–1 Co per subunit	Mutation
Bromoperoxidase	Bacteria	≈0.35 Co per 2 subunits	Bromination
Cobalt-porphyrin-containing protein	Bacteria	1 Co-porphyrin per protein	Electron carrier

[a]Lysine-2,3-aminomutase also contains an iron–sulfur cluster, zinc, and PLP as cofactors.
Source: Adapted from Kobayashi and Shimizu (1999).

of 263 residues that bind two Co^{2+} ions in its active site. The two subfamilies of cobalt-containing methionine aminopeptidase are a prokaryotic class (type I) and a human class (type II).

Prolidase (or praline dipeptidase) specifically cleaves Xaa–Pro dipeptides. In concert with other endopeptidases and exopeptidases, prolidase is thought to be involved in the terminal degradation of intracellular proteins and may also function in the recycling of proline (Ghosh *et al.*, 1998).

Nitrile hydratase catalyzes hydration of nitriles to amides (see below) and is a key enzyme involved in the metabolism of toxic compounds. The presence of cobalt as an essential cofactor may be explained by the effective catalysis of CN-triple bond hydration as well as a requirement for the protein folding.

Glucose isomerase catalyzes the reversible isomerization of D-glucose to D-fructose. Although this enzyme requires divalent cations for the activity, its specific requirement depends on the source of the enzyme.

Most metals that play essential roles as cofactors in biological processes have to be actively incorporated into cells against concentration gradients. COT1 acts as a cobalt toxicity suppressor in *Saccharomyces cerevisiae* by sequestration or compartmentalization within the mitochondria of cobalt ions that cross the plasma membrane (Conklin *et al.*, 1992). The cobalt transporter NhlF mediates cobalt incorporation into the cell in an energy-dependent manner. The sequence of this transporter shows eight putative hydrophobic membrane-spanning domains. NhLF contains nine histidine residues and two cysteine residues. One histidine (His306) and one cysteine (Cys301) residue are located on the fourth outside loop and may comprise a cobalt-binding site for the initial fixation of the metal. The transmembrane segments could form a cobalt channel in which the four histidines might function as transient cobalt-binding ligands.

The biotin-containing enzyme methylmalonyl–CoA carboxytransferase (transcarboxylase) is a complex multisubunit enzyme that catalyzes the transfer of a carboxyl group from methylmalonyl–CoA to pyruvate to form propionyl–CoA and oxaloacetate.

Aldehyde decarboxylase converts a fatty aldehyde to hydrocarbon and carbon monoxide. This enzyme is responsible for a key step in the biosynthesis of hydrocarbon compounds. Lysine-2,3-aminomutase catalyzes the reversible isomerization of l-lysine to l-β-lysine, a reaction in which the hydrogen on the 3-pro-R position of lysine is transferred to the 2-pro-R position of β-lysine and the 2-amino group of lysine migrates to carbon-3 of β-lysine. The enzyme contains three cofactors: pyridoxal phosphate, Fe–S centers, and cobalt or zinc.

Bromoperoxidase catalyzes the formation of a carbon–bromine bond in the presence of peroxides. More studies, however, are needed to elucidate the function of bromoperoxidase. Other proteins contain cobalt–porphyrins in plants and in sulfate-reducing bacteria. The cobalt–porphyrins are not covalently bound to the proteins. The prosthetic groups of these cobalt-containing proteins are found to be cobalt isobacteriochlorins.

3. Nickel

Nickel-containing enzymes are involved in at least five metabolic processes, including the production and consumption of molecular hydrogen, hydrolysis of urea, reversible oxidation of carbon monoxide under anoxic conditions, methanogenesis, and detoxification of superoxide anion radicals. The active sites of the relevant enzymes harbor unique metallocenters, which are assembled by auxiliary proteins. Different ligand environments are involved in the coordination of nickel in the various metalloenzymes (Eitinger, 2000). The reactions catalyzed by nickel-dependent enzymes are summarized in Figure 11.

Some transporters are potentially able to transport nickel; however, CorA, MgtA, and MgtB have affinities that are too low to be of physiological importance (Ragsdale, 1998). Two different types of high-affinity nickel transporters have been identified. One is a mul-

a. Superoxide dismutase

$$2H^+ + 2O_2^{-\bullet} \rightarrow H_2O_2 + O_2$$

b. Urease

$$H_2N-CO-NH_2 + 2H_2O \rightarrow 2NH_3 + H_2CO_3$$

c. Hydrogenase

$$2H^+ + 2e^- \leftrightarrow H^+ + H^- \leftrightarrow H_2$$

d. Methyl-CoM reductase

$$CH_3-CoM + CoB-SH \rightarrow CH_4 + CoM-S-S-CoB$$

e. CO dehydrogenase

$$CO + H_2O \rightarrow 2H^+ + CO_2 + 2e^-$$

f. Acetyl-CoA synthase

$$CH_3-CFeSP + CoA-SH + CO \rightarrow CH_3-CO-SCoA + CFeSP$$

FIGURE 11 Reactions catalyzed by nickel-dependent enzymes.

ticomponent ATP-binding cassette (ABC) transporter system such as NikABCDE that uses ATP. The other is a one-component transporter such as NixA, UreH, HupN, and HoxN. NixA and HoxN are integral membrane proteins that have eight transmembrane-spanning helices and a sequence motif that is essential for function. HypB can sequester nickel and release it for incorporation into apoproteins when nickel becomes limiting (Ragsdale, 1998).

A nickel–superoxide dismutase (Ni–SOD) has been isolated from *Streptomyces*. The protein is a homotetramer of four 13-kDa subunits with little sequence similarity to earlier known SODs. Nickel induces its expression, represses the FeZn–SOD, and is involved in maturation of a precursor polypeptide (Ragsdale, 1998).

Urease plays a key role in the nitrogen metabolism of plants and microbes and acts as a virulence factor for some human and animal pathogens (Mobley *et al.*, 1995). There are two nickel ions in the enzyme, and CO_2 is required to generate a carbamylated lysine bridge between the two nickel ions.

Hydrogenases are of different types. Some hydrogenases involve Ni, Fe, and Se; some Ni and Fe. In addition, some depend only on Fe. In fact, one hydrogenase has no association with metals at all (Hartmann *et al.*, 1996).

Methyl-coenzyme M reductase is a remarkable enzyme composed of six subunits forming a heterohexamer. Three unusual coenzymes are embedded in a long channel between the subunits. Of particular interest is the binding of coenzyme F430, a Ni–porphinoid that occurs exclusively in this enzyme. As substrates, the enzyme binds methyl-coenzyme M (methyl-thioethane sulfonate) and coenzyme B (7-thioheptanoyl threoninephosphate). Structural studies of this enzyme have been hampered by the low activity of the purified enzyme; however, substantial improvements in this respect have been seen in recent years (Finazzo *et al.*, 2003).

Carbon monoxide (CO) dehydrogenase oxidizes CO to CO_2 in the half reaction shown in Figure 12 using a Ni–Fe cluster. Another enzyme, a hydrogenase, also contains a Ni–Fe cluster and uses the electrons in the first half reaction to reduce protons to hydrogen gas: $2H^+ + 2e^- \leftrightarrow H_2(g)$ (Watt & Ludden, 1999). Carbon monoxide dehydrogenases are bifunctional enzymes that perform reversible CO oxidation and also function to synthesize or degrade acetyl–CoA. The bifunctional characteristics of this enzyme led to changing its name to CO–dehydrogenase/acetyl–CoA synthase (Hausinger, 1993). Both catalytic sites for the individual reactions require nickel for catalysis. Methanogenic

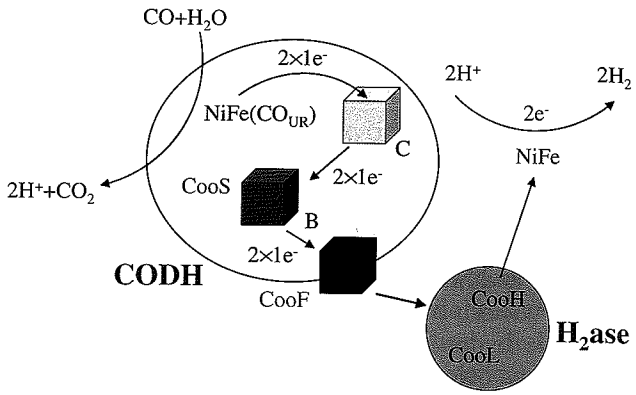

FIGURE 12 CO-dehydrogenase oxidizes CO to CO_2 using a NiFe cluster and a nickel-dependent hydrogenase (H2ase) to produce hydrogen gas. (Adapted from Watt and Ludden, 1999.)

bacteria convert acetate to methane and CO_2 and couple acetate degradation to ATP synthesis. This metabolic process uses the CO–dehydrogenase/acetyl–CoA synthase (Watt & Ludden, 1999).

Nickel compounds are recognized as human carcinogens. It has been suggested that the molecular mechanism of the genotoxicity underlying the induction of carcinogenesis is the delivery of nickel into a cell from particulates outside the cell. Water-soluble nickel salts penetrate cells poorly. Following phagocytosis, the particles are contained in vacuoles that become highly acidified, and this greatly enhances the dissolution of soluble nickel from the particles. This increases the intracellular load of nickel, which can subsequently attack chromatin and particularly histones and produce effects associated with carcinogenic activity (Zoroddu *et al.*, 2002).

B. Chromium, Molybdenum, and Tungsten

This triad of trace elements comprises group 6 in the periodic table; consequently, they should have some chemical properties in common. An intriguing property of these trace elements is their relationship with the non-metal elements of group 16; hence, the trace elements of group 6 often appear covalently bonded and not as simple metal ions.

1. Chromium

Chromium(III) was already established as an essential dietary component in the 1950s. Many studies have been conducted to elucidate the biological role of chromium but have had a low degree of success. A major

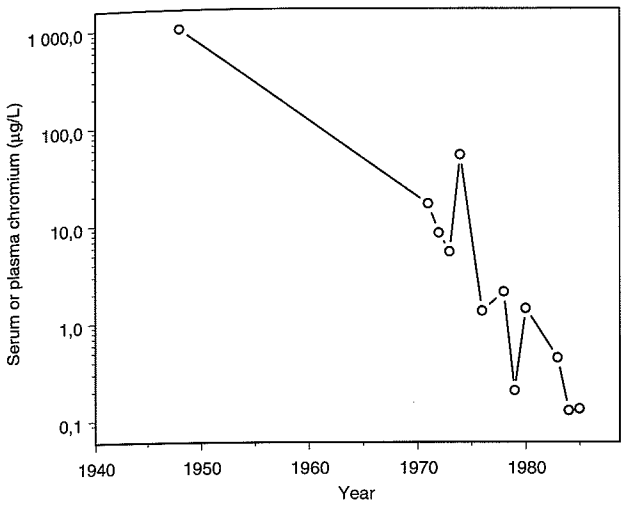

FIGURE 13 "Normal" serum levels of chromium over 30 to 40 years.

problem is the low concentration in tissues and body fluids and the high risk of contamination of samples. It was not until the 1980s that reliable analytical methods were developed. Over the course of 30 to 40 years of analytical efforts (Figure 13), the so-called normal serum level of chromium in humans has been decreasing by orders of magnitude. It has been suggested that chromium is integral to the glucose tolerance factor (GTF). Efforts to purify this factor have led to the detection of nicotinic acid, glycine, glutamic acid, and cysteine, as well as chromium (Mertz *et al.*, 1974).

Recent years have witnessed a plethora of activity related to the elucidation of a potential role for trivalent chromium in mammalian carbohydrate and lipid metabolism at a molecular level. In the 1980s, the isolation and characterization of a unique chromium-binding oligopeptide known as low-molecular-weight chromium-binding substance (LMWCr) or chromodulin were reported (Yamamoto *et al.*, 1987). The oligopeptide has a molecular weight of about 1500 Da and is comprised of only four types of amino acid residues (*i.e.*, glycine, cysteine, glutamate, and aspartate). Despite its small molecular weight, it binds four equivalents of chromic ions, apparently in a tetranuclear assembly, as necessitated by charge balance arguments (Vincent, 2000). Chromodulin has an intriguing ability to potentiate the effects of insulin on the conversion of glucose into carbon dioxide or lipid. No other naturally occurring chromium-containing species potentiates insulin action in this manner (Vincent, 2000).

How chromium is absorbed and transported is still uncertain. It appears that transport is mediated by transferrin, the main iron-transporting protein (molecular weight, 80 kDa). This may be due to the fact that transferrin usually carries only about a 30% load of iron so it has unused transportation capacity. Transferrin is also thought to be a transporter of various trace elements. Recent reports on the effects of insulin on iron transport and the relationship between hemochromatosis and hepatic iron overload and diabetes suggest that transferrin may actually be the major physiologic chromium transport agent (Vincent, 2000).

Hexavalent chromium compounds have been established as being carcinogenic. Chromate easily enters cells through the sulfate channel and is quickly reduced by, for example, glutathione. The ultimate step of the metabolic pathway yields Cr(III) inserted within the cell nucleus, where it cross-links DNA to proteins. Recent results indicate that glutathione is not only a primary target for oxidation by chromate but also acts as an efficient ligand-stabilizing Cr(V) in a dimeric bridged cluster (Gaggelli *et al.*, 2002).

2. *Molybdenum*

Molybdenum is found in the second row of transition metals in the periodic table. It is the only metal in this row that is required by most living organisms. Although only a minor constituent of the Earth's crust, molybdenum is readily available to biological systems because of the solubility of molybdate salts in water. In fact, molybdenum is the most abundant transition metal in seawater. It is not, therefore, surprising that molybdenum has been incorporated widely in living organisms. Molybdenum is redox active under physiological conditions (ranging between oxidation states VI and IV). The V valence state is also available, and molybdenum can act as a transducer between obligatory two- and one-electron oxidation–reduction systems such as the hydroxylation of carbon centers under more moderate conditions than are required by other systems (Hille, 2002).

An important feature of molybdenum-containing enzymes is the molybdenum cofactor (Moco) that is able to associate with different apoenzymes to form the Mo–holoenzymes where, depending on the type of apoenzymes, molybdenum catalyzes redox reactions on C, N, and S atoms. The only exception is bacterial nitrogenases that contain an FeMo cofactor not related to Moco (Mendel, 1997). It has been shown that in Moco molybdenum is complexed by a pterin with a four-carbon alkyl side chain containing a Mo-coordinating dithiolene group and a terminal phosphate ester (Mendel, 1997). This pterin has been named molybdopterin (Figure 14).

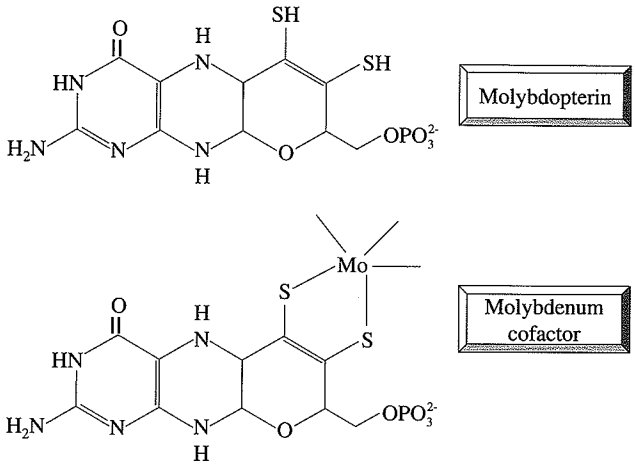

FIGURE 14 Molybdopterin and molybdenum cofactor.

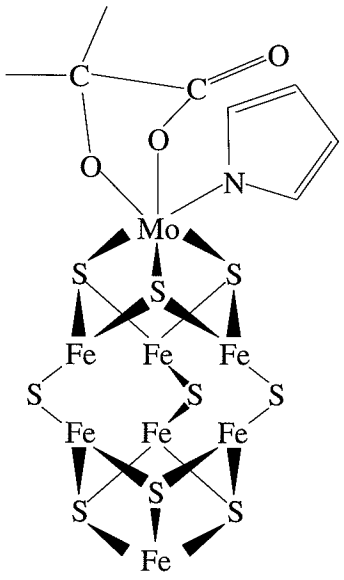

FIGURE 15 Multinuclear center with iron, sulfur, and molybdenum in nitrogenase.

There are two different kinds of molybdenum enzymes. One is exemplified by the nitrogenase enzyme family, which is characterized by a multinuclear center with iron, sulfur, and molybdenum (Figure 15). In fact, molybdenum can be replaced by vanadium or iron. Nitrogenases catalyze the reaction from dinitrogen to ammonia and are the basic components in nitrogen fixation. Indeed, most cycling of nitrogen in the biosphere depends on the trace element molybdenum. The second type of molybdenum enzyme is characterized by a dependence on a mononuclear center, which is associated with molybdopterin. Three enzyme families are included in this group: xanthine oxidases, sulfite oxidases, and dimethylsulfoxide DMSO reductases. Examples of molybdenum enzymes of these families are discussed below.

Xanthine oxidases comprise the largest family of molybdenum enzymes, with up to 20 members. The xanthine oxidase catalyzes purine or pyrimidine catabolism by inserting oxygen and/or removing hydrogen from the substrates. It is a well-studied enzyme that exists as a dimer with a molecular weight of about 300 kDa. Each subunit contains one molybdenum, one flavin adenine dinucleotide, and two Fe_2S_2 centers. The reaction catalyzed is xanthine + H_2O → uric acid + $2H^+$ + $2e^-$.

Sulfite is a highly reactive and potentially toxic compound. Like other reduced inorganic sulfur compounds such as hydrogen sulfide or thiosulfate, it occurs in nature as a consequence of geological and industrial processes and the anaerobic mineralization of organic matter by dissimilatory sulfate reduction (Kappler & Dahl, 2001). There is no doubt that sulfite oxidases have developed during evolution as a response to sulfite reactivity. Sulfite oxidase contains only a single prosthetic group, a heme group, in addition to the Moco-derived center. The holoenzyme is a dimer with a molecular weight of 120 kDa. The enzyme can oxidize sulfite by using oxidized cytochrome *c*, ferricyanide, or dioxygen as an electron acceptor. Sulfite oxidation occurs at the molybdenum center, with the heme center serving to couple this two-electron oxidation to the reduction of two molecules of cytochrome *c*. DMSO reductase catalyzes the reduction of dimethylsulfoxide to dimethylsulfide and liberates the oxygen atom of DMSO as water. The reducing equivalents come from a specific pentaheme cytochrome. In addition to the enzymes described here are a few other molybdenum enzymes, such as pyridoxal oxidase, xanthine dehydrogenases, and pyropallol transhydrolases.

3. Tungsten

Tungsten is by far the heaviest metal with a biological function (*i.e.*, an essential trace element). The three classes of tungsten enzymes are aldehyde ferredoxin–oxidoreductase, formate dehydrogenase, and acetylene hydratase. Formate is a common metabolite in most life forms. In most cases, its production and consumption involve formate dehydrogenase (FDH), which catalyzes the reversible two-electron conversion of CO_2 to formate according to:

$$CO_2 + 2H^+ + 2e^- \rightleftharpoons HCOO^-$$

Not all FDHs are tungsten dependent. On the other hand, most FDHs of aerobic organisms do not contain metals or other cofactors. The tungsten-dependent FDHs have mostly been identified in Chlostridiae (*e.g.*, *C. thermoaceticum*, *C. formioaceticum*, and *C. acidiurici*) (Kletzin & Adams, 1996).

Formyl methanofuran dehydrogenase (FMDH) catalyzes the reversible formation of *N*-formylmethanofuran from CO_2 and methanofuran (MFR). The first step in CO_2 utilization by methanogens is thus:

$$CO_2 + MFR^+ + H^+ + 2e^- \rightleftharpoons CHO\text{-}MFR + H_2O$$

However, FMDH was found to be a molybdoenzyme in the first methanogens examined (Kletzin & Adams, 1996). Tungsten-dependent FMDHs have been isolated from moderate thermofils such as *Methanobacterium wolfei* and *M. thermoautotrophicum*.

Aldehyde-oxidizing enzymes catalyze oxidation of aldehydes of one type or another and again have been found in microorganisms. The reaction catalyzed is

$$CH_3CHO + H_2O \rightleftharpoons CH_3COO^- + 3H^+ + e^-$$

Among the aldehyde-oxidizing enzymes are carboxylic acid reductase (CAR) from acetogens which was the second tungsten-dependent enzyme isolated. Another tungsten-dependent enzyme is aldehyde ferredoxin oxidoreductase (AOR), from *Pyrococcus furiosus*, which catalyzes the oxidation of a range of aliphatic and aromatic aldehydes and reduces ferredoxin. Formaldehyde ferredoxin oxidoreductase (FOR) has also been demonstrated in *P. furiosus*. These enzymes also use ferredoxin as the physiological electron carrier and are maximally active at temperatures above 95°C. In contrast to AOR, however, they oxidize only C1–C3 aldehydes and are of much lower specific activity (Kletzin & Adams, 1996).

The enzyme glyceraldehyde-3-phosphate ferredoxin oxidoreductase (GAPDH) has been found in the peculiar hyperthermophilic archaeon *P. furiosus* and is thought to play a role in gluconeogenesis. A fourth tungsten-containing enzyme from *P. furiosus* has recently been characterized (Roy & Adams 2002). This enzyme, preliminarily named WOR 4, is thought to play a role in SO reduction. It has an interesting structure in that it contains approximately one W atom, three Fe atoms, three or four acid-labile sulfides, and one Ca atom per subunit (Roy & Adams, 2002). Aldehyde dehydrogenases (ADH) have been shown in *Desulfovibrio gigas*.

Acetylene hydratase (AH) converts acetylene to acetaldehyde according to:

$$H\text{-}C \equiv C\text{-}H + H_2O \rightarrow H_3C\text{-}HC = O$$

which is a hydration reaction.

In addition to availability a key factor in tungsten utilization appears to be its redox properties relative to molybdenum. When tungsten substitutes for molybdenum in molybdoenzymes, some will be inactive, probably because the tungsten site has a lower reduction potential as compared to the molybdenum site. Conversely, to catalyze a reaction of extremely low potential, tungsten should be preferred over molybdenum (Kletzin & Adams, 1996).

C. Manganese: Photosynthesis and Defense Against Oxygen

Although fairly abundant in the biosphere, manganese is found only in trace amounts in living organisms. Manganese exhibits the widest range of oxidation states of any of the first row *d*-block metals. The lowest states are stabilized by π-acceptor ligands, usually in organometallic complexes. Most of the biochemistry of manganese can be explained by two properties: It is redox active, and it is a close but not exact analog of Mg^{2+}. Manganese plays many roles in biological systems ranging from acting as a simple Lewis acid catalyst to being an element that can transverse several oxidation states to carry out water oxidation. The presence of Mn^{2+} in the cytoplasm in significant concentrations would pose a serious problem for aerobic eukaryotes. One reason is that manganese is considered to be mutagenic, and it binds relatively weakly to proteins. Prokaryotes, on the other hand, may make use of this property to promote mutations to generate variation. Manganese in aerobic eukaryotic cells, then, has to be pumped into various organelles. Examples of such organelles are presented in Table VIII.

TABLE VIII. Manganese-Containing Vesicles

Vesicle	*Known Mn protein*
Chloroplast	Superoxide dismutase
Golgi apparatus	Glycosyl-transferases
Lysosome (acid)	Acid phosphatase (Mn(III))
Mitochondria	Superoxide dismutase
Thylakoid (acid)	Generation of molecular oxygen
Vacuole (acid)	Mn(II) is free

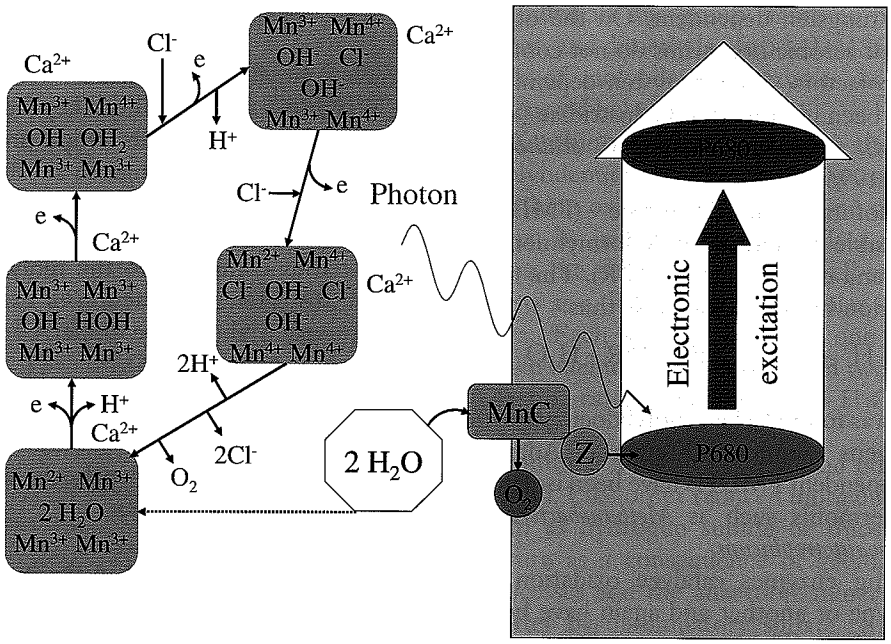

FIGURE 16 A partly hypothetical model of the splicing of water.

1. Generation of Dioxygen from Water

Higher plants and algae complete oxygenic photosynthesis whereby water is oxidized by a cluster of manganese ions. This process takes place in Photosystem II (PS II), which is a multisubunit complex embedded in the thylakoid membranes. This photosynthetic enzyme catalyzes the most thermodynamically demanding reaction in biology, the splitting of water into dioxygen and reducing equivalents (Barber, 2003). Light is captured by antennae chlorophyll and funneled to the primary reaction center, which contains chlorophyll (P680). The oxidized P680 first oxidizes a redox-active tyrosine, hydrogen bonded to a histidine (Yz). Subsequent oxidation of the manganese cluster occurs via this generated tyrosine radical. The enzyme can exist in five oxidation levels named S_n states, where S_0 is the most reduced and S_4 the most oxidized. Oxygen is liberated upon the $S_3 \rightarrow S_4 \rightarrow S_0$ transition (Yocum & Pecoraro, 1999). A partly hypothetical model of this process is depicted in Figure 16. It is interesting to note that the oxidation of water to dioxygen takes place in the thylakoid membranes of plants and algae. These membranes correspond to the mitochondrial membrane of eukaryote cells, in which the reverse process of reducing dioxygen to water takes place. Even more intriguing is that manganese plays important roles in both processes.

2. Defense for a Life with Oxygen

The enzyme most commonly associated with manganese is superoxide dismutase, which is a scavenger of the very reactive superoxide anion produced during reduction of oxygen in cellular respiration. As the name suggests, the enzyme acts on the superoxide anion produced in the first step in the reduction of dioxygen to water. It catalyzes the following reaction:

$$\mathrm{Mn(III) + O_2^- \rightleftharpoons [Mn(III)\text{-}O_2^-] \rightarrow Mn^{2+} + O_2}$$

$$\mathrm{Mn^{2+} + O_2^- \rightleftharpoons [Mn^{2+}\text{-}O_2^-] + 2H^+ \rightarrow Mn(III) + H_2O_2}$$

The end product, hydrogen peroxide, although not a radical, is a reactive oxygen species. MnSODs are widespread in bacteria, plants, and animals. In most animal tissues and yeast, MnSOD is largely confined to mitochondria (Fridovich, 1995). It is conspicuous that, although manganese is deeply involved in the generation of oxygen from water, it is also necessary in scavenging reactive oxygen species when dioxygen is metabolized in mitochondria. MnSOD is the most phylogenetically widespread manganese-dependent enzyme (Kehres & Maguire, 2003).

There are some aspects of redox biochemistry that have to be taken into account to understand the unexpected tolerance of relatively high concentrations of

Mn^{2+}. Similar to iron, manganese can cycle readily *in vivo* between the 2+ and 3+ oxidation states; however, the reduction potential of any molecule depends on its ligand environment. Another important feature is that manganese is less reducing than iron under most biological conditions (Kehres & Maguire, 2003). "Free" (solvated) Mn^{2+} has a reduction potential too high to reduce H_2O_2 in aqueous solution; however, replacing one or two inner hydration shell waters with hydrogen carbonate results in rapid catalase activity by a Mn^{2+}-dependent disproportionation reaction. That the reducing potential of manganese is smaller than that of iron can be explained by the different $3d$ electron occupancies of the two cations. The electrochemical consequence is that $Mn^{3+} + e^- \rightarrow Mn^{2+}$ has a standard reduction potential of +1.51 V, while $Fe^{3+} + e^- \rightarrow Fe^{2+}$ has a standard reduction potential of +0.77 V.

Two consequences of this redox chemistry are obvious. The similarity between Mn^{2+} and Fe^{2+} is that their intrinsic reduction potentials are close enough to those of many common biological molecules that each metal can take part in biologically important redox catalysis. In addition, the critical difference between the two metals is that the higher reduction potential of Mn^{2+} renders the free (*i.e.*, solvated) Mn^{2+} innocuous under conditions (notably in an aerobic environment) where free Fe^{2+} would actively generate toxic radicals. Cells can thus tolerate very high cytoplasmic concentrations of Mn^{2+} with essentially no negative redox consequences. This is not the case with iron or with any other biologically relevant redox-active metal (Kehres & Maguire, 2003).

3. Similarity to Magnesium

The greatest similarity between these two metals is in the context of structure. Mg^{2+} is an ideal structural cation for biological molecules, especially phosphorylated ones such as nucleic acids and many intermediary metabolites. This is due to a complete lack of *d* electrons in Mg^{2+}, and its $2s^22p^6$ electron configuration confines it to strict octahedral liganding geometry with liganding bond angles very close to 90°. This geometry is useful in organizing the conformations of complex compounds or macromolecules (Kehres & Maguire 2003). The lack of *d* electrons is important because there is very little covalent interaction between Mg^{2+} and its ligands; therefore, Mg^{2+} is a labile and rapidly exchangeable cation that does not interpose itself in the way of other close intermolecular interactions. Mn^{2+}, with its relatively similar ionic radius and relatively minor involvement of its stable symmetric $3d^5$ shell electrons in bonding, readily exchanges with Mg^{2+} in most structural environments and exhibits much of the same octahedral, ionic, labile chemistry. However, less similarity is seen in catalysis. The $3d^5$ electrons of Mn^{2+} do interact to some extent with electrophilic ligands. Thus, Mn^{2+}-ligand bonds are generally much more flexible than Mg^{2+}-ligand bonds, in both length and angle (Kehres & Maguire, 2003).

4. Manganese and Reactive Oxygen Species

The manganese-dependent superoxide dismutase has already been mentioned. In addition to this enzyme is a set of enzymes involved in the detoxification of reactive oxygen species that are dependent on manganese. There is a family of manganese-dependent catalases that often are referred to as "non-heme catalases." They are structurally and mechanistically unrelated to conventional catalases that are cofactored by iron in a prosthetic heme group. Both classes operate on hydrogen peroxide. In addition to catalases, a family of catabolic heme enzymes known as manganese peroxidases couples the redox activity of H_2O_2 to the degradation of nutrients such as lignin via oxidation of a manganese bound to a propionate side-chain of the heme group. An intriguing phenomenon is that salens, synthetic chelators that are derivatives of *N,N'-bis*(salicylidene) ethylendiamine chloride, form complexes with Mn^{2+} that show efficacy as combined superoxide dismutase/catalase mimics. They have been shown, in their oxo forms, to oxidize nitric oxide and nitrite to the more benign nitrate *in vitro* (Kehres & Maguire, 2003). A summary of manganese-related enzymes and proteins can be found in Table IX.

D. Iron: Savior and Threat

Iron is the most important of all metals and is the fourth most abundant element in the Earth's crust. It is a *d*-block element that can exist in oxidation states ranging from −2 to +6. In biological systems, however, these oxidation states are limited primarily to the ferrous (+2), ferric (+3), and ferryl (+4) states. Fe^{3+} is quite water insoluble and significant concentrations of water-soluble Fe^{3+} species can be attained only by strong complex formation. The interconversion of iron oxidation states is not only a mechanism whereby iron participates in electron transfer but also a mechanism

TABLE IX. Manganese-Dependent Enzymes and Proteins

Enzyme/Protein	*Function*	*Occurrence*
MnSOD	Detoxify superoxide radical anion	Bacteria, archaea, and eukaryotes
Non-heme Mn-catalase	Detoxify hydrogen peroxide	Bacteria
Transcription factor (*mntR*)	Repress Mn^{2+} uptake transporter expression	Homologs in diverse bacteria; extent unknown
ppGpp hydrolase (*spoT*)	Hydrolyze RNA synthesis regulator ppGpp	Practically ubiquitous in bacteria
Protein phosphatases	Dephosphorylate many cellular proteins	All cells; highly conserved between prokaryotes and eukaryotes
Agmatinase	Synthesize osmoprotectant putrescine from angmatine (decarboxylated arginine)	Many Gram-negative bacteria
Aminopeptidase P	Hydrolyze typical X-Pro sequence	Enterobacteria, most other bacteria
Phosphoglyceromutase	Catalyzes interconversion of 3-phosphoglycerate and 2-phosphoglycerate	Enterobacteria, other bacteria, and plants
Fructose-1,6-BP phosphatase	Convert fructose-1,6-BP to fructose-6-phosphate	Enterobacteria; extent otherwise unknown
Adenyl cyclase	Synthesize cyclic AMP	*Mycobacterium tuberculosis*
Aromatic hydrocarbon metabolism	Oxidation of catechols and other aromatics	*Arthrobacter globiformis* and similar enzymes in many soil bacteria
Lipid phosphotransferases	Modify or remove polar headgroups on lipids	Enterobacteria, Gram-positive bacteria
Polysaccharide polymerases	Synthesize capsular or secreted polysaccharide	Some Gram-positive and -negative bacteria
Protein kinases	Phosphorylation of unknown proteins	Extent unknown
Pyruvate carboxylase	Catalyze carboxylation of pyruvate to oxaloacetate	Eukaryotes, *Bacillus licheniformis*, and *Mycobacterium smegmatis*
Ribonucleotide reductase	Convert ribonucleotides to deoxyribonucleotides	Most bacteria, eurkaryotes
Arginase	Convert arginine to urea+ornithine	Higher eukaryotes, liver, and macrophages/monocytes; *Bacillus* sp.
Concavalin A	Plant lectin binding	Plants
Mn-lipoxygenase	Synthesize lipoxins from fatty acids	Fungi
Mn-peroxidase	Degrade lignin	White- and brown-rot fungi
Photosynthetic reaction center	Convert H_2O to O_2	Photosynthetic bacteria and plants

Source: Adapted from Kehres and Maguire (2003).

whereby iron can reversibly bind ligands. Iron can bind to many ligands by virtue of its unoccupied *d* orbitals. The preferred biological ligands for iron are oxygen, nitrogen, and sulfur atoms. Iron(III) is a hard acid that prefers hard oxygen ligands, while iron(II) is on the borderline between hard and soft and favors nitrogen and sulfur ligands. The electronic spin state and biological redox potential (from +1000 mV for some heme proteins to −550 mV for some bacterial ferredoxins) of iron can change according to the ligand to which it is bound. By exploiting the oxidation state, redox potential, and electron spin state of iron, nature can precisely adjust iron's chemical reactivity (Beard, 2001). Biologically important iron-containing proteins carry out oxygen transport and storage, electron transfer, and substrate oxidation–reduction. Four major classes of protein carry out these reactions (Beard, 2001) in mammalian systems: (1) iron-containing, nonenzymatic proteins (hemoglobin and myoglobin); (2) iron–sulfur enzymes; (3) heme-containing enzymes; and (4) iron-containing enzymes that are non-iron-sulfur, non-heme enzymes (Figure 17).

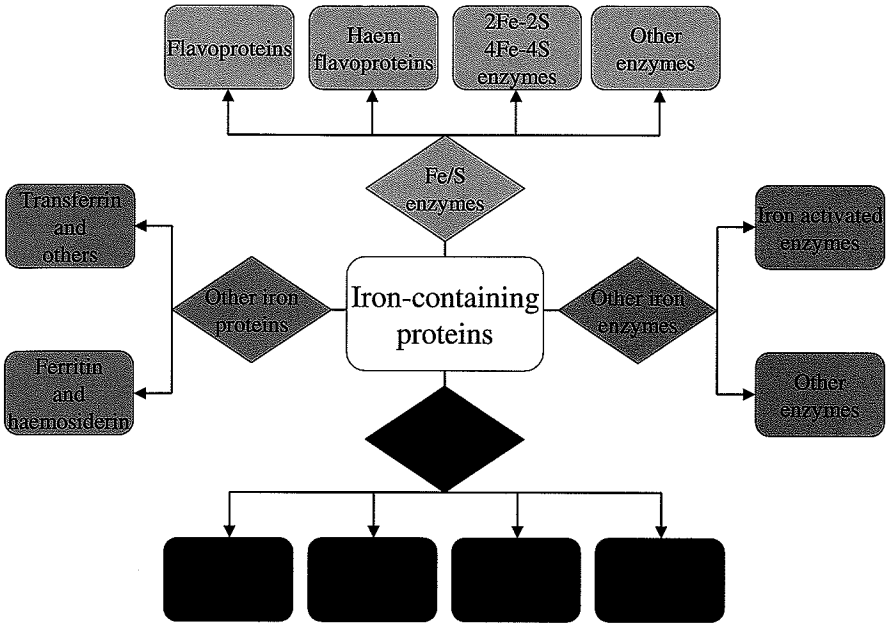

FIGURE 17 Iron-containing proteins.

1. Hemeproteins

Proteins with heme as the prosthetic group carry out important functions in biological systems; thus the biosynthesis of heme is very important. It begins with the synthesis of tetrapyrroles, where the term *tetrapyrrole* indicates compounds containing four linked pyrrole rings. Four classes of such compounds are very common in biology: (1) the widely distributed iron porphyrin, heme; (2) the chlorophylls of plants and photosynthetic bacteria; (3) the phycobilins, photosynthetic pigments of algae; and (4) the cobalamins, especially vitamin B_{12} and its derivatives. All tetrapyrroles are synthesized from a common precursor, δ-aminolevulinic acid (ALA). Figure 18 summarizes the relationships between the synthetic pathways.

2. Hemoglobin and Myoglobin

Evolution has provided animals with hemoglobin and myoglobin for oxygen transport and storage. Aerobic metabolism requires some kind of oxygen transporter because relying on diffusion would be adequate for only very small animals. Insects, however, have solved the problem with oxygen transport through their networks of tubes (tracheae) leading from the body surface to the inside tissues.

Oxygen transport and storage are tricky problems to solve. The molecules used must bind dioxygen without allowing oxidation of other substances, thereby reducing dioxygen. Are proteins suited for direct oxygen binding? The answer is no; however, *d*-block metals such as iron and copper in their lower oxidation states readily bind oxygen. Proteins can bind Fe(II) in various ways. In hemoglobin and myoglobin, iron is bound by the tetrapyrrole ring protoporphyrin IX (see Figure 19). When iron is bound to protoporphyrin IX, the system is referred to as ferroprotoporphyrin or heme. In addition to its use in hemoglobin and myoglobin, the heme group is a prosthetic group in a variety of proteins (see below). This makes the biosynthesis of heme very important. Myoglobin is a smaller molecule relative to hemoglobin and is the principal oxygen storage protein.

An interesting property of hemoglobin, in addition to its oxygen-binding ability, is its enzymatic activity. It has been thought that the oxygen carrier function was so specialized that globins were not recruited to new tasks; however, it has recently been found that the globin of some marine worms (*Amphitrite ornata*) has evolved into a powerful peroxidase, more precisely dehaloperoxidase. This enzyme catalyzes the oxidative dehalogenation of polyhalogenated phenols in the presence of hydrogen peroxide at a rate at least ten times faster than all known halohydrolases of bacterial origin. *A. ornata* can thus successfully survive in an environment where other species secrete brominated aromatics and other halocompounds as repellents (Lebioda, 2000).

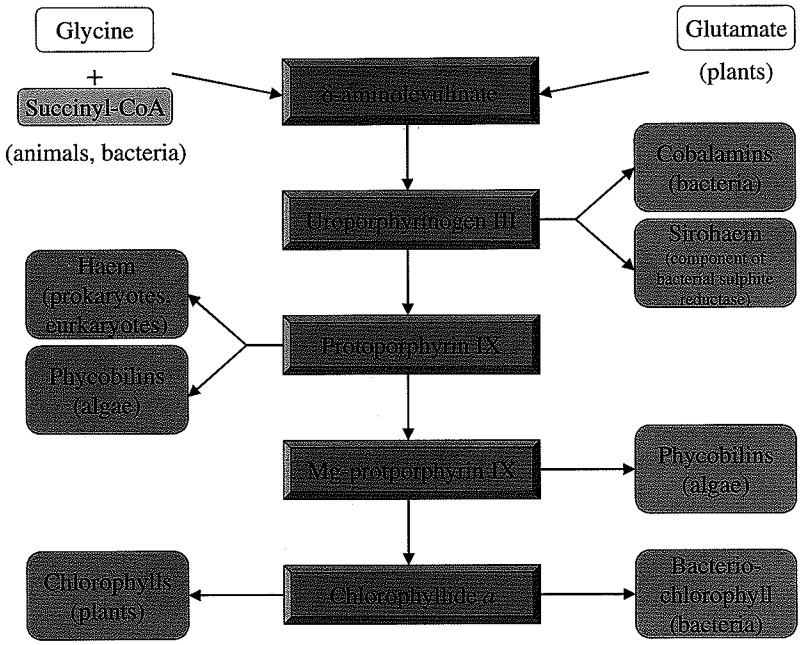

FIGURE 18 The principal steps of heme biosynthesis.

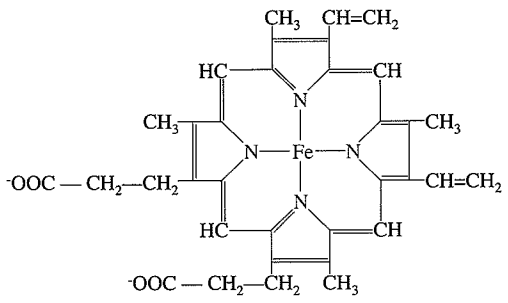

FIGURE 19 Iron binding in protoporphyrin IX. The complex is called *heme*.

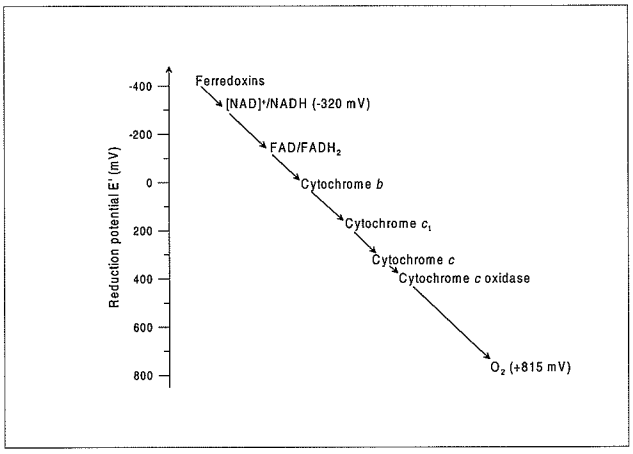

FIGURE 20 The mitochondrial electron-transfer chain.

3. *Cytochromes*

Cytochromes constitute a group of hemeproteins with distinctive visible-light spectra that function as electron carriers from biological fuels to oxygen. The major respiratory cytochromes are classified as *b*, *c*, or *a*, depending on the wavelengths of the spectral absorption peaks. They are vital members of the mitochondrial electron-transfer chain. Figure 20 shows a schematic representation of part of this electron-transfer chain. In addition, the cytochromes are also essential components in plant chloroplasts for photosynthesis. It is the ability of the iron center to undergo reversible Fe(III) ↔ Fe(II) changes to allow them to act as electron-transfer centers. In the mitochondrial electron-transfer chain, cytochrome *c* accepts an electron from cytochrome c_1 and then transfers it to cytochrome *c* oxidase. The electron is ultimately used in the four-electron reduction of dioxygen.

4. *Cytochrome P-450 Enzymes*

Cytochrome P-450 enzymes are hemeproteins that function as monooxygenases to catalyze the insertion of oxygen into a C—H bond of an aromatic of aliphatic hydrocarbon (*i.e.*, the conversion of RH to ROH). Examples of the biological functions of cytochrome P-450 are drug metabolism and steroid synthesis. Carbon monoxide adducts of cytochrome P-450 absorb at 450 nm; thus its name. One of the many interesting aspects of cytochrome P-450 enzymes is that some are inducible, which means that following exposure of the cell to an inducing chemical enzyme activity increases, in some cases several orders of magnitude. It has been proposed that the catalytic cycle begins with the enzyme in a resting state with iron present as Fe(III). The hydrocarbon substrate then binds, followed by one-electron transfer to the iron porphyrin. The resulting Fe(II) complex with bound substrate proceeds to bind O_2. A key reaction is the reduction of the porphyrin ring of the oxygen complex by a second electron, which produces the ring radical anion. Uptake of two H^+ ions then leads to the formation of the Fe(IV) oxo-complex, which attacks the hydrocarbon substrate to insert oxygen. Loss of ROH and uptake of an H_2O molecule at the vacated coordination position bring the cycle back to the resting state (Ortiz de Monetallano & De Voss, 2002).

5. *Iron-Activated Enzymes*

The most prominent example of this class of enzymes is heme oxygenase, which has evolved to carry out the oxidative cleavage of heme, a reaction essential in several physiological processes as diverse as iron reutilization and cellular signaling in mammals and synthesis of essential light-harvesting pigments in cyanobacteria and higher plants, as well as the acquisition of iron by bacterial pathogens (Wilks, 2002). Heme oxygenase (HO) is concentrated in both blood vessel endothelium and adventitial neurons which suggests that HO subserves functions that are handled by NO-generating enzymes. The gene of this cellular stress protein, mediating the catabolism of heme to biliverdin in brain and other tissues, is strongly induced by dopamine, oxidative stress, and metal ions. In the brain, it is primarily expressed in the astroglia, and, when upregulated, HO promotes mitochondrial iron deposition in these cells (Schipper, 1999). HO protects cells by degrading prooxidant metalloporphyrins and appears to facilitate iron efflux from the cell (Berg *et al.*, 2001). HO is responsible not only for iron export but also for the intracellular sequestration of iron by glial mitochondria (Schipper, 1999).

6. *Fe/S Enzymes*

The porphyrin ligand environment of iron that occurs in hemoglobin and myoglobin is also important in redox enzymes. Thus, in the large class of biochemically important heme proteins iron is coordinated to a porphyrin ligand. All other iron proteins are defined as non-heme. Those that contain iron in a tetrahedral environment of four sulfur atoms are particularly important. Iron–sulfur clusters, however, were not familiar to inorganic chemists prior to recognition of their biochemical importance. Iron–sulfur clusters are simple inorganic groups that are contained in a variety of proteins having functions related to electron transfer, gene regulation, environmental sensing, and substrate activation. Biological Fe–S clusters, however, are not formed spontaneously, but a consortium of highly conserved proteins is required for both the formation of Fe–S clusters and their insertion into various protein partners. The formation or transfer of Fe–S clusters appears to require an electron-transfer step (Frazzon *et al.*, 2002). Table X provides examples of proteins with Fe–S clusters and their function.

Although not all of the enzymes involved in the mitochondrial respiratory chain are iron–sulfur proteins, it is enlightening to look through the various parts of the chain. Electrons move from reduced nicotinamide adenine dinucleotide (NADH), succinate, or some other primary electron donor, through flavoproteins, ubiquinone, iron–sulfur proteins and cytochromes, and finally to O_2. Table XI summarizes the protein components of the mitochondrial electron-transfer chain.

In the context of iron–sulfur proteins, we can observe an intriguing link to another essential metal (namely, molybdenum) through xanthine dehydrogenase and xanthine oxidase. These are the most studied of the small but important class of molybdenum-containing iron–sulfur flavoproteins. Xanthine oxidoreductase catalyzes the hydroxylation of a wide variety of purine, pyrimidine, pterin, and aldehyde substrates. The active form of the enzyme is a homodimer of molecular mass 290 kDa, and each of the monomers acts independently in catalysis. Each subunit contains one molybdopterin group, two identical 2Fe–S centers, and one flavin adenine dinucleotide cofactor (Nishino & Okamoto, 2000).

7. *Transferrin, Lactoferrin, and Hemopexin*

Transferrin originally was the name of the serum protein that binds and transports iron for delivery to cells. Today, it is the name applied to a wider family of homologous proteins that includes serum transferrin,

TABLE X. Some Examples of Proteins and Enzymes with [Fe–S] Clusters and Their Function

Enzyme or Protein	*Function*	*Cofactors*
Rubredoxin	Electron transfer	Fe
Ferredoxin, Rieske ferredoxin	Electron transfer	[2Fe–2S]
Phthalate dioxygenase reductase	Electron transfer	[2Fe–2S], FAD
Naphthalene dioxygenase	O_2-activation	[2Fe–2S], Fe(II)
Adenine glycosylase, glutamine PRPP amidotransferase, endonuclease III	Structural stabilization	[4Fe–4S]
Aconitase	Electrophilic catalysis	[4Fe–4S]
IRP-1,[a] FNR,[b] soxRS[c]	Regulation of gene expression	[4Fe–4S], [2Fe–2S]
Dinitrogenase reductase, dinitrogenase	Nitrogen fixation	[4Fe–4S], FeMoCo, P-clusters

[a]Iron regulatory protein-1.
[b]Ferredoxin:NADP+oxidoreductase.
[c]Superoxide regulatory system.

TABLE XI. Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme Complex	*Mass (kDa)*	*Number of Subunits*	*Prosthetic Group(s)*
I NADH dehydrogenase	850	42	FMN,[a] [Fe–S]
II Succinate dehydrogenase	140	5	FAD,[b] [Fe–S]
III Ubiquinone: cytochrome *c* oxidoreductase	250	11	Hemes, [Fe–S]
Cytochrome *c*	13	1	Heme
IV Cytochrome oxidase	160	13	Hemes, Cu_A, Cu_B

[a]Flavin mononucleotide.
[b]Flavin adenine dinucleotide.

lactoferrin, ovotransferrin, and melanotransferrin (Baker *et al.*, 2003). Only serum transferrin has a proven transport function; however, transferrins seem to be involved in the homeostatic control of free iron in all the places where it might be found. The serum transferrin has two structurally similar lobes. Both lobes contain one iron-binding center that is very specific for iron(III) and has a binding constant of about 10^{20}. It is only when transferrin is loaded with two ferric ions that it binds strongly to the receptor for internalization. In addition to iron(III), transferrin can bind strongly to a range of other metal ions. Many such complexes are still recognized by the transferrin receptor, and some bacteria have transferrin receptors. Transferrin, therefore, holds promise for use in the development of antimicrobial therapies (Andrews *et al.*, 2003).

Lactoferrin is an iron-binding protein that binds iron even more tightly than transferrin. It is present in milk, many other exocrine secretions, and white blood cells (Baker *et al.*, 2003). One of the first functions attributed to lactoferrin was the ability to inhibit bacterial growth and viral infection. It is thought that lactoferrin is able to sequester iron from certain pathogens, which inhibits their growth. Another important function is its ability to stimulate the release of the neutrophil-activating polypeptide interleukin-8 (IL-8). This suggests that lactoferrin may function as an immunomodulator for activating the host defense system (Kruzel & Zimecki, 2002).

Hemopexin is a recycler and transporter of heme. Turnover of heme proteins, notably hemoglobin, leads to the release of heme into extracellular fluids with potentially severe consequences. Like free iron, heme is a source of essential iron for invading bacterial pathogens and is highly toxic because of its ability to catalyze free-radical formation. Protection is given by hemopexin, a 60-kDa serum glycoprotein that sequesters heme with very high affinity from the bloodstream; transports it to specific receptors on liver cells, where it undergoes receptor-mediated endocytosis; and releases the bound heme into cells. It thus serves both to protect against heme toxicity and to conserve and recycle iron (Baker *et al.*, 2003).

8. Ferritin and Hemosiderin

Ferritins constitute a class of iron storage proteins found in bacterial, plant, and animal cells. They form hollow, spherical particles in which up to 4500 iron atoms can be stored as iron(III). Although ferritins are quite small molecules about 8 to 12 nm in diameter, they are very effective iron stores. Additionally, they can provide iron on demand. The biosynthesis of ferritin is controlled by the level of iron in the cell via the iron regulatory protein (Andrews *et al.*, 2003). Hemosiderin is another iron-storage complex; however, knowledge of its structure is minimal. It is found solely in cells, in contrast to ferritin, which can also be found in the circulation. It has been suggested that hemosiderin could be a complex of ferritin, denatured ferritin, and some other material. Iron present in hemosiderin deposits is poorly available to provide iron on demand. The storage complex is found in macrophages and appears to be especially abundant following hemorrhage; thus, its formation might be related to phagocytosis of red blood cells and hemoglobin (Trinder *et al.*, 2000).

G. Copper: The Master of Oxidases

Historical records show that copper and copper compounds had been used medicinally at least as early as 400 BC (Mason, 1979). Many copper compounds were used to treat a variety of diseases during the nineteenth century, and the presence of copper in plants and animals was recognized more than 150 years ago. For quite some time it has been widely accepted that copper is an essential trace element required for survival by all organisms, from bacterial cells to humans (Linder & Hazegh-Asam, 1996). Copper ions undergo a unique chemistry due to their ability to adopt distinct redox states, either oxidized [Cu(II)] or in the reduced state [Cu(I)]. Consequently, copper ions serve as important catalytic cofactors in redox chemistry for proteins that carry out fundamental biological functions; however, copper provides a challenge to biological systems. The very properties that make copper indispensable to biology become toxic when copper is present in excess. Copper's outstanding redox properties, however, such as the transitions between Cu(II) and Cu(I), can in certain circumstances result in the generation of reactive oxygen species such as superoxide radicals and hydroxyl radicals. Susceptible cellular components can be damaged by these reactive species if an effective scavenging mechanism is not in operation. Copper can also bind with high affinity to histidine, cysteine, and methionine residues of proteins. This may result in the inactivation of the proteins (Camarakis *et al.*, 1999). Consequently, there is a great need for effective homeostatic mechanisms controlling the cellular concentration of copper.

1. Copper Proteins

Copper is present in three different forms in proteins: (1) blue proteins without oxidase activity (*e.g.*, plastocyanin), which function in one-electron transfer; (2) non-blue proteins that produce peroxidases and oxidize monophenols to diphenols; and (3) multicopper proteins containing at least four copper atoms per molecule and acting as oxidases (*e.g.*, ascorbate oxidase and laccase). Table XII provides an arbitrary selection of copper-dependent proteins to emphasize the versatility of copper proteins (Peña *et al.*, 1999; Fraústo da Silva & Williams, 2001).

2. Intracellular Distribution of Copper

To be distributed in the cell, copper has to be taken up in the cell. This is accomplished by copper transport 1 (Ctr1p), which is a membrane-spanning protein. Ctr1p specifically transports Cu(I), not Cu(II) or any other metals. A conspicuous finding was that the gene *CTR1* (Cu transport 1) was first discovered not as a gene for copper but as a gene essential for iron transport in *Saccharomyces cerevisiae* (Harris, 2000). The copper was required for Fet3, a multicopper ferroxidase that catalyzed the oxidation of Fe(II) to Fe(III).

The intracellular environment is generally reducing. This means that Cu(II) is rapidly reduced to Cu(I). Glutathione was identified early as being involved in copper transport. Glutathione (GSH) is a cysteine-containing tripeptide present more or less exclusively within cells at concentrations in the millimolar range. Copper(I) reacts directly with the internal cysteine sulfhydryl group of glutathione; however, it is less likely that Cu(II) binds to glutathione because of the propensity of Cu(II) to catalyze the oxidation of sulfhydryl groups. The formation of Cu(I)–GSH is a spontaneous reaction probably independent of enzyme involvement. Besides transferring copper to metallothionein, glutathione is required for biliary excretion of copper. In addition, there is evidence that a Cu–GSH complex can mediate stable Cu(I) binding to apocuproproteins (Harris, 2000).

Copper chaperones are a family of cytosolic peptides. They are usually referred to as metallochaperones and serve as an intracellular shuttle service for metal ions. In the case of copper, they form transient complexes with Cu(I). These chaperones escort copper ions on

TABLE XII. Examples of Copper-Dependent Proteins

Protein	*Function*
Cytochrome oxidase	Reduction of O_2 to H_2O
Laccase	Oxidation of phenols
Ceruloplasmin	Oxidation of Fe(II) to Fe(III), Cu transport
Hemocyanin	Transport of O_2
Lysine oxidase	Cross-linking of collagen
Ascorbate oxidase	Oxidation of ascorbate
Galactose oxidase	Oxidation of primary alcohols to aldehydes in sugars
Amine oxidase	Removal of amines and diamines
Blue proteins	Electron-transfer (many kinds)
Superoxide dismutase	Superoxide dismutation (defense)
Nitrate reductase	Reduction of NO_2^- to NO
Nitrous oxide reductase	Reduction of N_2O to N_2
Metallothionein	Cu(I) storage
Dopamine monooxygenase	Hydroxylation of Dopa
Co-proporphyrin decarboxylase	Production of protoporphyrin IX
Ethylene receptor	Hormone signaling
Methane oxidase	Oxidation to methanol
Terminal glycine oxidases	Production of signal peptides
Tyrosinase	Melanin production
Clotting factors V and VII	Blood clotting
Angiogenin	Induction of blood vessel formation
Hephaestin	Iron egress from intestines
CP-x type ATPase	Copper pump
Atx-1 (Lys 7)	Copper transfer

their way into proteins that require copper. The prototype of metallochaperones is MerP, a small soluble mercury-binding protein that transports Hg^{2+} to a membrane transporter and eventually to a reductase that reduces Hg^{2+} to the volatile Hg^0 as part of a detoxifying process (Lund & Brown, 1987). The best known metallochaperone protein is Atx1 (antioxidant 1), which was originally isolated as an antioxidant protein in *Saccharomyces cerevisiae* and functions in a high-affinity iron uptake pathway in yeast. It works together with Ccc2, a copper-transporting P-type ATPase. The cooperation of these two proteins is necessary for copper loading of the multicopper oxidase Fet3, which is required for the high-affinity iron uptake (Huffman & O'Halloran, 2001).

The human homologs of Ccc2 are the Wilson's (Atp7ab) and Menkes' (Atp7a) disease proteins, and the human homolog of Fet3 is ceruloplasmin. Cytochrome oxidase is essential for cellular respiration and requires

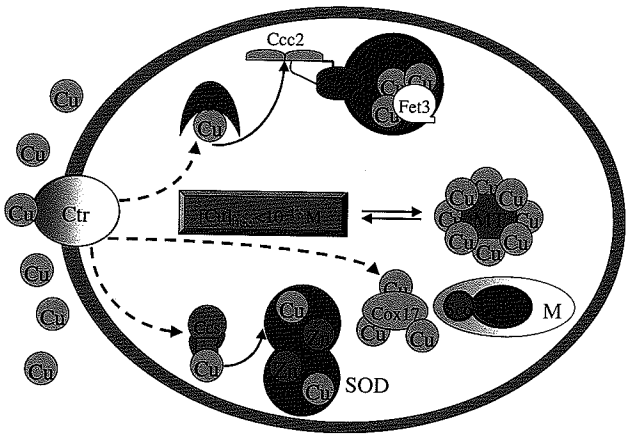

FIGURE 21 A schematic view of copper localization in the cell.

three copper atoms for proper function. Two are situated in a dinuclear site in one subunit and one in another subunit next to heme a_3. It has been suggested that Cox17, a small 8-kDa protein, functions in a copper trafficking pathway of cytochrome oxidase (Huffman & O'Halloran, 2001). The CCS metallochaperone seems to be necessary for the incorporation of copper into the radical scavenging enzyme Cu,Zn–superoxide dismutase. A schematic view of copper uptake and cellular localization is shown in Figure 21. A few intriguing examples of copper-binding proteins are presented in the next section.

3. *Ceruloplasmin*

This protein was first isolated from plasma and characterized as a copper-containing protein as early as 1948 (Holmberg & Laurell). It was later discovered that the concentration of this protein was low in patients with Wilson's disease. The first proposed physiological role of the protein was in iron homeostasis and as a ferroxidase (Hellman & Gitlin, 2002). Ceruloplasmin belongs to the group of multicopper oxidases. This group of proteins has three distinct copper sites, which are type I copper sites. Charge transfer between the cysteine ligand sulfur and the copper at these sites results in strong absorption at 600 nm. This is why they are called blue proteins. A single copper of type II is coordinated by four imidazole nitrogens and is in close proximity to two antiferromagnetically coupled type III copper ions absorbing at 330 nm. The type II and type III coppers form a trinuclear copper cluster, which is the site of oxygen binding during the catalytic cycle (Hellman & Gitlin, 2002).

Multicopper oxidases use the smooth electron chemistry of bound copper ions to couple substrate oxidation

with the four-electron reduction of molecular oxygen. Electrons pass from the substrate to the type I copper and then to the trinuclear copper cluster and subsequently to the oxygen molecule bound at this site (Messerschmidt *et al.*, 1989). In addition to ceruloplasmin, several multicopper oxidases have been identified that play a critical role in iron homeostasis. Fet3 is a ferroxidase essential for iron uptake in yeast, and hephaestin is a ceruloplasmin homolog required for efficient iron efflux from the placenta and enterocytes in mammals (Vulpe *et al.*, 1999). Ceruloplasmin is an acute-phase reactant, and its serum concentration increases during inflammation, infection, and trauma largely as a result of increased gene transcription in hepatocytes mediated by the inflammatory cytokines (Hellman & Gitlin, 2002).

The liver largely exercises copper homeostasis, and the hepatocytes are the primary site of copper metabolism. Hepatocytes are highly polarized epithelial cells that regulate copper excretion in the bile dependent on the intracellular copper concentration. The copper chaperone Atx1 is required for the delivery of copper to ceruloplasmin; however, the mechanism of copper incorporation into ceruloplasmin is not well understood. Studies of *Saccharomyces cerevisiae* show that both the H^+-transporting V-type ATPase and the CLC chloride channel Gef1 are necessary for copper incorporation in the homologous multicopper oxidase Fet3 (Gaxiola *et al.*, 1998). Ceruloplasmin is capable, *in vitro*, of catalyzing oxidation of a number of different substrates. This has caused some confusion as to the physiologic role of this protein. Ceruloplasmin from human serum has considerable ferroxidase activity necessary for the oxidation of the ferrous iron and incorporation of ferric iron in apotransferrin (Osaki *et al.*, 1971).

4. Superoxide Dismutase

Superoxide dismutase (SOD) is a member of the oxidoreductase family of enzymes. There are several forms of superoxide dismutase, including Mn–SOD, Fe–SOD, and Cu,Zn–SOD. The Mn–SOD is found exclusively in the mitochondria, whereas the Fe–SOD is generally found in prokaryotes. The Cu,Zn–SOD is active in the cytoplasm of eukaryotes and is the most abundant form of SOD. Cu,Zn–SOD is a dimer of identical subunits with a molecular weight of 16 kDa. One Cu(II) and one Zn(II) are included in each dimer. The role of zinc ions in SOD is structural, and the copper ions take part in the catalytic process. It is based on a redox process, and zinc does not take part in those reactions. SOD catalyzes the disproportion of the superoxide anion ($O_2^{-\bullet}$) to a less dangerous reactive oxygen species. SOD catalyzes the conversion of the superoxide anion according to the following process:

$$Cu^{2+} + O_2^{\bullet -} \rightarrow Cu^{+} + O_2$$

$$Cu^{+} + O_2^{\bullet -} + 2H^{+} \rightarrow Cu^{2+}H_2O_2$$

$$\text{Net reaction: } 2O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2$$

Recently, a Ni-containing SOD was found in *Streptomyces griseus* and *S. coelicolor* (Youn *et al.*, 1996). The subunits of Cu,Zn–SOD are stabilized by an intrachain disulfide bond but associated by noncovalent forces. This enzyme requires copper and zinc for its biological activity, and the loss of copper results in its complete inactivation, which can lead to the development of human diseases.

F. Zinc: The Ubiquitous Trace Element

Zinc has been known to be essential to life since 1869, when it was discovered that it was required by *Aspergillus niger*. It differs chemically from its neighbors in the transition metal area of the periodic table. Zinc does not take part in redox reactions, but it is a good Lewis acid. In fact, it could be called Nature's Lewis acid. It has a hard metal center and is ideally suited for coordination of N- and O-donors. It is also highly polarizing, and the activity of Zn(II)-dependent enzymes is due to the Lewis acidity of the metal center. In addition, it is characterized by fast ligand exchange. The first enzyme to be recognized as a zinc metalloenzyme was carbonic anhydrase, an enzyme essential for respiration in mammals. At present, zinc metalloenzymes have been recognized in all classes of enzymes. Today, more than 300 enzymes are known to be dependent on zinc for catalytic, structural, regulatory, and noncatalytic functions. Examples of enzymes in which zinc plays a catalytic role include carbonic anhydrase, carboxypeptidase, thermolysin, and aldolases. Zinc stabilizes the quaternary structure of oligomeric holoenzymes. It dimerizes *Bacillus subtilus* α-amylase without affecting its enzymatic activity and stabilizes the pentametric quaternary structure of asparatate–transcarbamylase. Zinc acts as an activator of bovine lens leucine aminopeptidase and inhibits the activity of porcine kidney leucine aminopeptidase and fructose-1,6-bisphosphatase.

1. Zinc and Enzymes

Zinc has three types of roles with regard to enzymes: catalytic, cocatalytic, and structural (see Table XIII). Carbonic anhydrases are a widely expressed family of enzymes that catalyze the reversible reaction $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$. These enzymes therefore both

TABLE XIII. Examples of Zinc Metalloproteins

Enzyme	*Role of Zinc*	*Enzyme*	*Role of zinc*
Class I: Oxidoreductases		Carboxypeptidase (other)	Catalytic
Alcohol dehydrogenase	Catalytic, non-catalytic	Carboxypeptidase A	Catalytic
D-Lactate cytochrome reductase	?	Carboxypeptidase B	Catalytic
D-Lactate dehydrogenase	Catalytic	Collagenase	Catalytic
Superoxide dismutase	Structural (copper catalytic)	Creatinase	?
		Cytidine deaminase	Catalytic
Class II: Transferases		D-Carboxypeptidase	Catalytic
Aspartate transcarbamylase	Structural	DD carboxypeptidase	Catalytic
Cobalamin-dependent methionine synthase	Catalytic	Dihydropyrimidine aminohydrolase	?
		Dipeptidase	Catalytic
Cobalamin-independent methionine synthase	Catalytic	Elastase	?
		Fructose-1,6-bisphosphatase	Regulatory
DNA polymerase	Catalytic	Neutral protease	Catalytic
Mercaptopyruvate sulphur transferase	?	Nuclease P_1	?
Nuclear poly(A) polymerase	Catalytic	Nucleotide pyrophosphatase	Catalytic
Phosphoglucomutase	?	Phosphodiesterase (exonuclease)	Catalytic
Protein farnesyltransferase	Catalytic	Phospholipase C	Catalytic
Reverse transcriptase	Catalytic	Procarboxypeptidase A	Catalytic
RNA polymerase	Catalytic	Procarboxypeptidase B	Catalytic
Terminal dNT transferase	Catalytic	Thermolysine	Catalytic
Transcarboxylase	?		
		Class IV: Lyases	
Class III: Hydrolases		δ-Aminolevulinic acid dehydratase	Catalytic
α-Amylase	Structural	Carbonic anhydrase	Catalytic
α-D-Mannosidase	?	Fructose-1,6-bisphosphatase adolase	Catalytic
β-Lactamase II	Catalytic	Glycoxalase	Catalytic
Adenosine deaminase	Catalytic	L-Rammulose-1-phosphate adolase	Catalytic
Alkaline phosphatase	Catalytic, noncatalytic		
Aminocyclase	?	Class V: Isomerases	
Aminopeptidase	Catalytic, regulatory	Phosphomannose isomerase	?
Aminotripeptidase	Catalytic		
AMP deaminase	?	Class VI: Ligases	
Angiotensin-converting enzyme	Catalytic	Pyruvate carboxylase	?
Astacin	Catalytic	TRNA synthetase	Catalytic

Source: Data from Prasad (1995) and McCall *et al.* (2000).

produce HCO_3^- for transport across membranes and consume HCO_3^-, which has been transported across membranes (Sterling *et al.*, 2001). Erythrocytes of mammals have two isoenzymes of carbonic anhydrase: CAI, which has a low activity, and CAII, which has high activity. The molecular weight of human CAI is 30,000 Da, and it contains one atom of zinc per molecule. Human CAII has 259 amino acids, while human CAI has 260 amino acids, and the two isoenzymes share 60% sequence homology. In carbonic anhydrase, zinc is catalytic.

An example of the structural role of zinc in enzymes is aparatate transcarbamylase (ATCase). This enzyme catalyzes the first step in pyrimidine biosynthesis, condensation of aspartate, and carbamyl phosphate. It is an allosterically regulated enzyme, and its activity is inhibited by cytidine triphosphate (CTP) and activated by ATP. Both responses seem to make sense from a physiological perspective. If CTP levels are already high, additional pyrimidines are not needed, and high ATP signals offers both a purine-rich state and an energy-rich cell condition under which DNA and RNA

synthesis can be active. There are similarities with hemoglobin in that the allosteric regulation of ATCase involves changes in the quaternary structure of the molecule. A change in the molecule from the tense state to the relaxed state involves a major rearrangement of subunit positions (Purcarea *et al.*, 1997).

Leucine aminopeptidase (LAP) is a prototypic dizinc peptidase that has been studied intensely. The enzyme is present in animals, plants, and bacteria and has various tissue-specific physiological roles in the processing or degradation of peptides. Human LAP has been shown to catalyze postproteosomal trimming of the N terminus of antigenic peptides for presentation on major histocompatibility complex class I molecules (Beninga *et al.*, 1998; Sträter *et al.*, 1999). The two zinc atoms bound to LAPs work in two different ways: One has a catalytic function, and the other regulates the activity induced by the zinc atom at the first site.

Alcohol dehydrogenase is a zinc-dependent enzyme. At least in some species, zinc may be essential for protection against oxidative damage (Tamarit *et al.*, 1997). In humans, there are at least nine different forms of the enzyme, most of which are found in the liver. It should be noted that an unusual iron- and zinc-containing alcohol dehydrogenase has been identified in the hyperthermophilic archaeon *Pyrococcus furiosus* (Ma & Adams, 1999). Alcohol dehydrogenase is our primary defense against alcohol intoxication. It catalyzes the following reaction:

$$\text{Ethanol} \xrightleftharpoons{\text{Alcohol dehydrogenase}} \text{Acetaldehyde} \xrightleftharpoons{\text{Aldehydede hydrogenase}} \text{Acetic acid}$$

In fact, alcohol dehydrogenase catalyzes a transformation to a yet more toxic product, acetaldehyde. So, this toxic molecule is transformed in the next step by aldehyde dehydrogenase to acetic acid and other molecules (Duester, 1998) that can be used by the cells. Alcohol dehydrogenase also catalyzes the transformation of retinol in the eye to retinaldehyde and by aldehyde dehydrogenase to retinoic acid. This first line of defense against alcohol, however, is beset with some dangers because alcohol dehydrogenase also modifies other alcohols, often producing dangerous products. For instance, methanol is converted into formaldehyde, which causes damage to proteins and possibly cancer. Small amounts of methanol cause blindness when the sensitive proteins in the retina are attacked, and larger amounts lead to widespread damage and death (Barceloux *et al.*, 2002).

The alcohol dehydrogenases are NAD(H) dependent and have two subunits. Each subunit contains two zinc atoms and binds one molecule of NAD(H). One zinc atom is essential for the catalytic effect, and the role of the second is largely still unknown; however, it does not seem to be necessary for structure in many cases. There is evidence that zinc may be of importance for the conformational stability of yeast alcohol dehydrogenase (Yang & Zhou, 2001).

2. *Zinc and Gene Expression*

It is by now well established that zinc plays a very important role in gene expression. The importance can be appreciated from the fact that about 25% of the zinc content of rat liver is found in the nucleus, and a significant amount of zinc is incorporated into nuclei *in vitro* (Cousins, 1998). One subject of major importance is genetic stability. The correct sequence of nucleotides in DNA is essential for proper replication, gene expression, and protein synthesis. Zinc is involved in the processes of genetic stability and gene expression in a variety of ways, including the structure of chromatin, replication of DNA, and transcription of RNA through the activity of transcription factors and RNA and DNA polymerases, as well as playing a role in DNA repair and programmed cell death (Falchuk, 1998).

Until the late 1980s, DNA-binding proteins were not well represented among the nearly 300 zinc-containing proteins known at that time. The multisubunit bacterial RNA polymerases were found to be zinc dependent in the early 1970s. Following these findings, the eukaryotic RNA polymerases, containing many more subunits than the bacterial enzymes, were also found to be zinc enzymes. Replicative DNA polymerases are essential for the replication of the genome of all living organisms. They catalyze the chemical reaction of DNA synthesis:

$$\text{template} - \text{primer} - (\text{dNMP})_n + \text{dNTP} \rightarrow$$
$$\text{template} - \text{primer} - (\text{dNMP})_{n+1} + \text{PP}_i$$

where dNMP and dNTP are deoxynucleoside 5′-monophosphate and 5′-triphosphate, respectively. During the reaction, inorganic pyrophosphate is released. The reaction requires a 3′-hydroxyl group of the primer for the nucleophilic attack on the α-phosphate of the incoming dNTP. The released pyrophosphate is hydrolyzed, thus providing energy for the reaction.

The sequence similarities may be used to classify them into three types. Type A polymerases are homologous to bacterial polymerases, type B includes archaeal DNA polymerases and eukaryotic DNA polymerase α, and type C is made up of the bacterial polymerase III class. The catalytic mechanism of all three types

involves two metal-binding acidic residues in the active site.

One very important aspect of zinc is the so-called zinc fingers, which were first discovered as the transcription factor IIIA (TFIIIA). TFIIIA is a site-specific DNA-binding protein that plays a central role in controlling the transcription of 5S ribosomal RNA genes in the African toad *Xenopus laevis*. This protein is slightly unusual because not only does it recognize the internal control region of about 45 bp in the center of the 5S RNA gene, but also TFIIIA itself is bound to the product. The name "zinc fingers" was introduced because of the specific interaction between the amino acids cysteine and histidine and a zinc ion responsible for the formation of the characteristic loop structure (Figure 22). Zinc fingers are generic protein motifs that can mediate DNA-binding and are both widespread and multifunctional. Since first being discovered in the early 1980s, several more zinc-finger proteins have been identified. More than 50 zinc-finger proteins are known today.

Zinc-finger domains are common, relatively small protein motifs that fold around one or more zinc ions. In addition to their role as DNA-binding modules, zinc-finger domains have recently been shown to mediate protein:protein and protein:lipid interactions (Ladomery & Dellaire, 2002). This small zinc-ligating domain, often found in clusters containing fingers with different binding specificities, can facilitate multiple, often independent intermolecular interactions between nucleic acids and proteins. Classical zinc fingers, typified by TFIIIA, ligate zinc via pairs of cysteine and histidine residues, but there are at least 14 different classes of zinc fingers, which differ in the nature and arrangement of their zinc-binding residues. Some types of zinc fingers can bind to both DNA and a variety of proteins. Thus, proteins with multiple fingers can play a complex role in regulating transcription through the interplay of these different binding selectivities and affinities (Ladomery & Dellaire, 2002). Other zinc fingers have more specific functions, such as DNA-binding zinc-finger motifs in the nuclear hormone receptor proteins and small-molecule-binding zinc fingers in protein kinase C. Some classes of zinc fingers appear to act exclusively in protein-only interactions. It has also been suggested that zinc fingers, in addition to the functions described above, play a protective role through their prevention of chemical attack by, for example, radicals or reactive oxygen species (Dreosti, 2001).

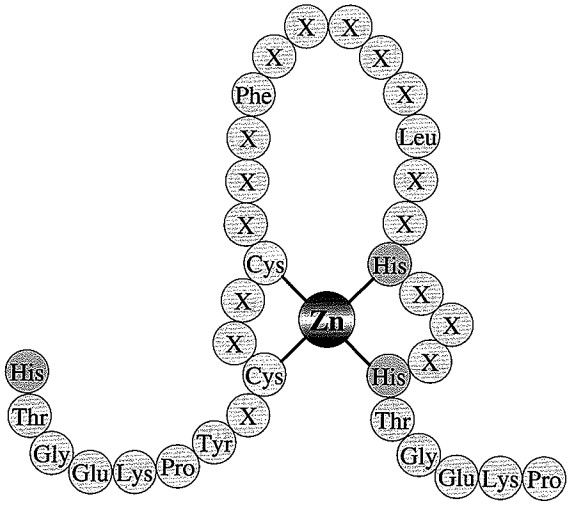

FIGURE 22 General structure of zinc fingers.

3. *Zinc and Metallothionein*

Metallothionein is a generic name for a superfamily of ubiquitous proteins or polypeptides possessing sulfur-based metal clusters. These proteins consist of a single polypeptide chain of 61 to 62 amino acids that contains 20 cysteine residues that, in turn, contain several bivalent cations such as zinc bound through metal–thiolate linkages. These clusters are usually formed through the preferential coordination of d^{10} metal ions by the cysteine thiolate ligands. Currently, four isoforms of metallothionein have been identified. Not all of them are expressed in all organs of mammals. Quite substantial differences exist among species. Metallothionein isoforms I and II have a ubiquitous tissue distribution, with particular abundance in liver, pancreas, intestine, and kidney, whereas isoforms III and IV are found principally in brain and skin (Davis & Cousins, 2000).

The zinc coordination in metallothionein is exceptional. The structure is a dumbbell-shaped molecule with two domains, in each of which zinc is bound in a cluster. In one domain, three zinc atoms are bound to nine cysteines; in the other domain, four zinc atoms are bound to 11 cysteines. In this way, each zinc atom is bound tetrahedrally to four cysteines, but overall there are fewer than the maximum number of possible ligands for the seven metals; consequently, some of the cysteines form ligand bridges that form an extensive zinc–sulfur network. The protein envelops the zinc atoms in a manner that effectively shields them from the environment and leaves only a few of the sulfur ligands partially exposed to solvent. Because both protein structure and tetrahedral zinc coordination preclude access of ligands to zinc, it would seem that a conformational

change of the protein is necessary to release zinc (Maret, 2000).

It is challenging that very few suggestions as to the biological functions of metallothionein have emerged, although the protein has been extensively studied for decades. The main consensus seems to be that it has a role in the detoxification of metal. The biological function of metallothionein is likely related to the physiologically relevant metals that it binds. Recent studies have produced strong evidence to support the idea that metallothionein functions as a metal chaperone for the regulation of gene expression and for the synthesis and functional activity of metalloproteins and metal-dependent transcription factors (Xun & Kang, 2002). Vital roles for this pleiotropic protein in more primitive life forms involve the sequestration of environmental metals such as cadmium and mercury.

It has been suggested recently that a biological function of metallothionein is to provide redox functions to the cells. The association of zinc with only sulfur ligands and the biological significance of the peculiar cluster of metallothionein and its purpose have not been elucidated in detail; however, Maret (2000) provided a chemical solution to this challenge: The cluster unit operates via a novel mechanism that allows the cysteine sulfur ligands to zinc to be oxidized and reduced with the concomitant release and binding of zinc. This results in an oxidoreductive process exercised by the ligands of the otherwise redox-inert zinc atom. Thus, metallothionein can become a redox protein in which the redox chemistry originates not from the metal atom but rather from its coordination environment.

Although zinc, copper, cadmium, mercury, gold, and bismuth are all metals that induce metallothionein, zinc is the primary physiological inducer. Zinc and copper are essential trace elements, and the other metals are environmental toxicants; however, copper in nontoxic concentrations does not induce metallothionein (Coyle & Phicox, 2002). Metal regulation of metallothionein genes is a complex process involving several steps. In short, the binding of zinc to metal transcription factor 1 (MTF-1) allows the protein to bind to metal-responsive elements (MREs) in the promotor region, which subsequently initiates metallothionein gene transcription. It has been proposed that MTF-1 regulates the free zinc concentration by controlling the expression of metallothionein as well as that of a zinc-transporter protein, ZnT-1. Basal expression of MTF-1 may be controlled by a zinc-sensitive inhibitor, which prevents MTF-1 binding to MREs. Zinc dissociates the inhibitor from MTF-1, thereby promoting transcription of metallothionein. MTF-1 is important in the regulation of a number of genes that play a role in cellular response to various stresses (Coyle & Phicox, 2002). Figure 23 illustrates a model of the complex interactions controlling the expression of metallothionein.

4. Zinc and Inflammation

There is no single factor regulating metallothionein synthesis in inflammation; instead, a complex interrelationship exists between factors that, in combination and in different tissues, act synergistically on metallothionein gene transcription. Nucleotide sequences other than MREs respond to glucocorticoids, interleukin-6 (IL-6), phorbol esters, and hydrogen peroxide. Many of the acute-phase proteins appear to be regulated by combinations of the same factors that include catecholamines and glucocorticoids as well as the cytokines IL-6, IL-1, TNF-α, and γ-interferon. Unlike other acute-phase proteins, metallothionein induction by inflammatory mediators has been found to be conditional upon the presence of zinc. Reactive oxygen species generated during the inflammatory response may induce metallothionein through multiple pathways, including directly stimulating an antioxidant response element and specific MREs in the promoter region as well as by events associated with various second-messenger protein kinase pathways.

5. Zinc, Insulin, and Diabetes

Several forms of disordered glucose metabolism are collectively referred to as diabetes. Although all diabetes mellitus (mellitus = "sweet as honey") syndromes have some degree of hyperglycemia in common, this is a symptom rather than the metabolic error itself. In insulin-dependent diabetes mellitus (IDDM), there is a destruction of the beta cells of the islets of Langerhans in the pancreas, most often on an autoimmune basis, which results in no insulin being produced. Without insulin, muscle, fat, and liver cells cannot transport glucose from the blood to the intracellular space. Intracellular starvation ensues, with fats becoming the primary intracellular energy source. This form of energy generation results in the production of ketone bodies and organic acids, primarily acetoacetic and beta hydroxybutyric acids, resulting in the development of severe metabolic acidosis (Chausmer, 1998).

With non-insulin-dependent diabetes mellitus (NIDDM), the pancreatic islet cells are capable of producing large quantities of insulin, at least at the beginning of the disease. In the healthy individual, insulin binds to a cell membrane receptor and, through several pathways, results in the transport of glucose across the

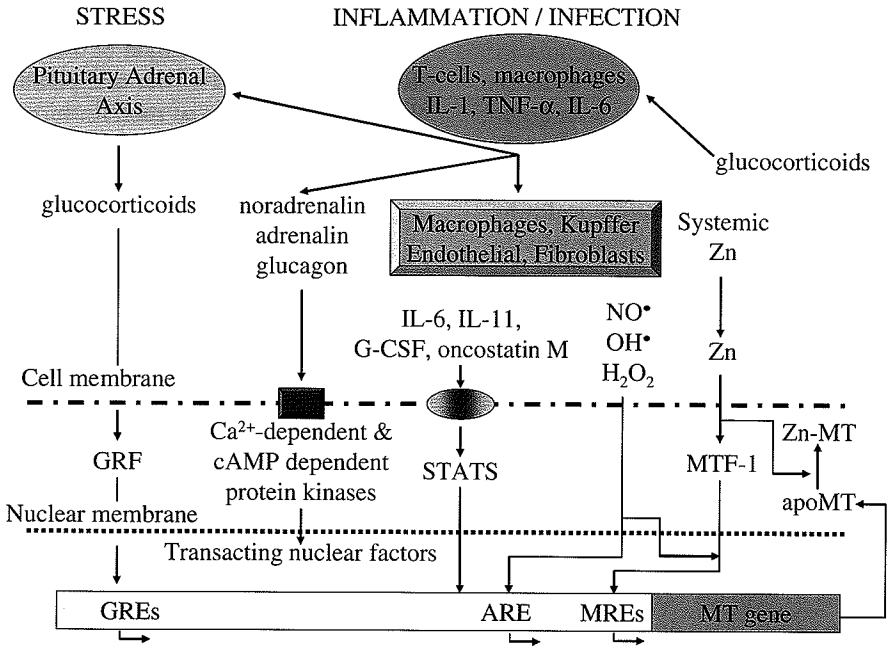

FIGURE 23 A model for the complex interactions both extra- and intracellularly controlling the expressions of MT. (Adapted from Coyle *et al.*, 2002.)

membrane. The intracellular events associated with the activation of glucose transport, after the signal from the insulin–receptor complex is received, are referred to as postreceptor events. To a great extent, it is the failure of the postreceptor events that results in hyperglycemia. In response to the hyperglycemia, the pancreatic islets produce greater and greater quantities of insulin, resulting in downregulation of the number of insulin receptors on the cell membrane and compounding the problem. This results in both hyperglycemia and hyperinsulinemia (Chausmer, 1998).

There is an intriguing relation between zinc and insulin regarding the storage of insulin in granulae of the pancreatic islets. Although insulin circulates in the blood and binds to its receptor as a monomer, it forms dimers at micromolar concentrations, and in the presence of zinc ions it further assembles to hexamers. The insulin monomer itself consists of two chains: an A chain of 21 amino acids and a B chain of 30 amino acids. Insulin is synthesized in the β cells of the islets of Langerhans. The β cells are characterized by two features associated with cells that export proteins: rough endoplasmatic reticular surfaces and well-defined storage vesicles. In this case, the vesicle typically contains microcrystals of packaged insulin. The presence of zinc is crucial for the stability of this storage system. On release into the blood, the insulin microcrystals experience a change of pH from about 5.5 to 7.4. This causes the hexamers and therefore the crystal to disintegrate rapidly (Dodson & Steiner, 1998).

The predominant effect on zinc homeostasis of diabetes is hypozincemia, which may be the result of hyperzincuria or decreased gastrointestinal absorption of zinc or both. Whereas the evidence for increased zinc excretion is uniform, the data supporting decreased absorption of zinc are less clear cut. It appears that hyperzincuria is more a result of hyperglycemia than of any specific effect of endogenous or exogenous insulin on the renal tubule (Chausmer, 1998).

The zinc–metallothionein complex in the islet cells may provide protection against radicals produced in the cell from any cause, and certainly the immune-mediated, cytokine-provoked oxidative stress would be a significant oxidative stress. The more depleted the intracellular zinc stores, the less able the cell is to defend itself against this oxidative load. This provides a potential mechanism for zinc deficiency to affect the progress of IDDM. With NIDDM, there is no good evidence for oxidative stress as a major factor in the development of either insulin deficiency or islet cell damage; however, there is clear evidence for increased secretion of insulin, at least early in the progress of the disease. Because zinc leaves the cell with insulin, the greater secretion of insulin causes depletion of zinc. The cell

can make more insulin, but it cannot make more zinc. With hypozincuria and decreased retention, the zinc is more likely to be excreted and not available for reuptake into the cellular pool. Zinc deficiency may therefore negatively affect the progress of NIDDM (Chausmer, 1998).

6. Zinc and the Immune System

It is by now well recognized that nutritional factors are important for the function of the immune system. In the case of zinc, the situation currently of interest is its deficiency and subsequent adverse effects on immune functions. Studies in young adult mice have shown greatly depressed responses to both T-lymphocyte-dependent and -independent antigens. Both primary and secondary antibody responses have been reported to be lowered in zinc-deficient mice. Declines in *in vivo*-generated cytotoxic T-killer activity as well as decreased natural killer (NK) cells have been reported in zinc-deficient mice. All these effects of zinc deficiency on immune functions in mice can be reversed with zinc supplementation (Dardenne, 2002).

Malabsorption of zinc occurs in the hereditary disease acrodermatitis enteropathica, in which patients experience thymic atrophy, anergy, reduced lymphocyte proliferative response to mitogens, selective decrease in $T4^+$ helper cells, and deficient thymic hormone activity. All of these symptoms may be corrected with zinc supplementation. Less severe cellular immune defects have been reported in patients who become zinc-deficient while receiving total parenteral nutrition. Controversial and partly contradictory results have been obtained when zinc intakes were high. In experimental models, high-zinc diets have been shown to reinforce immune functions above basal levels. Other studies have demonstrated the adverse effects of zinc excess; therefore, caution should be exercised when taking large zinc supplements for prolonged periods of time (Dardenne, 2002).

Several possible hypotheses can be offered regarding the mode of action of zinc on immune function. Zinc may be necessary for the activity of some immune system mediators such as thymulin, a nonapeptidic hormone secreted by thymic epithelial cells that requires the presence of zinc for its activity. This peptide promotes T-lymphocyte maturation, cytotoxicity, and IL-2 production. Thymulin activity in both animals and humans is dependent on plasma zinc concentrations. Zinc could also be critical for the activity of some cytokines; for example, it has been demonstrated that the production or activity of IL-1, IL-2, IL-3, IL-4, IL-6, IFN-γ, and TNF-α are affected by zinc deficiency. Zinc could contribute to membrane stabilization by acting at the cytoskeletal level. Additionally, zinc is a major intracellular regulator of lymphocyte apoptosis. Thus, it is becoming evident that the thymic atrophy and lymphopenia that accompany zinc deficiency are primarily due to an alteration in the production of lymphocytes and the loss of precursor cells via an apoptotic mechanism (Dardenne, 2002).

G. Selenium and Iodine: Young and Old Trace Elements

These trace elements are found in the non-metal area of the periodic table. Although they are neighbors, significant differences exist in their chemical behavior. Selenium is a nonmetal with semiconductor properties, and iodine is a halogen. The biological history of iodine can be traced back to the beginning of the nineteenth century when a physician named Jean-Francois Coindet (1821) used various iodine solutions to treat goiter. Another halogen, although more reactive than iodine, fluorine was also quite early connected to goiter. It was shown that feeding a dog with sodium fluoride caused goiter to appear (Maumené, 1854). The human essentiality of iodine was established in 1850.

The scientific community's appreciation of the trace element selenium has more or less undergone a metamorphosis. The toxic effects of selenium were first discovered in the 1930s when livestock ate certain plants with unfortunate results. This problem, mistakenly called "alkali disease," occurred in an acute form following the consumption by range animals of some wild vetches of the genus *Astragalus*, which accumulated toxic amounts of selenium from the soil (Moxon, 1937). An historical aside is that it is thought that General Custer might have survived his trip to the Little Bighorn if reinforcements had not been delayed by pack animals that were apparently suffering from selenium-induced lameness. In 1943, selenium was even considered to be a carcinogenic element (Nelson, 1943). It was some years before selenium was recognized as an essential trace element (Schwartz & Foltz, 1957). In the late 1960s, research suggested an anticarcinogenic effect of selenium (Shamberger & Frost, 1969).

1. Iodine Biochemistry

The thyroid, the largest endocrine gland in the body, is located in the neck. The normal gland consists of two lobes connected by a narrow isthmus and is composed of numerous functional units called *follicles*. Only one

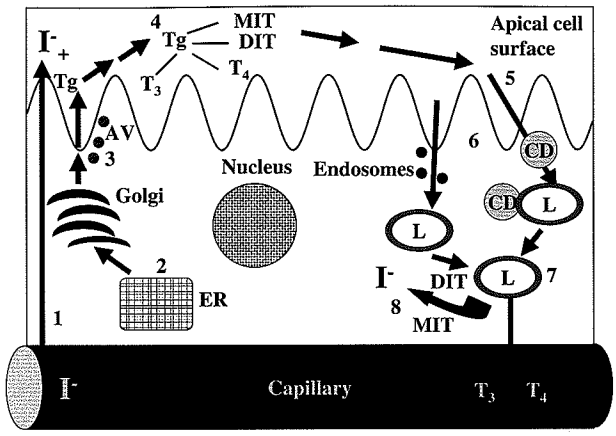

FIGURE 24 An overview of iodine metabolism.

clearly established function has been demonstrated: to synthesize its hormones, thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3). Iodine is an integral part of T_4 and T_3 that contributes 65 and 59% to their respective molecular sizes (Dunn & Dunn, 2001). Figure 24 provides an overview of iodine metabolism, which includes thyroglobulin (Tg) iodination, hormone formation, hormone release, and factors that influence these processes. Details regarding iodine metabolism have been known for some time; however, new information still appears quite often in the literature. Of special interest, of course, are the cell trafficking and regulatory aspects.

Iodine provides the raw material for hormone synthesis. In order to accomplish this function, iodine must be transported across the basal membrane of the thyroid by the sodium iodide symporter (Shen *et al.*, 2001). The major factors involved in this process are thyroperoxidase (TPO), hydrogen peroxide, nicotinamide adenine dinucleotide phosphate (NADPH), pendrin, cell-trafficking proteins (the molecular chaperones), and Tg itself (Dunn & Dunn, 2001).

Iodine is ingested in a variety of chemical forms. Most ingested iodine is reduced in the gastrointestinal tract and absorbed almost completely. Some iodine-containing compounds (*e.g.*, thyroid hormones) are absorbed intact. Iodate, widely used in many countries as an additive to salt, is rapidly reduced to iodide and completely absorbed. Once in the circulation, iodide is removed by the thyroid gland and the kidney. The thyroid selectively concentrates iodide (see above and Figure 24) in amounts required for adequate thyroid hormone synthesis. Most of the remaining iodine is excreted in the urine. Several other tissues can also concentrate iodine, including salivary glands, breast tissue, choroid plexus, and gastric mucosa. Other than the lactating breast, these are minor pathways of uncertain significance.

The NIS sodium iodide symporter in the thyroidal basal membrane is responsible for iodine concentration. It transfers iodide from the circulation into the thyroid gland at a concentration gradient of about 20 to 50 times that of the plasma to ensure that the thyroid gland obtains adequate amounts of iodine for hormone synthesis.

Iodine in the thyroid gland participates in a complex series of reactions to produce thyroid hormones. Thyroglobulin, a large glucoprotein weighing 666 kDa, is synthesized within the thyroid cell and serves as a vehicle for iodination. Iodide and Tg meet at the apical surface of the thyroid cell. There, TPO and H_2O_2 promote the oxidation of the iodide and its simultaneous attachment to tyrosyl residues within the Tg molecule to produce the hormone precursors diiodotyrosine and monoiodotyrosine. Thyroperoxidase further catalyzes the intramolecular coupling of two molecules of diiodotyrosine to produce T_4. A similar coupling of one monoiodotyrosine and one diiodotyrosine molecule produces T_3. Mature iodinated Tg is stored extracellularly in the lumen of thyroid follicles, with each consisting of a central space rimmed by the apical membranes of thyrocytes (Dunn & Dunn, 2001).

Thyroglobulin, which contains the thyroid hormones, is stored in the follicular lumen until needed. The endosomal and lysosomal proteases digest Tg and release the hormones into the circulation. About two-thirds of Tg iodine is in the form of the inactive precursors monoiodotyrosine and diiodotyrosine. The iodine is not released in the circulation but instead is removed from the tyrosine moiety by a specific deiodinase (see below) and then recycled within the thyroid gland. This process is an important mechanism for iodine conservation, and individuals with impaired or genetically absent deiodinase activity risk iodine deficiency.

Once in the circulation, T_4 and T_3 rapidly attach to several binding proteins synthesized in the liver, including thyroxine-binding globulin, transthyretin, and albumin. The bound hormone then migrates to target tissues where T_4 is deiodinated to T_3, which is the metabolically active form. The responsible deiodinase contains selenium, and selenium deficiency may impair T_4 conversion and hormone action. The iodine of T_4 returns to the serum iodine pool and follows the cycle of iodine again or is excreted in the urine.

Thyroid-stimulating hormone (TSH) is the major regulator of thyroid function. The pituitary secretes this protein hormone, which has a molecular weight of

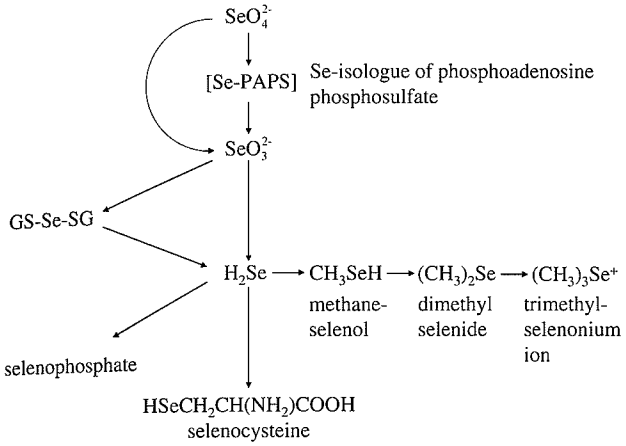

FIGURE 25 The assimilation of selenate.

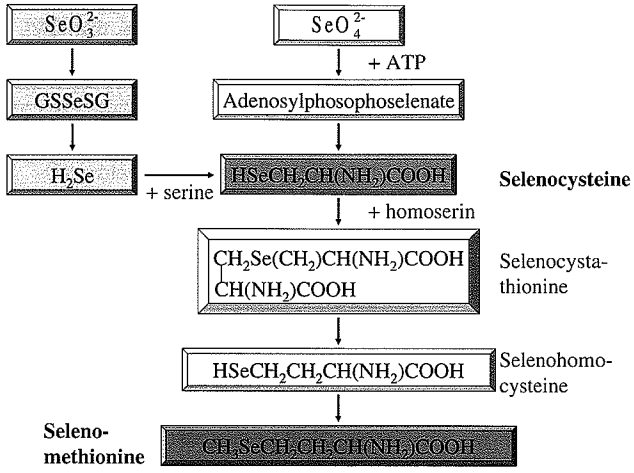

FIGURE 26 The major pathway of selenomethionine synthesis by plants, marine algae, and yeast.

about 28 kDa, in response to circulating concentrations of thyroid hormone: TSH secretion increases when levels of circulating thyroid hormone decrease. TSH affects several sites within the thyrocyte. The principal actions are to increase thyroidal uptake of iodine and to break down Tg in order to release thyroid hormone into the circulation. An elevated serum TSH concentration indicates primary hypothyroidism and a decrease in TSH concentration reflects hyperthyroidism.

2. *Selenium Biochemistry*

Selenium is primarily taken up from the soil by plants as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}). The assimilation of selenate appears to follow the sulfate reduction pathway common to higher plants (Figure 25). Analogous to sulfur metabolism, selenate is presumed to be activated by ATP sulfurylase to adenosine phosphoselenate, which then is reduced to selenite. Selenite reacts nonenzymatically with glutathione to form selenodiglutathione, which is readily reduced to selenide by flavine-dependent disulfide reductases, such as glutathione or thioredoxin reductase. In mammals, selenite may also be directly reduced by thioredoxin reductase. Selenide is the central metabolite in the utilization and excretion of selenium. It serves as a substrate for the biosynthesis of selenocysteine by cystein synthases, and it is transformed into selenophosphate, which is required for selenoprotein biosynthesis. Alternatively, it can be methylated by *S*-adenosylmethionine-dependent methyl transferases, which leads to excretion or volatilization of selenium (Ellis & Salt, 2003).

Cereals and forage crops convert selenium into mainly selenomethionine and incorporate it into protein in place of methionine because $tRNA^{Met}$ does not discriminate between methionine and selenomethionine. The major pathway of selenomethionine synthesis by plants, marine algae, and yeast is shown in Figure 26. There are some doubts, however, that marine algae can reduce selenate. It is also unclear whether higher animals can make use of selenate without the support of intestinal flora (Birringer *et al.*, 2002). Selenomethionine is not required for growth by plants but is produced along with methionine in quantities depending on the amount of selenium available (Schrauzer, 2000). Practically all small organic selenium compounds in plants, bacteria, yeast, and animals are the isologs of corresponding sulfur compounds (Table XIV). Most of the known selenoproteins contain one or more selenocysteine residues integrated into the main polypeptide chain. The incorporation of selenocysteine is a cotranslational process that makes use of the microsomal machinery of protein synthesis; however, the process is unique in several respects (Birringer *et al.*, 2002).

An intriguing phenomenon, usually referred to as the hierarchy of selenoproteins, has been observed in mammals. It recognizes that the individual selenoproteins respond differently to selenium availability when it is limiting (Birringer *et al.*, 2002).

3. *Selenoproteins*

The selenium-containing proteins identified thus far can be divided into three groups: proteins into which selenium is incorporated nonspecifically, specific selenium-binding proteins, and specific proteins that contain selenium in the form of genetically encoded selenocysteine and that have been defined as seleno-

TABLE XIV. Distribution (%) of Inorganic and Organic Selenium Compounds in Garlic and Yeast Extracts, Determined by HPLC-ICP-MS

Compound	*Garlic (296 mg kg^{-1} Se)*	*Yeast (1922 mg kg^{-1} Se)*
Selenate	2	ND
Selenite	ND	1
Selenocystine	0.5	0.5
Selenocystathione	0.5	1
Se-methylated selenocysteine	3	0.5
γ-Glutamyl-Se—methylselenocysteine	73	0.5
Selenomethionine	13	85
γ-Glutamylselenomethionine	4	ND
Se-Adenosylselenohomocysteine	ND	3
Selenolanthionine	ND	1.5
Total selenium (%)	96	93

Note: ND = not determined.
Source: From Kotrebai, M. *et al.*, *Analyst*, 125, 71–78, 2000.

proteins. In addition, there are proteins in which selenium has been demonstrated but for which no information regarding function is yet available (Behne & Kyriakopoulos, 2001).

Although there are intriguing differences between the selenoproteins found in prokaryotes and eukaryotes, especially mammals (Tables XV and XVI), this chapter deals exclusively with mammalian proteins in this context. In fact, at present it seems that selenophosphate synthetase is the only protein belonging both to prokarya and eukarya.

Selenium exerts its biological function through certain proteins containing the element. In this situation, it is in the form of covalently bound selenocysteine. The incorporation of selenium into these proteins requires a set of specific factors. Among others, incorporation of sulfur instead of selenium must be prevented. These elements share similar chemical and physical properties, and sulfur is much more abundant in the biosphere than selenium.

All selenoproteins identified thus far are enzymes in which the selenocysteine residues are responsible for their catalytic functions. Their metabolic importance is based on the fact that in contrast to the thiol in cysteine-containing enzymes, the selenol is fully ionized at normal physiological pH and that under comparable conditions it is of much higher reactivity than the thiol group (Behne & Kyriakopoulos, 2001).

4. Glutathione Peroxidases

Glutathione peroxidases (GSH-Px) catalyze the reduction of hydrogen peroxide and organic hydroperoxides. This is an important component of the cellular defense against reactive oxygen species. As can be expected, glutathione usually serves as the electron donor; however, in some cases other thiols are oxidized to fulfill a specific biological function. At present, four selenocysteine-containing GSH-Pxs have been identified (Table XVI):

- **Cytosolic or classical glutathione peroxidase**—As the name indicates, it is found in the cytosol of cells. It is present in almost all tissues but is not homogeneously distributed. Cytosolic GSH-Px consists of four identical selenocysteine-containing subunits of about 22 kDa. It catalyzes the reduction of hydrogen peroxide and various soluble organic peroxides. In this way, it contributes to the antioxidant defense against reactive molecules and complements the effects of vitamin E. This system seems to be quite insensitive to low activities of cGSH-Px, and it seems that the protective effects of cGSH-Px are of particular importance when the system is exposed to additional stress factors (Behne & Kyriakopoulos, 2001).
- **Gastrointestinal glutathione peroxidase**—This enzyme is thought to protect mammals from the toxicity of ingested lipid hydroperoxides. It is similar to cGSH-Px in that it is a cytosolic enzyme consisting of

TABLE XV. Selenoproteins in Prokaryotes

Protein	*Function*
Glycine reductase	Formation of a selenoether
Glycine/sarcosine/betaine reductase	Redox function
Glycine reductase selenoprotein B	Formation of a selenoether
Sarcosine reductase selenoprotein B	Formation of a selenoether
Betaine reductase selenoprotein B	Formation of a selenoether
Proline reductase	Redox function, formation of a selenoether
Heterosulfide reductase	Redox function
Selenoperoxiredoxin	Redox function (peroxidase)
Putative redox active selenoprotein	Redox function
Formate dehydrogenase	Hydrogen donor
Formylmethanofuran dehydrogenase	Redox function
NiFeSe-hydrogenase	Hydrogen donor
F420 nonreducing hydrogenase	Redox function
F420 reducing hydrogenase	Redox function
Selenophosphate synthetase	Selenoprotein synthesis
CO dehydrogenase	Formation of a carbon oxide selenide
Nicotinic acid hydroxylase	Unknown
Xanthine dehydrogenase	Unknown

Source: Data from Köhrle *et al.* (2000) and Birringer *et al.* (2002).

TABLE XVI. Mammalian Selenoproteins

Glutathione peroxidases (GSH-Px)
- Cytosolic or classical GSH-Px (cGSH-Px, GSH-Px-1)
- Gastrointestinal GSH-Px (GI-GSH-Px, GSH-Px-GI, GSH-Px-2)
- Plasma GSH-Px (pGSH-Px, GSH-Px-3)
- Phospholipid hydroperoxide GSH-Px (PhGSH-Px, GSH-Px-4)

Iodothyronine deiodinases
- 5′-deiodinase, type I (5′DI)
- 5′-deiodinase, type II (5′DII)
- 5-deiodinase, type III (5-DIII)

Thioredoxin reductases
- Thioredoxin reductase (TrxR)
- Testicular thioredoxin reductase (TrxR-2)
- Mitochondrial thioredoxin reductase (TrxR-3)
- Thioredoxin reductase homologues

Selenophosphate synthetase-2

Functionally undefined
- 15-kDa selenoprotein of T cells
- Selenoprotein P10
- Selenoprotein P12
- Selenoprotein W
- Selenoprotein R
- Selenoprotein T
- Selenoprotein X
- Selenoprotein N

Source: Data from Köhrle *et al.* (2000) and Birringer *et al.* (2002).

four identical selenocysteine-containing subunits weighing slightly below 22 kDa. In animal studies, selenium deficiency decreases the enzyme activity; however, no effect of human gastrointestinal (GI) GSH-Px has been reported. Gastrointestinal glutathione peroxidase is the most important selenoprotein antioxidant in the colon. Oxidative stress is a critical event in tumorigenesis. It is therefore likely that the antioxidant function of GI-GSH-Px is important in the early defense against colon cancer (Brown & Arthur, 2001).

• **Phospholipid hydroperoxide glutathione peroxidase**—This protein has been shown to have a membrane-associated as well as a cytosolic location. It is responsible for the reductive destruction of lipid hydroperoxides, and it was the second mammalian selenoenzyme to be discovered. The enzyme is a monomer of 19.7 kDa. The activity of the enzyme is preserved in preference to cGSH-Px when dietary selenium is low. It reacts with phospholipid hydroperoxides as well as small soluble hydroperoxides. It is also capable of metabolizing cholesterol and cholesterol ester hydroperoxides in oxidized low-density lipoprotein. Consequently, it is well recognized as being essential to the destruction of fatty acid hydroperoxides, which, if not reduced to hydroxyl fatty acids, will lead to uncontrolled radical chain reactions that are deleterious to the integrity of membranes (Brown & Arthur, 2001). It has also been suggested that the enzyme may have important functions in the redox regulation of a variety of processes, such as inflammation and apoptosis, although it is not known to what extent the other GSH-Pxs are involved in these reactions (Behne & Kyriakopoulos, 2001). Of note is that a significant role in spermatogenesis is exclusively fulfilled by this enzyme.

• **Plasma glutathione peroxidase**—Extracellular glutathione peroxidase (pGSH-Px) is another protein with antioxidant potential. Again, we are confronted with a tetrameric GSH-Px with subunits of about 23 kDa. The significant difference between pGSH-Px and cGSH-Px, as well as GI-GSH-Px, is that pGSH-Px is

a glucoprotein and is present in extracellular fluids. It is secreted into the extracellular fluids from tissues where it is expressed. The kidney is the main site of production for this enzyme. Similar to other tetrameric GSH-Pxs, pGSH-Px catalyzes the reduction of hydrogen peroxide and various organic peroxides when glutathione is used as a substrate. The biological function of this enzyme still has not been unveiled.

5. Thioredoxin Reductases

Thioredoxin reductases are a recently identified family of selenoproteins that catalyze the NADPH-dependent reduction of thioredoxin and therefore play a regulatory role in its metabolic activity. This is a family of homodimeric flavoenzymes present in various tissues. In addition to the flavin and the active site of the prokaryotic homologs with their redox-active disulfide, they also contain selenocysteine as the penultimate C-terminal amino acid residue, which is indispensable for their enzymatic activity (Gromer *et al.*, 1998). A description of the thioredoxin reductases identified so far follows:

- **Thioredoxin reductase 1**—TrxR1 was the first thioredoxin reductase to be identified. It is a dimer with two identical 50-kDa subunits. It is a ubiquitous cytosolic enzyme in contrast to the other types.
- **Thioredoxin reductase 2**—The second thioredoxin reductase to be discovered, it was named mitochondrial thioredoxin reductase 2 (TrxR2). The biological role of TrxR2 in the mitochondria is not known, but it seems to be involved primarily in the protection against mitochondria-mediated oxidative stress (Behne & Kyriakopoulos, 2001).
- **Thioredoxin reductase 3**—A third selenocysteine-containing thioredoxin reductase, here named thioredoxin reductase 3 (TrxR3), is preferentially expressed in the testes. The deduced sequence of the human enzyme shows 70% identity to that of TrxR1. It contains a long N-terminal extension and has a higher molecular mass (about 65 kDa) than the two other isozymes (Behne & Kyriakopoulos, 2001).

6. Selenophosphate Synthetase 2

Selenophosphate synthetase catalyzes the reaction of selenide with AMP. The product, selenophosphate, acts as the selenium donor for the biosynthesis of selenocysteine. In addition to selenophosphate synthetase 1, which contains threonine in its active center, a selenocysteine-containing homolog of about 50 kDa has also been identified in various human and mouse tissues. The detection of a selenoenzyme that is involved in the production of the selenoproteins is of special interest with regard to regulation of mammalian selenium metabolism (Behne & Kyriakopoulos, 2001).

7. Iodothyronine Deiodinases

Comparable to the prokaryotic and the methanococcus world, most of the eukaryotic selenocysteine-containing proteins with identified function are also involved in redox reactions (Köhrle, 1999). The deiodinases were discovered in the 1990s. The iodothyronine deiodinases are a large group of selenoproteins. Three iodothyronine deiodinases regulate the conversion of thyroxine (T_4) to 3,3′,5-triiodothyronine (T_3): the active thyroid hormone or reverse triiodothyronine (rT_3), the inactive thyroid hormone. Type I deiodinase (IDI) is expressed in liver, kidney, brain, pituitary, and brown adipose tissue of ruminants. Type II deiodinase (IDII) has been present in the brain and pituitary of all species examined thus far and in brown adipose tissue of humans. IDII catalyzes conversion of T_4 to T_3 within tissues that cannot utilize circulating T3. Type III deiodinase (IDIII) converts T_4 to rT_3 and T_3 to diiodothyronine; is found in brain, skin, and placenta; and functions to deactivate thyroid hormones. The role of selenium in iodothyronine deiodinases implies that some of the consequences of selenium deficiency may be directly attributed to disturbances in thyroid hormone metabolism. The type I enzyme deiodinates 4′-*O*-sulfates of T_4 and T_3 at the tyrosyl ring by 5-deiodination. The type II enzyme acts as a heterotrimeric complex of about 200 kDa containing a 29-kDa subunit that interacts with filamentous actin (Köhrle, 2000). In contrast to IDI, IDII is strictly a phenolic-ring 5′-deiodinase that, as a substrate, prefers T_4 to rT_3. The type III enzyme inactivates T_4 and its metabolites by removal of iodine atoms at the tyrosyl ring. The holoenzyme structure of this enzyme, containing a 32-kDa substrate binding subunit, is not yet known (Köhrle, 2000).

8. Selenoprotein P

The existence of selenoprotein P was reported in 1973 (Burk, 1973); however, it was not until ten years later that it was recognized as a selenoprotein. The P stands for its presence in blood plasma. Selenoprotein P has been purified from humans and rats by a procedure that includes immunoaffinity chromatography. Six typical glycosylation sites in the deduced amino acid sequence of the human proteins have been detected; however, no characterizations of the bound carbohydrates has been reported. Selenoprotein P is the first and so far the only protein described to contain more than one selenium atom per polypeptide chain. Ten selenocysteine residues were predicted (Persson Moschos, 2000). Selenopro-

teins with known enzymatic activity are redox enzymes that contain selenocysteine in their active sites. A key issue concerning selenoprotein P that remains to be revealed is its catalytic function. It has been suggested that it acts as an extracellular oxidant defense or as a transport protein. It seems less likely to be a transporting protein because the selenium is covalently bound in the protein (Persson Moschos, 2000). It is currently unknown whether selenoprotein P is able to react with certain phospholipid hydroperoxides under physiological conditions (Saito & Takahashi, 2000). Recent work suggests that selenoprotein P in plasma diminishes the oxidizing and nitrating reactivity of peroxynitrite, a reactive intermediate formed by the reaction of nitrogen monoxide and superoxide anion. Due to the association with endothelial membranes, it has been speculated that endothelial cells are protected against peroxynitrite toxicity by selenoprotein P (Saito & Takahashi, 2000).

9. Selenoprotein W

Selenoprotein W contains both a selenocysteine residue that is encoded by a UGA codon in the open reading frame of the mRNA as well as a bound glutathione molecule. The protein is localized predominantly in the cytoplasm (Whanger, 2000; Dae-won *et al.*, 2002); however, a small portion of the total selenoprotein W is associated with the cell membrane. Selenoprotein W was expressed in all tissues examined in selenium-supplemented animals including muscle, heart, testis, spleen, kidney, intestine, tongue, brain, lung, and liver (Gu *et al.*, 1999). The loss of the protective effect of selenoprotein W against hydrogen-peroxide-induced cytotoxicity in cells treated with an inhibitor of glutathione synthesis indicates that the protein is a glutathione-dependent antioxidant *in vivo* (Dae-won *et al.*, 2002). Glutathione and its redox cycle play a critical role in catabolizing hydrogen peroxide and other peroxides through enzymatic coupling reactions. Additionally, glutathione is important for the detoxification of electrophiles and for protection of the thiol groups from oxidation. Glutathione is also required for regeneration of the glutathione peroxidase and glutaredoxin system (Dae-won *et al.*, 2002).

10. Selenoprotein R

Selenoprotein R is a small protein with a molecular mass of about 12 kDa. It contains selenocysteine. Homologs of this protein have been identified in bacteria, archaea, and eukaryotes, and, with the exception of vertebrate selenoprotein R, all homologs contain cysteine in place of selenocysteine (Kryukov *et al.*, 2002). Bioinformatic analyses have suggested a functional linkage of selenoprotein R to a pathway of methionine sulfoxide reduction as well as a role of selenoprotein R in protection against oxidative stress and/or redox regulation of cellular processes (Kryukov *et al.*, 2002). Methionine sulfoxide reduction is an important process by which cells regulate biological processes and cope with oxidative stress (Hoshi & Heinemann, 2001). Methionine sulfoxide reductase is a protein that has been known for decades. It is involved in the reduction of methionine sulfoxides in proteins. It has been shown that methionine sulfoxide reductase is only specific for methionine-*S*-sulfoxides. The fact that oxidized methionines occur in a mixture of R and S isomers *in vivo* raised the question of how methionine sulfoxide reductase could be responsible for the reduction of all protein methionine sulfoxides. The study of Kryukov and coworkers (2002) explained this puzzle. It appeared that a second methionine sulfoxide reductase exists specific for methionine-*R*-sulfoxides. This reductase was selenoprotein R, and, in addition, these researchers showed that it contains zinc.

11. Selenium and Cancer

Chemoprevention is a recently introduced and strongly growing area within oncology. The number of studies of chemoprevention has increased drastically during recent years. The reason for this is, of course, that the possibility to prevent or hinder cancer is generally attractive. A great number of agents have been tested for prevention of different forms of cancer (Decensi & Costa, 2000). Tamoxifen has been used as a chemoprevention agent against breast cancer as well as raloxifen and synthetic retinoides and combinations. Several nonsteroidal antiinflammatory preparations have been used against colorectal cancer, as have cyclooxygenase-2 and prostanoides. Even calcium has been used in this way, as well as α-difluoromethylornithine, which is an irreversible inhibitor of the enzyme ornithine decarboxylase; also, beta-carotene and retinol have been used against lung cancer.

The first intervention study of prevention of human cancer with selenium was performed in Qidong, an area north of Shanghai with a high incidence of primary liver cancer. In an urban area, 20,847 inhabitants received table salt supplemented with 15 ppm of selenium as sodium selenite. Those individuals were thus supplemented with about 30 to 50 μg of selenium per day over the course of 8 years. Supplementation resulted in a decrease of the incidence of primary liver cancer to 27.2 per 100,000 inhabitants, while it remained at 50.4 per 100,000 inhabitants in four surrounding areas. When selenium was no longer added to the table salt, the inci-

dence began to increase (Yu *et al.*, 1991, 1997). In another trial, 2474 members of families with a high risk of primary liver cancer were administered 200 μg of selenium as yeast with a high concentration of selenium or placebo. During the 2-year trial, 1.26% of the controls developed primary liver cancer in contrast to 0.69% in the treated group ($p < 0.05$). Out of 226 bearers of HBVsAg, 7 of 113 individuals in the placebo group developed primary liver cancer, while none of the 113 individuals in the treated group developed cancer during the same period ($p < 0.05$).

The effects of supplementation of vitamins and trace elements on cancer incidence and mortality were investigated in an intervention study in Linxian, China (Li *et al.*, 1993). This area is known to have the highest mortalities of esophagus and ventricular cancer. Among those who received supplementation with beta-carotene, vitamin E, and selenium, total mortality was reduced by 9% and cancer mortality by 13%. Mortality due to ventricular cancer decreased significantly (20%), while mortality in other forms of cancer decreased by 19%.

Within this context, prevention of skin cancer assumes a prominent position, although the outcome of chemoprevention has not always been positive. Two excellently designed studies evaluated the preventive effects of selenium or retinol on skin cancer (Clark *et al.*, 1996; Moon *et al.*, 1997). The selenium study (Clark *et al.*, 1996) showed negative results for the prevention of squamous cell cancer and basilioma. This study involved 1312 patients that had a history of skin cancer of the nonmelanoma type and lived in areas of the United States with a naturally low intake of selenium. The investigation, presented as a post hoc observation, showed a significant preventive effect on prostate, lung, and colon cancer. The cancer incidences were as follows: prostate cancer (selenium, $n = 13$; placebo, $n = 35$; RR (relative risk) = 0.37; 95% CI (confidence interval); 0.18–0.71; $p = 0.002$), lung cancer (selenium, $n = 17$; placebo, $n = 31$; RR = 0.54; 95% CI; 0.30–0.98; $p = 0.04$), and colorectal cancer (selenium, $n = 8$; placebo, $n = 19$; RR = 0.42; 95% CI; 0.18–0.95; $p = 0.03$). The study also showed a decreased total mortality without statistical significance, although the total mortality in cancer decreased significantly.

SEE ALSO THESE CHAPTERS

Chapter 5 (Uptake of Elements from a Chemical Point of View) · Chapter 8 (Biological Responses of Elements)

FURTHER READING

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