CHAPTER 3

METAL METABOLISM AND TOXICITIES

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INTRODUCTION

The heavy metals such as lead, cadmium, and mercury have long been known for their potential toxicities; however, there are trace elements known to have toxic effects that are also essential dietary nutrients. Several of these elements have specific deficiency symptoms and can lead to death of the organism. These same essential elements are toxic when an organism has an increased intake, whether by dietary means or other exposure routes. Trace metals such as copper, zinc, iron, and selenium are known for their deficiencies. With respect to selenium, early work focused on its toxicity, especially in the livestock industry where selenosis in specific regions of the world is likely to occur due to the naturally high selenium content of the soil. On the other hand, copper, zinc, and iron have been primarily evaluated in regard to deficiency aspects. Toxicity due to these minerals is known to occur via industrial and environmental exposures. Also, due to the propensity of many individuals to consume nutrient supplements, toxicity symptoms and specific levels known to lead to these symptoms have been of increased interest.\textsuperscript{1,3}

Toxic effects of trace elements depend on many factors: chemical form, exposure level, route of intake, and organ and subcellular storage sites within the body that are targeted. The storage site under “normal” levels of intake for the essential trace minerals often determine the type and extent of toxic effects when excess levels of the metals are acquired by the organism. Many of the essential trace elements are components of enzymes, and consequently the potential toxicities may be influenced by disturbances in the activities of these enzymes.

A potential confounding factor in the study of trace metal toxicology includes the influence of other trace elements upon the relative toxicities. For example, increased zinc intake may lead to decreased copper absorption by the gut or other organs. This leads to the question as to whether the observed toxic effects of high metal intake are due to a secondary influence of the lack of another mineral or to a high level of the metal per se. Exposure of organisms to large intakes of some of the macroelements may lead to a decreased uptake of some of the trace metals, and other dietary components may influence their relative toxicities. For instance, excess calcium, fiber, and fat may decrease the bioavailability of the metals for absorption and could theoretically lessen potential toxicities if excess levels of these other nutrients are consumed.

Toxicities of these elements may also be related to an inability to eliminate these trace elements or to regulate their absorption from the diet. Genetic disorders of metabolism may lead to a build-up of some metals in specific organs, such as what happens with liver copper levels in patients afflicted with Wilson’s disease.\textsuperscript{4} Similarly, iron uptake enhanced by a failure to regulate mucosal absorption is known to result in hemochromatosis. In both of these examples, “normal” levels of these minerals lead to toxicities. The heavy metals such as lead, mercury, and cadmium produce well-defined toxic effects in humans. While these elements
are not essential to the health and existence of the organism, each of these metals has specific storage sites, upon which the toxic effects they exert is in part dependent. Additionally, the chemical forms of the metal to which the organism is exposed will often determine the toxic effects. This is because the storage sites may be altered by different chemical forms. A good example of this is mercury. Methylmercury and other organic forms are highly toxic to humans, with the nervous system being a primary target due to the enhanced lipid solubility of this chemical form. This is in contrast to the lower toxicity of inorganic mercury, where most of this form is deposited in the kidney and liver. Another aspect to consider is that many of these metals are pro-oxidants, and the production of free radicals may exert their toxic influences. Recent concern with respect to iron supplementation and the potential influence upon heart disease is an illustration of this as a practical consequence. To understand the physiological forces that impact the mineral toxicities, “normal” metabolism in terms of how the body absorbs, utilizes, stores, and eliminates each of the trace metals will be considered for each metal.

2 COPPER

Copper toxicity is usually due to an imbalance between intake into and subsequent excretion from the body. Exposure of humans to copper in the environment can occur via water, food, soil, or air. Many times the chemical form of copper is copper-sulfate. Copper may exist as Cu$^{+1}$ or Cu$^{+2}$, with very small amounts as Cu$^{+3}$. Cu$^{+}$ is insoluble and often complexed. Normally it is the +2 form of copper that is found in plant and animal cells, and it is this form that is usually complexed to metalloenzymes. (See Table 1.)

2.1 METABOLISM

Copper is found in foods such as nuts, shellfish, organ meats, and legumes. Grains and grain products, as well as chocolate, have appreciable levels of copper. While these food items are good to excellent sources of copper, the absolute amount of copper absorbed may be
influenced by other dietary components. Excess dietary iron may decrease copper absorption; conversely, too much copper may cause an iron deficiency. Zinc excess may also decrease copper absorption. The mechanism by which dietary zinc inhibits the uptake of copper involves the intestinal protein metallothionein (MT), a small-molecular-weight peptide of about 3000. As dietary zinc levels increase, MT synthesis in the mucosal cells of the small intestine increases. This results in a binding and trapping of zinc in the mucosal cell. The binding is not easily dissociated and when mucosal cells are sloughed off in the normal turnover, zinc is lost through the fecal compartment. However, the MT peptide has a greater affinity for copper. Thus, when excess zinc is consumed, the increased MT production will cause copper to be sequestered in the mucosal cells.

Molybdenum excess can produce copper deficiency, in addition to its own inherent toxicity. Ruminants have been reported to have this occur. With respect to humans, vitamin C supplementation results in decreased copper status, and in rats, large doses of vitamin C can lead to copper deficiency. Other dietary components have an influence upon copper status, but not necessarily absorption. Feeding rats either sucrose or fructose, as opposed to glucose or cornstarch, decreases copper status and exacerbates the signs of copper deficiency.

Copper may be absorbed by both the stomach and small intestinal mucosa, with most absorbed by the small intestine. Active transport and passive uptake mechanisms are thought to be responsible for the absorption, with MT regulating some of the absorption as illustrated above. The average level of copper stored in the body is from 50 to 120 mg. Most of this is found in the liver bound to albumin or transcuprin, and the copper is incorporated into the liver protein ceruloplasmin. Excess copper could bind to MT, thereby acting to detoxify the actions of too much copper, which suggests that the MT role for copper homeostasis is similar to that of other trace metals. Excess copper can also lead to increased kidney levels; however, little copper is excreted via the urine. Copper excreted through the glomerulus may be reabsorbed by the kidney tubules. Copper is usually transported from the liver to other organs in the form of ceruloplasmin, a blood copper containing protein synthesized in the liver.

Most copper is excreted via the bile that is released into the gastrointestinal tract, with minimal copper reabsorbed by intestinal cells. The uptake of copper and elimination through the bile allows copper to be conserved and tightly regulated.

Copper is utilized by most cells as a component of the enzyme cytochrome C oxidase and superoxide dismutase. Copper is also a constituent of many other cuproenzymes, including lysyl oxidase, involved in crosslinking of connective tissue proteins elastin and collagen fibrils; dopamine β hydroxylase, involved in the conversion of dopamine to norepinephrine; and ceruloplasmin. It is not surprising that some influences of copper toxicity are in part due to an influence upon these enzymes.

### 2.2 Toxicity

As indicated, copper uptake is regulated at the gut level and excreted through the bile. An increase in tissue copper to toxic levels is caused by an imbalance among these two processes. Normally the first organ deleteriously affected by these increased levels is the liver; however, there appears to be species differences in terms of the relative levels of copper required to induce a toxic response. Sheep, for instance, are animals perhaps the most sensitive to copper toxicity (in terms of dietary copper), with a narrow tolerance range between deficiency and toxicity in comparison to other mammals; hemolytic crisis often occurs in these animals. On the other hand, rats appear to be the most resistant to the toxic effects of copper, being able to withstand on a per weight basis several magnitudes more copper than sheep. At 250 μg Cu per g diet, no toxicity signs have been reported. Pigs and cattle are moderately tolerant to large dosages of copper; however, goats are more similar to sheep in terms of their levels of toxicity.
Serum levels of glutamic oxaloacetic transaminase, liver-specific arginase, glutamate dehydrogenase, and sorbitol dehydrogenase are increased. Lysosomes appear to increase in relation to copper content, and during the subsequent hemolytic crisis, blood levels of methemoglobin and blood urea nitrogen increase. Release of lysosomal enzymes may contribute to the copper toxicity in the liver. Kidney tubule breakdown follows subsequent to this release.\textsuperscript{15}

While the majority of toxicity studies appear to focus on diet intake, another potential route of copper intake is through the lung. Such a route is problematic in that the regulatory systems of the gastrointestinal tract are essentially bypassed, leading to the potential for increased toxicity; inhalation of copper dust does irritate the upper airways.\textsuperscript{16,17} Copper intake through the lungs may occur via the use of copper-containing pesticide sprays. Romeo-Moreno et al.\textsuperscript{18} reported that inhalation of copper sulfate in Wistar rats was similar to injecting rats with copper sulfate in terms of copper concentration among selected organs. In both instances, the copper appeared to bind to metallothionein in both the liver and kidneys.

Hirano et al.\textsuperscript{11} reported that acute inhalation of copper oxide in rats resulted in metallothionein induction within 1/2 to 3 days after exposure. The level of MT produced was proportional to the dose. A half-time for elimination was determined to be 37 hr. Indices of lung inflammation, such as the number of macrophages, lactate dehydrogenase, and \( \beta \)-glucuronidase activities of lavage fluid, resulted in a peak within a 1/2 day after exposure and up to 3 days after, depending on the inhalation dose. Followup studies indicated that copper sulfate exposure gave results similar to exposure to copper oxide. Metallothionein probably played a limited role in the handling of the copper because most of the copper was solubilized and rapidly cleared from the lung.

With respect to the effect of copper upon liver toxicity, Sokol et al.\textsuperscript{19} suggested that mitochondria lipid peroxidation and decreased cytochrome C oxidase activity could partly explain the toxic effects. Mitochondria respiration was decreased in rats given in excess of 2000 \( \mu \)g Cu per g diet for 8 wk. Decreases in both state 3 respiration and the respiratory control ratio were reported. The authors suggested that peroxidation of mitochondria cardiolipin, which regulates cytochrome C oxidase activity, may be a factor. An inhibition of the oxidoreductase function may have resulted in the decreased state 3 respiration observed.

Copper toxicity has an apparent affect upon liver lysosomes. Copper becomes bound to the MT in the lysosomes, and normally the copper is released from the lysosomes by exocytosis to the bile. The exocytosis of the lysosomal copper is a major excretory pathway for copper elimination. The lysosomes may sequester copper, which may result in lysosomal damage via increased fragility.\textsuperscript{20-23} Lysosomal membrane fluidity increases and membrane lipid peroxidation increases, along with an increase in polyunsaturated fatty acids (PUFA) content and a decrease in saturated fatty acid (SFA) content of lysosomal membranes. The increased peroxidation is thought to account for these observations and the altered lysosomal morphology. Copper in the free intracellular form may react with hydrogen peroxide to generate hydroxyl radicals.\textsuperscript{23}

Other organ systems may be susceptible to copper toxicity. Cardiovascular disease has been the focus of much research as it relates to copper deficiency.\textsuperscript{24-26} However, excess copper also has been shown to cause cardiac dysfunction. Rabbits infused with copper sulfate up to levels of 10 mg/kg body weight had decreased contractile force, heart rate, and pulse pressure, leading eventually to shock at the highest dose.\textsuperscript{27} Liu and Ceci\textsuperscript{28} demonstrated that excess copper (100 mg/kg diet) significantly increased systolic blood pressure in Wistar and in the Spontaneously Hypertensive rat.

The effect of copper toxicity as a teratogen has been studied in cattle, pigs, and sheep without apparent effects on offspring.\textsuperscript{29} Copper injections in lab animals have been found to be teratogenic.\textsuperscript{30,31} Copper-containing intrauterine devices do not demonstrate any teratogenic effects in laboratory animals.\textsuperscript{32,33} Wilson’s disease patients experience complications during pregnancy.\textsuperscript{34,35}

Female rats fed 0.185% copper acetate in drinking water for 7 weeks and then mated demonstrated liver and renal inflammation, and the embryos had moderate growth retardation.
and differentiation, particularly with regard to the neural tube. A reduced number of ossification centers in the vertebrae, sternum, and fore and hind limb phalanges were reported.

2.3 Genetic Conditions of Copper Toxicity

Two well-known genetic diseases affecting copper metabolism should be addressed. Menkes’ kinky-hair disease, characterized and reported in the early 1960s, is a problem with copper transport or absorption. Wilson’s disease, described in the early part of the 1900s, is characterized by increased liver copper content, leading to severe hepatic damage followed by increased brain copper levels and neurological problems. Menkes’ disease, however, results in a pathology resembling copper deficiency as opposed to copper toxicity, as in the case for Wilson’s disease. Fortunately, animal models exist to study these genetic conditions. The brindled mouse is a well-researched model for Menkes’ disease. Another model, the macular mutant mouse, is also a useful model for the disease. In both of these models, the copper levels are high in the kidney and intestine and correspond to elevations in renal copper metallothionein. Shiraishi et al. demonstrated that the macular mutant mouse was more sensitive to high doses of copper, in terms of toxicity, as compared to the normal mouse. Intraperitoneal injection of copper into 6 to 8 day old macular mice at relatively high doses resulted in nearly 100% mortality within 10 days. The mortality rate for normal mice injected with 28 mg Cu per kg was 38% after 1 day, and 83% for the macular mutant mice. Heterozygote mice had an intermediate mortality rate of 47% 1 day after injection. There were no differences among the three strains of mice with respect to MT synthesis nor MT-1 mRNA levels after copper injection. The different mortalities due to copper toxicity among the three strains was not readily explained by these results.

A gene has been reported to be deleted in subjects with Menkes’ disease. The gene apparently codes for an ATPase of the P type, which involves cation transport. Copper toxicity of Wilson’s disease has several animal models. The Bedlington terrier has excess liver copper bound to lysosomal metallothionein. In both Wilson’s disease patients and in the Bedlington terrier, there may be an inability to degrade MT. There is very little copper content in the bile produced; however, Wilson’s disease patients have little ceruloplasmin, whereas the terriers appear to have normal levels. Wilson’s disease patients appear to accumulate the copper in the liver cytoplasm, in contrast to the lysosomes for the dogs, as indicated previously. As already mentioned, sheep are very sensitive to copper intake levels and toxicity, which could be due to the limited capacity of sheep to synthesize MT. Another more recently studied model is the Long-Evans Cinnamon (LEC) mutant rat, which has a single autosomal recessive mutation. Hepatitis along with jaundice and anemia are clinical outcomes, and the condition is highly lethal. Okavasu et al. reported high levels of hepatic copper compared to normal Long-Evans rats, and serum copper levels and ceruloplasmin activity were significantly lower than control Long-Evans rats at 4 months of age. However, kidney levels of copper did not increase until a much later age in the LEC rats (e.g., 12 months of age). For further discussion on copper toxicity, the reader is referred to the review of Awing and Metra.

3 Zinc

3.1 Metabolism

Most of the research concerning zinc and human health has centered on deficiency, marginal diet intakes, and the consequences of these aspects. Furthermore, zinc requirements as influenced by age, gender, health status, physical activity, and other dietary components
have been the subject of many investigations. A basic aspect, and perhaps a fundamental problem as well, is the issue that zinc is a co-factor for more than 200 enzymes, many dealing with various aspects of protein synthesis or hormone function of some type. While the studies pertaining to outright zinc deficiency, the consequences of marginal dietary zinc consumption, and health aspects often relate to the effect zinc has upon these enzymes, zinc excess may not have a direct influence upon these target enzymes and in fact could produce secondary problems. This is well documented in the previous discussion on copper and zinc antagonism. Excess diet zinc may be secondary in its ability to lead to a copper deficiency, which has much different symptoms. A challenge, therefore, is to separate out the direct effects of zinc toxicity from the indirect or secondary effects that it may have by perturbing the balance of another essential element. (See Table 2.)

In humans, most of the body’s zinc is in the skeletal muscle (about 60%) and one third in the bone. Skin, liver, brain, kidneys, and the heart have small total amounts in this regard. Zinc is intracellular in its primary distribution, and the concentration of zinc in extracellular fluids is low. These distributions are apparently age dependent. Shaw documented that liver zinc in a newborn infant was greater than for an adult man. Widdowson reported that 25% of the zinc in newborns is found in the liver. More zinc is probably also found in the bones of newborns as compared with adults.

Hormonal balance may influence the zinc distribution between extracellular and intracellular fluids. Cousins has reported that insulin, glucagon, and glucocorticoids will likely influence liver zinc levels. For example, Failla and Cousins reported earlier that glucocorticoids stimulated zinc uptake in hepatic cultured cells, and Henkin et al. also suggested this for the human liver in vivo. Glucagon may stimulate zinc uptake by liver cells. Other hormones for which zinc plays a fundamental role include growth hormones. It is well known that zinc deficiency leads to dwarfism in humans.

There are two known genetic disorders of zinc metabolism. Danbolt and Closs reported a decreased ability of zinc to be absorbed by the small intestine, thereby leading to acrodermatitis enteropathica; zinc supplements can reverse this. The familial disorder, hyperzincaemia, results in elevated serum zinc levels but apparently does not produce any toxicity. Zinc overdoses that result in similar plasma zinc levels have been known to be fatal.

Humans are able to regulate the uptake of zinc such that there is relatively little variation in body zinc in proportion to the variation in dietary zinc. If zinc intake is low, proportionately more dietary zinc is absorbed and vice versa. Jackson et al. reported that elevated dietary

**TABLE 2**

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zinc results in reduced fractional absorption of zinc but an increased rate of gastrointestinal zinc excretion.

There is a point, however, when the body is unable to respond to excess zinc intake. Excess loss of zinc may lead to more zinc deposited in hair. Excess zinc intake also may lead to increased hair zinc levels but other studies dispute this. Prior zinc status and actual diet levels most likely account for such different results. Zinc is usually bound to albumin when it is transported in the plasma; some is bound to amino acids. Zinc uptake from plasma to hepatocytes appears energy dependent. How it gets into other tissues from the plasma remains obscure.

3.2 Toxicity

With respect to human toxicity of zinc, the incidence appears infrequently with some acute toxicities reported. Much of our knowledge of zinc toxicities comes from animal studies and through supplementation with mineral preparations. Using animals that are given high diet zinc results in a large decrease in food intake, perhaps due to unpalatability. Growth rate and even weight loss follow, but this is due to the decreased food intake. However, excess zinc consumed by animals has been reported to result in rough hair, achromotrichia, emphysema, diarrhea, arthritis-like symptoms, abortive fetuses, stillbirths, etc. A microcytic anemia occurs, but this could be due to a decreased uptake of either copper or iron, both of which, if limited, can lead to this type of anemia. Hemolytic anemia is not uncommon. Renal fibrosis, fatty liver, and liver necrosis have been reported. Hypercholesterolemia sometimes occurs with excess zinc, which may be due to a copper-deficiency. Excess zinc normally would produce symptoms that mimic deficiencies of copper, iron, manganese, and/or calcium. When one or more of these elements are increased in the diet, the signs of zinc toxicity appear markedly reduced.

Acute toxic effects of zinc in humans have been reported; intakes above 15 mg/l in water can produce nausea (see below). As the level of zinc increases, vomiting and diarrhea result. Tachycardia, hemolytic anemia, pancreatitis, renal damage, and death have been reported on occasion. Acute intakes of 4 to 28 g/day have resulted in a variation of these toxic effects.

Subchronic and chronic effects have been investigated, but to a limited extent. Impaired immune response has been reported in response to excess diet zinc by Chandra. Other basic studies pertaining to chronic toxicity have focused on the decrease in high-density-lipoprotein (HDL) cholesterol reported by several laboratories and on copper deficiency as a consequence of excess zinc intake. Zinc supplements can lower HDL cholesterol levels in serum, as demonstrated by Hooper et al. Zinc levels as low as 50 mg Zn per day for 12 weeks has resulted in decreased HDL cholesterol levels. This level is often found in over-the-counter mineral supplements. Zinc supplements have been known to produce nausea and vomiting in subjects. Brown et al. documented vomiting and diarrhea when foods and beverages were contaminated by zinc from galvanized containers. When 28 g of zinc sulfate were ingested by a woman, vomiting, tachycardia, hyperglycemia, and eventually death resulted, due to pancreatic hemorrhage and renal damage. Ingestion of 12 g of zinc by an adolescent male resulted in lethargy, lightheadedness, and altered gait.

Copper deficiency is a likely result of pharmacological zinc doses in the range of 100 to 300 mg Zn per day. Low blood copper levels, anemia, and neutropenia result. Lowering zinc levels and/or increasing copper may alleviate this problem. The sensitive influence of zinc intake upon copper balance was studied by Sandstead and Festa et al. Less than 20 mg zinc per day may lead to increased fecal copper and thereby decreased copper retention in the human body. 50 mg of zinc as zinc gluconate has been shown to depress erythrocyte Cu, Zn superoxide dismutase (SOD) activity in human males. Low serum iron and copper levels leading to anemia have been reported by several groups with excess zinc intakes.
Some recent attention has been paid to the potential neurotoxicity of zinc.\textsuperscript{83} Zinc has been reported to impair the neuroexcitation of the $N$-methyl-$d$-aspartate receptors and increase the alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors.\textsuperscript{84,85} Attenuation of gamma amino butyric acid (GABA) receptors has been reported.\textsuperscript{85} Corticol neurons have been reported to be destroyed by excess zinc intake.\textsuperscript{86,87}

### 4 IRON

With regard to a physiological iron imbalance in humans, it is the deficiency state that receives the most attention. Iron-deficiency anemia is one of the most recognized nutrition-related diseases worldwide,\textsuperscript{88} however, the prevalence of hereditary-based iron overload makes it one of the most common metal-related toxicity disorders.\textsuperscript{89} (See Table 3.)

Iron is found in food and the human body in the ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) state. Iron’s oxidation-reduction properties make it ideal for participation in complex cellular systems such as mitochondrial electron transport. Likewise, it is probably iron’s oxidation-reduction potential properties that result in the pathological alterations associated with overload.

#### 4.1 METABOLISM

While there have been reports of certain occupational environments in which iron dust may be inhaled, under most situations iron is introduced into the human body by ingesting iron-containing foods. Since iron does not have a major excretory pathway, human iron
balance is regulated at the point of absorption to offset daily losses of approximately 1.0 mg for adult men and 1.5 mg for menstruating women.\textsuperscript{98}

The iron content of the Western diet has been estimated at about 7 mg of iron per 1000 kcals.\textsuperscript{91} Iron is present in foods in one of two forms: heme iron and nonheme iron. Heme iron is derived from animal sources such as meat, fish, and poultry and is part of hemoglobin, myoglobin, cytochromes, and other heme-containing molecules. Heme iron crosses the luminal membrane of enterocytes intact; once inside the cell, the iron is liberated from the protoporphyrin ring by the action of heme oxygenase.\textsuperscript{92} Nonheme iron is derived from both plant and animal sources and includes the iron complexed into ferritin, hemosiderin, and iron-containing enzymes and salts. Nonheme iron is released from food components by digestive secretions; two suggested mechanisms for the movement of ionic iron into enterocytes are (1) a facilitative transport system mediated by a 160,000-\textit{M}_r glycoprotein,\textsuperscript{93} and (2) integrin-involved movement of iron through the plasma membrane to a 56,000-\textit{M}_r protein in the intracellular compartment.\textsuperscript{94,95} Gastric and intestine luminal factors such as gastric acid, ascorbic acid, and components of meat, fish, and poultry appear to increase the efficiency of nonheme iron absorption.\textsuperscript{91} Once inside the enterocyte, iron derived from either diet form may be retained by cellular ferritin or transferred across the basolateral membrane to plasma transferrin for systemic distribution.\textsuperscript{96}

Iron absorption is directly related to physiological iron need.\textsuperscript{91} Absorption may be regulated systemically by the level of plasma transferrin receptors and the rate of erythropoiesis,\textsuperscript{97} although some evidence exists for local regulatory factors in mucosal tissues as well.\textsuperscript{98} Iron is transported in the plasma to various tissues bound to transferrin, a 79,550-\textit{M}_r glycoprotein.\textsuperscript{99} Transferrin can accommodate two atoms of iron; however, only about 30% of the iron-binding sites are occupied with iron under normal conditions.\textsuperscript{100}

The total body content of iron is about 2 to 5 g depending on gender, diet, size, and menstrual status,\textsuperscript{101} and it is distributed between either metabolic or structural and transport compartments. Iron is engaged in the metabolic operations in all human cells. Greater than 60% and up to 10% of the iron in the human body can be found in erythrocyte hemoglobin and muscle myoglobin, respectively, while other heme and nonheme enzymes contribute about 2 to 4% of body iron.\textsuperscript{101}

Iron transport as transferrin and storage in the form of ferritin and hemosiderin make up the remaining 20 to 30% of body iron.\textsuperscript{101} Iron bound to transferrin makes up a very small portion (3 to 4 mg) of this compartment. Apoferritin, a spherical 440,000-\textit{M}_r protein, contains approximately 24 subunits and can hold approximately 4500 atoms of iron.\textsuperscript{102} The primary sites of ferritin synthesis are the liver, spleen, bone marrow, and intestine. Some tissue-derived ferritin can be found in the plasma and is used to gauge body iron stores, as 1 \( \mu \text{g} \) of ferritin/1 of serum equals 10 mg of iron stores.\textsuperscript{103}

The other iron storage protein is hemosiderin, which may be derived from ferritin. The ferritin:hemosiderin ratio in the liver is believed to reflect iron storage, as the ratio increases with decreasing cellular iron content and vice versa.\textsuperscript{89,91}

### 4.2 Iron Overload

Iron toxicity has been reported in both humans and animals. Human leukocyte antigen (HLA)-linked hemochromatosis appears to be one of the most common inborn errors in metabolism among Caucasians of European descent.\textsuperscript{104} The hemochromatosis locus is linked to the HLA region on the short arm of chromosome 6 and is an autosomal recessive trait.\textsuperscript{100} The prevalence of this genetic abnormality may be as high as 12 in 1000\textsuperscript{105} and is characterized by excessive iron absorption, elevated plasma iron concentration and transferrin saturation, and high iron content in liver parenchyma cells.\textsuperscript{100} Contrarily, macrophage iron content is relatively low.
Congenital atransferrinemia is an extremely rare disorder characterized by a nearly complete lack of transferrin. This disorder is probably an autosomal-recessive anomaly and is accompanied by hypochromic anemia and iron overload involving the liver, heart, and pancreas and an almost complete lack of iron in bone marrow. This disorder along with a mouse model of hypotransferrin are very suggestive that plasma transferrin is not necessary for the transport of absorbed iron.

More isolated examples of human genetic disposition for iron overload have also been described. One third of the members of a large Melanesian family have been reported to have developed iron overload. Although many characteristics are similar to HLA-linked hemochromatosis, the mode of inheritance appears to be an autosomal-dominant transmission. Another instance of inherited iron overload hereditary was reported in two siblings in a Yemenite Jewish family. This inherited trait is also believed not to be HLA-linked hemochromatosis.

Iron overload has been reported in at least 15 sub-Saharan African countries. The overload is the result of drinking locally brewed beer with a high iron content. The histological alterations to the liver are distinct from alcohol-related insult, and iron accumulates in both hepatic parenchyma cells and macrophages. Necropsy evaluation estimated the incidence of iron overload-induced liver cirrhosis to be greater than 10% in these geographic regions. Further investigation suggested that most likely there is an underlying genetic factor concomitant with a high dietary iron consumption.

The forms of inherited anemia — homozygous β-thalassemia, β-thalassemia/hemoglobin E, and hemoglobin H disease — all result in ineffectual erythropoiesis in bone marrow. Although the mechanisms are unclear, the ineffective erythropoiesis ultimately leads to augmented iron absorption. The treatment of these diseases involves multiple blood transfusions which contribute even more iron to these individuals. Initially the overloading of iron results in deposits in the liver; however, with time, iron accumulates in other organs such as the heart and pancreas.

Other forms of anemia associated with ineffective erythropoiesis can increase iron absorption and potentially lead to overload. These anemias include congenital dyserythropoietic anemias, a number of sideroblastic anemias, and many anemias associated with poor iron incorporation into hemoglobin.

Iron overload may also be induced clinically by frequent blood transfusions in patients with aplastic anemia, pure red cell anemia, Blackfan-Diamond syndrome, myelodysplasia, and sickle cell disease. The iron is derived primarily from erythrocyte hemoglobin, and excessive iron initially accumulates in macrophages and then liver parenchyma cells. Neonatal iron overload has been described as being associated with certain perinatal metabolic disorders such as hypermethionemia and fatal liver disease.

Animal models have been developed to study iron overload and its related pathology. Rats fed a diet enriched with 2 to 3% elemental (carbonyl) iron over a period of 2 to 4 months develop hepatic nonheme iron concentrations 50 to 100 times normal. Excessive iron deposition in cardiac and pancreatic tissue is modest, and nonhepatic organ toxicity is not evident.

4.3 Toxicity

The exact mechanisms of toxicity from iron overload are not completely understood; however, many investigators agree that the pathological alterations associated with iron overload are probably the result of increased free radical activity initiated by excessive iron. Under normal situations, iron is almost entirely found bound to proteins; however, unbound iron in the reduced ferrous form is believed to contribute to free radical activity by participating in the Fenton reaction which results in the production of the highly reactive hydroxyl radical (OH·):
Fe$^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}\cdot$

Many investigators have reported the products of lipid peroxidation in various tissue, including liver,$^{121,129}$ plasma,$^{127,130}$ kidney,$^{128,129}$ spleen,$^{131}$ muscle,$^{128}$ and skin.$^{128}$ Britton et al.$^{89}$ suggested a paradigm for iron overload associated hepatic tissue pathology. Increased iron absorption results in hepatic iron overload, which ultimately leads to organelle dysfunction and injury; lipocyte collagen synthesis leading to fibrosis; and possibly alterations in hepatic DNA initiating tumor formation.$^{89}$

Although iron deposition during overload deposits in many tissue such as the heart, lung, kidney, and brain, the liver has received the most investigative attention, most likely because of its prominent involvement in iron storage and also because cirrhosis is recognized as one of the most common causes of death with genetic-based hemochromatosis in humans.$^{132}$ Rats fed a diet enriched with 2 to 3% carbonyl for 2 to 4 months develop hepatic iron concentrations of 3 to 6000 µg Fe per g liver.$^{120}$ The iron preferentially deposits in perportal hepatocytes similar to early HLA-linked hemochromatosis and African iron overload.$^{120,121}$ The iron-overloaded rats present direct evidence of mitochondrial and microsomal lipid peroxidation,$^{121,123}$ along with an increase in the low-molecular-weight pool of catalytically active iron.$^{133}$ Further, at a hepatic iron concentration at which lipid peroxidation is observed, specific mitochondrial membrane-associated activities such as oxidative metabolism and Ca$^{2+}$ sequestering are decreased.$^{123,133,134}$ Similarly, microsomal membranes demonstrate decreased cytochrome concentrations, enzyme activities, and Ca$^{2+}$ sequestration.$^{124,134,135}$

Iron overload results in excessive accumulation of iron in hepatocellular lysosomes and appears to increase their fragility.$^{136,137}$ This increase in fragility results in the release of hydrolytic enzymes into the cytosol of hepatocytes and initiates cellular damage. Myers et al.$^{126}$ reported that experimental iron-overloaded rat hepatocytes presented lysosomes that were more fragile, enlarged, and misshapen.$^{126}$ These membranes also demonstrated decreased fluidity and increased lipid peroxidation as determined by malondialdehyde content.

At liver iron concentrations similar to those observed in HLA-linked hemochromatosis (3000 to 6000 µg Fe per g liver), experimental iron-overloaded rat mitochondria show increased lipid peroxidation, as demonstrated in vivo by the presence of conjugated dienes in phospholipid extracts.$^{121,123}$ These investigative efforts also resulted in the determination of a hepatic iron concentration threshold for the presence of lipid peroxidation in mitochondria (1000 to 1500 µg Fe per g liver) and microsomes (3000 µg Fe per g liver).$^{122}$ It has also been reported that mitochondrial malondialdehyde content is also increased several fold in experimental iron-overloaded rats and that this increase in malondialdehyde is likely due not only to increased lipid peroxidation but also to an impairment in malondialdehyde metabolism.$^{125}$ Furthermore, at modest increases in hepatic iron concentration, there was a significant impediment of mitochondrial electron transport as exemplified by a 70% reduction in cytochrome C oxidase activity and a 48% decrease in cellular oxygen consumption.$^{138}$

Iron overload also results in hepatic fibrosis.$^{89}$ The mechanisms of fibrogenesis in this condition are poorly understood, as efforts with experimental iron-overloaded rats and baboons have failed to demonstrate a consistent relationship with prolyl hydrolase activity.$^{138}$ Morphological investigation of experimental iron overload has revealed that hepatic fibrosis is recognized at 8 months, and by 1 year periportal fibrosis is pronounced and concomitant to the identification of cirrhosis in some animals.$^{120}$ Investigators have found that the hepatic levels of type I procollagen mRNA are augmented$^{139,140}$ and that nonparenchymal cells are predominantly involved, most likely activated lipocytes.$^{141}$

Iron overload is also associated with a greater incidence of cancer; humans with HLA-linked hemochromatosis are at about a 200 times greater risk of hepatocellular carcinoma.$^{132,142}$ Experimental iron overload rats have presented evidence of an increase in DNA strand breaks with a liver iron concentration of 3130 µg/g tissue but not at lower liver iron.
concentrations (≈ 600 μg/g). Further, a synergistic carcinogenic effect was reported with the combination of iron in conjunction polychlorinated biphenyls.

Recent concern has been over the reported association of serum ferritin levels with increased myocardial infarction. In a study of over 1900 Finnish males from 40 to 64 years of age, serum ferritin levels greater than 200 μg/l had a 2.2 times greater risk of myocardial infarct compared to males with lower levels. This was after adjustment for other known risk factors such as cigarette smoking, higher systolic blood pressure, lipoprotein cholesterol levels, etc. In fact, those males with a serum low-density-lipoprotein (LDL) cholesterol level greater than 193 mg/100 ml had even a greater risk with the added high serum ferritin levels. The mechanism apparently may be related to the role of iron in free radical generation, as reviewed above. Oxidation of LDL cholesterol is known to result in greater cholesterol uptake by macrophages, which is a key mechanism in foam cell production and subsequent plaque formation.

5 SELENIUM

5.1 METABOLISM

Selenium is efficiently absorbed in the gastrointestinal tract in several organic forms; however, two distinct chemical forms, selenomethionine and selenite, have been traced in humans using stable isotopes. Selenomethionine is a selenium analog of a sulfur-containing amino acid. Selenium and sulfur are exchanged due to their chemical similarities, and selenium is absorbed as selenite. The major site of absorption is the duodenum, although some selenium is absorbed in the ileum and jejunum. Selenium absorption does not occur in the stomach; there is no known regulatory mechanism for selenium absorption. A range of 50 to 100% absorption of these two forms has been demonstrated.

In the form of selenium dioxide, a white crystalline material that melts at 340°C, selenium can be absorbed in the respiratory tract. It is then reduced to selenium metal, which can cause liver damage if prolonged exposure occurs. Symptoms of overexposure to selenium dioxide include a garlic odor of perspiration and breath.

When selenium is absorbed as selenomethionine, it is incorporated into a plasma protein called selenoprotein P. This protein functions to transport and store selenium. As selenite, selenium becomes incorporated into the metalloenzyme glutathione peroxidase. Glutathione peroxidase functions to reduce organic and hydrogen peroxides. This is especially important for phagocytic cells such as leukocytes and macrophages. In these cells, peroxides are the byproducts of the oxidative destruction of foreign matter; therefore, glutathione peroxidase protects these cells from being destroyed as they function.

Another site of glutathione peroxidase activity is at the platelet. Here it acts in an antiaggregative capacity. This metalloenzyme reduces fatty acid peroxide formation, and the ratio of prostacyclin (an antiaggregating factor) to thromboxane (a proaggregant) becomes increased. Through this mechanism, selenium is linked to cardiovascular disease by decreasing platelet aggregation which reduces clots and atherosclerosis.

Of selenium excreted, 50 to 60% is through the urine, the remaining 40 to 50% of selenium being excreted through the feces. Body stores of selenium greatly influence renal clearance of this mineral; hence, the kidneys appear to be the regulatory mechanism for selenium homeostasis. Endogenous selenium is lost through the feces, which was demonstrated by showing that fecal selenium excretion remains the same regardless of dietary intake. At toxic intakes of dietary selenium, volatile selenium compounds such as dimethylselenide are exhaled and can escape through the skin. (See Table 4.)
5.2 Toxicity

There are three forms of selenium toxicity: acute selenosis, subacute selenosis, and chronic selenosis. Acute selenosis occurs when excess amounts of selenium are ingested over a short length of time. Symptoms of acute selenosis include an unsteady gait, cyanosis of the mucous membranes, and difficulty breathing which can lead to death. Autopsy reports of acute selenosis describe liver congestion, endocarditis, myocarditis, and smooth muscle degeneration in the gastrointestinal tract, gallbladder, and bladder. Long bone erosion was also reported in these cases.\textsuperscript{154}

When large doses of selenium are ingested over a long time frame, subacute selenosis is observed. Symptoms of subacute selenosis include neurologic dysfunction such as vision impairment, ataxia, and disorientation; respiratory distress is often seen as well. Subacute selenosis is commonly seen in livestock that graze on selenium-accumulating plants. These seleniferous plants are concentrated in the western U.S. — Montana, Colorado, Wyoming, New Mexico, and Arizona.\textsuperscript{155}

Chronic selenosis occurs when moderate doses of selenium are ingested over a considerable length of time. This condition is characterized by skin lesions and dermatitis such as alopecia and hoof necrosis (in livestock), emaciation, chronic fatigue, anorexia, gastroenteritis, liver dysfunction, and spleen enlargement.\textsuperscript{156}

The most toxic forms of selenium are noted to be sodium selenite, sodium selenate, selenomethionine, and selenodiglutathione;\textsuperscript{157, 158} however, there are wide variations in selenium toxicity with respect to the valence state of the molecule. Recently, a multitude of selenium compounds have been synthesized as chemopreventive/anticarcinogenic substances. These are being tested for their toxic effects.\textsuperscript{158}

An area of China has unusually high concentrations in the soil of selenium\textsuperscript{159-161} which becomes incorporated into the food supply. Residents were evaluated for clinical and biochemical indications of selenium intoxication. The average daily selenium intake was estimated to be 1.4 mg for adult males and 1.2 mg for adult females. When comparing this group to those whose selenium intakes were 0.07 and 0.06 mg, respectively, for men and women, increased clotting time and reduced serum glutathione were observed. Clinical signs that were observed consisted of garlic odor in the breath and urine, brittle or lost nails, lowered hemoglobin levels, and nervous system problems such as peripheral anesthesia, acroparesthesia, and pain in the extremities.\textsuperscript{158}

In livestock, symptoms of selenium toxicity are observed in the nervous system as ataxia, tremors, hypersensitivity, and convulsions. In humans, nervousness, chills, numbness, impaired nerve conduction, and peripheral anesthesia are symptoms. Mottled teeth have been observed

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in humans with selenium toxicity. In animals, the liver has been demonstrated to have steatosis and necrosis associated with selenium intoxication; however, this has not been reported in humans.\textsuperscript{158,162}

Kidney problems such as congestion, necrotizing nephrosis, and calcinosis have been reported only in animals. The heart appears to be affected by selenium toxicity, resulting in myocarditis in rats and bradycardia in humans. Only livestock have shown respiratory disturbances such as congestion, edema, respiratory distress, and hydrothorax. The skin is affected by selenium toxicity, seen as thick, streaked, brittle nails; dry, brittle hair; hair loss; red, swollen hands and feet. In animals, cracked hooves are noticed, as well as dermatosis and alopecia. Anemia, increased prothrombin time, and decreased hemoglobin are hematological parameters observed in both humans and animals. Decreased immune function has been demonstrated in rats with selenium toxicity. In both animals and humans with selenium toxicity, loose stools, diarrhea, excessive salivation, and dyspepsia have been observed. Deformities of fetal chicks and ducks have been documented in selenium toxicity.\textsuperscript{162}

5.3 \textsc{Interactions Between Selenium and Other Nutritional/Environmental Substances}

Vitamin E has been shown in animals to spare selenium and reduce the amount necessary in the diet. Other factors with such a role have also been identified and include: decreased food intake, high protein intake, high levels of vitamin A and vitamin C, and synthetic antioxidants. Conversely, there are substances which are known to antagonize dietary selenium: heavy metals, sulfate, mercaptans, and chlorinated hydrocarbons as well as deficiencies of vitamin E, riboflavin, vitamin B-6, and methionine.\textsuperscript{162}

There are substances known to affect selenium toxicity. These may act as methyl donors which synthesize selenium metabolites and are simply excreted. The methyl donors include methionine, betaine, choline, creatinine, and amidinoglycine. Also, the heavy metals mercury, cadmium, lead, silver, and arsenic along with the trace elements copper, zinc, and iron substitute for selenium and reduce the toxic potential of selenium. Furthermore, antioxidants such as vitamin E, diphenyl-p-phenylene diamine (DPPD), and beta-hydroxy-toluene (BHT) help the antioxidant role of glutathione peroxidase and thereby reduce toxic effects of selenium.\textsuperscript{162}

6 \textsc{Mercury}

6.1 \textsc{Chemical Form as Related to Toxicity and Sources of Exposure}

Mercury toxicity is enhanced in the organic chemical form. Organomercurial concentration in food chains was first discovered and reported in Japan in 1959. Shellfish contaminated with mercury were consumed by a small Japanese population in the area of Minimata Bay. This resulted at the time in 46 fatalities along with disorders such as mental depression and tremors in other affected individuals. Minimata disease\textsuperscript{163} is the term applied to this chronic alkylmercury poisoning. In the U.S., some instances of mercury poisoning have been reported, such as the well-known case of a New Mexico farmer who accidentally poisoned his family when he fed contaminated waste seed to his hogs, which in turn were eaten by family members.\textsuperscript{164}

Mercury has been used for centuries, but it was not until the industrial revolution that its use became extensive.\textsuperscript{165} The occurrence of mercurials in the environment can generally be traced to three major sources: (1) alkylmercurials, found in agricultural pesticides; (2)
arylmercurials, which occur in paints and are used in the manufacture of paper; and (3) inorganic divalent mercury from chlor-alkali plants.166 Many of these compounds find their way into aquatic systems through run-off and discharge of liquid effluences into surface waters. The U.S. Environmental Protection Agency (USEPA) banned the use of mercurials in pesticides and fungicides in 1972.167

The organic mercurials are the most toxic known to humans and other vertebrates. These forms of mercury have a lower water solubility than the inorganic ones but a higher lipid solubility. The alkyl compounds have a greater water solubility and volatility than aryl compounds possessing the same X or side groups.165,168 Among the methyl and ethyl compounds, the most toxic are the phosphate derivatives, followed by the chloride and cyano forms.165

(See Table 5.)

6.2 Toxicity

The toxic effects of the mercurials are apparently related to the excretion rates. Phenyl- and alkoxyalkylmercurials are not persistent in vertebrates and have biological half-lives of approximately 3 to 4 days, which is about the same as for inorganic mercury.168 Alkylmercury compounds are usually more stable and excreted more slowly, showing half-lives of 15 days in the rat and 23 to 27 days in poultry.168 Excretion rates in rats of aryl and inorganic mercury are similar to one another. Excretion rates of methylmercury are lower than rates observed for aryl and inorganic mercury.163

Among higher vertebrates, including humans, inorganic and alkoxyalkyl compounds cause kidney damage, which usually leads to death. Some uptake of inorganic mercury by kidney cells suggests that active transport is involved, since energy is required, but most is by diffusion.169 Chronic poisoning by elemental mercury is characterized by progressive renal and central nervous system damage. Symptoms include mental depression, irritability, and tremors. Chronic levels of alkylmercury compounds produce different effects. There is a latency period that lasts from one to several weeks, during which no symptoms are apparent. Then, damage to the central nervous system expressed as poor muscular coordination, loss of a sense of positioning and equilibrium, and impaired hearing are observed.163,168

Methylmercury compounds appear to be the most toxic of the mercurials, tending to be retained in the body, especially in the brain.163,168 Sensory malfunctions include paresthesia, astereognosis, and constriction of visual field. Motor manifestations range from impairment of fine coordination to gross ataxia, depending on degree of exposure.170 Neonatal exposure to mercury vapors in rats results in behavioral changes, such as increased locomotive activity,
but decreased rearing of young when tested at 4 months of age. Learning ability is impaired as well. Prenatally exposed rats demonstrated similar findings when tested at similar later ages. Some of the neurotoxicity of mercury may be due to decreased central nervous system (CNS) glutamate uptake by astrocytes and spinal cord, as demonstrated in cell culture experiments. The efficient absorption of methylmercuric and phenylmercuric compounds from food may be due to the lipid solubility of their chloride complexes. Various organomercurials are converted to inorganic mercury once in the body, but the precise mechanism appears unclear.

The intracellular distribution of mercurials varies with the type of mercurial present. Lysosomes may be one of the main organelles to concentrate mercuric chloride, followed by the mitochondria and microsomes. The microsomal fraction usually has a greater amount of methylmercury.

The biological properties of the short-chain alkylmercury compounds are related to their ability to cross cell membranes and to their slow conversion to inorganic mercury in the body. The almost complete absorption of these compounds from food and their subsequent rapid passage across the blood-brain and placental barriers accounts for damage to the CNS in both adult and fetal mammals. Low levels of inorganic mercury have been shown to be mutagenic in Chinese hamster ovary cells; these levels were not cytotoxic. The apparent neurotoxicity of methylmercury appears related to the catalyzed hydrolysis of phospholipids composing the cell membranes of neurons. In the liver and kidney, the high affinity of mercury to thiol groups appears to be the most significant chemical property explaining their toxic effects. While they have a high specificity in terms of sulphydryl groups, these compounds are nonspecific in terms of proteins they target, since almost all proteins contain sulphydryl groups that are metal reactive. Mercurials are consequently potent but nonspecific inhibitors of enzymes. The consequence of these properties is apparent inhibition of energy metabolism, formation of cell structural proteins, and a variety of cellular processes. The mercury ion is known to promote oxidation of kidney cells and to disrupt renal mitochondrial function. Increased H₂O₂ production by rat renal mitochondria is an indirect effect of inorganic mercury. Renal mitochondria from rats treated with mercuric chloride (1.5 mg/kg i.p.) have a twofold increase in H₂O₂ but reduced glutathione content. Thiobarbiturate reactive substances were increased by more than two thirds. Depolarization of the inner mitochondrial membrane was reported. Hyperpolarization of cultured renal cell membranes exposed to mercury ions have been reported previously along with an increase in cell membrane potassium selectivity. However, another study supported the concept that mercury thiol complexes in the kidney possess redox activity and promote porphyrinogen oxidation, leading to excess porphyrins in the urine. The affinity of mercury for thiol groups accounts for the accumulation of large amounts of inorganic mercury in the kidneys without much damage on a metabolic basis. However, there has been evidence that some damage may occur to the extent that both renin- and angiotensin-I-converting enzyme activities were reduced and may modify systemic hemodynamics. Metallothionein in the kidney may afford some degree of protection from the toxic effects of mercurials.

Recent studies have contended that mercury may have deleterious influences upon lymphocytes and may be related to renal autoimmune disease in genetically predisposed animals. A human study reported increased lymphocyte micronuclei in mercury-exposed chloralkali workers. The influence of mercury upon endocrine function appears minimal. McGregor and Mason reported in a population exposed to mercury vapors a lack of relation between pituitary and thyroid endocrine function with blood and urinary mercury levels. Moszczynski et al. studied 89 men, 21 to 57 years old, and grouped them into levels according to the length of mercury exposure. No relation among various clinical, hematological, and biochemical measures was noted in these subjects. All appeared clinically healthy.
7 CADMIUM

7.1 SOURCES OF EXPOSURE

The interest in cadmium toxicity has centered on occupational exposure of workers and exposure of a local population through some type of industrial activity. Exposure to cadmium is common for workers engaged in such occupations as electroplating (coating steel, iron, copper, brass, etc., in order to make it corrosion resistant) and the manufacture of cadmium batteries, plastics, paints, textiles, and phosphate fertilizers. Mining activities can lead to the exposure of workers to cadmium, and the surrounding area could be exposed to large amounts of cadmium through runoff. Accidental exposure of a Japanese population through rice contaminated by runoff from a mine upstream to the paddy is perhaps the best publicized case of cadmium toxicity. (See Table 6.)

Cigarette smoke is a large source of cadmium intake. Between 0.1 and 0.2 µg of cadmium per cigarette has been reported, but the amount inhaled is dependent on the number of puffs and inhalation pressure. Furthermore, more cadmium is found in the particulate phase than in the gaseous phase.\(^{187}\)

In the nonsmoking population, food is the major source of cadmium intake.\(^ {188}\) Some areas of Japan have the largest intake of foodborne cadmium of any other area studied. In the U.S., the amount of cadmium in diets was fairly constant from 1920 to 1945. From 1945 to 1975, a 20% decline in the average daily cadmium intake was observed. This decline is thought to reflect changes in the dietary patterns of Americans rather than a change in the total amount of available cadmium in the environment.

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### Table 6

<table>
<thead>
<tr>
<th>Metabolic Aspects</th>
<th>Affected Site/Action</th>
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<tbody>
<tr>
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<tr>
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<td>Storage</td>
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<tr>
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<tr>
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<tr>
<td>Symptoms</td>
<td>—</td>
<td>187, 196, 197</td>
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<tr>
<td>Cobalt</td>
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### Toxicity

#### Cardiovascular
- Hypertensive effects | 203–210 |
- No blood pressure effects | 211, 212 |
- Biphasic blood pressure effects | 213, 214 |
- Hypertensive mechanisms | 215, 216 |
- Epidemiology evidence and hypertension | 196, 197, 218, 219, 221, 222 |

#### Reproductive
- Testicular and prostate tumors | 187, 223, 224 |
- Testicular pathology | 225–230 |
- Sex hormones | 231 |
- Fetal development | 187, 232 |
- Anemia | 236–238 |
Water contributes slightly to cadmium intake. Water concentrations below 10 parts per billion (ppb, or \(\mu g/l\)) will contribute little to daily intake. At a concentration of 20 ppb, a daily intake of 20 to 40 \(\mu g\) is expected if water consumption is in the amount of 1 to 2 l per day. Plumbing is a factor that must be considered in assessing cadmium intake, because both metal and plastic pipes contain some cadmium.\(^{187}\)

Whatever the source, cadmium usually accumulates as the body ages, up to 50 years. At this age, one who has essentially been unexposed to cadmium may have accumulated 20 to 30 mg of cadmium in the body. In human newborns, the total body content of cadmium is less than 1 \(\mu g\).\(^{187}\)

### 7.2 Metabolism

Experimental animal studies have led to the estimate that less than 10% of an oral dose of cadmium is absorbed. Many studies report an absorption rate of 2%. Inhalation may result in the uptake of more cadmium, with studies reporting 10 to 40% retention of inhaled cadmium.\(^{187}\) Absorption will occur regardless of the total body burden of cadmium, and little of the absorbed cadmium is excreted through the urine or intestinal tract. For example, less than 2 \(\mu g\) of cadmium per day is excreted in the urine by the average person.\(^{187}\) An estimated biological half-life for cadmium of between 16 and 33 years has been computed.

Following absorption, cadmium is transported primarily to the liver, where it is bound to metallothionein, a protein composed of sulfur, copper, mercury, zinc, and other metals.\(^{189}\) The protein has a low molecular weight of approximately 3000. Metallothionein is thought to be composed of three protein subunits that strongly bind cadmium and one protein subunit that loosely binds zinc. Cadmium, therefore, has a greater affinity to metallothionein.\(^{190}\)

After cadmium is sequestered by the liver, small amounts of metallothionein-bound cadmium appear in the plasma, where it is cleared efficiently by the kidney. Cadmium will accumulate in the renal tubules,\(^{191}\) where it may interfere with zinc-dependent enzymes (e.g., leucine-aminopeptidase, which is thought to play a role in renal handling of protein). With increased renal cadmium content, less protein will be catabolized or reabsorbed, causing tubular proteinuria. When this occurs, cadmium excretion will increase because less metallothionein will be reabsorbed.\(^{187}\) When cadmium exposure is moderate, renal concentration rises slowly to about 200 \(\mu g\) of cadmium per gram of tissue. At this concentration, renal tubular injury may occur. Cadmium-exposed workers usually have greater concentrations of \(\beta\)-2-microglobulin in the urine and greater urine and plasma metallothionein concentrations, which suggest renal damage.\(^{191}\)

Cadmium toxicity is also known to affect calcium metabolism, but the metabolic antagonism between calcium and cadmium may not be a mimicking phenomenon. Excess loss of calcium in the urine through renal injury can lead to calcium mobilization from skeletal stores to maintain serum calcium levels leading to osteomalacia. This condition is often termed “itai-itai disease” and was first discovered in Japan as a result of cadmium toxicity. Translated, it means “ouch-ouch” disease. The victims are often of a decreased height, and deformities and numerous microfractures develop in the skeleton with increasing brittleness due to the loss of calcium and phosphate. Pregnant women are more susceptible to this condition, because they have increased calcium requirements, and an inadequate supply of dietary calcium may further aggravate the problem. Lack of sunlight could result in diminished vitamin D synthesis required to aid in calcium absorption. Cadmium may also have a direct effect on bone,\(^{192}\) but further studies are needed to confirm this.

Pulmonary damage is known to occur in rats intratracheally treated with cadmium chloride. Polymorphonuclear leukocyte numbers and permeability increased.\(^{193}\) Pulmonary tumors have been reported to develop in genetically susceptible mice when exposed to cadmium.\(^{194}\) Chronic exposure to cadmium can cause lung emphysema.\(^{195}\)
7.3 CLINICAL EVALUATION

How can the cadmium burden of an individual be evaluated? This is difficult to answer. As mentioned previously, the concentration of cadmium in the urine may not indicate exposure, because little of the element is eliminated through the kidney until renal injury has occurred. Blood cadmium levels may be helpful but, again, may represent transient levels. It is not clear whether blood levels reflect short- or long-term accumulation of the element. Hair concentration of cadmium and of other elements has been suggested as a viable alternative, because it may represent a longer term of exposure than blood does and may act as a recording filament. One difficulty arises from the lack of information on normal values. The use of hair to evaluate the exposure of populations to cadmium has been studied; however, the use of hair elemental analysis for assessing cadmium burden for an individual has not been established. Until further research has been carried out, the use of hair analysis for clinical evaluation should be viewed with caution.

When chronic cadmium poisoning occurs (e.g., itai-itai disease), several subjective symptoms may become apparent. Back and joint pain, lumbago, disturbance of gait, restriction of spinal movement, decreased height, and pain when pressure is applied to an area are some of these. Roentgenograms will often reveal Milkman’s pseudofractures, thinned bone, cortex decalcification, deformation, and fish-bone vertebrae. A urinalysis may reveal proteinuria and glucosuria but a decreased phosphorus/calcium ratio. Analysis of the serum may reveal an increase in alkaline phosphatase and a decrease in serum inorganic phosphate. In many cases, detectable roentgenographic signs of osteomalacia are not observable in the early stages of the disease, but analysis of serum may demonstrate changes.

7.4 RELATIONSHIP WITH OTHER TRACE ELEMENTS

A discussion of any trace element is not complete unless the interrelationship with other elements is considered, and cadmium is no exception. Zinc deficiency can increase cadmium toxicity, whereas extra zinc may protect against this negative effect to some extent. Selenium may also protect against cadmium toxicity through the formation of strong metal-selenium bonds.

In the absence of copper and iron, 25 ppm of cadmium caused mortality in chicks. When these two elements were added to normal levels, 200 ppm of cadmium were required before an increase in mortality was achieved. The addition of zinc appeared to reverse some of the toxic effects of cadmium, such as growth depression and gizzard abnormalities.

Pregnant mice given cadmium in drinking water exhibited fetal growth retardation and anemia, but iron-supplemented diets prevented these effects. The addition of vitamin C to quail diets lowered the toxic effects of cadmium due to increased iron uptake. Iron-deficient rats given intragastric doses of $^{109}$CdCl$_2$ exhibited greater cadmium uptake than did animals with normal iron status. Similar results were reported in mice. Cadmium inhibited cobalt uptake in a similar manner. In humans, cadmium absorption is greater for those with lower body iron stores than for those with normal body stores. Thus, it appears that subjects with lower iron and zinc status could have increased cadmium uptake.

7.5 CARDIOVASCULAR TOXICITY

This area of cadmium research has perhaps generated the most data and interest. Several studies on rats have demonstrated the blood pressure-elevating effects of cadmium administration, either through the drinking water or by intraperitoneal injections. Cadmium at a concentration of 5 ppm in drinking water produced systolic hypertension, and a zinc chelate
reversed the effect in rats.\textsuperscript{203,204} Doses as low as 1 ppm of cadmium have produced hypertension in rats, and concentrations as low as 0.1 ppm of cadmium in drinking water had a pressor effect on rats.\textsuperscript{208} Studies with monkeys have produced similar findings.\textsuperscript{209,210} Not all cadmium-feeding trials, however, have produced hypertension in experimental animals.\textsuperscript{211} Rats made hypertensive by means of unilateral nephrectomy and given 1\% saline as drinking water had lower blood pressure when injected with cadmium as compared to rats not injected with cadmium. In fact, cadmium-treated rats remained normotensive.\textsuperscript{212} The results of these studies are in apparent conflict with studies in which blood pressure elevation was found.\textsuperscript{203-208}

Some of the inconsistencies as to whether cadmium can raise blood pressure may be explained by the work of Kopp et al.\textsuperscript{213} Results from their laboratory revealed that at lower cadmium concentrations in drinking water, comparable to environmental exposure (e.g., 0.01 to 0.5 ppm), blood pressure was elevated in rats. Exposure to cadmium concentrations from 0.5 to 50 ppm lowered rather than raised blood pressure. A series of studies on cardiac function and tissue metabolism documented greater changes at 1 ppm of cadmium than at 5 ppm of cadmium in drinking water, whereas liver metabolism appeared to be unaffected. These data suggest that at lower doses, cadmium accumulates to concentrations that affect cardiovascular tissues without the appearance of overall systemic toxicity. Earlier work by Perry and Erlanger\textsuperscript{214} supported the conclusion that larger doses of cadmium are hypotensive or vasodepressive, whereas small doses have a hypertensive effect.

Several ideas on the mechanisms of cadmium-induced hypertension have been suggested. For example, sodium ions accumulate in the kidney due to cadmium deposition, which could influence the excretion and reabsorption of renal sodium, resulting in elevated blood pressure.\textsuperscript{215} In another mechanism affecting the kidney, Perry and Erlanger\textsuperscript{216} suggested that cadmium increases circulating renin activity leading to elevated blood pressure. Revis\textsuperscript{217} reported that two enzymes, monoamine oxidase and catechol-o-methyl transferase, which are involved in norepinephrine and epinephrine catabolism, were inhibited in the aortic tissue of rats receiving cadmium, either through injection or in their drinking water. The binding of norepinephrine to aortic membranes was stimulated in these animals. Thus, cadmium could exert its hypertensive effect by inhibiting the catabolism of norepinephrine while promoting norepinephrine binding in the aorta.

Another study\textsuperscript{211} revealed that sodium chloride intake was greater for rats given 5 ppm of cadmium in their drinking water for 23 to 42 weeks as compared to controls. This result suggests that the control mechanism for fluid intake could be altered in cadmium exposure.

The question remains whether or not cadmium is involved in either hypertension or heart disease in humans. Much of the data are epidemiological. For example, populations living in hard water areas have a lower incidence of heart disease. This is thought to be due to ions, such as calcium, blocking the uptake of cadmium and other deleterious elements or compounds. Borgman et al.\textsuperscript{196} demonstrated a positive correlation between cadmium concentration in the hair of adolescents in South Carolina and the incidence of heart disease in their respective home counties. Our laboratory has observed elevated hair cadmium concentrations of adult black hypertensive women as compared to weight- and age-matched black normotensive women.\textsuperscript{197} Hair cadmium levels in the babies of hypertensive mothers have been reported to be three times as high as in their hypertensive mothers.\textsuperscript{218}

Lead and cadmium may interact and lead to increased risk for heart disease. Voors et al.\textsuperscript{219} demonstrated elevated liver cadmium and aortic lead levels in North Carolina among victims dying from cardiovascular disease. Furthermore, Revis et al.\textsuperscript{220} demonstrated that lead and cadmium could induce aortic atherosclerosis and hypertension in pigeons.

Not all human studies, however, have suggested a link between cadmium and hypertension. Ostegaard\textsuperscript{221} reported in postmortem analysis that hypertensives between the ages of 45 and 65 years had lower renal cadmium concentrations than did normotensives who were
accident victims. In France, one study evaluated blood cadmium concentrations in 29 hypertensive men matched to controls for sex, age, and smoking habits. The results indicated no difference in blood cadmium concentrations between the two groups.

In view of the study by Kopp et al., the experimental approach to evaluating the effects of cadmium on human hypertension must be addressed. Since a biphasic rather than a linear response may be operating, negative data may have to be re-evaluated. It is apparent that studies on humans must be better designed in order to address these problems.

7.6 Reproductive Toxicity

Cadmium is known to have an adverse effect on reproduction. Epidemiological studies on occupational exposure to cadmium have suggested excessive deaths due to prostate cancer. Injections of large amounts of cadmium into experimental rats can cause sarcoma at the injection sites or testicular damage and eventually testicular tumors. Long-term exposures at low cadmium doses, however, have not resulted in testicular or prostate tumors in experimental animals in some studies, but more recent studies suggest findings to the contrary.

Cadmium can concentrate in the testes and prostate during heavy exposure and cause a decrease in testosterone synthesis. Excess exposure may also interfere with a zinc/hormone relationship in the prostate. Evidence suggests that direct action of cadmium on prostate cells is unlikely, and it is also unlikely that low-level exposure to cadmium is a causative factor for prostate cancer.

Mice given a subcutaneous injection of \( \text{CdCl}_2 \) exhibit effects such as karyolysis on the seminiferous epithelium. Degenerative spermatids with vacuolated nuclei are often observed, but Leydig’s cells appear to be unaffected. Some studies have suggested effects on testicular blood vessels. Berlinder and Jones-Witters, using electron microscopy, demonstrated that in gerbils cadmium acts on the interstitial capillary bed rather than on the seminiferous tubules. It does appear that scrotal testes are more sensitive to cadmium than cryptorchid testes. There have also been conflicting studies suggesting that estrogen and progesterone offer some protection against the toxic action of cadmium on the male reproductive system.

Studies on the effect of cadmium on the fetus have produced mixed results. Some animal experiments have suggested that the placenta constitutes a barrier against transfer of cadmium when small doses are given. When large doses are given, however, cadmium may destroy the placental barrier and enter the fetus. A study of 102 mothers and their newborns revealed decreased birth weight and an increase in hair cadmium levels of newborns. This was especially evident with cases of placental calcification. The reader is encouraged to refer to Robards and Worsfold for a comprehensive review of cadmium toxicology.

8 Lead

Lead is a divalent metal and often competes with other divalent ions such as iron, calcium, and zinc with respect to absorption and biochemical physiological processes. It may also substitute for the “normal” roles some of these other ions have, but with deleterious consequences. Overall, lead appears clinically to exert its toxic effects more in some tissues as opposed to others. The nervous, renal, and circulatory system appear to be sites where lead appears to have its greatest toxic impact. (See Table 7.) Furthermore, much like some of the other minerals discussed, lead can cross the placenta and have consequences upon the developing fetus. Age appears to be a strong factor in predicting the relative toxicity of lead. Children are much more sensitive to lead’s toxic effects than are adults. Of special concern
has been the influence of even small levels of lead exposure in young children and later effects upon learning processes, which are impaired.\textsuperscript{235}

Lead often leads to anemia by several mechanisms: (1) competing for absorption with the ferrous iron form; (2) inhibiting heme synthesis, as detailed below; and (3) altering the relative composition of cell membranes, including red blood cells, that make them more fragile and likely to hemolyze when passing through tiny capillary spaces. Besides the impact that lead has upon the red blood cells, it may also affect white blood cells and impair immune function. Separately from this, lead can bind tightly to antibodies, compromising the ability to ward off infections.\textsuperscript{235}

As with the other trace elements discussed, the toxicity of lead depends upon other dietary factors, including the levels of other metals. Low calcium diets may result in greater lead levels of various organs. This could be due to lack of competition for uptake in the small intestine. The amount of lead absorbed is usually greater when the stomach is empty. Iron deficiency, severe or mild, probably has the largest impact on the potential toxicity of lead. Less dietary iron conceivably allows more lead to be absorbed. The added lead to hemopoietic cells will decrease heme synthesis, which has already been compromised by iron deficiency.

While lead may be present in food, it is the industrialization of society that has been the prime factor leading to an increased incidence of lead intoxication. Lead-based paints, pesticides, auto emissions, and other industrialized byproducts contribute to everyday lead exposure. Much of this environmental lead can end up in soils, leading to its accumulation in plants and animals.

\begin{table}
\centering
\caption{Lead Metabolism and Toxicity}
\begin{tabular}{lll}
\hline
\textbf{Organ} & \textbf{Pathology} & \textbf{Ref.} \\
\hline
Blood & Heme biosynthesis & 236–238 \\
Kidney & Vitamin D\textsubscript{3} impairment, protein-lead complexes, gout & 238–240 \\
 & Nephron & 238, 241–243 \\
 & Protein and nucleic acids & 234, 246–251 \\
Cardiovascular & Hypertension & 252–259 \\
 & Cardiac conduction & 261, 262 \\
 & Cardiac calcium influx & 234 \\
 & Electrocardiograms & 264 \\
 & Cardiac Na/K ATPase & 265 \\
Bone & Dental development & 267 \\
 & Skeletal development & 268 \\
 & Osteocalcin and protein synthesis & 269, 270 \\
 & Cellular activity and homeostasis & 271–286 \\
Central nervous system & Memory loss and learning difficulties & 287–290, 296–298, 308–310 \\
 & Hippocampal and cortical effects & 291, 292, 296–298 \\
 & Visual effects & 293–295 \\
 & Brain cellular morphology & 299–300 \\
 & Functional deficits & 293, 301–303 \\
 & Pre-synaptic neurotransmitter release & 304–307 \\
 & Encephalopathy & 301, 310, 311 \\
 & Cognitive intelligence & 312–317 \\
 & Dopamine, acetylcholine, and GABA release & 318–321 \\
 & Second messenger substitution effects, Protein C kinase & 322–326 \\
 & Edema & 327–329 \\
Reproductive & Spontaneous abortions & 236, 320 \\
 & Sperm morphology and count & 331–335 \\
 & Pregnancy, gestation period, implantation influences & 337–339 \\
\hline
\end{tabular}
\end{table}
8.1 Lead Effects Upon Blood

Anemia is a classic indication of lead toxicity. Heme biosynthesis impairment results because lead can inhibit the enzyme delta-aminolevulinic acid dehydratase. This enzyme is involved in synthesis of the porphyrin units. As an overview, porphyrin biosynthesis begins with the condensation of glycine and succinyl CoA, a tri-cyclic acid (TCA) cycle intermediate, to form α-amino-β-ketoadipic acid in the presence of the enzyme aminolevulinic acid synthase and vitamin B6. The complex undergoes decarboxylation to form delta-aminolevulinic acid. This reaction occurs in the mitochondria. Subsequently, the compound goes to the cytoplasm where two molecules of delta-aminolevulinic acid condense to form porphobilinogen via the dehydratase enzyme. This porphobilinogen synthesis is significantly impaired by lead toxicity. This enzyme is also a zinc-requiring enzyme. The porphyrin heme ring is formed essentially by condensation of four monopyrroles synthesized from the porphobilinogen. Thus impairment of porphobilinogen by lead will decrease heme biosynthesis by this mechanism.

Another mechanism by which lead may cause anemias is through its inhibition of the mitochondrial enzyme ferrochelatase. This enzyme facilitates the transfer of the iron in ferritin into the protoporphyrin ring to produce heme. The protoporphyrin accumulates in the red blood cells of human subjects and leads to intoxication.

8.2 Renal Toxicity

One target organ of lead exposure is the kidney, which given sufficient lead exposure levels over a sufficient length of time may succumb to renal failure. Goyer and Ryne reported that the proximal tubules of the nephron are lead sensitive. Furthermore, it is well known that the active form of vitamin D₃ (1,25-dihydroxycholecalciferol) is produced in the proximal tubules. Decreased calcium absorption by the gut is one result that will affect bone; however, as discussed later, lead has other effects upon bone. Rosen et al. reported that this activation is impaired at blood levels of 25 μg/100 ml blood. Higher blood lead levels can lead to protein-lead complexes in the tubules which appear as dense accumulations. Gout may be a symptom of such toxicity due to increased reabsorption of uric acid. Continued accumulation of lead by the kidneys often leads to an increased accumulation of fibrotic connective tissue. Typical measures of renal failure (e.g., blood urea nitrogen, creatinine) are elevated as a consequence of this lead-induced renal failure.

The mitochondria appear to be altered histologically in the proximal tubules as a result of lead accumulation. Vascular lesions and atrophy of various portions of the nephron may appear, and inclusion of bodies are commonly reported upon histological examination of the glomeruli. Apparently, other tissue sites in the body have the same appearance of these inclusion bodies upon lead exposure. Osteoclasts and neuroblastoma cells often have these features as a result of lead toxicity. Subsequent studies have revealed that agents such as cyclohexamide and actinomycin-D impair the formation of these bodies, suggesting that lead leads to protein synthesis processes of these bodies. RNA and DNA levels may also increase. Lead-binding proteins are thought to be induced in both cytoplasmic and nuclear portions of tubular cells, as reviewed elsewhere.

Unlike some of the other metals reviewed, lead does not appear to induce metallothionein synthesis in the kidney; however, liver metallothionein appears to increase. Goering and Fowler reported that lead will bind to renal metallothionein after its induction by cadmium.

While it is not entirely clear as to why these various proteins are increased in the synthesis, a protective effect may be exerted by the production of these lead-binding proteins by the kidneys. The toxic effects of lead upon the kidney may lead to problems with other organ systems, such as hypertension and deleterious alterations in both circulating hormones and bone metabolism. The influence of other metals (e.g., calcium, copper, zinc, etc.) has an
impact upon kidney damage as a result of lead toxicity. Therefore, when discussing the two pathologies below, the toxic influences of lead upon the renal system and the diet of the individual must be considered in the final analysis.

8.3 Cardiovascular Toxicity

The association of lead with hypertension has been linked by some epidemiological studies. Pirkle et al.\textsuperscript{252} reported that even low lead levels are associated with elevated blood pressure. Increased lead absorption leading to hypertension was reported in one study.\textsuperscript{253} Some of lead's influence upon blood pressure may be related to the associated renal toxicity reviewed previously. Low levels of lead exposure have been reported to result in hypertension when the toxicity signs are absent\textsuperscript{254,256} and have been reported by animal studies.\textsuperscript{253,257,258} One study with rats suggested that while chronic lead ingestion may lead to increased blood pressure, a concomitant ingestion of sucrose as the major source of carbohydrates potentiates the response.\textsuperscript{259} They suggested that, in contrast to other proposals, renal involvement did not appear to be significant due to lack of urine protein. Blood urea nitrogen and serum creatinine did not increase in the rats receiving lead in drinking water. Aviv et al.\textsuperscript{260} earlier reported significant renal damage in rats consuming similar lead levels. Thus, it appears that while there may be an association between lead-induced renal damage with hypertension, a clear cause-and-effect relationship has yet to be established.

Rats exposed to lead in drinking water have decreased cardiac conduction\textsuperscript{261} and increased sensitivity to arrhythmias produced by catecholamine administration.\textsuperscript{262} Lal et al.\textsuperscript{263} using rats as a model, reported only minor changes in the heart itself in response to graded lead levels. Calcium influx across papillary and atrial muscle appeared increased when lead was given to rats. Electrocardiogram (ECG) abnormalities were significantly altered in the lead-exposed rats for this same study, which could be due to the influence upon calcium transport; Myerson and Elsenhauer\textsuperscript{264} earlier had reported similar findings in humans. Goyer\textsuperscript{265} postulated that the inhibition of Na/K ATPase by lead may alter the intracellular concentrations of sodium and calcium. This is thought to elevate plasma renin and cause hypertension. A similar influence of lead upon Na/K ATPase in cardiac myocytes cannot be discounted.

Calcium and lead are known to have antagonistic biochemical roles, as reviewed elsewhere;\textsuperscript{234} however, the influence of calcium upon lead-induced increases in blood pressure appears paradoxical. Feeding rats a high level of calcium does not appear to block or dampen the effect of lead hypertension. Furthermore, Bogden et al.\textsuperscript{266} reported that such a combination actually led to increased renal tumors and nephrocalcinosis.

8.4 Bone Integrity

Lead has a high affinity for bone, partly due to its antagonistic relationship with calcium. The greatest deposit site for lead in the body is the skeleton, which acts as a reservoir. The influence of lead upon bone must be considered from the perspective of vitamin D\textsubscript{3}, the metabolism of which is significantly altered in lead toxicity. Lead is known to influence the various biochemical and physiological events involved with bone remodeling.

Lead may accumulate in bone beginning in fetal life. Young children with growing bones are apparently more susceptible to the toxicity of lead than are adults. Impaired dental development and delayed skeletal maturation have been reported in congenital lead poisoning cases.\textsuperscript{267} In children, the NHANES II (second National Health and Nutrition Examination Survey) study documented decreased height and chest circumference with increased blood lead levels for children 7 years and younger.\textsuperscript{268} Levels of plasma osteocalcin, a bone protein, are lower in children exposed to toxic levels of lead.\textsuperscript{269}
Bone matrix synthesis is impaired with low to high lead exposure. Decreased bone formation rate and radial closure were reported in beagle dogs by Anderson et al.\textsuperscript{270} Osteoblastic activity is enhanced, in addition to a decreased bone formation rate.\textsuperscript{271} Trabecular bone appears less dense in rabbits exposed to lead. Many of the processes of bone turnover are not only attributed to the direct influences of lead on bone dynamics but also indirect mechanisms, as lead may influence hormones targeting calcium and bone homeostasis.\textsuperscript{272} The active form of vitamin D\textsubscript{3}, 1,25-dihydroxycholecalciferol, appears to be lower from children with elevated blood lead levels.\textsuperscript{273,274} These observations have also been reported for rats fed lead acetate in drinking water which resulted in depressed plasma 1,25-dihydroxycholecalciferol levels.\textsuperscript{275} Children with elevated blood levels have demonstrated increased parathyroid hormone levels and a concomitant decline in blood ionized calcium.\textsuperscript{273} The antagonistic hormone, calcitonin, which inhibits bone resorption, inhibits the hypercalcemia induced by high lead levels.\textsuperscript{276-278} As mentioned already, the osteoid proteins are decreased in bone from animals and humans exposed to lead. Type I collagen synthesis is impaired by lead.\textsuperscript{271,279-282} Osteocalcin plasma levels are lower from children with toxic lead levels, but treatment with EDTA returns them to a normal range.\textsuperscript{269} Lead inclusion bodies have been reported in osteoblasts and osteocytes.\textsuperscript{283,284} These bodies appear similar to other organ sites reviewed earlier in this section. Carbonic anhydrase facilitates the acid environment of the osteoclasts, and this activity appears reduced by lead when measured outside of the osteoclasts; however, it is unknown how this observation would be if tested on osteoclasts directly.\textsuperscript{285,286} Many studies have been conducted to investigate calcium and lead interactions. While calcium homeostasis is clearly perturbed by lead, as reported above, the mechanisms are not always direct. Plasma membrane calcium channels, mitochondrial calcium pump, and calcium-activated ATPase changes are direct effects of lead. Changes in adenylate cyclase and Na/K ATPase are indirect, and decreased heme levels may be secondary. For an excellent review of these mechanisms, the reader is encouraged to read the review of Pounds et al.\textsuperscript{272}

8.5 CENTRAL NERVOUS SYSTEM TOXICITY AND BEHAVIOR AND LEARNING CONSEQUENCES

Exposure to low lead levels in growing organisms (e.g., children) lead to behavioral and learning difficulties. The ability of lead to have such consequential effects at relatively low levels gives testimony to its reputation as a potent neurotoxin. Memory loss and learning difficulties have been the subject of numerous reports.\textsuperscript{287-290} Both the hippocampus and cortical brain regions appear to be targeted by lead.\textsuperscript{291,292} Rodents and children may also exhibit impaired visual function.\textsuperscript{293-295} The hippocampus is the region of the brain most often associated with the cellular basis of memory and learning.\textsuperscript{296-298} Learning was impaired along with hippocampal long-term potentiation of afferents when rats were exposed to lead in prenatal life or during early postnatal development, with continued exposure until adulthood.\textsuperscript{299} If exposure to lead began at day 16 of life, no such impairment due to lead exposure was revealed. Hotzman et al.\textsuperscript{299} reported morphology alterations in the glial cells by lead exposure. Swelling of astrocytes and the presence of cytoplasmic electron-dense bodies and intranuclear inclusions are cell responses to lead toxicity. A study with rats in which lead acetate was given to pups at day 7 (via the mother’s milk) was continued for up to 90 days. Starting at day 60, hippocampal astrogial alterations were noted, thereby demonstrating that even postnatal exposure to lead could cause brain alterations.\textsuperscript{300} While the developing nervous system is sensitive to lead, even when exposure has ceased, the functional effects are likely long term and may lead to a permanent deficit.\textsuperscript{293,301,302} Rodent studies in which lead exposure is introduced at the early postnatal stage demonstrated significant decrements in the motor skills and exploratory behavior of rats.\textsuperscript{303} Reduced forepaw grasping ability and ambulation
and rearing in an open field were reported at lead levels that were similar to some doses reported for children.

The mechanism by which lead may affect brain physiology and biochemistry could be due either to a direct influence upon nerve endings or by influence on neurotransmitter release in some fashion. Low lead concentrations enhance the release of neurotransmitters from presynaptic endings.304-307

Depending on the level of lead exposure, children have been reported to have symptoms such as ataxia, convulsions, headache, and learning disabilities and tend to exhibit hyperactive behaviors.308-310 Encephalopathy has been reported in lead-toxic children. Learning and behavioral deficits in children have been reported.301,310,311 Blood levels of up to 1.5 μM, which are comparatively low, could result in such dysfunctions in children.

Blood lead levels have demonstrated an inverse relation to the neuropsychological performance of children.312-316 Children 2 to 4 years of age had lower mental development as blood lead levels increased, even after adjustment for confounding factors.301,317 In the same studies, children with blood lead levels of 1.45 μM had a decrease of 3.3 points on the Bayley Mental Development Index (3.2%) at 2 years of age, and 7.2 points (6.7%) on the McCarthy General Cognitive Index at 4 years of age, when compared to children of similar ages with blood lead levels of 0.48 μM.301,317 A followup study on 494 of these children at age 7 revealed an inverse relation between IQ scores and blood lead levels determined antenatally and postnatally. This was significant even after adjustment for socioeconomic factors, maternal IQ, birth weight, birth order, method of infant feeding, gender, parent’s education level, etc.

Lead has been shown to stimulate the release of dopamine, acetylcholine, and gamma-aminobutyric acid (GABA).318-320 Some of these effects may be due to the ability of lead to alter calcium entry into nerve cells or by an increase in the intracellular calcium level.308 Lead may enter the cells through calcium channels.321 Calcium may also competitively inhibit lead uptake in nonexcitatory cells such as the adrenal medulla and cannot be discounted as a potential mechanism in nerve cells.321 Calcium channel blockers likewise may inhibit lead uptake in the same cells.

Lead is known to substitute for calcium as a second messenger and can bind to calmodulin. In fact, calmodulin has a greater affinity for lead than for calcium.322,323 The calcium-calmodulin complex may activate a kinase referred to as calmodulin protein kinase which is high in nervous tissue and may regulate neurotransmitter release.324 Synapsin I when phosphorylated is believed to have a role in neurotransmitter release.325 If lead acts as calcium in the activation of this kinase, this may explain the ability of lead to result in neurotransmitter release.325

As indicated previously, lead appears to impair hippocampal voltage potentials. Protein kinase C can regulate this activity. Lead may serve to activate this kinase and consequently inhibit this potential, thereby impairing learning in children.326

The encephalopathy induced by lead toxicity is most likely due to a compromise in the blood-brain barrier. Brain edema occurs in the interstitial area and appears due to compromised blood vessel integrity. The brain capillaries and blood vessels have endothelial cells that contain tight junctions and act as a seal or barrier that excludes many plasma proteins and organic molecules and impedes Na and K exchange.327 Elevated lead levels disrupt these vessels, and plasma proteins such as albumin enter the interstitial spaces, as do some ions. This increases osmotic pressure, and water accumulates in response. The lack of lymphatic structures within the central nervous system means that the fluid flows into the cerebrospinal fluid. This edema causes an increase in intracranial pressure and restricts blood flow to the brain, resulting in ischemia.328,329 The direct mechanism by which the blood-brain barrier and blood vessels that compose the barrier may be compromised may be due to the astrocytes appearing to be vulnerable to the toxic effects of lead. The astrocytes cover the vascular walls of the brain vessels, and lead can injure these structures, as reviewed earlier.
8.6 REPRODUCTIVE TOXICITY

Lead toxicity is known to influence male and female reproductive organs in both laboratory animals and humans. An increased incidence of spontaneous abortions have been documented in female lead workers and also in the wives of male lead workers.\textsuperscript{336,330} Male lead workers with blood lead concentrations of 53 to 75 µg/dl have been reported to have decreased sperm counts as well as altered morphology of the sperm.\textsuperscript{331-333} Several animal studies with both laboratory rodents and nonhuman primates support these findings. A long-term study of lead exposure to rats did not reveal any morphological alterations in the testis and epididymis after 9 months of consuming 1% lead acetate in drinking water; however, there was significantly less spermatozoa in all regions of the epididymis as compared to control rats.\textsuperscript{334} The spermatozoa also demonstrated reduced oxidative-reductive enzyme activities in the midsection. Other researchers have failed to demonstrate reproductive effects in rats (e.g., structural alterations of gonads, fertility) either by diet (0.3 mg lead acetate in drinking water) or inhalation (5 mg/m³ lead oxide) after 70 days of exposure.\textsuperscript{335} Male Cynomolgus monkeys administered lead acetate in gelatin capsules over different periods of the life cycle (infancy, post-infancy, and lifetime exposure) all demonstrated increased lipid droplets within the secretory cells of the seminal vesicles. The infancy and post-infancy period were the most affected by lead exposure.\textsuperscript{336}

In rats, injecting pregnant dams daily with lead acetate did not appear to alter the gestation period or number of pups per litter.\textsuperscript{337} Mice, on the other hand, have been observed to have reduced pregnancy and implantation.\textsuperscript{338} One study on rats has suggested that inhalation of lead by rats during pregnancy has minimal effects upon the reproductive function of male offspring.\textsuperscript{339} No change in litter size, death rate, or malformations were reported in the same study. Despite some differences among rodent studies, it would appear from human studies that lead does appear to affect reproductive performance and fertility, and males appear sensitive to the toxic effects of lead.

9 SUMMARY

The trace minerals discussed all exert toxic effects. The distinguishing characteristics are that copper, zinc, iron, and selenium are essential nutrients for humans, whereas mercury, lead, and cadmium are not. Attention historically has been paid to the latter three elements because of known environmental releases and subsequent deleterious actions upon the environment. Selenium originally was investigated because of its toxic effects in an earlier part of this century, and later its role in normal nutrition and interest in the deficiency aspects began to receive attention. Copper, zinc, and iron toxicities have also been studied but have not had the same level of public awareness as some of these other elements. However, given the propensity for the American adult population to use vitamin and mineral supplements and also some genetic conditions, more attention has been focused on potential toxicities. Clearly, these three trace elements exert toxic effects upon humans. More research information would be of value, especially in determining some of the upper levels of safe intake for these elements.

For all of the above elements reviewed, there appears to be a lack of studies reporting on the reversibility of many of these toxic effects. Such information would be of clinical value and would help evaluate the toxic implications as related to the burden placed on the healthcare system for potential treatments, both short and long term.

The above list of elements is by no means exhaustive. Other trace elements are known to have toxic effects upon human health. Iodine, fluoride, and chromium have been studied, but many of the investigations have focused on requirements and deficiency aspects. Iodine-induced hyperthyroidism has been known to occur, as reviewed by Clugston and Hetzel.\textsuperscript{340} Chromium is thought to be rather nontoxic to humans, but cases of skin irritations have
been reported in some individuals through external contact. Nickel, arsenic, and molybdenum also have received increased interest in terms of their human toxicity. Arsenic has enjoyed the dubious distinction of being a highly toxic element, but in reality it is rather nontoxic in terms of the absolute amounts needed and is less toxic than selenium. For more complete discussions on these and other trace elements as related to human toxicity, the reader is referred to the reviews of Nielsen.

REFERENCES


189. Fox, M. R. S., Cadmium metabolism — a review of aspects pertinent to evaluating dietary cadmium intake

190. Shaikh, Z. A. and Lucis, O. J., Cadmium and zinc binding in mammalian liver and kidney,

191. Nordberg, G. F., Garvey, J. S., and Chang, C. C., Metallothionein in plasma and urine of cadmium workers,

192. Bryce-Smith, D.,


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<thead>
<tr>
<th>Reference</th>
<th>Title</th>
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