UNIT 8

Trace Minerals

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I. OVERVIEW

In addition to the minerals already described as members of the macromineral class of nutrients, there are two groups of minerals needed in far smaller amounts. These groups fall into the general class of microminerals. One of these is the trace mineral group that includes iron, copper, and zinc, while the other group, the ultratrace minerals, includes chromium, manganese, fluoride, iodide, cobalt, selenium, silicon, arsenic, boron, vanadium, nickel, cadmium, lithium, lead, and molybdenum. Of these minerals, only four, iron, zinc, iodide, and selenium, have been studied sufficiently to provide a database upon which a recommended dietary allowance (RDA) has been made. Table 1 gives the RDAs for these elements.

Table 1 Recommended Dietary Allowances (RDA) for Iron, Zinc, Iodide, and Selenium

		RDA			
Group	**Age**	**Iron (mg)**	**Zinc (mg)**	**Iodide (mg)**	**Selenium (μg)**
Infants	0–6 months	6	5	40	10
	7–12 months	10	5	50	15
Children	1–3 years	10	10	70	20
	4–7 years	10	10	90	20
	8–11 years	10	10	120	30
Males	12–14 years	12	15	150	40
	15–18 years	12	15	150	50
	19–24 years	10	15	150	70
	25–50 years	10	15	150	70
	51+ years	10	15	150	70
Females	12–14 years	15	12	150	45
	15–18 years	15	12	150	50
	19–24 years	15	12	150	55
	25–50 years	15	12	150	55
	51+ years	10	12	150	55
Pregnancy		30	15	175	65
Lactation	0–6 months	15	19	200	75
	7–12 months	15	16	200	75

Table 2 Safe and Adequate Intakes for Selected Minerals

Mineral	Intake
Copper (Cu)	1.5–3.0 mg/day
Fluoride (F)	1.4–4.0 mg/day
Manganese (Mn)	2.0–5.0 mg/day
Chromium (Cr)	50–200 µg/day
Molybdenum (Mo)	75–250 µg/day

For some of the elements listed above we have an intake recommendation that is "generally recognized as safe and adequate" — abbreviated GRSA. GRSA recommendations (Table 2) have been made for copper, fluoride, manganese, chromium, and molybdenum. While cobalt is known to be needed for microbial synthesis of vitamin B_{12}, an essential micronutrient (see Unit 4, Section IX), an intake recommendation for this mineral has not been made. No intake recommendations have been made for the remaining minerals and it should be acknowledged that many of these are toxic when large exposures occur. Table 3 summarizes these minerals, some of which will be discussed in detail later in this chapter.

Almost all of the trace and ultratrace minerals can be found in the bones and teeth. These minerals are deposited in the organic matrix of these structures as is calcium phosphate. Mineralization is not solely a macromineral event, but one which also involves the other 18 minerals known to be consumed by humans and other animals. These minerals are present in far lower amounts, but nonetheless they comprise part of the mineral apatite that characterizes the skeletal system. These minerals can be mobilized from the bones but the extent of this mobilization is variable. To a large degree, the mobilization of the trace elements is very slow indeed. This results in a long residence time and long half-life. When coupled with a low absorption efficiency, this could be regarded as part of a system designed to protect the body from excess mineral exposure, i.e., mineral toxicity. For many of the trace elements, their discovery as essential nutrients was preceded by their recognition as toxic elements.

II. TOXICITY OF MICROMINERALS

Inadvertent exposure to a variety of minerals, whether it be via inhalation, absorption through the skin, or ingestion with food or drink, can elicit a toxic response. The first line of defense is offered by the gastrointestinal system: vomiting and diarrhea. Through vomiting, contaminated food is expelled. Through diarrhea, malabsorption as well as excretion of a recirculated (via bile) mineral is facilitated, reducing the intestinal exposure and subsequent uptake of the mineral. Failing to ablate the toxic state, the kidney tubules will attempt to reduce the body load; however, some minerals, e.g., copper, iron, zinc, and lead, are not as subject to renal filtration as are other minerals such as magnesium, calcium, molybdenum, etc. Reduction of the body load that is in circulation then is accomplished by depositing the excess mineral in the bones. Bone mineral content has been used to document cases of suspected toxicity. Accidental or intentional poisoning can sometimes be masked by other nonspecific symptoms, but bone analysis can provide the documentation needed to support or deny a supposition of toxicity.

To a lesser extent, hair analysis together with blood analysis can reveal the mineral status of an individual. The difficulty in using these analyses is that the mineral content can be transient. That is, blood levels of trace minerals represent the immediate mineral intake rather than long-term exposure, whereas hair mineral content can represent not only food or drink mineral but also airborne mineral. Hair mineral can be contaminated by shampoos and other hair treatments.

The adverse effects of trace minerals are as diverse as the minerals themselves. Each mineral has its preferred target in the body. For some, the target is DNA. Certain minerals (copper, arsenic,

Table 3 Trace Elements and Their Key Features

Micromineral	Function	Remarks
	Trace Minerals	
Copper	Essential cofactor for a variety of enzymes involved in iron use, collagen synthesis, energy metabolism, and antioxidants.	Wilson's disease and Menckes disease are genetic disorders characterized by disturbed copper homeostasis.
Iron	Essential for hemoglobin synthesis, cytochrome activity, urea cycle activity, lipogenesis, and cholesterogenesis.	Hemochromatosis results from excess intake. Anemia is the major sign of inadequate intake.
Zinc[a]	Essential to the function of over 70 enzymes; important component of DNA binding proteins.	Poor growth in children with renal disease due to excess zinc loss during dialysis. Deficiency may occur with use of diuretics. Zinc loss is increased in traumatized patients.
	Ultratrace Minerals	
Arsenic	Needed for growth and optimal iron use.	Toxic; well-known metabolic poison.
Chromium	Needed for optimal action of insulin at target tissue.	Widely dispersed in a variety of foods. Brewer's yeast and beer are good sources. Deficiency is unlikely.
Cobalt	Important component of vitamin B_{12}.	Deficiency symptoms are those of B_{12} deficiency.
Fluorine	Increases the hardness of bones and teeth, activates adenylate cyclase.	Fluorosis (mottling of teeth) results from excess intake.
Iodine[a]	Needed for thyroid hormone synthesis.	Goiter and cretinism result from inadequate intake.
Manganese	Cofactor in a wide variety of enzymes; essential to reactions using ATP or UTP.	Widely distributed throughout the body. Deficiency is unlikely.
Molybdenum	Activates adenylate cyclase; cofactor in sulfite oxidase and xanthine oxidase.	Widely distributed in the food supply. Deficiency is unlikely.
Nickel	The need for this element has not been shown for humans.	Widely distributed in the food supply. Deficiency is unlikely.
Selenium[a]	Essential to glutathione peroxidase and thyroxine deiodinase.	Toxicity and need are influenced by environmental factors.
Silicon	The need for this element has not been shown for humans but has been shown for animals.	Widely distributed in the food supply. Deficiency is unlikely.
Vanadium	The need for this element has not been shown for humans but has been shown for animals.	Widely distributed in the food supply. Deficiency is unlikely.

[a] An RDA has been set for this mineral (see Table 1).

nickel, chromium) bind to DNA in a cross-link fashion. The binding is a covalent one and produces either a nonfunctional DNA or a DNA which can not repair itself. Evidence of this cross-linking has been demonstrated *in vitro* using a variety of cell types. Chinese hamster cells were used to show copper-induced, chromium-induced, and nickel-induced DNA cross-linking, while human fibroblasts and epithelial cells have been used to show arsenic-induced cross-linking.

The concept of chemically induced cross-linkage of DNA as a factor in carcinogenesis has been proposed to explain the role of asbestos and the development of mesothelioma. Mesothelioma is a malignant growth of the pleural and peritoneal cavities. These tumors are stimulated to grow by the presence of asbestos fibers which act as artificial linkers of DNA, resulting in mutations within the pleural and peritoneal cells. Changes in the CDKN2 (p16) gene seem to be involved. This gene is either lost or mutated. Its gene product is a regulator of the phosphorylation of protein 105, a tumor suppressor. Unphosphorylated p105 can inhibit passage from the G1 to the S phase of the cell cycle whereas phosphorylated 105 permits this passage. Passage inhibition is a common feature of cancer cell initiation. Thus, any substance that interferes with this passage could be regarded as a carcinogen. Minerals in excess can have this effect and excess intakes of some have been linked

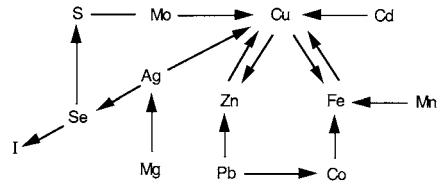


Figure 1 Trace mineral interactions.

with certain forms of cancer. Iron for example has been linked to colon cancer. The mechanism whereby excess iron has its effect is far from clear. It may induce DNA cross-linking as described above, but it may also act as an ion that stimulates free radical formation and attacks cells and their DNA, causing them to mutate. In either scenario, excess iron and colon cancer are associated.

III. ANTAGONISMS AND INTERACTIONS AMONG TRACE MINERALS

No general discussion of trace minerals would be complete without mention of mineral interactions. Numerous antagonisms and synergisms have been reported. This should be expected as one realizes that many of the trace minerals have more than one charged state and that living cells have preferences with respect to these states. For example, the uptake of iron is much greater when the iron is in the ferrous (2+) state than when in the ferric (3+) state. Minerals that keep iron in the ferric state will interfere with its absorption and use. Minerals that do the reverse will enhance iron uptake. Such is the beneficial action of copper on iron. The copper ion (Cu^{2+}) keeps the ferrous ion from losing electrons and becoming the ferric ion. The interactions of essential minerals are best illustrated in Figure 1. To the nutrition scientist seeking to design a purified diet providing all the known nutrients in needed amounts, these mineral interactions can be quite a problem. Sometimes, the so-called purified ingredients contain (or fail to contain) minerals unknown to the producer or user. This is especially a problem in the protein portion of the diet. Proteins can bind minerals (as described in the sections on each of the minerals) and these minerals can be found in the proteins. Soybean protein can contain phytate-bound phosphorus and magnesium; casein and lactalbumin, depending on origin, contain variable amounts of calcium, magnesium, and selenium. Unless the investigator determines the mineral content of the diet ingredients there is the possibility that an unexpected mineral imbalance could occur which could affect the outcome of the experiment. The specifics of these imbalances and interactions will be detailed as each of the minerals is discussed.

IV. IRON

A. Overview

Iron, element 26 in the periodic table, is the fourth most prevalent mineral in the earth's crust. It has an atomic weight of 56. Neolithic man learned to mine iron and to forge tools from iron. The Romans used iron preparations as tonics but the clinical recognition of iron as an essential nutrient was not accomplished until the seventeenth century. Sydenham was the first to propose that chlorosis (a sickness in adolescent females, characterized by a pale skin color) was due to iron deficiency anemia. He showed that iron salts were an effective treatment.

In 1713, Remmery and Jeffrey demonstrated the presence of iron in the mineral matter of blood and in 1852 Funke showed that this mineral was contained by the red cell. Thus, it was learned that iron and the number of red cells were related and that the red cell's function of carrying oxygen depended on its hemoglobin content.

Iron is present in a variety of inorganic salts in the environment. It exists mainly in a trivalent form as ferric oxide or hydroxide or its polymers. Absorption of these salts is very limited unless they can be solubilized and ionized by the intestinal contents. Both ferric and ferrous salts are present in the diet but only the ferrous salts are absorbed from the gastrointestinal tract. Ferric compounds must be reduced in the gastric juice in order to be absorbed.

The availability of iron from food depends on its source. Soybean protein, for example, contains an inhibitor of iron uptake. Diets such as those in Asia contain numerous soybean products and iron absorption is adversely affected by this soybean inhibitor. Tannins, phytates, certain fibers (not cellulose), carbonates, phosphates, and low-protein diets also adversely affect the apparent absorption of iron. In contrast, ascorbic acid, fructose, citric acid, high-protein foods, lysine, histidine, cysteine, methionine, and natural chelates, i.e., heme, all enhance the apparent absorption of iron. Zinc and manganese reduce iron uptake by about 30 to 50% and 10 to 40%, respectively. Excess iron reduces zinc uptakes by 13 to 22%. Stearic acid, one of the main fatty acids in meat, also enhances iron uptake.

In foods, as well as in animal tissues, iron is present in a variety of metalloproteins which include hemoglobin, myoglobin, cytochromes, transferrin, ferritin, and a variety of other iron-binding proteins. In the heme proteins, iron is coordinated within a tetraporphyrin moiety which, in turn, is bound to a polypeptide chain. Hemoglobin is a tetramer with a molecular weight of 64,500 Da and which contains two α-subunits and two β-subunits that give the protein allosteric properties in the uptake and release of oxygen. Each polypeptide subunit contains 1 atom of ferrous iron which amounts to 0.34% of the protein by weight.

B. Absorption, Metabolism, Excretion

Iron uptake by the gut, iron use and reuse, and iron loss comprises a system that for all intents and purposes is a closed system. The gain through the gut is very inefficient and there is virtually no mechanism aside from blood loss that rids the body of its iron excess. This system is shown in Figure 2. The total iron content of the body averages 4.0 g in men and 2.6 g in women. As shown in Table 4, there are two groups of iron-containing compounds that are considered essential to life. Essential iron compounds in the body include hemoglobin, myoglobin, and the cytochromes. In addition, there are a number of enzymes whose active site has an iron-sulfur center. Hemoglobin is the most abundant and easily sampled of the heme proteins and accounts for greater than 65% of body iron. The second group of molecules are those involved in iron transport (transferrin) and storage (ferritin, hemosiderin).

Transferrin is the iron-transport protein that carries ferric iron between the sites of its absorption, storage, and utilization. It is a β-glycoprotein of 76,000 Da which binds 2 atoms of ferric iron per mole. Iron is transferred from the intestinal mucosa to transferrin and is carried through the blood to peripheral tissues containing receptor sites for transferrin. Transferrin is synthesized in the liver, brain, and testes as well as other tissues. The regulation of the gene for transferrin varies from cell type to cell type and each cell type has its own array of promoters and transcription factors that control the amount of transferrin synthesized. The amount of transferrin synthesized is inversely related to the iron supply. In times of low intake, more transferrin is produced so as to optimize iron availability.

Once iron enters the cell it is chelated to a protein which is called ferritin. The enzyme that catalyzes this chelation is called ferrochelatase. This reaction then represents the ultimate destination for the majority of the iron that enters the cell. Chelation of iron to its storage protein occurs at the outer aspect of the mitochondrial membrane. Ferritin has a molecular weight of 450,000 Da. It is composed of 24 subunits which form an outer shell within which there is a storage cavity for polynuclear hydrous ferric oxide phosphate. Over 30% of the weight of ferritin may be iron. It is present in the liver, gut, reticuloendothelial cells, and bone marrow. Its synthesis is highly regulated

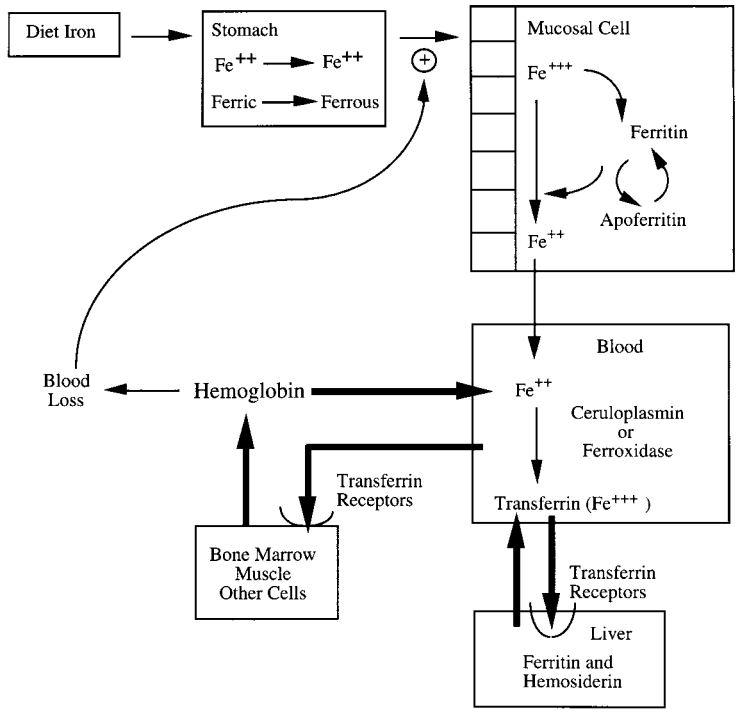


Figure 2 Overview of iron uptake and use showing the apparently closed system which indicates the recycling and conservation of iron once absorbed from the gut.

Table 4 The Body Content of Iron

Types of Iron	Male (70 kg)	Female (60 kg)
Essential Iron	3.100 g	2.100 g
Hemoglobin	2.700	1.800
Myoglobin, cytochromes, and other enzymes	0.400	0.300
Storage and transport iron	0.900	0.500
Ferritin, Hemosiderin	0.897	0.407
Transferrin	0.003	0.003
Total iron	4.000	2.600

by iron at the level of translation. When iron is present, the mRNA is available for translation. In the absence of iron the mRNA folds up on itself such that the start site for translation is covered up. Hemosiderin is a form of denatured ferritin which contains significantly less (~66%) iron.

1. Iron-Containing Materials in the Body

The cytochromes are enzymes involved in the electron transport system which is located principally in the mitochondria. Cytochrome P-450, a specialized cytochrome, is used to oxidize organic compounds. This cytochrome is located in the endoplasmic reticulum. While the cytochrome P-450 enzymes are active in the detoxification of drugs and chemicals, these enzymes also activate carcinogens. Cytochrome P-450 I has this function while P-450 IIE has a propensity to form oxygen radicals which are both cytotoxic and carcinogenic. Other cytochromes generate oxygen radicals

by futile cycling. In some respects, the ability to generate peroxides has a protective effect in that peroxides will kill invading pathogens. Peroxide formation is, in fact, the first line of defense against such an invasion. Other enzymes in which iron is not bound to heme include iron-sulfur proteins, metalloflavoproteins, and certain glycolytic enzymes.

Since the lifetime of a red cell is about 120 days in humans, the flow of iron through the plasma space amounts to about 25 to 30 mg/day in the adult (about 0.5 mg per kilogram of body weight). This amount of iron corresponds to the degradation of about 1% of the circulating hemoglobin mass per day. Iron is conserved in males and postmenopausal females to a great degree; only 10% being lost per year in normal males, or about 1 mg/day. This loss of 1 mg/day has to be made up by absorption of iron from the diet, which is only about 10% efficient, thus requiring about 10 mg of dietary iron per day. In menstruating females the loss is increased to 2 mg/day during the menstrual period of the estrus cycle. This means that the intake and absorption of iron must be increased to avoid iron deficiency anemia.

In contrast to the turnover of hemoglobin in the red cell, tissue iron compounds which include the cytochrome enzymes and a variety of other nonheme enzymes, are heterogeneous in respect to the life span. Furthermore, these compounds are subject to degradation at exponential rates similar to the rate of turnover of the subcellular organelle with which they are associated. For example, in rats, mitochondrial cytochrome C has a half-life of about 6 days, whereas hemoglobin has a half-life of about 63 days.

C. Recommended Dietary Allowance

The apparent absorption of iron, i.e., the amount absorbed from food, can vary from less than 1% to more than 50%. The percentage that is absorbed depends on the nature of the diet, on the type of iron compound in the diet, and on regulatory mechanisms in the intestinal mucosa that reflect the body's physiological need for iron.

Two types of iron are present in the food: namely, heme iron which is found principally in animal products and nonheme iron which is inorganic iron bound to various proteins in plants. Most of the iron in the diet, usually greater than 85%, is present in the nonheme form. The absorption of nonheme iron is strongly influenced by its solubility in the upper part of the intestine. Absorption of nonheme iron depends on the composition of the meal and is subject to enhancers of absorption such as animal protein and by reducing agents such as vitamin C. On the other hand, heme iron is absorbed more efficiently. It is not subject to these enhancers. Although heme iron accounts for a smaller proportion of iron in the diet, it provides quantitatively more iron to the body than dietary nonheme iron.

The regulation of iron entry into the body takes place in the mucosal cells of the small intestine. Its iron gate is very sensitive to the iron stores, so if the iron stores are low, which is true for most women and children, the intestinal mucosa increases its iron uptake efficiency, particularly that of the nonheme iron. On the other hand, if the body is replete with iron, as is typical of healthy men and postmenopausal women, then the percentage of iron absorbed is low. This mechanism offers some protection against iron overload. In infancy, lactoferrin, an iron-binding protein in human milk, promotes the absorption of iron through lactoferrin receptors on the surface of the intestinal mucosa of infants. This may explain why the small amount of iron that is in milk is well absorbed by breast-fed infants. Milk is not usually considered a good source of iron, but for the breast-fed infant this lactoferrin-iron mechanism is important. As the infant grows, however, this mechanism may be inadequate. Iron deficiency can develop, particularly if the hepatic iron stores are insufficient at birth. This can happen in infants of malnourished mothers who themselves are iron deficient.

On the average, only about 10% of dietary iron is absorbed. In order to be absorbed the iron must be in the ferrous state, but upon entry into the enterocyte it is incorporated into ferritin as ferric ion. When the iron is transported from the mucosal to the serosal side of the enterocyte it is

transported in the ferrous state, probably bound to cytoplasmic proteins. When the iron is pumped out of the enterocyte it must be oxidized to the ferric state in order to bind to transferrin. This is accomplished by ceruloplasmin (160,000 Da) which contains 8 copper ions in the divalent state. Ceruloplasmin copper is reduced by the iron, resulting in the formation of cuprous ions in ceruloplasmin and ferric iron in transferrin.

As mentioned earlier, transferrin is recognized in the periphery by cells that have transferrin receptors. The transferrin receptors vary, depending on the tissue and the condition. Tissue such as erythroid precursors, placenta, and liver have a large number of transferrin receptors and have a high uptake of iron. When these cells are in an iron-rich environment, the number of receptors decreases and, conversely, when they are in an iron-poor environment the number of receptors increases. The up- and down-regulation of transferrin receptors is accomplished at the genetic level. Measurements of messenger RNA for the transferrin receptors indicate that there can be as much as a 20-fold change in messenger RNA and, presumably, as much as a 20-fold difference in transcription of the transferrin receptor gene. The change in mRNA and its transcription is related to the concentration of iron.

After iron is delivered to an erythroid precursor cell in the bone marrow, the ferric iron has again to be reduced to the ferrous state in order to be incorporated into the heme prosthetic group. This reduction is accomplished by an NADH-dependent reductase and the insertion of iron in the heme ring is accomplished by another enzyme known as chelatase. When the ferrous iron is inserted into heme associated with specific subunits in hemoglobin, the various subunits polymerize to form the tetramer of hemoglobin A.

The presence of oxygen, of course, tends to oxidize a certain small percent of the iron each day and the formation of ferric iron in the hemoglobin molecule results in its conversion to methemoglobin, which has no capacity to take up and release oxygen. In order to minimize this effect of the oxidation of ferrous iron in hemoglobin by cellular oxygen concentrations, methemoglobin reductase, which is also an NADH-dependent enzyme, reduces the ferric iron in methemoglobin back to the ferrous state which, in turn, regenerates the oxygen-carrying hemoglobin.

1. Iron Needs

A normal red cell lasts for about 120 days in the human circulation and is then taken up by the reticuloendothelial system and degraded. The hemoglobin is degraded into bile pigments to which iron is attached. These pigments are secreted via the bile into the intestinal lumen, contributing iron to the intestinal pool. Iron is recirculated to the extent of about 25 mg/day. Thus, the turnover of iron within the body is 10 to 20 times the amount absorbed. A similar small amount, about 1 mg/day is lost by the sloughing of GI cells and by skin cells. Fecal losses of iron are about 0.6 mg/day. Urinary losses are essentially nil.

In menstruating women, or in individuals with hemorrhage, the iron losses can be considerable and anemia can occur as a result of menstrual losses or bleeding. This is the basis for chlorosis in adolescent girls, who were first identified as iron-deficient in the seventeenth century.

During the childbearing years, females must replace the iron lost in menstrual blood, which over a month amounts to about 1.4 mg/day. During infancy and childhood, about 40 mg of iron are required for the production of essential iron compounds associated with the gain of 1 kg of new tissue. Obviously, the iron needs are great in infants and adolescents. The needs of pregnant women are also great; a total of about 1.0 g of iron is needed to cover both fetal and maternal needs during the course of pregnancy and delivery. It is difficult to obtain this amount of iron from the usual diet. It is estimated that about 30 mg/day of elemental iron is needed in the diet to provide 4 mg/day for absorption.

The RDA for iron varies between 10 and 15 mg/day for different groups, except in pregnancy when it is 30 mg/day. Table 1 gives the RDAs for various groups of males and females.

D. Deficiency Disease

Iron deficiency is probably the most common nutritional deficiency present in the world population. This is true because iron is poorly absorbed and because many diets, especially those consumed by Third World populations, are iron poor. Diets which contain whole grain cereals and legumes contain only nonheme iron which is poorly absorbed. Furthermore, women and children are at constant risk for iron deficiency. Assessment of deficiency includes the determination of levels of tissue and serum ferritin, transferrin, transferrin receptor activity, heme iron, red cell number and size (mean corpuscular volume), hematocrit, and hemoglobin levels.

Clinical iron deficiency anemia occurs in three stages: the first involves depletion of iron stores as measured by a decrease in serum ferritin which reflects the ferritin supply (iron stores) in the body, without loss of essential iron compounds and without any evidence of anemia. The second stage is characterized by biochemical changes that reflect the lack of iron sufficient for the normal production of hemoglobin and other iron compounds. This is indicated by a decrease in transferrin saturation levels and an increase in erythrocyte protophyrin — so-called iron deficiency without anemia. In the final stage, iron deficiency anemia occurs, with depressed hemoglobin production and a change in the mean corpuscular volume of the RBC to produce a microcytic hypochromic anemia. This is expressed clinically as pallor and weakness. There are also changes in the nails, which take on a spoon shape when the iron-deficient state is severe.

In rats made iron deficient, several changes in intermediary metabolism have been reported. These include an increase in peripheral tissue sensitivity to insulin, an increase in hepatic glucose production (gluconeogenesis), a decrease in the conversion of thyroxine to triiodothyronine, decreased heat production, impaired fatty acid oxidation and ketogenesis, an increased need for carnitine, evidence of oxidative damage to the erythrocyte membrane, abnormal monoamine metabolism in the brain (increased dopamine synthesis and down-regulation of dopamine receptors), increased serum triglycerides and cholesterol, and slightly less pentose shunt activity. In addition to these metabolic changes, iron-deficient rats had an impaired immune response to a pathogen challenge. There was a decrease in antibody production and a decrease in the natural killer cell population. Whether these same responses also exist in iron-deficient humans is not certain; however, it is likely that there are numerous similarities.

E. Pharmacological Action

The treatment of iron deficiency anemia is a pharmacologic activity and involves giving large doses of iron, usually equivalent to 60 mg of elemental iron or 300 mg of ferrous sulfate, once or twice a day. It is usually given with meals to minimize gastrointestinal side effects and maximize uptake. Fortunately, the more severe the anemia, the greater will be the percentage of iron absorbed. Iron supplementation is usually continued for 2 to 3 months to normalize hemoglobin levels and iron stores. These should be monitored until satisfactory values are obtained.

F. Toxicology

Iron toxicity is a result of excess iron intake. This can occur acutely in children who ingest iron pills or iron-vitamin supplements, not realizing that they can be toxic. Severe iron poisoning is characterized by damage to the intestine with bloody diarrhea, vomiting, and sometimes liver failure. Systemic effects include hemorrhage, metabolic acidosis, and shock. Lethality occurs at doses in excess of 200 to 250 mg/kg. Effective treatment includes induced emesis (vomiting), food and electrolyte treatment to prevent shock, and the use of iron-chelating agents to bind the iron. This treatment has substantially decreased the mortality from about 50% in 1950 to less than a few percent in recent years.

Chronic overload of iron can result either from chronic excess intake (hemosiderosis, abnormally high levels of hemosiderin) or from a genetic disorder, hemochromatosis. This is the most common form of chronic iron overload. It is a genetic disorder carried on chromosome 6. The disorder is characterized by an increased iron absorption with damage to the pancreas, liver, and heart, and which results in diabetes, liver failure, and heart failure. There is evidence that heterozygous individuals also have increased iron absorption but tissue pathology does not result. The frequency of the homozygous condition is about 0.3 to 0.4% while that of the heterozygous condition is about 10%. Certain populations in the African sub-Saharan regions have a higher percentage of their population with the disorder. The symptoms can vary widely depending both on diet and on whether one or two copies of the aberrant gene are present. Transferrin saturation is a helpful screening test. Greater than 60% saturation is a good indication of the disorder. Phlebotomy on a regular basis can be helpful in reducing tissue damage.

Chronic iron overload has also been found in the South African Bantu tribesmen. In this instance it was of dietary origin. Their traditional maize beer is very high (40 to 80 mg/l) in iron due to the iron containers used to make the brew. Bantu may also be genetically susceptible to iron overload but the possibility requires further study.

Frequent blood transfusions can also lead to iron overload. One unit of blood contains about 200 to 250 mg of iron in its hemoglobin. This is roughly equivalent to 150 to 200 times the usual daily intake; thus 6 to 12 transfusions could lead to iron overload. Persons with hemochromatosis are at greater risk for hepatocellular carcinoma.

In addition to the development of hemochromatosis, there is a growing body of evidence that suggests that chronically high iron intakes are associated with the development of cancer, in particular, colon cancer. Two lines of evidence have been put forth that support this suggestion. The first line concerns the production of free radicals. Iron, in excess, can catalyze the production of free radicals which in turn can damage cell membranes as well as act as mutagens through damaging DNA. At low exposures to mutagens the DNA can repair itself, but free radical damage could be so great that such repair might be inadequate. Free radical damage to circulating lipid-protein complexes, especially the low-density lipoproteins, has also been proposed as a response to iron overload. The second line concerns the fact that cancer cells, like normal cells, require iron as an essential ingredient of metabolism. Having a surplus of iron in the system could increase the survival and proliferation of the cancer cell. Several population studies have provided support for this line of thought. In these studies a dose-response relationship, that is, a correlation of iron intake, ferritin levels, and the development of colon cancer, was found that was highly suggestive of a causal role for excess iron intake in colon cancer development.

Carcinogenesis can also be instigated by other minerals. Nickel subsulfide, for example, is a potent carcinogen having the renal tissue as a target. In the presence of high to moderate iron levels, the activity of the nickel compound is increased. In copper excess due to a genetic disorder involving the protein that transports copper, hepatic cancer develops and is potentiated by high iron levels. It would appear in these last examples that the role of iron is that of a cancer promoter rather than that of an initiator as described for colon cancer.

Although high iron intakes can be harmful, it should be noted that optimal iron intakes can protect against lead toxicity. Lead competes with iron for uptake by the enterocyte. If the transporter is fully saturated by its preferred mineral, iron, then the lead will be poorly absorbed and excreted in the feces. Well-nourished individuals with respect to iron nutriture are at less risk for lead toxicity than are those whose iron intake is marginal or deficient. Unfortunately, humans at risk for lead toxicity are frequently those whose diets are less than optimal. Lead intoxication has anemia as a characteristic because lead substitutes for iron in the hemoglobin molecule and kills the red cell. As the heme is being synthesized lead competes with iron for placement yet does not have the divalent characteristic of the iron so the heme is nonfunctional. Lead-induced anemia therefore is a direct effect of the lead, not a sign of iron deficiency per se. Nonetheless, lead toxicity and iron deficiency frequently coexist.

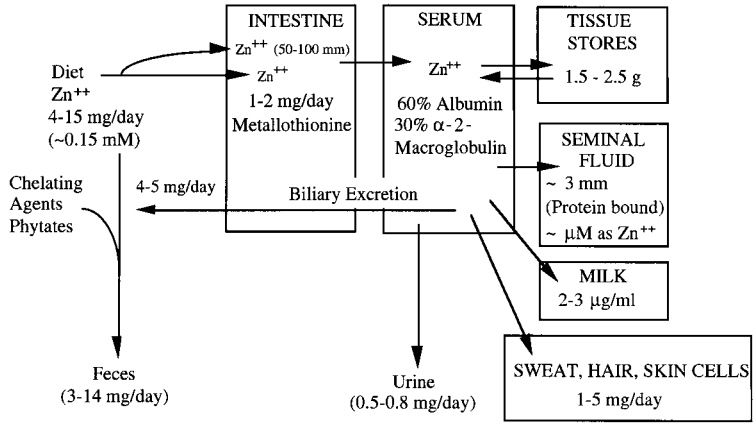


Figure 3 Zinc balance in normal adult humans.

V. ZINC

A. Overview

Zinc is the last transition element in the series of the fourth period of the periodic table. It has an atomic number of 30 and an atomic weight of 65.4. There are 15 isotopes of which ^{65}Zn is the most useful. This radioisotope has a half-life of 245 days.

Zinc is a good reducing agent and will form stable complexes with other ions as well as form a wide range of salts with members of the halogen family as well as with carbonates, phosphates, sulfates, oxalates, and phytate.

B. Absorption, Metabolism, Excretion

Like iron, zinc absorption is relatively poor. Of the approximately 4 to 14 mg/day consumed, only 10 to 40% is absorbed. Absorption is decreased by the presence of binding agents or chelating agents which render the mineral unabsorbable. Zinc binds to ligands that contain sulfur, nitrogen, or oxygen. Zinc will form complexes with phosphate groups (PO_4^{2-}), chloride (Cl^-), and carbonate groups (HCO_3^-) as well as with cysteine and histidine. Buffers such as N-2-hydroxyethyl-pysera-zine-N′-2-ethanesulfonic acid (HEPES) have little effect on zinc binding to these ligands. Clay, a mixed mineral soil fragment, for example, can render zinc unavailable. So too can fiber, phosphate, and phytate (inositol hexaphosphate). Zinc bound in this fashion is excreted via the feces. People who are geophagic (pica) and/or who consume large amounts of phytate-containing foods (mainly cereal products) are at risk for developing zinc deficiency. Oberleas has calculated that diets having a phytate to zinc ratio greater than 10 will induce zinc deficiency regardless of the total zinc content in these diets.

Unlike iron, zinc exists in only one valence state: Zn^{2+}. The normal 70-kg human absorbs 1 to 2 mg/day (Figure 3) using both a nonsaturable and a saturable process. The former is passive diffusion while the latter may involve the zinc-binding metallothionein protein and/or a cysteine-rich intestinal protein. Studies on the mechanisms of zinc absorption by the enterocyte have shown that fast zinc uptake is attributable to extracellular binding of zinc followed by internalization of the zinc ligand mediated by an unknown molecular entity. After entry into the enterocyte the zinc is bound to a cysteine-rich intestinal protein (CRIP) which in turn transfers the zinc to either metallothionine or through the serosal side of the enterocyte to albumin which carries it to its site

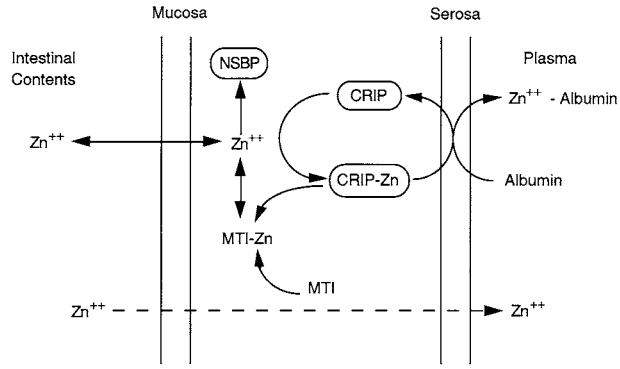


Figure 4 Intestinal zinc absorption. Passive diffusion is shown at the lower part of the diagram while mediated transport involving metallothionen I (MTI), the cysteine-rich protein (CRIP), and the nonspecific binding proteins (NSBP) is shown in the upper portion of the diagram.

Table 5 Enzymes Requiring Zinc as a Cofactor. These Are Representative of the Many Requiring Zinc for Activity

Alcohol dehydrogenase	δ-Aminolevulinate dehydrase
Lactate dehydrogenase	Fructose-1,6-bisphosphatase
Alkaline phosphatase	Transcarboxylases
Angiotensin converting enzyme	Reverse transcriptase
Carbonic anhydrase	Leukotriene hydrolase
Carboxypeptidase A, B, and DD	Phosphodiesterase
Cytoplasmic superoxide dismutase (also requires copper)	Elastase
DNA and RNA polymerases	Adenosine deaminase
Pyruvate dehydrogenase	5′-Nucleotidase
Proteases and peptidases	Glyoxalase
Aspartate transcarbamylase	Transcription factor Sp1
Thymidine kinase	Thymulin

of use. Figure 4 shows this proposed uptake system. Vitamin D enhances zinc uptake probably due to an effect on the synthesis of metallothionein. From the enterocyte it is transferred to the plasma where ~77% is loosely bound to albumin, ~20% is tightly bound to an α-2-macroglobulin, and 2 to 8% is ultrafilterable. This ultrafiltrate is excreted either in the urine (0.5 to 0.8 mg/day) or in the feces via biliary excretion.

The liver appears to be a major site of Zn^{2+} uptake after it has been absorbed. There is both rapid uptake ($t_{1/2}$ = 20 s) and a slower linear uptake.

C. Function

Zinc has two important functions. One is that of serving as an essential cofactor for more than 70 enzymes. Table 5 provides a partial list of these enzymes. In this role, zinc binds to the histidine and cysteine residues of the enzyme proteins and in so doing stabilizes and exposes the active sites of these enzymes such that catalysis of the reaction in question can take place. Figure 5 illustrates this binding. Even though these enzymes require zinc as a cofactor, these enzymes appear to function at near normal levels in deficient animals. In part, this occurs because these enzymes are intracellular enzymes and tenaciously retain the zinc so as to continue to function. There obviously is a hierarchy of zinc need by the living body. Tissue stores of zinc are raided well before the intracellular zinc stores needed by these enzymes. Furthermore, deficient states are characterized by an increase in zinc absorption efficiency, further protecting the system from self destruction.

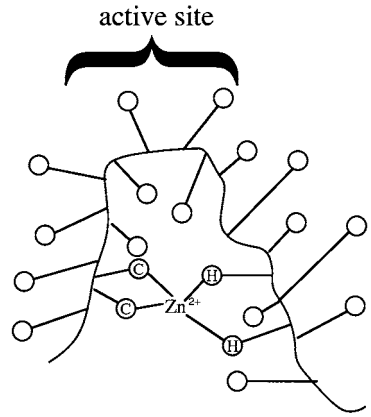


Figure 5 Zinc binds to cysteine (C) and histidine (H) residues of an enzyme protein stabilizing it so as to expose its active catalytic site. The rods and balls are used to represent the various amino acid residues sticking out of the amino acid chain.

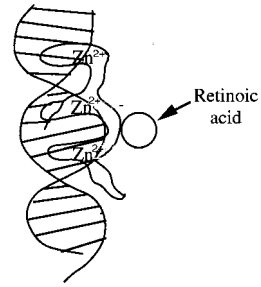


Figure 6 A zinc-DNA binding protein in action. In this instance retinoic acid bound to its zinc-containing DNA binding receptor protein is shown.

Equally important is the binding of zinc to specific DNA binding proteins found in the nucleus. In this role, zinc binds again to histidine and cysteine residues in the same fashion as shown above in Figure 5. This binding to the linear portions of the molecule give it a shape like a string of sausages or a bunch of fingers. These proteins, with zinc attached, are called zinc finger proteins or simply zinc fingers. More than one zinc is attached so that the DNA binding protein can envelop the DNA on both sides. Up to 37 zinc fingers can be part of these transcription factors. Figure 6 provides a visual representation of a zinc finger as it binds to both sides of the double-stranded DNA helix.

A number of nutrients, e.g., vitamin A and vitamin D, and hormones such as the steroids, insulin-like growth factor I, growth hormone, and others have their effects on the expression of specific genes because they can bind to very specific zinc fingers which in turn bind to very specific DNA regions. These DNA binding proteins (transcription factors) also contain leucine in sequence so as to form a hydrophobic region around the binding site for the DNA. Because more than one transcription factor is usually involved in turning on (or turning off) the synthesis of new messenger RNA, the hydrophobic regions allow these factors to associate along the DNA without interacting yet enhancing or suppressing RNA polymerase II action in mRNA synthesis. Each of these transcription factors has a preferred base sequence on the DNA. Retinoic acid as well as steroid hormones bind to factors that prefer the GGTCA sequence. This is the sequence that these proteins recognize and bind. Other transcription factors similarly have preferred base sequences.

If there is a mutation in the genes which encode these DNA binding proteins such that they lack the requisite two residues each of histidine and cysteine in the linear part of their structure, then the functional attributes of these vitamins and hormones at the genetic level will be ablated. Instances of such mutations have been published as well as instances where these zinc fingers have been purposely modified as a therapeutic approach to disease control. Zinc-containing transcription factor Zif268 has been modified with the result of a loss in sequence-specific recognition of DNA by viruses, thus ablating the viral invasion and takeover of their target cells. Although this modification was done *in vitro* and not tested in whole animals, this approach might have therapeutic application in the future as a means to avert the consequences of viral diseases such as AIDS.

Zinc as part of a zinc finger or by itself stimulates the expression of its own transporter protein, metallothionein. Metallothionein exists as two distinct yet related compounds termed MT-1 and MT-2. These proteins are hydrophilic, low molecular weight proteins (6 to 7 kDa) containing a high percentage of cysteine residues (23 to 22 mol%). The function of the cysteine is to bind heavy metals via clusters of thiolate bonds. The synthesis of metallothionein is regulated by zinc through its action on the expression of the genes for these proteins. The level of MT-1 is a very sensitive indicator of zinc deficiency. The metallothionein gene was identified by Palmiter et al. By combining the results of DNA sequence information with the results of deletion mapping studies, unique short sequences of DNA were found that mediated the role of zinc in metallothionein gene expression. The metal response elements were sites for trans-acting transcription factors that bind and enhance the basal rate of transcription of the genes for metallothionein. In the absence of dietary zinc, gene transcription is impaired and metallothionein levels are low. In addition, in zinc-deficient animals numerous breaks in single-strand DNA have been observed. This can be reversed when dietary zinc is restored. Incidentally, transgenic mice that overexpress metallothionein I are very resistant to zinc deficiency. The overexpression of metallothionein increases the absorption efficiency of these mice thus compensating for inefficient absorption.

The cytokine, interleukin 1, has been shown to direct and regulate zinc metabolism in the traumatized or septic individual as well as in normal persons. Interleukin 1 increases the expression of the metallothionein gene thereby increasing zinc uptake through the gut and its transport to, and uptake by, bone marrow and thymus, with relatively less zinc taken up by other body components. Trauma and sepsis both require zinc for new protein synthesis for tissue repair and both conditions are characterized by a rise in interleukin 1 levels in the blood.

Zinc is stored in the β cells of the islets of Langerhans in the pancreas. There, zinc is incorporated into the hormone insulin. Insulin contains two to four atoms of zinc as part of its crystalline structure. Zinc may play a role in insulin release but the details of this role have not been completely elucidated. Pharmaceutical preparations of insulin needed by diabetics for hormone replacement therapy contain zinc. It should be noted that not all species incorporate zinc in the insulin structure.

Zinc can sometimes be displaced on the zinc fingers by other divalent metals. Iron, for example, has been used to displace zinc on the DNA binding protein that also binds estrogen. This protein binds to the estrogen response element of the DNA in the promoter regions encoding estrogen-responsive gene products. When this occurs in the presence of H_2O_2 and ascorbic acid, damage to the proximate DNA, the estrogen response element, occurs. It has been suggested in this circumstance of an iron-substituted zinc finger that free radicals are more readily generated, with the consequence of genomic damage. This suggestion has been offered as an explanation of how excess iron (iron toxicity) could instigate the cellular changes that occur in carcinogenesis.

In excess, cadmium can also substitute for zinc in the zinc fingers. In this substitution, the resultant fingers are nonfunctional. Because of the importance of these fingers in cell survival and renewal, a cadmium substitution is lethal. Cadmium toxicity is an acute illness with little lag time needed for the symptom of cell death to manifest itself.

In addition to its function in metallothionein transcription and as a component of numerous enzymes and in the zinc fingers, zinc also is important for the stabilization of membranes and

provides structural strength to bone as part of the bone mineral apatite. Excess zinc intake can adversely affect copper absorption and also affect iron absorption. Further, excess zinc can interfere with the function of iron as an antioxidant and can interfere with the action of cadmium and calcium as well. Ferritin, the iron storage protein, can also bind zinc. In zinc excess, zinc can replace iron on this protein. Other interactions include the copper-zinc interaction. Copper in excess can interfere with the uptake and binding of zinc by metallothionein in the enterocyte. In humans consuming copper-rich diets the apparent absorption of zinc is markedly reduced. In part this is due to a copper-zinc competition for enterocyte transport and in part due to a copper effect on metallothionine gene expression. Metallothionein has a greater affinity for copper than for zinc and thus zinc is left behind while copper is transported to the serosal side of the enterocyte for export to the plasma, whereupon the copper rather than the zinc is picked up by albumin and transported to the rest of the body. Fortunately, excess copper in the normal diet is not common. Zinc is usually present in far greater amounts and this interaction is of little import in the overall scheme of zinc metabolism.

The metallothionein protein, in addition to binding zinc and copper, also binds other heavy metals such as lead, mercury, and cadmium. This occurs when the individual is acutely exposed to toxic levels of these metals.

D. Deficiency

Until the early 1960's, zinc had not been demonstrated as an essential nutrient for humans. Prasad in 1961 and Halsted in 1958 and 1963 described conditions in humans later found to be due to inadequate zinc intake. Among the symptoms were growth failure, anemia, hypogonadism, enlarged liver and spleen, rough skin, and mental lethargy. These features can be attributed both to the loss of zinc as a cofactor in many enzymatic reactions *and* to the loss of zinc as an essential component of the DNA binding zinc fingers. Detailed studies of populations having these symptoms among its members revealed the custom of clay eating as well as diets that were very low in animal protein and high in cereal products. Geophagia (clay eating) can affect the bioavailability of not only zinc, but also iron and other minerals needed for optimal growth and development. In Iran, Prasad and Halsted found that the provision of iron and protein supplements corrected the anemia and the enlarged spleen and liver. Pubic hair and the gonads also began to develop. It was difficult to explain all of the clinical features (and their reversal) solely on the basis of iron deficiency and/or protein deficiency since other investigators had not reported the features of hypogonadism as part of the iron- or protein-deficient state. However, studies in animals showed that this feature is characteristic of zinc deficiency.

Later, Prasad in Egypt reported on growth retardation and testicular atrophy in young men. Geophagia was not a custom in this group nor were there signs of enlarged spleens and livers. The dietary patterns were similar to those found in Iran in that they were high in cereals and low in animal protein. Zinc concentrations in hair, plasma, and red cells were lower than normal. Zinc turnover using ^{65}Zn was increased above normal and the 24-hr exchangeable pool of zinc was smaller than expected. There were no signs of liver disease or any other chronic disease that would affect zinc status except that the Egyptian subjects were infected with schistosomiasis and hookworms. Studies of populations in Egypt where these infections were absent but where the diets were similar lead to the conclusion that the growth failure and hypogonadism were indeed signs of zinc deficiency. This was proven without a doubt when zinc supplements were provided and these signs were reversed.

The zinc deficiency signs of growth failure and sexual immaturity are the result of an individual's inability to support cell division and hence tissue growth. The skin symptoms are the most obvious because skin cells turn over very rapidly (~7 days). These symptoms include a moist eczematoid dermatitis found in the nasolabial folds and around other body orifices. There is a failure in zinc-deficient individuals to replace these routinely desquamated cells. In infants and young children,

inadequate zinc intake can result in abnormal CNS development as well as impaired skeletal development. In the latter instance, zinc deficiency results in an impaired calcium uptake probably due to a decreased synthesis of intestinal calbindin. Impaired immune response and impaired taste sensitivity also characterize the deficient state. These features again relate to the role of zinc in cell turnover. Immunity requires antibody synthesis involving zinc fingers while the taste sensation involves short-lived epithelial cells on the surface of the tongue and oral cavity. The features of zinc deficiency have been reported in infants having an autosomal recessive mutation in one of the genes which encode the zinc-carrying metallothionein found in the enterocyte (acrodermatitis enteropathica). This condition is treated with oral zinc supplements which, through mass action, provides enough zinc to the enterocyte by passive diffusion. Because only one of the metallothionine genes has mutated, this strategy overcomes the inherent zinc-deficient state.

Other zinc-deficient states have been reported in severely traumatized individuals and in patients with end-stage renal disease with or without dialysis. In renal disease, more zinc is lost via the kidneys than normal. In renal failure, excess zinc is lost through the dialysate when patients are maintained on regular dialysis treatment. Children who are anephric and on dialysis must be monitored with respect to their zinc status. Failure to do so will result in growth failure and lack of sexual maturation. Again, a zinc supplement can reverse these symptoms.

E. Status

Zinc status can be difficult to assess sensitively. Plasma and neutrophil zinc levels can give a static measure of status; however, these blood levels can only evaluate the amount of zinc in transport, not the functional state of the individual. Measurement of alkaline phosphatase, carbonic anhydrase, or 5′-nucleotidase activity is quite useful because these are zinc-requiring enzymes and are sensitive to zinc deprivation more so than the level of metallothionein I in blood. Hair zinc levels have been suggested as indicators of chronic zinc status; however, hair samples are frequently contaminated by zinc-containing shampoos or zinc-containing water. Hair growth rate could also influence hair zinc content. Hence, assessing zinc status using hair samples probably is not very useful. Currently, the intake recommendation is set at 15 mg/day (see Table 1). Assuming that the individual consumes a wide variety of foods and that the intake recommendation for good quality protein is met, the average individual should be well nourished with respect to zinc.

F. Toxicity

Zinc toxicity is not a common occurrence. In some instances it has occurred as a result of food contamination from galvanized food containers. Food or drink can pick up significant quantities of zinc as it leaches from the container into the food. This is particularly a problem when the food or drink is slightly acidic and the storage is prolonged. Symptoms of acute toxicity include nausea, vomiting, epigastric pain, abdominal cramps, and diarrhea. In severe cases the diarrhea can be bloody. Central nervous system symptoms (lethargy, light-headedness, staggering gait, and difficulty with fine finger movement) have been reported in an individual consuming elemental zinc in large quantities.

Chronic excess zinc ingestion in the range of 100 to 300 mg/day in the absence of adequate copper intake can also result in symptoms of toxicity that more nearly replicate those of copper deficiency. These symptoms include low blood copper levels, anemia, leukopenia, and neutropenia. The use of a copper supplement will reverse the condition. Interestingly, once the zinc supplements were discontinued the copper supplement was no longer necessary. At risk for this zinc-copper imbalance are those persons medicating themselves with zinc supplements. Even small supplements (15 to 100 mg zinc per day) may elicit adverse symptoms which may include not only anemia as described above but also disturbances in the serum lipoprotein profile. Decreases in high-density

lipoprotein have been reported in such individuals and these decreases were reversed when the supplement was withdrawn. The mechanism of this zinc-copper interaction probably involves the metallothionein in the mucosal cell. This metal-binding protein has a higher affinity for copper than for zinc, and it may sequester this mineral in preference to zinc, creating a mineral imbalance.

VI. COPPER

A. Overview

Since the Bronze Age, copper alloyed with tin has been used to fabricate a vast array of useful items. However, it has only been in the last few decades that it was recognized as an essential nutrient for humans and other animals. In the 1920s it was recognized that copper, in addition to iron, was needed for hemoglobin synthesis. This came about when anemic animals supplemented with iron did not improve unless copper was also provided. The pioneering work of Cartwright and associates showed the relationship between the two metals in heme biosynthesis. Other roles for copper have since been elucidated.

Copper is a transition metal in the fourth period of the periodic table. It has a molecular weight of 63.4 Da, an atomic weight of 29, and two oxidation states, cuprous (Cu^{+}) and cupric (Cu^{2+}). The 2+ form is biologically active and found in most living systems. There are two naturally occurring isotopes, Cu^{63} and Cu^{65} and two radioisotopes, ^{64}Cu and ^{67}Cu. The former has a half-life of 12.7 hr while the latter has a half-life of 62 hr.

Copper is present in nearly all foods in varying amounts. Dairy products are poor sources of copper while legumes and nuts are rich in this mineral. Raisins, whole grains, beef liver, shellfish, and shrimp are excellent sources as well. Surveys of foods consumed by a variety of population groups in the U.S. indicate a range of intake from 0.7 to 7.5 mg/day. On low intakes, absorption is markedly more (56% of intake) than when intake is high (12% of intake). High levels of zinc, tin, ascorbic acid, and iron adversely affect copper absorption.

B. Absorption, Metabolism, Excretion

Copper absorption takes place in the small intestine and to a limited extent in the stomach. Copper status affects absorption; where need is great, uptake is high. The amount absorbed also depends on the food mixture consumed and on the presence of other divalent minerals which may compete for uptake. Absorption efficiency is low, with an average uptake of 12%. Copper absorption is not affected by phytate. That which is not absorbed is excreted in the feces. Also excreted in the feces is copper transported to the intestine from the liver via the bile. About 2 mg/day is excreted via the biliary route. Copper is also excreted in the urine and lost through the skin and hair. The percent lost via the urine, feces, skin, and hair is between 12 and 43% of the intake. The daily urinary copper is quite small, amounting to 10 to 50 μg/day.

Once copper is absorbed by the enterocyte, it passes to the blood where it is bound to either albumin or transcuprein. The half-life of albumin-bound copper is on the order of 10 min. The copper is then delivered to the liver whereupon it is incorporated into an α-globulin transport protein called ceruloplasmin. Ceruloplasmin can carry six atoms of copper. It has been estimated that from 60 to 95% of plasma copper is transported by this protein. A computer-based prediction model suggests that, in humans, 62 to 72% of the plasma copper is carried by ceruloplasmin. Blood levels of copper are about 1 μg/ml while ceruloplasmin varies from 15 to 60 mg/dl. Ceruloplasmin is not only useful in transporting copper to all parts of the body but also has enzyme activity as a ferroxidase, an amide oxidase, and as a superoxide dismutase. As a ferroxidase it is an active participant in the release of iron from its liver storage sites to transferrin in the plasma. It is active in the conversion of iron from the ferrous to ferric state and in the linkage of the ferric iron to

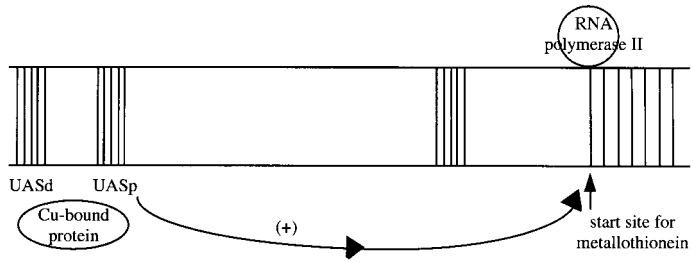


Figure 7 Copper plays a role in the expression of the gene for the cysteine-rich metallothionein by being a part of a DNA binding protein. This protein has several zinc fingers which attach to the UASd and UASp sites. When bound it stimulates transcription.

apotransferrin to form transferrin which, in turn, transports the iron to the reticulocyte for hemoglobin synthesis.

Although ceruloplasmin may indeed be the most active of the copper transporters, other transporters also participate in the delivery of copper to the cells which use and/or store this mineral. Albumin, as mentioned, can serve in this function as can a recently discovered 270-kDa protein (transcuprein) and certain of the amino acids, notably histidine. The liver is the major user and repositor of copper. The copper levels in the liver remain relatively constant. Biliary excretion and ceruloplasmin release are the major mechanisms used to maintain copper levels in this tissue. Ceruloplasmin has a high copper content and accounts for 70 to 90% of the approximately 1 μg/ml copper in the plasma.

Transcuprein has been shown to compete for copper with albumin in the intestine, yet functions in the portal circulation as a donor of copper to albumin. The existence and function of transcuprein in the maintenance of copper status has not been fully explored.

While the transport of copper in the blood has received considerable attention, its transport into the cell has not been as well studied. Copper passes through the plasma membrane via fixed membrane transporter proteins. These membrane proteins may either reversibly bind the copper or form channels through which the copper passes. The kinetics of copper transport have been studied. The Km values are uniformly in the low micromolar range, whereas the Vmax is highly variable depending on cell type, incubation conditions, and media used.

C. Function

Copper, zinc, and iron are all involved in the regulation of the expression of the genes for the metal-binding proteins, the cysteine-rich metallothioneins. These genes have metal response elements that are specific for each of these metals. The one for copper is called CUPI. The gene it affects is the one that encodes metallothionein, a 6570-Da protein which binds heavy metals, particularly copper. The promoter region for this gene does not respond directly to copper. Rather, it responds to an upstream activating sequence (UAS) present as a tandem sequence designated UASp and UASd located between –105 and –108 bp from the transcription start site. It is this sequence that is bound by the zinc and copper DNA binding protein. It would appear that transcription of the metallothionine is a function of both copper and zinc since this metal-binding protein can only be synthesized in the presence of both. This cascade-like process for the expression of the copper-responsive metallothionein is illustrated in Figure 7.

Using the messenger RNA differential display method, ten other genes have been shown to have a copper response element needed for expression. Seven of these had substantial homology with ferritin mRNA, fetuin mRNA, mitochondrial 12S and 16S rRNA, and with mitochondrial tRNA for phenylalanine, valine, and leucine. These homologies suggest roles for copper in mitochondrial gene expression which in turn relate to the observation of decreased oxidative phosphorylation in

Table 6 Enzymes Requiring Copper as a Cofactor

Cytochrome c oxidase
Lysyl oxidase
Tyrosinase
Dopamine-β-hydroxylase (DOPA-4-monoxygenase)
Tyrosine oxidase
Cytoplasmic superoxide dismutase (Cu/Zn SOD)
Amine oxidase
Diamine oxidase
Monoamine oxidase
α-Amidating enzyme
Ferroxidase II
Ascorbate oxidase
Phenylalanine-4-monooxygenase
Metallothionein
Ceruloplasmin

copper-deficient rats. This observation has also been related to the requirement for copper by one of the mitochondrial respiratory enzymes, cytochrome c oxidase. Of the remaining RNAs identified as having a copper response element, no gene product has yet been found. It is possible that these products might be enzymes requiring copper as a cofactor (Table 6) or may be copper transport proteins. This would follow the paradigm of gene expression found with zinc and iron.

D. Deficiency

Copper deficiency is rare in humans consuming a wide variety of foods. One of the major characteristics of copper deficiency is anemia and poor wound healing, similar to that observed in vitamin C deficiency. The anemia is not responsive to iron supplementation. Weakness, lassitude, joint ache, osteoporosis, small petechial hemorrhaging, and arterial aneurysms can all be attributed to the vital role of copper in the synthesis of connective tissue and, in particular, collagen synthesis. Hypertrophy of the heart followed by rupture is a frequently reported feature of copper-deficient rats. Central nervous system degeneration can be related to a decline in respiratory chain activity and ATP synthesis that is essential to neural activity. Reduced immune response has also been reported. Copper-deficient animals have been shown to have decreased T lymphocyte and neutrophil activities.

Other signs of the deficient state include elevated levels of plasma cholesterol, neutropenia, achromatism, twisted kinky hair, and hemacytic hypochromic anemia. Adrenal steroidogenesis is impaired as is catecholamine synthesis. The latter is due to a deficiency of the copper-containing enzyme dopamine beta hydroxylase. Chronic diarrhea and malabsorption have been reported in infants fed copper-deficient formulas. In male rats fed purified diets, the use of pure sugars (mono- and disaccharides) in a high-carbohydrate diet accelerate the development of the copper-deficient state. Probably this has to do with the purity of this dietary ingredient rather than on any copper-sugar interaction at the cell or subcellular level.

E. Abnormal Copper Status

In normal humans, copper intake excess is rare. Although copper toxicity can develop if the exposure is high enough and long enough, the body can protect itself from occasional excess intake by lowering its absorption and increasing excretion via bile and urine. The normal human should consume 1.5 to 3.0 mg/day of copper to maintain an optimal nutritional status. There is some discrepancy between the figure given in the RDA table as an estimated safe and adequate dietary intake (Table 2) and the population studies which indicate that the usual intake is between 1 and 1.5 mg/day. At intakes of 1 to 1.5 mg/day no signs of deficiency have been observed. Turnlund has

reported that young men consuming between 0.75 to 7.53 mg/day were able to attain positive copper balance regardless of intake. Likely, the figure given for optimal intake is a little high because of the paucity of data on copper status under controlled conditions.

There are two rare genetic disorders that have assisted scientists in understanding the function and metabolism of copper. These two disorders affect copper status in opposite directions. In one, Menkes syndrome, copper absorption is faulty. This is an X-linked genetic disorder. Patients with Menkes disease have the symptoms of copper deficiency, i.e., depigmentation of skin, kinky hair, brain damage, muscle and connective tissue abnormality in the absence of anemia, and neutropenia. Intestinal cells absorb the copper but can not release it into the circulation. Parenteral copper can overcome intestinal malabsorption but cannot correct the problem with respect to neural tissue uptake. Infants with Menkes disease are characterized by cerebral degeneration and rarely survive infancy.

Another genetic disorder in copper status is Wilson's disease. This disease is an autosomal recessive disorder associated with premature death and associated excess copper accumulation. It is due to an impaired incorporation of copper into ceruloplasmin and decreased biliary excretion of copper. The genetic defect, like that of Menkes disease, involves the P-type ATPase cation transporters. These transporters play a role in the intracellular transport of copper. Human and mouse gene mapping studies have placed the ATPase cation transporter defect associated with Wilson's disease on chromosome 8 for the mouse and chromosome 13 for the human. This results in an accumulation of copper in the liver and brain. Early signs of Wilson's disease include liver dysfunction, neurological disease, and deposits of copper in the cornea manifested as a ring that looks like a halo around the pupil. This lesion is called the Kayser-Fleischer ring. Renal stones, renal aciduria, neurological deficits, and osteoporosis also characterize Wilson's disease. Wilson's disease can be managed by providing copper-chelating agents such as D-penicillamine and by increasing the intake of zinc which interferes with copper absorption.

F. Copper Need

There is no RDA for copper. However, there are sufficient data that document the need for this mineral. Thus there is a recommendation for an intake that should be safe and adequate. This is shown in Table 2.

VII. SELENIUM

A. Overview

Selenium is one of the "newer" minerals discovered during this century as being both required and toxic, with a relatively narrow range of intake between the two. The existence of selenium as a metal was first reported by Berzelius in 1817 and can occur in nature in a variety of forms and colors. In nature, selenium is frequently found in combination with lead, copper, mercury, and silver. These combinations are called selenides. Its chemistry is similar to that of sulfur. Selenium is an allotropic metal in group 6 of the fourth period of the periodic table. Its molecular weight is 78.96 Da and its atomic weight is 34. Although selenium has 26 isotopic forms, only five of these are naturally occurring: Se^{76}, Se^{78}, Se^{77}, Se^{80}, Se^{82}. Of the radioisotopes, ^{75}Se is the most useful, having a half-life of 118 days. Selenium has three valence states: Se^{2+}, Se^{4+}, and Se^{6+}. It can form selenides, as described above, and selenate. Because it will react with sulfur and oxygen it will form selenomethionine, selenocystine, methylselenocysteine, and dimethylselenide. These compounds are volatile.

Although the presence of selenium in the earth's mineral matter was known, its recognition as an essential nutrient did not occur until Schwarz and Foltz showed that a form of liver necrosis in

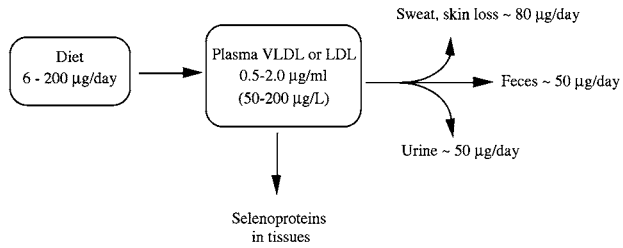


Figure 8 Daily selenium flux in a 70-kg man.

rats could be cured if either vitamin E *or* selenium were administered. This report and others showed that for some purposes these two nutrients were interchangeable. It was this interchangeability that interfered with the identification of selenium as an essential nutrient. In hindsight, we now realize that selenium and vitamin E both play important roles in the detoxification of peroxides and free radicals.

B. Absorption, Metabolism, Excretion

Absorption is very efficient, with most of the ingested selenium absorbed readily from a variety of foodstuffs. The source of the mineral can have effects on its absorption. Figure 8 provides an overview of the daily selenium flux in a normal human.

Selenium is transported from the gut on the very-low- and low-density lipoproteins. Red cells, liver, spleen, muscle, nails, hair, and tooth enamel all contain significant quantities of this mineral. Normal blood values for adults range from 55 to 72 µg/l. People with disorders that are characterized by increased oxidative stress to the red cell, i.e., β-thalassemia, diabetes, and/or smoking tend to have slightly higher blood selenium levels, whereas pregnant and lactating women tend to have lower selenium levels. Excess (greater than 10 ppb in water or 0.05 ppm in food) intake is toxic, but unlike iron and copper which have inefficient excretory pathways, selenium is actively excreted in the urine. The urinary system functions to maintain optimal selenium status.

Figure 9 shows how selenium is utilized. The metabolic use of selenium follows that of sulfur. It involves the formation of a selenium-sulfur bond using the SH group of either cysteine or methionine. From there it is incorporated into one of a group of proteins called selenoproteins. Under normal intake conditions (50 to 200 µg Se per day), about 50 µg can be found in the feces

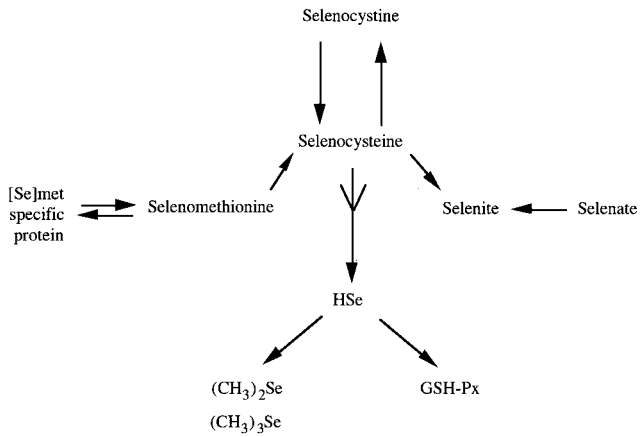
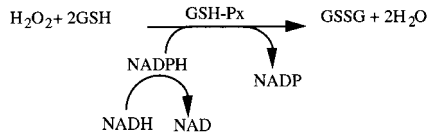


Figure 9 General scheme for selenium metabolism. Selenium is used by almost all cell types because of its vital role in glutathione peroxidase.



GSH-Px	glutathione peroxidase (Requires Se)
GSH	reduced glutathione
GSSG	oxidized glutathione
H_2O_2	hydrogen peroxide

Figure 10 The reduction of oxygen radicals in the red cell. Protection from free radical attack preserves the function of the red cell membrane and the hemoglobin.

and a similar amount is excreted in the urine. Sweat and desquamated skin cells account for another 80 µg/day. These losses can vary depending on dietary conditions. When intakes are low, losses are low, and when intakes are high there are corresponding rises in fecal and urine losses. Diets which include whole grain products, seafood, and organ meats will provide an optimal amount of selenium. In addition to these rich food sources, selenium in lesser amounts can be found in a wide variety of foods. In the U.S., selenium deficiency is rare. This is not the case in China or in some parts of New Zealand and perhaps other parts of the world where the variety of foods available for consumption is severely constrained or reduced to those items produced locally. If the locality is a selenium-poor area, humans as well as domestic animals may become deficient.

C. Function

Selenium is an essential element in a group of proteins called the selenoproteins. The synthesis of these selenoproteins involves sulfur-containing amino acids and selenium. These are joined via selenophosphate to form selenocysteine. The reaction is catalyzed by the enzyme selenophosphate synthetase. Vitamin B_6 serves as a coenzyme in this synthetic reaction.

Of the selenoproteins, glutathione peroxidase (GSH-PX or GPX, EC 1.11.1.9) has been the most widely studied. Glutathione peroxidase catalyzes the reduction of various organic peroxides as well as hydrogen peroxide. This reaction is shown in Figure 10. Glutathione furnishes the reducing equivalents in this reaction. Since membranes contain readily oxidizable unsaturated fatty acids, the stability of these membranes (and hence their function) is dependent on the activity of the antioxidant system of which this selenium-containing enzyme is a part.

If the diet is marginal in selenium but adequate in copper, iron, and vitamins A, E, and C, which also serve as antioxidants, then cell damage by free radicals will be minimized. These other antioxidants can and do suppress free radical formation. While vitamin E serves an important role in suppressing free radical production, its site of action is separate from that of selenium in its role as an essential component of glutathione peroxidase. Studies of selenium-deficient rats given a vitamin E supplement showed that these rats had no change in enzyme activity. Similarly, vitamin E-deficient rats show little improvement in red cell fragility with selenium supplements despite the overlap in antioxidant function of the two nutrients. The antioxidant properties of these cell components, however, are of particular interest to the pharmacologist because many drugs are, in themselves, oxidizing agents that work by disrupting the membranes of invading pathogens.

The aging process as well as carcinogenesis may well be related to the adequacy of the body's antioxidant system. Since free radicals damage cells, these cells may not be replaced at the same rate as the rate of destruction or the damage. That, in turn, may make the cell vulnerable to invasion by cancer-producing viruses or agents that initiate cancer growth. Anticancer drugs, as mentioned, may be strong oxidizing agents. Normal as well as infected cells of the host will be affected by

drug treatment. Should the host's antioxidant system be compromised either by nutritional deficiency or because of some genetically determined deficiency in the maintenance of intracellular redox states, cell survival and normal function will be compromised. Other enzymes shown in Figure 10 include catalase and superoxide dismutase (SOD). Superoxide dismutase is found in both cytoplasm and mitochondria and requires different divalent ions as cofactors. Manganese, zinc, copper, and magnesium function in this respect. Glutathione peroxidase is of major importance to the maintenance of red blood cell redox state. The red cell lacks mitochondria (found in other cell types) which function in these cells in the maintenance of an optimal redox state. The red cell, because it contains the oxygen-carrying hemoglobin, must regulate the redox state so that this hemoglobin can release oxygen in exchange for CO_2. In this exchange, there are opportunities for peroxide formation. These must be suppressed, and glutathione peroxidase does just this.

Glutathione peroxidase is less active in deficient animals than in normally nourished animals. In fact, a decline in enzyme activity is a sensitive indicator of selenium status. At least four isozymes exist and these have been isolated and characterized. The four enzymes are numbered 1 through 4 as GPX1, GPX2, GPX3, and GPX4. Two of these, GPX1 and GPX4, are expressed in most tissues. GPX1 is found in large quantities in red blood cells, liver, and kidney, whereas GPX4 is found largely in testes. GPX2 is found mainly in the gastrointestinal tract and GPX3 is expressed mainly in kidney, lung, heart, breast, and placenta. There are species differences in the expression of these isozymes. In humans, GPX2 and 3 are expressed in the liver. In rodents this is not the case. Rodent liver expresses only GPX1, not GPX2 or 3.

Selenocysteine in proteins is encoded by either a UGA opal codon or the TGA triplet which is normally a stop codon. In order to read the TGA codon as selenocysteine there must be a stem-loop structure in the 3′-untranslated region of the selenoprotein genes. Although this stem-loop structure appears to be absolutely required for the incorporation of selenocysteine into a protein, documentation of this requirement is not fully complete. The active site of the selenium dependent glutathione peroxidase contains a selenocysteine encoded by a UGA opal codon. However, not all of the isozymes are selenium dependent. There is an androgen-induced epididymal cell enzyme that shares sequence homology with GPX3 but is not selenium dependent nor does it have the UGA codon in its mRNA. The GPX2 maps to chromosome 14 while GPX3 and GPX4 map to chromosomes 5 and 19, respectively. GPX1 maps to human chromosome 3 and has sequences which are homologous to those found in chromosome 21 and the X chromosome.

Several drugs have been developed that have antiinflammatory properties and that also contain selenium. They work by catalyzing the degradation of peroxides, much like glutathione peroxidase, or by reducing the production of leukotriene B. Both actions serve to reduce inflammation.

Although approximately 36% of the total selenium in the body is associated with glutathione peroxidase, a number of other proteins in the body also contain this mineral. Table 7 provides a list of these proteins. There are 13 different selenoproteins ranging in weight from 10 to 71 kDa that have been identified. Several of these are glutathione peroxidase isozymes and several have been isolated from a variety of cell lines. One, having thioredoxin reductase activity, has been isolated from human lung adenocarcinoma cells. Another, selenoprotein W, is thought to be responsible for white muscle disease when this protein is not made due to deficient selenium intake.

Table 7 Selenoproteins of Biological Importance

Cytosolic glutathione peroxidase
Phospholipid hydroperoxide glutathione peroxidase
Gastrointestinal glutathione peroxidase
Extracellular glutathione peroxidase
Selenoprotein W
Selenoprotein P
Iodothyronine deiodinase
Sperm capsule selenoprotein

Selenoprotein P, a protein that accumulates in plasma, may be a selenium transport protein but its true function has yet to be elucidated.

Selenium is an integral part of the enzyme, type 1 iodothyronine deiodinase (DI, EC 3.8.1.4) which catalyzes the deiodination of the iodothyronines, notably the deiodination of thyroxine (T_4) to triiodothyronine (T_3), the most active of the thyroid hormones. This deiodination is also catalyzed by type II and type III deiodinases, which are not selenoproteins. While all the deiodinases catalyze the conversion of thyroxine to triiodothyronine, there are differences in the tissue distribution of these enzymes. The pituitary, brain, central nervous system, and brown adipose tissue contain types II and III, whereas type I is found in liver, kidney, and muscle. These two isozymes (II, III) contribute very little triiodothyronine to the circulation except under conditions (i.e., starvation) that enhance reverse triiodothyronine (rT_3) production. In selenium-deficient animals type I synthesis is markedly impaired and this impairment is reversed when selenium is restored to the diet. Under these same conditions, the ratio of T_3 to T_4 is altered. There is more T_4 and less T_3 in the deficient animals and the ratio of the two is reversed when selenium is restored. Because type II and III deiodinase also exist, these enzymes should increase in activity so as to compensate for the selenium-dependent loss of function. However, they do not do this because their activity is linked to that of the type I. When T_4 levels rise (as in selenium deficiency), this rise feeds back to the pituitary, which in turn alters (reduces) TSH release. The conversion of T_4 to T_3 in the pituitary is catalyzed by the type II deiodinase yet TSH release falls. T_4 levels are high because the type I deiodinase is less active. Whereas the deficient animal might have a T_3/T_4 ratio of 0.01 the sufficient animal has a ratio of 0.02, a doubling of the conversion of T_4 to T_3.

The effect of selenium supplementation on the synthesis and activity of the type I deiodinase probably explains the poor growth of deficient animals. Sunde and co-workers have reported significant linear growth in selenium-deficient rats given a single selenium supplement. This growth was directly related to the supplement-induced increase in type I deiodinase activity and to the conversion of thyroxine to triiodothyronine. In turn, the observations of changes in selenium status coincident with changes in thyroid hormone status provided the necessary background for establishing the selenium-iodide interaction that today is taken for granted. Selenium as part of type I deiodinase clearly explains the selenium-iodide interaction. Humans lacking both selenium and iodine show impaired thyroid gland function which in turn results in poor growth, poor mental capacity, and shortened life-spans. In this situation the deficiencies have cumulative effects on the patient. Of interest is the observation that in this dual state there is no thyroid gland enlargement as is typical of simple iodide deficiency.

Other trace mineral interactions also exist. Copper-deficient rats and mice have been shown to have reduced glutathione peroxidase activity. Copper deficiency increases oxidative stress yet oxidative stress affects all of the enzymes involved in free radical suppression. Even though glutathione peroxidase does not contain copper, the expression of the genes for this enzyme and for catalase is reduced in the copper-deficient animal. Other trace minerals are also involved as required components for SOD. Copper, zinc, magnesium, and manganese are part of the antioxidant system as is NADPH and NAD (niacin-containing coenzymes). The NAD, although not usually shown as part of the system, is involved because it can transfer reducing equivalents via the transhydrogenase cycle to NADP. Clearly, there are numerous nutrient interactions required for the maintenance of the optimal redox state in the cell. This is important not only because it stabilizes the lipid portion of the membranes within and around the cells but also because it optimizes the functional performance of the many cellular proteins.

D. Deficiency

Selenium deficiency can develop in premature infants and in persons sustained for long periods of time by selenium-free enteral or parenteral solutions. Symptoms characteristic of deficiency in humans include a decline in glutathione peroxidase activity in a variety of cell types, fragile red

Table 8 Signs and Symptoms of Selenium Deficiency in Animals

Poultry	Exudative diathesis, skeletal myopathy, encephalomalacia, pancreatic necrosis, reduced growth, reduced egg production, reduced fertility, reduced feather growth.
Bovines	Reduced growth, skeletal and cardial myopathy, embryonic death, retained placenta.
Equines	Skeletal myopathy, reduced performance, foals also have muscle steatitis.
Ovines	Skeletal and cardial myopathy, poor growth, reduced fertility, embryonic death.
Porcines	Poor growth, skeletal myopathy, "mulberry heart disease", gastric ulcers, hepatic dysfunction.
Rodents	Skeletal myopathy, erythrocyte hemolysis, testicular degeneration, fetal death and resorption, increased hepatic malic enzyme and glutathione reductase activity.

blood cells, enlarged heart, cardiomyopathy, growth retardation, cataract formation, abnormal placenta retention, deficient spermatogenesis, and skeletal muscle degeneration. Some of these characteristics are shared with other species as shown in Table 8. In China, selenium deficiency is called Keshan disease. It appears mainly in children and is marked by degenerative changes in the heart muscle (cardiomyopathy). This develops in children whose intakes are less than 17 μg/day. Selenite-enriched salt has been shown to reverse this deficiency and the time needed for this reversal in all cells depends on the half-life of the affected cells. Those cells that turn over rapidly will quickly show signs of deficiency and just as quickly show signs of reversal. Should cells that are not normally replaced quickly be affected by the deficient state, that effect will not be quickly reversed by selenium supplementation.

E. Toxicity

Selenium intake in excess of 750 μg/day is toxic. Toxicity does not usually occur unless the individual is exposed not only to high diet levels but also to industrial conditions (smelters, selenium-rich smoke, etc.) that increase entry of the mineral into the body. Selenium toxicity in farm animals has been observed when the food supply for these animals consists of selenium-rich pastures and grain. Selenium toxicity in these animals is characterized by hoof loss and a neuromuscular condition known as "blind staggers." Damage to the liver and muscle is observed as well. Excess selenium intake interferes with zinc absorption and use, reduces tissue iron stores, and increases copper level in heart, liver, and kidney. Clearly, selenium excess upsets the normal trace element balance in the body.

F. Recommended Dietary Allowance

Data are still being gathered for the establishment of the human requirement for selenium. There are, however, sufficient reports in the literature that support the essentiality of its intake. Thus, the NAS-NRC has recommended that an intake no less than 50 and no more than 200 μg/day should be sufficient to meet the needs of the average adult. The RDA is shown in Table 1.

VIII. IODINE

A. Overview

The essentiality of iodine has been recognized since the late 1800s. Its relationship to the production of thyroxine was not fully realized, but even the ancient medical literature recommended the consumption of seaweed or burnt sponges (both of which are rich in iodine) for the treatment of goiter. Iodine deficiency used to be endemic in all but the coastal regions of the world. Presently, it is frequently observed in Third World nations whose access to iodine is limited. With the advent and use of iodized salt and the development of means to process and distribute frozen seafoods, this once common nutritional disorder has all but disappeared.

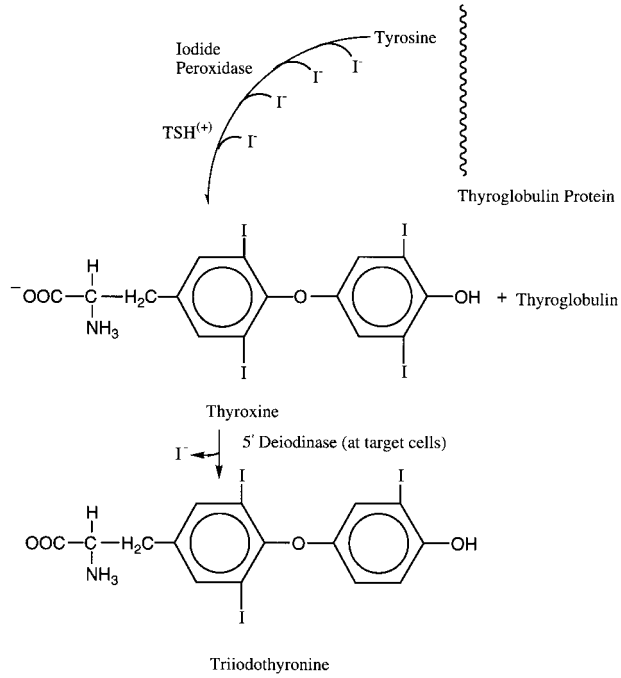


Figure 11 Schematic representation of thyroid hormone synthesis.

Iodine is a member of the halogen family which includes fluorine, chlorine, and bromine. These appear as Group VII in the fifth period of the periodic table. Iodine has an atomic number of 53 and an atomic weight of 127. There are no naturally occurring isotopes but two radioisotopes are useful in biological systems. These are ^{131}I with a half-life of 8 days and ^{125}I with a half-life of 60 days. Iodine is very labile but forms stable salts, the most common is NaI. The iodide ion has a negative one valence, I^-.

B. Absorption, Metabolism, Excretion

The average human in the U.S. consumes between 170 to 250 µg/day. The iodine is converted to the iodide ion (I^-) and is easily absorbed. Once absorbed, it circulates in the blood to all the tissues of the body. Salivary glands, the gastric mucosa, choroid plexus, and the lactating mammary gland as well as the thyroid gland can concentrate iodine. Of the iodine consumed, 80% is trapped by the thyroid gland which uses it for the synthesis of thyroxine. As shown in Figure 11, thyroxine is synthesized through the stepwise iodination of thyroxine. The gland produces and releases thyroxine when stimulated by the pituitary thyroid stimulating hormone (TSH). TSH acts on the tyrosine-rich thyroglobulin, serving to unravel this protein to make tyrosine available for iodination via the enzyme, iodide peroxidase. First, monoiodothyronine is produced, then diiodothyronine, triiodothyronine, and then thyroxine. TSH stimulates the thyroid to release thyroxine which is transported to its target tissues (all the cells of the body) by way of a transport protein called thyroid binding protein. Upon delivery to the target cell the thyroxine is carried into the cell and deiodinated to triiodothyronine. The enzyme catalyzing this reaction is 5′-deiodinase, a selenium-containing enzyme. Triiodothyronine is the active form of the hormone, having at least 10 times the activity of thyroxine. The iodide released by this deiodination is conserved and sent back into the blood-stream for further use by the thyroid gland. Iodide not used or sequestered is either excreted as organic iodine in the feces or as free iodine in the urine. The urinary loss is 40 times greater than the fecal loss. Figure 12 illustrates the endocrine pathway for thyroid hormone metabolism.

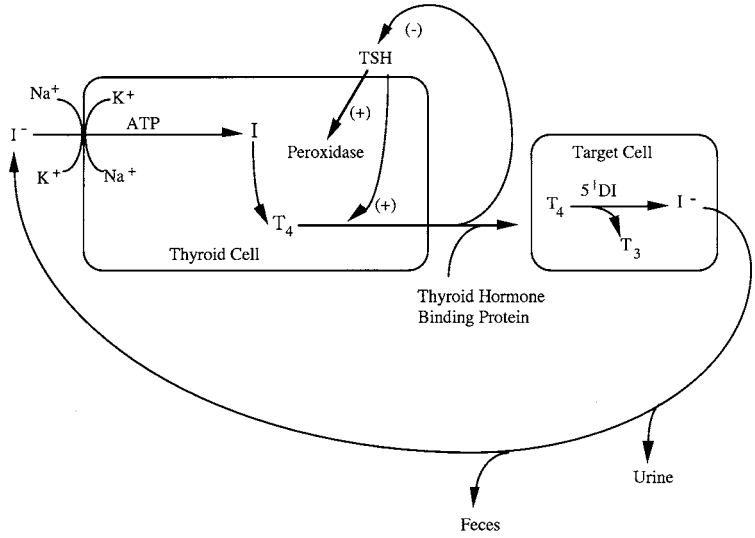


Figure 12 Overview of thyroid hormone production: TSH, thyroid stimulating hormone from pituitary; T_4, thyroxine; T_3, triiodothyronine; 5′ DI, 5′-deiodinase. The thyroid gland is stimulated to produce and release thyroxine by thyroid stimulating hormone (TSH). Thyroxine is carried by a binding hormone to all cells of the body whereupon it is deiodenated to its active form, T_3.

C. Deficiency

The enlargement of the thyroid gland (goiter) is characteristic of iodine deficiency and is marked by a hypertrophic, hyperplasic change in the gland's follicular epithelium. These epithelial cells synthesize thyroxine and their growth is stimulated by the pituitary hormone, thyroid stimulating hormone (TSH). The cells become enlarged when iodine is not available to complete the thyroxine synthetic process. A goiter is not only unsightly but may also cause obstruction of the airways and damage the laryngeal nerves. The synthesis of thyroxine is the only known function of iodine. Thyroid hormone production can be influenced not only by iodine deficiency but also by selenium deficiency. In the latter instance, the active thyroid hormone, triiodothyronine, is not produced in adequate amounts because the deiodination of thyroxine is dependent on the selenoprotein, 5′-deiodinase.

A diet deficient in iodine can cause a number of illnesses depending on the age at exposure. The developing embryo or fetus of an iodine-deficient woman will suffer a neurological deficit that is quite profound. If they survive and are born, these infants are called cretins. Infants and children consuming a deficient diet fail to grow normally and develop intellectually. Children and adults having inadequate iodine intakes develop enlarged thyroid glands and the condition is called goiter. The thyroid hormone producing cells enlarge (hypertrophy) and increase in number (hyperplasia) as a response to the pituitary hormone, TSH. The level of TSH rises as the body senses a need for thyroxine. Pregnant women may lose their pregnancies as the fetus fails to develop and dies in utero.

The deficient state can develop in instances where the population subsists on a marginal iodine intake and consumes a diet rich in vegetables of the *Brassica* genus (Cruciferae family). Although cabbage and related vegetables have been shown to contain goitrogens (compounds that inhibit thyroxine release by the thyroid gland or that inhibit iodine uptake by the gland) other vegetables contain these substances as well.

D. Recommended Dietary Allowance

Because iodine is conserved very efficiently and is not toxic even at 10 to 20 times the required intake, iodine is considered to be a benign (but essential) trace element. An intake of 1 to 2 μg/kg

should be adequate for most adult humans. Pregnant females need slightly (10 to 20%) more. Thus, the Food and Nutrition Board of the National Academy of Sciences has recommended an intake of 150 µg/day for adults, 6 µg/kg/day for infants, 5 µg/kg/day for young children, 4 µg/kg/day for older children, and 175 µg/day for pregnant and lactating females. Table 1 provides the details of these RDAs.

IX. MOLYBDENUM

A. Overview

Molybdenum as an essential nutrient for humans and animals was first recognized in 1953. Its importance in relation to copper and iron was appreciated in that an excess of molybdenum interfered with copper and iron absorption. Molybdenum also interferes with the binding of copper by ceruloplasmin. In ruminants, a molybdenum excess when combined with a high sulfur intake results in a copper-deficient state. The three elements (Mo, Cu, S) combine to form cupric thiomolybdate complexes. In humans, excess molybdenum intakes occur rarely. This is because most human foods that contain molybdenum also contain copper in amounts that exceed that of molybdenum.

Molybdenum is a transition metal in the fifth period of the periodic table. It has an atomic weight of 95.94 and an atomic number of 42. There are several naturally occurring isotopes ranging in weight from 92 to 100; however, Mo^{96} is the most useful in biological research. There are 8 radioisotopes with weights ranging from 88 to 93. Molybdenum has four charged states: 3+, 4+, 5+, and 6+. The most common is the 6+ state. Where this mineral serves as a cofactor in enzymatic reactions, it vacillates between 4+ and 6+.

B. Absorption, Excretion, Function

Molybdenum is readily absorbed from most foods. Between 40 and 100% of the ingested mineral will be absorbed by the epithelial cells of the gastrointestinal system and will be found in the blood in a protein-bound complex. A plasma concentration range of 0.5 to 15 µg/dl has been reported and molybdenum may be found in very small amounts (0.1 to 1.0 µg/g) in most cell types. Some accumulation occurs in the liver, kidneys, bones, and skin. Molybdenum is readily excreted in the urine with small amounts excreted via the biliary route. The average intake of 300 µg/day is 1/3 to 1/5 that of copper.

Molybdenum's function is as a cofactor for the iron- and flavin-containing enzymes, xanthine oxidase, aldehyde oxidase, and sulfite oxidase. The molybdenum cofactor common to these enzymes is a pterin structure shown in Figure 13. This pterin is a monophosphate ester susceptible to cleavage by alkaline phosphatase. One molecule of pterin phosphatase is associated with each molybdenum atom in sulfite oxidase and xanthine dehydrogenase. Molybdenum also serves to activate adenylate cyclase in brain, cardiac, and renal tissue, and erythrocytes. It has no effect on testicular adenylate cyclase nor does it have an effect on adenylate cyclase previously stimulated with fluoride or GTP.

C. Food Sources, Recommended Intake

Molybdenum is found in very small amounts in most foods. The germ of grain is a good source of this mineral; however, much of this germ is lost when the grain is milled. No recommended

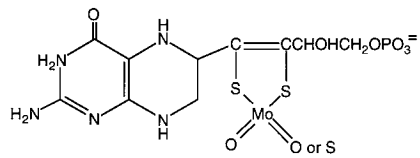


Figure 13 Pterin structure with molybdenum as part of its molecule.

dietary allowance has been set but the Food and Nutrition Board of the National Academy of Sciences has estimated that an intake of 75 to 250 μg/day for adults should be safe and adequate. For infants the recommendation is 15 to 40 μg/day, and for children an intake of 25 to 75 μg/day should suffice. Table 2 shows these recommendations.

X. MANGANESE

A. Overview

Although manganese (Mn) was recognized as an essential nutrient in 1931, its ubiquitous role as a cofactor in a variety of enzymatic reactions has only recently been appreciated. Structural abnormalities in growing birds and animals are the chief symptoms of deficiency. Depression of mucopolysaccharide synthesis and decreased mitochondrial manganese superoxide dismutase activity accompany these skeletal abnormalities, as does congenital ataxia due to abnormal inner ear development and abnormal brain function. Biochemical abnormalities were hard to document because many of the requirements for manganese as a cofactor in enzymatic reactions could be met by magnesium. Nonetheless, bone and connective tissue growth are abnormal in manganese-deficient animals. Few cases of human manganese deficiency have been reported.

Manganese, like magnesium, is a transition element. It can exist in 11 oxidation states. However, in mammalian systems it usually exists in either the 3+ or 2+ state. In the 2+ state it is less easily chelated than in the 3+ state. The 3+ state is one which interacts with the ferric ion (Fe^{3+}); it is also the state needed for service as a cofactor in the Mn superoxide dismutase enzyme.

In contrast, manganese toxicity with symptoms resembling Parkinson's disease has been reported to occur in up to 25% of miners and metal mill workers in Russia, North Africa, Chile, and the former Yugoslavia. The inhalation of mineral dust in mines and mills is the main route of excess exposure.

B. Absorption, Excretion, Function

Manganese is poorly absorbed through the gut. Between 2 and 15% of that ingested in the food appears in the blood. This has been traced using radioactive ^{54}Mn. As with many minerals, the route of excretion is via resecretion into the small intestine. That which is unabsorbed appears in the feces as does that which is excreted from the body via the bile. The mechanism whereby manganese is transported from the site of absorption to the site of use is not fully known. It may share transport with iron using transferrin or another protein, notably α-2-macroglobulin. Manganese circulates throughout the body at a level of 1 to 2 μg/dl (~2 μmol/l). Widely varying blood levels of manganese have been reported. Smith et al. contend that this wide variation has to do with contamination introduced by both sampling technique and analytical technique. Contamination can double the amount of manganese in the food, blood, or tissue sample. It is for this reason that balance studies to determine apparent absorption are not useful. Actually this problem is common to many of the trace minerals. They are present in such small amounts in biological samples that extreme precautions must be taken to control contamination. Once transported from the gut to the liver almost all of the manganese is cleared from the blood. In the liver it enters one of several pools. It can be found in the bile canaliculi from which it can be exported via the bile back into the small intestine, or it can enter the hepatocytes whereupon it is used by its various organelles (lysosomes, mitochondria, nucleus) as a cofactor for several enzymes, or it can remain in the cytoplasm.

Table 9 Enzymes Requiring Manganese

Pyruvate carboxylase
Acetyl CoA carboxylase
Isocitrate dehydrogenase
Mitochondrial superoxide dismutase
Arginase
Glucokinase
Galactose transferrase
Hydroxymethyl transferase
Superoxide dismutase

Manganese is present in the bone as part of the mineral apatite, and in the lactating gland and liver to a greater extent than in other tissues. However, no tissue is free of this mineral. The turnover of manganese in the body varies depending on location. It is very short (~3 min half-life) in hepatic mitochondria, and in bone its half-life is about an hour. The pool size likewise varies from 2 mg/kg in liver to 3.5 mg/kg in bone. As mentioned, it is an essential cofactor in a wide variety of enzymatic reactions. Listed in Table 9 are some of the many enzymes shown to require manganese.

Manganese and calcium share a uniport mechanism for mitochondrial transport. It can accumulate in this organelle because it is cleared very slowly. Mitochondrial manganese efflux is not sodium dependent and in fact appears to inhibit both the sodium-dependent and -independent calcium efflux. This manganese effect on calcium is not reciprocal; calcium has no effect on manganese efflux.

It has been reported that manganese deficiency in experimental animals causes the down-regulation of the mitochondrial manganese containing superoxide dismutase (SOD) at the level of the activation of transcription of the gene which encodes this protein. Superoxide dismutases are a class of metalloproteins that catalyze the dismutation of the superoxide radical (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2). These enzymes play a critical role in protecting cells against oxidative stress, particularly that produced by drugs. Most mammalian cells contain two forms of this enzyme: one in the cytosol requiring zinc and copper and the other requiring manganese. It is generally thought that the latter, present in the mitochondria, protects that organelle from potential damage by the superoxide radical that could possibly be produced through the activity of the respiratory chain. The important role of Mn SOD has been demonstrated using cell cultures. Those cultures in which the gene for this enzyme was altered, or in which the enzyme was inhibited, died. Apoptic cell death has been reported in cultured spinal neurons and in PC12 neuronal cells. Maintenance of mitochondrial manganese-dependent SOD is especially important if a drug is used that disrupts the control or function of the respiratory chain. Redox active drugs, e.g., antibiotics, tetracycline, or pesticides such as paraquat, have this effect. When these compounds are given to animals or cells in culture, the activity of the manganese superoxide dismutase increases. Interleukin-1 and tumor necrosis factor also increase the activity of this enzyme. Some tumor cells respond to chemotherapeutic agents by increasing the activity of SOD. As a result, these tumors are resistant to certain cytotoxic agents. This is particularly true for melanoma cells and may explain the resistance of this cancer to many of the therapies found successful for other tumor cell types.

C. Food Sources, Recommended Intake

Rich food sources include nuts, whole grains, and leafy vegetables. Meats, milk, seafood, and other animal products are poor sources. The germ of grains can contain up to 14 ppm. The average diet consumed in the U.S. contains about 3 to 4 mg/day, the range suggested by the National Academy of Sciences as being safe and adequate (see Table 2). A recommended dietary allowance has not been established for this nutrient.

XI. COBALT

A. Overview

The discovery of cobalt as an essential nutrient for mammals was first made in Australia by animal scientists seeking to understand the pathophysiology of a wasting disease in sheep and cattle. At first it was thought to be an iron deficiency disorder because iron supplements seemed to cure the condition. The iron supplements, however, were rather impure substances. In the mid 1930s Underwood and associates discovered that it was not the iron per se but an impurity in the supplement that cured the condition. That impurity was cobalt.

The essentiality of cobalt as a nutrient for humans was assumed based on the results of the above animal studies. Many human foods, especially green leafy vegetables, contain cobalt. Meats, including the organ meats, provide cobalt as a component of vitamin B_{12}. Of the free cobalt found in these foods, very little is used although it is absorbed by the enterocytes. The results of tracer studies have shown that almost 100% of ingested cobalt appears in the urine. Very little appears in the feces and very little is retained in the tissues. As discussed in Unit 4, Section IX, the absorption of vitamin B_{12} (and its cobalt component) is dependent on the presence of an intrinsic factor in the stomach. Whereas in ruminants the consumption of cobalt cures the wasting disease (pernicious anemia) it does this not because the ruminant can absorb and then use this metal, but because the rumen flora can synthesize vitamin B_{12} which in turn is absorbed. This then is the basis for understanding why vegetarians consuming large amounts of cobalt-rich green leafy vegetables are at risk for developing pernicious anemia. While they obtain sufficient cobalt they can not synthesize vitamin B_{12}. Cobalt has no other known function aside from its central action in vitamin B_{12} function.

B. Toxicity

Although cobalt is readily absorbed by the enterocyte, it is just as readily excreted by the kidney. Thus, toxicity in the usual sense is not a significant problem with respect to environmental exposures. Excess cobalt (1000 times normal) can be tolerated by a variety of species with little ill effect. However, cobalt does interfere with the absorption of iron and in fact can completely block iron uptake. Symptoms of iron deficiency anemia as well as symptoms of disturbed thyroid function (enlarged hyperplasic thyroid gland, myxedema) and congestive heart failure have been observed when excess cobalt was consumed accidentally as a contaminant of beer. Cobalt salts were once used in the production of beer as a foaming agent. While the occasional beer would not provide toxic amounts of cobalt, large intakes consumed regularly could and have done so. Once this was recognized, this production practice was discontinued. Actually, it was not the cobalt content of the beer alone that was responsible for the toxic response. The alcohol in the beer plus the likelihood of inadequate protein, iron, and B vitamin intake were also part of the problem.

C. Requirement

Because cobalt serves no other function than as a constituent of vitamin B_{12}, there is no requirement for this mineral. If the vitamin B_{12} requirement is met, then the cobalt need is met.

XII. OTHER MINERALS

Shown in Table 3 are a variety of minerals which have been shown to be essential to one or more species, not necessarily humans. Included in this list is fluorine, an element known to provide hardness to teeth and bones, and which also inhibits tooth decay. In excess, it is toxic. Arsenic, a known poison, has been shown to be essential to chickens, rats, pigs, and goats, but its biological

function in humans is not known. It is thought to have a role in bone metabolism. Animals with arsenic deficiency exhibit depressed growth, myocardial degeneration, and premature death. Boron, likewise, is an essential nutrient for rats but its function is not known. Deficient rats have a reduced stress response which probably relates to a change in brain electrical activity. Boron-copper and boron-calcium-phosphate interactions have been reported. These interactions have to do with increased brain copper concentrations in the former and increased calcium and phosphate concentrations in the latter in boron-deprived animals.

Chromium was shown by Schwartz and Mertz to be essential for optimal peripheral insulin action with respect to glucose uptake. Studies of elderly humans with noninsulin-dependent diabetes mellitus showed an improvement in glucose tolerance in about half of the subjects following a period of chromium supplementation. The mechanism of action of chromium as a potentiator of insulin action has yet to be elucidated. Nonetheless, because a chromium supplement has been shown to be of benefit to some persons with diabetes, a safe and adequate recommendation for intake (Table 2) has been made.

Nickel is another mineral whose function has not been defined, yet animals fed nickel deficient diets fail to thrive. Nickel is a component of the urease enzyme family and although not found in mammalian urease has been found in ureases isolated from lower life forms. Similarly, nickel has been found associated with hydrogenases in lower life forms and, in these species has a role in oxidation-reduction reactions as well as in methane formation.

Silicon has been found essential to chickens and rats and appears to be involved in bone formation. Skeletal abnormalities typify silicon deficiency in these species.

Vanadium is also needed by chickens for appropriate pigmentation; however, whether this need is a true need or one induced by the particular diet ingredients used to compose the deficient diet is subject to discussion.

Aluminum, boron, tin, cadmium, lead, germanium, lithium, and rubidium have all been examined with respect to their essentiality. Data are lacking that document a need for these elements in humans.

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