All elements that have not been assigned separate chapters in this volume fit into the category of elements that have become known as the ultratrace elements. Ultratrace elements are those with estimated dietary requirements usually <1 µg/g and often <50 ng/g of diet for laboratory animals. At least 18 elements have been suggested to be ultratrace elements: aluminum, arsenic, boron, bromine, cadmium, chromium, fluorine, germanium, iodine, lead, lithium, molybdenum, nickel, rubidium, selenium, silicon, tin, and vanadium. The quality of the experimental evidence supporting the suggestion of nutritional essentiality varies widely among these elements. The evidence for the essentiality of three elements, iodine, molybdenum, and selenium, is quite substantial and noncontroversial; specific biochemical functions have been defined for these elements. Iodine and selenium are discussed in Chapters 36 and 31, respectively; molybdenum will be discussed here. Specific biochemical functions have not been identified for the other 15 elements. Thus, their essentiality is based on circumstantial evidence; that is, a dietary deprivation consistently results in a suboptimal biological function that is preventable or reversible by an intake of physiological amounts of the element in question. The circumstantial evidence for essentiality is substantial for arsenic, boron, chromium, nickel, silicon, and vanadium; thus, except for chromium, which is discussed in Chapter 34, they will be discussed in detail here. The evidence for essentiality of the other elements is generally limited to a few gross observations in one or two species by one or two research groups. Because it was judged premature to discuss these elements in detail here, these elements will be only briefly mentioned in table form at the end of this chapter. However, fluoride, which has a well-known beneficial pharmacologic property (anticariogenic), is discussed in Chapter 32.

Arsenic

History. Although arsenic has been considered synonymous with poison for centuries, its bad reputation did not prevent it from becoming an important pharmaceutical agent. By 1937, the pharmacologic actions of 8000 arsenicals had been recorded. Arsenicals were considered at various times to be specific remedies for the treatment of anorexia and other nutritional disturbances, syphilis, neuralgia, rheumatism, asthma, chorea, malaria, tuberculosis, diabetes, various skin diseases, and numerous hematologic abnormalities. The use of arsenicals for these disorders has either fallen into disrepute or been replaced by more effective alternatives.

Reports describing attempts to produce a nutritional arsenic deficiency first appeared in the 1930s. The first substantial evidence for arsenic essentiality was published in 1975 and 1976. Arsenic deprivation signs were described for rats, pigs, and goats. Subsequently, signs also were described for chickens and hamsters. Thus, it is only recently that arsenic has been studied from the biochemical, nutritional, and physiological, and not only the toxicological or pharmacological, points of view.

Chemistry and method of analysis. Both the trivalent and pentavalent states of arsenic exist in biologic material. The most biochemically important organic arsenic compounds are those that contain methyl groups. The methylation of inorganic oxyarsenic anions occurs in organisms ranging from microbial to mammalian. The methylated end products include arsenocholine, arsenobetaine, dimethylarsinic acid, methylarsonic acid, trimethylarsine oxide, and tetramethylarsonium ion.

Other arsenic compounds of interest are those possibly formed when arsenate replaces phosphate in biologic molecules. The relatively unstable nature of arsényl esters apparently is the reason that only indirect evidence exists for compounds such as glucose-6-arsenate and adenosine diphosphate-arsenate. Nonetheless, arsenate ester might be the form of arsenic that performs an essential function.

A comprehensive review of arsenic chemistry and biochemistry has been published. One of the most precise and sensitive methods for the determination of arsenic in biological material involves measuring arsine generated from dry combusted samples by graphite-furnace atomic absorption spectrometry.

Absorption, transport, storage, and turnover. Absorption of inorganic arsenic from the gastrointestinal tract correlates well with the solubility of the compound.
The form of organic arsenic also determines how well it is absorbed. For example, >90% of an oral dose of arsenobetaine was recovered in the urine of hamsters; 70–80% of an oral dose of arsenocholine was recovered in the urine of mice, rats, and rabbits; and 45% of an oral dose of dimethylarsinic acid was recovered in the urine of hamsters. In contrast, >90% of an oral dose of sodium-p-N-glycolylarsenilate was recovered in the feces of rats or humans within 3 days of administration; urinary excretion accounted for only 4–5% of the dose. Also, most orally administered arsenosugars are not absorbed from the gastrointestinal tract.

Arsenate and phosphate, despite structural similarities, do not share a common transport pathway in the duodenum. The absorption of arsenate can be separated into two components. First, arsenate becomes sequestered primarily in or on the mucosal tissue. Eventually, the sites of sequestration become filled, with concomitant movement of arsenate into the body. The absorption of arsenate apparently involves a simple movement down a concentration gradient. In rats, some forms of organic arsenic are absorbed at rates directly proportional to their intestinal concentration over a 100-fold range. This finding suggests that organic arsenicals are absorbed mainly by simple diffusion through lipid regions of the intestinal boundary.

Once absorbed, inorganic arsenic is transferred to liver, where it is methylated. Thus, blood contains both inorganic (probably protein bound) and methylated forms of arsenic. In 56 healthy volunteers consuming a diet high in organic arsenic, mean blood total arsenic was 7.3 µg/L and was 73% trimethylated arsenic, 14% dimethylated arsenic, and 9.6% inorganic arsenic. Before arsenate is methylated, it is reduced to arsenite via the use of glutathione. Methylation takes place in liver with S-adenosylmethionine as the methyl donor. In humans, the final product, dimethylarsinic acid, results from the methylation of the monomethylarsenic acid precursor formed from arsenite. The methylation of arsenic can be modified by changing the glutathione, methionine, and choline status of the animal.

The fate of absorbed organic arsenic depends on its form. For example, arsenobetaine passes through the body into the urine without biotransformation. Some orally ingested arsenocholine appears in the urine, and some is incorporated into body phospholipids similarly to choline; however, most is biotransformed to arsenobetaine before being excreted in urine.

If the ingestion of arsenic is low, no tissue has significant accumulation of arsenic. The highest amounts of arsenic are usually found in skin, hair, and nails, probably the result of arsenite binding to SH groups of proteins that are relatively plentiful in these tissues.

The metabolism of arsenic in some animal species is quite unusual. For example, rats, unlike other mammals, concentrate arsenic in their erythrocytes. Marmoset monkeys are unable to methylate arsenite, which is a major reaction in the elimination of arsenic from the body for most animals. Studies with rabbits, hamsters, and chickens seem to give findings on the metabolism of arsenic most applicable to humans.

The excretion of ingested arsenic is rapid, principally in urine. Only minor amounts are removed through sweat, loss of hair and skin, and bile. A reported example of the proportions of the forms of arsenic in human urine after an oral dose of inorganic arsenic is 51% dimethylarsinic acid, 21% monomethylarsinic acid, and 27% inorganic arsenic. The proportions are quite different, however, with the consumption of organic arsenic. For example, an analysis of urine from 102 Japanese students who consumed luxuriant amounts of organic arsenic in seafood revealed 9.4% inorganic arsenic, 3.0% monomethylarsinic acid, 28.9% dimethylarsinic acid, and 58.2% trimethylated arsenic compound. Similar findings were obtained in another study of 56 healthy volunteers.

**Physiological (biochemical) function.** The evidence suggesting that arsenic is essential does not clearly define its biochemical function. Recent findings suggest that arsenic affects the formation of various metabolites from methionine (e.g., S-adenosylmethionine, S-adenosyl homocysteine, cysteine, and taurine) and arginine (e.g., putrescine, spermidine, and spermine) or affects labile methyl-group metabolism. Arsenic deprivation depressed the concentrations of putrescine, spermidine, and spermine in liver of rats fed marginal amounts of methionine and depressed the taurine concentration in the plasma of hamsters.

Perhaps arsenic has a role in some enzymatic reactions. As an enzyme activator, arsenic as arsenate probably substitutes for phosphate. As an inhibitor, arsenic as arsenite apparently affects enzymes by reacting with sulfhydryl groups.

Arsenic may also regulate gene expression. Arsenite can induce the cellular production of certain proteins known as heat-shock or stress proteins. The production of these proteins in response to arsenite apparently is controlled at the transcriptional level and may involve changes in the methylation of core histones. Recent findings have shown that arsenic deprivation in the rat, chick, and hamster affects labile methyl metabolism. Also, arsenic enhances DNA synthesis in unsensitized human lymphocytes and in those stimulated by phytohemagglutinin.
In goats, miniature pigs, and rats, the most consistent signs of arsenic deprivation were depressed growth and abnormal reproduction characterized by impaired fertility and elevated perinatal mortality. Other notable signs of deprivation in goats were depressed serum triacylglycerol concentrations and death during lactation. Myocardial damage was also present in lactating goats. The organelle of myocardium most affected was the mitochondrion, which was affected at the membrane level; in advanced stages, the membrane actually ruptured. Other signs of arsenic deprivation have been reported. Listing these signs is problematic because studies with chicks, rats, and hamsters have revealed that the nature and severity of the signs of arsenic deprivation are affected by several dietary manipulations, including variations in the concentrations of zinc, arginine, choline, methionine, taurine, and guanidoacetic acid (a methyl-depleting agent). The signs of arsenic deprivation were changed and generally enhanced by nutritional stressors that affected sulfur amino acid or labile methyl-group metabolism. However, some recently reported responses to arsenic deprivation that may be significant are decreased glutathione S-transferase activity and increased kidney calcium concentrations in female rats fed the AIN-76 diet.40

Requirement. Only data from animal studies are available for estimating the arsenic need of humans. An arsenic requirement of <50 ng/g and probably =25 ng/g was suggested for growing chicks and rats fed an experimental diet containing 20% protein, 9% fat, 60% carbohydrate, 11% fiber, minerals, and vitamins.42,43 Thus, the arsenic requirement is apparently between 6.25 and 12.5 µg/4.18 MJ (6.25 and 12.5 µg/1000 kcal). From these data a possible arsenic requirement for humans eating 8.37 MJ (2000 kcal) would be =12–25 µg/day.42,43 A safe upper limit of arsenic intake most likely will be 140–250 µg/day.42,43

Food and other sources. The reported arsenic content of diets from various parts of the world indicates that the average daily intake of arsenic is generally 12–40 µg.44,46 However, the dietary arsenic intake by a typical Japanese person (high-seafood diet) was found to be 195 µg/day (range 16–1039 µg/day).18 Fish, grain, and cereals contribute most of the arsenic to the diet.

Excess (toxicity). Because of mechanisms for the homeostatic regulation of arsenic, its toxicity through oral intake is relatively low; it is actually less toxic than selenium, an ultratrace element with a well-established nutritional value. Toxic quantities of inorganic arsenic generally are reported in milligrams. For example, the estimated fatal acute dose of arsenic trioxide for humans is 70–180 mg, or =0.76–1.95 mg As/kg body weight.47

The ratio of the toxic to the nutritional dose for rats apparently is near 1250. Some forms of organic arsenic are virtually nontoxic; for example, a 10 g/kg body weight dose of arsenobetaine (common form of arsenic in food) depressed spontaneous motility and respiration in male mice, but these signs disappeared within 1 hour.48 Arsenocholine is slightly more toxic than arsenobetaine; a dose of 5.8 g/kg body weight caused death in some rats, but a dose of 4.8 g/kg did not.49

Briefly, the signs of subacute and chronic high exposure of arsenic in humans include the development of dermatoses of various types (hyperpigmentation, hyperkeratosis, desquamation, and loss of hair); hematopoietic depression; liver damage characterized by jaundice, portal cirrhosis, and ascites; sensory disturbances; peripheral neuritis; anorexia; and weight loss.50–52

Results of numerous epidemiologic studies have suggested an association between chronic arsenic overexposure and the incidence of some forms of cancer. Although the role of arsenic in carcinogenesis remains controversial, arsenic does not seem to act as a primary carcinogen and is either an inactive or extremely weak mutagen.53

Summary. Until more is known about the biochemical and physiological functions of arsenic, it is inappropriate to associate specific disorders with deficient arsenic nutrition. At present, it is important to recognize the likelihood that arsenic is essential for humans. Thus, the belief that any form or amount of arsenic is unnecessary, toxic, or carcinogenic is unrealistic, if not potentially harmful.

Boron

History. In the 1870s it was discovered that pharmacological amounts of borax and boric acid could be used to preserve foods. For about the next 50 years, borates were considered some of the best preservatives for extending the palatability of foods such as fish, meat, cream, and butter. In 1904, however, Wiley54 reported that human volunteers consuming >500 mg of boric acid per day for 50 days displayed disturbed appetite, digestion, and health. Subsequent to this report, the opinion that boron posed a risk to health gained momentum; by the middle 1950s boron was essentially forbidden throughout the world as a food preservative.

In 1923 Warrington55 showed that boron is an essential element for plants. About 15 years later, attempts to demonstrate boron essentiality for higher animals began; these attempts were unsuccessful.56–60 Thus, before 1980, students of biochemistry and nutrition were taught that boron was a unique element because it was essential for plants but not for higher animals. In 1981 it was reported that boron stimulated growth and partially prevented leg abnormalities present in cholecalciferol-deficient chicks.61 Since then, evidence has been accumulating indicating
that boron is an essential nutrient for higher animals including humans.

**Chemistry and methods of analysis.** Boron exists in biological material mainly bound to oxygen. Thus, boron biochemistry is essentially that of boric acid. Dilute aqueous boric acid solutions comprise \(\text{B(OH)}_3\) and \(\text{B(OH)}_4^-\) species at the pH of blood (7.4); because the \(pK_a\) of boric acid is 9.2, the abundance of these two species should be 98.4% and 1.6%, respectively.62

Boric acid forms ester complexes with hydroxyl groups of organic compounds; this preferably occurs when the hydroxyl groups are adjacent and cis.63 Among the hydroxylated substances of biological interest with which boron complexes are adenosine-5-phosphate, pyridoxine, riboflavin, dehydroascorbic acid, and pyridine nucleotides. Formation of these complexes may be biologically important because, in vitro, it results in the competitive inhibition of some enzymes.62 These include oxidoreductases that require cis-hydroxyl–containing pyridine or flavin nucleotides as cofactors.

The added stabilization of hydrogen bonding between hydroxyls bound to boron and hydrogen of imidazole or amido groups allows complexes to be formed between borate and compounds containing single hydroxyl groups. Through forming this type of complex, borate and boronic acid derivatives can form transition analogues that inhibit the activity of some enzymes.64 For example, serine hydrolases are inhibited when a tetrahedral complex is formed between the serine hydroxyl group and boron, with hydrogen bonding to an imidazole ring of an adjacent histidine adding stabilization.62

Two naturally occurring organoboron compounds have been identified; they contain boron bound to four oxygen groups. These compounds are aplanomycin, a novel ionophoric macrolide antibiotic isolated from strain ss-20 of *Streptomyces griseus*, and boromycin, an antibiotic synthesized by *Streptomyces antibioticus*.55,65 Boromycin can encapsulate alkali metal cations and increase the permeability of the cytoplasmic membrane to potassium ions.

Only recently have methods been developed that can determine low concentrations of boron in biological substances with acceptable accuracy. Development of such methods has been difficult because many boron compounds volatilize at temperatures far below those required for most dry or wet ashing procedures, and most forms of glassware and chemical reagents contain significant amounts of boron. Procedures that have been developed to digest biological substances with minimal boron loss or contamination include a low-temperature wet digestion in semiclosed teflon tubes and a teflon bomb digestion in a microwave oven.57,64 Inductively coupled argon plasma spectroscopy is generally used to determine the boron content of the digestates.67,68

Prompt gamma activation analysis and neutron activation–mass spectrometry (NA-MS) techniques have been developed that can accurately measure the usual or normal concentration of boron in biomaterials.69 One major advantage of these techniques is that they do not require the destruction of the organic matrix containing boron. For example, with NA-MS, a freeze-dried sample is irradiated to generate \(^{4}\text{He}\) from \(^{10}\text{B}\); the \(^{4}\text{He}\) is measured by mass spectrometry. The sophistication and cost of the equipment precludes either of these methods from becoming of general laboratory use.

**Absorption, transport, storage, and turnover.** Sodium borate, boric acid, and possibly food boron are rapidly absorbed and are excreted largely in the urine. Because there is no usable radiisotope of boron, the study of its metabolism has been made difficult. However, it is likely that most ingested boron is converted to \(\text{B(OH)}_3\), the normal hydrolysis end product of most boron compounds and the dominant inorganic species at the pH of the gastrointestinal tract. It is postulated that boron is absorbed and secreted mainly as undissociated \(\text{B(OH)}_3\). The mechanism by which boron is transported through the body has not been defined. Recently, an inductively coupled plasma–mass spectrometry method using the ratio of the two stable isotopes, \(^{11}\text{B}/^{10}\text{B}\), was developed to study boron metabolism.70 This method was used to show that boron in broccoli, intrinsically enriched with \(^{10}\text{B}\), was absorbed as well as extrinsic boron \(^{10}\text{B}\) in boric acid from a test meal by rats. When 20 µg of \(^{10}\text{B}\) isotope were fed to rats, 95% of this isotope was detected in the urine and 4% in the feces after 3 days. This agrees with other urinary recovery findings indicating that >90% of ingested boron is usually absorbed.71,72

Boron is distributed throughout soft tissues and fluids of animals and humans at concentrations mostly between 0.015 and 0.6 µg/g fresh tissue.52,73-75 Bone, fingernails, hair, and teeth usually contain several times these concentrations.

Evidence showing that boron is homeostatically controlled includes the rapid urinary excretion of absorbed boron, the lack of accumulation of boron in tissues, and the relatively narrow range of boron concentrations in blood of apparently healthy individuals. In a group of 50 blood samples collected from hospitals and clinics in the United Kingdom, the serum boron concentration ranged from 0.77 to 4.45 µmol/L (8.4 to 48.1 ng/mL), with a median of 2.06 µmol/L (22.3 ng/mL).76 In postmenopausal women, an increase in dietary boron from 0.36 mg/day (probably deficient) to 3.3 mg/day (luxuriant) did not increase plasma boron concentrations when dietary magnesium was 340 mg/day; however, a 2.4-fold increase occurred when dietary magnesium was 109 mg/day (C.D. Hunt and F.H. Nielsen, unpublished data, 1987). Increasing dietary boron from 0.465 (deficient) to 2.465 (luxuriant) mg/kg diet increased the plasma boron concentration by only 50% in cholecalciferol-deficient chicks.76,77 As with other mineral elements, overcoming homeostatic mechanisms by high boron intakes will elevate tissue boron concentrations.
Physiological (biochemical) function. A biochemical function for boron has not been elucidated, even for plants for which boron has been known for 70 years to be essential and for which boron deficiency has a multiplicity of effects.\textsuperscript{78,79} Two hypotheses recently advanced for the biochemical function of boron in higher animals accommodate a large and varied response to boron deprivation and the known biochemistry of boron. Hunt\textsuperscript{77} proposed that boron is a metabolic regulator through complexing with a variety of substrate or reactant compounds in which there are hydroxyl groups in favorable positions. On the basis of the knowledge that two classes of enzymes are competitively inhibited in vivo by borate or its derivatives and his findings showing dietary boron can alter the in vivo activity of a number of these enzymes, Hunt hypothesized that the metabolic regulation by boron is mainly negative; that is, boron controls a number of metabolic pathways by competitively inhibiting some key enzyme reactions. Nielsen\textsuperscript{80} hypothesized that boron has a role in cell membrane function or stability such that it influences the response to hormones by modulating transmembrane signaling or transmembrane movement of regulatory cations or anions. This hypothesis is supported by the recent findings that boron influences the transport of extracellular calcium and the release of intracellular calcium in rat platelets activated by thrombin and that boron influences redox actions involved in cellular membrane transport in plants.\textsuperscript{79,81}

Deficiency signs. The listing of the signs of boron deficiency is difficult because most boron-deficiency studies have used stressors to enhance the response to changes in dietary boron. Thus, it has been found that the response to boron deprivation varies as the diet varies in its content of nutrients such as calcium, phosphorus, magnesium, potassium, and cholecalciferol.\textsuperscript{82} However, although the nature and severity of the changes may vary with dietary composition, many findings indicate that boron deprivation impairs calcium and energy metabolism. For example, a boron supplement of 3 μg boron/g alleviated the cholecalciferol-deficiency-induced distortion of marrow sprouts of chick proximal tibial epiphyseal plate and elevated the number of osteoblasts within the marrow sprouts.\textsuperscript{76} Boron also substantially alleviated or corrected cholecalciferol-deficiency-induced elevations in plasma glucose, changes in energy substrate use, and depressions in growth.\textsuperscript{77}

Brain composition and function are also affected by dietary boron. Boron deprivation was found to systematically influence brain electrical activity assessed by an electrocorticogram in mature rats; the principal effect was on the frequency distribution of electrical activity.\textsuperscript{45} In this study, brain copper concentrations were higher in boron-deprived than in boron-supplemented rats. Furthermore, calcium concentrations in total brain and in brain cortex, as well as the phosphorus concentration in the cerebellum, were found to be higher in boron-deprived than in boron-supplemented rats fed a cholecalciferol-deficient diet.\textsuperscript{84}

Some of the preceding findings may reflect an effect of dietary boron on macromineral metabolism. The apparent absorption and balance of calcium, magnesium, and phosphorus were found to be higher in boron-supplemented (2.72 μg boron/g diet) than in boron-deprived (0.158 μg boron/g diet) rats fed a cholecalciferol-deficient diet.\textsuperscript{84}

Findings involving boron deprivation of humans have come mainly from two studies in which men over the age of 45, postmenopausal women, and postmenopausal women on estrogen therapy were fed a low-boron diet (0.25 mg/8.37 MJ [0.25 mg/2000 kcal]) for 63 days and then fed the same diet supplemented with 3 mg boron/day for 49 days.\textsuperscript{81,85-89} These dietary intakes were near the low and high values in the range of dietary boron intakes (0.5–3.1 mg/day) found in a limited number of surveys.\textsuperscript{90} In the first experiment the diet was low in magnesium (115 mg/8.37 MJ) and marginally adequate in copper (1.6 mg/8.37 MJ) throughout the study.\textsuperscript{85,86} In the second experiment the diet provided 300 mg magnesium and only 1.7 mg copper/8.37 MJ for the first 32 days; from day 33 onward, the diet was supplemented to contain 2.4 mg copper/8.37 MJ.\textsuperscript{87,88} Thus, the major differences between the two experiments were the intakes of copper and magnesium; in one experiment they were marginal or inadequate, in the other they were adequate. Among the effects of boron supplementation after 63 days of boron depletion in these experiments were the following: an effect on macromineral and electrolyte metabolism evidenced by increased serum 25-hydroxycholecalciferol and decreased serum calcitonin (with low dietary magnesium and copper);\textsuperscript{90} an effect on energy substrate metabolism suggested by decreased serum glucose (with low dietary magnesium and copper) and increased serum triglycerides (with adequate dietary magnesium and copper);\textsuperscript{85,88} an effect on nitrogen metabolism indicated by decreased blood urea nitrogen and serum creatinine and increased urinary hydroxyproline excretion;\textsuperscript{81,85,87,88} an effect on oxidative metabolism indicated by increased erythrocyte superoxide dismutase and serum ceruloplasmin;\textsuperscript{81,85} and an effect on erythropoiesis and hematopoiesis suggested by (all with adequate dietary magnesium and copper) increased blood hemoglobin and mean corpuscular hemoglobin content but decreased hematocrit, platelet number, and erythrocyte number.\textsuperscript{89} Boron supplementation after depletion also enhanced the elevation in serum 17β-estradiol and plasma copper caused by estrogen ingestion, altered electroencephalograms such that they suggested improved behavioral activation (e.g., less drowsiness) and mental alertness, and improved psychomotor skills and the cognitive processes of attention and memory.\textsuperscript{80,89}

Requirement. For normal development, chicks apparently require ≈1 μg boron/g diet.\textsuperscript{91} In the human studies
composition of the diet. Recent surveys indicate that the daily intake of molybdenum is 50-350 µg. However, most diets apparently supply =50-100 µg molybdenum/day; thus, many diets do not meet the minimum level of the suggested safe and adequate intake. The richest food sources of molybdenum include milk and milk products, dried legumes, organ meats (liver and kidney), cereals, and baked goods. The poorest sources of molybdenum include vegetables other than legumes, fruits, sugars, oils, fats, and fish.

**Excess (toxicity).** Large oral doses are necessary to overcome the homeostatic control of molybdenum. Thus, molybdenum is a relatively nontoxic element; in nonruminants an intake of 100-5000 mg/kg of food or water is required to produce clinical toxicity symptoms. Ruminants are more susceptible to elevated dietary molybdenum. The mechanisms of molybdenum toxicity are uncertain. Most signs are similar or identical to those of copper deficiency (i.e., growth depression and anemia) or indicate abnormal sulfur metabolism. In humans, both occupational and high dietary exposure to molybdenum have been linked through epidemiologic methods to elevated uric acid in blood and increased incidence of gout.

**Summary.** The essentiality of molybdenum is unquestioned. Biochemical functions have been defined for molybdenum, and signs and symptoms of molybdenum deficiency have been described. However, except for the molybdenum-responsive patient with "acquired molybdenum deficiency" resulting from long-term use of total parenteral nutrition, there is no indication that molybdenum is clinically important. The search for possible molybdenum-responsive syndromes in humans is still warranted because situations may be occurring where molybdenum nutriture is important. For example, low dietary molybdenum might be detrimental to human health and well-being through an effect on the detoxification of xenobiotic compounds. Molybdenum deprivation depresses the activity of the molybdenum hydroxylases without any apparent overall detrimental effect in animals; perhaps the same phenomenon occurs in humans. Low molybdenum hydroxylase activity may have undesirable consequences when a person or animal is stressed by high intakes of xenobiotics. The molybdenum hydroxylases apparently are as important as the microsomal monoxygenase system in the metabolism of drugs and foreign compounds.

**Nickel**

**History.** Although nickel was first suggested to be nutritionally essential in 1936, strong evidence for essentiality did not appear until 1970. Studies between 1970 and 1975, however, gave inconsistent signs of nickel deprivation, probably because of suboptimal experimental conditions. Since 1975, diets and environments that allow optimal growth and survival of laboratory animals have been used in studies of nickel nutrition and metabolism. Thus, most of the significant biochemical, nutritional, and physiological studies of nickel have appeared subsequent to 1975.

**Chemistry and method of analysis.** Monovalent, divalent, and trivalent forms of nickel apparently are important in biochemistry. Like other ions of the first transition series, Ni⁺ can complex, chelate, or bind with many substances of biological interest. The binding of divalent nickel by various ligands, including amino acids (especially histidine and cysteine), proteins (especially albumin), and a macroglobulin called nickelo- plasmin, probably is important in the extracellular transport, intracellular binding, and urinary and biliary excretion of nickel. Ni²⁺, in a tightly bound form, is required for the activity of urease, an enzyme found in plants and microorganisms. In the microbial enzyme, methyl coenzyme M reductase, nickel is present in a chromophore called factor F₄₃₀. Coenzyme M, which is involved in methane formation in anaerobic bacteria, is 2,2'-dithiodiethane sulfonic acid. Factor F₄₃₀ is a tetrapyrrole similar in structure to vitamin B₁₂, and the formation of factor F₄₃₀ also requires the presence of Ni²⁺.

Ni²⁺ apparently is essential for enzymatic hydrogenation, desulfurization, and carboxylation reactions in mostly anaerobic microorganisms. In some of these reactions, the redox action of nickel may involve the 1+ oxidation state, especially in that of methyl-coenzyme M reductase. Nickel also acts as a structural component in some enzymes.

The determination of nickel in biological material after appropriate collection and preparation is most precisely done with great analytical sensitivity through the use of electrothermal atomic absorption spectrometry. This high absorption is depressed by certain foodstuffs and simple substances, including milk, coffee, tea, orange juice, ascorbic acid, and ethylene diamine tetraacetic acid (EDTA). Foods such as those found in a typical Guatemalan meal or in a North American breakfast suppress the absorption of nickel to <1%. Thus, nickel is often poorly absorbed (<10%) when ingested with typical diets. Nickel absorption is enhanced by iron deficiency, pregnancy, and lactation. Pigs were found to absorb >19% of nickel ingested from day 21 of pregnancy until parturition.

The mechanisms involved in the transport of nickel through the gut have not been conclusively established. Becker et al. reported that the transport of nickel across the mucosal epithelium apparently is an energy-driven process.
process rather than simple diffusion and suggested that nickel ions use the iron transport system located in the proximal part of the small intestine. On the other hand, Foulkes and McMullen\textsuperscript{143} presented evidence that indicates no existence of a specific nickel carrier mechanism at the brush-border membrane; thus, nickel absorption probably depends upon the efficiency of mucosal trapping through charge neutralization on the membrane. This suggests that nickel crosses the basolateral membrane through passive leakage or diffusion, perhaps as part of an amino acid or other low-molecular-weight complex. The passage as a lipophilic complex is a possibility because nickel affects the absorption of ferric ions, which probably traverse biomembranes as lipophilic complexes.\textsuperscript{144} Oral intakes of lipophilic nickel-pyridinethione complexes markedly increased the concentrations of nickel in tissues of mice.\textsuperscript{145}

The extracellular transport of nickel is probably through a variety of ligands; however, the principal ligand in blood apparently is serum albumin.\textsuperscript{151} The remaining nickel in serum is associated with the amino acid L-histidine and with $\alpha$-macroglobulin.\textsuperscript{131,146}

No tissue significantly accumulates orally administered physiological doses of nickel. Recently reported reference values for nickel concentrations in some human tissues are (mean $\mu$g/kg dry weight) lung, 173; thyroid, 141; adrenal, 132; kidney, 62; heart, 54; liver, 50; brain, 44; spleen, 37; and pancreas, 34.\textsuperscript{147} The physiological significance of the relatively high nickel concentrations in thyroid and adrenal glands is unknown.

Although fecal nickel excretion (mostly unabsorbed nickel) is 10–100 times as great as urinary excretion, the small fraction of nickel absorbed from the intestine and transported to the plasma is rapidly excreted via the kidney as urinary low-molecular-weight complexes. In human renal cytosol the low-molecular-weight fraction contains two nickel-binding components; these are anionic oligosaccharides that bind 70% of the nickel and an acidic peptide that binds the remaining 30%.\textsuperscript{148} High-molecular-weight proteins ranging from 10 to 13 kDa were also fractionated from renal cytosol and microsomes.\textsuperscript{149} The role of these proteins in the renal handling of nickel needs clarification.

Although urine is the major excretory route of absorbed nickel, significant amounts are lost through sweat and bile.\textsuperscript{150,147,150} The nickel content of sweat is high, which indicates active nickel secretion by the sweat glands.\textsuperscript{150} The loss of nickel through the bile has been estimated at 2-5 $\mu$g/day.\textsuperscript{147}

**Physiological (biochemical) function.** A defined biochemical function for nickel in higher animals, and thus humans, has not been described. Recently, however, functional roles for nickel have been defined for bacteria, fungi, plants, and invertebrates. These roles may provide clues as to the nature of the biological function of nickel in higher animals; thus, some of them are described here.

Since the discovery in 1975 that jackbean urease is a nickel-containing enzyme, evidence has accumulated indicating that nickel is a universal component of ureases (urea amidohydrolases, EC 3.5.1.5).\textsuperscript{132} Nickel has been found in ureases from bacteria, mycoplasma, fungi, yeast, algae, higher plants, and invertebrates. Highly purified urease contains two Ni\textsuperscript{2+} ions per 96.6-kDa subunit. An elegant model proposed for the urease mechanism of action involves the polarization of the urea carbonyl by one nickel ion that allows nucleophilic attack by an activated hydroxyl anion associated with the second nickel ion.\textsuperscript{132}

The hydrogenases are an extremely heterogeneous group of enzymes.\textsuperscript{151} All known hydrogenases contain iron-sulfur clusters. In addition, some hydrogenases also contain nickel, or a nickel-selenocysteine bond; these have been designated as (NiFe) hydrogenases and (NiFeSe) hydrogenases. Hydrogenases containing nickel have been identified for over 35 species of bacteria, including methanogenic, hydrogen-oxidizing, sulfate-reducing, phototrophic, and aerobic $N_2$-fixing bacteria. Nickel may be a common constituent of hydrogenases that function physiologically to oxidize rather than to evolve $H_2$. The oxidation state of nickel in hydrogenase is a point of controversy. However, all parties in the controversy agree that nickel is redox active and apparently interacts with the substrate.

In addition to its redox role, nickel also has a regulatory role in the production of hydrogenase. Evidence has been presented that nickel is required for the synthesis of the hydrogenase mRNA in *Bradyrhizobium japonicum*.\textsuperscript{152} For the hydrogenase gene to be expressed, $O_2$ and $H_2$, when diffused into the cell, affect the redox state of the nickel bound to a nickel-containing, DNA-binding protein, which in turn leads to transcriptional regulation of the hydrogenase message.

Carbon monoxide dehydrogenase (carbon monoxide: [acceptor] oxidoreductase, EC 1.2.99.2; CODH), which oxidizes CO to CO$_2$, is a nickel enzyme that has been found in acetogenic, methanogenic, phototrophic, and sulfate-reducing anaerobic bacteria.\textsuperscript{153,154} In addition to oxidizing CO to CO$_2$, CODH in acetogenic bacteria catalyzes the reduction of CO to CO and the synthesis and degradation of acetyl-CoA, and thus can also be designated as an acetyl-CoA synthase.

Methyl-S-coenzyme-M reductase is the terminal enzyme in the conversion of CO$_2$ to methane in methanogenic bacteria.\textsuperscript{133-135} The enzyme catalyzes the reductive cleavage of CH$_3$SCoM to methane and coenzyme M. The enzyme contains factor $F_{430}$ which is thought to be the site of substrate reduction. Factor $F_{430}$ has been called a tetrahydrocorphin because of its hybrid relationship to corrin and porphyrin
macrocystic structures; nickel-corphin has been suggested to be a missing link between iron porphyrin and cobalt corrin systems.

Another nickel porphynoid, tunichlorin, was isolated from the Caribbean tunicate Trididemnum solidum. Tunichlorin is a blue-green pigment and is identified as nickel(II) 2-devinyl-2-hydroxymethylpyropheophorbide A. The function of tunichlorin is unknown but it is suspected to be involved in a reductive process similar to that occurring with methyl-S-coenzyme-M reductase.

Thus, nickel participates in hydrolysis and redox reactions, regulates gene expression, and, possibly, stabilizes certain structures. In these roles, nickel forms ligands with sulfur, nitrogen, and oxygen and exists in oxidation states of 3+, 2+, 1+, and perhaps 0 and 4+. Because nickel is so dynamic in lower forms of life, it most likely has an essential functional role in higher forms of life, including humans. Supporting this supposition is the response of experimental animals when they are deprived of dietary nickel. Findings indicate that vitamin B-12 status affects signs of nickel deprivation in rats and that vitamin B-12 must be present for optimal nickel function. Nickel may have a function in higher animals that involves a pathway using vitamin B-12.

Deficiency signs. The reported signs of nickel deprivation for six animal species—chickens, cows, goats, pigs, rats, and sheep—are extensive and have been listed in several reviews. Unfortunately, the described signs probably will have to be redefined because recent studies indicate that many of the reported signs of nickel deprivation may be manifestations of pharmacological actions of nickel. That is, high dietary nickel was alleviating an abnormality caused by something other than nickel deficiency, or changing a variable that was not necessarily subnormal. The suggestion that some of the reported signs of nickel deprivation are misinterpreted manifestations of a pharmacological action does not necessarily detract from the conclusion that nickel is an essential element. Several studies that apparently examined nickel physiologically indicate that signs of nickel deprivation include depressed growth, reproductive performance, and plasma glucose concentrations. Nickel deprivation also affects the distribution and proper functioning of other nutrients, including calcium, iron, zinc, and cobalamin (vitamin B-12). Also, the nature and severity of nickel deprivation signs are affected by diet composition and nutritional stressors. For example, vitamin B-12 deprivation seemed to depress growth in nickel-supplemented rats but enhanced growth in rats depressed by nickel deprivation. As a result, there was no difference in growth between nickel-deprived and -supplemented rats fed a diet deficient in vitamin B-12. This is one other similar findings suggest that in higher animals, vitamin B-12 is necessary for the optimal expression of the biological role of nickel.

Requirement. Because of the strong circumstantial evidence indicating that nickel is essential for several animals, a reasonable hypothesis is that nickel is also required by humans. Some animal studies provide some idea about the amount of nickel possibly required by humans. Most monogastric animals have a dietary nickel requirement of <200 µg/kg diet. If it is assumed that adult humans consume 500 g of a mixed diet daily (dry basis), then the dietary nickel requirement of humans would be <100 µg/day. A nickel requirement for humans of 25–35 µg/day has been suggested.

Food and other sources. Total dietary nickel intakes of humans vary greatly with the amounts and proportions of foods of animal (nickel-low) and plant (nickel-high) origin consumed. Rich sources of nickel include chocolate, nuts, dried beans and peas, and grains; diets high in these foods could supply >900 µg nickel/day. Conventional diets, however, often provide <100 µg/day. Examples of reported intakes are 69–162 µg/day in the United States and 130 µg/day (range 60–260) in Denmark.

Excess (toxicity). Life-threatening toxicity of nickel through oral intake is unlikely. Because of excellent homeostatic regulation, nickel salts exert their toxic action mainly by gastrointestinal irritation and not by inherent toxicity. Generally, >2250 µg nickel/g diet is required to produce signs of nickel toxicity (such as depressed growth) in rats, mice, chickens, rabbits, and monkeys. If animal data can be extrapolated to humans, a daily dose of 250 mg of soluble nickel would produce toxic symptoms in humans.

Some findings, however, suggest that oral intake of nickel in moderate doses could adversely affect health under certain conditions. Moderate amounts of dietary nickel exacerbate signs of severe iron deficiency and copper deficiency in rats. Nickel may act similarly in humans. Some evidence suggests that the ingestion of small amounts of nickel may be more important than external contacts in maintaining eczema caused by nickel allergy. An oral dose as low as 0.6 mg nickel as nickel sulfate given with water to fasting subjects (thus nickel was highly available) produced a positive reaction in some nickel-sensitive individuals. This dose is only a few times higher than the human daily requirement postulated from animal studies.

Summary. A biochemical function for nickel in higher animals has not been defined. However, multiple defined functions in lower forms of life, the response of experimental animals to low dietary intakes, and nickel's dynamic stimulation in vitro of some enzymes strongly suggest that nickel has an essential functional role in higher animals, including humans.
Silicon

History. In 1901 it was reported that high concentrations of silicon were present in tendons, aponeuroses, and eye tissues. As early as 1911, researchers suggested that silicon might have an antiatheroma action. Until 1972, however, silicon was generally considered non-essential, except in some lower classes of organisms (diatoms, radiolarians, and sponges) in which silica serves a structural role. In that year, the first substantial evidence was published that silicon is an essential element for chickens and rats. Most of the limited studies on the biochemical, nutritional, and physiologic roles of silicon have been published since 1974.

Chemistry and method of analysis. The chemistry of silicon is similar to that of carbon, its sister element. Silicon forms silicon-silicon, silicon-hydrogen, silicon-oxygen, silicon-nitrogen, and silicon-carbon bonds. Thus, organosilicon compounds are analogues of organocarbon compounds. The substitution of silicon, however, for carbon, or vice versa, in organocompounds results in molecules with different properties because silicon is larger and less electronegative than carbon.

In animals, silicon is found both free and bound. Silicic acid probably is the free form. The bound form has never been rigorously identified. Silicon may be present in biologic material as a silanolate, an ether (or ester-like) derivative of silicic acid. R₁-O-Si-O-R₂ or R₁-O-Si-O-Si-O-R₃ bridges may play a role in the structural organization of some mucopolysaccharides.

Inductively coupled argon plasma emission and graphite-furnace atomic absorption spectrometric methods have been used to obtain apparently accurate measures of silicon in biological material.

Absorption, transport, storage and turnover. Little is known about the metabolism of silicon. Increasing silicon intake increases urinary excretion up to fairly well-defined limits in humans, rats, and guinea pigs. However, the upper limits of urinary silicon excretion apparently are not determined exclusively by the excretory ability of the kidney because urinary excretion can be elevated above these limits by peritoneal injections of silicon. Thus, the upper limits apparently are set by the rate and extent of silicon absorption from the gastrointestinal tract.

The form of dietary silicon determines whether it is well absorbed. In one study, humans absorbed only ~10% of a large single dose of an alumina-silicate compound, but absorbed ~70% of a single dose of methylsilanetriol salicylate, a drug used to treat circulatory ischemias and osteoporosis. Further evidence that some forms of silicon, including those in food, are well absorbed is that in rats and humans urinary excretion can be a high percentage (close to 50%) of daily silicon intake. Silicon absorption is affected in rats by age, sex, and the activity of various endocrine glands. The mechanisms involved in the intestinal absorption of silicon are unknown.

Connective tissue (including aorta, trachea, tendon, bone, and skin) and its appendages contain much of the silicon that is retained in the body. The high silicon content of connective tissues may be the result of its presence as an integral component of the glycosaminoglycans and their protein complexes that contribute to structural framework.

Silicon is not protein bound in plasma; it is believed to exist in plasma almost entirely in the undissociated monomeric silicic acid form, Si(OH)₄. The elimination of absorbed silicon is mainly via the urine, where it probably exists as magnesium orthosilicate.

Physiological (biochemical) function. The distribution of silicon and the biochemical changes caused by silicon deprivation in bone indicate that silicon influences bone formation by affecting cartilage composition and ultimately cartilage calcification. Silicon is localized in the active growth areas or the osteoid layer and within the osteoblasts in young bone of mice and rats. In bone of silicon-deficient animals, hexosamine (glycosaminoglycans) and collagen concentrations are depressed whereas macromineral composition of bone mineral is not markedly affected. Extraction and purification procedures have shown silicon to be chemically combined with the glycosaminoglycan fraction of several types of connective tissues. Silicon is required for maximal bone prolylhydroxylase activity, which is important for collagen formation. Additionally, silicon was suggested to be involved with phosphorus in the organic phase in the series of events leading to calcification. Silicon may be involved in allowing an association between phosphoprotein-mucopolysaccharide macromolecules and collagen, which play a role in the initiation of calcification and the regulation of crystal growth.

The finding that silicon affects gene expression in some diatoms suggests that a similar role may also exist in higher animals.

Deficiency signs. Most of the signs of silicon deficiency in chickens and rats indicate aberrant metabolism of connective tissue and bone. Chicks fed a semisynthetic silicon-deficient diet exhibited skull structure abnormalities associated with depressed collagen content in bone and long-bone abnormalities characterized by small, poorly formed joints and defective endochondral bone growth. Tibias of silicon-deficient chicks exhibit depressed contents of articular cartilage, water, hexosamine, and collagen. In optimally growing chickens, growth is not significantly retarded by silicon deficiency. In rats, humerus hexose is increased and hydroxyproline is decreased, plasma amino acid and bone mineral composition is altered, and femur alkaline and acid phosphatase are decreased by silicon deprivation. Growth of rats
is not markedly affected by silicon deprivation.\textsuperscript{184,185} Signs of silicon deprivation can be influenced by low dietary calcium and high dietary aluminum.\textsuperscript{185,187} Rats fed a diet low in calcium and silicon and high in aluminum accumulated high amounts of aluminum in brain.\textsuperscript{187}

**Requirement.** Although a biochemical function for silicon is unknown, animal findings strongly suggest that silicon is required by humans. However, postulating a silicon requirement for humans is difficult because no appropriate human data and only limited usable animal data are available. Rats fed about 4.5 mg silicon/kg diet, mostly as the very available sodium metasilicate, do not differ from rats fed about 35 mg silicon/kg diet; both prevent, equally well, silicon deficiency signs exhibited by rats fed =1.0 mg silicon/kg diet.\textsuperscript{189} Animal diets contain =4000 kcal/kg. The food an average person consumes daily often contains between 8.37 and 10.46 MJ (2000 and 2500 kcal). Thus, if dietary silicon is highly available, on the basis of animal data, the human requirement for silicon is quite small, perhaps in the range of 2–5 mg/day. However, silicon as found in most diets probably is not absorbable or as available as sodium metasilicate; significant amounts probably occur as aluminosilicates and silica from which silicon is not readily available.\textsuperscript{188} Factors such as aging and low estrogen status apparently decrease the ability to absorb silicon.\textsuperscript{189} Thus, the recommended intake of silicon may be found to be between 5 and 10 mg/day.

**Food and other sources.** Total dietary silicon intake of humans varies greatly with the amount and proportions of foods consumed and the amounts of refined and processed foods in the diet.\textsuperscript{190,190,191} Normally, refining reduces the silicon content of foods. However, in recent years, silicate additives have been increasingly used in prepared foods and confections as anticaking or anti-foaming agents.\textsuperscript{192} Although this increases total dietary silicon, most of it is not bioavailable. The silicon content of drinking water, and beverages made thereof, shows geographical variation; silicon is high in hard-water and low in soft-water areas. The richest sources of silicon are unrefined grains of high fiber content, cereal products, and root vegetables.\textsuperscript{90,190,191}

Average daily intakes of silicon apparently range from =20 to 50 mg/day. The calculated silicon content of the FDA total diet was 19 mg/day for women and 40 mg for men.\textsuperscript{191} A human balance study indicated that the oral intake of silicon could be =21-46 mg/day.\textsuperscript{17} The average British diet was estimated to supply 31 mg silicon/day.\textsuperscript{190}

**Excess (toxicity).** Most silicon compounds are essentially nontoxic when taken orally. Magnesium trisilicate, an over-the-counter antacid, has been used by humans for >40 years without obvious deleterious effects. Other silicates are food additives used as anticaking or anti-foaming agents.\textsuperscript{192} However, antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, were reduced in rats fed high amounts of sodium metasilicate.\textsuperscript{193} Additionally, ruminants consuming plants with a high silicon content may develop siliceous renal calculi. Renal calculi in humans may also contain silicates.\textsuperscript{169}

**Summary.** Ample circumstantial evidence exists to indicate that silicon is an essential nutrient for higher animals, including humans. Findings from animals indicate that silicon nutrition affects macromolecules such as glycosaminoglycans, collagen, and elastin. Although more should be known about the physiological or biochemical function and requirement for silicon before doing so, speculation has materialized on the possible involvement of silicon deprivation in the occurrence of several human disorders, including atherosclerosis, osteoarthritis, osteoporosis, hypertension, and Alzheimer’s disease.\textsuperscript{90,194} This speculation indicates the need for more work to clarify the consequences of silicon deficiency in humans.

**Vanadium**

**History.** In 1876, Priestley and Gamgee reported on the toxicity of sodium vanadate in frogs, pigeons, guinea pigs, rabbits, dogs, and cats.\textsuperscript{195} However, the paper considered to be the classic for pharmacological and toxicological actions of vanadium appeared in 1912.\textsuperscript{196} It was also at this time that high vanadium concentrations were discovered in the blood of ascidian worms.\textsuperscript{197,198} A surge of interest in vanadium started in 1977 when Cantley et al.\textsuperscript{199} reported that vanadate, which inhibits ATPases, was a contaminant of commercially available ATP. The interest was maintained subsequently by the finding in the early 1980s that vanadium is an insulin mimetic agent.\textsuperscript{200} The first vanadium-containing enzyme, a bromoperoxidase, was discovered in the blood of ascidian worms.\textsuperscript{197,198} A surge of interest in vanadium started in 1977 when Cantley et al.\textsuperscript{199} reported that vanadate, which inhibits ATPases, was a contaminant of commercially available ATP. The interest was maintained subsequently by the finding in the early 1980s that vanadium is an insulin mimetic agent.\textsuperscript{200} The first vanadium-containing enzyme, a bromoperoxidase, was discovered in the blood of ascidian worms.\textsuperscript{197,198} A surge of interest in vanadium started in 1977 when Cantley et al.\textsuperscript{199} reported that vanadate, which inhibits ATPases, was a contaminant of commercially available ATP. The interest was maintained subsequently by the finding in the early 1980s that vanadium is an insulin mimetic agent.\textsuperscript{200} The first vanadium-containing enzyme, a bromoperoxidase, was discovered in the blood of ascidian worms.\textsuperscript{197,198} A surge of interest in vanadium started in 1977 when Cantley et al.\textsuperscript{199} reported that vanadate, which inhibits ATPases, was a contaminant of commercially available ATP. The interest was maintained subsequently by the finding in the early 1980s that vanadium is an insulin mimetic agent.\textsuperscript{200}
states apparently are the most important forms of vanadium.\textsuperscript{205,206} The tetravalent state appears most simply as the vanadyl cation, VO\textsuperscript{2+}. The vanadyl cation behaves like a simple divalent aquo ion and competes well with Ca\textsuperscript{2+}, Mn\textsuperscript{2+}, Fe\textsuperscript{2+}, etc., for ligand binding sites. Thus VO\textsuperscript{2+} easily forms complexes with proteins, especially those associated with iron, such as transferrin or hemoglobin, which stabilize vanadyl against oxidation. The pentavalent state of vanadium is known as vanadate (H\textsubscript{2}VO\textsubscript{4} or more simply VO\textsubscript{3}\textsuperscript{-}). Vanadate forms complexes with other biological substances, including those that result in it being a phosphate transition-state analogue, and thus competes with or replaces phosphate in many biochemical processes. Vanadyl is easily reduced by ascorbate, glutathione, or NADH. For example, with certain cells (e.g., adipocytes), vanadyl enters through nonspecific anionic channels and is reduced and complexed by glutathione.\textsuperscript{207-209}

Another form of vanadium has been discussed as being responsible for many biological actions of vanadium, including its insulin mimetic action and haloperoxidase role; this is the peroxo form.\textsuperscript{209-211} Vanadyl can interact with O\textsubscript{2} formed by NADPH oxidase to generate peroxovanadyl [V(IV)-O-O]. Peroxovanadyl can in turn remove hydrogen from NADPH to yield vanadyl hydroperoxide [V(IV)-O-OH]. Peroxo (hetero-ligand) vanadyl is suggested to be a useful model for the active-site vanadium involved in bromide oxidation in haloperoxidases.\textsuperscript{211}

Heydorn\textsuperscript{212} reviewed analytical methods for the determination of vanadium in the low amounts found in tissues, blood, and urine. For this task, especially for human plasma and serum, methods using atomic emission spectrometry, particle-induced x-ray emission, flame atomic absorption spectrometry, and catalysis were found to be inadequate. Methods that apparently can determine vanadium accurately in low amounts are electrothermal atomic absorption spectrometry (ETAAS), neutron activation analysis with radiochemical separation (RNAA), and neutron activation with preirradiation separation (NAA).\textsuperscript{213-216} RNAA and NAA are not available to most laboratories; thus, ETAAS is the method of choice for analysis of samples that have been dried, wet-digested, or bomb-digested in a microwave.\textsuperscript{215-216} As with all trace elements, contamination of samples is a concern, but apparently for vanadium this is not as much of a concern as it is for some other trace elements.\textsuperscript{212,214-216}

Absorption, transport, storage, and turnover. Most ingested vanadium is unabsorbed and is excreted in the feces. Because very low concentrations of vanadium, generally <0.8 μg/L, are found in urine, compared with the estimated daily intake of 12-30 μg and the fecal content of vanadium, apparently <5% of vanadium ingested is normally absorbed.\textsuperscript{214,217,218} Byrne and Kosta\textsuperscript{217} estimated that ≤1% of vanadium normally ingested with the diet is absorbed. Curran et al.\textsuperscript{219} reported that =0.1-1.0% of vanadium in 100 mg of very soluble diammonium oxytartarvandate was absorbed by the human gastrointestinal tract. Animal studies generally support the concept that vanadium is poorly absorbed.\textsuperscript{220-222} However, two studies with rats indicated that vanadium absorption can exceed 10%.\textsuperscript{223,224} These studies suggest caution in assuming that ingested vanadium always will be poorly absorbed from the gastrointestinal tract. Factors such as fasting and dietary composition probably had an influence on the percentage absorbed from the intestine in these studies.

Kinetic modeling of whole-body vanadium metabolism in sheep indicates that much of vanadium absorbed is absorbed in the upper gastrointestinal tract.\textsuperscript{225} Most ingested vanadium probably is transformed in the stomach to VO\textsuperscript{2+} and remains in this form as it passes into the duodenum.\textsuperscript{226} However, in vitro studies suggest that vanadate can enter cells through phosphate or other anion transport systems. This may be the reason that VO\textsuperscript{3-} is absorbed 3 to 5 times more effectively than VO\textsuperscript{2+}. Thus, the different absorbability rates, the effect of other dietary components on the forms of vanadium in the stomach, and the speed at which it is transformed into VO\textsuperscript{2+} apparently markedly affect the percentage of ingested vanadium absorbed.\textsuperscript{226} Supporting this concept are the reviewed findings showing that a number of substances can ameliorate vanadium toxicity, including ascorbic acid, EDTA, chromium, protein, ferrous iron, chloride, and aluminum hydroxide.\textsuperscript{204}

Based on studies using intravenous or intraperitoneal injections of the element in animals, vanadium is rapidly removed from the blood plasma and is retained in highest amounts in the kidney, liver, testes, bone, and spleen. For example, at 96 hours, 30-46% of an intravenous dose of \textsuperscript{48}V was found in the urine and 9-10% was found in the feces of rats.\textsuperscript{217,218} Thirty minutes after an intraperitoneal injection of \textsuperscript{48}V, rats retained 7.2% in the kidney and 2.1% in bone; at 48 hours, the kidney retained 1.6% and bone 3.45% of the dose.\textsuperscript{222}

Much evidence suggests that the binding of the vanadyl ion to iron-containing nonheme proteins is important in vanadium metabolism. For example, vanadium in milk of lactating rats injected with \textsuperscript{48}V was found mainly in the protein fraction and apparently was associated with a transferrin-like protein, perhaps lactoferrin.\textsuperscript{229} Nursing pups absorbed a significant amount of \textsuperscript{48}V from the milk, perhaps as lactoferrin-vanadium complexes in plasma and body fluids.\textsuperscript{226,227,230,231} One study showed that 1 day after intravenous administration of \textsuperscript{48}VO\textsuperscript{3-}, 29% of \textsuperscript{48}V incorporated in rat liver
cytosol existed as a vanadium low-molecular-weight complex (<5000 mol wt).\textsuperscript{232} By day 9, however, the low-molecular-weight complex had disappeared and vanadium was present only as vanadyl-ferritin (15\%) and vanadyl-transferrin (85\%) in rat liver cytosol. It remains to be determined whether vanadyl-transferrin can transfer vanadium into cells through the transferrin receptor or whether ferritin is a storage vehicle for vanadium.

Under normal conditions, the body burden of vanadium is low (=100\(\mu\)g); most tissues contain <10 ng vanadium/g wet weight.\textsuperscript{204} However, tissue vanadium is markedly elevated in animals fed high dietary vanadium. In rats, liver vanadium increased from 10 to 55 ng vanadium/g wet weight when dietary vanadium was increased from 0.1 to 25 \(\mu\)g/g.\textsuperscript{223} In sheep, bone vanadium increased from 220 to 3320 ng/g dry weight when dietary vanadium was increased from 10 to 270 \(\mu\)g/g.\textsuperscript{233} Thus, bone apparently is a major sink for excessive retained vanadium.

On the basis of studies in which vanadium is administered parenterally, urine is the major excretory route for absorbed vanadium.\textsuperscript{234,237,228} Both high- and low-molecular-weight complexes have been found in urine;\textsuperscript{227,228} one of these may be transferrin. A significant portion of absorbed vanadium may be excreted through the bile. Byrne and Kost\textsuperscript{217} found 0.65, 0.55, and 1.85 ng vanadium/g of human bile. In two studies, 8-10\% of an injected dose of \(^{48}\text{V}\) was found in the feces of rats.\textsuperscript{224,228} The form of vanadium in bile apparently has not been determined.

**Physiological (biochemical) function.** A defined biochemical function for vanadium in higher animals, and thus humans, has not been described. Numerous biochemical and physiological functions for vanadium have been suggested on the basis of its in vitro and pharmacological actions; these have been discussed in several reviews and are too extensive to discuss in detail here.\textsuperscript{206,207,234,235} In vitro studies with cells and pharmacological studies with animals have shown that vanadium has insulin-mimetic properties; numerous stimulatory effects on cell proliferation and differentiation; effects on cell phosphorylation-dephosphorylation; inhibitory effects on the motility of sperm, cilia, and chromosomes; effects on glucose and ion transport across plasma membranes; interfering effects on intracellular ionized calcium movement; and effects on oxidation-reduction processes. In vitro cell-free systems have shown that vanadium inhibits numerous ATPases, phosphatases, and phosphoryl transfer enzymes.\textsuperscript{234} The pharmacological action of vanadium receiving the most attention recently is its ability to mimic insulin.\textsuperscript{260} Functional roles for vanadium were recently defined for some algae, lichens, fungi, and bacteria. These roles may provide clues as to the nature of the actual biochemical role of vanadium in humans; thus, they are briefly described here.

Haloperoxidases catalyze the oxidation of halide ions by hydrogen peroxide, thus facilitating the formation of a carbon-halogen bond. In 1984, vanadium was found essential to enzymatic activity of a bromoperoxidase from the brown algae Ascophyllum nodosum.\textsuperscript{201} Since then, vanadium-dependent bromoperoxidases have been found in a number of marine brown algae, marine red algae, and a terrestrial lichen.\textsuperscript{201,238} Vanadium-dependent iodo­peroxidases were also detected in brown seaweeds, and a chloroperoxidase was identified in the fungus Curvularia inaequalis.\textsuperscript{201,236,237}

The mechanism of action of vanadium in the haloperoxidases has not been firmly established. However, findings to date do not favor a mechanism in which \(V^{5+}\) is reduced to \(V^{4+}\) or \(V^{3+}\) and reoxidized to \(V^{5+}\) by \(H_2O_2\). Rather, in the bromoperoxidases, \(H_2O_2\) reacts with vanadium as \(V^{4+}\) to form a dioxigen species, which reacts with bromide to yield an oxidized bromine species, the intermediate that forms the carbon-halogen bond.\textsuperscript{238}

Conversion of atmospheric nitrogen to ammonia by nitrogen-fixing microorganisms is catalyzed by the enzyme nitrogenase. Vanadium-dependent nitrogenases were recently reviewed.\textsuperscript{239} The reduction of dinitrogen by nitrogenase involves the sequential MgATP-dependent transfer of electrons from an iron-protein to a vanadium-iron-cofactor center at the substrate-binding site in nitrogenase.

Vanadium is found in high concentrations in some species of the mushroom genus Amanita. The isolation and structure determination of a vanadium-containing compound found in mushrooms and named amavadin was reviewed.\textsuperscript{240} The physiological function of amavadin is unknown but has been suggested to be a cofactor with a protective oxidase or peroxidase action.\textsuperscript{211} The electrochemistry of amavadin is such that it may function in electron-transfer reactions through a \(V^{3+}/V^{4+}\) redox couple.\textsuperscript{241}

**Deficiency signs.** Between 1971 and 1985 several research groups described possible signs of vanadium deficiency for some animals.\textsuperscript{202} However, most of the early studies were performed with animals fed unbalanced diets which resulted in suboptimal health and growth. The diets used often had widely varied contents of protein, sulfur-containing amino acids, ascorbic acid, iron, copper, and perhaps other nutrients that affected, or were affected by, vanadium metabolism.\textsuperscript{202} Thus, pharmacological responses may have been induced by the high-vanadium supplements fed. As a result, it is difficult to determine whether the deficiency signs in early experiments with questionable diets were true deficiency signs, indirect changes caused by an enhanced need for vanadium in some metabolic function, or manifestations of a pharmacological action of vanadium. Vanadium deficiency signs for humans have not been described.
Table 1. Ultratrace elements needing further study to confirm nutritional importance

<table>
<thead>
<tr>
<th>Element</th>
<th>Reported deficiency signs</th>
<th>Apparent deficient dietary intake</th>
<th>Other apparent beneficial or physiological action</th>
<th>Dietary sources for humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum (Al)</td>
<td>Goat: Increased spontaneous abortions, depressed growth, incoordination and weakness in hind legs, and decreased life expectancy&lt;sup&gt;250&lt;/sup&gt;</td>
<td>Goat: 162 µg/kg</td>
<td>Activates adenylate cyclase&lt;sup&gt;252&lt;/sup&gt; enhances calmodulin activity&lt;sup&gt;253&lt;/sup&gt; stimulates DNA synthesis in cell cultures&lt;sup&gt;254&lt;/sup&gt; stimulate osteoblasts to form bone through activating a putative G-protein coupled cation sensing system&lt;sup&gt;255&lt;/sup&gt;</td>
<td>Baked goods prepared with chemical leavening agents (e.g. baking powder) processed cheese, grains, vegetables, herbs, tea, antacids, buffered analgesics&lt;sup&gt;256&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Chick: Depressed growth&lt;sup&gt;251&lt;/sup&gt;</td>
<td>Chick: not given</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bromine (Br)</td>
<td>Goat: Depressed growth, fertility, milk fat production, hematocrit, hemoglobin and life expectancy, and increased spontaneous abortions&lt;sup&gt;257,258&lt;/sup&gt;</td>
<td>Goat: 0.8 mg/kg</td>
<td>Alleviates growth retardation caused by hyperthyroidism in mice and chicks&lt;sup&gt;259,260&lt;/sup&gt; substitutes for part of chloride requirement for chicks&lt;sup&gt;261&lt;/sup&gt; insomnia exhibited by many hemodialysis patients associated with bromide deficit&lt;sup&gt;262&lt;/sup&gt;</td>
<td>Grains, nuts, fish&lt;sup&gt;263&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>Rat: Depressed growth&lt;sup&gt;264,265&lt;/sup&gt;</td>
<td>Rat: &lt;4 µg/kg</td>
<td>Has transforming growth factor activity or stimulates growth of cells in soft agar&lt;sup&gt;267&lt;/sup&gt;</td>
<td>Shellfish, grains—especially those grown on high-cadmium soils, leafy vegetables&lt;sup&gt;268&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Goat: Depressed growth&lt;sup&gt;266&lt;/sup&gt;</td>
<td>Goat: 20 µg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorine (F)</td>
<td>Rat: Depressed growth and incisor pigmentation&lt;sup&gt;269&lt;/sup&gt;</td>
<td>Rat: 0.04–0.46 mg/kg</td>
<td>High dietary fluoride improves fertility, hematopoiesis and growth in mice and rats&lt;sup&gt;272,273&lt;/sup&gt; is anticariogenic&lt;sup&gt;274&lt;/sup&gt; can be antiosteoporotic&lt;sup&gt;274&lt;/sup&gt; prevents phosphorus-induced nephrocalcinosis&lt;sup&gt;275,276&lt;/sup&gt;</td>
<td>Fish, tea, fluoridated water&lt;sup&gt;274&lt;/sup&gt;</td>
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<td></td>
<td>Goat: Depressed growth and life span, histological changes in kidney and endocrine organs&lt;sup&gt;270&lt;/sup&gt;</td>
<td>Goat: &lt;0.3 mg/kg</td>
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<tr>
<td>Germanium (Ge)</td>
<td>Rat: Altered bone and liver mineral composition, and decreased tibial DNA&lt;sup&gt;271&lt;/sup&gt;</td>
<td>Rat: 0.7 mg/kg</td>
<td>Reverses changes in rats caused by silicon deprivation;&lt;sup&gt;184&lt;/sup&gt; some organic germanium compounds have antitumor activity&lt;sup&gt;277,278&lt;/sup&gt;</td>
<td>Wheat bran, vegetables, leguminous seeds&lt;sup&gt;263&lt;/sup&gt;</td>
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<td>Lead (Pb)</td>
<td>Rat: Depressed growth, anemia, disturbed iron metabolism, decreased liver glucose, triglycerides, LDL-cholesterol phospholipids, glutamic-oxalic transaminase activity and glutamic-pyruvate transaminase activity, increased liver cholesterol and alkaline phosphatase activity, increased serum ceruloplasmin, and decreased blood catalase&lt;sup&gt;279–282&lt;/sup&gt;</td>
<td>Rat: 200 µg/kg&lt;sup&gt;279&lt;/sup&gt;</td>
<td>Alleviates iron deficiency signs in young rats&lt;sup&gt;264&lt;/sup&gt;</td>
<td>Seafood, plant foodstuffs grown under high-lead conditions&lt;sup&gt;283&lt;/sup&gt;</td>
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<td></td>
<td>Rat: 18–45 µg/kg&lt;sup&gt;280–282&lt;/sup&gt;</td>
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Table continues on next page
<table>
<thead>
<tr>
<th>Element</th>
<th>Reported deficiency signs</th>
<th>Apparent deficient dietary intake</th>
<th>Other apparent beneficial or physiological action</th>
<th>Dietary sources for humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Depressed growth, and elevated serum cholesterol, phospholipids, and bile acids(^{283})</td>
<td>Pig: 30–32 µg/kg</td>
<td>Stimulates growth of some cultured cells;(^{289}) exhibits insulinomimetic action;(^{290}) incidence of violent crimes higher in areas with low-lithium drinking water;(^{291}) hair lithium low in violent criminals, learning-disabled subjects, and heart disease patients(^{292})</td>
<td>Eggs, meat, processed meat, fish, milk, milk products, potatoes, vegetables (content varies with geological origin)(^{296})</td>
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<tr>
<td>Lithium (Li)</td>
<td>Goat: Depressed fertility, birth weight, life span, liver monoamine oxidase activity, and serum isocitrate dehydrogenase, malate dehydrogenase, aldolase, and glutamate dehydrogenase activities, and increased serum creatine kinase activity(^{286})</td>
<td>Goat: &lt;1.5 mg/kg</td>
<td>Factor R, which prevents hind leg paralysis, swelling of abdomen and death may be rubidium(^{284})</td>
<td>Coffee, black tea, fruits and vegetables (especially asparagus), poultry, fish(^{299})</td>
</tr>
<tr>
<td>Rat</td>
<td>Depressed fertility, birth weight, litter size, and weaning weight(^{287,288})</td>
<td>Rat: 0.6–15 µg/kg</td>
<td></td>
<td>Canned foods(^{101})</td>
</tr>
<tr>
<td>Rubidium (Rb)</td>
<td>Goat: Depressed food intake, growth, and life expectancy, and increased spontaneous abortions(^{293})</td>
<td>Goat: 180 µg/kg</td>
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<tr>
<td>Tin (Sn)</td>
<td>Rat: Depressed growth, alopecia, response to sound, feed efficiency, heart zinc and copper, tibial copper and manganese, muscle iron and manganese, spleen iron, kidney iron and lung magnesium; increased lung calcium(^{286,297})</td>
<td>Rat: 17 µg/kg</td>
<td>Influences heme oxygenase activity(^{298,299}) associated with thymus immune and homeostatic function(^{100})</td>
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</table>
The uncertainty about vanadium deficiency signs stimulated new efforts to produce deficiency signs in animals fed diets apparently containing adequate and balanced amounts of all known nutrients. Anke et al. found that, when compared with controls fed 2 μg vanadium/g diet, goats fed <10 ng vanadium/g diet exhibited a higher rate of spontaneous abortion, and animals that delivered offspring produced less milk during the first 56 days of lactation. Forty percent of kids from vanadium-deprived goats died between days 7 and 91 of lactation, with some deaths preceded by convulsions; only 8% of kids from vanadium-supplemented goats died during this time. Vanadium-deficient goats had only 55% the life span of control goats. Also, skeletal deformations were seen in the forelegs, and forefoot tarsal joints were thickened.

Uthus and Nielsen reported that, when compared with controls fed 1 μg vanadium/g diet, vanadium deprivation (2 ng vanadium/g diet) increased thyroid weight and the ratio of thyroid weight to body weight and tended to decrease growth of rats. Vanadium deprivation also depressed erythrocyte glucose-6-phosphate dehydrogenase and cecal total carbonic anhydrase. Uthus and Nielsen also found that, as dietary iodine increased from 0.05 to 0.33 to 25 μg/g, thyroid peroxidase activity decreased, and the decrease was more marked in the vanadium-supplemented than the vanadium-deprived rats. Also, as dietary iodine increased, plasma glucose increased in the vanadium-deprived rats but decreased in the vanadium-supplemented rats. These vanadium-deprivation studies probably have found some true deficiency signs. It is unlikely the diets lacked any nutrient that caused such marked deficiency signs, which were prevented by pharmacological action of the small vanadium supplements used.

Requirement. If vanadium is essential for humans, its requirement most likely is small. The diets used in animal deprivation studies contained only 2–25 ng V/g; these often did not markedly affect the animals. Vanadium deficiency has not been identified in humans, yet diets generally supply <30 μg vanadium/day and most supply only 15 μg/day. Thus, a daily dietary intake of 10 μg of vanadium probably will meet any postulated vanadium requirement.

Food and other sources. Foods rich in vanadium include shellfish, mushrooms, parsley, dill seed, black pepper, and some prepared foods. Beverages, fats and oils, and fresh fruits and vegetables contain the least vanadium (<1 to 5 μg/g).

Excess (toxicity). Vanadium is a relatively toxic element. The threshold level for toxicity apparently is near 10–20 μg/day, or 10–20 μg/g of diet; this is supported by animal findings and the following human findings. Schroeder et al. fed 15 patients 4.5 and 9 mg vanadium/day as diammonium oxysulfatovanadate for 6–16 months without apparent detrimental effect. However, serum cholesterol was reduced slightly by the treatment, so the vanadium supplement was not inactive. Curran et al. fed each of five subjects 13.5 mg/day in three divided doses as diammonium oxysulfatovanadate for 6 weeks; no sign of intolerance or toxicity was found. Somerville and Davies gave each of 12 patients 13.5 mg vanadium/day for 2 weeks and then 22.5 mg vanadium/day for 5 months; five patients exhibited gastrointestinal disturbances and five patients exhibited green tongue. Dimond et al. gave ammonium vanadyl tartrate orally to six subjects for 6–10 weeks in amounts ranging from 4.5 to 18 mg vanadium/day; green tongue, cramps, and diarrhea were observed at the larger doses.

From their in-depth study of vanadium toxicity, Proesch et al. concluded that vanadium is a neurotoxic and hemorrhagic-endotheliotoxic poison with nephrotoxic, hepatotoxic, and probably leukocytotoxic components. Thus, it is not surprising that a variety of toxicity signs exist and that they can vary among species and with dosage. Some of the more consistent signs include depressed growth, diarrhea, depressed food intake, and death.

Summary. Although it has numerous in vitro and pharmacological properties that suggest essentiality, the importance of vanadium in nutrition remains to be determined. Identification of a specific biochemical role for vanadium is necessary to disentangle pharmacological importance of vanadium and to determine its safe and adequate intakes. Because vanadium is so pharmacologically active, a beneficial pharmaceutical role for this element may be found.

Other Elements
As indicated in the introduction, the evidence for essentiality of aluminum, bromine, cadmium, fluorine, germanium, lead, lithium, rubidium, and tin is quite limited. Findings that have led to some researchers suggesting that these elements are essential are summarized in Table 1.

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