Ann. Rev. Nutr. 1984. 4:21–41

ULTRATRACE ELEMENTS IN NUTRITION[1]

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INTRODUCTION

Since 1970, scientists have suggested that at least 11 elements could be added to the list of trace elements that are essential in animal nutrition. Estimated dietary requirements for those elements usually are less than 1 μg/g, and often are less than 50 ng/g dry diet. They have been designated ultratrace elements. Those so proposed are arsenic, boron, bromine, cadmium, fluorine, lead, lithium, nickel, silicon, tin, and vanadium. The quality of the experimental evidence supporting the suggestion of nutritional essentiality varies widely among these elements.

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In this chapter, an element is considered essential if a dietary deficiency of that element consistently results in a suboptimal biological function that is preventable or reversible by physiological amounts of the element. Deficiency in humans has not been described for any of the 11 ultratrace elements. Thus, their possible importance in human nutrition (except for the special case of fluorine) can be only inferred from the results of animal studies. Generally, extrapolation of experimental findings from animals to humans is difficult. For the major trace elements that are clearly required by humans, however, signs of deficiency often correspond closely with signs observed in experimental animals. Possibly, therefore, most of the ultratrace elements that are essential for other animals also are essential for humans. Furthermore, some of the deficiency signs and requirements described for animals might have counterparts for humans. Those who are concerned with human nutrition should be cognizant of this possibility and recent advances in research on ultratrace element nutrition are of broad interest. However, information about recent advances is not readily available because many of the studies of ultratrace elements have been reported in abstracts, unedited proceedings of specialist symposia, book chapters, reviews, or obscure journals. This chapter is an attempt to bring together and critically appraise the diverse literature on ultratrace element nutrition. Although the speculative nature of some conjectures is recognized, it has been judged useful to attempt such a synthesis.

Although they fulfill the definition for an ultratrace element, chromium, molybdenum, and selenium are not discussed in this chapter. For these elements, the evidence for essentiality (initially appearing in the 1950s) is substantial and noncontroversial, and information about recent research advances is readily available. Moreover, these elements were subjects of extensive reviews (28, 73, 85).

SIGNS OF DEFICIENCY AND POSSIBLE BIOLOGICAL FUNCTIONS

Arsenic

Reviews summarizing the signs of arsenic deprivation in four animal species—chicken, goat, miniature pig, and rat—were recently published (52, 56, 82). In the goat, miniature pig, and rat, 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 triglycerides and death during lactation. Histologic examination revealed myocardial damage in the lactating goats that died.

Studies with chicks indicated that the extent, severity, and direction of the signs of arsenic deprivation were affected by the arginine and zinc status of the animal (82). Moreover, arsenic deprivation in chicks markedly influenced the

effects of high levels of dietary arginine and/or zinc deprivation. Interactions among arsenic, arginine, and zinc affected parameters such as growth, activities of kidney arginase and plasma alkaline phosphatase, and levels of plasma uric acid and urea.

The effect of dietary arsenic on growth depended upon the zinc status of the chick. Arsenic deprivation did not markedly affect growth when dietary zinc was luxuriant (>50 μg/g), but depressed growth when dietary zinc was marginally adequate (25–40 μg/g). In severe zinc deficiency, growth was more markedly depressed in arsenic-supplemented than in arsenic-deprived chicks.

In chicks fed a normal level of arginine (14 mg/g diet) and adequate zinc (40–50 μg/g diet), arsenic deprivation elevated kidney arginase activity and did not affect plasma urea. In zinc-deficient chicks, on the other hand, kidney arginase activity was higher in arsenic-supplemented than in arsenic-deprived chicks because zinc deficiency elevated kidney arginase more markedly in arsenic-supplemented than in arsenic-deprived chicks. When dietary arginine was increased to 30 mg/g, kidney arginase and plasma urea were substantially elevated. Zinc deficiency alleviated the elevation in both parameters in arsenic-deprived chicks, but enhanced the elevation in arsenic-supplemented chicks.

The findings with chicks suggest that arsenic has a biological function in the metabolism of arginine and zinc. This function may involve amino acid or protein metabolism. Recent findings support the possibility that arsenic has a role in the utilization of amino acids for protein synthesis, or in protein degradation. Arsenic deprivation elevated plasma uric acid in chicks fed a normal level of arginine and luxuriant zinc (82) but depressed plasma uric acid in chicks fed a normal level of arginine and less than 40 μg zinc/g of diet. Chicks usually eliminate excess amino acid nitrogen as uric acid. Arsenic deprivation depressed total microsomal protein in the livers of rats (12) and chicks (W. E. Cornatzer, personal communication) and the "raw" protein level in minipigs (3). Arsenic deprivation depressed the level of arginine and elevated the level of lysine in the plasma of chicks (E. O. Uthus, personal communication).

Although a specific biochemical function for arsenic is unknown, ample evidence indicates that arsenic is an essential nutrient. Of course, only data from animal studies are available for estimating the amount of arsenic required by humans. An arsenic requirement of less than 50 ng/g of diet and probably near 25 ng/g was suggested for chicks and rats fed an experimental diet containing 20% protein, 9% fat, 60% carbohydrate, and 11% fiber, minerals, and vitamins (52). Thus, the arsenic requirement apparently was somewhere between 6.25 and 12.5 μg/1000 kcal. Extrapolating from animal data, one might conclude that a possible arsenic requirement for humans eating 2000 kcal/day would be about 12–25 μg daily.

Boron

Between 1939 and 1944, several attempts to induce a boron deficiency in rats were unsuccessful, although the diets fed apparently contained only 155–163 ng boron/g (32, 58, 79). In 1945, Skinner & McHargue (75) reported that supplemental dietary boron enhanced survival and maintenance of body fat and elevated liver glycogen in potassium-deficient rats. Those findings were not confirmed by Follis (23), who fed a different diet with an unknown boron content and different levels of boron supplementation.

After those reports, boron was generally accepted as being essential for plants but not for animals. In 1981, however, evidence was reported that indicated boron might be an essential nutrient for chicks. In some early studies on arsenic nutrition in the laboratory of Hunt & Nielsen (34), chicks exhibited poor growth and leg abnormalities even though all known essential nutrients had been added to the diet. Studies designed to determine the shortcoming of the diet showed that boron supplementation stimulated growth. Subsequently it was found that the diet contained inadequate cholecalciferol, because the indicated potency of the cholecalciferol supplement was incorrect. After that deficiency was corrected, growth of chicks improved and was not markedly stimulated by boron supplementation. The findings indicated that boron and cholecalciferol interacted, but factorially arranged experiments did not show a statistically significant interaction. However, the relationship between boron and cholecalciferol nutriture was confirmed. Depressed growth and abnormal bone development were more marked in boron-deprived (<0.2 μg/g diet) chicks fed a cholecalciferol-deficient diet than in those fed a cholecalciferol-luxuriant diet. Hunt & Nielsen (34) suggested that cholecalciferol deficiency enhanced the need for boron and that boron might interact, in some manner other than through an effect on cholecalciferol metabolism, with the metabolism of calcium, phosphorus, or magnesium. Subsequent experiments were consistent with an interaction between boron and magnesium, but not between boron and either calcium or phosphorus (C. D. Hunt, F. H. Nielsen, unpublished observations). When fed a diet containing marginal magnesium (250 mg/kg) and cholecalciferol (250 IU/kg), boron-deprived chicks exhibited depressed growth and rachitic-like long-bone histology. Those abnormalities were not found in boron-deprived chicks fed adequate dietary magnesium (500 mg/kg) and cholecalciferol (2500 IU/kg).

The establishment of boron as an essential nutrient requires further evidence. Meanwhile, suggestion of a possible dietary boron requirement for humans would be inappropriate. Nevertheless, tablets containing magnesium carbonate and sodium borate are touted as a remedy for arthritis (47).

Bromine

Only weak evidence indicates that bromine is an essential nutrient for animals. This evidence includes (*a*) bromide can substitute for part of the chloride

requirement of chicks (39); and (*b*) trace amounts of dietary bromide elicited a small, but significant, growth response in chicks and mice with hyperthyroid-induced depressed growth as a result of being fed a semi-synthetic diet containing iodinated casein (6, 33).

In 1981, Oe et al (57) found unusually low bromide concentrations in serum and brain of patients subjected to chronic hemodialysis; apparently the artificial kidney removed bromine. They associated the insomnia exhibited by many hemodialysis patients with the bromine deficit. Subsequently, they did a double blind trial in which either bromide or chloride was added to the dialysate of four patients on maintenance hemodialysis. Quality of sleep improved markedly in the two patients who received bromide but not in those who received chloride. Those findings are not the first association between bromide and quality of sleep. Before barbiturates were used, doctors prescribed bromide for sleep.

Nonetheless, the findings of Oe et al (57) indicate that the possible essentiality of bromine should be studied further. Bromine, however, is ubiquitous in the biosphere, and signs of deprivation might be hard to induce in experimental animals.

Cadmium

The evidence, from two laboratories, suggesting that cadmium is an essential nutrient does not fulfill the definition for essentiality. Anke et al (4) found in one experiment that, after 100 days of dietary treatment, growth was slower in goats fed 20 ng cadmium/g diet than in goats fed 250 ng cadmium/g diet. They stated, however, that their findings were inconclusive because the experiment was not repeated and two of five cadmium-deprived goats died from unknown causes. Schwarz & Spallholz (72) found that a dietary supplement of cadmium slightly stimulated growth of suboptimally growing rats but did not result in optimal growth. Apparently, the suboptimal growth was mainly due to riboflavin deficiency (45, 72). It is unknown whether a deficiency of cadmium depresses growth in rats that are not riboflavin-deficient. Criticisms of the experiments of Schwarz & Spallholz (72) are reported in the discussion of tin below. Because findings did not show that cadmium deficiency consistently impaired any function of animals, cadmium cannot be considered an essential nutrient at this time.

Fluorine

The beneficial function of fluoride in preventing human dental caries was discovered in the late 1930s. Subsequently, epidemiologic findings suggested that fluoride is beneficial for the maintenance of a normal skeleton in adults. A recent review (1) summarized those findings, and a number of reports describing the treatment response of patients, suffering from osteoporosis and other demineralizing diseases, who were given substantial amounts of sodium

fluoride. In some patients, back pain, bone density, and calcium balance were improved.

In the early 1970s, scientists suggested that fluoride is necessary for hematopoiesis, fertility, and growth in mice and rats. The suggestion, however, was based on experiments in which animals were not fed optimal diets. Schwarz & Milne (70) reported that a dietary supplement of fluoride slightly improved the growth of suboptimally growing rats but did not result in optimal growth. The shortcomings of that study were the same as those described for cadmium (above) and tin (below). Messer et al (44) fed a marginally iron-deficient diet when they showed that high dietary fluoride (50 mg/l of drinking water) improved hematopoiesis and fertility in mice. Subsequent studies showed that very high levels of dietary fluoride can improve iron absorption or utilization from a diet that is marginally sufficient in iron (78, 83). To date, most of the findings that often are accepted as evidence for fluoride essentiality reflect a pharmacologic, not physiologic, action of fluoride. That is, high levels of orally administered fluoride alleviated a disorder caused by something other than fluoride deficiency. If tooth mottling is evidence of toxicity, near-toxic amounts of fluoride prevent tooth decay caused by bacterial plaque. Also, near toxic levels of fluoride are needed for preventive or therapeutic action against osteoporosis, which probably is a disorder of calcium-phosphorus metabolism. Extremely high levels of fluoride, i.e. 50 μg/ml of drinking water, probably acted through a pharmacologic mechanism to overcome depressed hematopoiesis and impaired reproduction caused by a marginal iron deficiency in mice.

At present, signs of fluoride deficiency have not been described for any animal, and no evidence shows unequivocally that fluoride is an essential nutrient for animals or humans. Lack of evidence, however, does not mean an essential function will not be found for fluoride. Furthermore, fluoride still must be recognized as a trace element with beneficial properties.

Lead

Findings suggesting that lead is an essential nutrient come from two laboratories. Schwarz (68) found that lead slightly improved the growth of suboptimally growing rats but did not result in optimal growth. In a series of reports summarized recently, Kirchgessner & Reichlmayr-Lais (35) reported that lead deprivation resulted in depressed growth and hypochromic microcytic anemia in rats. Also, lead deprivation apparently disturbed iron metabolism and altered the activities of several enzymes (e.g. catalase, ceruloplasmin, alkaline phosphatase) and the levels of several metabolites in liver (e.g., cholesterol, glucose, triglycerides).

Both studies on lead essentiality have shortcomings. The study of Schwarz (68) has the same faults as the studies on the essentiality of cadmium (above) or

tin (below). In the study of Kirchgessner & Reichlmayr-Lais (35), the most convincing evidence came from only a small number of very young rats in one of three experiments. In their first experiment, weaning rats were fed diets containing either 45 ng or 1 μg lead/g. After 30 days, dietary lead had no effect on the growing rats (65). In the second experiment, the experimental diets were fed to weanling female rats that were allowed to reproduce. The only positive finding in this experiment was that hematopoiesis was slightly depressed in 21-day-old lead-deprived pups. However, neither the dams nor the remainder of the pups killed at age 48 days were affected by lead deprivation. In the third experiment, lead-deprived and -supplemented rats were allowed to reproduce through two generations (35, 64, 66). The lead-deprived rats were fed either 18 ng (F_1 generation) or 30 ng (F_2 generation) of lead/g of diet (64). The primary evidence of lead essentiality was from eight lead-deprived and ten lead-supplemented F_1 female rats examined 17 days after weaning (aged 38 days). Lead deprivation markedly depressed growth and disturbed iron metabolism in this selected group of rats. In the other F_1 pups examined at older ages, however, the apparent effects of lead deprivation on growth, hematopoiesis, and iron disappeared. In the F_2 generation, no pups at any age exhibited signs of lead deprivation as marked as those exhibited by the eight female F_1 pups. Upon reviewing the data, the following questions seem appropriate: (*a*) Why did lead deprivation, contrary to the expected trend, affect F_2 pups less severely than F_1 pups? (*b*) Why did lead deprivation have only a transitory effect on rats? Organisms generally require essential nutrients throughout their lifetimes. Possible answers suggested by Reichlmayr-Lais & Kirchgessner (66) to those questions were: (*a*) The lead-deficient diet fed to older and F_2 rats contained more lead than the lead-deficient diet fed to F_1 rats; and (*b*) older rats homeostatically adapted to low dietary lead. On the other hand, perhaps lead had a pharmacologic effect, like that described for flouride (see above), on iron metabolism in young rats. The lack of sufficient data to provide conclusive answers to the questions indicates that the role of lead in nutrition and health should be clarified by additional studies. Until such studies are done, lead probably should not be considered essential.

Lithium

Findings suggesting that lithium is an essential nutrient come from two laboratories. Anke et al (2) found that lithium deprivation (2–3 μg lithium/g diet) depressed growth, fertility, and longevity of goats. In O'Dell's laboratory (7, 60), rats fed a low-lithium diet (5–15 ng/g) grew adequately, but fertility was depressed in F_2 and F_3 dams. In addition, the lithium content was depressed in testes, seminal vesicles, and epididymis. Lithium concentrations were relatively high in the pituitary and adrenal glands and remained constant through two generations regardless of dietary lithium.

Thus, two independent studies, in which the same sign of lithium deprivation—depressed fertility—was induced in two animal species, indicate that lithium is an essential nutrient. Furthermore, analyses of tissues suggested that lithium might have a role in endocrine function. Interpretation of the findings, however, is confounded by the wide difference between the two studies in the lithium content of the deficient diets (2–3 μg/g vs 5–15 ng/g). Possibly, the difference could be explained either by species differences in lithium requirement or by an error in lithium analyses. Nevertheless, concerns about the uncertainty of what constitutes a deficient diet and the lack of marked deprivation signs preclude stating conclusively that lithium is an essential nutrient.

Nickel

Nickel deprivation in animals was first described in 1970. The early studies, however, were conducted under conditions that produced suboptimal growth in the experimental animals (52). Also, some of the reported signs of nickel deprivation were shown inconsistently. Retrospective consideration of the methodology used in those studies indicated that much of the inconsistency was probably related

for, the experimental animals. Thus, most of the early findings apparently represented nickel deficiency modified by dietary and environmental conditions.

Since 1975, diets and environments that allow optimal growth and survival of experimental animals have been used in studies of nickel nutrition and metabolism. Signs of nickel deprivation have been described for six species—chick, cow, goat, minipig, rat, and sheep. The most prominent and consistent signs include depressed growth and hematopoiesis and changes in the levels of iron, copper, and zinc in the liver. The signs of nickel deprivation for each of the six species are listed in reviews (36, 49, 52, 53). This review focuses mainly on findings that could help identify the biological function of nickel. Some findings indicate that nickel functions either as a cofactor or structural component in specific metalloenzymes, or as a bioligand cofactor facilitating the intestinal absorption of the ferric ion.

Development of the hypothesis that nickel functions as an enzyme cofactor or structural component has been stimulated by the discovery of several nickel-containing enzymes in plants and microorganisms. Urease from jack bean *(Canavalia ensiformis)* was the first natural nickel metalloenzyme discovered (17, 18). Subsequently, ureases from several other plants and microorganisms were identified as nickel metalloenzymes (26, 61, 62, 76). Jack bean urease (EC 3.5.1.5) contains stoichiometric amounts of nickel, 2.00 ± 0.12 g-at/mol of 96,600-dalton subunits (17, 18). The nickel is tightly bound at the active site, apparently in an octahedral configuration (17). The active-site sulfhydryl groups of urease exhibit relatively low reactivity, which suggests

coordination of the active-site nickel with the unreactive cystine. Urease catalyzes the reaction:

$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3. \quad 1.$$

Apparently, binding of the substrate urea to a nickel ion in urease is an integral part of the mechanism in the hydrolysis reaction. A carbamato-enzyme intermediate involving active-site nickel and the oxygen of urea has been proposed (5). Nucleophilic attack by a suitable active-site group would then lead to an active-site, nickel-ammonia complex. Jack bean urease was the first enzyme to be crystallized. After 50 years, nickel was identified in the structure of urease.

More than 50% of the nickel taken up by methanogenic bacteria is incorporated into a low-molecular-weight compound with an absorption maximum at 430 nm (14, 80). This nickel-containing compound, named factor F_{430}, apparently contains a nickel tetrapyrrol structure. A nickel-containing degradation product of factor F_{430} has an absorption spectrum, in the visible region, resembling that of vitamin B_{12}. Also, biosynthetic studies indicate that, per mol of nickel, 8 mol of δ-aminolevulinic acid are incorporated into factor F_{430} (13). Factor F_{430} apparently is component C of methyl coenzyme M methylreductase, which reduces CH_3–S–CoM to methane and HS–CoM (20, 21). Component C has been identified as the (methylthio)ethanesulfonic acid methylreductase.

In acetogenic bacteria, an energy-source reaction

$$8[H] + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad 2.$$

proceeds via formate, formyl tetrahydrofolate, methenyl tetrahydrofolate, methylene tetrahydrofolate, and methyl tetrahydrofolate. The reductive carboxylation of the methyl tetrahydrofolate to acetate is catalyzed by a multienzyme complex, which has a moiety with carbon monoxide dehydrogenase activity. The synthesis of the moiety requires nickel (15, 16, 19, 80). Furthermore, analyses of the multi-enzyme complex indicate that the moiety is a protein with a nickel-containing prosthetic group with porphyrin-type properties (63, 80). Ni(III) apparently is involved in the interaction between the enzyme and a radical species formed from CO or HCO_3^-/CO_2 (63).

In bacteria that derive energy from the conversion of H_2 to CH_4 (methanogenic bacteria) or H_2O (Knallgas bacteria), the hydrogenase involved contains nickel, or requires nickel for its synthesis (24, 25, 38, 59, 80). In the hydrogenases of *Methanobacterium bryantii* and the sulfate-reducing bacterium, *Desulfovibrio gigas,* a substantial amount of the nickel is in the Ni(III) state (38, 40). LeGall et al (40) suggested that redox-sensitive nickel is an

important catalytic component of the *D. gigas* hydrogenase, and may represent the binding site for the substrate, H_2.

The finding of nickel metalloenzymes in plants and microorganisms (summarized in Table 1) suggests that nickel may have a similar function in

Table 1 Nickel metalloenzymes

Enzyme	Species from which isolated	Reference	Properties of nickel in enzyme
Urease (EC 3.5.1.5)	Jack bean	(5, 17, 18, 22)	Tightly bound at the active site; apparently in an octahedral configuration; 2.00 ± 0.12 g-at/mol[a] of 96,000-dalton subunit
	Soybean	(61, 62)	
	Rice, Tobacco	(62)	
	Lemna paucicostata	(26)	
	Ruminal	(76)	
Factor F_{430}—Component C of methyl coenzyme M methylreductase	Methanogenic bacteria	(13, 14, 20, 21, 80)	2 g-at/mol of enzyme; F_{430} may be a nickel tetrapyrrole
Hydrogenase			
Soluble	*Alcaligenes eutrophus*	(24, 25)	1–2 g-at/mol of enzyme
Particulate	*Alcaligenes eutrophus*	(24, 25)	
Periplasmic	*Desulfovibrio gigas*	(8, 40, 46)	About 1 g-at/mol of 89,500-dalton enzyme apparently present in a redox-sensitive form—Ni(III)
	Methanobacterium thermoautotrophicum	(27)	1 g-at/mol of enzyme
Membrane	*Vibrio succinogenes*	(81)	1 g-at/mol of enzyme with a mol wt of 100,000
Hydrogenase I	*Desulfovibrio desulfuricans*	(37)	0.6 g-at/mol of enzyme with a mol wt of 77,600
Hydrogenase II	*Desulfovibrio desulfuricans*	(37)	0.6 g-at/mol of enzyme with a mol wt of 75,500
Carbon monoxide dehydrogenase	*Clostridium thermoaceticum*	(16, 19, 63)	Enzyme with a mol wt of 440,000 and composed of three each of two different subunits $(\alpha\beta)_3$; two g-at nickel/mol dimer; at active site Ni(III) may be present in a porphyrin-like factor
	Acetobacterium woodii	(15)	

[a](gram-atom)/mole

animals. Nickel can activate many enzymes in vitro, but a role as a specific cofactor for any animal enzyme has not been shown (50).

The hypothesis that nickel may function as a bioligand cofactor facilitating the intestinal absorption of Fe^{3+} was supported by findings showing that, depending upon the form of dietary iron, nickel interacts with iron in the rat. Nickel synergistically interacted with iron to affect hematopoiesis in rats fed dietary iron as ferric sulfate only, but not as a mixture of ferric and ferrous sulfates (48, 50). Furthermore, when low levels of ferric sulfate only were fed, whole body retention of $^{59}Fe^{3+}$ was less in nickel-deprived rats, even though they were more anemic, than in nickel-supplemented rats, whereas $^{59}Fe^{2+}$ retention was not affected by dietary nickel (51, 52).

The mechanism through which nickel enhances Fe^{3+} absorption is unclear. Because Fe^{3+} tends not to stay in a soluble form in the duodenum, it needs to be complexed or converted to the more soluble Fe^{2+} form for efficient absorption (42). According to May et al (42), only ligands, such as porphyrin-like molecules, that form high-spin complexes and thereby increase the electrode potential stabilize Fe^{2+} over Fe^{3+}. Most other bioligands lower the electrode potential and thus enhance the stability of the Fe^{3+} state. Therefore, the preferred chelated state of iron in vivo is probably Fe^{3+} and the reduction to Fe^{2+} occurs spontaneously only in the presence of high local concentrations of a reducing metabolite, or under the influence of special enzyme mechanisms. The idea that nickel might act in an enzyme mechanism that converts Fe^{3+} to Fe^{2+} for absorption is attractive because of the recent finding of redox-sensitive nickel in enzymes of microorganisms. However, the possibility that nickel promotes the absorption of Fe^{3+} per se by enhancing its complexation to a molecule that can be absorbed cannot be overlooked. Such a molecule could be similar to the nickel tetrapyrrol molecule found in microorganisms.

By extrapolation from animal data, it is reasonable to postulate that nickel is required by humans. Moreover, the nickel requirement of animals should give a general idea of the amount of nickel that may be required by humans. For rats and chicks, the nickel requirement apparently is about 50 μg/kg of diet, or 16 μg/1000 kcal (52). For cows and goats the requirement is higher (>100 μg/kg of diet), probably because some rumen bacteria use nickel as part of their enzyme urease. Calculated from data for monogastric animals, a suggested dietary nickel requirement of humans would be near 35 μg daily.

Silicon

Early studies describing signs of silicon deprivation in chicks and rats have been summarized (9). Most of the signs indicated aberrant metabolism of connective tissue and bone. Animals in early studies were fed crystalline amino acid diets that did not give optimal growth in controls. Carlisle (10, 11) recently developed a semi-synthetic, silicone-deficient diet that produces near optimal growth in chicks. With this diet, in contrast to amino acid diets, silicon

deprivation did not affect chick growth or outward appearance but did affect connective tissue and bone. Abnormalities included structural abnormalities of the skull associated with depressed collagen content in bone, and long-bone abnormalities, characterized by small, poorly formed joints and defective endochondrial bone growth. Silicon-deficient chick tibiae exhibited depressed contents of articular cartilage, water, hexosamine, and collagen. Thus, although some of the early evidence for the essentiality of silicon may have been disputable because of the poor growth of the experimental animals, Carlisle's more recent findings clarify the issue.

Both the distribution of silicon in the organism and the effect of silicon deficiency on connective tissue form and composition support the view that silicon functions as a biological cross-linking agent and contributes to the architecture and resilience of connective tissue. Schwarz (67) found that silicon is a constituent of certain glycosaminoglycans and polyuronides where it is apparently bound to the polysaccharide matrix. In his review of the possible biochemistry of silicon, Schwarz (67) concluded that silicon is present as a silanolate, an ether-like derivative of silicic acid, in mucopolysaccharides. Thus, silicon could link portions within the same polysaccharide chain, different polysaccharides to each other, or acid mucopolysaccharides to proteins. Unfortunately, none of the proposed structures have been rigorously identified.

Proteins in connective tissue, notably collagen and elastin, also contain bound silicon (9). Carlisle (10, 11) found that levels of collagen were depressed in silicon-deficient chick skull, tibia, and tibial cartilage. Loeper et al (41) found that silicon preserved the integrity of elastic tissue in rabbits fed an atherogenic diet. Those findings suggest that silicon plays a fundamental role in the cross-linking mechanism in collagen and elastin.

Silicon apparently is involved in bone calcification (9), but the exact mechanism remains unclear. Some findings suggest a catalytic function for silicon. On the other hand, the marked influence of silicon on collagen and mucopolysaccharide formation and structure suggests that the influence of silicon on bone calcification is an indirect consequence of changes in these bone matrix components. Support for the latter view is that, in silicon-deficient animals, the formation of organic matrix, whether cartilage or bone, is apparently affected more severely than the mineralization process (11).

Ample evidence indicates that silicon can be accepted as an essential nutrient, but more work is needed to clarify the consequences of silicon deficiency in animals and humans. Carlisle (9), however, speculated that silicon might be involved in several human disorders including atherosclerosis, osteoarthritis, hypertension, and the process of aging. Those speculations demonstrate the critical need for study of the importance of silicon nutrition, especially in aging humans.

Although the essentiality of silicon was suggested more than ten years ago,

little is known about the nutritional requirements for silicon and silicon metabolism. The form needed and minimum requirement of silicon have not been ascertained for any animal, so there is no basis for conjecture concerning possible human requirements. The estimated requirement of chicks for silicon, as sodium silicate, is in the range of 100–200 μg/g diet, or approximately 26–52 mg/1000 kcal of an experimental diet (52). Schwarz (67) indicated that he found other silicon compounds that were 5–10 times as effective, per atom of silicon, as the silicate in preventing nutritional deficiency. Thus, the absolute requirement for chicks probably is much lower than 26–52 mg/1000 kcal.

Tin

The only evidence that supports essentiality of tin is that a dietary supplement of tin slightly improved the growth of suboptimally growing, apparently riboflavin-deficient (45) rats but did not result in optimal growth (71). The use of riboflavin-deficient rats in tin essentiality studies would be of particular concern because the oxidation-reduction potential of $Sn^{2+} \rightleftarrows Sn^{4+}$ is 0.13 V, which is near the oxidation-reduction potential of flavine enzymes. Attempts to show that tin deprivation depressed growth in riboflavin-adequate rats were unsuccessful, even though animals and dietary materials used were from the same sources as those used in the study with riboflavin-deficient rats (F. H. Nielsen, unpublished observations). Thus, it cannot be stated unequivocally that tin deprivation reproducibly impairs a function from optimal to suboptimal.

The study (71) that suggested the essentiality of tin can also be criticized as follows: (*a*) The small growth difference between "deficient controls" and tin-supplemented rats (about 5–7 g after 25 to 30 days on experiment) may be of questionable physiologic meaning. Because it was so small, perhaps the growth response was just an indication that tin prevented the breakdown of some essential nutrient (e.g. riboflavin), substituted for some trace element lacking in the diet, or acted as an antibiotic. Those possibilities would indicate that the action of tin is pharmacologic, rather than physiologic. (*b*) The addition of tin to the diet was of no apparent benefit to deficient controls in subsequent studies. For example, the deficient controls gained about 1.3–1.9 g/day; tin-supplemented rats, 1.7–2.2 g/day. However, even with the addition of tin and some other possibly essential elements, such as fluorine, to the same diet (70), the deficient controls in a lead essentiality study (68) still gained only 1.5–2.1 g/day, and lead-supplemented rats gained 1.6–2.2 g/day. Subsequent to the lead study, deficient controls and cadmium-supplemented rats exhibited similar daily weight gains (72). The finding that deficient controls gained at the same rate in each study—of tin, fluorine, lead, and cadmium—was unexplained, even though the deficient controls would be expected to grow better in the latter studies because their diets contained additional essential elements.

Without more conclusive evidence, tin should not be considered an essential trace element. Moreover, the description of any biological function or nutritional requirement would be inappropriate.

Vanadium

The essentiality of vanadium is difficult to categorize because reported evidence is inconclusive.

In 1971, Strasia (77) reported that rats fed less than 100 ng of vanadium/g of diet exhibited slower growth, higher plasma and bone iron, and higher hematocrits than controls fed 0.5 μg of vanadium/g of diet. Williams (84), however, could not duplicate those findings in the same laboratory under similar conditions. Schwarz & Milne (69) reported that a vanadium supplement of 25–50 μg/100 g of diet gave a positive growth response in suboptimally growing rats. On the other hand, Hopkins & Mohr (30) reported that the only effect of vanadium deprivation on rats was impaired reproductive performance (decreased fertility and increased perinatal mortality) that became apparent only in the fourth generation.

Studies with chicks also gave inconsistent signs of deficiency. Hopkins & Mohr (31) found that vanadium-deprived chicks exhibited significantly depressed wing- and tail-feather development, depressed plasma cholesterol and triglycerides at age 28 days, and elevated plasma cholesterol at age 49 days. Nielsen & Ollerich (55) reported that vanadium deprivation depressed growth, elevated hematocrits and plasma cholesterol, and adversely affected bone development in chicks.

Nielsen (49, 50) became concerned about the inconsistency of the effect of vanadium deprivation on chicks and rats, and attempted to establish a reproducible set of signs of vanadium deprivation in these species. In 16 experiments, in which chicks were fed several diets of different composition, vanadium deprivation adversely affected growth, feathering, hematocrit, plasma cholesterol, bone morphology, and the amounts of lipid, phospholipid, and cholesterol in liver. In several experiments with rats, vanadium deprivation adversely affected perinatal survival, growth, physical appearance, hematocrit, plasma cholesterol, and lipids and phospholipids in liver. Unfortunately, no sign of deficiency was found consistently throughout all experiments.

Recently, however, Shuler & Nielsen (74) found that the iron and cystine status of experimental animals must be controlled before data from different studies of vanadium nutrition can be compared. In moderately iron-deficient rats, for example, vanadium deprivation depressed hematocrit when dietary cystine was 4.65 mg/g and did not markedly affect hematocrit when dietary cystine was 10.15 mg/g. Vanadium deprivation did not affect hematocrit in rats fed adequate iron.

Possibly the effect of cystine indicated that differences in dietary amino acid composition (e.g. the ratio between essential sulfur amino acids), rather than the absolute cystine level, affect vanadium metabolism. When the total dietary level of sulfur amino acids was held nearly constant, changing the ratio of methionine to cystine altered the response of chicks to vanadium deprivation (54). The toxicity of vanadium, as measured by growth depression, was decreased by increasing dietary protein (29).

Differences in levels of dietary cystine (perhaps amino acid composition in general) and iron may explain some of the inconsistencies in vanadium deprivation signs found in earlier studies. Strasia (77), who found that vanadium deprivation depressed growth and elevated hematocrits in rats, fed a diet that contained only 20 μg iron/g and 269 g vitamin-free casein/kg. On the other hand, Williams (84), who found that vanadium deprivation did not affect growth and hematocrits, fed a diet that contained 35 μg iron/g and 175 g vitamin-free casein/kg. Possibly those differences in iron and protein levels were large enough to account for the differences in the findings of the two investigators. Similar comparisons could be made for the studies of Hopkins & Mohr (30, 31), Schwarz & Milne (69), and Nielsen et al (54, 55).

Recent interest in vanadium has been aroused by the finding in vitro that vanadate (vanadium in the +5 state) is a potent inhibitor of (Na^+,K^+)-ATPase and other phosphoryl-transfer enzymes. Vanadium is present in tissues at concentrations that might inhibit phosphoryl-transfer enzymes in vivo. Macara (43) therefore hypothesized that vanadium functions in vivo as a regulator of (Na^+,K^+)-ATPase and thus the sodium pump. That hypothesis should be tested in vivo. Also, the proposal of a regulatory function for vanadium would be strengthened by evidence of an in vivo mechanism whereby vanadium, which apparently exists in tissue as the relatively inactive 4+ oxidation state complexed to protein or small molecules, would be converted to vanadium in the 5+ state. The studies suggesting a regulatory function for vanadium are more completely summarized elsewhere (50, 52).

Failure to define the conditions that induce reproducible deficiency in animals has prevented the establishment of vanadium essentiality, but emerging evidence of its physiological function suggests that vanadium is essential for humans. However, findings to date can only allude to a possible vanadium requirement. Diets containing 4–25 ng vanadium/g adversely affect rats and chicks under certain conditions (49). Apparently any human requirement for vanadium would be very small. However, since food contains very little vanadium, the metabolism of which apparently is affected profoundly by other dietary components, there is a possibility that vanadium intake is not always optimal. That possibility demonstrates the need to clarify the biological function of vanadium, the conditions that produce vanadium deficiency, and the dietary components and their mechanisms that affect vanadium metabolism.

Table 2 Classification summary of the ultratrace elements

Element	Evaluation of essentiality	Selected reported deficiency signs[a]	Possible function	Suggested requirements for species studied
Arsenic	Essential	Depressed growth (C, R, P, G); abnormal reproduction characterized by impaired fertility and elevated perinatal mortality (R, P, G); myocardial damage (G); depressed serum triglycerides (G); altered plasma amino acid profile (C)	Necessary for the normal metabolism of an amino acid or a protein that influences the urea cycle	25 mg/g of diet (C, R)[a]
Boron	Further study required to establish essentiality; probably essential	Depressed growth and abnormal bone development with marginal magnesium and/or cholecalciferol nutriture (C)	Acts in concert with magnesium in some aspect of major mineral (Ca, P, Mg) metabolism	None
Bromine	Weak evidence for essentiality; should not be considered essential at present	Insomnia (H)?	No available evidence conducive to speculation	None
Cadmium	Weak evidence for essentiality; should not be considered essential at present	Depressed growth (G, R)?	No available evidence conducive to speculation	None
Fluorine	Weak evidence for essentiality; should not be considered essential at present; should be recognized as an element with beneficial pharmacologic properties	Depressed growth (C, R)? Depressed hematopoiesis and fertility (M)? Caries?	Pharmacologic actions suggest fluoride has a structural role in some calcified tissues	None

Lead	Evidence for essentiality has shortcomings; probably should not be considered essential at present	Depressed growth; hypochromic micocytic anemia; disturbed iron metabolism (R)	Facilitates iron absorption	None
Lithium	Further study required to establish essentiality; probably essential	Depressed fertility (R, G); depressed growth and longevity (G)	Regulation of some endocrine function	None
Nickel	Essential	Depressed growth (G, P, R, S, B); depressed hematopoiesis (C, R, S, G); liver ultrastructural abnormalities (C, R); disturbed iron metabolism (R); disturbed zinc metabolism (G); concentration changes in liver of iron, copper, and zinc (G, P, R, S); unthriftiness (R, P, G); depressed ruminal urease (B)	Cofactor or structural component of a metalloenzyme; bioligand cofactor facilitating ferric iron absorption or metabolism	50 ng/g of diet (C, R)
Silicon	Essential	Skull structural abnormalities (R, C); long-bone abnormalities characterized by small, poorly formed joints and defective endochondrial bone growth (C); depressed collagen and hexosamine in bone (C)	Biological cross-linking agent contributing to the architecture and resilience of connective tissue	Not accurately determined to date
Tin	Weak evidence for essentiality; should not be considered essential at present	Depressed growth (R)?	No available evidence conducive to speculation	None
Vanadium	Further study required to establish essentiality; probably essential	None consistently found	Regulation of some phosphoryl-transfer enzyme	None

[a]Letter in parenthesis indicates species: B = bovine (cow), C = chicken, G = goat, H = human, M = mice, P = minipig, R = rat, S = sheep.

CONCLUDING REMARKS

A summary of the preceding discussion is presented in Table 2. This table shows that a review of the experimental evidence supporting the suggestion of nutritional essentiality for 11 ultratrace elements indicated that only arsenic, nickel, and silicon meet the definition of essentiality. Some evidence suggests that boron, lithium, and vanadium are also essential nutrients. Only limited evidence supports the nutritional essentiality of bromine, cadmium, fluorine, lead, and tin. However, certain benefits of fluorine are well known.

Knowledge about the physiologic functions and optimum intakes of the ultratrace elements that have been identified as essential should be extended and refined. Further research might establish essentiality for some or all of the other eight proposed elements and may even identify new candidates. Improvements in the sensitivity and accuracy of analytical and histological methods and in the management of experimental animals should enhance the specificity with which the physiologic functions of ultratrace elements can be identified initially in animals and eventually in humans. Research may reveal that minute amounts of obscure elements are more important in human health than is now generally acknowledged.

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