Zinc: Health Effects and Research Priorities for the 1990s

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This review critically summarizes the literature on the spectrum of health effects of zinc status, ranging from symptoms of zinc deficiency to excess exposure. Studies on zinc intake are reviewed in relation to optimum requirements as a function of age and sex. Current knowledge on the biochemical properties of zinc which are critical to the essential role of this metal in biological systems is summarized. Dietary and physiological factors influencing the bioavailability and utilization of zinc are considered with special attention to interactions with iron and copper status. The effects of zinc deficiency and toxicity are reviewed with respect to specific organs, immunological and reproductive function, and genotoxicity and carcinogenicity. Finally, key questions are identified where research is needed, such as the risks to human health of altered environmental distribution of zinc, assessment of zinc status in humans, effects of zinc status in relation to other essential metals on immune function, reproduction, neurological function, and the cardiovascular system, and mechanistic studies to further elucidate the biological effects of zinc at the molecular level. — Environ Health Perspect 102(Suppl 2):5–46 (1994).

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The Spectrum of Health Effects Related to Zinc Status: From Deficiency to Excess

There is considerable evidence that zinc deficiency in humans is a serious worldwide problem and outweighs the potential problem of accidental, self-imposed, or environmental exposure to zinc excess. Acute deficiency (*1*) and chronic deficiency (*2*) are well-known entities in human populations and are probably much more common than generally recognized. The importance of zinc for human health was first documented in 1963 (*3*). During the past 25 years, deficiency of zinc in humans due to nutritional factors and several disease states has now been documented throughout the world. Prevalence of zinc deficiency is high in populations that consume large quantities of cereal proteins containing high amounts of phytate, an organic phosphate compound. Alcoholism, malabsorption, sickle cell anemia, chronic renal disease, and other chronically debilitating diseases are now known to be predisposing factors for zinc deficiency in humans (*4*).

Zinc deficiency is reflected in clinical syndromes which affect men and women of all ages and all socioeconomic and cultural classes in the United States. It is neither prevalent in any specific area of the United States nor associated with any specific or definitive biochemical marker, which can make its identification difficult and confusing. Its presence is manifested by a wide spectrum of symptoms, from acute, life threatening problems to mild subclinical or marginal disorders which may only vaguely disturb well being. The acute problems are often seen in profoundly ill patients treated in hospitals, whereas subclinical problems may be so vague that patients seek assistance outside traditional medical practice.

Based upon clinical data and using traditional, epidemiologic techniques, Henkin and Aamodt (*5*) have reclassified zinc deficiency into three syndromes; these are a) acute, b) chronic, and c) subacute zinc deficiency. Acute zinc deficiency is relatively uncommon and follows parenteral hyperalimentation or oral L-histidine administration. Chronic zinc deficiency is more common, usually resulting from chronic dietary lack of zinc. Subacute or latent zinc deficiency is the most common of these syndromes. It is estimated that there are 4 million people in the United States with this syndrome, the initial symptom being dysfunction of taste and olfaction; treatment with exogenous zinc restores taste and smell but this usually requires months before these functions are returned to normal (*6*). Diagnosis of these disorders is most efficacious following oral administration of zinc tracers such as ^{65}Zn, ^{67}Zn, or ^{70}Zn with subsequent evaluation of the kinetics of transfer of the isotope into various body tissues, the formulation of the data by compartmental analysis, and the integration of the data by a systematic model of zinc metabolism. Obviously, these techniques are complex and technically difficult, not a routine means readily applicable to assessing zinc status in individuals. In fact, there are no simple means for assessing the status of zinc in the human population.

Clinical symptoms of human zinc-deficiency states exhibit a spectrum ranging from mild to severe and may even be fatal if unrecognized and not corrected (*4*). The clinical manifestations of severely zinc deficient subjects include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and intercurrent infections due to cell-mediated immune disorders. These severe signs are seen in patients with acrodermatitis enteropathica secondary to an inborn error of zinc absorption, patients receiving total parenteral nutrition without zinc, and patients receiving penicillamine therapy. Growth retardation, male hypo-

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gonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing are usual manifestations of moderate deficiency of zinc. Recent studies show that a mild or marginal deficiency of zinc in humans is characterized by neurosensory changes, oligospermia in males, decreased serum testosterone in males, hyperammonemia, decreased serum thymulin activity, decreased IL-2 production, decreased natural killer cell activity, alterations in T cell subpopulations (*4*), impaired neuropsychological functions (*7*), and decreased ethanol clearance (*8*). All the above manifestations are correctable by zinc supplementation.

Zinc is generally considered a relatively nontoxic metal (*9*). This classification is based on several characteristics: a) zinc is a metal essential to hundreds of biological processes and must be consumed in the diet for optimum health; b) zinc is relatively abundant in the natural environment; c) the recommended daily allowance (RDA) of zinc in the human population is 8 to 15 mg higher than many other essential metals; d) zinc does not appear to accumulate in the body with age; e) there are no known genetic abnormalities which result in excessive accumulation of zinc in the body, unlike metals such as copper (Wilson's disease) and iron (hemochromatosis); f) homeostatic mechanisms regulate the body burden of zinc such that increased intake is associated with decreased absorption and increased excretion; g) zinc may have antioxidant effects and does not participate in oxidation–reduction cycles like iron and other transition elements; h) administration of zinc for therapeutic purposes in man at doses above the RDA have not produced significant pathology; and i) administration of zinc to experimental animals in doses more that 100 times the RDA have not produced significant pathology.

Some zinc salts, such as zinc chloride, in sufficient concentration can injure epithelial tissue. Inhalation, exposure of the skin, or ingestion can produce local pathological effects. In addition, intake of excess zinc has been reported in human studies to affect levels of pancreatic enzymes (*10*) and lipoproteins in serum (*11,12*), to alter the metabolism of copper (*13–15*) and iron (*16*), and to alter immunological function (*12*).

The industrial use of zinc affects the environmental distribution of this metal; zinc is frequently found in industrial waste sites. Currently, regulatory agencies in the United States are concerned with the possible significance of increased exposure to zinc through environmental sources such as increases in drinking water, particularly through leaching of zinc into ground water surrounding waste sites. Excess exposure to zinc is also potentially a hazard for industrial workers, especially through inhalation of welding fumes and exposure in smelting operations. Oral intake at levels above the RDA is also of concern in individuals who self medicate with dietary supplements of zinc and those who are treated with these preparations for therapeutic purposes.

The purposes of this paper are to a) review data on the zinc status of the US population, including critical dietary and physiological determinants; b) summarize current understanding of the role of zinc in biochemical and physiologic processes, as revealed in part by studies of zinc deficiency; c) evaluate the available literature regarding effects of increased exposure to zinc; and d) recommend areas of investigation for enhancing understanding of the health effects of zinc over the spectrum of zinc status from deficiency to excess.

Zinc Status in the US Population

Zinc Intake in the Diet

Zinc status of humans is generally determined by the quality and quantity of zinc in the diet and the physiologic condition of the individual. For persons with fully functional homeostatic mechanisms, quality and quantity of the diet are the major determinants of zinc adequacy. Diet quality and quantity are determined by the economic resources of the person, the availability of foods in the market place and individual food choices. Quality of the diet determines the sources and bioavailability of dietary zinc.

The zinc content of omnivorous North American diets is about 10–15 mg daily (*17*). A survey of US foods found 4.63 mg Zn/1000 kcal (*18*). Some reported dietary intakes of zinc are shown in Table 1 (*19–30*). The zinc contents of food products are altered by cooking, and zinc bioavailability is dependent on other components of the diet. Consequently, intakes of zinc based on calculations from food tables are estimates only.

Zinc is not evenly distributed in quantity or availability among foods. Table 2 indicates the total zinc content in ordinary size portions of foods. The major source of readily bioavailable zinc in the US diet is beef (*32,33*). The second most important source is pork (*34*). Current data suggest that meat provides nearly 50% of the zinc in the average US diet, that dairy products provide about 20% and that cereals and legumes provide the remainder (*35*) (Table 3). In contrast to omnivores, lacto-ovo-vegetarians obtain the majority of zinc from cereals (16%), beans and nuts (26%), and milk and eggs (18%) (*36*).

The critical importance of the bioavailability of dietary zinc to zinc nutriture was first shown in experimental animals

Table 1. Zinc intakes in diets of children, adults, and vegetarians.

Age	Sex	*n*	Zinc intake, mg		Population	Reference
			Mean	SD		
1 month	M, F	35	1.90	0.20	Breast-fed, Canada	(*19*)[a]
1 month	M, F	25	3.60	0.60	Bottle-fed, Canada	(*19*)[a]
6 months	M, F	16	2.70	0.50	Breast-fed, Canada	(*19*)[a]
6 months	M, F	23	4.60	0.70	Bottle-fed, Canada	(*19*)[a]
2–7 years	M, F	96	6.30	0.44	Middle income, USA	(*20*)[b]
2–6 years	M, F	85	5.00	—[m]	Low income, USA	(*21*)[c]
3–5 years	M, F	5	6.36	0.83	Norwegian children	(*22*)[d]
10–13 years	M, F	6	10.16	1.42	Norwegian preteens	(*22*)[d]
14–16 years	F	15	12.00	5.00	High school, USA	(*23*)[e]
18–30 years	F	21	13.80	7.27	College women, USA	(*23*)[e]
14–64 years	M, F	22	8.60	0.52	Middle income, USA	(*24*)[f]
30 years	F	100	10.10	3.30	Canadians, omnivorous	(*25*)[g]
Adult	F	5	6.40	1.69	Vegetarians, USA	(*26*)[h]
Adult	M	5	20.30	8.80	Military, USA	(*27*)[i]
81.7 years	M, F	21	5.53	—[m]	Geriatric hospital	(*28*)[j]
85.5 years	M, F	24	8.90	2.53	Healthy elderly at home	(*29*)[k]
25–48 years	M, F	112	8.5	4.5	Vegetarians (Punjabi), Canada	(*30*)[l]

[a]3-day records taken at home visits. [b]24-hr recall and food checklist. [c]24-hr recall and food checklist. [d]2-week food records. [e]Weighed 24-hr diets with duplicate food composite. [f]14-day food record and 132 diet composites. [g]3-day food records and 24-hr food composite. [h]3-day food records. [i]6 days of duplicate chemical diet composites. [j]Duplicate chemical diet composites. [k]Duplicate chemical diet composites. [l]3-day weighed records and thirty 1-day duplicate composites. [m]Value not reported.

Table 2. Zinc content of common household portions of selected foods[a]

Food	Portions	Zinc, mg
Fish, light poultry meat, shell fish (except crab and oyster)	3 oz	< 2.0
Poultry liver, dark chicken meat	3 oz	2.0/3.0
Pork, veal, crab, dark turkey meat, ground beef, 77% lean	3 oz	3.0/4.0
Beef liver, beef	3 oz	4.0/5.0
Oyster	3 oz	> 5.0
Egg, whole	1	0.5
Peanut butter	2 Tbsp	0.9
Mature dried beans, lentils, chickpeas, split peas (boiled, drained)	1/2 cup	0.9/1.0
Cow peas, black eyed peas (boiled, drained)	1/2 cup	1.5
Milk: Whole, fluid	1 cup	0.9
Canned, evaporated	1/2 cup	1.0
Dried, nonfat, instant	1/3 cup	1.0
Ice cream	1 1/2 cup	1.0
Cheddar cheese	3 slices (1 1/2 oz)	1.6
Cooked oatmeal	1 cup	1.2
Cooked whole wheat cereal	1 cup	1.2
Wheat flakes	1 oz	0.6
Bran flakes, 40%	1 oz	1.0
Wheat germ, toasted	1 Tbsp	0.9
Corn flakes	1 oz	0.08
Cooked corn meal	1 cup	0.3
White wheat bread	1 slice	0.2
Whole wheat bread	1 slice	0.5
Cooked brown rice, hot	1 cup	1.2
Cooked white rice, hot	1 cup	0.8
Precooked white rice, hot	1 cup	0.4

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(*37,38*). Phytate, present in grain and vegetable components of the diet, was shown to inhibit zinc absorption. Subsequent research showed that zinc absorption in humans was also impaired by phytate (*39–42*). Zinc forms insoluble complexes with phytate at alkaline pH, which are even more insoluble as a calcium–phytate–zinc complex (*43*). Other dietary inhibitors of zinc absorption include lignin, certain hemicelluloses, products of nonenzymatic Maillard browning, calcium phosphopeptides from the digestion of casein, and calcium (*43–46*).

The availability of zinc from diets rich in foods prepared from unrefined cereals tends to be poor. This was first illustrated by balance studies on Iranian subjects fed diets rich in bread prepared from whole grain wheat flour (*39,40,47*). Subsequently, others used analysis of plasma zinc to show that foods rich in phytate, fiber, and lignin reduce apparent absorption of zinc (*44,48*). Of particular interest was the effect of tortilla on the increase in plasma zinc that occurs after ingestion of 120 g of oyster (*44*). The increase was completely prevented by simultaneous consumption of 120 g of tortilla. Other studies found that as little as 26 g daily of wheat bran incorporated into 180 g of bread in a mixed western diet reduced the net retention of zinc (*49*). The importance of the inhibition of zinc retention by high cereal diets is illustrated by the Prasad-Halsted syndrome, a condition of growth retardation and delayed sexual maturation among adolescents in Egypt and Iran who subsist on diets that are based on whole wheat unleavened bread and little or no meat (*3,50–52*). More recent findings in East African children, among whom short stature is common, support the concept that diets high in phytate can cause serious impairment in zinc nutriture of populations. The principle finding was an inverse relation of phytate–zinc and calcium–phytate–zinc molar ratios to the concentration of zinc in hair from the children (*53*). Although hair zinc is a relatively insensitive indicator of zinc status, it is a useful index because it may be low while plasma levels are within the normal range (*54–56*).

The binding of zinc by calcium phosphopeptides derived from the peptic digestion of casein (*45*) may affect the bioavailability of zinc to humans and thus affect zinc nutriture. This suggestion is supported by the finding of lower zinc retentions in 18 men fed diets that provided 15% of energy from protein, of which about 40% was from dairy products, as compared to zinc retention when the dietary protein was 8% of energy and the dairy products were not added (*49*). A nearly 50% reduction in zinc retention in postmenopausal women fed cow's milk compared to water also indicates that cow's milk can decrease zinc bioavailability (*57*). Others have found that dairy products added to a meal that contained turkey meat lowered zinc retention (*58*). Zinc-tolerance tests in humans fed milk or cheese also indicate impaired zinc absorption (*48,59*).

Zinc Intake in Drinking Water

Under usual conditions, drinking water is a minor source of zinc intake. According to a review by the National Research Council of the National Academy of Sciences (NAS/NRC), the highest observed concentrations of zinc in drinking water are 1.3 to

Table 3. Contribution of foods to dietary intake of zinc.

Food group	Zinc content as purchased, mg/kg	Zinc consumed/ person/day, mg	Percent of total food intake
Milk, cheese, ice cream	4.3	2.5	20
Meat, poultry, fish	18.6	5.5	43
Dry beans, peas, nuts	24.7	0.5	4
Eggs	12.7	0.6	5
Dark green and deep yellow vegetables	2.8	0.1	1
Citrus fruit, tomatoes	1.8	0.3	2
Potatoes	2.4	0.3	2
Other vegetables, fruit	2.0	0.7	6
Cereal, pasta	16.3	0.8	7
Flour, mixes	2.8	0.1	1
Bread	7.2	0.6	5
Other bakery products	6.0	0.4	3
Fats, oils	1.8	0.1	1
Sugar, sweets	0.6	0	0
Total food		12.5	100

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Table 4. Recommended dietary allowances (RDA) for zinc.[a]

	Infant		Children	Males	Females	Pregnant	Lactating	
Age, years	0–0.5	0.5–1.0	1–10	11–51 +	11–51 +		1st 6 mo	2nd 6 mo
Zinc RDA, mg	5	5	10	15	12	15	19	16

[a]From National Research Council (*62*).

1.5 mg/l (*9*). Thus a person drinking 2 l daily would consume a maximum of 3 mg/day from this source. The usual dietary intake of most adults does not exceed 12 mg daily. Therefore, consumption of drinking water containing the maximum observed zinc concentration would result in a total intake of 15 mg/day, which is the current RDA for adult men. The US Environmental Protection Agency standard for maximum zinc concentration in drinking water is 5 mg/l (*60*), which is based on the aversive taste associated with higher zinc concentrations in water (*61*).

Zinc Dietary Requirements

It is difficult to precisely determine the requirement of zinc in various populations, inasmuch as many dietary factors affect the bioavailability of zinc and environmental and physiological factors alter the requirements of zinc in different age groups. Nonetheless, the current US guidelines for RDAs of zinc (Table 4) appear reasonable. A substantial fraction of the population may consume less zinc than recommended (*31,63,64*).

Growing infants, children, adolescents and pregnant mothers require more zinc per kilogram than mature adults. In early lactation human milk provides about 2 to 3 mg zinc daily. The amount gradually decreases to about 0.6 mg/day at 6 months (*65*). During lactation, the zinc content of milk remained constant in spite of 60 mg zinc supplementation per day for 2 weeks (*66,67*).

Zinc is less available from cow's milk formula than from human milk. Growth of infants fed zinc-enriched (5.8 mg/l) cow's milk formula was better than that of infants fed an unenriched formula that contained an amount of zinc (1.8 mg/l) similar to human milk (*68*). Since that report was published, formulas have been supplemented to provide 5 mg zinc daily, assuming consumption of 750 ml of formula (*17*). Because of the phytate content, zinc availability from soy-based formulas is lower than from cow's milk or human milk (*69*).

Provisional factorial estimates of zinc requirements of infants (Table 5) are 1.1 to 1.25 mg daily up to 1 year (*70*). The US NAS/NRC RDA of zinc infants is 5 mg daily, assuming 20% bioavailability (*17*). The minimal requirement of dietary zinc for premature infants weighing 670 to 2420 g during the period from 36 to 40 weeks postconceptional age was determined to be 0.8 mg/kg/day (*71*). The current recommendation for zinc administration in infants receiving total parenteral nutrition is 97.5 µg/kg/day. This amount of zinc may not be adequate for infants who have excessive gastrointestinal fluid losses, such as those who have recently undergone surgery and had excessive gastrointestinal drainage (*72*).

Factorial estimates (Table 5) suggest that preadolescent children require about 1.6 mg of absorbed zinc daily for growth and maintenance (*70*). The mean dietary requirement is therefore about 8.0 mg daily for diets containing 20% bioavailable zinc. If one assumes a 15% coefficient of variation and an allowance of 30% above the mean requirement, the recommended intake for preadolescent children is 10.4 mg daily. Based on different data and calculations, the RDA committee recommended 10 mg daily (*17*). Average zinc intakes of US children ages 1to 3 years are about 75% of the RDA, or 7.5 mg daily (*35*). Data in Table 1 are consistent with the US Department of Agriculture report. One early study showed that with dietary intakes of 4 to 6 mg/day, zinc balance was negative in preschool children (*73*). If one assumes the RDA is 30% above the average dietary requirement, the probability approach for evaluating dietary data indicates that more than 50% of US children are at risk of zinc deficiency (*74*).

It is evident from the above that quantitative knowledge of the zinc requirements of children is limited. One suspects that children from poor families, such as those cited in Table 1, who were from poor Hispanic families in Denver and displayed improved growth when supplemented with zinc (*21*), are at substantial significant risk of zinc deficiency. Studies in Middle Eastern schoolboys have also demonstrated retardation of growth and maturation (*75,76*), ameliorated by zinc supplementation (*77*).

Because of accelerated growth, the zinc requirement of adolescents, as noted above, is greater than that of preadolescents. For this reason the RDA for this age group is the same as that for adults (12 mg for women and 15 mg for men) (*17*). Factorial estimates (Table 5) of requirement are 14 mg daily for males ages 11 to 17 years and 13.3 mg daily for females ages 10 to 13 years, with 20% bioavailability of dietary zinc (*70*). Assuming a 15% coefficient of variation in requirement, the recommended intake from the factorial estimates is 18.2 mg for boys, and 17.3 mg daily for girls. At present it is unknown how close

Table 5. Provisional dietary requirements for zinc in relation to estimates of retention, losses, and availability.

Age	Peak daily retention, mg	Urinary excretion, mg	Sweat excretion, mg	Total required, mg	Milligrams necessary in daily diet if content of available zinc is 10%	20%	40%
Infants							
0–4 months	0.35	0.4	0.5	1.25	12.5	6.3	3.1
5–12 months	0.2	0.4	0.5	1.1	11.0	5.5	2.8
Males							
1–10 years	0.2	0.4	1.0	1.6	16.0	8.0	4.0
11–17 years	0.8	0.5	1.5	2.8	28.0	14.0	7.0
18+ years	0.2	0.5	1.5	2.2	22.0	11.0	5.5
Females							
1–9 years	0.15	0.4	1.0	1.55	15.5	7.8	3.9
10–13 years	0.65	0.5	1.5	2.65	26.5	13.3	6.6
14–16 years	0.2	0.5	1.5	2.2	22.0	11.0	5.5
17+ years	0.2	0.5	1.5	2.2	22.0	11.0	5.5
Pregnant women							
0–20 weeks	0.55	0.5	1.5	2.55	25.5	12.8	6.4
20–30 weeks	0.9	0.5	1.5	2.9	29.0	14.5	7.3
30–40 weeks	1.0	0.5	1.5	3.0	30.0	15.0	7.5
Lactating women	3.45	0.5	1.5	5.45	54.5	27.3	13.7

From Sandstead (*31*) based on WHO Report (*70*); reprinted with permission.

the factorial estimates (*70*) are to the true requirement. If one assumes that dietary zinc intakes of adolescents are similar to those reported for adults (Table 1), the great majority consume less zinc than is recommended by the RDA or that is calculated as needed from the factorial estimates of requirement. Information is insufficient to resolve these issues. As a practical application of this knowledge, one suspects the observation on low-income adolescents of improved pregnancy outcome subsequent to zinc supplementation was related to insufficient zinc in their habitual diets (*78*).

With secession of growth the zinc requirements of adults decrease to amounts required for tissue repair and general maintenance (Table 5). At 20% bioavailability the factorial estimate of need is 11.0 mg daily for both men and women (*70*). The RDA is slightly higher, consumption of 15 mg/day by adult males (Table 4). Other government advisory bodies have set different recommendations for zinc intake. The Canadian Bureau of Nutritional Sciences has recommended that a lower zinc intake (9 mg/day for adult males and 8 mg/day for females) is safe and adequate.

The effects of dietary composition on the requirement for zinc are illustrated by data from 30-day balance studies carried out in healthy men fed omnivorous diets (*79*). In these studies the relationship between dietary intake of zinc (y) and zinc balance (x) was examined to determine the intake required to maintain equilibrium (y intercept). The diets provided 8% of energy as protein and were low in fiber and phytate. An intake of 7.18 ± 0.09 (SE) mg zinc daily was sufficient for equilibrium, which suggests the dietary zinc was more than 30% bioavailable. When 26 g of various cereal brans were added to the diet in 30 balance studies, the zinc requirement for equilibrium increased to 9.13 ± 0.37 (SE) mg daily. When the protein intake was increased to 15% of energy by adding dairy products to the diet, equilibrium intake was 12.17 ± 0.40 (SE) mg zinc on a diet without fiber and phytate and 14.74 ± 0.33 (SE) mg zinc ($p<0.01$) with a diet containing 26 g of cereal. Estimates of zinc requirements in relation to protein and phosphorus intake, based on data from the above metabolic studies, are indicated in Table 6.

The factorial estimate (*70*) of zinc requirement at 20% bioavailability suggests a recommended intake of 14.3 mg for both men and women, assuming a 15% coefficient of variation. The RDA for men is 15 mg daily and for women is 12 mg daily. Reported self-selected adult intakes of zinc are listed in Table 1. According to US Department of Agriculture data, 60% of women ages 19 to 50 years consume <9.0 mg zinc daily (*35*), or 75% of the RDA. This suggests that more than 50% of these women are at risk of zinc deficiency (*74*).

Among the elderly, zinc intakes are about 9 mg daily with a wide range (*80*). Because the capacity to absorb zinc from the intestine is reduced with aging (*81*), it seems likely that the elderly are at increased risk of zinc deficiency, an impression that appears supported by data (*80*). The current zinc RDA for the elderly is the same as for younger adults. Relatively few elderly consume those amounts of zinc. Bunker et al. (*82*) have recently published a study illustrating these points. Five-day metabolic balance studies for zinc were carried out in 20 housebound elderly 70- to 85-year-old subjects who ate self-selected diets. The mean daily intake for zinc was significantly lower than the RDA for this age group. Zinc balances were negative; leukocyte zinc levels (but not plasma zinc) were decreased compared to controls and correlated with zinc balance.

In conclusion, evaluation of the risk of zinc deficiency by the probability approach, using estimates of zinc requirements from factorial calculations and balance studies, the 1989 RDA, and reported intakes of zinc suggest that many Americans are at risk of zinc deficiency (*31,63,64*). Zinc deficiency has been documented in low-income children who participated in a Head-Start program (*83*), in poor children in the Southeastern United States (*63,64,84*), in elderly persons from the United States (*4,85*), and in so-called "normal healthy subjects" from the United States who showed zinc-related immunological abnormalities (*86,87*). In the United States, although severe dietary deficiency of zinc in the absence of other factors is unlikely, mild and marginal deficiency of zinc is likely in subjects who consume diets rich in phytate, dietary fiber, and calcium phosphopeptides from dairy products, and low in red meat. The risk of zinc deficiency (moderate or severe) is substantially increased by conditioning factors such as intestinal malabsorption, catabolic illness, alchoholism, cirrhosis, hemolytic anemias, renal failure, certain medications (diuretics, steroids and acetylcholinesterase inhibitors), blood loss by parasitic infestations, geophagia, and an excessive loss of zinc due to sweating (*3,4,50,88,89*).

Biochemical Properties of Zinc

Chemistry of Elemental and Salt Forms of Zinc

Zinc, an element with an atomic weight of 65, is classified as a Group IIB post-transition member of the periodic table. The Group IIB metals below zinc in the periodic table are cadmium and mercury, nonessential metals of greater toxicity. Key chemical characteristics of zinc are a) a tendency to lose two electrons and as the +2 cation form salts of varying solubility in aqueous solution and b) a tendency to form relatively stable coordinate bonds with electronegative ligands such as nitrogen, oxygen, and sulfur. Zinc, unlike other transition elements, is relatively stable in the divalent state and does not undergo redox changes.

Reactions in Aqueous Solution

Zinc in aqueous solution as the +2 cation becomes hydrated at low pH and at high pH forms zincate anions, possibly $Zn(OH)_4^{-2}$. Various zinc compounds differ significantly in their aqueous solubility.

Table 6. Relationship of dietary zinc requirements to dietary phosphorus and protein intakes of men fed fixed American diets.[a]

Protein intake, g	Dietary zinc requirements, mg[a]			
	40	60	80	100
1000	5.27 (2.48–8.07)[b]	6.91 (4.11–9.70)	8.54 (5.74–11.33)	10.17 (7.38–12.96)
1500	9.11 (6.32–11.91)	10.27 (7.47–13.06)	11.42 (8.62–14.21)	12.57 (9.78–15.36)
2000	12.95 (10.16–15.75)	13.63 (10.83–16.42)	14.30 (11.50–17.09)	14.97 (12.18–17.76)
2500	16.79 (14.00–19.59)	16.99 (14.19–19.78)	17.18 (14.38–19.97)	17.37 (14.58–20.16)

[a]These data are exclusive of the zinc loss in sweat, which was 0.50 ± 0.38 mg for 88 twenty-four-hour collections on 13 men under temperate conditions. [b]Estimated 95% confidence interval. Table from Sandstead (*31*); reprinted with permission.

For example, the solubility of zinc chloride (mw 136) in water at 25°C is 432 g/100 ml whereas that of zinc oxide (mw 81) at 29°C is 0.00016 g/100 ml (*90*).

Interactions with Proteins

Cellular Binding Ligand–Metallothionein. Metallothionein, found in most mammalian tissues, is a significant macromolecular ligand for zinc. This small protein (mw 6700) is primarily an intracellular cytosolic molecule, although the protein is detectable in extracellular fluids such as plasma and urine. Metallothionein, unique in its high cysteine content, binds 7 moles of zinc per mole of protein. The zinc atoms are now known to be distributed in 2 clusters, 1 containing 3 zinc atoms and 9 cysteine molecules and the other 4 zinc and 11 cysteines (*91*). The normal function of this protein is not clear but may include a role as a free-radical scavenger (*92*). *In vitro* studies have shown metallothionein to be a potent binder of hydroxyl radicals (*93*)
. Whether it also plays a role in the provision of zinc to apometalloenzymes is as yet unconfirmed. It does play a prominent role in the tissue localization of zinc, as the major intracellular binding site, and possibly in the gastrointestinal absorption of zinc. Metallothionein is also important in the "detoxification" of certain nonessential metals such as cadmium. Factors which promote increased expression of metallothionein, such as stress, infection, heat shock proteins, glucocorticoids, and nonessential metals such as cadmium and mercury, may increase zinc content of the liver and decrease its plasma concentration (*92*).

Enzymes. Zinc is required for the optimum function of as many as 300 enzymes (*91*). The role of zinc in these metalloenzymes includes participation in catalytic functions, maintenance of structural stability, and regulatory functions (*94*). Zinc metalloenzymes are involved in the formation or hydrolysis of each of the major classes of endogenous compounds (proteins, lipids, carbohydrates, etc.) and includes representatives from each of the six categories of enzymes of the International Union of Biochemistry. The crystal structure has been identified for 12 zinc enzymes (*91*), including alcohol dehydrogenase, carboxypeptidases A and B, alkaline phosphatase and carbonic anhydrases I and II. Current evidence indicates that the zinc binding sites vital to catalysis include three amino acid residues and an "activated" water molecule. The amino acids can include combinations of histidine, glutamine, aspartate, or cysteine. Histidine is the most frequently observed and is often coordinated with an oxygen group of a nearby amino acid, most notably the carboxylate in aspartate (*95*). Analysis of the coordination sites of zinc in enzymes where its role is structural indicates bonding to sulfur ligands in cysteines to form a tetradentate complex. Vallee and Auld (*91*) have recently proposed, based on analysis of matrix zinc enzymes such as procollagenase, that one mechanism of enzyme activation may entail proteolytic cleavage of a segment with a cysteine zincbinding site. The residual protein would contain a zinc with only tridentate binding characteristics, thus rendering a fourth site available to act in a catalytic role.

DNA-binding Proteins. The interaction of zinc with proteins helps explain another critical function of zinc in biological systems, the regulation of DNA and RNA synthesis (*96–99*). By coordination with cysteine and histidine residues in certain proteins, zinc confers on the complex a tertiary structure which has affinity for unique stretches of DNA in promoter gene regions. The configurations include the zinc finger, the most common zinc motif, and the more recently characterized zinc thiolate cluster, seen in GAL4 (two zinc, six cysteine). A model of the 3-dimensional interaction of a zinc-finger binding protein with DNA, based on X-ray crystallographic analysis, has recently been published (*100*). The α-helix of the finger loops was found to sit in the major groove of β-DNA, one loop per groove, with the primary contact at a three base-pair subsite. Binding at these sites enhances DNA transcription and increases synthesis of the protein product for that messenger RNA sequence. Quantitative analysis for zinc content has definitively established the presence of zinc in the DNA binding proteins, TFIIA, the glucocorticoid receptor, GAL4, and g32P. The amino acid sequences of many other DNA binding proteins, estimated at more than 150 (*91*), contain homologous cysteine- and/or histidine-containing sites, suggestive of a critical structural role of zinc.

Hormone-Receptor Interactions. Zinc is also required for the optimum activity of growth hormone. Recently, Cunningham et al. (*101*) demonstrated that zinc markedly enhances the affinity of human growth hormone for the extracellular binding domain of the human prolactin receptor. The K_D value of zinc (0.4 μM) is in the range of free zinc in serum which supports the physiological significance of this observation. Analysis of binding constants for mutants suggests that zinc forms a tetradentate complex through coordination with two histidine sites and one glutamine site on growth hormone and one histidine on the prolactin receptor. Remarkably, zinc does not play a role in the interaction of human growth hormone with the human growth hormone receptor. Cunningham et al. (*101*) have suggested the importance of examining the role of zinc in binding interactions of hormones with receptors of the cytokine superfamily which are homologous to the prolactin receptor.

Neurotransmitter Receptors. Recent evidence indicates that zinc plays a modulating role in synaptic transmission by interacting with specific sites on ionotropic neurotransmitter receptor proteins. Celentano et al. (*102*) have demonstrated in spinal cord neurons that extracellular zinc inhibits γ-aminobutyric acid (GABA) receptor function by an allosteric mechanism. The zinc interaction site differs from that for other known modulatory substances but is recognized by certain divalent cations like cadmium. Zinc has also been shown to interact with the *N*-methyl-D-aspartate (NMDA) receptor at a unique extracellular site distinct from other modulators like magnesium or glycine. In cultured hippocampal neurons, zinc, in micromolar concentrations, acts as a noncompetitive antagonist of NMDA-induced increased membrane conductance (*103*). Zinc in normal physiological conditions is believed to affect synaptic transmission in certain areas of the central nervous system, such as the hippocampus where it is concentrated in presynaptic vesicles and on release inhibits $GABA_B$ receptor activation (*104*). The normal role of zinc in the brain is clearly complex, since it acts as an inhibitory modulator at receptors for both inhibitory (GABA) and excitatory, potentially neurotoxic (glutamate/NMDA) neurotransmitters.

Transduction Mechanisms–Protein Kinase C. Several investigators have reported interactions of zinc with protein kinase C, a critical enzyme in the signal transduction pathway elicited by membrane receptor activation followed by enhanced phosphoinositol turnover. The literature is at present not consistent with respect to the effects of zinc. Murakami et al. (*105*) reported that zinc activated and then inhibited the catalytic activity of this enzyme, as the concentration increased. Other investigators demonstrate only an inhibitory effect of zinc and argue that the activation observed by others is an artifact of calcium

release in the experimental model (*106*). The site and mechanism of zinc-induced inhibition has not been thoroughly established; data from the work of Speizer suggest that zinc does not interact with the cation (Ca, Mg) or diacylglycerol regulatory sites but may impair stimulation of the enzyme by fatty acids (*106*). Other work has focused on the translocation of protein kinase C which is involved in its activation (*107*). These studies suggest that zinc enhances interaction of the enzyme with "phospholipid and membrane cytoskeletal protein," which may play a role in a specific functional response in human B lymphocytes.

Antioxidant Activity. Under *in vitro* conditions in biological systems, zinc can act as an antioxidant by interacting with sulfhydryl groups of macromolecules, thereby inhibiting their oxidation and by competing for binding sites on membranes with metals such as copper and iron, thereby decreasing the electron transfer capabilities of the latter (*108*). *In vivo* at least one mechanism of zinc-induced antioxidant effect is the induction of metallothionein which is an effective free-radical quencher. Zinc administration can inhibit the toxicity of agents such as carbon tetrachloride, ethanol and ionizing radiation, which act in part through oxidative injury. These effects of zinc, however, may not be directly linked to an antioxidant effect and may be attributable to other mechanisms.

Membrane Interactions. Another property of zinc which has been hypothesized to play a role in normal physiological function of zinc is its capacity to "stabilize membranes" (*109,110*). This phenomenon is presumably a consequence of the binding of zinc to ligands in membranes which are essential for maintenance of the normal structural geometry of the protein and lipid components. Experimental systems where this property of zinc has been observed include erythrocytes where zinc decreases osmotic fragility and protects against various inducers of hemolysis such as osmotic gradients and bacterial toxins, and hepatic lysosomes where zinc elevated *in vitro* or *in vivo* enhances their stability as indicated by a reduction in β-glucuronidase release.

Utilization and Interactions

Absorption, Distribution, and Elimination of Zinc

Dermal Absorption. There is a paucity of literature on the dermal absorption of zinc. Information is lacking on the rate and extent of transport of different zinc salts and on the various factors likely to influence this process such as zinc-binding proteins in the skin. Published studies have limited applicability because they are of limited scope. Keen and Hurley (*111*) observed that 8- or 24-hr application of zinc chloride in corn oil to the skin of pregnant rats on a zinc-deficient diet increased the plasma concentration of zinc to normal or slightly above normal levels, respectively. The fraction of the applied dose which was absorbed was not determined. A study by Hallmans (*112*) suggested that serum and urine zinc levels increased following application of adhesive tape containing zinc oxide onto second and third degree burn wounds. These data do not permit estimation of the magnitude or rate of dermal absorption of zinc. Skog and Wahlberg (*113*) have reported estimates of the percutaneous uptake of zinc chloride following topical application to the dorsal skin of the guinea pig. Their data were based on monitoring the decline of radioactivity emitted by ^{65}Zn in at least 10 trials for each concentration. Concentrations of 0.08 to 4.87 M $ZnCl_2$, with pH ranging from 1.8 to 6.1, were tested. Their method of data presentation makes interpretation difficult. It appeared that in most trials the loss of radioactivity after 5 hr was less than 1%, except for the trial with the lowest pH where the mean may have been between 1 and 2%. Kapur et al. (*114*) examined radioactivity remaining after 6 or 24 hr in the block of rabbit skin to which one of four zinc compounds in a glycerin:propylene glycol vehicle had been applied. This study entailed two animals sacrificed at different times, so the data are extremely limited.

Gastrointestinal Absorption. Zinc uptake by high molecular weight proteins in the intestinal mucosa is an active process requiring ATP (*115*). Under conditions of normal dietary zinc uptake, the initial uptake of zinc by the brush-border membrane of the mucosal cell appears to depend on its net availability for uptake to, and transfer by, a membrane associated carrier. The uptake increases significantly with zinc depletion. The net availability of zinc for uptake into the mucosal cell depends on the relative zinc binding affinity of zinc-binding ligands in the intestinal lumen and the membrane carrier.

Recently, the intestinal site of zinc absorption in humans has been determined with the triple-lumen steady state perfusion technique (*116*). Seventeen healthy subjects participated in the study. During intestinal perfusion of a balanced electrolyte solution containing 0.1 mM of zinc acetate, zinc absorption occurred throughout the entire small intestine. However, the jejunum had the highest rate of absorption (357 ± 14 nM/min/40 cm) compared to the duodenum (230 ± 33 nM/min/40 cm) and ileum (84 ± 10 nM/min/40 cm). A linear increase in the rate of zinc absorption was observed when the concentration of zinc infused into the jejunum varied from 0.1 to 1.8 mM. Intestinal absorption of zinc was significantly stimulated by the addition of glucose (20 mM). Conversely, zinc (0.9 mM) also enhanced the absorption of glucose. The enhanced absorption of zinc in glucose was not accompanied by any increase in absorption of water and sodium. In contrast, increasing the concentration of zinc in the perfusate resulted in decreased absorption of sodium and water in a dose-related manner. Thus, these studies demonstrated that zinc absorption is concentration-dependent, occurs throughout the small intestine with a maximum rate in the jejunum, and is probably carrier-mediated.

Factors known to influence the net gastrointestinal absorption of zinc are listed in Table 7 and include: a) ligands (zinc may be bound to one or more ligands, some of which will impede and others enhance absorption); b) zinc status (absorption is increased during zinc deficiency); c) intracellular transport (the active transport mechanism of zinc appears to be under metabolic control); and d) endogenous zinc secretion (a significant amount of zinc is secreted into the intestinal lumen via the epithelial cells, bile, and pancreatic secretion.

LIGANDS. Most of the information concerning zinc absorption is limited to experimental animal model studies. During digestion, zinc is released from its dietary ligands (mostly proteins) and becomes associated with intestinal low molecular weight ligands which make zinc available to the intestinal microvilli. Some of these ligands such as histidine and other amino acids are of dietary origin but others such as metallothionein may be of endogenous origin. Wapnir et al. (*117*) observed that amino acids increased zinc absorption in the colon, a site not considered as important for absorption of zinc in humans.

Putative ligands in milk include citric acid, picolinic acid, immunoglobulins and lactoferrins. Whether picolinic acid or citric acid is the predominant low molecular weight zinc ligand in human milk has not been settled (*119–121*). The possibility also exists that the association of zinc with citric or picolinic acid is an artifact of isolation procedures. Although both citric acid

Table 7. Factors associated with decreased zinc absorption.[a]

Dietary factors
Calcium
Copper (in animals ingesting large quantities)
Iron
Phytate (greatest inhibition in presence of high calcium)
Fiber (certain hemicelluloses)
Lignin
Products of Maillard Browning
Alcohol
Polyunsaturated fatty acids
Absence of appropriate absorption ligands
Acrodermatitis enteropathica (AE)
Cystic fibrosis
Pancreatic dysfunction
Breast milk vs milk formulas (phosphopeptide products of casein digestion in presence of calcium)
Phenylketonuria
Hypothyroidism
Gastrointestinal dysfunction
Intestinal mucosal disease
Malabsorption syndrome
Gastrointestinal surgery

[a]Modified from Cunnane (*118*).

and picolinic acid enhance zinc absorption, their physiological relevance is not clear.

Although human milk contains less zinc than cow's milk, the availablility of zinc in human milk is significantly higher (*122*). Some investigators have suggested that human milk contains a specific protein ligand for zinc, which is not present in significant quantity in cow's milk (*123,124*). In one study, a human milk protein was isolated which enhanced zinc absorption (*123*). Other compounds in milk which may or may not be ligands per se also facilitate zinc absorption. These include histidine, D-penicillamine, essential fatty acids and prostaglandins (*118*).

Prostaglandin E has been reported to increase zinc absorption. In zinc-deficient rats, a positive correlation between zinc and prostaglandin E content of the gut has been observed (*125*). Cottonseed oil improved the clinical condition of three cases of patients with acrodermatitis enteropathica, a fatal disease prior to the advent of zinc therapy (*126*). Linoleic acid and γ-linolenic acid increased the phospholipids of the intestinal mucosa from zinc-deficient rats. Although zinc is not bound to prostaglandins or essential fatty acids, they may facilitate its absorption by unknown mechanisms.

Zinc absorption was measured in 37 children with malnutrition using the oral zinc tolerance test (22 mg elemental zinc) and the data were compared with those of a group of healthy control subjects (*127*). The increase in plasma zinc was significantly lower in patients with marasmic kwashiorkor than in the control group which may reflect decreased absorption. The zinc tolerance test was, however, normal in marasmic patients.

Plant proteins, such as soy, increase zinc requirement in comparison to proteins of animal origin in experimental animals. Excessive intake of phytate, a component of plant protein (myoinositol 1,2,3,4,5,6-hexakisdihydrogen phosphate), has been implicated as an important factor responsible for zinc deficiency in Middle Eastern dwarfs (*128*). Negative zinc balance as a result of fiber supplementation has been demonstrated (*129*). This effect may have been secondary to phytate and lignin in the diet. However, the effect has not been consistently observed in humans or in animals which may be due to different effects of soluble and insoluble fiber (*26,36,130*). In vegetarians, phytate and fiber intake is significantly higher than in individuals eating an omnivorous diet, and it is likely that their combined effect on zinc absorption is clinically more important.

Alcohol is known to decrease zinc absorption in the rat (*131,132*). In humans, the effect of alcohol on zinc absorption is not well defined. Sullivan et al. (*133,134*), by using a zinc tolerance test, showed that zinc absorption was decreased by concurrent alcohol intake. Absorption of ^{65}Zn has been shown to be lowered in patients with alcoholic cirrhosis in comparison to controls (*135*). In alcoholics consuming a standard meal, absorption of 50 mg but not 25 mg of zinc was decreased (*136*). Alcoholics exhibit low levels of plasma zinc and also hyperzincuria, which might be an important factor in the pathogenesis of zinc deficiency (*134*).

Zinc absorption has also been examined in nonalcoholic cirrhotic patients with the oral zinc (22.5 mg elemental zinc) tolerance test (*137*). The increase in plasma zinc was significantly lower in cirrhotic patients compared to the control group in the first, second, and fourth hour post ingestion. Zinc malabsorption may in part be the result of pathological changes in the intestinal mucosa and lamina propria, documented in biopsies

EDTA, which is used during the processing of vegetables, has been shown to reduce zinc absorption in humans (*44,138*). EDTA appears to have two effects, one to decrease dietary zinc content and the other to decrease its absorption. Thus, depending upon the frequency and quantity of EDTA being used, one may observe a negative effect on zinc homeostasis in humans.

ZINC STATUS. Zinc absorption is increased in zinc-deficient rats and decreased in rats fed excess zinc (*118,139,140*). Zinc absorption is also increased in pregnant rats and reverts to normal after parturition. In humans an 11-fold increase in zinc ingestion, from a normal dietary intake of 8 to 13 mg daily to diet plus a 100 mg zinc supplement daily (as $ZnSO_4$), produced only a 37% increase in plasma zinc (*141*). These results could be explained in part by a drop in absorption from 43 to 9% of the ingested zinc load.

INTRACELLULAR TRANSPORT. The mechanism by which zinc is transferred to or across the mucosal surface of the microvilli is not known. Once in the intestinal cell, zinc becomes associated with metallothionein and perhaps other proteins (*142*). This process appears to be dependent on protein synthesis, since cycloheximide and actinomycin D inhibit mucosal zinc uptake (*143,144*). The complexing of zinc with the intracellular metalloprotein is an energy-dependent process. The precise role of intestinal metallothionein in zinc absorption is not well understood.

Transport studies using pig small intestinal brush-border membrane vesicles showed that zinc uptake was augmented by highly permeable anions such as thiocyanates, suggesting that movement of zinc involved complex formation with negative species (*145*). The imposition of an outwardly directed K^+ gradient (negative inside) did not affect the maximum value (J_{max}) of saturable zinc uptake but increased the concentration for half maximal uptake (K_m) significantly. This suggests that at least a portion of zinc which crosses the membrane does not do so in a cationic form. The presence of calcium had no effect on zinc entry into the vesicles (*145*).

In one study (*146*), the addition of an excess of folate, histidine or glucose had no effect on zinc (5 μM) uptake by porcine intestinal brush-border membrane vesicles, whereas addition of picolinate, citrate and phytate to the incubation medium significantly reduced zinc uptake. The inhibitory effects of these ligands may have been due to the formation of zinc ligand complexes which are either insoluble, or which reduce the binding of zinc to its mucosal receptor. Although this model is useful for comparing the effects of potential zinc binding ligands in the diet, these data do not provide an insight into the effects of various ligands on zinc absorption.

Zinc uptake was investigated in membranes derived from small intestines of rats fed zinc-adequate and zinc-deficient diets

Table 8. Zinc concentrations in body fluids, hair, and blood cells of humans.

Fluid	Zinc concentration[a]	Reference
Plasma	110.7 ± 14.8 μg/dL	(148)
Erythrocytes	40.67 ± 3.60 μg/g Hb	(148)
Lymphocytes	50.9 ± 5.9 μg/10^{10}cells	(154)
Granulocytes	45.9 ± 6.4 μg/10^{10} cells	(154)
Platelets	3.3 ± 0.3 μg/10^{10} cells	(154)
Hair	193 ± 18 μg/g	(155)
Urine	643 ± 198 ug/d	(155)
Milk[b]	1.2 ± 3.6 μg/ml	(156)
Bile	171 ± 106 μg/ml	(157)
Whole sweat	115 ± 30 μg/dl	(88)
Cell-free sweat	93 ± 26 μg/dl	(88)
Seminal plasma	692 ± 24 μg/ml	(158)
Cerebrospinal fluid	0.03 ± 0.02 μg/ml	(159)

[a]Mean ± SD. [b]During lactation.

(*147*). Zinc uptake into basolateral membrane vesicles exhibited a saturable phase within the zinc concentration range of 5 to 625 μM. Zinc uptake also showed a nonsaturable (diffusion) phase. Kinetic analysis of the saturable component showed a K_m at 1 min of 24 μM zinc and J_{max} of 17 nmoles/mg protein/min. These studies demonstrated that the basolateral membrane zinc transport system proceeds principally by a carrier-mediated mechanism. These studies also suggest that zinc transport by the basolateral membrane may be ATP dependent.

Zinc in Plasma and Tissues. Values for the plasma zinc in normal subjects obtained by different investigators using various techniques are with few exceptions in reasonably good agreement. Better methods of avoiding sample contamination and more precise analytical tools now provide accurate data for plasma zinc. The plasma zinc concentration (mean ± SD) in normal subjects has been reported as 110.7 ± 14.8 μg/dl (*148*) (Table 8).

Plasma zinc levels in the newborn are in the same range as in adults (*65*). The levels fall to just below adult level within the first week of life and continue to decline until 3 months; the adult level is reachieved at about 4 months of age. Some investigators have shown decreasing plasma zinc values with increasing age over 60 years (*150,151*), which may reflect lower dietary intake and decreased absorption. Whether or not this decrease implies an increased requirement for zinc in elderly subjects remains to be established.

Transferrin, albumin, and α-2-macroglobulin have been proposed as likely serum proteins involved in the transport of zinc in blood subsequent to its absorption (*152*). The binding of zinc to amino acids and serum protein has been studied *in vitro* by Prasad and Oberleas (*152*). Following incubation of ^{65}Zn with pooled native human serum, ultrafilterable zinc was determined to be 2 to 8% of the total serum zinc, when the zinc/albumin molar ratio was varied from 0.33 to 2.5. Under similar conditions, 0.2 to 1.2% of zinc was ultrafiltrable when predialyzed serum was used. In physiological concentrations, addition of amino acids to predialyzed serum increased ultrafilterable ^{65}Zn severalfold. Histidine, glutamine, threonine, cystine, and lysine showed the most marked effect in this regard. It was suggested that the amino-acid-bound fraction of zinc may have an important role in biological transport of this element. In predialyzed serum, the endogenous zinc content was determined to be highest in the albumin fraction with smaller concentrations in α-, β-, and γ-globulins. The results obtained by using ^{65}Zn-incubated predialyzed serum, however, indicated a difference in the behavior of exogenous zinc as compared with the endogenous zinc bound to various serum proteins. *In vitro* studies using predialyzed albumin, haptoglobin, ceruloplasmin, α-2-macroglobin, transferrin, and IgG, incubated with ^{65}Zn, revealed that zinc was bound to all these proteins, and that the binding of zinc to IgG was electrostatic in nature. Whereas amino acids competed effectively with albumin, haptoglobin, transferrin and IgG for binding of zinc, a similar phenomenon was not observed with respect to ceruloplasmin and α-2-macroglobulin, suggesting that the latter two proteins exhibited a specific binding property for zinc.

The uptake of transferrin-bound zinc by human lymphocytes is stimulated *in vitro* by several agents such as prostaglandin E_1, epinephrine, glucagon, histamine, and serotonin (*153*). Prostaglandin $F_{2\alpha}$, however, is inhibitory. The mechanism is not known.

Body fluid, hair, and blood cell levels of zinc in humans are shown in Table 8. Zinc in lymphocytes, granulocytes, and platelets appear to be a sensitive indicator of zinc status in humans. Table 9 shows the concentrations of zinc in various human and animal tissues.

Peripheral Utilization. Apart from studies of zinc uptake by the liver (*160–162*), very little is known about the utilization of zinc by peripheral tissues. ^{65}Zn uptake by isolated rat hepatocytes in culture showed a rapid initial phase which was not carrier-mediated, and a slower second phase. Neither phase was affected by cyanide, prostaglandins or sex steroids, suggesting that active transport was not involved (*160,163*). High (65,000) and low (metallothionein, about 6000) molecular weight proteins were involved in zinc uptake by hepatocytes. When excess zinc was available, the lower weight proteins played a greater role.

A kinetic analysis of zinc uptake by isolated rat liver parenchymal cells defined two intracellular pools (*161,162*). In 1 pool zinc was bound relatively weakly and equilibrated rapidly with the medium at 37°C (labile pool). In the other pool zinc appeared to be bound tightly and equilibrated slowly. Zinc uptake was temperature dependent and was inhibited by both N-ethylmaleimide and iodoacetamide, suggesting that sulfhydryl groups may be involved in one or more steps in the translocation/binding process. In contrast

Table 9. Zinc concentrations in human and animal tissues (mg/kg dry weight)[a]

Tissue	Human	Rat	Calf	Pig
Liver	141–245	101 ± 13	101	150.8 ± 12
Kidney	184–230	91 ± 3	73	97.8 ± 3.0
Lung	67–86	81 ± 3	81	
Muscle	197–226	45 ± 5	86	
Pancreas	115–135			139.5 ± 4.0
Heart	100	73 ± 16		
Bone	218	168 ± 8	78	95 ± 1.8
Prostate				
Normal	520			
Hyperplasia	2330			
Cancer	285			
Eye				
Retina	571			
Choroid	562			
Ciliary body	288			
Testis		176 ± 12	79	54 ± 2.0
Esophagus		108 ± 17		88.1 ± 3.0

[a]Mean ± SD. [b]From Prasad (*155*); reprinted with permission.

to earlier studies, metabolic inhibitors such as azide, cyanide, and oligomycin inhibited uptake of zinc. The factors that augmented the uptake/exchange of zinc, namely glucocorticoids, glucagon, epinephrine, and dibutyryl cyclic AMP, were also those that stimulated metallothionein gene expression in hepatocytes. Changes in zinc flux into intracellular pools were directly related to the metallothionein content of hepatocytes.

In vitro ^{65}Zn uptake by human retinal pigment epithelium has also been studied (*164*). The data were consistent with a facilitated type of transport and demonstrated the ability of human retinal pigment epithelium to accumulate and retain zinc.

It has been suggested that the peripheral utilization of zinc is abnormal in patients with myotonic dystrophy, thereby causing localized zinc depletion in cardiac and skeletal muscle (*165*). Muscle catabolism is also an important mechanism by which zinc can be released to maintain fetal growth in zinc-deficient pregnant rats (*166*). In pregnancy, there is reduced maternal fecal zinc excretion suggesting that peripheral utilization of zinc by the fetoplacental unit may be increased (*167*).

Excretion. Zinc excretion is primarily via the feces (*168*). Daily fecal excretion ranges from 5 to 10 mg/day and depends upon the dietary zinc. In pathological conditions accompanied by diarrhea and malabsorption excessive fecal loss of zinc may rapidly result in negative zinc balance. Fecal zinc is comprised of unabsorbed dietary zinc and endogenous zinc loss from bile, pancreatic fluid and cells of the intestinal mucosa. In man studies of ^{65}Zn administered intravenously indicate that secretion of zinc into the intestine is as high as 18% of the administered tracer (*169,170*). The pancreatic secretion of zinc into the intestinal lumen has been studied in animals (*171,172*). Mateseche et al. (*173*) have demonstrated that even under "normal" conditions nearly as much zinc could be secreted from the pancreas as was absorbed from the intestine. The secretion of endogenous zinc is increased when the dietary protein is of plant origin in comparison to when the protein source is of animal origin.

Basal and cholecystokinin-stimulated pancreaticobiliary secretion of zinc has been studied in normal subjects on zinc-adequate and zinc-deficient diets and in patients with Wilson's disease before and after zinc therapy (*153*). Following intravenous infusion of cholecystokinin (CCK8) (40 ng/kg/hr), the pancreaticobiliary secretion of zinc increased from a basal of 283 ± 76 nM/min to a peak of 717 ± 175 nM/min in normal subjects on a zinc adequate diet. Normal subjects on a zinc deficient diet had both lower basal (67 ± 16 nM/min) and stimulated (560 ± 31 nM/min) pancreaticobiliary secretion of zinc. In contrast to the markedly reduced pancreaticobiliary secretion of copper, patients with Wilson's disease, not treated with zinc, had normal basal (227 ± 126 nM/min) and stimulated (729 ± 196 nM/min) zinc secretion. These studies demonstrate that a considerable amount of zinc is being secreted in pancreaticobiliary fluid in healthy subjects and in patients with Wilson's disease. These data also indicate that pancreaticobiliary secretion of zinc is dependent on zinc status and may therefore play a role in zinc homeostasis.

Zinc is also excreted in the urine. In humans approximately 200 to 600 μg zinc is lost per day in the urine, generally less than 10% of the amount consumed in the diet. Urinary zinc excretion appears to be sensitive to alterations in zinc status (*4,141*). An 11-fold increase in zinc ingestion in humans, by supplementation with $ZnSO_4$, has been shown to produce a 37% increase in plasma zinc, but a 188% increase in daily zinc excretion in the urine (*141*). These results indicated enhanced renal clearance in response to an increase in plasma levels, possibly due to increased filtration and/or decreased reabsorption.

Zinc clearance studies in anesthetized dogs have been performed during hydropenia, mannitol infusion, and infusion of mannitol plus $ZnSO_4$, $ZnCl_2$, or cysteine (*174*). Under control conditions the clearance of ultrafiltrable endogenous zinc was about one-quarter that of inulin, indicating substantial net reabsorption. $ZnSO_4$ infusion increased filtered zinc 13-fold and zinc excretion only 6-fold, indicating increased net zinc reabsorption. Stop-flow studies localized zinc reabsorption to the distal nephron during infusion of mannitol and mannitol plus $ZnSO_4$ or $ZnCl_2$. Cysteine infusion increased urinary zinc excretion 86-fold, indicating net tubular zinc secretion, some of which derived from nonplasma sources. Zinc secretion was shown to occur in the proximal tubule with reversal of the distal reabsorption pattern seen during $ZnSO_4$ and $ZnCl_2$ infusion. These experiments demonstrate that the nephron under these experimental conditions is capable of both proximal secretion and distal reabsorption of zinc. There is no known capacity in mammals to store zinc with the possible exception of zinc stored in the bone. Homeostasis of zinc thus depends upon the balance between absorption and excretion. Zinc excretion is decreased when animals or humans are zinc deficient (*175–177*). Zinc excretion in both urine and feces may be extremely low in zinc-deficient rats. In addition, zinc redistribution from bone into muscle may occur (*178,179*). Conversely, in zinc-supplemented animals, endogenous secretion of zinc is increased which helps maintain zinc homeostasis.

Interactions with Other Metals

Zinc and Copper. The interaction between zinc and copper may be considered to be mutually antagonistic. In zinc-deficient experimental animals levels of copper in liver and bone have been observed to increase (*180–184*). In zinc deficient dairy cows copper excretion in milk is increased (*185*). In contrast, excessive dietary zinc in experimental animals produces copper deficiency, as manifested by reduced copper concentrations in liver, heart, and serum, and decreased activities of copper metalloenzymes such as ceruloplasmin, cytochrome oxidase, and superoxide dismutase (*186–190*). The effective dose level of zinc varies over a wide range from about 100 to more than 1000 mg/kg dietary dry matter, because various dietary constituents influence the availability of the dietary zinc and, thereby, the effects on copper metabolism. In humans chronic, elevated intake of zinc, 100 mg or more per day prescribed or self-administered, has been shown to induce copper deficiency (*15,191*).

Changes in copper status are reversed by raising dietary copper in order to narrow the zinc:copper ratio. Similarly, the zinc supply may be raised as a protective measure against copper toxicity in livestock (*192–194*).

Imbalances between zinc and copper may occur because of either deficient or excessive copper intake, or excessive intake of zinc relative to copper. When the copper supply is low and zinc intake is adequate, zinc will be present at a relatively high level compared to copper and, in this situation, the zinc status is not affected, but copper status may be impaired (*190,195*). Only a slight increase in zinc concentration in the liver and bone may be noted in copper-deficient animals (*196*). The effects of high copper intake when the zinc intake is normal are not well defined.

Mode of Zinc–Copper Interactions. Zinc and copper inhibit each other's intestinal absorption under certain condi-

tions. In rats excess copper decreases zinc absorption in zinc-adequate, but not in zinc-deficient, rats (*197–200*). In zinc deficiency, absorption of both zinc and copper is increased. In contrast, in copper deficiency, copper absorption is increased but zinc absorption is not.

A completely satisfactory explanation for the zinc–copper interactions at the site of absorption is not available. Intestinal metallothionein level may play an important role. The level of metallothionein in the intestine is directly related to zinc status; zinc induces the synthesis of metallothionein in intestinal cells (*201*). Inasmuch as metallothionein has a higher affinity for copper than zinc, copper absorbed by the intestinal mucosal cells is bound to metallothionein, does not enter the body and is returned to the intestinal lumen with the turnover of the intestinal mucosal cells (*143*). No such relationship between metallothionein and reduced zinc absorption after excess copper exposure has been reported (*202*).

Zinc and Copper Interactions in Humans. Copper deficiency is rare in human adults and is manifested by leukopenia and anemia (*15*). In infants and children, nutritional copper deficiency resulting in hypochromic microcytic anemia and neutropenia has been reported (*203*). Occasionally, total parenteral nutrition without copper supplementation has resulted in copper deficiency in adults (*204,205*).

Hypocupremia and hypoceruloplasminemia were observed in an adult with sickle cell anemia who received zinc (25 mg elemental zinc every 4 hr for 2 years) as an antisickling agent (*15*). The hypocupremia was associated with microcytosis and relative neutropenia. Administration of copper corrected these abnormalities. Hypoceruloplasminemia of varying degrees in several other sickle cell anemia patients who were receiving oral zinc therapy has been observed (*15*).

These observations prompted Brewer et al. (*206*) to treat patients with Wilson's disease by oral administration of zinc. Wilson's disease is an autosomal, recessively inherited, inborn error which causes low excretion of copper by the liver and toxic accumulation in the liver, brain, and other tissues. Conventional therapy with the chelator penicillamine can cause a variety of side effects so severe as to prevent its use in about 10% of patients (*207*). The need for alternative therapies led to trials with zinc by Brewer. Supportive of his approach were reports of Hoogenraad (*208*) which suggested that zinc might be efficacious in this disease.

Brewer et al. have studied a large series of patients since their original report (*206,209–211*). Their findings demonstrate the efficacy of zinc (50 mg, 3 times daily) in the treatment of Wilson's disease. Use of the copper tracer ^{64}Cu acetate in cow's milk has documented that zinc therapy markedly reduces copper absorption from the intestine into blood from about 6% to <1% (*212*). Further studies have revealed a decline to normal levels in 24-hr urinary copper excretion and in nonceruloplasmin plasma copper in zinc-treated patients with Wilson's disease (*209*). Liver biopsies suggested that zinc therapy also prevented further hepatic accumulation of copper (*210*).

Interactions of Zinc and Copper in Other Clinical Conditions. A reciprocal relationship between plasma levels of zinc and copper has been observed in various clinical conditions. A decreased plasma zinc level and an increased plasma copper level have been reported in pregnancy, women on oral contraceptives, acute infection, malignancy, cardiovascular disease, renal disease, schizophrenia, and certain endocrine diseases such as acromegaly and Addison's disease.

The interference of zinc with copper retention in humans is illustrated by clinical reports and investigations of subjects taking zinc supplements. Anemia and neutropenia developed in a man who took excess amounts of nonprescribed zinc gluconate for 2 years (*13*). Hyperzincemia, hypocupremia, low serum ceruloplasmin, and ringed sideroblasts in normoblasts in the bone marrow were observed. All the above manifestations were reversed following withdrawal of zinc. Presumably, these manifestations were caused by copper deficiency induced by excessive ingestion of zinc. Patterson et al. (*213*) also reported sideroblastic anemia and leukopenia associated with copper deficiency in a young man who had ingested more than 50 mg elemental zinc orally for 2 years. A woman studied by Hoffman et al. (*14*) who took 440 to 660 mg of zinc sulfate (110–165 mg elemental zinc) daily for 10 months for aphthous ulcers of her mouth and tongue developed copper deficiency with anemia and neutropenia. Samman and Roberts (*214*), using a double-blind crossover design, compared similar amounts of zinc supplementation, 150 mg zinc/day for 6 weeks, to placebo in healthy men and women. Plasma copper levels and hematocrits were not altered. However, in a subsequent report, these investigators (*215*) indicated that in females, but not males, the zinc treatment significantly reduced ferroxidase activity of serum ceruloplasmin, antioxidant activity of erythrocyte superoxide dismutase and copper–zinc erythrocyte superoxide dismutase. Plasma zinc levels were higher in females than in males, which possibly could explain the sex-dependent effect of zinc on copper status.

Fischer et al. (*216*) studied the effects of a lower level of zinc supplementation (25 mg twice daily) on copper metabolism in 26 healthy adult men, also over a 6-week interval. Plasma zinc was significantly increased by week 2. No change occurred in plasma copper. However, by 6 weeks Cu, Zn-superoxide dismutase in red blood cells was decreased about 10% in the zinc-treated group, suggestive of decreased copper status. Whether enzyme levels were altered in other tissues was not tested, although studies with rats by the same group (*189*) have shown decreases in liver superoxide dismutase as zinc intake increased. Balance studies in humans also suggest that copper requirements for equilibrium are increased as dietary zinc is elevated (*217*)

In summary, several studies indicate that zinc may reduce the activity of erythrocyte superoxide dismutase, probably in response to a change in copper status. The physiological significance of this effect has not been established, either in primary copper deficiency or secondary to elevated zinc levels. This enzyme converts superoxide to hydrogen peroxide and oxygen and therefore modulates the cellular toxicity of superoxide generated in xenobiotic biotransformation or oxidant and nonoxidant stress (*218*). Various factors such as nonlethal stress have been shown to induce increased synthesis of this enzyme (*219*). Implications of the protective effect of this enzyme against chemically induced cancer are offered in studies such as that by Werts and Gould (*220*) who demonstrated higher enzyme activity in mammary gland tissues from aged, pregnant or multiparous Sprague–Dawley rats, physiological states with decreased susceptibility to dimethylbenzanthracene-induced mammary cancer. Other factors, however, may be more critical determinants. To date there is no evidence that excess intake of zinc is associated with mutagenicity or carcinogenicity. Consequently, it is not clear that pathological significance can be attributed to the zinc-induced decrease in Cu,Zn-superoxide dismutase, secondary to copper deficiency.

Iron Impairment of Zinc Absorption. Recently, a concern has been raised that an antagonistic effect of iron on zinc absorption may have a deleterious effect on zinc nutrition, particularly in population groups that are routinely supplemented with iron (*16,221*). Both iron and zinc appear in the first transition series of the periodic table, and they share an identical outer electronic configuration with manganese, cobalt and nickel. Zinc and iron are essential for normal growth and development and are similar in amounts normally ingested.

In rodents, the capacity of the intestine to absorb iron is greatly increased by feeding a low iron diet. In iron-deficient rats and mice, the oral absorption of zinc is also greatly increased, suggesting a shared transport pathway (*222–224*). Hill and Matrone (*225*) have proposed that ions with similar electronic configuration might compete for binding ligands that mediate their intestinal absorption and subsequent transport into the blood. Numerous studies, with only one exception (*226*), have subsequently demonstrated that iron inhibits zinc absorption into plasma when aqueous solutions of the minerals are tested (*227–234*). Consistent with the electronic configuration hypothesis, ferrous iron produces greater inhibition of zinc uptake than ferric iron (*225*).

The mechanisms of zinc and iron absorption are not completely similar. Iron absorption is restricted to the duodenum, whereas zinc is absorbed throughout the small intestine and colon (*140,223,229*). Zinc appears to be a less effective inhibitor of iron absorption than iron is of zinc absorption. Although the capacity to absorb iron is increased equally by either bleeding or by feeding a low-iron diet, the absorption of zinc is increased only in rats and mice fed low-iron diets (*222*). Finally, in mice with sex-linked anemia (sla), the genetic lesion affects the absorption of iron but not zinc (*235*).

In human studies, it has been observed that inorganic iron added to test solutions of zinc salts in Fe/Zn ratios of 2.25 significantly lowered zinc absorption (*229, 231,232,234*). In contrast to rodents, human subjects with an increased capacity to absorb iron do not absorb increased amounts of zinc given as zinc chloride (*234*). This observation suggests that zinc and iron probably do not interact in humans to the same extent as observed in rodents and could explain why higher Fe/Zn ratios were required to inhibit zinc absorption in the human studies (*221*).

In contrast to the above results in humans, in three studies where iron was given with food, no effect on zinc absorption was observed. When oysters, providing about 54 mg "organic" zinc, were consumed with 100 mg of ferrous iron, plasma uptake of zinc was not altered (*231*). When turkey meat containing 4 mg of zinc was consumed with either 17 mg or 34 mg of ferric iron, zinc absorption was unchanged (*234*). Finally, when ferrous iron at a Fe/Zn ratio of 25 was added to a composite meal containing 2.6 mg of zinc, the absorption of zinc was not significantly changed (*229*).

It appears, therefore, that under usual conditions human zinc absorption is determined largely by the nature and extent of zinc complex formation with food in the intestinal tract, and normally the influence of iron on zinc absorption may not be significant. Under unusual circumstances, however, if large iron supplements are ingested in the absence of food, it is likely that iron could impair zinc absorption. A number of clinical studies support this point (*16*). For one, the study of Prasad et al. (*175*) followed four human volunteers for several months on a semisynthetic soy protein-based zinc-deficient diet containing 3.5 mg of zinc per day. The fall in plasma zinc was more pronounced in the first two subjects who received 130 mg of iron per day (Fe/Zn ratio of 37:1), in comparison to the other two who received 20.3 mg of iron per day (Fe/Zn ratio of 8:1). These results suggested that the iron excess enhanced the zinc deficiency.

A second study measured growth rates of healthy middle-class white infants receiving zinc, 1.8 mg/l cow's milk formula, vs those receiving zinc, 5.8 mg/l, plus iron, 12 mg/l. The results suggested that more zinc was required in the presence of iron for achieving optimal growth in the infants (*16*). However, in another study of healthy infants who were zinc sufficient, iron supplementation (up to 30 mg/day) did not appear to affect zinc nutriture (*236*). This observation needs further confirmation.

In healthy nonpregnant women, a ratio of iron to zinc increasing from 0.1 to 3.1 given in aqueous solution (zinc kept constant at 25 mg) caused a progressive decrease in the plasma response to the ingested zinc (*231*). In pregnant women, zinc absorption is decreased by oral iron supplements (*167,237*). Hambidge et al. (*167*) reported an inverse relationship between the level of daily iron supplement and the plasma zinc level in the first and third trimester of pregnancy. Campbell-Brown et al. (*238*) also observed that three women who supplemented 100 mg or more of iron daily had the lowest plasma zinc levels in comparison to other pregnant women who supplemented less iron daily. Breskin et al. (*239*) showed that prenatal supplements of 30 mg or more iron were associated with significantly lower plasma zinc levels in women in comparison to those who received no iron supplement or less than 30 mg per day. In contrast to the above observations, other investigators found no effect of iron supplementation (160 mg of iron daily) on plasma zinc level in pregnant women (*16*).

Iron, 300 mg administered with 25 mg of zinc, decreased the peak plasma concentration of zinc in uremic subjects (25% decrease vs 40% decrease in controls). The apparently decreased zinc absorption was associated with lower fasting plasma zinc and was exacerbated with aluminum intake (*240*). Inasmuch as high aluminum intake is common in hemodialyzed patients, the above interactions are important for long-term management of these cases.

Effect of Zinc on Iron Nutriture. A recent study has shown that zinc impaired the intestinal absorption of iron in humans (*241*). Mahloudji et al. (*242*) reported that growth of iron-deficient Iranian schoolboys was greater when supplementation was 20 mg of iron daily, rather than a combination of 20 mg of iron and 20 mg of zinc. These results suggested that oral zinc may have decreased the absorption of iron in these children. The ^{59}Fe absorption studies of Aggett et al. (*228*) lend support to this view.

A high level of zinc supplementation may also affect iron storage. Toxic levels of zinc in the diet may shorten the life-span of red blood cells and cause anemia because of the faster iron turnover (*243*). Zinc is known to interfere with iron uptake by the liver so that there is a decrease in the storage of iron as ferritin (*244*). Decreased iron levels in the liver and kidneys in response to excessive zinc supplementation has been observed (*188,245–247*). Inasmuch as transferrin in plasma is known to transport both iron and zinc, the interaction of iron and zinc may be due in part to competition at the transport level.

In women supplemental zinc, 50 mg daily for 10 weeks, has been shown to alter indices of iron status. Yardick et al. (*190*) measured the effects of 50 mg zinc daily or 50 mg zinc and 50 mg iron daily on zinc, iron and copper indices in 18 women, 25 to 40 years of age, over a 10-week interval.

Zinc lowered serum ferritin, hematocrit, and erythrocyte superoxide dismutase after 10 weeks compared to baseline. Zinc treatment increased serum zinc but had no effect on hemoglobin, ceruloplasmin, or salivary zinc. In the zinc–iron treatment group, superoxide dismutase and salivary zinc decreased. Serum ferritin and serum zinc increased significantly, but other indices did not. These data suggest that supplemental zinc at a level of 50 mg daily impairs both iron and copper status. Simultaneous iron supplementation protected iron status.

Iron absorption and distribution is altered as well by zinc deficiency. A marked increase in iron and a decrease in zinc concentration in various organs such as liver, bone, pancreas, and testes has been observed in zinc-deficient animals in comparison to pair fed controls (*180,181, 248,249*). These changes are reversed following zinc supplementation. Absorption of iron is enhanced in animals fed zinc-deficient diets, which may explain in part the increased organ levels of iron (*250*).

Health Effects of Zinc Deficiency and Excess

Effects on Organ Systems

Gastrointestinal Tract. HUMAN STUDIES. Gastrointestinal effects of higher than normal levels of oral intake of zinc compounds have been described in a number of cases of accidental or self-administered exposures and in prospective studies. Some of these reports will be described in this section.

Chobanian (*251*) related the case of a man who accidentally ingested about 3 oz of liquid zinc chloride solution. There were local caustic effects including erosive pharyngitis and esophagitis; in addition, there was burning pain in the mouth and throat along with nausea, vomiting, and abdominal cramps. Chelation therapy was instituted and within a few days the clinical and biochemical effects of overdose were reversed. The patient was discharged after 5 days. Since the ingested material was a solution, exact dosage of zinc chloride could not be established.

Burkhart et al. (*252*) reported the case of a 16-year-old boy who ingested approximately 50 zinc sulfate tablets (500 mg). Following ipecac-induced emesis and orogastric lavage, an abdominal radiograph performed 4 hr after ingestion still demonstrated most of the tablets within the stomach but with three pills within the colon. Whole-bowel irrigation with a polyethylene glycol lavage solution administered through a nasogastric tube produced a rectal effluent that contained pills. Significantly, the patient remained asymptomatic throughout the whole bowel irrigation. Stool guaiac tests were negative, but serum chloride increased from 105 to 127 mEq/l. Follow-up radiographic assessment revealed complete clearance of the zinc tablets from the gastrointestinal tract during the next 24 hr.

Murphy (*253*) has also reported on a single oral dose of zinc in a 16-year-old boy who ingested 12 g of elemental zinc in an attempt to hasten healing of a minor laceration. Lethargy occurred for several days after the ingestion, accompanied by an elevated zinc concentration in whole blood. Chelation therapy promoted dramatic clinical improvement, and a fall in blood zinc levels. Gastrointestinal disturbance was not reported in this case.

In a prospective study, Henkin et al. (*6*) gave 80 volunteers zinc sulfate, 100 mg as zinc ion, in four divided doses daily for 3 to 6 months. Only one patient experienced a mild degree of diarrhea as the only gastrointestinal disturbance. In contrast, Samman and Roberts (*214*) observed gastrointestinal symptoms in 26 of 47 healthy volunteers who ingested 150 mg of zinc sulfate in three divided doses daily for 6 weeks. Symptoms in the latter cases included headache, nausea, stomach cramps, and diarrhea. The form of the zinc salt, the sulfate per se, similar to iron sulfate, may have had a direct effect on the gastrointestinal mucosa, but exact mechanisms are unknown. Zinc gluconate and zinc acetate are reported to be less irritating to the gastrointestinal tract than other forms, but this has not been clearly confirmed (*16*).

ANIMAL STUDIES. Maita et al. (*254*) reported ulcers of the forestomach in mice fed zinc sulfate at levels from 1500 to 3900 mg/kg/day for 13 weeks. However, there were no observed adverse effects in mice exposed to 150 to 390 mg/kg/day (3000 ppm) for 13 weeks. Some mice given 30,000 ppm dietary zinc sulfate exhibited other gastrointestinal lesions including ulcers at the junction of the forestomach and the glandular portion, and increased mucus secretion in the upper small intestine near the glandular pyloric region. The study by Straube et al. (*255*) wherein ferrets reacted adversely with intestinal hemorrhages and death is difficult to evaluate. There were only three or four animals per group, the diet may have been inadequate, and no dose-response studies were reported.

The overall impression of reported studies of higher than normal oral exposure to zinc compounds is that most are subject to criticism and questioning with respect to human risk. In some cases the numbers of experimental animals were exceedingly small. Furthermore the levels of exposure were far in excess of that to be expected in human situations; ferrets received 4.25 mg zinc oxide/kg/day for 3 weeks. In some cases levels of exposures (e.g., sheep study detailed below in the lung section) were not clear, since some of the sources were environmental (*256*). The reported ulcerations in mice (*254*) exposed to zinc sulfate stands alone and is difficult to interpret in view of lack of confirming studies. It must be recognized, however, that a potential exists for gastrointestinal toxicity from accidental or intended intake of large amounts of zinc compounds.

Pancreas. NORMAL ROLE OF ZINC AND EFFECTS OF DEFICIENCY. Zinc is a required element for the normal exocrine and endocrine function of the pancreas. Its concentration in this tissue is many fold higher than that of plasma, and in some species pancreatic secretion into the gut may be an important elimination route for this metal (*171,257*). A key determinant of its accumulation in this site appears to be the level of metallothionein, the binding site for 70 to 80% of pancreatic zinc in the chick (*258*). In rats 24 hr after injection of a single sc dose of zinc (20 mg Zn/kg as $ZnSO_4$), levels of the metal and of metallothionein and its mRNA were higher in the pancreas than in liver and kidney (*259*). The increased expression of this protein occurred in both endocrine and exocrine cells of this organ (*260*).

In the pancreas zinc is found in highest concentrations within the secretory granules of islet β-cells, a distribution pattern similar to that of numerous secretory cells and neurons (*110*). Zinc in these granules and vesicles is believed to facilitate the stabilization of the structure of intravesicular proteins. In the case of insulin, the crystalline precipitate contains two zinc atoms within the hexameric structure, bound to histidines in each monomer, with an additional 10 to 12 zinc atoms more loosely associated with the complex. Removal of zinc from these sites by certain zinc-chelating agents may explain their ability to cause diabetes. Model studies with liposomes indicate that the chelation of zinc produces acidification of intravesicular contents and solubilization of insulin (*261*). These phenomena lead to an increase in vesicular osmotic pressure that results in

rupture of vesicles, loss of insulin, and possible damage to β-cells.

The glucose-stimulated secretion of insulin is markedly decreased from *in vitro* perfused pancreas of rats fed a zinc-deficient diet; the degree of inhibition appeared to increase with a greater degree of zinc deficiency (1 ppm vs 5 ppm vs 30 ppm zinc in diet). The level of insulin mRNA was unchanged, leading the authors to speculate that either translation was impaired or more likely the degradation of insulin was enhanced (*262*). Another possible explanation was an inhibition of insulin release; Huber and Gershoff (*263*) have reported a decrease in glucose-induced insulin release from pancreatic slices of zinc-deficient rats but no change in the pancreatic content of insulin. A decrease in plasma insulin levels in response to intraperitoneal injection of glucose has been observed in obese rats fed a zinc-deficient diet (<1 ppm); the degree of impairment increased over the 8 weeks of the study (*264*). No change, however, was reported in lean animals of the same strain (LA/N–cp). This importance of phenotype may explain in part the fact that other investigators have found a decrease or no change in glucose tolerance tests in zinc-deficient animals.

A markedly zinc-deficient diet in rats has been shown to significantly reduce the total pancreatic content of zinc within 2 days. This loss of zinc is associated with more than a 50% decrease in the activity of γ-glutamyl hydrolase in pancreatic tissue (*265*). Rapid loss in activity of pancreatic carboxypeptidase, a zinc metalloenzyme, has been demonstrated under similar conditions (*266*). It has been suggested that the amount of apoenzyme is diminished (*267*), based on data provided by Kirchgesser et al. (*268*). Studies have not been reported on whether the loss of activity results from a decrease in the synthesis of the enzyme, increased degradation or reduction in catalytic activity due to perturbation in structure or cofactor function associated with insufficient zinc. In rats the decrease in pancreatic activity of γ-glutamyl hydrolase is of physiological significance; hydrolysis of pteroylpolyglutamates in the gut lumen is decreased and therefore the absorption of monoglutamylfolate also is decreased, since the pancreas is the source of the enzyme. In man, this enzyme is associated with the intestinal brush border, is a zinc-activated protein as well, and is decreased in experimentally induced zinc deficiency in humans (*269*)

EFFECT OF ZINC EXCESS. Changes in the structure, biochemistry and function of the pancreas have also been observed in animals and in man following increased intake of zinc under experimental conditions or inadvertent exposures. In a chronic study in mice, Aughey et al. (*270*) administered zinc sulfate in the drinking water (0.5 g Zn/l) for 14 months. Plasma levels of zinc increased to a plateau about 1.5- to 2-fold over controls within the first 30 days. Levels of zinc in liver, spleen and skin were unchanged. The investigators concluded that pancreatic islet cells in zinc-treated animals were hypertrophied and contained an increased number of secretory granules. Their speculation as to mechanism was an increase in pituitary release of GH and ACTH, which could also explain the hypertrophy observed in the adrenal cortex. Their conclusions are difficult to confirm from their paper based on subjective evaluation of histology with no attempt at quantitation. Furthermore, no functional consequences were found, in that insulin and glucose levels in plasma were not different from controls. Only one concentration of zinc supplementation was tested. No other literature has been found which reports histological changes in islet cells in zinc excess. Even diets containing 30,000 ppm of zinc sulfate fed for 13 weeks to mice and rats did not reportedly alter the morphology of pancreatic islets and caused no change in blood glucose levels (*254*).

The more frequently reported response to marked elevation in zinc intake is an increase in the serum levels of the pancreatic enzymes, amylase and lipase, and alterations in the histology of pancreatic acinar cells. There are several studies in humans which report an increase in serum amylase. In patients with Wilson's disease treated with zinc acetate (25–50 mg elemental zinc 3 times a day chronically, or 200 mg 4 times a day for 10 days), serum levels of amylase and lipase increased with a significantly greater response in the high dose group (*271*). The values were normal before the initiation of zinc therapy, increased to slightly above normal after a few weeks of therapy, and later stabilized at the high normal range after 1 year of zinc therapy. Very large doses of zinc (800 mg/day) caused even greater elevation of serum amylase and lipase. These changes, however, were not associated with symptoms suggestive of pancreatitis such as pain or diarrhea. The relevance of these findings to subjects with normal copper levels, i.e., without Wilson's disease in which copper levels are elevated, is unclear. Copper deficiency which can be induced by excess zinc is known by itself to produce pancreatic atrophy with disorganization of acini (*272*).

Several case reports of excessive zinc intake in humans indicate possible effects on the pancreas. In a patient who ingested 3 oz of zinc chloride solution, serum zinc was 146 μg/dl and serum amylase and fasting blood sugar were elevated to 183 and 221 mg/dl, respectively (*251*). The zinc solution had a corrosive effect on the pharynx and esophagus, and the patient experienced persistent nausea and vomiting. In another patient ingestion of 12 g (150 mg/kg) of elemental zinc over a 2-day period produced an increase in serum lipase and amylase, measured 8 days later; this patient did not experience "gastrointestinal distress" (*253*). An approximately 10-fold increased intake of zinc (estimated 25 mg zinc/l TPN solution as sulfate, dose not indicated) through 26 to 60 days of total parenteral nutrition elevated serum zinc from 140 to 490 μg/dl in seven patients and increased serum amylase from the normal range of 130 to 310 to peaks of 557 to 1850 Klein units; reportedly, none of these patients exhibited clinical signs of pancreatitis. It should be noted that all of these patients had preexisting gastrointestinal abnormalities which necessitated TPN (*10*).

Studies of the effect of excessive zinc exposure on exocrine function of the pancreas have been carried out in several animal models. More detailed mechanistic studies have been carried out in avian than mammalian species. In the chick, a 7-fold increment in the zinc content of a purified diet to 500 mg/kg (500 ppm), added as zinc oxide, resulted in an increase in plasma and pancreatic zinc, to about 2 and 10 times that of control, respectively (*258*). After 9 days, the pancreas appeared firmer and paler with changes in acinar structure, decreased zymogen granule density, some evidence of "periacinar fibrotic infiltration," and no change in islet cells (*258*). The plasma amylase activity rose precipitously in the first day and then dropped to a level which remained above normal. The time-course of this drop reflected a substantial decrease in pancreatic content of amylase to 20% of control on day 2. Studies with radiolabeled leucine indicated that this decline was associated with a significant decrease in the rate of synthesis of amylase in the pancreas. Amylase levels in the duodenal lumen were decreased, which was of functional significance as indicated by the decreased breakdown of starch in the large intestinal

contents (*273*). Similar dose-response curves were observed for decreases in pancreatic trypsinogen, chymotrypsinogen, and lipase with detectable effects at the lowest dose tested, 100 mg/kg. In contrast, either no change or an increase was observed in pancreatic content of procarboxypeptidase A and B and RNase. The authors noted that only the latter are zinc-containing enzymes, and that zinc may differentially influence various steps of gene expression for these proteins (*258*).

With respect to the environmental significance of these zinc exposures, it is critical to note that in chicks fed a purified diet (based on corn and soybean meal) decreases in the three pancreatic enzymes tested were observed only with the highest dose, 2000 mg/kg. The level of effect was about equivalent to that of the 100 mg/kg zinc dose administered in a purified diet (*273*). Zinc is therefore about 20-fold less potent in altering pancreatic enzymes when administered in a nonpurified diet and must be fed in extremely high doses. The basis for this difference is not known, but it illustrates the important influence of dietary compostion on dose-response relationships for this metal.

More extensive studies on histological changes in the pancreas following zinc exposure have recently been reported by Kazacos and Van Vleet (*274*). Their experiments entailed feeding 2500 ppm zinc (as $ZnSO_4.H_2O$) to male ducklings from 1 to 56 days of age. This level of zinc reduced food intake, so a control group on a restricted diet was included. Histological analysis was carried out every 2 to 7 days. Altered acinar cell structure was evident after 3 days of zinc supplementation. The major early change appeared to be the development of cytoplasmic vacuoles containing zymogenlike material, altered mitochondria, intracisternal sequestration of rough endoplasmic reticulum, autophagocytosis, and apoptosis as the major form of acinar cell death. With time, atrophy of the pancreas occurred primarily because of decreased number and size of acinar cells. Ductlike structures appeared which the authors suggested may develop from "atrophic, dedifferentiated" acinar cells. Several hypotheses as to the mechanism of this effect were presented including zinc-induced copper deficiency, and excessive pancreatic stimulation through zinc-induced cholecystokinin or glucocorticoid release, all of which have been observed to produce similar histological changes in the pancreas.

The histological changes observed in the pancreas of avian species following excess zinc exposure have been observed in mammals as well. In mice and rats changes in acinar cells of the pancreas were only found after 13 weeks of feeding diets supplemented with 30,000 ppm of $ZnSO_4$ (*25*). The report implies but does not explicity state that the 10- and 100-fold lower levels of zinc supplementation did not alter pancreatic morphology. At the highest dose (30,000 ppm), the changes included degeneration and necrosis of acinar cells, ductlike metaplasia and in rats interstitial fibrosis. There were no reports of physiological changes associated with this effect, i.e., no diarrhea, and no reference to islet cell abnormality. At the highest dose blood glucose levels were decreased in mice but not in rats. At this high-dose food intake was also decreased in all groups; the magnitude and time dependence was species and sex dependent. Body weight was decreased in males of both species and in female mice. There were no food-deprived controls in this study. The basal content of zinc in the diet, a pulverized chow, was not reported. The daily intake of this highest dose was about 2-fold higher in mice than in rats, consistent with the degree of effect, and about equivalent to 3.9 and 1.5 g Zn/kg/day, respectively. These doses are more than a 100-fold higher than the usual intake of zinc from a normal natural product diet.

In a very limited study in sheep (*256*), high doses of zinc sulfate, 2 g zinc/day for 13 days by intubation in solution or 0.8 g zinc/day for 12 days incorporated into the diet (2000 ppm), produced more than a 10-fold increase in the pancreatic content of zinc. On gross inspection the pancreas was altered in color. Histology revealed changes in acinar cell structure including decrease in basophilia, vacuolization of the cytoplasm, some necrosis, as well as evidence of neutrophil infiltration and interstitial fibrosis. Atrophy and necrosis of pancreatic acinar tissue was also observed following high doses of zinc fed to veal calves (706 μg/g milk replacer) for 1 month (*275*).

Cats (3.5 kg) fed 0.25 to 0.5 g of zinc oxide per day for 12 to 16 weeks accumulated up to 480 μg/g zinc in the pancreas, about seven times normal control values (*276*). Pancreatic weight dropped, total insulin content remained the same and no changes were observed in islet cells. However, there were marked changes in histology including fibrotic tissue replacing and surrounding acinar cells. These findings were consistent with results from earlier studies by Drinker et al. (*277*) in three cats receiving the highest doses, 680 to 1000 mg zinc oxide per day for 13 weeks. No pancreatic changes were found in this study in other cats or in dogs on slightly lower doses.

Only limited studies have been carried out in mammalian models to investigate the mechanism of increased serum amylase and lipase. One report indicates that zinc stimulated the release of amylase from isolated rat pancreatic acini (*278*). The effect of only one concentration of zinc was reported; this study focused primarily on the mechanism of copper-induced amylase release. A study in dogs indicated that the increase in pancreatic output of protein following intraduodenal administration of zinc sulfate may be mediated in part through stimulation of cholecystokinin release (*279*)

It should be noted that levels of another serum enzyme, alkaline phosphatase, also change with zinc status, decreasing in zinc deficiency and increasing with zinc replenishment or excess in both human and animal studies (*3,175,187,280*). A decrease in the activity of serum alkaline phosphatase with the induction of zinc deficiency and its reversibility to normal levels following zinc supplementation have been observed in otherwise normal human subjects (*175*). In zinc-deficient subjects reported from the Middle East (*3*), serum alkaline phosphatase was observed to increase severalfold following supplementation with zinc. A consensus sequence for the transcription factor Sp1, which contains several zinc fingers, has been found in the promoter region of the gene coding for the intestinal form of serum alkaline phosphatase (*281*). It is thus possible that the promoter region of other alkaline phosphatase isoenzymes also contains a sequence or metal-responsive element similar to genes of other metalloproteins such as metallothionein (*282*). It is, therefore, probable that zinc induction of serum alkaline phosphatase is a continuum in that zinc deficiency produces below normal values, whereas zinc supplementation produces higher than normal levels. The major organ source, characterization of the isozymes affected by zinc status, and the mechanism of this effect have, however, not been determined.

In summary, zinc is required for normal function of the pancreas, in part through its critical role in enzymes like γ-glutamyl hydrolase and carboxypeptidase, and also in the stabilization of insulin. Studies in experimental animals indicate that zinc inges-

tion of a 100- to a 1000-fold above normal may produce degenerative abnormalities in acinar cells with release of pancreatic enzymes into plasma. Clinical case reports following overdoses also suggest that marked increases in zinc intake may elevate plasma amylase and lipase. The mechanisms of these effects have not been definitively established.

Liver. There is limited reference to altered structure or function of the liver in the literature on zinc deficiency and zinc excess. Studies in mice and rats fed diets supplemented with 30,000 ppm zinc sulfate (more than a 1000-fold in excess of the normal dietary intake) did not reveal changes in the histology of the liver or changes in the liver weight to body weight ratio after 13 weeks (*254*). Plasma GOT and GPT were not increased, indicating that zinc did not produce plasma membrane damage in the liver. In a study with chicks fed zinc oxide (500 or 1000 mg/kg purified diet containing a basal zinc level of 70 mg/kg), no significant change in PGPT was found after 14 days; PGOT was significantly elevated in a dose-dependent manner with a 33.3% elevation at the highest dose which the authors considered of doubtful physiological significance (*273*).

Limited study has been conducted on effects of zinc on the activity of intracellular hepatic enzymes. Zinc deficiency in rats impairs the biotransformation of certain pharmacologic agents (*283*). In a clinical study a significant correlation was observed between the degree of zinc deficiency (as assessed by leukocyte zinc content) and reduction of the total plasma clearance of antipyrine in subjects with hepatic cirrhosis (*284*). Other investigators have demonstrated a correlation between leukocyte zinc content and degree of impaired "hepatic functional reserve" as assessed by biochemical indices and clinical symptoms (*285*). Whether zinc deficiency per se contributes to impaired hepatic biotransformation of xenobiotics is unclear.

An increase in zinc exposure has been reported to enhance biotransformation mediated by hepatic mixed-function oxidases. Specifically, Kadiiska et al. (*286*) found that zinc sulfate (100 mg/kg/day for 30 days via the drinking water) decreased hexobarbital-induced sleeping time in rats. An increase in hepatic level of cytochrome P_{450} was found in association with an increase in the activity of ALA synthetase and decrease in heme oxygenase. The activity of several cytochrome P_{450} enzymes was increased. The zinc content in the animals was not determined and only one level of zinc exposure was examined. In contrast, Cho et al. (*287*) have reported that the hepatic concentration of cytochrome P_{450} was decreased in a dose-dependent manner by zinc (20 and 40 mg/kg once each day for 5 days by gastric intubation). *In vitro* studies have demonstrated that addition of zinc (*17*) (70 μM) to hepatic microsomes impaired NADPH-dependent monooxygenase activity (*288,289*).

Limited studies have also suggested that zinc status influences the hepatic activity of glutathione S-transferases. Zinc deficiency in rats is reported to decrease the activity of this enzyme system in the liver (*290*). A single dose of zinc in mice (30 mg/kg sc, as $ZnSO_4$) was reported to significantly increase the hepatic activity 5 hr later (*291*). No change in glutathione levels were observed. Parenthetically, Seagrave et al. (*292*) have shown that *in vitro* exposure to 100 μM zinc chloride in certain cells such as the Chinese hamster ovary (CHO) cell line increases glutathione levels and possibly increases glutathione S-transferase activity. The authors speculate that these reponses may play a role in the ability of zinc to protect against the cytotoxic effect of the alkylating agent melphalan in certain cell lines. In none of these studies have the individual isozymes of glutathione S-transferase been examined.

Administration of zinc in animal models has also been shown to increase the synthesis of metallothionein in the liver. In rats, for example, a single ip injection of a high dose of zinc chloride (10 mg Zn/kg) produced about a 5-fold increase in metallothionein concentration in the liver within 24 hr (*293*).

In 13 subjects treated with 600 mg zinc sulfate per day for 18 weeks, no significant changes were observed in liver-function tests measured every 6 weeks; the nature of the tests was not specified in the publication (*294*).

In summary, animal and clinical studies indicate that increased intake of zinc, even in extremely high doses, is not likely to be hepatotoxic. Data on the effect of increased zinc exposure on the activity of enzyme systems such as the cytochrome P_{450}s are conflicting and require further investigation.

Kidney. As with the liver, there is limited reference to the kidney in the literature on zinc deficiency and zinc excess. Zinc status is known to influence the expression of metallothionein in the kidney (*293*). Studies of this phenomenon have dealt primarily with the role of zinc status on the mechanism and magnitude of renal injury from cadmium. In the clinical study referred to above (*294*), in which subjects received 600 mg zinc sulfate per day, urinalyses were carried out and the authors only imply that no abnormalities were observed.

Studies in animals have been limited. A small study using ferrets indicated that a diet containing 18-fold higher levels of zinc than normal (500 ppm, added as zinc oxide to "tinned dog food") fed for 48 to 191 days to three animals produced no changes in renal histology, serum urea nitrogen, or urinalysis (Boehringer-Mannheim Combur eight test strips) (*255*). Higher levels of supplementation in seven animals (54- and 108-fold above normal) produced evidence of "diffuse nephrosis" (evidence not provided) and abnormalities in urinalyses. The ferrets had marked anorexia, decrease in body weight, and pronounced anemia with significant intestinal hemorrhages. In three sheep, 2 g of zinc per day for 13 days (as zinc sulfate intubated in drinking water) produced a 16-fold increase in kidney zinc but no change in renal histology (*256*). In two sheep fed 0.8 g per day for 12 days and then 1.2 g for 44 or 72 days (about 2000 ppm zinc), renal zinc increased 14-fold and a variety of abnormalites were observed. In mice fed 30,000 ppm zinc for 13 weeks, "regressive changes of the renal cortex" and an increase in kidney weight was observed in females (*254*). No changes in renal histology were observed in male mice or male or female rats treated with these levels of zinc or in animals fed one-tenth or one-hundredth these amounts. Blood urea nitrogen was unaltered in all groups at all levels of zinc.

In summary, unlike numerous nonessential metals, excess zinc exposure appears not to cause significant nephrotoxicity except with extremely high doses ingested by experimental animals.

Cardiovascular System. CARDIOVASCULAR FUNCTION. The literature on zinc administration in humans, in doses above dietary requirements, suggests that zinc does not produce major changes in the function of the cardiovascular system. Reports of cases of acute overdoses do not refer to alterations in blood pressure or cardiac function. Detailed clinical studies addressing this issue are very limited.

Two recent prospective studies tested the effects of zinc on cardiovascular function in humans. One double-blind prospective study involved 24-week administration of placebo or zinc (220 mg $ZnSO_4$ orally 3 times a day) (*296*). Subjects (14 placebo, 16 zinc) were institutionalized, over 65 years of age and had senile dementia due to atherosclerosis. Blood pressure and heart rate were

measured weekly, and EKGs performed at 0, 12, and 24 weeks. Zinc treatment increased plasma zinc from 84 μg/dl to 124 μg/dl by 2 weeks with a plateau after 4 weeks at about 150 μg/dl. No significant changes were detected in pulse rate, blood pressure, or EKG characteristics (or in hematologic or chemical evaluation of blood samples).

A second prospective study examined the effect of zinc (30 mg/day of $ZnSO_4$ orally for 4 days followed by 600 mg/day for 4 days more) on plasma renin actvity, serum aldosterone, and diastolic and systolic blood pressure in normal subjects (*297*). Zinc treatment produced a dose-dependent rise in plasma renin and aldosterone but no significant changes in blood pressure, or plasma and urinary sodium and potassium. No tests of cardiac performance were included in this study. The data from this study are compromised because there were no placebo controls and only six subjects.

In a retrospective study from Dunedin, New Zealand of 69 noninstitutionalized elderly subjects taking zinc supplements for at least 1 year (20 to 150 mg/day), there was no evidence that increased zinc intake was associated with changes in the electrocardiogram or frequency of cardiovascular disease based on questionnaire responses (heart failure, heart attack, angina, claudication, hypertension, medication use) (*295*). Among the deficiencies of this study are a lack of verfication of reported zinc supplementation, lack of information on dietary zinc content, concomitant intake of vitamins not matched in controls, and lack of data on other cardiovascular risk factors such as level of exercise.

Analysis of data from the National Health and Nutrition Examination Survey II (NHANES II) has indicated that serum zinc is inversely correlated with diastolic and systolic blood pressure in both men and women (*298*). This observation was revealed through use of a multiple stepwise regression model, which adjusted blood pressure values for known covariables (age, race, body mass index). The range of serum zinc levels in this data base was not stated in the report, which primarily focussed on the hypertensive effects of lead.

Several recent studies have examined the effect of zinc on components of the cardiovascular system under *in vitro* conditions. Unlike other metals tested, zinc chloride, even at cumulative concentrations above 83 μM, did not change the tension of vascular smooth muscle *in vitro* (endothelium-free ring of ventral aorta from the dogfish shark) (*299*). In physiologically relevant concentrations (IC_{50} of 9–35 μM but without protein in buffer) zinc has been shown *in vitro* to selectively inhibit the binding of platelet activating factor (PAF) to human platelet membranes and inhibit PAF-induced platelet secretion and aggregation (*300*). Inhibition of aggregation by other stimulants such as collagen and thrombin has been demonstrated with platelets from dog and rabbit, respectively. Whether zinc may attenuate thrombus formation *in vivo*, or modify other effects of PAF related to ovulation, implantation, parturition or inflammation is not known.

A study with the isolated rat heart preparation indicates several effects of zinc, administered in the perfusate as the histidine complex (*301*). Decreases in cardiac rate, contractile force, and peak systolic pressure were observed; for each of these effects there was a significant dependence on zinc concentration (12.5 μM in 2-fold increments to 400 μM). Inspection of the data suggests that for each of these end points the maximum effect was achieved with 100 μM zinc. Coronary flow was unaltered. Negative inotropy and chronotropy have been reported by other investigators using isolated cardiac preparations exposed to 1 to 5000 μM zinc (*302,303*). In the study by Powell et al. (*301*) zinc was also found to protect the heart during regional ischemia as indicated by a decrease in ventricular fibrillation during reperfusion and a decrease in release of lactic dehydrogenase. The authors argue, not too convincingly, that the antiarrythmic effect of zinc cannot be fully explained by the negative inotropy and chronotropy (which decreases myocardial oxygen demand and therefore the degree of injury during coronary occlusion) and suggest that zinc may inhibit free radical formation during ischemia. They noted the work of others, indicating that zinc protects against catecholamine-induced cardiotoxicity (*304,305*).

SERUM LIPIDS. There is evidence that serum lipid concentrations can be altered by both deficient and excess intake of zinc. Prasad (*306*) observed that total serum cholesterol decreased following experimentally induced zinc deficiency in two human volunteers. To investigate the mechanisms underlying impaired intestinal absorption of lipids in zinc deficiency, Koo et al. (*307*) measured the apo-B content and chemical composition of chylomicrons for marginally zinc-deficient rats fed 2.8 ppm of dietary zinc, and the results were compared with those for pair-fed and *ad libitum*-fed control rats. The authors concluded that the intestinal synthesis of apo-B, essential for chylomicron formation, may be impaired in zinc deficient rats which may account for the impaired intestinal absorption of lipids.

High levels of zinc ingestion by rats are associated with an increase in serum cholesterol (*195*). Most studies suggest that this effect is due to copper deficiency which may be induced by excess zinc intake (*308,313*).

Alterations in serum lipids with increased oral intake of zinc in humans have been reported, but the results are not consistent. Black et al. (*314*) conducted a placebo-controlled trial with dietary restrictions and record keeping. They observed a significant decrease in HDL cholesterol in young men (19 to 29 years old) after 12 weeks of daily supplementation with 50 mg zinc (as the gluconate). A decrease in HDL cholesterol occurred at 6 and 12 weeks in men receiving a slightly higher dose, 75 mg zinc per day. These decreases were based on comparisons to both a placebo group and baseline values for each subject. It should be noted, however, that the magnitude of this effect was small; the mean (± SE) values of HDL cholesterol at week 12 in the 50- and 75-mg groups, 1.38 ± 0.05 and 1.39 ± 0.08 mM, were similar to the baseline value in the placebo group, 1.44 ± 0.09, and higher than the placebo group at week 8, 1.31 ± 0.07. Furthermore, in the 75-mg group although a decrease was observed at 6 and 12 weeks, no difference from placebo was found at the 8- and 10-week samplings. No significant changes in other lipid parameters, i.e., serum triglycerides or total, LDL, VLDL cholesterol, occurred in the zinc-supplemented group. Freeland-Graves et al. (*315*) observed only transient changes in HDL cholesterol when she supplemented women with 100 mg of elemental zinc per day.

Hooper et al. (*11*) administered higher doses, 160 mg per day of elemental zinc as sulfate, to 12 healthy adult men for 35 days and observed a decrease in HDL cholesterol (from 40.5 mg/dl to 33.5 mg/dl, $p < 0.001$); total serum cholesterol showed no change. These results were not observed in a subsequent study by Samman and Roberts (*215*) using a double-blind crossover design. They studied the effect of zinc supplementation (150 mg zinc per day for 6 weeks) on plasma lipoproteins and copper status in 26 healthy young females, mean age 27 years, and 21 males, mean age 28 years. Plasma was analyzed only at the beginning and end of the 6-week trials so no time-dependent trend can be assessed

from the study. Plasma zinc increased significantly in both groups, as expected, with a greater rise in females. Plasma total and HDL cholesterol remained unchanged in both sexes. In females the mean LDL cholesterol decreased significantly (9%), thus decreasing a major risk factor for cardiovascular heart disease. There was a trend for total HDL cholesterol to be redistributed in that HDL_2 increased and HDL_3 fell slightly in the females. Zinc supplementation altered copper status in females, but not in males, as described earlier in this text. Inasmuch as the females were lighter than the males but received the same dose of zinc, a dose-response effect rather than a sex difference could not be ruled out.

In a study with even higher doses, 300 mg zinc per day for 6 weeks in 11 healthy men, an increase in LDL cholesterol and a decrease in HDL cholesterol were observed, although triglyceride and total cholesterol concentrations did not vary significantly (*12*); this study lacked a placebo control.

Laitinen et al. (*316*) studied the association between serum lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and levels of serum zinc, serum copper, and their ratio in 3373 subjects (3-, 6-, 9-, 12-, 15-, and 18-year-old Finnish girls and boys). Serum zinc was positively related to total cholesterol, HDL cholesterol, and LDL cholesterol, whereas serum copper related negatively to HDL cholesterol. The results of this study, however, suggested only a weak relationship between serum lipids and serum levels of zinc and copper.

Shah et al. (*317*) conducted a controlled study in order to evaluate the effect of zinc supplementation on serum lipids in patients with ischemic heart disease. Ten stabilized patients were given 200 mg of zinc sulfate orally three times a day for 1 month. Ten other subjects received placebo. The zinc-supplemented group showed a significant decrease in serum cholesterol β-lipoproteins, a significant increase in α-lipoproteins, and no change in triglycerides. Control subjects on placebo showed no significant changes in any of the above parameters. Inasmuch as serum α-lipoproteins have a protective effect against atherosclerosis and serum β-lipoproteins denote a risk factor for cardiovascular heart disease, these studies show a beneficial effect of zinc supplementation in patients with ischemic heart disease.

Goodwin et al. (*318*) investigated the relationship between zinc intake, physical activity, and blood levels of HDL cholesterol in a healthy elderly population. These investigators observed that physical activity was positively associated with HDL cholesterol only for those subjects who were not taking supplemental zinc. In those subjects who were taking supplemental zinc (29.1 ± 11.8 mg/day), the effect of exercise on HDL cholesterol was not observed. The same group of investigators completed an experimental trial of zinc supplementation (50 mg of zinc/day for 8 weeks) in young trained runners and found no change in HDL cholesterol. It is not clear as to why there is a difference between the elderly and the younger subjects. It should also be pointed out that although a significant interaction between zinc intake and physical activity with respect to the effects upon HDL cholesterol was observed by using multiple regression analysis, the level of correlation was low, and it is not clear if these effects are physiologically relevant to the development of cardiovascular disease.

Brewer et al. (*319*) have recently reported the effect of zinc administration (50 mg of elemental zinc as acetate three times per day) on lipid profiles in 24 subjects (11 females and 13 males) with Wilson's disease. In male patients receiving zinc for an average of almost 2 years, both total cholesterol level and HDL cholesterol were significantly reduced by zinc. The reduction in HDL cholesterol was proportionally a little greater than total cholesterol, resulting in a slight increase in average cholesterol to HDL cholesterol ratio from 3.9 to 4.6. This change was, however, not significant statistically. In females, this ratio changed from 4.36 to 3.83 following zinc treatment for almost 2 years. As interpreted by the authors (based primarily on the Framingham cardiovascular studies), a ratio of total cholesterol to HDL cholesterol of 5.0 will give a coronary heart disease risk factor of 1.0, i.e., an average risk for developing coronary heart disease within 4 years. Thus, the coronary heart disease risk factor was not changed significantly by zinc therapy in either sex and, further, it remained below average in these patients after zinc therapy for almost 2 years. The authors have concluded that zinc therapy in Wilson's disease is not atherogenic (*319*).

In the Dunedin, New Zealand, study (referred to earlier) elderly subjects who consumed zinc supplements (20 to 150 mg/day for more than 1 year) did not differ from controls in laboratory values commonly associated with cardiovascular disease including total cholesterol and triglycerides (*295*). Thus, none of the indices examined provided any association of increased risk of developing cardiovascular disease in participants who regularly took zinc supplements.

Kok et al. (*320*) performed a case-control analysis of data obtained in a Dutch prospective follow-up study in order to investigate the association of serum copper and zinc with mortality from cancer and cardiovascular disease. Cancer (n = 64) and cardiovascular disease (n = 62) deaths and their matched controls were taken from a cohort of 10,532 persons examined during 1975 to 1978. Trace elements were assayed in baseline serum samples which had been stored during the 6 to 9 years of follow up. The adjusted risk of death from cancer and cardiovascular disease was 4 times higher for subjects in the highest serum copper quintile (serum copper >143 mg/dl) compared with those with normal levels. An excess mortality was also observed in subjects with low copper status, suggesting a U-shaped relationship. A protective effect of high zinc status on the risk of cancer and cardiovascular disease was compatible with their data (*320*).

Nervous System. DISTRIBUTION AND FUNCTION OF ZINC IN THE BRAIN. A comprehensive review of the neurobiology of zinc has recently been published by Frederickson (*110*). The following information concerning metabolism of zinc in the brain is from Frederickson's review, unless indicated otherwise.

The zinc content of brain tissue is about 13 to 17 μg/g of tissue water. The zinc content of gray matter on a dry weight basis is about 2.5 times the level in white matter because of the high fat content of white matter. The zinc content of gray matter from all regions of the brain is about 50 to 80 μg/g with slightly higher levels in the hippocampus.

In the brain, in addition to its general functions as an essential component of more than 50 functionally diverse metalloenzymes, and in the structure of macromolecules including DNA and RNA, zinc appears involved in neurotransmission, the formation of microtubules, and is bound to hormones such as insulin and 7-S-nerve growth factor.

Intracellular zinc content is maintained within a fairly narrow range in spite of increases or decreases in extracellular zinc. The mechanism of zinc uptake by brain is incompletely understood. Ultrafilterable zinc in plasma rapidly enters the cerebrospinal fluid where its concentration is similar to that of plasma (10 μg/l). Under

usual conditions zinc in spinal fluid is slowly taken up by brain cells against a concentration gradient and bound to proteins in the subcellular fractions. In rats the half-time for whole brain zinc turnover is about 22 days, and the zinc content of most brain regions is little affected by zinc deficiency (*321,322*). Exceptions are seen in the hippocampal neuropil, a region where zinc is concentrated in mossy fiber boutons and olfactory bulbs (*322*). In liver disease, a condition that can cause substantial losses of zinc (*323*), decrements in brain zinc may be substantial.

Certain neurons "sequester histochemically reactive zinc in their axonal boutons" (*110*). For example, granular cells of the hippocampus are considered "zinc neurons" because their stimulation by implanted electrodes causes zinc to accumulate in regions they innervate. This accumulation of zinc is greater in older rats than young rats with incompletely developed mossy fiber systems. In addition, mossy fiber boutons have lower stainable zinc after electrophysiological activity. The release of zinc from mossy fiber boutons appears to occur by exocytosis of zinc-rich vesicles. Mapping studies suggest that "virtually all of the major zinc-containing systems of the brain are of limbic or cerebrocortical origin." Zinc neurons have also been identified in the thalamus, brain stem and spinal cord. Though no neuroactive substance has been identified uniquely in association with zinc-containing neurons, studies of receptors suggest that zinc-containing neurons are a subclass of glutamate-aspartate neurons (*110*).

The functions of zinc in nerve terminals are unclear (*110*). One putative function is stabilization of secretory macromolecules. Its stabilization of the hormones insulin, 7-S-nerve growth factor, histamine–heparin, and thymulin is consistent with such a function. Another proposed function is modulation of synaptic receptors. Research on this hypothesis has produced mixed results. Zinc-deficient rats displayed lower hippocampal electrophysiological responses to stimuli (*324*). *In vitro* studies found inhibition of the uptake of amino acid neurotransmitters and increased uptake of monoamines by brain tissue. Low (10 μM or less) and high (100 μM or more) concentrations of zinc inhibited receptor binding of certain amino acids. In other studies the activity of glutamate decarboxylase was inhibited by zinc, resulting in a decrease in GABA.

IMPORTANCE OF ZINC TO BRAIN DEVELOPMENT AND FUNCTION: ANIMAL STUDIES. The essentiality of zinc for growth of rats was described in the mid 1930s (*325–327*). *In vivo* evidence of the essentiality of zinc for maturation of the brain was provided by studies in chicks (*328,329*) and rats (*330,331*), which demonstrated a variety of malformations in brains of offspring that had been deprived of zinc early in gestation. Inhibition of DNA synthesis in neural crest cells is believed to be one of the causes of the malformations (*332*). In humans some studies have shown an association between low maternal plasma zinc in early gestation and neural tube defects in infants (*333*).

Further evidence of the essentiality of zinc for development of the brain was found through studies of zinc-deprived suckling rats. Incorporation of thymidine into DNA was suppressed in 10-day-old sucklings (*334*). Histologic examination of the cerebellum on the 21st postnatal day found retarded maturation (*335*) and impaired division and migration of external granular cell neurons (*336,337*). In addition the arborization of Purkinje, stellate and basket cell dendrites was decreased and morphologically grossly abnormal; the number of boutons was decreased and their shapes were strikingly abnormal (*336,337*). Presumably similar abnormalities occur in other regions of the brain when zinc is deficient during the critical time of maturation.

The long-term functional significance of zinc deficiency in the fetus and neonate was studied in rats deprived of zinc during late gestation and/or suckling. Severe maternal zinc deprivation (<1 ppm Zn in the diet) from days 14 through 20 of gestation caused stunting and a decrease in brain cell number in fetuses (*338*). Active avoidance of shock was impaired in nutritionally rehabilitated male offspring while littermate females performed as well as the controls (*339*). When compared to controls, rehabilitated offspring also showed an increased aggressive response to shock that was most evident in females (*340,341*).

Severe maternal zinc deprivation throughout nursing impaired growth of suckling pups and subsequently increased errors by nutritionally rehabilitated offspring on a relatively simple maze (*342*). Food motivation of previously zinc-deprived offspring was increased compared to ad libitum controls, but was less than that of food-restricted controls (*343*). Reference or long-term memory, 40 days after exposure to shock on days 18, 19, 20, and 21 of nursing, was also impaired (*344*). Adult nutritionally rehabilitated offspring also displayed impaired working memory of a 17-arm radial maze (*345*).

Experiments in mildly zinc-deprived dams fed 10 ppm Zn throughout pregnancy and lactation, a level of intake that did not appear to impair pregnancy or lactation and had only a small effect on growth of pups, found deficits in working memory of a 17-arm radial maze when the offspring had been nutritionally rehabilitated and were 300 days of age (*346*). These findings indicate the rat brain is highly susceptible to injury from zinc deficiency during its critical period of development, and that sequelae from the injury include persistent abnormalities in behavioral function.

Severe zinc deprivation in weanling rats caused higher brain levels of catecholamines, copper and manganese than were present in pair-fed control rats (*321,322,347*). The concentration of zinc was decreased in the olfactory bulb. Zinc deprivation caused anorexia and cyclic pattern of feeding. Food intake was inversely related to the level of plasma zinc but not to plasma amino acids, glucose, and fatty acids (*348*). Brain tryptophan, tyrosine (*349*), and serotonin (*350*) were increased compared to pair-fed control animals.

Findings in chronically zinc-deprived rats suggest that electrophysiological function is impaired. Responses recorded from the mossy fiber region of the hippocampus by electrodes were abnormal (*324*). Behavioral functions were also impaired (*351,352*). Further evidence of the essentiality of zinc for hippocampal function was provided from acute zinc depletion studies in which the zinc was removed by perfusion of the hippocampus with zinc binding chelators (DDC, Na_2S) (*110*). Immediate transitory impairment of spatial memory function was produced. Function returned with clearance of the chelator and precipitated zinc.

Research on a small number of nonhuman primates (rhesus) has shown that severe maternal zinc deprivation (<1 ppm dietary zinc) throughout most of the third trimester caused acrodermatitis in the dam and a subsequent reduction of exploration and play in infants during weaning (*353*). Later study of these animals found impaired ability to solve complex learning sets at 300 and 700 days, but not at 1000 days (*354*). In other studies rhesus dams fed about 4 ppm zinc throughout gestation and lactation displayed acrodermatitis, while their infants displayed abnormal behaviors (*355*).

IMPORTANCE OF ZINC TO BRAIN DEVELOPMENT AND FUNCTION: HUMAN STUDIES. In humans, evidence that zinc deficiency can impair brain function is limited. The most compelling evidence was provided by an experimental depletion study in which severe deficiency was induced by treating the subjects with large amounts of histidine, a potent chelator of zinc (*1*). Following massive zincuria, the subjects displayed ataxia, dysgeusia, dysosmia, nyctalopia, and disturbed mentation. Zinc repletion restored function.

Effects of mild zinc deficiency are less clear. A small study (*356*) of nine men who were fed about 3.5 mg zinc daily found an inverse relationship between accuracy of backward recall of digits and plasma zinc. Retention of zinc measured by balance technique was inversely related to accuracy and speed of immediate recall of verbal and visual information. These latter findings disappeared during the repletion of zinc nutriture.

Further evidence that mild zinc depletion can affect cognitive function was provided by two studies that were presented at the 1991 meeting of the American Institute of Nutrition. Fourteen men fed low zinc diets displayed evidence of impaired function (*7*). The men were housed under well-controlled conditions for 7 months and fed diets that provided 1, 2, 3, 4, or 10 mg Zn daily, in random order, for intervals of 35 days. Significant ($p < 0.05$) decreases in function were found in two sensorymotor tasks (pursuit and trials), two attention tasks (orienting and misdirection), three perceptual tasks (search, time estimate, and Sternberg), two memory tasks (shape and cube recognition), and one spatial task (maze). Abnormalities were evident at all levels of zinc deprivation, although there were no decreases in plasma or leukocyte zinc (DB Milne, personal communication, 1991).

Supportive of the above findings were results of a study of premenopausal women, aged 19 to 40 years (*357,358*). An 8-week randomized controlled trial of zinc treatment (30 mg daily with a vitamin–mineral mixture that was based on NRC guidelines) found that zinc-treated women (n=11) displayed increases over baseline in scores on tests of short-term recall of visual images (17.5%, $p < 0.004$) and word pairs (11.4%, $p < 0.07$), while women given vitamins and minerals alone (n=6) showed little or no change in scores on these tests (part of the Wechsler Memory Scale No.1 and 2).

The relation of zinc nutriture to seizure disorders, senile dementia, anorexia nervosa, schizophrenia and other neurological conditions is unclear (*110*). Clinical findings do not distinguish whether low levels of serum zinc or brain zinc reported are primary, or secondary to complications of the condition. Appropriately designed, randomized, controlled zinc treatment trials have not been reported.

NEUROLOGICAL FUNCTION AND EXCESS ZINC. Clinical evidence suggests that neurological abnormalities do not typically occur in animals or humans exposed to higher than usual levels of zinc in air, water or food. For example, neurological phenomena, other than headache, are not considered a part of the zinc fume fever syndrome (*359*) and workers chronically exposed to zinc fumes and dust do not display characteristic neurological findings (*360*). Neurological findings are not part of the typical illness of humans exposed to levels of zinc in food or drink sufficient to cause nausea, vomiting, and diarrhea (*361*). Neurological abnormalities were not noted in ruminants naturally or experimentally exposed to amounts of zinc in feed sufficient to cause pancreatic degeneration (*256*).

Isolated reports have raised questions about possible associations of excess zinc with development of neurological diseases. For example, Stein et al. (362) in 1987 described a statistically significant increase in the incidence of multiple sclerosis (MS) (11 cases over 10 years) in workers in a manufacturing plant where zinc was a primary raw material. However, plasma and erythrocyte zinc levels in subjects with MS did not differ from controls. Furthermore, there was no relationship between the length of employment at the site and the time of onset of symptoms. There is thus no convincing evidence that excess zinc contributes to the known role of risk factors such as genetics, sex, and geography in the etiology of this immunologically based myelin disorder of the nervous system. Animal models with CNS pathology analogous to MS are available (*363*), which might be used to examine the effect of increased zinc intake on disease progression.

Unlike the clinical data, neurological effects of excess zinc have been observed in animal studies of CNS administration and in cell culture. Injection of 10 µg zinc or more into the ventricles of the brain of rats increased motor activity in a dose dependent manner; activity of ATPase was reduced in the hippocampus (*364*). Other investigators found that motor activity caused by intraventricular injection of 0.3 µmole zinc could be prevented by administration of 0.4 µmole GABA acid (*365*). The phenomenon was associated with lower activity of glutamic acid decarboxylase in hippocampus, but not in other brain regions that were assayed. A recent study, consistent with these observations, has demonstrated allosteric inhibition of the GABA response of voltage clamp spinal cord neurons by 10 to 100 µM zinc (*102*).

When zinc wires were placed in brain, degenerative changes occurred in proximitry to the wire. The inflammatory response included astrocytic scarring, lymphocyte cuffing of capillaries and demyelination (*366*). In addition, mitochondria were enlarged, and swollen and cytoskeletal changes were found in neurons. Abnormalities also included loss of neurotubules, the formation of structures that were morphologically similar to zinc-tubulin aggregates, accumulation of lipofuscin inclusion bodies, and other degenerative changes. Exposure to platinum, nickel, cobalt, or magnesium did not produce such changes.

Studies in tissue culture also indicate that high concentrations of extracellular zinc are neurotoxic. Exposure to 250 µM zinc overnight caused death of cortical neurons (*367,368*). In contrast, 100 and 200 µM caused minimal injury. Yokoyama et al. (*367*) speculated that these findings are significant for human and animal health because concentrations of zinc released during neurotransmission are believed to reach 250 µM at least temporarily. The validity of this speculation is unclear. In their experiments exposures to high levels of zinc were prolonged. *In vivo* zinc is presumably retrieved by neurons from the extracellular space, and that not retrieved is soon diluted by the extracellular fluid.

Duncan et al. (*369*) have also demonstrated the toxicity of zinc chloride (100 µM) to cultured cerebellar granule and ventral mesencephalic cells (with sparing of astrocytes). Their study was designed to identify neurotoxins in cycad-derived flours, a putative environmental factor in the etiology of amyotrophic lateral sclerosis parkinsonism dementia. These investigators provide evidence of high zinc content in traditionally prepared flours (possibly from soaking of seed in galvanized containers) and data to suggest that the zinc was the causative factor for *in vitro* neurotoxicity of extracts from these flours. No *in vivo* studies were performed to test whether oral intake of these flours would produce neurological effects. Based on the zinc content, the authors speculated that consumption of zinc would in-

crease to 100-fold the daily requirement. Other studies described in this report with zinc supplements of this level in animal models (e.g., 100 ppm dietary zinc for 100 g rat consuming 20 g diet) have not revealed neurological effects.

SUMMARY. Zinc is essential for ontogeny of the brain and for brain function. Among its normal roles are participation in neurotransmission of mossy fiber neurons of the hippocampus. Zinc deficiency impairs development of the brain and has been shown to cause long-term behavioral sequelae in rats. In humans as well, severe zinc deficiency causes abnormalities in neurophysiological function. Recent research suggests that mild zinc deficiency also can impair neurophysiological function in humans. Clinical evidence does not support the thesis that exposures to high concentrations of zinc, such as occur in metal fume fever, cause acute or persistent neurophysiological abnormalities. However, experimental studies in which zinc was injected into brain ventricles, introduced as a wire, or incubated at high levels with cultured neurons have demonstrated neuronal injury.

Lung. RESPIRATORY EFFECTS IN HUMANS. There were no reports in the accessible literature of respiratory effects from ingestion of zinc. On inhalation exposure to zinc compounds adverse effects may occur in situations where individuals are exposed to relatively high concentrations for long periods of time, such as in mining and smelting, or from exposure to very high concentrations for short periods of time, as with exposure to smoke bombs (*370–372*). Thus, the response in cases of human exposure to high levels of toxic forms of zinc is predictable and largely preventable by precluding excessive exposure in such occupations as mining, smelting, or welding, or exposure to smoke bombs without protective gear.

Acute exposure to high concentrations of zinc chloride or zinc oxide, e.g., 600 mg/m^3 for 5 hr, results in "metal fume fever," a well-recognized syndrome which results from inhaling the compound, largely zinc chloride, contained in smoke bombs (*373–375*). The common army smoke bomb contains a mixture of zinc oxide or zinc chloride/hexachloroethane and small quantities of calcium silicide and potassium nitrate (*376*). This type of exposure results from release of zinc oxide or zinc chloride and hexachloroethane in closed spaces or where little ventilation is available. The resulting irritation to the lungs, often referred to as smoke bomb pneumonitis (*377*), is readily reversible except under extreme conditions of exposure. The reaction between zinc oxide and hexachloroethane, in the presence of other agents, produces zinc chloride particles small enough to reach the deeper airways of the lung. Zinc chloride is relatively caustic when in contact with moist areas of the body, particularly the deeper recesses of the lung (*371*), which further enhances the toxic effects of these compounds.

Typically, the syndrome of metal fume fever begins 4 to 12 hr after the initiating exposure (*373*). The response is manifested initially by a metallic taste in the mouth, accompanied by dryness and irritation of the throat. The attacks have been described as a flulike illness accompanied by shaking chills, muscle and joint aches, weakness, sweating, and a high fever (*374*). Coughing, fatigue, and shortness of breath may be present and the entire process, when uncomplicated, runs its course in 24 to 48 hr (*375*).

Respiratory tract irritation occurs in both human and experimental animals following exposure to zinc oxide or zinc chloride. Experimental studies have demonstrated effects of exposure to inhaled zinc oxide/hexachloroethane (11,580 $mg/min/m^3$) or to intratracheally instilled zinc chloride (2.5 mg/kg body wt). Most laboratory animals and humans exhibited pulmonary congestion, peribronchial leukocytic infiltration, respiratory distress and pneumonitis (*370,371,378*).

Evans (*376*) reported on casualties following exposure to smoke bombs in 1945. During World War II on the Island of Malta, smoke generators stored near the entrance to Corradino tunnel were ignited, and the smoke generated filled the tunnel in a short time. Over 100 people were in the tunnel at the time, and most of them were exposed to high levels of the zinc chloride generated by the smoke bombs. Ten of the 34 treated patients died. This is an extreme case in which individuals were exposed to an unusually large amount of zinc compound in a closed space. It was estimated that exposure was about 0.2 lb/cubic yard near the generators. While as noted this was a most unusual case, it illustrates the potential for significant numbers of people to be exposed to the adverse effects of zinc oxide and zinc chloride in combination with other compounds and under unusual conditions.

Most reports in the literature describe metal fume fever in individual cases where the clinical history of the exposure and its sequelae may be of interest. Pare and Sandler (*377*) described the case of a soldier who was exposed to a cannister smoke bomb for about 10 min, without a respirator. The patient had an undulating course over a period of several weeks with severe clinical symptoms including shortness of breath, high temperature, epistaxis, pain in chest and side, rales in right lung and patchy consolidation of many areas of both lungs. Within about 6 weeks the patient had effectively recovered with no residual signs of the exposure. The case described here is not unusual in its clinical course, either in individuals exposed to smoke bombs or in welders (noted below) exposed to fumes generated during the welding process (*371,377*). The salient feature, despite the rather severe symptoms, is the pneumonitis which in a large majority of exposed individuals is reversible with or without treatment.

In some cases assumed to be metal fume fever, there are additional signs and symptoms which diverge from the norm. For example, Farrell (*379*) described anaphylactoid reactions wherein the patient developed rales and angioedema after working in a zinc smelting plant for 12 years. Although the patient was an engineering technician and his office was separated by a considerable distance from the smelting mill, he entered the mill about once weekly. In addition, he occasionally helped in the use of a oxygen–acetylene torch to cut metal and also did limited electric welding on zinc-coated items. Thus, there was a long history of on-going exposure.

There are many reports in the literature regarding metal fume fever all of which play on a central theme with some usually modest variation on either the conditions of exposure or the parameter(s) examined. Brown (*359*) described the radiologic appearance of the chest cavity of an individual following exposure to zinc fumes in a shipyard job. There were multiple nodules in both lung fields measuring 3 to 4 mm, following a few days of exposure. Based on clinical features of headache, dyspnea, arthralgia, lymphocytosis, and the lung lesions, a diagnosis of zinc fume fever was made. After 3 days of hospitalization the symptoms had cleared, and a day later the chest fields had cleared.

Others have measured lung function and found generally minimal to moderate declines in function which returned to normal within 2 to 3 days after cessation of exposure to the metal fume. Blanc et al. (*380*) designed a human model of metal fume fever using volunteer welders recruited through public advertisements. The

exposures to zinc oxide fume from welding over a 15- to 30-min period (in a specially designed 512 ft^3 exposure chamber with a ventilation rate of 273 ft^3/min) were designed to exceed 10 mg/m^3 over 15 min. Actual exposure to zinc oxide was measured using personal sampling pumps. The mean cumulative exposure to zinc oxide for the 14 participants was reported as 2.3 ± 1.7 g min/m^3 (range 0.6–5.1) for 15 to 30 min of welding. Dividing this value by the shortest (15 min) and longest (30 min) exposure times indicates a mean exposure level ranging from 77 to 153 mg/m^3 and a minimum exposure of 20 to 40 mg/m^3. Data for individual exposures were not reported. Based on bronchioalveolar washings and measurements of lung volume, air flow, and diffusion capacity for carbon monoxide, these investigators demonstrated minimal to mild changes which were transitory and returned to normal within two days. Results of another study in which welders were exposed to fumes from zinc-coated steel indicate no effects on lung function (*372*). Many additional reports in the literature confirm that, except for the most extreme exposures, zinc metal fumes produce a generally mild pulmonary response that is self-limiting.

A recent experimental study examined pulmonary function in four subjects who inhaled ultrafine zinc oxide particles for 2 hr at the current 8-hr threshold limit value (TLV, 5 mg/m^3) (*386*). All subjects experienced clinical symptoms of metal fume fever such as fever and chest tightness; however, no changes occurred in the 24-hr observation period in specific airway resistance, diffusion capacity of carbon monoxide, forced vital capacity, or maximal forced expiratory volume.

Nemery (*381*) has published an excellent, extensive review on metal toxicity and the respiratory tract, documenting that other metals, including cadmium, manganese, mercury, copper, vanadium, chromium, and nickel can also cause metal fume lung disease. The consequences of exposure to these metals are often more serious than the syndrome associated with zinc metal fume fever. For example, cadmium, chromium, and nickel are documented human carcinogens (*382–384*); manganese and mercury cause serious CNS disturbances which can be irreversible.

Studies in Experimental Animals. There are numerous reported studies on pulmonary toxicity of zinc based on animal experimentation. Since a majority of them describe similar results, only a few of the more recent and typical ones will be summarized here.

Amdur et al. (*385*) have examined effects in guinea pigs of zinc oxide fumes at a concentration of about 1 mg/m^3 for 1 hr, a relatively low exposure. The degree of irritant response was evaluated by measuring pulmonary mechanics in unanesthetized guinea pigs, including intrapleural pressure, tidal volume and rate of flow of gas in and out of the respiratory system. The exposure produced a slight but statistically significant decrease in compliance 1-hr postexposure. In a second group of guinea pigs, observed 2 hr after exposure, there was a progressive decrease in compliance between the 1- and 2-hr periods, indicating an escalating effect with time of zinc oxide fumes on lung distensibility. There were no changes observed in tidal volume.

The most recent work by Amdurs group (*386*) examined pulmonary lavage fluid at 0, 4, and 24 hr after 3 hr inhalation of ultrafine zinc oxide particles by guinea pigs, rats, and rabbits. Zinc oxide concentrations were 2.5 and 5.0 mg/m^3 (TLV for 8-hr exposures in the industrial setting). At both exposure levels, changes in lavage fluid from guinea pigs and rats, but not rabbits, were consistent with development of an acute inflammatory response.

Marrs et al. (*387*) exposed female mice, rats and guinea pigs to smoke produced by ignition of a zinc oxide/hexachloroethane pyrotechnic composition for 1 hr/day, 5 days/week at three different dose levels. Mice and rats received 100 daily exposures but guinea pigs were exposed to only 15 doses because of high mortality during early exposures. The exposure concentrations in the three species were the same and ranged from a low of 1.3 mg/m^3 to a medium of 12.8 mg/m^3 to a high of about 120 mg/m^3 as zinc. Following exposure to the smoke, the animals were held for up to 18 months for observations. Mortality at the high dose was significant in all three species with mice sustaining the greatest losses (Table 10). Table 11 lists results of observations, mainly histological, recorded at the end of the study. The most significant observation in mice was the increase in alveologenic carcinoma in the high dose group. Other lesions have been reported previously, and since there was no significant chronic injury reported, only lymphocytic infiltration is shown for each of the three species. There were no lung tumors in either rats or guinea pigs. Lung lesions, i.e., macrophages and other signs of chronic injury, were not remarkable and were similar to those described in other studies (*388,389*). Lymphocytic infiltration in the lung as shown in this study can be associated with age, as well as with exposure to zinc oxide smoke.

The results of the study by Marrs et al. (*387*) deserve further comment. First, the design and conduct of the study were excellent examples of toxicological investigations which provide reliable results in a difficult area of research. Second, despite the extensive and intensive nature of the multispecies, chronic investigation, essentially nothing new was revealed. Aside from the increased incidence of lung tumors in a susceptible strain of mice, the results were less severe than might be expected based on knowledge of human exposure and experimental animal data. The only significant observation was the increased incidence of lung tumors in the high-dose group of mice. There are a number of problems in attempting to relate zinc toxicity to the lung tumors observed as follows:

- The increase occurred only at the high-dose, not in the medium or low dose.
- Only one sex (female) of mouse was used.
- The high dose of smoke caused a mortality of about 50%, much higher than is usual for such a study.
- The alveologenic lung tumors in the mouse strain studied are not generally regarded as a reliable end point for lung cancer studies by many carcinogenesis experts because of the high incidence and variability of this type of tumor in control untreated animals.

These points illustrate the question of the significance of the observed increase in tumor incidence in the treated mice and relative difficulty in interpretation of such observations. The extreme treatment, confirmed by high mortality, undoubtedly contributed to lung damage, and this effect may have contributed to an indigenous process already extant in the mouse lung. This observation cannot be used to ascribe a carcinogenic role for zinc.

Two other studies warrant mention here. One study (*378*) exposed rats to inhaled zinc oxide/hexachloroethane smoke (11,580 mg/min/m^3) or to intratracheally instilled zinc chloride (2.5 mg/kg/body weight). Both the smoke and the instilled zinc chloride produced similar responses including pulmonary edema, alveolitis, and, in some, a late stage fibrosis. The additional imposition of oxygen had little effect on the development or progression of the pathological changes.

Another study used sheep as the test animal, exposed either to a single bolus dose

of welding fume solution or to 5 weeks daily exposure by inhalation (*390*). The metals in the fume from the welding electrode included by percentage: iron 14.1; manganese, 4.0; titanium, 0.56; magnesium 0.17; zinc, 0.02; copper, 0.02; and aluminum, 0.50. In the acutely exposed, sheep were anesthetized, a bronchoscope inserted into the trachea and a suspension of welding fume, 0.5 g suspended in 50 ml normal saline, was instilled into the right main bronchus. The 0.5 g was calculated from a welding fume exposure of 5 to 10 mg/m^3 for 3 effective working hours, an average welder's exposure to welding fume. For the long-term exposure (25 to 33 days, 174 to 181 min per day, 31 to 51 mg/m^3), sheep were exposed via tracheal tubes to welding fumes, sucked from a bench where welding was performed in a mixing chamber. Particle size, determined by an optical particle counter in combination with a particle meter and scanning electron microscopy, averaged less than 1.0 microns (range 0.5–10).

In both acute and chronic exposures fume metal particles accumulated, as determined histologically, and in the chronic exposure there was fibrosing pneumonitis. In the acutely exposed sheep there was accumulation of iron, magnesium and manganese in the lungs as well as elevated pulmonary arterial pressure. In the animals exposed daily for 5 weeks, there were increases in iron and, particularly, in manganese which was retained at high concentrations (40 times more than in unexposed sheep) following exposure. The concentration of zinc in the lungs of exposed sheep was about the same as the untreated controls. Metals are found in organs and tissues of welders with disease symptoms (*390,391*); the observations in sheep correlated with those in welders with chronic diseases and suggested that accumulation and retention of manganese and iron can result in negative health effects. Zinc, however, was not implicated.

Skin. Zinc accumulates in skin, 3- to 7-fold relative to plasma (*392*), and is required for normal function of this tissue. Zinc deficiency is characterized by rash, alopecia, hyperkeratosis, parakeratosis, and hypopigmentation (*393*). These symptoms are observed in acrodermatitis enteropathica (AE), a hereditary hypozincemia (*392*), and in dietary zinc insufficiency in humans as well as in animal models (*267*). In four patients with AE, levels of zinc in the skin were about one-third to one-half normal levels (*392*). The reasons for development of skin lesions from zinc deficiency are not known. As reviewed by Bettger and O'Dell (*109*), in animal models the development of these lesions can be retarded by high dietary levels of antioxidants such as vitamin E. This observation and others support the concept that enhanced lipid peroxidation contributes to development of dermal symptoms.

Dermal effects of increased zinc in the skin in response to elevated plasma levels have not been examined. There is literature on dermal effects of topical exposure to zinc salts. Studies purporting to demonstrate enhanced wound healing following application of zinc in humans are controversial; the weight of evidence suggests that in man and animals zinc accelerates wound healing only in the zinc deficient (*267,394*).

Certain of the zinc salts have irritant properties when applied in sufficiently high concentrations (*395*). For a series of zinc compounds tested in a single concentration, the degree of dermal irritation based on macroscopic observations was similar in a variety of mammals (rabbit, mouse, and guinea pig). In this study the zinc compounds in a 0.5 ml volume were applied to shaved skin on dorsal sites once a day for 5 days. Twenty-four hours after the last application, mice were administered vincristine (0.1 mg, ip) to arrest mitoses; after 4 hr the mitotic index was determined by assessment of the number of cells in mitosis per 1000 cells in the stratum germinativium and first layer of the stratum spinosum. In all species zinc chloride (1% w/v in deionized water) consistently produced irritant effects, including ulceration, acanthosis, parakeratosis, hyperkeratosis, and inflammatory responses. In mice the epidermal mitotic index was significantly elevated. Zinc acetate induced similar effects, somewhat less in magnitude, when tested at a 20-fold higher concentration than $ZnCl_2$. Other compounds tested, 1% zinc sulfate, 20% zinc oxide suspension, 20% zinc undecylenate suspension, and 20% zinc pyrithione suspension, did not alter the mitotic index and did not definitively alter the epithelial histology.

In summary, alterations in epidermal structure are an important component of zinc deficiency, although the biochemical mechanism is not understood. Zinc compounds, applied topically in high enough concentration to the skin, may also induce pathology. This effect is highly dependent on the specific form of the zinc compound with the chloride salt being the most potent. Zinc oxide in contrast is used therapeutically for its topically protective effect on the skin.

Effects On Immunological Function

Immunopathology of Zinc Deficiency. Clinicians have frequently noted that suboptimal nutriture predisposed their patients to increased risk of infection and disease (*396*). Further, the severity of the disease was enhanced in malnourished patients such that recovery and even survival were threatened. Indeed, the mortality rate of undernourished children in Third World countries to ordinary childhood diseases is 50 to 100 times that of well-fed children (*396*). Suboptimal intake of dietary zinc is one of the most common worldwide nutritional problems and has been shown to exist in low-income, institutionalized, pregnant teenagers and elderly Americans as well (*397,398*).

However, poor quality diet and insufficient amounts of food are not the only causes of nutritionally acquired immunodeficiency states. A number of Western diseases, including various cancers, AIDS, gastrointestinal disorders, renal disease, sickle cell anemia, and chronic alcoholism, alter the intake and assimilation of nutrients. Suboptimal zinc is also noted in all of these diseases (*398*). Reduced food intake and weight loss accompany these diseases as

Table 11. Histological changes in lungs of mice, rats, and guinea pigs exposed to zinc oxide/hexachloroethane smoke.[a]

	Dose group			
	Control	Low	Medium	High
Alveologenic carcinoma				
Mice	6/78	7/74	8/76	15/50
Rats	0/43	0/44	0/48	0/44
Guinea pigs	0/45	0/39	0/46	0/39
Lymphocytic infiltration				
Mice	26/78	26/74	17/76	17/50
Rats	27/43	32/44	10/48	7/44
Guinea pigs	44/45	38/39	44/46	39/39

[a]Low, 1.3 mg/m^3; medium, 12.8 mg/m^3; high, 120 mg/m^3 as zinc; 1 hr/day, 5 days/week for 20 weeks (mice and rats) or 3 weeks (guinea pigs). Abridged from Brown et al. (*378*).

well as heightened susceptibility to respiratory illness, sepsis and other secondary infections. All indicate that malnutrition and impaired immunity are significant factors in the morbidity of these diseases.

There is little question that the effect of dietary zinc deficiency on immune function of rodents represents the best developed paradigm regarding the effects of a nutritional deficiency on host defense systems. The ubiquitous nature of zinc deficiency (*398*) and the high reliability of the mouse as an immunological model for humans makes these in-depth studies of substantial interest to those interested in malnutrition and its effects on immunity. Furthermore, they indicate that zinc is essential to maintaining integrity of the immune system. Information gathered to date indicate that a 30-day period of suboptimal intake of zinc in the young adult mouse, which produced a 20 to 25% weight loss, reduced the thymus to a quarter normal size and depleted the lymphocytes and macrophages in the spleen 50 to 70% (*399–401*). Thymic atrophy, which is a benchmark of both zinc deficiency and protein-calorie deficiency, was also accompanied by loss in activity of thymulin, a thymic hormone dependent on zinc for function (*402*). Antibody-mediated responses to both T-cell dependent and T-cell independent antigens were reduced 50 to 70% as demonstrated by several labs (*399–401*). Antibody-mediated responses are key to immune defense against a variety of pathogens and tumors, thus, such reductions explain the increased incidence of disease and infection observed in zinc-deficient subjects. Delayed-type hypersensitivity skin reactions, cell-mediated responses to tumors, and the function of natural killer cells were significantly reduced in zinc-deficient mice (*399,401*). Recent work indicates, nevertheless, that the residual splenocytes of zinc-deficient mice are normally distributed. In other words, the proportion of T-cells to B-cells and subsets thereof remained normal, although the absolute number of lymphocytes declined (*403*). Likewise, the functional capacity of these residual lymphocytes appeared to be normal. The ability of lymphocytes to proliferate and produce interleukins and antibody in response to mitogenic stimuli was normal. This remained the case even when care was taken to avoid possible repair of function by avoiding the high levels of zinc present in conventional culture media (*404*). The overall picture which emerges suggests that much of the loss of host defense capacity created by suboptimal zinc is due to the overall loss in the total numbers of leukocytes of the peripheral immune system. The underlying mechanism for this loss in cellularity appears to involve the inability of the bone marrow to produce adequate numbers of new lymphocytes.

Since substantial characterization of effects of zinc deficiency on the rodent immune system are in place, it is appropriate to begin to compare these results with those obtained with zinc-deficient humans. Unfortunately, the data collected to date for zinc-deficient humans are diverse with regard to disease state, age, overall nutritional status, etc. Further, almost no data are available on effects of zinc deficiency on the number and types of leukocytes present in the peripheral blood of the murine system, whereas a great deal of the human immunologic data have come from studies of the blood. Nevertheless, some encouraging commonality can be found between the rodent studies and patients suffering from acrodermatitis enteropathica, a genetic defect in the assimilation of zinc (*405*). These patients have increased incidence of secondary infections, lymphopenia, and reduced natural killer and delayed type hypersensitivity responses. Prasad's group has noted similar changes in patients with sickle cell anemia and other subjects who were zinc deficient (*398,401*). An increase in T_{101}^{-}, sIg-cells, and decreases in T4+/T8+ ratio, in serum thymulin activity, and in IL-2 production by mononuclear cells were observed in an experimental human model during specific, mild zinc depletion, induced by dietary means; changes were corrected after zinc repletion (*86*). A patient primarily zinc-deficient due to prolonged artificial feeding of an oral supplement containing insufficient zinc exhibited decreased delayed type hypersensitivity reaction and reduced lymphocytic response to phytohemagglutinin, a T-cell mitogen (*406*). Both functions were rapidly restored by zinc repletion. Another group studying patients receiving total parenteral solutions without sufficient zinc observed lymphopenia and reduced natural killer function, but elevated monocyte activity (*407*). For the most part these observations match the findings for the murine model. However, human data which conflict with the murine model can be found for the reasons already discussed (*401*).

Potential Modulation of Immune Function by Excess Zinc. As is evident from other sections of this report, the average US citizen is at far greater risk of becoming zinc deficient than experiencing adverse health effects as a result of exposure to high zinc. Further, data collected from both human and various animal models clearly indicate that the integrity of all aspects of the immune system is highly dependent on the availability of adequate levels of zinc. What effects excess zinc has on immune integrity is more problematic because exposure of subjects to high zinc is infrequent and the available literature is limited.

When considering potential effects of excess zinc on immune function, the route of exposure of the subject to this metal will control how the host defense system may be altered. For example, inhalation of zinc compounds could adversely affect the mucosa where responses of macrophages, neutrophils, mast cells and other components of the secretory immune system might be altered. Skin contact would involve phagocytic cells and the T-helperlike cells involved in delayed contact sensitivity and perhaps mast cells. Oral intake of high zinc via water or diet would have a more systemic effect that might alter circulating leukocytes and immune cells found in the primary and secondary lymph nodes. In addition, considerable variation in the observed immune defects would be expected depending on exposure duration.

In this regard, a set of related practical questions must also be addressed to properly evaluate the current literature as well as design meaningful future experiments. Under what circumstances, how frequently, and for what durations of time might industrial workers be exposed to zinc inhalation? It is these individuals who are at greatest risk of experiencing immunotoxic effects as a result of exposure to zinc. In what situations would individuals receive milligrams of zinc in water and/or diet as a result of "pollution" and for what lengths of time? Which animal species are good predictors of potential immunotoxic effects of zinc for the human population and which are poor models? These important variables, given little consideration in reports concerned with potential immunotoxic effects of zinc, must be addressed, if future research in this area is to provide meaningful data.

Effects of High Intake of Oral Zinc on Host Defense. The 1984 study of Chandra (*12*) is frequently cited as evidence that zinc is toxic to the immune system. In this case, 11 adult men were given 300 mg of elemental zinc once a day for 6 weeks. This constituted pharmacological levels of zinc, being 20 times above the RDA. Because high levels of intake of zinc can cause copper deficiency (*398*), a major flaw in this

study was the failure to monitor serum copper levels of the subjects. There is a high probability that the subjects were moderately deficient in copper with this prolonged regime of high intake of zinc. Thus were observed changes in immune function (and rising HDL levels) noted in this study due to high zinc or low copper? The seriousness of this flaw alone precludes the usefulness of the results of this study in assessing the effect of excess zinc on immunocompetence.

A second major flaw concerns the measures of immune status, most of which were performed *in vitro* over a period of 2 to 4 days, using culture medium which contained normal levels of zinc. It is unclear how these responses, tested in a normal zinc environment, relate to those in the host environment with a 2-fold elevation in serum zinc. Possible metabolic or physiological aberrations resulting from elevated zinc in the host environment were obviously not mimicked in tissue culture. Thus, the cells of the immune system might have repaired or performed better in the *in vitro* test environment than *in vivo*. Mitogens mimic antigens and cause certain resting lymphocytes to become activated and proliferate. The degree of proliferation can be measured by quantitating the amounts of ^{3}H-thymidine incorporated into the DNA of the actively dividing cells. In this study one mitogen (phytohemagglutinin) at a single dosage was used as a test of lymphocyte immune function. This assessment is too restrictive; proper immunology necessitates that a dose-response curve be determined. Proliferative responses thought to be suboptimal may have been normal at different doses of mitogen. Nonetheless, a depressed proliferative response was observed 4 weeks into the study that was presumed to be due to the high level of oral zinc.

Zinc itself is a well-known mitogen when present at high levels (*408*). The background response of the supplemented individuals might well have been high as a result of exposure to high zinc. If so, this would give a false negative because of the way the data were expressed. In this study, the data were reported only as a stimulation index (SI) rather than absolute counts (SI = absolute counts of stimulated cells divided by background counts of unstimulated cells). Thus, a high background in cells from zinc-treated subjects causing a lower SI would have been interpreted as a reduced mitogen-induced proliferative response when that was not really the case. Direct addition of zinc to culture medium in this study as an additional means of looking at "zinc toxicity" simply confirmed results noted in earlier studies which showed that high levels of zinc can, in some cases, activate lymphocytes (*408*). The subsequent observed influx of Ca^{++} into lymphocytes, which was reported, is also a common event noted early in the activation process of these cells.

A reduction in chemotaxis and ability to engulf or phagocytose bacteria was also noted in these studies. However, these assessments were also performed *in vitro* so all of the aforementioned concerns about *in vitro* studies apply to these data as well. The absolute number of lymphocytes and proportion of T- and B-cells were not altered in these subjects. It is interesting that there was no change in this important parameter that is often greatly altered in other nutritionally related immunodeficiency diseases (*396*).

The immunological data are further confounded by the fact that there were no placebo controls. Baseline assessments were made of the subjects prior to supplementation. However, the various immune assessments made are all subject to a great deal of biological variation giving different absolute values on different test days. Thus, there was no control for the experimental variation that can occur over time.

Since more than 50% of adults would find the doses of zinc used in the above study to be highly irritating to the stomach and would generally stop taking zinc, it is interesting that all 11 subjects apparently tolerated this zinc preparation. This well-known side effect of high zinc was not discussed. Indeed, few discussions of problems associated with oral intake of truly high levels of zinc consider that stomach disorders would be an early indicator of excess zinc and would probably cause subjects to seek alternate water and food sources (*61*). Zinc at 15 mg/l produces an undesirable taste in water, so it is doubtful that its presence would go unnoticed (*61*).

The elderly represent a subset of the population that may be more sensitive to the effects of certain toxic substances. A plethora of data indicates that many of the elderly (>70 years of age) may be marginally zinc deficient (*397,409*). As a result, a number of studies examined the effects of zinc supplementation on zinc status and degree of immunocompetence of the elderly. These studies are of interest because they are in conflict with the findings of Chandra (*12*). In each of the studies of the elderly which will be discussed, they were supplemented for substantial periods of time with doses of zinc well above the RDA. In one case, modest improvement in immune function was noted, while in another very well-controlled study, no change was noted.

In the first study, Duchateau et al. (*410*) gave elderly subjects doses of zinc for 1 month which were clearly pharmacological (440 mg zinc sulfate per day). Unfortunately, there were only 15 supplemented and 15 unsupplemented subjects. Furthermore, their zinc status was not assessed at the outset of the study. Nevertheless, seven of the subjects showed some increase in responsivity to delayed type hypersensitivity antigens and immunization with tetanus toxoid after zinc supplementation. However, the latter are recall responses which are easier to rejuvenate than virgin immune responses. Nevertheless, they indicate that high dietary zinc may have some enhancement capacity for certain human populations. Whether these subjects became marginally copper deficient as a result of this regime was not determined.

Bogden's laboratory initiated more careful studies of the elderly. Degrees of mild zinc deficiency were noted in 100 elderly subjects (*397*). Some 40% of these individuals gave poor delayed type hypersensitivity skin responses. Subsequently, some of these subjects were given 100 mg of zinc for 3 months (*411*). These individuals exhibited a modest increase in serum zinc from 13.1 to 16.8 μmole/l with no change in plasma copper. Although no enhancement of immune function was noted by these investigators, the number of tests made was necessarily limited. Further, no attempt was made to stratify subjects according to zinc status and compare the immunological responses of zinc supplementation between the two groups. What is clear is that levels of zinc about 6 times above the RDA had no adverse effect. Interestingly, Winchurch et al. (*412*) found that he could substantially increase the antibody mediated responses of splenocytes (lymphocytes from spleens) from old mice by simply incubating them with high quantities of zinc (0.15 mM $ZnCl_2$) *in vitro*. Many nutritionists as well as scientists at the US Food and Drug Administration feel that further investigation of possible efficacious effects of high quantities of oral zinc supplementation of the elderly are needed. In sum, prolonged exposure to oral intake of high levels of zinc had no adverse immunological or physiological effects on the elderly in these

studies, which further detracts from the validity of the 1984 study of Chandra (*12*).

Effects of Zinc Inhalation on Immune Defense Systems of the Lung. The small concentration of zinc routinely found in the air suggests that the average American citizen is at little risk of adverse pulmonary effects from zinc. However, inadvertent exposure to high levels of zinc either on an acute or a chronic basis in the industrial arena appears to have the potential for damaging immune defense systems in the lung. This supposition is based on literature containing less-than-desirable experimental protocols. Those at risk appear to be industrial workers, involved in welding galvanized steel or processing zinc from ore, who could become victims of metal fume fever (*413*).

At the onset, it must be recognized that understanding of pulmonary immunology is quite limited. Lavage of the lung reveals the presence of extensive numbers of macrophages, some neutrophils and components of the secretory immune system vital to host defense in the mucosa. Some lymphocytes and various classes of immunoglobulin are also present in the lung. The lung is continuously exposed to a variety of air- and bloodborne agents that might trigger inflammation, activate various immune processes, or enhance the probability of infection. For example, it is known that exposure to organic dusts can induce hypersensitivity pneumonitis; so it is possible that metals such as particulate zinc may do the same. Indeed the symptoms of metal fume fever parallel those of hypersensitivity pneumonitis (*413*). In the acute phase of pneumonitis, symptoms appear 4 to 6 hr after exposure and include a nonproductive cough, dyspnea, fever, chills, malaise, etc. These symptoms along with increased numbers of neutrophils and immune complexes are often noted in the lungs of patients with metal fume fever (*413*). In more delayed reactions of pneumonitis, mononuclear cells, granulomas and other signs of ongoing inflammation of the lung become apparent. Wheezing, cough, respiratory rales, etc. and general malaise can accompany this phase. Thus, it is important to keep in mind that this well-known disease or inflammatory lung response and metal fume fever have the same general characteristics.

At a very early date numerous studies of fume fever noted the rise in body temperature and increased leukocytosis in the lung among welders and those involved in smelting operations (*414*). One of the earliest attempts to further document immune inhalation effects of zinc oxide was an animal study conducted by Drinker and Drinker in 1928 (*415*), which gave mixed results. These investigators exposed cats, rats, and rabbits in a gas cabinet to zinc oxide in concentrations ranging from 110 to 600 mg/m^3 for several hours. The cats exhibited excessive lung secretions and "varying degrees" of infiltration of the lungs with neutrophils, but surprisingly, no rise in temperature was noted for the subsequent 29 hr. The lungs of rats and rabbits subjected to the same treatment appeared normal. The dichotomy in effects among these species was an early warning that there are significant species variations in responsivity to inhalation of zinc oxide. As a result, animal experimental models appropriate to humans should be selected with extreme care. In a later related study, it should be noted that guinea pigs exhibited a resistance to repeated inhalation exposure to zinc sulfate but not to zinc oxide (*385*). The latter data help make the point that it would indeed be important to know if humans become more sensitive or more resistant upon repeated exposure to zinc oxide in the air.

More recent studies of fume fever in humans affirm the already well-known attributes of the phenomenon, but unfortunately they do not provide much further illumination on the condition. Vogelmeier and colleagues (*416*) monitored a 26-year-old individual who experienced metal fume fever as a result of welding in improper conditions. He exhibited the usual fever, sweating, and shortness of breath within a few hours of the accident with no observable signs of illness after a few days time. Six months later the investigator had him weld for 1 hr again under conditions that purposefully reexposed him to high zinc. One day later the subject was free of overt symptoms of fume fever; however, a lung lavage indicated the presence of 10 times as many leukocytes as normal. Many of the additional cells appeared to be neutrophils. Nevertheless, cell and differential counts in the lung were normal when checked 7 weeks later. It would have been helpful if additional time points had been taken to see if the neutrophils continued to rise several days after inhalation of zinc. Nevertheless, this and other studies suggest that the effects of inhalation of zinc on the lung may be transient. The 10-fold increase in cellularity of the lung upon reexposure to zinc also suggests the possibility of a heightened response upon reexposure to zinc among humans.

The most current study available regarding metal fume fever was published recently by Blanc et al. (*380*). Though part of this study was well-controlled, the immunological analyses have weaknesses. Fourteen welders were placed in a special chamber and asked to weld without necessary respiratory protection for a 15- to 30-min period such that they were exposed to 2.3 g zinc oxide min/m^3. Bronchial respiratory rates were measured 6 and 20 hr later. Changes in immune components of the lung were measured 8 and 22 hr later using bronchoalveolar lavages. In the 8- to 22-hr span, the cells found in the lung lavages increased 3-fold with the proportion of neutrophils increasing from 9 to 37%. No increase in the production of cytokines by infiltrating leukocytes was noted in the lung fluids. These studies reaffirm that fume fever markedly increases the neutrophils in the lung, but the duration of these increases is not known. Too much reliance was placed on commercial kits for measuring the cytokines which could initiate fever and inflammation. What controls, if any, the investigators used to test the efficacy of these kits was not provided. Functional assays of the various cytokines were not carried out and could have been informative. Further, 22 hr might have been too soon to examine lung fluids for these particular substances. Worse, these investigators apparently never tested for signs of fever in the subjects which was a major oversight. Indeed, the subjects were provided with acetaminophen to use. As a result only two subjects reportedly exhibited fever. It is not clear to what extent the low incidence of fever was a result of the subjects taking acetaminophen. This unfortunate variable introduced into the study may well have altered the investigators' ability to measure the actual degree of inflammation created by zinc oxide. Thus, the immunology regarding effects of zinc oxide on host defense systems of the lung remains wanting and in need of further experimentation.

Additional Studies of Effects of Zinc Inhalation. In 1987, Farrell (*379*) demonstrated that it is also possible to develop dermal hypersensitivity responses after inhalation of high amounts of zinc in the air. A subject experienced some signs of fume fever (e.g., malaise and fever) a few hours after welding. The next day hives and itching of the face, lips, and throat were apparent. These observations suggest a delayed allergic response to zinc was in operation. Since the dermal sensitivity of some individuals to the metals found in jewelry, es-

pecially earrings, watches, etc. has been recognized for some time, it may be possible that this individual had a dermal allergic response to zinc. This report is interesting only in that it documents that a hypersensitive dermal response might be expected among a small fraction of those suffering from metal fume fever. Stated another way, the types of hypersensitive states induced by exposure to high quantities of airborne zinc may vary among individuals and may not be limited to the lung.

There are also studies which document lung injury among those exposed to smoke bombs containing zinc chloride. Because of the presence of other highly toxic chemicals such as phosgene in such smoke bombs, it is not clear that all observed symptoms can be ascribed to zinc. Further, it would seem that the number of individuals at risk to such exposure today are minuscule. Nevertheless, a study by Matarese and Mathews (*417*) of a 20-year-old male exposed to a smoke bomb for at least 5 min in a confined area noted that he suffered acute respiratory distress. Four months later there was little residual evidence of the accident. Once again, these studies point out that the degree and duration of changes in pulmonary immunity are key to whether or not zinc inhalation causes significant and prolonged impairment of defense systems in the lung.

Summary of Effects of Zinc Inhalation on Pulmonary Immunology. The febrile response associated with metal fume fever (*413*) certainly indicates probable involvement of cells of the immune system and synthesis of immune cytokines known to induce inflammation and fever such as interleukin 1 (IL-1) and tumor necrosis factor (TNF). Infiltration of the lung with neutrophils and other leukocytes after exposure to zinc oxide has frequently been noted in humans and some animal species as discussed. Macrophages, in particular, can produce high quantities of IL-1 and TNF that would induce fever and cause malaise and loss of appetite. They may well be released into the fluid of the lung as a result of exposure to zinc. In addition, the phagocytic cells can release prostaglandins, thromboxanes, and leukotrienes that initiate inflammation, cause tissue injury, and facilitate edema. Depending on the nature of the interaction of phagocytic cells with particles of zinc, the oxygen burst might also be initiated which causes the release of toxic oxygen radicals such as the superoxide anion, peroxide, and hydroxyl radical into the surrounding environment, causing tissue injury and inflammation. Complement factors are known to be chemotactic, enhancing infiltration of neutrophils and macrophages into the lung after such an insult. Quantitation of the extent of production of cytokines, inflammatory factors, complement factors, etc., would reveal the degree of inflammation and disease process initiated by fume fever and also serve as hallmarks to determine the duration of damage and injury caused by fume fever. Unfortunately, examination of lungs for the presence of these important biological products has yet to be done in a satisfactory way subsequent to inhalation of zinc.

Effects on Reproduction

Role of Zinc in Reproduction. Zinc nutriture affects specific processes that control and facilitate genetic expression, DNA replication, and RNA and protein synthesis; the efficient utilization of food; the metabolism of carbohydrates and lipids; and processes that mediate metabolism of some vitamins, including retinol, pyridoxine, and folate. Therefore, it is not surprising that severe zinc deficiency is either lethal or injures the embryo and fetus of experimental animals (*418*).

A review of research from the 1975 to 1985 by Apgar (*418*) found the evidence of adverse effects from zinc deficiency in human pregnancy incomplete. In contrast, evidence from animal experiments conclusively showed zinc essential for conception (*419*), blastula development and implantation (*420*), organogenesis (*328–330*), fetal growth (*338,421*), prenatal survival (*330*), and parturition (*422*).

An extensive 1987 review of the topic by Swanson and King (*333*) found associations between maternal zinc deficiency and adverse outcomes, but noted that understanding of the phenomenon was incomplete. They attributed the lack of understanding in part to the lack of a sensitive and generally available method for assessment of zinc status.

An important contribution of Swanson and King (*333*) was their factorial estimation of zinc requirements during pregnancy. Using published data on zinc concentrations of tissues, they estimated that an average daily retention of zinc of 1.2, 3.7, 8.1, and 11.2 μmole/day during successive quarters of pregnancy was sufficient to satisfy the added needs of pregnancy. They also estimated about 9.2 μmole (about 600 μg) was needed daily during the last half of pregnancy. This was lower than a previous estimate of 11.5 μmole (about 750 μg) daily (*63*). Swanson and King (*333*) estimated the mean dietary requirement for zinc is 10.5 mg zinc daily at 25% bioavailability. Assuming a 15% coefficient of variation, this suggests that 13.6 mg daily will meet the needs of most normal women who consume diets from which zinc is 25% bioavailable (*74*). It also indicates that 50% of women who consume 10.5 mg zinc daily are at risk of zinc deficiency, unless homeostatic adjustments increase the amount of zinc they retain. Relevant to this interpretation, Apgar (*418*) found published dietary zinc intakes of pregnant women were 8 to 11 mg daily in eight studies and 12 to 14 mg daily in two studies. Thus it appears the occurrence of zinc deficiency in human pregnancy is probably far greater than is currently recognized clinically.

The first comprehensive study to show an association between an index of zinc nutriture and human pregnancy outcome was published by Jameson (*423,424*). He found that low serum zinc at about the 14th week of gestation was predictive of outcome. Women with low plasma zinc were found to have a significantly increased incidence of complications of pregnancy, including teratology. Others cited by Apgar (*418*) and Swanson and King (*333*) also reported an association between low indices of maternal zinc status and malformations, including neural tube defects in babies.

Supportive of Jameson's (*423*) findings was a study (*425*) from Ohio that found a highly significant increase in pregnancy complications, including fetal distress and maternal infections, among women with low plasma zinc during the latter half of pregnancy. More recently Neggers et al. (*426*) confirmed Jameson's finding that plasma zinc levels in early pregnancy are predictive of outcome. They studied low-income women from Alabama and found lowest quartile plasma zinc at about 16 weeks gestation associated with an 8 times greater likelihood of delivery of a low birth weight infant compared to highest quartile plasma zinc. Supportive of this finding was the report of Meadows et al. (*427*) of an association of low maternal leukocyte and muscle zinc at term with low birth weight, and a report by Campbell-Brown et al. (*238*) of the association of low zinc intakes in Hindu women with low birth weight.

Some zinc treatment trials found evidence that improved zinc nutriture was beneficial for pregnancy outcome in some women. A double-blind, randomized controlled trial of 30 mg zinc daily given to

poor teenagers found zinc eliminated the need for assisted respiration in the newborn infants and reduced the incidence of premature delivery among mothers who were of normal weight (*78*). Another study found that 20 mg zinc daily lowered the overall incidence of pregnancy-induced hypertension (*428*). Related to these observations are Jameson's (*429*) findings that zinc treatment reduced the perinatal mortality in 598 women to 2, compared to 13 in 633 control women ($p<0.001$). In addition, delivery before 33 weeks of gestation was decreased to 2/598 as compared to 14/633 ($p< 0.001$). Spontaneous abortions were less frequent among women who received zinc supplements before the 22nd week of gestation compared to women who were untreated before the 22nd week ($p< 0.05$). Consistent with the reported benefits of zinc supplements were results of an incompletely controlled study by Kynast and Saling (*430*) which found improved pregnancy outcome in women given zinc. In contrast a randomized controlled intervention trial from Britain was "negative" (*431*).

Excess Zinc and Reproduction. Reported evidence that high intakes of zinc cause harm to the embryo and fetus is limited. Studies in hamsters found intravenous injection of 25 mg zinc sulfate/kg caused about 12% fetal resorptions and 6% malformations, including exencephaly and rib malformations (*432*). In contrast, 2 mg cadmium/kg caused a nearly 60% rate of malformations which were prevented by dosing with 2 mg zinc/kg simultaneously. In contrast to the observations in animals, there is essentially no interpretable data indicating that zinc can impair human pregnancy outcome. This is probably because very high exposures to zinc are unusual in human pregnancy. The only evidence of possible harm from zinc supplements is an unpublished report from the Indian National Nutrition Institute (*433*) that found administration of 300 mg daily during the third trimester was associated with the occurrence of three premature births and one stillbirth, which caused the research project to be discontinued.

Summary. In animal species, zinc deficiency during gestation causes a variety of adverse effects in the fetus, including abortion, malformations and growth failure. Evidence in humans is consistent with the animal data. Current evidence in animals suggests there is a wide range between required doses of zinc and toxic doses in pregnancy. Clear evidence of zinc toxicity in human pregnancy has not been reported.

Genotoxicity

There were no reports in the accessible literature on genotoxic effects of zinc compounds in human populations.

Zinc salts do not appear to be genotoxic when administered to animals, even at very high doses. Zinc sulfate was negative in a sex-linked lethal assay in Drosophila. This form of zinc was also negative in a mouse bone marrow micronucleus assay (*434*). Other studies (*435*) have shown that zinc chloride induced chromosomal aberrations in bone marrow cells of calcium-deficient mice but not in normal calcium-supplemented mice. These investigators administered high doses (5000 ppm) of zinc chloride for 1 month in the diet without observable genotoxic effects in calcium-replete animals.

A single report (*436*) on chromosomal aberrations in mice exposed to zinc oxide by inhalation is difficult to interpret and not considered significant; the reported cytogenetic effects of inorganic and acetate compounds of tungsten, zinc, cadmium, and cobalt in animal and human somatic cells are not convincing.

The results of mutagenicity studies of zinc, based on *in vitro* genotoxicity tests, are mixed. In an extensive survey of metal mutagenicity, Nishioka (*437*) reported moderately positive recombinant-deficient (rec) effects for arsenic and cadmium, but cobalt, magnesium, nickel, lead, and zinc chloride were negative. The assay used by these investigators compared differential growth inhibition by the various chemicals in wild and recombination-deficient (rec) strains of *Bacillus subtilus.* In contrast to cadmium, nickel, and transplatinum (II) diaminedichloride, which significantly increased the absolute number of trifluorothymidine-resistant mutants, zinc chloride did not. The mutagenicity of the metals generally paralleled their carcinogenicity.

Others have also failed to find that zinc salts are mutagenic. Marzin and Vo (*438*) observed that zinc sulfate in water at doses of 10 to 3000 nmole/plate, the latter the threshold toxic dose, was negative in the *Salmonella typhimurium* TA102 assay. Doses were in logarithmic progression up to insolubility or toxicity. Zinc chloride has been reported to be capable of disrupting or breaking chromosomes in lymphocytes (*439*). However, Amacher and Paillet (*440*) found that zinc chloride did not induce mutations at the thymidine kinase locus in L5178Y/TK mouse lymphoma cells.

In another study of the genotoxicity of zinc in four short-term mutagenicity assays, Thompson et al. (*441*) observed that the result depended on the assay used to evaluate genotoxicity. Zinc acetate produced dose-related positive responses in the L5178Y mouse lymphoma assay and in an *in vitro* cytogenetic assay using Chinese hamster ovary cells (CHO), but was negative in the Salmonella mutation assay and did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes. Zinc-2,4-pentanedione produced frameshift mutations in Salmonella tester strains TA1538 and TA98, but did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

The cytotoxicity of zinc *in vitro* has been examined by Borovansky and Riley (*442*). These investigators tested effects of zinc acetate on three mammalian cell lines and found that growth was inhibited in all three by zinc in concentrations ranging from 0.125 to 0.15 mM Zn^{2+}. This growth inhibition could be suppressed by either iron or calcium ions. There appears to be at least two routes of zinc entry into the cell, one of which can be blocked by iron, the other by calcium. The authors suggest that the toxic action of zinc requires entry into the cell and that zinc shares cell entry channels with iron and calcium ions.

In summary, these observations, similar to those reported by Hansen and Stern (*443*), indicate that *in vitro* and *in vivo* genotoxicity test results vary widely and that the weight of evidence supports the conclusion that zinc is not genotoxic in the systems examined. However, zinc salts can be cytotoxic at high concentrations, as noted in numerous studies.

Carcinogenicity

Epidemiological Studies

There are very few references in the literature relative to the risk for cancer from zinc exposure in human populations. Stocks and Davies (*444*) correlated cancer mortality with the zinc and copper content of soil in 12 districts of England and Wales. These investigators found higher zinc levels and higher ratios of zinc to copper in the soil of vegetable gardens near houses where a resident had died from gastric cancer compared to houses where a death had occurred from other causes. A combined study in France and Italy (*445*) on the role of zinc and copper in breast cancer inci-

dence, using hospital-based case-controls as subjects, showed that blood zinc levels were consistently higher in cancer patients than in controls. Dietary intake of zinc was reported to be equivalent in all subjects.

Schrauzer et al. (*446,447*) evaluated food intake in several countries and reported a direct correlation between estimated zinc intake and age-adjusted mortality from leukemia and cancers of the skin, breast, prostate, and intestine. The authors speculated that increased dietary zinc interfered with absorption or utilization of dietary selenium, producing a relative deficiency of this latter essential trace element. A relative deficiency of selenium produced in this manner may have contributed to the increased cancer incidence, but the data provided by these investigators did not support this hypothesis.

In contrast to the interpretation of Schrauzer et al., Van Rensburg (*448*) observed that in many countries where wheat and corn are primary staples there is a high risk for esophageal cancer. After examining the correlations between dietary intake of selected nutrients, this investigator proposed that diets low in micronutrients, particularly zinc, magnesium, and nicotinic acid, might be etiologically related to cancer of the esophagus. While there were no data to support such an assumption, the dietary deficiencies may have modified the metabolism of carcinogens in the environment by changing the activity of cytochrome P_{450} enzymes which activate or detoxify the chemicals; such inferences have been drawn from zinc deficiency studies in animals (*449*).

Strain et al. (*450*) compared zinc and copper levels in serum of patients with bronchogenic carcinoma to levels of these elements in serum of controls. Zinc levels were comparable, but copper levels were lower in controls, resulting in higher ratios of zinc to copper in the bronchogenic cancer patients. In contrast to the observations by Strain et al. (*450*), Davies et al. (*451*) had reported earlier that zinc levels in plasma of bronchogenic patients were lower than in patients with other types of cancer and lower than normal laboratory reference values. Similar results were reported by Gyorkey et al. (*452*).

Lin et al. (*453*) examined serum, hair and other tissues from Chinese men in Hong Kong for zinc and copper concentration. These investigators found that zinc concentrations in serum, hair and apparently tumor-free esophageal tissues from esophageal cancer patients were significantly lower than concentrations in patients with other types of cancer or in patients dying from other causes. Analyses of esophageal tumors and apparently tumor-free esophagi showed that the zinc concentrations (μg/g dry weight) in the tumors from 72 to 111 (mean 95) were much lower than those in healthy tissues from 114 to 159 (mean 148). Esophageal tissues in normal adults contained 150 to 197 (mean 178) μg zinc/g. Caution is recommended, however, in the interpretation of results from serum concentrations of zinc, since many factors including infectious disease can influence these levels.

Logue et al. (*454*) studied workers in nine electrolytic zinc and copper refining plants. In two of the plants the employees were exposed to zinc or zinc and copper. There were no correlations between zinc exposure and any form of cancer; however, the numbers of people dying of cancer were small. Similar observations were reported by Newberger and Hallowell (*455*) with respect to lung cancer mortality in a subpopulation living in an area where an old lead/zinc mining and smelting operation had previously existed. Lung cancer incidence was elevated in the area but it could not be traced to the smelting operation and consequent exposure to environmental contamination by zinc or lead.

Studies in Experimental Animals

A number of reliable reports associate zinc status with susceptibility to cancer. Most of these studies examine zinc deficiency as opposed to zinc excess. In studies of zinc excess, exposure has primarily been by inhalation (*387,388*). For example, rats, mice, and guinea pigs were exposed to a zinc oxide/hexachloroethane smoke mixture in concentrations up to approximately 125 mg zinc/m^3. Exposure was 1 hr/day, 5 days/week for 18 months. There was a statistically significant increase in frequency of alveologenic carcinoma in mice exposed to the concentration of 125 mg zinc/m^3, but no tumorigenic effect was noted in either rats or guinea pigs subjected to similar exposure protocols. However as pointed out above, lung tumors are common in many strains of mice and an increased incidence of this type tumor in one strain, one sex and at the highest dose of exposure cannot be taken as evidence for carcinogenicity of zinc. Moreover, as noted, this study used only female animals, and the exposure was to a mixture of chemicals, not to zinc only; the contribution of other chemicals to the end result is unknown.

A number of reports from experimental animal studies suggest that zinc deficiency inhibits growth of transplanted tumors in animals as well as prolonging survival time. Petering et al. (*456*) and DeWys et al. (*457*) showed that growth of transplanted Walker 256 carcinoma in rats was significantly inhibited by zinc deficiency. Other studies have demonstrated an effect of zinc deficiency on leukemias and lung carcinoma (*458*), Ehrlich ascites tumor (*459*), and plasmacytoma (*460*) in rats and mice. The inhibitory effects of zinc deficiency on these tumors can be explained in part by the well-known influence of zinc on tumor cells: rapidly growing tumor cells require relatively large amounts of zinc for maintenance of growth.

The reports of other investigators seemingly contradict the results of studies alluded to above. Fong et al. (*461*) observed that the incidence of esophageal tumors induced by methylbenzylnitrosamine (MBN) was significantly higher in rats maintained on diets low in zinc (3 ppm) compared to normal, zinc-supplemented (60 ppm) animals. It is likely that the dramatic effect of zinc deficiency on the esophageal epithelium (*462*) contributes to enhanced susceptibility to cancer. The epithelium is severely damage by the deficiency alone, leaving it more sensitive to the carcinogen or its activated metabolite. There is also some evidence that metabolism of the carcinogen (MBN) is modified by zinc deficiency (*463*).

Others have shown that zinc intake greatly exceeding nutritional requirements (more than 10 times) suppressed carcinogenesis induced by dimethylbenzanthracene (DMBA) in Syrian golden hamsters (*464*). Duncan and Dreosti (*465*) have observed similar effects in rats exposed to azo dye which induces liver cancer in rats.

The contradictory nature of the two types of experiments described above would appear to result in part from different systems and approaches to studies in carcinogenesis. The studies wherein zinc deficiency inhibited tumors used transplantable tumors or tumor cells introduced into the zinc-deficient animal. The host, serving essentially as a graft recipient, simply could not support the growth of tumor cells since immediately after transplant of the tumor or cell lines they require a source of zinc in relatively large amounts. In the case where zinc deficiency enhanced the chemical induction of tumors, the host provided a limited supply of zinc which was sufficient for development of the tumor but not enough zinc to permit resistance to tumor development, perhaps via immune mechanisms.

Other investigators have shown that zinc offers a protective effect against such carcinogens as nickel (*466*) and cadmium (*467*). These investigators gave zinc in doses of 1.0 mmole zinc acetate/kg *sc*, 6 hr before, simultaneously, and 18 hr after cadmium injection, or 100 ppm zinc acetate in drinking water throughout the 100 week study. Diplock (*468*) has discussed mineral insufficiency, including zinc, and a possible relationship between minerals and cancer.

The impression gained from results of genotoxic studies as well as epidemiologic and experimental cancer studies is that, while there may be some conflicting and contradictory results, the weight of evidence is that zinc, although potentially toxic at high levels of exposure, is clearly essential for optimum resistance to cancer, especially in mounting an immune defense against developing tumors. Furthermore, there is no evidence for enhanced cancer risk by higher than normal dietary zinc.

Summary of Health Effects

In summary, this review supports the consensus statement from the NAS/NRC report (*9*) on "Drinking Water and Health" which stated that: "In view of possible deficiency in United States diets, it is prudent to maintain all dietary sources of zinc... The possibility of detrimental health effects arising from zinc consumed in food and drinking water is extremely remote." The evidence for significant toxic effects of zinc in human subjects is restricted almost entirely to accidental exposure to high concentrations of zinc compounds, such as those in smoke bombs, or from accidental or intended overdosage of zinc in therapeutic forms. Notably, these kinds of exposures are acute sudden exposures to high concentrations. Even so, these effects are almost always reversible.

There are unequivocal results from both human and animal observations, which document that acute, high level exposure to zinc compounds can produce respiratory and gastrointestinal toxicity. However, these effects are largely self-limiting and require only modest medical attention. Exposure to smoke from bombs containing zinc compounds can cause illness, generally transient, as can acute or chronic exposure to fumes from acetylene or electric welding of zinc-containing metals. Exposure to excess zinc in tablets intended for human consumption, controllable for the most part, can also result in clinical changes, such as decreased activity of erythrocyte superoxide dismutase and increased activity of serum alkaline phosphatase and pancreatic enzymes. Other reported effects such as changes in serum lipoproteins and immunological status are controversial and require more definitive assessment. There is no compelling evidence supporting a cause for concern about zinc in the environment as a putative toxic agent.

Research Priorities for the 1990s

Identification of Subjects at Risk of Altered Zinc Status

Environmental Studies. Nutritional studies have confirmed that inadequate zinc intake occurs in subsets of the United States population and can lead to adverse health effects. Toxicity from increased intake of zinc has been documented following acute inhalation by industrial workers, overdose of oral supplement preparations and excessive levels in total parenteral nutrition fluids. Currently, there is not good evidence that a subset of the population is at risk of adverse health effects from increased zinc intake as a result of local environmental contamination at waste sites or from industrial discharges. A first priority is to establish that drinking water levels or dietary content of zinc are increased in populations adjacent to environmental sites with unusually high zinc content. This rationale is based on concern that research dollars should not be devoted to study of the high end of the zinc dose-response curve without clear evidence of its relevance. Reasons for considering that there is unwarranted concern about increased intake of zinc include the well-known aversive taste of drinking water with elevated zinc levels and uncertainty that crops and other dietary sources would accumulate zinc in significantly greater levels from leaching of the metal from waste sites. Research is needed to identify populations suspected of increased intake of zinc from contamination of drinking water and foods, before further work is justified to evaluate the effects of elevated zinc intake in experimental studies. Environmental studies should entail sampling of ground water, drinking water, and the diet. Care must be taken to evaluate the contribution to tap water of zinc from water-supply piping, as opposed to environmental waste sites.

Epidemiological Studies. If environmental studies reveal that segments of the population are indeed at risk of zinc ingestion significantly above the RDA, epidemiological studies may prove useful in determining whether there are associated health effects. These types of analyses would not necessarily reveal effects of zinc alone, since under environmental conditions contaminations might potentially include multiple metal and organic pollutants and require extensive data collection to account for confounding variables. The difficulty arises in that although zinc at certain dose levels may produce symptomalogy, it may also be protective against effects of other agents. Questionnaire–based surveys of populations in areas of unusually high or altered distribution of zinc, in comparison to a well-chosen control group, might consider end points such as reproductive history, infection rates, behavioral assessments, and cardiovascular morbidity and mortality. Such a study obviously would require careful attention to dietary histories for assessment of zinc intake and of course would be most productive with some measure of zinc status in individuals (see below).

Development of Methods for Assessing Zinc Status in Humans

Research should be supported to develop improved methodology for determining zinc status in individual subjects. More practical, inexpensive, sensitive and physiologically valid methods are needed to permit estimating the total body burden of zinc and its compartmental distribution within the body. This methodology is needed to improve research on the dose-response relationship between zinc levels in the body and effects at various target sites. Since plasma zinc concentrations have been criticized as a relatively insensitive indicator of zinc status, other approaches including reliable biomarkers must be developed, especially for large-scale screening studies. Possibilities that deserve expanded consideration include: zinc in sampling sites such as hair and nails, shed teeth from children, and urine. A more sophisticated approach for clinical studies that warrants further validation is the determination of the turnover rate of the readily mobilizable pool of zinc, about 10% of total body burden. There is evidence that this parameter is inversely correlated with zinc status. This relationship should be more clearly established by determination of turnover rates in subjects with controlled or estimated zinc intakes. Turnover rate appears to be most accurately estimated by iv administration of the stable isotope ^{67}Zn, followed by measurements of 30- to 60-min plasma levels using plasma emission mass spectrometry. The most well-controlled, albeit expensive, study would entail placing subjects in a metabolic ward and sequentially providing diets varying in zinc content below, at, and above the RDA. A less well-controlled approach

would be to study subjects whose dietary history permitted classification as marginal, normal, or zinc-supplemented. Another posssible indicator of total body burden of zinc may be provided by *in vivo* neutron activation analysis of zinc in bone, a concept that also deserves research development.

In addition to zinc measurements for assessing zinc status, research should be directed, as noted above, to the development of valid biomarkers. Among possible candidates are erythrocyte superoxide dismutase, metallothionein, serum alkaline phosphatase, and serum amylase or lipase, although none of these alone would be sufficient. Research in this area must include studies that further elucidate the mechanisms of zinc-induced changes in these parameters, especially in relation to alterations secondary to changes in other essential metals such as copper.

Studies of the Health Effects of Zinc Status: Interactions with Other Trace Elements

Rationale. Further research on the health effects of zinc should primarily address questions related to the critical role of this metal in physiological processes. Studies should therefore encompass the dose-response relationship ranging from mild deficiency to mild excess. Research is encouraged in areas of immunology, reproduction, multigenerational studies, neurology, and lipoproteins and cardiovascular function. Studies should address the role of zinc-induced perturbations in other essential metals, especially copper and iron, in the production of biological effects. *In vivo* dose-response experiments with zinc should therefore include the monitoring of copper and iron status by measures of these metals in serum and tissues and of relevant biomarkers such as erythrocyte superoxide dismutase and serum ceruloplasmin for copper and serum ferritin for iron. The effect of increased copper or iron intake on zinc dose-response curves should be determined.

Interactions of zinc and toxic heavy metals are also fertile fields for investigation, especially because of the likely multiple components of environmental waste sites. The well-known toxicity of both lead and cadmium seem to be, under some circumstances, ameliorated by zinc. The in teractions of zinc, lead, and cadmium on biological systems appear to be poorly understood. As a minimum, end points that should be examined include immunocompetence and reproduction.

Studies feeding massive doses such as 100 and 1000 times the RDA, as in some work described earlier in this text, do not warrant support. Long-term carcinogenesis studies with high doses in rodents also are not recommended, primarily because of the negative results in earlier studies.

Immunological Function. CAUSES OF IMMUNE DEFECTS IN ZINC DEFICIENCY. The average American is at far greater risk of experiencing immune defects due to suboptimal intake of zinc rather than defects induced by high zinc. There is a definite need for more extensive human research to elucidate further the nature and degree of change that suboptimal zinc status makes in host defense. Additional studies of the immune defects using rodent models are also necessary to identify the underlying mechanism(s) that cause the rapid demise of the immune system by zinc deficiency. Animal models have not been used to good advantage to elucidate how cytokines, nutritional repletion, etc., might be used to help rejuvenate immune systems impaired by inadequate zinc. Rodent models (since extensive *in vivo* assessments can be performed) would be vital to these studies as well.

It is now clear that deficiencies in zinc alter endocrine function and lymphopoietic processes in the mouse. More studies are needed regarding the nature and extent of induction of glucocorticoids and the stress-endocrine axis by suboptimal zinc. Likewise, it is now clear that the glucocorticoids induce programmed cell death or apoptosis in the immature cells of the immune system. This induction of cell suicide and its role in the rapid atrophy of the thymus and decline in lymphopoiesis and production of new lymphocytes in the bone marrow need to be further characterized for the zinc-deficient mouse, since it appears to be a seminal mechanism.

The elderly appear to be mildly deficient in zinc. Whether this is a normal and acceptable outcome of áging or a deviation that should be addressed by supplementation is a topic of key current interest. The studies of Bogden et al. (*397*) indicate that extensive zinc supplementation of the elderly may have little effect on immune competence. However, this is an important problem that might well be addressed again with better attention given to the immune tests used and appropriate controls. It is well documented that T-helper function and production of IL-2 decline with age. This might be exacerbated by mild zinc deficiency and might also be quite sensitive to the beneficial effects of supplementation. There has been virtually no examination of the capacity of monocytes and neutrophils to carry out phagocytosis and microbicidal killing in the elderly. These cells, the first line of immune defense, may be impaired by zinc deficiency (*398*). Vaccinations of the elderly and subsequent analysis of antibody titers (e.g. influenza) would be an excellent way of comparing host defense capacity in zinc-supplemented and unsupplemented groups.

CHARACTERIZATION OF IMMUNE EFFECTS FROM ZINC INHALATION. Purposeful, oral supplementation of various subjects, including the elderly, with levels of zinc significantly in excess of the RDA have provided no substantive evidence of immune toxicity. However, the repeated observation that the lungs of welders exposed to metal fume fever are infiltrated with PMNs provides evidence that exposure to high levels of zinc in the air may have possible adverse immune effects. Additional experimentation in this area would be of value providing the following caveats are considered.

Human Studies. a) Experiments should closely mimic the amounts and extent to which workers are exposed to zinc oxide and use well controlled conditions and appropriate inhalation chambers. Obviously smokers, asthmatics and other individuals with respiratory diseases should be eliminated from such studies.

b) Future studies should take greater care to examine the duration of the changes in immune parameters of lungs after exposure to zinc inhalation. It is undoubtedly important to monitor pulmonary immunological changes for at least several days after signs of metal fume fever have subsided, to determine if effects are transient or potentially long lasting.

c) Examination of lung fluids, post inhalation of zinc should include: 1) total and differential counts of all major leukocytic cell groups; 2) flow cytometric quantitation of changes in the phenotypic distribution of subsets of the major classes of leukocytes if the proportions are markedly changed; 3) test of lung fluids for proteins and other molecules indicative of initiation of fever, inflammation, etc. (e.g., IL-1, TNF, immune complexes, complement factors, leukotrienes, prostaglandins); 4) the capacity of PMNs and macrophages prepared from such lungs to carry out phagocytosis and/or initiate an oxygen burst should also be determined since these functions are vital to protection of the lung. It is not enough to simply determine the number of

PMNs, or macrophages present in the lung. Rather it must be ascertained whether they can still carry out vital functions.

d) Whether or not repeat exposure to inhalation of zinc oxide increases or decreases the sensitivity of the lung is also important to ascertain. For example, if sensitivity increases markedly, the effect of sequential exposure on pulmonary immune function could prove to be far more serious than results from a single exposure would suggest.

Animal Studies. Inhalation studies should be conducted in several animal species until definitive assessment is made of the most appropriate model for mimicking the human response to zinc inhalation. Because the mouse has served as a highly reliable immunological model for humans, it should serve well for predicting immunological effects of excess exposure to oral intake of zinc. The optimum experimental facsimile for oral intake would seem to be to provide excess zinc to mice via their water supply rather than adding it to their diet, in order to mimic the most likely source in man of increased zinc from environmental contamination.

To ascertain the effects of excess intake of zinc on immune function, care must be taken to measure host defense response *in vivo*. This approach will avoid the artificiality of the *in vitro* or tissue culture environment that contains normal levels of zinc, metabolites, hormones, etc., and which can facilitate repair of defective functions. For this purpose mice provided normal and excess amounts of zinc should be periodically challenged by *in vivo* immunization with T-cell dependent and independent antigens using a standard Jerne plaque assay to evaluate the proportion of splenocytes responding in the two treatment groups. In this manner, actual differences in antibody-mediated response capacity can be readily detected. In addition, mice should be challenged with subacute levels of live pathogens to determine if immune defense systems are indeed intact.

To ascertain the status of cell-mediated responses, mice should also be challenged *in vivo* with small numbers of solid tumor cells and evaluated for their ability to eliminate the tumor. Balb/c mice inoculated with myeloma cells are especially useful for such studies. Finally, since leukopenia and altered distribution of leukocytes accompany many changes in nutritional status, the peripheral blood, spleen, thymus lymph nodes, and bone marrow should be evaluated for the absolute numbers of the major classes of leukocytes. The proportion of the major classes and subclasses of leukocytes should be determined by flow cytometry. These evaluations would provide a solid preliminary determination of whether or not excess intake of zinc significantly altered antibody or cell-mediated responses, key to host defense.

Reproduction and Teratology. Multigenerational studies to assess possible effects of zinc on fertility and reproduction should be carried out in a rodent model. These studies should emphasize analysis of doses ranging from mild deficiency to moderate excess. The highest priority is determination of effects of doses above the RDA, since effects of deficiency are better studied. Zinc should be administered in the diet or drinking water but only at levels that do not alter food or water intake. The protocol for these studies should follow EPA guidelines. End points should include, as a minimum, number of conceptions, percent live births, teratologic assessment, and birth and growth weights of pups. Studies should include analysis of both male and female fertility. Attention should be directed to potential effects secondary to induced deficiency of other essential metals, such as the reduction in litter size and increased neonatal mortality associated with copper deficiency (*469*).

Neurological Function. ANIMAL MODELS. Further research is important to characterize the role of zinc in neurological function, especially development and function of the brain. Mechanistic studies of the mediators of release of zinc from nerve terminals and receptor modulation are critical to understanding the normal role of endogenous zinc. Of higher priority with respect to effects of zinc excess are *in vivo* experiments in rodent models. These studies should entail dose-response analyses of offspring from pregnant rats fed increased levels of zinc before, through and after gestation. Offspring should be investigated for zinc concentrations in brain regions, histological assessment of neural architecture and myelination, and long-term behavioral effects. Because of the known effects of copper deficiency on neural development, especially reduced myelination (*470*), copper status in offspring should be assessed as well. Studies of adult rats fed excess zinc are of lesser priority because the literature suggests that behavioral effects are not likely to be observed.

STUDIES OF ZINC NUTRITURE AND COGNITION IN HUMANS. *Premenopausal Women.* Premenopausal women are at risk of zinc deficiency from food selection and menstrual loss. A pilot, randomized controlled trial in women with low serum ferritin and lower serum zinc than women with normal ferritin found that zinc repletion was associated with improved short-term recall of visual design compared to baseline, while control women showed no improvement. Findings from this pilot study are consistent with experimental findings in zinc depleted men and in experimental animals. Studies are needed to validate and expand on these pilot observations. Three groups should be included in this study: women with documented zinc deficiency, women with adequate zinc status, and women supplementing with zinc at 50 mg or more daily. In each group assessments of cognitive function should be carried out before and after treatment with zinc, 50 mg/day orally.

Offspring of Women Supplemented with Zinc during Pregnancy. The long-term effects of zinc status during gestation on neurological function in children is not known. Animal studies clearly indicate that zinc deficiency during pregnancy adversely affects fetal development and cognitive function of offspring. Studies in women indicate fetal growth failure and increased incidence of complications of pregnancy. A two-center study, which examined the relation between the nutritional status of low-income teenagers and pregnancy outcome, included a randomized controlled trial of zinc (30 mg/day) from the middle to end of pregnancy. Zinc reduced the incidence of pregnancy complications. The maternal records from this study are available. Follow-up studies of the offspring offer a pilot approach for determining the effect of zinc supplementation during pregnancy on long-term cognitive function in offspring. Critical data on children would include estimates of nutritional status after birth and indices of cognition that test short and long-term memory, problem-solving and motor skills.

Serum Lipoproteins and Cardiovascular Effects. The dose-response relationship between zinc intake and serum lipoproteins should be further explored. Primary emphasis should be on a) definitive determination of the effect of increased oral intake of zinc on lipoprotein particles of significance to atherosclerosis, b) where changes are found, mechanisms should be evaluated by studies of clearance and synthesis of the particles and the major relevant apoproteins, c) the effects of zinc on serum lipoproteins and induction rates of atherosclerosis in animal models of hyperc-

holesterolemia such as the Watanabe rabbit. Careful evaluation of the role of zinc-induced changes in copper status should be included in these studies because of the documented relationship between mild copper deficiency and hypercholesterolemia.

There is no evidence that excess zinc impairs cardiovascular function. However, copper deficiency has been shown to produce cardiac arrythmias, impair ATP formation in the heart and increase aortic fragility secondary to reduced lysyl oxidase activity (*471*). Studies of the effect of excess zinc intake on these parameters should be explored in relation to copper intake.

Hematologic Effects: Sideroblastic Anemia. Excess intake of zinc has been shown in humans to induce sideroblastic anemia. In this condition ferric iron accumulates in mitochondria of erythrocytes. The mechanism of this effect is unknown. One hypothesis is that the symptoms result from zinc-induced copper deficiency. The pathogenesis of this effect should be studied, which may facilitate understanding of mechanisms of sideroblastic anemias and possibly management strategies, both of which are lacking at present.

Mechanistic Studies

FACTORS INFLUENCING ZINC STATUS. ***Regulation of Absorption and Excretion.*** Research should be directed to enhance understanding of the molecular mechanisms of zinc absorption and excretion and their homeostatic regulation under conditions of mild deficiency and moderate excess. The transport process of zinc through the brush border membrane of gut mucosal cells and its interaction with intracellular ligands that influence the extent of absorption needs better definition. Pancreaticobiliary secretion is known to be a major excretory route of zinc. Research is needed to improve understanding of the regulation of these secretory pathways, the form of zinc in these fluids e.g., "packaging," the extent of intestinal reabsorption from this source and the of altered secretion by this route in homeostatic regulation.

CLONING OF GENES THAT INFLUENCE ZINC TRANSPORT. Study of the molecular mechanisms of the known genetic disorders which affect zinc status should provide critical knowledge about zinc transport under normal conditions. These disorders include acrodermatitis enteropathica in humans, characterized by impaired zinc absorption, and lethal milk syndrome in mice, characterized by decreased zinc transport into milk. Research should be directed to determination of the altered genes, cloning of these genes and identification of the normal gene products.

Functional Significance of Zinc-induced Metallothionein Expression. Further research should be supported to determine the significance of zinc-induced expression of metallothionein isozymes, especially in relation to zinc disposition, hydroxy radical scavenger activity and metal donor function for metalloenzyme activation.

Zinc Status and the Function of Ezymes and DNA-binding Proteins. The molecular mechanism for the essential role of zinc in biological processes has been facilitated by basic studies on the interaction of zinc with enzymes and more recently with DNA-binding proteins. Further work in this area is likely to be highly fruitful. Studies should be supported to determine dose-response relationships between zinc status and function at the molecular level, such as the activity of critical regulatory transcription activators.

Mechanism of Zinc-induced Copper Deficiency: Regulation of Ceruloplasmin Expression. The molecular mechanisms underlying the interactions of zinc with other trace elements requires better definition. As indicated above, the inverse relationship between zinc and copper levels in the body appears significant to understanding the health effects of zinc status. Studies should be supported to determine the basis for the effect of zinc on copper status. The competition between these metals demonstrated in intestinal mucosal cells is likely to explain the reciprocal relationship between zinc status and copper bioavailability. Other interaction sites should be determined. For example, ceruloplasmin, the major copper-binding protein in plasma which is synthesized in the liver, also exhibits an inverse relationship to zinc status. Factors which decrease plasma zinc such as stress and acute infection, mediated in part by IL–1, also increase plasma ceruloplasmin. Studies should be carried out to determine whether zinc is a negative or positive regulator of the hepatic synthesis of this protein.

REFERENCES

1. Henkin RI, Patten BM, Re PK, Bronzert DA. A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. Arch Neurol 32:745–751(1975).
2. Prasad AS. Discovery of human zinc deficiency and studies in an experimental human model. Am J Clin Nutr 53:403–412(1991).
3. Prasad AS, Miale A Jr. Farid Z, Sandstead HH, Schulert AR. Zinc metabolism in patients with syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. J Lab Clin Med 61:537–549(1963).
4. Prasad AS. Clinical spectrum and diagnostic aspects of human zinc deficiency. In: Essential and toxic trace elements in human health and disease (Prasad AS, ed.). New York:Alan R. Liss, 1988;3–53.
5. Henkin RI, Aamodt RL. A redefinition of zinc deficiency. In: Nutritional bioavailability of zinc, chap. 6 (Inglett GE, ed.). Washington:American Chemical Society, 1983;83–105.
6. Henkin RI, Schechter PH, Friedewald WT, Demets DL, Raff M. A double blind study of the effects of zinc sulfate on taste and small bowel dysfunction. Am J Med Sci 272:285–299(1976).
7. Penland JG. Cognitive performance effects of low zinc (Zn) intakes in healthy adult men. FASEB J 5:A938(1991).
8. Milne DB, Johnson PE, Gallagher SK. Effect of short-term dietary zinc intake on ethanol metabolism in adult men. Am J Clin Nutr 53:25(1991).
9. NAS/NRC. Drinking water and health. The contribution of drinking water to mineral nutriton in humans. Washington:National Academy of Sciences, 1980;265–403.
10. Faintuch J, Faintuch JJ, Toledo M, Nazario G, Machado MCC, Raia AA. Hyperamylasemia associated with zinc overdose during parenteral nutrition. J Parenter Enteral Nutr 2:640–645(1978).
11. Hooper PL, Visconti L, Garry PJ, Johnson GE. Zinc lowers high density-lipoprotein-cholesterol levels. JAMA 244:1960–1961(1980).
12. Chandra RK. Excessive intake of zinc impairs immune response. JAMA 252:1443–1446(1984).
13. Simon SR, Brande RF, Tindle BH, Burns, SL. Copper deficiency and sideroblastic anemia associated with zinc ingestion. Am J Hematol 28:181–183(1988).
14. Hoffman HN, Phylisky RL, Fleming CR. Zinc induced copper deficiency. Gastroenterology 94:508–512(1988).
15. Prasad AS, Brewer GJ, Schoomaker E, Rabbani P. Hypocupremia induced by zinc therapy in adults. JAMA 240:2166–2168(1978).
16. Solomons NW. The iron:zinc interaction in the human intestine. Does it exist? An affirmative view. In: Essential and toxic trace elements in human health and disease (Prasad AS, ed). New York:Alan R. Liss, 1988;509–518 .

17. Food and Nutrition Board Recommended Dietary Allowances. Washington:National Academy of Sciences, 10th ed, 1989;205–213,284.
18. Pennington J, Wilson DB, Newell RF, Harland BF, Johnson RD, Vanderveen JE. Selected minerals in food surveys, 1874 to 1981–82. J Am Diet Assoc 84:771–780(1984).
19. MacDonald LD, Gibson RS, Miles JE. Changes in hair zinc and copper concentration of breast fed and bottle fed infants during the first six months. Acta Pediatr Scand 71:785–789(1982).
20. Hambidge KM, Chavez MN. Brown RM, Walravens PA. Zinc nutritional status of young middle-income children and effects of consuming zinc-fortified breakfast cereal. Am J Clin Nutr 37:2532–2539(1979).
21. Walravens PA, Krebs NF, Hambidge KM. Linear growth of low income preschool children receiving a zinc supplement. Am J Clin Nutr 38:195–201(1983).
22. Schlage C, Wortberg B. Zinc in the diet of healthy preschool and school children. Acta Pediatr Scand 61:421–425(1972).
23. White HS. Inorganic elements in weighed diets of girls and young women. J Am Diet Assoc 55:38–43(1969).
24. Holden JM,Wolf WR, Mertz W. Zinc and copper in self-selected diets. J Am Diet Assoc 75:23–28(1979).
25. Gibson RS, Scythes CA. Trace element intakes of women. Brit J Nutr 48:241–248(1982).
26. King JC, Stein T, Doyle M. Effect of vegetarianism on the zinc status of pregnant women. Am J Clin Nutr 34:1049–1055(1981).
27. Milne DB, Schnakenberg DD, Johnson HL, Kuhl GL. Trace element mineral intake of enlisted military personnel. J Am Diet Assoc 76:41–45(1980).
28. Thomas AJ, Bunker VW, Hinks LJ, Sodha N, Mullee MA, Clayton BE. Energy, protein zinc and copper status of twenty-one elderly in patients: analyzed dietary intake and biochemical indices. Brit J Nutr 59:181–194(1988).
29. Bunker VW, Hinks LJ, Lawson MS, Clayton BE. Assessment of zinc and copper status of healthy elderly people using metabolic balance studies and measurement of leukocyte concentrations. Am J Clin Nutr 40:1096–1102(1984).
30. Bindra GS, Gibson RS, Thompson LU. [Phytate] [calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr Res 6:475–483(1986).
31. Sandstead HH. Availability of zinc and its requirement in human subjects. In: Clinical, biochemical and nutritional aspects of trace elements (Prasad AS, ed). New York:Alan R. Liss, 1982;83–101.
32. Welsh SO, Marston RM. Zinc levels of the US food supply 1909–1980. Food Technol 36:70–76(1982).
33. Welsh SO, Marston RM. Trends in levels of zinc in the United States food supply, 1909–1981. In: Bioavailability of zinc (Inglett GE, ed). ACS Symposium Series 210, Washington:American Chemical Society, 1983;15.
34. Pekarinen M. World food consumption patterns. In: Man, food, and nutrition (Rechcigl M, ed). Boca Raton, FL:CRC Press, 1973;15–34.
35. United States Department of Agriculture Nutrition Monitoring in the United States. A progress report from the Joint Nutrition Monitoring Evaluation Committee. Hyattsville, MD:United States Department of Agriculture, 1986, DHHS publ no 1255 .
36. Anderson BM, Gibson RS, Sabry JH. The iron and zinc status of long-term vegetarian women. Am J Clin Nutr 34:1042–1048 (1981).
37. O'Dell BL, Savage JE. Effect of phytic acid on zinc availability. Proc Soc Exp Biol Med 103:304–306(1960).
38. Oberleas D, Muhrer ME, O'Dell BL. Effects of phytic acid on zinc availability and parakeratosis in swine. J Anim Sci 21:57–61(1962).
39. Reinhold JG, Nasr K, Lahimgarzadeh A, Nasr K, Hedayati H. Effects of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus, and nitrogen in man. Lancet (1):283–88(1973).
40. Reinhold JG, Faradji B, Abadi P. Ismail-Beigi F. Decreased absorption of calcium, magnesium, zinc and phophorus by humans due to increased fiber and phophorus consumption as wheat bread. J Nutr 106:493–503 (1976).
41. Turnland JR, King JC, Keyes WR, Gong B, Michel MC. A stable isotope study of zinc absorption in young men: effects of phytate and alph-cellulose. Am J Clin Nutr 40:1071–1077(1984).
42. Sandström B, Almgren A, Kivisto B, Cederblad A. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale, and whole wheat. J Nutr 117:1898–1902(1987).
43. Mills CF. Dietary interactions involving trace elements. Ann Rev Nutr 5:173–193(1985).
44. Solomons NW, Jacob RA, Pineda O, Viteri FE. Studies on the bioavailability of zinc in man. II Absorption of zinc from organic and inorganic sources. J Lab Clin Med 94:335–343(1979).
45. Harzer G, Kauer H. Binding of zinc to casein. Am J Clin Nutr 35:981–990(1982).
46. Lykken GI, Mahalko J, Johnson PE, Milne D, Sandstead HH, Garcia WJ, Dintzes FR, Inglett GE. Effect of browned and unbrowned corn products intrinsically labeled with zinc on absorption of zinc in humans. J Nutr 116:795–801(1986).
47. Reinhold JG, Faradji B, Abadi P, Ismail-Beigi F. Binding of zinc to fiber and other solids of wholemeal bread. In: Trace elements in human health and disease (Prasad AS, ed). New York:Academic Press, 1976;163–180.
48. Pecoud A, Donzel P, Schelling JL. Effect of foodstuffs on the absorption of zinc sulfate. Clin Pharmacol Ther 17:469–473(1975).
49. Sandstead HH, Dintzis FR , Bogyo T, Milne DB, Jacob RA, Klevay LM. Dietary factors that can impair calcium and zinc nutriture of the elderly. In: Nutrition and aging (Princeley DM, Sandstead HH, eds). New York:Alan R. Liss, 1990;241–262.
50. Prasad AS, Schulert AR, Miale AJ, Farid Z, Sandstead HH. Zinc and iron deficiencies in male subjects with dwarfism but without ancylostomiasis, schistosomiasis, or severe anemia. Am J Clin Nutr 12:437–444(1963).
51. Sandstead HH, Prasad AS, Schulert AR, Farid Z, Miale A Jr, Basilly S, Darby WJ. Human zinc deficiency, endocrine manifestations, and response to treatment. Am J Clin Nutr 20:422–442(1967).
52. Halsted JA, Ronaghy HA, Abadi P, Haghshenass M, Amerhakemi GH. Zinc deficiency in man: the Shiraz experiment. Am J Med 53:277–284(1972).
53. Ferguson E, Gibson R, Thompson L, Ounpuu S. Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. Am J Clin Nutr 50:1450–1456(1989).
54. Hambidge KM, Hambidge C, Jacobs M, Baum JD. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatn Res 6:868–874(1972).
55. Gibson RS, Smit Vanderkooy PD, MacDonald AC, Goldman A. A growth-limiting, mild zinc-deficiency syndrome in some Southern Ontario boys with low height percentiles. Am J Clin Nutr 49:1266–1273(1989).
56. Ruz M, Cavan KR, Bettger WJ, Thompson L, Berry M, Gibson RS. Development of a dietary model for the study of mild zinc deficiency in humans and evaluation of some biochemical and functional indices of zinc status. Am J Clin Nutr 53(5):1295–1303(1991).
57. Wood R, Hanssen D. Effect of milk and lactose on zinc absorption in lactose-intolerant postmenopausal women. J Nutr 118:982–986 (1987).
58. Flanagan P, Cluett J, Chamberlain M, Valberg, L. Dual-isotope method for determination of human zinc absorption: the use of a test meal of turkey meat. J Nutr 115:111–122(1985).
59 Oelshlegal F, Brewer G. Absorption of pharmacological doses of zinc. In: Zinc metabolism: current aspects in health and disease (Prasad A, Brewer G, eds). New York: Alan R. Liss, 1977;299–311.
60. (Abernathy CO. CantilliRH, eds). Zinc: an environmental and health effects assessment. Washington:United States Environmental Protection Agency, Health Effects Branch, 1984.
61. Fox MRS. Zinc excess. In: Zinc in human biology (Mills CF, ed). New York:Springer-Verlag,1989;365–370.
62. NRC. Recommended dietary allowances. 10th ed. Washington:National Academy of Sciences, 1989.
63. Sandstead HH. Zinc nutrition in the United States. Am J Clin Nutr 26:1251–1260(1973).
64. Sandstead HH. Nutritional role of zinc and effects of deficiency. In: Adolescent nutrition (Winicle M, ed). New York:John Wlley, 1982;97–924.

65. Hambidge KM, Casey CE, Krebs NF. Zinc. In: Trace elements in human and animal nutrition (Mertz W, ed). Orlando, FL: Academic Press, 1986;1–137.
66. Feeley RM, Eitenmiller RR, Jones JB, Barnhart H. Copper, iron and zinc contents of human milk at early stages of lactation. Am J Clin Nutr 37:443–448(1983).
67. Moore MEC, Moran JR, Greene HL. Zinc supplementation in lactating women — evidence for mammary control of zinc secretion. J Pediatr 105:660–602(1984).
68. Walravens P, Hambidge K. Growth of infants fed a zinc supplemented formula. Am J Clin Nutr 29:1114–1121 (1976).
69. Lönnerdal B, Cederblad Å, Davidsson L, Sandström B. The effect of individual components of soy formula and cow's milk formula on zinc bioavailability. Am J Clin Nutr 40:1064–1070(1984).
70. Anonymous. Zinc. In: Trace element requirements. Technical Report, vol 532. Geneva:World Heath Organization, 1973;9–16.
71. Higashi A, Ikede T, Iribe K, Matsude I. Zinc balance in premature infants given the minimal dietary zinc requirement. J Pediatr 112:262–270(1988).
72. Shulman RJ. Zinc and copper balance studies in infants receiving total parenteral nutrition. Am J Clin Nutr 49:879–883(1989).
73. Scoular FL. A quantitative study, by means of spectrographic analysis, of zinc in nutrition. J Nutr 17:103–113(1939).
74. Gibson, R. Probability approach to evaluating nutrient data. In: Principles of nutritional assessment, 1st ed, New York:Oxford University Press, 1990;148–152.
75. Prasad AS. Metabolism of zinc and its deficiency in human subjects. In: Zinc metabolism (Prasad AS, ed). Springfield, Illinois:Charles C. Thomas, 1966;250–303.
76. Coble YD, Schulert AR, Farid Z. Growth and sexual development of male subjects in an Egyptian oasis. Am J Clin Nutr 18:421–425(1966).
77. Ronaghy HA, Reinhold JG, Mahloudji M, Ghavami P, Fox MRS, Halsted JA. Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. Am J Clin Nutr 27:112–121(1974).
78. Cherry FF, Sandstead HH, Rojas P, Johnson LK, Batson HK, Wang XB. Adolescent pregnancy: associations among body weight, zinc nutriture, and pregnancy outcome. Am J Clin Nutr 50:945–954(1989).
79. Sandstead HH. Are estimates of trace element requirements meeting the needs of the user? In: Trace elements in man and animals, vol. 5 (Mills CF, Bremner I, Chesters JK , eds). Farnham Royal, Commonwealth Agricultural Bureaux, 1985;875–878.
80. Sandstead HH, Henrickson L, Greger J, Prasad AS, Good R. Zinc nutriture in elderly in relation to taste acuity, immune response and wound healing. Am J Clin Nutr 36:1046–1059(1985).
81. Turnland J, Durkin N, Costa F, Margin S. Stable isotope studies of zinc absorption and retention in young and elderly men. J Nutr 116:1239–1247(1986).
82. Bunker VW, Hinks LJ, Stansfield MF, Lawson MS, Clayton BE. Metabolic balance studies for zinc and copper in housebound elderly people and the relationship between zinc balance and leukocyte zinc concentrations. Am J Clin Nutr 46:353–359(1987).
83. Hambidge KM, Walravens PA, White S, Anthony ML, Roth ML. Zinc nutrition of preschool children in the Denver Head Start Program. Am J Clin Nutr 29:734–738(1976).
84. Price NO, Bunce GE, Engel RW. Copper, manganese and zinc balance in preadolescent girls. Am J Clin Nutr 23:258–260(1970).
85. Greger JL. Dietary intake and nutritional status in regard to zinc of institutionalized aged. J Gerontol 32:549–553(1977).
86. Prasad AS, Meftah S, Abdallah J, Kaplan, J, Brewer GJ, Bach JF, Dardenne M. Serum thymulin in human zinc deficiency. J Clin Invest 82:1202–1210 (1988).
87. Meftah S, Prasad AS. Nucleotides in lymphocytes of human subjects with zinc deficiency. J Lab Clin Med 114:114–119(1989).
88. Prasad AS, Schulert AR, Sandstead HH, Miale A Jr, and Farid Z. Zinc, iron, and nitrogen content of sweat in normal and deficient subjects. J Lab Clin Med 62:84–89(1963).
89. Sandstead HH, Vo-Khactu KP, Solomons N. Conditioned zinc deficiency. In: Trace elements in human health and disease, part I (Prasad AS, ed). New York:Academic Press, 1976;33–49.
90. Lide DR, ed. CRC handbook of chemistry and physics. Boca Raton, FL:CRC Press, 1990:4/116–4/117.
91. Vallee BL, Auld DS. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 29:5647–5659(1990).
92. Bremner I, Beattie JH. Metallothionein and the trace minerals. Annu Rev Nutr 10:63–83(1990).
93. Thornalley PJ, Vasak M. Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanisms of its reaction with superoxide and hydroxyl radicals. Biochim Biophys Acta 827:36–44(1985).
94. Vallee BL Zinc: biochemistry, physiology, toxicology and clinical pathology. Biofactors 1:31–36(1988).
95. Christianson DW, Alexander RS. Carboxylate-histidine-zinc interactions in protein structure and function. J Am Chem Soc 111:6412–6419(1989).
96. Wu FY-H, Wu C-W. Zinc in DNA replication and transcription. Annu Rev Nutr 7:251–272 1987).
97. Evans RM, Hollenberg SM. Zinc fingers: gailt by association. Cell 52:1–3(1988).
98. South TL, Summers MF. Zinc fingers. Adv Inorg Biochem 8:199–248(1990).
99. Berg JW. Zinc finger domains: hypotheses and current knowledge. Annu Rev Biophys Biophys Chem19:405–421(1990).
100. Pavletich NP, Pabo C. Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 Å. Science 252:809–817(1991).
101. Cunningham BC, Bass S, Fuh G, Wells JA. Zinc mediation of the binding of human growth hormone to the human prolactin receptor. Science 250:1709–1712(1990).
102. Celentano JJ, Gyenes M, Gibbs TT, Farb DH. Negative modulation of the γ-aminobutyric acid response by extracellular zinc. Mol Pharmacol 40:766–773(1991).
103. Westbrook GL, Mayer ML. Micromolar concentrations of Zn2+ antagonize NMDA and GABA responses of hippocampal neurons. Nature 328:640–643(1987).
104. Xie X, Smart TG. A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. Nature 349:521–524(1991).
105. Murakami K, Whiteley MK, Routtenberg A. Regulation of protein kinase C activity by cooperative interaction of Zn^{2+} and Ca^{2+}. J Biol Chem 262:13902–13906(1987).
106. Speizer LA, Watson MJ, Kanter JR, Brunton LL. Inhibition of phorbol ester binding and protein kinase C activity by heavy metals. J Biol Chem 264:5581–5585(1989).
107. Forbes IJ, Zalewski PD, Giannakis C. Role for zinc in a cellular response mediated by protein kinase C in human B lymphocytes. Exp Cell Res 195:224–229(1991).
108. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 8:281–291(1990).
109. Bettger WJ, O'Dell BL. Minireview: A critical physiological role of zinc in the structure and function of biomembranes. Life Sci 28:1425–1438(1981).
110. Frederickson C. Neurobiology of zinc and zinc-containing neurons. In: International review of neurobiology, vol 31 (Smythies J, Bradley R, eds). New York:Academic Press,1989;145–238 .
111. Keen CL, Hurley LS. Zinc absorption through skin: correction of zinc deficiency in the rat. Am J Clin Nutr 30:528–530(1977).
112. Hallmans G. Treatment of burns with zinc-tape. Scand J Plast Reconstr Surg 11:155–161(1977).
113. Skog E, Wahlberg JE. A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110}mAg, ^{115}mCd, ^{203}Hg. J Invest Dermatol 43:187–192(1964).
114. Kapur SP, Bhussry SR, Harmuth–Hoene E. Percutaneous uptake of zinc in rabbit skin. Proc Soc Exp Biol Med 145:932–937(1974).
115. Menard MP, Cousins RJ. Zinc transport by brush border membrane vesicles for rat intestine. J Nutr 113:1434–1442(1983).
116. Lee HH, Prasad AS, Brewer GJ, Owyang C. Zinc absorption in human small intestine. Am J Physiol 256:G87–G91(1989).
117. Wapnir RA, Khani DE, Bayne MA, Lifshitz F. Absorption of zinc by the rat ileum: effects of histidine and other low-molecular ligands. J Nutr 113:1346–1354(1983).
118. Cunnane SC. Zinc: clinical and biochemical significance. Boca

Raton, FL:CRC Press,1988;69–78 .
119. Evans GW, Johnson EC. Zinc concentration of liver and kidneys from rat pups nursing dams fed supplemental zinc dipicolinate or zinc acetate. J Nutr 110:2121–2124 (1980).
120. Hurley LS, Lonnerdal B. Zinc binding in human milk: citrate versus picolinate. Nutr Rev 40:65–71(1982).
121. Johnson WT, Evans GW. Tissue uptake of zinc in rats following the administration of zinc dipicolinate or zinc histidinate. J Nutr 112:914–919(1982).
122. Casey CE, Walravens PA, Hambidge KM. Availability of zinc in loading tests with human milk, cow's milk and infant formulae. Pediatrics 68:394–396(1981).
123. Eckhert CD. Isolation of a protein from human milk that enhances zinc absorption in humans. Biochem Biophys Res Commun 130:264–269(1985).
124. Eckhert CD, Sloan MV, Duncan JR, Hurley LS. Zinc binding: a difference between human and bovine milk. Science 195:789–790(1977).
125. Song MK, Adham NF. Relationship between zinc and prostaglandin metabolism in plasma and small intestine of rats. Am J Clin Nutr 41:1201–1209(1985).
126. Cash R, Berger CK. Acrodermatitis enteropathica: defective metabolism of unsaturated fatty acids. J Pediatr 74:717–729(1969).
127. Atalay Y, Arcasoy A, Kurkcuoglu M. Oral plasma zinc tolerance test in patients with protein energy malnutrition. Arch Dis Child 64:1608–1611(1989).
128. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency, anemia, hepatosplenomegaly, dwarfism, hypogonadism and geophagia. Am J Med 31:532–546(1961).
129. Latta D, Liebman M. Iron and zinc status of vegetarian and nonvegetarian males. Nutr Rep Int 30:141–149(1984).
130. Swanson CA, Turnlund JR, King JC. Effect of dietary sources and pregnancy on zinc utilization in adult women fed controlled diets. J Nutr 113:2557–2567(1983).
131. Antonson DL, Vanderhoff A. Effect of chronic ethanol ingestion on zinc absorption in rat small intestine. Dig Dis Sci 28:604–608(1983).
132. Silverman B, Kwaitkowski D, Pinto J, Rivlin R. Disturbances in zinc binding to jejunal proteins induced by ethanol ingestion in rats. Clin Res 27:555A(1979).
133. Sullivan JF, Jetton MM, Burch RE. A zinc tolerance test. J Lab Clin Med 93:485–492(1979).
134. Sullivan JF, Williams RV, Burch RE. Metabolism of zinc and selenium in cirrhotic patients during 6 weeks of zinc ingestion. Alcoholism 3:235–239(1979).
135. Valberg LS, Flanagan PR, Brennan J, Chamberlain MJ. Does the oral zinc tolerance test measure zinc absorption? Am J Clin Nutr 41:37–42(1985).
136. Dinsmore WW, Callender ME, McMaster D, Love AHG. The absorption of zinc from a standardized meal in alcoholics and in normal volunteers. Am J Clin Nutr 42:688–693(1985).
137. Karayalcin S, Arcasoy A, Uzunalimoglu O. Zinc plasma levels after oral zinc tolerance test in nonalcoholic cirrhosis. Digest Dis Sci 33:1096–1102(1988).
138. Spencer H, Rosoff B, Lewin I, Samachson J. Studies of zinc-65 metabolism in man. In: Zinc metabolism (Prasad AS, ed). Springfield, Illinois:Charles C. Thomas, 1966;339–362.
139. Evans GW, Grace CI, Votava HJ. A proposed mechanism for zinc absorption in the rat. Am J Physiol 228:501–505(1975).
140. Flanagan PR, Haist J, Valberg LS. Zinc absorption, intraluminal zinc and intestinal metallothionein in zinc deficient and zinc replete rodents. J Nutr 113:962–972 (1983).
141. Babcock AK, Henkin RI, Aamodt RL, Foster DM, Berman M. Effects of oral zinc loading on zinc metabolism in humans II: *In vivo* kinetics. Metabolism 31:335–347(1980).
142. Cousins RJ. Theoretical and practical aspects of zinc uptake and absorption. Adv Exp Med Biol 249:3–12(1989).
143. Richards MP, Cousins RJ. Metallothionein and its relationship to the metabolism of dietary zinc in rats. J Nutr 106:1591–1599(1976).
144. Richards MP, Cousins RJ. Zinc-binding protein: Relationship to short term changes in zinc metabolism. Proc Soc Exp Biol Med 153:52–56(1976).
145. Tacnet F, Watkins DW, Ripoche P. Studies of zinc transport into brush-border membrane vesicles isolated from pig small intestine. Biochem Biophys Acta 1024:323–330(1990).
146. Turnbull AJ, Blakeborough P, Thompson RPH. The effects of dietary ligands on zinc uptake at the porcine intestinal brush-border membrane. Brit J Nutr 64:733–741(1990).
147. Oestreicher P, Cousins RJ. Zinc uptake by basolateral membrane vesicles from rat small intestine. J Nutr 119:639–646(1989).
148. Whitehouse RC, Prasad AS, Rabbani PI, Cossack ZT. Zinc in plasma, neutrophils, lymphocytes, and erythrocytes as determined by flameless atomic absorption spectrophotometry. Clin Chem 28:475–480(1982).
149. Lindeman RD, Bottomley RG, Cornelison RL Jr, Jacobs LA. Influence of acute tissue injury of zinc metabolism in man. J Lab Clin Med 79:452–460(1972).
150. Wilden EG, Robinson MRG. Plasma zinc levels in prostatic disease. Brit J Urol 47:295–299(1975).
151. Hallbook T, Hedelin H. Zinc metabolism and surgical trauma. Brit J Surg 64:271–273(1977).
152. Prasad AS, Oberleas D. Binding of zinc to amino acids and serum proteins *in vitro*. J Lab Clin Med 76:416–425(1970).
153. Phillips JL. Uptake of transferrin-bound zinc by human lymphocytes. Cell Immunol 35:318–329(1978).
154. Wang H, Prasad AS, DuMouchelle EA. Zinc in platelets, lymphocytes, and granulocytes by flameless atomic absorption spectrophotometry. J Micronutrient Anal 5:181–190(1989).
155. Prasad AS, ed. Zinc. In: Trace elements and iron in human metabolism. New York:Plenum Press, 1976;251–346.
156. Fransson GB, Lonnerdal B. Distribution of trace elements and minerals in human and cow's milk. J Pediatr 17:912–915(1983).
157. Strain WH, Macon WL, Pories WJ, Perim C, Adams FD, Hill OA. Excretion of trace elements in bile. In: Trace element metabolism in animals, vol 2 (Hoekstra WH, Suttie JW, Ganther HE, Mertz W, eds). Baltimore:University Park Press, 1974;644.
158. Schoenfeld C, Amelar RD, Dubin L, Numeroff M. Prolactin, fructose and zinc levels found in human seminal plasma. Fertil Steril 32:206(1979).
159. Agarwal RP, Henkin RI Zinc and copper in human cerebrospinal fluid. Biol Trace Elem Res 4:117–124(1982).
160. Failla ML, Cousins RJ. Zinc accumulation and metabolism in primary cultures of adult rat liver cells. Biochem Biophys Acta 543:293–304(1975).
161. Pattison SE, Cousins RJ. Kinetics of zinc uptake and exchange by primary cultures of rat hepatocytes. Am J Physiol 250:E677–E685(1986).
162. Pattison SE, Cousins RJ. Zinc uptake and metabolism by hepatocytes. Fed Proc 45:2805–2809(1986).
163. Stacey NH, Klaassen CD. Zinc uptake by isolated rat hepatocytes. Biochem Biophys Acta 640:693–697(1981).
164. Newsome DA, Rothman RJ. Zinc uptake *in vitro* by human retinal pigment epithelium. Invest Ophthalmol Vis Sci 28:1795-1799(1987).
165. Horrobin DF, Morgan RD. Myotonic dystrophy: A disease caused by functional zinc deficiency due to an abnormal zinc-binding ligand. Med Hypoth 6:375–384(1980).
166. Masters DG, Keen CL, Lonnerdal B, Hurley LS. Release of zinc from maternal tissues during zinc deficiency or simultaneous zinc and calcium deficiency in the pregnant rat. J Nutr 116:2148–2154(1986).
167. Hambidge KM, Krebs NF, Jacobs MA, Favier A, Guyette L, Ikle DN. Zinc nutritional status during pregnancy: a longitudinal study. Am J Clin Nutr 37:429–442(1983).
168. Spencer H, Osis D, Kramer L, Norris C. Intake, excretion and retention of zinc in man. In: Trace elements in human health and disease (Prasad AS, ed). New York:Academic Press,1976;345–361.
169. Spencer H, Rosoff B, Feldstein A, Cohn SH, Gusmano E. Metabolism of zinc-65 in man. Radiatr Res 24:432–445(1965).
170. Spencer H, Vankinscott V, Lewin I, Samachson J. Zinc-65 metabolism during low and high calcium intake in man. J Nutr 86:169–177(1965).
171. Montgomery ML, Sheline GE, Chaikoff IL. Elimination of administered zinc in pancreatic juice, duodenal juice, and bile of dog as measured by its radioactive isotope. J Exp Med 78:151–159(1943).

172. Davies NT. Studies on the absorption of zinc by rat intestine. Br J Nutr 43:189–203(1980).
173. Mateseche JW, Phillips SF, Malagelada JR, McCall JT. Recovery of dietary iron and zinc from the proximal intestine of healthy man. Studies of different meals and supplements. Am J Clin Nutr 33:1946–1953(1980).
174. Abu–Hamdan DK, Migdal SD, Whitehouse R, Rabbani P, Prasad AS, McDonald FD. Renal handling of zinc: effect of cysteine infusion. Am J Physiol 241:F487–F494(1981).
175. Prasad AS, Rabbani P, Abbasi A, Bowersox E, Spivey Fox MRS. Experimental zinc deficiency in humans. Ann Intern Med 89:483–490(1978).
176. Coppen DE, Davies NT. Studies on the effect of dietary zinc dose on zinc-65 absorption *in vivo* and the effects of zinc status on zinc-65 absorption and body loss in young rats. Br J Nutr 57:35–44(1987).
177. Wada L, Turnlund JR, King JC. Zinc utilization in young men fed adequate and low zinc intakes. J Nutr 115:1345–1354(1985).
178. Guigliano R, Millward DJ. Growth and zinc homeostasis in the severely zinc deficient rat. Br J Nutr 52:545–560(1984).
179. Jackson MJ, Jones DA, Edwards RHT. Tissue zinc level as an index of body zinc status. Clin Physiol 2:333–343(1982).
180. Moses HA, Parker HE. Influence of dietary zinc and age on the mineral content of rat tissues. Fed Proc 23:132(A)(1964).
181. Prasad AS, Oberleas D, Wolf P, Horwitz JP, Miller ER, Luecke RW. Changes in trace elements and enzyme activities in tissues of zinc deficient pigs. Am J Clin Nutr 22:628–637(1969).
182. Petering HG, Johnson MA, Horwitz JP. Studies of zinc metabolism in the rat. Arch Environ Health 23:93–101(1971).
183. Burch RE, Williams RV, Hahn HKJ, Jetton MM, Sullivan JF. Serum and tissue enzyme activity and trace element content in response to zinc deficiency in the pig. Clin Chem 21:568–577(1975).
184. Roth HP, Kirchgessner M, Zum Gehalt von Zink Kupfer Eisen Mangan und Calcium in Knochen and Lebern von an Zink depletierter and repleiterter Ratten. Zbl Vet Med A 24:177–188(1977).
185. Kirchgessner M, Schwarz FJ, Schnegg A. Interactions of essential metals in human physiology. In: Clinical, Biochemical, and Nutritional Aspects of Trace Elements (Prasad AS, ed). New York:Alan R. Liss, 1982;477–512 .
186. Duncan GD, Gray LF, Daniel LJ. Effect of zinc on cytochrome oxidase activity. Proc Soc Exp Biol Med 83:625–627(1953).
187. Van Reen R. Effects of excessive dietary zinc in the rat and the interrelationship with copper. Arch Biochem Biophys 46:337–344(1953).
188. Cox DH, Harris DL. Effect of excess dietary zinc on iron and copper in the rat. J Nutr 70:514–520(1960).
189. L'Abbe MR, Fischer PWF. The effects of dietary zinc on the activity of copper–requiring metalloenzymes in the rat. J Nutr 114:823–828(1984).
190. Yardick MK, Kenney MA, Winterfeld EA. Iron, copper and zinc status: response to supplementation with zinc or zinc and iron in adult females. Am J Clin Nutr 49:145–150(1989).
191. Porter KG, McMaster D, Elmes ME, Love AHG. Anemia and low serum-copper during zinc therapy. Lancet 2:774(1977) (ltr.).
192. Suttie NF, Mills CF. Studies on the toxicity of copper to pigs. 1: Effects of oral supplements to zinc and iron salts at the development of copper toxicosis. Br J Nutr 20:135–148(1966).
193. Suttie NF, Mills CF. Studies on the toxicity of copper to pigs. 2: Effects of protein source and other dietary components on the response to high and moderate intakes of copper. Br J Nutr 20:149–161(1966).
194. Bremner I, Young BW, Mills CF. Protective effect of zinc supplementation against copper toxicosis in sheep. Br J Nutr 36:551–561(1976).
195. Klevay LM. Interactions among dietary copper, zinc and the metabolism of cholesterol and phospholipids. In: Trace elements metabolism in animals, vol 2 (Hoekstra WG, Suttie JW, Ganther HE, Mertz W, eds). Baltimore:University Park Press, 1974;553–556.
196. Schwarz FJ, Kirchgessner M. Experimentelle Untersuchungen zur Interaktion Zwischen den Spurenelementen Zink und Mangan. Z Tierphysiol Tierernahr Futtermittelkde 43:272–282(1980).
197. Evans GW, Grace CI, Han C. The effect of copper and cadmium on ^{65}Zn absorption in zinc-deficient and zinc-supplemented rats. Bioinorg Chem 3:115–120(1974).
198. Schwarz FJ, Kirchgessner M. Intestinale Cu-Absorption *in vitro* nach. Fe-oder Zn-depletion. Z Tierphysiol Tierernahr Futtermittelkde 31:91–98(1973).
199. Schwarz FJ, Kirchgessner M. Absorption von Zink-65 und Kupfer-64 im Zinkmangel. Int J Vit Nutr Res 44:258–266(1974).
200. Schwarz FJ, Kirchgessner M. Wechselwirkungen bei der intestinalen Absorption von ^{64}Cu, ^{65}Zn and ^{59}Fe nach Cu-, Zn- oder Fe-depletion. Int J Vit Nutr Res 44:116–126 (1974).
201. Richards MP, Cousins RJ. Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. Biochem Biophys Res Comm 64:1215–1223(1975).
202. Hall AC, Young BW, Bremner I. Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. J Inorg Biochem 11:57–66(1979).
203. Graham GG, Cordano A. Copper deficiency in human subjects. In: Trace elements in human health and disease, vol I (Prasad AS, ed). New York:Academic Press, 1976;363–372.
204. Dunlap WM, James GW, Hume DM. Anemia and neutropenia caused by copper deficiency. Ann Intern Med 80:470–476(1974).
205. Vilter RW, Bozian RC, Hess EV, Petering HG, Zellner DC. Manifestations of copper deficiency in a patient with systemic sclerosis on intravenous hyperalimentation. N Engl J Med 291:188–191(1974).
206. Brewer GJ, Hill GM, Prasad AS, Cossack ZT, Rabbani P. Oral zinc therapy for Wilson's disease. Ann Intern Med 99:314–320(1983).
207. Hirschman SZ, Isselbacher KJ. The nephrotic syndrome as a complication of penicillamine therapy of hepatolenticular degeneration (Wilson's disease). Ann Intern Med 62:1297–1300(1965).
208. Hoogenraad TU, Van den Hamer CJA, Koevoet R, de Ruylir Korver EGWM. Oral zinc in Wilson's disease. Lancet II:1262(1978).
209. Brewer GJ, Hill GM, Dick RD, Nostrant TT, Sams JS, Wells JJ, Prasad AS. Treatment of Wilson's disease with zinc. III. Prevention of reaccumulation of hepatic copper. J Lab Clin Med 109:526–531(1987).
210. Brewer GJ, Hill GM, Prasad AS, Dick RD. Treatment of Wilson's disease with zinc. IV. Efficacy monitoring using urine and plasma copper. Proc Soc Exp Biol Med 184:446–455(1987).
211. Hill GM, Brewer GJ, Prasad AS, Hydrick CR, Hartmann DE. Treatment of Wilson's disease with zinc. I. Oral zinc therapy regimens. Hepatology 7:522–528(1987).
212. Hill GM, Brewer GJ, Juni JE, Prasad AS, Dick RD. Treatment of Wilson's disease with zinc. II. Validation of oral 64-copper with copper balance. Am J Med Sci 292:344–349(1986).
213. Patterson WK, Winkelmann BA, Perry MC. Zinc-induced copper deficiency:megamineral sideroblastic anemia Ann Intern Med 103:385–386(1985).
214. Samman S, Roberts DCK. The effects of zinc supplements on plasma zinc and copper levels and the reported symptoms in healthy volunteers. Med J Australia 146:246–249(1987).
215. Samman S, Roberts DCK. The effect of zinc supplements on lipoproteins and copper status. Atherosclerosis 70:247–252(1988).
216. Fischer PWF, Giroux A, L'Abbe MR. Effect of zinc supplements on copper status in adult men. Am J Clin Nutr 40:743–746(1984).
217. Sandstead HH. Copper bioavailability and requirements. Am J Clin Nutr 35:809–814(1982).
218. Canada AT, Calabrese EJ. Superoxide dismutase:its role in xenobiotic detoxification. Pharmacol Ther 44:285–295(1989).
219. Hassan HM. Biosynthesis and regulation of superoxide dismutases. Free Radic Biol Med 5:377–385(1988).
220. Werts ED, Gould MN. Relationship between cellular superoxide dismutase and susceptibility to chemically induced cancer in the rat mammary gland. Carcinogenesis 7:1197–1201(1986).
221. Flanagan PR, Valberg LS. The intestinal interaction of zinc and iron in humans: does it occur with food? In: Essential and toxic trace elements in human health and disease (Prasad AS, ed). New York:Alan R Liss, 1988;501–507.
222. Pollack S, George JN, Reba RC, Kaufman RM, Crosby WJ. The absorption of nonferrous metals in iron deficiency. J Clin Invest 44:1470–1473(1965).
223. Forth W, Rummel W. Iron absorption. Physiol Rev 63:724–792(1973).

224. Hamilton DL, Bellamy JEC, Valberg JD, Valberg LS. Zinc, cadmium and interactions during intestinal absorption in iron deficient mice. Can J Physiol Pharmacol 56:384–389(1978).
225. Hill CH, Matrone G. Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. Fed Proc 29:1474–1481(1970).
226. Payton KB, Flanagan PR, Stinson EA, Chrodiker DR, Chamberlain MJ, Valberg LS. Technique for determination of human zinc absorption from measurement of radioactivity in a fecal sample of the body. Gastroenterology 83:1264–1270(1982).
227. Abu-Hamdan DK, Mahajan SK, Migdal SD, Prasad AS, McDonald FD. Zinc absorption in uremia: effects of phosphate binders and iron supplements. J Am Coll Nutr 3:283(1984).
228. Aggett PJ, Crofton RW, Khin C, Gvozdanovic S, Gvozdanovic D. The mutual inhibitory effects on their bioavailability of inorganic zinc and iron. In: Zinc deficiency in human subjects (Prasad AS, Cavder AO, Brewer GJ, Aggett PJ, eds). New York:Alan R Liss,1983;117–124.
229. Sandström B, Davidson L, Cederblad A, Lonnerdal B. Oral iron, dietary ligands and zinc absorption. J Nutr 115:411–414(1985).
230. Solomons NW. Competitive mineral: mineral interactions in the intestine: implications for zinc absorption in humans. In: Nutritional bioavailability of zinc. ACS Symposium Series, Washington:American Chemical Society, 1983;247–271.
231. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in man. Effects of heme and non-heme iron on the absorption of zinc. Am J Clin Nutr 34:475–482(1981).
232. Solomons NW, Pineda O, Viteri F, Sandstead HH. Studies on the bioavailability of zinc in humans: mechanisms of the intestinal interaction of nonheme iron and zinc. J Nutr 113:337–349(1983).
233. Solomons NW, Marchini JS, Duarte-Favaro RM, Vannuchi H, Dutra de Oliveira JE. Studies on the bioavailability of zinc in humans. VI. Intestinal interaction of tin and zinc. Am J Clin Nutr 37:566–571(1983).
234. Valberg LS, Flanagan PR, Chamberlain MJ. Effects of iron, tin, and copper on zinc absorption in humans. Am J Clin Nutr 40:536–541(1984).
235. Flanagan PR, Haist J, MacKenzie I, Valberg LS. Intestinal absorption of zinc: competitive interactions with iron, cobalt, and copper in mice with sex-linked anemia (sla). Can J Physiol Pharmacol 62:1124–1128(1984).
236. Yip R, Reeves JD, Lonnerdal B, Keen CL, Dallman PR. Does iron supplementation compromise zinc nutrition in healthy infants. Am J Clin Nutr 42:683–687(1985).
237. Meadows NJ, Grainger SL, Ruse W, Keeling PWN, Thompson RPH. Oral iron and the bioavailability of zinc. Br Med J 287:1013–1014(1983).
238. Campbell-Brown M, Ward R, Haines, A, North, W, Abraham R, McFadyen I. Zinc and copper in Asian pregnancies— is there evidence for a nutritional deficiency? Br J Obstet Gynaecol 92:875–885(1985).
239. Breskin MW, Worthington-Roberts BS, Knopp RH, Brown Z, Plovie B, Mottet NK, Mills JL. First trimester serum zinc concentration in human pregnancy. Am J Clin Nutr 38:943–953(1983).
240. Ab-Hamdan DK, Mahajan SK, Migdal SD, Prasad AS, McDonald FD. Zinc tolerance test in uremia. Effect of ferrous sulfate and aluminum hydroxide. Ann Intern Med 204:50–52(1986).
241. Crofton RW, Gvozdanovic D, Gvozdanovic S, Khin CC, Brunt PW, Mowat NAG, Aggett PJ. Inorganic zinc and the intestinal absorption of ferrous iron. Am J Clin Nutr 50:141–144(1989).
242. Mahloudji M, Reinhold JG, Haghasenass M, Ronaghy HA, Spivey-Fox MRS, Halsted JA. Combined zinc and iron supplementation of diets of 6- and 12-year-old school children in southern Iran Am J Clin Nutr 28:721–725(1975).
243. Settlemire CT, Matrone G. *In vivo* effect of zinc on iron turnover in rats and life span of the erythrocyte. J Nutr 92:159–164(1967).
244. Settlemire CT, Matrone G. *In vivo* interference of zinc with ferritin iron in the rat. J Nutr 92:153–158(1967).
245. Magee AC, Matrone G. Studies on growth, copper metabolism and iron metabolism on rats fed high levels of zinc. J Nutr 72:233–242(1960).
246. Kang HK, Harvey PW, Valentine JL, Swendseid ME. Zinc, iron, copper and magnesium concentrations in tissues of rats fed various amounts of zinc. Clin Chem 23:1834–1837(1977).
247. Hamilton RP, Fox MRS, Fry BVE Jr, Jones AOL, Jacobs RM. Zinc interference with copper, iron and manganese in young Japanese quail. J Food Sci 44:738–741(1979).
248. Prasad AS, Oberleas D, Wolf P, Horwitz JP. Studies on zinc deficiency: changes in trace elements and enzyme activities in tissues of zinc deficient rats. J Clin Invest 46:549–557(1967).
249. Prasad AS, Oberleas D, Wolf P, Horwitz JP. Effect of growth hormone on nonhypophysectomized zinc deficient rats and zinc on hypophysectomized rats. J Lab Clin Med 63:486–494(1969).
250. Hahn CJ, Evans GW. Absorption of trace metals in the zinc deficient rat. Am J Physiol 228:1020–1023(1975).
251. Chobanian SJ. Accidental ingestion of liquid zinc chloride: local and systemic effects. Ann Emerg Med 10:91–93(1981).
252. Burkhart KK, Kulig KW, Kumack B. Whole-bowel irrigation as treatment for zinc sulfate overdose. Ann Emerg Med 49:1167–1170(1990).
253. Murphy JV. Intoxication following ingestion of elemental zinc. JAMA 212:2119–2120(1970).
254. Maita K, Hirano M, Mitsumori K, Takahashi K, Shirasu Y. Subacute toxicity studies with zinc sulfate in mice and rats. J Pestic Sci 6:327–336(1981).
255. Straube EF, Schuster NH, Sinclair AJ. Zinc toxicity in the ferret. J Comp Pathol 90:355–361(1980).
256. Allen JG, Masters HG, Peet RL, Mullins KR, Lewis RD, Skirrow SZ, Fry J. Zinc toxicity in ruminants. J Comp Pathol 93:363–377(1983).
257. Lee HH, Hill GM, Sikha VKNM, Brewer GJ, Prasad AS, Owyang C. Pancreaticobiliary secretion of zinc and copper in normal persons and patients with Wilson's disease. J Lab Clin Med 116:283–288(1990).
258. Lu J, Combs GF Jr, Fleet JC. Time-course studies of pancreatic exocrine damage induced by excess dietary zinc in the chick. J Nutr 120:389–397(1990).
259. Suzuki CAM, Ohta H, Sinclair AJ. Induction of metallothionein synthesis by zinc in cadmium pretreated rats. Toxicology 63:273–284(1990).
260. Andrews GK, Kage K, Palmiter-Thomas P, Sarras MP Jr. Metal ions induce expression of metallothionein in pancreatic exocrine and endocrine cells. Pancreas 5:548–554(1990).
261. Epand RM, Stafford AR, Tyers M, Nieboer E. Mechanism of action of diabetogenic zinc-chelating agents. Model system studies. Mol Pharmacol 27:366–374(1985).
262. Jhala US, Baly DL. Zinc deficiency results in a post transcriptional impairment in insulin synthesis. FASEB J 5:A941(1991).
263. Huber AM, Gershoff SN. Effect of zinc deficiency in rats on insulin release from the pancreas. J Nutr 103:1739–1744(1973).
264. Zwick D, Frimpong NA, Tulp OL. Progressive zinc-induced changes in glycemic responses in lean and obese LA/N-cp rats. FASEB J 5:A941(1991).
265. Canton MC, Cremin MF. The effect of dietary zinc depletion and repletion on rats: Zn concentration in various tissues and activity of pancreatic gamma-glutamyl hydrolase as indices of Zn status. Br J Nutr 64:201–209(1990).
266. Mills CF, Quarterman J, Williams RB, Dalgarno AC, Panic B. The effects of zinc deficiency on pancreatic carboxypeptidase activity and protein digestion and absorption in the rat. Biochem J 102:712–718(1967).
267. Prasad A.S Clinical, biochemical and pharmacological role of zinc. Annu Rev Pharmacol Toxicol 20:393–426(1979).
268. Kirchgessner M, Roth HP, Wiegand E. In: Trace elements in human health and disease. (Pradad AS, ed). New York:Academic Press,1976;1–20.
269. Tamura T, Shane B, Baer MT, King JC, Margen S, Stokstad ELR. Absorption of mono- and polyglutamyl folates in zinc-depleted man. Am J Clin Nutr 31:1984(1978).
270. Aughey E, Grant L, Furman OL, Dryden WF. The effects of oral zinc supplementation in the mouse. J Comp Pathol 87:1–14(1977).
271. Yuzbasiya-Gurkan V, Brewer GJ, Abrams GD, Main B, Giacherio D. Treatment of Wilson's disease with zinc. V. Changes in serum levels of lipase, amylase, and alkaline phosphatase in patients with Wilson's disease. J Lab Clin Med 114:520–526(1989).

272. Fell BF, King TP, Davies NT. Pancreas atrophy in copper-deficient rats: histochemical and ultrastructural evidence of a selective effect on acinar cells. Histochem J 14:665–680(1982).
273. Lu J, Combs GF Jr. Effect of excess dietary zinc on pancreatic exocrine function in the chick. J Nutr 118:681–689(1988).
274. Kazacos EA, Van Vleet JF. Sequential ultrastructural changes of the pancreas in zinc toxicosis in ducklings. Am J Pathol 134:581–595(1989).
275. Graham TW, Holmberg CA, Keen CL, Thurmond MC, Clegg MS. A pathologic and toxicologic evaluation of veal calves fed large amounts of zinc. Vet Pathol 25:484–491(1988).
276. Scott DA, Fisher AM. Studies on the pancreas and liver of normal and of zinc-fed cats. Am J Physiol 121:253–260(1938).
277. Drinker KR, Thompson PK, Marsh M. An investigation of the effect of long-continued ingestion of zinc, in the form of zinc oxide, by cats and dogs, together with observations upon the excretion and the storage of zinc. Am J Physiol 80:31–64(1927).
278. Otsuki M, Williams JA. Copper stimulates amylase release from isolated rat pancreatic acini independent of Ca++. Gastroenterology 83:84–91(1982).
279. Inoue K, Fried GM, Wiener I. Effect of divalent cations on gastrointestinal hormone release and exocrine pancreatic secretion in dogs. Am J Physiol 248:G28–G34(1985).
280. Sadasivan V. Studies on the biochemistry of zinc. Biochem J 52:452–455(1952).
281. Millan JL. Promoter structure of the human intestinal alkaline phosphatase gene. Nucleic Acids Res 15:10599(1987).
282. Stuart GW, Searle PF, Palmiter RD. Identification of multiple metal regulatory elements in mouse metallothionein–I promoter by assaying synthetic sequences. Nature 317:828–831(1985).
283. Becking GC, Morrison AB. Hepatic drug metabolism in zinc deficient rats. Biochem Pharmacol 19:895–902(1970).
284. Barry M, Keeling PWN, Feely J. Tissue zinc status and drug elimination in patients with chronic liver disease. Clin Sci 78:547–549(1990).
285. Goode HF, Kelleher J, Walker BE. Relation between zinc status and hepatic functional reserve in patients with liver disease. Gut 31:694–697(1990).
286. Kadiiska M, Stoytchev T, Serbinova E. Effect of some heavy metal salts on hepatic monooxygenases after subchronic exposure. Arch Toxicol, Suppl 8:313–315(1985).
287. Cho CH, Chen SM, Ogle CW, Young TK. Effects of zinc and cholesterol/choleate on serum lipoproteins and the liver in rats. Life Sci 44:1929–1936(1989).
288. Chvapil M, Ludwig JC, Sipes IG, Misiorowski RL. Inhibition of NADPH oxidation and related drug oxidation in liver microsomes by zinc. Biochem Pharmacol 25:1787–1791(1976).
289. Jeffery EH. The effect of zinc on NADPH oxidation and monooxygenase activity in rat hepatic microsomes. Mol Pharmacol23:467–473(1983).
290. Lee JSK, Fong LYY. Decreased glutathione transferase activities in zinc–deficient rats. Carcinogenesis 7:1111–1113(1986).
291. Cho CH, Fong LYY. The interaction of ethanol and zinc on hepatic glutathione and glutathione transferase activity in mice. Agents Actions 29:382–385(1990).
292. Seagrave J, Tobey RA, Hildebrand CE. Zinc effects on glutathione metabolism: relationship to zinc-induced protection from alkylating agents. Biochem Pharmacol 32:3017–3021(1983).
293. Wong KL, Klaassen CD. Relationship between liver and kidney levels of glutathione and metallothionein in rats. Toxicol 19:39–47(1981).
294. Hallbook T, Lanner E. Serum-zinc and healing of venous leg ulcers. Lancet: 780–782(1972).
295. Hale WE, May FE, Thomas RG, Moore MT, Stewart RB. Effect of zinc supplementation on the development of cardiovascular disease in the elderly. J Nutr Elderly 8:49–57(1988).
296. Czerwinski AW, Clark M, Serafetinides EA, Perrier C, Huber W. Safety and efficacy of zinc sulfate in geriatric patients. Clin Pharm Ther 15:436–441(1974).
297. Calero M, Sampalo A, Millan JE, Freire J, Senra A, Zamora E. Changes in plasma renin activity and aldosterone induced by progressively increasing zinc sulphate administration in normotensive individuals. Med Clin (Barc.) 92:729–732(1989).
298. Harlan WR. The relationship of blood lead levels to blood pressure in the United States population. Environ Health Perspect 78:9–13(1988).
299. Evans DH, Weingarten K. The effect of cadmium and other metals on vascular smooth muscle of the dogfish shark, Squalus acanthias. Toxicology 61:275–281(1989).
300. Nunez D, Kumar R, Hanahan DJ. Inhibition of [3H]platelet activating factor (PAF) binding by Zn^{2+}: a possible explanation for its specific PAF antiaggregating effects in human platelets. Arch Biochem Biophys 272:466–475(1989).
301. Powell S, Saltman P, Uretzky G, Chevion M. The effect of zinc on reperfusion arrhythmias in the isolated perfused rat heart. Free Radic Biol Med 8:33–46(1990).
302. Ciofalo FR, Thomas LJ. The effects of zinc on contractility, membrane potentials, and cation content of rat atria. J Gen Physiol 48:825–839(1965).
303. Nayler WG, Anderson JE. Effects of zinc on cardiac muscle contraction. Am J Physiol 209:17–21(1965).
304. Chvapil M, Owen JA. Effect of zinc on acute and chronic isoproterenol induced heart injury. J Mol Cell Cardiol 9:151–159 (1977).
305. Singal PK, Dhillon KS, Beamish RE, Dhalla NS. Protective effect of zinc against catecholamine-induced myocardial changes. Lab Invest 44:426–433 (1981).
306. Prasad AS. Zinc in human studies. In: The biomedical role of trace elements in aging (Hsu JH, Davis RL, Neithamer RW, eds). Florida:Eckerd Coll, 1976;15–33.
307. Koo SI, Lee CC, Norvell JE. Effect of marginal zinc deficiency on the apolipoprotein–B content and size of mesenteric lymph chylomicrons in adult rats. Lipids 22:1035–1040(1987).
308. Klevay LM. Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Am J Clin Nutr 26:1060–1068(1973).
309. Klevay L. Coronary heart disease: The zinc/copper hypothesis. Am J Clin Nutr 28:764–774(1975).
310. Petering HG, Murthy L, O'Flaherty E. Influence of dietary copper and zinc on rat lipid metabolism. J Agric Food Chem 25:1105–1109(1977).
311. Looney MA, Lei KY. Dietary fiber, zinc and copper: effects on serum and liver cholesterol levels in the rat. Nutr Rep Int 17:329–337(1978).
312. Caster WO, Doster JM. Effect of dietary zinc/copper ratio on plasma cholesterol level. Nutr Rep Int 19:773–775(1979).
313. Fosmire GJ. Zinc toxicity. Am J Clin Nutr 51:225–227(1990).
314. Black MR, Medeiros DM, Brunett E, Welke R. Zinc supplements and serum lipids in young adult white males. Am J Clin Nutr 47:970–975(1988).
315. Freeland-Graves JH, Han WH, Friedman BJ, Shorey RL. Effect of dietary Zn/Cu ratios on cholesterol and HDL-cholesterol levels in women.Nutr Rep Int 22:285–29(1980).
316. Laitinen R, Vuori E, Viikari J. Serum zinc and copper: associations with cholesterol and triglyceride levels in children and adolescents. Cardiovascular risk in young Finns. J Am Coll Nutr 8:400–406(1989).
317. Shah DR, Singh PP, Gupta RC, Bhandari TK. Effect of oral zinc sulphate on serum lipids and lipoproteins in human subjects. Indian J Physiol Pharmacol 32:47–50(1988).
318. Goodwin JS, Hunt WC, Hooper P, Garry PJ. Relationship between zinc intake, physical activity and blood levels of high-density lipoprotein cholesterol in a healthy elderly population. Metabolism 34:519–523(1985).
319. Brewer GJ, Yuzbasiyan-Gurkan V, Johnson V. Treatment of Wilson's disease with zinc. IX. Response of serum lipids. J Lab Clin Med 118:466–470(1991).
320. Kok FJ, Van Duijn CM, Hofman A, Van der Voet GB, de Wolff FA, Paays CH,Valkenburg HA. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. Am J Epidemiol 128:352–359(1988).
321. Wallwork JC, Milne DB, Sims RL, Sandstead HH. Severe zinc deficiency: Effects on the distribution of nine elements (potassium, phosphorus, sodium, magnesium, calcium, iron, zinc, copper and manganese) in regions of the rat brain. J Nutr 113:1895–1905(1983).
322. Wallwork JC, Milne DB, Sandstead HH. Distribution of minerals

and catecholamines in rat brain: Effects of zinc deficiency. In: The Neurobiology of Zinc. Part B: Deficiency, toxicity, and pathology. (Frederickson CJ, Howell GA, Kasarskis EJ, eds). New York: Liss, 1984;49–64.
323. Sandstead HH. Zinc in human nutrition. In: Disorders of mineral metabolism–2 (Bronner F, Coburn J, eds). New York:Academic Press, 1981;93–157.
324. Hesse G. Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. Science 205:1005–1007(1979).
325. Todd WR, Elvehjem CA, Hart EB. Zinc in the nutrition of the rat. Am J Physiol 107:146–156(1934).
326. Hove E, Elvehjem CA, Hart EB. The physiology of zinc in the nutrition of the rat. Am J Physiol 119:768–775(1937).
327. Hove E, Elvehjem CA, Hart EB. Further studies on zinc deficiency in rats. Am J Physiol 124:750–758(1938).
328. Blamberg DL, Blackwood UB, Supplee WC, Combs WG. Effect of zinc deficiency in hens on hatchability and embryonic development. Proc Soc Exp Biol Med 104:217–220(1960).
329. Kienholz WE, Turk DE, Sunde ML, Hoekstra WG. Effects of zinc deficiency in the diets of hens. J Nutr 75:211–221(1961).
330. Hurley LS, Swenerton H. Congenital malformation resulting from zinc deficiency in rats. Proc Soc Exp Biol Med 123:692–696(1966).
331. Warkany J, Petering HG. Congenital malformation of the central nervous system in rats produced by maternal zinc deficiency. Teratology 5:319–334(1972).
332. Swenerton H, Shrader R, Hurley L. Zinc deficient embryos: Reduced thymidine incorporation. Science 166:1014–1015(1969).
333. Swanson C, King J. Zinc and pregnancy outcome. Am J Clin Nutr 46:763–771(1987).
334. Sandstead HH, Gillespie DD. Zinc deficiency: effect on brain of suckling rat. Pediatr Res 6:119–125(1972).
335. Buell SJ, Fosmire GJ, Ollerich DA, Sandstead HH. Effects of postnatal zinc deficiency on cerebellar and hippocampal development in the rat. Exp Neurol 55:199–210(1977).
336. Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. I. Impaired acquisition of granule cells. Brain Res 271:217–226(1983).
337. Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. II. Impaired maturation of Purkinje cells. Brain Res 16:11–20(1984).
338. McKenzie JM, Fosmire GJ, Sandstead HH. Zinc deficiency during the later third of pregnancy: effects on fetal rat brain, liver, and placenta. J Nutr 105:1466–1475(1975).
339. Halas ES, Rowe MC, Johnson OR, McKenzie JM, Sandstead HH. Effects of intrauterine zinc deficiency on subsequent behavior. In: Trace elements in human health and disease (Prasad AS, ed). New York:Academic Press, 1976;327–343.
340. Halas ES, Hanlon MJ, Sandstead HH. Intrauterine nutrition and aggression. Nature 257:221–222(1975).
341. Halas ES, Reynolds GM, Sandstead HH. Intra-uterine nutrition and its effects on aggression. Physiol Behav 19:653–661(1977).
342. Lokken PM, Halas ES, Sandstead HH. Influence of zinc deficiency on behavior. Proc Soc Exp Biol Med 144:680–682(1973).
343. Halas E, Burger P, Sandstead HH. Food motivation of rehabilitated malnourished rats: implications for learning studies. Anim Learn Behav 8:152–158(1980).
344. Halas ES, Heinrich MD, Sandstead HH. Long term memory deficits in adult rats due to postnatal malnutrition. Physiol Behav 22:991–997(1979).
345. Halas ES, Eberhardt M.J., Diers MA, Sandstead HH. Learning and memory impairment in adult rats due to severe zinc deficiency during lactation. Physiol Behav 30:371–381(1983).
346. Halas ES, Hunt CD, Eberhardt MJ. Learning and memory disabilities in young adult rats from mildly zinc deficient dams. Physiol Behav 37:451–458(1986).
347. Wallwork JC, Botnen JH, Sandstead HH. Influence of dietary zinc on rat brain catecholamines. J Nutr 112:514–519(1982).
348. Wallwork JC, Fosmire GJ, Sandstead HH. Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat. Br J Nutr 45:127–136(1981).
349. Wallwork JC, Sandstead HH. Effect of zinc deficiency on appetite and free amino acid concentration in rat brain. J Nutr 113:47–54(1983).
350. Sandstead HH, Wallwork JC, Halas ES, Tucker DM, Dvergsten CL, Strobel DA. Zinc and central nervous function. In: Biological aspects of metals and metal related diseases (Sarkar B, ed). New York:Raven Press, 1983; 225–241.
351. Hesse G, Hesse K, Catalanotto F. Behavioral characteristics of rats experiencing chronic zinc deficiency. Physiol Behav 22:211–215(1979).
352. Massaro TF, Mohs M, Fosmire G. Effects of moderate zinc deficiency on cognitive performance in young adult rats. Physiol Behav 25:117–121(1982).
353. Sandstead HH, Strobel DA, Logan GM Jr, Marks EO, Jacob RA. Zinc deficiency in pregnant rhesus monkeys: effects on behavior of infants. J Clin Nutr 31:844–849(1978).
354. Strobel DA, Sandstead HH. Social and learning changes following prenatal or postnatal zinc deprivation in rhesus monkeys. In: The neurobiology of zinc. Part B: Deficiency, toxicity, and pathology (Frederickson CJ, Howell GA, Kasarskis EJ, eds). New York:Alan R. Liss, 1984; 121–138.
355. Golub MS, Gershwin ME, Hurley LS, Hendrickx AG, Saito WY. Studies of marginal zinc deprivation in rhesus monkeys: infant behavior. Am J Clin Nutr. 42:1229–1239(1985).
356. Tucker DM, Sandstead HH. Neuropsychological function in experimental zinc deficiency in humans. In: The neurobiology of zinc. Part B: Deficiency, toxicity, and pathology (Frederickson CJ, Howell GA, Kasarskis EJ, eds). New York:Alan R. Liss, 1984; 139–152.
357. Darnell LS, Sandstead HH. Iron, zinc and cognition of women. Am J Clin Nutr 53(3):16(1991).
358. Sandstead HH, Darnell LS, Alcock NW. Association of iron and zinc nutriture. FASEB J 5:A1291(1991).
359. Brown JJL. Zinc fume fever. Br J Radiol. 61:327–329(1988).
360. Batchelor R, Fehnel J, Thomson R. A clinical and laboratory investigation of the effect of metallic zinc, of zinc oxide, and of zinc sulphide upon health of workmen. J Ind Hyg 8:322–363(1926).
361. Brown M, Thorn J, Otth G. Food poisoning involving zinc contamination. Arch Environ Health 8:657–660(1964).
362. Stein EC, Schiffer RB, Hall WJ, Young N. Multiple sclerosis and the workplace: report of an industry-based cluster. Neurology 37:1672–1677(1987).
363. Wisniewski HM, Keith AB. Chronic relapsing experimental allergic encephalomyelitis: an experimental model of multiple sclerosis. Ann Neurol 1:144–148(1977).
364. Donaldson J, Pierre T, Minnich J. Seizures in rats associated with divalent cation inhibition of Na^+-K^+-ATPase. Can J Biochem. 49:1217–1224(1971).
365. Itoh M, Ebadi M. The selective inhibition of hippocampal glutamic acid decarboxylase in zinc-induced epileptic seizures. Neurochem Res 7:1287–1289(1982).
366. Kress Y, Gaskin F, Bronsnan C. Effects of zinc on the cytoskeletal proteins in the central nervous system. Brain Res. 220:139–149(1981).
367. Yokoyama M, Koh J, Choi D. Brief exposure to zinc is toxic to cortical neurons. Neurosci Lett 71:351–35(1986).
368. Choi D, Yokoyama M, Koh J. Zinc neurotoxicity in cortical cell culture. Neuroscience 24:67–79(1988).
369. Duncan MW, Marini AM, Watters R, Kopin IJ, Markey SP. Zinc, a neurotoxin to cultured neurons, contaminates cycad flour prepared by traditional Guamanian methods. J Neurosci 12:1523–1537(1992).
370. Hjortso E, Quist J, Bud M, Thomsen JL, Andersen AB, Wiberg-Jorgensen F, Jensen NK, Jones R, Reid LM, Zapol WM. ARDS after accidental inhalation of zinc chloride smoke. Intensive Care Med 14:17–24(1988).
371. Milliken J, Waugh D, Kadish ME. Acute interstitial pulmonary fibrosis caused by a smoke bomb. Can Med Assoc J 88:36–39 (1963).
372. Marquart H, Smid T, Hiederi D, Maarten V. Lung function of welders of zinc-coated mild steel. Am J Ind Med 16:289–296(1989).
373. Key MM, Henschel AF, Butler J, Ligo RN, Tabershaw IR. Occu-

pational diseases: a guide to their recognition. Washington:U S Dept Health Education Welfare,1977; 408–410.
374. Daig AT, Challen PJR. Respiratory hazards in welding. Ann Occup Hyg 7:223–231(1964).
375. Rom WN. Environmental and occupational medicine. Boston:Little Brown and Co, 1983.
376. Evans EH. Casualties following exposure to zinc chloride smoke. Lancet 2:368–370(1945).
377. Pare CM, Sandler M. Smoke bomb pneumonitis: description of a case. J Royal Army Med Corp 100:320–322(1954).
378. Brown RFR, Marrs TC, Rice P, Masek, LC. The histopathology of rat lung following exposure to zinc oxide/hexachloroethane smoke or instillation with zinc chloride followed by treatment with 70% oxygen. Environ Health Perspect 85:81–87(1990).
379. Farrell FJ. Angioedema and urticaria as acute and late phase reactions to zinc fume exposure with associated metal fume fever-like symptoms. Am J Ind Med 12:331–337(1987).
380. Blanc P, Wong H, Bernstein MS, Boushey HA. An experimental human model of metal fume fever. Ann Intern Med 114:930–936(1991).
381. Nemery B. Metal toxicity and the respiratory tract. Eur Respir J 3:202–219(1990).
382. Lemen RA, Lee JS, Wagoner JK, Blejer HP. Cancer mortality among cadmium production workers. Ann NY Acad Sci 271:273–279(1976).
383. Sunderman FW Jr. Carcinogenic effects of metals. Fed Proc 37:40–46(1978).
384. Sunderman FW Jr. Recent progress in nickel carcinogenesis. Ann 1st Super Sanita 22(2):669–679(1986)
385. Amdur MO, McCarthy JF, Gill MW. Respiratory response of guinea pigs to zinc oxide fume. Am Ind Hyg Assoc J 4:887–889(1982).
386. Gordon T, Chen LC, Fine JM, Schlesinger RB, Su WY, . Pulmonary effects of inhaled zinc oxide in human subjects, guinea pigs, rats, and rabbits. Am Ind Hyg Assoc J 53:503–509(1992).
387. Marrs TC, Colgrave HF, Edginton JAG, Brown RFR, Cross NL. The repeated dose toxicity of a zinc oxide/hexachloroethane smoke. Arch Toxicol 62:123–132(1988).
388. Marrs TC, Clifford WE, Colgrave HF. Pathological changes produced by exposure of rabbits and rats to smokes from mixture of hexachloroethane and zinc oxide. Toxicol Lett 19:247–252(1983).
389. Karlson N, Cassel G, Fangmar KI, Bergman FA. Comparative study of the acute inhalation toxicity of smoke from pyrotechnic mixtures. Arch Toxicol 59:160–166(1986).
390. Naslund PE, Andreasson S, Bergstrom RL, Smith L, Risberg B. Effects of exposure to welding fume: an experimental study in sheep. Eur Respir J 3:800–806(1990).
391. Kleinfeld M, Messite I, Kooyman O, Shapiro J. Welders siderosis. Arch Environ Health 19:70–73(1969).
392. Hambidge KM, Walravens PA, Neldner KH. The role of zinc in the pathogenesis and treatment of acrodermatitis enteropathica. In: Zinc metabolism: current aspects in health and disease (Prasad A, Brewer G, eds). New York:Alan R. Liss, 1977;329–340.
393. Arlette JP. Zinc and the skin. Pediatr Clin of North Am 30:583–596(1983).
394. Sandstead HH, Lanier VC, Shephard GC .Zinc and wound healing. Am J Clin Nutr 23:514–519(1970).
395. Lansdown ABG. Interspecies variations in response to topical application of selected zinc compounds. Food Chem Toxic 29:57–64(1991).
396. Bogden JD, Oleske JM, Munves EM, Lavenhar MA, Bruening KS, Kemp FW, Holding KJ, Denny TN, Louria DB. Zinc and immunocompetence in the elderly: baseline data on zinc nutriture and immunity in unsupplemented subjects. Am J Clin Nutr 46:101–109(1987).
397. Chandra RK, Newberne, PM. Immunocompetence in undernutrition. In: Nutrition, immunity and infection. New York:Plenum Press, (1977); 67–126, 181–196.
398. Endre L, Beck FW, Prasad A. The role of zinc in human health. J Trace Elem Exp Med 3:337–375(1990).
399. Fernandes G, Nair M, Onoc K, Tonaka T, Floyd R, Good RA. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc Natl Acad Sci USA 76:457–461(1979).
400. Fraker PJ, DePasqual-Jardieu P, Zwick CM, Luecke RW. Regeneration of T-cell helper function in zinc-deficient adult mice. Proc Natl Acad Sci USA 75:5660–5664(1978).
401. Fraker PJ, Gershwin ME, Good RA, Prasad A. Interrelationships between zinc and immune function. Fed Proc 45:1474–1479(1986).
402. Dardenne M, Savino W, Wade S, Kaiserlion D, Lemmonier D, Bach JF. *In vivo* and *in vitro* studies of thymulin in marginally zinc deficient mice. Eur J Immunol 14:454–458(1984).
403. King LE, Fraker PJ. Flow cytometric analysis of the phenotypic distribution of splenic lymphocytes in zinc-deficient adult mice. J Nutr 121:1433–1438(1991).
404. Cook-Mills JM, Fraker PJ. Functional capacity of the residual lymphocytes in zinc deficient mice. Br J Nutr 69:835–848(1992).
405. Editors, Nutrition reviews zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Nutr Rev 39:168–170(1981).
406. Pekarek RS, Sandstead HH, Jacob RA, Barcome DF. Abnormal cellular immune response during acquired zinc deficiency. Am J Clin Nutr 32:1466–1471(1979).
407. Allen JI, Perri RT, McClain CJ, Kay NE. Alteration in human natural killer cell activity and monocytic cytotoxicity induced by zinc deficiency. J Lab Clin Med 102:577–589(1983).
408. Hart D. Effect of zinc chloride on hamster lymphoid cells: mitogenicity and differential enhancement of lipopolysaccharide stimulation of lymphocytes. Infect Immun 19:457–461(1978).
409. Swanson CA, Mansourian R, Dirren H, Rapin CH. Zinc status of healthy elderly subjects: response to supplementation. Am J Clin Nutr 48:343–349(1988).
410. Duchateau J, Delepesse G, Vrijens R, Collet H. Beneficial effects of oral zinc supplementation on the immune response of old people. Am J Med 70:1001–1004(1981).
411. Bogden JD, Oleske JM, Lavenhar MA, Munves EM, Kemp FW, Bruening KS, Holding KJ, Denny TN, Guaino MA, Krieger LM, Holland BK. Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. Am J Clin Nutr 48:655–663(1988).
412. Winchurch RA,Thomas DJ, Adler WH, Lindsay T. Supplemental zinc restores antibody formation in cultures of aged spleen cells. J Immunol 133:569–571(1984).
413. McCord C. Metal fume fever as an immunological disease. Indust Med Surg 29:101–107(1960).
414. Sturgis CC, Drinker P, Thomson RM. Metal fume fever I. Clinical observations of the effect of the experimental inhalation of zinc oxide by two apparently normal persons. J Indust Hyg 9:88–97(1927).
415. Drinker KR, Drinker P. Metal fume fever: V. Results of the inhalation by animals of zinc and magnesium oxide fumes. J Ind Hyg 10:56–72(1928).
416. Vogelmeier G, Konig G, Bencze K, Fruhmann G. Pulmonary involvement in zinc fume fever. Chest 92:946–948(1987).
417. Matarese SL, Matthews JI. Zinc chloride (smoke bomb) inhalation lung injury. Chest. 89:308–309(1986).
418. Apgar J. Zinc and reproduction. Ann Rev Nutr 5:43–68(1985).
419. Swenerton H, Hurley L. Zinc deficiency in rhesus and bonnet monkeys, including effects on reproduction. J Nutr 110:575–583(1980).
420. Hurley LS, Shrader RE. Abnormal development of preimplantation rat eggs after three days of maternal dietary zinc deficiency. Nature (London) 254:427–429(1974).
421. Fosmire G, Greely S, Sandstead HH. Maternal and fetal response to various suboptimal levels of zinc intake during gestation in the rat. J Nutr 107:1543–1550(1977).
422. Apgar J. Effect of zinc repletion late in gestation on parturition in the zinc-deficient rat. J Nutr 103:973–981(1973).
423. Jameson S. Effects of zinc deficiency in human reproduction. Acta Med Scand Suppl 593:4–89(1976).
424. Jameson S. Zinc status and pregnancy outcome in humans. In: Clinical application of recent advances in zinc metabolism (Prasad AS, Dreosti EI, Hetzel BS, eds). New York:Alan R. Liss, 1982; 39–52.
425. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, Johnson LK, Milne DB, Stelling HP. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. Am J Clin Nutr

40:496–507(1984).
426. Neggers YH, Cutter GR, Acton RT, Alvarez JO, Bonner JL, Goldenber RL, Go R, Roseman JM. A positive association between maternal serum zinc concentration and birth weight. Am J Clin Nutr 51:678–684(1990).
427. Meadows NJ, Ruse W, Smith MF. Zinc and small babies. Lancet 2:1135–1136(1981).
428. Hunt IF, Murphy NJ, Cleaver AE, Faraji B, Swendseid ME, Coulson AH, Clark, VA, Browdy BL, Cabalum MT, Smith JC Jr. Zinc supplementation during pregnancy: effects on selected blood constituents and on program and outcome of pregnancy in low income women of Mexican descent. Am J Clin Nutr 40:508–521(1984).
429. Jameson S, Berström M, Hellsing K. Zinc status in pregnancy, the effect of zinc therapy on perinatal mortality. In: Proc. 7th International Symposium on Trace Elements in Man and Animals: Zagreb, IMI (Momcilovic B, ed). Institute for Medical Research and Occupational Health, University of Zagreb, 1991, 4.8–4.9.
430. Kynast G, Saling E. Effect of oral zinc application during pregnancy. Gynecol Obstet Invest 21:117–123(1986).
431. Mahmoud K, James D, Golding J, McCade R. Zinc supplementation during pregnancy: a double blind randomized trial. Br Med J 299:826–830(1989).
432. Ferm V, Carpenter S. The relationship of cadmium and zinc in experimental mammalian teratogenesis. Lab Invest 18:429–432(1968).
433. Anon Annual Report. Hydrabad, India:National Institute of Nutrition,1975.
434. Gock E, King MT, Echardt K, Wild D. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat Res 90:91–109(1981).
435. Deknudt G, Gerber GB. Chromosomal aberrations in bone marrow cells of mice given a normal or a calcium-deficient diet supplemented with various heavy metals. Mutat Res 68:163–168(1979).
436. Voroshilin SI, Platko EG, Fink T, Nikiforova VJA. Cytogenetic effects of inorganic and acetate compounds of tungsten, zinc, cadmium and cobalt in animal and human cells. Tsitol Genet 12:241–243(1978).
437. Nishioka H. Mutagenic activities of metal compounds in bacteria. Mutat Res 31:185–189(1975).
438. Marzin DR, Vo PH. Study of the mutagenicity of metal derivatives with Salmonella typhimurium TA102. Mutat Res 155:49–51(1985).
439. Deknudt G, Deminatti M. Chromosome studies in human lymphocytes after *in vitro* exposure to metal salts. Toxicology 10:67–75(1978).
440. Amacher DE, Paillet SC. Induction of trifluorothymidin-resistant mutants by metal ions in L5178Y/TK/cells. Mutat Res 78:279–288(1980).
441. Thompson ED, McDermott JA, Zerkle TB, Skare JA, Evans BLB, Cody DB. Genotoxicity of zinc in 4 short-term mutagenicity assays. Mutat Res 223:267–272(1989).
442. Borovansky J, Riley PA. Cytotoxicity of zinc *in vitro*. Chem Biol Interact 69:279–291(1989).
443. Hansen K, Stern RM. A survey of metal-induced mutagenicity *in vitro* and *in vivo*. J Am Coll Toxicol 3:381–430(1984).
444. Stocks P, Davies RI. Zinc and copper content of soils associated with the incidence of cancer of the stomach and other organs. Br J Cancer 18:14–24(1964).
445. Cavallo F, Gerber M, Marubini E, Richardson S, Barbieri A, Costa A, DeCarli A, Pujol H. Zinc and copper in breast cancer. Cancer 67:738–745(1991).
446. Schrauzer GN, White DA, Schneider CJ. Cancer mortality correlation studies. III. Bioinorg Chem 7:23–24(1977).
447. Schrauzer GN, White DA, Schneider CJ. Cancer mortality correlation studies. IV. Bioinorg Chem 7:35–56 (1977).
448. Van Rensburg SJ. Epidemiologic and dietary evidence for a specific nutritional predisposition to esophageal cancer. J Natl Cancer Inst 67:243–251(1981).
449. Barch DC, Fox CC. Dietary zinc deficiency increases the MB-induced formation of O^6-methylguanine in the esophageal DNA of the rat. Carcinogenesis 8:1461–1464(1987).
450. Strain WH, Mansour EG, Flynn A, Pories WJ, Tomaro AJ, Hill OA Jr. Letter to the editor: plasma-zinc concentrations in patients with bronchogenic cancer. Lancet 1:1021–1022(1972).
451. Davies IJT, Musa M, Dormandy TL. Measurements of plasma zinc. Part II. In malignant disease. J Clin Pathol. 21:363–365 (1968).
452. Gyorkey F, Min KW, Huff JA, Gyorkey P. Zinc and magnesium in human prostate gland: normal, hyperplastic and neoplastic. Cancer Res. 27:1348–1353(1967).
453. Lin J, Chan WC, Fong YY, Newberne PM. Zinc levels in serum, hair and tumors from patients with esophageal cancer. Nutr Rpts Internat 15:635–643(1977).
454. Logue J, Koontz M, Hattwick A. Historical prospective mortality study of workers in copper and zinc refineries. J Occup Med 24:398–408(1982).
455. Newberger J, Hallowell J. Lung cancer excess in an abandoned lead-zinc mining smelting area. Sci Total Environ 25:287–294(1982).
456. Petering HG, Buskirk HH, Grin JA. The effect of dietary mineral supplements to the rat on the antitumor activity of thiosemicarbazone. Cancer Res 27:1115–1121(1967).
457. DeWys WW, Pories WJ, Richter MC, Strain WH. Inhibition of Walker 256 carcinosarcoma growth by dietary zinc deficiency. Proc Soc Exp Biol Med 135:17–22(1970).
458. DeWys WW, Pories W. Inhibition of a spectrum of animal tumors by dietary zinc deficiency. J Natl Cancer Inst 48:375–381(1972).
459. Barr DH, Harris JW. Growth of the P388 leukemia as an ascites tumor in zinc-deficient mice. Proc Soc Exp Biol Med 144:284–287(1973).
460. Fenton MR, Burke JP, Tursi FD, Arena FP. Effect of a zinc-deficient diet on the growth of an IgM-secreting plasmacytoma. J Natl Cancer Inst 65:1271–1272(1980).
461. Fong LYY, Sivak A, Newberne PM. Zinc deficiency and MBN-induced esophageal cancer in rats. J Natl Cancer Inst 61:145–150(1978)
462. O'Dell BL, Newberne PM, Savage J. Significance of dietary zinc for the growing chicken. J Nutr 65:508–512(1959).
463. Fong LYY, Lee JSK, Chan WC, Newberne PM. Zinc deficiency and the induction of esophageal tumors by methylbenzylamine and sodium nitrite. In: N-nitro compounds—occurrence and biological effects IARC Scientific Publications, vol 41(Bartsch H, O'Neil IK, Castegnaro M, OkadaFM, eds). Lyon: International Agency for Research on Cancer, 1983;:679–683.
464. Poswillo DE, Cohen B. Inhibition of carcinogenesis by dietary zinc. Nature 231:447–448 (1971).
465. Duncan JR, Dreosti IE. Zinc intake, neoplastic DNA synthesis and chemical carcinogenesis in rats and mice. J Natl Cancer Inst 55:195–196(1975).
466. Kasprzak KS, Kovatch RM, Poirier LA. Inhibitory effect of zinc on nickel subsulfide carcinogenesis in Fischer rats. Toxicology 52:253–262(1988).
467. Waalkes MP, Rehm S, Riggs CW, Bare RM, Devor DE, Poirier LA, Wenk ML, Henneman JR. Cadmium carcinogenesis in male Wistar rats: dose–respones analysis of effects of zinc on tumor induction in the prostate, testes and at the injection site. Cancer Res 49:4282–4288(1989).
468. Diplock AT. Mineral insufficiency and cancer. Med Oncol Tumor Pharmacother 7:193–198 (1990).
469. DiPaolo RV, Newberne PM. Copper deficiency in the newborn and postnatal rat with special reference to phosphatidic acid synthesis. In: Trace substances in environmental health—V (Hemphill DD, ed).Columbia: University of Missouri,1972; 177–188.
470. DiPaolo RV, Newberne PM. Copper deficiency and myelination in the central nervous system of the newborn rat: histological and biochemical studies. In: Trace substances in environmental health—VII. (Hemphill DD, ed). University of Missouri, Columbia, 1974; 213–223.
471. Hunt CE, Carlton WW, Newberne PM. Interrelationships between copper deficiency and dietary ascorbic acid in the rabbit. Br J Nutr 24:61–69(1970).