Evaluation of Publicly Available Scientific Evidence Regarding Certain Nutrient-Disease Relationships:

2. Zinc and Immune Function in the Elderly

December 1991

By
William R. Beisel, M.D.

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under
FDA Contract No. 223-88-2124
Task Order #9
EVALUATION OF PUBLICLY AVAILABLE
SCIENTIFIC EVIDENCE REGARDING
CERTAIN NUTRIENT-DISEASE RELATIONSHIPS:

2. ZINC AND IMMUNE FUNCTION IN THE ELDERLY

December, 1991
By
William R. Beisel, M.D.

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under
FDA Contract No. 223-88-2124
Task Order #9

Life Sciences Research Office
Federation of American Societies
For Experimental Biology
9650 Rockville Pkwy
Bethesda, Maryland 20814
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon literature reviews and the scientific analyses by knowledgeable investigators engaged in work in specific areas of biology and medicine.

This report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), in accordance with the provisions of Task Order #9 of Contract No. 223–88–2124. Potential authors and reviewing consultants were identified by the LSRO based on their qualifications, experience, and freedom from conflict of interest, with due consideration for balance and breadth in appropriate disciplines. The author and reviewing consultants were selected with the concurrence of the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB).

On March 14, 1991, the FDA requested submission of scientific data and information on the ten specific topics for which health claims might be made (Federal Register 56:12932–12933). The scientific data and information provided in response to this request were considered by LSRO in preparing this report. Copies of the submitted materials are available for public inspection at the Dockets Management Branch, FDA (Docket No. 91N–0102). Copies of documents cited in this report are available for public inspection at LSRO, FASEB.

William R. Beisel, M.D., Adjunct Professor, Department of Immunology and Infectious Disease, Johns Hopkins School of Hygiene and Public Health, Frederick, MD should be cited as the author of this report. The LSRO acknowledges the efforts of William R. Beisel, M.D. and also the critical assistance of Pamela J. Fraker, Ph.D., Professor, Department of Biochemistry, Michigan State University, East Lansing, MI, and M. Eric Gershwin, M.D., Professor of Medicine, Department of Internal Medicine, University of California, Davis, CA, who reviewed several drafts of the manuscript. The appendix tables were prepared by the LSRO staff and author and were critically reviewed by the author and reviewers. Subsequently the draft report and tables were revised by the author, edited by the LSRO scientific staff, and received final concurrence from the author and reviewing consultants.

The evaluation of scientific literature, data, and information submitted to the LSRO was made by the author, reviewers, and the LSRO independently of FDA or any other group, governmental or non-governmental. The author and LSRO accept responsibility for the accuracy of the report conclusions and its appendix table(s). This final report was reviewed and approved by members of the LSRO Advisory Committee under authority delegated by the Federation Board. The LSRO Advisory Committee members who reviewed this report were free of conflicts of interest in regard to the subject matter under policies established by the Federation. Upon completion of these review procedures, the report was approved by the Executive Director, FASEB, and transmitted to FDA.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

December 31, 1991
Date

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
TABLE OF CONTENTS

FOREWORD ................................................................. iii

I. INTRODUCTION ......................................................... 1

II. BACKGROUND INFORMATION ........................................ 3
    A. The Physiologic Role and Normal Metabolism of Zinc ............... 3
        1. Body zinc content ........................................... 3
        2. Zinc absorption and excretion .............................. 3
        3. Zinc values ................................................... 4
    B. Medical Conditions Known to be Associated with Zinc Deficiency ... 4
        1. Studies of immunological dysfunction in states of severe deficiency 5
        2. Studies of immunological dysfunction in states of mild zinc deficiency 6
    C. Zinc and Immune System Interrelationships .......................... 6
    D. Zinc Nutrition in the Elderly ...................................... 8
        1. Zinc intake in the elderly .................................... 8
        2. Zinc values in plasma, cells, tissues, or urine of the elderly 9
    E. Immunosenescence in the Elderly .................................... 11
        1. Consequences of immunosenescence ............................ 11
        2. Recent studies of the effect of age on immune function .......... 11
        3. Possible mechanisms for immunosenescence .................... 12

III. ZINC INTERVENTION STUDIES IN HEALTHY ELDERLY SUBJECTS .... 15

IV. DISCUSSION OF AVAILABLE DATA ..................................... 19
    A. How Much Information Is Available About Zinc as a Nutrient and a Component of Foods? 20
    B. What Are the Zinc Requirements for a Beneficial Effect?
        What Data are Available? ...................................... 20
    C. What Are the Optimal Levels of Zinc Consumption? ............... 21
D. Are the Zinc Effects on Immunity Generalizable to the Total US Population of Elderly Persons? ........................................... 21

E. What Other Positive and Negative Factors Impact on the Consumption of Important Zinc-Containing Foods by the Elderly? ......................... 22

F. Are There Safety Concerns About Reasonable or Excess Zinc Intakes? .... 22

G. Are There Differences Between Zinc in Foods and Zinc in Supplements? ... 23

H. Are There Critical Gaps in Knowledge? ........................................... 23

V. SUMMARY AND CONCLUSIONS ......................................................... 25

VI. BIBLIOGRAPHY ............................................................................. 27

APPENDIX ............................................................................................ A-1
I. INTRODUCTION

A considerable body of clinical and experimental evidence attests to the facts that:

- Deficits of body zinc are detrimental to immune system function.
- Some degree of zinc deficiency may exist in many elderly persons.
- Impairment of immunological functions (immunosenescence) accompanies the aging process.

Potential interactions between these variables can be viewed conceptually as the sides of an equilateral triangle, with zinc nutrition, aging, and immunity being located at the three angles, as shown in Figure 1.

\[ 	ext{Zinc Nutrition} \]

\[ \text{Body Zinc Deficiency} \rightarrow \text{Zinc's Essential Role in Immune System Functions} \]

\[ \text{Aging} \rightarrow \text{Immunosenescence} \rightarrow \text{Immunity} \]

**Figure 1.** This simplified figure depicts the conceptual relationships among zinc nutrition, aging, immunity, and their highly complex interactions.
The Surgeon General's Report on Nutrition and Health (U. S. Department of Health and Human Services, 1988) states that deficits or excesses of body zinc may impair the immune system. It also discusses the possible interactions between malnutrition and immunosenescence, but states that these relationships in elderly persons have yet to be clarified.

The report of the Committee on Diet and Health of the National Research Council (1989a) states that zinc deprivation has been shown in earlier reports to alter many facets of immunocompetence in experimental animals as well as humans. The report recommends that effects of trace metals, such as zinc and copper, on the immune system be studied further so that plausible mechanisms for their association with chronic disease in humans can be offered.

It is generally believed that immunosenescence is an inevitable component of the aging process, but some aspects of immunosenescence may result from deficits or imbalances of one or more micro-nutrients, with or without an accompanying disease process (Bogden et al., 1987; Chandra, 1991; Duchateau et al., 1981). It is not certain that deficits of body zinc in elderly subjects are sufficient to produce abnormal results in laboratory tests of immune function, or more importantly, to cause overt clinical dysfunctions in host immunity or resistance to infectious illnesses. Further, subtle abnormalities in laboratory tests of immune function may not be reflected in meaningful changes in resistance against infectious diseases in the elderly, or in immune system-related risks for the development of malignancies. There is thus some uncertainty as to whether zinc supplements given to the elderly can prevent or correct problems associated with immunosenescence.
II. BACKGROUND INFORMATION

A. THE PHYSIOLOGIC ROLE AND NORMAL METABOLISM OF ZINC

Zinc is an essential constituent of more than 200 metalloenzymes or zinc–enzyme complexes. Many metalloenzymes, including thymidine kinase, RNA polymerases, ribonucleases, and the nucleoside phosphorylases, play important roles in nucleic acid metabolism, cell replication, tissue repair, and growth. Zinc is required for both DNA and RNA synthesis, and plays a role in polysome formation and polynucleotide conformation (Prasad, 1985). Zinc functions in certain regulatory proteins, signaling proteins, and in glucocorticoid receptors facilitating binding to DNA. Zinc stabilizes the tertiary structure of nucleic acids, protein macromolecules, and cellular membrane components, in part because of its high affinity for protein sulfhydryl groups (Marozzi et al., 1986).

In addition, zinc has many biologically important interactions, participating in the production, storage, secretion, and activities of hormones (Chandra and Chandra, 1986; Gershwin et al., 1985; Hambidge, et al., 1986, National Research Council, 1989a,b). The thymic production (by non-lymphocytic epithelial cells) of hormones necessary for the normal functioning of the immune system is dependent, in part, on adequate concentrations of cellular zinc. In addition, full activity of the thymic hormone, thymulin, requires saturation by zinc after its secretion by the thymus (Dardenne et al., 1982; Fabris et al., 1988; Franceschi et al., 1985).

1. Body zinc content

The adult body content of zinc totals about 1 to 2 grams, contained mainly in bone matrix and within cells of muscle and some other tissues. However, the main stores are not in rapid equilibrium with those of other tissues. In contrast, the labile body pool of extracellular zinc is relatively small and has a rapid turnover rate. Accordingly, a continuing adequate intake of zinc is necessary to prevent deficiencies in cell growth and repair (National Research Council, 1989b).

Zinc circulates in plasma, bound mainly to proteins (albumin and α-2-macroglobulin), and to amino acid microligands (histidine and cysteine). If hypoalbuminemia is present, plasma zinc concentrations are reduced.

2. Zinc absorption and excretion

Intestinal zinc absorption is subject to relatively strong homeostatic regulation by mucosal cells. The absorption of zinc is thought to occur mainly in the duodenum and proximal jejunum. Because only small amounts of dietary zinc are actually absorbed each day, the excretion of fecal zinc is proportional to dietary intake. Small amounts of zinc are absorbed more efficiently than large amounts, and persons with low stores absorb more efficiently than those in good status (National Research Council, 1989b). Despite these physiologic restrictions on intestinal absorption, the ingestion of zinc in excessive quantities can lead to high serum concentrations and signs of toxicity.

A high concentration of dietary phytate (myoinositol hexaphosphate), in the dietary absence of sufficient animal products, can be a major cause of zinc deficiency (O'Dell, 1969; Fraker et al., 1982). Many other dietary factors, including high intakes of minerals such as calcium, copper, iron, tin, and phosphorus, dietary fiber, polyunsaturated fatty acids in large amounts, chelating agents and other drugs may adversely affect zinc absorption, as may diarrhea (Behrens et al., 1990; Greger, 1989;
Marozzi et al., 1986; National Research Council, 1989a,b; Sandstead et al., 1990). The elderly, as a group, are the largest consumers of pharmaceuticals and other medications, but few studies have explored the effects of these agents on zinc absorption, or conversely, the effects of zinc deficiency on drug actions, especially in the elderly. Because yeast fermentation destroys phytate, zinc in leavened bread is more available than in unleavened bread (Marozzi et al., 1986). Although milk is a good source of dietary zinc, milk also appears to impair the intestinal absorption of Zn\textsuperscript{62} in postmenopausal women (Wood and Hanssen, 1988). The consumption of refined foods and a reduced intake of red meats are other factors that could contribute to reduced zinc intakes by the elderly.

Zinc microligands can be filtered by the renal glomerulus to carry zinc to the urine. Normally, adults excrete 0.3 to 0.5 mg/day of zinc. Urinary losses of zinc are increased by diuretics and by chelating agents such as penicillamine. Cisplatin therapy of malignancies also enhances excretion of body zinc, and leads to secondary hypozincemia (Sweeney et al., 1989). Disease- or trauma-induced acute-phase reactions that cause rapid catabolism of body proteins also cause increased plasma amino acid concentrations of histidine and cysteine, and thus lead to hyperzincuria. Zinc is also lost in desquamating skin and hair, in sweat, and in other body secretions (Greger, 1989; Prasad, 1985).

3. Zinc values

In individuals, plasma zinc exhibits a small circadian periodicity, but over the larger population there is an extremely wide range of values (variously estimated at 60–150, 69–121, 72–136, or 76–170 \( \mu \)g/dL) in fasting morning values of normal adult subjects (Greger, 1989). Wide ranges also characterize the normal zinc content of various blood cells and hair. However, during acute-phase reactions of infectious illnesses and trauma, the induction of intracellular zinc-binding metallothioneins, initiated by various cytokines causes an important sequestration of zinc in hepatocytes, bone marrow cells, lymph nodes, and the thymus (Cousins and Leinart, 1988). This sequestration causes a rapid and dramatic lowering of plasma zinc values.

Because of these variables, zinc concentrations in plasma and blood cells are generally deemed inadequate for assessing the true nutritional status of zinc in single individuals (National Research Council, 1989a). Moreover, there currently exists no acceptable clinical method for establishing with certainty the status of zinc nutrition in an individual patient. Although low serum or blood cell concentrations appear useful in identifying groups whose zinc status should be further investigated (Pilch and Senti, 1984), serum zinc values are not considered definitive for the assessment of zinc nutritional status.

B. MEDICAL CONDITIONS KNOWN TO BE ASSOCIATED WITH ZINC DEFICIENCY

In humans, deficiencies of body zinc are caused by or associated with a number of diseases and abnormal conditions. These include acrodermatitis enteropathica (an autosomal recessive genetic defect in intestinal zinc absorption), sickle cell anemia, Down's syndrome, uremia, cirrhosis, inflammatory bowel disease and malabsorption syndromes, osteoporosis, chronic alcoholism, idiopathic hypoguesia, chronic or repeated diarrheas, and some malignancies. Zinc deficiency often coexists with protein energy malnutrition and severe immunological dysfunctions of patients with cachexia appear to reflect the combined deficiencies of these nutrients. Zinc deficiency can also be caused by renal hemodialysis, by intravenous hyperalimentation, and by long-term enteral feedings (Behrens et al., 1990; Chandra and Chandra, 1986; Gershwin et al., 1985; Gershwin and Hurley, 1987; Goode et al., 1990; Hendricks and Walker, 1988; Herzberg et al., 1990; Kenny et al., 1989; Morley et al., 1988; Prasad, 1985).
Abnormal keratogenesis of the tongue caused by zinc deficiency impairs taste bud functions, and leads to hypogeusia and anorexia. Villous atrophy of the intestinal mucosa associated with zinc deficiency can impair digestion, absorption, and metabolic utilization of a wide range of other nutrients, such as vitamin A and protein (Gershwin and Hurley, 1987). Zinc deficiency causes deranged production of adrenal steroids, growth hormone, and thymic hormone. Human zinc deficiency leads to parakeratosis, hypogonadism, sexual dysmaturity, infertility, and possibly to zinc–reversible impotence in the elderly (Gershwin et al., 1985; Prasad, 1976).

Zinc is required for normal wound healing. Administration of supplemental zinc may aid in the resolution of peripheral vascular ulcers in elderly persons with zinc deficiency (Morley et al., 1988). Studies by Barry et al. (1990) suggest that zinc is also required for the hepatic elimination of some drugs. These authors studied 15 male patients (mean age = 52 years) with chronic cirrhosis and 15 healthy age–matched controls. Plasma zinc of the patients averaged only 72 percent of control values, and leukocyte zinc averaged 85 percent (p <0.05). Abnormalities in the hepatic clearance of antipyrine correlated with deficiencies of leukocyte zinc, but not with values for plasma zinc or with estimates of the severity of liver pathology.

Since zinc content is high in eye tissue and zinc metalloenzymes are important for function of the chorioretinal complex, Newsome et al. (1988) examined the effects of supplementation with 200 mg/day of zinc sulfate (80 mg/day of zinc) on the rate of macular degeneration in 151 elderly subjects (mean age of 68). In a blind, random–placebo controlled preliminary study those individuals receiving supplemental zinc had significantly less degeneration than controls over a 24–month period.

Because zinc metalloenzymes are important in nucleic acid metabolism, manifestations of zinc deficiency are observed most readily in rapidly proliferating cells, including surface epithelium, gastrointestinal mucosal cells, and lymphocytes, with resultant alopecia, acrodermatitis, growth retardation, diarrhea, hypogonadism in males, loss of hair, mental disturbances, delayed wound healing, and immunological dysfunctions giving rise to infections by opportunistic organisms (Chandra and Chandra, 1986).

The effects of zinc supplementation on immune response have been examined in a number of diseases known to be associated with zinc deficits. These studies have been conducted for the most part in infants, children, and young adults.

1. Studies of immunological dysfunction in states of severe zinc deficiency

The adverse immunological consequences of a clinically pure form of severe zinc deficiency have been characterized in children with acrodermatitis enteropathica. This disease produces symmetrical progressive bullous pustular dermatitis of the extremities and mucocutaneous junctions, generalized alopecia, diarrhea, growth failure, and secondary infections. Immunological dysfunctions include impaired delayed dermal hypersensitivity (DDH) reactions, reduced T4–helper cell numbers, poor in vitro lymphocyte responses to mitogens, and early death from severe infections (Chandra, 1980, 1991). Zinc supplementation (35–150 mg elemental zinc/day) reversed these conditions in children (Barnes and Moynahan, 1973; Oleske et al., 1979). Numerical data showing correction of immunological defects (Chandra, 1991), and clinical data showing the control of infections (Moynahan, 1981), all demonstrate the validity of oral zinc therapy in such children.
2. Studies of immunological dysfunction in mild zinc deficiency

A paper by Prasad et al. (1988) sheds considerable light on the problems associated with mild deficiencies of body zinc. Deficiency was defined as zinc concentrations 1 SD below the mean in at least two types of blood cells in subjects with normal plasma zinc values. Three groups were studied: healthy young adult volunteers with experimentally induced zinc deficiency, adult sickle cell anemia patients, and apparently healthy volunteers in whom zinc deficiency was diagnosed during screening studies. Mildly zinc-deficient subjects exhibited depressed serum thymulin activities, decreased interleukin-2 (IL-2) activities, and a decrease in the T4+/T8+ lymphocyte ratio. There was an increase in lymphocytes lacking surface immunoglobulins and the T-cell–specific markers reactive with the T101 monoclonal antibody. After 12 weeks of supplementation with 27 mg/day of ionic zinc, all laboratory abnormalities had returned to normal. Infection was not mentioned as a problem in any of these patients.

Diarrhea can reduce dietary zinc intake, impair intestinal absorption, and increase fecal losses of endogenous zinc stores. Behrens et al. (1990) reported a randomized, double-blind, controlled trial in 64 infants under 2 years of age admitted to a Bangladesh hospital with acute diarrhea. Infants supplemented for two weeks with 15 mg/kg/day of zinc acetate (7.8 mg/kg/day of zinc) responded with a 25 percent increase in linear growth over a 9-week period. In a similarly controlled study with marasmic infants in Chile (Castillo–Duran et al., 1987), supplementation with 2 mg/kg/day of ionic zinc significantly increased weight gain and reduced both the number of infections and the number of anergic skin test responses.

Franceschi et al. (1985) gave 21 children with Down’s syndrome 1 mg/kg/day of zinc sulfate (0.4 mg/kg/day of ionic zinc) for two months. This led to significant increases in plasma zinc values (from 87 to 126 μg/dL), plasma thymulin activity, and T-lymphocyte numbers. This dose of zinc (approximately twice the RDA for small children) provoked no significant side effects. The incidence of infections in these children was not mentioned.

The immunological abnormalities associated with the zinc deficiency of sickle cell anemia were studied by Prasad et al. (1989) before and after therapy with 45 mg/day zinc acetate (23 mg/day of ionic zinc). With the correction of zinc concentrations in neutrophils, neutrophil functions improved significantly or returned to normal, as did erythrocyte nucleoside phosphorylase activity. DDH responsiveness also showed improvement, and serum thymulin activity was restored to normal.

Allen et al. (1985) noted that patients with bronchiogenic cancer often had low plasma zinc concentrations, sometimes accompanied by high urinary losses of zinc. In 75 cancer patients, mean plasma zinc was 67 μg/dL (versus 96 μg/dL for normal controls), and urinary losses averaged 1.385 mg/day (versus 0.392 mg/day in normal controls). Plasma and urinary zinc concentrations in individuals correlated highly with depressed in vitro responses of their T lymphocytes to phytohemagglutinin, but blood natural killer (NK) cell activity did not show a similar correlation. Supplementation with 660 mg/day zinc sulfate (264 mg/day of ionic zinc) for 6 weeks in 7 of the patients with hyperzincuria caused normalization of T-cell responses to phytohemagglutinin, but there was no apparent effect on NK cell activity.

C. ZINC AND IMMUNE SYSTEM INTERRELATIONSHIPS

Clinical zinc deficiency in humans causes many immune system dysfunctions, including thymic atrophy, lymphopenia, anergic DDH responses, and reduced NK cell activity (Cunningham–Rundles
et al., 1990; Fraker et al., 1986; Prasad, 1985). At least in children and young adults some of these immunological dysfunctions can be corrected by administering sufficient zinc, over time, to eliminate the deficiency state (Chandra and Chandra, 1986).

The dysfunctions noted above may be critical for aged persons, because recurrent pulmonary and urinary infections as well as secondary infections of stasis ulcers are a major cause of patient morbidity (Gershwin and Hurley, 1987). Interrelationships among essential micronutrient deficiencies, immune system dysfunctions, and infection, however, need clarification. There is no doubt, that frank zinc deficiency impairs immunocompetence. The immunologic consequences of zinc deficiency appear to be quite similar to those of protein energy malnutrition (PEM) and of aging (Thompson et al., 1987). Cell-mediated immunity, especially that involving the helper/inducer population of T cells, is primarily depressed, as shown by anergy or depressed DDH responses to skin test antigens, and poor lymphocyte blastogenesis after mitogen stimulation (Keen and Gershwin, 1990; Thompson et al., 1987). In contrast, effects of zinc deficiency on humoral immunity, or on B–cell numbers and their functions have not been as extensively studied. Studies in mice suggest that defects in these reactions may be sizable (Fraker et al., 1986). Since zinc seems necessary for normal humoral responses to T–cell dependent antigens, as well as for B–cell functions, zinc deficiency may also impair the formation of antibodies against some types of infection. Zinc is also important in the function of phagocytic cells, including neutrophils and macrophages (Chandra, 1991; Hambidge et al., 1986).

Recent in vitro evidence shows that zinc participates in regulating the secretion of key cytokines from peripheral blood leukocytes (Scuderi, 1990). Although IL–1 and IL–2 trigger and modulate immune responses and IL–1, IL–6, and TNF initiate some beneficial aspects of the acute–phase febrile response, these cytokines can also have harmful effects. These include the accelerated consumption of body nutrients, and sometimes, the induction of shock in both Gram–negative and Gram–positive infections (Dinarello, 1991). The possible role of zinc deficiency in influencing these complex cytokine interactions in human infections has yet to be elucidated.

Because the body pool of labile zinc is small, and urinary losses are continuous and relatively large, it is easier to produce an isolated deficiency of zinc in laboratory animals or humans than it is to produce an experimental deficiency of any other micronutrient (with the possible exception of iron). These experimental findings indicate that elderly subjects taking in an inadequate quantity of dietary zinc are at risk for developing some degree of body zinc deficiency.

Animal models clearly show that experimental zinc deficiency causes thymic atrophy, impaired thymic hormone production, lymphopenia, reductions of cytolytic T–cell responses and NK cell activities, and alterations in the proportions of the various subsets of lymphocytes and mononuclear phagocytes (Fraker et al., 1986). As a consequence, antibody–mediated responses to both T–cell dependent and T–cell independent antigens are reduced, and DDH reactions are depressed. Immunological dysfunctions associated with zinc deficiency in experimental animals are greater than those seen with deficiencies of any other micronutrient (Chandra, 1991).

In humans, zinc is essential for the blast transformation of both T and B lymphocytes, for cell mediated immunity, including DDH responses to a large number of test antigens, for antibody–mediated responses to both T–cell dependent and T–cell independent antigens, for the development of normal distribution of lymphocyte subsets including NK cells, for some aspects of humoral immunity, and for the proper function of phagocytic cells (Gershwin et al., 1985; Greger, 1989; Meydani, 1990; Prasad, 1985; Tapazoglou et al., 1985).

Lowered content of zinc in lymphocytes of patients with zinc deficiency can diminish the activity of their zinc metalloenzymes, including nucleoside phosphorylase (NPase), a purine catabolic pathway enzyme. NPase deficiency in lymphocytes can lead, in turn, to toxic accumulation of nucleotides
within these cells and to abnormalities in lymphocyte functions (Meftah and Prasad, 1989), as demonstrated experimentally in volunteer subjects.

Franceschi et al. (1985) described a marked decrease of thymic hormonal activity in patients with Down's syndrome, and in the elderly. The low thymulin activity found in plasma of both groups was ascribed to the presence of inhibitory substances, rather than to a primary defect in thymic hormone production. The inhibitory substances appeared to be thymic hormones which were not associated with bound zinc but which could block hormone receptor sites on target cell membranes. The in vitro addition of zinc salts caused an activation of serum thymulin and a disappearance of inhibitory effects. The authors suggested that zinc malnutrition might be diagnosed by measuring plasma thymulin, in the presence and absence of added zinc salts. Such an approach would presumably measure a deficiency in plasma zinc available for interaction with thymulin.

D. ZINC NUTRITION IN THE ELDERLY

Table I, and Tables which follow, attempt to summarize original data from the several studies or surveys of elderly individuals published later than those reviewed by the National Research Council (1989a,b) or those in the Surgeon General's Report on Nutrition and Health (U. S. Department of Health and Human Services, 1988). Pertinent or unique data from a few earlier studies are also included.

1. Zinc intake in the elderly

The RDA for zinc of 15 mg/day for adults affords some margin for safety and reflects the estimate that zinc available in the food supply consumed by healthy adults amounts to 12.3 mg/day per capita (National Research Council, 1989a). Severe, moderate, and marginal zinc deficiencies have been reported in the United States (National Research Council, 1989a,b). These benchmark conclusions are amplified by Greger (1989) who reviewed reports by at least 17 different groups concerning zinc intake of the elderly. These surveys have yielded consistent information: elderly women generally consume less dietary zinc than do elderly men; institutionalized subjects generally consume less than persons living at home. Intakes of zinc seem to diminish as elderly persons advance in age (Meydani, 1990; Moser–Veillon, 1990).

Because of altered dietary habits, and often some degree of anorexia, the elderly appear to have a high incidence of mild deficiency in body zinc (Morley et al., 1988; Prasad, 1985; Sandstead et al., 1990). Diets that are low in energy content also tend to be low in zinc (Moser–Veillon, 1990). Thompson et al. (1987) reported that the average estimated dietary zinc intake of 18 healthy centenarians was 7.5 mg, with a range of 5–15 mg. Intakes of zinc below the RDA can lead to negative zinc balances, and to losses from the nonlabile stores of zinc in tissues such as bone and kidney (Morley et al., 1988). In addition, the intestinal capacity to absorb zinc is reduced with age (Sandstead et al., 1990; Turnlund et al., 1988).

Wagner et al. (1983) found the dietary zinc intake of 173 individuals, aged 60 to 97 years and from rural, low-income households, to average only 7.3 mg/day. The plasma zinc concentrations correlated significantly with their poor intake of zinc.
Bunker et al. (1987) and Bunker and Clayton (1989) found that 20 housebound elderly people (7 men, averaging 78.0 years, and 13 women, averaging 78.8 years) with stable chronic diseases had a suboptimal zinc intake averaging only 5.9 mg/day, and that they were losing an average of 1.05 mg/day of body zinc. In contrast, 24 apparently healthy aged subjects (11 men, averaging 78.2 years, and 13 women, averaging 75.8 years) had an average zinc intake of 9.0 mg/day and were in slightly positive zinc balance. Thomas et al. (1988) reported an average dietary zinc intake of 5.6 mg/day in 21 elderly (mean age = 81.7 years) long-term inpatients, many of whom had chronic leg ulcers or pressure sores. These three British papers contain important information about dietary zinc intake and plasma zinc content of house-bound, hospitalized, and healthy elderly control subjects.

In a study of total minerals in the diet (Penington and Young, 1991), zinc was found to be low in the diets of older women, averaging only 8.7 mg/day, in contrast to 12.9 mg/day for older men. Major zinc-contributing components of the diet were meats (47 percent), grains (37 percent), mixed dishes (10 percent) and vegetables (8 percent). A similar study of 260 institutionalized elderly subjects with a mean age of 80.5 years, conducted by Sahyoun et al. (1988), showed intakes of zinc generally below RDA. The dietary intake of zinc for men averaged 11 mg/day, and 49 percent of subjects consumed one-third or less of the RDA; comparable figures for women were 10 mg/day zinc intake with 58 percent consuming one-third of the RDA. These large-scale surveys contain excellent data on the zinc intake of a wide variety of elderly subjects.

In a study of 44 apparently healthy Dutch lacto-ovo-vegetarians whose ages ranged from 65 to 97 years, Brants et al. (1990) found that the average daily intake of zinc was 8.5 mg for men, and 7.6 mg for women. No plasma or cellular values for zinc were published in this study. Flint et al. (1981) determined the dietary zinc intake in elderly Australians. Twenty-four healthy subjects living in the community (mean age = 76.2 years) averaged 11.0 mg zinc intake/day, but in contrast, the zinc intake for 66 institutionalized subjects with a mean age of 82.2 years was only 7.6 mg/day. Despite the differences in intake, plasma zinc averaged 89 and 92 µg/dL respectively in these two groups. These papers help demonstrate the differences in dietary zinc intake observed in different populations of elderly individuals.

Turnlund et al. (1986) studied zinc balance, absorption, and retention using stable isotopes (70Zn and 67Zn) in six healthy young men (aged 22 to 30 years) and six healthy elderly men (age range = 65 to 74 years) during a 12-week stay in a metabolic unit. On a dietary zinc intake of 15 mg/day, zinc absorption averaged 17 percent in elderly men, in contrast to 31 percent in young men. However, endogenous zinc losses were smaller in the elderly than in the young men, and as a result, zinc balances did not differ between groups. Based on these findings, Turnlund et al. (1986) could not determine if the elderly had a lower requirement for absorbed zinc, or if their diminished loss of body zinc reflected a lower intestinal absorption. This study is important because it is the only one using isotopic methodology to estimate body zinc retention and loss in elderly subjects.

Recommendations about food consumption patterns to increase the intake of dietary zinc (National Research Council, 1989b) apply equally well to aged individuals and include: using zinc-rich animal foods like poultry, lean meats, and low-fat (or non-fat) dairy products, using whole grain products and legumes, and increasing physical activity to moderate levels to maintain adequate daily food consumption.

2. Zinc values in plasma, cells, tissues, or urine of the elderly

Senapati et al. (1989) measured zinc in plasma and leukocytes of elderly hospitalized patients, healthy elderly patients, and younger controls. In 19 healthy controls (mean age = 51.3 years), plasma zinc averaged 118 µg/dL and leucocyte zinc averaged 58.2 ng/mg dry weight. The 25 healthy home-living elderly patients (mean age = 77.7 years) had comparable values in plasma of 105 µg/dL and in cells of
56.1 ng/mg. Significantly lower concentrations were found in two groups of hospitalized elderly patients. Mean values of plasma zinc in 30 "long stay" patients (mean age = 80.8 years) in a geriatric hospital, and in 34 patients with decubitus of the leg or other areas (mean age = 81.3 years) were 84 and 83 µg/dL respectively. Their mean values for leukocyte zinc (51.3 and 52.6 ng/mg) were also significantly lower. It is impossible to tell if the low plasma zinc values in the two patient groups were due to a deficiency of body zinc or to a disease-induced sequestering of zinc. The lowered leukocyte zinc values suggest that a deficiency of body zinc was probable.

In 20 housebound elderly patients, 70 to 85 years of age, who were receiving less than 15mg/day of zinc, and who were in negative zinc balance (Bunker et al., 1987), plasma zinc concentrations (mean of 74 µg/dL), whole blood values (674 µg/dL) and 98 pmol/10^6 leukocytes were found. In a control group of healthy elderly subjects (Bunker and Clayton, 1989), average plasma zinc was similar (71.9 µg/dL), but leukocyte zinc was significantly higher, averaging 120 pmols/10^6 cells. Comparable values in 21 hospitalized patients studied by the same group (Thomas et al., 1988) included a mean plasma zinc of 70 µg/dL, and a mean leucocyte zinc of 91 pmol/10^6 cells.

Paterson et al. (1985) reported zinc values in 99 elderly patients (mean age = 79) admitted consecutively to a geriatric assessment unit. Mean plasma zinc was 72 µg/dL, erythrocyte zinc was 1.27 µg/10^9 RBC, and urinary zinc excretion was 0.59 mg/g creatinine. Findings were compared with published control values. The somewhat low plasma zinc values and the somewhat high urinary zinc excretions may not be representative of healthy elderly subjects. They may have been due to alterations in zinc metabolism caused by the illnesses that led to hospitalization.

At the time of their hospital admission, 79 elderly (mean age = 77.9 years) patients were found by Wilson and Myskov (1985) to have depressed plasma zinc concentrations, which averaged 69 µg/dL. An attempt to raise these values by administering 300 mg/day of zinc orotate (94 mg/day of ionic zinc) was successful in 12 of 18 attempts. Failure to correct plasma zinc values with up to six weeks of supplementation in the unresponsive patients was attributed to patient variability induced by their diseases, which were not reported.

A pilot study of 20 healthy subjects (age range 65 to 95 years) was reported by Haboubi et al. (1988). Zinc concentrations increased from a mean of 75 to 103 µg/dL in men and from 72 to 106 µg/dL in women three months after oral supplementation of an unstated daily dose of zinc. Immunological responses were not reported.

Craig et al. (1990) found the plasma zinc concentration to be below 69 µg/dL (the bottom of the locally used reference range for healthy adults) in 38 percent of 107 patients (mean age = 72 years) consecutively admitted to a geriatric ward, in 69 percent of 51 "long stay" geriatric patients (mean age = 82 years), and in 19 percent of a "control" group of 102 elderly patients (mean age = 75 years) from medical and surgical wards who had normal serum albumin values. The differences in zinc values for these groups could not be explained by serum α-2-macroglobulin, transferrin, or albumin concentrations. Again, plasma zinc concentrations reported in this study could have been lowered by the zinc-sequestering effects of acute phase responses associated with their underlying diseases.

Elderly women with osteoporosis were found by Herzberg et al. (1990) to have significantly higher losses of zinc in the urine than non-osteoporotic controls. Urinary zinc in excess of 0.8 mg/g creatinine was observed and appeared to be a direct consequence of bone loss.

The studies reviewed in this section are of importance because they reveal the very common occurrence of depressed concentrations of zinc in the plasma and cells of different groups of elderly
subjects, and also, because they illustrate the continuing lack of consensus among investigators concerning the types (and numbers) of body cells which should be assayed to define the nutritional status of zinc in patients.

E. IMMUNOSENESCENCE IN THE ELDERLY

Certain immune functions tend to decline with aging (Fletcher, 1986; Kay, 1985;), in a process termed immunosenescence. Aging is associated with a 90 percent reduction in thymic mass, a 50 percent reduction in splenic mass, and decreased quantities of circulating thymic hormones (Chandra, 1984a; Fletcher, 1986). The major changes appear to occur primarily in T lymphocytes and cellular immune responses (Thompson et al., 1987), although changes in stem cells, B–cells, and null cells also occur (Kay, 1985). Alterations occur in T–cell subsets, and in their in vitro responses to mitogens (Rao et al., 1979). DDH responses are diminished. Changes in humoral immunity include decreased antibody responses to immunization (e.g. tetanus toxoid, rabies vaccine, influenza vaccine), but in contrast, there are increases in autoantibodies (Fletcher, 1986). Age–related defects in neutrophil function have not been reported.

These effects of aging can also be demonstrated experimentally in the cells and tissues of animals. As examples, abilities of stem cells to expand clonally, to repair X–ray induced damage, and to home to the thymus all decline with age, as do rates of cell division and B–cell formation (Kay, 1985). B–cell responses to mitogens and primary antibody responses decline in aging mice. Macrophage functions seem to be preserved.

1. Consequences of immunosenescence

As immunologic vigor decreases in the aged, the incidence of anergy, infections (including those due to commensal microorganisms), autoimmune and immune–complex diseases, amyloidosis, paraproteinemia, and cancer increases. Decreased T–cell responsiveness and immune complex deposition have also been implicated as causative factors in arteriosclerosis (Lattime and Strausser, 1977).

2. Recent studies of the effect of age on immune function

In humans, T–cell dependent cell–mediated functions decline with age, as demonstrated by impaired or anergic DDH reactions. The proliferative capacity of T cells decreases in response to mitogens or allogeneic target cells. The autologous mixed lymphocyte reaction of T cells to autologous non–T mononuclear cells decreases with age in humans. The number of circulating T cells may remain normal or decrease progressively after humans reach adulthood, but the proportions of T–lymphocyte subpopulations undergo dramatic changes (Kay, 1985; Thompson et al., 1987).

The number of human helper/inducer T cells decreases with age, whereas lymphocytes associated with suppression/killing either increase or remain constant. Immature lymphocytes of the T–lineage and the percentage of apparently activated T cells bearing the Ia and immature thymic phenotypic markers increase with age (Thompson et al., 1987). Cells from elderly humans are less capable of responding to mitogens, allogeneic lymphocytes, or soluble antigens (Thompson et al., 1987).

In a follow–up to an earlier study (Franceschi et al., 1985) by the same group, Licastro et al. (1990) compared 40 elderly patients with Alzheimer type dementia and 43 age and sex matched controls (mean plasma zinc values were 77 and 83 µg/dL, respectively) with 15 young controls. The elderly groups showed similar decreases in lymphocyte proliferation after mitogen stimulation, and similar
decreases in IL-2–induced cell activation. Basal levels of active plasma thymulin were significantly and similarly depressed in both groups, and there was an impairment of thymulin reactivation in both groups, but significantly greater in the dementia group.

The absolute number of colony forming, circulating T cells decreases markedly in the elderly with respect to that found in young adults. Electron microscopy has shown swollen mitochondria, containing myelinated structures and reduced numbers of cristae in T cells of elderly persons (Kay, 1985). Since the involution of the thymus begins at about the time of sexual maturity, and antedates the age–related decline in T–cell dependent immune responses, a 'cause–and–effect' relationship has been suspected.

In a study of 17 healthy centenarians, 100–103 years of age, Thompson et al. (1984) found normal total lymphocyte numbers and serum immunoglobulin concentrations. However, there was a 50 percent depression in the number of T4+ helper/inducer lymphocytes, a decrease in the production and response to IL–2, and a decrease in mitogen responsiveness of T cells. In contrast, there were normal numbers of T8+ suppressor/killer lymphocytes, and slight increases in percentages of null cell, immature B cells, and immature NK cells. In this oldest group of subjects to be studied, discriminating T–lymphoid functions were reduced in association with an apparent failure of some T, B, and NK cells to differentiate to functional maturity (Thompson et al., 1984).

Murasko et al. (1986) studied immune responses in 260 noninstitutionalized elderly free–living subjects with a mean age of 84.5 years. They presented evidence that T–lymphocyte function was decreased, with decreased in vitro responses to phytohemagglutinin and to concanavalin A, but with normal responses to pokeweed mitogen. No response to any of the 3 mitogens was found in 13 percent of those under 89, but all subjects aged 90 to 106 showed some response, and in fact, responded poorly only to phytohemagglutinin. Based on these findings, Murasko et al. (1986) suggested that the decreased immune response is no longer age–related after the age of 70, and that there may be a selection process in which subjects who live past the age of 90 are those in whom the least decrease in immune response is demonstrated. This interpretation might also apply to the Thompson et al. (1984) study of healthy centenarians.

Studies reviewed in this section illustrate recent attempts to evaluate the immune system by measuring: a) lymphocyte subset numbers, b) the in vitro ability of lymphocytes to respond to mitogens or to produce lymphokines, c) delayed dermal hypersensitivity responses, and d) plasma thymulin activity. The scarcity of studies measuring antibody responses to new or familiar antigens should also be noted.

3. **Possible mechanisms for immunosenescence**

Possible non–nutritional mechanisms responsible for the age–related decline in immune function include defects in the thymic–hypothalamic–pituitary communication network, leading to changes in the thymus and extrathyroid hormonal environment, as well as to a general decline of endocrine functions (Fletcher, 1986; Kay, 1985). These mechanisms may include decreased production of lymphokines, including IL–2, in elderly individuals (Thompson et al., 1987; Ventura et al., 1986).

Changes in cell membranes which disrupt inter– and intracellular communication could lead to decreased ability of lymphoid cells to respond to specific stimuli. Also, there could be genetically determined decreases in the proliferative potential of lymphoid cells (Fletcher, 1986; Kay, 1985). It is not certain that these possible abnormalities could account for the increased incidence of infections in the elderly. Conversely, age–associated chronic illnesses and infections could well have an adverse effect on immune functions, as would lowered mucosal barriers to detrimental substances from the environment (Fletcher, 1986).
Poor nutrition, including protein/energy, vitamin, and trace element deficiencies, is an important cause of immune system dysfunction, and one that is playing an important role in immunosenescence. Reports by Duchateau et al. (1981) and by Bogden et al. (1988) indicate that to a modest extent some of the immunological test abnormalities generally ascribed to immunosenescence can be reversed by micronutrient supplements. Sandstead et al. (1990) suggested (on the basis of dietary data) that a mild deficiency of zinc in elderly subjects might account for the high incidence of these immunological abnormalities. Thompson et al. (1987) pointed out that zinc deficiency also leads to anorexia, which would, in turn, reduce the dietary intake of immunologically important proteins, vitamins, and essential micronutrients.

A few studies have examined possible association of immunological anomalies with zinc nutriture. Baseline data in 100 subjects aged 60 to 89 (mean = 72.1 years), studied by Bogden et al. (1987) showed a 41 percent incidence of anergy to a panel of seven skin test antigens, and a 21.3 percent incidence of subnormal or nonresponders when their lymphocytes were studied in vitro for proliferative responses to three mitogens. Poor dermal hypersensitivity responses in this group correlated significantly with low plasma zinc values, and poor lymphocyte proliferative responses correlated with low platelet zinc values, but also with high zinc concentrations in mononuclear cells. It was shown earlier by Liechтенstein et al. (1982) that 94 percent of healthy elderly subjects exhibited at least one positive DDH reaction to four skin test antigens.

Kaplan et al. (1988) reported that elderly subjects with mild zinc deficiency (defined as normal plasma zinc values, but a depressed content of zinc in blood cells) also showed an impairment in IL-2 production by lymphocytes.
III. ZINC INTERVENTION STUDIES IN HEALTHY ELDERLY SUBJECTS

A limited number of recent studies have attempted to determine if the supplemental administration of zinc would improve the immunological functions of elderly subjects. These studies have differed considerably in a number of aspects, including the ages and medical condition of the subjects, the amounts of zinc given, the duration of supplementation, the tests used to detect immunological responses, and the apparent results. These studies are summarized in Table II, with criteria for inclusion as previously noted.

1) Duchateau et al. (1981) studied 38 institutionalized but healthy subjects over 70 years of age. A large supplement (220 mg of zinc sulfate twice daily; i.e., 176 mg/day zinc) was given to 15 subjects for a month, with an equal number of age (mean ages of 81 and 80) and sex matched controls. A few immunological parameters in the subjects receiving zinc showed an improvement, including delayed dermal hypersensitivity responses to PPD, candidin, and streptokinin–streptodornase and the serum IgG antibody response to tetanus toxoid.

No in vitro lymphocyte response was found to several mitogens, and numbers of circulating leukocytes or total lymphocytes did not change. No measurements of zinc concentration in plasma or blood cells were made, either before or after the study. Transient nausea and diarrhea occurred in five subjects receiving this pharmacologic dose of zinc.

This study is important because of the immune system improvements in immunological recall and memory effects reported in zinc supplemented elderly subjects, and because it stimulated other groups to investigate the apparent benefits of zinc supplementation of immunoresponsiveness in the elderly.

2) Eight healthy non–institutionalized males, ranging in age from 65 to 78, were given a supplement of 60 mg elemental zinc daily for 4.5 months in a study by Cossack (1989). They were selected for supplementation because initial test values were suggestive of zinc deficiency. Significant increases of plasma zinc (75 to 115 μg/dL), erythrocytes (39 to 43 μg/gHbg), lymphocytes (35 to 51 μg/10^10 cells) and neutrophils (48 to 67 μg/10^10 cells) were noted. The number of positive DDH responses increased from 53 percent to 78 percent and the mean diameter of positive responses increased significantly from 8 to 14 mm. Corresponding mean DDH reactions in 13 healthy age and sex matched controls were 85 percent positive with a mean diameter of 17 mm. In the supplemented group, the activity of the zinc metalloenzyme, nucleoside phosphorylase, in erythrocytes also rose significantly.

3) Soltesz et al. (1988) studied six elderly nursing home patients (ages not stated) who had no medical problem known to be associated with zinc abnormalities. Their intake of zinc ranged from 45–86 percent of the RDA, and their plasma zinc values were all in the normal range. After 28 days of supplementation of 15 mg/day of zinc no appreciable changes were noted in plasma zinc concentrations, but DDH reactions to four skin–test antigens (candida, mumps, trichophyton, and PPD) increased significantly from a mean of 1.8 to 2.8 positive reactions per subject (p = .034). Although this study is small and poorly controlled, it suggests that some improvement in immunological parameters can be achieved in elderly subjects by zinc supplementation within RDA limits.

4) Five healthy elderly subjects (aged 64–76) who were found to be anergic to four skin tests (candida, trichophyton, mumps, and PPD) were given a 55 mg/day zinc supplement for four weeks by Wagner et al. (1983). An average increase in plasma zinc of 18 μg/dL was found, and
all subjects developed DDH responsiveness to one or more of the antigens. No adverse effects of the supplementation were described. These three papers contribute limited evidence for the positive immunologic effects of zinc supplements in possibly zinc deficient, but otherwise healthy elderly subjects. It should be noted, however, that improvements in DDH responses (that are subject to recall effects) need not translate into improved host defenses against infection.

5) Swanson et al. (1988) found that the average zinc intake of 53 healthy elderly subjects (mean age = 77 years) was 9.2 mg, and that 65 percent of the subjects had intakes of less than two-thirds of the RDA. Their mean plasma zinc concentration (85 μg/dL) and urinary excretion (0.46 mg/day) values were considered normal, as were the zinc contents of platelets, mononuclear cells, and polymorphonuclear cells.

These subjects were placed in 3 groups, and given no treatment, a placebo, or a zinc supplement of 30 mg/day for 28 days. In 17 subjects given supplemental zinc, the mean plasma value increased significantly to 108 μg/dL and urinary zinc excretion increased significantly to 1.15 mg/day. Blood cell values for zinc did not change in these subjects, and no changes were noted in serum IgG, IgM, or IgA concentrations.

This limited study indicates that elderly patients do absorb extra zinc from supplements of 30 mg/day. Unfortunately, no important immunological parameters were studied. Data on total serum immunoglobulin concentrations are of little value for this review, since their concentrations would not be expected to show a response to alterations in zinc status.

6) Zinc supplementation (50 mg/day ionic zinc) was given to half of a group of 60 healthy elderly (mean age = 74 years) subjects in a study conducted by Bracker et al. (1988). The others received a daily placebo. After one month, all were injected with an influenza vaccine, and the oral medications were continued. No differences in serological responses were found. Unlike the studies by Bogden et al. (1988, 1990), all subjects were instructed to discontinue any vitamin-mineral supplementation a month before this study began.

7) In contrast to the above studies, three recent reports by Bogden et al. (1987, 1988, and 1990) are of major importance because of their thoroughness, their inclusion of controls, their long duration and excellent follow up, and because they failed to support the conclusions of Duchateau et al. (1981). Results of zinc supplementation (as zinc acetate) in elderly, free-living subjects were reported after 3 and 12 months, and at 16 months after four additional months of washout without zinc supplements. All subjects were given a zinc-free multivitamin-multimineral preparation at breakfast, and at supper, a capsule containing either a placebo, or 15 or 100 mg of zinc.

The baseline mean plasma zinc value (84 ± 16 μg/dL) of the subjects was at the lower end of the normal range, and 90 percent had dietary zinc intakes below the RDA (Bogden et al., 1987). Dietary intakes were also below the RDA for total calories, and for many other essential micronutrients including folate, pyridoxine, α-tocopherol, copper, and magnesium.

At three months (Bogden et al., 1988), approximately equal numbers of subjects were in each study group, which were matched for age (means of 71 years) and sex (61 and 67 percent female). Pill counts showed 89.1 percent consumption of the vitamin-mineral capsules, and 86.9 percent of the zinc/placebo capsules. No side effects were reported.

Plasma zinc concentrations rose significantly (to a mean of 110 ± 23 μg/dL) only in the group receiving 100 mg zinc/day (Bogden et al., 1988). No changes from mean baseline zinc values
were seen in red blood cells, neutrophils, mononuclear cells, or platelets, or in serum alkaline phosphatase, total serum cholesterol or HDL cholesterol, or plasma copper after three months. Although DDH responses tended to improve in all groups, changes were not statistically significant, and no changes were noted in blastogenic responses of lymphocytes to the mitogens or antigens studied.

After one year (Bogden et al., 1990), only the group receiving 100 mg zinc/day showed a significant increase above baseline plasma zinc (to a mean of 110 μg/dL), but after four additional months without the supplemental zinc, plasma zinc had fallen back to baseline values. Again, no differences were found in zinc concentrations for blood cells except for a transient increase in neutrophil zinc in the group receiving 15 mg zinc/day. Serum cholesterol and copper concentrations remained unchanged in all groups.

DDH responses (both number and size) increased continuously during the course of the 16 month study to about double baseline values, and surprisingly, the increases seen in the placebo group were significantly greater than those in the zinc-supplemented groups. Thus, these increases in DDH responses were clearly not due to zinc supplementation (Bogden et al., 1990). Lymphocyte responses to mitogens did not differ among groups. NK activity increased transiently only in the group receiving 100 mg zinc/day.

In these studies conventionally dosed multivitamin–mineral supplements were administered to all subjects, but this inclusion had fortuitous consequences, in that immune recall responses were enhanced in DDH testing (Bogden et al., 1988, 1990; Louria, 1990). Virtually all of the administered micronutrients are known to be required for the normal function of the immune system (Chandra, 1984a; 1991), but the role of individual constituents in the DDH effects is not clear. Selenium (Peretz et al., 1991), has proven to be immunostimulatory when given as a supplement to elderly subjects. Placebo-controlled studies have shown that supplementation with vitamin E can improve DDH response and enhance the in vitro mitogenic response to the T-cell mitogen, concanavalin A (Meydani et al., 1990) in elderly subjects. Vitamin B-6 has been shown to enhance the mitogenic responses of peripheral blood lymphocytes to T- and B-cell mitogens and to increase IL-2 production (Meydani et al., 1991).

Another possible cause for the progressive increase in DDH responses in all groups might be a booster effect of repeated skin tests with multiple antigens, an effect largely discounted by Bogden et al. (1990). But the fact that the improvements in DDH responses of zinc-supplemented groups were less than those in placebo controls raises still other questions about possible adverse interactions between zinc and one or more of the many other micronutrients given to all subjects (Louria, 1990).

This study also provided valuable information about the rather transient effects of 12 months of zinc supplementation at doses as high as 100 mg/day.
IV. DISCUSSION OF AVAILABLE DATA

Interpretations of data concerning the relationships between zinc nutrition and immune functions in the elderly continue to be based on the assumptions that: a) deficits (or excesses) of body zinc are detrimental to immune system functions, b) zinc deficiency undoubtedly exists in many elderly patients, and c) various forms of malnutrition can contribute to immunosenescence.

Several important constraints influence the interpretation of data under review:

- Despite the title and objectives of this report, the elderly do not represent a homogeneous population. Papers reviewed herein include subjects with ages ranging over five decades, some healthy, vigorous, alert, and free living; some sickly, frail, somewhat mentally obtunded, and institutionalized. Any given finding, or recommendation, may not be generally applicable.

- Problems in quantitating zinc status in individual elderly patients remain paramount. No currently acceptable method exists that can accomplish this easily or certainly. As noted earlier, many factors can change plasma zinc values without altering body zinc content. Cellular zinc is perhaps the best guide, but cell populations vary, and measurements are hard to standardize.

- The major adverse consequences of immunosenescence include severe infectious diseases, malignancies, and autoimmune diseases. However, none of these have been linked directly in elderly patients to zinc deficiency. No epidemiological evidence for association of these disorders with zinc status in the elderly was encountered in this survey. Infectious diseases in the elderly are often life threatening. A causal relationship between zinc deficiency and increases in susceptibility to, and severity of, infectious diseases has been shown experimentally in laboratory animals (Fraker et al., 1982) and clinically in infants and children (Moynahan, 1981; Hambidge et al., 1986). Although a similar relationship probably exists in elderly patients, this remains to be proved.

- Problems in evaluating the immune status of the elderly remain an important issue. Early studies can be faulted because currently-used tests were not available, or because they used methodologies currently considered unreliable. Early cell separation techniques were inadequate. In vitro tests of lymphocyte mitogenesis are especially suspect, because of the different mitogens, different doses, different cell separation methods, and different cell-culture conditions employed. Culture media containing normal (zinc-containing) serum can produce false negative tests by improving the responses of zinc-deficient cells. Further, abnormal immunological findings reported in the elderly cannot be interpreted, with certainty, to indicate an increased susceptibility to infectious diseases. Few studies in the elderly have attempted to assess adequacy of their immune responses to new antigens.

- Perhaps the most important immunological abnormalities reported in the elderly are anergic reactions to DDH testing. In studies under review, a wide and inconsistent variety of test antigens were used, and the sizes of reactions said to indicate a positive response varied widely between studies. These tests are highly dependent on the skill and competence of the reporting clinician, are hard to standardize, require an ability by the subject to generate an inflammatory reaction, and, as previously noted, do not yield information about the ability of the patient to respond to new antigens.
Relatively few studies in the elderly relate zinc deficiency and immune functions. Although such studies in infants, children, young adults, or laboratory animals may not have specific relevance for the elderly, their findings should not be ignored. Practical and/or ethical problems may preclude the generation of data needed in elderly subjects, especially with regard to infectious diseases. Quantitative data can be obtained in animals with varying degrees of induced zinc deficiency.

With the foregoing interpretational constraints as a necessary background, several pertinent questions can be addressed.

A. HOW MUCH INFORMATION IS AVAILABLE ABOUT ZINC AS A NUTRIENT AND A COMPONENT OF FOODS?

Zinc is an essential nutrient. As reviewed in section B–2 of this report, it is vital in the synthesis of all DNA, RNA, and protein molecules in body cells. The metabolic role of zinc, the consequences of its deprivation and its content in various foods have been reviewed by Hambidge et al. (1986), Prasad (1976), and Sandstead et al. (1990).

The content of zinc in specific foods and diets is variable. Red meats, liver, and seafoods (especially oysters) are the best food sources of available zinc. But eggs, hard cheeses, milk, yogurt, legumes, nuts, and whole-grain cereals are also good sources (National Research Council, 1989b).

Most of the zinc consumed by Americans is provided by animal products, especially red meats. Cereals are the major source of zinc from plant products. Zinc in drinking water makes a negligible contribution to total intake. Additional bioavailability factors in foods play an important role in limiting the intestinal absorption of zinc. As reviewed in section II–A–2 of this report, the consumption of dietary zinc can be inadequate because of the amounts or the types of food being consumed.

B. WHAT ARE THE ZINC REQUIREMENTS FOR A BENEFICIAL EFFECT? WHAT DATA ARE AVAILABLE?

The current RDA for zinc, 15 mg/day for adults, was devised to maintain zinc status equivalent to that of healthy young adults, even in those individuals whose absorption of zinc was reduced to 20 percent by diets high in fiber and low in meat (National Research Council, 1989b). The RDA should thus be satisfactory for many of the elderly. In animals some parameters of the immune response are sensitive to only marginal zinc deficiencies (Fletcher, 1986). If dietary insufficiency of zinc is a factor in the immunosenescence which becomes increasingly evident with human aging, it might be argued that the current RDA is inadequate for the aged. Most studies indicate that many elderly persons are not meeting this RDA, and that many have depressed zinc concentrations in plasma or cells. It cannot be certified, however, that these subjects were zinc deficient, since many were healthy despite low plasma zinc, and findings discussed earlier (Bunker and Clayton, 1989) showed that apparently healthy elderly subjects remained in positive zinc balance with an intake of only 9 mg/day. It is therefore impossible to define concentrations for zinc in plasma or cells, which clearly indicate the need for zinc supplementation. This remains an issue of medical judgement in individual patients. Healthy elderly individuals consuming diets containing normal RDA amounts of zinc do not present evidence of body zinc deficiency.

Although methods for diagnosing or confirming a deficiency of body zinc in individual subjects are not entirely adequate, there can be no doubt that a severe deficiency of body zinc is life threatening (see
section II-B-1). In young adults certain laboratory tests of immune function seem sensitive to lesser deficiencies in body zinc. Thus individuals with cellular zinc levels about 11 to 22 per cent below the mean of a control population had lowered levels of zinc-bound plasma thymulin, and volunteers receiving 3 mg/day of zinc had cellular zinc levels about 50 per cent of normal with concomitant changes in T-cell function (Prasad et al., 1988).

Negative zinc balances are known to accompany acute and chronic illness (see section II-A-2), but data in this regard are quite inadequate, especially for the elderly. Many questions about such zinc losses and the necessity for correcting zinc deficits during acute or chronic illnesses, or in the convalescence period, remain unanswered.

A wide range of doses of zinc have been used therapeutically in medical problems known to be associated with zinc deficiency (see section II-B). With sufficient zinc given over a sufficient period of time, the restoration of plasma and cellular values for zinc to their normal ranges is generally accompanied, in children and young adults, by a correction or improvement of zinc-related immunological dysfunctions (see sections II-B-1 and 2). Evidence remains uncertain whether the restoration of body zinc values to normal in elderly adults will improve abnormal immunological function tests (see section III).

It is apparent, as discussed in section IV-F which follows, that excesses of body zinc, or excessive levels of zinc supplementation can be harmful. Few studies have been conducted to determine when zinc intake is excessive and more research is needed. The detailed studies of Bogden et al. (1990) imply that zinc intakes only slightly above the RDA may slightly diminish the enhancement of DDH that has been ascribed to multivitamin supplements. Evidence for this diminution is clearer with prolonged supplementation of 100 mg/day of elemental zinc, but these effects are transitory and can be reversed by discontinuing the supplementation (Bogden et al., 1990).

C. **WHAT ARE THE OPTIMAL LEVELS OF ZINC CONSUMPTION?**

As indicated by the preceding paragraphs, available evidence supports the RDA of 15 mg/day as the optimal level of zinc consumption in young adult subjects with normal body stores of zinc. Additional documentation is needed to assure that this RDA is satisfactory for the elderly, but in the absence of such documentation, this RDA remains the goal for zinc intake in elderly patients with disease-induced inadequacies of zinc absorption, with disease-induced excesses in fecal or urinary zinc losses, or in those deficient in body zinc from other causes. There is no conclusive evidence, however, that zinc supplementation confers any immunological benefit to a subject whose body zinc stores are adequate.

D. **ARE THE ZINC EFFECTS ON IMMUNITY GENERALIZABLE TO THE TOTAL US POPULATION OF ELDERLY PERSONS?**

Since zinc is essential for normal immunocompetence, all persons would appear to face an approximately equal risk if zinc deficiency should occur. The elderly population, however, is heterogeneous and it is not known if quantitative relationships between zinc status and immunocompetence are comparable in all individuals of that population. The risks to an elderly person of dietary zinc insufficiency vary considerably and are clearly higher in women, the poor, and those who live alone or are institutionalized. The redistribution of zinc associated with illness puts the sick, chronically ill, and debilitated at particular risk of zinc deficiency.
E. WHAT OTHER POSITIVE AND NEGATIVE FACTORS IMPACT ON THE CONSUMPTION OF IMPORTANT ZINC-CONTAINING FOODS BY THE ELDERLY?

Inadequate dietary consumption of zinc containing foods is influenced by a number of factors in the elderly. These include economic status, psychosocial changes associated with aging, poor dental health, chronic diseases, and the intake of drugs that might unfavorably affect zinc metabolism (Meydani, 1990). Some elderly persons eat low-calorie diets for weight reduction, some are vegetarians, and some are taking multiple medications that can alter the intestinal absorption or metabolism of essential micronutrients such as zinc (Louria, 1990).

Publicity concerning the dangers of high-fat, high-cholesterol foods, and the importance of high-fiber diets is causing elderly patients to switch from red meats to white meats, to avoid eggs and cheeses, and to eat cereals and dark breads with a high fiber content. As a consequence, many elderly people are turning away from foods that are high in bioavailable zinc, and are replacing them with foods low in available zinc. The relative cost of some of the best zinc sources such as oysters and seafoods also limits intake by elderly persons.

Alcoholism is associated with reduced serum zinc values and with high losses of zinc in the urine (Akar and Arcasoy, 1990). These findings are particularly clear in alcoholic cirrhotics. The intestinal villous atrophy and functional abnormalities associated with alcohol abuse are improved markedly following zinc therapy (Akar and Arcasoy, 1990). Diabetes is also said to reduce the body content of zinc, and the anorexia that accompanies many diseases serves to reduce the intake of all foods, including the zinc-containing ones.

F. ARE THERE SAFETY CONCERNS ABOUT REASONABLE OR EXCESS ZINC INTAKES?

Zinc is one of the least toxic of the essential trace elements (Hambidge et al., 1986; Prasad, 1976). Deaths have occurred after ingestion of 45 g of zinc sulfate (Prasad, 1976). An emetic dose of zinc sulfate is generally 1 to 2 g. Acute zinc toxicity (manifested by gastrointestinal irritation, diarrhea and vomiting, epigastric pain, lethargy, and fatigue, and/or by CNS symptoms including staggering and difficulty in writing) has been observed following the ingestion of 2 to 12 g of zinc sulfate, amounts clearly in the toxic range (Fosmire, 1990; Hathcock and Rader, 1990).

Although long-term intakes of moderate amounts of zinc are necessary for the therapy of some zinc deficiency states, chronic ingestion of moderate or large doses of zinc supplements may have adverse effects (Hathcock and Rader, 1990). The more subtle effects of moderately elevated intakes of zinc are of greater concern because they may not be detected easily (National Research Council, 1989b). Zinc compounds are readily available in "health food" stores in 30 to 50 mg capsules, and self-imposed intakes well above the RDA are not uncommon. Chandra (1991) has recently reviewed dangers of excesses as well as deficits of essential micronutrients.

Patients given 150–450 mg/day of zinc for several months have developed anemia, hypocupremia, microcytosis, and neutropenia (Fosmire, 1990; Hathcock and Rader, 1990; National Research Council, 1989b). The copper deficiencies induced by excess zinc have been quite difficult to correct (Fosmire, 1990). Effects of zinc excesses on iron metabolism are less clear (Fosmire, 1990).

Broun et al. (1990) described two patients with sideroblastic anemia, neutropenia, and bone marrow dysplasia secondary to zinc-induced copper deficiency. This was caused in one patient by an excessive chronic ingestion of zinc supplements and in the other by swallowing large numbers of zinc containing
penny coins. Plasma zinc values in these two patients were 238 and 300 μg/dL respectively. Hematologic values of both patients returned to normal after the source of the zinc excess was removed.

Zinc supplements of 80–150 mg/day have also been shown to decrease high-density serum lipoproteins and to slightly increase low-density lipoproteins within several weeks, with resultant adverse effects on the LDL/HDL cholesterol ratio (Chandra, 1984b; Fosmire, 1990; National Research Council, 1989b).

Excessive intakes of zinc may also have undesirable immunological consequences, although evidence for this comes essentially from effects on in vitro tests. Supplementation of healthy adults with 300 mg of zinc per day for six weeks was associated with a reduction in the in vitro lymphocyte stimulation response to phytohemagglutinin, as well as a reduction in chemotaxis and phagocytosis by neutrophils (Chandra, 1984b; National Research Council, 1989b). The immunosuppressive effects of excessive zinc on lymphocyte blastogenic responses to mitogens was also demonstrated in vitro by Rao et al. (1979), who found that following zinc addition to human lymphocyte cultures, stimulation by concanavalin A demonstrated an age-dependent effect. Lymphocytes from young adults showed either no change or an enhancement in the mitogenic response, whereas blastogenesis of lymphocytes from healthy aged donors was suppressed by zinc. Recent studies related to these effects are summarized in Table III.

Because of this evidence for subtle toxic effects at intakes above 15 mg/day, the National Research Council (1989b) recommended against any chronic ingestion of zinc above the RDA without adequate medical supervision. This recommendation has not been challenged and indicates that a 15 mg/day intake of zinc by healthy adults is reasonable.

G. ARE THERE DIFFERENCES BETWEEN ZINC IN FOODS AND ZINC IN SUPPLEMENTS?

The ionic zinc in various zinc salts used in supplements is the same as the ionic zinc in the many various organic molecules found in dietary foods. The body takes up ionic zinc from the intestinal tract by actively regulated mechanisms involving the intestinal mucosa cells.

The absolute amount of zinc contained in different foods is variable. In addition, apparent differences in the amounts of zinc absorbed from different food sources are due primarily to other factors in foods. These bioavailability factors (such as phytates and fiber, some proteins, and some minerals, such as iron and certain forms of phosphate) can bind, or unite with, ionic zinc within the intestinal contents, and thereby reduce its availability for absorption (National Research Council, 1989b).

H. ARE THERE CRITICAL GAPS IN KNOWLEDGE?

As with all scientific knowledge, many uncertainties continue to exist about the three-way interrelationships among zinc nutrition, immune function, and aging. Several of these uncertainties can be posed as questions.

- Would additional zinc supplementation of healthy, zinc-sufficient elderly subjects be helpful or harmful, and would such supplementation have any immunological consequences? Attempts to answer such questions will obviously require reliable data on dose-related responses.

- If immunosenescence is not an inevitable consequence of aging or genetic predisposition, how might it be prevented or reversed, and what are the roles of dietary nutrients? The studies of Bogden et al. (1988, 1989) suggested that the supplementation of dietary intake in the
elderly with a conventional multivitamin/multimineral capsule, but one lacking zinc, could reverse some of the findings of immunosenescence. If so, what is the specific role of zinc in this multiple interaction?

- Does a gradual decline in the zinc concentrations of plasma (see Pilch and Senti, 1984) and blood cells, or in the body pool of mobilizable zinc, occur as a normal consequence of aging? And as a correlated question, does the slowed genesis of new body cells in aged persons reduce the need zinc below 15 mg/day?

- What is the purpose of the sequestration of zinc in certain body cells during acute phase reactions? Does this phenomenon induce a functional zinc deficiency state in other cells? Does the sequestered zinc play a protective role by abetting functions in cells which sequestered zinc? Would attempts to reverse body losses of zinc during acute illnesses prove helpful or harmful?

- Can clear evidence of body zinc deficiency in elderly persons be equated with an increase in incidence (or severity) of infectious diseases? No such data have been published.

- Can animal models be developed to provide useful data about zinc and immune system interrelationships specifically for the elderly?
V. SUMMARY AND CONCLUSIONS

- Zinc is of profound importance for proper immune system functions. Too much or too little zinc can induce immunological dysfunctions. Steps to return zinc nutriture to normal serve to correct such dysfunctions.

- Elderly persons are at risk for developing zinc malnutrition. Zinc deficiency is prone to occur in elderly persons who are poor, who live alone or in institutions, who are female, who have acute or chronic illnesses or deilities, or are of advanced ages.

- Immunological dysfunctions in the elderly have been improved by nutritional means, but the role of zinc in this remains unclear. Reported immune system improvements induced by supplemental zinc (given in a wide range of doses) generally occurred in subjects with preexisting dietary or laboratory evidence of zinc deficiency.

- RDA intakes of zinc may not correct preexisting zinc deficiencies in the elderly. Even large supplements may not improve low plasma zinc values if disease-induced zinc-sequestering mechanisms are active.

- There is no definitive evidence to suggest that the current RDA for zinc is inadequate for healthy elderly individuals. Elderly subjects who are truly zinc deficient may benefit immunologically from medically supervised nutritional rehabilitation.
VI. BIBLIOGRAPHY


*This bibliography contains all reference citations that are either in the text or the tables or both.


APPENDIX

CRITERIA FOR INCLUSION OF ARTICLES IN APPENDIX TABLES

Articles in peer-reviewed journals related to the topic of this review were selected primarily on the basis of date and content. In general, papers appearing in 1987 or thereafter were included, provided that they presented original data from studies in humans. Certain items tabulated for the sake of completeness may not have been cited in the body of the text if their weight or relevance did not add significantly to development of the author's argument. Reviews have not been listed except as they included new data or useful meta-analyses.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Number &amp; Age of Subjects</th>
<th>Factors Affecting Interpretation</th>
<th>Zn Value Results</th>
<th>Dietary Intakes of Zn/Day</th>
<th>Immunological Findings</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bugrien et al., 1987</td>
<td>Baseline data gathering, comparison with young adults</td>
<td>100 35 yrs, 65 yrs</td>
<td>Healthy, free living</td>
<td>64.8 μg/dL RBC, WBC, Platelet, Hair had average values within normal ranges</td>
<td>92.9–95.7% &lt;15mg/day &lt;10.1 mg/day = 7.8 mg/day</td>
<td>41% increase to 7 DDH tests, others poor. Values correlated with plasma Zn. 18 were non- or poor responders to lymphocyte stimulation.</td>
<td>Quite thorough. Best available.</td>
</tr>
<tr>
<td>2. Brants et al., 1990</td>
<td>Uncontrolled survey</td>
<td>44 18 yrs, 26 yrs</td>
<td>Healthy Lacto-ovo-Vegetarians</td>
<td>Not measured</td>
<td>&lt;8.5 mg/day = 7.8 mg/day</td>
<td>None measured</td>
<td>Diet evaluated by check list. No other data.</td>
</tr>
<tr>
<td>3. Bunker et al., 1987</td>
<td>Metabolic balance studies (5 day)</td>
<td>20 7 yrs, 13 yrs</td>
<td>Housebound, with chronic stable disease</td>
<td>74 μg/dL Low whole blood WBC values</td>
<td>5.9 mg/day with 1.05 mg daily loss</td>
<td>None measured</td>
<td>Daily loss of body Zn was reflected by low plasma and cell values.</td>
</tr>
<tr>
<td>4. Bunker &amp; Clayton, 1989</td>
<td>Control subjects for above study</td>
<td>24 11 yrs, 13 yrs</td>
<td>Apparently healthy</td>
<td>72 μg/dL WBC = 120 pmol/10^6 cells</td>
<td>9.0 mg/day, with 0.1 mg daily gain</td>
<td>None measured</td>
<td>Healthy controls not in neg balance; they had signif. higher WBC Zn values.</td>
</tr>
<tr>
<td>5. Craig et al., 1990</td>
<td>Controlled survey of elderly subjects with acute &amp; chronic illnesses</td>
<td>107 acute 51 long-stay 102 controls</td>
<td>Controls were patients with normal serum albumin values</td>
<td>70 μg/dL = Acute Illness 62 μg/dL = Long Stay (Chronic) 83 μg/dL = Controls</td>
<td>Not measured</td>
<td>None measured</td>
<td>Plasma Zn values reflect disease effects.</td>
</tr>
<tr>
<td>6. Herzberg et al., 1999</td>
<td>Controlled survey</td>
<td>94 34 controls</td>
<td>#Patients with osteoporosis vs. non-osteoporotic # controls. Some receiving Vit D &amp; Calcium</td>
<td>Urinary Zn&lt;0.1 μg/g Creatine vs. 58 μg/g Creatine in controls</td>
<td>Not measured</td>
<td>None measured</td>
<td>High loss of Zn in urine was associated with osteoporosis.</td>
</tr>
<tr>
<td>7. Kaplan et al., 1988</td>
<td>Controlled survey</td>
<td>23 elderly 25 sickle cell 13 controls</td>
<td>Healthy, ambulatory, low socioeconomic, elderly patients</td>
<td>Eight elderly had low lymphocyte and PMN Zn values</td>
<td>Elderly = 7.6 mg/dL</td>
<td>Lymphocytes from elderly patients with low cellular Zn produced less IL-2 after PHA stimulation.</td>
<td>Small number of subjects. Not clear if thymulin and/or Zn in test media influenced IL-2 yields.</td>
</tr>
<tr>
<td>8. Lecastro et al., 1999</td>
<td>Controlled survey</td>
<td>40 senile dementia 43 elderly controls 15 young controls</td>
<td>Elderly controls lived in a retirement home.</td>
<td>T74 μg/dL vs 83 μg/dL in elderly controls and 165 μg/dL in young controls</td>
<td>Not measured</td>
<td>In vitro thymulin was low in elderly and partially corrected by Zn addition to media. In vitro response of lymphocytes to mitogens was poor in elderly.</td>
<td>More evidence of poor immune response in elderly, who had low plasma Zn values.</td>
</tr>
</tbody>
</table>
Table I. Survey Data, Zinc Concentrations and Immunological Findings in the Elderly (Continued).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Number &amp; Age of Subjects</th>
<th>Factors Affecting Interpretation</th>
<th>Zinc Value Results</th>
<th>Dietary Intake of Zn/Day</th>
<th>Immunological Findings</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>9. Murasko et al., 1986</td>
<td>Controlled cross-sectional survey</td>
<td>250</td>
<td>70-106 yrs. 23-35 yrs.</td>
<td>Subjects grouped into decades. Elderly were living independently</td>
<td>Not measured</td>
<td>Not measured</td>
<td>All elderly decades &lt;controls in mitogen stimulated lymphocytes. No significant differences in NK activity between aged and controls.</td>
</tr>
<tr>
<td>11. Pennington &amp; Young, 1981</td>
<td>Dietary survey</td>
<td>8 groups studied</td>
<td>60-65 yrs.</td>
<td>Cross sectional survey</td>
<td>Not measured</td>
<td>Elderly &lt;12.9 mg, &lt;8.7 mg.</td>
<td>None measured</td>
</tr>
<tr>
<td>12. Salih et al., 1988</td>
<td>Controlled survey</td>
<td>260</td>
<td>80.5 yrs. 72 yrs.</td>
<td>Long-term care patients free of terminal or wasting illness</td>
<td>&lt;96.1 µg/dL, &lt;89.6 µg/dL, &lt;96.4 µg/dL</td>
<td>&lt;11 mg 49% below 10 mg, &lt;10 mg 58% below 10 mg</td>
<td>None measured</td>
</tr>
<tr>
<td>13. Senapati et al., 1989</td>
<td>Controlled survey</td>
<td>34 with chronic ulcers</td>
<td>81.3 yrs. 60.8 yrs.</td>
<td>25 free living 77.7 yrs. 19 healthy younger 61.3 yrs.</td>
<td>83 +/-2 µg/dL, 65.6 mg/mg dry wt. * 84 +/-2 µg/dL, 61.3 mg/mg dry wt. * 105 +/-9 µg/dL, 66.1 mg/mg dry wt. * 118 +/-11 µg/dL, 58.2 mg/mg dry wt. * WBC</td>
<td>Hospital diet 9.5 mg Zn/d</td>
<td>None measured</td>
</tr>
<tr>
<td>14. Thomas et al., 1988</td>
<td>Uncontrolled survey</td>
<td>21 long-stay pts.</td>
<td>61.7 yrs. 14 had chronic ulcers</td>
<td>70 µg/dL Zn=9µmol/10^6 WBC</td>
<td>5.6 µg/dL</td>
<td>None measured</td>
<td>None measured</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Number &amp; Age of Subjects</td>
<td>Factors Affecting Interpretation</td>
<td>Zinc Value Results</td>
<td>Dietary Intake of Zn/Day</td>
<td>Immunological Findings</td>
<td>Assessment of Study</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------</td>
<td>--------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>15. Thompson et al., 1984</td>
<td>Controlled survey</td>
<td>17 centenarians 100–103 yrs.</td>
<td>All healthy, T in long-care facility. Comparisons made with unstated controls.</td>
<td>Plasma Zn: Not measured</td>
<td>Not measured</td>
<td>Unusually high incidence of Class I HLA antigens on blood leucocytes. T-helper-inducer cell numbers decreased more than two-fold. T-suppressor-killer cell numbers normal, so T-4/T-8 ratio was low. NK cells and activated immature T cells were increased, as were early B cells. In vitro lymphocyte response to mitogens was depressed. T cell production of IL-2 was depressed.</td>
<td>Excellent study of certain immunological parameters in healthy centenarians, but no relationships to Zn status were measured. Young T,B, and NK cells appear to fail to differentiate to normal maturity.</td>
</tr>
<tr>
<td>16. Thompson et al., 1987</td>
<td>Review with some survey data</td>
<td>7♂ 11♀ 100+ yrs.</td>
<td></td>
<td>0.7 mg/d +7.8 mg/d</td>
<td></td>
<td></td>
<td>Supplied Zn intake data missing from earlier study (#16).</td>
</tr>
<tr>
<td>17. Turnlund et al., 1986</td>
<td>Controlled prospective study of Zn absorption and retention</td>
<td>6 elderly (♂) 65–74 yrs. 6 control (♀) 22–30 yrs.</td>
<td>All healthy, n studied with stable isotopes (67 Zn and 115Zn) during 12 wks. on a metabolic balance ward.</td>
<td>Changes in Zn from start to end of study. Serum 78–98 μg/dL (♂) 67–86 (♂) 105–113 μg/dL (♀) 69–94 (♀) Fecal mg/d 14.2–14.6 (♂) 15.3–14.1 (♀)</td>
<td>15.4 mg/d 17–18 (♂) 15.4 mg/d 29–33 (♀)</td>
<td>Absorbed</td>
<td>Well done study showing smaller absorption of dietary Zn in elderly, but also smaller losses of endogenous Zn. Zn in mg/d Balance: (♂) +0.6–0.1 (♀) +0.8–0.4 Absorbed: (♂) 2.7–2.8 (♀) 4.6–5.1 Endogenous Loss: (♂) 1.9–2.2 (♀) 3.8–4.5</td>
</tr>
<tr>
<td>18. Wagner et al., 1983</td>
<td>Uncontrolled survey</td>
<td>173 60–97 yrs.</td>
<td>Rural and urban low-income subjects.</td>
<td>92 mg/dL 140 mg/g hair</td>
<td>7.3 mg/d</td>
<td>27/121 subjects were anergic on DDH tests with 4 antigens. Plasma Zn correlated with intake of Zn. Plasma Zn lowest in anergic subjects. See #7 in Table 2.</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Number &amp; Age of Subjects</td>
<td>Factors Affecting Interpretation</td>
<td>Zinc Value Results</td>
<td>Dietary Intake of Zn/Day</td>
<td>Immunological Findings</td>
<td>Assessment of Study</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------</td>
<td>--------------------------</td>
<td>----------------------------------</td>
<td>--------------------</td>
<td>--------------------------</td>
<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>19. Lichtenstein et al., 1982</td>
<td>Uncontrolled survey</td>
<td>47 70 yrs.</td>
<td>All were healthy</td>
<td>Not measured</td>
<td>Not measured</td>
<td>2-4/7 failed to show &gt;0.6 mm induration to any of 5 antigens used for DTH responses</td>
<td>Percentage of anergic subjects roughly equivalent to that of other studies</td>
</tr>
<tr>
<td>20. Wood &amp; Hansen, 1988</td>
<td>Controlled study</td>
<td>162 45-75 yrs.</td>
<td>All postmenopausal Some lactose intolerant</td>
<td>Not measured</td>
<td>7.0 mg/dl Zn absorption depressed equally by milk and lactose-free milk in both groups as compared to water or lactose in water</td>
<td>Not measured</td>
<td>Solid evidence for the suppression of elemental Zn absorption by milk.</td>
</tr>
<tr>
<td>21. Flint et al., 1981</td>
<td>Semi-controlled survey</td>
<td>Community 24 70.1 yrs. Institutionalized 66 82.2 yrs.</td>
<td>Both community-based and institutionalized</td>
<td>88.3 μg/dL (C) 91.5 μg/dL (I) Plasma Zn correlated with serum albumin</td>
<td>11 μg/dL (C) 7.6 μg/dL (I) Plasma Zn not correlated with Zn intake</td>
<td>Not measured</td>
<td>Demonstrates low Zn intake in institutionalized elderly.</td>
</tr>
</tbody>
</table>

Abbreviations:
- DDH: Delayed dermal hypersensitivity
- PMN: Polymorphonucleocyte
- RBC: Red blood cells
- WBC: White blood cells
### Table II. Zinc Supplementation of Healthy Elderly Subjects.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design/Duration</th>
<th>Number &amp; Age of Subjects</th>
<th>Source &amp; Identity of Test Supplements</th>
<th>Dosage</th>
<th>Base Diet</th>
<th>Additional Treatments</th>
<th>Adverse Effects</th>
<th>Other Factors Affecting</th>
<th>Interpretation</th>
<th>Results</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Duchateau et al., 1981</td>
<td>Clinical intervention trial with controls.</td>
<td>30 total 70+ 15 test = 81±5 yrs. 15 control = 80±4 yrs.</td>
<td>Zinc sulfate</td>
<td>220mg 2x/day 175mg/d Zn</td>
<td>Usual institutional food</td>
<td>All testing was blinded. Tetanus vaccination</td>
<td>Transient nausea; mild diarrhoeas</td>
<td>Healthy institutionalized subjects. No measurement of Zn status</td>
<td>Treated group showed improved DH1 responses and post-vaccine titers, but no change in mitogen responses.</td>
<td>Small but definite immunologic test improvement, in recall and memory effects, but no data on prior Zn status.</td>
<td></td>
</tr>
<tr>
<td>2. Bogden et al., 1988</td>
<td>Same as above but double blinded. 36 months</td>
<td>104 total 36 70.9 yrs. 31 71.4 yrs. 36 71.3 yrs.</td>
<td>Zinc acetate capsules</td>
<td>15mg elemental Zn/d 100mg elemental Zn/d</td>
<td>8.5mg/d 8.81mg/d 8.3mg/d (Estimated from uncontrolled diet at home)</td>
<td>All subjects received multi-vitamin/multi-mineral (~Zn) supplement daily.</td>
<td>Free living healthy subjects. Many had poor lymphocyte responses to mitogens and some had skin test reactivity.</td>
<td>Plasma Zn increased only in 100mg group. Cellular Zn unchanged. Slight increase in DH1 of all groups. Slight increase in mitogen response of all groups.</td>
<td>None of the immune function improvements could be ascribed to Zn. A carefully done study.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Bogden et al., 1990</td>
<td>Same as above, but double blinded. 12 months with 4 additional month follow-up with all subjects receiving placebo.</td>
<td>63 total 20 71.0 yrs. 19 72.1 yrs. 24 71.2 yrs.</td>
<td>Same as above</td>
<td>Uncontrolled diet at home. Zn estimated</td>
<td>Same as above, plus supplement continued for 16 months.</td>
<td>Same as above</td>
<td>Plasma Zn increased at 3, 6, and 12 months only in 100 mg group. Cellular Zn unchanged. DH1 doubled in placebo group. Increase was suppressed by Zn. Increase in lymphocyte response to mitogens was significantly greater in placebo group. A transient increase in NK activity occurred only at 3 months in the group receiving 100mg Zn.</td>
<td>Immune functions were helped by multi-vitamin/mineral supplements, but this was slightly suppressed by Zn. This is the most complete study reported to date.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Hattoubi et al., 1988</td>
<td>Uncontrolled intervention trial 3 months</td>
<td>20 total 10+ 65 yrs. 10+ 95 yrs.</td>
<td>Oral Zn supplement. Form not stated.</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Studies of erythropoiesis and testosterone</td>
<td>None mentioned</td>
<td>No immunological studies</td>
<td>Increase in plasma Zn from 75 to 103 μg/dL in 72 to 106 μg/dL in 9.</td>
<td>Of little value. Unknown Zn dose. No immunological data.</td>
<td></td>
</tr>
</tbody>
</table>
Table II. Zinc Supplementation of Healthy Elderly Subjects (Continued).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design/Duration</th>
<th>Number &amp; Age of Subjects</th>
<th>Source &amp; Identity of Test Supplements</th>
<th>Dosage</th>
<th>Base Diet</th>
<th>Additional Treatments</th>
<th>Adverse Effects</th>
<th>Other Factors Affecting Interpretation</th>
<th>Results</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Cassack, 1989</td>
<td>Clinical intervention trial with controls.</td>
<td>8 yr 66-78 yrs. 13 agerilx-matched controls</td>
<td>Zn acetate capsules</td>
<td>60mg/d elemental Zn.</td>
<td>Uncontrolled diet at home.</td>
<td>2 baseline treatments</td>
<td>None mentioned</td>
<td>Low socioeconomic status of subjects selected for study because of low Zn values and anergy.</td>
<td>Increase in Zn values. Plasma 75-115µg/dL. RBC 30-43µg/dL Hgb Lymphocytes 35-61µg/dL cells PMNs 48-67µg/dL cells Increased RBC count Increased DDH number 55-78% Increased DDH size 7.8-14.1 mm.</td>
<td>Supplementation of Zn deficient elderly men improved Zn values and DDH although the skin tests did not fully reach control values. A well done study.</td>
</tr>
<tr>
<td>6. Soltesz et al., 1988</td>
<td>Uncontrolled intervention trial with before/after analysis.</td>
<td>6 total “elderly” 37, 39</td>
<td>Zn Ghaonate</td>
<td>5mg 2x/d = only 3.8mg elemental Zn/d</td>
<td>Nursing home food, weighed and recorded.</td>
<td>Dietary Zn range 6.8-13mg/d</td>
<td>None mentioned</td>
<td>Nursing home subjects. All patients carefully screened for any confounding problem. DDH called negative if &lt; 6mm.</td>
<td>Slight increase in DDH reaction number, barely significant increase in plasma Zn (88-102µg/dL).</td>
<td>A poor study. Dose of Zn was only 3.8mg/dL. Results were not impressive.</td>
</tr>
<tr>
<td>7. Swanson et al., 1988</td>
<td>Controlled intervention trial.</td>
<td>53 total 77 yrs. including 17 supplemented 77.5 yrs. 10 placebo 76.4 yrs.</td>
<td>Zn acetate capsules</td>
<td>20mg elemental Zn/d</td>
<td>Institutional food evaluated for Zn.</td>
<td>None mentioned</td>
<td>Healthy residents of a public housing facility. No initial difference in plasma or urine Zn. Both normal.</td>
<td>Supplemented group increased plasma Zn (87-106µg/dL) and urinary Zn but cellular Zn was unchanged.</td>
<td>A limited study. Subjects can absorb extra Zn at this dose. No immunological data.</td>
<td></td>
</tr>
</tbody>
</table>
### Table II. Zinc Supplementation of Healthy Elderly Subjects (Continued).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design/Duration</th>
<th>Number &amp; Age of Subjects</th>
<th>Source &amp; Identity of Test Supplements</th>
<th>Dosage</th>
<th>Base Diet</th>
<th>Additional Treatments</th>
<th>Adverse Effects</th>
<th>Other Factors Affecting Interpretation</th>
<th>Results</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. Wagener et al., 1983</td>
<td>Questionably controlled, small intervention study. 1 month</td>
<td>173 total 74±7 +42% 5 anergic subjects given Zn supplements</td>
<td>Zn sulfate 55mg/d +2mg/d elemental Zn</td>
<td>Regular household food. Zn intake based on 24-hour recall, average 7.3mg</td>
<td>None</td>
<td>None mentioned</td>
<td>Subjects from poor rural households. 22% anergic on DDH testing.</td>
<td>Dietary Zn correlated with plasma Zn but not with hair Zn. An increase of 18μg/dl after supplementation in 5 subjects and anergy was reversed.</td>
<td>22μg of elemental Zn reversed DDH anergy in 5 subjects.</td>
<td></td>
</tr>
<tr>
<td>9. Wilson &amp; Mykow, 1985</td>
<td>Small intervention study. 1-6 weeks supplementation given 18 yrs.</td>
<td>150± 75.6 yrs. 84± 78.5 yrs.</td>
<td>Zn citrate 300 μg/d +24 μg elemental Zn given to 18 patients</td>
<td>Hospital food</td>
<td>None</td>
<td>None mentioned</td>
<td>No immunologic testing. No information on diseases of patients.</td>
<td>Plasma Zn normalized in 12, improved in 4, but fell in 2 despite Zn.</td>
<td>Variability in response may have been related to disease.</td>
<td></td>
</tr>
<tr>
<td>10. Brucker et al., 1988</td>
<td>R, double blinded, vaccine study. 8 weeks</td>
<td>41 healthy, 64-90 yrs. 23 supplemented 18 controls</td>
<td>Zn gluconate 50 mg Zn/d</td>
<td>Uncontrolled diet at home</td>
<td>Trivalent influenza vaccine at 4 wks.</td>
<td>None mentioned</td>
<td>All mineral-vitamin supplements were stopped 3 months prior to study.</td>
<td>Serum Zn unchanged in control, Inc. 45% in treated group, no effect on serum Cu. No effect of Zn supplementation on serum antibody response to influenza vaccine.</td>
<td>No effect of Zn supplement on influenza immunization.</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:**

DDH: Delayed dermal hypersensitivity

R: Random
Table III. Zinc Toxicology.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Number &amp; Age of Subjects</th>
<th>Factors Affecting Interpretation</th>
<th>Zinc Value Results Plasma Zn</th>
<th>Dietary Intake of Zn/day</th>
<th>Immunological Findings</th>
<th>Assessment of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chandra, 1984b</td>
<td>Controlled prospective study</td>
<td>11 yr adults non-supplemented. Controls mentioned but not detailed.</td>
<td>All healthy volunteers given 150 mg ionic Zn 2x/day for 6 wks. as Zn sulfate. Copper status of subjects not measured</td>
<td>83 μg/dL →→ 101 at 2 wks. of Zn 182 at 4 wks. of Zn 200 at 6 wks. of Zn 167 at 2 wks. post Zn 90 at 10 wks. post Zn</td>
<td>10.1-12.0 mg/day</td>
<td>632→→585 PMN migration &amp; 492→→386 PMN Phagocytosis at 2 wks. →→255 PMN migration →→272 PMN Phagocytosis at 4 wks. →→292 PMN migration →→251 PMN Phagocytosis at 6 wks. →→361 PMN migration →→198 PMN Phagocytosis at 8 wks. Post →→576 PMN migration →→459 PMN Phagocytosis at 10 wks. Post</td>
<td>Excellent study clearly showing inhibitory effects by 300 mg daily ionic Zn on PMN migration and phagocytosis and on lymphocyte mitogenesis. Supplements caused a 24% increase in plasma Zn. No significant changes in total blood lymphocyte numbers, or in Bc, T4+ or T8+ percentages.</td>
</tr>
<tr>
<td>3. Bugden et al., 1988 &amp; 1990</td>
<td>See Table 2</td>
<td>See Table 2</td>
<td>See Table 2</td>
<td>See Table 2</td>
<td>See Table 2</td>
<td>See Table 2</td>
<td>Although not planned as a Zn toxicity study, 15 mg/d &amp; definitely 100 mg/d inhibited some of the positive immunological effects of the multimineral placebo.</td>
</tr>
</tbody>
</table>