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THE EFFECTS OF DIETARY ZINC DEFICIENCY ON THE HUMORAL IMMUNE RESPONSE OF THE YOUNG ADULT A/J MOUSE

By

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ABSTRACT

THE EFFECTS OF DIETARY ZINC DEFICIENCY
ON THE HUMORAL IMMUNE RESPONSE
OF THE YOUNG ADULT A/J MOUSE

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Suzanne Marie Haas

Despite the devastating effects of disease in malnourished populations, little attention has been focused on the relationship of malnutrition and immunity. This thesis will investigate this relationship by studying the effects of dietary zinc deficiency on the humoral immune response of the young adult A/J mouse.

Mice were injected with KLH-ars on the same day that they were placed on a deficient diet. The titer of the deficient group to the antigen was identical to that of controls, indicating that B cells could produce antibody once stimulated by healthy T cells. However, when mice were injected with SRBC after three weeks on a zinc deficient diet, the mice had a reduced indirect plaque forming ability in the primary and secondary responses to antigen, which could be significantly restored by reconstitution with thymocytes. This suggested that T cell helper activity was impaired in zinc deficient animals.

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LIST OF SYMBOLS AND ABBREVIATIONS

BGG	bovine gamma globulin
BGG-ars	bovine gamma globulin-p-azophenylarsonate
DEAE-cellulose	Diethylaminoethyl cellulose
KLH	keyhole limpet hemocyanin
KLH-ars	keyhole limpet hemocyanin-p-azophenylarsonate
MEM	Eagle's Minimum Essential Medium with Earle's Balanced Salt Solution
PCM	protein calorie malnutrition
PFC	plaque forming cells
RIA	radioimmunoassay
SRBC	sheep red blood cells
TCA	trichloroacetic acid

INTRODUCTION

The devastating effects of disease in malnourished populations have been well known for many years. In one study in rural Guatemala, an enhanced fatality rate, greater frequency of complications, and an exaggerated clinical course were observed in malnourished children with the common childhood diseases of measles, whooping cough, mumps, rubella, and chickenpox when compared to well-fed children of higher socio-economic status (1). However, despite the publication of many similar reports from epidemiological studies of other malnourished populations in the world, few clear-cut immunological studies have been attempted to delineate the role of nutritional deficiencies on immunity. As a result, little is known about the effects of nutritional status on an individual's immune response, thus emphasizing the need for further research in this area.

The aim of this thesis was to initiate a series of investigations to explore the relationship of malnutrition and immunity by studying the effects of dietary zinc deficiency on the immune response. Investigation of zinc deficiency was chosen because zinc is an important trace metal in mammalian metabolism and it also can be easily monitored by atomic absorption spectroscopy. In addition, the increasing importance in the world's diet of cereal grains, which contain many zinc chelating compounds, suggested the possibility of large scale zinc deficiency in the future. Therefore, studies are needed to

define all the consequences of zinc deficiency, including its effects on the immune system.

Published reports on experimental zinc deficiency in animals have demonstrated the wide range of known effects of zinc deficiency. Zinc deficient rats (2) and pigs (3, 4) have exhibited poor growth, anorexia, skin lesions, a decrease in spermatogenesis, and in some cases, a decrease in specific activity of zinc metalloenzymes in the deficient tissues. Hurley (5) has shown that maternal zinc deficiency in rats resulted in a decreased zinc content of the fetuses, smaller litter size, a decreased birth weight, and an increase in the incidence of severe congenital malformations in the offspring.

Zinc deficiency has also been described in man, although it is not as yet a major nutritional problem in the world. A study of 338 persons in the Denver, Colorado area has documented the existence of 10 children with low levels of zinc in their hair who were small for their age, had a history of poor appetite, and decreased taste acuity, symptoms characteristic of experimental zinc deficiency in animals (6).

The major study to date on zinc deficiency in human subjects was a report by Sandstead (7) in 1967 on zinc deficiency in male dwarfs in the Middle East. In addition to low levels of zinc in the hair, sweat, urine, and plasma, the adolescent boys studied by Sandstead exhibited retarded growth, hepatosplenomegaly, hypogonadism, and a delayed closure of the long bones. Supplementation of the diet of these children with zinc sulfate produced a reversal of these symptoms with dramatic increases in growth and sexual development. Sandstead has hypothesized that the diet of these children which consisted almost entirely of cereal grains, contained large amounts of

phytic acid, which chelated zinc, forming insoluble complexes which were excreted from the body. With a world population that must of necessity become more dependent on economically efficient cereal grains as a primary food source, increasing numbers of zinc deficient individuals may be found with severe growth retardation and sexual development similar to that observed in the Middle East.

Despite these reports enumerating the many serious effects of zinc inadequacy on the deficient animal and man, the growing numbers of recognized zinc deficient individuals, and the depression of immunity in malnourished persons, little is known about the effects of zinc deficiency on immunity. In two separate studies, weanling pigs (4) and rats (8) on zinc deficient diets were found to have a severe atrophy of the thymus that was absent in the pair fed controls. Brummerstedt (9) has also described an autosomal recessive trait of cattle characterized by thymic atrophy, loss of hair, and parakeratotic lesions. Oral zinc oxide therapy resulted in an increase in weights and the recovery of the skin lesions. He has hypothesized that these calves were unable to utilize zinc in their food and that the presence of zinc was important for the development of the thymus. Since the thymus has been shown to be responsible for the development of immunologically competent T cells, which play an important role in both cellular and humoral immunity, the wasting of this organ by zinc deficiency suggested that T cells and therefore the immune response was being impaired.

A depression of immunity in human zinc deficiency was indicated in a study of a patient with terminal acrodermatitis enteropathica, thought to be a genetic deficiency of zinc absorption (10). Analysis of the serum of this patient showed undetectable levels of immunoglobulins.

Death resulted from infection with E. coli and C. albicans. Postmortem studies on the same child showed no germinal centers in the spleen, a lack of lymph nodes, and no tonsils or Peyer's patches. The thymus consisted of epithelial cells with a few thymocytes and Hassal's corpuscles. Although these isolated reports in the literature have suggested a relationship between decreased immune response and zinc deficiency, no actual tests of immune function were performed.

Other investigators have attempted to define the effects of a variety of nutritional deficiencies on the immune response. Several researchers have recently shown that metal deficiencies may result in a depressed immune response. Magnesium deficiency in rats decreased both agglutinin and hemolysin titers to sheep red blood cells (11). Nalder (12) reported that iron deficiency in rats caused a depression of anti-tetanus toxoid titer in proportion to the degree of iron depletion. In a study of 20 iron deficient children, Chandra (13) found no differences in IgG levels and antibody titers to tetanus toxoid and S. typhi antigens when these children were compared to well-fed children of the same age. However, cell mediated immunity, measured by bactericidal activity of macrophages, and cutaneous delayed type hypersensitivity to C. albicans and other antigens was impaired.

A. E. Axelrod has investigated the effects of specific vitamin deficiencies on the immune response in several animal models. An impairment of antibody formation following a single injection of diptheria toxoid was found in pantothenic acid, riboflavin, pyridoxine, biotin and vitamin D deficiencies in rats (14). No significant differences between experimental and control animals were found in Vitamin A or thiamine deficiency, nor could the observed results be attributed to reduced intake

in the experimental animals. Vitamin B_6 deficient guinea pigs studied by the same investigator had a reduced cutaneous delayed type hypersensitivity response to BCG which was reversible by administration of the missing vitamin (15). Rats deficient in B_6 had a high percentage of successful skin transplants (16). Axelrod's results have indicated that both the humoral and cell mediated limbs of the immune response may be affected by vitamin deficiencies. However, the variety of model systems used and the lack of detailed and quantitative experiments on the effects of single vitamin deficiencies on the humoral and cell mediated responses of these animals have generated a vague and unclear picture of the immunological effects of vitamin inadequacies.

Amino acid deficiencies have also produced deleterious effects on the immune response of rats. Jose (17) investigated the effects of a decrease of single essential amino acids to 50%, 25% and 10% of their required levels in mice on the primary response to DBA/2 mammary adenocarcinoma cells. A 25% reduction of phenylalanine-tyrosine, valine, threonine, methionine, cysteine, isoleucine and tryptophan produced large decreases in hemagglutinating and blocking antibody activity. Cell mediated immunity, measured by in vitro assay, was not affected until amino acid levels were reduced to 10%. However, only a slight depression of immunity was observed with arginine, histidine, and lysine. Leucine was unique in that cell mediated responses were lowered before a depression in humoral response could be detected. Individual amino acid deficiencies appeared to be complex to study because the extent of immunological impairment seemed to be dependent on the particular amino acid that was absent and the level of deficiency. Although this report has provided interesting data, no attempt was made by the authors to explain their results or determine the

specific effects of the individual amino acid deficiencies on the components of immunity.

Because it is the world's most pressing nutritional problem, protein calorie malnutrition (PCM) has been the most thoroughly studied nutritional deficiency. Many reports have shown that PCM also produced adverse effects on immunity. Several investigators have observed atrophy of the lymphoid organs and reduced lymphoid germinal centers in protein or calorie depleted animals (18, 19). Winick (20) has investigated the effects of three weeks of calorie deprivation and subsequent refeeding on organ weight in the neonatal, weanling and adult rat. Although most organs showed signs of wasting in growing rats, only the thymus, the site of maturation of immunologically important T cells, was permanently atrophied by calorie deprivation in all of the age groups studied. Although the reduced size of this organ suggested immunological impairment, no ascessment of immunological status was attempted.

The humoral response of protein calorie deficient animals to a number of different antigens has also been studied. Marasmic pigs produced antibody titer that was equal to that of controls using tetanus toxoid, ΦX174, or sheep red blood cells as antigen, but produced reduced amounts of antibody following injection of erythrocyte A antigen (18). No differences were noted between the two groups with respect to IgG or IgM concentration.

A decrease in the number of plaque forming cells (PFC) in response to a single injection of sheep red blood cells has been observed by many investigators with PCM induced in a number of different animal strains (19, 21, 22). Impairment of T cells by PCM was suggested in the study by Mathur (19), in which injection of syngeneic thymocytes improved the number of plaques observed significantly over similarly treated controls.

Chandra (23) observed a decrease in PFC not only in calorie deprived rats, but also in the F_1 and F_2 generations of these rats. These results have important clinical implications for the malnourished woman and strongly emphasize the importance of adequate nutrition during pregnancy.

Several indeces of cell mediated immunity have been studied in animals with PCM. Skin allograft survival was increased from 9 days in control mice to 16 days in the experimental groups, indicating an impairment of T cells (21). Macrophage clearance of carbon particles was reduced in mice (24) as was delayed type hypersensitivity to DNCB and tuberculin antigens in marasmic pigs (18). Conversely, the results of Cooper (25) indicated that protein depletion had an enhancing effect on cell mediated immunity as shown by a decrease in survival time of skin grafts, an increase in PHA stimulation, an increased ability to ingest bacteria, and an enhanced resistance to pseudorabies virus in mice. Although the results of these investigations did not agree with the impairment of immunity observed in other animal systems, the authors did not attempt to explain this discrepancy.

The contradictory results obtained by these investigators have pointed out even more strongly the importance of good immunological studies in nutritionally deficient model systems. However, the isolated studies in this area, the variety of animal strains and species employed, and the lack of specific investigations on the effects of nutritional deficiencies on animals of different ages have made it difficult to construct a clear picture of the link between nutrition and immunity. Important studies such as the effects of marginal deficiencies, and the long range effects of deficiencies produced at various ages and for different lengths of time have also been lacking in these model systems, and are

essential to an understanding of the consequences of various states of human malnutrition. Therefore, rigorous immunological studies in a well-defined system are needed to define more precisely the role of malnutrition in the immune response.

In addition to exploring the effects of PCM in animal models, some investigators have also studied the effects of PCM in human subjects from underdeveloped nations, although these studies have been limited because ethical standards do not permit the maintenance of chronic deficiency in malnourished patients. The startling implications of these reports have further emphasized the deleterious effects of malnutrition on the lymphatic system.

The histopathology of PCM in Ugandian children was studied in detail by Watts (26). Postmortem reports compiled on a number of children with marasmus and kwashiorkor by this investigator showed a decrease in the ratio of thymus weight to body weight. Histological reports revealed that the cells of the thymus were replaced by fibrous tissue. Another postmortem study of South African children by Schonland (27) confirmed the presence of the severe atrophy of the thymus seen by Watts and also noted the occurence of lower spleen weights, reduced tonsilar area, decreased thickness of Peyer's patches in the intestine, and reduced cross-sectional thickness of the appendix in children diagnosed as suffering from kwashiorkor or marasmus. Other lymphatic structures such as the paracortical areas of the lymph nodes and the germinal centers of the various lymphatic organs were also depleted. These results have provided histological support for the impairment of immune function seen in nutritionally deficient populations.

Despite the establishment of large scale immunization programs in many undernourished countries, few studies have attempted to evaluate

the effectiveness of immunization in these individuals who have shown a reduced ability to combat disease. Ugandian children studied by Brown and Katz produced a normal response to smallpox virus (28) whereas antibody production was decreased as compared to adequately nourished controls when immunized with yellow fever vaccine (29). Serum immunoglobulins were increased in 76 undernourished children studied in Ghana, although the antibody titers were identical between these children and controls to the antigen keyhole limpet hemocyanin and pneumococcal polysaccharide (30). Opposite results were obtained by Coovadia (31) who showed that serum IgG was within normal limits in South African children with marasmus and kwashiorkor, as was the percentage of B cells in peripheral blood. However, antibody levels to specific antigens were reduced. The variety of results obtained by these investigators has emphasized the difficulties of studying these children, especially since PCM differs qualitatively and quantitatively in different parts of the world and may be complicated by the presence of infections.

Various parameters of cell mediated immunity have also been assayed in children with kwashiorkor and marasmus. Neumann (30) found that malnourished children in Ghana had decreased cutaneous delayed type hypersensitivity to Monilia, streptokinase-streptodornase, and phytohemagglutinin both in terms of smaller diameters of inflammatory sites and a higher percentage of nonreactors. In vitro stimulation of lymphocytes by phytohemagglutinin, a mitogen specific for T cells, was also reduced in some individuals with PCM (30, 32, 33), although not affected in other cases (31, 34, 35). Interpretation of these conflicting results was difficult because these studies were performed on subjects of various ages and heterogeneous nutritional and immunological backgrounds.

Douglas (36) investigated the effects of PCM on phagocytic function in 16 children with kwashiorkor. Phagocytic activity, the activity of hexose monophosphate shunt enzymes, and morphological studies by electron microscopy showed no differences between controls and depleted individuals. However, bactericidal activity was reduced in the malnourished individuals. Similar results were reported by Seth (37).

Although these reports have indicated that the immune response was depressed in malnourished populations, conflicting results due in part to complications such as infection and varying degrees of malnutrition have made difficult an analysis of the specific effects of PCM on the humoral and cell mediated aspects of immunity in man. In addition, few investigators have explored the nutritional and immunological backgrounds of the individuals under study or have performed follow-up studies on the consequences of malnutrition present in these children to their immunological status as an adult and the effects on their offspring.

The lack of thorough, comprehensive studies on any nutritional deficiency in man or animals, as demonstrated by the review of the literature given here, has resulted in a very unclear picture of the nature of the relationship of malnutrition and immunity. This has pointed out the need for detailed immunological studies of a well-monitored dietary deficiency produced in a defined population. This thesis attempted to initiate such studies by investigating the effects of dietary zinc deficiency on the immune response of the inbred A/J mouse. Inbred mice were used as a model system because they assured a homogenous population for study and eliminated the problems of individual variability, complicating infections, and heterogeneous nutritional backgrounds present in

many of the earlier nutritional studies. The use of inbred mice will also make possible the ultimate goal of monitoring the effects of zinc deficiency on the neonatal, weanling, and adult mouse to construct a complete picture of the consequences of inadequate zinc on the humoral and cell mediated responses of essentially a single individual. In addition, other questions, such as the effects of marginal deficiency and the effects of short periods of nutritional inadequacy can be easily explored in this system.

This thesis has investigated the effect of dietary zinc deficiency on the humoral response of the young adult A/J mouse. The adult was chosen for these initial experiments because the effects of zinc deficiency were more easily defined in a mouse with a fully developed immune system. The results of these studies are important since they will provide a framework for future research toward elucidating the role of malnutrition in the immune response.

MATERIALS AND METHODS

Materials

Zinc standard solutions were obtained from Harleco Corp., Philadelphia, Pennsylvania. Freund's adjuvant was purchased from Difco Laboratories, Detroit, Michigan. Miles Laboratories, Kankakee, Illinois were the source of bovine gamma globulin and non-hemolytic guinea pig complement. Sheep red blood cells were obtained from Cordis Laboratories, Miami, Florida. Seakem plaque agarose was purchased from Marine Colloids Inc., Rockland, Maine. Calbiochem, San Diego, California, was the source of keyhole limpet hemocyanin. Arsanilic acid was obtained from Eastman Chem. Co., Rochester, New York, and recrystallized from water and ethanol before use. All other chemicals were analytical or reagent grade and purchased from a variety of commercial sources. ^{125}I was obtained from New England Nuclear, Boston, Massachusetts.

Mice of the A/J strain were derived from inbred stocks, maintained and distributed by Jackson Laboratories, Bar Harbor, Maine. Rabbits were purchased from the Center for Laboratory Animal Resources, Michigan State University, E. Lansing, Michigan.

Animals

Female mice of the A/J strain were used for all experiments. The mice were housed in acid washed polycarbonate cages with stainless steel tops. Diets and deionized water, zinc free by atomic absorption assay,

were provided ad libitum in all experiments.

Diet Preparation

The diet used for these experiments was an egg white based, biotin fortified diet, designed by Luecke (2) for the rat. Zinc was added to the diet in the form of zinc carbonate.

Preparation of the vitamin-glucose mixture. (Table 1) The choline chloride was premixed with an equal amount of glucose monohydrate to assure even mixing of these components and to avoid clumping of the diet. Small increments of glucose monohydrate were combined with the mixture until 200 to 300 grams of the glucose were added. The rest of the vitamins and glucose monohydrate were then added to the choline chloride-glucose mixture, stirred well by hand, placed in a Hobart mixer, and stirred on speed 1 for 20 minutes in the dark.

Mixing of the mouse diet. (Table 2) The liquid ingredients of the diet, corn oil, vitamins A, D, E, and K, and santoquin antioxidant were combined and mixed with a glass stirring rod. The dry components of the diet were placed in a large bowl on the Hobart mixer and stirred at speed 1. While mixing, the liquid ingredients were slowly added to the rest of the diet. Mixing was continued for an additional 20 minutes. The diets were stored in acid washed plastic jars at 4°C for the duration of each experiment.

Zinc Assay by Atomic Absorption Spectroscopy

A known weight of sample to be assayed for zinc content was added to a preweighed acid washed flask. 25 ml of concentrated nitric acid was added to the flask and the sample was slowly digested over low heat to a volume of approximately 5 ml. 2 ml of perchloric acid and 25 ml of concentrated nitric acid were added to the sample and the digestion repeated,

Table 1

Vitamin-glucose mixture

	g/kg
Vitamin B_{12} in Mannitol	0.01
d-Biotin	0.10
Folic Acid	0.02
Pyridoxine-HCl	0.10
Riboflavin	0.20
Thiamine Mononitrate	0.20
Calcium Pantothenate	0.50
Nicotinic Acid	0.50
Inositol	2.00
p-Aminobenzoic Acid	1.00
Ascorbic Acid	1.00
Choline Chloride	30.00
Glucose Monohydrate	964.40

Table 2

Diet Ingredients

	g/kg
Egg White	200
Glucose Monohydrate	569
Corn Oil	100
Vitamin-Glucose Mixture[1]	50
Mineral Mixture ± Zinc[2]	45
Cellulose	30
Vitamins A, D, E, and K	5
Santoquin Antioxidant	1

[1]Table 1.

[2]Phillips-Hart Salt Mixture No. 4 (38).

evaporating the liquid to near dryness. The residue remaining after this treatment was diluted to a known weight with 1% hydrochloric acid. Blanks containing only the acid reagents were prepared with each series of samples that were assayed.

Zinc concentration of the digested samples was determined with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer, using a single electrode zinc cathode tube. Absorption values were determined at 2138 angstroms. A standard curve for zinc absorption was obtained by dilution of a commercial zinc standard solution in 1% hydrochloric acid. This method was accurate to 0.2 ppm zinc.

Coupling of the Diazonium Salt of Arsanilic Acid to Proteins

The coupling of arsanilic acid to proteins was performed by the method of Nisonoff (39). One volume of a 1.2 mg/ml solution of the diazonium salt of arsanilic acid in 1 N hydrochloric acid was added dropwise to ten volumes of a 20 mg/ml solution of the desired carrier protein, with vigorous stirring at 4°C, maintaining the pH between 9.0 and 9.5. After addition of all reagents, the reaction mixture was allowed to stir for an additional hour. The reaction product was dialyzed for a week against several changes of borate saline buffer to remove all unreacted arsanilic acid. The conjugate was finally dialyzed into phosphate saline buffer for injection purposes and stored at 0°C in small aliquots. Coupling efficiency of this method was reported by Nisonoff to be about 35% for KLH and most gamma globulins.

Preparation of Rabbit Anti-Mouse Gamma Globulin

Gamma globulin was isolated from the ascites fluid of Balb/c mice innoculated with MOPC-21 and LPC-1 tumor cells (40). The gamma globulin was precipitated from the ascites fluid by bringing the fluid to a final

concentration of 18% sodium sulfate. The resulting precipitate was resuspended in a small volume of 0.04 M phosphate buffer pH 8.0, dialyzed in the same buffer for several hours, and applied to the top of a column of DEAE cellulose, which was equilibrated in 0.04 M phosphate buffer and contained 1 cc DEAE for every 7 mg protein applied. The column was then washed with 0.04 M phosphate buffer pH 8.0 and the absorbance of 1 ml column fractions was monitored at 280 nm. The gamma globulin was eluted with approximately one column volume of buffer. Following pervaporation of the pooled gamma globulin fractions to a concentration of 5 mg/ml, the gamma globulin was dialyzed into phosphate saline buffer for injection purposes. The purity of the isolated gamma globulin was checked by immuno-electrophoresis.

The purified gamma globulin was injected intramuscularly into several rabbits at a dose of 4 mg every two weeks. They animals were bled at regular intervals and the serum was pooled, decomplemented, and stored at 0°C.

Protein Iodination

Iodination of proteins was performed by a modification of the Chloramine T method of Hunter (41). The iodination mixture of 100 μl contained 100 μg of protein, 30 μg of cold potassium iodide and 3 X 10^7 cpm Na ^{125}I in borate saline buffer. The reaction was initiated by the addition of 4 μg of Chloramine T and allowed to stir for 10 minutes at 4°C. 4 μg of sodium metabisulfite was then added, followed by stirring for an additional 10 minutes. A small aliquot of the iodinated protein was removed for counting purposes, added to 1 mg of bovine serum albumin and precipitated with 10% TCA in the cold for 20 minutes. The protein pellet was washed 3 times in 10% TCA, dissolved in 1N sodium hydroxide

and assayed for protein bound ^{125}I counts in a Beckman Biogamma Counter. 5 mg of bovine gamma globulin was added to the rest of the iodinated protein as a carrier and the sample was dialyzed for a week with daily changes of borate saline buffer prior to use.

Jerne Plaque Assay

The number of plasma cells producing anti-SRBC antibody was quantitated by a modification of the Jerne Plaque assay (42). This assay measured the formation of plaques, clear areas in a lawn of SRBC produced by diffusing hemolytic antibodies from a single plasma cell, in agar suspensions of SRBC and spleen cells from mice immunized with SRBC.

Preparation of cells for the assay. Several days after injection of SRBC, the mice were anesthetized and swabbed with 70% alcohol. Spleens to be assayed for plaquing capacity were removed from each mouse and placed in cold sterile Eagle's Minimum Essential Medium (MEM) with Earle's Balanced Salt Solution. Each spleen was separately trimmed, minced with scissors and passed through an 80 gauge stainless steel screen into fresh MEM. The cells were washed several times in MEM, resuspended in a volume of 1 ml and placed on ice.

Sheep red blood cells from the same source used for injection of the mice were washed several times in phosphate saline buffer, resuspended in MEM at a concentration of 2×10^9 cells/ml, and placed on ice.

Quantitation of plaque forming cells. 0.9 ml aliquots of a solution of 0.6% agarose in MEM were distributed in culture tubes placed in a 56°C water bath. Both the spleen cells and SRBC were removed from the ice and warmed slightly. 0.1 ml aliquots of both the spleen cells and the SRBC suspension were pipetted into a culture tube containing the agar solution, mixed well, and quickly poured into a Petri dish containing a base layer

of 1.6% agarose in MEM. The top layer containing the SRBC and spleen cells was allowed to solidify and the plates were immediately placed in a 37°C humidified incubator in an atmosphere of 95% air, 5% CO_2 for 90 minutes. To assay for direct plaques, or IgM producing plasma cells, 0.5 ml of non-hemolytic guinea pig complement, diluted 1 to 10 in complement buffer, was pipetted over the top layer. The plates were incubated for an additional half hour. The number of plasmacytes producing anti-SRBC antibody was quantitated by counting on each plate the total number of plaques.

To assay indirect plaques, or IgG producing plasmacytes, 0.5 ml of non-hemolytic rabbit anti-mouse gamma globulin, previously absorbed with μ chains, and diluted to a strength producing maximum plaques was added to each plate. After a 30 minute incubation, excess liquid was discarded and 0.5 ml of non-hemolytic guinea pig complement was added to each plate followed by another 30 minute incubation. Plaques were counted immediately. Results were expressed as the number of plaque forming cells per spleen.

All assays were done in duplicate or triplicate. Controls consisted of unimmunized animals. Background plaques, produced by these animals, were subtracted when significant.

Direct plaques were considered a measure of IgM producing plasma cells. The number of direct plaques was subtracted from the number of indirect plaques to obtain the number of IgG producing plasma cells.

Statistics

The data were examined by analysis of variance with statistical significance of treatment differences being determined by the multiple range test of Duncan (43).

RESULTS

Establishment of a Diet for Zinc Studies in the Adult Mouse

In order to study the effects of zinc deficiency on immunity, it was first necessary to establish for the mouse, the adequacy of the egg white based, biotin fortified diet of Luecke (2), originally designed for zinc studies in the rat, and the approximate minimum requirement of the mouse for zinc.

Three week old A/J females were placed in several dietary groups receiving the diet supplemented with 0.4 , 4, 12, 37, or 70 ppm zinc in the form of zinc carbonate. Another age matched group of mice received a diet of Wayne Mouse Breeder Blox which contained 65 ppm zinc. The weights and diet consumption of all of the mice were monitored for a three week period. Since the mouse has been shown to double in weight from the age of three weeks to six weeks, any deficiencies in the synthetic diet should result in a decreased growth rate of the mice on this diet when compared to control mice fed the commercial diet.

As seen in Figure 1, mice fed a diet containing 12, 37, or 70 ppm zinc had growth rates which closely paralleled that of the mice raised on the stock diet. The small difference between the final weights of the mice on the synthetic diet and the stock diet could be attributed to a difference in the palatability of the two diets. The mice receiving a diet of 4 ppm zinc appeared to have a slightly reduced rate of growth, whereas the mice on the deficient diet failed to thrive. These mice also exhibited patchy

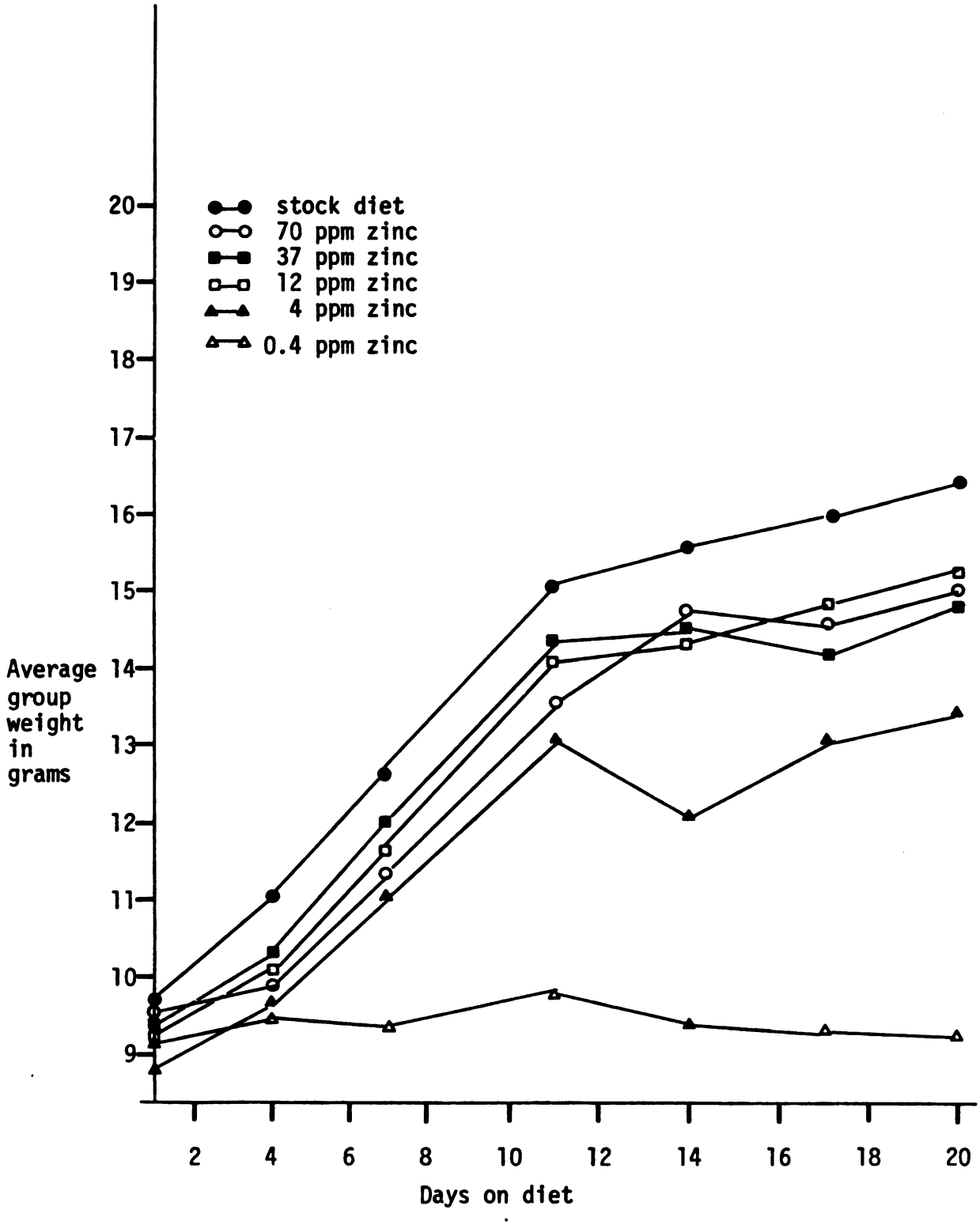

Figure 1. Growth curves of A/J mice fed diets supplemented with different levels of zinc.

coats, eye lesions, and appeared emaciated, all symptoms of zinc deficiency.

The final organ weights of the mice in the different dietary groups are presented in Table 3. The mice on the synthetic diet containing 0.4 ppm zinc had significantly lower organ weights than any of the other groups. When the same organ weights were expressed as a percentage of final body weight, the thymus of the deficient group showed a seven fold reduction in size. The spleen was reduced to approximately half in the deficient mice. The liver and kidney, on the other hand, showed no signs of wasting in these animals. Therefore, in the growing mouse, the lymphatic organs appeared to be preferentially atrophied as a result of zinc deficiency.

This study indicated that the synthetic diet was sufficient for normal growth of the mouse when supplemented with adequate zinc. The results also showed that the minimum zinc requirement of the mouse lies between 5 and 12 ppm zinc. Dietary zinc deficiency resulted in a rapid atrophy of the lymphatic tissues, especially the thymus, in the A/J mouse. This confirmed and extended the earlier observations of thymic atrophy in the zinc deficient pig (4) and rat (8).

The Zinc Content of the Thymus of the A/J Mouse

Before beginning additional studies, it was of interest to determine if the thymus contained an unusual amount of zinc compared to other organs. Various organs, including the thymus of the six week old adult mouse, were wet ashed and analyzed for zinc content by atomic absorption spectroscopy. The results, presented in Table 4, indicated that the thymus contained levels of zinc that were similar to that of other organs. However, this data did not indicate the importance of zinc to the thymus

Table 3

Organ weights of six week old A/J mice fed diets supplemented with different levels of zinc for three weeks

Dietary Zinc ppm	Thymus	Spleen	Liver	Kidney
0.4	0.003(0.03)[1]	0.021(0.23)	0.606(6.76)[aa]	0.161(1.81)
4	0.039(0.25)[aa]	0.076(0.49)[a]	1.039(6.76)	0.252(1.64)
12	0.034(0.20)[aa]	0.081(0.48)[a]	1.129(6.73)	0.251(1.50)
37	0.038(0.23)[aa]	0.076(0.45)[a]	1.063(6.38)	0.264(1.59)
70	0.036(0.21)[aa]	0.084(0.50)[a]	1.052(6.20)	0.249(1.47)
stock 65	0.043(0.23)[aa]	0.071(0.39)[a]	0.946(5.18)	0.287(1.56)

[1] Average organ weight in grams and in parentheses, percentage of body weight with statistical comparison of the latter.

[a] Significantly higher than the least value ($p < 0.01$).

[aa] Significantly higher than the least value ($p < 0.001$).

Table 4

Zinc analysis of the organs of the six week old A/J mouse

Organ	Zinc analysis ppm wet weight of tissue
Liver	28
Kidney	20
Brain	15
Heart	20
Lung	21
Spleen	21
Thymus	22

or the requirement of the various subpopulations of cells in the thymus for zinc.

The Effects of Zinc Deficiency on the Humoral Response of the Young Adult A/J Mouse

The atrophy of the thymus observed in the zinc deficient rat (8), pig (4), and mouse suggested that there might be impairment or destruction of T cells that are important to humoral and cell mediated immune responses. However, despite this evidence, no previous studies have attempted to investigate the effects of zinc deficiency on the immune response. In this thesis, the effects of zinc deficiency on the immune response were examined in the young adult A/J mouse. The young adult mouse was chosen as the subject of these studies since the effects of zinc deficiency would be less severe and therefore would be easier to define in a mouse with a fully developed immune system. The humoral response of the zinc deficient young adult was evaluated by measuring the response of these animals following injection of KLH-ars, a hapten-carrier conjugate, and sheep red blood cells, both of which are dependent on the function of T helper cells.

The response of the young adult A/J mouse to KLH-ars. In this experiment, the serum of mice fed diets containing different levels of zinc was assayed for antibody formation following injection of the hapten-carrier conjugate KLH-ars, in which arsanilic acid groups were coupled to keyhole limpet hemocyanin, a very antigenic protein in the mouse.

Six week old adult mice were fed diets containing 0.8, 4 or 69 ppm zinc. The three groups represented zinc deficient, marginally deficient and zinc adequate diets respectively. Each mouse was injected on day 0 with 500 μg of KLH-ars intraperitoneally in Freund's complete

adjuvant, followed by a second and third injection of 250 μg of KLH-ars in Freund's incomplete adjuvant at ten day intervals. Weights and dietary intakes were monitored throughout the study. One week after the final injection, the mice were anesthetized with ether and bled out by severing of the subclavian artery. The mice were then killed by cervical dislocation and the kidney, spleen, liver, and thymus were removed and weighed.

The body and organ weight data from this experiment is presented in Table 5. The adult mice on the deficient diet weighed approximately six grams less than the controls at the end of the experiment. As expected, the organ weights of the deficient mice were significantly lower than the weights of the same organ in the zinc supplemented groups. However, when organ weights were determined relative to individual body weights, only the thymus showed significant atrophy in the deficient mice. The thymus was reduced to one fifth that of the control animals, whereas the spleen, liver, and kidney remained a constant percentage of body weight in all of the dietary groups. Thus the effect of zinc deficiency on the thymus of the adult mouse was similar to that observed in the rat (8) and pig (4).

The response of the A/J mouse to KLH-ars was determined by sensitive radioimmunoassays which were developed to quantitate the levels of antibody in the serum of each mouse to both KLH, the carrier protein, and to arsanilic acid, by coupling the hapten to a carrier that did not cross-react with KLH.

The protocol of the individual RIA was as follows. The desired amount of serum from the mouse to be tested was added to a 0.3 ml volume of borate saline buffer with sufficient A/J normal mouse serum to make the

Table 5

Growth and organ weights of ten week old mice fed diets supplemented with different levels of zinc for four weeks

	Dietary zinc level		
	Zn deficient 0.8 ppm	Marginal Zn 4 ppm	Adequate Zn 69 ppm
Body weight			
Initial wt., g	18.04 ± 0.38[1]	18.39 ± 0.51	18.19 ± 0.24
Final wt., g	14.51 ± 0.51	20.49 ± 0.40[a]	21.12 ± 0.23[a]
Organ weights, g (%)[2]			
Thymus	0.005(0.03)	0.028(0.14)[a]	0.032(0.15)[aa]
Spleen	0.064(0.44)	0.120(0.58)	0.119(0.56)
Liver	0.720(4.90)	1.140(5.50)	1.110(5.30)
Kidney	0.220(1.50)	0.230(1.10)	0.250(1.20)

[1]Mean ± standard error of the mean.

[2]Average organ weights in grams and in parentheses, percentage of body weight with statistical comparisons of the latter.

[a]Significantly greater than the least value ($p < 0.01$).

[aa]Significantly greater than the least value ($p < 0.001$).

total volume of serum 15 μl. To each tube was also added 0.01 μg of either ^{125}I labelled KLH, to test for anti-KLH antibody, or 0.01 μg of ^{125}I BGG-ars, in which arsanilic acid was coupled to BGG, a protein that did not cross-react with KLH, to assay for anti-arsanilic acid antibody. This reaction mixture was incubated for an hour at 37°C. A slight excess of rabbit anti-mouse IgG was then added to each tube to cause complete precipitation of the mouse IgG, followed by another incubation for one hour at 37°C. The assay tubes were left overnight at 4°C. The resulting pellets were washed three times in borate-saline buffer and dissolved in 1 N sodium hydroxide. The supernatant and pellet washes were pooled. Both the supernatant and pellet were counted for ^{125}I in a Beckman Biogamma Counter.

Following subtraction of background counts, the counts in the supernatant and pellet were added and the percentage of the total counts that were in the pellet was calculated. Blanks representing non-specific precipitation of antigen, which were never greater than 7%, were subtracted from this value to obtain the percentage of labelled antigen specifically bound to the antibody. The antigen binding capacity of the serum of each mouse was established by incubating 0.01 μg of either ^{125}I-KLH or ^{125}I BGG-ars with increasing dilutions of serum. The percentage of labelled antigen bound to antibody was plotted against the volume of serum assayed. Titer for each mouse was defined as the amount of serum required to precipitate 30% of the labelled antigen, midway in the binding curve.

The results of the radioimmunoassays for the mice injected with KLH-ars are presented in Table 6. The titer of the mice to arsonate was essentially identical in all of the groups. The response of the

Table 6

Titer of mice immunized with keyhole limpet hemocyanin-p-azophenylarsonate

Dietary zinc level	Average volume of serum in μl required to precipitate 30% of ^{125}I KLH (0.01 μg)	Average volume of serum in μl required to precipitate 30% of ^{125}I BGG-ars (0.01 μg)
Zinc deficient 0.8 ppm	0.21 ± 0.02[1]	0.16 ± 0.03
Marginal Zinc 4 ppm	0.10 ± 0.01	0.12 ± 0.03
Adequate Zinc 69 ppm	0.11 ± 0.01	0.12 ± 0.02

[1]Mean ± standard error of the mean

mice to the carrier protein KLH showed a slight depression of the titer, although not statistically significant, in the zinc deficient mice.

These results indicated that despite the marked atrophy of thymic tissue, the deficient mice were able to respond to antigenic stimulus by KLH-ars. The slightly reduced titer to the carrier protein KLH, a response which has been shown by Mitchison and others (44) to be more dependent on helper T cells than the response to hapten, suggested the possibility that T cell helper function was slightly impaired in the deficient mice.

However, since the immunization program in this experiment was begun on day 0 when a healthyT cell helper population was present, an adequate response was generated in the deficient mice apparently because the B cells were able to continue to proliferate and produce antibody once they were stimulated by antigen. This occurred despite the development of zinc deficiency and thymic atrophy and indicated that in the young adult the B cells were not severely affected by zinc deficiency for the period of time studied. This point will be demonstrated more clearly by a subsequent reconstitution experiment.

It could be argued that the reduced weight of the thymus in this experiment could be attributed to growth interruption as a result of reduced intake in the deficient animals and not zinc deficiency. Although the deficient mice in this experiment consumed, on the average, less of the diet per day than the mice in the other dietary groups (Table 7), their intake was approximately 70% of the controls, indicating that they were not starving. Moreover, the wasting of the thymus reported by Miller (4) in the zinc deficient pig was absent in the pair fed controls.

Table 7

Dietary intake of adult A/J mice fed diets supplemented with different levels of zinc for four weeks

Dietary zinc level	Average total diet consumed, g
Zinc deficient 0.8 ppm	37.2 ± 3.6[1]
Marginal Zinc 4 ppm	52.4 ± 2.3[a]
Adequate Zinc 69 ppm	53.7 ± 1.9[a]

[1]Mean ± standard error of the mean.

[a]Significantly greater than the least value ($p < 0.05$).

The primary response of the young adult A/J mouse to SRBC. Since zinc deficiency had no severe effects on the humoral response of the mouse to an antigen injected when a healthy population of T cells was present, it was of interest to determine the effects of zinc deficiency on the primary response of the mouse to a more T cell dependent antigen such as sheep red blood cells, after the thymus had become totally or partially atrophied.

Six week old mice were fed either zinc deficient diets (0.5 ppm) or zinc adequate diets (25 ppm). After three weeks on the diet, when the thymuses of the deficient mice were 40% of the controls, all of the mice were injected intraperitoneally with 1×10^8 SRBC. Seven days after immunization, the mice were anesthetized, spleens were removed and assayed for plasma cells producing anti-SRBC antibody by the Jerne plaque assay (42).

The results of this experiment are presented in Table 8. The number of direct plaques, representing IgM forming plasma cells, was reduced to approximately half in the zinc deficient mice, although this difference was not statistically significant. However, the number of IgG forming plasma cells elicited by the zinc deficient mice was one seventh of the number of plaques produced by the positive controls, a difference which was highly significant.

These results indicated that the IgM response, which is relatively T independent, was similar in the zinc deficient and control mice. However, it was in the switch from IgM to IgG antibody formation, a process more dependent on T cell-B cell cooperation (45), that impairment of the immune response was detected. Hence the decrease in IgG antibody formation of the zinc deficient mouse following a single injection of SRBC

Table 8

Primary response of A/J mice to sheep red blood cells

Dietary zinc level	Average number of plaque forming cells per spleen	
	IgM plaques	IgG plaques
Zinc deficient 0.5 ppm	2,286 ± 403[1]	3,861 ± 839
Zinc adequate 25 ppm	4,058 ± 684	21,952 ± 3110[a]

[1] Mean ± standard error of the mean.

[a] Significantly greater than the least value ($p < 0.05$).

suggested an impairment or destruction of helper T cells. A similar reduction of IgG plaque formation in response to SRBC has been reported in mice depleted in T cells due to either thymectomy (46) or congenital absence of the thymus (47).

The secondary response of the young adult A/J mouse to SRBC. Since zinc deficiency had produced a severe impairment of the primary IgG response to SRBC in the A/J mouse, the effects of zinc deficiency were also examined on the secondary response of the A/J mouse to SRBC. Since the antibodies formed in the secondary response are largely of the IgG class, this response was therefore a more rigid test of T cell helper function. In addition, to confirm the hypothesis that the reduced response to SRBC was a consequence of impairment or destruction of helper T cells, while B cell function remained relatively intact, part of the zinc deficient mice were reconstituted with syngeneic thymocytes prior to immunization with SRBC and their plaquing response compared to control animals.

Six week old adult mice were fed diets containing 0.5, 2 or 25 ppm zinc for 39 days during which intakes were monitored daily and weights were taken weekly. These three groups represented zinc deficient, marginally deficient and zinc adequate populations. After 27 days on these diets, half of the mice on the deficient diet received an intravenous injection of 6×10^7 thymocytes, prepared from healthy A/J donors of identical age. The following day, all of the mice were injected intraperitoneally with 1×10^8 SRBC, followed by a second injection of 10^8 cells one week later. Five days after the final injection, on day 39, the mice were anesthetized, organs were removed and weighed and the spleens were prepared for the Jerne plaque assay (42).

The body weights, thymus weights, and diet consumption of the mice in this experiment are presented in Table 9. Both zinc deficient groups lost weight while the mice on the marginal and zinc adequate diets gained similar amounts of weight in the same time period. The identical final body weights and the fact that both the marginally deficient and zinc adequate groups consumed the same amount of diet in the 39 days of the experiment indicated that the marginally deficient mice, although consuming a diet deficient in zinc, were not suffering from inanition. The zinc deficient groups consumed on the average 80% of the amount of the diet eaten by the zinc supplemented groups and had essentially no thymus remaining at the end of the experiment. The marginally deficient mice had thymuses of approximately half the size of the positive controls, indicating that these mice were zinc deficient.

The results of this experiment are presented in Table 10. The zinc deficient animals produced 6,000 indirect (IgG) plaques, one tenth the number produced by the positive controls. This indicated a further reduction of immunity in the zinc deficient mice in the secondary response to SRBC. Since the majority of the antibodies formed in the secondary response were of the IgG class, and therefore, more dependent on the presence of helper T cells, the ten fold reduction of plaque formation in the secondary response to antigen was additional evidence for T cell helper impairment in zinc deficiency.

However, in order to confirm that T cells were the prinicipal cell type affected by zinc deficiency, half of the zinc deficient mice were injected intravenously with 6×10^7 viable thymocytes, the cellular equivalent of a thymus, from healthy A/J donors prior to immunization with SRBC. The reconstituted mice formed 37,000 plaques which were significantly

Table 9

Growth, thymus weights, and intake of A/J mice fed diets containing different levels of zinc for thirty nine days

	Dietary zinc level			
	Zinc deficient	Zinc deficient plus thymocytes	Marginal zinc	Adequate zinc
	0.5 ppm	0.5 ppm	2 ppm	25 ppm
Body Weights				
Initial wt., g	16.6 ± 0.3[1]	16.4 ± 0.2	16.4 ± 0.2	16.3 ± 0.3
Final wt., g	14.5 ± 0.7	13.4 ± 0.8	19.9 ± 0.4[a]	20.7 ± 0.5[a]
Gain, g	-2.1 ± 0.7	-3.0 ± 0.8	+3.5 ± 0.3[a]	+4.4 ± 0.3[a]
Thymus wt., mg.	0.24 ± 0.04	0.27 ± 0.05	18.9 ± 0.7[a]	29.4 ± 0.6[a]
Average total diet consumed, g	70.7 ± 0.9	73.4 ± 4.8	85.9 ± 0.8[a]	89.3 ± 0.4[a]

[1] Mean ± standard error of the mean.

[a] Significantly greater than the least two values ($p < 0.01$).

Table 10

Secondary response of A/J mice to sheep red blood cells

Dietary zinc level	Average number of plaque forming cells per spleen IgG
Zinc deficient 0.5 ppm	6,180 ± 660[1]
Marginal zinc 2 ppm	15,380 ± 1840
Adequate zinc 25 ppm	61,490 ± 5900[a]
Zinc deficient 0.5 ppm reconstituted with 6×10^7 thymocytes	37,100 ± 6840[aa]

[1] Mean ± standard error of the mean.

[a] Significantly greater than all other values ($p < 0.01$).

[aa] Significantly greater than the least two values ($p < 0.01$).

higher than the plaques formed by the deficient mice and 60% of the number of plaques produced by the positive controls. Since one seldom fully restores the immune response by reconstitution (48), the significantly increased plaque forming response of the deficient mice following injection of T cells indicated that the T cell was the primary cell type affected by zinc deficiency. Although a slight impairment of B cells in zinc deficiency cannot be ruled out by this experiment, the data indicated that the B cell was able to proliferate and produce antibody despite 39 days on a deficient diet.

A/J mice fed a diet marginal in zinc produced an average of 15,000 plaques, one fourth of the response of the control mice. This suggested that reduced T cell helper function also occurred in this dietary group. Thus marginal zinc deficiency states also produced serious consequences in the development of an adequate immune response. The fact that this group of mice had a reduced plaque forming ability despite the same final body weight and daily intake as the zinc adequate group, indicated very clearly that zinc deficiency and not inanition was responsible for the drop in immunity.

SUMMARY AND CONCLUSIONS

The purpose of this thesis was to investigate the effects of dietary zinc deficiency on the humoral response of the young adult A/J mouse. These studies were the beginning of a series of investigations to explore the relationship between malnutrition and immunity by studying the effects of a single nutritional deficiency on the immune response of a well-defined population.

In summary, dietary zinc deficiency was found to produce a rapid atrophy of the thymus and a significant depression of the humoral immune response of the young adult mouse to T dependent antigens. The decreased number of indirect (IgG) plaques in both the primary and secondary responses of the deficient mice to SRBC, a response that is dependent on T cell-B cell interaction, suggested that the reduced number of plaques was due to impairment or destruction of T cell helper function. This interpretation was supported by the fact that significant restoration of the response to SRBC could be achieved by the injection of syngeneic thymocytes into the deficient mice prior to injection of antigen. Of additional significance was the finding that the marginally deficient mice also had a reduced plaque forming ability, indicating that these mice had a reduced T cell helper activity.

B cell function appeared to be relatively unaffected in the zinc deficient adult mouse. This was indicated by the similar antibody titers of the zinc deficient, marginally deficient and control mice to KLH-ars when the antigen was injected when a healthy population of helper T cells

was present. The significant restoration of the depressed response of the deficient mice to SRBC following injection of thymocytes also showed that B cells were able to proliferate and produce antibody despite 39 days on a deficient diet. Thus zinc deficiency appeared to depress the immune response of the young adult mouse by impairment or destruction of T cell helper function.

The reduced intakes of the mice on the zinc deficient diet suggested that the wasting of the thymus and the depression of the humoral response could be due in part to inanition in the deficient mice and not zinc deficiency. However, the reduced intake of the deficient animals was not unexpected since anorexia is common in zinc deprived animals (4). In addition, the development of thymic atrophy and the four-fold reduction in plaque forming ability in marginally deficient mice despite the similar intakes of the marginally deficient animals and the zinc adequate controls over a 39 day period was evidence that these severe effects on the immune response were due to zinc deficiency and not simply to a reduced diet consumption. Moreover, recent experiments with pair fed animals have shown that over a 39 day period, inanition accounted for only 25% of the total drop in immunity in the zinc deficient mice (49). Therefore, the detrimental effects of dietary zinc deficiency on the humoral immune response can be largely attributed to a lack of zinc and not starvation.

These results linking zinc deficiency and decreased immune function have important significance forpopulations like those in the Middle East who are zinc deficient due to the ingestion of diets composed of cereal grains. Diets of animal protein, rich in available zinc, are economically inefficient and will not be able to adequately feed an expanding world population. Therefore, in the future, larger proportions of the world's

population will rely on cereal grains as a primary foodstuff and thus the possibility of decreased immune function as a result of zinc deficiency may be increased. Furthermore, the finding that marginal zinc deficiency also causes a decrease in immune response has important implications for those individuals in our own country such as trauma patients, patients with liver disease, alcoholics, pregnant women and women taking oral contraceptives whose decreased plasma zinc levels are indicative of marginal zinc deficiency (50).

The possibility of a depressed immune response in these zinc deficient people and the observed depression of immunity in mice in this thesis has stressed the importance of future investigations to expand our knowledge of the relationship between zinc and immunity. Information is lacking on the effects of zinc deficiency on other immunological parameters such as delayed type hypersensitivity and graft rejection in the adult mouse as well as complete immunological studies on the effects of zinc deficiency in the neonatal and weanling mouse. The effects of zinc deficiency on the young mouse may or may not be similar to those observed in this thesis for the adult. Also unknown are the long range effects of zinc deficiency and the consequences of short periods of deficiency as well as the reversibility of these effects. Examination of the histology of the thymus as it begins to atrophy due to zinc deficiency and how this relates to wasting as a result of age, injury, or stress will also aid in clarifying the role of zinc in immunity.

Although the experiments in this thesis have explored the effects of zinc deficiency on immunity, they have not attempted to determine how zinc exerts its detrimental effects on the lymphoid cell. Therefore, future experiments are also needed to understand the mechanism of this

immune depression. The demonstration that the zinc content of the thymus was similar to that of other organs in the mouse indicated that the requirement of the thymus for zinc was not unusual. However, zinc depletion could cause a decrease in specific activity of critical zinc metalloenzymes in the thymus and thus affect lymphoid cell function. Thymic hormones, responsible for maturation of T cells and other immunological parameters, may also depend on the presence of zinc for activity. Hence the wasting effect of zinc deficiency on the thymus and its impairment or destruction of helper T cells, may be due to a greater sensitivity of biochemical processes in the thymus to zinc depletion than other organs.

Zinc deficiency could also produce deleterious effects on the immune system by affecting the levels of adrenocorticosteroid hormones in the adult mouse. Although no studies have been done in this area, the enlargement of the adrenal glands in zinc deficient rat (8) and pig (4) indicated that there may be a relationship between zinc deficiency and adrenocorticosteroid metabolism. Some of the effects of the adrenocorticosteroid hormone cortisone on the immune system, studied by the injection of cortisone acetate in experimental animal models, were thymic atrophy (51) and a depression of IgG formation in the Jerne plaque assay (52). These effects were similar to those produced by experimental zinc deficiency in the adult mouse. Therefore, these studies suggested a possible role of adrenocorticosteroid hormones in the immunological effects seen in the zinc deficient mouse.

These investigations of zinc deficiency on immunity have indicated the severity of the immunological effects that can be caused by a single nutritional deficiency. Although these studies have only emphasized a deficiency of zinc, the results suggested that other

nutritional inadequacies could cause equally significant impairment of the immune response. Thus, these results have important implications for future research into the bodily responses to malnutrition. This emphasizes the need for continuing studies to clarify the role of nutritional elements in immunity.

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