EFFECT OF ZINC DEFICIENCY ON CADMIUM-INDUCED IMMUNOPATHOLOGY

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CADMIUM - INDUCED IMMUNOPATHOLOGY

BY

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ABSTRACT

The heavy metal cadmium is a widely distributed naturally occurring toxic element. Zinc and cadmium have striking physical and chemical similarities. The fact that they compete with each other for the same metallothionein makes their biological interactions very likely. In this study the effect of zinc deficiency on potentiating the pathologic effects of cadmium on the immune system and the kidneys were studied. Four week old C57BL6 male mice were fed zinc deficient diet (Zn 1 ppm) for 4 weeks, then cadmium 50 ppm was added to the drinking water, and after 3 weeks they were fed a regular chow diet. Animals were sacrificed at 0, 3 and 6 weeks after cessation of treatment. In other groups, 50 ppm zinc was added to the regular chow diet.

The number of IgM and IgG antibody forming cells in the spleen was significantly lower after treatment with zinc-deficient diet compared to nontreated controls. Concurrent administration of cadmium antagonised that reduction. Proliferative response of spleen cells to the T cell-mitogen, phytohaemagglutinin decreased after treatment with zinc-deficient diet and cadmium administration tended to increase it. The number of CD4+ and CD8+ cells was decreased after treatment with zinc-deficient diet and a further reduction was observed after concurrent cadmium administration. Natural killer cell activity in the spleen decreased after treatment with zinc deficient diet. Concurrent cadmium administration caused more suppression.

Simultaneous Treatment with zinc deficient diet and cadmium did not alter spleen, thymus and kidney weights and lymphocyte count. However, lymphocyte

count in blood collected from the right atrium of the heart was significantly decreased in animals treated with zinc-deficient diet.

On electron microscopic examination, kidneys of cadmium-treated mice showed ultrastructural alterations in the proximal tubular epithelial cells. However, animals treated with low zinc diets showed cellular degeneration mainly in the cortical area of the thymus. Zinc concentrations in the kidney tended to decrease after treatment with zinc-deficient diet. Cadmium concentrations increased after treatment with zinc-deficient diet and further increase observed after concurrent cadmium administration.

INDEXING KEY WORDS: Cadmium, Zinc, Cellular Immunity

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BSS	-	balanced salt solution
^{o}C	-	celsius
C	-	complement
Chl	-	cholesterol
Cd	-	cadmium
Cr	-	chromium
CO2	-	carbon-dioxide
Con A	-	concanavalin A
Cu	-	copper
FITC	-	fluorescein isothiocyanate
g	-	gram
Ig	-	immunoglobulin
K	-	kelvin
Kg	-	kilogram
mg	-	milligram
MHc	-	major histocompatibility complex
ng	-	nanogram
NK	-	natural-killer
PBS	-	phosphate-buffered saline
PBS-T	-	phosphate-buffered saline-tween 20
pfc	-	plaque-forming cells
PHA	-	phytohemagglutinin
RPMI	-	Rosewell Park Memorial Institute
SCA	-	suppressor cell activity
SRBC	-	sheep red blood cells
WK	-	week
Zn	-	zinc

CHAPTER I

Introduction

I) Trace Elements

Until the beginning of the 20th century, it was not possible to measure the precise concentrations of many mineral elements that are present in living tissues in minute amounts. Therefore, they were described as "traces" and the term trace elements emerged to identify them. Although they can now be estimated in biological materials with considerable precision and accuracy, the designation "trace elements" has remained in popular usage.

As early as 1800, Copper was shown in snails to exist in combination with blood proteins, and copper-containing pigment, hemocyanin, was found to behave as a respiratory compound in various marine organisms. A french botanist published remarkable observations on the iodine content of soil, water and food and concluded that the occurrence of goiter in man was associated with a deficiency of environmental iodine (Chatin 1870).

It has been difficult in the last several decades to find a useful classification for trace elements or to draw a completely satisfactory line of demarcation between those so designated and the so called "major" elements. Even the discovery of such substances did not rapidly attract enough scientific curiosity to stimulate studies of possible wider significance.

A permanent and logical classification of trace elements may be based on chemical characteristics. At the present time, more than 90 naturally occurring elements are known to exist. Out of these, 15 are known to be essential or beneficial

elements for humans or animals. These are: arsenic, chromium, cobalt, copper, fluorine, iodine, iron, molybdenum, nickel, selenium, silicon, tin, vanadium, manganese and zinc (Mertz, 1981, Chandra, 1983). The essentiality of such elements is established when a further reduction below the range of tolerable level results in a consistent and reproducible impairment of a physiological function. A common property shared by most essential elements is that they exist and function in the living tissues at very low concentrations; some are expressed as parts per million (ppm) others as parts per billion (ppb). Practical importance in terms of toxicity of the trace elements is totally unrelated to essentiality. Some essential elements such as manganese are not being of any as-yet-known concern to human nutrition, whereas, others such as zinc affect both human nutrition and immunity.

Other trace elements referred to as "non-essential", for example, cadmium, arsenic or mercury, do not appear to serve any vital function in man, although recent observations in ruminants do point to some biological role. The existence of such elements in the living organisms acquired by inhalation, ingestion or injection even in small to moderate amounts presents potential hazards thus they are labelled "toxic" elements.

Essential trace elements serve a wide range of functions. They may act as catalysts in enzyme systems in cells that vary from a weak ionic effect to highly specific associations in metalloenzymes. Such functions are physiologically maintained by specific proteins (e.g. transferrin and metallothionein) that regulate trace element absorption, distribution, metabolism and excretion and also carry them to specific sites where recognition and dissociation takes place (Chandra and Dayton, 1982).

The essential function of trace elements in the defence mechanisms against microbiological, chemical, viral and oxidative insults has become well recognized (Diplock A. 1981 - Chandra and Dayton, 1982 - Bendich and Chandra 1990) linking deficiencies of some trace elements not only with risk of infection but also with chronic degenerative and neoplastic diseases.

Deficiency of some of the essential trace elements tends to be common world wide problems. Deficiency is defined as "a state in which the concentration of an essential nutrient at its sites of action is inadequate to maintain the nutrient-dependent function at its optimum level". Since the number of functions that are accepted as criteria of nutritional status increases every day with every new discovery, this definition is considered as a wide ranging spectrum of clinical and metabolic disturbances. A new proposal has been submitted (Mertz, 1986) that characterizes the gradual changes that occur as a result of suboptimal intake of nutrient supplies in diet and environment into four phases: Phase I, changes in the metabolism of the element itself as a compensation for suboptimal intake during a certain period of time so that no change in biological structure or function is detected. This phase may revert back to normal status with an increasing intake of the nutrient. Phase II, "compensated metabolic phase" is characterized by an impairment of certain specific biochemical functions such as trace element - dependent enzyme activities. Phase III "decompensated metabolic phase", characterized by the appearance of defects in functions important for health, such as: metabolic, immunological, cognitive, emotional, developmental and those related to work capacity. Signs and symptoms of this phase are not clinical and are

detected only by specialized tests. Phase IV "clinical phase" characterized by appearance of disease and with increasing severity of deficiency, by death.

Iron is an essential element that acts as a main component of oxygen carrying protein haemoglobin, a deficiency of which leads to anemia especially in high risk persons, such as: infants, adolescents, elderly, pregnant and lactating women. Iron is also essential for optimum immune responses, so that its deficiency impairs T-cell function, lymphocyte proliferation, response to different mitogens, macrophage migration inhibition factor and delayed cutaneous hypersensitivity responses (Chandra, 1983). Zinc is an essential co-factor in many metalloenzymes and, its severe deficiency is seen in the inherited malabsorption syndrome, acrodermatitis enteropathica, which is characterized by frequent bacterial, fungal and viral infections. Zinc is also important for the integrity of the immune system, its deficiency resulting in rapid and marked atrophy of the thymus and loss of its activity (Chandra and Au 1980 - Dardenne et.al., 1984).

Copper is a key regulator of lysyl oxidase activity. Reduction of its availability produces neutropenia and impaired reticuloendothelial function and antibody response to heterologous red cells (Prohaska et.al., 1983 - Vyas and Chandra, 1983).

The subject of interactions between trace elements with other dietary elements has attracted the attention of many investigators (The Task Group on Metal Interaction, 1978 - Levander and Cheng 1980 - Hoadley and Cousins, 1985). Recently the implications of these interactions on the immune system have started to emerge (Chowdhury and Chandra, 1987).

Zinc and Cadmium are two recognized members of the trace elements group, that have similar physical and chemical properties because they both belong to the second group of the periodic table. This makes them compete for the same metallothionein. However, zinc is an essential element while cadmium is a toxic metal.

In a subsequent section of this chapter the metabolic and biological features of zinc and cadmium will be discussed with particular emphasis on their effect on the immune system.

II) Zinc

Zinc was clearly established as an essential element for high forms of plant life more than 150 year back, (Somner and Lipman 1926). Before that, in the previous century it was only considered essential in the nutrition of the fungus Aspergillus niger.

Considerable progress towards clarification of the practical importance of zinc in human nutrition followed the studies of Prasad and co-workers (1961) which provided the first evidence for the occurrence of nutritional zinc deficiency in man. The essentiality of zinc has been proven to be for almost every organ system of the body as it is widely distributed in the tissues. An adult body is estimated to contain 1.4 - 2.3g of zinc, 20% of the total body content is present in the skin and considerable proportions exist in the bones and teeth. Adult human blood contains less than 0.5% of the total body zinc (National Research Council, 1978) 75-88% is in the red cells, 12-22% in the plasma and less than 3% in the leukocytes and platelets.

Zinc metabolism:

The mechanism and control of zinc absorption is not fully understood. Conflicting results from experimental studies in vitro or in vivo may arise in part from differences in experimental conditions, as zinc absorption appears to be readily influenced by a wide variety of host and environmental factors (Solomons and Cousins 1984).

In rats, zinc is absorbed mainly from the duodenum, jejunum and ileum with very little being absorbed from the stomach (van Campen and Mitchell, 1965-Davies

1980). Intestinal absorption of zinc has been proposed in four phases (Cousins, 1982): first, uptake by the intestinal cells, the details of this mechanism have not been defined yet; second, movement through the mucosal cell; third, transfer to the portal circulation, this appears to be a carrier - mediated process which probably involves interaction with the metal in a chelated form. The availability of zinc for uptake by the brush border may be determined by factors such as pH and its relative distribution among small ligands and larger molecules within the intestinal lumen. The transfer of zinc from the mucosal wall to the portal circulation is slower than the uptake and accumulation within the cell and appears to be the rate limiting step in absorption in rats (Davies 1980). In humans, at low zinc levels, much of the available supply is transported to the plasma, but as the zinc content increases, less is taken up into the body; fourth, secretion of endogenous zinc back into the intestinal wall. To study the mechanisms of zinc absorption in animals previously fed a zinc-adequate or zinc-deficient diet, Steal and Cousins (1985) used a vascular perfusion preparation in rats that included most of the intestine. From 1 cm distal to the bile duct to the ileocecal valve, they found that zinc absorption (transfer from the lumen to portal circulation) was saturable in both groups and its uptake was increased by zinc deficiency. Zinc depleted animals also showed a more rapid rate of absorption at all lumen zinc concentrations and higher cytosolic concentrations after zinc perfusion, suggesting that enhanced uptake, rather than increased basolateral transport may account for some of the differences in absorption rates.

Absorbed zinc is carried from the intestine to the liver in the portal circulation bound to albumin (Smith et.al. 1979). The liver extracts about 30% of the zinc entering the hepatic venous supply and releases it back to the blood. Circulating

zinc is carried by high molecular weight proteins into various extrahepatic tissues in which there are different rates of zinc turnover (Aamodt et.al., 1979). The most rapid accumulation and turnover of retained zinc is seen in the pancreas, liver, kidney and spleen (McKenney et.al., 1962). However, zinc uptake by the central nervous system and the bones is relatively slow, and it remains firmly bound for long periods. Zinc entering the hair also becomes unavailable to the tissues and is lost as the hair is shed.

The liver is the major organ involved in zinc metabolism as it contains various binding components of different molecular weight in proportions that vary with different zinc levels (Mason, et. al., 1981). Metallothionein has an integral role in hepatic zinc metabolism. It is a low molecular weight protein that is regulated in the liver under the control of dietary and hormonal signals and its synthesis is indirectly stimulated by glucocorticoids, catecholamines, effect of zinc transport into the hepatocytes, stress, acute infection and shock (Etzel and Cousins, 1981 - Brady and Helving, 1985 - Disilvestro and Cousins, 1984 - Oh, et.al., 1978). The synthesized metallothionein binds to heavy metals and is degraded with a half-life of 18-20 hours. The degradation rate may be increased by low-zinc status (Disilvestro and Cousins, 1983).

In the healthy adult human, about 90% of zinc excretion occurs in the feces (Weigand and Kirchgessner, 1980) (Bunker, et.al., 1982) and the remainder 10% in the urine, sweat and skin. Fecal excretion includes both unabsorbed dietary and endogenous zinc. Endogenous fecal losses arise from the zinc that is secreted into the gut from the body and is not subsequently reabsorbed, and this varies according to the balance between true absorption and metabolic needs. The actual amount of

endogenous zinc excretion from human and animals depends on zinc intake and status. In humans, it may range from <1mg to several milligrams daily (Matseche, et.al., 1980).

Zinc excretion in the urine ranges between 300 to 500 ug/day (Hambidge, 1983). This level varies according to dietary intake of zinc adequate or zinc deficient diet, renal tubular function, and also catabolic states such as severe burns, major surgery or trauma. However, urinary excretion of zinc may be above normal as in the case of alcoholic cirrhosis or administration of chelating agents such as EDTA (Prasad, et.al., 1965) (Spencer and Rosoff, 1966).

Biological function of zinc:

The selective property related to zinc biological functions evolved from its ability to form stable complexes with the side chain of proteins. Zinc is an essential component of different enzymes in human and animal tissues. Carbonic anhydrase was the first zinc metalloenzyme to be identified (Keilin and Mann, 1940). Subsequently, its involvement was recognized in alcohol dehydrogenase, alkaline phosphatase, procarboxypeptidase and in nucleic acid metabolism enzymes such as DNA polymerase, RNA polymerase, reverse transcriptase and nucleoside phosphorylase (Lieberman, et.al., 1963).

Zinc has also an important structural role in the retina as it contains the zinc metalloenzyme, retinene reductase which is required for the reconstitution of retinene (Vitamin A aldehyde) during the rhodopsin cycle (Harper, et.al., 1979). If related enzymes from different species are included, then over 200 zinc metalloenzymes are now on record (Vallee, 1983) (Prasad, 1984). Zinc supplementation may be effective

to maintain normal concentrations of vitamin A in the plasma as it is required for mobilization of vitamin A from the liver when its concentration in the plasma is lower than normal and unresponsive to vitamin A therapy.

Zinc forms complexes with insulin which makes it possible for the crystalline zinc insulin molecule to increase the duration of insulin action when given by injection. Zinc insulin complexes are also present in the (B)ß cells of the pancreas and there is evidence suggesting that its existence is important for storage and release of insulin as required.

Many authors have addressed the important structural role of zinc in forming mercaptides with the thiol group of proteins, in linking the phosphate moiety of phospholipids and its interactions with the carboxyl groups of sialic acids or proteins on plasma membrane resulting in a change in its fluidity and stabilization (Chvapil, 1976).

Substantial quantities of zinc are essential for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecular synthesis (Hambidge et.al., 1986). It stabilizes the three dimensional structure of DNA binding domain to some regulatory proteins involved in gene expression (Kadonage et.al., 1987). Zinc-deficiency alters plasma membrane functions and nucleic acid metabolism. In animals, the incorporation of labelled thymidine into DNA was impaired a few days after starting a zinc-deficient diet (Prasad, and Oberleas, 1974).

Zinc deficiency and immunity

The importance of zinc in the development and maintenance of the immune system is now widely accepted. Prasad and co-workers first recognized zinc

deficiency in 1961. Absolute deficiency of dietary zinc is uncommon in human or animals except under experimental conditions. Relative deficiency has been noted among children of low income families (Hambidge, et. al., 1976), institutionalized individuals, elderly (Roebothan and Chandra 1990) and pregnant teenagers (Sandstead, 1973).

The pathogenesis of several diseases such as sickle cell anemia, renal disease, alcoholism, certain gastrointestinal disorders, burns and the genetic disorder acrodermatitis enteropathica can create deficiencies in zinc (Chandra and Dayton, 1982) (Good, and Fernandes, 1979).

Clinical manifestations of zinc deficiency vary with different zinc serum levels. These may include: bullous-pustulardermatitis, alopecia, diarrhea, mental disturbances, male hypogonadism, poor appetite, decreased taste acuity, abnormal dark adaptation, poor wound healing, anergic response to skin sensitization and recurrent infections due to distorted cell mediated immunity (Hambidge et.al., 1986).

The effect of zinc deficiency on immune function have been well characterized (Chandra, 1980) (Fraker et. al., 1982) (Tapazoglou et.al., 1985) (Chandra and Puri 1985). Zinc deficiency affects mainly cell mediated immunity in man and laboratory animals with associated loss of the general lymphoid tissue. The thymus in particular is affected more often than other organs in the body (Fraker et.al., 1977) (Chandra and Au, 1980). Although a persistent severe zinc deficiency state will eventually lead to atrophy of the entire organ, there is preferential loss from the cortex and thymus dependent areas of peripheral lymphoid tissues.

The critical role of zinc in lymphocyte function and the specific mechanism(s) underlying the thymic atrophy associated with zinc deficiency is as yet unclear. Good

and associates (1982) suggested that decreased activity of terminal deoxyribonucleotidyl transferase as zinc containing DNA polymerase which is nearly exclusive to the thymus and immature thymocytes (McCaffrey et.al., 1973) will lead to an arrest in the growth, division and differentiation of precursor cells into immunocompetent thymocytes. This theory may account for the unique sensitivity of the thymus to zinc deficiency. As a result, lymphopenia, alterations in the proportions of T-cell lymphocytes and subsets (helper/inducer and suppressor/cytotoxic) and mononuclear macrophages occur. Furthermore, lymphocytes from zinc-deficient subjects or animals show reduced blastogenic response to T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A).

A critical role of zinc in normal T-cell function is provided by the effect of zinc deficiency on delayed type hypersensitivity (DTH) responses (Fraker et.al., 1982). Adult mice fed zinc-deficient diet and, subsequently, percutaneously sensitized with dinitrofluorobenzene (DNFB) gave significantly lower DTH responses compared to both pair fed and ad-libitum control mice with T helper cells. In the same experiment repletion of the zinc-deficient mice resulted in completely normalized DTH responses. Similar observations have been noted in human clinical literature, depressed DTH responses with severe zinc deficiency and positive responses after zinc supplementation. Such responses have been seen in cases of acrodermatitis enteropathica (Chandra, 1980), prolonged hyperalimentation with inadequate zinc (Allen et.al., 1981) obesity (Chandra and Kutty, 1980) (Rodin and Goldman, 1969) and in cases of Down's syndrome in which patients are known to have increased susceptibility to infections and low serum zinc for unclear reasons. Bjorksten and

associates (1980) were able to correct much of the immune abnormalities with oral zinc supplementation, thus confirming the nature of the abnormality and its relationship to zinc deficiency.

The impact of zinc deficiency on hurmoral immunity and antibody production is much less unclear. Zinc has been considered to be an effective B-cell activator as well as being capable of acting synergistically with B-cell mitogens (Cunningham-Rundles et.al., 1980). Other investigators found that B-cell mitogenesis is unaffected by zinc deficiency in vitro (ZanZonico et.al., 1981).

Zinc deprivation reduces primary (IgM) and secondary (IgG) antibody responses to heterologous antigens. In 1986, Fraker et.al. suggested that zinc-deficient mice produced 40% as many IgM and IgG plaque forming cells (PFC) per spleen in response to sheep red blood cells (SRBC), a T-cell-dependent antigen, compared to adequately fed mice. In the same experiment, the ratio of T-cells to B-cells was unaltered in the zinc-deficient mice and there was nearly double the proportion of B-cells bearing high amounts of surface IgM. These data suggesting that augmented in vitro responses by splenocytes from zinc-deficient mice to mitogens thought to stimulate the more immature B-cells (dextran sulfate and lipopolysaccharides), indicated that a greater proportion of immature B-cells might be accumulating in the spleens of the zinc-deficient mice (Fraker, 1977).

Natural killer cell (NK) activity is also affected by suboptimal intake of dietary zinc or in patients receiving total parenteral nutrition without zinc supplementation. A significant depression of NK cell activity was demonstrated in zinc-deficient patients with sickle cell anemia as compared to control subjects (Tapazoglou, et.al., 1985).

Lack of zinc is especially harmful to the in utero development of the immune response. Studies in mice have shown persistent states of immunodeficiency in the offspring that can even be transferred to subsequent generations (Beach, et.al., 1982).

The critical role played by zinc in functional neutrophil activity has been reviewed. As a result of zinc deficiency, actual intracellular quantity is reduced (Chvapil, 1976) and chemotactic response of polymorphonuclear leukocytes is reduced which can be corrected after replenishment (Weston, et.al., 1977).

Excessive zinc intake by healthy adults caused significant impairment of polymorphonuclear function together with reduced lymphocyte activity and response to mitogens. The theory which has been proposed by Chandra (1984) is that altered lipid profiles in the blood and leukocyte membranes were responsible in part for the immunosuppression associated with zinc excess. Other possible mechanisms for this finding include changes in membrane fluidity, interaction with copper and alterations in calcium flux. Duchateau, et.al., (1981) found that substantial doses of zinc (220mg twice daily) in immunosuppressed and normal elderly subjects increased the number of circulating T-cells, and improved skin reactivity and the antibody response to tetanus toxoid. These data suggest that the pretreatment zinc status and metabolic state have an important effect on the utilization of zinc and its effect, beneficial or adverse, on immune responses.

In spite of the extensive studies that started in the early days of this century, the impact of zinc on immunity is not fully understood. Several hypotheses have been put forward and some of these have been proven experimentally. Experiments suggest that the essentiality of zinc as a co-factor for a variety of metalloenzymes

that are essential for cellular multiplication may result in deficiency of cellular multiplication and decreased number of T- and B-cells. Other experiments suggest that zinc is an essential co-factor for thymulin (one of the putative thymic hormones), and its suboptimal intake decreases thymic hormone activity and, subsequently leads to adverse immunological effects (Chandra, 1985) (Dardenne, et.al., 1984). Recently, in acquired immune deficiency syndrome (AIDS), it has been suggested that decreased zinc saturation may be responsible in part for the reduction of the thymic hormones and subsequent immunological defects (Farbis et.al., 1988).

III) Cadmium

The heavy metal cadmium (Cd) is a widely distributed, naturally occurring toxic element. At birth, cadmium is virtually absent from the human body. Thereafter, its concentration tends to increase gradually until age 50. Beyond that age the levels of renal cadmium remain essentially constant or decrease (Kjellstrom, 1979). The total body burden of cadmium in a healthy middle-aged person varies from 5-20mg (Kowal et.al., 1979) (Vahterm, 1982), with large concentrations in the liver and the kidney and minute amounts distributed in other tissues including testis, lung, pancreas, spleen and various endocrine organs.

In 1942 Nicaud, et.al. reported an unusual number of patients with severe osteoporosis, pseudofractures and associated impairment of general health in a group of alkaline accumulator factory workers, and cadmium was suggested to be the causative agent. A few years later Friberg, 1950 reported an unusual number of cases of lung and kidney damage among workers in an electrical battery plant where they were exposed to cadmium-oxide.

The greatest concern over cadmium pollution was triggered by Hagino, (1957) from Japan who reported that cadmium poisoning is not restricted to industrial workers only, but food contamination can constitute a health hazard to the general population. In areas polluted by cadmium, a high prevalence of proteinuria was noted, but in other areas the exposure was high enough to cause severe bone disease, the Itai-Itai disease (Hagino, 1957).

Epidemiological observations and laboratory experiments have underlined the importance of cadmium as a toxic agent and focussed light on the importance of

other nutrients such as zinc, iron and calcium in modifying the metabolism and toxic effects of cadmium in man and animals (Fox, 1979).

Exposure to cadmium:

Cadmium is widely distributed in the environment. Human exposure occurs mainly through inhalation and oral intake.

In the past 30 years industrial use of cadmium has increased its level in the atmosphere (Fleischer et.al., 1974) where it contaminates rain water, deposited on land and natural waters, and subsequently gains entry to the food chain of man and animals. Thus, food is normally the major source of cadmium for the average person.

In 1979 Harvey and co-workers reported in England that a small population in a Somerset village were exposed to unusual levels of cadmium through consumption of vegetables grown in cadmium contaminated garden soil. The cadmium was brought to the surface from a nearby zinc mine, and contaminated the soil throughout the village (Harvey et.al., 1979).

Cigarette smoke is an additional source of cadmium, especially for heavy smokers. The relationship between smoking and cadmium levels in the kidneys, liver, adipose tissue and lung have been demonstrated in a study of smokers and nonsmokers (Mussalo-Rauhamaa et.al., 1986). A higher body burden of cadmium has also been found in smokers after analysis of necropsy materials (Hahn et.al., 1987). Another study that was carried out on pregnant women who smoked, demonstrated a significant elevation of cadmium levels in maternal blood, placenta and cord blood (Kuhnert et.al., 1982).

Since little or no recycling of cadmium occurs, the amount entering the environment roughly equals the amount produced or used. This steady accumulation in the environment is of considerable concern when estimating cadmium exposure.

Health Effects of Cadmium:

Data suggesting that cadmium might be an essential element are very limited. In 1976 Schwarz and Spallholz reported a growth supporting effect of cadmium in rats, but no confirmatory reports have analyzed these findings.

Cadmium is toxic to virtually every system in the human or animal body, whether ingested, injected or inhaled. There are large differences between the effects of high, single exposures and of chronic exposure to smaller doses of cadmium. Present data suggest that the toxicity of cadmium is possibly determined by the capacity of the tissues to synthesize metallothioneins.

Vast literature on various aspects of cadmium effect on general health has been published but is of limited value in evaluation of human or animal health.

In humans, inhaled cadmium can produce active bronchial and pulmonary irritation and sometimes fatal edema and shock (Stanescu et.al., 1977). Such instances may occur in workers involved in industrial processes like welding, smelting or soldering, where cadmium is heated at a very high temperature and inhalation of cadmium-oxide fumes occurs.

Oral exposure to cadmium predominantly produces gastrointestinal reactions. In Sweden, Nordberg et.al., 1973 reported on some school children who had ingested fruit juices from a vending machine which had a cadmium-plated reservoir.

Toxicity symptoms appearing minutes after ingestion, included, nausea, vomiting, salivation, abdominal cramps and diarrhea.

In animals, single injections of high cadmium doses have a special impact on the reproductive organs and the nervous system with minor effect on other organs. Gabbiani, et.al., (1974), reported necrosis of the sensory ganglia after injection of high doses of cadmium, while Parizek, (1983) observed testicular and placental necrosis and suggested that testicular necrosis can also be induced by relatively low doses that do not damage other organs.

Long term exposure to cadmium affects almost all organs in the body. Kidney is the primary organ to be affected. Renal tubular proteinuria occurs regardless of whether exposure is oral, by inhalation or by repeated injections. Data on the mechanism of kidney damage by cadmium and relation to metallothionein have been reviewed by many authors (Squibb and Fowler, 1984) (Piscator, 1984).

Cadmium mainly damages the proximal tubules, resulting in decreased reabsorption of proteins especially low molecular weight proteins like $ß_2$ microglobulin and lysozyme. Experimental animals treated with cadmium demonstrated mitochondrial abnormalities and electron dense materials (probably cadmium - metallothionein) in the proximal tubular cells (Borgman and Chandra, 1987).

The mechanism by which renal toxicity develops during cadmium toxication is still unclear. Many investigators believe that kidney damage is prevented as long as the kidney can produce enough metallothionein. Beyond that stage, the non-metallothionein bound cadmium ions become very toxic (Norberg 1978 - Homiyama and Nomiyama, 1982).

The link between cadmium and hypertension is not fully understood. It has been observed that selenium, copper, and zinc may counteract the hypertensive action of cadmium. Data from several animal studies reveal that hypertension can be induced on exposure to low doses of cadmium (0.1 - 2 ppm) in drinking water while hypotension will occur if the doses exceed this range (Perry and Erlanger, 1974) (Kopp et.al., 1982).

In humans, some authors have suggested cadmium induced hypertension (Schroeder et.al., 1965) (Schroeder, 1974), while another group of scientists from Japan (Shigematsu et.al., 1979) could not prove the hypertensive effects of cadmium when they conducted a study on a very large sample of population living in cadmium polluted areas.

Attention to the possible role of cadmium in inducing bone disease was enhanced when a meeting was held in Japan in 1979 on cadmium induced osteopathy (Shigematsu and Nomiyama, 1979). Most of the findings obtained from animal studies indicated that cadmium can induce osteomalacia or osteoporosis. Mineral disturbances which are caused by renal dysfunction eventually might be the cause of renal stones or osteomalacia.

In humans long term exposure to cadmium has not been known to increase its concentration in the bone. Itai-Itai disease was an extreme example of osteomalacia that affected middle aged multiparous women with typical features of bony pain, walking difficulty and spontaneous fractures (Friberg et.al., 1974).

Data about association between exposure to cadmium and lung or prostatic cancer have been obtained mostly from experimental animals. Rats exposed to cadmium chloride for 18 months by inhalation had acquired lung carcinoma

(TaKenaka et.al., 1983) and injection of cadmium chloride in the prostate have produced prostatic tumours (Scott and Aughey, 1978). The risk of acquiring lung cancer in humans after exposure to cadmium depends on its percentage in the atmosphere as well as presence of other metals such as arsenic (Holden, 1980). This confirms the report of Thun et.al (1985) who noted an increased risk of lung cancer in cadmium smelter workers. However, other authors have noticed an insignificant risk of lung cancer in alkaline battery factory workers (Kjellstrom et.al., 1979).

Interpretation of studies on humans have been difficult. Some of the studies have used small numbers of subjects while others did not take in consideration the airborne concentration of cadmium compounds that might lead to cancer of the lung or the prostate.

Damage of reproductive tissues such as gonads and uterus is considered to be one of the most critical effects of cadmium. A single parenteral injection of cadmium salts was reported to produce haemorrhagic necrosis of the testes (Parizek, 1983), while in female rats, cadmium injection caused hyperemia of the ovary, atresia of the follicles and features similar to toxemia of pregnancy (Furuta, 1978). Teratogenic effects of cadmium have not been identified. It has been hypothesized that occupationally exposed women have offspring with lower birth weights than in controls. Some workers have reported that cadmium may have a role in the development of pregnancy induced hypertension (Chisolm and Handorf, 1985) but this report has not yet been confirmed.

Several investigators have demonstrated that high doses of cadmium induces congenital developmental anomalies and fetal death in laboratory animals (Ferm and

Carpenter, 1968) (Ferm, 1971) (Chernoff, 1973) and low levels of maternal dietary cadmium fed throughout gestation cause fetal growth retardation (Webster, 1978). Placental transfer of cadmium has been investigated but with conflicting results. Early studies lead to speculations that the placenta constitutes a barrier against fetal cadmium accumulation (Berlin and Ullberg, 1963). A few years later Sonawane, et.al., (1975) indicated that with intravenous administration in rats, cadmium chloride crossed the placenta with relative ease. However, when cadmium chloride was administered orally to pregnant rats fetal accumulation of cadmium was extremely low (Ahokas and Dilts, 1979). Thus it is not known whether the embryotoxic effect of cadmium is a function of the amount transferred to the fetus or an indirect action on the embryo.

Cadmium and immunity

Cadmium has the potency to interfere with the immune system (Kranje et.al., 1983). Significant suppressive effect had been observed on both humoral and cellular immunity. The responses vary according to the species, route, dose and time of cadmium exposure in relation to antigen challenge (Koller, 1980) (Muller et.al., 1979).

Primary (IgM) and secondary (IgG) antibody responses were not altered after antigen challenge of oral cadmium (25 - 50mg) treated mice (Muller, et. al., 1979). These results were in contrast to those reported by Koller et.al., 1975. However, Malave and DeRuffino (1984) claimed a 30% increase in the IgM of cadmium treated as compared to control mice, while Blakley (1985) found a decrease in responses

with the same low oral doses. The same results were obtained later by Borgman, et.al., 1986.

The parenteral route of cadmium administration affected different levels of antibody production. Koller et.al. in 1976 reported an increase in primary (IgM) and secondary (IgG) antibody responses after injection of 0.15 mg of cadmium, but when doses of 1.8 mg/kg body weight was injected, suppressed antibody responses occurred due to direct B-cell inactivation (Fujimaki, 1985).

The effects of cadmium on cell - mediated immunity have been reviewed by many authors. Delayed type hypersensitivity was evaluated by Muller, et.al. (1978) as this is a classical test for determination of cell-mediated immunity. A group of cadmium treated mice and a control group were sensitized with sheep RBC and challenged by a single intracutaneous foot pad injection of the same antigen. The reaction was inhibited in cadmium treated mice in relation to the control ones. Inhibition was related to cadmium concentration detected in the serum of the animals. Another way of estimating cell-mediated immunity is evaluation of T and B lymphocyte function. Cadmium treated mice gave significantly higher responses to T-cell mitogen, phytohemagglutinin (PHA) and concanavalin A (Con A). Similar but less pronounced effects were observed by stimulating spleen cells with, lipopolysaccharide (LPS), a ß lymphocyte activator (Muller et.al., 1978). Similar results were obtained by Malave and De Ruffino (1984) with increased T-cell activity to PHA and Con A. However, a decreased response was observed by Gaworski and Sharma (1978), and no change in responses was claimed by Wesenberg and Wesenberg (1983). The variations and the conflicting results obtained from different experiments might be due in part to using different strains of mice as CBA mice were

found to be high responders while C57 BL/6 mice are low responders to PHA or Con A, and to different experimental protocols. Low responsiveness might be a consequence of the inhibitory activity of adherent cells (Malave, 1981). On the other hand, variations in the procedure to evaluate lymphocyte proliferation could also contribute to the differences in the reported results.

Cadmium as an environmental contaminant alters antibacterial defense mechanisms (Fassett, 1975) (Gardner et.al., 1977). This alteration seems to be due specially to an impairment of the function of phagocytic cells (Gardner et.al., 1977). It was reported that in cadmium treated animals the phagocytic capacity of macrophages and polymorphonuclear leukocytes has been reduced (Loose et.al., 1977). Also macrophage migration inhibitor factor was found to be subnormal (Kiremidjan-Schumacher et.al., 1981). Not only are antibacterial defense mechanisms affected by cadmium treatment, but antiviral and antiprotozoal defence mechanisms are also affected, with generalized immunosuppression and increased susceptibility to infections (Exon et.al., 1979).

Interaction between cadmium and zinc:

Zinc is known as an essential element and cadmium as a toxic heavy metal. Both have striking physical and chemical similarities that place them in group II of the periodic table. The fact that they compete with each other for the same metallothionein makes their biological interactions very likely, also raising the possibility that cadmium toxicity can be treated or prevented by zinc.

In 1957 Parizek declared that the destructive effect of cadmium ion on the testicular tissue can be prevented by administration of large doses of zinc. These observations were confirmed by Gunn et.al. (1961) and Webb (1972).

Petering et.al. (1971) could not prove that the prevention of cadmium induced pathological changes by zinc may have been because cadmium had been orally administered in small doses as opposed to other workers who had used it in large parenteral injections. However, they agreed on the same principle of the antagonizing effect between zinc and cadmium even at low cadmium doses. They found that the growth rate of rats was markedly affected when zinc was given orally in low doses (zinc:cadmium ratio 1:1), whereas there was no effect when zinc intake was higher (zinc:cadmium ratio 4:1).

Cadmium and zinc interactions also appear to be involved in other biological areas such as teratogenecity (Ahokas et. al. 1979). When pregnant rats were given low drinking water levels of cadmium (0, 10, 100 ng/ml) during gestation it resulted in a decrease of all maternal parameters throughout the period of pregnancy. Even term fetal weight was significantly less than that of control subjects. Growth retardation was a result of decreased cell division (DNA) and cell growth (protein/DNA ratio). Dietary zinc supplementation (5.0 ug/ml) of cadmium fed dams

increased maternal food consumption, fetal weight, DNA, protein/DNA ratio and fetal zinc levels.

It is well known that lack of dietary zinc results in severe impairment of growth and substantially increased protein and amino acid catabolism and urinary nitrogen excretion (Hsu and Anthony, 1975). The decreased feed efficiency observed with cadmium may be a manifestation of zinc deficiency induced by competitive interaction of cadmium with dietary zinc. This mechanism had an impact on nephrotoxicity (Watanabe, et. al. 1985), carcinogenicity (Gunn, et. al 1963) and immunity (Chowdhury, et. al. 1987) where selective immunotoxic effect of relatively low doses of cadmium was prevented by large doses of zinc in drinking water. The specific mechanism(s) by which cadmium decreases appetite and nutrient metabolism requires further investigation.

In higher mammals such as man and horse, zinc concentration in the renal cortex has been shown to increase on an equimolar basis with the increase of cadmium up to a cadmium level of about 50-70 ng/g. Above this level the increase of zinc is less pronounced (Piscator, 1975 - Elinder, et. al., 1977). In 1973 Nordberg, et.al postulated that the increase of zinc is believed to be a compensation for the increase of cadmium through formation of metallothionein which binds both zinc and cadmium on a molar ratio of 1:1. Webb (1975) stated that after long term exposure, about 78% of the cadmium in the kidney and liver is bound to metallothionein. Three forms of metallothionein have been identified, one binding equimolar amounts of cadmium and zinc, a second binding mainly cadmium, and a third binding mainly zinc. In large farm animals, the finding of an equimolar increase of zinc with cadmium in the renal cortex seems to indicate that it is the form of metallothionein

which binds equal amounts of zinc and cadmium that is present there. On the other hand, the limited increase of zinc despite greater increases of cadmium in the renal cortex of most laboratory animals might indicate that the forms of metallothionein binding higher amount of cadmium are produced.

In experimental animals, cadmium exposure causes increases in hepatic and renal zinc concentrations which occur at the expense of decreased zinc concentration in other organs (Roberts et. al. 1973). Other in vitro studies showed that cadmium reduced the activity of several zinc dependent enzymes (Vallee and Ulmer, 1972). This might explain the tubular proteinuria that occurs in chronic cadmium poisoning, which was postulated to be due to decreased activity of certain zinc requiring enzymes like alkaline phosphatase and leucine aminopeptidase, and believed to be involved in tubular reabsorption of proteins (Wachsmuth and Torhorst, 1974).

It should be pointed out that the exposure potential for humans and farm animals is usually quite different from that of laboratory animals. Laboratory animals are usually given excessive doses of cadmium or zinc during a short time, while humans have a low daily intake.

CHAPTER II

Rationale and Objectives

Cadmium, is a widely distributed, naturally occurring toxic element. With increasing industrial use, pollution of the environment and cigarette smoking, its toxicity to humans has become a matter of worldwide concern. In moderate to high doses, cadmium is an immunosuppressive agent and enhances susceptibility to bacterial, protozoal and viral infections.

On the other hand, zinc is an essential trace element. Its deficiency was thought to be exceedingly rare, but recently it has been suggested that milder forms of this condition are sufficiently common to be of potential public health concern. This may, in part, be due to increasing consumption of highly processed and refined foods that results in loss of zinc.

Zinc and cadmium have striking physical and chemical similarities. Both belong to Group II of the periodic table and compete for the same metallothionein in various tissues. Thus it was suggested that cadmium toxicity can be prevented or ameliorated by zinc administration. Both toxicity of cadmium and deficiency of zinc affect all systems of the body. Their impact on the immune system has been extensively investigated in man and laboratory animals with variable results depending on the dose, duration and route of administration of the two elements.

The principal objective of the present study is to investigate the possible effect of zinc deficiency on potentiating the pathologic effect of cadmium on the immune system and the kidney of mice. The mouse was chosen as the animal model as it

has a well characterized immune system that closely parallels the human immune system at all developmental stages.

Primary Objective:

To study the effect of a relatively low dose of oral cadmium treatment on the immune system of zinc deficient mice.

Secondary Objectives:

1. To study the effect of cadmium on renal morphology;

2. To study the effect of zinc deficiency on the immune system of mice;

3. To study the immunopathological effect of cadmium on zinc supplemented mice.

CHAPTER III

Design of the Study

Treatment Schedules:

Male 5-6 week old C57BL/6 mice were used in the study. Mice were divided into six experimental groups. Each group received a different treatment but housing conditions were identical in all groups. The design of the experiment is shown in Fig. 3-1.

Group I: No treatment

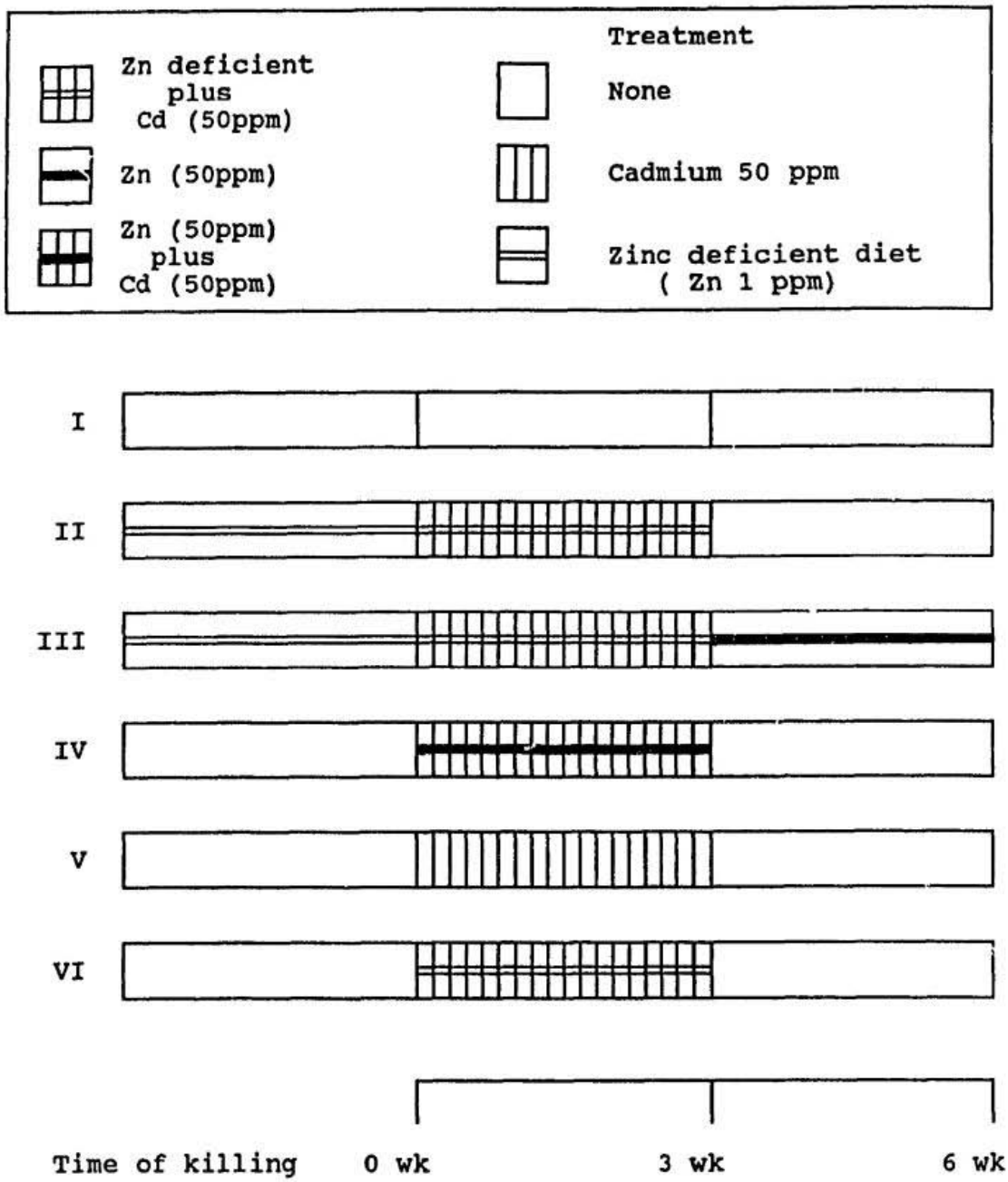
Group II: Zinc deficient diet (Zn 1 ppm) for 4 weeks, followed by the administration of cadmium (50 ppm) (cadmium chloride, No. B10064-34, BDH chemicals, Toronto) in deionized distilled water for the next 3 weeks, and no treatment for the last 3 weeks.

Group III: Same protocol as Group II but during the last 3 weeks, zinc (50 ppm) (zinc chloride, No. Z.0150, Sigma Chemical Company, St. Louis, Missouri) was added in deionized distilled water.

Group IV: No treatment for 4 weeks, zinc (50 ppm) and cadmium (50 ppm) added to deionized distilled water for the next 3 weeks and no treatment for the last 3 weeks.

Group V: No treatment for 4 weeks, cadmium (50 ppm) added to deionized distilled water for the next 3 weeks, and no treatment for the last 3 weeks.

Group VI: No treatment for 4 weeks, zinc deficient diet (1 ppm) + cadmium (50 ppm) in deionized distilled water for the next 3 weeks, and no treatment for the last 3 weeks.



Design of the study

Animals were killed for examination at the end of each treatment period (4th, 7th and 10th weeks) these are termed 0, 3 and 6 weeks of observation respectively. In each treatment period for all six groups, there were 8 to 10 mice, however, at 0 week, only 2 groups were sacrificed, because at that time period groups I, IV, V and VI were identical, and groups II and III were identical as well. This design allowed study of the effects of zinc deficient diet on the immune system of mice before and after receiving low dose of cadmium. The no treatment group (Group I) provided control data and baseline values.

Zinc deficient diet containing zinc 1 ppm was chosen as previous studies have shown that the immune system of mice was affected by such a diet after 4 weeks (Fraker, et al. 1987; Fraker and Gershwin, 1986). A low dose of cadmium (50 ppm) and oral route of exposure were chosen as these are more akin to possible human cadmium toxicity. Since cadmium has a long biological half life, the study period was extended after cessation of cadmium treatment. Cadmium was used at a dose of 50 ppm as previous studies have shown that at this dose the immune system of mice is affected (Borgman et.al., 1986), but the animals in this study did not show any clinical manifestations of toxicity, as was also observed by a previous study (Chowdhury et.al., 1987). Zinc treatment was chosen at a dose of 50 ppm because zinc and cadmium compete for the same metallothionein, and at an identical concentration, it is possible that zinc may ameliorate the toxic effects of cadmium.

Experimental protocol:

The experimental period was spread out between 6 to 10 weeks and the total number of animals was 96.

Mice from all groups were monitored for weight and food consumption once a week at the same time and hour. After killing each animal, the kidneys, spleen and thymus were weighed.

The total number of lymphocytes was counted in the blood, spleen and thymus. Direct and indirect plaque - forming cell responses which assay IgM and IgG antibody production respectively by spleen cells against sheep red blood cells were measured. Proliferation response of spleen cells to T cell mitogen (phytohemaglutinin - PHA) was also assayed. Number of T cells and subsets (helper and suppressor) was counted by direct immunofluorescence staining by using appropriate monoclonal antibodies. Natural killer cell activity was measured by chromium release assay in isolated splenic lymphocytes. Transmission electron microscopic examination of kidney and thymus sections were performed. Kidney was analyzed for cadmium and zinc levels.

Detailed description of the animals studied, diet, housing, and laboratory methods is given in Chapter IV.

CHAPTER IV

Methods

4.1 Housing and feeding the mice:

Male C57BL/6 mice (Charles River Canada, Montreal, Quebec) were maintained in the Animal Care Facility of the Faculty of Medicine, Memorial University of Newfoundland.

In group I and V mice were housed singly in plastic cages with bedding while in group II, III, IV and VI mice were also marked individually but in stainless steel cages with wire mesh bottoms to avoid mice treated with zinc deficient diet from having an access to the bedding.

Distilled water was provided in plastic bottles with siliconized rubber stoppers and stainless steel tubing. Food pellets were placed in stainless steel feeders. The temperature in the animal rooms was controlled at 22-24^{o}C, relative humidity at 45-55% and lighting hours at 0800 to 0200. Standard guidelines for care and use of experimental animals were followed throughout (Canadian Council on Animal Care, 1984) and the protocol was approved by the University President's Committee . . . Animal Care Committee.

For groups I, II and III mice were purchased at 3 weeks of age while for groups IV, V and VI they were purchased at age 6-7 weeks. In the Animal Care Facility they were acclimatized for 1 or 2 weeks during which they had free access to ordinary drinking water and food, then each was assigned randomly to one of the experimental groups. Chow food pellets that were used by some groups (Rodent Chow No. 5012, Ralston Purina, St. Louis, Missouri) were analyzed for trace element

contents and found to contain on average 0.09 mg cadmium/g and 43.3 mg zinc/g. Other constituents are shown in Table 4-1, and constituents of the zinc deficient food pellets are shown in Table 4-2. During the treatment period mice had free access to drinking water. On a weekly basis throughout the whole experiment general health, weight and food consumption were monitored and recorded.

TABLE 4-1

CONSTITUENTS OF THE REGULAR CHOW DIET

Analysis

Crude protein	not less than	22.2%
Crude fat	not less than	4.0%
Crude fiber	not less than	5.0%
Ash	not less than	3.0%

Ingredients

Ground extruded yellow corn, soybean meal, fish meal, cane molasses, wheat middings, dehydrated alfalfa meal, ground oats, brewers dried yeast, wheat germ meal, dried beet pulp, soybean oil, dicalcium phosphate, calcium carbonate, salt, vitamin B-12 supplement, calcium pantothenate, choline chloride, riboflavin supplement, thiamin, niacin supplement, DL methionine, vitamin A supplement, D activated animal sterol (source of vitamin D-3), vitamin E supplement, calcium iodate, manganous oxide, ferrous carbonate, cobalt carbonate, copper sulfate, zinc sulfate.

TABLE 4-2

CONSTITUENTS OF ZINC DEFICIENT DIET

Composition:

Egg White	18.00%
Corn Oil	10.00%
Corn Starch	44.30%
Sucrose	20.00%
Alphacel Hydrolyze	3.00%
Choline Bitartrate	0.20%
A1N-76C Vitamin Mixture	1.00%
A1N-76 Mineral Mixture	3.50%
Biotin	20 mg/kg

4.2 Sacrificing the mice and collecting their tissues:

Animals were sacrificed in the animal care procedure rooms. Each mouse was anesthetized with ether and killed by cervical dislocation, the abdominal skin was wetted with 70% ethanol and the cavity was opened, aseptic precautions were maintained to avoid contamination of the spleen that was used for culture work. Sterilized scissors were used to cut through the peritoneal membrane, spleen was removed and transferred to a tube containing sterile culture medium, subsequent handling of this organ was done in a level A laminar flow hood (Envirco, Biodynamics Inc., Albuquerque, New Mexico), this followed by removal of the kidney and thymus. Organs were transferred to preweighed containers which were weighed again to obtain the organs weights. A single electronic balance (Mettler PC 180, Greinfensee, Zurich, Switzerland) was used throughout the experiment.

In animals whose kidney or thymus sections were prepared for electron microscopic examination, the required organ was removed first to maintain the ultrastructural integrity of the organ.

4.3 Lymphocyte counts in the blood, thymus and spleen:

(A) Lymphocyte Count in the blood:

Immediately after killing, the right atrium of the heart was opened and 10 ul of blood was collected and transferred to 190 ul of Turk's solution (0.1 gentian violet, 10.0 ml 95% ethanol and 0.5 ml acetic acid in 98.5 ml distilled water). A small aliquot of that diluted blood was transferred to a Neuber Counting Chamber, and the lymphocytes counted with a light microscope.

(B) Lymphocyte count in the thymus:

A known volume of normal saline was added to a petridish, then the thymus was gently homogenized using a tissue pestle on E-C cellector tissue sieve (Mandel Scientific, Rockwood, Ontario) with a mesh size 50 and opening size 0.28 mm, that allowed the contents to pass through and leave the capsule behind. Large debris were broken down by gentle agitation with a pasteur pipette, a single cell suspension was obtained bypassing the solution through graded needles (21G and 26G). 10ul of the final solution was added to 190 ul gentian violet, small aliquot of it was transferred to a Neuber counting chamber and lymphocyte counted with a light microscope. Results are expressed as total lymphocyte count in the thymus.

(C) Lymphocyte count in the spleen:

The entire procedure for handling spleen cells was carried out with total aseptic precautions in a laminar flow hood.

A known volume of sterile RPMI 1640 with L-glutamine without sodium bicarbonate (N0. 1060120, Flow Laboratories, McClean, Virginia) was placed in a petri dish. Spleen was gently teased with two curved needles. This maneuver allowed to squeeze out the contents and leave the capsule behind. Large cellular clumps were broken down by gentle agitation with pasteur pipette and debris removed by sedimentation for 10 minutes. Supernatant was collected and a single cell suspension obtained by passing the solution through graded needles (21G and 26G). 10 ul of the final solution was diluted in 190 ul gentian violet and lymphocytes were counted in a Neuber chamber.

4.4 In vitro stimulation of spleen cells with mitogens:

(A) Preparation of splenic cell suspension:

Spleen cells was prepared as described in section 4.3.C. Cells were washed three times in sterile RPMI 1640 (Flow Laboratories, McClean, Virginia) at 200 xg for 10 minutes each. After the final wash cells were counted and resuspended to 1 x 10^6 lymphocytes/ml in sterile RPMI 1640 supplemented with sodium bicarbonate, 10 ul of penicillin, 100 mg/ml of streptomycin, 250 ug/ml of fungizone (Gibco Laboratories, Chagrin Falls, Ohio) and 5% heated inactivated (57^oc for 30 minutes) fetal calf serum (Bocknek Organic Material, Rexdale, Ontario). Aseptic precautions were maintained for all the previous and subsequent procedures.

(B) Stimulation of spleen cells with phytohoemaglutinin (PHA)

A flat bottom microculture plate was used (Flow Laboratories, McClean, Virginia). The procedures were set up in triplicate for each mitogen and control culture. For mitogen stimulation, 200 ul of spleen cell suspension (1 x 10^6) lymphocytes/ml was placed in each well + 20 ug of phytohaemaglutinin (PHA) (Wellcome, Diagnostics, Dartford, England), while for control culture 20 ul of normal saline was delivered in each well instead of the PHA.

The culture plate were incubated for 72 hours at 37^oC in a humidified atmosphere containing 5% $C0_2$ in air. In the final 4 hours of incubation 0.5u Ci of [^{3}H] thymidine with a specific activity of 2Ci/mM (New England Nuclear, Boston, Massachussets) was added in each well to assess DNA synthesis, cells were then harvested on glass fiber filters (Mandel Scientific, Rockwood, Ontario) with a multiple

automated cell harvester (Titertek, Flow Laboratories, Rockville, Maryland). Filter papers were left for 2 days to be completely dry then the discs were put in scintillation vials and 8 ml of scintillation fluid was added (BDH chemicals, Toronto, Ontario) and counted for radioactivity by liquid scintillation system, fullerton (California). Results were expressed as stimulation index:

$$\text{Stimulation index} = \frac{\text{Mean counts per minute of triplicate stimulated culture}}{\text{Mean counts per minute of triplicate control culture}}$$

4.5 Direct and indirect splenic plaque-forming cell response:

Plaque forming cell (PFC) in response to sheep red blood cells was assayed by a modified (Kappler 1974) Jerne plaque assay.

(A) Immunization of the mice:

For estimation of direct (IgM) or indirect (IgG) plaque forming cells, mice were injected 4 or 8 days intraperitoneally with 0.2 ml of sheep red cells before sacrificing. Sheep red cells were stored in Alsever solution (Woodlyn Laboratories, Guelph, Ontario), were washed three times in normal saline at 450 xg, 10 minutes each, buffy coat was removed after each time. After the last wash cells were resuspended to 20% in normal saline.

(B) Reverse hemolytic plaque assay:

1. Spleen cells were prepared as in section (4.3.C). Cells were washed 3 times in Hank's balanced salt solution (No. 18104, Flow Laboratories, McClean,

Virginia). For the next wash the balanced salt solution was added and the process repeated. After the 3rd wash supernatant was aspirated and cells were resuspended in BSS containing 5% heat inactivated (57^{o}c for 30 minutes) fetal calf serum, then cells were adjusted to 2 x 10^{6} lymphocytes/ml.

2. Sheep red cells were prepared to be used as target for lysis in the plaque assay. Cells were washed 3 times in normal saline, each time cells centrifuged at 450 x g for 10 minutes, buffy coat was removed and normal saline was added to bring up the volume for the next wash. After the 3rd wash the concentration of the sheep red cells was adjusted to 15% in BSS. 40 microliter of the 15% solution was added to 1 ml BSS to make a 0.57% solution.
3. Guinea pig complement (Gibco Laboratories, Grand Island, New York) and rabbit anti-mouse IgG (Miles Scientific, Rexdale, Ontario) were absorbed over sheep red blood cells to prevent non-specific binding. The sheep red cells were washed three times at 450 xg, 10 minutes each, buffy coat was removed after each time. 2 ml packed sheep red cells was added to 10 ml guinea-pig complement or anti-mouse - IgG and incubated for 30 minutes at 4^{o}C with continuous rotation at 10 cycles/minute. After incubation packed cells were centrifuged at 450 x g for 10 minutes, supernatant removed and incubated with sheep blood cells again. The process was repeated 3 times. After the absorption, 500 ml volume of the guinea-pig complement or anti-mouse - IgG was aliquoted and stored at -70^{o}C. For the plaque assay, 1/10 dilution of guinea-pig complement and 1/40 dilution for anti-mouse - IgG was used.
4. For performing direct (IgM) antibody plaque assay, flat bottom microculture plates were used (Flow Laboratories, McClean, Virginia). In each well of the

plate 40, 30, 20 ul spleen cell suspension (8, 6, 4 x 10^6 lymphocytes) along with 50 ul of 0.57% sheep red cells and 50 ul of 1/10 dilution of guinea-pig complement were delivered. The final volume in each well was brought up to 190 ul by adding an appropriate amount of culture medium. Constituents of each well are shown in Table 4-3. The assay was set up in triplicates for each sample and cell dilution. Plates were centrifuged at 55 x g for 3 minutes to obtain a monolayer and incubated at 37^oC with 5% CO_2 in air. The number of plaques were determined with an inverted microscope. Under the microscope, the plaque looks like a clear zone of red cell lysis with a lymphocyte in the center [Fig. 4-1]. Results are expressed as plaques/10^6 spleen cells and plaques/spleen.

5. For indirect (IgG) plaque assay, a similar procedure as for direct (IgM) assay (step number 4) was followed, but an additional 50 ul of 1/40 rabbit-anti-mouse IgG was added to each well. To get the number of IgG producing cell count, direct PFC number was subtracted from indirect plaque forming cells.

TABLE 4-3

Direct and Indirect Plaque Assay

Constituents of Culture Wells	Lymphocytes/Well			Lymphocytes/Well		
	$8x10^6$	$6x10^6$	$4x10^6$	$8x10^6$	$6x10^6$	$4x10^6$
	Direct (IgM) Plaque			Indirect (IgG) Plaque		
Spleen cell suspension ($2x10^6$ ml)	40 ul	30 ul	20 ul	40 ul	30 ul	20 ul
Sheep red blood cells (0.57%)	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul
Guinea-pig complement (1/10)	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul
Mouse anti-IgG	-	-	-	50 ul	50 ul	50 ul
Culture medium	50 ul	60 ul	70 ul	50 ul	60 ul	70 ul

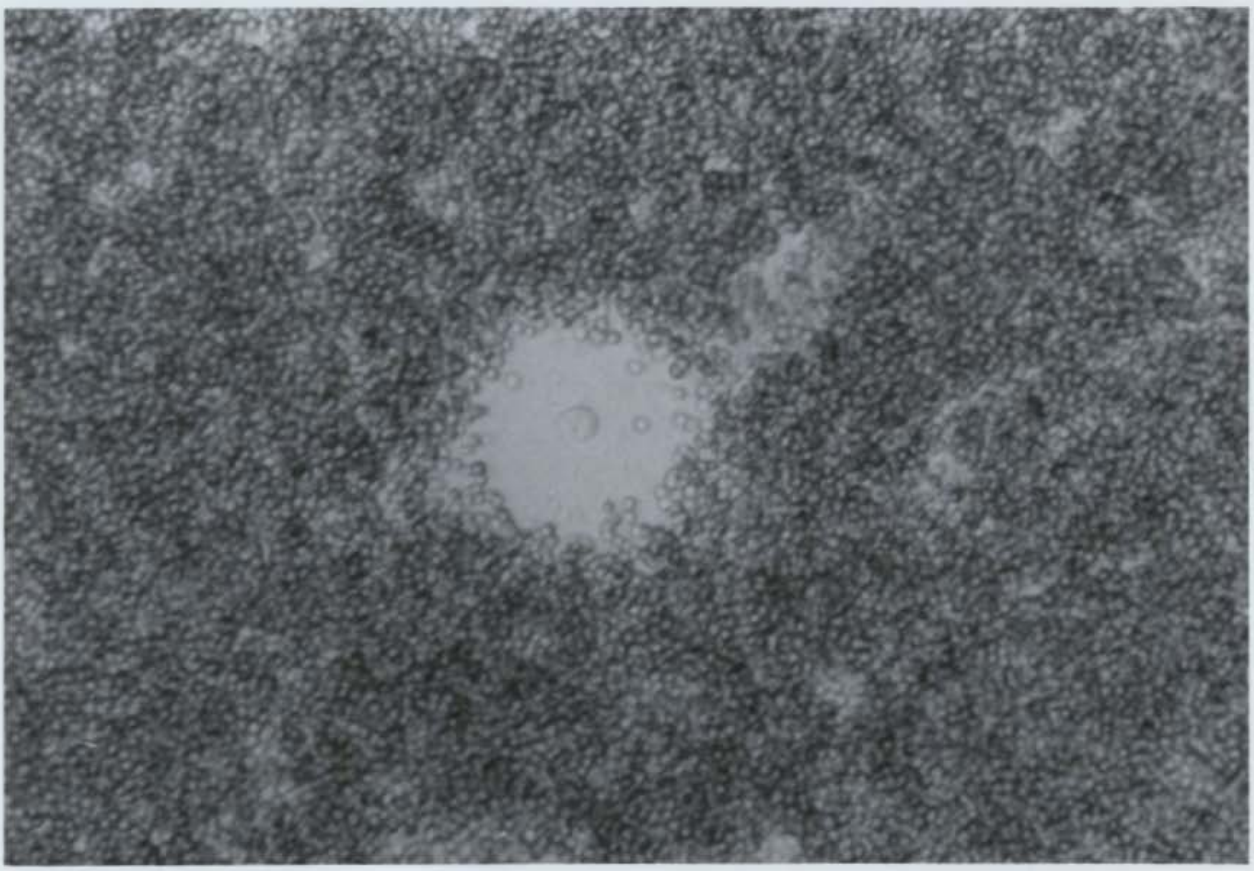


FIG 4-1

Antibody producing lymphocyte. The clear area of red cell lysis around a lymphocyte is counted as one positive plaque.

4.6 Assay for natural killer cell activity:

Natural killing is based on the fact that unsensitized lymphocytes from normal individuals are spontaneously cytotoxic for a variety of target cells (Ortaldo and Herberman, 1984).

(1) Preparation of the splenic cell suspension is similar to that described in section 4.3.C. Lymphocytes were isolated by density gradient centrifugation. The suspension of spleen cells was layered over Ficoll-Hypaque (Sigma Chemical Company, St. Louis, Missouri) with a specific gravity of 1.09 and centrifuged at 400 xg for 30 minutes at room temperature. A visible interface which formed of lymphocytes between the two layers was collected and washed 3 times in RPMI - 1640 at 200 xg for 10 minutes each, then counted and adjusted to a concentration of 20×10^6 cells/1 ml in RPMI 1640 with sodium bicarbonate, 100 ul/ml of penicillin, 100 mg/ml of streptomycin, 250 mg/ml of fungizone (Gibco Laboratories, Chagrin Falls, Ohio) and 10% heat inactivated (57% for 30 minutes) fetal calf serum (Bocknek Organic Material, Rexdale, Ontario) this suspension was used as the effector cells in the natural killer cell assay.

(2) For labelling of target cells, Molony virus induced mouse lymphoma cell line YAC-1 (American type tissue culture, Rockville, Maryland) maintained in continuous culture in complete culture medium was used as target cells. 200 $\mu Ci^{51}Cr$ as sodium chromate (New England Nuclear Corp. Boston, Mass.) was added to $5x10^6$ tumour cells in 0.5 ml of culture medium and incubated for 60 minutes at 37^oC with continuous rotation at 10 cycles/minutes, then cells were

washed twice in assay medium, counted and resuspended to the final concentration ($0.8x10^6$ cells/ml).

(3) For setting up the chromium release assay, a V-bottom microculture plate was used (Flow Laboratories, McClean, Virginia). In each well 50 ul labelled target cell suspension (0.8×10^6/ml) and 100 ul effector cell suspension (20×10^6/ml) was delivered with resultant of 50:1 effector target ratio.

Spontaneous ^{51}Cr release from target cells was measured in the absence of effector cells and total release was measured by treating the target cells with 100 ul/well of 1% Nonidet P40 (Sigma Chemical Company, St. Louis, Missouri). In each well, volume was maintained at 200 ul by adding 50 ul of culture medium. Constituents of the culture wells are shown in Table 4-4.

Culture was performed in triplicate, plate was incubated at 37^oC in 5% CO_2 air atmosphere for 4 hours. After incubation the plate was centrifuged at 400 xg for 5 minutes and 100 ul of supernatant was collected from each well. Radioactivity in the supernatants was counted in a gamma counter (Beckman Instruments, Fullerton, California). The percentage of isotope released from the target cells was calculated by the following formula:

$$\text{Percent release} = \frac{\text{Mean count per minute of experimental release} - \text{Mean count per minute of spontaneous release}}{\text{Mean count per minute of total release} - \text{Mean count per minute of spontaneous release}} \times 100$$

TABLE 4-4

Assay of Natural Killer Cell Activity

	50:1 Target/Effector Ratio	Total Release	Spontaneous Release
	ul/well	ul/well	ul/well
Effector cell ($?0x10^6$/ul)	100	-	-
Target cell ($0.8x10^6$/ul)	50	50	50
Culture medium	50	50	150
1.0% nonidet P40	-	100	-

4.7 Enumeration of T-lymphocytes and subsets in the spleen:

(1) Spleen cell suspension was prepared as explained before in section (4.3.C). Washed 3 times at 200 x g for 10 minutes each, after the last wash the supernatant was removed and the total volume of the packed cells was between 4-5 ml.

(2) Lysis of the red blood cells was obtained by hypotonic shock (B.B. Mishell and S.M. Shiigi, 1979), while slowly vortexing the packed cells, 1 ml of distilled water was added slowly drop by drop followed by 1 ml of double concentration (2 x RPMI) slowly, then suspension was centrifuged at 200 x g for 10 minutes and cells adjusted to 2 x 10^6/ml in RPMI 1640.

(3) T-lymphocytes and subsets in the spleen were enumerated by direct immunofluorescence staining (Becton Pickinson Procedures). This method uses a fluorescein conjugated antibody directed to a specific antigen for the demonstration of cellular localization of that specific antigen. Antibodies are fluorescein conjugate anti-mouse Thy 1-2 for T cells, phycoerythrin conjugate anti-mouse L_3T_4 for helper/inducer and fluorescein conjugate anti-mouse Lyt-2 for suppressor/cytotoxic cells (all from Becton Dickinson immunocytometry systems, Mountain View, California).

(4) 50 microliter of the stock was added to 20 ul of fluorescien conjugate anti-mouse Thy 1-2, phycoerythrin conjugate anti-mouse L_3T_4 or fluorescein conjugate anti-mouse Lyt-2 for T cell, helper/inducer and suppressor/cytotoxic cells respectively.

(5) The mixture was incubated for 30 minutes at 4°C, washed 3 times with Dulbecco-phosphate-buffer saline (PBS) (No. 450-1300, Gibco laboratories, Grand Island, New york)containing 0.1% sodium azide at 300 xg for 5 minutes each at 4°C.

(6) After the last wash cells were resuspended in 0.5 ml cold medium and 1 ml paraformaldehyde 2% (Gibco Laboratories) was added to fix the cells prior to analysis. Analysis of the different cells was carried out by EPICS CD flow cytometry system (Coulter Corporation, Hiapeah, FL, USA).

4.8 Electron microscopy of the kidney and the thymus:

Electron microscopic procedure was performed in the electron microscopy department, Faculty of Medicine, Memorial University of Newfoundland. Kidney and thymus sections were examined at 0, 3 and 6 weeks of observations. Six animals from each group were examined.

Kidney and thymus were collected immediately after sacrificing the animal. All steps for tissue examination were carried out in a fume hood at room temperature while tissues were placed on a rotator.

Tissue was diced into small pieces (1 cubic mm) and immersed for 20 minutes in a mixture of paraformaldyde and glutaraldehyde in 0.2M sodium cacodylate buffer (PH 7.4) for fixation, then the tissue was washed thoroughly with 0.1M sodium cacodylate buffer and post fixed for 10 minutes with 1% osmium tetroxide in 0.2M sodium cacodylate buffer, washed with 0.1M sodium cacodylate buffer and dehydrated by passing through serial concentrations (70%, 95% and 100%) of ethanol for 3 minutes in each concentration with two changes, then after, for 10

minutes for absolute ethanol with two changes. After dehydration, the tissues were immersed in absolute acetone for 10 minutes with two changes, transferred to a 50:50 mixture of absolute acetone and Epon 812 embedding resin for 10 minutes, then to 100% resin for 10 minutes with two changes. Finally, the tissues were embedded with resin in capsules and polymerized at 70^{o}C for 16 hours in a Rechert KT-100 oven.

Sections 0-5 um were cut with a LKB Huxley ultramicrotome and ultra-thin silver sections were prepared with a Reichert OMU_3 ultratome. Sections were stained with lead citrate and uranyl acetate and observed under a Phillips 300 transmission electron microscope.

For observation under light microscope, sections with the same thickness were stained with 1% toluidine blue in 1% sodium borate.

4.9 Trace Element Analysis:

Portions of the kidney were weighed, freeze dried for 24 hours and stored at 4^{o}C for cadmium and zinc analyses. The analyses were done in the laboratory of Dr. Bo Lonnerdal, Department of Nutrition, University of California, Davis. The tissues were ashed at 500^{o}C for 16 hours in porcelain crucibles. The ash was dissolved for 30 minutes in 0.5 ml pure HNO_3 and 4.5 ml 1 N Hcl and the resulting solution was made up to 10 ml with deionized distilled water. Acid washed containers were used in all stages of the procedure. The samples were analysed by atomic absorption spectroscopy (Perkin Elmer, Norwalk, Connecticut) (Beaty, 1978). Control mice tissues were analyzed with a HGA-300 graphite atomizer with deuterium arc background correction, while remaining samples were analysed by

flame with a single slot 4-inch burner head. All results are reported as ug/g wet weight of tissue.

4.10 Statistical analysis:

Data were stored on EDT file in the VAX/VMS main frame computer system of the Memorial University of Newfoundland (EDT Editor Manual, 1980) and analyzed by the statistical packages MINITAB (Ryan, Joiner and Ryan 1985 a; Ryan, Joiner and Ryan 1985 b) and SAS (Cody and Smith 1985). Data were stored in a two-dimensional matrix suitable for handling by the statistical packages. A one way analysis of variance was performed to compare the groups. When a difference was found to be present at $P<0.05$, treatment effect was inferred and Duncans' multiple-range test was performed to make multiple comparison between the means. Significance levels used were 0.05 or less. When analysis of variance did not reveal any significant difference no further analysis was done.

CHAPTER V

Results

5.1 General health of mice:

The mice were examined once a week throughout the duration of the experiment for signs of cadmium toxicity, zinc deficiency, failure to gain weight, altered food intake and death. No toxic manifestations were seen in the cadmium treated animals. Mice given a low zinc diet showed loss of hair from all over the body especially the tail (Fig 5-1) at the second observation period (week 3). None of the animals died during any of the treatment schedules. On dissection, the thymus in 3 of the mice treated with zinc deficient diet was enlarged and hypertrophied. Other organs did not have any gross pathological changes. Weights of the animals taken weekly on the same day of the week and at the same hour for the four time periods are shown in Table 5.1. All the animals under different treatment schedules had a tendency to lose weight, more so for those treated with the zinc deficient diet. However, there were no statistically significant differences between various groups for weight loss. It was also seen that after cessation of treatment weight gain was similar in all groups.

Food consumption was measured weekly at the same time. Animals housed in wire-meshed hanging cages lost considerable amounts of food because as the size of the pellets became small they tended to drop from the cage. Food waste was measured and subtracted from the weekly food disappearance to calculate net food consumption. Table 5.2 reflects food consumption during the 3 observation periods. The results did not differ among the six treatment groups during the different observation periods.

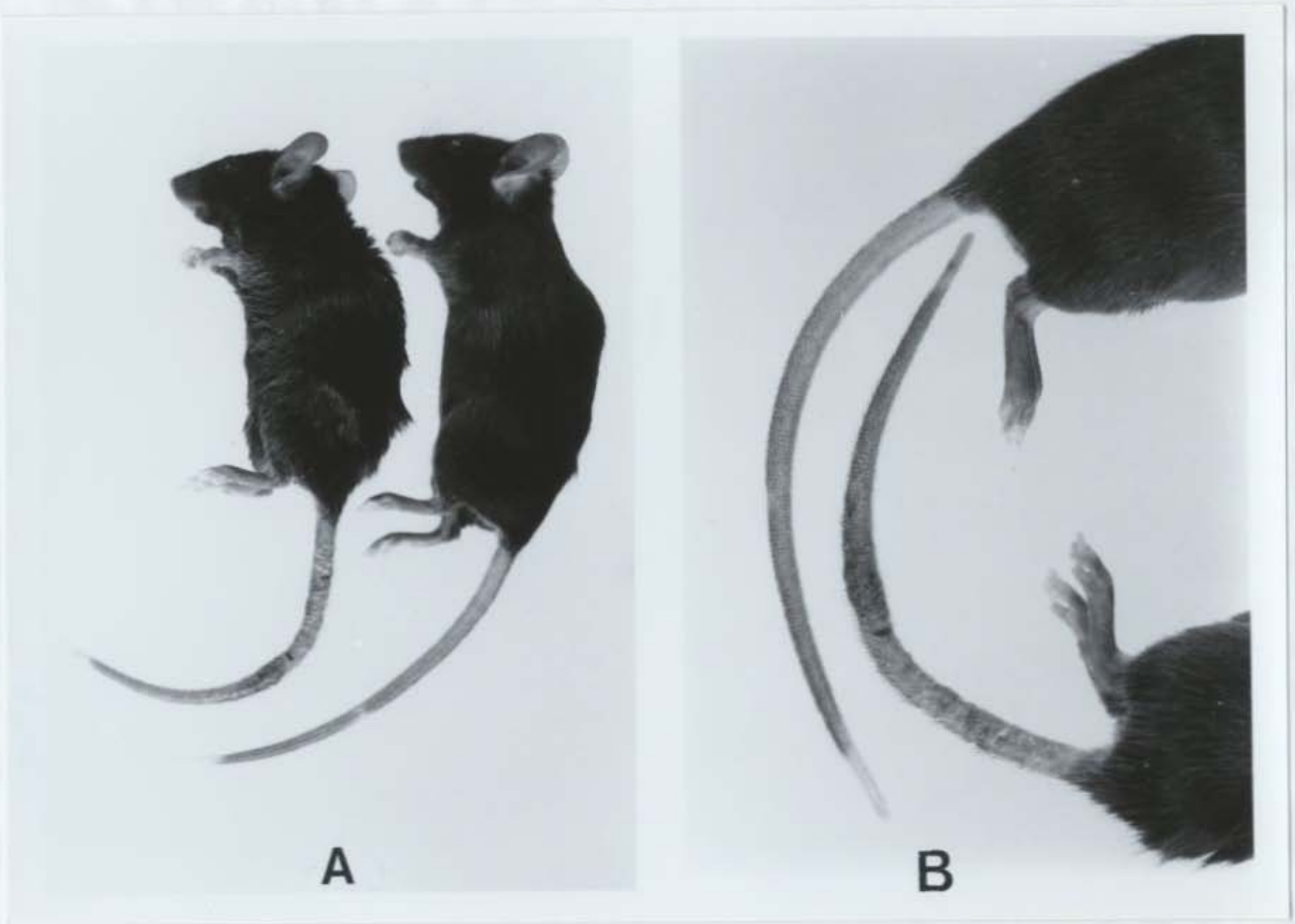


Figure 5.1

Fig. (A) shows control mouse (right) and low zinc-treated mouse (left) with loss of hair from all over the body especially the tail.

Fig. (B) shows more details of the tail sections from the control mouse (left) and low zinc-treated mouse (right) with loss of hair and bleeding spots.

TABLE 5-1

WEIGHT OF THE MICE (gm)

Group	Treatment	Before Treatment	0 Week	3 Week	6 Week
1	No treatment	15.8 ± 1.69	22.17 ± 1.52	25.08 ± 1.31	27.42 ± 1.30
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	15.0 ± 1.48	21.50 ± 3.82	22.92 ± 4.74	26.25 ± 1.40
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	15.0 ± 1.47	21.50 ± 3.82	22.92 ± 4.74	27.58 ± 2.15
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	16.2 ± 1.89	20.66 ± 1.07	24.08 ± 0.99	26.83 ± 1.50
5	None for 4 wks Cd for 3 wks None for 3 wks	16.8 ± 1.77	20.75 ± 1.80	24.58 ± 1.50	27.91 ± 0.99
6	None for 4 wks Zn^- + Cd for 3 wks None for 3 wks	16.5 ± 1.78	19.75 ± 1.76	22.50 ± 1.88	26.58 ± 1.83

There were 8 animals in each group.
Results are expressed as weight (gm) given as mean ±SD.

There are no statistically significant differences ($p > 0.05$) between the treatment groups by one-way analysis of variance.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-2

FOOD CONSUMPTION (gm/day)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	3.32 ± 0.43	3.40 ± 0.49	4.14 ± 0.58
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	3.21 ± 0.62	3.18 ± 0.37	3.81 ± 0.63
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	3.27 ± 0.56	3.24 ± 0.38	4.04 ± 0.61
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	3.29 ± 0.35	3.28 ± 0.34	4.45 ± 0.71
5	None for 4 wks Cd for 3 wks None for 3 wks	3.22 ± 0.38	3.25 ± 0.46	4.18 ± 0.61
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	3.12 ± 0.39	3.20 ± 0.52	4.33 ± .0.59

Results are expressed as mean ± SD.

There are no statistically significant differences ($p > 0.05$) between the treatment groups by one way analysis of variance.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

5.2 Organs weights:

After sacrificing the animals, the spleen, thymus and kidney were removed and weighed immediately. Tables 5.3, 5.4 and 5.5 reflect the weight of different organs at the 3 observation periods (0,3 and 6 weeks). None of the organs showed significant weight loss and there was increasing weight gain with age at all observation periods. However, animals treated with zinc deficient diets had less weight gain in the different organs, even after cessation of treatment in comparison with the control group. However, this difference did not reach the level of statistical significance.

5.3 Lymphocyte counts in the blood, spleen and thymus:

Lymphocytes were counted in 10ul of blood collected from the right atrium of the heart and from all suspensions prepared from homogenized spleen and thymus. Results are shown in Tables 5.6, 5.7 and 5.8.

Animals treated with zinc deficient diet had slightly decreased numbers of lymphocytes in the blood after 4 weeks of treatment (week 0 observation). However, concurrent administration of cadmium caused a significant decrease in the lymphocyte counts in comparison with the control group. Even animals treated with cadmium only had a significant decrease in the lymphocyte counts. Lymphocyte count of the spleen cells and thymus did not differ among the treatment groups at any point. However, animals treated with zinc deficient diet had a tendency to decrease lymphocyte counts in comparison with other treatment groups, but this did not reach levels of statistical significance.

TABLE 5-3

WEIGHT OF SPLEEN (gm)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	0.087 ± 0.022	0.111 ± 0.015	0.127 ± 0.018
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	0.098 ± 0.026	0.105 ± 0.038	0.123 ± 0.023
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	0.113 ± 0.106
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	0.118 ± 0.202	0.119 ± 0.017
5	None for 4 wks Cd for 3 wks None for 3 wks	-	0.112 ± 0.010	0.128 ± 0.018
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	0.097 ± 0.234	0.109 ± 0.006

8 animals in each group. Values are expressed as mean ± SD

There are no statistically significant differences ($p > 0.05$) between the treatment groups by one-way analysis of variances.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd:Cadmium 50 ppm

TABLE 5-4

WEIGHT OF THYMUS (gm)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	0.069 ± 0.030	0.083 ± 0.029	0.090 ± 0.020
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	0.053 ± 0.018	0.077 ± 0.016	0.072 ± 0.021
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	0.093 ± 0.031
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	0.073 ± 0.028	0.091 ± 0.017
5	None for 4 wks Cd for 3 wks None for 3 wks	-	0.090 ± 0.016	0.110 ± 0.531
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	0.070 ± 0.023	0.87 ± 0.020

Values are expressed as mean ± SD

There are no statistically significant differences ($p > 0.05$) between the different groups by one-way analysis of variance.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-5

WEIGHT OF KIDNEY (gm)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	0.282 ± 0.195	0.356 ± 0.052	0.350 ± 0.060
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	0.274 ± 0.046	0.338 ± 0.099	0.349 ± 0.083
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	0.317 ± 0.104
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	0.359 ± 0.057	0.420 ± 0.040
5	None for 4 wks Cd for 3 wks None for 3 wks	-	0.357 ± 0.023	0.353 ± 0.066
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	0.271 ± 0.024	0.349 ± 0.066

Values are expressed as mean ±SD

There are no statistically significant difference ($p < 0.05$) between the treatment groups by one-way analysis of variance.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd:Cadmium 50 ppm

TABLE 5-6

LYMPHOCYTES COUNT IN BLOOD X 10^6/ML

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	4.160 ± 0.651	3.922 ± 0.424	4.343 ± 0.762
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	3.932 ± 1.009	3.202 ± 0.357*	3.736 ± 1.283
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	4.553 ± 0.753
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	4.157 ± 0.509	4.270 ± 1.843
5	None for 4 wks Cd for 3 wks None for 3 wks	-	3.658 ± 0.567*	3.853 ± 0.970
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	3.837 ± 0.296	4.202 ± 0.633

Values are expressed as mean ±SD

*Significantly different at $p < 0.05$ from other values in the same Column by Duncan's multiple-range test.
Zn^-: ..inc 1 ppm, Zn^+: zinc 50 ppm, Cd: cadmium 50 ppm

TABLE 5-7

LYMPHOCYTE COUNTS IN SPLEEN x 10^6

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	181.01 ± 37.66	234.67 ± 12.59	276.72 ± 86.94
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	166.78 ± 17.73	181.25 ± 73.11	204.37 ± 69.73
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	210.15 ± 86.93
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	230.76 ± 58.55	278.43 ± 41.44
5	None for 4 wks Cd for 3 wks None for 3 wks	-	231.56 ± 29.72	254.80 ± 24.84
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	188.42 ± 39.64	197.85 ± 41.88

Values are expressed as mean ±SD

There are no statistically significant differences ($p < 0.05$) between the treatment groups by one-way analysis of variance.
Zn^-: zinc 1 ppm, Zn^+: zinc 50 ppm, Cd: cadmium 50 ppm

TABLE 5-8

LYMPHOCYTE COUNT IN THE THYMUS x 10^6

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	85.80 ± 18.83	148.35 ± 66.80	114.15 ± 70.71
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	71.90 ± 14.57	128.68 ± 27.36	127.50 ± 53.23
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	151.62 ± 36.65
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	146.81 ± 27.92	152.39 ± 63.86
5	None for 4 wks Cd for 3 wks None for 3 wks	-	119.09 ± 41.08	138.43 ± 42.47
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	88.09 ± 42.14	172.83 ± 64.27

Values are expressed as mean ±SD

There are no statistically significant difference ($p < 0.05$) between the treatment groups by one-way analysis of variance.
Zn^-: zinc 1 ppm, Zn^+: zinc 50 ppm, Cd: cadmium 50 ppm

5.4 Stimulation of lymphocytes with PHA mitogens:

Spleen cell cultures were stimulated with T cell mitogen phytohemagglutinin (PHA) for 72 hours and during the last 4 hours radioactive thymidine incorporation was measured. Table 5.9 presents the stimulation index (ratio of thymidine uptake between stimulated (i.e. PHA containing) and non-stimulated cells). Animals treated with a zinc deficient diet for 4 weeks (week 0 observation) had a significant decrease in the mean stimulation index in comparison with the control group. Concurrent administration of cadmium tended to increase the mean stimulation index with a greater increase after cessation of treatment. However, the difference was not statistically significant. Animals treated with zinc deficient diet and cadmium for 3 weeks (Group 6) had a significant decrease in the mean stimulation index that continued even after cessation of treatment. Animals which had received cadmium and zinc together had values similar to the nontreated controls, while those receiving cadmium alone had values that tended to be higher. This difference, however, did not reach levels of statistical significance.

5.5 Assay of natural -killer cell activity:

Natural-killer cell activity was measured by assay of 4 hour chromium release from target cells (Moloney virus induced lymphoma cell line YAC-1 labelled with 51Cr). Table 5-10 presents the results at the different observation periods. Animals treated with zinc deficient diet had a significant decrease in natural-killer cell activity. Three weeks after concurrent cadmium administration (week 3 observation) more significant depression was observed. After cessation of treatment there was tendency of increased activity and greater increase with concurrent zinc administration. This difference still was statistically significant in comparison with the nontreated controls. Groups treated with cadmium alone

TABLE 5-9

STIMULATION OF THE LYMPHOCYTES WITH PHA MITOGENS

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	6.35 ± 2.22	6.40 ± 2.07	6.54 ± 1.34
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	*4.32 ± 1.34	5.85 ± 1.90	6.33 ± 2.15
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	5.26 ± 2.35
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	6.21 ± 1.80	6.51 ± 1.14
5	None for 4 wks Cd for 3 wks None for 3 wks	-	7.89 ± 3.38	7.11 ± 1.87
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	*2.07 ± 0.32	*4.24 ± 0.72

Values are expressed as mean ±SD

*Significant difference at 0 week for group 2, 3 and at 6 week for group 6 from other values in the same column by Duncan's multiple-range test.
Zn^-: zinc 1 ppm, Zn^+: zinc 50 ppm, Cd: cadmium 50 ppm

TABLE 5-10

NATURAL-KILLER CELL ACTIVITY

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	24.00 ± 5.94	21.28 ± 6.32	23.14 ± 8.80
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	*12.71 ± 3.03	*2.71 ± 1.38	*12.29 ± 5.82
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	*13.38 ± 3.81
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	18.00 ± 5.31	23.29 ± 6.80
5	None for 4 wks Cd for 3 wks None for 3 wks	-	*8.38 ± 4.90	*12.75 ± 6.62
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	*1.0	* 9.00 ± 5.70

*Statistical significant difference at $p < 0.05$ form other values in the same column by Duncan's multiple range test.
Zn^-: zinc 1 ppm, Zn^+: zinc 50 ppm, Cd: cadmium 50 ppm

had significant decreased activity that continued after cessation of treatment. However, animals treated with zinc and cadmium had values similar the control ones. Thus zinc may have prevented the cadmium induced reduction in natural-killer cell activity.

5.6 Direct plaque-forming cell response:

Direct plaque-forming cell responses were assayed by injecting sheep red cells 4 days before killing. Then the number of splenic lymphocytes producing IgM antibody against sheep red cell antigens were counted. Results were expressed as plaques/10^6 spleen cells and plaques/spleen and are shown in Tables 5-11 and Fig. 5-2 respectively. Mice treated with a low zinc diet for 4 weeks (week 0 observation) had a significant decrease in the number of pfc/10^6 spleen cells. Concurrent administration of cadmium tended to antagonise the depressing effect of zinc deficiency that was still maintained 3 weeks after cessation of treatment (both with no statistical significance). However, other animals treated with zinc and cadmium together had similar values compared to the nontreated controls. Mice treated with cadmium only had a significant increase in the number of pfC, that was even maintained after cessation of treatment for 3 weeks. The number of IgM plaque - forming cells/spleen followed the same pattern with significant decrease after treatment with zinc deficient diet, however, concurrent cadmium administration tended to increase the number of pfC but it was still significantly lower than the non-treated controls, that was also maintained for 3 weeks after cessation of treatment. Animals treated with cadmium only had significant increase of pfC. Even after cessation of treatment it was significantly higher than the control group (Fig. 5-2).

TABLE 5-11

DIRECT PLAQUE FORMING CELL RESPONSE [IgM PLAQUE/10^6 SPLEEN CELLS]

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	169.87 ± 5.60	173.33 ± 7.16	178.46 ± 5.69
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	131.32 ± 6.27*	174.33 ± 7.81	169.92 ± 16.67
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	172.42 ± 4.67
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	179.14 ± 38.38	164.08 ± 16.98
5	None for 4 wks Cd for 3 wks None for 3 wks	-	285.21 ± 41.08*	204.73 ± 10.86*
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	178.15 ± 15.44	188.03 ± 29.16

*Statistical significant difference at $p < 0.05$ from other values in the same column by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

Direct Plaque Forming Cells Response (IgM)

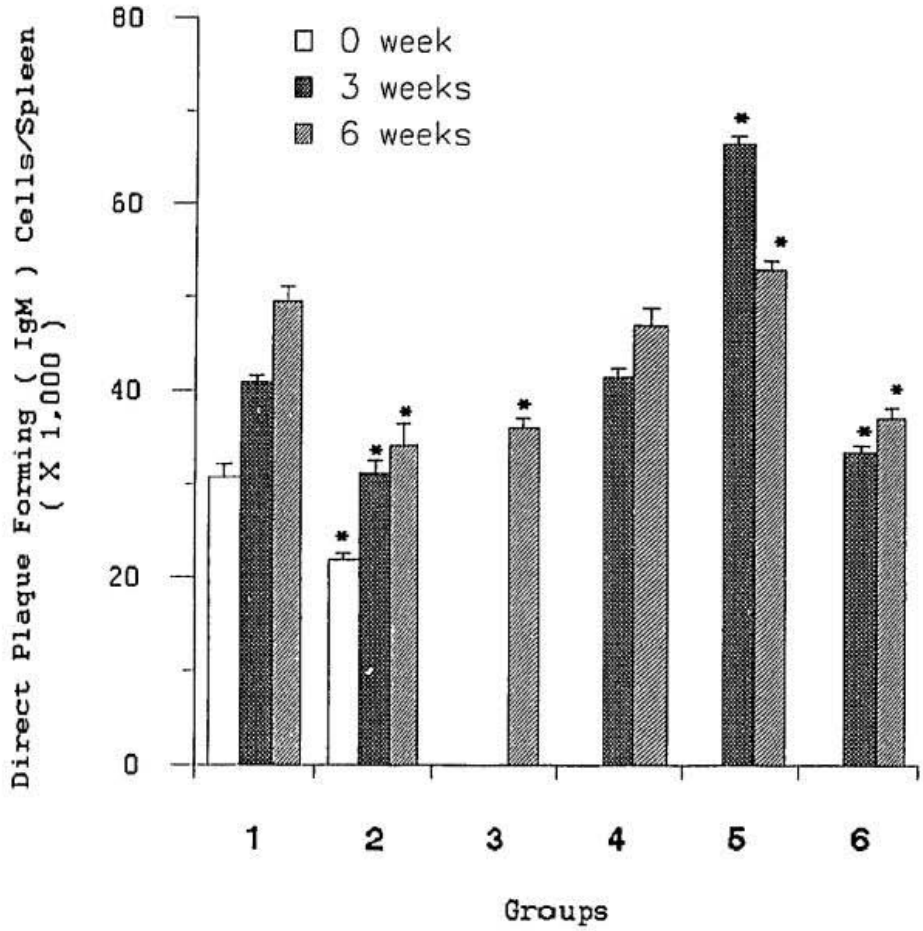


Fig. 5.3

Direct plaque forming (IgM) cells response: Group 1 (Control) no treatment.
Group 2: Zn (1ppm) 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
Group 3: Zn (1ppm) 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - Zn (50ppm) 3 weeks.
Group 4: None, 4 weeks - Zn (50ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
Group 5: None, 4 weeks - Cd (50ppm); 3 weeks - none 3 weeks.
Group 6: None, 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
* Statistical significant difference at $p < 0.05$ from the control group at the same point of observation by Duncan's multiple-range test.

5.7 Indirect plaque-forming cell response:

Indirect plaque-forming cell response were assayed by injecting sheep red cells 8 days prior to killing. Then the number of splenic lymphocytes producing IgG antibody against the sheep red cell antigen were counted. Results are expressed as plaques/10^6 spleen cells and plaques/spleen in Table 5-12 and Fig. 5-3 respectively. Results followed the same pattern as for the IgM plaque-assay. At week 0 of observation, mice treated with a zinc deficient diet had significantly lower numbers of IgG plaque forming cells in comparison with the untreated controls. However, after 3 weeks of concurrent cadmium administration (week 3 observation), the number of IgG PFC were similar to that of the control group. Similar values were observed in mice treated with zinc and cadmium together and lower values in those treated with a zinc deficient diet and cadmium for 3 weeks (group 6). Thus cadmium antagonized the reducing effect of a zinc deficient diet in group 2 and zinc prevented the cadmium-induced increase of IgG PFC response in group 4. However, mice treated with cadmium only had significantly higher IgG pfC numbers than all the other treatment groups and even 3 weeks after cessation of treatment those animals still tended to have higher IgG PFC number than the other five groups. This difference was, however, not statistically significant.

5.8 Total T-lymphocyte in the spleen:

T-lymphocytes were counted by direct immunofluorescence staining using conjugated monoclonal antisera. Results are shown in Table 5-13. There were no significant differences among the groups on different treatment schedules. However, mice treated with a zinc deficient diet for 4 weeks (week 0 observation) tended to have lower total T-cell counts, and with further decrease in values observed after concurrent cadmium

TABLE 5-12

INDIRECT PLAQUE-FORMING CELL RESPONSE [IgG PLAQUES/10^6 SPLEEN CELLS]

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	1291.0 ± 28.8	1276.2 ± 51.4	1281.6 ± 293.2
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	786.0 ± 23.3*	1290.0 ± 49.9	1260.8 ± 57
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	1266 ± 56.2
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	1271.3 ± 51.8	1277.5 ± 53.4
5	None for 4 wks Cd for 3 wks None for 3 wks	-	1865.3 ± 57.1*	1288.5 ± 58.2
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	1258.3 ± 61.3	1262.8 ± 58.6

Values expressed as mean ±SD

*Statistical significant difference at $p < 0.05$ from other groups on the same column by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

Indirect Plaque Forming Cells Response (IgG)

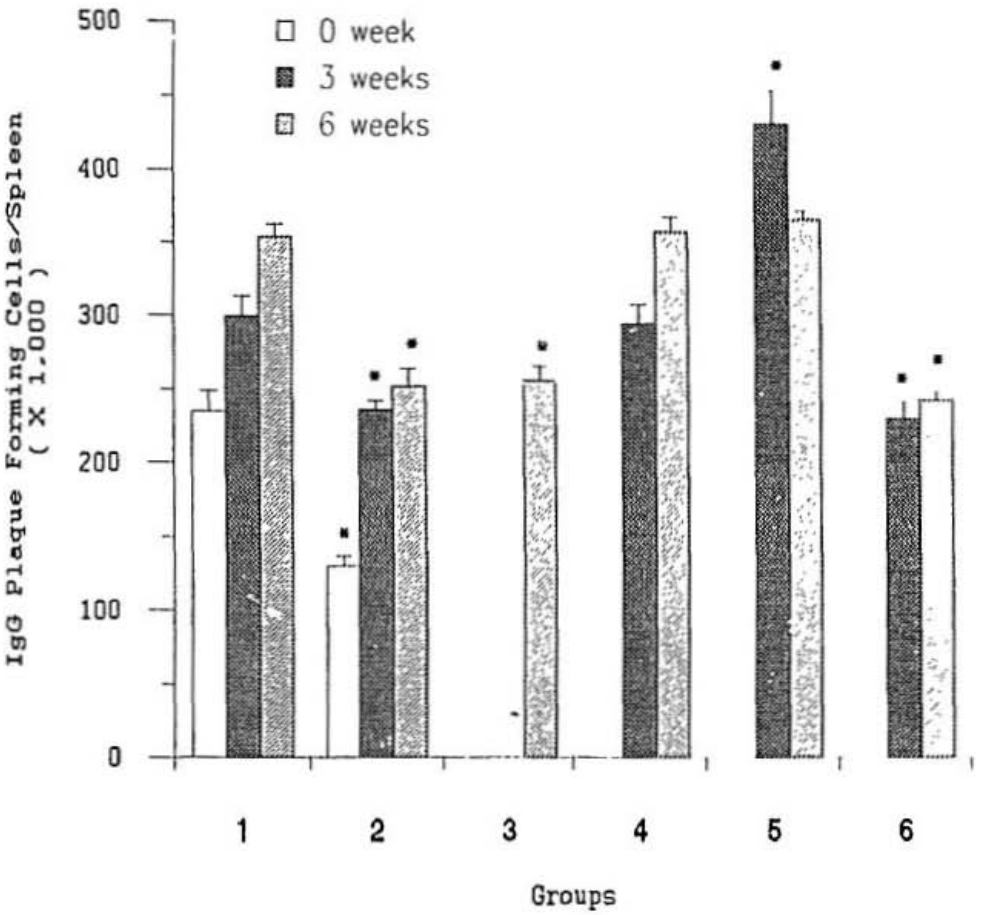


Fig. 5.3

Indirect plaque forming (IgG) cells response: Group 1 (Control) no treatment.
Group 2: Zn (1ppm) 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
Group 3: Zn (1ppm) 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - Zn (50ppm) 3 weeks.
Group 4: None, 4 weeks - Zn (50ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
Group 5: None, 4 weeks - Cd (50ppm); 3 weeks - none 3 weeks.
Group 6: None, 4 weeks - Zn (1ppm) + Cd (50ppm); 3 weeks - none 3 weeks.
* Statistical significant difference at $p < 0.05$ from the control group at the same point of observation by Duncan's multiple-range test.

treatment for 3 weeks. However, 3 weeks after cessation of treatment, total values were similar to that of the nontreated controls, however, none of the readings reached a significant level. Mice treated with cadmium and zinc together had similar total T-cell counts, while those treated with cadmium only tended to have lower values in comparison with the nontreated controls.

5.9 Assay of T-lymphocyte subsets in spleen:

T-lymphocyte subsets were counted by direct immunofluorescence staining procedures, using monoclonal anti-L_3T_4 and anti LyT-2 antibodies that identify the murine lymphocyte differentiation antigens CD4+ for helper/inducer and CD8+ for suppressor/cytotoxic T cells. Results are shown in Tables 5-14 and 5-15. Table 5-16 shows the ratio of CD4+ and CD8+ cells. The percentage of helper (CD4) cells was significantly lower in animals treated with zinc deficient diet at week 0 observation. Three weeks after concurrent treatment with cadmium, helper cells showed even lower values. However, mice treated with a zinc deficient diet and cadmium and those treated with cadmium only had significant lower percentages of CD4+ cells in comparison with the nontreated controls. However, animals treated with cadmium and zinc together had similar percentages as the control groups. Thus zinc prevented the cadmium induced reduction of CD4+ cell number. The number of suppressor T cells (CD8+) followed the same pattern as for helper cells. However, 3 weeks after cessation of all treatments, animals treated with a zinc deficient diet and cadmium (group 2) had a significantly different values in comparison with the control group. Even concurrent administration of zinc tended to increase the percentage of CD8+ cells and the difference was still statistically significant. The ratio for CD4+ and CD8+ cells, which reflects the balance of immunoregulatory T-

TABLE 5-13

T-LYMPHOCYTE IN THE SPLEEN

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	61.143 ± 6.362	59.167 ± 4.956	59.429 ± 9.572
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	56.857 ± 4.947	54.286 ± 2.632	57.429 ± 9.981
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	58.143 ± 4.670
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	58.714 ± 4.231	61.714 ± 8.480
5	None for 4 wks Cd for 3 wks None for 3 wks	-	53.713 ± 8.361	55.857 ± 4.018
6	None for 3 wks Zn^+ + Cd for 3 wks None for 3 wks	-	53.857 ± 4.741	57.143 ± 3.024

7 - 8 animals in each group.

No significant differences among the groups on different treatment.
Schedules by one-way analysis of variance ($P > 0.05$).

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-14

T-CELL SUBSETS (HELPER CD4+) %

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	35.14 ± 5.20	33.67 ± 5.78	31.66 ± 5.24
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	25.66 ± 4.10*	17.65 ± 1.63*	30.4 ± 6.17
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	32.21 ± 2.90
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	34.57 ± 3.82	31.30 ± 2.90
5	None for 4 wks Cd for 3 wks None for 3 wks	-	26.28 ± 3.49*	30.90 ± 3.40
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	20.56 ± 3.82*	29.80 ± 2.68

Values are expressed as mean ±SD

*Statistical significant difference at $p < 0.05$ from other values on the same column by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-15

T-CELL SUBSETS (SUPPRESSOR CD8+)%

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	27.57 ± 3.69	28.33 ± 4.32	28.20 ± 2.42
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	19.66 ± 1.36*	10.20 ± 1.78*	16.83 ± 3.86*
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	17.60 ± 3.20*
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	27.23 ± 3.82	26.71 ± 2.81
5	None for 4 wks Cd for 3 wks None for 3 wks	-	15.30 ± 1.49*	20.12 ± 6.96
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	17.14 ± 3.43*	21.83 ± 2.13

Results are expressed as mean ±SD

*Statistical significant difference at $p < 0.05$ from other values on the same column by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-16

HELPER/SUPPRESSOR

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	1.27 ± 0.19	1.18 ± 0.18	1.12 ± 0.19
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	1.30 ± 0.21	1.73 ± 0.36*	1.80 ± 0.28*
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	1.83 ± 0.21*
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	1.27 ± 0.11	1.17 ± 0.15
5	None for 4 wks Cd for 3 wks None for 3 wks	-	1.71 ± 0.39*	1.54 ± 0.13
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	1.19 ± 0.17	1.37 ± 0.12

Results are expressed as mean ±SD

*Statistical significant difference at $p < 0.05$ from other values in the same column by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

lymphocytes was higher in groups treated with cadmium after zinc deficient diet and other groups treated with cadmium only, while animals treated with zinc and cadmium together did not show that increase. Thus zinc prevented the cadmium induced imbalance of immunoregulatory T-lymphocytes. However, when zinc was given after treatment with zinc deficient diet for 7 weeks (group 2) and concurrent treatment with cadmium in the last 3 weeks, it did not prevent this increase.

5.10 Trace element levels in the kidney

Kidney samples were frozen at -20^{o}C and later analyzed for zinc and cadmium levels by atomic absorption spectroscopy. Results are shown in Tables 5-17 and 5-18. Animals treated with zinc deficient diet had lower zinc concentrations in comparison with the nontreated controls. Concurrent treatment with cadmium increased zinc concentrations. However, the difference did not reach statistically significant levels in both observation periods. Three weeks after cessation of treatment, there was a significant increase in zinc concentration. Significantly higher values were observed after zinc administration (group 3). Mice treated with cadmium only had similar values of zinc concentration as the control group. However, after cessation of treatment, zinc levels tended to increase. Animals treated with cadmium and zinc had slight increases in zinc levels at week 3 observation, however, these were not statistically significant.

Mice treated with a low zinc diet for 4 weeks had a slight increase in cadmium levels. However, concurrent administration of cadmium for 3 weeks increased cadmium levels significantly and even after cessation of treatment, cadmium levels were still highly significant in comparison with the nontreated controls. Concurrent administration of zinc

TABLE 5-17

ZINC CONCENTRATION IN THE KIDNEY (ug/g)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	22.15 ± 2.60	22.29 ± 0.90	22.53 ± 2.04 ^{bc}
2	Zn^- for 4 wks Zn^- + Cd for 3 wks None for 3 wks	20.90 ± 0.50	22.63 ± 0.96	24.01 ± 1.60 ^{c}
3	Zn^- for 4 wks Zn^- + Cd for 3 wks Zn^+ for 3 wks	-	-	26.05 ± 2.20 ^{a}
4	None for 4 wks Zn^+ + Cd for 3 wks None for 3 wks	-	23.77 ± 2.19	22.53 ± 1.20 ^{b}
5	None for 4 wks Cd for 3 wks None for 3 wks	-	22.97 ± 1.70	25.79 ± 2.40 ^{c}
6	None for 3 wks Zn^- + Cd for 3 wks None for 3 wks	-	22.09 ± 1.54	24.01 ± 2.50 ^{c}

Values expressed as mean ±SD

At 6 week observation, values not sharing a common superscripts letter are significantly different at $p < 0.05$ by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

TABLE 5-18

CADMIUM CONCENTRATIONS IN THE KIDNEY (ug/g)

Group	Treatment	0 Week	3 Week	6 Week
1	No treatment	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.11 ± 0.29 [a]
2	Zn^- for 4 wks Zn^- +Cd for 3 wks None for 3 wks	0.51 ± 1.19 [a]	9.42 ± 3.71 [b]	6.21 ± 0.61 [b]
3	Zn^- for 4 wks Zn^- +Cd for 3 wks Zn^+ for 3 wks	-	-	5.10 ± 1.34 [b]
4	None for 4 wks Zn^+ +Cd for 3 wks None for 3 wks	-	3.73 ± 0.78 [c]	3.60 ± 0.70 [c]
5	None for 4 wks Cd for 3 wks None for 3 wks	-	8.30 ± 0.96 [b]	6.80 ± 2.34 [b]
6	None for 3 wks Zn^- +Cd for 3 wks None for 3 wks	-	18.50 ± 3.20 [d]	11.19 ± 4.66 [d]

Values are expressed as means ±SD

In vertical rows values not sharing a common superscripts letter are significantly different at $p < 0.05$ by Duncan's multiple-range test.

Zn^-: Zinc 1 ppm, Zn^+: Zinc 50 ppm, Cd: Cadmium 50 ppm

(group 3) tended to decrease cadmium concentrations but the difference was still significantly different from the control group.

Similar but slightly lower values were observed in animals treated with cadmium only, as they had significant differences at 3 and 6 weeks of observations. Mice receiving zinc and cadmium together had significantly higher cadmium levels in comparison with the nontreated groups, but at the same time these were significantly lower than those receiving cadmium alone. Animals receiving a low zinc diet and cadmium for 3 weeks had significantly higher cadmium levels than all other treatment groups, including those receiving cadmium alone. This significant increase continued 3 weeks after cessation of treatment (week 6 observation).

5.11 Electron microscopy of kidney and thymus:

Ultrastructural alterations in kidney and thymus sections were examined by transmission electron microscopy. Four animals from each group were examined at 0, 3 and 6 weeks of observation. In kidney sections, whole nephron and interstitial tissues were examined. Animals treated with 50 ppm cadmium in drinking water for 3 weeks, showed abnormalities of the proximal tubular epithelial cells, as compared to the non-treated animals. The mitochondria were increased in number, swollen and the cisterna distorted (Fig. 5.4). Similar changes were also seen 3 weeks after cessation of treatment. Other areas of kidney appeared normal. Animals fed with a low zinc diet only (Zn 1 ppm) or cadmium and low zinc diet together did not show any abnormalities.

In the thymus sections, the cortex and medulla were examined. Animals treated with cadmium only for 3 weeks and those treated with low zinc diet for 4 weeks showed

increased interepithelial connective tissue. However, animals treated with low zinc diet only also showed lymphoid cellular degeneration, more prominant in the cortical epithelial cells, with loss of mitochondria, cytoplasmic envelope and fragmentation of the cytoplasm in the interepithelial space (Fig. 5.5). Mice treated with cadmium and low zinc diet together did not show ultrastructural changes of the thymus.

ELECTRON MICROSCOPY OF KIDNEY SECTIONS

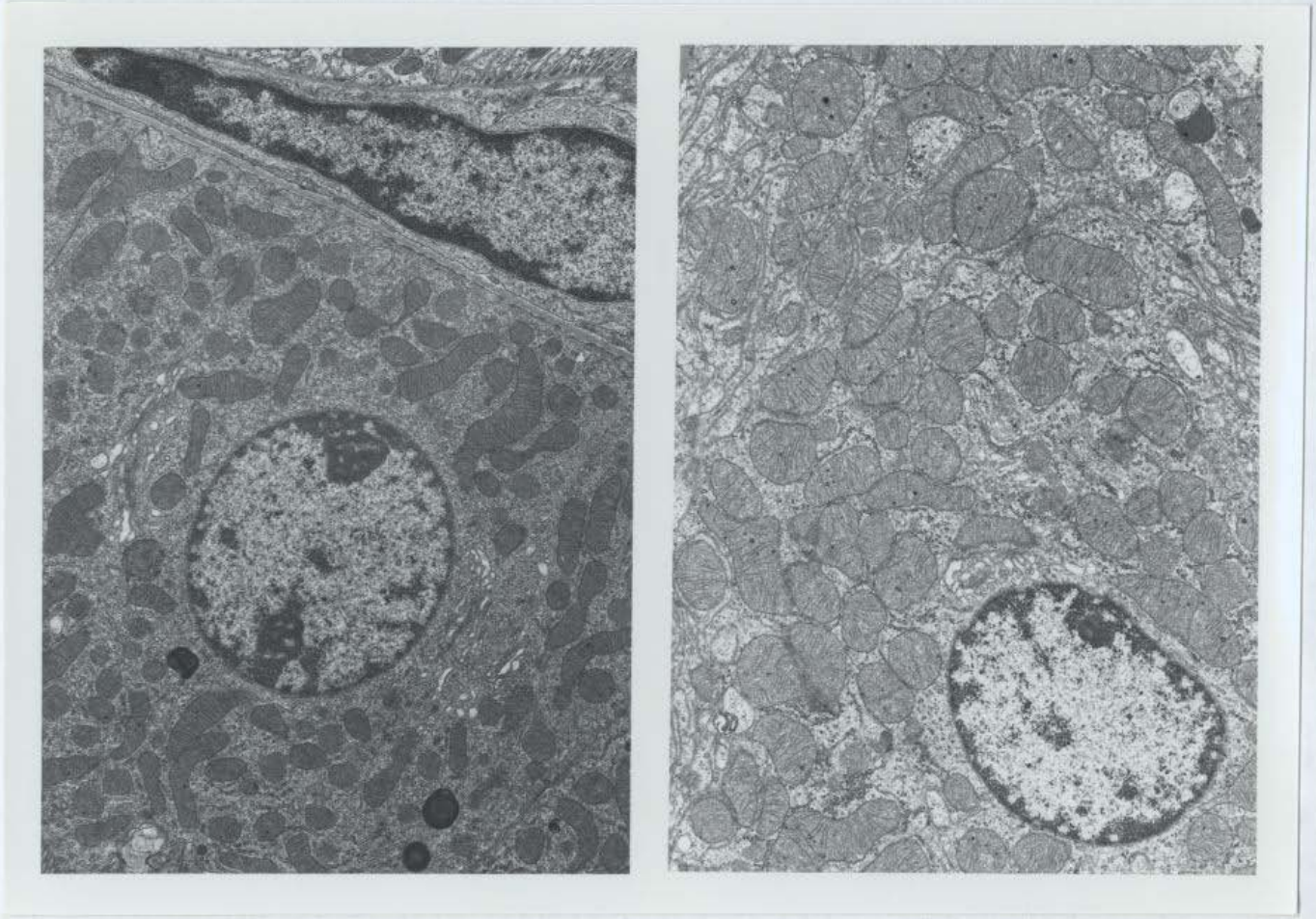


Fig. 5.4

Electron microscopic appearance of proximal tubular cell from a control mouse (left) and cadmium treated mouse (cd 50 ppm in drinking water for 3 weeks (right). The mitochondria in the treated animal are increased in number, swollen and the cisternae are distorted (x12,000).

ELECTRON MICROSCOPY OF THYMUS SECTIONS

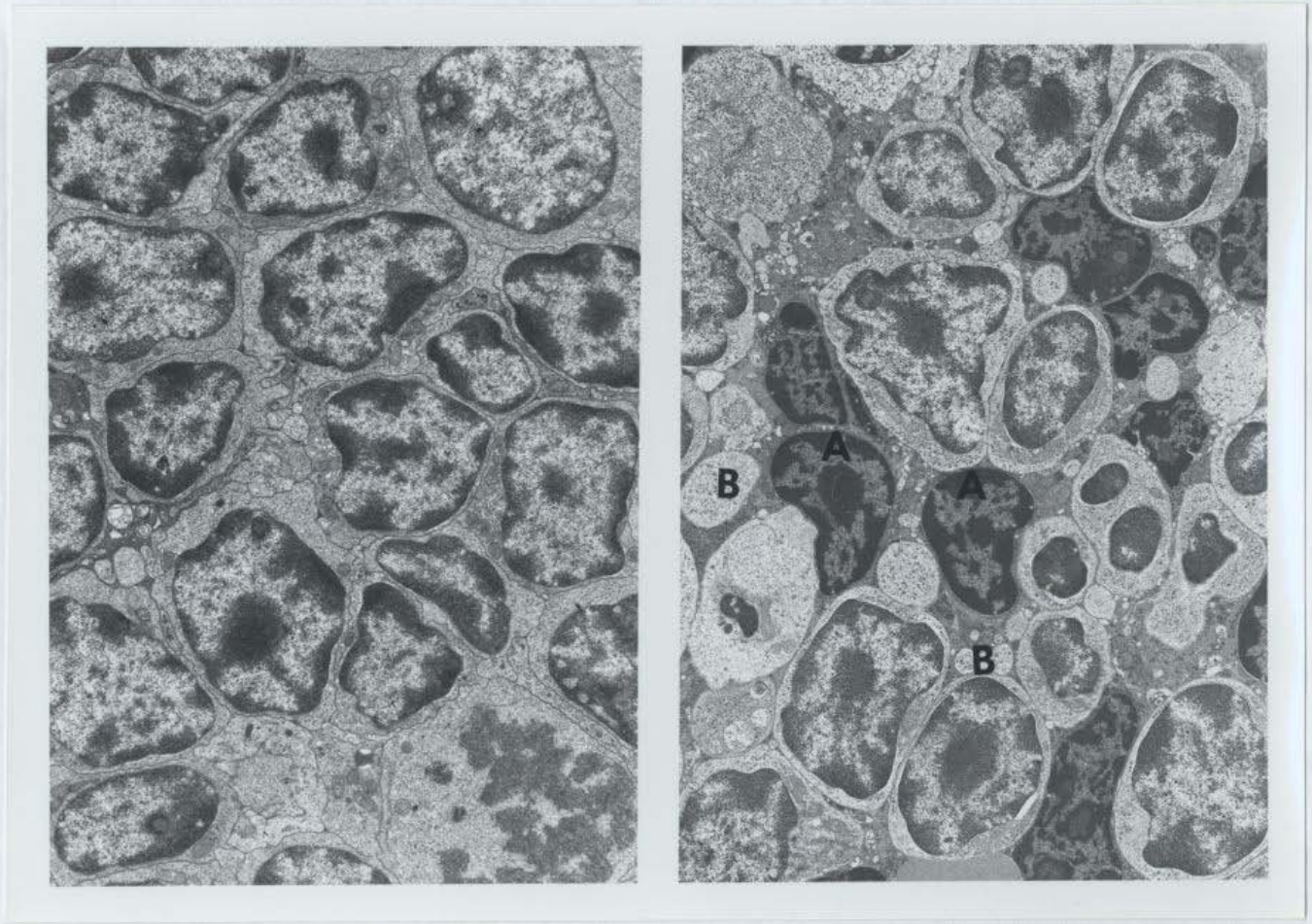


Fig. 5.5

Electron microscopic appearance of cortical part of the thymus from control mouse (left) and low zinc diet-treated mouse (right). Treated mouse showed loss of the cytoplasmic envelope (A) and fragmentation of the cytoplasm in the interepithelial space (B) (x12,000).

CHAPTER VI

Discussion

In the present study, the effects of a relatively low dose of oral cadmium treatment and the effects of the interaction between cadmium and zinc on the immune system of mice fed a low zinc diet were studied. To produce a deficient state, the concentration of zinc used in the diet was very low (1 ppm). Since there are no body stores of zinc, it was hoped that such a concentration would dramatically affect the immune system in a short period. Animals treated with low zinc diets exhibited loss of hair from all over the body especially the tail. This is a recognized effect of zinc deficiency. There was, however, no weight loss. Serum zinc levels were reduced in animals treated with a low zinc diet in comparison with the control group. However, these were pooled samples which is not appropriate for statistical analysis. The same low zinc concentrations were used before by Hoaldley and Cousins in 1985, with significant depression of serum zinc levels, but they did not report any changes in the general health of the animals. Fraker et al, 1987 used the same concentrations with fall in body weight and serum level. However, it is important to note that serum, plasma and urine zinc levels changed rapidly during the first few weeks of deficiency and remain stable afterwards (Prasad 1984 - Allen et al 1981 - Chandra 1980). In this study, animals received zinc-deficient diets for 4 weeks.

The dose of cadmium used was approximately comparable to possible human exposure. Animals treated with cadmium only did not show any changes of weight or food consumption. Similar doses were used in other studies (Chowdhury

and Chandra 1987 - Borgman et.al., 1986 - Blakley 1985 - Muller et.al., 1979) and no general health effects were reported. However, a tendency for body weight reduction was reported by Malave and DeRuffino (1984) at cadmium doses of 50 ppm with more discernable weight loss after the third week of treatment. In the present study cadmium treatment was discontinued after 3 weeks.

Zinc treatment using a dose of 50 ppm did not affect the general health of the mice when given alone or together with cadmium.

6.1 Effect of cadmium on the immune system:

Cadmium at oral doses of 50 ppm for 3 weeks did not alter the weight or lymphocyte counts in the spleen and thymus. Similar findings were obtained by Chowdhury and Chandra (1987) - Borgman et.al. (1986) and Malave and DeRuffino (1984) who also used similar doses of oral cadmium. However, in this study, a significant decrease was observed in the blood lymphocyte counts when cadmium was given alone and when given as a concurrent treatment with zinc-deficient diet. Other investigators who used either a different route for cadmium delivery or a bigger dose, reported other findings in lymphoid organs such as thymus atrophy and spleen enlargement (Suzuki et.al., 1981 - Yamada et.al., 1981). At identical dose levels, the morphologic effects are more pronounced with IV or intraperitoneal administration. Smaller amounts would be absorbed and delivered to the immune system following oral treatment and such small amounts are probably not sufficient to cause major pathological and structural alterations in different organs.

The effect of cadmium on lymphocyte functions was investigated by different tests. Firstly, to characterize the proliferative response of spleen cells to a T-cell

mitogen (phytohemagglutinin - PHA) in a 72 hour culture system at which time the mitogen produces its maximal effect on DNA synthesis. This was accomplished by pulse labelling the cultures with tritiated thymidine which is incorporated into newly synthesized DNA. Secondly, enumeration of T- lymphocytes and subsets, one of the indices of immunocompetence, was performed by direct immunofluorescence which using specific monoclonal antibodies.

Thirdly, the effect of cadmium on IgM and IgG antibody producing cell responses to sheep red cells was estimated by direct (IgM) and indirect (IgG) plaque forming cell response (Jerne et al 1974).

Finally, natural killer cell activity was also estimated based on the fact that unsensitized lymphocytes from normal individuals are spontaneously cytotoxic for a variety of target cells (Ortaldo and Herberman 1984).

In the present study, cadmium at doses of 50 ppm for 3 weeks increased IgM and IgG plaque-forming cell responses (Table [5-10, 5-11] Fig. [5-2, 5-3]). Three weeks after cessation of treatment, the responses tended to be higher than in nontreated controls. These observations are in agreement with those of Chowdhury et.al., (1987) and Malave and DeRuffino (1984) who also used 50 ppm cadmium in drinking water. However, Borgman et.al., (1986) reported that the same dose of cadmium in drinking water caused depression of the formation of splenic plaque-forming cells in response to SRBC, though Muller, et.al, (1979) could not show any significant difference in the number of plaque-forming cells after chronic oral exposure of mice to 600 ppm of cadmium. The differences might be due to the different strains of mice used, as the expression of heavy metal toxicity is known to

vary in different species and strains of laboratory animals (Malave 1981), as is true of the immune response itself. In the present study and also in the study of Chowdhury, et al (1987) and Malave and Deruffino (1987) C57BL/6 mice were used, however, Borgman, et al (1986) used CD1 mice.

Other authors who used other non-oral routes of cadmium treatment obtained different results, as parenteral administration of cadmium (0.2 ug/day) reduced the number of plaque forming cells (Bozelka et.al., 1978). A similar suppression was produced when cadmium was administered by inhalation (Koller et.al, 1975) and injection (Fujimaki 1985). The absorption of air borne cadmium depends greatly on its physiochemical form as well as the subsequent fate of cadmium deposited in the respiratory tract. The extent of deposition is a function of the particle size and solubility (Health Assessment Document for Cadmium, 1981). However, its overall absorption is more complete than from the gut.

Absorption of ingested cadmium differs according to animal species and type of compounds. A small proportion of dietary cadmium is absorbed and the rest excreted in the faeces (Rahola et al 1972). Cadmium is taken up from the blood into the liver where it is incorporated into metallothionein and rendered nontoxic. It is then slowly released from the liver into the blood for transport to other organs.

Following parenteral administration, cadmium by-passes the portal circulation. Similarly, when it is given by intramuscular or intraperitoneal injection, immediate rise in tissue level occurs. Following inhalation, its absorption from the lung is proportionately more complete than from the gut. Thus in studies where cadmium

was administered by routes other than oral treatment, a more toxic form was delivered to the immune cells than in the present study.

For further investigation of the effects of cadmium on the immune system, proliferative response of spleen cells to T-cell mitogen phytohemagglutinin (PHA), T-cell lymphocyte counts and subsets were studied.

In this study, cadmium at the dose of 50 ppm in drinking water tended to increase the proliferative response of spleen cells to T-cell mitogen - PHA, but with no significant difference from the nontreated controls. This observation is in agreement with the data of Chowdhury, et al (1987). However, Malave and DeRuffino (1984) observed increased proliferative response of T-cells to PHA mitogens after 4 weeks of oral cadmium treatment. An even greater increase was noted after 11 weeks. However, in the present study cadmium treatment was discontinued after 3 weeks. It is possible that different strains of mice have different responses as C57BL/6 are low responders, while others such as CBA mice are high responders to T-cell mitogens (Malave and DeRuffino, 1984). The number of helper/inducer and suppressor/cytotoxic T-cells identified by the surface markers CD4+ and CD8+ respectively were significantly reduced after 50 ppm cadmium treatment. This is in agreement with Chowdhury, et al (1987) who used the same dose of cadmium treatment. T-helper clones could be divided into two types: TH1 makes interleukin-2 (IL-2) and interferon-gamma (IFNY), and TH2 secretes B-cell stimulating factor (BSf-1), both TH1 and TH2 produce IL-3. It is possible that changes in lymphokine production are due to reduced TH1, a subset of T-helper cells that synthesize these lymphokines. Cadmium is known to adversely affect

enzyme function and protein synthesis in various tissues (Kostial 1986) it could cause reduction of IL-2 and IFNy which are important for T-helper cell proliferation and killing of intracellular organisms. The selective reduction by cadmium on the suppressor cell activity may provide another explanation. 50 ppm of cadmium in drinking water significantly reduces suppressor cell activity after 3 weeks (Chowdhury et al 1987 - Malave and DeRuffino 1984).

Natural-killer cell activity was also studied. Natural-killer cells are a heterogeneous group of large granular lymphocytes that manifest spontaneous cytotoxicity against a variety of target cells without prior sensitization. In the present study, cadmium significantly reduced natural-killer cell activity in mice at 4 hours. This is in agreement with Chowdhury and Chandra (1987) who also used the same dose of cadmium treatment and observed a tendency for reduced NK activity at 4 hours and a larger reduction at 12 hours. Natural-killer cells recognize and lyse a wide variety of tumour cells and other cell lines in vitro. Glycoproteins on the surface of the target cells are important for recognition by putative NK cell receptors. After lysis of the target cell, the killer cell survives the encounter and proceeds to kill again (Young and Cohn 1988). Reduction of NK activity in cadmium-treated animals may be due to decreased total numbers of NK cells (Chowdhury and Chandra 1989), as animals failed to increase NK activity between 4 - 12 hours. It is also possible that reduced NK activity may be due to cadrnium-induced inhibition of calcium uptake by epithelial cells (Verbost et al 1987) as the process of target cell lysis by NK cells occurs by $Ca^{2}+$ dependent mechanisms.

6.2 Effect of zinc deficiency on the immune system of mice:

In the present study, the effect of dietary zinc deficiency on the immune system of young adult mice was investigated. Mice were maintained on a low zinc diet (1 ppm) for 4 weeks. Animals started to shed hair from all over the body especially the tail by the end of the fourth week. Zinc at a concentration of 1 ppm in the diet for 4 weeks did not alter weight of the mice and that of different organs. It also did not alter lymphocyte counts in the blood, thymus and spleen. However, there was a tendency for weight reduction at the end of the fourth week, when the treatment was discontinued. Fraker, et al (1987) reported a 26% reduction of body weight as well as marked reduction of thymus weight and splenic lymphocyte number. This differences may be due to the form of diet provided as the powdered form is more rapidly absorbed than the pellet form, with more pronounced effects. It may also be strain dependent, as in the present study C57BL/6 mice were used while Fraker's study used different strains of mice named N = NIH.

Loss of appetite is one of the first signs of zinc deficiency and occurs rapidly after introduction of zinc-restricted diet (Chester and Quarterman, 1970). The rate of food consumption in the present study did not change for 3 weeks. However in the fourth week of feeding a low zinc diet, the mice started to lose their appetite. It was also noted by Hoaldley and Cousins (1985) that zinc depletion did not markedly depress food consumption.

Thymus of animals fed a low zinc diet showed moderate lymphoid cellular degeneration on electron microscopy with more pronounced effect in the cortex than the medulla. There was loss of mitochondria, cytoplasmic matrix and

fragmentation of the cytoplasm in the interepithelial space. In 1985, Chandra reported rapid and marked atrophy of the thymus and paucity of the cortical lymphoid cells in zinc deficient animals. It is possible that zinc protects cells from injury by stabilizing cytoplasmic microtubules, cell surface membrane morphology, calcium flow across these membranes and inhibiting calmodulin function (Maro and Bornes, 1979).

In the electron microscopic pictures of thymus, counting the number of cortical epithelial cells of animals treated with a low zinc diet and comparing them with those of control ones with the same magnification, the number of cells were found to be reduced in the former. However, the number and morphology of medullary cells were normal. Also the total T-lymphocyte count in the spleen was normal. It is possible that the normal number of lymphoid cells in the medulla compensated the moderately reduced number of cells in the cortex with resultant normal total T-lymphocyte count.

Direct (IgM) and indirect (IgG) plaque forming cell responses were significantly reduced in mice treated with zinc-deficient diets. These observations are in agreement with Chandra and Au (1980) Fraker, et al (1987), Chandra and Puri (1985). IgM and IgG plaque-forming cell responses after SRBCS are injected during a course of zinc deficiency is relatively poor. A possibility is that a state of zinc deficiency might stimulate more immature B-cells to accumulate in the spleen bearing high amounts of surface IgM and IgG (Fraker et al 1987). Or, it may be that zinc deficiency could alter member responses already in force.

For further investigation of the effect of zinc deficiency on the immune system, T-cell subsets (helper and suppressor) and proliferative response of spleen cells to T-cell mitogen (phytohemagglutinin - PHA) were investigated. After 4 weeks of treatment with zinc-deficient diet, mice showed significant reductions in the numbers of both T-cell subsets (helper/inducer and suppressor/cytotoxic). These observations are in agreement with Pekarek, et al (1979), Fernandes, et al (1979), Fraker et al (1982) and Chandra and Dayton (1982). Zinc is required for both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis. Zinc deficiency in animals impairs the incorporation of labelled thymidine into DNA. It is also possible that the reduction in DNA synthesis may be due to decreased activity of deoxythymidine kinase (Prasad and Oberleas 1974). Recently, zinc finger motifs have been demonstrated in bacterial DNA (Nagai et al 1988) suggesting a crucial role of zinc in DNA synthesis and cell multiplication.

The proliferative response of the spleen cell to T-cell mitogen (PHA) was significantly reduced in mice treated with zinc-deficient diet. The unique sensitivity of the thymus to zinc- deficiency with a resultant decrease in the number and proliferative response of spleen cells to T-cell mitogens may be explained partially by the enzyme leucocyte terminal deoxynucleotidyl transferase (TdT) which is present in large quantities in early stages of thymocyte differentiation. In protein energy malnutrition (in which different nutrients including zinc are deficient), thymic hormone activity is reduced (Chandra 1982) with resultant maturation defects in thymic lymphocytes, greater proportions of immature (null) cells with increased level of TdT enzyme (Chandra 1980) and decreased fully differentiated T-cells. Effect of

zinc deficiency on various hormones such as growth hormone might be another possibility that requires further investigations. Further evidence for the critical role of zinc in normal T-cell function is provided by the effects of zinc deficiency on delayed-type hypersensitivity responses. In children with acrodermatitis enteropathica, these responses are impaired and are restored to normal after several weeks of zinc therapy (Chandra 1980). Fraker, et al (1982) demonstrated lower DTH responses in zinc-deficient mice who were subsequently sensitized with dinitrofluorbenzene. Similar observations have been reported in Down's syndrome (Bjorksten et al 1980) and prolonged hyperalimentation with inadequate zinc intake (Oleske et al, 1979).

Natural-killer cell activity is an important aspect of the immune response for protection against early phases of infections and tumours. Mice treated with zinc-deficient diets for 4 weeks showed a significant reduction of NK cell activity. This is in agreement with Chandra and Puri (1985) and Fraker, et al (1985) who also used young adult mice fed with zinc-deficient diets. It is possible that reduced NK cell activity is due to disturbances in the zinc-calcium relationship. Zinc appears to play a role in regulating the function of membrane proteins and its deficiency affects cell microtubules and microfilaments (Brewer et al 1979). Lysis of the target cell by natural-killer cells appears to be a calcium dependent mechanism by which release of the extracellular granules (perforin or cytolysin) occurs into the space between the two cells (Young and Cohn 1988).

6.3 Effect of cadmium administration on the immune system of zinc-deficient mice

Cadmium and zinc have several physiochemical similarities. Cadmium is a nonessential toxic metal. However, zinc is an essential element that counteracts a number of the toxic effects of cadmium (Gunn et al 1963). In this study, the effect of a relatively low dose of cadmium on the immune system of zinc-deficient mice was investigated. In addition, the effect of zinc administration after cessation of treatment was observed. Treatment of mice with cadmium and zinc-deficient or zinc-adequate diets did not affect the general health, weight of the mice, weight of the different organs or lymphocyte counts in the spleen and thymus. However, the lymphocyte counts of blood (collected form the right atrium of the heart) were significantly decreased in mice treated with zinc-deficient diet and in those given cadmium for 3 weeks following 4 weeks of zinc-deficient diet. Concurrent administration of a moderate amount of zinc prevented the cadmium-induced reduction in lymphocyte number. Lymphocyte stimulation response to PHA mitogen was significantly reduced in mice treated with zinc-deficient diet for 4 weeks. However, concurrent administration of cadmium for 3 weeks compensated this reduction with no significant differences from the non-treated controls. Zinc administration did not affect lymphocyte stimulation response and mice treated with cadmium and zinc had values comparable with the control group.

It is possible that low zinc intake potentiate higher cadmium absorption and retention (Campbell et al, 1978). Dietary zinc status is also a controlling factor for metallothionein gene expression in the intestine (Menard et al 1981) and the liver (McCormick et al 1981). Hence, in a state of zinc deficiency, metallothionein

synthesis decreases with subsequent decrease of metallothionein bound cadmium and increased non-bound toxic cadmium.

Direct (IgM) and indirect (IgG) plaque forming cells decreased significantly in mice treated with zinc-deficient diet. Concurrent cadmium administration tended to increase plaque-forming cell numbers. However, zinc administration prevented that increase. Mice treated with zinc and cadmium together demonstrated the antagonizing effect of both metals with values similar to the non-treated controls. These observations are in agreement with those of Malave and De Ruffino (1984) who reported an increase in the IgM antibody response after treatment with 50 ppm cadmium. Zinc administration (50 ppm) was seen to prevent that increase. However, Chowdhury et al (1987) used the same low dose of cadmium, and demonstrated increases in primary and secondary antibody response which were prevented by large doses of zinc (500 ppm). Meanwhile, Shippee, et al (1983) did not observe any protective effect of zinc on cadmium induced alteration of immune responses. It is possible that large doses of cadmium when delivered by injection, produced irreversible toxicity to the immune cells, in contrast to small oral doses that caused temporary alteration of the immune function with gradual recovery after cessation of treatment.

It was also demonstrated in this study that an important step where the antagonism between cadmium and zinc occurred was at the level of T-cell subsets. Treatment with zinc-deficient diet significantly reduced the number of helper/inducer and suppressor/cytotoxic cells. However, concurrent administration of cadmium caused more reduction of both subsets. Zinc administration prevented the reduction

of helper (CD4+) cells and tended to prevent that of suppressor cells (CD8+) but the number of suppressor was still significantly lower than the non-treated controls. The CD4+/CD8+ ratio which reflects the immunoregulatory balance, increased significantly after treatment with zinc-deficient diet and cadmium. However, mice treated with zinc and cadmium together showed total number of T-cell subsets and ratio to be comparable with the non-treated controls.

Chowdhury, et al (1987) demonstrated cadmium-induced reduction of suppressor cell number, as well as an increased CD4+/CD8+ ratio with 50 ppm cadmium in drinking water.

Zinc deficiency decreases the activity of zinc-containing DNA polymerase with resultant decrease of the DNA content of the thymus (Ku et al 1970). This would affect growth, division and differentiation of T-lymphocytes.

When the suppressive effects of zinc-deficiency and cadmium were combined together they dramatically affected the numbers of the T-cell subsets and ratio. This was probably due to interactions between the two metals as cadmium treatment alone altered T-cell subsets and zinc-deficiency alone also caused similar alterations, but excess zinc did not affect T-cell subsets or ratio. This observation might also confirm that zinc-deficiency potentiates cadmium retention and toxicity.

Natural-killer cell activity in a 4-hour assay was significantly depressed after treatment with zinc-deficient diet. Concurrent cadmium administration caused a greater depression. Zinc administration tended to prevent the suppression induced by zinc-deficiency and cadmium, but the activity was still significantly lower than in

the non-treated controls. Mice given zinc and cadmium together, had NK activity comparable to that of the non-treated controls.

Chowdhury, et al (1987) demonstrated decreased NK cell activity in a 4-hour assay and an increase in mean target cell lysis between 4 and 12 hours with cadmium treatment. It is possible that the biochemical interaction of cadmium and zinc and their relationship with calcium is responsible for suppression of NK activity. Young and Cohn (1988) proposed that lysis of the target cell by NK cell appears to be a Ca^{+}-dependent mechanism. Zinc-deficiency has been indicated to increase cadmium absorption (Campbell et al 1978); cadmium has an inhibitory effect on calcium absorption from the gastrointestinal tract (Gruden 1977 - Chertok et al 1981) as it directly causes physical and subsequently functional damage to the gut epithelial cells.

6.4 Cadmium and zinc in the kidney

As might be expected, cadmium treated mice had significantly high cadmium concentrations in the kidney. However, after cessation of treatment and probably due to the long half life of cadmium, its concentrations were still significantly higher than in the non-treated controls. Similar observations were reported by Borgman, et al in 1986 and Chowdhury et al in 1987. Cadmium concentrations in the kidney tended to increase in animals receiving zinc-deficient diet. Concurrent cadmium treatment increased its levels further, reaching a statistically significant difference. Even after cessation of treatment, the levels were significantly higher than the non-treated controls. This is in agreement with Tandon and Khandelwal (1987) who

noted increased deposition of cadmium in kidneys of zinc-deficient rats and higher concentrations after concurrent cadmium treatment.

Zinc administration (50 ppm) tended to decrease cadmium concentrations. This is similar to findings reported by Campbell, et al in 1978 and Petering, et al in 1971.

Animals treated with a zinc-deficient diet for 4 weeks, showed a tendency for decreased zinc concentrations in the kidney. However, concurrent cadmium treatment tended to increase zinc concentration. On the other hand, animals treated with cadmium only also showed increased zinc concentrations. Similar observations were noted by Tandon and Khandelwal (1987).

Administration of zinc significantly increased its concentrations in the kidney (Table 5-17). However, a tendency for increased zinc concentrations was observed in all the groups after cessation of treatment. Cadmium is absorbed from the gastrointestinal tract, then incorporated with metallothionein in the liver, released to the circulation, filtered through the glomeruli, reabsorbed from the proximal tubules and excreted mainly in urine (Kostial 1986). Zinc is absorbed in the intestine, carried to the portal circulation where it is bound to albumin, then secreted back into the intestine and excreted in feces (Bunker et al 1982).

It also appears that during treatment with cadmium, there is a continuous incorporation with metallothionein in the liver, while after cessation of treatment, there is gradual loss of cadmium from the liver and continuous uptake by the kidney (Friberg 1984). This effect was greater in the proximal tubules, as in the present study, electron microscopy showed increased mitochondria and distorted cisternae

in the proximal tubular cells. The mechanisms by which cadmium damages the proximal tubules may originate in the reabsorption of cadmium-metallothionein from the tubular lumen or a direct effect of toxic non-metallothionein - unbound cadmium. Mice treated with zinc-deficient diets did not show any ultrastructural changes in the kidney.

6.5 Cadmium and zinc interaction:

Zinc and cadmium have striking physical and chemical similarities. They both belong to group II of the Periodic Table. Zinc is an essential element, however, cadmium is a toxic metal. Toxicity of cadmium can be prevented or treated by zinc. Various reports have tried to explain the mechanism(s) behind cadmium and zinc interaction and the effects on the immune system.

Metallothionein has been considered a key component in the cellular detoxification system for cadmium and zinc. Three forms have been identified, one binding equimolar amounts of cadmium and zinc, a second binding mainly zinc and a third binding mainly cadmium (Elinder, 1977). It is known that metallothionein synthesis is regulated by heavy metals such as cadmium, copper, zinc and mercury. It is possible that zinc deficiency causes reduced metallothionein synthesis, especially the type that binds firmly to cadmium. As a result, levels of non-bound toxic cadmium are increased and concurrent cadmium treatment increases the toxicity further. However, zinc administration may reverse this phenomenon.

Studies on experimental animals showed that cadmium reduces the activity of zinc dependent enzymes (Vallee and Ulmer 1972). It is possible that the competition between cadmium and zinc at cofactor sites in enzymes requiring zinc

is responsible for the toxicity of cadmium. On the other hand, cadmium may displace zinc from certain enzymes crucial for natural killer cell activity. In the present study, treatment with a zinc-deficient diet tended to increase cadmium concentrations in the kidney, and decrease NK activity. However, concurrent cadmium treatment caused further suppression of NK activity and zinc administration tended to prevent this selective suppressive effect of cadmium on NK activity. Suzuki and Cherian (1987) noted that pretreatment with zinc prevented the cadmium-induced alterations in some enzyme activities.

Another possibility is that zinc may interfere with cadmium metabolism. This was hypothesized by Jones et.al., 1988 who reported that zinc treatment, slowed the cadmium distribution process. So in zinc deficiency cadmium might be distributed in tissues more widely with resultant increased toxicity.

6.6 Summary and conclusion:

In the present study, it was demonstrated that zinc deficiency (1 ppm) for 4 weeks, had a suppressive effect on the immune system. Concurrent cadmium treatment at doses of 50 ppm in drinking water caused more alterations of the immune cell function. General health of the mice, weight of different organs or lymphocyte counts in the spleen and thymus were not altered by either zinc-deficient diet or cadmium treatment. The number of T-cell subsets (helper CD4+ and suppressor CD8+) and natural-killer cell activity were reduced. IgM and IgG antibody production decreased with zinc deficiency and increased with cadmium treatment. Zinc in oral doses of 50 ppm antagonized cadmium-induced immunopathology.

Kidneys were affected by cadmium treatment, as evidenced by ultrastructural abnormalities. However, a low zinc diet affected the electron microscopic appearance of the cortical area of the thymus but not the medulla. It is possible that low zinc levels for such short periods might be enough to be absorbed by different tissues, but not released back to the circulation which may be explained by low lymphocyte counts in the blood, but not in the spleen or thymus. Further investigations with longer lengths of zinc deficiency might prove this hypothesis.

Results of this study demonstrated that symptoms of cadmium toxicity were similar to those of zinc deficiency. It is possible that the cytotoxic effects of cadmium were partially due to conditional zinc deficiency inside the cells. Nevertheless, zinc deficiency might potentiate cadmium toxicity by direct interference with its metabolism or by interference with metallothionein synthesis.

This study has focused attention on only a part of the immune system. It would be worthwhile to test other specific and non-specific responses such as suppressor cell activity, B-cells in the spleen, and interleukin-2. It would also be helpful to investigate the effects of cadmium and zinc deficiency on other organs such as the liver and on other metals, such as calcium and copper.

Finally, the ultimate testing of immunocompetence is to examine the outcome challenge following of zinc deficient animals with tumour implants and infectious organisms that are cleared by various host defences such as Listeria monocytosis, Staphylococcus aureus and Escherichia coli.

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