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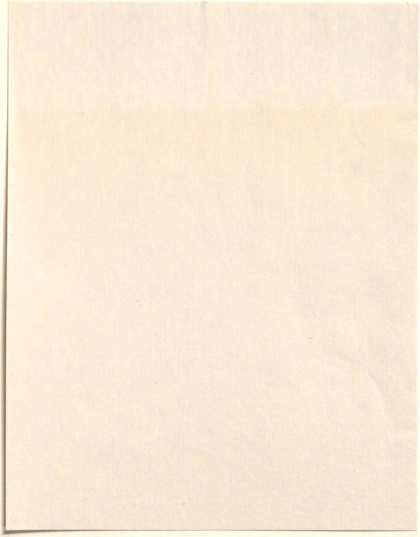

BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF ZINC DEFICIENCY IN YOUNG AND AGING MALE HOLTZMAN RATS

By

Elizabeth Fabri Gordon

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ABSTRACT

BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF ZINC DEFICIENCY IN YOUNG AND AGING MALE HOLTZMAN RATS

By

Elizabeth Fabri Gordon

An animal model was used to study the physiological and behavioral effects of feeding a low zinc diet to young and aging male Holtzman rats. The following parameters were evaluated: (1) food intake, (2) body weight, (3) plasma zinc concentration, (4) open field behavior, including latency to leave the center circle of the field, grooming, freezing, numbers of sectors entered, rearing and defecation, and (5) discrimination learning (Study 1 only).

Study 1: Three hundred day-old male Holtzman rats were randomly assigned to one of three treatment groups as follows: Group ZDA rats were fed ad libitum, a diet deficient in zinc; Group ZSP (control) rats were pair-fed a diet supplemented with zinc so that the daily intake for each animal was equal to the mean number of grams consumed by Group ZDA in the previous 24 hour period; Group ZSA (control) rats were fed ad libitum a diet supplemented with zinc. Animals were weighed weekly and food intake recorded daily. Plasma zinc

levels were determined following fifteen weeks of treatment and behavioral testing was begun.

The ZDA and ZSP groups had a significantly lower intake than the ZSA group following two weeks of feeding. The ZDA group weighed significantly less than the ZSP or ZSA group following five weeks of feeding. The ZDA animals had a significantly lower plasma zinc level than the ZSP or ZSA animals. Results from the successive black-white discrimination learning task (T-maze) revealed slightly more errors for the ZDA group compared to the two control groups. These differences were not significant. Responses in a circular open-field demonstrated the following: (1) A significantly longer latency to leave the center circle for the ZDA group compared to the ZSP or ZSA group, (2) the ZDA group spent significantly less time grooming than the two control groups and (3) the ZDA group entered significantly fewer sectors than the controls.

Study 2: Thirty-five day-old male Holtzman rats were randomly assigned to treatments and fed as described in study one. Plasma zinc concentrations were determined following seven weeks of feeding and behavioral testing was started. The ZDA and ZSP groups had a significantly lower intake than the ZSA group that was apparent following eight days of feeding. The ZDA animals weighed significantly less than the ZSP or ZSA animals and the ZSP animals weighed significantly less than the ZSA animals. These differences

were apparent following eight days of feeding. Responses in the open-field revealed the following: (1) the ZDA animals had a significantly longer latency to leave the center circle of the field, (2) significantly fewer rearings, and (3) entered a significantly fewer number of sectors compared to the control animals.

Apparent physical changes for the young ZDA animals included ruffled coats, hair loss including bald patches, inflamed paws in 50 percent of the ZDA animals and irritated conjunctiva. All of the ZDA animals had urethrititis and skin lesions. Thirty percent of the ZDA group died prior to the conclusion of the study, apparently from severe zinc deficiency. None of these physical changes were apparent in the aging ZDA animals.

Results of the present studies are compared with those of previous research in the area of zinc deficiency in living organisms and comparison of differences between the two age groups are made.

Study 1 is the only study, to date, that has used an animal model to assess zinc deficiency in aging organisms, and, therefore previous research findings in this area have not permitted a comparison of the detrimental effects of feeding low-zinc diets for these two age groups.

DEDICATION

To all of my family members who have been most responsible for my deep appreciation of the concept of life-long human development.

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INTRODUCTION

The study of zinc and its effects on the living organism has been of great interest to biologists for over one hundred years. This has gradually progressed through the disciplines of microbiology, agriculture, nutrition, medicine and more recently psychology which has begun to investigate the behavioral correlates of zinc deficiency. With the addition of psychology to these investigations we are now starting to form a comprehensive picture of the consequences of zinc deficiency for humans.

Background

Knowledge of the biological importance of zinc dates to 1869 when Raulin showed that the element was necessary for the growth of the black mold Aspergillus niger (Raulin, 1869). Since that time extensive studies in a number of different disciplines in biology and medicine have demonstrated the importance of zinc in basic biochemical processes and in its more applied aspects in agriculture and in pathophysiology of disease. Zinc was discovered later than many of the other metals and it derives its name from the German "of unknown origin" (Vallee, 1959). It is twenty-fifth on earth in order of abundance and usually

occurs in combination with sulfur or oxygen. It is in group IIB in the periodic table of the elements, has the atomic number 30 and atomic weight of 65.38 (Martz, 1970; Cordas & Holland, 1977). In addition to being found on the earth it was also present in samples of moon rock but in less concentration than on earth (Halsted et al., 1974). Zinc was first used as an alloy with copper in the manufacture of brass. Its medical uses began almost 2,000 years ago when the Egyptians used zinc oxide or calamine to aid in the healing of burns and wounds (Cordas and Holland, 1977).

The occurrance of zinc in biological materials was first described by LeChartier and Bellamy in 1877 and was demonstrated to be present in human liver in the same year (Raoult and Breton, 1877). The influence of zinc and manganese on A. niger was investigated by Bertrand and Javillier in 1911 who conformed the earlier zinc work of Raulin who was a student of Pasteur. The essentiality of zinc for higher forms of plant life was demonstrated by Maze' in 1914 (Maze', 1914) and later by Somer and Lipman (Somer & Lipman, 1926). The impetus for interest in the study of the importance of the element in the biological processes of man came about through toxicological investigations of metal fume fever in the 1920s when it was shown that zinc fumes were toxic to industrial workers (Papp, 1968). These investigations aided in the development of theories relating to the role of zinc in biological functions (Vallee, 1974).

During the period that the toxicologists were investigating the untoward effects of zinc fumes on humans the agricultural chemists were finding that zinc in solution was indispensible in the growth of barley, sunflowers, buckwheat, beans and vetch in solution cultures (Somer & Lipman, 1926). As early as 1927 the application of zinc in the field was found to be beneficial for field and vegetable crops in Florida (Viets, 1966). The problem of deficiency of zinc in crops has been noted to be worldwide. The zinc content of plants averages 23 parts per million (ppm) but some plants may have a very high concentration of up to 16 percent of their ash weight (Halsted et al., 1974).

Lutz studied the biological distribution of zinc in the rat and found it to be present in all organs (Lutz, 1926). The element was shown to be an essential in the diet of this animal by Todd and coworkers (Todd et al., 1934) and this work was later confirmed by Day and McCollum in 1940 (Day & McCollum, 1940). Hove, Elvehjem and Hart showed in 1937 that rats on a synthetic diet low in zinc experienced acceleration of the growth rate when zinc salts were added to the diet (Hove, Elvehjem & Hart, 1938) and were the first to describe the development of abnormalities of the coat of fur of animals on zinc deficient diets. Subsequent research has confirmed the significance of dietary zinc in the growth of chickens (O'Dell et al., 1958) and calves (Miller & Miller, 1962). Early studies of the effect

of zinc were also carried out in mice with some of the first conclusive growth studies (Day, 1942); however in recent years the rat has been most popular for investigation of the effects of zinc deficiency on rodents. At least fifteen animals including humans have now been shown to require zinc (Halsted et al., 1974).

Biochemical studies of the importance of zinc in physiological processes became of widespread interest following the isolation and purification of the enzyme carbonic anhydrase by Keilin and Mann in 1944 which was a compound known to contain zinc as part of its molecule (Keilin & Mann, 1944). Numerous investigations of the role of the metal have now been carried out and it is known to be an integral constituent and cofactor for more than 20 metalloenzymes. Zinc and its sister metal copper are believed to keep the polymerases on track in winding or unwinding DNA (Cordas & Holland, 1977). Zinc deficiency has also been shown to inhibit the synthesis of both DNA and RNA (Halsted et al., 1974). The chemical relationships of zinc and other elements have been investigated in some detail revealing interactions between zinc and cadmium (Schroeder et al., 1967), zinc and calcium (Forbes, 1960), and copper and zinc (Kinnamon, 1966; Gray & Ellis, 1949; Murthy, Klevay & Petering, 1974). Zinc has in addition been shown to have a protective effect against cadmium toxicity in the testes of rats (Gunn, Gould & Anderson, 1961; 1963).

The possible role of zinc deficiency in the diet of the pregnant female in causing congenital malformations has been studied in rats by Hurley and Swenerton (1966). The initial work of these investigators revealed that with severe dietary restriction reproduction was impossible. Dams given a diet somewhat less restricted in zinc had fetuses were noted to be small in size, have an abnormally shaped head, clubbed feet, fused or missing digits and malformations of the jaw and tail. Sandstead and associates (Sandstead et al., 1972; McKenzie et al., 1975) found that the major biochemical effect of zinc restriction during pregnancy was the impairment of DNA and protein synthesis in brain, liver and the epiphyseal plate of bones. This work was carried out by Swenerton and associates using radiolabeled thymidine which showed that 12-day-old embryos had a reduced uptake of the material and indicated that the gross congenital abnormalities resulting from zinc deficiency were probably caused by a basic defect in the synthesis of DNA (1969).

These studies have been extended to the effect of dietary zinc deficiency on suckling rats. Dams who received a normal diet during pregnancy were postnatally given zinc deficient diet and the pups then developed smaller forebrains and a reduced number of brain cells than controls (Fosmire et al., 1975). Studies of the effect of zinc deficiency during pregnancy on the behavior of progeny

have been carried out (Halas et al., 1975; Halas & Sanstead, 1975). These initial evaluations revealed that prenatally zinc deficient rats are more aggressive and tolerate electric shock less well than controls. Caldwell and associates have carried out similar studies and showed long term effects in male but not female progeny (Caldwell et al., 1976).

The experimental pathology of zinc deficiency in the rat was first described by Todd and coworkers in 1934 (Todd et al., 1934) where growth failure and loss of hair was noted. The original autopsy studies of zinc deficient rats carried out in 1940 (Follis et al., 1941) have been reconfirmed many times (Follis, 1966). These consist of decrease in the size of the spleen and thymus and histological changes in the skin and lining of the esophagus. The testes often show reduction in tubular diameter and other changes in their structure.

The influence of zinc deficiency on the functioning of the immune system has been of interest for many years. This dates to the observation of the Egyptians that zinc oxide has a healing effect on wounds. Early studies with experimental wounds in rats in 1953 and with surgical patients who had excision of pilonidal sinuses demonstrated that zinc supplementation of the diet promoted healing (Pories & Strain, 1966; 1974). Basic studies of the interrelationships of zinc and infection were carried out at the

germ warfare center by Beisel and coworkers (Pekarek & Beisel, 1971; Beisel, 1977) and the results have generally indicated a fall in serum zinc levels in a variety of infectious illnesses and in bacterial endotoxemia. Extensive studies have been carried out; however zinc therapy has never been shown to produce a beneficial effect in any infectious disease studied in animal models.

More basic studies have involved specific limbs of the immune system and have shown that the administration of zinc in an incubation medium stimulates the DNA synthesis of lymphocytes within six to seven days. In addition, the thymus has been shown to be a zinc sensitive tissue (Prasad, 1978). More recent studies of the effects of zinc deficiency on the immune response of mice indicate that perhaps both humoral and cellular immunity are affected and that refeeding of zinc deficient mice may restore T-cell helper function (Fraker et al., 1978).

Interest in the possibility of zinc deficiency in humans was shown many years ago and early experiments on the absorption and excretion of zinc were carried out (Drinker et al., 1927); however studies of McCance and Widdowson (1942) failed to reveal deficiencies; however, they did state that some of the individuals they identified might become deficient "if they lived long enough." They further stated that "symptoms of zinc deficiency in human beings have, however, never been described." Tribble and Scholar

(1953) carried out early studies on the zinc metabolism of young college women on self-selected diets and various other nutritional investigations were continued over the years yet as late as 1962 Underwood stated that "an uncomplicated dietary deficiency of zinc has never been observed in man." The recommended daily allowance for zinc was not established until 1974 (Food and Nutrition Board, 1974).

The major impetus for investigation of the importance of zinc in human nutrition came about with the description by Prasad and associates of the first zinc-responsive syndrome in Egypt. They found that treatment of a group of patients from the farming class who were chronically ill with parasitic disease and anemia and who ingested diets prepared with unleavened bread lead to dramatic increase in longitudinal growth and sexual maturation (Prasad et al., 1963a, 1963b). Since that time many investigations of the nutritional effects of zinc deficiency have been carried out including the study of phytate concentrations of bread as a possible mechanism for zinc deficiency through binding (Reinhold, 1971, 1972; Ismail-Beigi et al., 1977). Zinc responsive dwarfism caused by problems with intestinal absorption of zinc has been described and workers have shown that some children with growth problems may have impairment of taste acuity. Zinc supplementation has yielded improvement in taste ability, appetite and growth in these children (Hambidge, 1972).

The widespread use of total intravenous nutrition led to the description of a clinical syndrome consisting of dermatitis, diarrhea, hair loss and pronounced depression. Increased excretion of zinc in the urine was demonstrated and it was noted that a rapid clinical response to zinc supplementation occurred and, therefore, the illness was linked to acute zinc deficiency (Kay & Tasman-Jones, 1975; van Rij & McKenzie, 1977).

Other conditions that have been shown to be related to deficiency of zinc include the rare autosomal recessive genetic disease acrodermatitis enteropathica. Symptoms include diarrhea and vomiting, skin lesions and hair loss along with growth retardation. The condition manifests itself in the period following weaning and was often fatal until the administration of zinc was found to be therapeutic (Neldner, Hambidge & Walravens, 1978). Conditions that also involve zinc deficiency but that are less well defined include impotency in renal failure (Antoniou, Shalhoub & Sudhaker, 1977), chronic liver disease (Sullivan & Burch, 1976) and acne (Hillstrom et al., 1977).

The other area of research involving humans is that related to the possible role of zinc in psychiatric disease. The early work with manganese administration in schizophrenics (English, 1929; Hoskins, 1934) is the basis for the speculation regarding the use of zinc in treatment of psychiatric disorders.

Dietary Intake of Zinc

There is a wide range of values for zinc content reported in the literature for the same foods and this is thought to be due to differences in analysis, source and variety of the food (Halsted et al., 1974). Zinc concentrations in food are listed as total zinc and studies indicate that foods and diets high in protein are also high in zinc while foods and diets that are mostly carbohydrate are much lower in zinc and nitrogen content (Osis et al., 1972). The National Academy of Sciences has recommended the following daily dietary allowances for zinc: infants, 3-5 mg.; children, 1-10 years, 10 mg.; males, 10-51+ years, 15 mg.; pregnant women, 20 mg.; and lactating women, 25 mg. (Underwood, 1977). Murphy and coworkers have supplied provisional tables on the zinc content of foods and showed that the major dietary sources of zinc are foods of animal origin such as meat, fish, shellfish, poultry, eggs and dairy products (1975). The zinc content of oysters is usually reported as being high but may range from 9 mg. for Pacific species to 207 mg. per 100 grams for the Atlantic variety (Murphy et al., 1975; Sandstead, 1973).

The dietary intake of zinc is influenced by the foods consumed and their origin. Underwood (1977) reviewed the literature on dietary intake according to country of residence and found that the U.S. adult mixed diet supplied 12-15 mg. per day while the daily intake of zinc in adults in

nine different regions of India ranged from 14-24 mg. per day. Tribble and Schoular (1954) carried out one of the first studies of zinc metabolism using modern techniques and a sizeable number of subjects and found that self-selected diets in thirteen college women supplied from 9.8 to 14.4 mg. of zinc daily. Price and associates (1970) studied the intake of zinc in fifteen preadolescent girls and found it to range from 4.53 to 6.93 mg. per day. White and Gynne (1971) carried out a similar study in nine college women and found a mean intake of zinc of 11.5 mg. per day.

A major study of dietary intake of zinc was carried out by Murphy and associates (1971) who investigated trace element intake through type A school lunches. Lunches were collected that were to be served to children in the sixth grade from 300 schools in nineteen states. Samples of food were analyzed by spectroscopy and the amount per lunch ranged between 1.77 and 7.68 mg. with a mean of 3.91 mg. Zinc content in these diets correlated well with the content of copper, manganese and strontium. Additional studies of the dietary intake of zinc have been carried out to investigate the zinc content of hospital diets. Osis and coworkers (1972) studied the dietary intake of zinc at a Veterans Administration hospital including nine different diets and found that the total zinc content ranged from 7.0 to 16.3 mg. per day with an average of 11.3 mg. zinc and confirmed that diets high in protein had more zinc. Zinc levels have

also been studied in human colostrum and range between 10 and 20 mg. per liter while the mild concentration remains at 3 mg. per liter during the first month of lactation and then declines with prolonged lactation (Hambidge, 1979).

Factors that affect the concentration of zinc in foods particularly include the methods of processing and preservation. Schroeder (1971) reviewed the topic and found that when a food is divided into its components by refinement or extraction, a majority of the trace metals go with one part or the other. Zinc was found to be largely contained in the germ and bran of wheat, a marked loss of trace metals occurred when rice was polished and refined sugar was found to have a minimal amount of zinc when compared with raw sugar or sugar cane. Basic studies of the zinc available in grains and animal products were carried out to determine the growth response of chickens by O'Dell and coworkers (1972). The results indicated that in general zinc in plant seeds was less available than that in animal products. Another factor relating to the availability of zinc in foods is the amount of phytate or inositol hexaphosphate present in cereal grains. Reinhold (1971) studied the phytate concentration of unleavened bread in Iranian villages and found it to be considerably higher than in the leavened flat breads in commercial bakeries in cities of the same region. He postulated that the excessive intakes of phytate may explain the occurrence of overt zinc deficiency in villagers

and its absence in the cities. Further investigation by Reinhold and associates (1973) was carried out in thirteen villages in Iran in an area where a high phytate diet is ingested. Following admission to a metabolic ward the phytate intake fell to one-fourth to one-third of the usual intake and a positive balance of zinc occurred in the majority of subjects.

On a more basic level O'Dell and Savage (1960) investigated the possible role of phytic acid in soy protein in making zinc unavailable to laboratory chicks. They found that the zinc in isolated soy protein is less available than in casein and this is additional evidence in support of a relationship between zinc deficiency and phytic acid in the diet. Taking a somewhat different approach laboratory studies were carried out (Ismail-Beigi et al., 1977) to determine whether the unleavened bread ingested by Iranian villagers had any direct binding effect on zinc and iron. In these studies it was found that the removal of phytate from tanok (unleavened bread) did not reduce its binding capability. Investigators speculated that zinc or iron can form complexes with wheat fiber and that this binding can help explain why deficiencies of these metals are prevalent in the middle eastern countries and that phytate alone may not be the whole explanation.

Physiological and Biochemical Aspects of Zinc Metabolism

Zinc is found in the biological state complexed to organic ligands rather than free in solution as metallic ion (Vallee, 1959). Important aspects of the physical chemistry of zinc include the observation that in the blood serum zinc exists in two fractions as a firmly bound and loosely bound zinc. Several proteins have been suggested as being involved in the plasma binding of zinc including transferrin and alpha-2-macroglobulin. Additionally, several amino acids have been found to bind zinc and it is possible that this amino acid bound fraction may play a significant role in biological transport (Halsted et al., 1974). A recent review reports that approximately 50 percent of plasma zinc is freely exchangeable being loosely bound to albumen, 7 percent is bound to amino acids such as histidine and cysteine and the remainder is tightly bound to the macroglobulin and other serum proteins (Walravens, 1979). Besides binding to the components of serum, zinc has been demonstrated to bind to specific sites on the hormone insulin. Whether this is of definite biological significance is still undecided since insulin can be produced that is free of zinc and other metals, yet is physiologically active.

Cadmium is an element that is similar to zinc and has been found to replace it in several biological systems which may vary from a partial retention of zinc-dependent

activity to total inhibition of it (Chesters, 1978). Recent studies of physico-chemical aspects of zinc have revealed that zinc has a high affinity for sulfhydryl groups which are important determinants of protein structure and for the activity of metalloenzymes (Chesters, 1978). In addition it has been shown that zinc can alter the properties of membranes *in vivo*. Zinc has also been found to be a trace element that is essential in vitamin A metabolism (Smith *et al.*, 1973). Zinc has been shown to be involved in the metabolism of polynucleotides in RNA and DNA synthesis and in addition laboratory studies in rats have demonstrated that zinc may be involved in carbohydrate and fat metabolism.

Zinc has an important role in the metalloenzymes including especially carbonic anhydrase, carboxypeptidase A and B, alkaline phosphatase, alcohol dehydrogenase, retinene reductase and lactic-, glutamic- and d-glyceraldehyde-3-phosphate dehydrogenase (Walravens, 1979). Zinc is involved in an enzyme that also has copper, superoxide dismutase or erythrocuprein and in addition it is thought to act as a stabilizer of biological membranes. The absorption of zinc occurs mainly in the duodenum and proximal small intestine, and the mechanism probably involves active transport.

Extensive studies have been carried out on the distribution of zinc in the body through autopsy studies of subjects dying of accidents or of various diseases (Halsted *et al.*, 1974). The findings from many studies as summarized

by these investigators reveal that the highest concentration of zinc in the human is in the retina (571 mg./kg. dry weight) and significant concentrations in the range of 150 to 245 mg./kg are found in liver, kidney and muscle. The concentration in hyperplastic tissue of the prostate gland was noted to be quite elevated at 2,330 mg./kg. It is of note that zinc concentrations in the kidney are often not decreased even when there is zinc deficiency. The concentration of zinc in serum is said to be higher than in plasma. Modern studies have revealed zinc concentrations in the range of 63 to 160 mg./11 ml. in adults in plasma or serum (Halsted et al., 1974).

Another factor of importance in the study of the metabolic role of zinc is the demonstration of a circadian variation in normal volunteers (Lifschitz & Henkin, 1971). Results from the study revealed that serum zinc was above the mean from 10 AM to 10 PM and below the mean at 2 AM and 6 AM. The ceruloplasmin level tended to follow serum copper concentrations which were also being monitored and suggested that the regulation of metal binding protein may be important in the circadian pattern. Other factors that may affect serum concentration of zinc include pregnancy, oral contraception, myocardial infarction, extensive surgery and acute infection (Halsted et al., 1974). In diseases of the thyroid gland, zinc levels have been reported to be high in hyperthyroidism and low in hypothyroidism (Hartoma et al., 1979). Other ions that may interact with zinc include calcium

(Forbes, 1960) and it has been implied that interference in zinc function by calcium occurs at the cellular level. Zinc has been determined to be present in a concentration of 175 ug./gram in hair and 125 in sperm among others studied (Walravens, 1979).

Hartoma and associates (1979) studied fourteen healthy male volunteers with primarily high or low serum zinc. In all subjects extensive laboratory studies were carried out showing that blood picture, carbohydrate metabolism, fat and protein metabolism were all normal. Significant differences between those with low zinc levels, 7.50 ± 21.01, and high levels, 166.12 ± 24.64 were in the alpha globulin protein fraction. Serum thyroxine, effective thyroxine ratio and immunoglobulin IgA showed a tendency to lower levels in subjects with low serum zinc. The authors suggested there is a relationship between zinc and some metabolic functions in healthy subjects without symptoms of zinc deficiency.

Zinc Toxicity

The extent of tolerance to ingestion of large quantities of zinc depends greatly on the nature of the diet, particularly its content of calcium, copper, iron and cadmium with which it interacts in the process of absorption and utilization (Underwood, 1977). Experimental zinc intoxication in the rat leads to anemia, high levels of zinc and reduced levels of iron, copper, cytochrome oxidase and

catalase. The anemia is thought to be due to the induced iron and copper deficiencies (Cox & Harris, 1960). Weanling pigs fed large amounts of zinc had depressed growth and appetite, arthritis and internal hemorrhages (Brink et al., 1959). Again the type of diet is important since no evidence of toxicity was seen when a diet containing 2,000 parts per million (PPM) of zinc was added to corn-soybean, corn-fish meal, or sucrose-soybean diets fed for two weeks to baby chicks. The same amount of zinc added to a sucrose-fish meal diet was more toxic.

Human zinc toxicity through inhalation of metal fumes has been known for many years (Sayers, 1938) and is seen in welders. The clinical presentation in this case may be that of an upper respiratory infection, pneumonia or many other manifestations (Papp, 1968). A second type of zinc toxicity was described where oral ingestion of twelve grams of zinc sulfate over a two day period caused drowsiness, lethargy and increased serum lipase and amylase levels (Murphy, 1970). A third type of acute zinc toxicity was observed in a patient with renal failure who developed nausea, vomiting, fever and severe anemia when the water used to carry out his hemodialysis had been stored in a galvanized tank (Gallery et al., 1972). Callender and Gentzkow (1937) reported on zinc intoxication occurring in soldiers who drank limeade prepared in galvanized iron garbage cans and the illness consisted of gastrointestinal

distress and diarrhea. A complicating factor was the presence of significant amounts of antimony and iron in the metal. A more recent report by Brown and associates (1964) described mass food poisoning where several hundred people became ill after attending a celebration where food was stored in galvanized tubs. In another episode reported by the same investigators individuals became sick after ingesting punch stored in a galvanized container and chemical analysis of a five ounce serving contained 525 mg. of zinc.

Zinc in Infection and Immunity

The first studies of zinc in infection were carried out by Vikbladh in 1951 who noted that the serum zinc concentration was depressed in infection. The majority of studies of zinc metabolism in infection have been carried out by Beisel and associates (Pekarek & Beisel, 1971; Beisel, 1977). When infection is present zinc moves from serum to liver and in contrast copper moves from liver to the binding protein ceruloplasmin in the serum. When the zinc enters liver cells it binds to the metallothionines of the cell. The concentration of zinc in serum in humans is usually depressed in infectious diseases such as dysentery, typhoid fever and in tuberculosis and in experimentally induced endotoxemia (Smith et al., 1972). Similarly in experimental animals tularemia, rocky mountain spotted fever and other conditions cause a fall in serum zinc concentrations (Pekarek & Beisel, 1971; Powanda et al., 1976). It has been

postulated that a mediating substance for zinc depression is released by phagocytes of the host and has been shown to be produced in vitro by activated neutrophiles and macrophages. This has been investigated in experimental animals where isotopic zinc tracers have been given demonstrating that there is an accelerated uptake of zinc into the liver (Beisel, 1977).

The role of zinc in host defense responses were summarized by Beisel as follows: zinc contributes to plasma membrane integrity and the functioning of binding sites; in high concentrations it inhibits phagocytic functions; high concentrations cause mitogenic effects in lymphocytes and low body zinc stores are associated with dysfunction of T cells. Zinc is also important for nucleic acid and protein synthesis, has a key role in the metalloenzymes with regard to cellular function, and is also known to have negative effects on the growth of microorganisms when not in the optimal concentration. Basic studies of the effect of zinc deficiency on the immune response have been carried out by many investigators (Frost et al., 1977; Miller et al., 1968; Haas et al., 1976; and others). Spleen and thymus weights have been known to decrease in deficient animals and thymic atrophy has been described in zinc deficiency in the rat. Peripheral lymphocyte counts are said to be reduced, there is a deficiency in the immunologic response to experimental antigens, and there is a reduction in serum immunoglobulins

as well. Experimental findings recently reported by Fraker and coworkers (Fraker et al., 1977; Luecke et al., 1978) have indicated that zinc deficiency may interfere with T cell helper function; however the severe dietary deficiency caused by prolonged feeding of a zinc deficient diet will also contribute to immunologic deficiency on a nonzinc related basis.

The earliest practical studies of the role of zinc in the inflammatory and healing response were surgical investigations carried out by Pories and coworkers (Pories & Strain, 1974) in 1951 where it was noted that wounded and burned rats maintained on diets accidentally enriched with zinc healed more rapidly than in animals fed control diets. Since that time numerous studies of the relationship of zinc and healing have been carried out and it has been shown that the turnover of zinc is high and that it moves rapidly into healing tissues. It has been postulated that the importance of zinc in healing lies in its essentiality in the basic processes of healing such as protein synthesis and cellular growth and replication. The enhancement of wound healing by zinc may stem from a heightened metabolic demand for the element in collagen synthesis yet this is not completely documented (Underwood, 1977).

Experimental Zinc Deficiency in Animals

The first attempt at the study of experimental deficiency was carried out in 1922 by Bertrand and Benzon

who fed mice a purified diet that was believed to be free of zinc. The animals lived for an average of only twenty days because no vitamins were added to supply those destroyed during purification of the ration. Another group of animals was fed the same purified ration to which a small quantity of zinc had been added and they found that animals in the latter group lived 25 to 50 percent longer. A later study by Hubbell and Mendel (1927) in which mice received a diet supplying only 0.005 mg. zinc per animal daily and additional vitamins showed no great differences in the growth of the animals. Todd and associates (1934) were among the first workers to utilize the rat as an experimental animal in zinc research. In their early work they fed a diet containing only 1.6 mg. of zinc per kg. of dry weight to weanling rats and found that growth and weight of experimental animals was considerably less than in another group of animals fed the same diet and added zinc in a concentration of 5 mg. of zinc per 100 grams of ration. Day and McCollum (1940) investigated the effect of acute zinc deficiency on the young rat using restriction of dietary intake to not more than two to four µg. of zinc per rat daily. They found that growth was greatly retarded after two to three weeks, there was marked eczema in two animals and alopecia or loss of hair as described by previous workers. Hove and associates (1937) investigated the physiology of zinc in the nutrition of the rat and made the following conclusions

regarding the studies at three to eight weeks of age: zinc deficiency is prevented when 40 µg. per day is included in the diet. There was a 50 percent decrease in growth rate when 22 µg. of zinc was given per day. They were unable to detect any disorders in carbohydrate metabolism through determination of urinary sugar, blood sugar, liver glycogen or glucose tolerance. They further concluded that there is a marked delay in intestinal absorption in the zinc deficient rat and that this delay is probably greater for nitrogenous products than carbohydrates. Zinc deficient rats have been shown to have a fall in creatinine and a normal urinary nitrogen excretion per gram of food eaten in spite of the demonstrated delayed absorption, thus suggesting the possibility of a lower rate of endogenous metabolism. Pituitary transplants carried out by injection of tissue by needle into muscle produced growth stimulation and increased efficiency in converting food to body weight. Researchers have postulated that zinc is involved in the production or utilization of some pituitary hormone which controls the motility and tone of the intestinal tract.

Numerous investigations of zinc deficiency in rats have been carried out, however other investigators have used the chicken (O'Dell et al., 1958) and calf (Miller & Miller, 1962). The zinc requirements of chickens and swine are considerably higher than those of rats when maintained on a zinc deficient diet for a period of four to six weeks

beginning at one day of age. These animals developed symptoms ranging from mild deficiency manifestations such as slow growth, shortening and thickening of the long bones, development of frizzled feathers, abnormal respiration and an unsteady gait when kept in galvanized batteries to a very severe deficiency when kept in galvanized batteries that were lacquered. The severely deficient animals exhibited the same symptoms but to a more severe degree and also developed keratosis of the skin and an increase in packed cell volume of the red cells. Microscopically there was parakeratosis in the esophagus and poor calcification of bone. Experimental zinc deficiency in calves was studied by Miller and Miller (1962). They used seven male Holstein calves in two experiments and fed a low-zinc purified diet with and without supplemental zinc in the two experiments. These animals developed a severe parakeratosis as originally described in swine by Tucker and Salmon (1955). Zinc deficiency symptoms in the calves included anorexia, dull and listless appearance, low weight gain, alopecia and various changes in the skin. It was also noted that the testicles were undersized and there was reduced blood zinc content and carbonic anhydrase activity. All of the conditions responded to zinc supplementation with the exception of the undersized testicles.

The effect of zinc deficiency on food intake and feeding patterns of rats was investigated by Chesters and

Quarterman (1970) who found that incipient zinc deficiency in the young rat was characterized by a cessation of growth followed by an extended period during which body weight varied very little. The voluntary food intake of zinc deficient rats fell to 70 percent of that of the controls. The day to day variation increased markedly and was associated with cyclical food consumption. When food was slightly restricted at this point the troughs of the cycles disappeared. Concurrent with the fall in food intake the zinc deficient rats ceased to gain weight and a similar change was found in the pair-fed controls. Additional findings of note were that force feeding of rats with 140 percent of their voluntary intake rapidly induced signs of ill health. The zinc deficient animals responded to a zinc supplemented diet within one to two hours by an increased food intake.

Chesters and Will (1973) studied additional factors controlling food intake in zinc deficient rats and found that when the food intake was examined for periods of two hours throughout the day the deficient rats were found to eat on fewer occasions than the control rats; however when the deficient rats did eat, the quantities eaten in two hours showed the same distribution of weights as did those for the zinc adequate diet. They also found that reduction of the environmental temperature would increase the deficient animals' intake of food. Statistical analysis of the patterns of plasma zinc concentration and food intake showed

the inverse correlation of food intake during a day and the plasma zinc concentration at the end of twenty-four hours was more significant than any of the other correlations tested.

McConnell and Henkin (1974) investigated changes in NaCl preference and in plasma and urinary zinc in weanling rats fed ad libitum zinc deficient or zinc supplemented diets and in rats pair-fed an amount of food equal to that eaten by rats fed a zinc-deficient diet. Sodium chloride preference in the zinc deficient rats was significantly greater than in pair-fed controls or in rats fed zinc supplemented diet ad libitum: this alternation in preference occurred within three days of initiation of the zinc deficient diet although anorexia was observed in these rats within the first two days. Both increased preference for NaCl and anorexia occur in zinc deficiency. Concentrations of plasma and urinary zinc were significantly lower in rats fed the zinc-deficient diet than in pair fed controls or those fed the zinc supplemented diet ad libitum. The concept of a physiological role for zinc in normal taste sensation together with the observation that hypogeusia (loss of taste acuity) and dysguesia (disordered taste or pica) that is correctable by zinc is now accepted (Underwood, 1977).

Walker and Kelleher (1978) studied the concentrations of plasma, whole blood and urine zinc in young adult rats fed zinc deficient and zinc supplemented diets for a

period of sixty-five days. They found that within forty-eight hours the plasma zinc concentrations fell to 25 percent of control values and remained at this level throughout the period of the study. Whole blood values for zinc did not change during the study. Urine zinc fell to very low values ten days after the beginning of the zinc deficient diet but after thirty days rose to control values. A substantial diurnal variation of zinc levels in plasma was found and the level was significantly lower in the fasting state.

An investigation of possible correction of experimental zinc deficiency in the rat was carried out by Keen and Hurley (1977) where pregnant rats consuming a zinc deficient diet had zinc applied topically as zinc chloride. Topical zinc supplementation by application to the skin resulted in a rise in plasma values similar to the control group fed a normal diet and significantly higher than those of rats receiving no zinc application to the skin. Animals given twenty-four, four hour applications demonstrated plasma zinc values that were higher than in any other group including controls.

The effect of zinc repletion late in gestation was studied by Apgar (1973) who gave zinc acetate by stomach tube to pregnant females at various times in late gestation. These experiments indicated that stressful parturition

could generally be avoided if the zinc was administered to deficient rats between day 19 and day 21.

Extensive studies of the effects of zinc deficiency on the enzymes in tissues of rats have been carried out. Kfoury and coworkers (196) investigated (1) alcohol and glutamic dehydrogenase in liver and kidney, (2) alkaline phosphatase in intestinal mucosa, kidney and plasma, (3) catalase in liver, kidney and blood, (4) xanthine oxidase in liver and kidney and (5) aspartic and alanine aminotransferases in liver in rats fed diets low in zinc or protein or both and in their pair-fed controls for 45 to 180 days. The alkaline phosphatase decreased in intestinal mucosa but not in plasma or kidney. Alcohol dehydrogenase decreased marginally in the livers of rats on diets with two to four PPM of zinc but not in plasma or kidney. A correlation existed between alcohol dehydrogenase activity in liver and zinc concentration in liver. Elevated concentrations of uric acid in plasma occurred infrequently. Prasad and Oberleas (1971) confirmed that alcohol dehydrogenase in liver and carboxypeptidase in pancreas were significantly reduced in the deficient rats. No changes were observed when isocitric dehydrogenase (manganese dependent) and succinic dehydrogenase (iron dependent) were studied in the tissues. These results were generally confirmed by Huber and Gershoff (1973) who also felt that decreased carbonic anhydrase in brain and red cells and the

decreased alkaline phosphatase in heart of zinc deficient animals seemed related in part to alterations in food intake.

Many investigators have studied the effect of zinc deficiency on the synthesis of nucleic acids. Fujioka and Lieberman (1964) carried out some of the earlier work regarding the requirement of zinc for DNA in rat liver. They found that use of EDTA chelation largely or completely prevents the increase in the rate of liver DNA synthesis that normally follows experimental partial hepatectomy in the rat. Sandstead and Rinaldi (1969) confirmed the observation of these workers demonstrating that dietary zinc deficiency in the rat will impair in vivo zinc deficiency in the rat liver parenchymal cell. Extensive work by Sandstead and Terhune (1974) also showed that zinc deficiency in suckling rats results in a decreased activity in liver nuclear DNA dependent RNA polymerase, that the sedimentation characteristics of liver RNA are altered by zinc deficiency and that the incorporation of uridine into liver RNA is decreased by deficiency of zinc. Experimental work in pigs by Prasad and Oberleas (1974) was carried out giving a zinc deficient diet and comparing deficient animals and pair-fed controls. Results showed that growth was retarded and parakeratosis of the skin became moderately severe by the end of the second week. Extensive biochemical and histochemical studies were carried out and it was shown that the DNA content of the

tissues per mg. of wet weight was the same for deficient animals or pair-fed controls. Significant differences were seen, however, when the zinc dependent enzymes were expressed as activity per mg. of DNA. They concluded that glutamic dehydrogenase and carbonic anhydrase are much less susceptible to zinc deficiency than alcohol dehydrogenase, alkaline phosphatase and carboxypeptidase.

Studies in zinc deficient rats using incorporation of radiolabeled cystine-S were carried out by Hsu (1974) who found that the incorporation of this material into skin protein was only one-seventh of that in zinc supplemented controls that had been pair-fed. He found no differences in the incorporation of the radiolabeled cystine into liver, kidney, testes or muscle but there was more incorporation of the cystine into pancreatic protein of the deficient rats which he attributed to a depletion in methionine in zinc deficient animals. Additional studies were done with multiple compounds and tritiated thymidine and he concluded also that there was slower rate of epithelial renewal as demonstrated by a decrease in thymidine incorporation into DNA and a decrease of amino acid incorporation into skin protein.

Extensive studies of the concentration of ash and zinc in bones were carried out by Swenerton and Hurley (1968) and both were reduced in the femurs of rats that were zinc deficient. More recent studies have been carried out

to determine if the net zinc content of bone was altered during the development of zinc deficiency in the growing rat (Brown et al., 1978). They utilized control, zinc deficient and pair-fed groups of weanlings. They noted that the plasma zinc concentration of the zinc deficient group decreased dramatically during the first twenty-four hours of the deficient diet and continued to decline over the next two days. Body weight gain stopped after one week. Femur zinc concentration decreased 61 percent during the experimental period of twenty-three days while the total femur zinc decreased 37 percent. Calcium and phosphorous content continued to increase and the ratio remained constant. Data indicate that zinc can be released from bone during periods of dietary zinc deficiency and furthermore the decline in zinc appears to be independent of calcium and phosphorous in the bone.

The interrelationships of zinc and other elements besides calcium and phosphorous have been investigated by a number of workers. Gray and Ellis (1950) were among the first investigators to study the association between copper, molybdenum, zinc and lead in the nutrition of the rat. They noted that addition of molybdenum to diets was toxic and retarded growth while zinc produced an anemia and the addition of copper corrected the anemia from excess zinc. Kinnamon (1966) studied the absorption of radiolabeled iron in the rat under the influence of dietary supplements of

copper or zinc. He found that dietary zinc or copper had no effect on absorption of radiolabeled iron but that diets high in zinc significantly lowered retention of iron stores.

Kinnamon suggested that iron absorbed from the digestive tract apparently followed the normal metabolic pathway but later deviated under conditions of zinc toxicity while copper had no effect.

A change in the ratio of zinc to copper ingested by rats was found by Klevay (1973) to cause an hypercholesterolemia and lead to an hypothesis that such a change in the balance might be involved in the genesis of atherosclerosis Further studies led Klevay to conclude (1975) that the ratio of copper and zinc may be the most preponderant factor in heart disease; although this hypothesis is not completely accepted (Kine & Sifri, 1976). Murthy and associates (1974) carried out extensive studies of the interrelationships of zinc and copper in the rat and improved upon methods of use of hair analysis to evaluate zinc and copper nutriture and metabolism. They concluded that liver copper was inversely related to serum zinc, aortic copper and hair copper were directly related to serum copper and hair zinc was directly related to serum zinc.

The major other area of investigation involving zinc and other metals has been in the observation (Gunn et al., 1960, 1963) that the toxicity of cadmium for the testes of the rat may be protected against by the

administration of zinc. The rat has long served as a useful animal for almost all aspects of experimental zinc deficiency or toxicity.

Teratogenic Effects of Intrauterine Zinc Deficiency

Studies with poultry were among the first attempts to look at the effect of zinc deficiency on intrauterine development. Turk et al. (1959) demonstrated that when hens were fed a zinc deficient diet they produced chicks that were weak and died soon after hatching. Other investigators (Blamberg et al., 1960; Keinholz et al., 1961) used more severe dietary restriction of zinc and noted gross malformations in the chick embryos such as skeletal defects, brain abnormalities and visceral herniation. Hurley and associates (Hurley et al., 1966, 1971) were the first to study the effect of zinc deficiency on the progeny of pregnant rats and noted frequent embryonic death, intrauterine growth retardation, and a high incidence of congenital malformations of all systems. These findings have been confirmed by numerous other workers as reviewed by Hurley (1974). More recently reported work from Hurley's laboratory (1977) described observations that lung abnormalities were frequently found in addition to the malformations of the central nervous system so often noted. Biochemical studies of lungs indicated that fetal lung lecithin was lower in zinc deficient fetuses and found that the

lecithin:sphinogomyelin ratio was lower in zinc deficient fetuses indicating that zinc is important for the functional and morphological development of the lung. Hurley and investigators have shown that the uptake of tritiated thymidine was severely depressed in zinc deficiency in twelve day old rat embryos. Autoradiography of the cerebral cortex from the embryos showed a similar effect with lower concentrations of the silver stain in the zinc deficient fetus (Swenerton et al., 1969). More recent studies (Hurley, 1977) indicate that the head is more vulnerable to deficiency of zinc than the rest of the body and incorporate less tritiated thymidine into DNA and RNA relative to the control than did the body region. Bell and associates (1975) have studied the effect of zinc deficiency on nucleic acid synthesis and its effect on chromosomes. Pregnant rats given a zinc deficient diet showed bone marrow and fetal liver cell chromosomal aberrations.

Additional studies of the effect of zinc deficiency on the brain of the suckling rat were carried out by Sandstead et al. (1972) using radiolabeled thymidine and it was shown that the zinc deficiency impaired DNA and protein synthesis in brain tissue. More recent studies of the effect of zinc deficiency on pregnancy were reported by McKenzie et al. (1975) who found that deficient dams were anorexic and lost weight. The fetuses showed intrauterine growth retardation as compared to controls, the brains were

smaller than controls and the livers were severely affected. McKenzie noted that the total cell number was reduced and there was an increase in cell size in the brains of deficient animals. The growth failure in zinc deficient liver resulted in less total DNA, RNA, protein and lipid and there was less incorporation of radiolabeled thymidine into DNA in the liver. Postnatal zinc deficiency during the suckling period has also been investigated by Fosmire et al. (1975) who found that there was a depression of normal growth and that the forebrains were smaller than those of control animals. There was reduction of total RNA because of the smaller brain size and the amount of protein per brain cell was also decreased.

Experimental Zinc Deficiency in Humans

Human zinc deficiency with clinical manifestations was first reported from the middle east in 1963 (Prasad et al., 1963) and in the past sixteen years the spectrum of illness related to this trace element has broadened considerably. The first experimental studies of zinc metabolism in humans were carried out in the 1920s and reviewed by Lutz (1926). Investigators had shown by that time that the usual human intake of zinc was in the range of 10 to 15 mg./day and that most of this was excreted in the feces. The classical studies of the absorption and excretion of zinc were carried out in 1938-1941 by McCance and Widdowson (1942). In these studies forty-five zinc balance

determinations were carried out on twelve people including the intravenous injection of zinc in ten of the subjects.

These studies were carried out before many studies of the response of animals to intravenous injections had been performed and it was stated that "the response of animals to zinc injections will be found to be similar to that of human beings" (McCance and Widdowson, 1942, p. 694). This is an unusual example of generalization of human results to animals rarely seen in the literature.

Zinc balance studies were subsequently performed in young college women (Tribble & Scoular, 1954) as described earlier in this review and more recently in thirty-six school children (Engel et al., 1966). These children were studied under controlled dietary conditions in a camp environment. Daily zinc intake was shown to range from 4.6 to 9.3 mg. and urinary zinc excretion was relatively constant (274 $\pm$ 69 ug/24 hours) and constituted 6.5 percent of the total zinc lost through urine and feces. Enhancement of gastrointestinal absorption of zinc was encountered in twelve subjects in one of three studies during conditions of low protein intake and was more predictable in two other studies reported in the same paper. Radiolabeled zinc has been utilized for study of the metabolism of zinc in humans and it has been found that most of the zinc of the body is in a state of constant movement with accumulation and turnover varying with the tissue being studied. It was found to

enter the skeleton slowly and remained firmly bound there for long periods (Prasad, 1966). The metabolism of radio-labeled zinc-65 in patients with cancers was studied by Ross and coworkers (1958) who found that the zinc rapidly appeared in leucocytes, reached a maximum in twenty-four to forty-eight hours and then decreased in concentration but persisted in the circulating leucocyte mass for several weeks. It was also found to be incorporated rapidly into red blood cells with a maximum concentration in ten days. When zinc-65 labeled erythrocytes were transfused into normal recipients the radioactivity disappeared at a rapid rate indicating that zinc-65 was not firmly bound to the red cell. The zinc was deposited into the liver in highest concentration (Ross, Ebaugh & Talbot, 1958). Prasad et al. (1963) studied the metabolism of zinc-65 in normal subjects and patients with the syndrome of iron deficiency anemia, hepatosplenomegaly (enlargement of liver and spleen), dwarf-ism and hypogonadism. The mean plasma zinc turnover rate for normal patients was 1.00 $\pm$ 0.09 mg./kg. The urinary excretion of zinc-65 in normals after thirteen days was 2.8 $\pm$ 0.56 percent of the administered dose. In normal subjects the fecal excretion of zinc-65 was 0.66 $\pm$ 0.19 percent of the administered dose/100 grams of stool. Spencer et al. (1966) also studied the metabolism of zinc-65 in humans following intravenous and oral administration of the com-pound. These investigators found that the intestine was

the major pathway of excretion of zinc-65. The zinc was found to be deposited mainly in the liver and reticulo-endothelial system and a high uptake of zinc-65 was noted to be present in the endocrine glands. Probably the most significant findings of this investigation was that approximately 50 percent of the orally administered zinc-65 was absorbed and this was not affected by the level of calcium taken in. It is of note that there was a significant difference ($p < 0.01$) between normal subjects and the dwarfs studied by Prasad et al. (1963) in the turnover rate, twenty-four hour exchangeable pool, and urinary excretion of zinc-65 while the excretion in stool in percentage of dose administered was significant at 0.05.

Spencer and coworkers (Spencer et al., 1974) extended their earlier studies of zinc metabolism in normal individuals and in patients with neoplasms and showed that there was no correlation between excretion of zinc and urinary calcium. The data also revealed that, although a ten to 20 fold increase in urinary calcium was induced by infusing calcium, this resulted in only a very slight increase in urinary zinc excretion. Administration of ammonium chloride given orally increased the urinary calcium excretion considerably more than the excretion of zinc. The effect of starvation on urinary and plasma zinc levels was investigated by these workers using a period of total caloric restriction which resulted in marked weight loss and marked

and sustained increase in the excretion of zinc in the urine. These excretions reached a maximum of 6.5 mg./day and represented an approximate increase in urinary excretion of zinc of ten fold. The plasma zinc level did not fall in spite of this urinary loss of zinc, and in the majority of patients it increased during fasting. When other subjects were maintained on a low protein-low calorie diet containing two mg./kg. of zinc per day there was a gradual weight loss and a similar loss of zinc via the kidney. The total zinc loss in two subjects studied for sixty and sixty-four days was 270 mg. during starvation and 250 mg. on a low protein diet respectively. These same investigators also studied the effect of tetracycline administration in a patient with an E. coli urinary tract infection and found that the plasma level and urinary excretion increased during the time of administration of this antibiotic. The studies by these workers have demonstrated that the amount of zinc necessary to attain equilibrium is approximately twelve mg. per day under normal conditions. The zinc balance in malnutrition became very positive on a normal intake and continued to be positive until the nutritional repletion was completed. Their studies during weight reduction have shown that the loss of zinc during weight reduction induced by a low caloric intake is as great as during total starvation.

Henkin et al. (1975) have investigated a syndrome of acute zinc loss that occurs when the amino acid histidine is administered to patients with systemic sclerosis (scleroderma). In six patients anorexia, dysfunction in taste and smell, mental changes and cerebellar dysfunction developed on an acute basis that was associated with significant decreases in serum zinc concentration and increases in urinary excretion. Findings indicated that the administration of zinc, even in the face of continuation of the histidine, resulted in return of signs and symptoms to or toward normal within eight to twenty-four hours at the time that correction of the zinc concentration in the blood occurred. The explanation of the authors for these findings has been that the preferential binding of zinc to histidine alters the normal equilibrium and leads to the stripping of zinc from albumin resulting in the excretion of a significant amount of zinc in the urine since the small molecular weight histidine-zinc ligands can easily pass into urine. It is of interest that the administration of histidine leads to the lowering of zinc levels with development of anorexia and then loss of smell. This was reversible with administration of zonc. This paper contributes a significant amount of information to the experimental zinc deficiency literature but the authors did not mention much about the role of the underlying disease scleroderma in the etiology of the syndrome.

More recently, Prasad and coworkers (Prasad et al., 1978) have intensively studied four patients in which they induced a marginal zinc deficiency using dietary means. They followed two subjects receiving a semi-purified diet based upon texturized soy protein for a period of fifty-two weeks and another two subjects similarly fed a special diet for sixty-four weeks. Both groups received an oral zinc supplement at some point during the experiment. The first two subjects received 2.7 mg. of zinc daily and complained of mild roughening of the skin and lethargy while the second group received 3.5 mg. of zinc daily and had no symptoms or complaints. There was a decrease in body weight in all four subjects which was attributed to dietary zinc restriction. Urinary excretion of zinc decreased in three out of four subjects as a result of zinc restriction; however in one of the four subjects there was no decrease in urinary excretion of zinc since he was also receiving a diuretic, hydrochlorothiazide, for mild hypertension during the study. One unexpected finding in the multiple chemical studies carried out was that the plasma ammonia level appeared to increase as a result of the zinc deficiency. There was also some suggestion that the zinc deficient state led to hypercatabolism of fat in the four subjects. Prasad and associates also believe that measuring the concentration of zinc in a twenty-four hour urine may be of help in diagnosing zinc deficiency provided cirrhosis of the

liver, sickle cell disease and chronic renal disease are ruled out.

Clinical Manifestations of Zinc Deficiency in Children

The syndrome of adolescent nutritional dwarfism reported from the Middle East by Prasad et al. (1963) was the first documented clinical condition shown to be caused by zinc deficiency. Since that time deficiency of zinc, or in one incidence, congenital elevation of serum zinc has been demonstrated to be linked to twelve syndromes in the pediatric population.

These conditions will be reviewed, beginning with the syndrome first reported, on adolescent nutritional dwarfism. Halsted (1977) described the events leading to the study of the original patient later demonstrated to be suffering from zinc deficiency and gives credit to Prasad for suspecting it. This young man of twenty-three was dwarfed, anemic and had underdeveloped gonads. Prasad soon moved to Egypt where in 1960 in Cairo he headed a U.S. Navy research unit where he found many cases similar to the ones in Iran. He found that this clinical picture had been described by Lemann (1910) who felt that this was infantilism related to hookworm disease. A number of studies have been reported describing the syndrome (Prasad et al., 1963a, 1963b; Sandstead et al., 1965; Sandstead et al., 1967; Eminians et al., 1967; Ronaghy et al., 1968; Say et al.,

1969) but these were not completely accepted as demonstrating that zinc deficiency was the cause of the syndrome until zinc supplementation was shown to affect clinical improvement or cure (Halsted et al., 1972; Ronaghy et al., 1974). The unraveling of the puzzle was quite involved since many factors apparently enter into the clinical situation. Complicating factors include the presence of parasites (often multiple) in the patients, a frequent history of geophagia (often clay or dirt eating) and the possible influence of high phytate concentration in the unleavened bread in the Middle East causing poor absorption of dietary zinc. Clinically the patients resemble patients with idiopathic hypopituitarism and treatment with zinc is followed by increased growth and sexual maturation which exceeds the changes resulting from adequate diet or treatment of iron deficiency. Extensive studies have shown that children with these clinical presentations do not always respond to zinc. Carter and associates (Carter et al., 1969) gave iron sulphate, zinc sulphate or a placebo by use of the double blind technique and saw no differences in growth or sexual development between the three groups after one year observation. The zinc and iron levels in the plasma returned to normal, however one criticism of this study is that the subjects actually received only five and one-half months of therapy.

Coble et al. (1969) carried out extensive studies of endocrine function of boys in Egypt with retarded growth,

delayed sexual maturation and zinc deficiency and found that although the clinical appearance was similar there were two specific endocrine patterns that were involved. One group had relatively normal function and one group had poor growth hormone responses to hypoglycemia. Thus a simple relationship of zinc deficiency and hormonal abnormality was not apparent in all the children although they looked the same.

Zinc supplementation studies carried out by Ronaghy et al. (1974) demonstrated significantly increased height, weight and bone age in the children receiving supplemental zinc, and the total serum protein and albumin concentration increased although the zinc levels remained abnormally low throughout most of the study. The results demonstrated a clearly defined stimulation of growth when a supplement was added to the diet that contained protein, vitamins and zinc; although a tendency toward acceleration of sexual development was noted in the boys no statistically significant changes occurred during the twenty month treatment period.

Hambidge et al. (1972) reported their work on determination of the concentration of zinc in 338 normal adults and children whose ages ranged from birth to forty years. Ten children were found to have hair levels of zinc of less than 70 ppm. Seven of these children had a history of poor appetite and eight had weights at or below the tenth percentile; this was not explicable on a familial basis. Taste acuity was tested in six of these children

using four chemicals representing salt, sweet, bitter and sour and five had objective evidence of hypogeusia or diminished taste acuity. After supplementation of the diet with zinc the taste acuity was normalized in each of the children and the hair zinc levels increased.

The discovery of the relationship of zinc deficiency to the etiology of the clinical manifestations of a lethal inherited disorder called acrodermatitis enteropathica is one of the most significant observations in pediatric aspects of zinc metabolism. This disease is a rare inherited disorder that is transmitted as an autosomal recessive trait which appears at weaning or earlier in the baby who is not breast fed. This disease manifests as a severe skin rash, abnormal bowel functions, profound psychic changes and growth failure that responded moderately well to the administration of a drug called diodoquin (di-iodohydroxyquinoline). Studies show that this drug chelates an abnormal factor (oligopeptide), that results from lack of the enzyme oligopeptidase, which binds zinc. It has now been demonstrated that the administration of zinc alone will very adequately treat this otherwise fatal condition (Moynahan, 1974). Recently it has been found that women with acrodermatitis enteropathica who had survived without zinc therapy may deliver infants with a high rate of congenital malformations (Hambidge et al., 1975).

Currently the possibility of zinc deficiency in relation to many other illnesses of the pediatric patient is being investigated. Impairment in cellular immunity is a good example and clinical research has shown that zinc deficient children will respond to zinc therapy with improvement in immunocompetance. This indicates that zinc deficiency may be a factor in the increased susceptibility to infections in conditions such as protein-energy malnutrition (Walravens, 1979).

Sickle cell disease and the syndrome of adolescent nutritional dwarfism described from the Middle East have many clinical characteristics in common. This partially relates to poor growth and delayed sexual development and led Prasad et al. (1977) to study the effect of treatment with zinc on the development of the picture and it is important to note that the plasma, red blood cell and hair levels of zinc were significantly decreased in sickle cell disease patients as compared to controls. A limited and uncontrolled trial of zinc therapy revealed beneficial effects of zinc on growth and development of secondary sexual characteristics in teenagers and young adults.

Zinc has been described as being of importance in the clinical picture of intestinal malabsorption (Sandstead, 1973) in the form of the adolescent dwarfism syndrome and similarly more recently with cystic fibrosis (Dodge & Yassa, 1978). In addition zinc may have a role in treatment of

rheumatoid arthritis since it has been found that serum zinc levels are low in that condition (Simkin, 1976). Two controlled studies have also indicated that oral zinc sulphate is useful in treatment of acne, a common pediatric problem (Michaelsson et al., 1977; Hillstrom et al., 1977). Hereditary hyperzincemia was described in 1976 (Smith et al., 1976) in a twenty-eight year old black man, his siblings, his son, and in one of two nieces. Although the only manifestation of the syndrome noted so far is abnormally high blood levels of zinc it is currently being looked at for pathological significance.

Clinical Aspects of Zinc Deficiency in Adults

Zinc deficiency in adults is more generally distributed throughout the various organ systems and is less specific than in children where it usually is directly related in some way to decreased intake in the diet. It is important to note that an extensive study of the molecular basis of taste and its diseases has been carried out (Henkin et al., 1969) and these workers have reported a decrease in taste acuity that accompanies many disease processes. It may well be that the zinc deficiency seen in some adult conditions (e.g., lung cancer) is due to decreased dietary intake of the element because of some toxicity on the taste mechanism rather than a more direct effect by the disease process on the metabolism of zinc itself.

Zinc levels may be decreased in a variety of conditions that are either acute or chronic in nature and these disorders associated with zinc deficiency are listed by Cordas and Holland (1977) as follows:

Nutritional
Alcohol and Cirrhosis
Renal Disease with Proteinuria
Malabsorption Syndrome
Burns
Iatrogenic
    (a) Copper Administration
    (b) Penicillamine Therapy
    (c) EDTA or other Chelating Compound
    (d) Hemodialysis
    (e) Total Parenteral Hyperalimentation
    (f) Oral Contraception
Acrodermatitis Enteropathica
Sickle Cell Disease
Porphyria
Malignancies, Especially Lung and Leukemia
Chronic Leg Ulcer
Postoperative Situation
Schizophrenia (Some Instances)
Postmyocardial Infarction
Post Cardiac Surgery
During Infection
Down's Syndrome
Atherosclerosis--Moderate to Advanced

In addition to this long list we can add several topics as follows: Diminished sexual function in men on hemodialysis for renal failure. When zinc chloride was added to the dialysis solution there was a striking increase in the testosterone and follicle stimulating hormone levels in plasma to normal values (Antoniou et al., 1977). In addition in another study of male subjects with oligospermia (decrease in sperm) zinc administration was accompanied by increased levels of plasma testosterone and increased sperm count (Hartoma et al., 1977).

Diabetes mellitus has been shown by Pidduck and coworkers (Pidduck et al., 1970) to have some abnormality in zinc metabolism. Diabetics were shown to have a significant elevation of zinc excretion in urine but investigations of the leucocyte contents did not demonstrate any difference in leucocyte zinc levels between patients and controls (Pidduck et al., 1971).

Plasma zinc and copper in obese patients before and after intestinal bypass surgery were investigated by Atkinson and coworkers (Atkinson et al., 1978) who found that obese patients showed significantly lower zinc levels than lean control subjects. After the intestinal bypass surgery their plasma zinc and copper levels were significantly lower than pre-operatively. One of the patients developed leucopenia (fall in white blood count) associated with a very low copper level but responded well to oral copper sulfate treatment. They recommended that careful observation and replacement therapy be carried out in all patients after intestinal bypass surgery.

Extensive studies of zinc metabolism in alcoholic and postalcoholic cirrhosis have been carried out by Sullivan and Lankford (1962) and by Sullivan and Heaney (1970). These workers found that there is an increased urinary excretion of zinc in most patients with postalcoholic cirrhosis. Approximately 50 percent of chronic alcoholics without cirrhosis also show excess excretion of

zinc in the urine. Halsted and coworkers (Halsted et al., 1968) investigated plasma zinc levels in patients with various lever diseases and noted that in all types of liver disease the plasma zinc is consistently less than in controls. They were unable to differentiate between the different types of liver disease, i.e., infection or cirrhosis due to alcoholism based upon the zinc levels.

Relatively few studies of the zinc status of the elderly have been carried out. Gregor (1977) investigated the dietary intake of sixty-five institutionalized aged subjects and found that the dietary intake was generally adequate compared to the recommended daily allowances except for zinc and magnesium. She found that 5 percent of the subjects had hair zinc levels indicative of deficiency. The levels were significantly correlated to protein intake in men and were significantly lower in women taking medication for coronary heart disease. Approximately one-fifth of the subjects had decreased taste acuity but neither dietary zinc intake, hair zinc levels nor smoking correlated to taste acuity.

Weismann and associates (Weissman et al., 1978) investigated the geriatric population of an institution with 585 inhabitants and found that twenty-six people with a mean age of eighty-two years had skin lesions suggestive of zinc deficiency. Ten of the patients had a subnormal plasma zinc concentration. This group was treated for four

weeks with oral zinc with no improvement of the skin condition and it was concluded that the skin changes were not caused by zinc deficiency. It was noted that the plasma albumin was subnormal in all patients and lower than the levels in patients with normal zinc concentrations. They concluded that the plasma zinc concentration may be low in the elderly because of low plasma albumin. Plasma zinc concentrations did rise however after oral administration of the zinc.

A recent study by Vir and Love (1979) determined the concentration of zinc in plasma and hair of 146 subjects aged 65 to 95 years. They found that the plasma zinc was less than 50 µg./100 ml. in 2.1 percent of subjects and hair zinc levels were less than 100 µg. in 2.7 percent of subjects. They found no correlation between zinc levels in hair and plasma. The administration of multivitamin supplements appears to increase hair zinc levels but this was significant only for females.

Zinc Deficiency and Behavioral Impairment in Laboratory Animals

The importance of zinc as it relates to behavior has not been extensively studied. This observation is interesting in that many studies make brief reference to the marked lethargy, slowing of behavior, apathy and decreased sexual activity in both males and females as part of the overall scenario of zinc deficiency (Apgar,

1968a; Ronaghy et al., 1969; Caldwell et al., 1970; Witenack et al., 1970; Miller & Stake, 1974). Animal models have typically been employed when research in this area has been carried out primarily because of ethical considerations but also because certain questions are not readily answerable from studies involving known zinc deficient humans. The rat has been the most popular but not the exclusive animal for carrying out investigations of the influence of zinc deficiency on behavior.

Eichelman and coworkers (1973) studied aggression in animals resulting from prenatal zinc deficiency; pregnant Long-Evans rats were given either a zinc supplemented or zinc deficient diet starting on day 14 and continuing until day 20 of pregnancy. The pups of the low zinc dams were markedly smaller for the first forty days of life; however by day 75 there were no differences between zinc deficient and control groups. Females were tested for aggressive behavior at seventy-five days. The zinc deficient animals were paired with those that were zinc supplemented. All rats received two levels of shock for 0.5 seconds and spaced 5.0 seconds apart. Aggressive attacks were defined as directed movement toward the opponent resulting in contact that included at least one of the following responses: biting, sparring, upright attack posturing or supine submissive posturing adopted by the attacked rat. The zinc deficient rats displayed a significantly higher percent of

aggressive attacks. The level of shock (1.3 mA or 1.6 mA) made no difference in number of aggressive attacks in either group of animals.

Peters (1977) studied the long term effects on aggression and affiliation behavior of male rats that had experienced either prenatal zinc deficiency or adequate prenatal nutrition. The animals were placed in a T-maze and their interactions observed. Zinc deficient animals were more aggressive than the well nourished animals and prenatally well nourished animals preferred to affiliate with similar animals.

Caldwell, Oberleas, Clancy and Prasad (1970) investigated the effects of severe, chronic zinc deficiency on postweaning age rats. Learning was measured by performance in a Lashley III water maze. Latency scores to traverse the maze, number of cul-de-sac entries, and number of alley re-traces were recorded. Emotionality was assessed by counting the number of squares traversed in a square, open-field.

Zinc deficient animals had significantly greater latencies in coursing the water maze and made a greater number of combined cul-de-sac and re-trace errors between the first and final maze trials compared to the zinc supplemented controls. Zinc supplemented animals were observed to be less emotional (i.e., had greater activity scores in the open field) than the zinc deficient animals. These

same workers next investigated the effects of chronic, mild zinc deficiency on gravid rats and in the behavior of their offspring (Caldwell, 1976). There was a high incidence of mortality in progeny of zinc deficient dams thus greatly reducing the number available for behavior testing. The results from those tested revealed similar behavioral impairment, although not of the magnitude reported in the first study. The evidence from these two studies suggests behavioral deficits occur whether the zinc deficiency occurs prenatally or postnatally. The following criticisms can be made of these studies: (1) plasma zinc concentrations of the animals were not determined in these studies thus making it necessary to define zinc deficiency based on intake alone; (2) the only control group employed was pair-fed zinc supplemented diet in the amounts consumed by the zinc deficient animals. Zinc deficient diets are known to cause anorexia thus decreasing intake in both groups. A second ad libitum control group would improve the design of this study since this would allow the zinc deficient group to be compared to an optimally nourished group. (3) it is impossible to state when testing physically debilitated animals whether the zinc deficiency actually resulted in decreased ability to learn or simply reflected animals less motivated to perform.

Lokken and coworkers (1973) sought to address this third issue by renourishing rats that had been deprived of

zinc from birth through twenty-one days of age and then adequately fed for twenty-three days prior to testing. They found that even after improving the zinc status of these animals acquisition of an elevated Tolman-Honzik maze was impaired compared to control animals, suggesting that zinc deficiency occurring during the critical period of brain development results in permanent learning deficits.

Halas and Sandstead (1975) employed an additional control group thus dealing with the second issue mentioned previously. Their study included a zinc deficient group, a zinc supplemented pair-fed group and a zinc supplemented ad libitum group. The zinc deficiency occurred prenatally and male animals were tested using a shock avoidance task. Performance was best for the ad libitum zinc supplemented group, somewhat less good for the pair-fed control and poorest for the zinc deficient group.

The effects of zinc deficiency on the brains and behavior of rats and Rhesus monkeys were recently reported by Sandstead and coworkers (1977). These investigators found that the brains of viable rat pups from zinc deficient mothers contained less DNA than those of pair-fed dams that were also given a controlled amount of zinc. Behavioral studies were carried out in deficient pups and it was found that active shock avoidance was impaired in the deficient males. Female pups did not display this impaired active shock avoidance. This study was unique in that sex

differences were demonstrated. Zinc deprivation in pregnant monkeys resulted in a rash and loss of hair and a decrease of plasma zinc to very low levels. Abortion was frequent and the pair-fed mothers tolerated the procedure poorly suggesting that the monkey is not a completely satisfactory animal for this type of study. Additional work with Rhesus monkeys by the same group (Sandstead et al., 1978) showed that the infants of the zinc deprived mothers played and explored less than the control infants and they associated with the mother a greater percentage of time and were less active.

Zinc Deficiency and Schizophrenia

There is a possible role of zinc in the etiology of psychiatric disease. The early work with administration of manganese in schizophrenia (English, 1929; Hoskins, 1934) is the basis for speculation regarding the potential use of zinc in treatment of psychiatric disorders. Blood histamine levels were noted by Pfeiffer and Cott (1974) to vary in schizophrenics and these authors postulated that abnormalities of trace metals might be related to these differences. Previous workers had found that brain autopsy specimens of schizophrenics contained approximately half of the amount of zinc as noted in brains of patients dying of other causes (Kimura & Kumura, 1965). Pfeiffer and Cott (1974) demonstrated that approximately 11 percent of schizophrenics studied had a low serum zinc level and that there were, in

addition, interrelationships between copper, manganese and zinc. Additional evidence suggesting that zinc deficiency might be related to schizophrenia has been provided in the use of penicillamine in the treatment of this psychiatric illness (Pfeiffer & Iliev, 1972). The reported improvement of these patients may be explained by the previously discussed relationships of zinc and copper. Presumably the penicillamine bound the copper and increased its excretion thus causing an elevation of zinc in body tissues possibly also including the brain.

Rationale

The importance of the role of zinc for living organisms is evident from the review of the research literature. Evident, also, is the paucity of research on the behavioral correlates of zinc deficiency in humans. The frequent mention of lethargy, apathy and decreased sexual activity appears in reports from investigations where the behavioral aspects of the syndrome were not of primary interest. Apgar (1973) investigated the effects of zinc repletion on parturition and included a brief statement about behavior. Henkin and coworkers (1975) reported mental changes as part of the overall scenario of acute zinc loss upon administration of the amino acid histidine, used in experimental treatment of scleroderma. Diminished sexual function was noted in men on hemodialysis for renal

failure (Antoniou et al., 1977) and this symptom was reversed when zinc chloride was added to the dialysis solution.

Pronounced depression was described as one of the characteristics found following the widespread use of total intravenous nutrition (Hambidge, 1972). Zinc supplementation resulted in rapid reversal of symptoms. Marginal zinc deficiency was induced experimentally in humans by Prasad and coworkers (1978) for the purpose of evaluating dietary requirements. Two levels of zinc were utilized; the two subjects on the lower level reported, among their many complaints, feeling lethargic.

Human zinc deficiency was first described in the Middle East (Prasad et al., 1963) but recent evidence indicates that zinc deficiency may be common in the United States and not limited to any specific age group.

No fewer than eleven syndromes related to zinc metabolism have been demonstrated in the pediatric population and most of these are related to dietary intake. Poor growth related to zinc has been described in infants and older children and low zinc levels are common in emotionally disturbed children (Cordas & Holland, 1977). A few studies have evaluated the zinc status of youngsters from low income families and found them to be more frequently affected (Hambidge et al., 1976).

Zinc deficiency has been reported in adults where the condition is often unrelated to intake but rather

occurs through various disease states including cirrhosis from alcoholism, atherosclerosis and malignancies, including lung cancer and leukemia. Decreased zinc levels have been noted in females taking contraceptive drugs, known to be in wide use (Cordas & Holland, 1977). Obese adults were found to have significantly lower zinc levels than lean controls and the levels were even lower following intestinal bypass surgery.

Low plasma zinc concentrations in aging individuals have been reported by Weismann and associates (1978) who found that levels rose with the oral administration of zinc. Vir and Love (1979) found low zinc levels in 2.7 percent of subjects aged sixty-five to ninety-five years, while Gregor (1977) found 5 percent of institutionalized aged subjects to be zinc deficient.

This brief summary demonstrates that although poorly defined there are behavioral consequences related to zinc deficiency and that this syndrome is more common than previously believed, affecting people of all ages.

Only a few studies to date have specifically investigated the behavioral aspects of zinc deficiency (Caldwell et al., 1970; Lokken et al., 1973; Halas & Sandstead, 1975; Sandstead et al., 1977). Animal models have typically been employed primarily because of ethical considerations; but also confounding present in human studies particularly in the elderly makes it difficult to attribute effects to the

treatment under investigation. The experimental designs of all of these studies involved at least one of these criticisms: (1) Plasma zinc levels were not always determined and zinc deficiency was inferred from low intake; (2) Typically a well-nourished control group was not used. This is extremely important since the zinc deficiency is known to cause a dramatic decrease in intake and the pair-fed control is thus possibly debilitated by low intake. Adding the free-fed control allows a comparison of the pair-fed groups to a normally nourished group; (3) All studies have failed to evaluate the open-field response other than number of squares entered; (4) Studies have assessed blatantly sick animals on learning tasks, rendering the results impossible to interpret since any difference could easily be attributed to physical disability rather than reduced learning capacity; (5) Most studies have assessed behavioral effects of prenatal and postnatal zinc deficiency and in fact no animals over three months old have been evaluated.

In the present studies:

(1) Young and aging rats were used. The strain, sex, laboratory conditions, diet composition and experimenter were the same to allow for comparisons including body weight and food intake changes over time.

(2) A free-fed control group was used in addition to the zinc deficient and zinc supplemented pair-fed groups.

(3) Body weight and intake data were collected throughout the studies so that the effects of feeding low zinc diets could be assessed.

(4) Plasma zinc concentrations were determined so that zinc status was not inferred from low-zinc intake.

(5) A learning task was used to evaluate learning in the older animals since these animals did not appear sick; however, learning was not assessed in the second study since the young zinc deficient animals did appear sick.

(6) Assessment of behavior in an open-field was used for both age groups. Latency, grooming, freezing, number of sectors entered, rearing and defecation responses were measured in the present studies so that comparisons on many specific behaviors could be made between zinc deficient and zinc supplemented animals.

STUDY 1

MATERIALS AND METHODS

Animals and Diets

The subjects were 150 day old male rats obtained from the Holtzman Company, Madison, Wisconsin, and housed in the animal quarters of the Psychology Research Building for the purpose of aging. Animals were maintained on a standard laboratory diet regimen until they were 300 days old. They were, then, randomly assigned to one of three treatment groups as follows: Group ZDA rats were fed, ad libitum, a diet deficient in zinc. Group ZSP (control) rats were pair-fed a diet supplemented with zinc so that the daily intake for each animal was equal to the mean number of grams consumed by rats in Group ZDA in the previous 24-hour period; Group ZSA (control) rats were fed, ad libitum, a diet supplemented with zinc.

The diets were commercially prepared by Teklad Test Diets, Madison, Wisconsin (Table 1). Diets were isocaloric, differing only in that the supplemented diet contained zinc carbonate ($ZnCO_3$) at a level of 0.089 g./kg. of diet whereas the zinc-deficient diet contained less than one ppm zinc and 636.49 g./kg. of dextrose.

Table 1

Zinc Deficient Test Diet*

Ingredient	g/Kg
Egg White Solids, Spray Dried	200.000
Dextrose	636.498
Corn Oil	100.000
Non-Nutritive Fiber (Cellulose)	30.000
Sodium Chloride	5.551
Potassium Phosphate	10.687
Calcium Phosphate	2.489
Magnesium Sulfate	1.651
Calcium Carbonate	9.944
Ferric Citrate	0.911
Potassium Iodide	0.026
Manganese Sulfate	0.008
Cupric Sulfate	0.009
Cobalt Chloride	0.001
Biotin	0.004
Vitamin B_{12}	0.020
Calcium Pantothenate	0.016
Choline Chloride	1.500
Folic Acid	0.001
Niacin	0.025
Pyridoxine HCl	0.004
Riboflavin	0.006
Thiamin HCl	0.010
Vitamin A Palmitate	0.020
Vitamin D_2	0.002
Vitamin E Acetate	0.220
Menadione	0.001
Chlortetracycline HCl	0.390

*In the Control Diet, Zinc Carbonate ($ZnCO_3$) is added at a level of 0.089 g/Kg of diet and the Dextrose is reduced to 636.409 g/Kg of diet (adds 50 ppm zinc).

The living environment of the animals was made as metal free as possible prior to the start of the study to control for contamination. All rats were transferred to stainless steel cages held on stainless steel racks. They were given free access to deionized water contained in glass bottles with vinyl tops and stainless steel water spouts. The diets were contained in glass food cups. All water bottles and food cups were soaked in 4N hydrochloric acid for 24 hours and rinsed with deionized water.

The laboratory was kept on a 12-hour photoperiod daily. Each animal was weighed and the weight recorded weekly and food intake weighed and recorded daily. Notes were kept regarding changes in physical appearance.

Apparatus

Testing equipment included a T-maze with black or white removeable interiors and a circular open-field similar to the one described by Price and Huck (1976), six feet in diameter, enclosed by a sixteen inch high steel wall. The floor had concentric circles equidistant apart and a circle with a diameter of fifteen inches was located in the center of the circle. A total of 47 sectors each had approximately the same dimensions. An incandescent light was used during field testing.

Procedure

Determination of Zinc Status

All animals had been under the dietary conditions for fifteen weeks prior to the determination of zinc status. Each rat was etherized and a 2 ml. blood sample obtained using the orbital sinus bleeding technique. The plasma zinc content was determined as described by Prasad et al. (1967) using atomic absorption spectrophotometry. This was a double-blind procedure in that the samples were coded and recoded prior to being sent to the animal nutrition laboratory so that there was no way for the testers to know which samples were from which animals.

Successive Black-White Discrimination Learning Task

Animals were approximately 415 days old when tested. The task was to learn to turn consistently to the right (left) alley when the interior of the maze was white and to the left (right) when the interior was black. The procedure was similar to that described by Davis (1957). Rats were habituated to the T-maze following 22 hours of water deprivation (drinking was permitted between the hours of 4:00 PM and 6:00 PM each day). Each rat was placed in the start box and allowed to explore freely for a period of five minutes on each of four successive days. The interior was white on days one and three and black on days two and four. On day five each animal was placed in one of the two goal

boxes where a deionized water reinforcer was available. This procedure was continued until all animals were drinking within one minute of being placed in the goal box. The animals were then placed in the start box and allowed free access to all parts of the maze. Water reinforcement was available in each goal box. This phase of the habituation procedure was continued until animals were drinking water within one minute of being placed in the start box. Habituation was completed on day 16. Animals were randomly assigned to be reinforced to either left-black, right-white or left-white, right-black to control for possible direction preference. Each rat received twelve trials per day for a total of 156 trials. The first eighteen trials were forced correct in that the incorrect alley was not accessible. A variable intertrial interval (ITI) was employed. The acquisition criterion was ten correct responses in twelve trials. The rate of habituation and number of errors were recorded.

Activity and Exploration

Animals were 435 days old at the time of testing. Each animal was placed in the center circle of the field and observed for five minutes. Six responses were measured and recorded as follows: (1) Latency to leave the center circle (seconds), (2) Grooming (seconds), (3) Freezing (seconds), (4) Number of sectors entered, (5) Number of rearings and (6) Defecation (number of boluses). Following

this test animals were saved to be used in an immunology study.

Statistical Analysis

A trend analysis was performed on the intake and weight data followed by univariate linear F tests. Means and standard deviations were determined for the weight intake data.

A multivariate analysis of variance was used to analyze the plasma zinc and open-field data followed by univariate linear F tests.

A minimum .05 level of significance was specified prior to the start of the study and nondirectional tabled values were used.

RESULTS

Food Intake

A multivariate test of significance revealed an overall significant treatment effect ($F_{2,17} = 78.26$; $p \leq .00001$) (Figure 1). The amount of variance accounted for by the treatment was approximately 90 percent.

The linear univariate F for all groups was significant ($F_{1,18} = 76.99$; $p \leq .00001$). The ZDA and ZSP groups had a significantly lower intake than the ZSA group.

Significant differences in food intake between groups were apparent following two weeks of feeding, and continued throughout the fifteen week study ($F_{1,18} = 8.78$; $p \leq .00832$).

Means and standard deviations for intake are reported in Appendix A.

Body Weight

A multivariate test of significance revealed an overall significant treatment effect ($F_{4,52} = 8.89$; $p \leq .00002$) (Figure 2). The amount of variance accounted for by the treatment was approximately 65 percent.

The linear univariate F for all groups was significant ($F_{2,27} = 15.36$; $p \leq .00004$).

Fig. 1.--Food intake of rats fed a zinc-deficient or zinc-supplemented diet for twenty weeks.

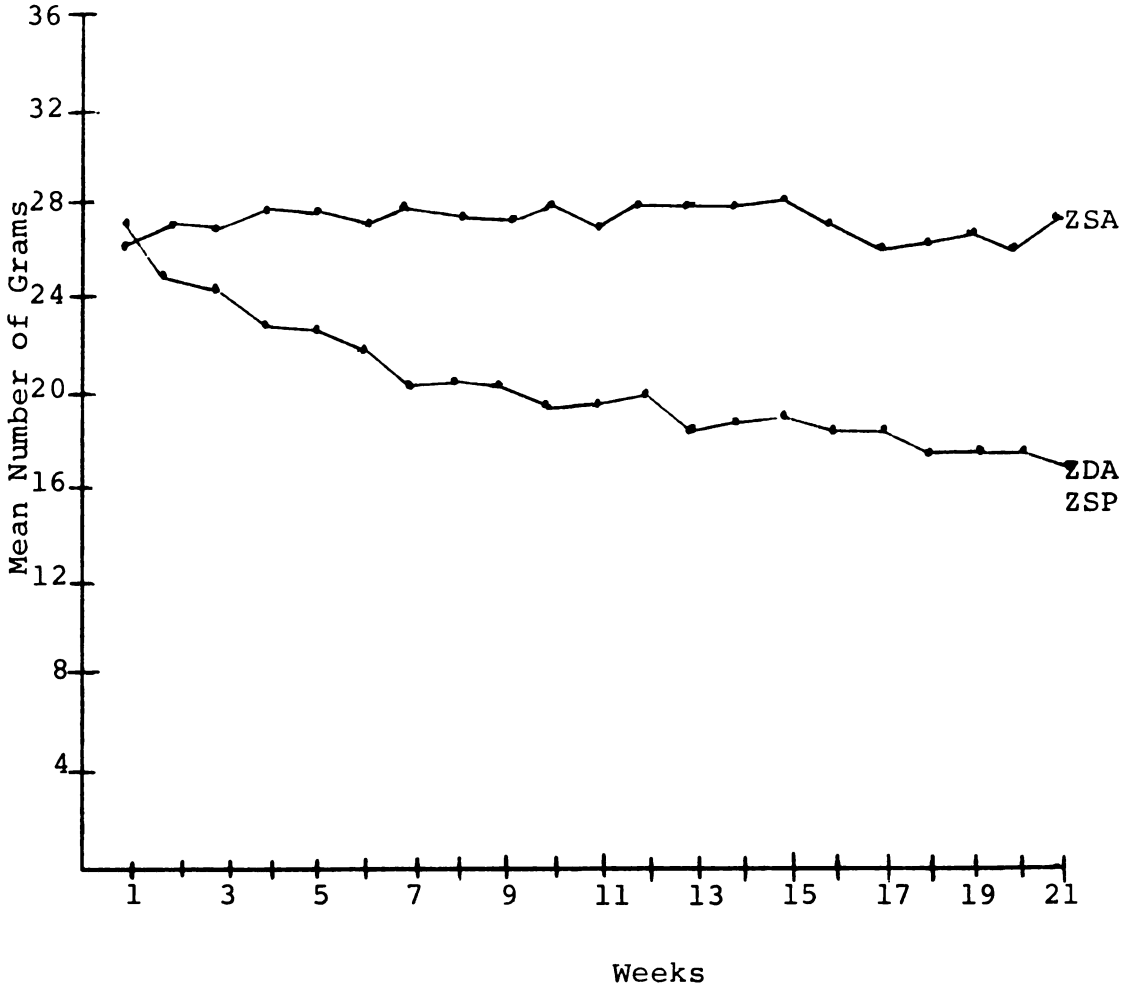
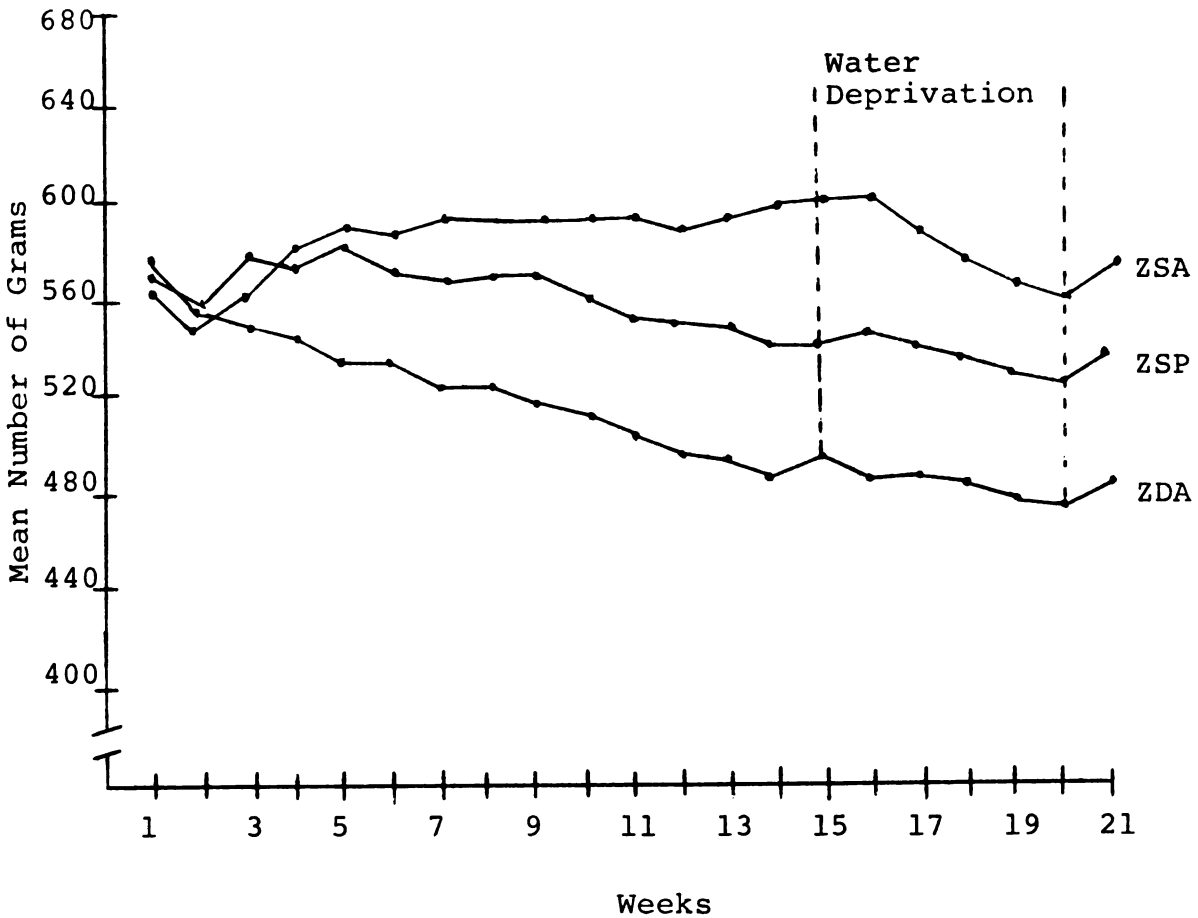

36
32
28
24
20
16
12
8
4
Mean Number of Grams
1
3
5
7
9
11
13
15
17
19
21
Weeks
ZSA
ZDA
ZSP

Fig. 2.--Body weight of rats fed a zinc-deficient or zinc-supplemented diet for twenty weeks.


680
640
600
560
520
480
440
400
Mean Number of Grams
Water
Deprivation
ZSA
ZSP
ZDA
1
3
5
7
9
11
13
15
17
19
21
Weeks

The ZDA group weighed significantly less than the ZSP group ($T_{18} = 4.35$; $p \leq .002$) and the ZSA group ($T_{18} = 5.21$; $p \leq .002$).

Significant differences in body weights were apparent following five weeks of feeding, at which time the ZDA group weighed significantly less than the ZSP group ($t_{18} = 2.75$; $p \leq .05$) and the ZSA group ($t_{18} = 7.19$; $p \leq .002$). Weights between groups ZSP and ZSA were not significantly different; however following seven weeks of feeding the ZSP group weighed significantly less than the ZSA group ($t_{18} = 2.94$; $p \leq .01$).

Means and standard deviations for body weight are reported in Appendix A.

Plasma Zinc Concentrations and Field Data

A multivariate test of significance was performed on the zinc and field data.

The overall F was significant ($F_{14,30} = 6.56$; $p \leq .00002$). The amount of variance accounted for by the treatment was approximately 94 percent.

Univariate F tests revealed the following: (1) Significant differences for zinc levels ($F_{2,21} = 67.55$; $p \leq .00001$); (2) Significant differences for latency to leave the center circle of the field ($F_{2,21} = 18.56$; $p \leq .00002$); (3) Significant differences for time spent grooming ($F_{2,21} = 6.04$; $p \leq .00846$); (4) Nonsignificant differences for freezing behavior ($F_{2,21} = 2.31$; $p \leq .12416$); (5) Significant

differences for number of sectors entered ($F_{2,21} = 3.12$; $p \leq .06479$); (6) Nonsignificant differences for number of rearings ($F_{2,21} = 2.065$; $p \leq .15174$); (7) Nonsignificant differences for number of boluses ($F_{2,21} = .363$; $p \leq .70023$).

Plasma Zinc - The ZDA group had significantly lower plasma zinc levels than the ZSP group ($t_{18} = 18.567$; $p \leq .002$) and the ZSA group ($t_{18} = 16.026$; $p \leq .002$). Differences between the ZSP and ZSA groups were not significant.

Latency - The ZDA group took significantly longer to leave the inner circle of the field than the ZSP group ($t_{14} = 9.423$; $p \leq .002$). Differences between the ZSP and ZSA groups were not significant.

Grooming - The ZDA group spent significantly less time grooming than the ZSP ($t_{14} = 3.29$; $p \leq .01$) and ZSA groups ($t_{15} = 6.012$; $p \leq .002$). The ZSA group spent significantly more time grooming than the ZSP group ($t_{14} = 2.72$; $p \leq .02$).

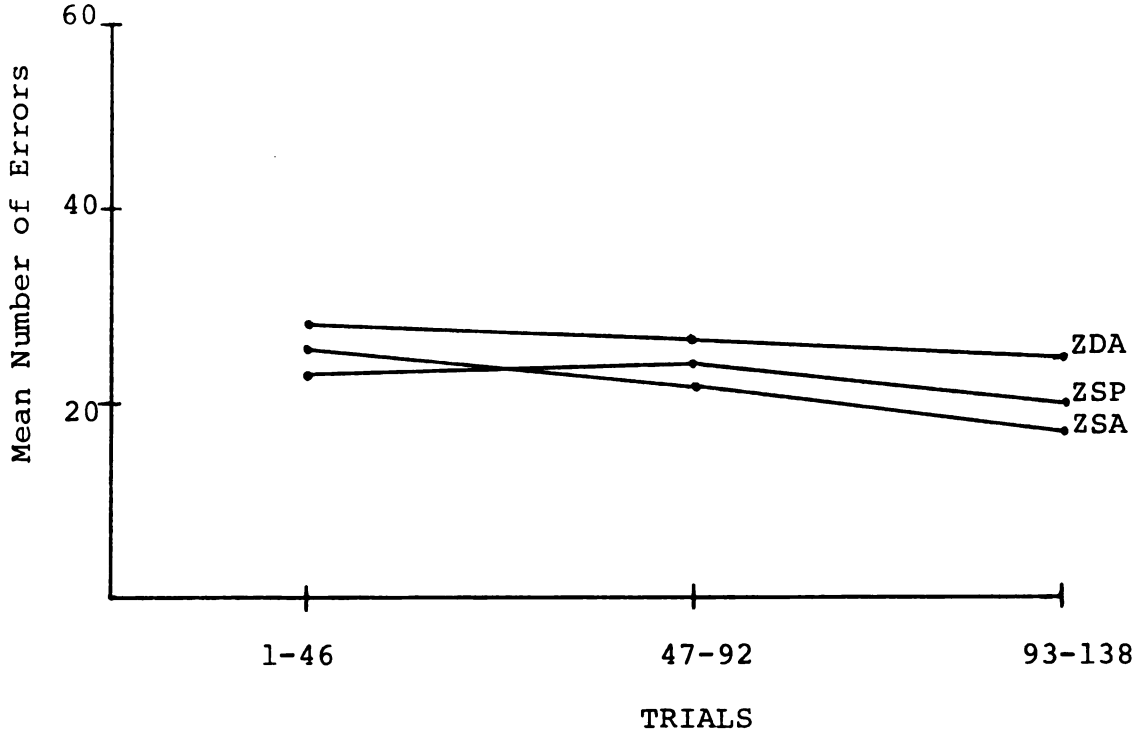
Sectors - The ZDA group entered significantly fewer sectors than the ZSP group ($t_{14} = 4.33$; $p \leq .002$) or the ZSA group ($t_{14} = 2.15$; $p \leq .05$).

Differences between the ZSP and ZSA groups were not significant.

Successive Black-White Discrimination Learning Time

The ZDA group made slightly more errors than the ZSP or ZSA groups, however differences were not significant (Figure 3).

Fig. 3.--Mean number of errors per forty-six trial blocks on a successive black-white discrimination learning task.


60
40
20
Mean Number of Errors
ZDA
ZSP
ZSA
1-46
47-92
93-138
TRIALS

DISCUSSION

The purpose of Study 1 was to induce zinc deficiency in aging rats and to evaluate the consequences on several physiological and behavioral parameters. No studies have reported behavioral data from aging zinc deficient laboratory animals.

Complete intake and body weight data were collected for each subject for twenty-one weeks: The intake of the animals free-fed a zinc deficient diet became significantly lower following two weeks of feeding. These data are in agreement with those of other investigators who have reported anorexia occurring as one of the symptoms of zinc deficiency (Day & McCollum, 1940; Swenerton & Hurley, 1968; Chesters & Quarterman, 1970); however the amount of feeding time prior to this effect was greater than that in other studies. This difference can probably be attributed to the fact that animals from the present study were older and zinc depletion would be expected to be slower in aging organisms because of increased bone concentration of zinc over time. Body weights of the zinc deficient animals were reduced significantly by the fifth week of treatment compared to both control groups. Retarded growth has been a

typical finding in studies with younger organisms (Hurley, 1974).

A study on experimental zinc deficiency in 55 to 65 year old humans resulted in weight loss (Prasad et al., 1978) in the subjects.

The differences found between the zinc deficient animals and the pair-fed controls is of interest in that intake differed only in amount of zinc and not calories or other nutrients. This strongly suggests that zinc plays a key role in metabolic processes. Plasma zinc concentrations were significantly less for the animals on the low zinc diet. Low hair zinc levels have been previously reported in aging humans (Gregor, 1978); however they were not correlated with dietary zinc intake. This could possibly be attributed to cardiovascular drug therapy since the medications may interact with zinc metabolism. Prasad's group (1978) also got reduced levels for the four subjects in their study; however intake changes were not reported. Results from Gregor's work demonstrate that low zinc levels can occur in the absence of reduced zinc intake while the data from the present study clearly show that reduction in zinc intake also results in low zinc levels in aging animals.

The zinc deficient animals made slightly fewer errors in the learning task, however these differences resulted from this group having a greater number of trials where no direction choice was made. None of the animals

approached the learning criterion. At least two explanations are possible. A deionized water reinforcer was used for correct responses. Zinc deficient animals are known to have decreased taste and olfactory acuity (Henkin et al., 1975); therefore to insure that the reinforcement had equal incentive value for all subjects, tasteless and odorless water was used. Deionized water may not be reinforcing to animals in any experimental condition. The animals were on a strenuous water deprivation schedule, drinking only two hours each day following testing, and thirst was evident as demonstrated by immediate drinking by rats in all groups upon being returned to the home cage. A second interpretation is that successive discrimination is too difficult a task for aging rats. Bitterman and Wodinsky (1953) compared single simultaneous, multiple simultaneous, and successive discrimination learning tasks and found the first to be least complex, the second to be most complex and the third at a level of complexity between the two.

When simple simultaneous discrimination tasks were used to compare young and old rats no age related differences were found; however, when multiple simultaneous learning tasks were presented the young rats made significantly fewer errors than the old ones (Field, 1953). Successive discrimination has not previously been tested in aging rats and is therefore difficult to state if the level of difficulty was too great for the animals in the study.

Behavioral responses in an open-field revealed a longer latency for the zinc deficient subjects prior to leaving the center circle, significantly less grooming and significantly fewer sectors entered. The highly significant longer latency suggests an initial tendency not to readily explore novel situations. The shorter grooming time and fewer number of sectors entered could be interpreted as decreased activity level in the zinc deficient animals. Caldwell and coworkers (1970) attributed the fewer number of squares entered by the zinc deficient group to increased emotionality. Use of the open-field to assess emotionality has been reviewed and criticized by Archer (1973). He reported that several types of evidence failed to support the validity of this measure for the assessment of emotionality and suggested that a variety of field behaviors be measured and interpreted without making the assumption that they represent a unitary major motivational construct. The present study attempted to use the field data responses in this way.

The results from the present study confirm the possibility of inducing zinc deficiency in aging lab animals, so that behavioral consequences can be evaluated. Both weights and intakes were lower and zinc deficiency was defined by lower plasma zinc levels in the rats fed a low zinc diet. Subsequent testing in an open-field demonstrated behavioral deficits on several parameters. Further

assessment using a less complex learning task with appropriate reinforcement would be of value in future studies as would rehabilitation and retesting for possible reversal of behavioral effects in the open field.

STUDY 2

MATERIALS AND METHODS

This section is the same as in Study 1 with the following exceptions.

Animals and Diets

The subjects were 28 day-old male weanlings obtained from the Holtzman Company, Madison, Wisconsin and housed in the animal quarters of the Psychology Research Building. Animals were maintained on a standard laboratory diet regimen for one week, and then randomly assigned to one of three treatment groups described in Study 1.

Apparatus

The T-maze was not used in Study 2.

Procedure

Determination of Zinc Status

All animals had been under the dietary conditions for seven weeks prior to the determination of zinc status.

Successive Black-White Discrimination Learning Task

This learning task was omitted in Study 2.

Activity and Exploration

Two judges were trained to measure the field behavior and the data collected by Judge 1 and Judge 2 were compared.

RESULTS

Food Intake

A multivariate test of significance revealed an overall significant treatment effect ($F_{2,17} = 65.39$; $p \leq .00001$) (Figure 4). The amount of variance accounted for by the treatment was approximately 88 percent.

The linear univariate F for all groups was significant ($F_{1,18} = 133.59$; $p \leq .00001$). The ZDA and ZSD groups had a significantly lower intake than the ZSA group.

Significant differences in food intake were apparent following eight days of feeding and continued throughout the seven week study ($F_{1,18} = 88.00$; $p \leq .00001$).

Means and standard deviations for intake are reported in Appendix B.

Body Weight

A multivariate test of significance revealed an overall significant treatment effect ($F_{4,52} = 138.95$; $p \leq .00001$) (Figure 5). The amount of variance accounted for by the treatment group was approximately 99 percent.

The linear univariate F for all groups was significant ($F_{2,27} = 1456.05$; $p \leq .00001$).

Fig. 4.--Food intake of rats fed a zinc-deficient or zinc-supplemented diet for seven weeks.

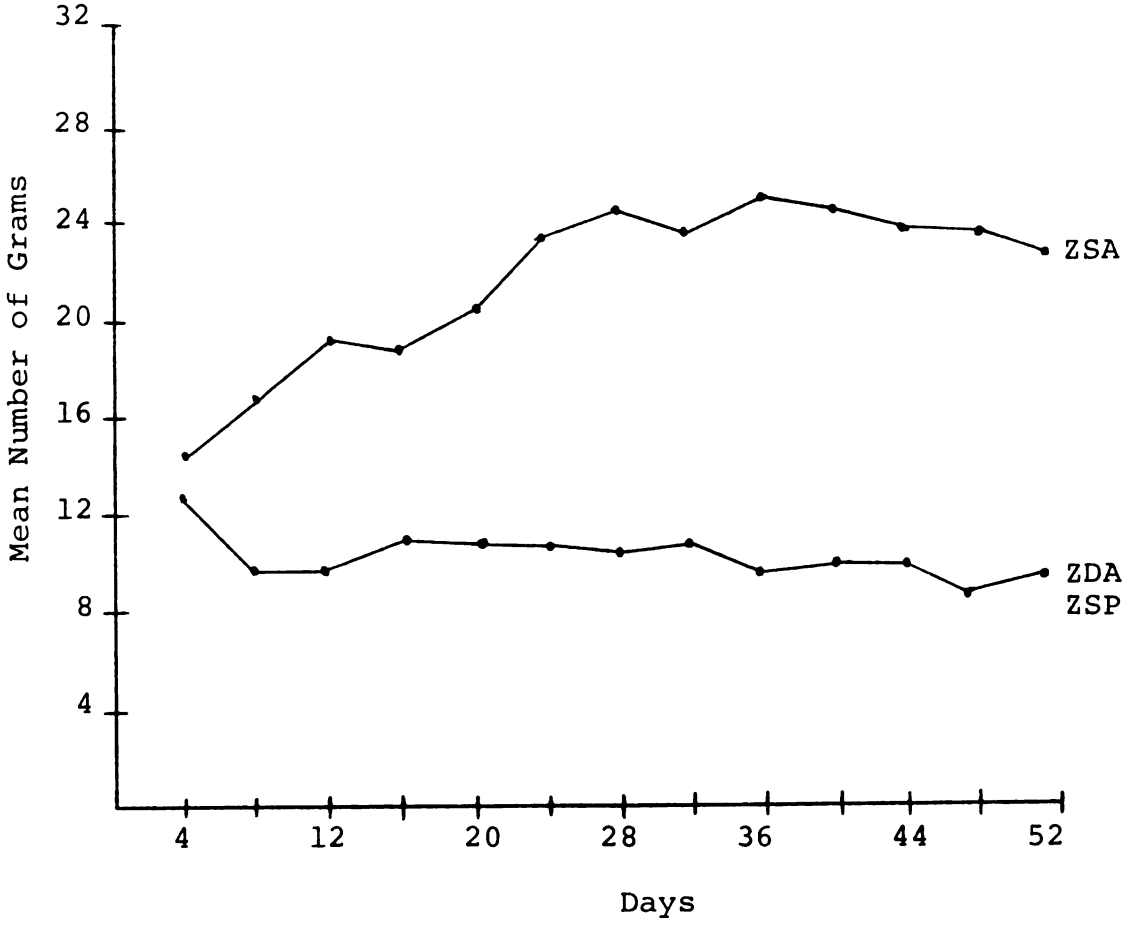
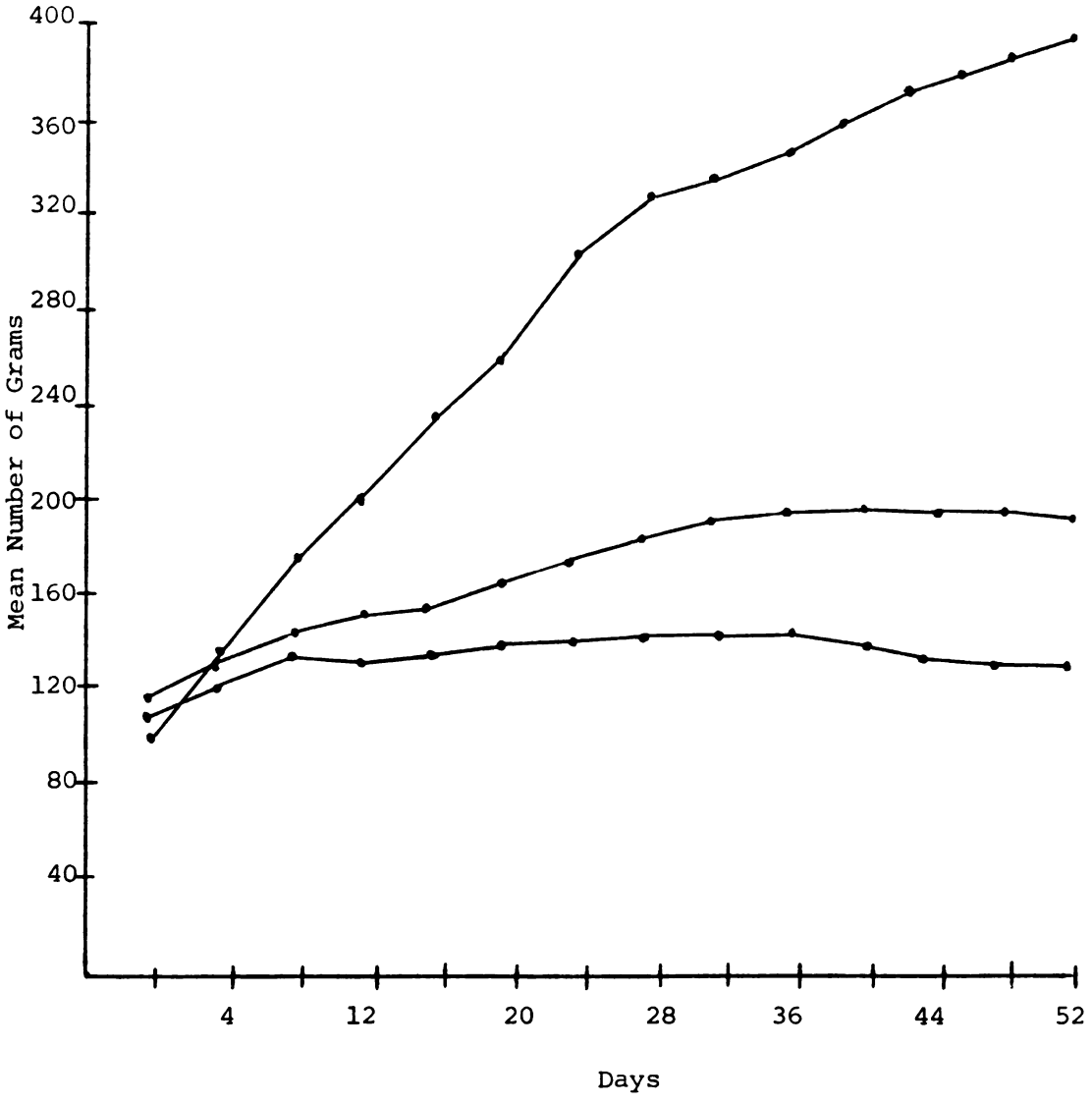

32
28
24
20
16
12
8
4
Mean Number of Grams
4
12
20
28
36
44
52
Days
ZSA
ZDA
ZSP

Fig. 5.--Body weight of rats fed a zinc-deficient or zinc-supplemented diet for seven weeks.


400
360
320
280
240
200
160
120
80
40
Mean Number of Grams
4
12
20
28
36
44
52
Days

The ZDA group weighed significantly less than the ZSP group ($t_{19} = 20.99$; $p \leq .002$) and the ZSA group ($t_{18} = 89.37$; $p \leq .002$).

The ZSP group weighed significantly less than the ZSA group ($t_{18} = 68.38$; $p \leq .002$).

Significant differences in body weight were apparent following eight days of feeding, at which time the ZDA group weighed significantly less than the ZSP group ($t_{18} = 2.32$; $p \leq .05$) and the ZSA group ($t_{18} = 13.75$; $p \leq .002$). The ZSP group weighed significantly less than the ZSA group ($t_{18} = 11.43$; $p \leq .002$).

Means and standard deviations for body weight are reported in Appendix B.

Plasma Zinc Concentrations and Field Data

A multivariate test of significance was performed on the field data.

The overall F was significant ($F_{10,42} = 6.00$; $p \leq .00002$). The amount of variance accounted for by the treatment was approximately 84 percent.

Univariate F tests revealed the following: (1) Significant differences for latency to leave the center circle of the field ($F_{2,24} = 14.76$; $p \leq .00007$); (2) Nonsignificant differences for time spent grooming ($F_{2,24} = 2.31$; $p \leq .1211$); (3) Nonsignificant differences for freezing behavior (no F's reported since no animals displayed this response); (4) Significant differences for number of sectors entered

($F_{2,24}$ = 20.07; $p \leq .00001$); (5) Significant differences for number of rearings ($F_{2,24}$ = 11.01; $p \leq .0004$); and (6) Nonsignificant differences for number of boluses ($F_{2,24}$ = 2.29; $p \leq .12341$).

Latency - The ZDA group took significantly more time to leave the inner circle of the field than the ZSP group (t_{16} = 6.82; $p \leq .002$). Differences between the ZSP and ZSA groups were not significant (t_{16} = .2178).

Number of sectors entered - The ZDA group entered significantly fewer sectors than the ZSP group (t_{16} = 31.83; $p \leq .002$) or the ZSA group (t_{16} = 19.64; $p \leq .002$). The ZSP group entered significantly more sectors than the ZSA group (t_{18} = 12.19; $p \leq .002$).

Rearing - The ZDA group reared significantly fewer times than the ZSP group (t_{16} = 10.51; $p \leq .002$) or the ZSA group (t_{16} = 5.10; $p \leq .01$). The differences between the ZSP and ZSA groups were significant. The ZSP group reared significantly more than the ZSA group (t_{18} = 7.41; $p \leq .002$).

The ratings of the two judges for the open field test were approximately the same (see Table 2).

Physical Changes

Apparent physical changes for the ZDA animals included ruffled coats (following fifteen days of treatment), hair loss and bald patches (following twenty-eight days of treatment), inflamed paws in 50 percent of the ZDA animals (following thirty days of treatment), a irritated conjunctiva

Table 2

Open-Field Data (Study 2)

		Judge One						Judge Two					
		Time (Sec.)			Number			Time (Sec.)			Number		
		Circle	Groom.	Freez.	Sect.	Rear.	Bol.	Circle	Groom.	Freez.	Sect.	Rear.	Bol.
ZDA	1	4	12	0	68	10	1	4	14	0	69	10	1
	2	15	4	0	76	14	0	13	4	0	75	15	0
	3	12	15	0	78	4	0	11	16	0	78	4	0
	4	24	0	0	90	4	3	23	0	0	95	4	3
	5	12	0	0	81	7	4	12	0	0	83	7	4
	6	9	0	0	114	7	0	9	0	0	115	7	0
	7	8	0	0	98	4	0	7	0	0	98	4	0
	8	10	8	0	79	3	0	11	8	0	79	3	0
ZSP	1	6	0	0	199	14	0	6	0	0	198	14	0
	2	6	4	0	172	23	0	6	4	0	168	22	0
	3	7	0	0	178	29	0	7	0	0	181	30	0
	4	5	3	0	234	14	0	5	3	0	243	15	0
	5	1	2	0	140	9	0	1	2	0	142	9	0
	6	2	0	0	254	29	0	2	0	0	254	29	0
	7	3	0	0	160	28	0	3	0	0	160	29	0
	8	5	0	0	141	9	0	4	0	0	141	9	0
	9	1	0	0	182	16	0	1	0	0	180	15	0
	10	3	5	0	204	39	0	2	6	0	197	39	0

Table 2

Continued

		Judge One						Judge Two					
		Time (Sec.)			Number			Time (Sec.)			Number		
		Circle	Groom.	Freez.	Sect.	Rear.	Bol.	Circle	Groom.	Freez.	Sect.	Rear.	Bol.
ZSA	1	3	10	0	106	4	0	3	9	0	108	4	0
	2	4	4	0	134	14	0	4	4	0	132	14	0
	3	5	8	0	123	10	0	5	8	0	124	10	0
	4	7	4	0	139	8	1	7	4	0	146	8	1
	5	2	15	0	174	13	1	2	15	0	176	14	0
	6	3	2	0	211	13	0	3	2	0	210	13	0
	7	3	8	0	183	17	0	3	8	0	187	16	0
	8	2	00	0	109	5	0	3	0	0	109	5	0
	9	1	8	0	172	17	0	1	8	0	173	1	0
	10	4	0	0	118	10	2	4	0	0	120	10	2

in all ZDA animals (onset from fourteen to thirty-six days). All of the ZDA animals had urethritis by six weeks of treatment and skin lesions were apparent on various parts of the body. Thirty percent of this group died prior to the conclusion of this study.

DISCUSSION

The goal of Study 2 was to produce zinc deficiency in young rats and assess resulting physiological and behavioral deficits and to employ an improved design compared to those used in previous behavioral research on zinc deficiency (Caldwell et al., 1976; Halas & Sandstead, 1975). Significantly lower intakes and body weights for the zinc deficient animals were apparent following eight days of feeding a low zinc diet. Decreased weights and intakes have been previously reported by Chesters and Quarterman (1970) who found that incipient zinc deficiency in young rats produced cessation of growth with food intake falling to 70 percent of free-fed controls. The differences were greater in their investigation compared to the present study probably because their rats were put on zinc-deficient diets at an earlier age. The differences in weight between the pair-fed groups suggest metabolic deficiency in low zinc animals. Tucker and Salmon (1955) reported weight loss and anorexia in calves fed low-zinc purified diets and Hambidge and coworkers (1972) reported that zinc deficient children and adults had weight loss and poor appetites. Comparison across these studies using rats, calves and humans indicates

that, at least for body weight and intake, there are similar findings across species. Plasma levels of zinc in zinc deficient rats (if lower) are in agreement with findings of Sandstead and coworkers (1977) and Kelleher (1978).

Behavioral responses in an open-field revealed a significantly longer latency to leave the center circle of the field, significantly fewer number of sectors entered and fewer rearings for the zinc deficient animals compared to the two control groups. The longer latency suggests an initial tendency not to readily explore novel environments. The zinc deficient animals were clearly less active as indicated by entering a fewer number of sectors. The differences for sectors entered were significant for the ZSP and ZSA groups in that the ZSP group was more active than the ZSA group. One explanation is that the ZSP was the starved group and were extremely hyperactive to the point of nearly jumping from the experimenter's hand when being placed in the field. The significantly lower number of rearings may have been related to physical weakness. These animals appeared sick and displayed "Kangaroo" like posture described by Swenerton and Hurley (1968) who studied severe zinc deficiency in male and female rats.

The time spent grooming in the zinc deficient animals was not significantly different, however it should be noted that their grooming was characterized by licking the front paws which were inflamed. This symptom of zinc

deficiency was previously reported (Follis, 1966). The control animals engaged in a variety of grooming behaviors.

Learning was not assessed in these young zinc deficient animals. Previous studies have used various learning tasks (Caldwell et al., 1970; Caldwell et al., 1976; Halas & Sandstead, 1975); however results from such tests are difficult to interpret since young zinc deficient animals are quite ill.

Lokkens and coworkers (1977) renourished animals deprived of zinc from birth to twenty-one days of age and then evaluated learning ability. The rehabilitated animals showed impaired performance compared to controls on an elevated Tolman-Honzik maze. These animals were immediately put on low zinc diets following birth. The present study utilized low zinc diets starting at thirty-five days of age. Future work in this area might look at the effect of rehabilitation on rats that were zinc deprived somewhat later than in previous studies to determine if learning deficits are greater when animals are deprived during the early, most critical period of brain development. Also of interest would be the comparison of performance of zinc deficient animals on tests varying in complexity. It may be that, given a simple task, no deficits will be evident and that only as the tasks become more complex will zinc deficient animals display reduced learning capacity.

GENERAL DISCUSSION

Comparisons of results of both studies indicate that, although physiological and behavioral deficits were present for each age group the aging animals were less affected than the young ones and zinc deficiency was induced in the young ones in a much shorter period of time.

Significant differences between the ZDA and ZSA groups in food intake and body weight were apparent following two weeks and five weeks respectively for the aging animals, while the differences for both of these parameters were significant by eight days for the younger rats.

One important consideration in evaluating these age-related differences is that the young animals were put on the low zinc diets during a period of rapid growth rate, and daily intake was not at a maximum. These effects are easily demonstrated by comparing weight and intake trends of the young zinc deficient animals to their free-fed zinc supplemented controls.

Many physical changes were apparent for the young zinc deficient animals that were not evident in the aging group. Fifteen days into the treatment the coats of the ZDA rats were ruffled and hair loss including bald patches was evidenced by twenty-eight days of treatment. The paws

of 50 percent of the young zinc deficient animals were inflamed and the conjunctiva were irritated. All of the zinc deficient animals had urethritis by six weeks of feeding and skin lesions appeared on various parts of the body. Two animals from this group died prior to testing and three more died the week following testing so that the overall mortality was 50 percent. No animals in the two control groups were lost and all animals in the aging study survived.

The field data revealed longer latency for both age groups of zinc deficient rats compared to controls. Grooming differences were significant in the first but not the second study; however, the aging zinc deficient animals did not have inflamed, irritated paws and grooming in the zinc deficient young animals consisted entirely of licking the sore paws. Both zinc deficient age groups were less active in the field as measured by the number of sectors entered. Rearings were not significantly different for aging zinc deficient animals compared to their controls but were for the young zinc deficient animals, who displayed "kangaroo" like posture possibly indicating neurological damage.

A comparison of data from studies of young and aging rats indicates that zinc deficiency results in physiological and behavioral deficits for both ages, but zinc deficiency induced in early life has greater detrimental effects for all parameters measured and has a high morbidity

and mortality rate, compared to the zinc deficient aging animals. The physiological and behavioral differences were caused by the decreased zinc intake in the ZDA animals as indicated by the very high amount of variance accounted for in each study.

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APPENDICES

APPENDIX A

TEST DATA FROM STUDY 1 AND MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT AND INTAKE

APPENDIX A

TEST DATA FROM STUDY 1 AND MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT AND INTAKE

Study I--Intake Data

		1	2	3	4	5	6	7	8	9	10	11
ZDA	1	26.3	24	24.3	23.9	22.3	21.9	21.3	21.9	19	18.3	18.9
	2	30	28	26.3	20	21.3	20	19.9	21.3	20	19.3	19.9
	3	29	25	24.3	24.9	25	24	20.9	20.3	19	21.3	20.3
	4	30.3	24.3	25	23.9	22.3	22.9	23.3	21.3	20.9	21	19.9
	5	29	24.9	23.9	24.4	25.4	22.5	22.9	20.9	20.3	21.3	19.9
	6	27.9	23.3	21	19.9	19.4	21.5	19	18.8	19.3	18.9	19.9
	7	25	24	24.9	23.5	23	21.9	18	18.4	19	17.9	17.3
	8	25.9	26.4	27.3	26	25	22.9	21.3	22.3	23	20.3	19.9
	9	26	27.3	25.3	23.9	24	22.5	20	21	23.3	19.9	21.9
	10	27.3	24.3	20.9	21	21	20.3	19.3	18.9	18.5	17	18.3
ZSP	1	27.7	25	24.3	23	22.9	22	20.5	20.5	20.2	19.5	19.6
	2											
	3											
	4											
	5											
	6											
	7											
	8											
	9											
	10											

		1	2	3	4	5	6	7	8	9	10	11
ZSA	1	26.3	27	25	26.9	27.3	26.3	27	26	25.3	27	26
	2	26	25	26.3	28	26.3	24	29	27	28.9	28	29
	3	25.9	26.3	27.4	27	25.9	28.3	27	28	29.3	26.9	27.3
	4	28.3	28	27.9	29.3	27.3	29	29.9	27.9	28.3	29	28.9
	5	27	26	26.5	25.3	26	25.9	27.3	26.3	25.4	25	26.9
	6	25.3	26.9	25.9	24.9	26.3	25	26.4	26.3	25	25.4	25
	7	30	29.3	30	30.3	29.9	28.3	29.9	30.3	28	29.3	27.9
	8	24.3	24.9	25.3	26	25.9	26.3	27	27.9	26.3	26	26.4
	9	28.3	29	28	28.3	28.9	30.4	29	28.3	28.9	30	27.9
	10	30.3	30	29	31.3	31.9	30	29.3	27.9	29	30.3	29.4
		27	27.2	27.1	27.7	27.5	27.4	28	27.6	27.4	27.7	27.5

		12	13	14	15	16	17	18	19	20	21
ZDA	1	19.3	17	18.9	19	17	16.9	15.3	16	15	15.3
	2	18.9	18	18.3	17.9	18.4	17.3	17	17.4	16.9	17.3
	3	22.3	20.9	19	19.3	18.9	19	20.3	18.9	17.3	17
	4	19.3	18.5	18	19.3	18	17.9	19.3	17.5	17	18.3
	5	21.3	20.5	21	19.9	20.3	19.9	18.3	19	20	18.5
	6	18.5	17.9	18.3	19	17.5	17	17.3	16.9	15.8	14.3
	7	18.8	17.3	17.9	16.9	18.3	19	19.3	18.4	18	17.9
	8	21.4	20	20.3	21.3	19.9	19.3	19.9	20.3	20	20.3
	9	19.9	20.3	19	19.9	19.3	20.9	20	18.9	19	19.3
	10	19.3	17	17.9	18.3	18	17	17	17.9	18.3	17.9
ZSP	1	19.9	18.7	18.9	19	18.6	18.4	18.3	18	17.7	17.6
	2										
	3										
	4										
	5										
	6										
	7										
	8										
	9										
	10										

		12	13	14	15	16	17	18	19	20	21
ZSA	1	26.9	27	27	27.3	25.9	25	25.3	25.9	26	27
	2	29.3	30	30	28.9	27.3	26.9	26.3	27.9	27.9	28
	3	28	26	26.9	27.3	25.9	26.3	26.9	27.4	26	25.9
	4	27.3	25.9	26.3	26	24.9	25.3	24	26.3	25.3	27.8
	5	27.3	27	28.9	28.3	26.9	27.3	26	24.9	24.9	27.3
	6	26.9	27.3	26	27.3	28.3	24	25.3	24.9	23	26.5
	7	28.3	28	28	29.3	27.9	26.3	26.5	25.9	25	26.9
	8	26.9	25.3	26.5	27.3	25.9	25.9	26.3	26.9	25.3	28
	9	29	30.3	30.9	31.9	28	26.3	27	26.3	25	29.9
	10	30	30.3	29.9	31.3	30	27	27.3	28.9	26	28.9
		28	27.7	28	28.5	27	26	26.1	26.6	25.4	27.6

Study 1--Weight Data

		1	2	3	4	5	6	7	8	9	10	11
ZDA	1	586	554	565	558	559	560	558	555	535	523	519
	2	602	572	565	571	578	576	554	554	553	566	553
	3	579	529	467	440	433	470	465	483	509	507	504
	4	564	553	559	550	520	537	533	530	528	527	515
	5	579	568	582	570	530	547	539	539	537	530	530
	6	585	569	584	580	575	538	538	538	539	530	525
	7	562	563	562	573	553	559	547	546	540	536	530
	8	604	578	577	562	542	528	520	495	479	436	426
	9	575	560	551	552	520	535	517	517	515	515	512
	10	533	519	500	500	498	496	490	480	462	444	429
	$\bar{x}$	576.9	556.5	551.2	545.6	530.8	534.6	526.1	523.7	519.7	511.4	504.3
ZSP	1	602	587	618	618	618	622	620	618	618	620	619
	2	564	549	581	572	583	566	570	570	578	580	577
	3	551	550	578	573	570	576	560	570	574	560	550
	4	542	545	601	582	587	586	586	584	585	570	558
	5	579	562	600	610	610	612	596	604	600	589	588
	6	607	577	572	580	586	594	593	598	588	565	554
	7	549	544	553	549	550	549	553	555	557	550	540
	8	615	615	620	620	618	610	615	612	614	607	600
	9	538	531	529	535	532	500	485	486	490	495	484
	10	581	577	562	550	570	574	570	562	558	533	511
	$\bar{x}$	572.8	563.7	581.4	578.9	582.4	578.9	574.8	575.9	516.2	566.9	558.1

		1	2	3	4	5	6	7	8	9	10	11
ZSA	1	568	547	575	592	578	570	587	595	601	590	590
	2	609	581	606	609	615	615	616	614	615	626	624
	3	609	564	609	618	620	624	620	618	620	625	625
	4	554	532	547	566	573	582	588	590	596	592	600
	5	585	570	572	562	560	550	557	550	550	531	528
	6	563	525	549	555	562	554	580	578	576	574	574
	7	563	535	545	597	593	596	602	600	600	607	605
	8	579	547	556	602	618	616	620	620	618	625	625
	9	580	557	543	578	582	587	590	588	586	596	597
	10	565	550	552	591	612	624	625	625	620	625	624
	$\bar{x}$	577.5	550.8	565.4	587.0	591.3	591.8	598.5	597.8	598.2	599.1	599.2

		12	13	14	15	16	17	18	19	20	21
ZDA	1	516	521	530	520	514	511	505	500	498	504
	2	545	550	553	546	533	536	542	534	528	538
	3	502	500	496	504	500	501	494	490	484	492
	4	504	493	488	500	495	491	478	470	465	474
	5	525	500	485	473	470	472	473	465	460	470
	6	523	524	529	533	530	539	544	535	532	540
	7	523	518	510	501	488	490	487	480	478	486
	8	418	415	414	444	440	441	430	424	420	427
	9	510	502	499	498	490	479	461	454	448	460
	10	408	401	395	417	424	432	426	420	415	426
	$\bar{x}$	497.4	492.4	489.9	493.6	488.4	489.2	484.0	477.2	472.8	481.7
ZSP	1	620	618	600	600	598	600	595	590	583	492
	2	578	576	580	580	575	575	570	567	565	574
	3	543	549	545	550	555	557	555	550	545	555
	4	554	558	557	564	568	570	574	570	565	575
	5	590	584	577	578	580	585	582	578	574	584
	6	545	544	537	539	545	544	548	540	540	549
	7	535	529	530	529	528	517	508	500	495	504
	8	595	587	589	590	587	580	574	566	560	570
	9	480	482	470	465	463	440	424	418	409	420
	10	509	500	498	500	500	509	508	500	495	503
	$\bar{x}$	554.9	552.7	548.3	549.5	549.9	547.7	543.8	537.9	533.1	542.6

		12	13	14	15	16	17	18	19	20	21
ZSA	1	585	588	590	587	581	565	548	540	537	545
	2	625	620	626	630	630	620	615	605	600	608
	3	625	630	628	632	634	628	620	611	602	611
	4	580	592	604	605	607	600	594	587	580	588
	5	523	539	555	567	580	568	560	552	547	557
	6	568	567	562	566	571	562	553	548	541	551
	7	607	609	614	615	615	609	603	594	586	597
	8	626	634	630	629	632	623	615	604	596	607
	9	596	590	593	592	593	562	536	524	518	531
	10	628	632	630	630	632	615	599	588	579	589
	$\bar{x}$	596.3	600.1	603.2	605.3	607.5	595.2	584.3	575.3	568.6	578.4

Study 1--Plasma Zinc Concentrations

	ZDA	ZSP	ZSA
1	0.418	1.337	1.056
2	0.502	1.528	1.429
3	0.455	1.253	1.245
4	0.502	1.561	1.337
5	0.890	1.321	1.626
6	0.399	1.650	1.185
7	0.455	1.287	1.412
8	0.716	1.561	1.228
9	0.305	1.279	1.270
10	0.361	1.168	1.326
$\bar{x}$	.500	1.39	1.326

Study 1--Successive Discrimination Learning Data

		Blocks		
		1	2	3
		1-46	47-92	93-138
ZDA	1	26	21	17
	2	28	25	19
	3	21	24	18
	4	25	22	21
	5	23	27	19
	6	21	23	22
	7	26	20	16
	8	23	24	18
	9	25	23	25
	10	21	24	25
	$\bar{x}$	23.9	23.3	20
	Mean # errors	67.2/138		
ZSP	1	24	23	20
	2	25	26	21
	3	24	22	16
	4	27	19	16
	5	22	22	15
	6	29	21	21
	7	24	26	17
	8	26	22	23
	9	28	24	18
	10	28	19	16
	$\bar{x}$	25.7	22.4	18.3
	Mean # errors	66.4/138		

		Blocks		
		1	2	3
		1-46	47-92	93-138
ZSA	1	26	29	24
	2	28	25	25
	3	25	30	27
	4	30	27	22
	5	29	28	26
	6	29	27	26
	7	30	28	21
	8	27	28	28
	9	25	27	25
	10	30	25	28
	$\bar{x}$	27.9	27.4	25.2
	Mean # errors		80.5/138	

Study 1--Field Behavior Data

		Time (Sec)			Number		
		Circle	Groom.	Freez.	Sect.	Rear.	Bol.
ZDA	1	16	2	0	162	22	0
	2	18	0	70	24	0	0
	3	2	5	0	147	16	0
	4	8	0	0	163	12	1
	5	13	0	0	142	11	0
	6	19	0	84	33	3	0
	7	12	0	0	148	20	0
	8	11	0	0	153	14	6
ZSP	1	1	6	0	183	15	2
	2	4	5	0	208	24	0
	3	7	2	0	161	10	0
	4	3	13	0	152	19	0
	5	1	2	0	132	29	0
	6	5	12	0	166	18	0
	7	2	0	0	162	12	1
	8	2	13	0	193	20	0
ZSA	1	3	20	0	132	19	0
	2	1	12	0	136	23	0
	3	1	8	0	174	18	0
	4	2	5	0	180	22	0
	5	1	4	0	151	11	1
	6	2	12	0	119	14	2
	7	3	2	0	123	12	0
	8	7	28	0	154	15	0

APPENDIX B

TEST DATA FROM STUDY 2 AND MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT AND INTAKE

APPENDIX B

TEST DATA FROM STUDY 2 AND MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT AND INTAKE

Study 2--Intake Data

		4	8	12	16	20	24	28	32	36	40	44	48	52
ZDA	1	12	8.5	9.75	10	11	11.5	8.75	10.25	8.75	10	9.5	8.25	8
	2	18	12.25	10.0	13.25	9	11.25	11	14	9.75	9	9	8.5	9.5
	3	13.75	8.75	9.5	9.75	9.25	13	9.25	10.5	8.75	10.75	9	8.75	9
	4	12.25	8.75	8	12.5	11.5	13.5	16.0	10.5	9.5	10	10.75	9.0	8.75
	5	9	8.5	4.4	12.25	11.5	9.25	6.5	11.5	10.25	9	9.5	9.25	9.75
	6	10	9.5	7.75	7.5	9.5	10.5	7.5	9.75	8.5	8	9.25	9.25	9
	7	13.25	10.25	10.75	9	9	12.75	9.5	9.75	9	10.75	11.25	9.0	10
	8	11.75	7.75	12.75	12.5	14.25	13.75	15.25	12.5	11.5	11	9.5	9.5	9.25
	9	17	9.25	12.5	13.25	11.75	13.25	12	12	9.8	10.75	10.25	10.25	9.5
	10	10.75	8.75	8.5	8	8.75	11	8.25	8.25	7.5	9.25	6.75	6.75	8.5
ZSP	1	12.78	9.23	9.39	10.8	10.55	10.6	10.4	10.9	9.33	9.85	9.48	8.85	9.12
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	9													
	10													

		4	8	12	16	20	24	28	32	36	40	44	48	52
ZSA	1	14	16	19.75	20.5	20.5	24.75	23.25	23.25	27	24.25	22.25	21.78	23
	2	13.25	14.25	20.75	19	18.25	23.75	22	20.5	24	23.75	23.5	23.5	21
	3	16.5	17.75	20	16.5	16	23	23	22.25	24	22.25	23	23	22
	4	17.5	18.75	19.5	18.75	21	23.25	24	23.5	22.5	24.25	26.25	25.5	24
	5	14	13.25	15.25	16	19.75	23.5	23	21.75	24.5	24.5	23.75	23.75	25
	6	13.25	17.25	19.5	20	21	21.25	27.25	25	26.5	25.75	23.75	23.75	24.5
	7	14.25	16.5	19.5	20	21	22.5	24	24.25	23	22.25	23.25	23.5	21.5
	8	14.25	17.75	18.75	19	19.75	24	23.5	22.75	25.75	24.5	22.75	22.75	20
	9	18.75	19.75	20.25	20.25	22.5	24.75	26.5	23.25	24.75	27	23.5	23.5	21.5
	10	13	14.75	16.75	18.75	21	23.5	25.25	22.75	25.5	24.5	23	23	20
	$\bar{x}$	14.88	16.6	19	18.89	20.08	23.38	24.2	22.9	24.75	24.3	23.5	23.4	22.25

Study 2--Weight Data

		1	2	3	4	5	6	7	8	9	10	11	12	13
ZDA	1	107	122	113	127	122	124	131	131	133	134	134	133	134
	2	119	141	130	133	150	143	144	147	152	156	152	153	154
	3	108	125	126	126	136	128	128	130	131	135	136	135	134
	4	110	124	121	152	137	132	130	134	136	140	142	144	145
	5	106	113	110	122	123	118	123	126	128	131	132	130	129
	6	118	127	127	137	124	128	132	132	129	130	136	137	139
	7	110	129	121	134	136	135	140	140	141	141	143	148	152
	8	124	129	139	142	145	147	151	155	157	159	157	156	151
	9	113	133	129	141	148	137	147	150	151	153	149	147	147
	10	97	107	110	118	123	116	116	118	119	121	119	121	124
	$\bar{x}$	111.2		122.6		134.4		134.2		137.7		140.0		140.9
ZSP	1	117	128	136	140	144	144	155	157	159	161	164	172	179
	2	119	129	121	129	131	131	143	146	148	149	155	162	170
	3	115	130	128	136	139	138	150	154	157	161	174	181	188
	4	129	140	139	146	147	145	153	156	158	160	169	170	178
	5	103	119	125	132	134	136	147	150	152	153	161	166	170
	6	127	141	147	153	151	152	159	158	155	155	165	172	178
	7	106	121	133	141	144	145	164	163	162	163	168	170	175
	8	112	126	133	141	140	139	153	156	158	159	164	169	172
	9	101	116	124	129	133	134	143	147	149	150	157	163	170
	10	126	137	137	142	144	145	155	160	161	164	172	176	179
	$\bar{x}$	115.5		132.3		140.7		152.2		155.9		164.9		175.9

	1	1	2	3	4	5	6	7	8	9	10	11	12	13
ZSA	1	104	122	133	153	169	188	203	220	239	258	265	287	312
	2	101	117	129	150	165	183	201	215	235	250	252	271	311
	3	106	120	141	160	175	193	199	206	211	214	225	243	278
	4	113	132	150	172	193	215	228	244	260	284	289	300	319
	5	97	110	123	138	150	155	167	178	194	216	232	244	279
	6	107	120	135	154	178	198	213	230	251	268	269	283	317
	7	109	125	139	160	181	199	219	233	249	269	279	284	316
	8	102	117	134	152	168	186	201	221	240	253	260	283	302
	9	110	125	143	163	185	202	215	235	253	266	275	279	314
	10	97	111	124	140	155	168	180	204	222	236	254	268	292
	$\bar{x}$	104.6		135.1		171.8		202.6		235.4		260.0		304.0

		14	15	16	17	18	19	20	21	22	23	24	25	26
ZDA	1	133	139	144	140	140	139	139	138	139	133	130	128	127
	2	156	160	165	164	163	163	164	162	163	165	160	154	150
	3	133	138	140	137	138	136	139	144	147	135	132	130	128
	4	139	133	138	137	138	139	135	130	124	112	110	110	108
	5	124	129	130	136	142	147	133	125	119	120	116	114	113
	6	138	137	135	138	139	141	140	139	136	135	134	134	135
	7	147	150	148	148	145	144	141	138	136	135	134	135	136
	8	162	158	169	164	161	157	156	152	149	147	149	150	153
	9	152	147	150	154	157	160	157	156	155	154	150	150	148
	10	125	121	123	123	121	122	120	122	121	119	118	118	117
	$\bar{x}$		141.2		144.1		144.8		140.6		135.5		132.3	131.5
ZSP	1	181	181	185	190	194	196	195	194	194	193	196	198	200
	2	168	173	178	179	178	181	183	183	185	182	184	186	187
	3	187	194	205	204	201	202	200	198	197	198	194	190	177
	4	178	180	192	194	196	198	198	201	200	200	200	198	195
	5	169	176	182	185	186	189	188	190	190	192	195	192	194
	6	184	183	192	195	198	203	205	205	207	204	205	203	202
	7	170	179	186	187	186	189	192	193	196	190	187	196	204
	8	169	181	189	191	194	196	199	201	203	201	200	204	207
	9	168	180	187	188	188	191	189	187	187	190	193	188	186
	10	178	185	190	194	197	202	200	201	199	202	199	200	197
	$\bar{x}$		181.2		190.7		194.7		195.3		195.2		195.5	194.9

		14	15	16	17	18	19	20	21	22	23	24	25	26
ZSA	1	322	327	330	335	346	352	354	357	360	376	380	385	390
	2	319	325	335	339	341	347	353	358	360	371	378	384	390
	3	292	298	304	315	321	326	339	345	356	359	365	370	377
	4	333	344	350	355	361	365	371	379	386	394	401	400	405
	5	291	298	309	309	311	312	325	334	346	355	360	364	369
	6	344	347	356	362	369	377	386	395	406	417	419	420	428
	7	330	338	345	352	354	356	363	371	377	380	390	389	391
	8	312	319	323	331	339	344	354	360	365	365	374	382	387
	9	336	342	347	354	356	360	374	380	390	394	400	405	413
	10	301	308	314	320	326	330	336	340	344	357	362	367	370
			324.6		337.2		346.9		361.9		376.4		386.6	392.0

Study 2--Field Behavior Data

		Judge One						Judge Two					
		Time (Sec.)			Number			Time (Sec.)			Number		
		Circle	Groom.	Freez.	Sect.	Rear.	Bol.	Circle	Groom.	Freez.	Sect.	Rear.	Bol.
ZDA	1	4	12	0	68	10	1	4	14	0	69	10	1
	2	15	4	0	76	14	0	13	4	0	75	15	0
	3	12	15	0	78	4	0	11	16	0	78	4	0
	4	24	0	0	90	4	3	23	0	0	95	4	3
	5	12	0	0	81	7	4	12	0	0	83	7	4
	6	9	0	0	114	7	0	9	0	0	115	7	0
	7	8	0	0	98	4	0	7	0	0	98	4	0
	8	10	8	0	79	3	0	11	8	0	79	3	0
ZSP	1	6	0	0	199	14	0	6	0	0	198	14	0
	2	6	4	0	172	23	0	6	4	0	168	22	0
	3	7	0	0	178	29	0	7	0	0	181	30	0
	4	5	3	0	234	14	0	5	3	0	243	15	0
	5	1	2	0	140	9	0	1	2	0	142	9	0
	6	2	0	0	254	29	0	2	0	0	254	29	0
	7	3	0	0	160	28	0	3	0	0	169	29	0
	8	5	0	0	141	9	0	4	0	0	141	9	0
	9	1	0	0	182	16	0	1	0	0	180	15	0
	10	3	5	0	204	39	0	2	6	0	197	39	0

		Judge One						Judge Two					
		Time (Sec.)			Number			Time (Sec.)			Number		
		Circle	Groom.	Freez.	Sect.	Rear.	Bol.	Circle	Groom.	Freez.	Sect.	Rear.	Bol.
ZSA	1	3	10	0	106	4	0	3	9	0	108	4	0
	2	4	4	0	134	14	0	4	4	0	132	14	0
	3	5	8	0	123	10	0	5	8	0	124	10	0
	4	7	4	0	139	8	1	7	4	0	146	8	1
	5	2	15	0	174	13	1	2	15	0	176	14	0
	6	3	2	0	211	13	0	3	2	0	210	13	0
	7	3	8	0	183	17	0	3	8	0	187	16	0
	8	2	0	0	109	5	0	3	0	0	109	5	0
	9	1	8	0	172	17	0	1	8	0	173	1	0
	10	4	0	0	118	10	2	4	0	0	120	10	2

Study 1--Means and SEM for Body Weight and Food Intake

	Group ZDA				Group ZSP				Group ZSA			
	Weight		Intake		Weight		Intake		Weight		Intake	
	Mean	SEM	Mean	SEM	Mean	Sem	Pair	Fed	Mean	SEM	Mean	SEM
1	576.90	6.53	27.60	.58	572.80	8.92	- -	- -	577.50	6.02	27.00	.63
2	556.50	5.98	25.00	.49	563.70	7.98	- -	- -	550.10	5.52	27.2	.55
3	551.20	12.00	24.20	.61	581.00	9.18	- -	- -	565.40	7.80	27.2	.51
4	545.60	13.69	23.10	.65	578.90	9.39	- -	- -	587.30	6.68	27.00	.57
5	530.80	13.52	22.70	.65	582.40	8.96	- -	- -	591.30	7.42	27.50	.67
6	534.60	9.90	21.90	.40	578.90	11.31	- -	- -	591.80	8.81	27.50	.61
7	526.10	9.27	20.50	.52	575.20	12.17	- -	- -	598.50	6.93	28.20	.41
8	523.70	9.01	20.40	.42	575.90	12.11	- -	- -	597.80	7.26	27.70	.36
9	519.70	9.20	20.10	.54	576.20	11.59	- -	- -	598.20	7.15	27.70	.53
10	510.40	12.43	19.40	.45	566.90	11.42	- -	- -	599.10	9.55	27.60	.60
11	504.30	13.46	19.60	.42	558.10	12.83	- -	- -	599.20	9.65	27.10	.37
12	488.90	16.52	19.80	.38	554.90	13.31	- -	- -	593.30	10.13	27.90	.34
13	492.40	15.03	18.60	.47	552.70	13.08	- -	- -	600.10	9.82	27.60	.58
14	489.90	15.78	18.80	.32	548.30	13.06	- -	- -	603.20	8.81	28.10	.56
15	493.60	12.45	19.00	.36	549.50	13.44	- -	- -	605.30	8.24	22.10	.62
16	485.90	11.36	18.40	.34	549.90	13.38	- -	- -	607.50	7.79	27.10	.45
17	489.20	11.19	18.40	.45	547.70	15.11	- -	- -	595.20	8.77	25.90	.31
18	484.00	12.68	18.30	.51	543.80	16.21	- -	- -	585.30	9.72	26.10	.34
19	477.20	12.51	18.00	.39	537.90	16.46	- -	- -	575.30	9.88	26.50	.40
20	472.80	12.60	17.70	.51	533.10	16.80	- -	- -	568.60	9.53	25.50	.40
21	481.70	12.45	17.40	.56	542.60	16.74	- -	- -	578.40	9.35	27.7	.36

Study 2--Means and SEM for Body Weight and Food Intake

	Group ZDA				Group ZSP				Group ZSA			
	Weight		Intake		Weight		Intake		Weight		Intake	
	Mean	SEM	Mean	SEM	Mean	Sem	Pair	Fed	Mean	SEM	Mean	SEM
1	111.20	2.43	12.80	.90	117.40	3.78	- -	- -	104.60	1.70	14.90	.17
2	122.60	3.00	9.40	.34	130.40	2.88	- -	- -	135.10	2.68	16.7	.70
3	134.40	3.45	9.50	.83	140.70	2.05	- -	- -	171.80	4.13	19.20	.57
4	134.20	3.50	10.90	.67	152.20	2.11	- -	- -	202.60	5.74	18.80	.51
5	137.70	3.87	10.70	.55	155.90	1.52	- -	- -	234.40	6.36	20.20	.61
6	140.00	3.49	12.10	.50	164.90	1.93	- -	- -	260.00	6.36	23.63	.37
7	140.90	3.29	10.59	.95	175.90	1.81	- -	- -	304.00	4.94	24.20	.53
8	141.20	3.94	11.10	.54	181.20	1.77	- -	- -	326.70	5.13	23.10	.34
9	144.10	4.17	9.60	.34	189.82	2.37	- -	- -	338.70	4.86	25.00	.47
10	144.80	3.93	9.90	.34	194.70	2.25	- -	- -	349.90	4.86	24.40	.49
11	140.60	4.15	9.60	.37	196.20	2.64	- -	- -	364.50	5.31	23.60	.34
12	135.50	5.18	8.90	.27	195.20	2.19	- -	- -	377.10	5.78	23.70	.33
13	132.30	4.90	9.30	.21	195.5	1.97	- -	- -	388.40	5.75	22.40	.58
14	131.50	4.99	- -	- -	194.90	2.93	- -	- -	393.80	5.41	- -	- -