1981

Effect Of Gestational And Lactational Zinc Deficiency On The Developing Dental Tissues And Incidence Of Dental Caries In The Rat.

Rashid Nouri Al-Hayali
University of Alabama at Birmingham

AL-HAYALI, RASHID NOURI

EFFECT OF GESTATIONAL AND LACTATIONAL ZINC DEFICIENCY ON THE DEVELOPING DENTAL TISSUES AND INCIDENCE OF DENTAL CARIES IN THE RAT

The University of Alabama in Birmingham PH.D. 1981

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print ✓
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

EFFECT OF GESTATIONAL AND LACTATIONAL ZINC DEFICIENCY ON THE DEVELOPING DENTAL TISSUES AND INCIDENCE OF DENTAL CARIES IN THE RAT

by

RASHID NOURI AL-HAYALI

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Pathology in The Graduate School of The University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1981

ABSTRACT OF DISSERTATION

GRADUATE SCHOOL, UNIVERSITY OF ALABAMA IN BIRMINGHAM

Degree Ph.D. Major Subject Oral Pathology

Name of Candidate Rashid Nouri Al-Hayali

Title Effect of Gestational and Lactational Zinc Deficiency on the Developing Dental Tissues and Incidence of Dental Caries in the Rat

This study is an attempt to investigate the effect of gestational and lactational zinc deficiency on developing dental tissue, and to provide morphological, biochemical data and caries incidence in a well controlled and highly defined animal model.

Using purified diet MIT #200, zinc deficiency was successfully induced in pregnant and lactating rats. Zinc deficiency was manifested by significantly low serum zinc concentration, aversion to diet and stress, difficulty in parturition, and poor weight gain. In this study we established that minimum dietary zinc during the last week of gestation and 18 days of lactation is 4 μg Zn/gm. Lower dietary zinc (2 μg Zn/gm) was associated with 50% mortality rate. It was observed also, that zinc deficiency during the last week of pre-natal life and 18 days of post-natal life was associated with high incidence of dental caries, smaller teeth and

significantly low zinc concentration in upper molars of 18 days old pups. Longitudinal investigations of the developing dental tissue from the first day to the seventeenth day of post-natal life showed that zinc deficiency imposed during the last week of gestation and seventeen days of lactation induced a retardation of two days in dentin apposition, enamel apposition, root formation, and degree of enamel maturation, and qualitative morphological abnormality in the dentin of the incisors.

Abstract Approved by: Committee Chairman _____

Program Director _____

Date 8/4/81 Dean of Graduate School _____

ACKNOWLEDGEMENTS

This dissertation was accomplished with the support, help and advice of many people. I am greatly indebted to the Iraqi Government, the University of Baghdad and College of Dentistry for all the support given to me throughout my graduate education. My appreciation and thanks to my graduate committee members, Dr. C. Hunt, Dr. G. Hand, Dr. M.G. Martinez (Chairman), Dr. V. Matukas, Dr. J. Navia, and Dr. K. Roozen.

I am very proud to mention that this research was conducted with the guidance and wisdom of a fine humane scientist, Dr. J. M. Navia, who always inspired me and everyone around him. I hope that this research will be the trigger of a continuous scientific future which will reflect his inspiration and wisdom. I wish to extend my appreciation and thanks to Dr. S. Hsieh who taught me and helped me in the analytical part of this work. Dr. S. Harris' contribution in correcting my English writing is unforgettable, and I appreciate her help. This acknowledgement would not be complete without mentioning Dr. H. Lopez' counseling and advice, my thanks to her.

I wish to acknowledge also the help and support that Dr. Matukas and Dr. Martinez have given me throughout my education.

My sincere appreciation, admiration and my gratefulness are extended to a fine friend, Dr. C. A. McCallum, for his continuous encouragement and support.

Finally to my wife Moazaz, my sons Mohammed and Marwan whom I love dearly, I extend my sincere thanks for their support, and understanding that has helped me endure the long separation.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

CHAPTER I

INTRODUCTION

The essentiality of zinc for normal growth and development of rats was observed as early as 1934 (Todd et al., 1934). Zinc deficiency in Iranian males was suspected by Prasad et al. in 1961. These findings were confirmed two years later with a study in Egypt (Prasad et al., 1963). The clinical manifestations in both Iranian and Egyptian patients were dwarfism, anemia, hypogonadism, hepatosplenomegaly, rough and dry skin, and mental lethargy. Geophagia were practiced by Iranian and Turkish patients (Cavdar and Arcasoy, 1972; Cavdar et al., 1977). In Shiraz, zinc supplementation to patients with chronic zinc deficiency enhanced their growth and hastened the onset of sexual functions as manifested by nocturnal emission in males and first menstrual periods in females (Halsted et al., 1972).

Zinc deficiency has been reported in preschool children from low income American families enrolled in a Denver Head Start Program (Hambidge et al., 1976), in elderly, black Americans from urban low-income households (Wagner, 1980), in pregnant Turkish women (Cavdar et al., 1980), and in aboriginal communities of North Western Australia (Holt, 1980). A survey in Western Scotland indicated that the average family (two adults, two children) diet

did not contain enough zinc to meet the recommended daily allowance for zinc (Lyon et al., 1979). In Tunisia, a nutritional study revealed that 20.2% of individuals in a selected sample from the Tunisian population were classified as severely zinc deficient (less than 75μgZn/100 ml plasma) and 59.5% were classified as being at some risk to become zinc deficient. Pregnant or lactating women had the highest incidence of zinc deficiency in Tunisia (Jacobs et al., 1980). Thus, zinc undernutrition is a significant public health problem in many parts of the world (Jacobs et al., 1980).

Zinc deficiency has been induced in various species of laboratory animals (Morrison and Sarett, 1958; O'Dell et al., 1958; Hurley and Swenerton, 1966; Miller et al., 1968; Miller and Miller, 1962). Skeletal abnormalities are a regular feature of experimental zinc deficiency in chicks (Morrison and Sarett, 1958; O'Dell et al., 1958), rats (Hurley and Swenerton, 1966), calves (Miller and Miller, 1962) and baby pigs (Miller et al., 1968). The exact mechanism of action of zinc in bone formation is still not known. Morphological and histochemical investigations indicate a reduction in osteoblastic activity and population, a decrease or failure in chondrogenesis associated with an increase in the amount of cartilage matrix (Hoekstra, 1969), and a reduction in bone alkaline phosphatase activity (Underwood, 1971).

Zinc deficiency severely affects rapidly growing tissue, such as the embryo (Hurley and Swenerton, 1966). This effect is most probably mediated through zinc dependent enzyme systems which influence nucleic acid metabolism such as DNA and RNA polymerase

and thymidine kinase (Hambidge, 1974; Prasad, 1976). Zinc deficiency seems to affect the developing dental tissue in the same way. The effect of zinc deficiency on the developing dental tissue and its relation to dental caries are not known. This investigation is an attempt to provide morphological, biochemical and caries incidence data on the effect of zinc deficiency in a well-controlled and highly defined animal model.

CHAPTER II

LITERATURE REVIEW

General Distribution Of Zinc In Tissues

Zinc distribution and concentration in biological tissues and fluids are quite variable. Tissues with high concentration of zinc are prostate, skin and appendages, choroid, liver, pancreas, bone and blood (Fisher, 1975). Total human body zinc in an adult male (1.4-2.3 gm) is about half that of iron, 10-15 times that of copper and more than 100 times that of manganase (Underwood, 1971).

Zinc In Blood

Laboratories using different techniques have not been consistent in reporting normal values for the zinc content of adult human plasma or serum (Mikac-Devic, 1970). The mean zinc serum concentration for normal individuals has been reported as 115 μg Zn/100 ml, and plasma zinc concentration 97 μg Zn/100 ml, which is 16% lower than that of serum (Vallee and Gibson, 1948). Values for whole blood are higher than plasma because erythrocytes are rich in zinc (Mikac-Devic, 1970). The erythrocytes contain 12-14 μg Zn/ml of packed cells, and leukocytes contain as much as 25 times that amount (Vallee and Gibson, 1948). Vallee and Gibson (1948)

indicated that 75-88% of total zinc in normal human blood is contributed by red blood cells, 12-22% by plasma and only 3% by white blood cells. Foley et al. (1968) reported that platelets contain zinc in an amount ranging from 0.20 to 0.45 μg Zn/10^9 platelets. About one third of plasma zinc is firmly bound to α_2-macroglobulin. The other 2/3 is loosely bound to albumin (Parisi and Vallee, 1970; Henkin et al., 1974). Zinc in erythrocytes occurs mostly as carbonic anhydrase (Keilin and Mann, 1940), an enzyme which has an important role in acid-base homeostasis of living organisms by catalizing reversible dehydration of carbon dioxide (Parisi and Vallee, 1969).

Zinc plasma concentration in animals varies according to physiological condition, species and genetic background. Wistar derived male (Gordon and O'Dell, 1980) and female (Hickory et al., 1979) rat plasma zinc concentration are reported to be 133 μg Zn/100 ml. This value dropped to 53 μg Zn/100 ml after the pregnant rats were fed a zinc deficient diet for two days and decreased further to 41 μg Zn/100 ml by day 14 (Hurley and Mutch, 1973). Fernandes et al. (1979) indicated that serum zinc concentrations ranged from 110 and 125 μg Zn/100 ml for C57BL/KS, A/Jax, CBA/H and AkR/H strains mice. Mean serum zinc concentrations for squirrel monkey (Saimiri sciureus) were reported to be 104.8 μg Zn/100 ml (Macapinlac et al., 1967). Guinea pig serum zinc concentration was reported to be 100 μg Zn/100 ml (Hsieh and Navia, 1980) and there was a slow reduction of serum zinc after feeding a zinc deficient diet.

Low plasma zinc concentrations have been observed in pregnant women (Jacobs et al., 1980). Serum zinc level in poorly nourished women tends to decrease as pregnancy progresses. It has been reported (Cavdar et al., 1980) that mean serum zinc concentration is 88 μg Zn/100 ml during the first trimester, 80 μg Zn/100 ml during the second trimester and dropped to 68 μg Zn/100 ml during the 3rd trimester compared to 177 μg Zn/100 ml, 108 μg Zn/100 ml and 100 μg Zn/100 ml, respectively, for well-nourished controls.

Rapid decrease in plasma zinc had been observed during acute stress such as surgery (Henzel et al., 1970; Flynn et al., 1973), burns (Larson et al., 1970; Sanchez-Agrenda et al., 1978), myocardial infarction (Wacker et al., 1956; Lindeman et al., 1973; Walker et al., 1978) and infections (Vikbladh, 1951; Halsted and Smith, 1970). This change in plasma concentration represents redistribution of zinc within the body, i.e., from plasma to liver mediated by the release of leukocytic endogenous mediator (LEM). This substance acts on the liver to increase the movement of zinc from plasma to hepatocyte (Beisel et al., 1976); it also stimulates the liver to synthesize a large number of acute phase reactant proteins.

Zinc In Bone

Zinc is present in relatively high concentration in bone (Prasad, 1966). Bertrand and Vladsco (1920) indicated that dry horse femur contained 405 μg Zn/gm of bone. McBean et al. (1972) showed that mean zinc concentration in human bones was

218 ± 94 μg Zn/gm dry weight. Mean zinc concentration in rat bone has been reported to be 162 ± 7 μg Zn/gm (Macapinlac et al., 1966). Zinc is deposited very slowly in the skeleton and teeth. It is bound for a relatively long time and exchanged slowly (Prasad, 1966; Orten, 1966).

Zinc concentration in bone is largely determined by the amount of dietary zinc. Zinc concentration in bone in pair-fed control rats, ad libitum fed control and purina chow fed controls, was 109 ± 11, 141 ± 30 and 162 ± 7 μg Zn/gm wet weight, respectively, while the zinc concentration of bones from deficient rats was 35 ± 4, which is significantly lower than all the control values (Macapinlac, 1966; Calhoun et al., 1973).

Zinc effect on bone metabolism, development and healing has been studied extensively in animal models (Hurley and Swenerton, 1966; Miller et al., 1968; Hoekstra, 1969). Calhoun et al. (1974) reviewed recently the research findings concerning the role of zinc in bone metabolism and physiology. Histological investigations, radiographic studies of Ca^{45} uptake in bone revealed that there was a significant increase in ^{65}Zn uptake in healing bones of rats and ^{65}Zn accumulation appeared to be correlated with bone formation and repair (Calhoun et al., 1970). Zinc supplementation to a normal diet resulted in improvement in early stages of extraction site alveolar bone healing (Mesrobian and Shklar, 1969). This last observation is not supported by recent findings by Norman et al. (1975a,b) which indicated that zinc supplementation in guinea pigs and rats did not affect the rate of healing or tensile strength of

incised wounds in either species. Some investigators have studied sites of bone healing after separating them from adjacent normal bone, and this has improved the evaluation of healing process (Klouda and Navia, 1974; Hsieh and Navia, 1980). Haumont (1961) and Haumont and McLean (1966) investigated histochemically and microradiographically zinc distribution in bone. They indicated that zinc is found in close proximity to sites of calcification and localized in layers of preosseous tissues where calcification is imminent. These findings were confirmed by Bergman (1970a,b).

Zinc deficiency in humans can affect skeletal growth and development. Zinc deficient patients from Egypt and Iran exhibited radiographically bone ages lower than their actual chronological ages (Halstead and Smith, 1970). Zinc supplementation for these patients effectively increased their height and bone skeletal maturity (Ronaghy et al., 1974; Ronaghy, 1974; Prasad, 1977).

Zinc Distribution In Other Tissues

High zinc concentration is encountered in horse testes, prostate of cattle and human, epididymis in rat and seminal vesicles in pigs. Prasad et al. (1967) reported that zinc deficient rat testes contained 132 ± 16 μg Zn/gm and showed atrophic testicular changes. Control rat testes contained 176 ± 12 μg Zn/gm zinc.

Human and animal spermatozoa, seminal fluid and prostate contain high concentration of zinc (Mawson and Fischer, 1952a,b, 1953). Prostate glands have the ability to take up more ^{65}Zn than

any other normal tissue (Mikac-Devic, 1970). The function of zinc in the prostate is largely unknown (Prasad and Oberleas, 1974).

Zinc distribution in the pancreas is mainly limited to the islets of Langerhans, in the cytoplasm of A & B cells (Miller et al., 1968). Prasad et al. (1967) indicated that zinc prolongs the physiologic action of insulin and corticotropin preparation by retarding the rate of absorption. Hove et al. (1937) indicated that rats fed zinc deficient diets manifested decreased glucose tolerance. This finding was confirmed later by Quarterman et al. (1966). A recent investigation by Brown et al. (1975) with rats indicated that "zinc deficiency per se had no effect on oral glucose tolerance or on pancreatic insulin concentration." This observation confirmed earlier work by Macapinlac et al. (1966) with albino rats. Halsted et al. and his co-worker (1974) in a recent review indicated that the exact role of zinc in carbohydrate metabolism needs to be further investigated.

Human milk contains 0.4-2.8 μg Zn/ml (Picciano and Guthrie, 1973; Johnson and Evans, 1978), which was much lower than that found in cow's milk, 2.3-5.0 μg Zn/ml. Even a higher zinc concentration was found in rat milk, 13-17 μg Zn/ml, which decreased when a zinc deficiency was imposed (Hurley and Mutch, 1973).

Zinc concentration in rat, cows and human hair reflect dietary intake (Miller et al., 1965; Reinhold et al., 1966, 1968). McKenzie (1979) indicated that healthy adult females had higher hair zinc concentration than males; furthermore, he concluded that measurement of zinc in serum, urine, hair and toe nails does not provide a sensitive indication of zinc status.

Reinhold et al. (1968) found that there was a substantial decrease in zinc concentration of rat hair within 40 days after starting to feed a low zinc diet. However, this decrease in zinc hair concentration diminished as the time of feeding deficient diet increased. Petering et al. (1971) postulated that the concentration of zinc and copper in human hair can be considered at two age groups: under 12 years of age and over 12 years of age. In either group, the concentration of these trace elements is directly related to the age of the individual; the relationship is linear when concentration and age are expressed as logarithms. There is an increase in zinc concentration in hair during childhood and a slow continuing decline after maturity. Zinc content of an infant's hair is higher than that of the mother's hair. The mean value of zinc decreased in mothers as parity increased, 160.6 μg Zn/gm in primiparity to 109.3 μg Zn/gm in multiparity (Baumslag et al., 1974). These findings may indicate a tendency toward nutritional insufficiency of zinc in multigravid mothers, for whom zinc supplementation might be beneficial. Zinc and other trace metal content of hair varies in different physiological states and in different populations as shown in a comparative study between Bushmen women, Bantu women and American inner city mothers (Baumslung and Petering, 1976).

Prasad et al. (1963) reported that hair zinc concentration in untreated Egyptian zinc deficient dwarfs was 54.1 ± 5.5 μg Zn/gm compared to 103.3 ± 4.4 μg Zn/gm in hair of normal Egyptians. Zinc supplementation increased hair concentration to a mean value of 125 μg Zn/gm (Strain and Pories, 1966).

The choroid of the eye has the highest zinc concentration of any living tissue (Underwood, 1971). Further dissection of the choroid disclosed that zinc is concentrated in the iridescent layer, tapetum lucidum, where values reach an extraordinary level of up to 8.5% (85.000 μg Zn/gm) in dog and up to 13.8% in the fox (Weitzel et al., 1954). The physiology of zinc in the eye is unexplored (Underwood, 1977).

Distribution Of Zinc In Teeth

Earlier reports indicate that human enamel contains 233 ± 27 μg Zn/gm dry weight (Cruickshank, 1936). Enamel from patients with tuberculosis has been reported to have higher zinc concentration than that of healthy individuals (Cruickshank, 1940). This finding is more likely to be associated with differences in amount of zinc ingested than with the specific disease (Brudevold et al., 1963). Concentration of zinc in surface layers of enamel in human teeth ranges from 430-2100 μg Zn/gm (Brudevold et al., 1963), with the highest concentration in the outer surface. The distribution pattern of zinc is similar to that exhibited by fluoride and lead. Dentin exhibits a similar gradient in zinc concentration, but the highest concentration occurs in areas adjacent to the pulp (Brudevold, 1963). Lower values for enamel were reported by Nixon et al. (1967) using neutron activation analysis (58-2000 μg Zn/gm), but the zinc distribution pattern was in agreement with Brudevold (1963) observations. In enamel, the major deposition of

zinc takes place before eruption and, in contrast to fluoride, posteruptive deposition appears to be irregular (Brudevold, 1963). Zinc deposition in bone and teeth is a relatively slow process, but once its incorporated into the mineralized tissue, it remains bound for a relatively long time and exchanged slowly (Orten, 1966; Prasad, 1966).

The mean zinc concentration for both deciduous and permanent teeth of children living in Oslo was reported as 130 μg Zn/gm with a range of 91-180 μg Zn/gm (Attramadal and Jonsen, 1976). A later report (Lappalainen and Knuuttila, 1979) from five different geological areas in Finland indicated that the mean zinc concentration of this sample is higher (182 ± 37.1 μg Zn/gm). Söremark and Samsahl (1961, 1962) reported the mean zinc concentration of 15 tooth samples from children 12-16 years of age was 276 ± 06 μg Zn/gm for enamel, and 199 ± 78 μg Zn/gm for dentin. There was no significant difference with respect to sex or mandibular versus maxillary teeth. This was confirmed in a later investigation in New Zealand (Curzon et al., 1975) which showed that enamel zinc concentration was lower than those reported for U.S. residents (Losee et al., 1974).

Geographical location, geological variability, food intake, water supply and racial factors may influence the amount of zinc deposited in the teeth. It has been shown that the mean zinc concentration in teeth from people living in four different localities in Japan (Annaka, Tokyo, Okitsue and Hachijo Island) was 198 ± 69 μg Zn/gm on dry weight basis, and the mean values of

zinc in teeth obtained from Hachijo Island were significantly higher than those for all other areas (Kaneko et al., 1974). Retief et al. (1978) indicated that enamel zinc concentration in the white population was significantly higher than in the black population of South Africa.

Animal studies have shown that zinc concentration in incisors of white and black hooded rats fed a control diet containing 47 μg Zn/gm, was higher in dentin (249 ± 132 S.E.) than in enamel (129 ± 23.5 S.E.) when expressed as μg Zn/gm of dry weight tissue (Huxley and Leaver, 1966). Calcium deficiency and excessive dietary zinc intake significantly increased the concentration of zinc in bone and dentin. A similar trend was observed in enamel, but the small number of individual analyses made these results less conclusive. Brudevold et al. (1963) showed that zinc was incorporated into synthetic hydroxyapatite and that it competes with calcium for position on the surface of the hydroxyapatite crystal.

Hove et al. (1933) reported a low zinc concentration in teeth from zinc deficient rats. However, Bergman (1970) could not detect significant differences in zinc concentration of incisors between zinc deficient and control rats. Recent studies have shown that zinc concentration in bone and teeth from zinc deficient rats are significantly lower than the values obtained from rats fed a zinc supplemented control diet. Feeding high amounts of zinc in the diet (108 μg Zn/gm) has resulted in significantly increased concentration of zinc in bones and teeth (Fang et al., 1980) compared to normals. Furthermore, jaw bones from zinc deficient rats

were reported by Fang et al. (1980) to be significantly lighter in weight than those from zinc sufficient pair-fed or ad libitum fed control rats.

Zinc And Oral Tissues

Zinc And Dental Caries

Epidemiologic investigations indicate that the prevalence of dental disease may be related in part to the variation in mineral content of soil, food and teeth (WHO, 1971). Sognnaes (1957) and Isaac et al. (1958) suggested that consideration should be given to trace elements other than fluoride as a factor affecting the resistance of enamel to dental decay. Losee (1966) emphasized the importance of diet in dental caries, including drinking and cooking water.

Helle and Haavikko (1977a) attempted to correlate the prevalence of dental caries with the presence of nine macro- and micro-minerals in drinking water. They found that fluoride showed the strongest negative correlation ($r = -0.484$, $p < 0.001$). Zinc was shown to have also a negative correlation with caries ($r = -0.179$, $p < 0.01$).

Analysis of zinc in water samples collected from 194 sources of public water supply in the U.S. was reported to range from 0.06-7.0 μg Zn/ml with a mean value of 1.33 μg Zn/ml (Taylor, 1963). Hadjimarkos (1967) believed that with the exception of fluoride water supplies contribute only an insignificant amount of trace elements to the daily diet compared with food-stuffs.

Therefore, he suggested that in conducting epidemiological studies of association between intake of trace elements and dental caries, determination of elemental concentration in water alone does not provide a reliable indicator of the total amount ingested daily by individuals.

There is a significant difference in the concentration of fluoride and strontium in human enamel between high and low caries areas in the U.S.A., but insignificant differences in concentration of other trace elements in enamel, including zinc (Curzon, 1977; Curzon, 1978). However, a similar study conducted in South Africa revealed that zinc concentration was significantly higher in the enamel from white population groups with higher caries incidence in comparison to black population groups with low caries incidence (Retief et al., 1978). An investigation conducted in Finland to correlate the macro- and micro-mineral composition of deciduous teeth from different geographical areas and dental caries indicated that zinc concentration in dentin correlated positively with caries prevalence (Helle and Haavikko, 1977b). This finding is in contradiction to previously mentioned observation by the same investigators (Helle and Haavikko, 1977a) and was explained by the fact that fluoride was high in the drinking water, which might be responsible alone for low caries prevalence and not necessarily be due to the presence of zinc and other trace elements. Curzon and Bibby (1970) indicated that zinc may stimulate dental caries. This has been substantiated in a recent study by Curzon and Crocker (1978).

Although Navia (1970) classified zinc as an element with doubtful effect on dental caries, and supplementation of diet fed to rats with zinc chloride or zinc sulphate was found to be ineffective in reducing dental caries (Navia et al., 1968; McClure, 1948), Hendershot and Forsaith (1959) found that when zinc was given to rats as zinc versenate, it had a moderately reducing effect on dental caries. More recently, it has been reported that (220 μg Zn/ml) zinc sulphate in drinking water of rats infected with S. mutans at the time of tooth eruption significantly reduced the buccal caries score (Bates and Navia, 1979a). Furthermore, they reported that topical application of 500 μg Zn/ml significantly reduced buccal caries in rats. Animal experiments have shown that supplementation with 250 μg/gm zinc, 10 μg/gm molybdenum and 4 μg/gm chromium enhanced the cariostatic action of carbamyl phosphate, and completely suppressed dental decay in rats fed a caries promoting diet (Steinman and Leonora, 1975).

The earliest studies of zinc deficiency and dental caries indicated that rats fed a low zinc, caries promoting diet exhibited a higher dental caries incidence than controls (Sortino and Palazo, 1971a,b). More recent investigations indicate that there is significantly greater severity of caries in enamel and dentin of pups nursed by zinc deficient rats for 20 or 21 days and then challenged with microbial and cariogenic diet for five weeks, than pups nursed by zinc adequate pair-fed and ad libitum rats (Brown et al., 1979). Furthermore, analysis of zinc concentration in maxillary molars after feeding a caries promoting diet for five weeks shows that

there is a significant difference between experimental groups and controls. The mean zinc concentration of molars was 124 ± 14 μg Zn/gm, 157 ± 16 μg Zn/gm and 150 ± 11 μg Zn/gm ($\bar{x}$ ± S.D.) for the zinc deficient, pair-fed and ad libitum groups, respectively. However, not all groups showed significant differences in zinc concentration in plasma nor in femurs after the experimental period. Carious lesions in rats suckled by zinc deficient dams showed greater penetration into the dentin than controls. However, there were no significant differences in caries scores between the ad libitum and the pair-fed groups (Brown et al., 1979).

Fang et al. (1980) investigated the effect of graded doses of dietary zinc on development and mineralization of bone and teeth and their susceptibility to dental caries in young growing rats, 21 days old. They reported that zinc deficient rats had significantly smaller tibia and jaws than zinc supplemented, pair-fed controls. Tibia, maxillary tooth and incisor zinc concentrations based on dry weight were significantly lower than those of pair-fed controls. The values of each group reflected the graded concentration of dietary zinc, being the lowest for zinc deficient group and highest for rats on 108 μg Zn/gm diet. Furthermore, a greater incidence of enamel lesions was observed in zinc-deficient rats than in pair-fed, zinc supplemented controls.

Zinc And Oral Microflora And Plaque

Zinc has been shown to have a definite antibacterial effect at low concentrations ranging from 8-32 μg Zn/gm. In vitro tests,

where S. mutans were grown in chemically defined media of known trace element composition, containing either 0.5, 5 or 16 parts 10^6Zn as $ZnSO_4$, revealed that zinc sulphate depressed S. mutans growth, initial plaque formation, and inhibition of acid production at Zn:cell ratio of 2 and 4 μg Zn/mg cell wet weight (Bates and Navia, 1979b). They suggested that zinc may inhibit the growth by interfering with cysteine metabolism.

Wildra (1964) reported a remarkable complete reversal of M-phase and overgrowth of Y-phase for Candida albicans by zinc ion in a defined culture medium. This finding was substantiated later by Yamaguchi (1975) who suggested that zinc participates in morphogenesis of a wide range of microorganisms. In contrast to these earlier reports, Bedell and Soll (1979) examined the effects of micromolar zinc concentration on growth and dimorphism of C. albicans, and reported that zinc neither depresses growth nor suppresses the formation of the invasive mycelium under all conditions.

Bacterial and monilial infections had been reported frequently in cases of acrodermatitis enteropathica (Campo and McDonald, 1976; Hambridge et al., 1977) which were successfully treated with 50 mg zinc sulfate twice daily, and oral candidiasis almost completely cleared within 48-72 hours of the beginning of zinc sulfate therapy (Campo and MacDonald, 1976). Bedell and Soll (1979) suggested that the increased intake of zinc in such patients caused a remission of symptoms of acrodermatitis enteropathica including reduction in

candida infection, which may be due to direct effect of increased zinc at the site of the infection or to stimulation of the host's defense mechanism, such as leukocytosis, phagocytosis, and cell-mediated immunity as well as inhibition of bacterial growth.

Hardwick and Martin (1967) using mass spectrometry for semi-quantitative estimation of trace element content of dental plaque reported that zinc concentration in plaque ranged from 100-1000 μg/gm. Schamschula et al. (1977) using flameless atomic absorption indicated that zinc content in dental plaque from children aged 9.7-13.0 years and lifelong residents of one of three cities in New South Wales, Kataomba, Sydney, and Yass, of dry weight was ($\bar{x}$ ± SD) 108.8 ± 47.0 μg/gm, 93.8 ± 52.2 μg/gm and 106.1 ± 37.1 μg/gm, respectively, and 103.5 ± 45.3 μg/gm for the whole population examined (n = 72). They further reported that the mean zinc concentrations of plaque were significantly higher for males (115.3 ± 51.8 μg Zn/gm) than for females (87.0 ± 27.2 μg Zn/gm). Higher, but comparable, zinc concentrations were reported in primitive populations in New Guinea (Schamschula et al., 1977). It is not clear if the presence of such amounts of zinc in dental plaque will modify its pathogenicity as far as dental caries or periodontal disease is concerned.

Zinc And Oral Mucosa

Most of our information about dietary zinc and the integrity of oral mucosa is derived from animal experimentation. No special attention was paid to the oral health status of zinc deficient

patients in Iran and Egypt described by Prasad et al. (1963), or in the more recent epidemiological study by Jacobs et al. (1980) which indicated that about 20.2% of a Tunisian subpopulation were classified as severely zinc deficient, and 59.1% were described as being at some risk to zinc deficiency. Toxic effect of zinc oxide has been observed by clinical and cytological examination of tissues from 290 workers in a zinc processing plant. It was reported that leukoplakia occurred more significantly in these workers than in unexposed controls. Furthermore, it was reported that cytological investigation failed to demonstrate presence of cells with neoplastic metaplasia (Chrusciel, 1975a,b).

Zinc deficient rats exhibited esophageal lesions consisting of epithelial hyperplasia, hyperkeratosis and parakeratosis (Diamond et al., 1971; Macapinlac et al., 1966; Follis et al., 1941) which were reversible by zinc repletion. Partial reversal was evident by day six, and complete disappearance of the lesion was evident after 15 day of zinc therapy (Diamond et al., 1971).

Alvares et al. (1968) observed that parakeratosis was seen consistently in circumscribed areas of the buccal mucosa, dorsal aspect of the tongue and in the posterior third of the ventral aspect of the tongue in zinc deficient rats, but not in the palatal mucosa, anterior half of the dorsal and ventral aspect of the tongue, or in the occlusal plane of the buccal mucosa. The susceptibility of the anterior third of the ventral surface of the tongue to parakeratosis appeared to be intermediate between that of the two regions described.

Zinc deficient rats have a thicker epithelium, almost twice that of controls, and have a higher mitotic rate. On the average they have six times the number of mitotic figures seen in control rats and there is widespread failure of nucleus disintegration in all keratinizing epithelia. These areas involved with parakeratosis show persistence of pericellular glycoproteins, cytoplasmic basophilia and nucleolar prominence in the upper cellular layer, and retention of the nuclei in the keratin layer (Alvarez et al., 1968). Furthermore, the buccal epithelium of zinc deficient rats exhibited increased dry weight per volume over that of controls (Meyer and Alvarez, 1974), and the migration rate of the cells from the basal layer toward the surface was faster (Alvarez and Meyer, 1974).

Cytochemical investigation showed that there was a decrease in nucleohistone in zinc-deficient rat buccal hyperplastic epithelium as compared to a control. An autoradiographic study showed that there was decreased incorporation of ^{3}H-arginine and ^{3}H-lysine in the hyperplastic epithelium (Chen, 1977). Ultramicrochemical assay of lactate dehydrogenase concentration in buccal epithelium of zinc deficient rats was reported to increase more than two-fold over that of control, but there was no change in the palatal epithelium (Gerson and Meyer, 1977).

Ultramicrochemical assay of acid phosphatase in zinc deficient rat buccal epithelium indicated an increase in this enzyme over that of control samples, and histochemical studies indicated that acid phosphatase was localized in the cell periphery of prickle

cell layers (Alvarez et al., 1973). Ultrastructural studies indicated that the acid phosphatase of zinc deficient rat buccal epithelium was distributed intercellularly in the prickle cell layers. Within the prickle cells acid phosphatase was present in recognizable Golgi systems (Rijhsinghani et al., 1975).

Electron microscopic investigation (Osmanski and Meyer, 1969) showed that the upper cell layers of buccal epithelium of zinc deficient rats exhibited an increase in concentration of ribonucleoprotein particles, and experimental changes in mitochondria and endoplasmic reticulum, but no changes could be detected in the lamina propria, basal and lower spinous cells. Tongues of zinc deficient monkeys develop thickening of the mucosal lining, which was particularly prominent over the anterior part of the dorsal surface and less marked on the ventral surface (Barney et al., 1967). There was also an increase in the number of cells in the mucosa and crowding of cells in the basal layer which exhibited hyperchromatic nuclei. The rete pegs extended deeply into the submucosa and had a clubbed appearance, keratohyaline granules were comparatively sparse or absent, and the underlying muscle fibers exhibited atrophic changes. Similar lesions have been described in zinc deficient lambs (Mann et al., 1974).

Grossly, there is atrophy of the body of the tongue and thickening of the dorsal epithelium with a marked increase in the length of filliform papillae in zinc deficient lambs. Fungiform papillae are present, but in many areas they are obscured by the elongated filliform papillae and by debris coating. Cytologically, cells

obtained from the tongue of a zinc deficient lamb in early stages were small, keratinized squamous epithelium with many bacteria adhering to their surfaces. In advanced stages of zinc deficiency scrapings of the tongue consisted of a cheesy mass of desquamated epithelial cells mixed with food particles and numerous free bacteria. The desquamated cells were heavily encrusted with cocci and rod-shaped bacteria. The cells have a tendency to be acutely angular or narrow and elongated, and smaller than cells from control sheep (15-40 μ diameter).

Histologically, the tongue exhibited markedly thickened epithelium and parakeratosis. Parakeratosis was most severe in the interpapillary spaces, and also involved the filliform papillae. The fungiform papillae were not involved. Bacteria were present on the surface of the epithelium and between the squamous layer. There was no evidence of inflammatory exudate in response to bacterial infiltration. Many cells in the deeper epithelial layers had para-nuclear vacuoles caused by collapse of ballooned nuclei. The lamina propria was thickened and there was a general increase in the amount of interstitial connective tissue. There was a considerable increase in the number of mononuclear cells and plasma cells in the lamina propria of deficient animals. Muscular atrophy was also present and muscle fiber degeneration was seen more frequently in the posterior part of the tongue. Other changes included arterial hypertrophy in the body of the tongue and vascular proliferation in the lamina propria. The terminal nerve fiber supplying straited muscle was described as shorter, thicker

and more tortuous in zinc deficient lambs, while in controls, the motor nerve ended in a spray of slender, naked nerve fibers. The myeline sheaths in zinc deficient animals were thin and irregular (Mann et al., 1974).

Zinc And Salivary Gland And Saliva

Mathur et al. (1977) showed that human adults have a mean zinc concentration in resting mixed saliva of 0.478 μg Zn/gm, which is ten times the zinc concentration of stimulated parotid saliva, but they could not demonstrate significant differences in zinc concentration between men and women, nor at different time intervals in the same individual due to the small size of their sample. Snowden and Freeland (1978) indicated that zinc concentration in human whole saliva showed circadian variation, ranging from 12-78 μg Zn/100 gm, and was correlated significantly to percentage of solids. They further indicated that the pattern of these changes was an initial decrease upon arising in the morning with a subsequent increase in late morning and eventually a decrease in the late afternoon and evening. Furthermore, they reported that there was no correlation between zinc dietary intake and zinc content of saliva. However, Freeland-Graves et al. (1981) showed that consumption of low zinc diet by female volunteers produced a significant decline in the level of salivary sediment zinc from 126 μg Zn/gm to 96 μg Zn/gm, and concentration of zinc in whole mixed saliva remained relatively stable during low-zinc diet

intake. They suggested that salivary sediment zinc may be a sensitive parameter for the assessment of zinc status in man. Zinc supplementation in older women significantly increased the zinc content of saliva, plasma and erythrocyte (Buchanan et al., 1980). Zinc supplementation in young women produced an increase in plasma zinc which was approximately double for older women, and the time at which maximum plasma zinc concentration occurred was significantly different between the young (5.5 weeks) and older women (7.9 weeks). However, the increase in the zinc content of saliva in response to zinc supplementation was not significantly different between young and older women (Geders et al., 1980). Henkin et al. (1975) indicated that parotid saliva can be analyzed quickly and reliably, without special handling and with little interference from other ions found in saliva by flameless atomic absorption. They demonstrated that parotid salivary zinc concentration in subjects with idiopathic hypogeusia was significantly lower than subjects with normal taste acuity. Greger and Sickles (1979) suggested that saliva sampling would be appropriate for assessing zinc status in a survey situation, as long as precautions were taken to prevent zinc contamination.

Zinc concentration of human salivary protein fractions were reported to be 49 μg/gm (Langmyhr and Eyde, 1979) and the values obtained were distributed over a wide range. The highest concentration was found in proteins of high molecular weight (Langmyhr and Eyde, 1979). Baratieri et al. (1979) investigated the distribution of zinc in human saliva and reported that zinc concentration in saliva pellet was 206 μg/gm, the concentration of

zinc, free or bound to low molecular weight substances, was 0.056 μg/gm and the concentration of zinc bound to proteins with molecular weight ≧10,000 was 0.022 μg/gm.

Everett and Apgar (1979) reported no difference between salivary zinc concentration in zinc deficient rats and zinc supplemented control rats. They suggested that salivary zinc is not as good an indicator of zinc status as plasma zinc in rats.

Histochemical investigation of rat salivary gland did not reveal significant differences between zinc content of glands from rats injected with zinc acetate and that of controls (Bulman and Cate 1962).

Frothy saliva production had been reported to be associated with early stages of zinc deficiency in lambs (Mann et al., 1974). Worthington (1978) reported that a reduction in the relative size of the parotid gland in the rat is associated with zinc deficiency.

Zinc And Taste

Henkin et al. (1967) established an association between trace elements and impairment of taste (hypogeusia). They reported that 23 out of 73 patients suffering from a variety of diseases treated with a chelating agent, D-pencillamine, manifested hypogeusia and developed copper deficiency. On the other hand, four out of 100 patients with Wilson's disease who were treated with D-pencillamine manifested hypogeusia. Copper administration to these patients with impaired taste acuity resulted in improvement of

hypogeusia, indicating that the chelating agent had depleted their copper supply. Furthermore, administration of Ni^{++} and Zn^{++} improved the taste acuity in patients with hypogeusia (Henkin and Bradley, 1970).

Hambidge et al. (1972, 1976) indicated that impaired taste acuity is a typical feature of zinc deficiency in children. Dietary supplementation of zinc sulphate (0.1 - 4.0 mg/kg body weight) reduced the hypogeusia in these children. In a single blind study involving 103 patients with hypogeusia, Henkin et al. (1974) reported that taste acuity improved with zinc therapy. Lasser microprobe studies (Henkin et al., 1974) revealed that zinc was found in the vallate papilla in association with strontium, barium and phosphorus. Furthermore, they showed that analysis of taste buds from patients with untreated idiopathic hypogeusia had no measureable zinc. In contrast to this, patients treated with 100 mg Zn/day recovered from hypogeusia and had measurable zinc in their taste buds.

Henkin et al. (1976) conducted a double blind study involving 106 subjects on the effect of placebo and zinc sulphate on smell and taste dysfunction, and reported that there were no significant differences between their taste acuity at the beginning of treatment and after 3 or 6 months of treatment for any group. These contradicting reports stem from the fact that hypogeusia could be caused by several aetiological factors, and one of these factors is zinc deficiency. Treatment with zinc compounds of hypogeusia caused by poor oral hygiene will be ineffective as that of

placebo. Zinc supplementation in older women (41-78 years) significantly increased the zinc content of saliva, plasma, erythrocyte, but there was no significant difference in taste acuity (Buchanan et al., 1980). Daily zinc supplementation given to healthy, normal females for 6 days produced a transient rise in plasma zinc and may have improved the ability to taste sweetness in this population. Slight improvement in the detection threshold for sodium chloride and sucrose was reported in a double blind study of aged, institutionalized groups after 95 days of zinc supplementation. However, recognition threshold for sodium chloride and sucrose were unaffected in this population (Greger and Geissler, 1978).

Henkin et al. (1975) isolated a zinc protein from subjects with normal taste acuity. The protein had a molecular weight of 37,000, and was composed of 8% histidine residues and had 2 moles of zinc per mole of protein. Shatzman and Henkin (1980) showed that patients with hypogeusia have approximately 1/5 as much gustin (the major zinc protein in saliva) and fraction II zinc (the fraction in which gustin is found on gel chromatography) although total parotid saliva zinc may be only 1/2 that of normal. Treatment of such patients with exogenous zinc for nine days improved the saliva zinc and gustin status as well as taste function.

Animal studies indicates that zinc deficient rats develop a strong preference for sodium chloride (Hastings, 1980; McConnell and Henkin, 1974) as compared to pair-fed or ad libitum-fed controls. In addition to increased preference for NaCl, zinc deficient rats showed significantly higher preference for

3.0×10^{-2} sucrose, 1.3×10^{-6}M quinine sulfate and 2.5×10^{-3}M hydrochloric acid as compared to controls (Catalanotto and Lacy, 1977). In an attempt to correlate the physiological alteration and pathological changes due to zinc deficiency, Catalanotto and Nanda (1978) studied the histology of zinc deficient rat tongue including the taste buds. They found that the epithelial cell lining the papilla appeared in disarray and exhibited loss of basal cell polarity. The taste buds appeared smaller, and the individual cell detail composing the taste buds was obscured. Mann et al. (1974) indicated that in zinc deficient lambs, the outer taste pores were not patent and the inner taste pores were small or vistigial. Taste cells were relatively few in number and in some instances appeared to be absent. Catalanotto (1978) suggested the depletion of zinc can lead to decreased taste acuity, but decreased taste acuity is not necessarily associated with depletion of zinc.

Zinc Metabolism

Absorption Studies

Absorption of zinc takes place mainly in the small intestine. Antonson et al. (1979) using *in vivo* intestinal perfusion in the rat provided evidence that 60.1% of dietary zinc is absorbed in the ileum, 20.2% in the jejunum and 19.1% in the duodenum. Evans (1976) proposed a mechanism for zinc absorption, whereby the pancreas secretes a zinc binding ligand (ZBL) into the intestinal lumen, which binds zinc. Then, the zinc-ligand complex is

transported through the microvilli into the epithelial cells, and the zinc is transferred to zinc binding sites on the basolateral plasma membrane. Metal-free albumin interacts with the binding site and removes zinc from the basolateral plasma membrane. Availability of metal-free albumin at the basolateral membrane regulates the amount of zinc that enters the body.

The ZBL was described by Hahn and Evans (1973) as a low molecular weight (<15,000 dalton) protein. Later, they characterized it as a peptide (Evans and Hahn, 1975). Further purification of the ligand led to the isolation of N, N, N^1-trimethyl-1,2-ethanediamine (Hahn et al., 1976). Song and Adham (1978) indicated that ZBL isolated from rat intestine is a prostaglandin E_2 (PGE_2). Further investigations concerning various PG effects on zinc transport across rat small intestine in vitro showed that PGE_2 and PGF_2 act as physiological regulators of zinc transport in the intestinal mucosa and that the PG effects are specific (Song and Adham, 1979).

ZBL in human milk was isolated and characterized as pyridin-2-carboxylic acid (picolinic acid), a bidentate chelating ligand which facilitates zinc absorption from the intestine (Evans and Johnson, 1980). The piconilic acid concentration in human milk is 38 µg/ml. However, Lönnerdal et al (1980) presented evidence that ZBL in human milk was neither prostaglandin, nor picolinic acid, but citrate in a concentration of 0.99 ± 0.12 mM in human milk ultrafiltrate.

According to Cousins (1979) zinc can enter intestinal cells from either the lumen or vascular supply (Smith et al., 1978). Transport across the brush border membrane into intestinal cells probably requires energy (Kowarski et al., 1974) and is related to an ATP-dependent system.

Many dietary factors can influence zinc absorption (Underwood, 1977), i.e., high copper levels reduce zinc absorption (Evans et al., 1974). Other divalent ions such as calcium, iron, cadmium, and chromium may act as antagonists of zinc, presumably competing with zinc for binding sites during absorption (Underwood, 1977). Phytate (inositol hexaphosphate) impairs zinc absorption in humans and animals (O'Dell and Savage, 1960; Reinhold et al., 1976). The bioavailability of dietary zinc seems to be related to the phytate/Zinc ratio (Morris and Ellis, 1980), as well as the level of dietary calcium. Morris and Ellis (1980) observed that growth of rats was not affected by a phytate/zinc ratio of 12 when dietary zinc and calcium were present at 10-12 μg/gm and 0.75%, respectively, but growth was depressed at ratios greater than six if level of calcium increased to 1.75%. Furthermore, they observed that higher phytate/zinc ratio did not depress growth if dietary zinc intake was increased also.

Recent studies indicate that high fiber intake is deleterious to zinc bioavailability (Reinhold et al., 1976). They reported that two men developed negative balance of calcium, magnesium, zinc and phosphorus, after consuming a high fiber diet in the form of bread made partly from wheaten wholemeal for 20 days. The negative

balances of the elements appeared to be due to increased fecal excretion.

Geophagia had been associated with zinc deficiency in Iranian and Turkish subjects (Prasad 1960; Cavdar and Arcasoy, 1972, 1977), therefore it was suspected that clay ingestion might interfere with zinc absorption. Animal experiments provided evidence that leached clay ingestion by zinc deficient rats was a beneficial and life saving measure. Leaching the clay with 3N HCl reduced the CaO from 25.4% to 5.5%, and increased the available zinc from 74 ± 14 to 112 ± 16 μg Zn/gm (Smith and Halsted, 1970). Furthermore, Halsted et al. (1974) deduced that the Iranian subjects had sought zinc through the ingestion of clay. The role of geophagia in zinc metabolism is not entirely clear at present. However, it is believed that the excessive amount of phosphate in the clay may make both dietary iron and zinc unavailable for absorption in man.

Intermediary Metabolism

Absorbed zinc is transported to the liver via the portal plasma bound to transferrin and albumin (Evans and Winter, 1975; Smith and Cousins, 1979). In venous plasma, zinc is mostly bound to albumin and to a lesser extent, to transferrin and α_2-macroglobulins (Underwood, 1977). Aamodt et al. (1979) reported that one hour after an intravenous injection of ^{69}Zn, plasma ^{69}Zn concentration decreased to less than 4% of the total injected dose, and after five hours, to less than 2%. The activity continued to decrease slowly, falling to nearly 1% by day four. Within 15

minutes, liver uptake represented 50% of the total injected ^{69}Zn. It increased to a maximum value of 60% at two hours, then decreased slowly. Thigh activity, representing muscle and bone uptake, reached 4.2% of the total injected dose by the fifth day. Oral administration of ^{69}Zn showed a distribution retention pattern similar to the intravenous administration. However, total body activity decreased more rapidly with oral administration than after intravenous administration, probably reflecting the rapid loss of unabsorbed activity from the gastrointestinal tract (Aamodt et al., 1979). These data confirmed earlier studies with ^{65}Zn (Spencer et al., 1976). In animals, intravenous injection of ^{65}Zn initially appeared in liver, then later the activity appeared in other tissue (Oh et al., 1978). Thus, liver seems to be the major organ involved in zinc metabolism (Underwood, 1977). Zinc in liver cytosol is associated with zinc binding protein (thionein), first isolated by Margoshes and Vallee (1957) from horse kidney and later characterized by Kagi and Vallee (1961). Metallothionein has been isolated from human kidney (Pulido et al., 1966), human fetal liver (Riordan and Richard, 1980), and other animal tissues (Cherian, 1977; Chen et al., 1977; Saylor et al., 1980). It has a molecular weight of 9,000-10,000 daltons (Johnson and Evans, 1980; Ogiso et al., 1979; Saylor et al., 1980), exhibits low absorbance at 280nm, has a high cystine content (30%), and no aromatic amino acids. Metallothionein probably plays an important role in hepatic storage and intrahepatic mobilization of dietary zinc (Cherian, 1977) and detoxification of metals such as cadimum (Kagi and Vallee, 1961).

However, Chen et al. (1977) indicated that one of the possible biological roles of metallothionein involves accumulation of excessive zinc, and as storage protein for zinc.

Excretion

Zinc is excreted mainly through feces. Fecal zinc consists mostly of unabsorbed dietary zinc with small amounts of endogenously originating zinc secreted by the small intestine (Underwood, 1977). Aamodt et al. (1979) reported that in 17 patients the urine-to-fecal ^{69}Zn ratio on the fifth day was 0.44 when zinc was administered intravenously and 0.018 by oral administration.

The urinary zinc excretion for a healthy individual is small ranging from 0.3-0.6 mg/day (Underwood, 1977). However, increased urinary zinc excretion has been reported in post alcoholic hepatic cirrhosis (Vallee et al., 1957) although these patients had low serum and hepatic zinc concentration. Hyperzincuria also has been reported in nephrosis (Fiarhall and Hoyt, 1929), hepatic porphyria (Prasad et al., 1965), postoperative procedures and burns (Henzel et al., 1970) and EDTA administration (Perry and Schroeder, 1957; Apgar, 1977).

Considerable amounts of zinc can be lost in sweat. The values for man are 1.15 mg/liter for whole sweat and 0.9 mg/liter for cell free sweat (Prasad et al., 1963). More recently, Ritchey et al. (1979) reported that the mean daily loss of zinc through sweat in preadolescent girls, ages seven to nine years, was 1.43 mg.

Negligible amounts of zinc are lost in the menstrual fluid. Underwood (1977) estimated zinc loss to be 450 μg/period. Lower estimations were reported by Hess et al. (1977).

Zinc Deficiency in Humans

Zinc deficiency was suspected for the first time in modern medicine by Prasad and his associates (1961) in Iranian males. The clinical syndrome consisted of iron deficiency anemia, hypogonadism, hepatosplenomegaly, and dwarfism (Prasad et al., 1961). Two years later, Prasad and coworkers documented such a syndrome in Egyptian patients due to zinc deficiency. None of the Egyptian patients practiced geophagia, but they suffered from parasitic infestations such as hookworm and schistosomiasis (Prasad et al., 1963). It is interesting that a similar syndrome was described 70 years ago as hookworm infantilism in a 22 year old white male from Biloxi, Mississippi (Lemann, 1910). The dietary histories of both Iranian and Egyptian patients were quite similar. The diet consisted mainly of bread and negligible amounts of animal protein. Biochemical investigations indicated that zinc concentrations in plasma, red cell and hair were consistently low in the dwarfs as compared with the normal subjects (Prasad et al., 1963). Furthermore, ^{65}zinc studies in these patients revealed that plasma zinc turnover was greater, the 24 hour exchangeable pool was smaller, and the excretion of zinc in stool and urine was less than for the control subjects (Prasad et al., 1963).

With zinc supplemention, the Egyptian patients exhibited a greater rate of growth than those supplemented with iron or animal protein diet alone. The increased growth was accompanied by appearance of pubic hair, normal genital size and development of secondary sexual characteristic (Sandstead et al., 1967). Similar observations were reported for zinc supplemented Iranian patients and malnourished school boys (Halsted et al., 1972; Ronaghy et al., 1974). Halsted et al. (1972) reported that zinc supplementations to male and female dwarfs greatly enhanced the growth and hastened the onset of sexual function as manifested by nocturnal emission in men and the first menstrual activity in females.

Hambidge et al. (1972) reported that a number of children living in Denver had hair zinc concentration less than 70 μg/gm, or more than 3 SD below the normal adult mean, with history of poor appetite and hypogeusia. Supplementation of 0.2-0.4 mg zinc/kg body weight per day normalized taste acuity, and increased hair zinc concentration.

Clinical manifestation of zinc deficiency is an established nutritional problem in many parts of the world. Probably sub-clinical human zinc deficiency is a worldwide frequently unrecognized health problem.

Acrodermatitis enteropathica (AE) is an autosomal recessive defect characterized by chronic diarrhea, alopecia, dermatitis and neuropsychiatric symptoms which develop in infants at weaning (Brandt, 1936). High doses of diiodohydroxyquin, a powerful chelating agent was discovered to be effective in the treatment of

AE by enhancing the absorption of zinc in gastrointestinal tract (Dillaha et al., 1953). Prior to that AE had been successfully treated with human milk (Sturtevant 1980), probably due to the presence of zinc binding ligand (Hurley et al. 1977). Almost twenty years later, Moynahan (1974) provided evidence that AE is an inherited zinc deficiency disease and recovery could be achieved with zinc supplementation (Moynahan and Barnes, 1973; Moynahan, 1974).

Klingberg et al. (1976) reported in detail the development of zinc deficiency in a male patient treated with penicillamine for Wilson's disease. The patient developed acral and periorificial parakeratosis, alopacia, keratitis and centracecal scotoma. Zinc deficiency was demonstrated in plasma, red blood cells, and hair. It is interesting to note that the patient's father, who was a farmer, observed the similarity between skin and hair involvement of the patient and swine parakeratosis due to zinc deficiency.

Human zinc deficiency has been associated with long-term total parenteral nutrition (TPN) (Kay et al., 1976; van Volten and Bos, 1978; Sivasubramanian and Henkin, 1978, Latimer et al., 1980). Patients developed clinical signs and symptoms reminiscent of AE, i.e., increased renal clearance of zinc, low zinc concentration in TPN solution, and increased tendency for zinc loss from chronic diarrhea (Kay et al., 1976; Latimer et al., 1980). Green (1977) suggested that intravenous supplements ranging between 0.02-0.04 mg Zn/kg/day would be sufficient to meet the zinc requirement of patients during TPN.

Low serum zinc concentration and other clinical indices of zinc deficiency have been described in patients with a variety of clinical disorders including alcoholic liver cirrhosis, nephrotic syndrome (Vallee et al., 1957, Lindeman et al., 1978), Crohn's disease (Solomons et al., 1977; McClain et al., 1980) and burns (Henzel et al., 1970, Cohen et al., 1973). Sandstead et al. (1976) listed a comprehensive list of various clinical disorders and the possible mechanisms for associated zinc deficiency in these situations.

Recently, marginal zinc deficiency by dietary means had been produced in four human volunteers (Prasad et al., 1978). Daily zinc intakes were 2.7 mg for two subjects, 3.5 mg for the other two subjects. All subjects showed significant decreases in plasma, red blood cells and leukocyte zinc concentration during restricted zinc intake. Plasma alkaline phosphatase and alcohol dehydrogenase activity, and tissue thymidine kinase activity decreased during restricted dietary zinc intake. However, the activity of plasma ribonuclease was increased during this phase of the experiment. Plasma ammonia concentration appeared to increase during zinc restriction. Other parameters evaluated during restricted zinc intake were total protein, total collagen and RNA:DNA ratio in subcutaneous implant sponges, all of which decreased. All these parameters showed complete reversal after supplementation of 30 mgZn daily.

Human zinc deficiency has been encountered in many clinical situations, TPN, AE, penicillamine therapy, and accompanied by

biochemical alteration and clinical manifestation, all of which can be reversed to normality by zinc therapy.

Zinc Deficiency in Animals

Zinc deficiency has been produced in rats (Todd et al., 1934), pigs (Tucker and Salmon, 1955), guinea pigs (Hsieh and Navia, 1980), chickens (Morrison and Sarett, 1958), squirrel monkeys (Macapinlac et al., 1967), and rabbits (Joseph et al., 1981).

The signs and symptoms of zinc deficiency in animals include anorexia, dermal lesions, alopecia, ocular lesions, testicular atrophy, retarded growth, and loss of weight. All these clinical manifestations are rapidly reversible with zinc therapy with the exception of testicular atrophy.

Zinc is an essential trace element to the well-being of plants, animals and human beings; however, it can act as a teratogenic agent when given in excess or in insufficient quantities during gestation in experimental animals.

Extremely low zinc concentrations in diets given to Sprague-Dawley female rats from the time of weaning to maturity resulted in complete failure to reproduce and severe disruption of the estrous cycle, and incapability to mate (Hurley and Swenerton, 1966). Rearing the same rat strain on a marginally zinc deficient diet from weaning to maturity resulted in mating, but 99% of the implantation sites were affected either by resorption or severe malformation, and in smaller litters than pair-fed or ad libitum zinc sufficient control. Offspring of zinc deficient mothers

weighed much less than controls and 98% showed gross congenital malformations. Grossly, they exhibited head anomalies, clubbed feet and fused short lower jaws. Skeletal examination after staining with alizarin red revealed fusion of the ribs, pronounced curvature of the spinal column, missing vertebrae in the tail, incomplete or retarded ossification in ribs and vertebrae, short or missing long bones, absence of ossification centers in the digits, doming of the skull and poor cranial bone ossification. The incidence of skeletal anomalies ranged between 28% for short or missing lower jaws, to 83% for curly or stubby tail, and 34% cleft palate. Soft tissue malformations manifested in the brain, eyes, heart, lungs and urogenital structures (Hurley and Swenerton, 1966).

When a zinc deficient diet (0.2-0.5 μg Zn/gm) was given to rats during the entire period of gestation (0-21 day), it caused resorption in 41% of the implantation sites, and the full term fetuses weighed almost half as control rats. Eighty percent of the fetuses exhibited gross malformation. Reducing the deficiency period to the first 14 days of gestation resulted in a 76% reduction in fetus malformations. Further shortening of the deficiency period to the first 12 days of gestation resulted in a 56% incidence of malformed offspring, and when the deficiency period was limited to the first 10 days of gestation, there was a noticeable decline in malformation observed to a level of 22% (Hurley et al., 1971).

Zinc supplementation after the first 12 days of maternal zinc deficiency prevented palatal anomalies, but failed to prevent occular malformations. The latter malformation was avoided by earlier zinc supplementation at the 10th day of gestation (Hurley et al., 1971).

Burch and Sullivan (1976) observed that the most frequent congenital anomalies associated with zinc deficiency in descending order of occurence were tail anomalies, clubbed feet, fused or missing digits (syndactyly), hydrocephalus and hydrencephalus, urogenital abnormalities, scoliosis and kyphosis, lung abnormalities, small or missing eyes, short or missing mandibles, hernias, and heart anomalies.

Zinc deficiency during the last week of gestation resulted in anorexia and weight loss of the pregnant dams (McKenzie et al., 1975), which substantiated earlier findings (Hurley et al., 1971). Fetuses from such dams displayed intrauterine growth retardation and smaller brains, earlier occurrence of anorexia with high severity associated with a very low level of zinc intake (Fosmire et al., 1977). Most of the offspring of zinc deficient Sprague-Dawley rats showed poor survival, which could not be attributed to inadequate postnatal zinc intake. Hurley and Mutch (1973) suggested that this poor survival might be attributed to congenital abnormalities or failure of the pups to suckle due to weakness at birth.

The outcome of the zinc deficiency during the suckling period in rats was a depression of normal growth, in part due to inanition experienced by the dams, and smaller brains with a reduction in

cell population of the forebrain in comparison to controls (Fosmire et al., 1975). Such central nervous system (CNS) changes were induced by zinc deficiency in the latter third of gestation in Wistar rats (Sandstead et al., 1975).

Congenital malformation of CNS can be produced in rats by subjecting pregnant females to a short period of dietary zinc deficiency at early stages of gestation (Warkany and Petering, 1972 and 1973; Sandstead et al., 1977; Hurley and Shrader, 1972). Histological examination of such fetuses revealed absence of eye balls and optic nerves, failure of the cranial bone to cover the forebrain and meninges, open neural tube (Warkany and Petering, 1972) and severe spinal cord disorganization (Hurley and Shrader, 1972). Biochemically, these CNS anomalies are manifested as a reduction in brain DNA, RNA and protein, and cerebellar lipid alleviation. In behavioral terms, these animals displayed impaired maze running as adults and shock avoidance behavior less well than controls (Halas and Sandstead 1975).

Stevenson et al. (1966) indicated that there is a high incidence of CNS malformation among Egyptians living in Alexandria (7.88/1000 births), and Damyanov and Dutz (1971) pointed out that Iranians living in Shiraz have a high incidence of anencephaly (1.6/1000 births). Sever and Emanuel (1972) suggested that this high incidence of CNS malformation in these two location might be related to zinc deficiency, which is a well-established, documented, nutritional deficiency in these two countries (Prasad et al., 1966; Prasad et al., 1963; Halsted et al., 1972; and Sandstead et al., 1967).

Difficulties, stress and long parturition have been associated with zinc deficiency in Sprague-Dawley rats (Apgar, 1968 and 1972). Zinc must be supplied through day 18 of pregnancy to avoid prolonged and difficult parturition in the rat. Earlier zinc deprivation caused moribundity at parturition. Apgar (1977) described the effect of low zinc diet and established that the requirement of this element for normal parturition was the same for Sprague-Dawley and Long Evans rats. There were no detectable differences in the degree of stress during parturition between the unsupplemented females of the two strains. An injection of 900 μg zinc on day 18 of gestation resulted in normal parturition in both strains.

Dietary administration of EDTA (2-3%) to Sprague-Dawley rats induced several congenital abnormalities similar to dietary zinc deficiency in pregnant rats. Swenerton and Hurley (1971) suggested that the several effects of EDTA might be due to an induced deficiency of zinc. The teratogenic action of EDTA was prevented by 100 μg/gm zinc supplementation in the diet. It is possible that the preventive effect of zinc might be due to binding of EDTA in the intestine, so that the chelating agent (EDTA) is unable to complex divalent essential elements. Similar congenital abnormalities have been reported recently in rats after oral administration of dithiocarbamates. These abnormalities can be reduced progressively by simultaneous administration of increasing amounts of zinc acetate. Dithiocarbamate may act as a chelating agent, like EDTA, trapping zinc and inducing its deficiency (Larsson et al., 1976).

Dietary zinc deficiency in pregnant rats from the first day of gestation to the 19th day was reported by Bell et al. (1975) to cause chromosomal anomalies in bone marrow of dams and liver cells of the fetuses. The most frequently observed chromosomal aberrations were gaps and terminal delations.

Schlicker and Cox (1968) investigated the effect of high zinc concentration in the diet of female rats on fetal development. These investigators pointed out that growth in terms of dry matter or variable degree of death and resorption occurred in fetuses from mothers fed 0.4% zinc. Complete resorption of 15 and 16 day-old fetuses occurred in mothers fed the high zinc diet for 21 days before breeding. Fetal development was normal when the mother's zinc intake was reduced to 0.2%.

Diamond and Hurley (1970) investigated the histology of full-term zinc deficient fetuses with a high incidence of congenital malformation. They reported that the anomalies consisted of stunting and tissue defects devoid of necrosis, inflammation, scarring or calcification. The severe stunting seen grossly was not associated with reduction in cell size. Instead, there was a reduction in cell population, which was well demonstrated in areas of endochondral bone formation. Microscopic examination of skin and gonads did not reveal any histological changes. A specific lesion was observed involving the esophageal and hypopharangeal areas, and to a lesser extent, the forestomach and undersurface of the tongue. Lesions were characterized by hypoplasia and dysplasia of the stratified squamous epithelium similar to the esophageal lesion characteristic of zinc deficiency in adult rats.

Zinc is an essential trace element for the well being of humans and animals. Zinc deficiency in laboratory animals leads to physiological, biochemical and pathological alterations. Loss of appetite, and reduced food intake, loss of body weight and failure to grow are early manifestations of a deficiency state. Reduced protein, RNA and DNA concentrations as well as other biochemical changes are constant consequences of zinc deprivation. Alopaecia, occular and dermal lesions and testicular atrophy are but few examples of the major pathological alterations.

CHAPTER III

MATERIALS AND METHODS

Animals

Timed pregnant Sprague-Dawley descended Crl:COBS rats were purchased from Charles River Laboratory, Wilmington, Massachusetts. Sexually mature male and female rats of the same strain were procured from ARS/Sprague-Dawley, Madison, Wisconsin.

Husbandry

Rats were housed individually in either suspended stainless steel cages or plastic tubs with stainless steel tops and hardwood chip bedding. Deionized water was provided ad libitum. Diet was provided ad libitum except for pair-fed rats, which received the same amount of diet consumed by zinc deficient rats on the previous day. Constant temperature (70-72°F) and humidity (50 percent) were maintained in the room with controlled lighting (12 hours light, 12 hours dark). Body weights were recorded at weekly intervals.

Anesthesia and Euthansia

Intramuscular injection of "Inovar-Vet" (Pitman-Moore, Inc., Washington Crossing, N.J.) was used as a short acting sedative with analgesic properties in caesarean section procedures and during

blood collection. Uthol solution (The Butler Company, Columbus, Ohio) administered intraperitoneally or carbon dioxide was used to sacrifice rats.

Blood Collection

Blood was collected from rats tails in 1.5 ml Eppendorf tubes containing heparin (Abbott Laboratories, North Chicago, Illinois) and centrifuged for 15 minutes in an Eppendorf Centrifuge 5412 (Eppendorf Geratebu, Hamburg, Germany). The serum supernatants were transferred with pasture pipets to small plastic tubes for further use and storage.

Diet

The composition of the basic zinc deficient gel diet (Table 1) is a modification of MIT #200 powdered diet (Navia et al., 1969). Lactoalbumin is the protein source and contained variable amounts of zinc as determined by atomic absorption spectropotometry (Model 251, Instrumentation Laboratory Inc., Lexington, Massachusetts). Therefore, the lactoalbumin was washed several times with deionized water to reduce its zinc content (Hsieh and Navia, 1980). If the zinc content was very high, the lactoalbumin was treated with 0.5% ethylenedinitrilotetraacetic acid (EDTA) for one hour, washed twice with deionized water, one-half hour each, treated again with 0.5% EDTA for one hour washed with deionized water four times for 30 minutes each and washed again for 20 minutes seven times or more with deionized water. Spot tests for EDTA (Fritz Feigl, 1966) were

Table 1

Zinc Deficient Diet MIT #200

Ingredient	gm/kg
Sugar	670.0
Lactoalbumin	200.0
Salt mix[1] (Zinc free)	30.0
Vitamin mix[2]	7.0
Choline, CH1	3.0
Cellulose (Alpha Cell)	40.0
Cottonseed Oil	30.0
Agar	20.0

[1]Salt mix was prepared in our laboratory, see Table 3.

[2]Vitamin mixture was prepared in our laboratory, see Table 3.

performed to ascertain that no EDTA was left in the protein. After washing, lactoalbumin was filtered, transferred to stainless steel trays and air dried in a hood for several days.

The gel diet was prepared as follows:

1. Dry, powdered ingredients were weighed and mixed thoroughly. Cottonseed oil was added and further mixing performed.
2. Agar was suspended in 500 ml of water in a stainless steel pot, and brought to boiling slowly, then mixed with the dry ingredients for a few minutes. At this stage, an aqueous solution of zinc sulfate was added to provide the desired zinc concentration in the diet.
3. The diet was transferred into plastic containers and refrigerated.

For caries studies, a moderate caries promoting purified powder diet MIT #305 (Navia et al., 1969) was offered to rats in glass jars. Table 2 shows the composition of this diet.

Analytical Methods

Serum Zinc Analysis

The serum zinc analysis used was essentially the same as that of Prasad et al. (1965) with a slight modification: 0.1 ml of serum was transferred to 5 ml disposable glass tubes, then 2.0 ml of 3% trichloro-acetic acid added to the sample, centrifuged and the supernatant transferred to disposable plastic tubes. Zinc determinations were performed by atomic absorption spectrophotometry.

Table 2

Caries Promoting Diet MIT #305

Ingredient	gm/Kg Diet
Confectionary Sugar	50
Cornstarch	620
Lactoalbumin	200
Cottonseed Oil	30
Cellulose (Alpha Cell)	60
Vitamin mixture[2]	10
Salt mixture[1]	30

[1]MIT Salt-mixture: contained the same ingredient as the salt in Table 3 plus 2.70 gm $ZnCo_3$.

[2]Vitamin mixture: contains the same ingredient as in Table 3.

Tissue and Diet Sample Zinc Analysis

A known amount of tissue sample was defatted, dried and ashed by the method of Niedermeier et al. (1971). Diets were not defatted, and the ash solutions were assayed for zinc by atomic absorption spectrophotometry.

Calcium and Phosphorus Analysis

Maxillary first molars were defatted, dried and ashed. Ash solutions were assayed for calcium by atomic absorption spectrophotometry, and phosphorus by a spectrophotometric method (Chen et al., 1956).

Dental Caries Model

Dental caries is a multifactorial disease involving microbial, host, and dietary factors (Navia and Narkates, 1980). Thus, it is imperative that all factors be present in the appropriate combinations for the disease to be expressed experimentally.

Caries Induction

Rats were weaned at 18 days of age and caged in groups of three in plastic tubs with stainless steel tops with sterilized hardwood chips bedding. All rats were fed moderate caries promoting diet MIT #305 in glass jars and deionized water was provided in glass bottles with stainless steel drinking tubes. On days 18 and 19, all rats were inoculated with S. mutans 6715, the degree of implantation tested by oral swabs on day 21 and the

recovery S. mutans were obtained on day 22. Further tests for the degree of S. mutans implantation were performed on day 40, before sacrifice by decapitation after Uthol IP injection.

Preparation of Mandibles

Heads were autoclaved for several minutes to facilitate defleshing of the mandibles. Lower jaws were removed, cleaned of soft tissues, stored in brown glass vials, and dried in a hood. Mandibles were stained with 10 ml murexide (Navia, 1979) for 24 hours. Stained jaws were removed, rinsed with distilled water, dried with paper towel, placed in small labeled envelopes, and left inside the hood overnight to dry.

Caries Score

Carious lesions in the buccal, sulcal, and interproximal molars surface were scored according to the procedure described by Keyes (1958).

Mesio-distal and Crown Height Measurement of Teeth

Mesio-distal distance of rat molar teeth and the crown heights of first molars were recorded with the aid of a stereomicroscope.

Histological Methods

All tissue were fixed in 10% neutral buffered formalin, processed in a Technicon and sectioned at 6μ thickness.

Hard tissues were decalcified in 22.5% formic acid-5% sodium citrate solutions. The end point of decalcification was tested chemically with 5% ammonium hydroxide and 5% ammonium oxalate (Luna, 1968). Decalcified tissues were washed with running water for 24 hours. Sections were stained routinely with H and E.

Statistical Methods

Means, standard deviations and standard error of the means were used for data reduction and descriptive purposes. Analysis of variance and Duncan multiple mean comparison test were done using Statistical Analysis System (SAS Institute, Raleigh, North Carolina), a computer software package in the computer facilities at UAB.

Table 3

Salt and Vitamin Mixtures for Diet MIT #200 and MIT #305

Salt Mixture	gm/kg of Mixture	Vitamin Mix	gm/kg of Diet
$CaHPO_4$	491.06	Thiamine	0.008
KCl	141.86	Riboflavin	0.006
NaCl	232.85	Pyridoxine	0.003
K_2SO_4	61.83	B_{12} (0.1%)	0.030
$MgSO_4$	19.82	Niacin	0.020
$MgCO_3$	27.28	Ca Pantothenate	0.030
Fe Citrate	18.00	PABA	0.200
$MnCO_3$	3.63	Inositol	2.000
$CuCO_3$	0.53	Biotin	0.0001
$ZnCO_3$ (1)	2.70	Menadione	0.003
KIO_3	0.05	Folic Acid	0.002
$AlCl_3 \cdot 6H_2O$	0.29	α-Tocopherol	0.100
$CoCl_2 \cdot 6H_2O$	0.09	Ascorbic Acid	0.100
		Vitamin A & D	0.040
		Choline	3.000
		Cellulose	4.458

(1) $ZnCO_3$ was omitted from all zinc deficiency experiments.

CHAPTER IV

EXPERIMENTAL RESULTS

Effect of Pregestational and Gestational Zinc Deficiency and Developing Embryos

Experimental Design

Thirty, sexually mature, female rats were divided into three groups, with three subgroups in group I. They were fed zinc deficient (0.6 μg Zn/gm) or zinc supplemented (50 μg Zn/gm) MIT #200 gel diet (Material & Methods) according to the schedule as shown in Table 4.

They were mated overnight with fertile males after the determinations of proestrus stage, and vaginal smears were examined the next morning for the presence of spermatazoa. Positive finding was considered as day "0" of pregnancy. Fetuses were recovered by cesarean section on the 20th day of gestation. The following parameters were evaluated: dam body weight and serum zinc concentration, fetal and dam liver zinc concentrations, fetal body weight, and transumbilical distances (TUD), Crown rump distances (CRD).

Table 4

Feeding Schedule for Female Rats

	Before Gestation		Gestation		
Groups	1st Week	2nd Week	1st Week	2nd Week	3rd Week
Experimental*** Group 1-A	-Zn*	-Zn	-Zn	-Zn	-Zn
Experimental Group 1-B	+Zn**	-Zn	-Zn	-Zn	-Zn
Experimental Group 1-C	+Zn	+Zn	-Zn	-Zn	-Zn
Pair Fed Group II (To Group I-A)	+Zn	+Zn	+Zn	+Zn	+Zn
Ad Libitum Group III	+Zn	+Zn	+Zn	+Zn	+Zn

*-Zn: Zinc deficient diet (containing 0.6 μgZn/gm diet)

**+Zn: Zinc sufficient diet (50 μgZn/gm diet)

***Six rats in each group and subgroup

Experimental Results

Mean Body Weight and Solid Diet Intake. All dams gained weight during the five weeks of the experiment (Figure 1); dams fed zinc deficient diet gained less body weight than controls, and their weight gain was inversely proportional to length of time of the deficiency (group I-A, 18 gram; group I-B, 21 gram; group I-C, 35 gram). Rats in the ad libitum group showed the maximum weight gain (76 gram), followed by rats in the the pair-fed group (49 gram). The mean body weights of ad libitum and pair-fed rats were not significantly different; however, the mean body weight of the ad libitum group was significantly higher than that of rats in groups I-A and I-B.

The solid diet intake for group I-A was monitored for 34 days, giving a mean intake of 13.8±2.9 ($\bar{\chi}$±SD) gram daily. The minimum intake, 3.6 grams, was recorded six days after the initiation of feeding the zinc deficient diet. Maximum diet intake was 18.4 gram.

Serum Zinc Concentration. Serum zinc concentration decreased from the basal line of 150 µg/100 ml to 30-60 µg/100 ml for all rats fed the zinc deficient diet after one week, and this low zinc concentration was maintained throughout the experiment (Figure 2). The mean serum zinc concentration of experimental rats was significantly lower than that of controls, ad libitum and pair-fed rats, but there was no significant difference between the pair-fed and the ad libitum mean serum zinc concentration.

Figure 1. Pregestational and gestational mean body weight of rats in experimental and control groups.

Pregestational and Gestational Mean Body Weight of Rats in Experimental and Control Groups

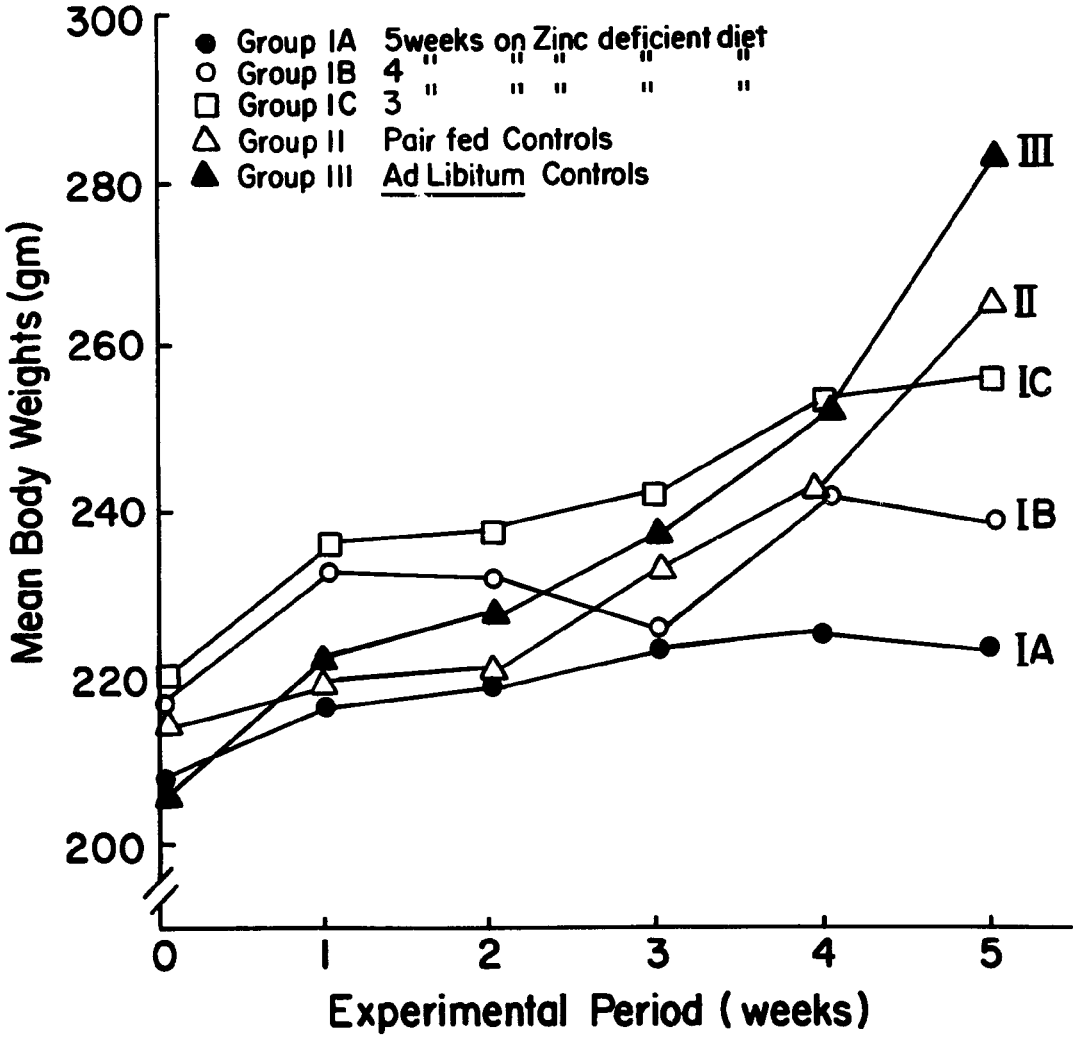
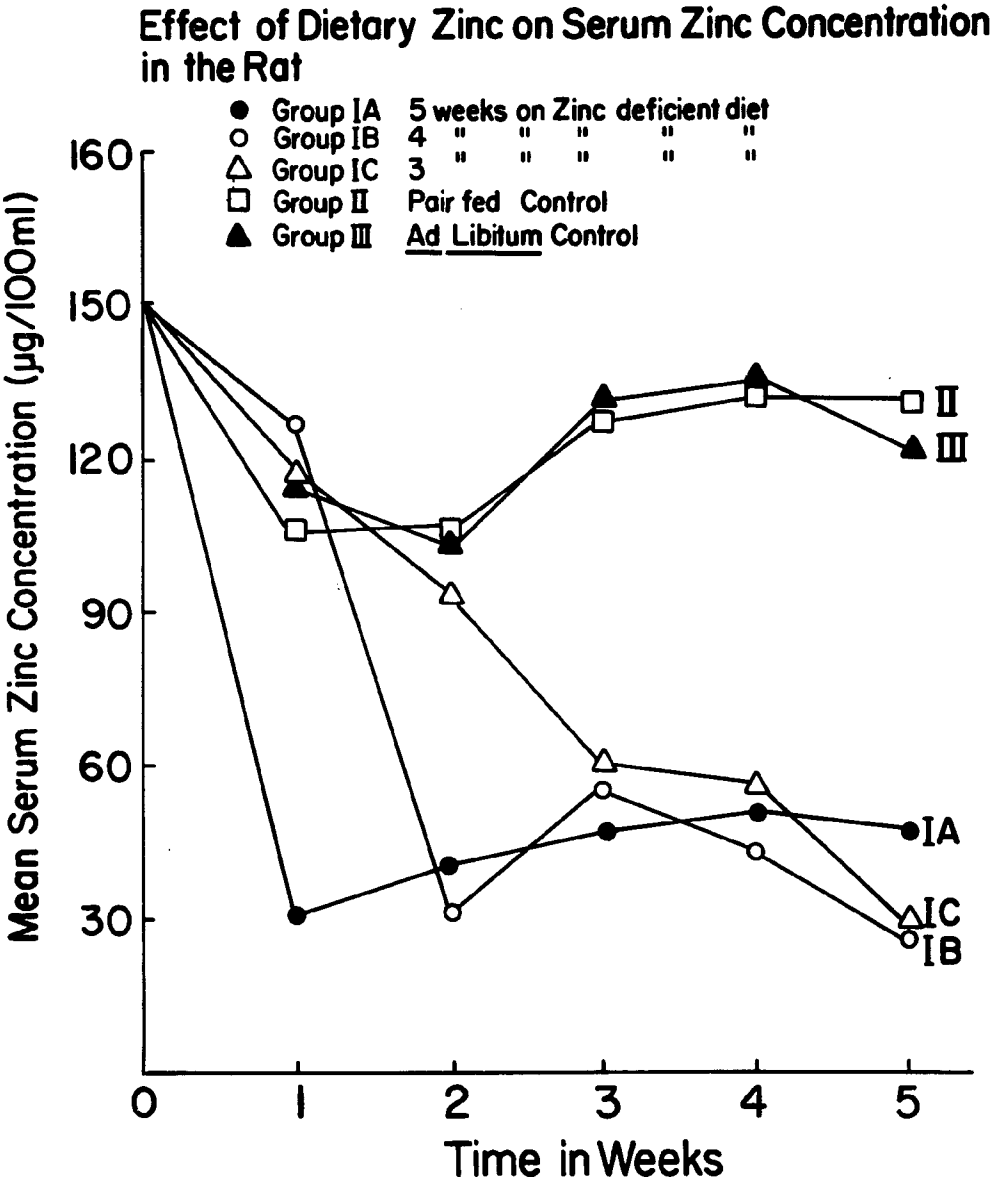


Figure 2. Effect of dietary zinc on serum zinc concentration in the rat.


Effect of Dietary Zinc on Serum Zinc Concentration in the Rat
Group IA 5 weeks on Zinc deficient diet
Group IB 4 " " " " "
Group IC 3 " " " " "
Group II Pair fed Control
Group III Ad Libitum Control
Mean Serum Zinc Concentration (µg/100ml)
160
150
120
90
60
30
0
1
2
3
4
5
Time in Weeks
II
III
IA
IC
IB

Mean Fetal Body Weight, Trans-umbilical Distance (TUD) and Crown-rump Distance (CRD). Statistical analysis of fetal weight indicated that there was a significant difference between mean weight of fetuses from zinc deficient dams and fetuses from both pair-fed and ad libitum dams (Table 5). Also, there was a significant difference between group I-A and I-C. Mean fetal weight of group I-B was not significantly different from group I-A or I-C. There was a significant difference in mean fetal weight between pair-fed and ad libitum groups.

There was also a significant difference in the fetal mean trans-umbilical distance and crown-rump distance between controls and experimental groups (Table 5). The controls, ad libitum and pair-fed, had greater TUD and CRD than rats in the experimental groups.

Liver Zinc Concentration. Fetal liver zinc concentrations in groups I-A, I-B, I-C, II and III were higher than the liver zinc concentrations for the dams in the same groups. The values were 180±56 μg Zn/gm dry weight, 128±24, 162±56, 231±51 and 245±44 ($\bar{\chi}$±SD) for fetal groups I-A, I-B, I-C, pair-fed and ad libitum respectively. However, liver zinc concentrations for the dams were 88.6±14 μg Zn/gm dry weight, 75.87±6.9, 92.0±3.4, 86±9.0 and 102.8±8.4 ($\bar{\chi}$±SD) for group I-A, I-B, I-C, pair-fed, and ad libitum, respectively (Figure 3).

Summary of Results. Feeding zinc deficient diets caused significant decline in serum zinc concentration, poor body weight gain, and intrauterine growth retardation as manifested by low body

Table 5

Physical Measurement of Twenty Days Fetuses

Group	Body Weight (gram)	CRD(mm)	TUD(mm)
I-A (35)	3.1±0.6*[c]	32.7±2.8[b]	10.7±1.1[b]
I-B (37)	2.9±0.4[d,c]	31.8±2.7[b]	10.5±0.9[b]
I-C (56)	2.8±0.5[d]	31.9±3.4[b]	10.2±1.1[b]
II (57)	3.7±0.5[a]	35.8±2.7[a]	11.5±1.0[a]
III (53)	3.5±0.3[b]	34.8±1.9[a]	11.3±0.7[a]

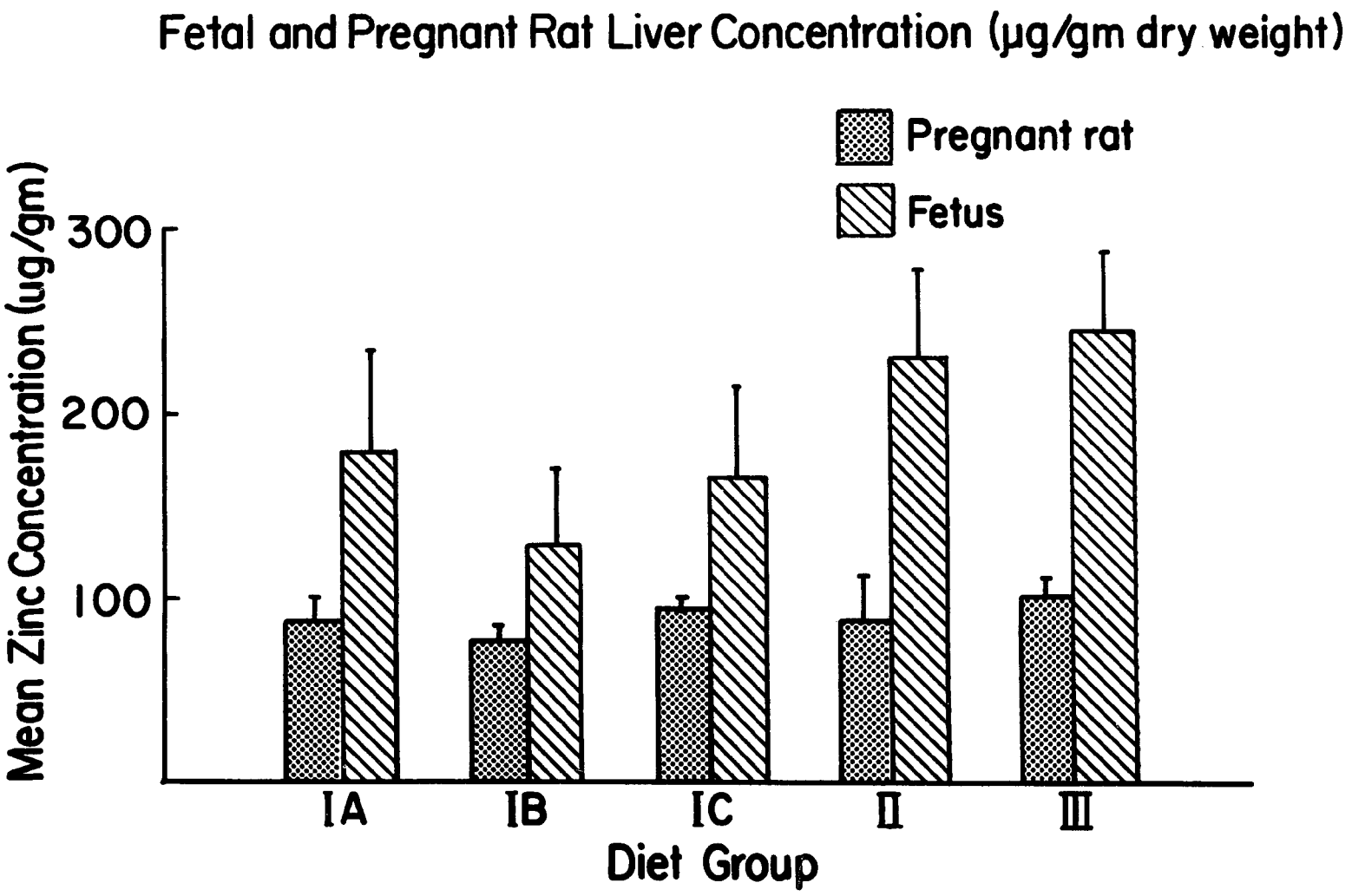
*Mean ± Standard Deviation.

Means with the same superscript in column are not significantly different. (P = 0.05).

Numbers in parentheses indicate number of rat fetuses per group.

CRD, Crown rump distance; TUD, trans-umbilical distance.

Figure 3. Fetal and pregnant rat liver concentration (μg Zn/gm dry weight).


Fetal and Pregnant Rat Liver Concentration (µg/gm dry weight)
Pregnant rat
Fetus
Mean Zinc Concentration (ug/gm)
300
200
100
IA
IB
IC
II
III
Diet Group

weight and shorter TUD and CRD. Fetal liver zinc concentrations in all groups were higher than the liver zinc concentrations for dams in the same groups.

Minimum Dietary Zinc Requirement During Last Week of Gestation and During 18 Days of Lactation

Experimental Design I

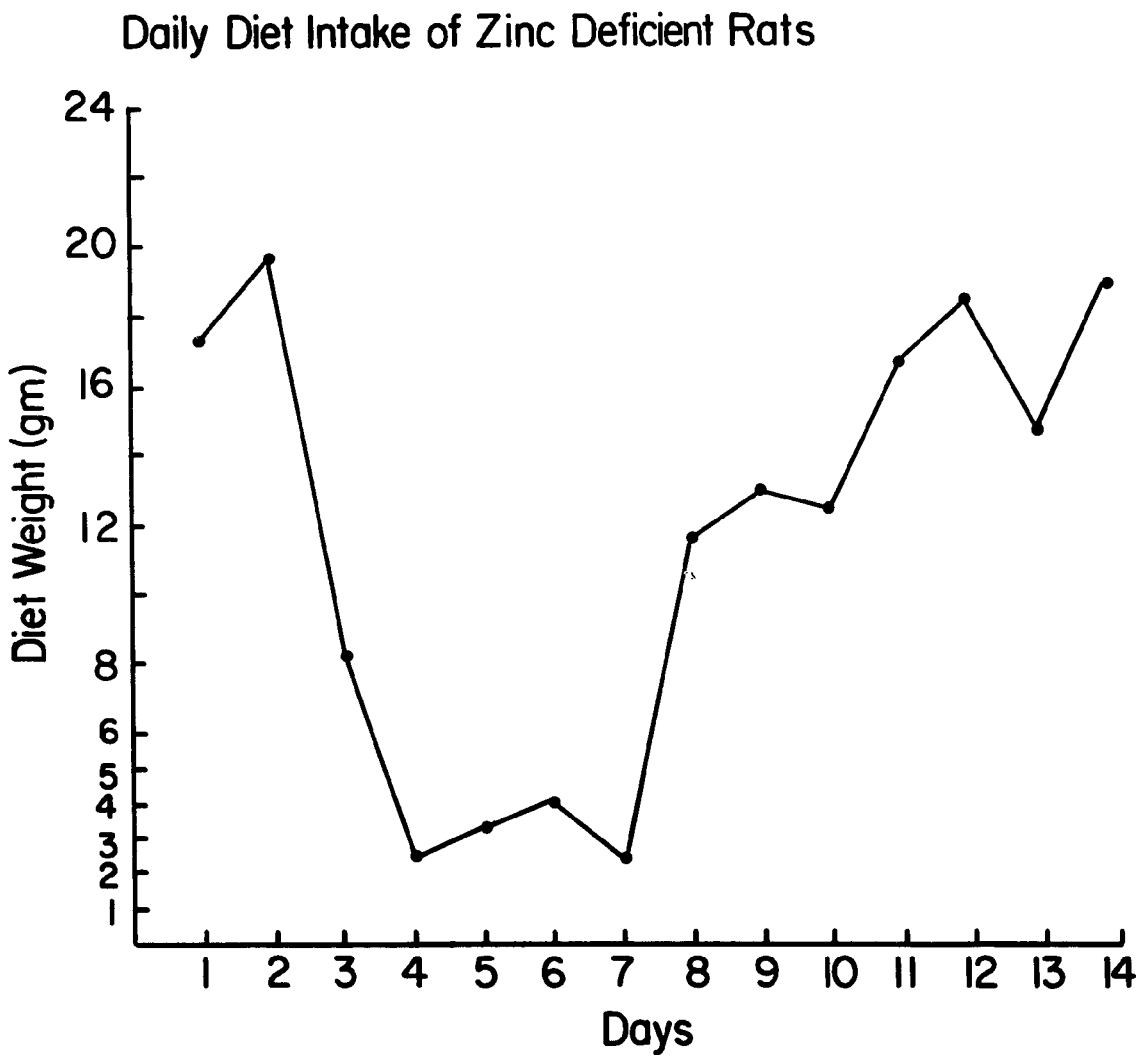
This experiment was designed originally to study the effect of zinc deficiency on the dental caries in rats; however, it only provided useful information on minimum dietary requirement for zinc. Twenty-one, timed pregnant rats (14 days) were randomly divided into three dietary groups (seven/group): group one was fed zinc deficient diet, MIT #200 in gel form (0.6 μgZn/gm), group two was pair-fed to group one with a zinc sufficient diet (50 μgZn/gm), group three was fed zinc sufficient diet ad libitum (50 μgZn/gm).

Dam body weights were monitored on a weekly basis and serum zinc concentrations were measured before the experiment and 36 hours after the first feeding of the experimental diet, then once weekly. Diet intake was measured daily.

Experimental Results I

Body Weight and Diet Intake. The mean solid intake for zinc deficient rats was 13.3±7.6 g ($\bar{\chi}$±SD), with a minimum and maximum intake of 2.5 and 26.9 gram, respectively (Figure 4). The lowest food intake was observed on the fourth and seventh day of experimental period which corresponds to two days before delivery and one day after delivery. The mean solid diet intake for the zinc sufficient ad libitum control was 22.1±2.7 gram.

Figure 4. Daily diet intake of zinc deficient rats.


Daily Diet Intake of Zinc Deficient Rats
Diet Weight (gm)
24
20
16
12
8
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days

All rats gained weight during the first four days of the experimental period (Figure 5). On the seventh day there was a significant difference in mean body weight, between groups ($P < 0.05$). The zinc deficient group had the lowest mean body weight (245 gm), ad libitum group had the highest (311 gm) and the pair-fed group was 270.5 gm. After delivery all rats lost weight, but the mean body weight remained significantly different between the groups. On days 14, 16, and 18 there were significant differences in mean body weights between the ad libitum group and the pair-fed and zinc deficient group, but there was no significant difference between the pair-fed and zinc deficient group.

Mortality Rate in Zinc Deficient Pups and Cannibalism Among Zinc Deficient Dams. A high mortality rate was observed among pups born to zinc deficient dams (Table 6). On day seven of lactation, the mortality rate reached 63.9% of the total number of pups. Cannibalism was prevalent among the zinc deficient dams. They destroyed 23 of their pups (31.9%) by the seventh day of lactation. Only three pups were alive by the end of the first week (4.1%). Thus the high mortality was due to behavioral changes in the dams, characterized by failure to clean and nurse the newly born pups, and cannibalizing them.

Serum Zinc Concentration. Mean serum concentration of zinc deficient dams dropped from 117.4 μg Zn/100 ml to 39.3 μg Zn/100 ml within the first two days after feeding the experimental diet. This low value was significantly different from that of pair-fed and ad libitum controls (Figure 6).

Figure 5. Dams body weight during gestation and lactation.

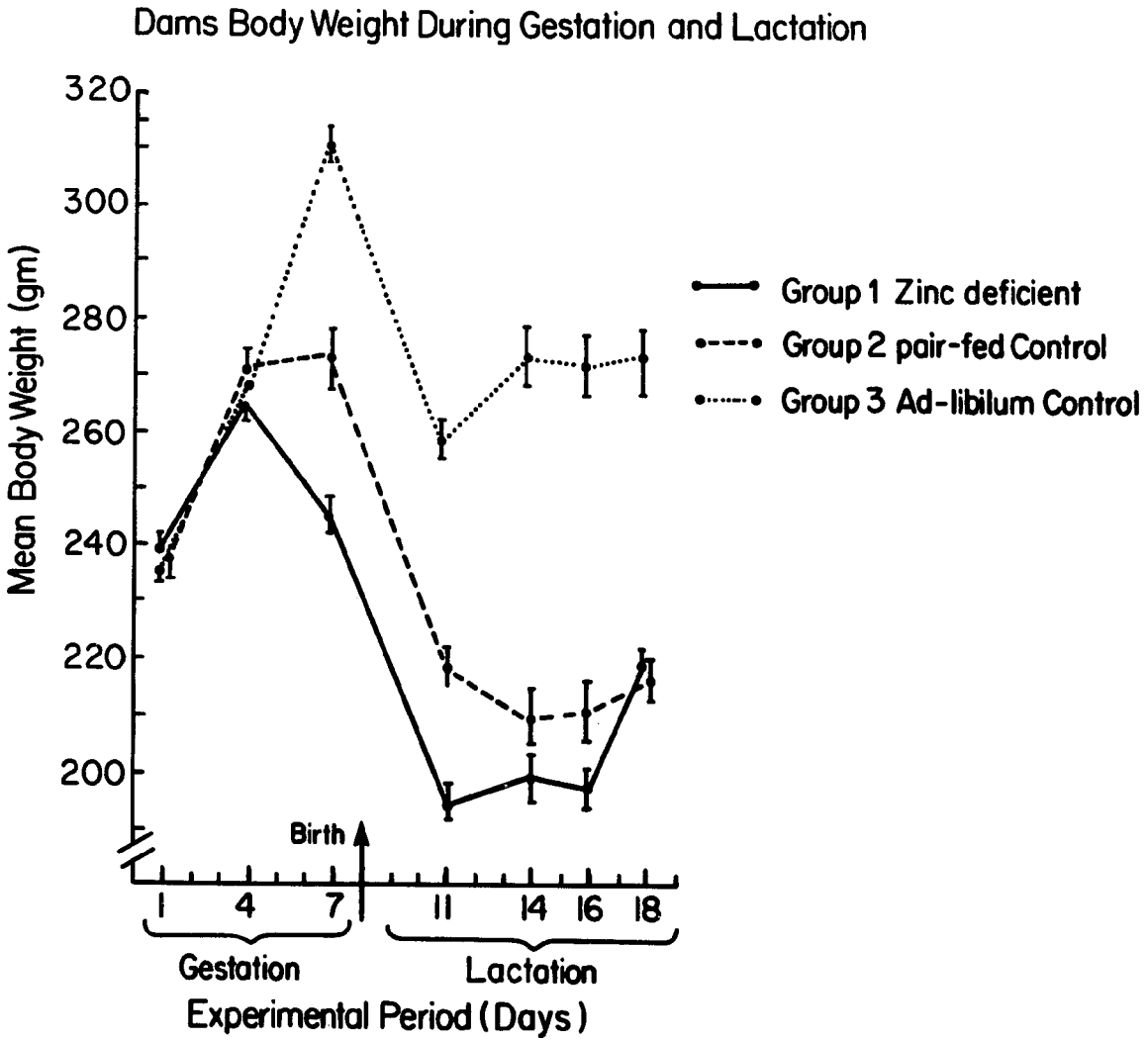

Dams Body Weight During Gestation and Lactation
Mean Body Weight (gm)
320
300
280
260
240
220
200
Birth
1
4
7
11
14
16
18
Gestation
Lactation
Experimental Period (Days)
Group 1 Zinc deficient
Group 2 pair-fed Control
Group 3 Ad-libilum Control

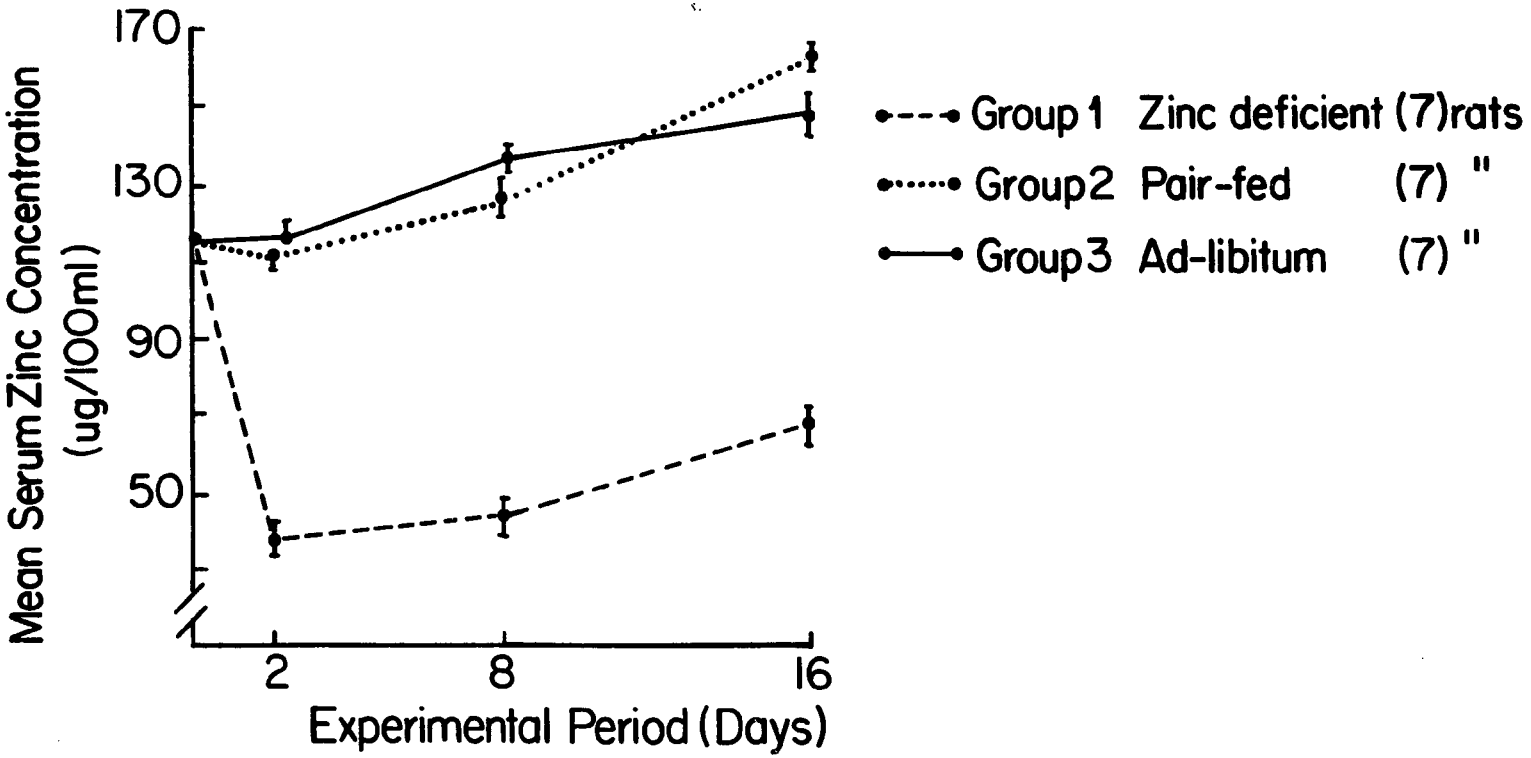
Table 6

Mortality Rate and Cannibalism of Pups Born to Zinc Deficient Dams

Age (days)	Living Pups		Dead Pups		Cannibalized Pups		Total	
	No. of Pups	%	No. of Pups	%	No. of Pups	%	No. of Pups	%
1	49	68.0	23	31.9	0		72	99.9
2	28	38.8	19	26.3	2	2.7		
3	21	29.1	0		7	9.7		
4	14	19.4	0		7	9.7		
5	7	9.7	0		7	9.7		
6	7	9.7	0		0			
7	3		4	5.5	0			
Total	3	4.1	46	63.9	23	31.9	72	99.9

Figure 6. Mean serum zinc concentration of experimental and control rats.

Mean Serum Zinc Concentration of Experimental and Control Rats



By day 16 the mean serum zinc concentration for the zinc deficient dams increased to 69.4 μg/ml, which was significantly lower than controls.

Summary of Experiment I Results. Feeding a diet extremely low in zinc to pregnant and lactating rats resulted in loss of appetite, significant decrease in zinc serum concentration within the first two days after feeding the experimental diet, poor body weight gain and very low survival rate, and high mortality rate of pups born to zinc deficient dams. These dams also tend to destroy their pups.

Experimental Design II

This experiment was designed to establish the minimum dietary zinc concentration which would induce a deficiency, but still support pup life until weaning at 18 days of age. Fourteen day pregnant Sprague-Dawley rats were assigned randomly into four groups and fed different concentrations of zinc according to the schedule as shown in Table 7.

Dams were housed individually in plastic tubs covered with stainless steel tops. Cages were changed every two days to minimize coprophagy and the build up of ammonia. All dams were provided with deionized water ad libitum. Forty-eight hours after birth pups were randomized within their respective group and litters reduced to seven pups. Pups were decapitated at 16 days of age.

Table 7

Experimental Design and Treatment of Pregnant and Lactating Rats

		Zinc Content of Diet MIT#200 (μg Zn/gm)	
Group	Number of Dams	Last Week of Gestation	18 Days of Lacation
A	3	2.0	0.6
B	3	2.0	2.0
C	3	4.0	4.0
D	2	6.0	6.0

Experimental Results II

Parturition. Dams in groups C and D gave birth at 1:20 pm of the expected day. Group B gave birth two hours later, and group A five hours later. Labor in groups A and B was accompanied by excessive bleeding.

Mortality, Survival and Cannibalism. Pups from groups C and D had a 100% survival rate (Table 8). Group A had a very poor survival rate (16.7%) with a high mortality rate (30.0%), and 53.3% of the pups were cannibalized by their dam. Group B had a better survival rate (48.3%) with a slightly lower mortality rate (27.5%) and fewer pups cannibalized (24.1%) by their mother than group A (Table 8).

Serum Zinc Concentration. Serum zinc concentrations for rats in groups A, B, and C were significantly lower than group D after two days of feeding the experimental diet (Table 9). On day 16, zinc concentrations were significantly different among all the groups reflecting the dietary zinc intake (Table 9).

Rat Pup Body Weights and Tibia Lengths. There was a significant difference in mean body weight between the groups (Table 10). Minimum body weights were recorded for group A, followed by group B, C, and D in ascending order. The longest tibias were those of group D (1.27±0.03 cm) followed by groups C, B and A in a descending order (Table 10) and these differences in mean tibia length were significant. Pups from groups A and B had rough, scanty fur, indicating zinc undernutrition.

Table 8

Mortality, Survival and Cannibalism Rates Among
Zinc Deficient Pups and Lactating Rat Dams

	Survival Rate		Mortality Rate		Cannibalism	
Group	No. of Pups	%	No. of Pups	%	No. of Pups	%
A*	5	16.7	9	30.0	16	53.3
B*	14	48.3	8	27.5	7	24.1
C**	21	100.0	0	0.0	0	0.0
D**	14	100.0	0	0.0	0	0.0

*Represent the total number of pups born

**Represent the number of pups after randomization

Table 9

Serum Zinc Concentration of Pregnant and Lactating Rat Dams

Diet Group	Serum Zinc Concentration After Initiating Experimental Diets (µg/100ml) Day 2	Day 8	Day 16
A (3)	59.57±13.64[b,1,2]	38.23±6.73c	42.58±4.72[d]
B (3)	53.6±15.47[b]	55.11±9.13[b]	64.98±4.61[c]
C (3)	65.39±13.67[b]	60.86±5.27[b]	79.32±5.08[b]
D (2)	102.76±6.32[a]	95.86±6.45[a]	99.32±6.68[a]

[1] $\bar{\chi}$ ± SD

[2] Values in the same column not sharing the same superscript letter are significantly different (P=0.05).

[3] Number between parentheses represent number of rats in each group.

Table 10

Body Weights and Tibia Lengths of 16 Day Old Pups

Group	Body Weight (gm)	Tiba Length (Cm)
A (5)	$10.2\pm0.4^{d*}$	0.94 ± 0.03^{d}
B (14)	15.1 ± 0.8^{c}	1.08 ± 0.03^{c}
C (21)	19.7 ± 2.6^{b}	1.13 ± 0.03^{b}
D (14)	28.7 ± 1.6^{a}	1.27 ± 0.03^{a}

Number between parentheses represents number of pups in each group.

*Means ±SD, values in the same column not sharing a common subscript letter are significantly different ($P < 0.05$).

Dietary Zinc and Dental Caries

Experimental Design

Zinc deficiency imposed preeruptively and posteruptively increases the incidence of dental caries in rats (Calhoun et al., 1979; Fang et al., 1980). While zinc deficiency was reported to be associated with increased incidence of caries in rat molars, none of these studies have used a model system in which zinc deficiency was imposed throughout tooth development.

This experiment is designed to quantitate the effect of zinc deficiency imposed during the last week of gestation and throughout lactation on the incidence of dental caries, and also on chemical composition and physical measurement of teeth.

Twenty-eight timed pregnant Crl:COBS rats of Sprague-Dawley strain, were divided randomly into five groups and fed diet MIT #200 in a gel form according to the schedule as shown in Table 11.

The pair fed control group D received the same amount of diet consumed by experimental group A in the previous day. Two days after delivery, pups were randomized within their respective groups. The size of litters was equalized to eight pups and litter weight recorded periodically. Dams in each group were rotated among the litters from day three to day 15.

At weaning sufficient numbers of pups were sacrificed and tissues dissected for chemical analysis. Pups were fed MIT #305, a moderately caries-promoting diet. All pups were challenged with Streptococcus mutans 6715 on days 18 and 19. Two days later, the oral cavity was swabbed to test the degree of bacterial

Table 11

Experimental Design and Treatment of Dams[1] for Caries Studies

Number of Dams	Group	Zn conc. (μg/gm) of diet fed during: Last Week of Gestation	18 Day of Lactation
7	Z.D. A	2	2
7	Z.D. B	4	2
5	Z.D. C	4	4
5	P.F. D	50	50
4	A.L. E	50	50

[1]MIT #200 Gel Diets containing different Zn concentrations fed to rats.

Z.D. - Zinc deficient groups.

P.F. - Pair-Fed group to Z.D. A group.

A.L. - Ad Libitum group.

implantation. At 40 days of age another test for the degree of S. mutans implantation was performed and the pups were killed on the same day (Table 12).

Mandibles were defleshed, stained with murexide (Navia, 1979), and mandibular molars scored for dental caries according to Keyes method (1958).

Experimental Results

Pregnant and Lactating Rats Body Weight Gains. There was no significant difference in mean body weight during the first week of the experimental period; meanwhile, all rats gained weight during this period. Group A gained the least amount of weight followed by groups B, C, D and E in ascending order. After delivery all rats lost weight, but group E, the ad libitum group, maintained a significantly higher body weight than pair-fed and experimental groups throughout lactation (Figure 7).

Litter Body Weight. Litter weights at two days of age were similar for all groups. At weaning, mean litter weight for group E control was significantly higher than for all other groups. The pair-fed control group D litter weight was significantly higher than the experimental groups A, B and C. Among the zinc deficient groups, mean litter weight for group C was significantly higher than for groups A and B (Figure 8).

Postweaning Rat Body Weight. From 23-40 days of age, mean body weight of ad libitum control group E was significantly higher than for all other groups. Group D, the pair-fed control mean body

Table 12

Experimental Design and Treatment of Pups

Days After Birth	Treatment
18	Weaned, fed moderately caries-promoting diet[1]
18, 19	S. mutans implanted
21	Swabbing the oral cavity to test implantation
40	Implantation test/Rats Sacrificed

[1]MIT #305 complete diet (powder form). This diet was used throughout the experimental period.

Figure 7. Mean body weight changes in zinc deficient and control rats during last week of gestation and 18 days of lactation.

Mean Body Weight Changes in Zinc Deficient and Control Rats
During Last Week of Gestation and 18 Days of Lactation

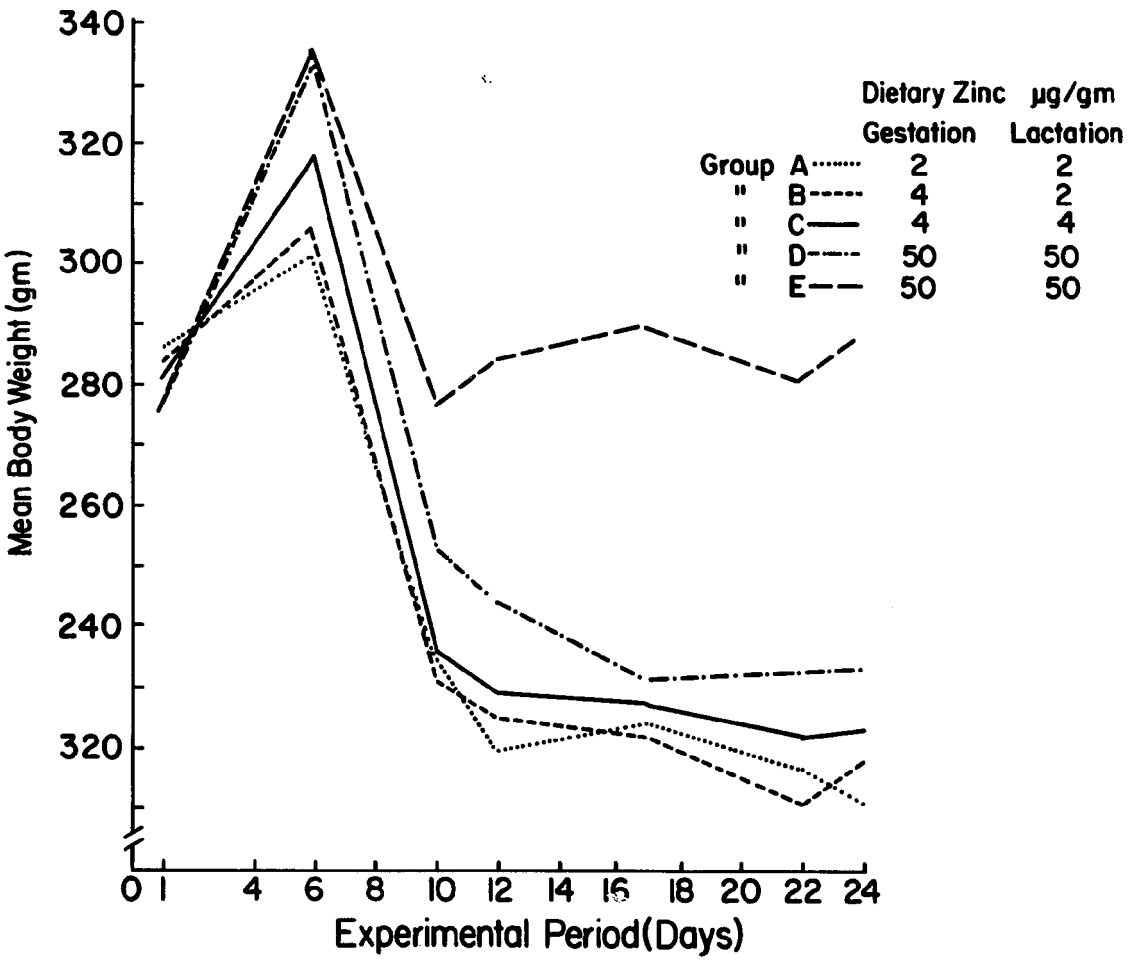
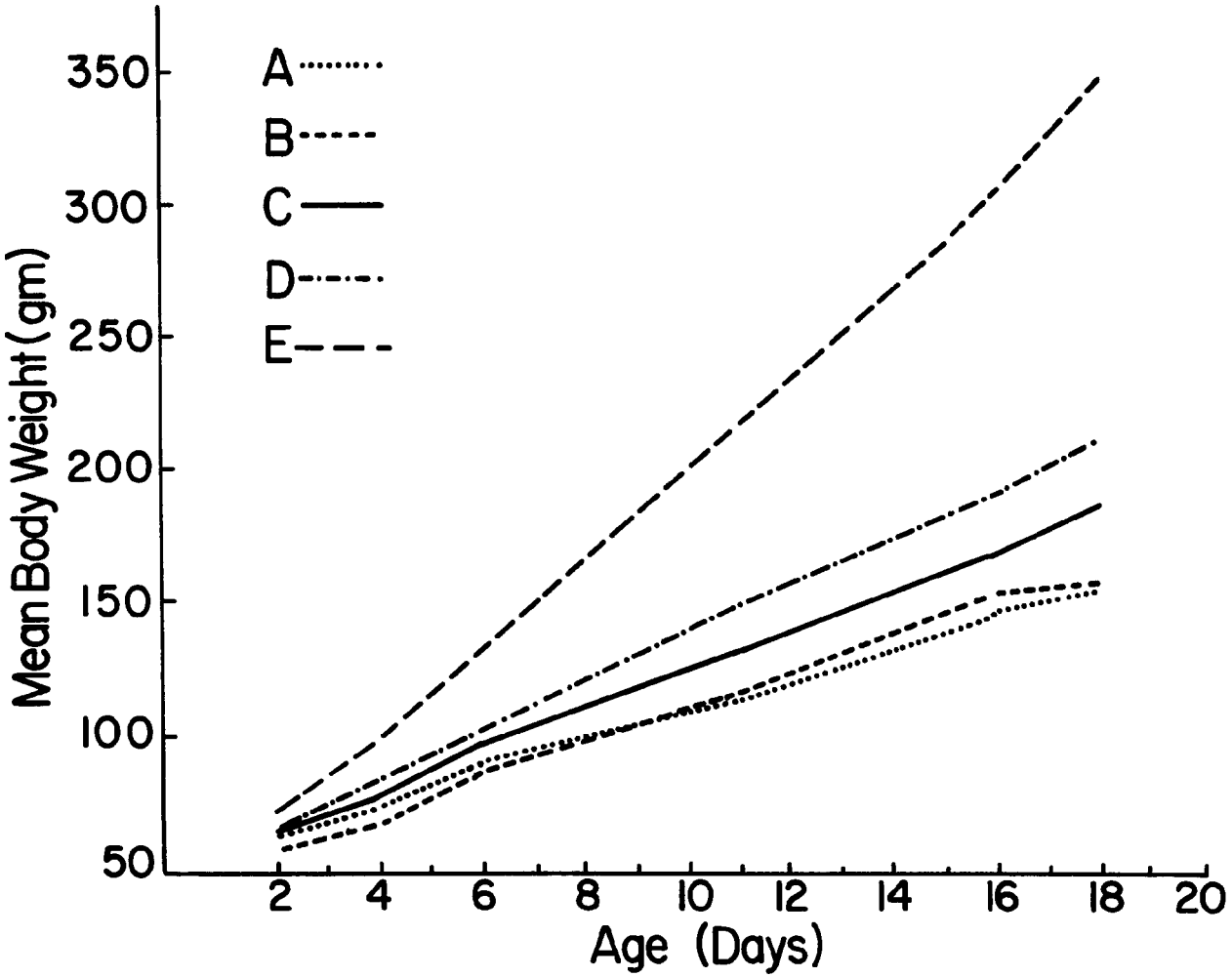


Figure 8. Mean litter (8 pups) body weight of zinc deficient rats in group A, B, C, and controls, pair-fed group D and ad libitum group E.

Mean Litter (8 pups) Body Weight of Zinc Deficient Rats in Groups A,B,C and Controls, Pair fed Group D and Ad Libitum Group E



weight was significantly higher than the zinc deficient groups A, B, and C, at 23 days of age. At the same age, group C mean body weight was higher than groups A and B. At the end of experimental period, group D pair-fed control and group C mean body weights were similar, but both of them were significantly higher than group A. Meanwhile, group B was not significantly different from group A, C and D (Figure 9).

Serum Zinc Concentration. Serum zinc analysis after two days of feeding the diet containing 2 μgZn/gm to group A was significantly lower than the serum zinc concentration of pair-fed rats in group D control and ad libitum fed group E control. The same significant difference was observed throughout the experimental period (Figure 10).

Mesio-distal Distance and Crown Height of Lower Molars. Statistical analysis of mesio-distal (MD) distance of lower molars from 40 day old rats shows that group E, the ad libitum controls, were significantly greater than all other groups (Table 13). Group D, pair-fed controls MD, was significantly greater than all zinc deficient groups A, B and C. Among zinc deficient groups, the MD distance for group C was significantly greater than for the other zinc deficient groups A and B (Table 13).

Crown height for group E, ad libitum controls crown height was significantly greater than for all other groups. However, group B was significantly shorter than pair-fed controls and the other zinc deficient groups, A and B (Table 13).

Figure 9. Mean body weight of zinc deficient rats in groups A, B, C, and control groups D (pair-fed) and E (ad libitum).

Mean Body Weight of Zinc Deficient Rats in Groups A,B,C, and Control Groups D (pair fed) and E (ad libitum).

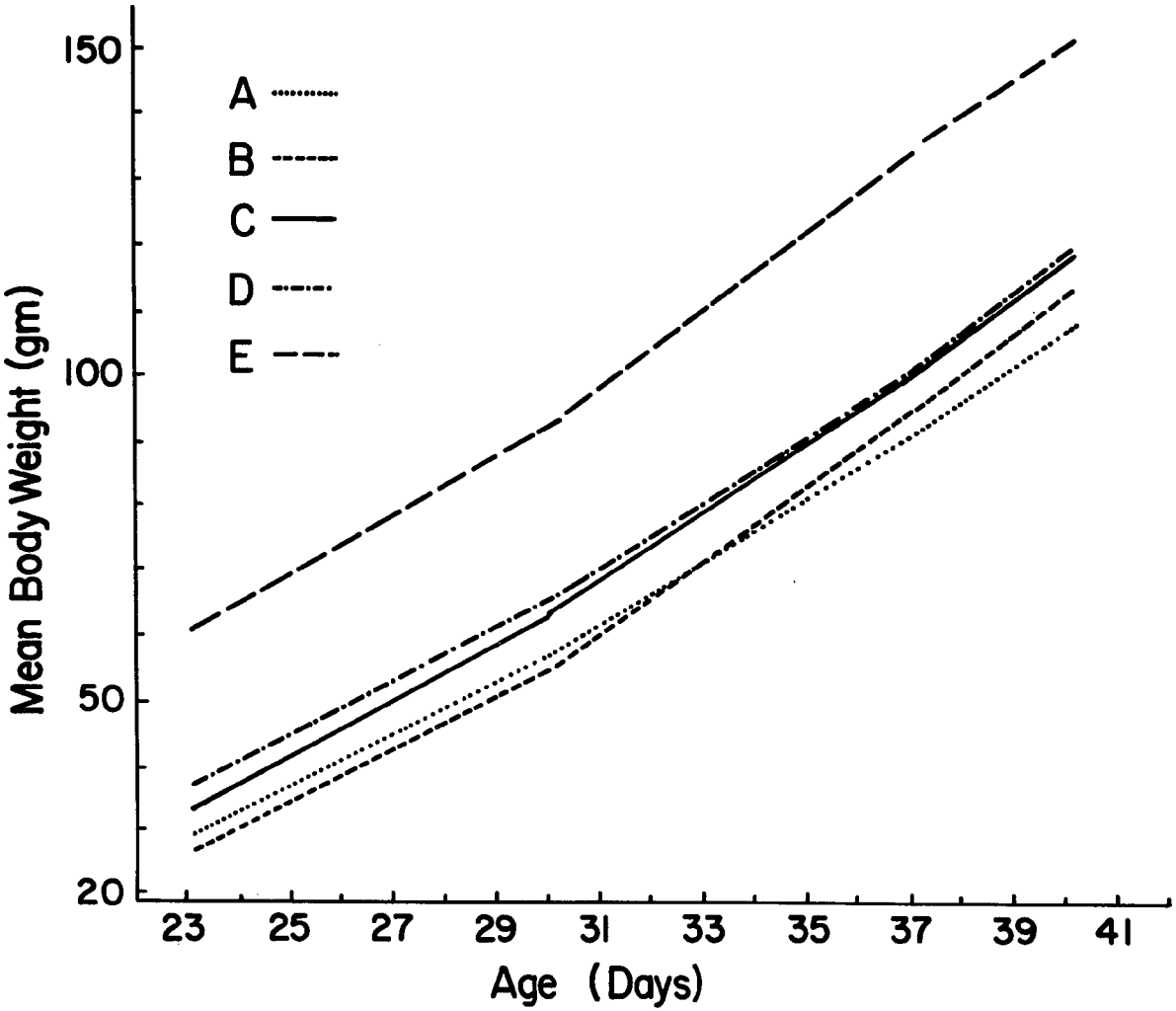


Figure 10. Serum zinc concentration of pregnant and lactating zinc deficient and control rats from day 2 to day 24.

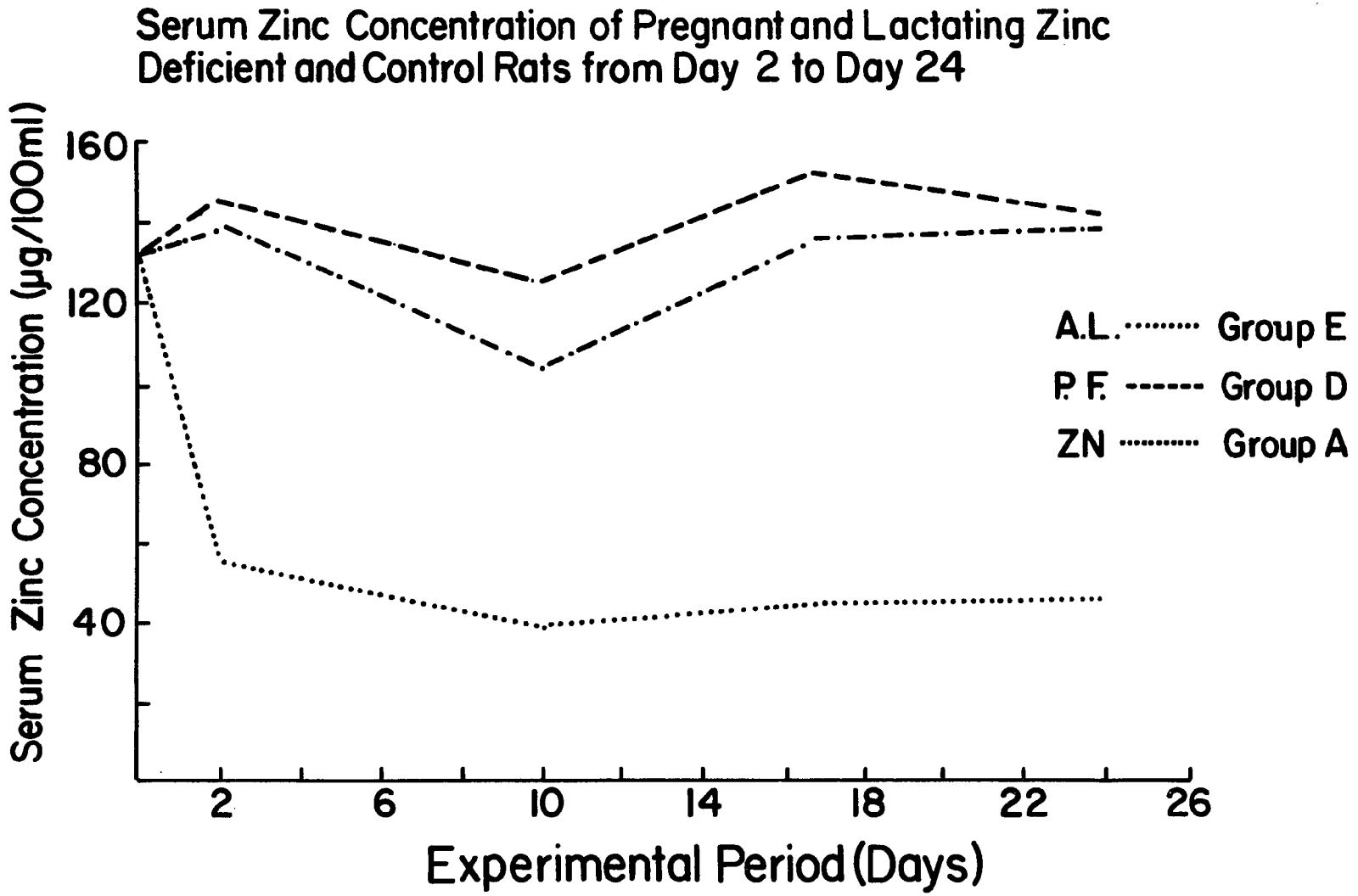

Serum Zinc Concentration of Pregnant and Lactating Zinc Deficient and Control Rats from Day 2 to Day 24
Serum Zinc Concentration (µg/100ml)
160
120
80
40
2
6
10
14
18
22
26
Experimental Period (Days)
A.L. ····· Group E
P.F. ------ Group D
ZN ····· Group A

Table 13

Mesio-distal Distance (MD) and Crown Height (CH) of Lower Molars[1,2]

Group	MD[3]	CH[4]
A (20)[5]	0.599 ± 0.015[a]	0.132 ± 0.010[a]
B (18)	0.602 ± 0.012[a]	0.123 ± 0.005[b]
C (30)	0.611 ± 0.014[b]	0.132 ± 0.012[a]
D (30)	0.620 ± 0.012[c]	0.136 ± 0.013[a]
E (22)	0.632 ± 0.008[d]	0.146 ± 0.012[c]

[1]Measured in cm and expressed as mean ± S.D.

[2]Values in the same column not sharing a common superscript letter are significantly different. ($p < .05$)

[3]Mesio-distal distance, MD.

[4]Crown height, CH.

[5]Number of observations.

Zinc, Calcium and Phosphorus Analysis. Chemical analysis of maxillary first molar crowns from 18 day old pups showed that zinc deficient groups A, B, and C contained significantly less zinc in their teeth than pair-fed and ad libitum controls (Table 14). However, rats in the pair-fed group contained significantly less zinc in their teeth than the ad libitum group E (Table 14).

Calcium content of teeth seemed to fluctuate. Teeth from group D, pair-fed controls contained significantly more calcium than ad libitum control group E, and zinc deficient group B. Groups A and C had similar calcium content in their teeth (Table 14).

All groups had similar phosphorus content in their teeth. Statistical analysis of calcium: phosphorus ratio (Ca:P ratio) showed that only group D had significantly higher Ca:P ratio than group B (Table 14).

Caries Scores. All zinc deficient rats, group A, B and C had significantly higher buccal Dm score than ad libitum group E controls. Two of the zinc deficient groups A and C had significantly higher buccal Dm scores than the pair-fed control. Buccal Dx scores of all zinc deficient groups A, B and C were significantly higher than the ad libitum controls, but compared to group D, only group A had significantly higher buccal Dx scores (Table 15).

Sulcal Dm scores for zinc deficient group A and B were significantly higher than the ad libitum control. There were no significant differences between groups C, D and E. Sulcal Dx score for zinc deficient groups A and B, and pair-fed group D are

Table 14

Zinc, Calcium and Phosphorus Contents of Teeth[1,2]

Element	Group A (8)[3]	B (6)	C (9)	D (6)	E (7)
Zn	59.8 ± 10.9[a]	59.1 ± 12.1[a]	60.3 ± 21.9[a]	106.3 ± 13.1[b]	129.7 ± 20.0[c]
Ca	262.7 ± 42.5[ab]	228.6 ± 58.7[b]	259.8 ± 21.9[a,b]	290.1 ± 35.7[a]	236.8 ± 15.8[b]
P	158.8 ± 18.7[a]	171.8 ± 5.2[a]	160.8 ± 9.2[a]	167.4 ± 3.6[a]	154.5 ± 24.1[a]
Ca:P Ratio	1.68 ± 0.38[a,b]	1.34 ± 0.38[b]	1.62 ± 0.12[a,b]	1.76 ± 0.22[a]	1.58 ± 0.33[a,b]

[1]Contents of Zn, Ca and P are expressed as μg/gm, mg/gm and mg/gm, respectively and as mean ± S.D.

[2]Values in the same line not sharing a common superscript letter are significantly different. ($p < .05$)

[3]Numbers between parentheses represents number of analyses (rats) per group.

Table 15

Effect of Developmental Zinc Deficiency on Rat Caries

		Caries Scores [2]				
		A(20)[1]	B(18)	C(30)	D(30)	E(22)
Buccal	Dm[3]	21.7 ± 5.3^{a}	17.8 ± 7.6^{a,b}	19.9 ± 6.0^{a}	15.7 ± 7.3^{b,c}	13.3 ± 5.3^{c}
	Dx	9.6 ± 3.1^{a}	7.8 ± 4.2^{a,b}	8.2 ± 4.5^{a,b}	6.6 ± 5.0^{b}	1.6 ± 3.7^{c}
Sulcal	Dm	9.6 ± 2.4^{a}	6.8 ± 3.9^{b}	5.9 ± 3.3^{b,c}	6.0 ± 4.0^{b,c}	4.0 ± 3.2^{c}
	Dx	0.6 ± 0.8^{a}	0.7 ± 0.8^{a}	0.4 ± 0.7^{a,b}	0.6 ± 0.9^{a}	0.1 ± 0.5^{b}
Proximal	Ds	1.7 ± 1.5^{a,b}	2.3 ± 1.4^{a}	1.2 ± 1.6^{b,c}	2.1 ± 1.8^{a,b}	0.7 ± 1.3^{c}

[1]Number between parentheses represent number of rats per group.

[2]Mean ± S.D. values in the same line not sharing a common superscript letter are significantly different ($p < 0.05$).

[3]Lesion severity: Ds lesion extending slightly into dentin; Dm, lesion pentrating half way into dentin; Dx, extensive dentin destruction, possibly reaching the pulp.

significantly higher than the ad libitum control. However, there were no significant differences among ad libitum control, group E and experimental group C which received 4 µg/gm zinc throughout gestation and lactation (Table 15).

Proximal Ds score for zinc deficient groups A and B, and pair-fed control group D were significantly higher than ad libitum control group E. Group C proximal Ds is similar to group E (Table 15).

Survival, Mortality Rates and Cannibalism. Pair-fed group D, and ad libitum group E controls had 100% survival rates. Group C had 91.7% survival, but group A had 50.7% survival rate, and an even lower survival rate (34.2%) was observed in group B. Mortality rate among zinc deficient groups was variable; group C had the lowest mortality rate (6.3%), followed by group A (27.4%), and surprisingly, group B had the highest mortality rate (47.4%). Probably this was due to the sudden change in dietary zinc from 4 µg Zn/gm during gestation to 2 µg Zn/gm throughout lactation. Cannibalism among zinc deficient rat dams in group A and B was almost similar; on the other hand, group C rat dams cannibalized only one pup (Table 16).

Zinc Deficiency and Development of Dental Tissues

Experimental Design

This experiment was designed as a longitudinal study to investigate the effect of zinc deficiency imposed during the last

Table 16

Survival, Mortality and Cannibalism Rates Among Zinc Deficient Pups and Dams

	Survival Rate		Mortality Rate		Cannibalism Rate		Total	
Group	No. of Pups	%	No. of Pups	%	No. of Pups	%	No. of Pups	%
A	37	50.7	20	27.4	16	21.9	73	100.0
B	26	34.2	36	47.4	14	18.4	76	100.0
C	44	91.7	3	6.2	1	2.1	48	100.0
D	51	100.0	-	-	-	-	51	100.0
E	37	100.0	-	-	-	-	37	100.0

week of gestation and also during 17 days of lactation on the early development of the dentition in the pups from the first day to 17 days of post-natal life.

Seven timed pregnant Crl:COBS rats of Sprague-Dawley strain were distributed randomly into three groups in their last week of gestation. Zinc deficient groups consisting of three dams were fed zinc deficient diet MIT #200 in a gel form containing 2 μg Zn/gm. Pair-fed control groups consisting of two dams were fed the same amount of diet (containing 50 μg Zn/gm) consumed by the ZD group on the previous day and the last group, ad libitum control group, consisted of two dams fed zinc adequate diet (50 μg Zn/gm).

After birth one pup was sacrificed from each litter on the first and also on the second day. Two days after delivery, pups were randomized within their respective groups. The size of the litter was equalized to eight pups.

One pup was sacrificed from each litter every other day, i.e., on days three, five, seven, nine, eleven, thirteen, fifteen and seventeen.

Heads were fixed immediately in neutral buffered formalin. Whole heads of rats killed at day seven to day seventeen were decalcified before sectioning (see Materials and Methods).

Experimental Results

Mean Body Weights of Pregnant and Lactating Rats. There was no significant difference in mean body weights recorded at the

beginning of the experiment. However, on the sixth day of the experimental period, the ad libitum group mean body weight was significantly higher than the mean body weight of zinc deficient groups. After delivery, the ad libitum group maintained a significantly higher body weight than pair-fed and zinc deficient groups throughout lactation (Figure 11).

Pregnant and Lactating Rats Daily Diet Intake. Diet intake was monitored daily for all groups. It was observed that zinc deficient group diet intake declined sharply on the second day of the experimental period. Diet intake by zinc deficient group dropped to zero on the seventh and eighth day of the experimental period. Food consumption by the ad libitum group dropped to 15 gm on the eighth day of the experimental period, then increased during lactation to a maximum intake on the 15th day (Figure 12).

Serum Zinc Concentration. Serum zinc concentration after two days of feeding the diet containing 2 μg Zn/gm zinc to zinc deficient rats was significantly lower than the serum zinc concentration of pair-fed control and ad libitum control rats. The same significant difference was observed throughout the experimental period (Table 17).

Histological Observation

One Day Old Pups. Sections from zinc deficient pups show irregular dentin formation in the incisors. The dentin appears to be thicker on the labial side than on the lingual side of the tooth. The labial surface appears to be covered by a very thin

Figure 11. Mean body weights of pregnant and lactating rats.

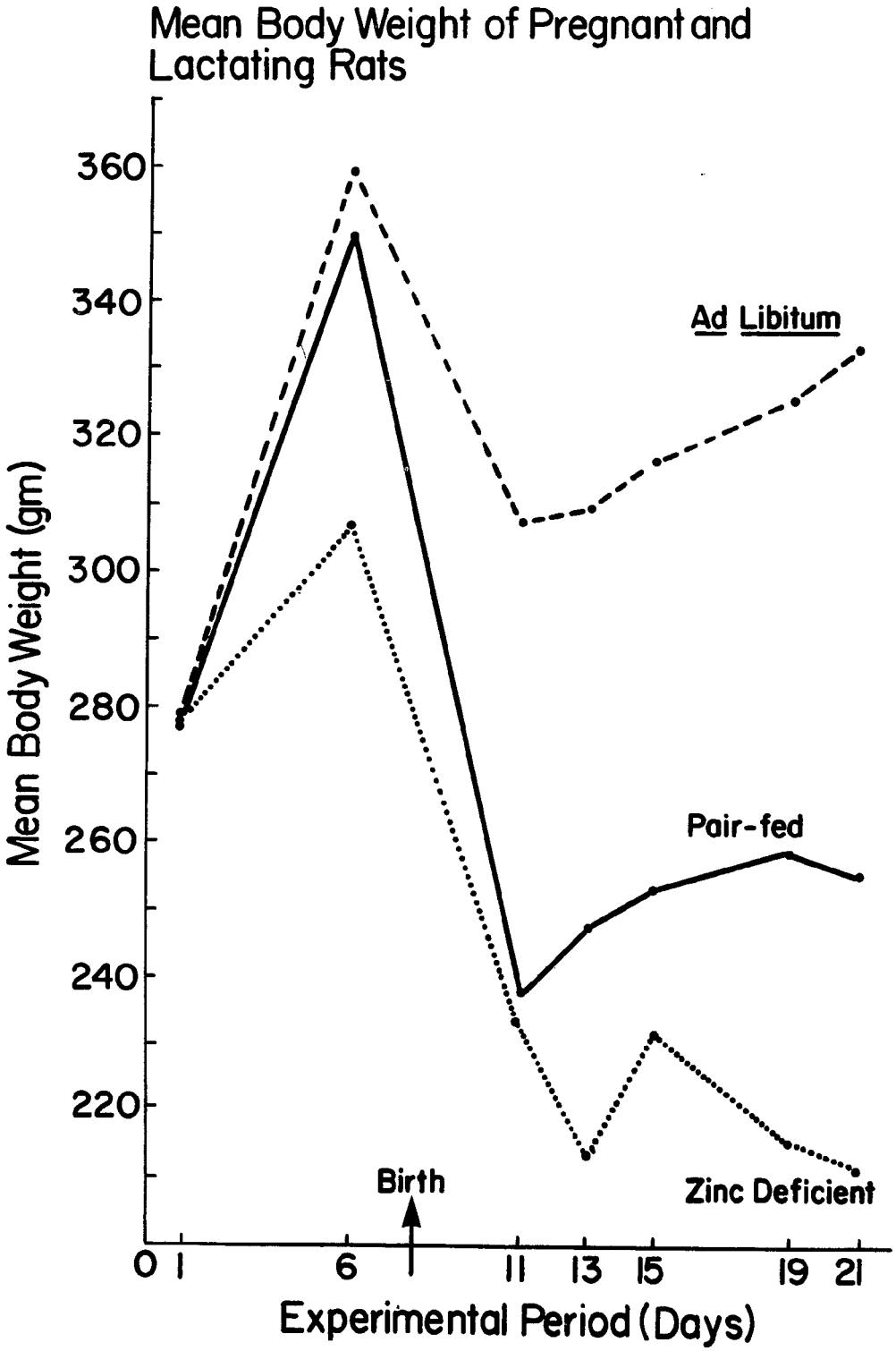

Mean Body Weight of Pregnant and Lactating Rats
Mean Body Weight (gm)
360
340
320
300
280
260
240
220
Ad Libitum
Pair-fed
Zinc Deficient
Birth
0 1 6 11 13 15 19 21
Experimental Period (Days)

Figure 12. Daily diet intake of zinc deficient and ad libitum control rats.

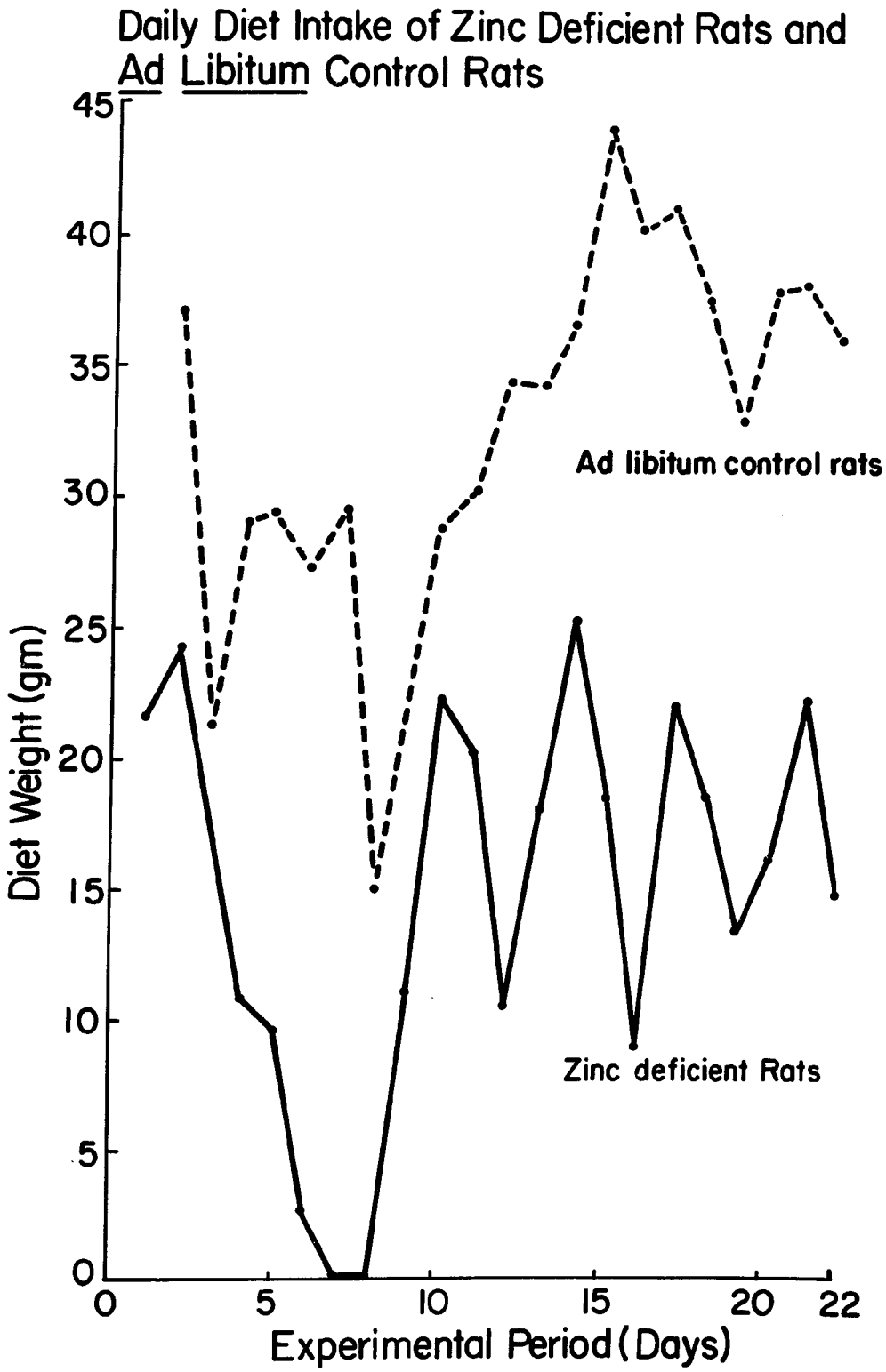

Daily Diet Intake of Zinc Deficient Rats and
Ad Libitum Control Rats
Diet Weight (gm)
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
22
Experimental Period (Days)
Ad libitum control rats
Zinc deficient Rats

Table 17

Zinc Deficient, Pair-fed, and Ad Libitum Rats Serum Zinc Concentration (μg/100 ml)

Group	Day 2	Day 10	Day 19	Day 23
Z.D. (3)	$54.7\pm4.7^{b*}$	39.5 ± 4.8^{c}	44.2 ± 7.7^{b}	44.5 ± 4.1^{b}
P.F. (2)	139.4 ± 11.6^{a}	114.3 ± 6.0^{b}	136.5 ± 15.9^{a}	137.8 ± 11.5^{a}
A.L. (2)	147.4 ± 11.3^{a}	135.5 ± 0.0^{a}	151.0 ± 5.2^{a}	145.9 ± 11.5^{a}

* Mean ±SD, values in the same column not sharing a common superscript letter are significantly different.

( ) Number of rats in group

Z.D. Zinc deficient group.

P.F. Pair-fed group.

A.L. Ad libitum group.

layer of enamel matrix. The first molar shows no signs of dentin matrix formation; it consists of enamel organ and dental papilla. Morphologically the enamel organ shows distinctively the following strata: outer enamel epithelium, stellate reticulum, few layers of stratum intermedium and inner enamel epithelium. The dental papilla consists of young vascular mesenchymal tissue.

Enamel matrix appears distinctively in the incisors of the ad libitum group and extends to a considerable distance over the labial surface of the tooth. In the first molar, dentin appears as slightly stained layer at the tip of the cusp (Figure 13).

Two Days Old Pups. Enamel matrix of the zinc deficient pups incisors appears to increase in thickness. The dentin at the tip of incisors seems to be irregular and exhibit abnormal morphology. The junction between dentin and predentin appears as an irregular line (Figure 14). In the first molar, odontoblasts are very well differentiated, and a very thin, faintly stained predentin appears at the height of the crown (Figure 15).

In the ad libitum group, the enamel matrix increases in thickness on the labial surface of incisors. The dentin also increases in thickness, but dentin, and the predentin layer on the labial surface is thicker than on the lingual surface (Figure 16). The first molar exhibited a very well stained area of predentin and dentin which extends to a considerable distance toward the cervical region, and the floor of the fissure. A very thin hair-like layer of enamel can be observed on the mesial slope of the distal cusp (Figure 17). In the pair-fed group, the findings are essentially the same as in the ad libitum group.

Figure 13. One day old ad libitum pup's first molar shows signs of dentin apposition at the tip of the cusp.

Figure 14. Two day old zinc deficient pup's incisor exhibit irregular and abnormal dentin morphology.

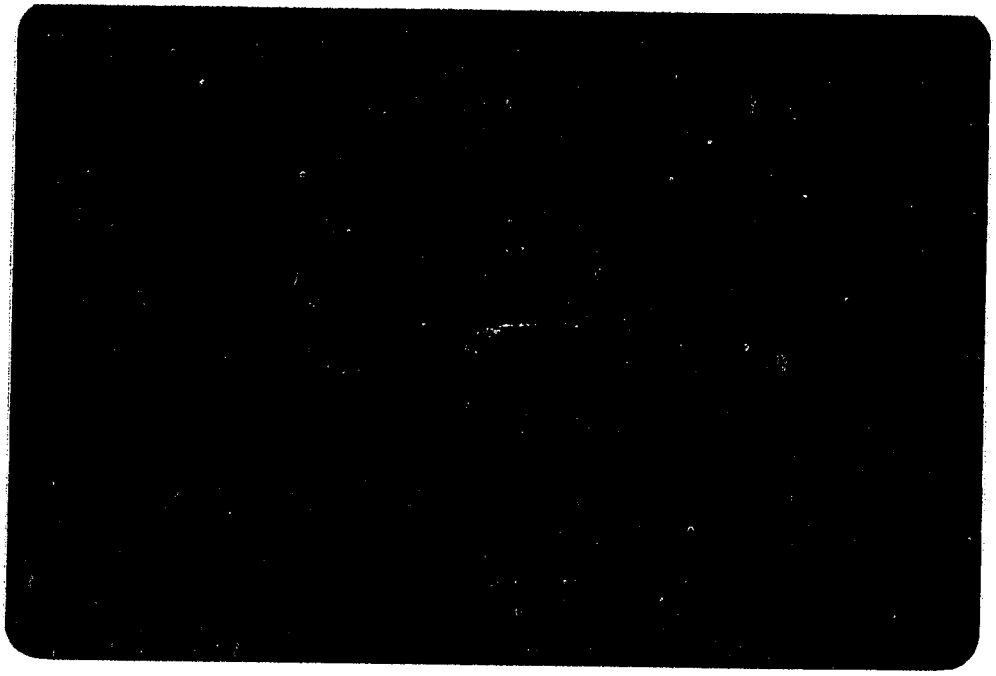


Figure 15. Two days old zinc deficient pup's first molar shows very thin, and faintly stained predentin.

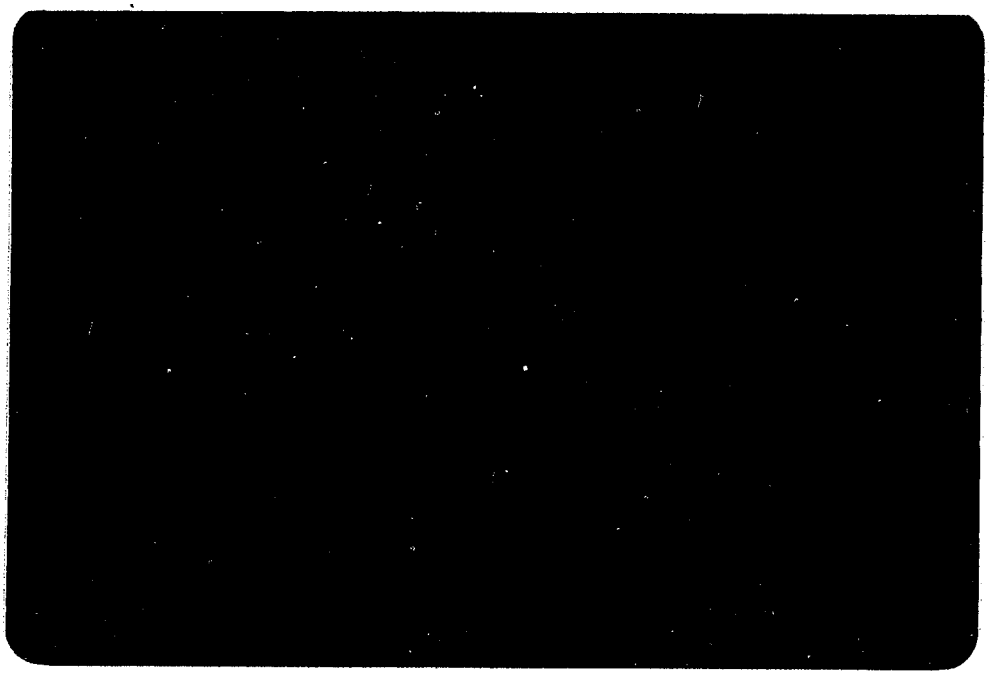
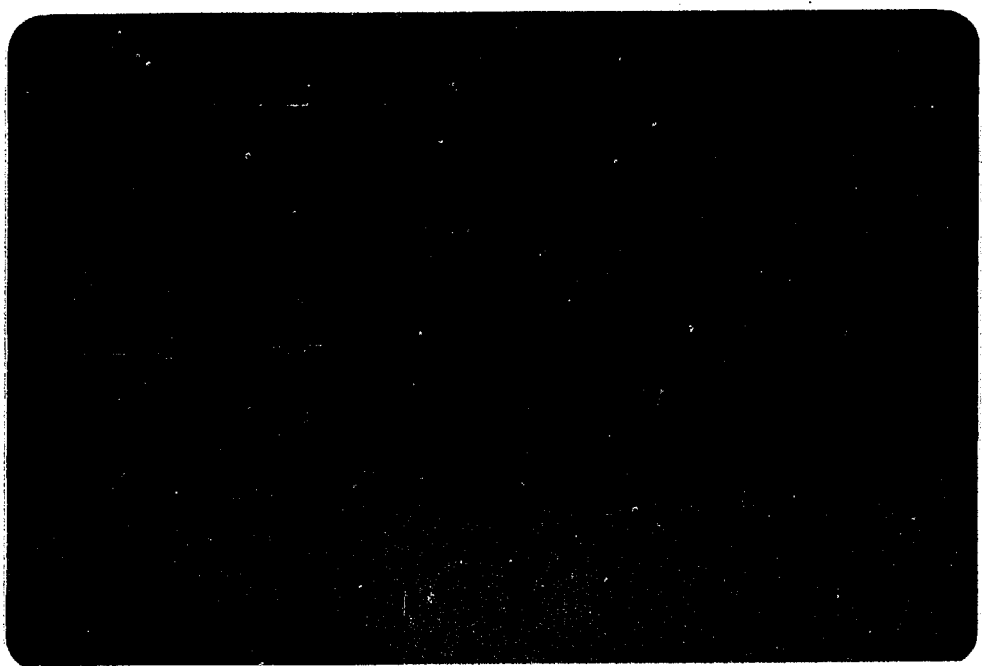


Figure 16. Two days old ad libitum pup's incisors shows increase in thickness of dentin and enamel matrix.

Figure 17. Two days old ad libitum pup's first molar shows extension of dentin toward the cervical region and the floor of the fissure and enamel matrix apposition on the mesial slope of the distal cusp.



Three Days Old Pups. The zinc deficient group at this age shows the presence of enamel, dentin and predentin in the first molar. In the pair-fed and ad libitum controls, the enamel matrix shows increase in thickness in the first molar; however, no matrix could be seen in the fissure floor.

Five Days Old Pups. In the five days old zinc deficient group, the enamel and dentin appear to increase in thickness (Figure 18). Sections from undecalcified pair-fed and ad libitum were lost during sectioning probably due to partial mineralization of enamel.

Seven Days Old Pups. Continuous apposition was observed in the first molar of zinc deficient group. The same process was observed in the first molars of control groups; furthermore, it was observed that the enamel matrix is covering the fissure floor at this age in the control animals.

Nine Days Old Pups. At this age apposition of enamel matrix in the fissure floor of the first molars of the zinc deficient group can be observed (Figure 19). The first molars of both pair-fed and ad libitum groups show increase in thickness of enamel and dentin. They also showed inward folding of the epithelial tissue at the cervical margin of the crown which indicates the formation of epithelial diaphragm prior to root formation (Figure 20).

Eleven Days Old Pups. The first molar of zinc deficient pups shows the appearance of the epithelial diaphragm (Figure 21) prior to beginning of root formation. The cuspid region of the enamel of

Figure 18. Five days old zinc deficient pup's first molar shows increase in dentin and enamel thickness.

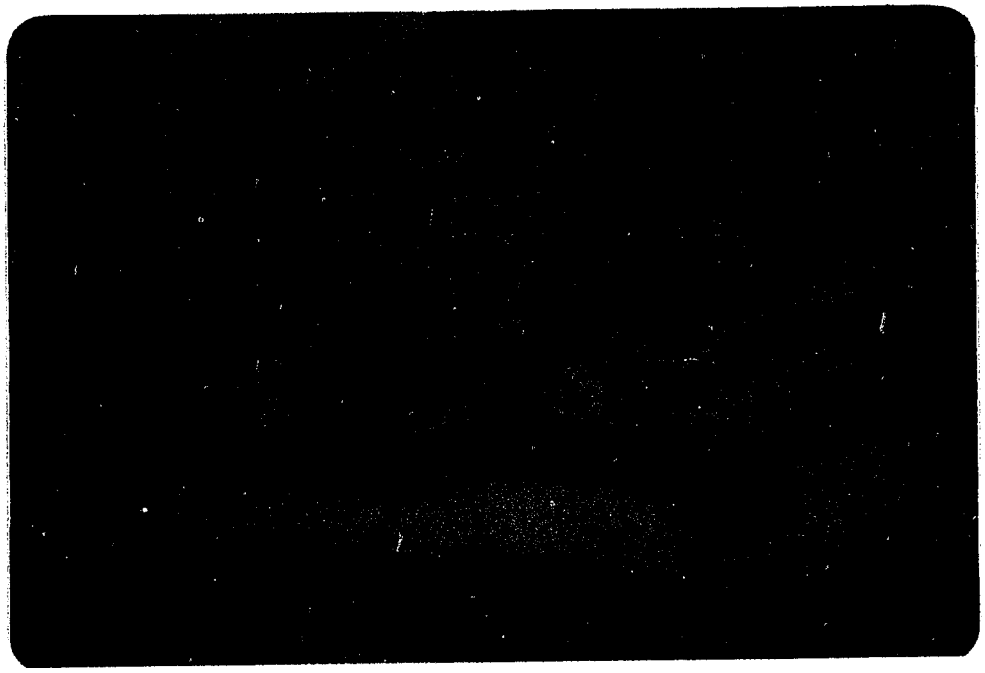
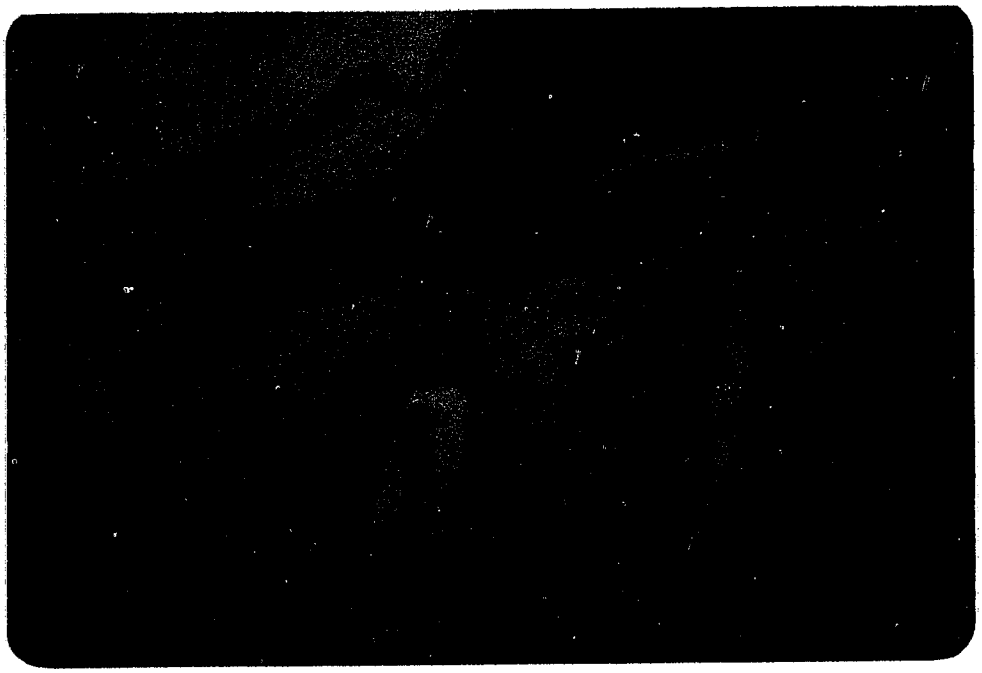


Figure 19. Nine days old zinc deficient pup's first molar shows apposition of enamel matrix on the fissure floor.

Figure 20. Nine days old pair-fed pup's first molar shows the formation of the epithelial diaphragm.

Figure 21. Eleven days old zinc deficient pup's first molar shows the formation of epithelial diaphragm.



the first molar in the pair-fed and ad libitum groups start to yield to the effects of decalcifying agents, which is an indication of beginning of mineralization and enamel maturation (Figure 22).

Fifteen Days Old Pups. Signs of enamel maturation were observed in cuspal region of the first molar in the zinc deficient group (Figure 23), while in the pair-fed and ad libitum groups, the enamel in the first molar had been decalcified completely, but enamel of the second molar was not affected by the decalcifying agent. The third molars shows the first signs of dentin apposition (Figure 24).

Seventeen Days Old Pups. The second molar from zinc deficient group shows intact enamel, and the appearance of dentin in the third molar (Figure 25). Both controls, pair-fed and ad libitum, lost most of the second molar coronal enamel, and the third molar shows a distinct area of enamel apposition approaching the cervical region (Figure 26).

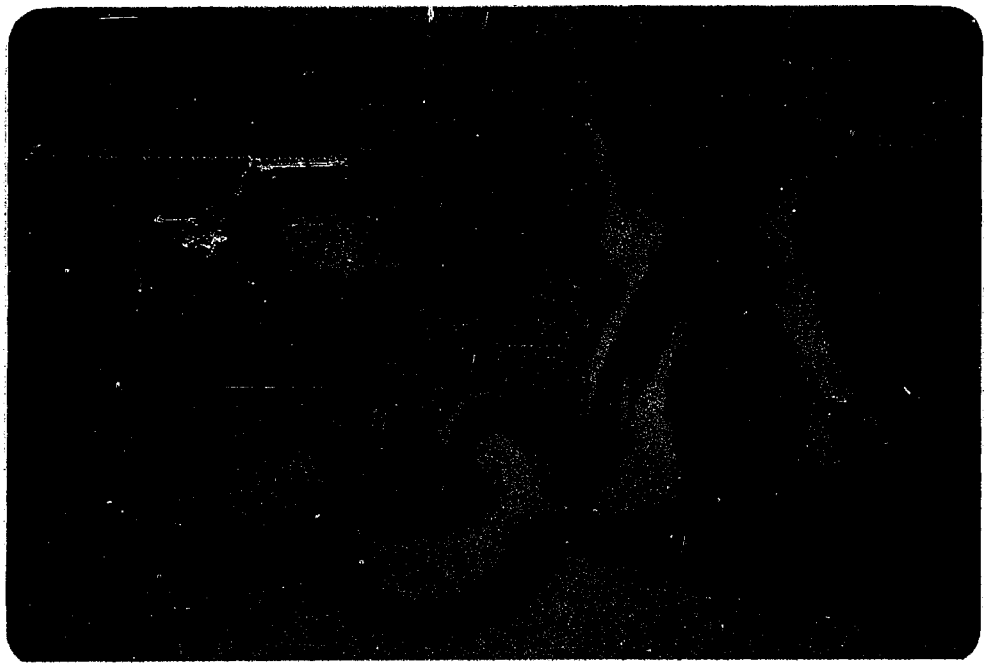
Figure 22. Eleven days old pair-fed pup's first molar yielding to the effect of decalcifying agent.

Figure 23. Fifteen days old zinc deficient pup's first molar yielding to the effect of decalcifying agent.

Figure 24. Fifteen days old ad libitum pup's second and third molars show the apposition of dentin in the third molar and the resistance of the second molar enamel to the action of decalcifying agent.

Figure 25. Seventeen days old zinc deficient pup's second and third molar shows the apposition of dentin in the third molar and the resistance of the second molar enamel matrix to the action of decalcifying agent.

Figure 26. Seventeen days old ad libitum pup's second and third molars show that most of the enamel is lost during decalcification, and the appearance of enamel matrix in the third molar.



CHAPTER V

DISCUSSION

Introduction

In all experiments conducted in this investigation, there were common denominators which appeared in every experiment. These were:

1. Reduced serum zinc concentration in zinc deficient rats.
2. Mean body weight changes.
3. Anorexia.

Serum Zinc Concentration

Using a purified diet MIT #200, it was possible to reduce its zinc content to 0.6 μgZn/gm, and to adjust the zinc content to different levels as indicated by the experiment protocol.

Measurements of the serum zinc concentration represented the best estimate of the rat zinc status and the dietary zinc intake of the animal. Serum zinc concentration decreased to less than 50 percent within less than 48 hours from the time of initiating the zinc deficient regimentation. Low values were maintained throughout the experimental periods. This rapid decrease in serum zinc concentration confirms an earlier report by Hurley and Mutch

(1973). It is clear that this early decline in serum zinc concentration was not achieved in the first experiment, only after one week from time of initiating the zinc deficient regimentation. Probably if the serum zinc concentration had been measured within the first 48 hours, it would have shown the same trend seen in all experiments.

Unlike human serum zinc concentration (McKenzie, 1979), the rat serum zinc concentration can be used as a reliable parameter for the assessment of zinc status in this species (Everett and Apgar, 1979). This parameter demonstrates the correlation with dietary zinc intake of the rat. The use of graded zinc concentration in the diet during lactation was reflected in the dam's serum zinc concentration (Table 9).

Mean Body Weight Changes

It was observed throughout this study that pregnant or lactating dams on zinc deficient diet gain less weight than pair-fed and ad libitum controls. Weight gain was inversely proportional to the duration of zinc deficiency (Figure 1). The highest weight gain during gestation and lactation was always recorded by the ad libitum group (Figures 1, 5, 7, 11). Zinc deficient rats tend to lose weight throughout lactation, probably due to the stress of nursing in addition to the zinc deficiency status.

Pups suckled by zinc deficient dams showed very poor growth rate and weight gain during lactation. The weight gain is almost half of that recorded by ad libitum group (Figure 8). Poor growth

had been associated with human zinc deficiency (Hambidge et al., 1972) and experimental zinc deficiency in laboratory animals (Mills et al., 1969). Weight changes and poor growth in this study confirmed these reports.

Zinc deficiency imposed during the last week of gestation and 18 days of lactation have a prolonged effect on the growth of the rat. Figure 9 showed that even after feeding a zinc adequate diet, the body weight gain of rats in the zinc deficient groups and the pair-fed controls were significantly lower than that of the ad libitum. The growth retardation demonstrated the effect of zinc in the biochemical and physiological processes in the body (Kirchgessner et al., 1976).

Anorexia

It was observed in our study that aversion to food occurs within two days in zinc deficient rats (Figures 4, 12). Food intake reached to its lowest level before and during parturition, then increased gradually to a maximum, which was lower than ad libitum controls. Food intake by zinc deficient rats showed a cyclic variation (Figure 12). These findings confirm the report of Mills et al. (1969). They showed that food consumption in rats drops abruptly about 3-5 days after receiving zinc deficient diet.

Following the first drop, there is a gradual increase of food intake until a maximum, which is always much lower than the control, is reached (Mills et al., 1969; Chesters and Quaterman, 1970). The cyclic variation in food intake has a frequency of

approximately 4 days. When refed with zinc, zinc deficient rats increase their food intake within 1-2 hours (Mills et al., 1969). The mechanism by which zinc deficiency affects appetite is unknown. With reduced food intake, growth rate also decreases. The retarded growth is further contributed to the poor food utilization efficiency, since pair-fed controls always gain more weight than the zinc deficient rats while both groups consume the same amount of food (Kirchgessner et al., 1976).

Effect of Pregestational and Gestational Zinc Deficiency and Developing Embryos

The first study of this dissertation concerns the effect of zinc deficiency on the development of fetus, with emphasis on the oral tissues. Hurley and Swenerton (1966) showed that feeding female rats a zinc deficient diet from the time of weaning to maturity resulted in complete failure to reproduce and severe disruption of the estrous cycle, and incapability to mate (Hurley and Swenerton, 1966). Rearing the same strain rat on a marginally zinc deficient diet from weaning to maturity resulted in mating, but 99% of the implantation sites were affected either by resorption or severe malformation, and also produced smaller litters than pair-fed or ad libitum zinc sufficient control. Offsprings of zinc deficient mothers weighed much less than those of controls and 98% showed gross congenital malformations. When a zinc deficient diet (0.2-0.5 μg Zn/gm) was given to rats specifically during the period of gestation (0-21 day), it caused resorption in 41% of the implantation sites, and the full term fetuses weighed almost half

as much as control rats. Eighty percent of the fetuses exhibited gross malformation. Reducing the deficiency period to the first 14, 12, or 10 days of gestation resulted in a 76%, 56% or 20%, respectively, reduction in fetus malformations. (Hurley et al., 1971). When the zinc deficient regime was imposed from day six to 14 of pregnancy, almost half of the pups were abnormal (Hurley et al., 1971; Hurley and Mutch, 1973). Similarly, congenital malformations of the brain also were observed in rats due to short term zinc deficiency during pregnancy (Warkany and Petering, 1972 and 1973). On the contrary, no gross abnormality was observed in rat pups when zinc deficiency was imposed during the last third (McKenzie et al., 1975; Sandstead et al., 1975) or the whole period of gestation (Fosmire et al., 1977). Similarly, when female rats were fed zinc deficient diet from day 12, 15 and 18 of gestation, no gross malformation in pups was observed (Apgar, 1972). In the same experiment (Apgar, 1972), only five out of 68, or 7.4%, of pups born to dams which were deprived of zinc during the whole gestation period were grossly malformed while Hurley et al. (1971) reported 80% of pups were malformed in a similar experiment. Retarded growth of the fetus, however, was consistently observed regardless of the presence or absence of malformation (Sandstead et al., 1975; McKenzie et al., 1975; Fosmire et al., 1977; Apgar, 1972).

In this study, retarded growth without malformation was observed in all three zinc deficient groups. The reduced growth was manifested by lower body weight, and shorter trans-umbilical

and crown rump distance than well fed controls (Table 5). The absence of congenital malformation in fetus observed in this study is in contrast to the results reported by Hurley and her associates (Hurley and Swenerton, 1966, Hurley et al., 1971) and by Warkany and Petering (1972, 1973), but is in agreement with those observed by Sandstead and his associates (McKenzie et al., 1975, Fosmire et al., 1977). The difference may be due to diet composition or other undefined nutritional factors. As protein source, Hurley's group used EDTA washed soybean protein, while Sandstead group used egg white protein and we used washed lactalbumin. Soybean protein, being high in phytate, increases the requirement of zinc (Oberleas and Prasad, 1976), which could possibly aggravate the zinc deficient status of dams. However, congenital malformation was also observed when egg white protein was used (Warkany and Petering, 1972, 1973). Meanwhile, using egg white protein diet, Sandstead's group did not observe gross abnomalities in fetus; neither did we observe such alterations in our study in which lactalbumin was used. Therefore, the reason for the discrepancy between the presence and absence of congenital malformation remains unresolved.

Zinc concentrations of liver for rat dams were not significantly different among all groups (Figure 3), in agreement with previous reports (Reis and Evans, 1977; Duncan and Hurley, 1978). However, zinc levels in fetal liver were significantly higher than those of respective dam liver (Figure 3). This is true also in the zinc deficient group, indicating that zinc was effectively

transported through placenta and possibly concentrated in liver during fetal development.

Minimum Dietary Zinc Requirement During Last Week of Gestation and During 18 Days of Lactation

The second study of this dissertation was originally designed to investigate the effect of zinc deficiency on dental caries. However, due to the unexpected high mortality rate of pups, the results were used to establish a minimal dietary zinc level required for survival of pups showing signs of zinc deficiency. Using egg white protein diet, Apgar (1968, 1972, 1977a) showed that zinc deficient dams had delayed parturition, excessive bleeding and failure to clean the pups and consume the afterbirths. In addition, when zinc deficiency was initiated on day one, 12, 15 or 18 of pregnancy, only the day 18 group showed normal delivery (Apgar, 1972), while three out of 12 dams in day 15 group experienced great stress during parturition. Reasoning that rats fed our lactalbumin based MIT #200 diet performed better than those fed egg white protein, we decided to impose the zinc deficient diet during the last week of gestation. Table 6 shows that 32% of pups in the zinc deficient group were born dead and still another 26% of pups died on the second day. Cannibalism was also observed in the zinc deficient dams, thus by the end of day five of lactation, 32% of pups were destroyed by mothers (Table 6). The second experiment of this study was then initiated to find a minimal dietary zinc level which would allow pups to survive before weaning (Table 7). Pups of group A had only 17% survival rate either due to death at birth

or cannibalism (Table 8). Approximately half of the group B pups survived, while pups of groups C and D had 100% survival rate. Thus, for all pups to survive to the end of lactational period, the minimal dietary zinc level would be 4 μgZn/gm.

Dietary Zinc and Dental Caries

This study shows the effect of zinc deficiency imposed during the last week of gestation and throughout lactation on the incidence of dental caries, and also the chemical composition and physical measurement of teeth found under these experiment conditions.

As a general trend, rats in zinc deficient groups show higher incidence of dental caries than controls. Similar observations have been reported by Brown et al. (1979) when zinc deficiency was imposed on rats preeruptively, during period of active maturation. Furthermore zinc deficiency imposed on weanling young rats increased the incidence of dental caries predominantly at the smooth surfaces of the molars (Fang et al., 1980).

Navia and Lopez (1977) indicated that the oral conditions prior to establishment of a cariogenic challenge are critically important to reduce caries susceptibility of the tooth. Duncan and Hurley (1978) reported an interaction between vitamin A and zinc in pregnant and fetal rats; plasma vitamin A concentration in both maternal and fetal animals was significantly reduced by low intake of zinc. Vitamin A deficient tissue implants had been demonstrated to contain less calcium than control (Harris, 1976). Furthermore

Harris and Navia (1979) have reported that vitamin A deficiency increased the susceptibility of rat molars to dental caries.

Although Navia (1970) classified zinc as an element with doubtful effect on dental caries, supplementation of diet fed to rats with zinc chloride or zinc sulphate was found to be uneffective in reducing dental caries (Navia et al., 1968; McClure, 1948). Hendershot and Forsaith (1959) found that when zinc was given to rats as zinc versenate, it had a moderately reducing effect on dental caries. White (1975) indicated that zinc decreases the solubility of enamel.

Zinc deficiency in animal experimentation induces alteration of the oral microflora (Mann et al., 1974) which might be expressed as an increase in the incidence of dental caries as this study has shown, as well as greater penetration of carious lesions (Brown et al., 1979).

More recently, it has been reported that 220 µg/ml zinc sulphate in drinking water of rats infected with S. mutans at the time of tooth eruption significantly reduced the buccal caries score (Bates and Navia, 1979). Furthermore, they reported that topical application of 500 µg/ml of zinc significantly reduced buccal caries in rats. Animal experiments have shown that supplementation with 250 µg/gm zinc, 10 µg/gm molybdenum and 4 µg/gm chromium enhanced the cariostatic action of carbamyl phosphate, and completely suppressed dental decay in rats fed a caries promoting diet (Steinman and Leonora, 1975).

Another avenue through which zinc deficiency might induce high incidence of caries lesions could be that zinc deficiency reduces nucleic acid, and protein synthesis in human and animal (Prasad et al., 1979; Duncan and Hurley, 1978). Menaker and Navia (1973) provided evidence that perinatal undernutrition increase the susceptibility of the rat dental caries.

In our study it was shown that zinc deficient rat teeth have significantly shorter MD distance and CH than controls, i.e., the teeth are smaller in size. A similar finding had been reported by Paynter et al. (1958) in vitamin A deficient upper molars. DiOri (1969) also reported that rats subjected to protein malnutrition had small teeth.

Chemical analysis of maxillary first molar crown shows all zinc deficient rats had significantly less zinc than controls, and the ad libitum control teeth contain significantly higher zinc than the pair-fed controls. These findings are in conformity with Brown et al. (1979) observation that the maxillary molars contains 124 ± 14 mg Zn/gm, 15 ± 11, 157 ± 16 for zinc deficient, pair-fed, and ad libitum control rats. Though Brown et al. showed higher zinc concentrations in their samples than the zinc concentration reported here, this was probably due to the difference in age between the two samples. The maxillary first molars used in this study were dissected from 18 days old rats, and in the Brown et al. (1979) study the samples were obtained from eight weeks old rats.

Phosphorus content of the teeth was similar for all groups (Table 14), but the phosphorus content reported here was higher

than that reported by Brown et al. (1979); again that could be due to the age difference. Calcium content of the teeth showed the same difference, and teeth in this study contain less calcium than had been reported by Brown and his associates (1979).

Histological Evaluation of Developing Dental Tissues

In the longitudinal study to evaluate developmental stages of dental tissues of rats from the first to the 17th day of post-natal life, we used the following criteria:

1. Initial apposition of dentin.
2. Initial apposition of enamel matrix, and cessation of the apposition as manifested by its appearance at the cervical region of the crown and simultaneous formation of the epithelial diaphragm indicating the beginning of root formation.
3. Degree of enamel maturity as demonstrated by its disappearance when treated with decalcifying agents.

Normally in rats, dentin appears between 20-21 days of intra-uterine life in the first molar and 1-2 and 13-14 days of life in the second and third molar, respectively (Schour and Massler, 1971).

In this histological investigation we followed the development of the first molar particularly and the incisors, second, and third molars occasionally. It was observed throughout this study that the zinc deficient dental tissue was developmentally one to two days behind that of the controls. This retarded development is

reminiscent of the delay in eruption due to perinatal undernutrition reported by Menaker and Navia (1973). Menaker and Navia (1973) observed that there was a one-day lag in eruption in pups born and suckled by dams fed 8% protein as compared to pups born and suckled by dams fed 25% protein. Similar findings were reported by DiOrio et al. (1969). They found that pups born to undernourished dams exhibited smaller teeth size which were delayed in eruption in comparison with adequately nourished controls. In our study we observed that dentin appeared in the first molar of one day old controls, and probably dentin began forming before birth, a time which approximates that of Schour and Massler (1971). It was also observed that dentin apposition took place at 13 days of age in the third molar of control rats which is in agreement with Schour and Massler (1971). Two days later, at 15 days of age, a very thin line of darkly stained enamel matrix appear on the third molar of control rats. End of amelogenesis in the first molar of control rats, as demonstrated by the apposition of enamel in the cervical region of the crown and the appearance of the epithelial diaphragm which indicates the beginning of root formation, took place at 15 days of age. This was also in agreement with Schour and Massler (1971) report. In control animals, enamel starts to yield to the action of decalcifying agents at 11 days of age which indicates that the tissue has started to mineralize. All the above mentioned events were delayed approximately two days in the zinc deficient rats.

The dentin of zinc deficient incisors shows abnormal morphology. It looked like an osteoid tissue lined by osteoblasts, and some of the cells are surrounded by the osteoid tissue as an osteocyte. The odontoblast was unobservable. The pulp cavity of the incisors contains masses of homogeneous material with unrecognizable structure. Similar changes in vitamin A deficient guinea pigs had been described by Harris et al. (1976) and more recently in vitamin A deficient rats by Tuchinda et al. (1981). Schour (1938) described a similar finding in vitamin A deficient rats, They attributed this alteration to the failure of odontoblasts to differentiate and persistent proliferation of odontogenic epithelium and subsequent invasion of the pulp, and stimulation of the pulpal mesenchymal tissue to form atypical dentin. In this investigation odontogenic epithelium was not observed in the pulp tissue. Furthermore, this osteoid-like tissue was not limited to the lingual side of the tooth; it involved both labial and lingual sides, and enamel was not observed on the labial side of the effected area. It is obvious that there is a complete failure of the pulpal mesenchymal tissue to respond to the induction effect of the inner enamel epithelium to differentiate into functioning odontoblasts to form dentin, and the subsequent impairment of the inner enamel epithelium to differentiate into physiologically active ameloblasts. The most probable explanation of this osteoid tissue is a de novo differentiation of the primitive pulpal mesenchyme into osteoblast destined to form osteoid tissue.

CHAPTER VI

CONCLUSIONS

1. Zinc deficiency was successfully induced in rat using a lactoalbumin based diet MIT #200. Zinc deficiency in pregnant rats was manifested by loss of appetite, low serum zinc concentration and various degrees of difficulty in parturition. In pups zinc deficiency was manifested by retarded growth and alopecia.
2. Pregestational and gestational zinc deficiency induced intrauterine fetal growth retardation.
3. Minimum dietary zinc requirement during the last week of gestation and throughout lactation was established as 4 µg Zn/gm. Dietary zinc concentration as low as 2 µgZn/gm can be used, but it was associated with low survival rate (50 percent).
4. Zinc deficiency imposed through last week of gestation and throughout lactation was associated with increased incidence of dental caries, smaller teeth, and significantly low zinc concentration in teeth.
5. Zinc deficiency induced a retardation of two day in dentin apposition, enamel apposition, root formation, and degree of enamel maturation.

REFERENCES

Aamodt, R.L., Rumble, W.F., Johnston, G.S., Foster, D., and Henkin, R.E. (1979). Zinc Metabolism in Humans after Oral and Intravenous Administration of Zn-69m. Amer. J. Clin. Nutr. 32:559-569.

Alvares, O. F., and Mayer, J. (1968). Regional Differences in Parakeratotic Response to Mild Zinc Deficiency. Arch. Derm. 98:191-21.

Alvares, O. F., and Meyer, J. (1974). Thymidine Uptake and Cell Migration in the Cheek Epithelium of Zinc-Deficient Rats. J. Oral Path. 3:86-94.

Alvares, O., Meyer, J., and Gerson, S. J. (1973). Activity and Distribution of Acid Phosphatase in Zinc Deficient Parakeratotic Rat Buccal Epithelium. Scand. J. Dent. Res. 81:481-488.

Antonson, D.L., Barak, A.J., and Vanderhoof, J.A. (1979). Determination of the Site of Zinc Absorption in Rat Small Intestine. J. Nutr. 109:142-147.

Apgar, J. (1968). Effect of Zinc Deficiency on Parturition in the Rat. Amer. J. Physio. 215:160-163.

Apgar, J. (1972). Effect of Zinc Deprivation from Day 12, 15, or 18 of Gestation on Parturition in the Rat. J. Nutr. 102:343-348.

Apgar, J. (1977a). Effects of Zinc Deficiency and Zinc Repletion During Pregnancy on Parturition in Two Strains of Rats. J. Nutr. 107:1399-1403.

Apgar, J. (1977b). Use of EDTA to Produce Zinc Deficiency in the Pregnant Rat. J. Nutr. 107:539-545.

Attramadal, A., and Jonsen, J. (1976). The Content of Lead, Cadmium, Zinc and Copper in Deciduous and Permanent Human Teeth. Acta Odont. Scand. 34:127-131.

Baratieri, A., Picarelli, A., and Piselli, D. (1979). Zinc Distribution in Human Saliva. J. Dent. Res. 58:540-541.

Barney, G. H., Macaplinlac, M. P., Pearson, W. N., and Darby, W. J. (1967). Parakeratosis of the Tongue - a Unique Histopathologic Lesion in Zinc Deficient Squirrel Monkey. J. Nutr. 93:511-517.

Bates, D., Alvarez, C., and Navia, J. M. (1978). Rat and Microbiological Caries Studies of Post-eruptive Zinc Administration. International Association for Dental Research, 56th General Session, pg. 110, Abstract #141.

Bates, D., and Navia, J. M. (1979a). Effect of Topical Zinc on Rat Oral Flora and Caries. International Association for Dental Research, 57th General Session, pg. 423, Abstract #1329.

Bates, D. G., and Navia, J. M. (1979b). Chemotherapeutic Effect of Zinc on Streptococcus Mutans and Rat Dental Caries. Arch. Oral Biol. 24:799-805.

Baumslag, N., and Petering, H.G. (1976). Trace Metal Studies in Bushman Hair. Arch. Environ. Health 31:254-257.

Baumslag, N., Yeager, D., Levin, L., and Petering, H.G. (1974). Trace Metal Content of Maternal and Neonate Hair. Arch. Environ. Health 29:186-191.

Bawden, J. W., and Hammarström, L.E. (1977). Autoradiography of ^{65}Zinc in Developing Rat Teeth and Bone. Arch. Oral Biol. 22: 449-454.

Bawden, J. W., and Wennberg, A. (1977). In-Vitro Study of ^{65}Zn Uptake in Developing Rat Enamel. Arch. Oral Biol. 22:693-697.

Bedell, G. W., and Soll, D. R. (1979). Effects of Low Concentrations of Zinc on the Growth and Dimorphism of Candida Albicans: Evidence for Zinc-Resistant and Sensitive Pathways for Mycelium Formation. Infection and Immunity 26:348-354.

Beisel, W. R., Pekarek, R. S., and Wannemacher, R. W., Jr. (1976). Homeostatic Mechanisms Affecting Plasma Zinc Levels in Acute Stress. In: Trace Elements in Human Health and Disease (ed. Prasad, A.A.), Vol. 1, Academic Press, Inc., New York.

Bell, L.T., Branstrator, M., Roux, C., and Hurley, L.S. (1975). Chromosomal Abnormalities in Maternal and Fetal Tissues of Magnesium- or Zinc-deficient Rats. Teratology 12:221-226.

Bergman, B. (1970a). The Zinc Concentration in Hard and Soft Tissues of the Rat. The Influence of Zinc Deficient Feeding. Acta Odontol. Scand. 28:427-439.

Bergman, B. (1970b). The Distribution of ^{65}Zn in the Endochondral Growth Sites of the Mandibular Condyle and the Proximal End of the Tibia in Young Rats. An Autoradiographic and Gamma Scintillation Study. Odontol. Revy 21:261-271.

Bergman, B., and Söremark, R. (1968). Autoradiographic Studies on the Distribution of Zinc-65 in Mice. J. Nutr. 94:6-12.

Bertrand, G., and R. Vladesco (1920). De la Repartition du Zinc dans 1' Organisme du Cheval. C.R. Acad. Sci. (Paris) 177:744. Cited by S. Haumont, and F.C. McClean (1966). Zinc and the Physiology of Bone. In Zinc Metabolism (ed. Prasad, A.S.), Charles C. Thomas, Springfield, Ill.

Brandt, T. (1936). Dermatitis in Children with Disturbance of the General Condition and Absorption of Food Element. Acta. Derm. Venerol. 17:513:546.

Brown, E.D., Calhoun, N.R., Larson, R.H., and Smith, J.C., Jr. (1979). An Effect of Zinc Deficiency on Dental Caries. Life Sci. 24:2093-2098.

Brown, E.D., Penhos, J.C., Recant, L., and Smith, J.C., Jr. (1975). Glucose Tolerance, Plasma Level and Pancreatic Insulin Level in Zinc Deficient Rats. Proc. Soc. Exp. Biol. Med. 150:557-560.

Brudevold, F., Steadman, L. T., and Smith, F.A. (1960). Inorganic and Organic Components of Tooth Structure. Ann. NY Acad. Sci. 85:110-132.

Brudevold, F., Steadman, L. T., Spinelli, A. A., Amdur, B.H., and Gron, P. (1963). A Study of Zinc in Human Teeth. Arch. Oral Biol. 8:135-144.

Buchanan, D., Geders, J., and Freeland-Graves, J. (1980). Response of Zinc Status Parameters to Zinc Supplementation in Older Women. Fed. Proc. 39:652, Abstr. #2047.

Bulman, J. S., and Cate, R. A. (1962). Experimental Investigation of Zinc in Saliva. J. Dent. Res. 41:1250-1260.

Bunyard, M.W. (1970). The Systemic Effects in the Rat of Cariogenic Diets with Varied Protein-Calorie Levels. I. Effects on Blood. Caries Research 4:319-324.

Burch, R. E., and Sullivan, J. F. (1976). Clinical and Nutritional Aspects of Zinc Deficiency and Excess. Med. Clinic North Amer. 60:675-685.

Calhoun, N.R., Becker, K.L. and Smith, J.C., Jr. (1973). Essentiality of Zinc for Bone Formation. Fed. Proc. 32:896, Abstr. #3812.

Calhoun, N. R., Brown, E. D., and Smith, J. C., Jr. (1979). Effect of Zinc Deficiency on Incidence of Dental Caries. International Association for Dental Research, 57th General Session, pg. 422, Abstr. #1328.

Calhoun, N. R., Campbell, S., Jr., and Smith, J. C., Jr. (1970). Accumulation of Labeled Zinc, Strontium and Calcium in Bone Injuries. J. Dent. Res. 49:1083-1085.

Calhoun, N.R., Smith, J.C., and Becker, K.L. (1974). The Role of Zinc in Bone Metabolism. Clin. Orthopaedic and Related Research 103:212-234.

Calonius, P.E.B., and Visapää, A. (1965). The Inorganic Constituents of Human Teeth and Bone Examined by X-Ray Emission Spectrography. Arch. Oral Biol. 10:9-13.

Campo, A. G., and McDonald, C. J. (1976). Treatment of Acrodermatitis Enteropathica with Zinc Sulfate. Arch. Dermatol. 112:687-689.

Catalanotto, F. (1978). The Trace Metal Zinc and Taste. Amer. J. Clin. Nutr. 31:1098-1103.

Catalanotto, F. A., and Lacy, P. (1977). Effect of Zinc Deficient Diet Upon Fluid Intake in the Rat. J. Nutr. 107:436-442.

Catalanotto, F.A., and Nanda, R. (1978). The Effect of Administration of Zinc Deficient Diet on Taste Acuity and Tongue Epithelium. J. Oral Pathol. 6:211.

Cavdar, A. O., and Arcasoy, A. (1972). Hematologic and Biochemical Studies of Turkish Children with Pica. A Presumptive Explanation for the Syndrome of Geophagia, Iron Deficiency Anemia, Hepatosplenomegaly and Hypogonadism. Clin. Pediat. 11:215.

Cavdar, A. O., Arcasoy, A., and Cin, S. (1977). Zinc Deficiency in Turkey. Amer. J. Clin. Nutr. 30:833.

Cavdar, A. O., Babacan, E., Arcasoy, A., and Ertem, U., (1980). Effect of Nutrition on Serum Zinc Concentration During Pregnancy in Turkish Women. Amer. J. Clin. Nutr. 33:542-544.

Chen, S. Y. (1977). Cytochemical and Autoradiographic Study of Nuclei in Zinc-Deficient Rat Buccal Epithelium. J. Dent. Res. 56:1546-1551.

Chen, P.S., Toribara, T.Y., and Warner, H. (1956). Microdetermination of Phosphorus. Anal. Chem. 28:1756-1758.

Chen, R.W., Vasey, E.J., and Whanger, P.D. (1977). Accumulation and Depletion of Zinc in Rat Liver and Kidney Metallothioneins. J. Nutr. 107:805-813.

Cherian, M.G. (1977). Studies on the Synthesis and Metabolism of Zinc-thionein in Rats. J. Nutr. 197:955-972.

Chester, J.K., and Quarterman, J. (1970). Effects of Zinc Deficiency on Food Intake and Feeding Patterns of Rat. Brit. J. Nutr. 24:1061-1069.

Chrusciel, H. (1975a). The Effect of Toxic Environmental Products on the Development of Leucoplakia in Workers in Zinc and Lead Processing Plants. Czas. Stomat. 28:109-110.

Chrusciel, H. (1975b). The Effect of Toxic Environmental Products During Work in Zinc and Lead Processing Plants on the Paradontium. Czas. Stomat. 28:116-118.

Cohen, I. K., Schechter, P. J., and Henkin, R. I. (1973). Hypogeusia, Anorexia, and Altered Zinc Metabolism Following Thermal Burn. JAMA 223:914-916.

Compton, F.H., and Beagrie, G. S. (1975). Inhibitory Effect of Benzethonium and Zinc Chloride Mouthrinses on Human Dental Plaque and Gingivitis. J. Clin. Periodont. 2:23-43.

Cousins, R. J. (1979). Regulatory Aspect of Zinc Metabolism in Liver and Intestine. Nut. Rev. 37:97-103.

Cruickshank, D. B. (1936). The Natural Occurrence of Zinc in Teeth. Preliminary Experiments. Br. Dent. J. 61:530-531.

Cruickshank, D. B. (1940). The Natural Occurrence of Zinc in Teeth. III. Variation in Tuberculosis. Br. Dent.J. 68: 259-271.

Curzon, M. E. J. (1977). Dental Caries and Trace Element Composition of Whole Human Enamel: Eastern United States. J. Amer. Dent. Assoc. 94:1146-1150.

Curzon, M. E. J. (1978). Dental Caries and Trace Element Composition of Whole Human Enamel: Western United States. J. Amer. Dent. Assoc. 96:819-822.

Curzon, M. E. J., and Bibby, B. G. (1970). Effect of Heavy Metal on Dental Caries and Tooth Eruption. J. Dent. Child. 37:463-465.

Curzon, M. E. J., and Crocker (1978). Relationship of Trace Elements in Human Tooth Enamel to Dental Caries. Arch. Oral Biol. 23:647-653.

Curzon, M. E. J., and Losee, F. L. (1975). Trace Element Content of Enamel Related to Dental Caries. International Association for Dental Research, 53rd General Session, pg. 451, Abstr. #453.

Curzon, M. E. J., Losee, F. L., and Macalister (1975). Trace Elements in the Enamel of Teeth from New Zealand and the U.S.A. N. Z. Dent. J. 71:80-83.

Damyanov, I., and W. Dutz (1971). Anencephaly in Shiraz, Iran. Lancet 1:82.

Derise, N. L., and Ritchey, S.J. (1974). Mineral Composition of Normal Human Enamel and Dentin and the Relation of Composition to Dental Caries. II. Micromineral. J. Dent. Res. 53:853-858.

Derise, N. L., Ritchey, S. J., and Furr, A.K. (1974). Mineral Composition of Normal Human Enamel and Dentin and the Relation of Composition to Dental Caries: I. Macrominerals and Comparison of Methods of Analysis. J. Dent. Res. 53:847-852.

Diamond, I., and Hurley, L. (1970). Histopathology of Zinc-Deficient Fetal Rats. J. Nutr. 100:325-329.

Diamond, I., Swenerton, H., and Hurely L. S. (1971). Testicular and Esophageal Lessions in Zinc-Deficient Rats and Their Reversibility. J. Nutr. 101:77-84.

Dillaha, C. J., Lorincz, A. L., and Aavik, O. R. (1953). Acrodermatitis Enteropathica. Review of the Literature and Report of a Case Successfully Treated with Diodoquin. JAMA 152: 509-512.

DiOrio, L.P. (1969). Influence of Protein Malnutrition on Growth and Development of Dental Structures of Infant Rats. M.S. Thesis, Massachusetts Institute of Technology.

Dreosti, I. E., and Hurley, L. S. (1975). Depressed Thymidine Kinase Activity in Zinc-Deficient Rat Embryos. Proc. Soc. Exp. Biol. Med. 150:161-165.

Duff, T. A., and Coleman, J. E. (1966). Macaca Mulata Carbonic Anhydrase: Crystallization and Physicochemical and Enzymatic Properties of Two Isoenzymes. Biochem. 5:2009-2018.

Duncan, J.R., and Hurley, L.S. (1978). Thymidine Kinase and DNA Polymerase Activity in Normal and Zinc Deficient Developing Rat Embryos. Proc. Soc. Exp. Biol. Med. 159:39-43.

Evans, G.W. (1976). Zinc Absorption and Transport. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.), Vol. I, Academic Press, Inc., New York.

Evans, G.W., Grace, G.I., and Hahn, C. (1974). The Effect of Copper and Cadmium on ^{65}Zn Absorption in Zinc Deficient and Zinc Supplemented Rats. Bioinorg. Chem. 3:115-120.

Evans, G.W., and Hahn, C.J. (1975). Copper- and zinc-binding components in rat intestine. In: Protein Metal Interactions (ed. Freidman, M.), Plenum Press, New York.

Evans, G. W., and Johnson, P. E (1980). Characterization and Quantitation of Zinc-Binding Ligand in Human Milk. Pediatr. Res. 14:816-880.

Evans, G.W., Majors, P.F., and Cornatzer, W.E. (1970). Mechanism of Cadmium and Zinc Antagonism of Copper Metabolism. Biochem. Biophys. Res. Commun. 40:1142-1148.

Evans, G.W., and Winter, T.W. (1975). Zinc Transport by Transferrin in Rat Portal Blood Plasma. Biochem. Biophys. Res. Commun. 66:1218-1225.

Everett, G. A., and Apgar, J. (1979). Effect of Zinc Status on Salivary Zinc Concentration in the Rat. J. Nutr. 109:406-411.

Fairhall, L.T., and Hoyt, L.H. (1929). The Excretion of Zinc in Health and Disease. J. Clin. Invest. 7:537-541.

Fang, M., Kilgore, L., and Lei, K. Y. (1978). Effects of Zinc Deficiency on Dental Caries and Immune Response in Rats. Fed. Proc. 37:1949, Abstr. #1954.

Fang, M. M., Lei, K. Y., and Kilgore, L. T. (1980). Effect of Zinc Deficiency on Dental Caries in Rats. J. Nutr. 110:1032-1036.

Feigl, F. (1966). Spot Test in Organic Analysis, Elsevier Publ. Co., Amsterdam.

Fernandes, G., Nair, M., Onoe, K. Tanaka, T., Floyd, R., and Good, R. (1979). Impairment of cell-mediated immunity functions by deficiency in mice. Proc. Natl. Acad. Sci. 76:457-461.

Fisher, G.L. (1975). Function and Homeostasis of Copper and Zinc in Mammals. Science Total Environ. 4:373-412.

Flynn, A., Strain, W. H., Pories, W. J., and Hill, O. A., Jr. (1973). Blood Serum Zinc as an Indicator of Acute Stress. In: Trace Substances in Environmental Health (ed. Hemphill, D. D.), Vol. VII, University of Missouri, Columbia.

Foley, B., Johnson S.A., Hackley, B.M., Smith, J.C., Jr., and Halsted, J.A. (1968). Zinc content of human platelets. Proc. Soc. Exp. Biol. Med. 128:265-269.

Follis, R.H., Day, H.G., and McCollum, E.V. (1941). Histological Studies of the Tissues of Rats Fed a Diet Extremely Low in Zinc. J. Nutr. 22:223-233.

Fosmire, G. J., Al-ubaidi, Y. Y., and Sandstead, H. H. (1975). Some Effects of Postnatal Zinc Deficiency on Developing Rat Brain. Pediat. Res. 9:89-93.

Fosmire, G. J., Greeley, S., and Sandstead, H. H. (1977). Maternal and Fetal Response to Various Suboptimal Levels of Zinc Intake During Gestation in the Rat. J. Nutr. 107: 1543-1550.

Freeland-Graves, J.H., Hendrickson, P.J., Ebangit, M.L., and Snowden, J.Y. (1981). Salivary Zinc as an Index of Zinc Status in Women Fed a Low-Zinc Diet. Am. J. Clin. Nutr. 34:312-321.

Friedman, B. J., Freeland-Graves, J.H., Han, W-S., Young, R., Schorlemer, M., and Geders, J. (1980). Effect of Zinc Supplementation on Taste Acuity in Young Women. Fed. Proc. 39:651, Abstr. #2038.

Geders, J., Freeland-Graves, J., King, J., and Buchanan, D. (1980). Difference in Response to Zinc Supplementation in Young and Older Women. Fed. Proc. 39:652 Abstr. #2048.

Gerson, S. J., and Meyer, J. (1977). Increased Lactate Dehydrogenase Activity in Buccal Epithelium of Zinc-Deficient Rats. J. Nutr. 107:724-729.

Gordon, P.R., and O'Dell, B.L. (1980). Short Term Zinc Deficiency and Hemostasis in the Rat. Proc. Soc. Exp. Biol. Med. 163:240- 244.

Green, H. L. (1977). Trace Metal in Parental Nutrition. In: Zinc Metabolism: Current Aspects in Health and Disease (eds. Brewer G. J., and Prasad, A. S.), Alan R. Liss, Inc., New York.

Greger, J. L., and Geissler, A. H. (1978). Effect of Zinc Supplementation on Taste Acuity of the Aged. Amer. J. Clin. Nutr. 31:633-637.

Greger, J.L., and Sickles, V.S. (1979). Saliva Zinc Levels: Potential Indicators of Zinc Status. Amer. J. Clin. Nutr. 32:1859-1866.

Hahn, C., and Evans, G.W. (1973). Identification of a Low Molecular Weight ^{65}Zn Complex in Rat Intestine. Proc. Soc. Exp. Biol. Med. 144:793-795.

Hahn, C.J., Severson, M.L., and Evans, G.W. (1976). Structure of a Zinc Ligand Isolated from Duodenum. Fed. Proc. 35:863.

Hadjimarkos, D. M. (1966). Trace Elements in Public Water Supplies and Dental Caries. Arch. Environ. Health 13:102-104.

Hadjimarkos, D. M. (1967). Effect of Trace Element in Drinking Water on Dental Caries. J. Pediat. 70:967-699.

Halas, E.S., and Sandstead, H.H. (1975). Some Effects of Prenatal Zinc Deficiency on Behavior of the Adult Rat. Pediat. Res. 9:94-97.

Halsted, J. A., Ronaghy, H. A., Abadi, P., Haghashenass,M., Amirhakemi, G. H., Barakat, R. M., and Reinhold, J. G. (1972). Zinc Deficiency in Man. The Shriz Experiment. Amer. J. Med. 53:277-284.

Halsted, J.A., Smith, C.J., Jr., and Irwin, M.I. (1974). A Conspectus of Research on Zinc Requirements of Man. J. Nutr. 104:345-378.

Halsted, J. A., and Smith, F. C., Jr. (1970). Plasma-Zinc in Health and Disease. Lancet 1:322-324.

Hambidge, K. (1974). Zinc Deficiency in Children. In: Trace Element Metabolism In Animals-2 (eds. Hoekstra, W.G., Suttie, J.W., Ganter, H.E., Mertz, W.), University Park Press, Baltimore, Md.

Hambidge, K. M., Hambidge, C., Jacobs, M., and Baum, J. D. (1972). Low Levels of Zinc in Hair, Anorexia, Poor Growth, and Hypogeusia in Children. Pediat. Res. 6:868-874.

Hambidge, K. M., and Walravens, P. A. (1976). Zinc Deficiency in Infants and Preadolescent Children. In: Trace Element in Human Health and Disease (ed. Prasad, A.S.) Vol. I, Academic Press, Inc., New York.

Hambidge, K. M., Walravens, P. A., Brown, R.M., Webster, J., White, S., Anthony, M., and Roth, M. L. (1976). Zinc Nutrition of Preschool Children in the Head Start Program. Amer. J. Clin. Nutr. 29:734-738.

Hambidge, K. M., Walravens, P. A., and Nelder, K. H. (1974). The Role of Zinc in the Pathogensis and Treatment of Acrodermatitis Enteropathica. In: Zinc Metabolism: Current Aspects in Health and Disease (eds. Brewer, G.J., and Prasad, A.S.), Alan R. Liss, Inc., New York, NY.

Hardwick, J. L., and Martin, C. J. (1967). A Pilot Study Using Mass Spectrometry for the Estimation of the Trace Element Content of Dental Tissue. Helva. Odont. Acta 2:62-70.

Harris, S.S. (1976). Biochemical Function of Vitamin A in Bone Formation. Ph.D. Dissertation, University of Alabama in Birmingham.

Harris, S.S., and Navia, J.M. (1980). Vitamin A Deficiency and Caries Susceptibility of Rat Molars. Arch. Oral Biol. 25:415-421.

Hastings, M. M. (1980). Adrenocortical Hormone Response to Sodium Deprivation in the Zinc-Deficient Rat. Fed. Proc. 39:896 Abstr. #3307.

Haumont, S. (1961). Distribution of Zinc in Bone Tissue. J. Histochem. Cytochem. 9:141-145.

Haumont, S., and McLean, H. C. (1966). Zinc and Physiology of Bone. In: Zinc Metabolism (ed. Prasad, A. S.), Charles C. Thomas, Springfield, Ill.

Helle, A., and Haavikko, K. (1977a). The Concentrations of Nine Macro- and Microminerals in Drinking Water Examined in Relation to Caries Prevalence. Proc. Finn. Dent. Soc. 73:76-86.

Helle, A., and Haavikko, K. (1977b). Macro- and Micromineral Levels in Deciduous Teeth from Different Geographical Areas Correlated with Caries Prevalence. Proc. Finn. Dent. Soc. 73:87-98.

Hendershot, L. C., and Forsaith, J. (1959). Effects of Metals on Rat Caries and Enamel Metal Levels. J. Dent. Res. 38:669.

Henkin, R. I., and Bradley, D. F. (1970). Hypogeusia Corrected by Ni^{++} and Zn^{++}. Life Sciences 9:701-709.

Henkin, R. I., Keiser, H. R., Jaffe, I. A., Sternlieb, I., and Scheinberg, I. H. (1967). Decreased Taste Sensitivity After D-Penicillamine Reversed by Copper Administration. Lancet 2:1268-1271.

Henkin, R. I., Lippoldt, R. E., Bilstad, J., and Edelhoch, H. (1975). A Zinc Protein Isolated From Human Parotid Saliva. Proc. Natl. Acad. Sci. U.S.A. 72:488-492.

Henkin, R.I., Mueller, C.W., and Wolf, R.O. (1975). Estimation of Zinc Concentration of Parotid Saliva by Flameless Atomic Absorption Spectrophotometry in Normal Subjects and in Patients with Idiopathic Hypogeusia. J. Lab. Clin. Med. 86:175-180.

Henkin, R. I., Schecter, P. J., Friedewald, W. T., Demets, D. L., and Raff, M. (1976). A Double Blind Study of the Effects of Zinc Sulfate on Taste and Smell Dysfunction. Amer. J. Med. Sci. 272:285-299.

Henkin, R. I., Schecter, P. J., Raff, M. S., Bonzert, P. A., and Friedewald, W. T. (1974). Zinc and Taste Acuity: A Clinical Study Including a Laser Microprobe Analysis of the Gustatory Receptor Area. In: Clinical Applications of Zinc Metabolism (ed. Pories, W.J., Strain, W.H., Hsu, J.H., and Woosley, R.L.), Charles C. Thomas, Springfield, Ill.

Henzel, J. H., Deweese, M. S., and Lichti, E. L. (1970). Zinc Concentration Within Healing Wounds. Arch. Surg. 100:349-357.

Hess, F. M., King, J. C., and Margen, S. (1977). Zinc Excretion in Young Women on Low Zinc Intake and Oral Contraceptive Agents. J. Nutr. 107:1610-1620.

Hickory, W., Nanda, R., and Catalanotto, F.A. (1979). Fetal Skeletal Malformations Associated with Moderate Zinc Deficiency During Pregnancy. J. Nutr. 109:883-891.

Hoekstra, W.G. (1969). Skeletal and Skin Lesions of Zinc-deficient Chickens and Swine. Amer. J. Clin. Nutr. 22:1268-1277.

Holt, A. B., Spargo, R. M., Iveson, J. B., Faulkner, G. S., and Cheek, D. B. (1980). Serum and Plasma Zinc, Copper and Iron Concentrations in Aboriginal Communities of North Western Australia. Amer. J. Clin. Nutr. 33:119-136.

Hove, E., Elvehjem, C. A., and Hart, E. B. (1933). Further Studies on Zinc Deficiency in Rats. Amer. J. Physiol. 124: 750-758.

Hove, E., Elvehjem, C.A., and Hart, E.B. (1937). The Physiology of Zinc in the Nutrition of the Rat. Amer. J. Physiol. 119:768-778.

Hsieh, H.S., and Navia, J.M. (1980). Zinc Deficiency and Bone Formation in Guinea Pig Alveolar Implant. J. Nutr. 110:1581-1588.

Hurley, L.S., Duncan, J.R., Sloan, M.V., and Eckhert, C.D. (1977). Zinc-binding Ligands in Milk and Intestine: A Role in Neonatal Nutrition? Proc. Natl. Acad. Sci. USA, 74:3547-3549.

Hurley, L. S., Gowan, I., and Swenerton, H. (1971). Teratogenic Effects of Short-Term and Transitory Zinc Deficiency in Rats. Teratology 4:199-204.

Hurley, L. S., and Mutch, P. B. (1973). Prenatal and Postnatal Development After Transitory Gestational Zinc Deficiency in Rats. J. Nutr. 103:649-656.

Hurley, L.S., and Shrader (1972). Congenital Malformation of the Nervous System in Zinc Deficient Rats. In: Neurobiology of the Trace Metal Zinc and Copper (ed. Pfeiffer C.C.), Int. Rev. Neurobiol. Supp. 1:7-51. Academic Press, Inc., New York.

Hurley, L. S., and Swenerton, H. (1966). Congenital Malformations Resulting from Zinc Deficiency in Rats. Proc. Soc. Exp. Biol. Med. 123:692-696.

Huxley, H.G., and Leaver, A.G. (1966). The Effect of Different Levels of Dietary Zinc and Calcium Upon the Zinc Concentration of the Rat Femur and Incisor. Arch. Oral Biol. 11:1337-1344.

Idris, Z. H., and der Kaloustian, V. M. Acrodermatitis Enteropathica. Clin. Pediat. 12:393-395.

Isaac, S., Brudevold, F., Smith, F. A., and Garner, D. E. (1958). Solubility Rate and Natural Fluoride Content of Surface and Subsurface Enamel. J. Dent. Res. 37:254-263.

Jacobs, R. M., Riza, R., Kallal, Z., Hanson, E., and Forbs, A. L. (1980). Zinc Nutrition of Tunisians. Fed. Proc. 38:897, Abstr. #3313.

Johnson, P.E., and Evans, G.W. (1978). Relative Zinc Availability in Human Breast Milk, Infant Formulas and Cow's Milk. Amer. J. Clin. Nutr. 31:416-421.

Johnson, P. J., Freeland, J., and Ebangil, M. L. (1978). Saliva: A Diagnostic Tool for Evaluating Zinc Status in Man. Fed. Proc. 37:253, Abstr. #217.

Johnston, W. T., and Evans, G. W. (1980). Isolation of a (Copper, Zinc) Thionein from the Small Intestine of Neonatal Rats. Biochem. Biophys. Res. Communication 96:10-17.

Joseph, C.E., Ashrafi, S.H., and Waterhouse, J.P. (1981). Structural Changes in Rabbit Oral Epilhelium Caused by Zinc Deficiency. J. Nutr. 111:53-57.

Kagi, J.H.R., and Vallee, B.L. (1961). Metallothionein: A Cadmium and Zinc-containing Protein from Equine Renal Cortex. II. Physicochemical properties. J. Biol. Chem. 236:2435-2442.

Kaneko, Y., Inamori, I., and Nishimura, M. (1974). Zinc, Lead, Copper, and Cadmium in Human Teeth from Different Geographical Areas in Japan. Bull. Tokyo Dent. Coll. 15:233-243.

Kay, R. G., Tasmen-Jones, J., Pylous J., Whiting R., and Black, H. (1976). A Syndrome of Acute Deficiency During Total Parenteral Alimentation in Man. Ann.Surg. 183:331-340.

Keilin, D., and Mann, T. (1940). Carbonic Anhydrase: Purification and the Nature of the Enzyme. Biochem. J. 34:1163-1176.

Keyes, P. H. (1958). Dental Caries in the Molar Teeth of Rats. Method for Diagnosis and Scoring Several Types of Lesions. J. Dent. Res. 37:1088-1099.

Kfoury, G. A., Reinhold, J. G., and Simonian S. J. (1968). Enzyme Activities in Tissues of Zinc-Deficient Rats. J. Nutr. 95: 102-110.

Kirchgessner, M., Roth, H.P., and Weigand, E. (1976). Biochemical Changes in Zinc Deficiency. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.), Academic Press, Inc., New York.

Klingberg, W. G., Prasad, A. S., and Oberleas, D. (1976). Zinc Deficiency Following Penicillamine Therapy. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.), Vol. I, Academic Press, Inc., New York.

Klouda, G.S., and Navia, J.M. (1974). An Improved Model System for the Study of Bone Repair. Calc. Tiss. Res. 14:245-249.

Knuuttila, M., Lappalainen, R., and Konlturi-Närhi, V. (1979). Concentrations of Ca, Mg, Mn, Sr, and Zn in Supra- and Subgingival Calculus. Scand. J. Dent. Res. 87:192-196.

Kowarski, S., Blair-Stanek, C. S., and Schanchter, D. (1974). Active Transport of Zinc and Identification of Zinc-Binding Protein in Rat Jejunal Mucosa. Amer. J. Physiol. 226:401-407.

Kreitzman, S. N. Nutrition in the Process of Dental Caries. Dent. Clin. North Amer. 20:401-505.

Langmyhr, F. J., and Eyde, B. (1979). Determination of the Total Content and Distribution of Cadmium, Copper and Zinc in Human Parotid Saliva. Anal. Chim. Acta 107:211-218.

Lappalainen, R., and Knuuttila, M. (1979). The Distribution and Accumulation of Ca, Zn, Pb, Cu, Co, Ni, Mn and K in Human Teeth From Five Different Geological Areas of Finland. Arch. Oral Biol. 24:363-368.

Larson, D. L., Maxwell, R., Abston, S., and Dobrkovsky, M. (1970). Zinc Deficiency in Burned Children. Plast. Reconstr. Surg. 46:13-21.

Larsson, K.S., Arnander, C., Cekanova, E., and Kjellberg, M. (1976). Studies of Teratogenic Effects of the Dithiocarbamates Maneb, Mancozeb. and Propineb. Teratology 14:171-184.

Latimer, J. S., McClain, C. J., and Sharp H. L. (1980). Clinical Zinc Deficiency During Zinc-Supplemented Parenteral Nutiriton. J. Pediat. 97:434-437.

Lease, J. G. (1975). Effect of Variations in Dietary Protein and Amino Acids on the Alkaline Phosphatase of the Zinc-Deficient Chick. J. Nutr. 105:385-392.

Lemann, I. I. (1910). A Study of the Type of Infantalism in Hookworm Disease. Arch. Int. Med. 6:139-146.

Lindeman, R.D., Baxter, D.J., Yunic, A.A., and Kraikitpanitch, S. (1978). Serum Concentrations and Urinary Excretion in Cirrhosis, Nephrotic Syndrome and Renal Insufficiency. Amer. J. Med. Sci. 275:17-31.

Lindeman, R. D., Yunice, A. A., Baxter, D. J., Miller, L. R., and Nordquist, J. (1973). Myocardial Zinc Metabolism in Experimental Myocardial Infarction. J. Lab. Clin. Med. 81: 194-204.

Lönnerdal, B., Stanislowski, A.G., and Hurley, L.S. (1980). Isolation of a low molecular weight zinc binding ligand from human milk. J. Inorg. Biochem. 12:71-78.

Losee, F. L. (1966). Trace Element Variables Related to Oral Health. In: Environmental Variables in Oral Disease (eds. Kreshover, S. J., and McClure, F. J.), Publication No. 81, American Association for the Advancement of Science, Washington, D. C.

Losee, F. L., Cutress, T. W., and Brown, R. (1974). Trace Elements in Human Dental Enamel. In: Trace Substance in Environmental Health VII (ed. Hemphill, D. D.), University of Missouri, Columbia.

Losee, F. L., Curzon, M.E.J., and Little, M.F. (1974). Trace Element Concentration in Human Enamel. Arch. Oral Biol. 19:467-470.

Luna, L.G., (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., McGraw-Hill Book Co., New York.

Lyon, T. D., Smith, H., and Smith, L. B. (1979). Zinc Deficiency in the West of Scotland? A Dietary Intake Study. Br. J. Nutr. 42:413-416.

Macapinlac, M. P., Barney, G. H., Pearson, W.N., and Darby, W. J. (1967). Production of Zinc Deficiency in the Squirrel Monkey (Saimiri sciureus). J. Nutr. 93:499-510.

Macapinlac, M. P., Pearson, N. W., and Darby, W. J. (1966). Some Characteristics of Zinc Deficiency in the Albino Rat. In: Zinc Metabolism (ed. Prasad, A.S.), Charles C. Thomas, Springfield, Ill.

Mann, S. O., Fell, B. F., and Dalgarno, A. C. (1974). Observations of the Bacterial Flora and Pathology of the Tongue of Sheep Deficient in Zinc. Res. Vet. Sci. 17:91-101.

Margoshes, M., and Vallee, B.L. (1957). A Cadmium Protein from Equine Kidney Cortex. J. Amer. Chem. Soc. 79:4813-4814.

Mathur, A., Wallenius, K., and Abudlla, M. (1977). Relation Between Zinc Content in Saliva and Blood in Healthy Human Adults. Scand. J. Clin. Lab. Invest. 37:469-472.

Mawson, C. A., and Fischer, M. I. (1952a). The Occurence of Zinc in the Human Prostate Gland. Can. J. Med. Sci. 30:336-339.

Mawson, C. A., and Fischer, M. I. (1952b). Carbonic Anhydrase in the Prostate Gland of the Rat and Rabbit. Arch. Bioch. Biophys. 36:485-486.

Mawson, C. A., and Fischer, M. I. (1953). Zinc and Carbonic Anhydrase in Human Semen. Bioch. J. 55:696-700.

McBean, L.D., Dove, J.T., Halsted, J.A., and Smith, J.C., Jr. (1972). Zinc Concentrations in Human Tissues. Amer. J. Clin. Nutr. 25:672.

McClain, C., Soutor, C., and Zieve, L. (1980). Zinc Deficiency: A Complication of Crohn's Disease. Gastroenterology 78:272-279.

McClure, F. J. (1948). Observation on Induced Caries in Rats. J. Dent. Res. 27:34-40.

McConnell, S. D., and Henkin, R.I. (1974). Altered Preference for Sodium Chloride, Anorexia and Changes in Plasma and Urinary Zinc in Rats Fed a Zinc Deficient Diet. J. Nutr. 104:1108-114.

McKenzie, J. M. (1979). Content of Zinc in Serum, Urine, Hair, and Toenails of New Zealand Adults. Amer. J. Clin. Nutr. 32: 570-579.

McKenzie, J. M., Fosmire, G. J., and Sandstead, H. H. (1975). Zinc Deficiency During the Later Third of Pregnancy: Effects on Fetal Rat Brain, Liver and Placenta. J. Nut. 105:1466-1475.

Menaker, L., and Navia, J.M. (1973). Effects of Undernutrition During the Perinatal Period on Caries Development in the Rats: IV. Effects of Differential Tooth Eruption and Exposure to a Cariogenic Diet on Subsequent Dental Caries Incidence. J. Dent. Res. 52:692-697.

Mesrobian, A. Z., and Shkler, G. (1969). The Effect of Dietary Zinc Sulfate Supplements on Healing of Experimental Extraction Wounds. Oral Surg. Oral Med. Oral Path. 28:259-265.

Meyer, J., and Alvares, C. F. (1974). Dry Weight and Size of Cells in the Buccal Epithelium of Zinc-Deficient Rats: A Quantitative Study. Arch. Oral Biol. 19:471-476.

Mikac-Devic, D. (1970). Methodology of Zinc Determination and the Role of Zinc in Biochemical Processes. Adv. Clin. Chem. 13:271-333.

Miller, E.R., Luecke, R.W., Ullrey, D.E., Baltzer, B.V., Bradley, B.L., and Hoefer, J.A. (1968). Biochemical Skeletal and Allometric Changes Due to Zinc Deficiency in the Baby Pig. J. Nutr. 95:278-286.

Miller, J.K., and Miller, W.J. (1962). Experimental Zinc Deficiency and Recovery of Calves. J. Nutr. 76:467-474.

Miller, W.J., Powell, G.W., Pitts, W.J., and Perkins, H.F. (1965). Factors Affecting Zinc Content of Bovine Hair. J. Dairy Sci. 48:1091-1095.

Mills, C.F., Quarterman, J., Chesters, J.K., Williams, R.B., and Dalgarno, A.C. (1969). Metabolic Role of Zinc. Amer. J. Clin. Nutr. 22:1240-1249.

Morris, E.R., and Ellis, R. (1980). Effect of Dietary Phytate Zinc Molar Ratio on Growth and Bone Zinc Response of Rats Fed Semipurified Diets. J. Nutr. 110:1037-1045.

Morrison, A. B., and Sarett, H. P. (1958). Studies of Zinc Deficiency in the Chick. J. Nutr. 65:267-279.

Moynahan, E. J. (1974). Acrodermalitis Enteropathica: A Lethal Inherited Human Zinc-Deficiency Disorder. Lancet 2:399-400.

Moynahan, E. J., and Barnes, P.M. (1973). Zinc Deficiency and Synthetic Diet for Lactose Intolerance. Lancet 1:676-677.

Navia, J. M. (1970). Effect of Minerals on Dental Caries. In: Dietary Chemicals vs. Dental Caries (ed. Harris, R. S.), Advances in Chemistry Series, No. 94, American Chemical Society, Washington, D.C.

Navia, J.M. (1977). Animal Models in Dental Research. The University of Alabama Press, University, Ala.

Navia, J. M., Cagnone, L. D., Lopez, H., and Harris, R. S. (1968). The Effect of $MgCl_2$, $ZnCl_2$, $MnCl_2$ and Na Trimetaphosphate on Dental Caries When Fed Alone or in Combinations. J. Dent. Res. 47:128, Abst. #361.

Navia, J. M., Lopez, H., and Harris, R. S. (1969). Purified Diet for Caries Research with Rats. J. Nutr. 97:133-140.

Navia, J.M., and Narkates, A.J. (1980). Dental Research in the Laboratory Rat (eds. Baker, H.J., Lindsey, J.R., and Weisbroth, S.H.), Vol. II, Academic Press, Inc., New York.

Niedermeier, W., Griggs, J.H., and Johnson, R.S. (1971). Emission Spectrometric Determination of Trace Elements in Biological Fluids. Applied Spectro. 25:53-56.

Nixon, G. S., Livingston, H. D., and Smith, H. (1967). Estimation of Zinc in the Human Enamel by Activation Analysis. Arch. Oral Biol. 12:411-416.

Norman, J.N., Rahmat, A., and Smith, G. (1975a). Effect of Supplements of Zinc Salts on the Healing of Incised Wounds in the Rat and Guinea Pig. J. Nutr. 105:822-826.

Norman, J.N., Rahmat, A., and Smith G. (1975b). Effect of Supplements of Zinc Salts on the Healing of Granulating Wounds in the Rat and Guinea Pig. J. Nutr. 105:815-821.

Oberleas, D., and Prasad, A.S. (1976). Factors Affecting Zinc Absorption. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.) Vol. I, Academic Press, Inc., New York.

O'Dell, B. L., Newberne, P.M., and Savage, J.E. (1958). Significance of Dietary Zinc for the Growing Chicken. J. Nutr. 65:503-523.

O'Dell, B. L., and Savage, J. E. (1960). Effect of Phytic Acid on Zinc Availability. Proc. Soc. Exp. Biol. Med. 103:304-306.

Oh, S.H., Deagen, J.T., Whanger, P.D., and Weswing, P.H. (1978). Biological Function of Metallothionein. V. Its Induction in Rats by Various Stresses. Amer. J. Physiol. 234:E282-E285.

Ogiso, T., Ogawa, N., and Miura, T. (1979). Inhibitory Effect of High Dietary Zinc on Copper Absorption in Rats. II. Binding of Copper and Zinc to Cytosol Proteins in the Intestinal Mucosa. Chem. Pharm. Bull. 27:515-521.

Orten, J.M. (1966). Biomedical Aspects of Zinc Metabolism. In: Zinc Metabolism (ed. Prasad, A.S.), Charles C. Thomas, Springfield, Ill.

Osmanski, C. P., and Meyer, J. (1969). Ultrastructural Changes in Buccal and Palatal Mucosa of Zinc Deficient Rats. J. Invest. Derm. 53:14-28.

Parisi, A. F., and Vallee, B. L. (1969). Zinc Metalloenzyme: Characteristics and Significance in Biology and Medicine. Amer. J. Clin. Nutr. 22:1222-1239.

Parisi, A.F., and Vallee, B.L. (1970). Isolation of a Zinc α2-macroglobulin from Human Serum. Biochemistry 9:2421-2426.

Paynter, K.J., and Grainger, R.M. (1956). Relation of Nutrition to the Morphology and Size of Rat Molar Teeth. J. Can. Dent. Assn. 22:519-531.

Pearez, A. G., Castro, C., and Franco, A. (1974). A Case of Optic Atrophy Possibly Induced by Quinoline in Acrodermatitis Enteropathica. Brit. J. Dermatol. 40:701-703.

Perry, H.M., Jr., and Schroeder, H.A. (1957). Leisons Resembling Vitamin B Complex Deficiency and Urinary Loss of Zinc Produced by Ethylendiamine Tetracetate. Am. J. Med. 22:168-172.

Petering, H.G., D.W. Yeager, and S.O. Witherup (1971). Trace Metal Content of Hair, I. Zinc and Copper Content of Human Hair in Relation to Age and Sex. Arch. Environ. Health 23:202-207.

Picciano, M.F., and Guthrie, H.A. (1973). Determination of Concentrations and Variations of Copper, Iron, and Zinc in Milk. Fed. Proc. 32:929.

Prasad, A. S. (1966). Metabolism of Zinc and Its Deficiency in Human Subjects. In: Zinc Metabolism (ed. Prasad, A.S.), Charles C. Thomas, Springfield, Ill.

Prasad, A.S. (1976). Deficiency of Zinc in Man and Its Toxicity. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.), Academic Press, Inc., New York.

Prasad, A. S. (1977). Zinc in Human Studies. In: The Biomedical Role of Trace Elements in Aging (eds. Hsu, J.M., Davis, R.L., and Neilhaman, R.W.), Eckerd College Gerontology Center, St. Petersburg, Fla.

Prasad, A.S., Fernand-Madrid, F., and Ryan, J.R. (1979). Deoxylhymidine Kinase Activity of Human Implanted Sponge Connective Tissue in Zinc Deficiency. Amer. Physiol. Soc. 236:E272-E275.

Prasad, A. S., Halstead, J. A., and Nadimi, M. (1961). Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Hypogonadism, Dwarfism and Geophagia. Amer. J. Med. 31:532-546.

Prasad, A. S., Maile, A., Jr., Farid, Z., Sandstead, H. H., and Schulert, A.R. (1963). Zinc Metabolism in Patient with the Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Dwarfism, and Hypogonadism. J. Lab. Clin. Med. 61:537-549.

Prasad, A. S., Oberleas, D., and Halsted, J. A. (1965). Determination of Zinc in Biological Fluids by Atomic Absorption Spectrophotometry in Normal and Cirrhotic Subjects. J. Lab. Clin. Med. 66:508-516.

Prasad, A. S., and Oberleas, D. (1974). Biochemical Effects of Zinc Deficiency in Experimental Animals. In: Clinical Applications of Zinc Metabolism (eds. Pories, W.J., Strain, W.H., Hsu, J.M., and Woosley, R.L.), Charles C. Thomas, Springfield, Ill.

Prasad, A.S., Oberleas, D., Wolf, P.L., and Horwitz, J. (1967). Studies on Zinc Deficiency. J. Clin. Invest. 46:549-557.

Prasad, A.S., Rabbani, P., Abassii, A., Bowersox, E., and Fox, M.R.S. (1978). Experimental Zinc Deficiency in Humans. Ann. Int. Med. 89:483-490.

Prasad, A. S., Schulert, A. R., Sandstead, H.H., Miale, A., Jr., and Farid, Z. (1963). Zinc, Iron and Nitrogen Content of Sweat in Normal and Deficient Subjects. J. Lab. Clin. Med. 62:84-89.

Pulido, P., Kagi, J.H.R., and Vallee, B.L. (1966). Isolation and Some Properties of Human Metallothionein. Biochem. 5:1768-1777.

Quarterman, J., Mills, C.F., and Humphries, W.R. (1966). The Reduced Secretion of, and Sensitivity to, Insulin in Zinc Deficient Rats. Biochem. Biophys. Res. Commun. 25:354-385.

Reinhold, J. G., Faradjii, B., Abadi, P., and Ismail-Beigi, F. (1976). Binding of Zinc to Fiber and Other Solids of Wholemeal Bread. In: Trace Element in Human Health and Disease (ed. Prasad, A. S.), Vol. I, Academic Press, Inc., New York.

Reinhold, J. G., and Kfoury, G. A. (1969). Zinc-Dependent Enzyme in Zinc Depleted Rats: Intestinal Alkaline Phosphatase. Amer. J. Clin. Nutr. 22:1350-1263.

Reinhold J.G., Kfoury, G.A., and Arslanian, M. (1968). Relation of Zinc and Calcium Concentrations in Hair to Zinc Nutrition in Rats. J. Nutr. 96:519-542.

Reinhold, J.G., Kfoury, G.A., Ghalambour, M.A., and Bennett, J.C. (1966). Zinc and Copper Concentration in Hair of Iranian Villagers. Amer. J. Clin. Nut. 18:294-300.

Reinhold, J.G., Kfoury, G.A., and Thomas, T.A. (1967). Zinc, Copper and Iron Concentrations in Hair and Other Tissues: Effects of Low Zinc and Low Protein Intakes in Rats. J. Nutr. 92:173-182.

Reis, B.L., and Evans, G.W. (1977). Genetic Influence on Zinc Metabolism in Mice. J. Nutr. 107:1683-1686.

Retief, D. H., Cleaton-Jones, P. E., Turkstra, J., and DeWet, W. J. (1971). The Quantitative Analysis of Sixteen Elements in Normal Human Enamel and Dentin by Neutron Activation Analysis and High Resolution Gamma Spectrometry. Arch. Oral Biol. 16:1257-1267.

Retief, D. H., Turkstra, J., Cleaton-Jones, P. E., and Biddlecombe, F. (1978). Mineral Composition of Enamel from Population Groups with High and Low Caries Incidence. J. Dent. Res. 15:150 Abstr. #302.

Rickli, E. E., and Edsall, J. T. (1962). Zinc Binding and the Sulfhydryl Group of Human Carbonic Anhydrase. J. Biol. Chem. 237:258-260.

Rijhsinghani, K., Squier, C. A., and Meyer, J. (1975). The Ultrastructural Localization of Acid Phosphatase in Zinc-Deficient Rat Buccal Epithelium. Arch. Oral Biol. 20:461-464.

Riodan, J. F. (1976). Biochemistry of Zinc. Med. Clin. North. Amer. 60:661-674.

Riodan, J.R., and Richards, V. (1980). Human Fetal Liver Contains Both Zinc- and Copper-rich Forms of Metallothionein. J. Biol. Chem. 255:5380-5383.

Ritchey, S. J., Korslund, M. I., Gilbert, L. M., Fay, D. C., and Robinson, M. F. (1979). Zinc Retention and Losses of Zinc in Sweat by Preadolescent Girls. Amer. J. Clin. Nut. 32:799-803.

Ronaghy, H. (1974). Dwarfism and Delayed Sexual Maturation Caused by Zinc Deficiency. In: Clinical Applications of Zinc Metabolism (eds. Proeis, W.J., Strain, W.H., Hsu, J.M., and Woosley, R.L.), Charles C. Thomas, Springfield, Ill.

Ronaghy, H., Reinhold, J. G., Mahlaudji, M., Ghavami, P., Fox, M.R.S., and Halsted, J. A. (1974). Zinc Supplementation of Malnourished School Boys in Iran: Increased Growth and Other Evidence of Effectiveness. Amer. J. Clin. Nut. 27:112-121.

Rubini, M. E., Montalvo, G., Lockhart, C. P., and Johnson, C. R. (1961). Metabolism of Zinc-65. Amer. J. Phys. 200:1345-1348.

Sanchez-Agreda, M., Gimorra, G. A., Mariona, M., and Garcia-Jalön, A. (1978). Trace Elements in Burned Patients: Studies of Zinc, Copper, and Iron Contents in Serum. Burns, 4:28-31.

Sandstead, H. H., Fosmire, G. J., McKenzie, J. M., and Halas, E. S. (1975). Zinc Deficiency and Brain Development in the Rat. Fed. Proc. 34:86-88.

Sandstead, H. H., Fosmire, G. J., Halas, E. S., Jacob, R. A., Strobel, D. A., and Markes, E. O. (1977). Zinc Deficiency: Effect of Brain and Behavior of Rats and Rhesus Monkeys. Teratology 16:229-234.

Sandstead, H. H., Prasad, A. S., Schulert, A. R., Farid, Z., Miale, A., Jr., Bassilly, S., and Dandy, W.J. (1967). Human Zinc Deficiency, Endocrine, Manifestation and Response to Treatment. Am. J. Clin. Nutr. 20:422-442.

Sandstead, H. H., Vo-Khactu, K.P., and Solomons, N. (1976). Conditioned Zinc Deficiency. In: Trace Elements in Human Health and Disease (ed. Prasad, A. S.), Vol. I, Academic Press, Inc., New York.

Saylor, W.W., Morrow, R.D. and Leach, R.M., Jr. (1980). Copper and Zinc-binding Proteins in Sheep Liver and Intestine: Effects of Dietary Levels of the Metal. J. Nutr. 110:460-468.

Schamschula, R. G., Agus, H., Bunzel, M., Adkins, L. B., and Barmes, D. E. (1977). The Concentrations of Selected Major and Trace Minerals in Human Dental Plaque. Arch. Oral Biol. 22:321-325.

Schlicker, S. A., and Cox, D. H. (1968). Maternal Dietary Zinc, and Development and Zinc, Iron and Copper Content of the Rat Fetus. J. Nut. 95:287-294.

Schour, I. (1938). Calcium Metabolism and Teeth. JAMA 110:870-877.

Schour, I., and Massler, M. (1971). The Teeth. In: The Rat in Laboratory Investigation (eds. Farris, E.J., and Griffith, J.Q.), Hafner Publishing Company, New York.

Sever, L.E., and Emanuel, I. (1972). Is There a Connection between Maternal Zinc Deficiency and Congenital Malformations of the Central Nervous System in Man? Teratology 7:117-118.

Shatzman, A. R., and Henkin, R. I. (1980). Saliva Zinc-Gustin-Taste Interaction. Fed. Proc. 39:897, Abstr. #3315.

Sivasubramanian, K. N., and Henkin, R. I. (1978). Behavioral and Dermatologic Changes and Low Serum Zinc and Copper Concentrations in Two Premature Infants After Parenteral Alimentation. J. Pediat. 93:847-851.

Smith, J.C., Jr., and Halsted, J.A. (1970). Clay Ingestion (Geophagia) as a Source of Zinc for Rats. J. Nutr. 100:973-980.

Smith, K.T., Cousins, R.J., Silbon, B.L., and Failla, M.L. (1978). Zinc Absorption and Metabolism by Isolated, Vascularly Perfused Rat Intestine. J. Nutr. 108:1849-1857.

Smith, K.T., and Cousins, R.J. (1979). Intracellular and Extracellular Aspects of Zinc Absorption by Isolated Vascularly Perfused Rat Intestine. Fed. Proc. 38:282.

Snowden, J., and Freeland, J. H. (1978). Circadian Rhythms of Zinc in Saliva. Fed. Proc. 37:890, Abstr. #3557.

Sobocinski, P. Z., Canterbury, W. J., Jr., and Powanda, M.C. (1977). Differential Effect of Parenteral Zinc on the Course of Various Bacterial Infections. Proc. Soc. Exp. Biol. Med. 156:334-339.

Sognnaes, R.F. (1957). Caries Research in Laboratory Animals. New Engl. J. Med. 256:1226-1229.

Solomons, N.W., Rosenberg, I.H., Sandstead, H.H., and Vo-Khactu, K.P. (1977). Zinc Deficiency in Crohn Disease. Digestion 16:87-95.

Song, M. K., and Adham, N. F. (1978). Role of Prostaglandin E_2 in Zinc Absorption in Rat. Amer. J. Physiol. 234:E99-105.

Song, K. M., and Adham, N. F. (1979). Evidence for an Important Role of Prostaglandins E_2 and F_2 in Regulation of Zinc Trans ports in Rat. J. Nutr. 109:2152-2159.

Söremark, R., and Samsahl, K. (1961). Gamma-Ray Spectrometric Analysis of Elements in Normal Human Enamel. Arch. Oral Biol. 6: 275-283.

Söremark, R., and Samsahl, K. (1962). Gamma-Ray Spectrometric Analysis of Elements in Normal Human Dentin. J. Dent. Res. 41:603-606.

Sortino, G., and Palazzo, U. (1971a). Zinco E Carie Sperimentale nel Ratto. Riv. Ital. Stomatol. 26:509-513.

Sortino, G., and Palazzo, U. (1971b). Azione Della Somministrazione di Dieta Priva di Zinco nei Ratti. Riv. Ital. Stomatol. 26:587-592.

Spencer, H., Osis, D., Kramer, L., and Norris, C. (1976). Intake, Excretion, and Retention of Zinc in Man. In: Trace Elements in Human Health and Disease (ed. Prasad, A.S.), Vol. I, Academic Press, Inc., New York.

Steinman, R. R., and Leonora, J. (1975). Effect of Selected Dietary Additives on the Incidence of Dental Caries in the Rat. J. Dent. Res. 54:570-577.

Stevenson, A.C., Johnston, H.A., Stewart, M.I.P., and Golding, D.R. (1966). Congenital Malformations. A Report of a Study of Series of Consecutive Births in 24 Centres. Bull. Wld. Hlth. Org., Suppl. 34:1-127.

Strain, W.H., and Pories, W.J. (1966). Zinc Levels of Hair as Tools in Zinc Metabolism. In: Zinc Metabolism (ed. Prasad, A.S.), Charles C. Thomas, Springfield, Ill.

Sturtevant, F. M. (1980). Zinc Deficiency, Acrodermalitis Enteropathica, Optic Atrophy, Subacute Mylo-Optic Neuropathy, and 5,7-dihalo-8-quinolinols. Pediatr. 65:610-613.

Swenerton, H., and Hurley, L.S. (1971). Teratogenic Effects of a Chelating Agent and Their Prevention by Zinc. Science 173:62-63.

Swenerton, H., and Hurley, L.S. (1980). Zinc Deficiency in Rhesus and Bonnet Monkeys, Including Effects on Reproduction. J. Nutr. 110:573-583.

Taylor, F.B. (1963). Significance of Trace Elements in Public Finished Water Supplies. J. Amer. Water Works Assoc. 55:619-623.

Todd, W. R., Elvehjem, C. A., and Hart, E. B. (1934). Zinc in the Nutrition of the Rat. Amer. J. Phys. 107:146-156.

Tuchinda, J., Hoffman, S., Harris, S.S., and Navia, J.M. (1981). International Association of Dental Research, 59th General Session, J. Dent. Res. 60:553, Abstr. #976.

Tucker, H. F., and Salmon, W. D. (1955). Parakeratosis or Zinc Deficiency Disease in the Pig. Proc. Soc. Exp. Biol. Med. 88:613-616.

Underwood, E.J. (1971). Trace Elements in Human and Animal Nutrition, 3rd ed. Academic Press, Inc., New York.

Underwood, E. J. (1977). Trace Element in Human and Animal Nutrition, 4th ed. Academic Press, Inc., New York.

Vallee, B.L., and Gibson, J.G. (1948). The Zinc Content of Normal Human Whole Blood, Plasma, Leucocytes, and Erythrocytes. J. Biol. Chem. 176:445-457.

Vallee, B. L., and Neurath, H. (1954). Carboxypeptidase, A Zinc Metalloprotein. J. Amer. Chem. Soc. 76:5006-5007.

Vallee, B.L., Wacker, W.E.C., Bartholomay, A.F., and Hoch, F.L. (1959). Zinc Metabolism in Hepatic Dysfunction. Ann. Int. Med. 50:1077-1091.

Vikbladh, I. (1951). Studies on Zinc in Blood. Scand. J. Clin. Lab. Invest. 3(Suppl. 2):1-74.

van Vloten, W. A., and Bos, L. P. (1978). Skin Lesions in Acquired Zinc Deficiency Due to Parenteral Nutrition. Dermatologica 156:175-185.

Wacker, W.E.C. (1976). Role of Zinc in Wound Healing: A Critical Review. In: Trace Elements in Human Health and Disease (ed. Prasad, A. S.), Vol. I, Academic Press, Inc., New York.

Wacker, W.E.C., Ulmer, D. D., and Vallee, B. L. (1956). Metalloenzymes and Myocardial Infarction. II. Malic and Lactic Dehydrogenase Activities and Zinc Concentrations in Serum. N. Engl. J. Med. 255:449-456.

Wagner, P. A., Krista, M. L., Bailey, L. B., Christakis, G. J., Jernigan, J. A., Araujo, P. E., Appledorf, H., Davis, C. G., and Dinning, J. S. (1980). Zinc Status of Elderly Black Americans from Urban Low-Income Households. Amer. J. Clin. Nutr. 33:1771-1777, 1980.

Walker, B. E., Hughes, S., Simmons, A. V., and Chandler, G. N. (1978). Plasma Zinc After Myocardial Infarction. Eur. J. Clin. Invest. 8:193-195.

Warkany, J., and Petering, H.G. (1972). Congenital Malformations of the Central Nervous System in Rats Produced by Maternal Zinc Deficiency. Teratology 5:319-334.

Warkany, J., and Petering, H. G. (1973). Congenital Malformations of Brain Produced by Short Zinc Deficiencies in Rats. Amer. J. Ment. Defic. 77:645-653.

Weitzel, G., Strecker, F. J., Buddecke, E., and Fretzdorff, A. M. (1954). Zinc in Tapetum Lucidum. Hoppe-Seyler's Z. Physiol. Chem. 296:19-30.

Westmoreland, N. (1971). Connective Tissue Alterations in Zinc Deficient Rats. Fed. Proc. 30:1001-1010.

Westmoreland, N., and Hoekstra, (1969). Histochemical Studies of Alkaline Phosphatase in Epiphyseal Cartilage of Normal and Zinc-Deficient Chicks. J. Nutr. 98:83-89.

Wildra, A. (1964). Phosphate Directed Y-M Variation in Candida Albicans. Mycopathol. Mycol. Appl. 23:197-202.

White, G.E. (1975). In: Dental Caries: A Multifactorial Disease, Charles C. Thomas, Springfield, Ill.

World Health Organization (1971). The Etiology and Prevention of Dental Caries, Report of a World Health Organization Scientific Group, Technical Report Series, No. 494.

Worthington, B. S., Alvares, O. F., and Ahmed, S. (1978). Effect of Protein Deficiency and Zinc Deficiency on the Parotid Gland of the Rat. Fed. Proc. 37:585, Abst. #1955.

Yamaguchi, H. (1975). Control of Dimorphism in Candida Albicans by Zinc: Effect on Cell Morphology and Composition. J. Gen. Microbiol. 86:370-372.

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Rashid Nouri Al-Hayali

Major Subject Pathology

Title of Dissertation Effect of Gestational and Lactational Zinc Deficiency on the Developing Dental Tissues and Incidence of Dental Caries in the Rat

Dissertation Committee:

________________, **Chairman**

Director of Graduate Program ________________

Dean, UAB Graduate School ________________

Date May 15, 1981

PS-1428