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Maze Acquisition in the Zinc Deficient Rat

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MAZE ACQUISITION IN THE ZINC DEFICIENT RAT

by

Paige M. Lokken

Bachelor of Philosophy, University of North Dakota, 1965

A Thesis

Submitted to the Graduate Faculty

of the

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for the degree of

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This thesis submitted by Paige M. Lokken in partial fulfillment of the requirements for the Degree of Master of Arts from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.

Edward S. Halas
(Chairman)

R H Kolstoe

Gary W. Evans

William Johnson
Dean of the Graduate School

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ACKNOWLEDGMENTS

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Bill Cornatzer raised the animals used in the first experiment. His cooperation as well as that of Dr. Ed Halas, Dr. Gary Evans, Dr. Ralph Kolstoe and Dr. Hilda Wing is gratefully acknowledged.

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ABSTRACT

Interest in the role played by trace minerals in brain development and the subsequent effect on learning is recent. Biochemically a great number of studies on zinc have been done, but in only one other laboratory have the behavioral consequences of a zinc-deficiency been looked at. The present study attempts to partially replicate the previous studies and to explore the behavioral effect of a zinc-deficiency in a different manner.

In the postnatal study, dams were made zinc-deficient from the day of delivery until weaning of the pups occurred (21 days). Paired animals were fed the same amount of food as that eaten by the zinc-deficient dams and a third group of dams were fed ad libitum. The pups were weaned at 21 days of life and fed ad libitum for 23 days at which time the behavioral study, using the elevated Tolman-Honzik maze, was begun. The results showed a significantly greater number of errors made by the formerly zinc-deficient animals when compared to the pair-fed and ad libitum-fed animals.

For the prenatal study, dams were made zinc-deficient, pair-fed or ad libitum fed from day 14 through day 19 of their pregnancy. Rehabilitation was started on day 20. Randomly selected pups from each of the three groups were studied for behavioral differences using the alley Tolman-Honzik maze. The results of the analysis were just below significance at the .05 level and were in the same direction as the results in the first experiment.

INTRODUCTION

The knowledge that trace minerals are a necessary part of man's diet is a relatively recent discovery. The study of zinc in relation to learning ability came about as a result of biochemical discoveries showing that zinc deficiency in a rat during the early neonatal period would impair growth of the brain as assessed by the incorporation of thymidine into DNA, sulfur into protein, and the total lipid concentrations.

The implications for people studying the area of mental retardation are important. If a relationship can be established between the intake of zinc of the mother and/or child and the presence or absence of retardation, the prevention of at least some forms of mental retardation may become possible.

This study was in two parts and investigated the effects of a zinc deficiency postnatally and prenatally. The first study investigated the effect of zinc deficiency on rats that were deprived of zinc from the day of birth to 21 days of age. The second study investigated the effect, on the pups, of a zinc deficiency of the dam from day 14 through day 19 of pregnancy. Learning ability was assessed in the postnatal study by comparing the number of errors committed by formerly zinc-deficient animals to those committed by control groups on the elevated Tolman-Honzik maze. The assessment of learning in the prenatal study was done in the same manner, but the alley Tolman-Honzik maze was used.

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CHAPTER I

LITERATURE SURVEY ON ZINC DEFICIENCY

The relationship of zinc deficiency and behavior is a new area
in which very little research has been done. This is in sharp contrast
to vitamin research in which well over 50 studies were reported in the
1930's and 1940's. More recently the interest has been in calorie and
protein deficiencies and behavioral studies in these areas have been
frequently reported during the past ten years. Trace minerals have
been largely neglected and it is only in recent years that a few
studies have begun to appear. Further behavioral studies in zinc
seem justified due to the brain mechanisms affected by zinc defi-
ciency and the scarcity of research in the area. The exact areas
of the brain affected by a zinc deficiency are unknown at the pre-
sent time.

In the study of the zinc-deficient suckling rat, it has been
found that zinc is essential for brain development during the critical
period for brain growth (Sandstead et al., 1972). It has also been
found (Hurley, 1969) that severe intrauterine zinc deficiencies are
teratogenic in the rat. These studies as well as the studies of
others (Todd et al., 1934; Macapinlac et al., 1967; Hurley, 1969;
Sandstead and Rinaldi, 1969; Somers and Underwood, 1969) indicate
the rapidity of onset of zinc deficiency.

According to H. H. Sandstead (1972):

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The biochemical correlates to these adverse effects on growth probably relate to zinc's participation in the synthesis of nucleic acids and protein (Winder and Denneny, 1959; Wacker, 1962; Schneider and Price, 1962; Lieberman et al., 1963; Fujioka and Lieberman, 1964; Hsu et al., 1969; Sandstead and Rinaldi, 1969; Somers and Underwood, 1969; Sandstead et al, 1972; Slater et al., 1971; Terhune and Sandstead, 1972; Sandstead and Terhune, 1972) which is inferred in the intact animal by the adverse effects of zinc deficiency on the incorporation of thymidine into DNA of rapidly growing tissues (Sandstead and Rinaldi, 1969; Sandstead et al., 1972) and the utilization of amino acids in the synthesis of protein (Hsu et al., 1969). This latter effect may be the result of an abnormality in the synthesis and/or degradation of ribonucleic acid. Support for this interpretation comes from the finding that RNAse activity is increased in testes of zinc deficient rats (Somers and Underwood, 1969), and the observation that E.coli DNA dependent RNA polymerase is a "zinc metalloenzyme" (Slater et al., 1971). Additional evidence consistent with the essentiality of zinc for formation of RNA is the finding that liver DNA dependent RNA polymerase activity is decreased in zinc deficient suckling rats (Terhune and Sandstead, 1972). Preliminary studies showing that sucrose density gradients of liver deoxycholate treated postmitochondrial supernatants from zinc deficient rats and mice resemble those produced by amino acid imbalance and that the net in vivo 16-hour incorporation of uridine into liver polysomes of mice is decreased in zinc deficiency (Sandstead and Terhune, 1972) are also supportive of the concept.

The outward manifestations of zinc deficiency have been well described. These include anorexia, seborrhea, loss of hair, growth retardation, testicular atrophy, parakeratosis (Prasad, 1966; Prasad and Oberleas, 1970) and slow wound healing (Sandstead et al., 1970). Behaviorally the symptoms exhibited are apathy, lethargy, decreased activity (Apgar, 1968a; 1968b; Caldwell et al., 1970), decreased sexual activity in both the male and female, sterility in adult males (Whitenack et al., 1970), loss of estrous cycle and reproductive failures (Hurley and Swenerton, 1966). The female lacks nest building behavior, does not clean the pups, consume the placenta, or nurse the pups when mildly or marginally zinc deficient (Apgar, 1968a; Apgar, 1973).

Clinically the first human zinc responsive syndrome was reported by Prasad et al. (1963a; 1963b) from Egypt. More complete details and the extended follow up observations were reported by Sandstead et al. (1967). It was found that Egyptian adolescent boys suffering from what was diagnosed z zinc insufficiencies showed severe growth retardation and hypogonadism. The results of the study indicated that treatment with zinc was followed by sexual maturation and growth which exceeded the changes found when an adequate diet or iron therapy were given.

Apparently animals are particularly susceptible to zinc deprivation during periods of rapid growth (Sandstead et al., 1967) and it would be reasonable to assume that this would also apply to children and adolescents. Two other cases (Caggiano et al., 1969; Sandstead, 1972) of dwarfism and hypogonadism have been reported as responding to zinc therapy following documentation of zinc insufficiency.

Poor eating habits (Hambidge et al., 1972) as well as gastrointestinal tract diseases may account for zinc deficiencies found in children in the United States from middle and upper, as well as, lower class homes. It is suggested (Sandstead, 1972) that pregnant women, teenagers, college women, institutionalized individuals, and some low income families may have a marginal intake of zinc since the most usable zinc is found in meat and seafood.

Postnatal behavioral studies (Caldwell et al., 1970) found significant behavioral differences between zinc deficient animals and animals pair-fed with the same diet but containing zinc. The animals were given the zinc-deficient diet at 30 days of age and maintained on it for 48 days and through the following two weeks of behavioral testing. Three methods of testing were used; they are the open field study,

the "platform box" which is a test of one way avoidance learning, and an eight blind water maze (modified Lashly III maze). Reduced emotionality levels were found using the open field to test the zinc supplemented subjects which differed from the zinc-deficient subjects significantly at <.05 level over 3 days of testing. The differences between the two groups on the "platform box" showed the zinc-supplemented animals as being superior but the results were not significant. The Lashly III water maze testing showed a difference in latency between the two groups significant at <.005 and a difference in both cul-de-sac and retrace errors significant at <.001. The zinc deficient animals were slower and made more errors.

The results of the above study are suspect as the animals were run while zinc-deficient. The fact that the zinc-deficient animals were less active than the control group in the open field study could perhaps be attributed as much to physical sickness as to the effects of zinc-deficiency on learning. The lethargy observed in the zinc-deficient animals may also have effected their performance in the maze study.

Oberleas et al. (1972) reported, in abstract, a study similar to that of Caldwell et al. (1970) in which he reported that rehabilitated zinc-deficient rats were inferior to normal rats in maze learning. However, the only measure used to investigate zinc deficiency in their study (Oberleas et al., 1972) was latency. Most investigators regard error scores as the best measure of maze learning (Munn, 1950). There is some question as to the value of the Oberleas et al. (1972) study because of their reliance on latency as their only measure of learning.

Oberleas et al. (1972) studied the effect of prenatal zinc deficiencies and obtained statistically significant differences in performance between treatment groups and also a significant zinc by litter interaction. Dams were maintained in a mildly zinc deficient state during pregnancy. Apparently all dams were fed the control diet during lactation and the pups were fed the control diet for three additional weeks before testing. When learning was measured by using the one-way avoidance conditioning test (platform box) only zinc deprived pups from large litters of zinc-deficient mothers responded poorly. The offspring from small litters of zinc deficient mothers responded normally. Formerly deficient pups differed from the controls in rate but not in level of performance. Their latency scores were slower but no statistical difference in the number of cul-de-sac and retrace errors was found.

This study is also questionable as the pups used came from several pregnancies of the zinc-deficient mothers. The fact that litter size must be controlled is evident in the significant zinc by litter interaction which was found. Latency was again reported as the major measure of learning even though error scores are generally accepted as being the better indicator of maze learning (Munn, 1950). The authors were very vague as to what they meant by a mild zinc-deficiency and as to the time and procedure used for rehabilitation.

The present study was done in part to replicate the Caldwell et al. (1970) postnatal study and the Oberleas et al. (1972) studies, as some of their methodology made the results appear questionable.

CHAPTER II

METHODOLOGY

Experiment I

In the first experiment six pregnant Sprague Dawley dams, obtained from Thorp Industries, White Bear Lake, Minnesota, were divided, at delivery, into three groups (zinc-deficient, pair-fed, and ad libitum-fed). They were housed in double wide plastic cages with plastic grate bottoms, fed a sprayed egg white diet (Luecke et al., 1968) containing <1.00 ppm zinc (Table 1) and given glass distilled water from the day of delivery. Zinc chloride was added to the glass distilled water (59 ug of Zn/ml) of the pair-fed and ad libitum-fed dams. Each zinc-deficient dam was paired with a pair-fed dam so that each pair-fed dam received only an amount of food equivalent to that eaten by her counterpart on the previous Day. As a result, the pups of the pair-fed dams were starved during the first 21 days of life and showed a growth retardation similar to that of the pups nursed by the zinc-deficient dams (Figure 1). The zinc-deficient and pair-fed dams nursed 6 to 9 pups each. The ad libitum-fed dams were allowed unlimited food and the growth rate of their pups was much more rapid than was the growth rate of pups in the other two groups.

All pups were weaned at 21 days of life, fed Purina Lab Chow ad libitum and given tap water. At 44 days of life, after 23 days of nutritional rehabilitation, behavioral studies were begun.

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TABLE 1

ZINC DEFICIENT TEST DIET

Formula:	g/kg
Egg White Solids, Spray Dried	200.00
Dextrose, Hydrate, Technical	630.108
Fiber, Non-Nutritive	30.00
Oil, Corn	100.00
Salt Mix (See Below)	
Vitamin Mix (See Below)	

Salt Mix

Calcium Carbonate ($CaCO_3$)	9.94405
Calcium Phosphate Dibasic ($CaHPO_4 \cdot 2H_2O$)	3.1489
Cobalt Chloride ($CoCl_2 \cdot 6H_2O$)	0.00185
Cupric Sulfate ($CuSO_4 \cdot 5H_2O$)	0.00945
Ferric Citrate ($FeC_6H_5O_7 \cdot 5H_2O$)	0.911542
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	3.38106
Manganese Sulfate ($MnSO_4 \cdot H_2O$)	0.008791
Potassium Iodide (KI)	0.026518
Potassium Phosphate Dibasic ($K_2HPO_4 \cdot 3H_2O$)	14.0044
Sodium Chloride (NaCl)	5.55198

Vitamin Mix

|	
Biotin	0.004
B_{12} (0.1% in Mannitol) Vitamin	0.020
Calcium Pantothenate	0.016
Choline Chloride	1.5
Folic Acid	0.0005
Menadione	0.00033
Niacin	0.025
Pyridoxine HCl	0.004
Riboflavin	0.006
Thiamine HCl	0.01
Inositol	1.00
	units/kg
Vitamin A Palmitate	10,000.000 IU
Vitamin D_2	1,250.000 IU
Vitamin E Acetate	110.000 IU
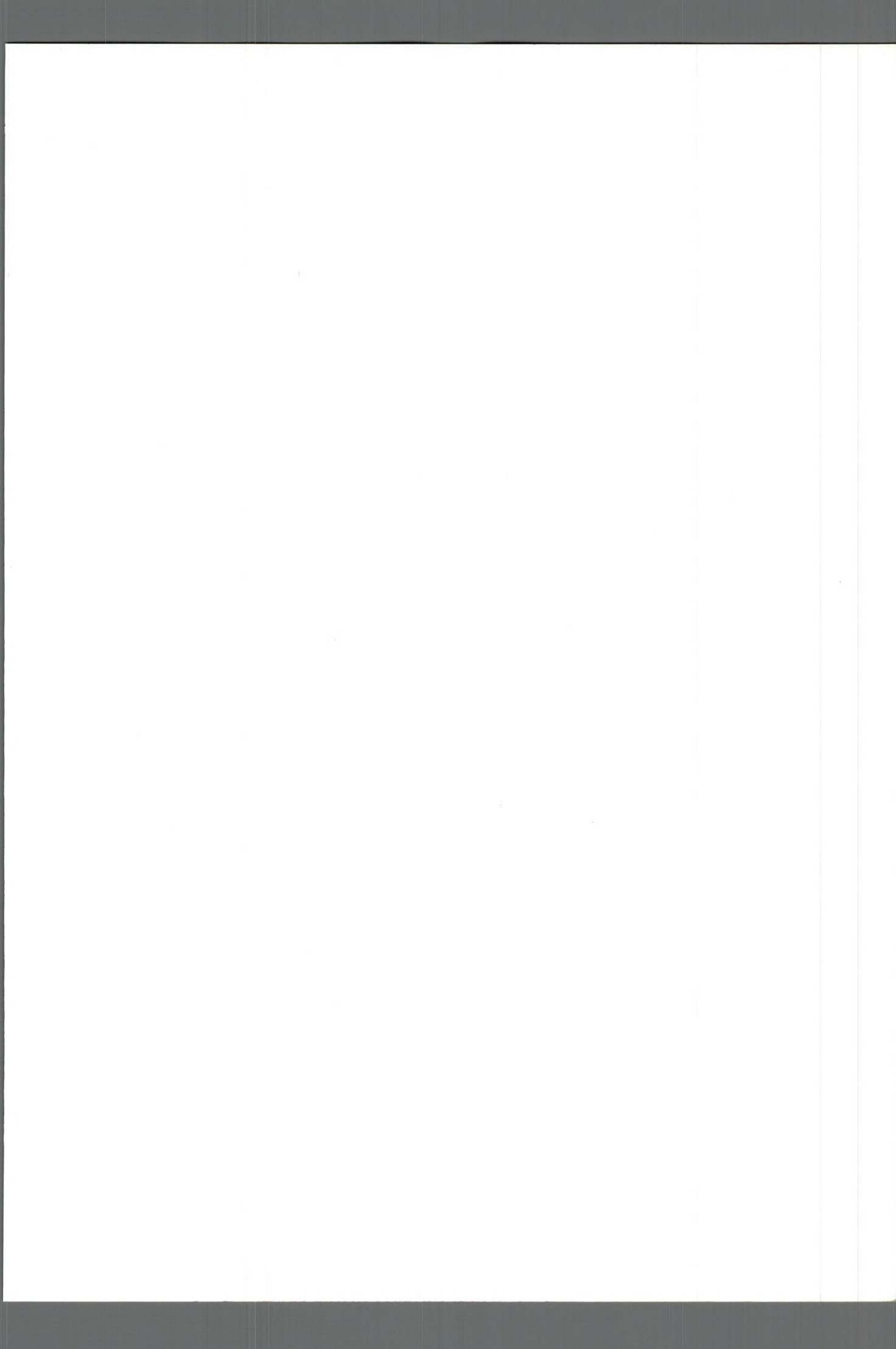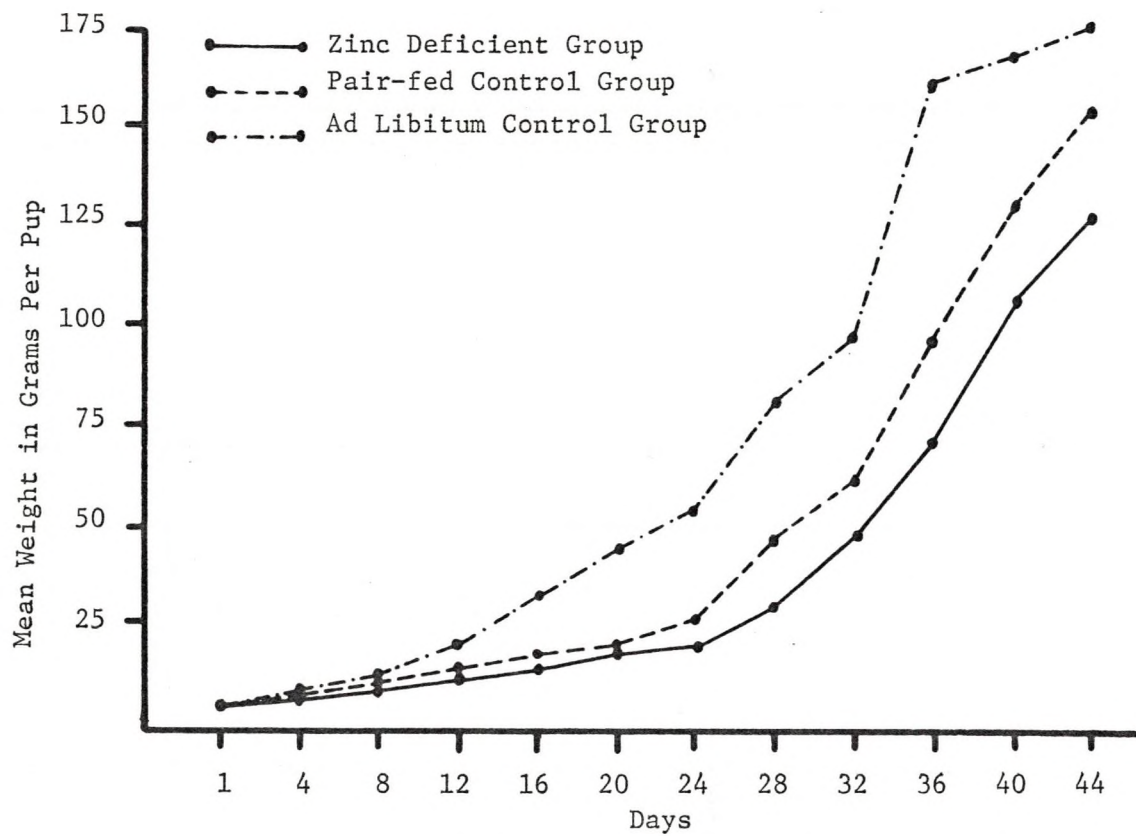
Training Conditions

All the animals (10 pair-fed, 10 zinc-deficient, and 4 ad libitum-fed) were exposed to 17 days of training on the 14 choice point elevated Tolman-Honzik maze (Tolman and Honzik, 1930). The maze (Figure 2) consisted of 15 sections each 2 ft long, 2 in wide, and 18 in high. The culs-de-sac were 6 in deep. The units were painted flat black. After each day's use the maze was wiped clean and disassembled. The maze was reassembled each day just prior to use. The all concrete experimental room had no windows and contained nothing but the maze and thus provided a minimum number of cues.

During the experimentation the animals were housed in metal cages in the animal quarters and transported in large plastic cages to the experimental room. The rats were food deprived for 23 hours prior to the beginning of training.

Maze Habituation.--The first three days of training consisted of maze habituation. All animals ran a 6 ft elevated runway, constructed of 3 units of the maze at a rate of 4 trials a day for 3 days. They were deprived of food for 23 hrs prior to the training. Each rat was allowed to eat a wet mash at the end of the runway for 15 sec before being placed at the start of the runway for the next trial. At the conclusion of the 4 trials the animal was placed in a holding cage with food and water for 45 min and then returned to its home cage.

Maze Training.--The remaining 14 days consisted of 1 trial per day on the elevated Tolman-Honzik maze. The rats were food deprived for 23 hrs prior to each day's trial. The time elapsed
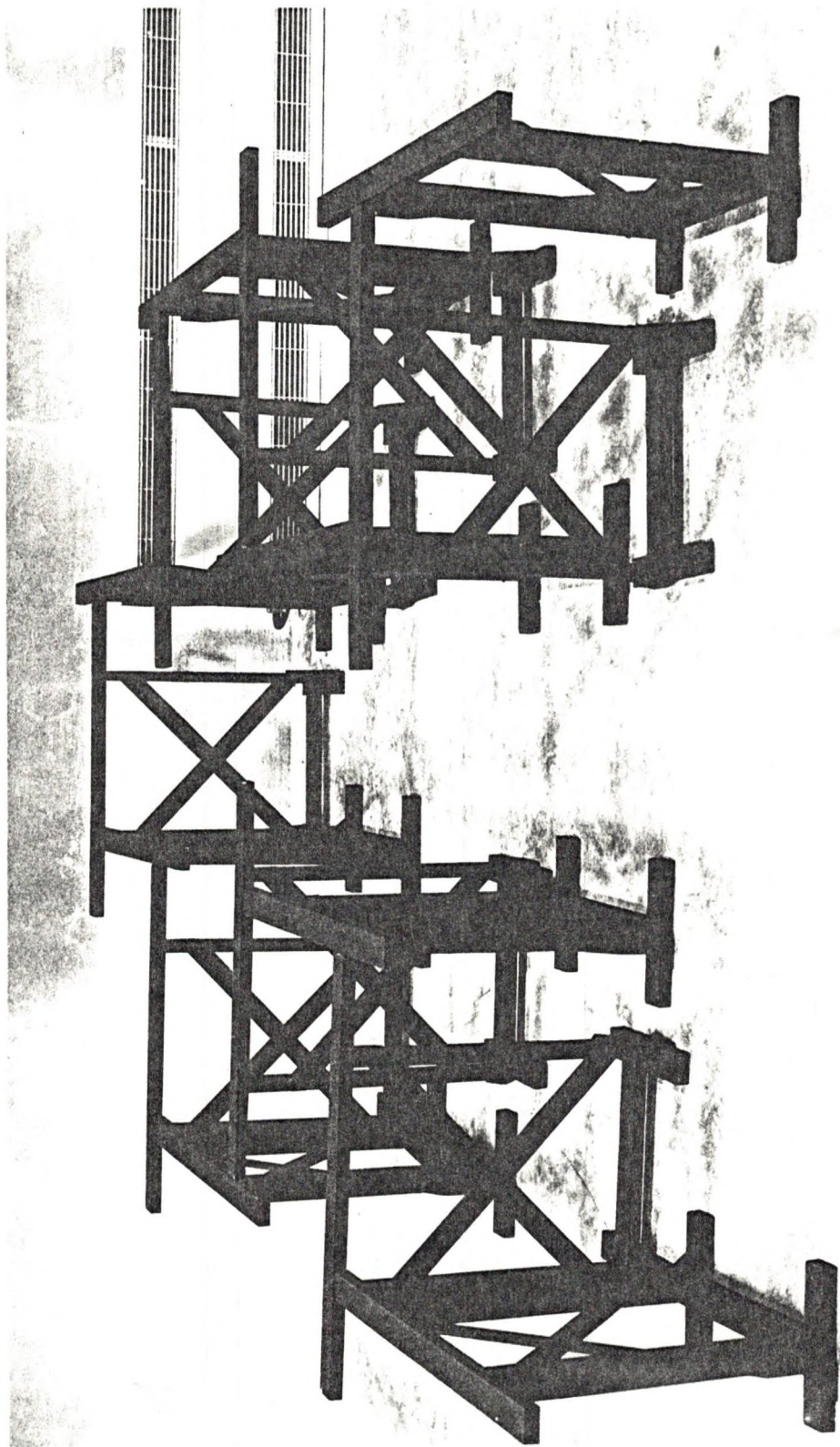
from the start of each trial to the moment the rat touched the wet mash
at the end of the maze was recorded. Each rat was allowed to eat the
wet mash for 15 sec and then was placed in a holding cage with food and
water for 45 min before being returned to its home cage. Whenever a
rat entered a cul-de-sac an error was recorded. Six types of errors
were recorded. Hesitation error, the rat paused before choosing the
correct turn; 1/4 body error, the rat's head and front legs entered
the cul-de-sac; 1/2 body error, the rat entered the cul-de-sac as far
as the middle of its body; 3/4 body error, the cul-de-sac was entered
as far as the rear legs; full body error, the rat entered the cul-de-
sac completely; retrace error, the rat passed the choice point, then
turned back and entered the cul-de-sac.

Experiment II

Thirty-one timed pregnant females obtained from Thorp Indus-
tries of White Bear Lake, Minnesota were used for the prenatal study.
At day 14 of pregnancy, the animals were randomly divided into 3
groups; 12 dams in the zinc-deficient group, 8 dams in the pair-fed
group, and 8 dams in the ad libitum-fed group. The animals were
housed in double wide plastic cages with plastic grate bottoms and
fed the same sprayed egg white diet used in Experiment I. They were
given super Q water in which the zinc content was not measurable.
Zinc chloride was added to the super Q water (50 ug of Zn/ml) of the
pair-fed and ad libitum-fed dams. The average amount of food eaten
each day by the zinc-deficient dams was fed to the pair-fed dams the
following day. As a result, the pair-fed dams were starved during a
critical period of their pregnancy. Their weight gain was similar

to that of the zinc-deficient dams. The ad libitum-fed dams were allowed
unlimited food and their weight gain was substantially greater than the
weight gain of the other two groups.

On day 19 of their pregnancy (day 20 for the pair-fed dams) the
animals were given Purina Lab Chow ad libitum and tap water. Rehabili-
tation was continued until weaning (30 days). The pups were continued
on the rehabilitation diet until behavioral studies were begun at 49 or
50 days of life.

The zinc-deficient pups, on the average, weighed less than either
the pair-fed or ad libitum-fed pups (Figure 3). The weight differences
between groups are not as striking as the differences between groups in
the postnatal study. At birth, there was only a 0.97 g difference between
the ad libitum and zinc-deficient groups and by day 40 the difference was
12.36 g as compared to the 50 g difference in the postnatal study by day
40. Data given in Table 6 in the appendix.

Training Conditions

Ten males were randomly selected from each of the three groups
and at day 49 or 50 of life they were exposed to 22 days of training on
the alley Tolman-Honzik maze (Tolman and Honzik, 1930). The maze (Fig-
ure 4) had 14 choice points. The alleys were 4 in wide. The starting
and end units were each 16 in long. Culs-de-sac 1, 5, 8, and 11 were
8 in deep and all other culs-de-sac were 15 in deep. Black curtains
were placed 6 in deep on both sides of all choice points to prevent
the animal from seeing which arm led to a dead end. The maze was
painted flat black and guillotine-type retrace doors were used. Heavy
cord attached to the doors allowed the experimenter to raise and lower
the doors. Eighth-inch plexiglas covered the alleys.
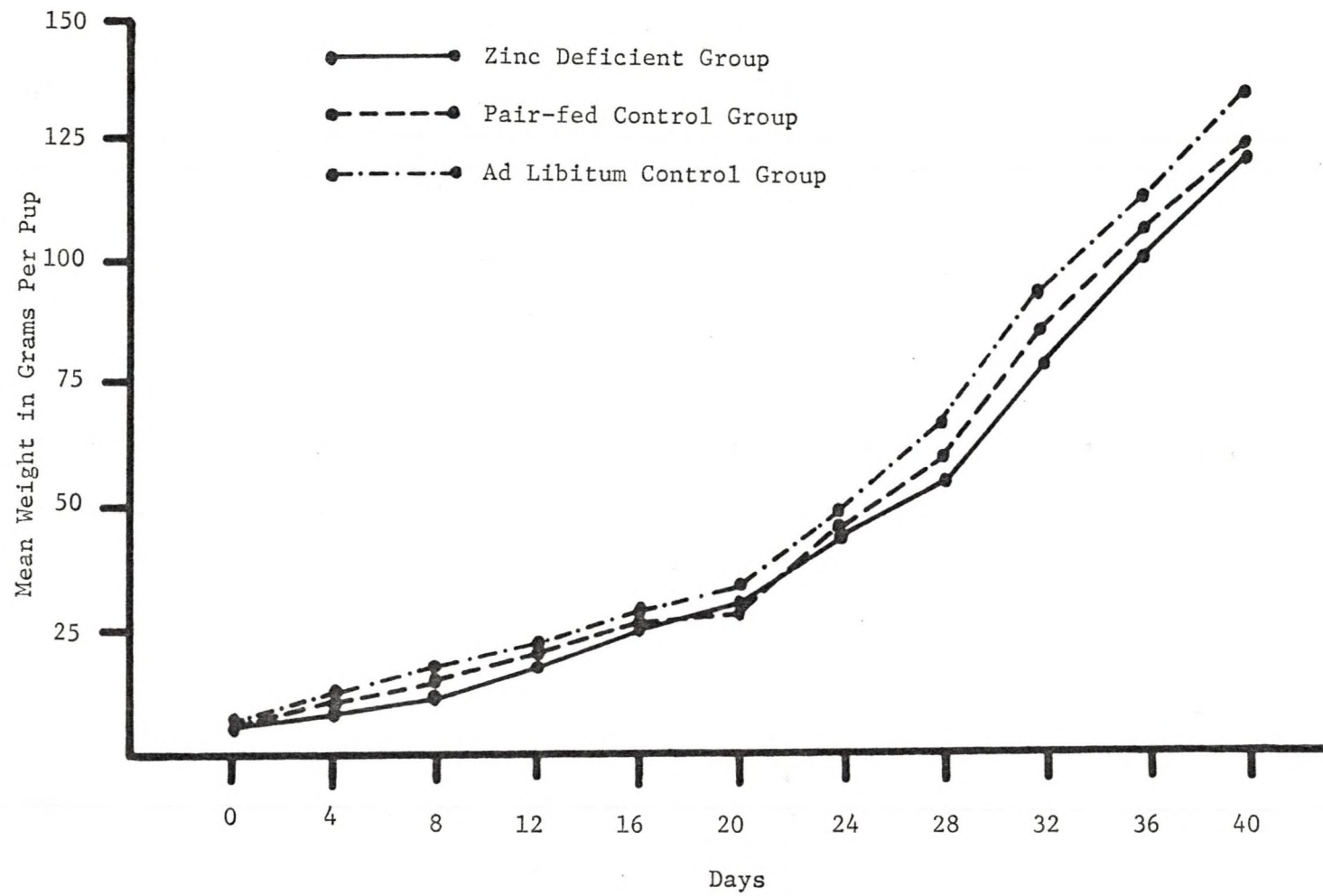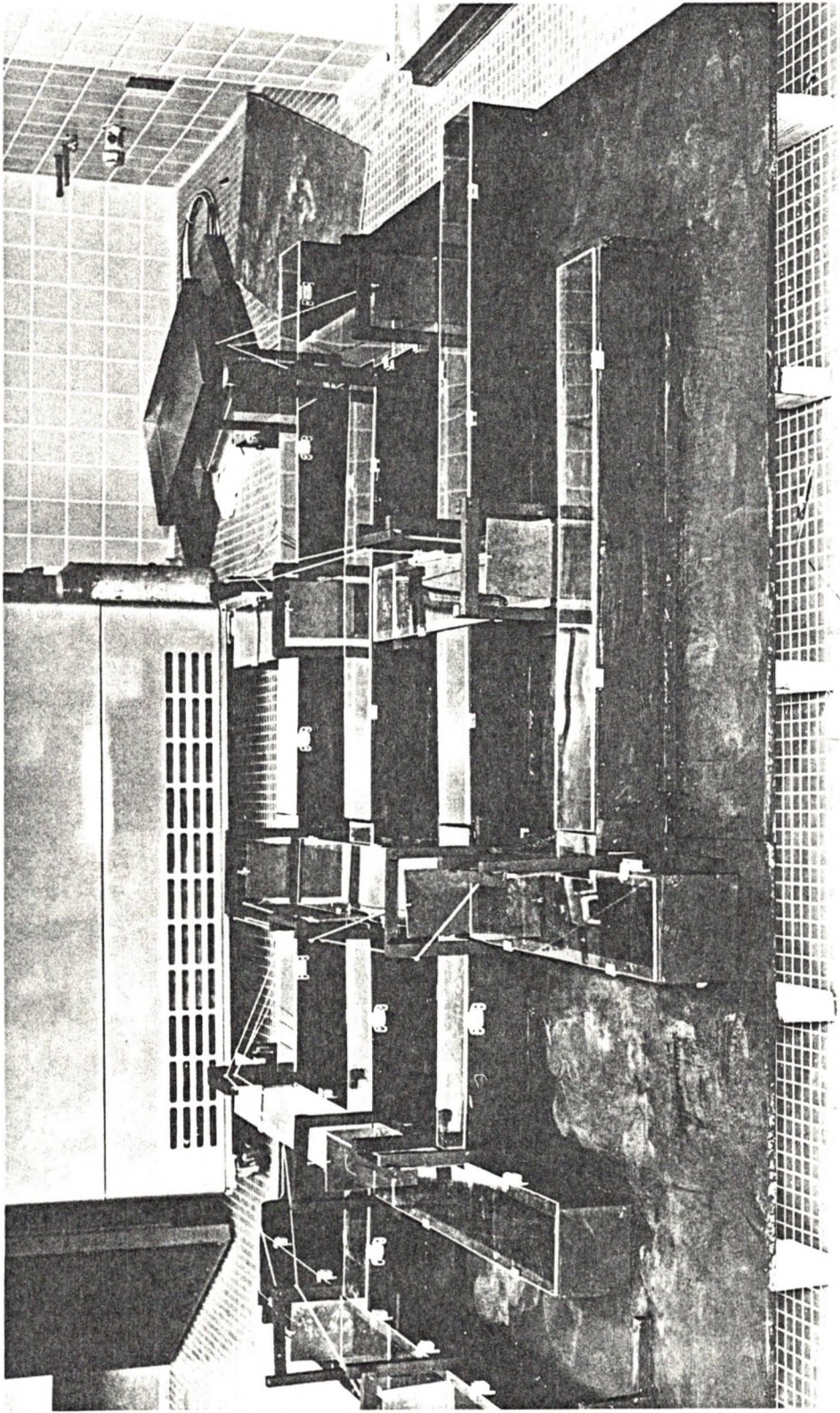
The animals were housed in metal cages in the animal quarters and transported to the experimental room as described in Experiment I. The rats were food deprived for 23 hrs prior to the beginning of training. Following the training period each animal was placed for 45 min in an individual cage which contained food and water and then returned to its home cage.

Maze Habituation.--During maze habituation each animal ran a straight 6 ft alley which contained 2 guillotine doors and 3 black curtains. The doors were closed each time behind the rat. Each rat ran the straight alley 3 trials per day for 4 days. They were deprived of food for 23 hrs prior to the start of training. Each rat was allowed to eat a wet mash at the end of the alley for 15 sec prior to being returned to the starting point for the next trial. After completing 3 trials each animal was immediately placed in a feeding cage and allowed to eat for 45 min before being returned to its home cage.

Maze Training.--Following habituation, the rats were given eighteen consecutive days of training in the alley maze at the rate of one trial per day. All animals were food deprived for 23 hrs preceding each trial. Time was recorded from the start of the trial to the moment at which the rat touched the wet mash at the end of the maze. Errors were counted in the manner described in Experiment I.

CHAPTER III

RESULTS

Experiment I

The results indicate that rats made zinc deficient from birth
until weaning, a critical period for brain growth, learned the maze
more slowly than did those rats that had been starved or adequately
fed during the same period. Postnatally zinc deficient animals made
more full body errors and more total errors than did animals in
either of the control groups (Figures 5 and 6).

Figure 5 shows that on the initial day of training all groups
made close to the same number of full body errors, but the pair-fed
control and the ad libitum-fed control groups decreased their error
rates more rapidly than did the zinc-deficient group. Even after 14
days of training, the zinc-deficient animals were still making a sub-
stantial number of errors.

Figure 6, which shows the average total errors, presents the
results even more dramatically. By day 14 the ad libitum-fed and
pair-fed animals were making, on the average, 1 or less errors per
animal while the zinc deficient animals were making 2 1/2 errors
per animal.

The running speed (latency) of the three groups (Figure 7)
shows numerous crossings and a very similar slope for all groups.
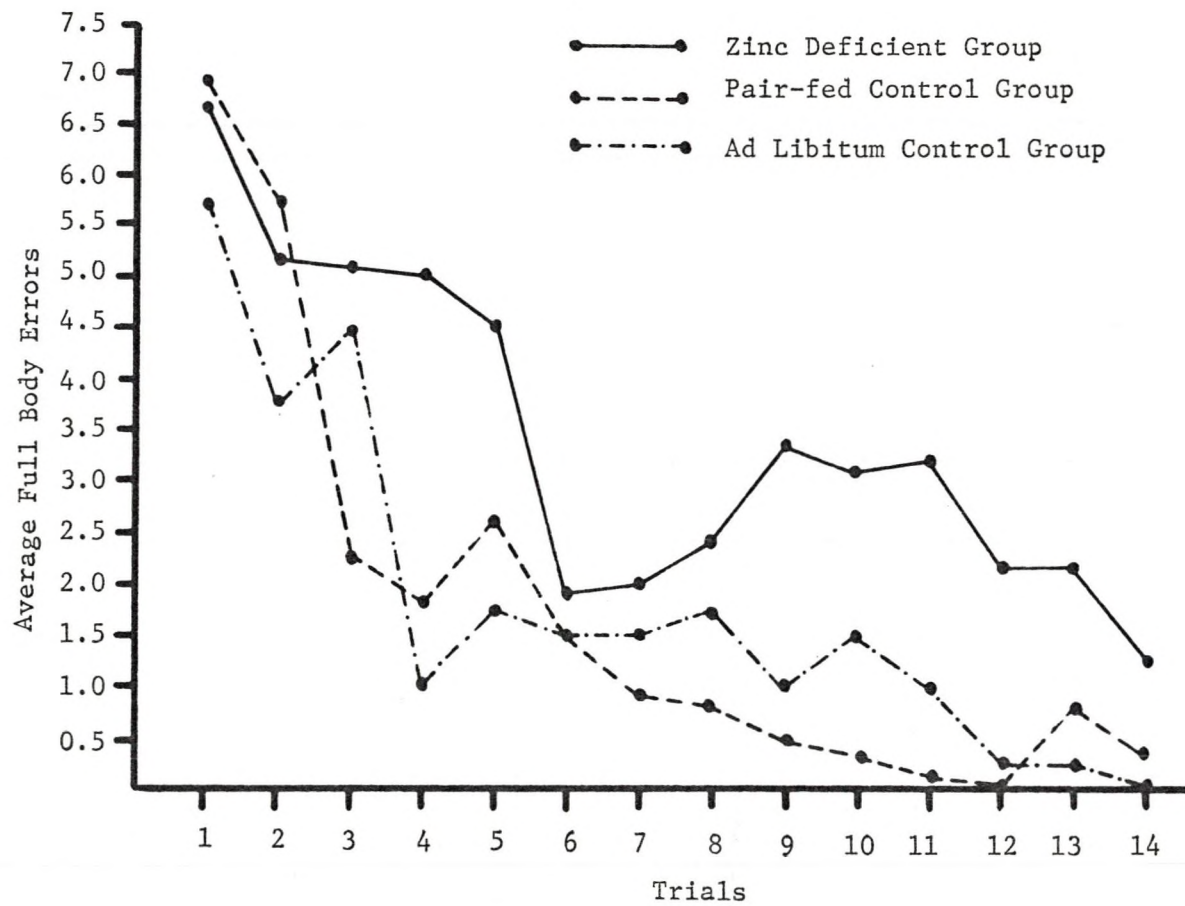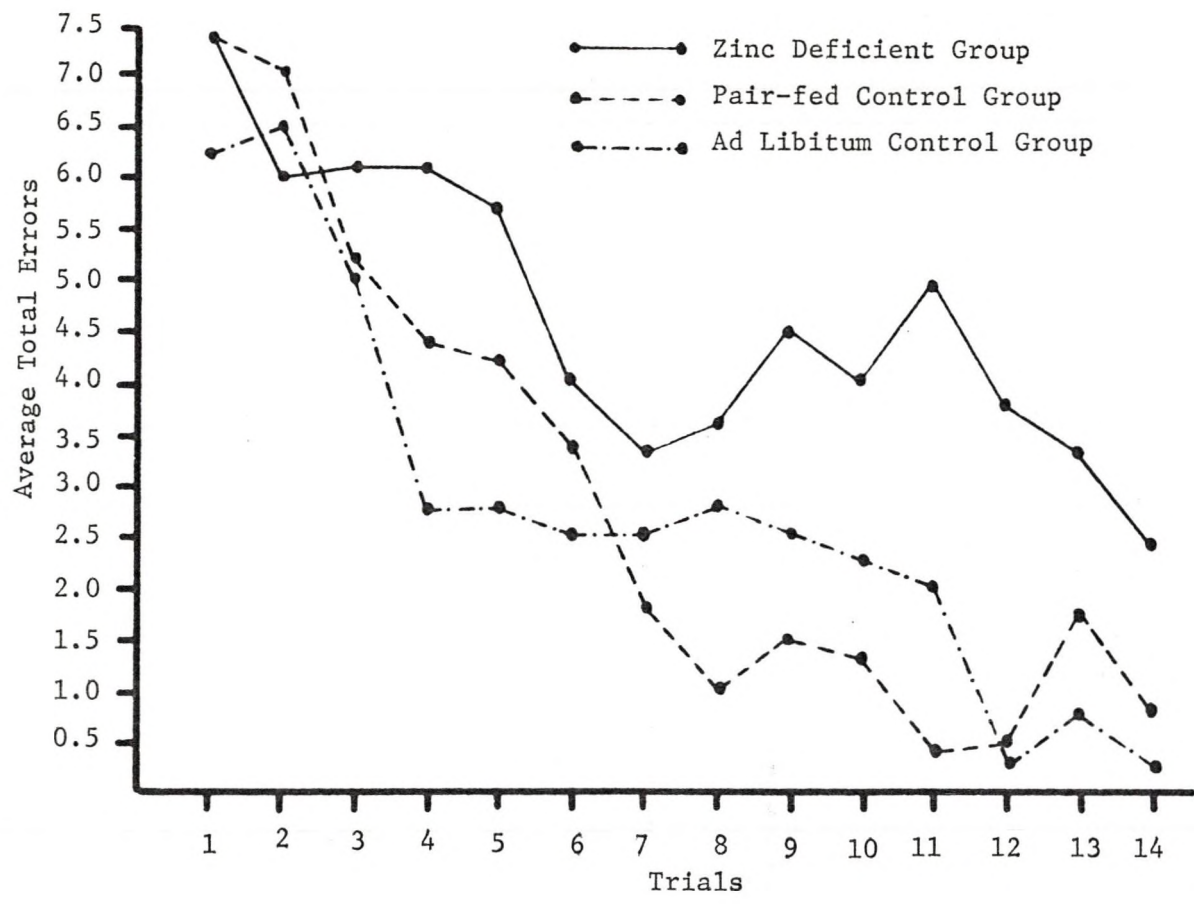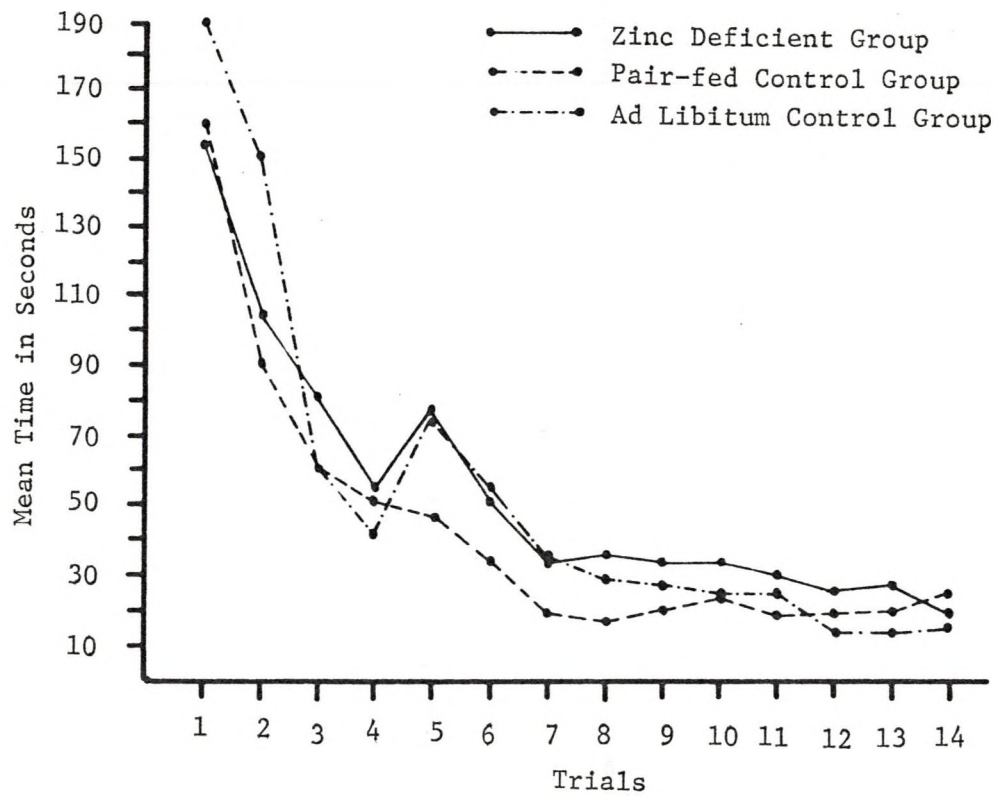
The slightly slower running speed of the zinc-deficient animals can easily be accounted for by the larger number of errors which they made.

Due to the unequal group sizes it was decided not to run a complex analysis of variance, but instead, the slopes of the regression lines were tested for significance. A common slope was fitted to all data and this slope was then compared to the individual slopes of each group. The analysis showed that for full body errors, the slope of the regression line for the zinc deficient group was significantly different from that for the pair-fed group (F=10.55 d.f. 1, 216; p <.01) and for the ad libitum-fed control group (F=15.79, d.f. 1, 151; p <.01). The difference between the slope of the regression line for the pair-fed and the ad libitum-fed controls was not significant. For total errors, the slope of the regression line for the zinc-deficient group was significantly different from that of the pair-fed group (F=11.26, d.f. 1, 216; p <.01) and for the ad libitum control group (F=17.31, d.f. 1, 151; p <.01). Again the F-ratio between the pair-fed and the ad libitum controls was not significant. None of the latency differences were statistically different.

Experiment II

The results indicate that the offspring of dams made zinc-deficient from the 14th day to the 19th day of pregnancy made more full body and total errors than did the offspring of pair-fed or ad libitum-fed dams (Figures 8 and 9).

Figure 8 shows that for most trials the average full body errors made by the zinc-deficient animals was greater than the average errors made by the other two groups. The average number
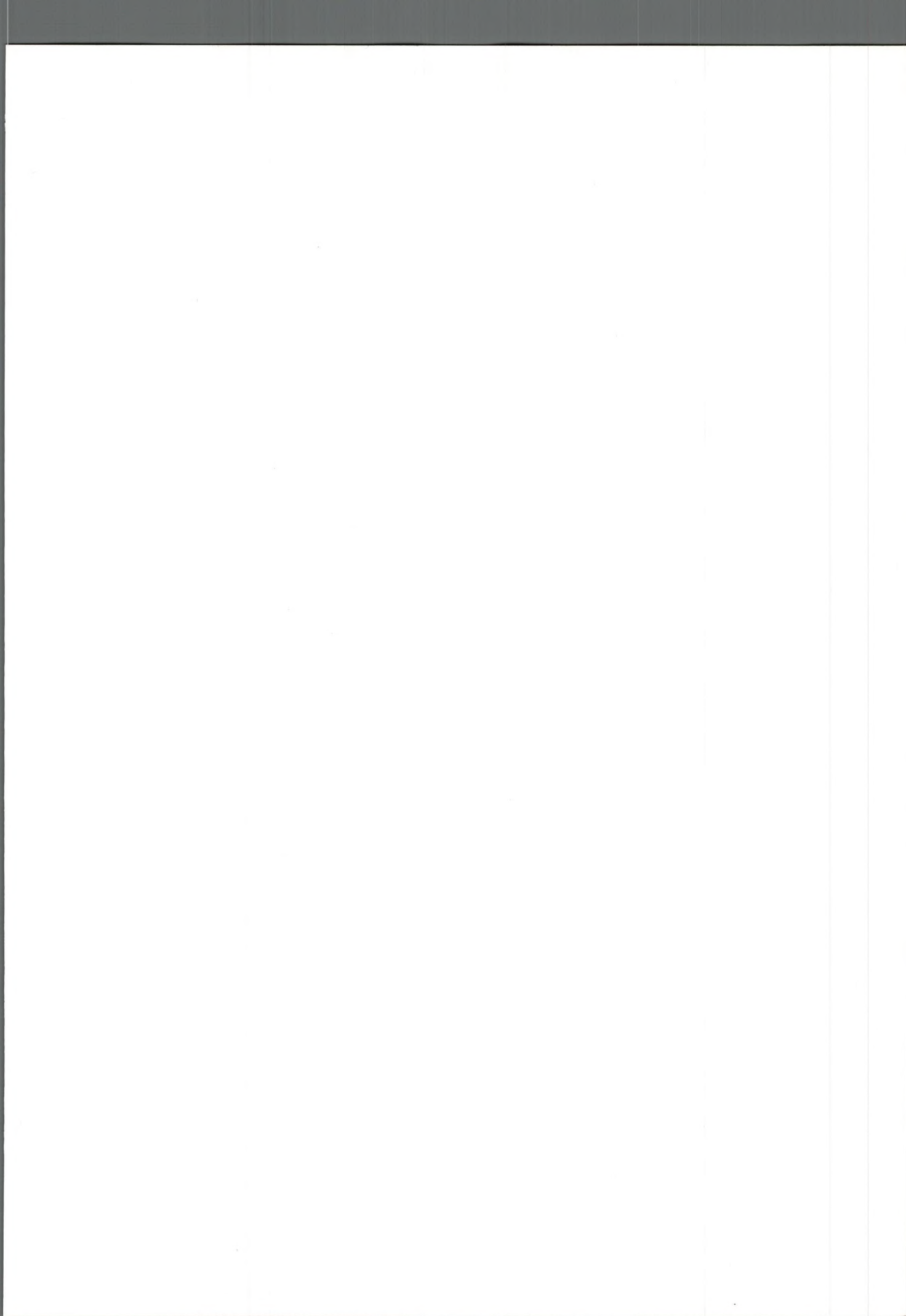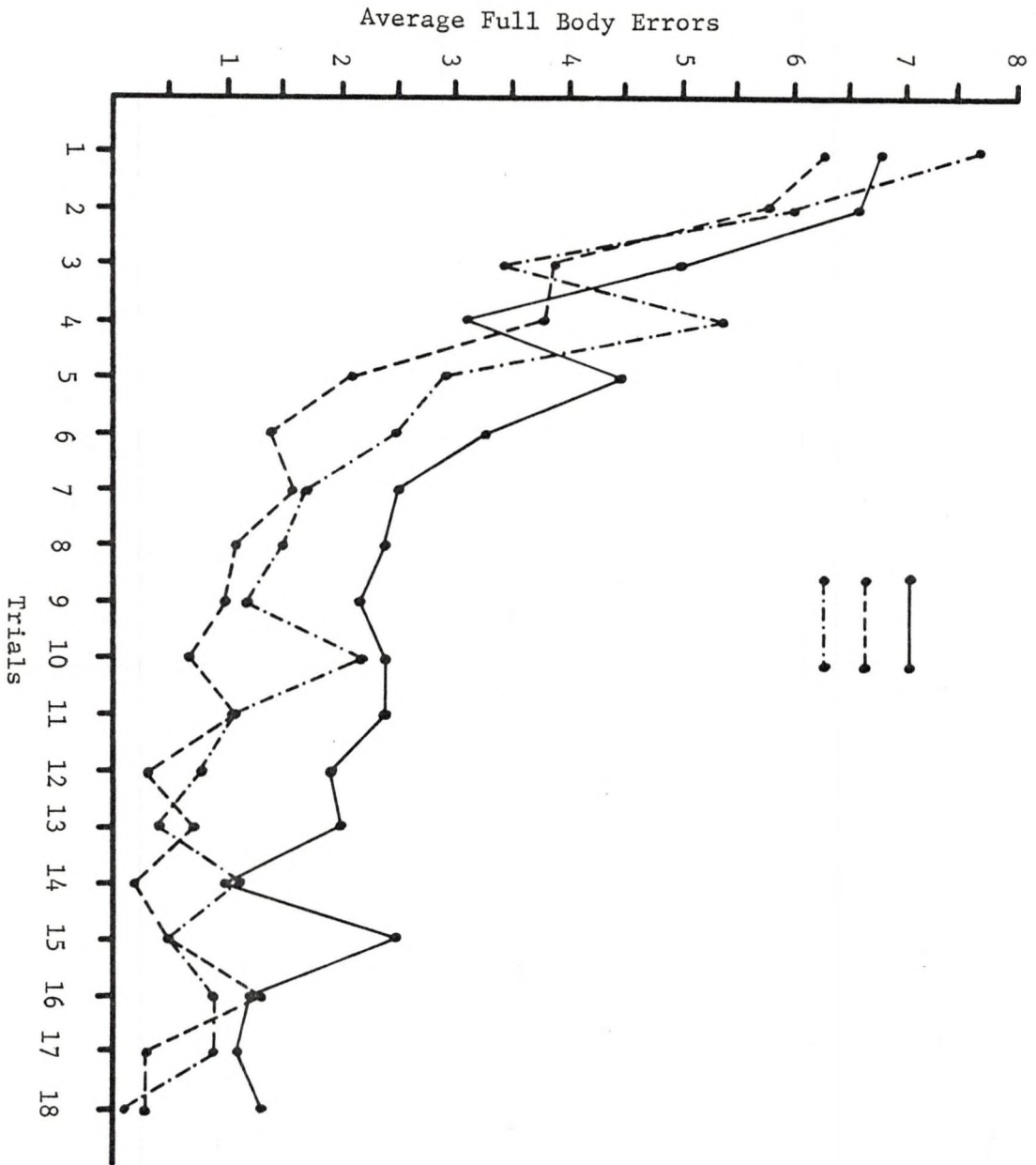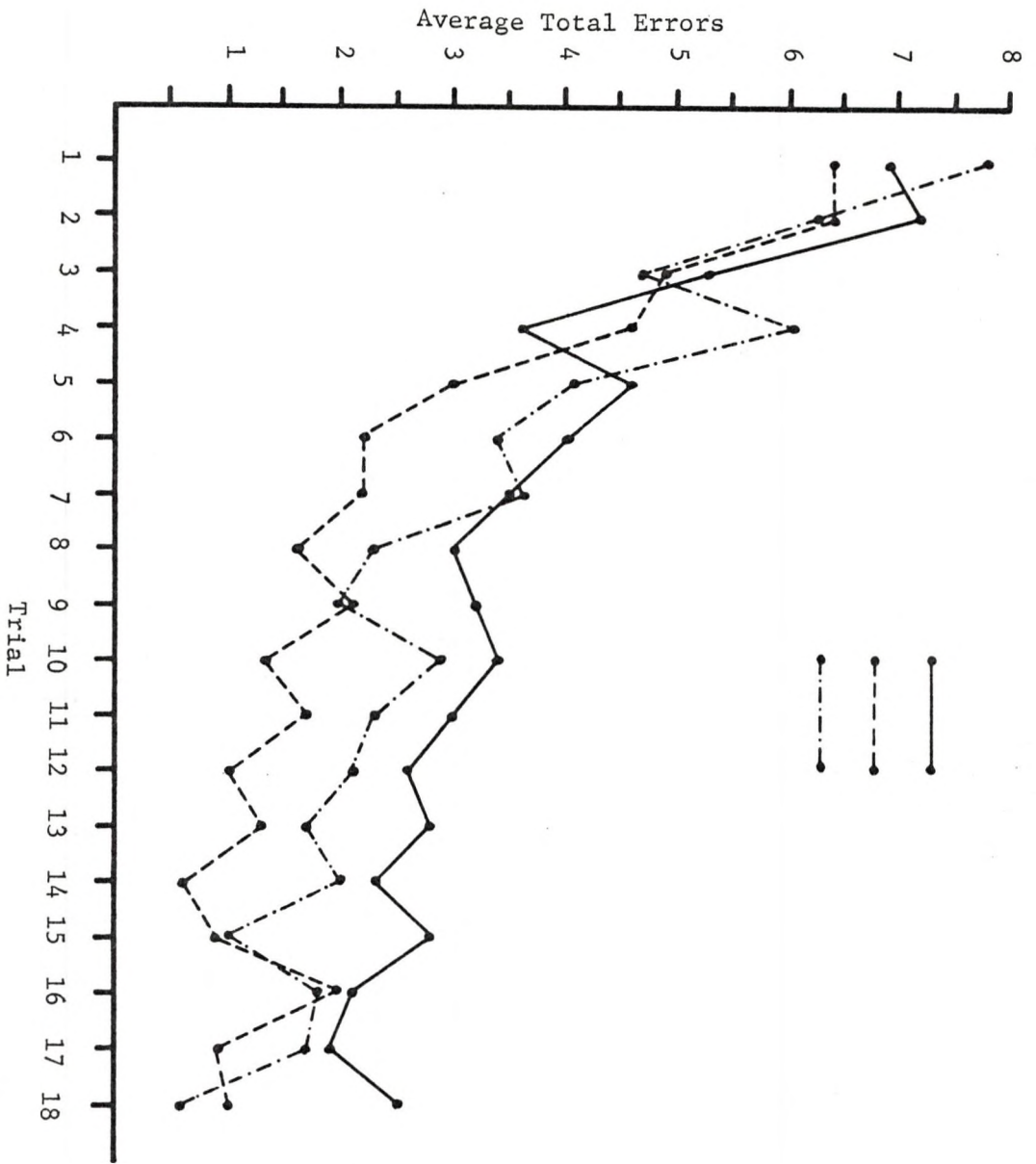
of errors made by the ad libitum-fed animals was less than the number
made by the zinc deficient group, but more than the errors made by the
pair-fed group.

Figure 9 is similar in composition to Figure 8. The average
total errors made by the zinc-deficient group was generally greater
than the average errors made by the two control groups. Again the
ad libitum-fed controls made an average number of errors intermediate
to the other two groups.

The running speed (Figure 10) of the three groups appears to
be very comparable after the first trial.

A statistical analysis was performed using an analysis of vari-
ance set up as a two-factor experiment with repeated measures on one
factor. The analysis of full body errors was not significant at the
.05 level for either between or within subjects (F=2.943, d.f. 2,27;
F=1.486, d.f. 34,459). The analysis of the total errors was also non-
significant at the .05 level for the between and within subjects
(F=2.119, d.f. 2,27; F=1.486, d.f. 34,459).

Further analysis was done between groups using t-tests. The
means over trials for each group were compared to the means over
trials for each of the other two groups for full body errors, total
errors, and time. None of the results were significant at the .05
level. These results are given in Table 2.
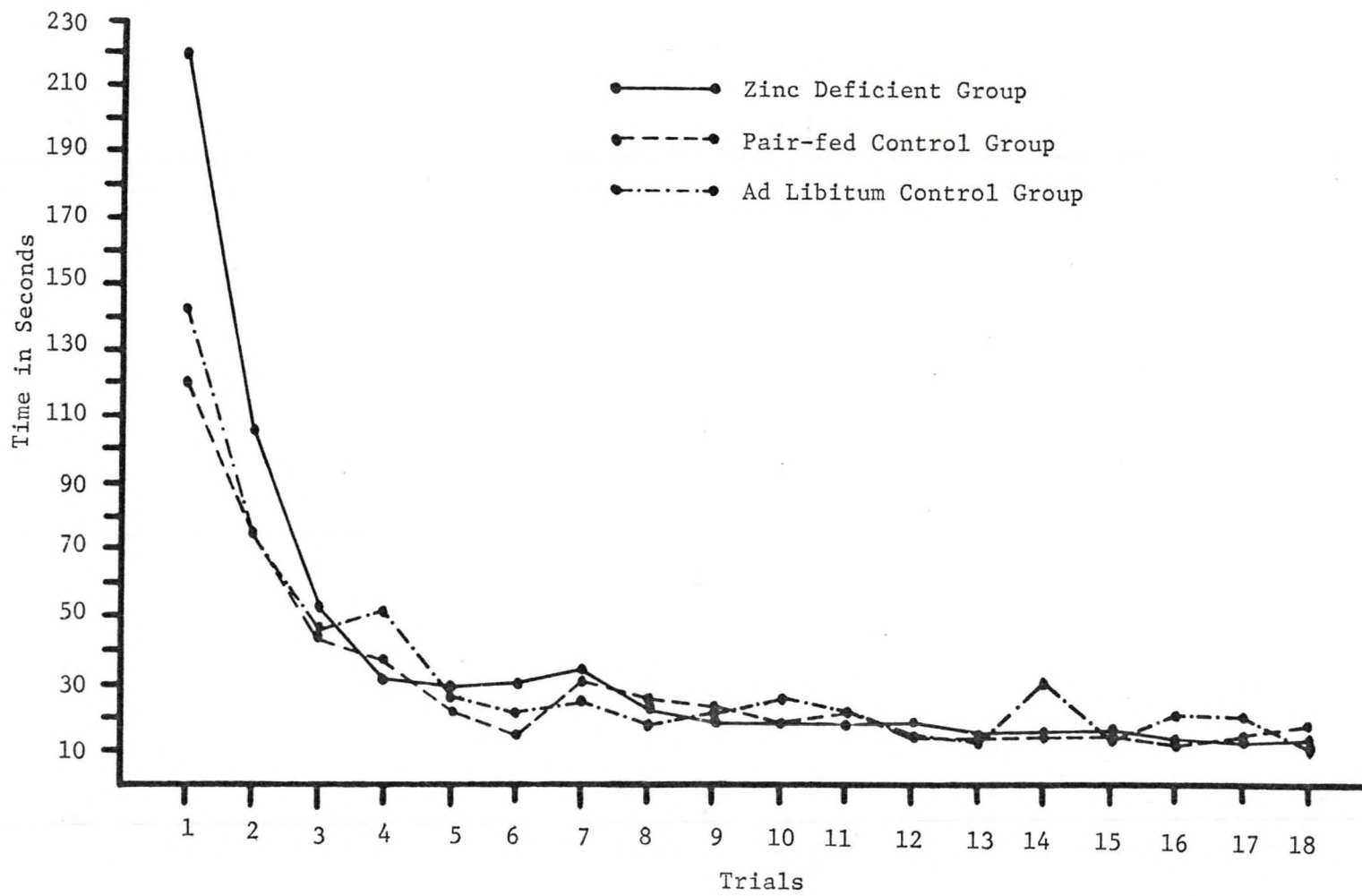
TABLE 2

t-VALUES[a]

	Zn-def vs. P-fed	Zn-def vs. Ad lib	Ad lib vs. P-fed
Full Body	1.86	1.09	0.66
Total Errors	2.02	0.78	1.07
Time	0.59	0.34	0.34

[a]df = 17

CHAPTER IV

DISCUSSION

The results were consistent with the initial hypothesis. The
formerly zinc-deficient pups from both the prenatal and postnatal
groups did make more errors on the elevated and the alley Tolman-
Honzik mazes.

The results of the postnatal study showed a significant dif-
ference (p <.01) in the average full body and total errors made by
the zinc-deficient animals when compared to the pair-fed and ad
libitum-fed groups. There was no significant difference between
the pair-fed and the ad libitum-fed groups on the errors made. The
analysis of the differences in running speed (latency) was nonsig-
nificant for all groups.

The results of the prenatal study, while not significant, were
in the right direction and for the total and the full body errors the
F's were very close to the .05 level of significance (p=.06). The
lack of significance in this study may be accounted for in part by
the possibility of a less severe deficiency having been created since
the dams were deficient for only 6 days. Figure 7 shows that the ini-
tial difference between the average weight of a zinc-deficient and
ad libitum-fed pup to be only 0.97 grams. The average weight differ-
ence between groups did become larger as the pups matured, but at 40
days there was a 12.36 g difference between the ad libitum-fed and
zinc deficient animals. This lack of differences in weights may

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indicate that a milder deficiency was obtained. In the light of a milder deficiency and an analysis which approached significance at the .05 level, the results would seem very consistent with the initial hypothesis and with the results of the postnatal study.

Other studies done in this laboratory (unpublished) found that pups made zinc-deficient from birth to 21 days of life and then rehabilitated for approximately 100 days before being tested on the alley Tolman-Honzik maze made no more errors than did the pair-fed and ad libitum-fed controls. Adult males made zinc deficient from about 45 days of life to day 98 of life and then rehabilitated before testing was begun on the alley Tolman-Honzik maze made no more errors than did the control animals.

The prenatal results differ from those of Oberleas et al. (1972) as they found, using the Lashly III water maze, that zinc-deficient animals were significantly slower in learning the maze but made no more cul-de-sac and retrace errors. Their results are opposite to those obtained in this experiment.

The postnatal study was impossible to compare with the Caldwell et al. (1970) postnatal study as they used animals made zinc-deficient on day 30 of life and then continued the zinc-deficiency through testing. Their animals were not deficient during a critical period for brain growth nor were they rehabilitated and presumably healthy during testing. Caldwell et al. (1970) did find a significant difference between zinc-deficient and zinc-supplemented animals. However, it would be difficult to determine whether that was a result of a reduced level of motivation due to illness of the zinc-deficiency or a reduction in intrinsic learning ability. The animals were reported to be

lethargic at the time of testing and this could certainly have accounted for the slower latency.

In the study mentioned above which used animals made zinc-deficient as adults and then rehabilitated, no difference was found in the number of errors made or the running time. This study would tend to discredit the Caldwell et al. (1970) results attributing a learning difference to zinc deficiency.

It should be noted that the performance of the zinc-deficient animals in the present study was in striking contrast to the effect of starvation which was sufficiently great to retard growth. Thus gross starvation or protein deficiency may not be directly responsible for mental retardation, but rather the lack of a specific dietary element may be responsible for mental deficiency. Therefore, it is possible that the starvation and the resultant mental retardation observed in human infants may not be due to insufficient protein in the diet, but rather due to a lack of zinc which prevents the proper utilization of what little protein may be available. In the experiments performed for this thesis, starved pups who were given high concentrations of zinc in their diet were able to learn the maze as well as normal rats. However, due to the many complex factors which may influence a rat's maze performance it can not be stated with certainty that the effect of the deficiency was on learning. It can be stated that a residual impairment in behavior was observed. Because the effects of zinc-deficiency on nucleic acid and protein synthesis and brain maturation as assessed by total lipid concentrations (Sandstead and Rinaldi, 1969; Sandstead et al., 1972; Terhune and Sandstead, 1972) are known

it would seem reasonable to assume that, in zinc-deficient rats, brain

maturation was retarded and that the retardation had an adverse effect

on their ability to acquire the maze.

APPENDIX

RAW DATA

TABLE 3

MEAN WEIGHT PER PUP FOR THE ANIMALS IN THE POSTNATAL STUDY

Days	Zinc-deficient N=10	Groups Pair-fed N=10	Ad libitum N=10
1	7.73	7.73	7.73
4	7.75	7.75	7.75
8	10.19	10.88	15.80
12	12.57	14.70	22.40
16	15.61	19.29	32.60
20	19.52	22.93	45.20
24	20.88	27.80	56.75
28	35.88	47.33	82.25
32	47.41	64.00	99.75
36	76.00	96.00	160.00
40	105.59	129.26	167.00
44	127.81	147.53	178.25

TABLE 4

MEAN FULL BODY AND TOTAL ERRORS MADE BY THE ANIMALS IN THE
POSTNATAL STUDY

	Zinc-deficient N=10		Groups Pair-fed N=10		Ad libitum N=4	
Trials	F.B.	Total	F.B.	Total	F.B.	Total
1	6.7	7.3	7.0	7.4	5.7	6.3
2	5.2	6.0	5.7	7.1	3.8	6.5
3	5.1	5.7	2.4	5.2	2.5	5.0
4	4.9	6.2	1.8	4.4	1.0	2.8
5	4.5	5.6	2.5	4.2	1.8	2.8
6	2.1	3.9	1.6	3.4	1.5	2.5
7	2.0	3.3	0.9	1.8	1.5	2.5
8	2.3	3.6	0.8	1.2	2.0	2.8
9	3.3	4.5	0.5	1.5	1.0	2.5
10	3.1	4.3	0.3	1.3	1.5	2.3
11	3.2	4.7	0.1	0.4	1.0	2.0
12	2.2	3.7	0.0	0.5	0.0	1.3
13	2.2	3.3	1.1	1.8	0.3	0.8
14	1.3	2.4	0.4	0.8	0.0	0.3

TABLE 5

MEAN RUNNING TIME IN SECONDS OF THE ANIMALS IN THE POSTNATAL STUDY

Trials	Zinc-deficient N=10	Groups Pair-fed N=10	Ad libitum N=10
1	157.7	154.6	176.5
2	103.8	78.6	151.25
3	81.3	61.3	61.0
4	59.0	52.8	42.5
5	69.7	47.8	74.5
6	54.3	34.8	54.5
7	31.3	19.9	33.5
8	35.2	17.0	28.5
9	34.1	21.7	26.5
10	33.8	22.7	23.5
11	100.5	17.9	25.0
12	26.2	18.6	16.25
13	28.0	20.8	15.75
14	19.6	24.6	16.5

TABLE 6

MEAN WEIGHT PER PUP FOR THE ANIMALS IN THE PRENATAL STUDY

Days	Zinc-deficient N=10	Groups Pair-fed N=10	Ad libitum N=10
0	5.37	5.61	6.34
4	7.07	8.04	9.48
8	11.85	13.11	14.85
12	17.88	18.88	19.40
16	24.43	25.22	26.12
20	31.72	30.67	32.25
24	40.55	42.52	45.50
28	57.05	60.82	65.12
32	78.15	82.5	88.33
36	100.95	105.23	110.05
40	119.68	122.50	132.04

TABLE 7

MEAN FULL BODY AND TOTAL ERRORS MADE BY THE ANIMALS IN THE
PRENATAL STUDY

Trials	Zinc-deficient N=10		Pair-fed N=10		Ad libitum N=4	
	F.B.	Total	F.B.	Total	F.B.	Total
1	6.8	6.9	6.3	6.4	7.7	7.8
2	6.6	7.2	5.8	6.4	6.0	6.3
3	5.0	5.3	3.9	4.9	3.4	4.7
4	3.1	3.6	3.8	4.6	5.4	6.1
5	4.5	4.6	2.1	3.0	2.9	4.1
6	3.3	4.0	1.4	2.2	2.5	3.4
7	2.5	3.5	1.6	2.2	1.7	3.6
8	2.4	3.0	1.1	1.6	1.5	2.3
9	2.2	3.2	1.0	2.1	1.2	2.0
10	2.4	3.4	0.7	1.3	2.2	2.9
11	2.4	3.0	1.1	1.7	1.1	2.3
12	1.9	2.6	0.3	1.0	0.8	2.1
13	2.0	2.8	0.7	1.3	0.4	1.7
14	1.0	2.3	0.2	0.6	1.1	2.0
15	2.5	2.8	0.5	0.9	0.5	1.0
16	1.2	2.1	1.3	2.0	0.9	1.8
17	1.1	1.9	0.3	0.9	0.9	1.7
18	1.3	2.5	0.3	1.0	0.1	0.6

TABLE 8

MEAN RUNNING TIME OF THE ANIMALS IN THE PRENATAL STUDY IN SECONDS

Trials	Zinc-deficient N=10	Groups Pair-fed N=10	Ad libitum N=10
1	220.80	121.40	142.30
2	106.60	75.50	73.00
3	52.30	43.10	45.30
4	31.90	38.80	51.00
5	29.40	22.00	28.60
6	30.30	16.29	21.90
7	34.10	30.60	26.90
8	22.60	25.90	19.70
9	19.50	23.30	22.30
10	19.30	19.70	26.00
11	19.30	21.20	21.30
12	19.40	14.30	15.60
13	15.50	14.30	14.10
14	16.40	15.50	31.30
15	16.50	15.70	15.00
16	13.80	12.30	20.60
17	12.60	13.80	20.10
18	14.90	16.70	13.30

REFERENCES

REFERENCES

Apgar, J. Effect of zinc deficiency on parturition in the rat. _American Journal of Physiology_, 1968a, 215, 160-163.

Apgar, J. Comparison of the effect of copper, manganese, and zinc deficiencies on parturition in rats. _American Journal of Physiology_, 1968b, 215, 1478-1481.

Apgar, J. Effect of zinc repletion late in gestation on paturition in the zinc-deficient rat. _Journal of Nutrition_, 1973, 103, 973-981.

Caggiano, V., Schnitzler, R., Strauss, W., Baker, R. K., Carter, A. C., Josephson, A. S., & Wallach, S. Zinc deficiency in a patient with retarded growth, hypogonadism, hypogammaglobulinemia and chronic infection. _American Journal of Medical Sciences_, 1969, 257, 305-319.

Caldwell, D.F., Oberleas, D., Clancy, J. J., & Prasad, A. S. Behavioral impairment in adult rats following acute zinc deficiency. _Proceedings of the Society for Experimental Biology and Medicine_, 1970, 133, 1417-1421.

Hambidge, K. M., Jambodge, C., & Jacobs, M. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. _Pediatric Research_, 1972, 6, 868-874.

Hurley, I. S. Zinc deficiency in the developing rat. _American Journal of Clinical Nutrition_, 1969, 22, 1332-1339.

Hurley, I. S., & Swenerton, H. Congenital malformations resulting from zinc deficiency in rats. _Proceedings of the Society for Experimental Biology and Medicine_, 1966, 123, 692-696.

Luecke, R. W., Olman, M. E., & Baltzer, B. V. Zinc deficiency in the rat: Effect on serum and intestinal alkaline phosphatase activities. _Journal of Nutrition_, 1968, 94, 344-350.

Macapinlac, M. P., Barney, G. H., Pearson, W. N., & Darby, W. J. Production of zinc deficiency in the squirrel monkey. _Journal of Nutrition_, 1967, 93, 499-510.

Munn, N. L. _Handbook of psychological research on the rat_. Boston: Houghton Mifflin, 1950.

Oberleas, D., Caldwell, D. F., & Prasad, A. S. Trace elements and behavior. _Neurobiology of the Trace Metals Zinc and Copper_. Edited by Carl C. Pfeiffer. New York: Academic Press, 1972.

Prasad, A. S., ed. Zinc metabolism. Springfield: Chas. C. Thomas, 1966.

Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., & Schulert, A. R. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. Journal of Laboratory and Clinical Medicine, 1963a, 61, 537-549.

Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., Schulert, A. R., & Darby, W. J. Biochemical studies on dwarfism, hypogonadism and anemia. Archives of Internal Medicine, 1963b, 111, 407-428.

Prasad, A. S., & Oberleas, D. Zinc: Human nutrition and metabolic effects. Annals of Internal Medicine, 1970, 73, 631-636.

Sandstead, H. H. Zinc nutrition in the U.S.A. Paper presented at a symposium of the American Institute of Nutrition, Atlantic City, N. J., April 9, 1972.

Sandstead, H. H., Gillespie, D. D., & Brady, R. N. Zinc deficiency: Effect on the brain of the suckling rat. Pediatric Research, 1972, 6, 119-125.

Sandstead, H. H., Lanier, V. C., Shepard, V. C., & Gillespie, D. D. Zinc and wound healing: Effects of zinc deficiency and zinc supplementation. American Journal of Clinical Nutrition, 1970, 23, 514-519.

Sandstead, H. H., Prasad, A. S., Schulert, A. R., Farid, Z., Miale, A., Jr., Bassilly, M. B., & Darby, W. J. Human zinc deficiency, endocrine manifestations and response to treatment. American Journal of Clinical Nutrition, 1967, 20, 422-442.

Sandstead, H. H., & Rinaldi, R. A. Impairment of deoxyribonucleic acid synthesis by dietary zinc deficiency in the rat. Journal of Cellular Physiology, 1969, 73, 81-83.

Somers, M., & Underwood, A. Ribonuclease activity and nucleic acid and protein metabolism in the testes of zinc deficient rats. Australian Journal of Biological Sciences, 1969, 22, 1277-1282.

Terhune, M. W., & Sandstead, H. H. Decreased RNA polymerase activity in mammalian zinc deficiency. Science, 1972, 177, 68-69.

Todd, W. R, Elvehjem, C. A., & Hart, E. B. Zinc in the nutrition of the rat. American Journal of Physiology, 1934, 107, 146-156.

Tolman, E. C., & Honzik, C. H. Introduction and removal of reward and maze performance in rats. University of California Publication in Psychology, 1930, 4, 257-275.

Whitenack, D. I., Luecke, R. W., & Whitehair, C. K. Pathology of the testes in zinc depleted and repleted mature and immature rats. Federation Proceedings, 1970, 29, 297.