12-1-1974

Early Postnatal Zinc Deficiency and Aggression in the Rat

Mark J. Hanlon

EARLY POSTNATAL ZINC DEFICIENCY AND AGGRESSION IN THE RAT

by
Mark J. Hanlon

Bachelor of Arts, Bemidji State College, 1972

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Arts

Grand Forks, North Dakota

December
1974

This thesis submitted by Mark J. Hanlon 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 L. Halas
(Chairman)

Harold D. Sandstead

James R. Antes

William Johnson
Dean of the Graduate School

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Permission

Title EARLY POSTNATAL ZINC DEFICIENCY AND AGGRESSION IN THE RAT

Department Psychology

Degree Master of Arts

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ACKNOWLEDGMENTS

I would like to acknowledge the patience and helpful suggestions of the members of my thesis committee: Dr. Ed Halas, Dr. Harold Sandstead, and Dr. Jim Antes. Special thanks are due to Dr. Halas and Dr. Sandstead for providing the opportunity to engage in this research, to Orris Johnson for building the experimental apparatus, to Maxine Rosenthal for caring for the animals and to my good friends Carl Westphal, Michael Rowe, Pete Peterson and Carrie Peterson for volunteering their time to be judges in this research.

DEDICATION

I would like to dedicate this thesis to Bee and Vern and Judy and Rance and Tin-Man.

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ABSTRACT

The effects of a transitory zinc deficiency on subsequent aggression were assessed. Eleven Sprague Dawley dams were assigned to three dietary conditions on days 1-21 postpartum. They were fed either a low zinc diet (less than 1 ppm) or an equal amount of the same diet but with zinc supplementation via their water supply, or unrestricted amounts of the zinc supplemented regimen. Within the first two days postpartum, litter sizes were equalized. All dams were returned to zinc adequate diets on day 22, and the pups were weaned on day 24. Food intake data for pups and dams were recorded as well as weights of dams and pups. After approximately 130 days of ad libitum access to a zinc sufficient diet, the pups were tested for levels of shock-elicited aggression. The animals were tested in pairs with each pair receiving 100 2 mA shocks. Human judges counted aggressive attacks between pairs, and their judgments were statistically compared for reliability. The judges viewed videotapes of the testing sessions. Pups whose dams were made zinc deficient on days 1-21 postpartum exhibited more aggressive attacks than pups of dams pair-fed or given ad libitum access to the zinc supplemented diet.

CHAPTER I

INTRODUCTION

Dietary Zinc Deficiency

Forty years ago, Todd, Elvehjem and Hart (1934) lowered the dietary zinc level of rats and after noting retarded growth and hair loss suggested "Zinc should be included in studies of the role of minor inorganic elements in nutrition" (p. 155). The essential nature of dietary zinc to various forms of life is now well recognized. Mice restricted to less than 0.3 ppm zinc exhibited emaciation, alopecia, a lower growth rate and higher mortality rate than controls (Day and Skidmore, 1947). Suckling mice nursed by foster mothers which were in later stages of lactation developed disorders including alopecia, hyperkeratinization, and retarded growth while those nursed by their own mothers and those nursed by foster mothers but supplemented with zinc did not (Nishimura, 1953). Supplementation of zinc to diets of pigs receiving 34-44 ppm zinc increased weight gain and reversed the symptoms of parakeratosis that the low-zinc diet produced (Tucker and Salmon, 1955). Growth retardation and various other debilities were demonstrated in other species given zinc deficient diets. These included growing chickens (O'Dell, Newberne, and Savage, 1958), Holstein calves (Miller and Miller, 1962), young lambs (Ott, Smith, Stob, and Beeson, 1964), and squirrel monkeys (Macapinlac, Barney, Pearson, and Darby, 1967). The teratogenic effects of zinc deficiency

were demonstrated in embryonic and newly hatched chicks, while zinc supplementations averted such anomalies (Kienholz, Turk, Sande, and Hoekstra, 1961).

The pathological states associated with zinc deficiency have been investigated, and some more recent research has been directed toward illuminating the mechanisms of such states. The gross symptoms of the zinc deficiency syndrome in the laboratory rat includes hyperkeratinized esophageal and skin lesions, retarded weight gain, alopecia, retarded genital maturation, abnormal estrous cycle, kangaroo-like posture, and increased mortality (Day and McCollum, 1940; Follis, Day, and McCollum, 1941; Hurley, 1969; Whitenack, Luecke, and Whitehair, 1970). Zinc deprived hooded Lister rats were found to eat significantly less food voluntarily than zinc supplemented controls and displayed a cyclic pattern in day to day food intake (Chesters and Quarterman, 1970). Female rats deprived of zinc during pregnancy produce pups with a greater incidence of congenital malformations than do pair-fed controls. Chronically zinc deficient dams lost weight during pregnancy, were less likely to produce living young, and produced fewer pups per litter, with those pups weighing less than pups of zinc supplemented controls (Hurley and Swenerton, 1966). Ninety-eight percent of these pups showed gross congenital malformations. Abnormally high rates of congenital malformations have also been reported for terms of zinc deficiency as short as six days (seventh-twelfth gestational day) during pregnancy (Hurley, Gowan, and Swenerton, 1971; Apgar, 1972; Warkany and Petering, 1973). Furthermore, zinc deficient dams take longer in parturition and are less likely to

eat afterbirths, retrieve pups, or assume a nursing position than pair-fed or ad libitum controls (Apgar, 1968a; Apgar, 1968b).

Studies of biochemical changes associated with zinc deficiency and the uptake of zinc by the brain have attempted to shed some light on the vital function of zinc. Zinc deficiency has been shown to impair DNA synthesis and the production of DNA-dependent RNA polymerase in the rat (Sandstead and Rinaldi, 1969; Williams and Chesters, 1970; Terhune and Sandstead, 1972). Of special interest is Sandstead, Gillespie, and Bradys' (1972) demonstration that a zinc deficiency impairs DNA and protein synthesis in the brain of the suckling rat. Brains of young rats made zinc deficient for three weeks after weaning took up a larger percentage of injected zinc than pair-fed or ad libitum zinc supplemented controls (O'Neal, Pla, and Spivey-Fox, 1970). The variation of zinc uptake in regions of mouse and rat brains was investigated by Czerniak and Haim (1971). In vitro and in vivo study of sixty rats injected with zinc chloride ZN-65 revealed regional changes in zinc uptake under phenothiazine injections. The occipitotemporal cortex, thalamus, and hippocampus proved most zincorphilic. The authors hint there may be a relationship between psychotic states and brain zinc cycles. Although these suggestions are at best speculative, the importance of zinc to the brain appears undeniable.

Human parallels to zinc deficiency in lower animals have been discovered and studied. Implications from these studies as well as laboratory studies involving lower animals have been extended to humans. A nutritional zinc deficiency in man which occurs in the middle east was extensively studied by a number of researchers (Prasad,

Miale, Farid, Sandstead, Schulert, and Darby, 1963; Sandstead, Prasad, Schulert, Farid, Miale, Bassilly, and Darby, 1967; Prasad and Oberleas, 1970). The condition is believed to be a result of excessive blood loss from hookworm and bilharzia infestation, excessive sweating, geophagia (in Iran), and a high dietary concentration of phytates and other vegetable compounds which bind zinc and make it unavailable for intestinal absorption. Human zinc deficiency manifestations include: retarded growth, hypogonadism, roughened skin, general lethargy, retarded bone growth and an oral glucose tolerance test suggestive of delayed absorption. Poor wound healing is noted in older persons suffering from this deficiency. Treatment of the condition with zinc supplementation produced reversibility of these symptoms in Egyptian (Sandstead, et al., 1967; Prasad and Oberleas, 1970) and Iranian subjects (Ronaghy, Spivey-Fox, Garn, Isreal, Harp, Moe, and Halsted, 1969). These effects were greater than either an adequate diet alone or an adequate diet with iron supplementation. Prasad (1969), in a review of the metabolic role of zinc, cites the importance of zinc in growth and gonadal development in man and evidence of zinc's role in RNA and protein synthesis. Prasad suggests that the possible relationship between zinc deficiency and human congenital malformations deserves attention. Similarly, epidemiological data concerning the incidence of central nervous system malformations and geographical areas of likely zinc deficiency is cited by Sever and Manuel (1973) who hypothesize a zinc deficiency-central nervous system malformation relationship.

Until recently, the behavioral effects of dietary zinc deficiency had been largely ignored. Open field behavior, one-way

avoidance learning, and maze learning were investigated by Caldwell and Oberleas (1969) using progeny of dams made mildly zinc deficient (10-14 ppm zinc) ten weeks before breeding. The zinc deficient pups were less active in the open field, produced fewer CRs in one-way avoidance, and had longer mean response latencies in the Lashley III maze than controls. Although the controls were not pair fed, it is reported that food intake was not significantly different between the groups. A later study subjected rats made zinc deficient (8 mg/kg diet) for 48 days to behavioral measures (Caldwell, Oberleas, Clancy, and Prasad, 1970). Zinc supplemented pair-fed controls were significantly superior in one-way avoidance learning and water maze learning. The pair-fed animals also exhibited more activity in the open field measure. In an unusual experiment, effects of chronic mild zinc deficiency on maternal behavior and behavior of offspring were studied (Caldwell, Oberleas, and Prasad, 1973). Dams were kept zinc deficient on 15 ppm zinc through the course of three pregnancies. Parturition and post partum maternal behavior was impaired by zinc deficiency as was the behavior of offspring. Learning deficits in offspring increased with subsequent pregnancies. Studies of Tolman-Honzik maze acquisition of pre- and postnatally zinc deprived rats have been carried out at the USDA Human Nutrition Laboratory at the University of North Dakota (Lokken, 1973; Lokken, Halas, and Sandstead, 1973). Zinc deficiencies were instituted either prenatally during the third trimester of pregnancy or postnatally during the first 21 days of life. Although maze running times did not differ between the zinc deficient groups and controls, the low zinc groups showed marked inferiority on an error criterion of maze performance. Rowe (1974) subsequently investigated maze and avoidance

learning in pre-natally zinc deprived female pups. His data failed to replicate Caldwell, et al. or Lokken, et al. results in that his zinc deficient group was inferior in neither maze nor avoidance learning to pair-fed or ad libitum controls. Rowe has suggested differential effects of zinc deficiency according to sex as a possible explanation of these discrepencies. Halas and Sandstead studied male rats exposed to a transitory zinc deficiency pre-natally on days 15-20 of gestation (Halas and Sandstead, 1974). These Ss were zinc rehabilitated and tested when they reached 60 days of age. The zinc deficient group proved inferior in avoidance conditioning to pair-fed controls who were in turn inferior to ad libitum controls using number of CRs as the criterion. The zinc deficient group also exhibited slower response latencies and, like the pair-fed control, fewer inter-trial responses than the ad libitum control group. Peterson (1974) investigated post-natal zinc deficiency in male rats using performance in an avoidance task as the dependent variable. He found more inter-trial responses during avoidance conditioning and extinction for his zinc deficient animals than pair-fed or ad libitum controls. No other main effects of nutrition on avoidance learning were obtained. These findings should be viewed with caution, however, as the particular experimental design included an additional variable--the injection of kryptopyrrole or saline solution. Thus, the interactions obtained may well have confounded and masked any nutritional effects.

In summary, behavioral research with rats made zinc deficient pre- and postnatally suggests subsequent deficits in maze and avoidance learning, possibly depending on the sex of the rat and the period of zinc deficiency. The present research addresses itself to this general

area of behavioral effects of zinc deficiency. Specifically, this project deals with the long-term consequences of transitory post-natal zinc deficiency. The particular consequence investigated is shock-elicited aggressive behavior.

Shock-Elicited Aggression

The laboratory investigation of aggressive behavior has received much attention within the last thirteen years. Primarily, this is a result of the controlled and reliable production of aggressive behavior experimentally. Thus the mere observation of paired rats and subsequent evaluation of aggression (Davis, 1933; Hall and Klein, 1942) has been replaced by counting the frequency of aggressive attacks between pairs of rats receiving scrambled electric shocks. This newer paradigm is generally referred to as shock-elicited aggression (SEA). The phenomenon was studied by Ulrich, Azrin, and others in the early sixties. Levels of aggressive behavior elicited by foot shock were found to be related to the enclosed floor area, shock intensity, shock frequency, and shock duration (Ulrich and Azrin, 1962). Scrambling the shock produced the most consistent levels of SEA in this early research. The SEA phenomenon was shown to exist in a wide range of species as well as between some species (Ulrich, Wolff, and Azrin, 1964; Ulrich, Hutchinson, and Azrin, 1965). Thus, shocked snakes may attack rats and shocked rats may attack cats.

Sex and age variables have been investigated, but their relationship to SEA is not yet clear. Reports of significant differences, males and older rats being more aggressive (Hutchinson, Ulrich, and Azrin, 1965; Powell and Creer, 1969; Hutzell and Knutson, 1972), and reports of

no differences (Powell, Silverman, Francis, and Schneiderman, 1970; Creer and Powell, 1971; Milligan, Powell and Borasio, 1973) appear in the literature. It is clear, however, that female Sprague Dawley rats display more consistent levels of SEA over trials than do males (Creer and Powell, 1971). Rats will exhibit a general increase in SEA over trials (Powell and Creer, 1969; Hutchinson, et al., 1965; Powell, et al., 1970), but exposures to inescapable shock with no opportunity for fighting depresses subsequent SEA (Powell and Creer, 1969; Maier, Anderson, and Lieberman, 1972). Castration or raising rats in isolation will lead to reduced levels of SEA (Hutchinson, et al., 1965; Hutzell, et al., 1972; Milligan, et al., 1973).

Other environmental and organismic factors which have been investigated in relation to SEA include sensory impairment, temperature, and the upright posture stereotypic of an attacking rat. Visual impairment in rats produces a lowered level of SEA, and the additional removal of the vibrissae lowers this level even further (Flory, Ulrich, and Wolff, 1965). Using 16 pairs of male Wistar rats, and temperatures of 40, 70, and 100 degrees Fahrenheit, Berry and Jack (1971) demonstrated the direct relationship between temperature and SEA. The upright attack posture commonly associated with SEA in rats was controlled in the SEA paradigm, but did not significantly effect levels of aggression (Knutson and Hynan, 1972). Finally, Lyon and Ozolins (1970) have demonstrated classical conditioning of SEA to an auditory stimulus repeatedly paired with shock onset.

No consistent relationship between food and water deprivation and SEA is yet described in the literature. Reports of both increases (Creer, 1973) and decreases (Hamby and Cahoon, 1971) in SEA with water

deprivation appear. Similarly, food deprivation has been reported as having no effect on SEA (Creer, 1973) and as increasing SEA (Cahoon, Crosby, Dunn, Herrin, Hill and McGinnis, 1971).

The shock parameters of SEA have been investigated for the purpose of describing optimal conditions for consistent and reliable aggressive responding. Ulrich and Azrin (1962) demonstrated a positive relationship between shock intensity and levels of aggression with 2 milliamperes producing optimal results. The intensity was then held constant at 2 ma. and a relationship between shock frequency and SEA was demonstrated. SEA attained maximal levels at a frequency of approximately 10 shocks per minute. Creer and Powell (1971) used 30 male Sprague Dawley rats, 90 days old, and found no differences between 1, 2, 3, and 4 milliampere shock intensities. Shock duration was also shown to be related to SEA. There is little increase in SEA for durations longer than 0.5 seconds, and the likelihood of physical debility may be increased with the longer periods (Azrin, Ulrich, Hutchinson, and Norman, 1964; Dreyer and Church, 1968).

Burr Eichelman at Stanford has used SEA extensively as a dependent variable maintaining close approximations of these suggested shock parameters. This work has demonstrated strain differences in SEA while showing no relationship between SEA and hypertension (Eichelman, Dejong and Williams, 1973). The strain differences remain to be systematically described. Brain lesions in septal and ventro-medial hypothalamic areas increased SEA in hooded Long-Evans male rats while hippocampal and amygdaloid lesions led to decreases (Eichelman, 1971). Male Sprague Dawley rats studied by Eichelman, Thoa and Ng

(1972) have demonstrated increases in SEA with intracisternal 6-hydroxydopamine injections as compared to saline injected controls.

SEA, then, has been widely investigated as a dependent variable. Research has clarified relevant variables which must be controlled such as temperature, age, rat strain, chamber size, and shock parameters. Finally, SEA has been shown to be useful as a dependent variable in investigations of brain-behavior relationships. The application of SEA to nutrition research is novel, but then behavioral measures in general are relatively new to the nutrition scene.

Statement of the Problem

As previously described, a zinc deficient diet will produce various malformations and behavioral debilities in offspring as well as physical and behavioral abnormalities in adult laboratory rats. Zinc adequate diet has been shown to reverse some of these effects while others appear permanent. In the present study, transitory post-natal zinc deficiency was imposed on the experimental animals. The Ss were allowed an extended period of zinc sufficient diet before measures of SEA were taken. Thus one long term behavioral effect of early zinc deficiency was investigated.

CHAPTER II

METHOD

Subjects

Twenty-four female albino rats of the Sprague Dawley strain were used as experimental subjects. These animals were progeny of eleven dams purchased from Thorp Industries, White Bear, Minnesota, and randomly assigned to three dietary conditions. The dams all delivered litters on September 1 or 2, 1973, at the Human Nutrition Laboratory in Grand Forks, North Dakota. The litters providing the Ss are summarized in Table 1.

TABLE 1

A SUMMARY OF THE LITTERS PROVIDING EXPERIMENTAL SUBJECTS

Pair Name	Dam Number	Original Litter Size	Number of Pups Used	Dietary Condition	Date of Parturition
AL1	750	12	2	AL	9/1/73
AL2	747	8	2	AL	9/2/73
AL3	753	13	2	AL	9/1/73
AL4	752	9	2	AL	9/2/73
ZN5	743	12	3	ZN	9/1/73
ZN6	739	11	3	ZN	9/1/73
ZN7	739 & 743	(See Above)	(See Above)	ZN	(See Above)
ZN8	742	10	2	ZN	9/2/73
PF9	748	6	2	PF	9/1/73
PF10	749	10	2	PF	9/1/73
PF11	745	13	2	PF	9/1/73
PF12	746	11	2	PF	9/2/73

The experimental design required twelve pairs of animals. Eleven of the twelve experimental pairs consisted of littermates with the twelfth pair from two different litters (#739 and #743). At the time these Ss were subjected to the experimental aggression procedures they were approximately 150 days old.

Apparatus

A clear plexiglass box with a hinged cover was constructed as an experimental aggression chamber. This chamber had inside dimensions of 32 cm. x 25.5 cm. x 30.5 cm. The floor of the chamber consisted of seventeen stainless steel rods 0.3 cm. in diameter lying parallel to one another and 1.9 cm. from center to center (see Figure 1). These rods were electrified by a Grason-Stadler Model 700 Shock Generator. Shock duration and intershock intervals were regulated by a Grason-Stadler Model 1180 Electronic Timer. The chamber was housed in a box 80 cm. high x 61 cm. deep x 80 cm. wide made of 3/4 inch plywood (see Figure 2). Two fans built into the box supplied ventilation and a constant noise level. The front of this box remained open during the experimentation. Three 20W flourescent tubes 60 cm. long supplied a constant level of illumination. Room temperature was maintained at 75-76 degrees Fahrenheit. Experimental sessions were recorded with a Shibaden Model FP 100 videotape camera and a Sony Model 3600 videotape deck. These tapes were viewed on a videotape monitor supplied by Instructional Communications at the University of North Dakota. Five graduate students including the experimenter were recruited and trained as judges to view the tapes and count aggressive attacks within pairs.

Fig. 1. The experimental aggression chamber.

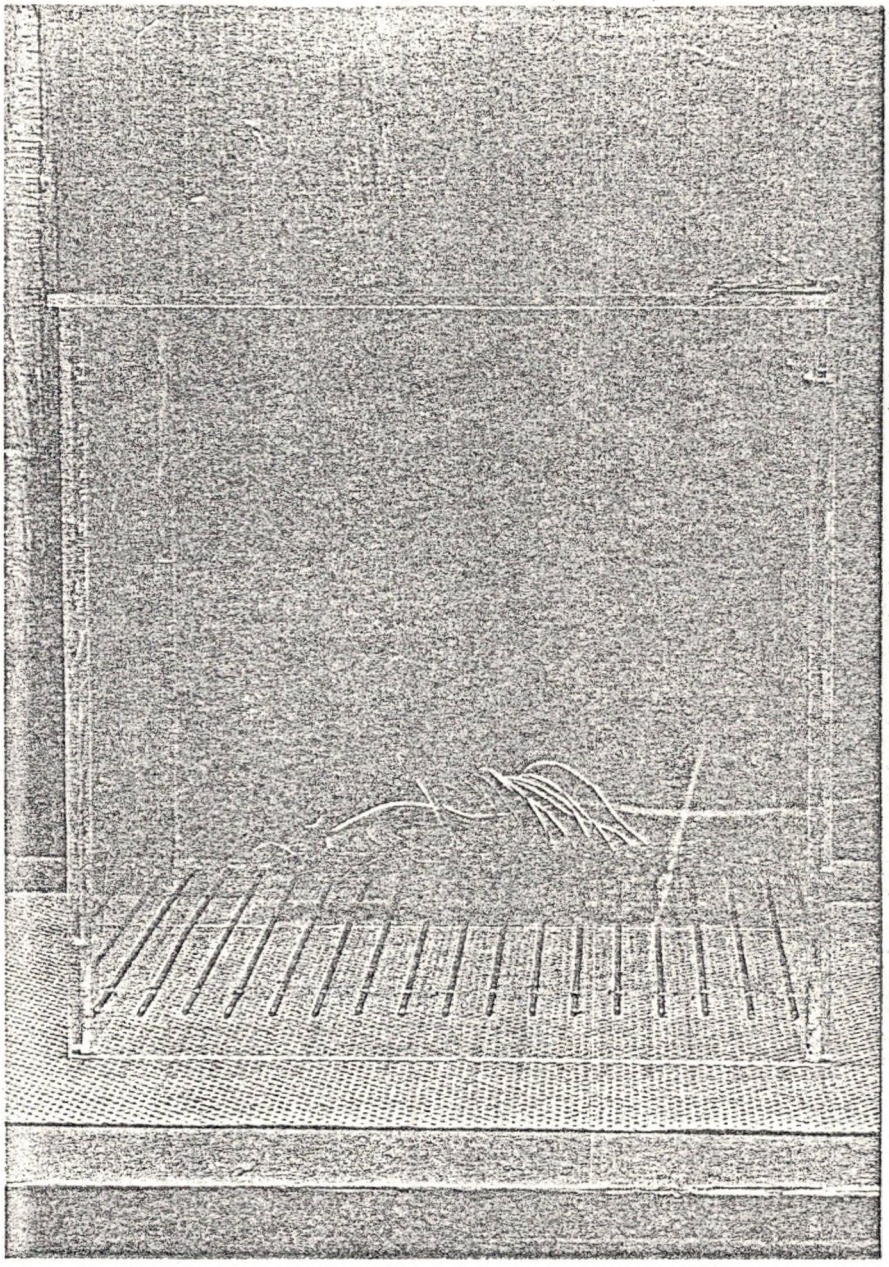
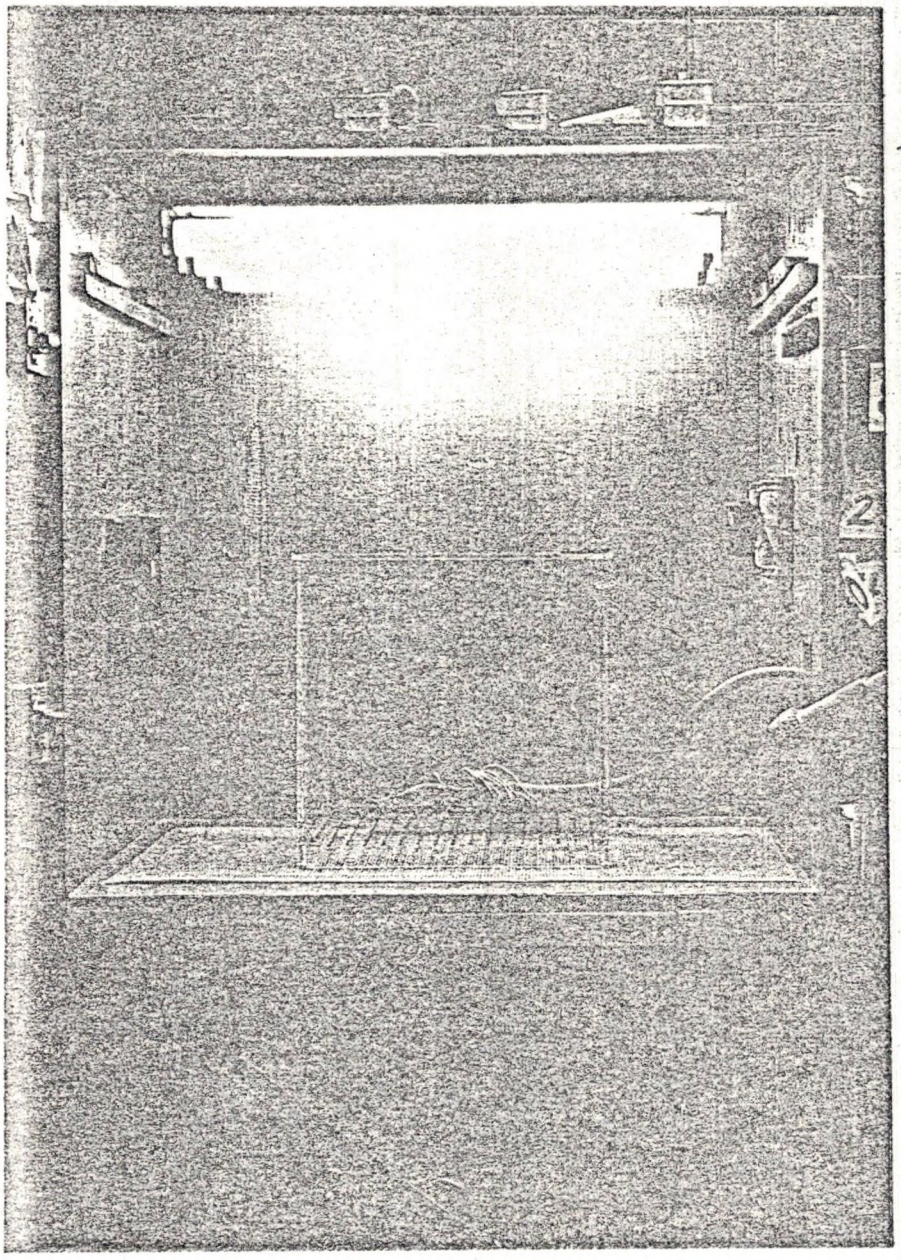


Fig. 2. The box housing the experimental aggression chamber.



Procedure

Eleven dams were maintained on controlled diets on days 1-20 postpartum. Litter sizes were balanced so that each dam suckled nine pups. This equalization of litter sizes took place in the first two days postpartum. When additions were necessary, they were made from litters of dams in the same dietary conditions. Three dams were maintained on zinc deficient diets produced at the USDA Human Nutrition Laboratory, Grand Forks, North Dakota, and described by Luecke, Olman and Baltzer (1968). This diet contains less than 1 ppm zinc (see Table 2). Eight female pups from these three litters made up the four zinc-deficient (ZN) pairs. Three of the pairs were littermate pairs while one was from two different litters. Four dams were given matched amounts (matched to the ZN group's food intake on the previous day) of the identical diet with the addition of 50 ppm of zinc chloride to their water supply (all dams were maintained on glass distilled water supplies). One pair of randomly chosen female pups from each of these four litters served as pair-fed controls (PF). Four dams were maintained on the same zinc-supplemented regimen as the PF dams but were given the diet ad libitum. One pair of female pups from each of these litters made up the ad libitum control group (AL). On day 21 postpartum, all Ss were given ad libitum access to Purina Lab Chow and tap water. On day 24, all Ss were weaned and maintained on tap water and chow ad libitum. All pairs were housed separately after day 40 and maintained on the same post-weaning diets. Experimental procedures began on approximately day 150 postpartum.

Each pair of rats was subjected to 100 2 ma. shocks of 0.5 seconds duration presented at a rate of one shock every five seconds.

TABLE 2

THE ZINC DEFICIENT DIET[a]

Formula	g/kg
Egg White Solids, Spray Dried	200.00
Dextrose, Hydrate, Technical	630.108
Fiber, Nonnutritive	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
Curpric 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} (p.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

[a]This diet was obtained from General Biochemicals of Chagrin Falls, Ohio. It is a modified TDF1305 with 1g/kg of inositol added in place of chlorotetracycline.

These 100 trials were distributed over 4 sessions, one session a day for 4 consecutive days. On days 1-3, each pair received 20 trials per session. On day 4 each pair received 40 trials. The order of testing of pairs was balanced within and between sessions (see Table 3).

TABLE 3

TESTING SEQUENCE OF THE TWELVE EXPERIMENTAL PAIRS

Serial Order	Day 1	Day 2	Day 3	Day 4
1st	AL1	PF10	AL3	PF4
2nd	ZN5	ZN6	ZN7	ZN8
3rd	PF9	AL2	PF11	AL4
4th	PF10	AL3	PF12	AL1
5th	ZN6	ZN7	ZN8	ZN5
6th	AL2	PF11	AL4	PF9
7th	AL3	PF12	AL1	PF10
8th	ZN7	ZN8	ZN5	ZN6
9th	PF11	AL4	PF9	AL2
10th	PF12	AL1	PF10	AL3
11th	ZN8	ZN5	ZN6	ZN7
12th	AL4	PF9	AL2	PF11

Pairs were placed in the aggression chamber for 15 seconds prior to the first shock and removed within 10 seconds after the last shock of each session. Sessions were recorded on videotape and shown to 5 pre-trained judges who counted aggressive attacks per pair per session. Aggressive attacks were defined as

> directed movement toward the opponent which resulted in contact, including at least one additional response of the following: biting, sparring, upright attack posturing, or supine submissive posturing adopted by the attacked rat (Eichelman, Dejong, and Williams, 1973, p. 302).

Judges were trained by comparing judgments of aggression on each trial of pilot-study animals until the experimenter was satisfied that

close concordance existed. The concordance of judgments was empirically validated in the subsequent analysis of the judgment data. Judges recorded a tally of aggressive attacks for each pair each session. These judgments were made independently and without knowledge of the experimental groups to which the pairs were assigned.

CHAPTER III

RESULTS

The Appendix contains the records of judgments of aggressive attacks on days 1–4. The four pairs labelled A, B, C, and D were another experimental group not relevant to this study. These tables include estimates of interjudge reliability for each day (Winer, 1971). These estimates ranged from 0.97 to 0.99 over the four testing sessions. Table 4 presents the number of aggressive attacks for each four-pair experimental group on each day. Table 5 is the conversion of these tallies to percentages of total trials on which aggressive attacks occurred.

TABLE 4

NUMBER OF AGGRESSIVE ATTACKS PER GROUP PER DAY AND TOTAL FOR ALL DAYS

Dietary Condition	Day 1	Day 2	Day 3	Day 4	Total
ZN	68	75	21	153	317
PF	49	44	24	117	234
AL	107	29	23	49	208

A total percentage of trials on which aggressive attacks occurred was computed for each group based on 400 trials per group (see Table 5). The percentages obtained were: ZN--15.85 percent,

PF--11.7 percent, and AL--10.4 percent. These data are graphically presented in Figure 3.

TABLE 5

PERCENTAGE AGGRESSIVE ATTACKS PER GROUP PER DAY AND TOTAL FOR ALL DAYS

Dietary Condition	Day 1	Day 2	Day 3	Day 4	Total
ZN	17.00%	18.75%	5.25%	19.13%	15.85%
PF	12.25	11.00	6.00	14.63	11.70
AL	26.75	7.25	5.75	6.13	10.40

These percentages were compared using a method described by Guilford (1965). The difference between PF and AL percentages of aggressive attacks was not significant, while the ZN group aggressed more frequently than either control group. The ZN group exhibited more aggressions than the AL control group ($p < 0.01$, one-tailed) and also more than the PF control group ($p < 0.04$, one-tailed). This method assumes the independence of each observation, an assumption which is not met in the present research. Violations of this assumption may spuriously inflate levels of significance between obtained differences. Clearly, a number of replications of the present study should be done before statistically defensible claims can be made concerning its validity, and the present findings should be interpreted with great care.

The three dietary conditions were compared two at a time on each of four dependent measures (pup weight, dam weight, pup food intake, and

Fig. 3. Percent total aggressive attacks for the three dietary conditions.

PERCENT AGGRESSIVE ATTACKS
0 %
5 %
10 %
15 %
DIETARY CONDITION
AL
PF
ZN
10.4%
11.1%
15.85%

dam food intake). These twelve comparisons were made by analyses of variance with repeated measures.

Mean dam weights from day 13 of pregnancy through weaning on day 24 for the dietary conditions are graphically presented in Figure 4. AL dams maintained higher overall weights during this period than PF ($p < 0.01$, $F = 15.55$, $df = 1/6$) or ZN ($p < 0.05$, $F = 14.64$, $df = 1/5$) dams. ZN and PF dams did not differ in overall weight or the course of weight changes during this period. Both groups lost weight while the AL dams did not show this marked change ($p < 0.01$, $F = 26.38$, $df = 8/40$, and $p < 0.01$, $F = 19.80$, $df = 8/48$, respectively).

Means of mean pup weights per litter on days 0-40 postpartum for the dietary conditions are depicted in Figure 5. AL pups showed greater overall weight than PF pups ($p < 0.01$, $F = 86.37$, $df = 1/6$) or ZN pups ($p < 0.01$, $F = 169.26$, $df = 1/5$). AL pups also showed the greatest weight gain over the forty days as compared to PF ($p < 0.01$, $F = 6.00$, $df = 10/50$) and ZN ($p < 0.01$, $F = 112.99$, $df = 10/50$) pups. PF pups maintained a higher overall weight than ZN pups ($p < 0.05$, $F = 10.64$, $df = 1/6$), and showed a greater weight gain over the forty days than ZN pups ($p < 0.01$, $F = 49.55$, $df = 10/60$).

Food intake of dams from parturition to day 19 postpartum was recorded and is presented in Figure 6. AL dams showed a greater overall food intake than ZN dams over this twenty day period ($p < 0.01$, $F = 81.38$, $df = 1/5$) as well as a greater increase in food intake ($p < 0.01$, $F = 4.25$, $df = 19/95$). This is as would be expected of zinc deprived Ss. It is also clear that fair approximations of ZN dams' food intake were made in feeding the PF dams.

Fig. 4. Mean dam weights from day 13 of pregnancy through weaning on day 24 postpartum.

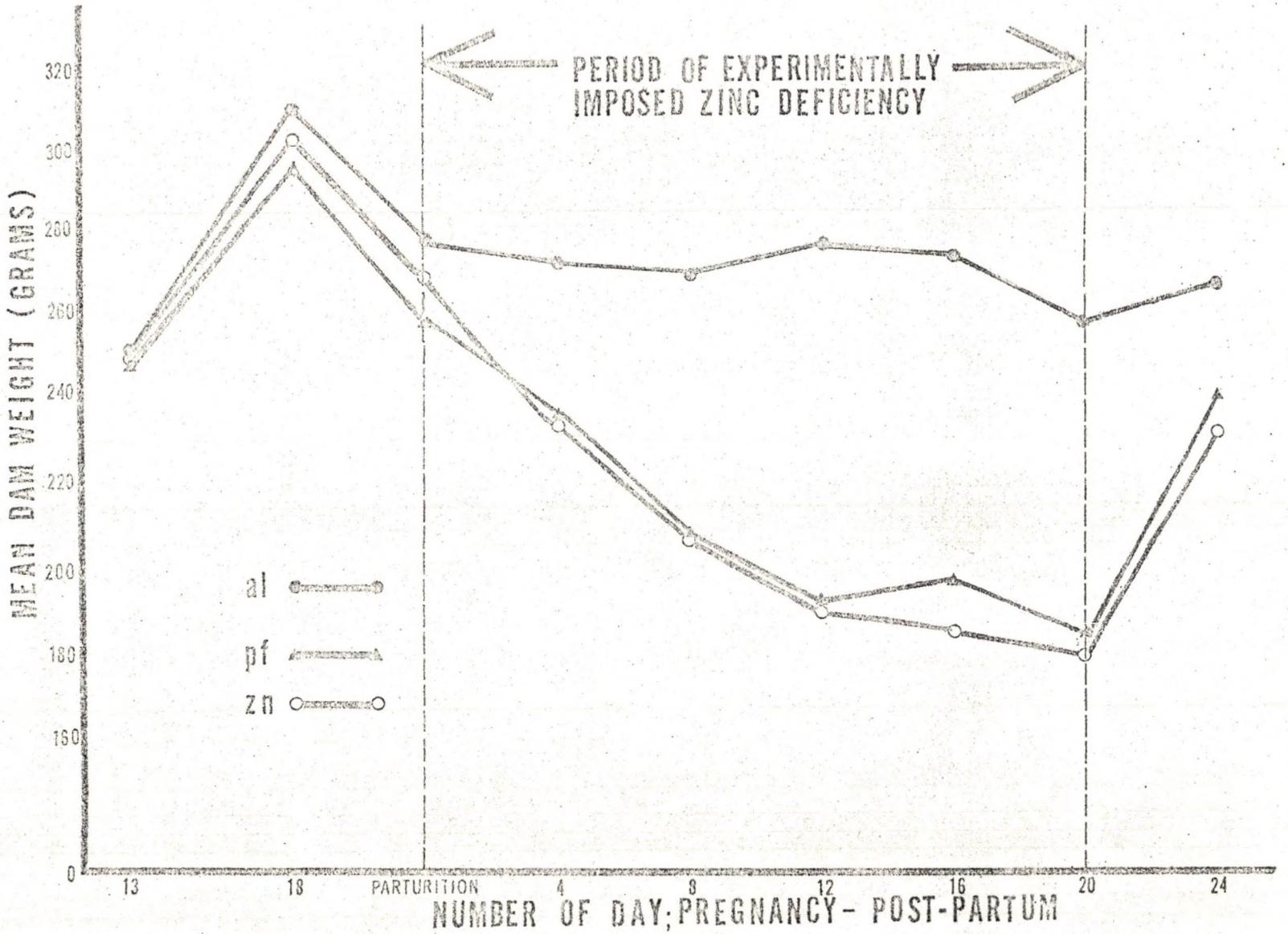

PERIOD OF EXPERIMENTALLY
IMPOSED ZINC DEFICIENCY
MEAN DAM WEIGHT (GRAMS)
320
300
280
260
240
220
200
180
160
0
al
pf
zn
13
18
PARTURITION
4
8
12
16
20
24
NUMBER OF DAY; PREGNANCY- POST-PARTUM

Fig. 5. Means of mean pup food intake per litter on days 24-39 postpartum.

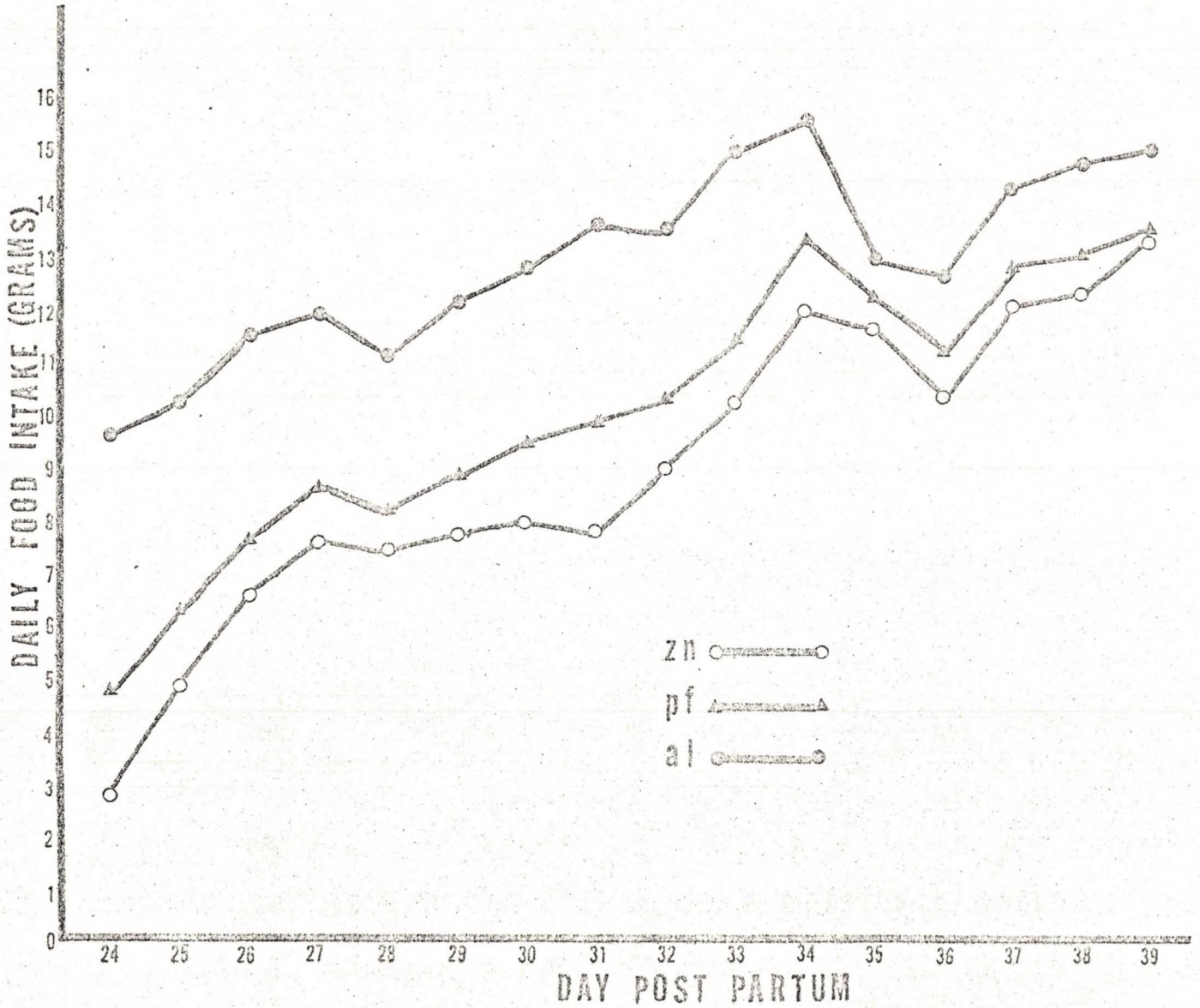
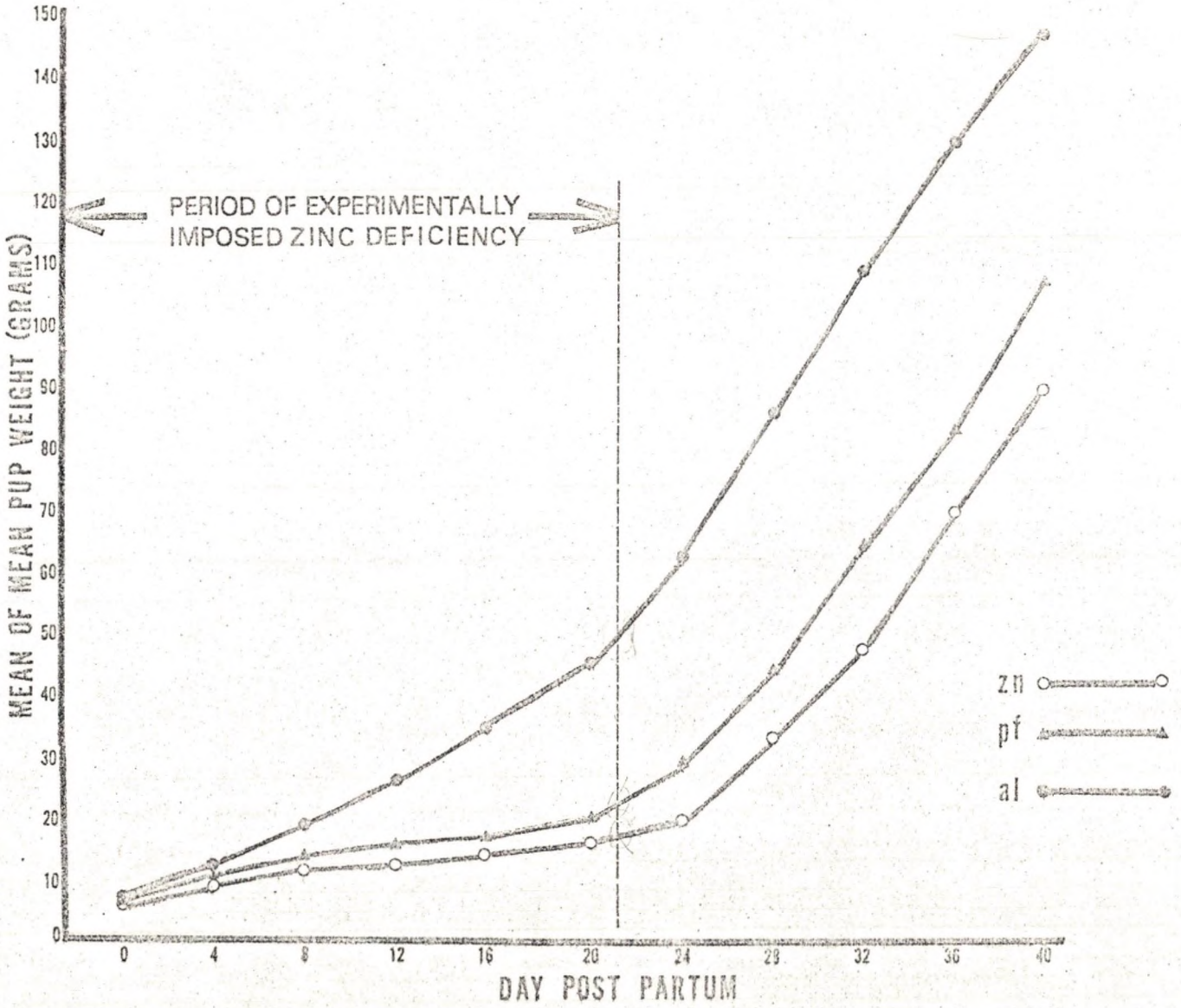

DAILY FOOD INTAKE (GRAMS)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
DAY POST PARTUM
zn
pf
al

Fig. 6. Mean food intake of dams on days 0-19 postpartum.

MEAN DAM FOOD INTAKE (GRAMS)
5
10
15
20
25
30
35
40
45
50
55
60
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
DAY POST PARTUM
Zn
pf
al

Figure 7 shows the means of mean food intake per pup per litter for days 24-39 postpartum. AL pups showed a higher food intake level than ZN ($p < 0.01$, $F = 71.85$, $df = 1/5$) or PF ($p < 0.01$, $F = 36.75$, $df = 1/6$) pups. However, ZN and PF pups showed a greater increase in food intake over this period than AL pups ($p < 0.01$, $F = 3.21$, $df = 15/75$, and $p < 0.01$, $F = 2.32$, $df = 15/90$, respectively). PF pups maintained higher overall food intake than ZN pups ($p < 0.05$, $F = 9.88$, $df = 1/5$), but their changes in food intake over the sixteen days did not differ. The profile of the three dietary groups supplied by these data generally verifies the existence of the zinc deficiency experimentally instituted. ZN dams lost weight during pregnancy (Hurley and Swenerton, 1966) and ate less than AL dams (Chesters and Quarterman, 1970), while ZN pups showed less weight gain than AL pups or PF controls (Day and Skidmore, 1947; Nishimura, 1953; Mutch and Hurley, 1974).

Fig. 7. Means of mean pup weights per litter on days 0-40 postpartum.


MEAN OF MEAN PUP WEIGHT (GRAMS)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PERIOD OF EXPERIMENTALLY
IMPOSED ZINC DEFICIENCY
0
4
8
12
16
20
24
28
32
36
40
DAY POST PARTUM
zn
pf
al

CHAPTER IV

DISCUSSION

The results of this research suggest the existence of a long term behavioral deficit which may result from transitory dietary zinc deficiency. The absence of PF-AL differences suggests transitory postnatal starvation did not affect subsequent aggression. Female Sprague Dawley rats made zinc deficient on days 1-21 postpartum were more aggressive than pair-fed or ad libitum controls. The long period of access to zinc sufficient diet could not attenuate this long term behavioral effect. Such insidious and irreversible effects have been suggested by other zinc researches. As a result of learning deficiencies in postnatally zinc deprived rats, Lokken (1973) speculated that mental retardation in humans may relate to a postpartum zinc deficiency. Halas and Sandstead (1974) suggest more generally that stress tolerance in young adult rats may be lowered as a result of zinc deficiency in the third trimester of gestation. These studies concerned prenatal deficiency and, like the present study, postnatal deficiency with one general finding present in all three: zinc deficiency in the later third of pregnancy or from birth to day 21 postpartum caused long term behavioral effects. The Halas-Sandstead stress hypothesis may be the best conceptual generalization available at this time.

The Peterson study (1974) is the only very comparable population to that of the present research. These animals, in fact, came from the same litters and were made zinc deficient during the same postnatal period. Peterson's zinc deficient animals showed a marked elevation of activity in open field and inter-trial response rates. His research, however, used males so that generalizations remain somewhat speculative. Hyperactivity and greater aggressiveness, then, were typical of this particular transitory zinc deficiency. Such findings may also lend themselves to the Halas-Sandstead hypothesis.

The speculative interpretations of these data suggest further research possibilities. The stress-tolerance question is now being experimentally examined. Other valuable directions include: replication of the present research with other rat strains and replication with males of the Sprague Dawley and other strains. Differences between rat strains in levels of SEA are known to exist (Eichelman, et al., 1973). It would be of value to test whether the effects demonstrated in the present research generalized to other strains as well. Similarly, sex differences in the effects of transitory deficiency have been suggested by Rowe (1974) and Halas, Rowe, Johnson, McKenzie, and Sandstead (1974). These differences must be clarified for zinc deficiency research to be understood and correctly interpreted. Future research should use more Ss so that differences are more easily demonstrated. It may well be that such research would find pair-fed, ad libitum differences as well as those of zinc deficiency.

A second general area of research is suggested by these results. That area is the study of brain relationships to the different levels of SEA exhibited. Levels of known neurotransmitters in the brain could be

compared for the groups. Eichelman, et al., (1972), for example, found depressed levels of norepinepherine in rats' brains injected with hydroxydopamine. These rats also proved more aggressive than saline injected controls. Besides such general levels, specific brain parts could be compared between groups for levels of neurotransmitters. It appears that the transitory zinc deficiency that was imposed somehow had a permanent effect on the brains of the Ss that was irreversible by zinc supplementation. Such an effect should be investigated to discover its mechanisms and to increase knowledge of brain behavior relationships.

The present experiment suggests a new avenue of research for explanations of human aggression. Nutritional factors may influence subsequent aggressive behavior in humans as it does in the rat. If this is so, the implications of this research for humans are clear. Human malnutrition and starvation are widespread. Dietary zinc deficiency may even occur on a broad scale in the United States. In a discussion of zinc nutrition in the U. S., Sandstead (1973) has suggested that "Some infants, pregnant women, teenage and college women, institutionalized individuals, and some living on low income diets have a marginal to deficient intake of zinc" (p. 1258). If the zinc deficiency-aggression relationship generalizes to humans, then this nutritional deficiency may well be a contributing factor in human violence that is often attributed to social and learned factors. The preceding suggestions are somewhat speculative, but in view of the importance of human aggression today, further research is certainly warranted.

APPENDIX

TABLE 6

JUDGMENTS OF AGGRESSIVE ATTACKS ON DAY 1 WITH AN ESTIMATE OF INTERJUDGE RELIABILITY

Pair Name	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5
A	0	0	0	2	2
B	1	3	2	1	2
C	8	11	9	7	7
D	1	1	1	1	0
AL1	12	12	11	11	8
AL2	3	3	2	2	2
AL3	8	9	9	8	7
AL4	0	0	0	0	0
ZN5	0	1	0	1	0
ZN6	6	6	7	5	6
ZN7	6	5	5	4	5
ZN8	2	2	3	2	2
PF9	3	4	6	6	11
PF10	0	0	0	1	0
PF11	0	0	0	0	0
PF12	4	4	5	2	3

r_5 = Interjudge Reliability Estimate = 0.97

TABLE 7

JUDGMENTS OF AGGRESSIVE ATTACKS ON DAY 2 WITH AN ESTIMATE OF INTERJUDGE RELIABILITY

Pair Name	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5
A	1	1	1	0	1
B	0	1	1	0	0
C	6	9	6	4	4
D	0	0	1	1	0
AL1	6	6	4	5	2
AL2	1	1	1	1	1
AL3	0	1	0	0	0
AL4	0	0	0	0	0
ZN5	4	4	5	4	4
ZN6	3	5	4	4	3
ZN7	7	8	7	8	6
ZN8	0	1	0	0	0
PF9	8	8	7	7	6
PF10	1	3	1	1	1
PF11	0	1	0	0	0
PF12	0	0	0	0	0

$r_5 = 0.98$

TABLE 8

JUDGMENTS OF AGGRESSIVE ATTACKS ON DAY 3 WITH AN ESTIMATE OF INTERJUDGE RELIABILITY

Pair Name	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5
A	0	1	0	0	0
B	0	0	0	0	0
C	0	0	0	0	0
D	1	1	1	0	0
AL1	3	5	4	3	3
AL2	0	0	0	0	0
AL3	1	2	0	1	1
AL4	0	0	0	0	0
ZN5	0	1	0	0	0
ZN6	0	0	0	0	0
ZN7	4	3	5	4	3
ZN8	0	0	1	0	0
PF9	3	5	3	4	3
PF10	0	2	1	0	1
PF11	0	0	0	1	1
PF12	0	0	0	0	0

$r_5 = 0.97$

TABLE 9

JUDGMENTS OF AGGRESSIVE ATTACKS ON DAY 4 WITH AN ESTIMATE OF INTERJUDGE RELIABILITY

Pair Name	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5
A	0	1	0	2	0
B	0	0	0	0	0
C	0	3	2	1	0
D	0	0	0	0	0
AL1	10	11	8	10	5
AL2	0	0	0	0	0
AL3	1	0	0	1	0
AL4	0	0	0	1	2
ZN5	7	11	13	9	8
ZN6	7	12	10	10	10
ZN7	0	3	0	1	0
ZN8	0	1	1	1	0
PF9	23	23	23	22	23
PF10	3	3	2	3	2
PF11	0	0	0	0	0
PF12	0	0	0	0	0

$r_5 = 0.99$

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