12-1-1974

# Behavioral Effects of Postnatal Zinc Deficiency and Kryptopyrrole in the Rat

Peter C. Peterson

# BEHAVIORAL EFFECTS OF POSTNATAL ZINC DEFICIENCY AND KRYPTOPYRROLE IN THE RAT

by
Peter C. Peterson

Bachelor of Arts, Huron 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 Peter C. Peterson 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 Commitee under whom the work has been done.

Edward S. Helas
(Chairman)

Harold W. Sandstead

James R. Antes

A. William Johnson
Dean of the Graduate School

## Permission

Title BEHAVIORAL EFFECTS OF POSTNATAL ZINC DEFICIENCY AND KRYPTOPYRROLE IN THE RAT

Department Psychology

Degree Master of Arts

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the Library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my thesis work or, in his absence, by the Chairman of the Department or the Dean of the Graduate School. It is understood that any copying or publication or other use of this thesis or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my thesis.

Signature Peter C Peterson

Date December 6, 1974

ACKNOWLEDGEMENTS

I would like to acknowledge the cooperation and help of the members of my thesis committee: Dr. Ed Halas, Dr. James Antes, and Dr. Harold Sandstead. I would like to give special thanks to Drs. Halas and Sandstead for allowing me the use of the facilities at the U.S.D.A. Human Nutrition laboratory, and to Orris Johnson, Mike Rowe, and my wife, Carrie. I would also like to acknowledge the help of Dr. Ralph Kolstoe.

DEDICATION

to Carrie

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Zinc deficiency has been shown to have a wide range of behavioral effects in the rat. The experiments presented below were conducted to determine the effects of postnatal zinc deficiency (birth-21 days) on avoidance learning and on measures of emotionality. Kryptopyrrole, a substance which has been shown to produce "catatonic" freezing behavior and atypical electrical activity in rat brains, was administered to half of the Ss to study possible zinc deficiency-kryptopyrrole interactions.

Dams were made zinc deficient from the day of delivery until day 21 on a diet contaning less than 1 ppm zinc. Two dietary control groups were used: a pair fed group which were fed an amount equivalent to that consumed by their zinc deficient counterparts and an ad libitum group allowed free access to food. 12 Ss were assigned to each dietary condition. Within each dietary condition 6 Ss were injected with 1 microliter of kryptopyrrole per 100g body weight; 6 Ss within each dietary condition served as saline injected controls. Behavioral testing was begun when the animals were 54-55 days of age.

Experiment I was an open field test of emotionality in which the Ss were not differentiated as to injection group. Data recorded were the number of defecation responses and number of squares entered per trial. Experiment I showed generally that the zinc deficient were not significantly more fearful of the open field. In fact, the ad libitum group ranked first in the number of defecation responses.

Experiment II was a two-way avoidance conditioning experiment consisting of 3 days of CS habituation, 5 days avoidance acquisition, 3 days extinction, and 5 days of reacquisition. The number of conditioned responses (CRs), number of intertrial responses (ITRs), and response latency were recorded. There were no significant differences with respect to CRs in all conditioning phases except in extinction in which the zinc deficient Ss exhibited slower extinction. The most clear difference was the extremely high rate of ITR responding shown by the zinc deficient group in avoidance acquisition, extinction, and reacquisition. Also observed were highly significant trials x nutrition x injection interactions in extinction and reacquisition in the number of ITRs. There were no significant differences with respect to response latencies except during extinction in which the zinc deficient group exhibited shorter latencies. The injection variable had no significant effect at the injection levels used.

Experiment III was a second open field experiment in which the Ss were differentiated with respect to injection condition, although they received no injections during the experiment. The zinc deficient Ss exhibited "hyperactivity" with repeated exposures to the open field.

# INTRODUCTION

Reasearch has shown the trace element zinc to be an essential nutrient for growth and development of animals. If animals are made zinc deficient during critical periods of development various deficits result. Sandstead, Gillespie, and Brady (1972) have shown that the molecular composition of the brain of the suckling rat is altered with zinc deficiency. If the chemical composition and micro-structure of the brain is altered, it is reasonable to assume these changes will be reflected in behavior. This study attempts to replicate previous zinc deficiency-avoidance learning findings, and will attempt to determine the effects of postnatal zinc deficiency on emotional behavior.

Kryptopyrrole has been shown to have toxic and psychoactive effects in experimental animals. Urinary excretion of kryptoprrole has been reported in both psychiatric and normal human populations. Experiments presented below will attempt to determine the effects of both postnatal zinc deficiency (days 1-20) and kryptopyrrole injection on the behavior of the rat. Two behavioral techniques--the open field test of emotionality and a two-way avoidance learning task--will be used to assess the effects of these two variables.

# CHAPTER I

## PART I: LITERATURE SURVEY ON ZINC DEFICIENCY

The importance of trace elements in plant and animal physiology has been recognized for over 100 years (Underwood, 1971). In the past two decades the essential nature of a variety of trace elements has become increasingly apparent. This literature survey will focus on one of these trace elements--zinc. The effects of zinc deficiency on behavior will be emphasized.

Biochemically, zinc plays an important role in many life processes. Although the exact role of zinc in these processes is largely unknown, experimentally produced zinc deficiencies have done much to further our knowledge. Zinc deficiency interferes with normal nucleic acid and protein synthesis (Winder & Denneny, 1959; Sandstead & Rinaldi, 1969; Williams & Chesters, 1970; Sandstead et al., 1972). The retardation of growth commonly associated with zinc deficiency may be due to impairment of RNA synthesis (Schneider & Price, 1962). Zinc has many important enzyme functions, in fact, many enzymes are zinc "metalloenzymes" which are found in many organ systems (Prasad, 1969). Experimentally produced zinc deficiency causes a reduction of the activities of many of these enzymes (Prasad, Oberleas, Wolf, & Horwitz, 1967; Terhune & Sandstead, 1972).

Certain signs and symptoms have been repeatedly observed in zinc deficient rats and mice including growth retardation, hair loss, hyper-

kertosis, parakeratosis, lethargy, and anorexia (Todd, Elvehjem, & Hart, 1934; Day & McCollum, 1940; Follis, Day, & McCollum, 1941; Day & Skidmore, 1947). Zinc deficiency drastically alters food intake and feeding patterns in rats. Forced feeding of anorexic zinc deficient rats induced lethargy, ataxia, abdominal distention, diarrhea, darkened urine, and a red pigment on nose and paws. Forced feeding of control animals showed none of these symptoms (Chesters & Quarterman, 1970). Zinc deficient Ss in the above study were able to discriminate between test diets containing 1 and 6 ppm zinc. Zinc deficiency has been observed in other species including chickens (O'Dell, Newberne, & Savage, 1958; Kienholz, Turk, Sande, & Hoekstra, 1961), sheep (Ott, Smith, Stob, & Beeson, 1964), cattle (Miller & Miller, 1962), pigs (Tucker & Salmon, 1955), squirrel monkeys (Macapinlac, Barney, Pearson, & Darby, 1967), and man (Prasad, Halsted, & Nadimi, 1961; Prasad, Miale, Farid, Sandstead, & Schulert, 1963; Prasad, Miale, Farid, Sandstead, Schulert, & Darby, 1963; Sandstead, Prasad, Farid, Miale, Bassilly, & Darby, 1967; Ronaghy, SpiveyFox, Garn, Israel, Harp, Moe, & Halsted, 1969; Prasad & Oberleas, 1970). Testicular atrophy in the male and sexual dysfunction and disruption of the estrous cycle in the female have also been associated with zinc deficiency (Hurley & Swenerton, 1966; Luecke, Olman, & Baltzer, 1968; Hurley, 1969; Whitenack, Luecke, & Whitehair, 1970).

In man the manifestations of zinc deficiency are growth retardation and hypogonadism. Associated manifestations observed in Iranian and Egyptian males which are not the direct result of zinc deficiency include: iron deficiency anemia, hepatosplenomegaly, and in some cases (Iranian), geophagia (Prasad et al., 1961; 1963a; 1963b; Prasad & Oberleas, 1970).

Zinc loss through perspiration in the tropical climate, schistomiasis or hookworm (which ingest erythrocytes which have high zinc content), and a vegetable diet high in phytate (phytate interferes with zinc absorption), were the probable etiological factors in the syndrome. A reversal of some symptoms, especially retarded sexual development, was accomplished with zinc supplementation (Sandstead et al., 1967; Ronaghy et al., 1969).

Although epidemiological data is scant, Sever and Emanuel (1973), have suggested a possible relationship between human congenital malformations of the central nervous system and maternal zinc deficiency. Human zinc deficiencies have been observed in Middle East ares which have relatively high rates of central nervous system malformations.

Zinc deficiency has an adverse effect on rapidly developing embryonic tissues and is important in the development of both the fetal and the neonatal brain. The teratogenic effects of zinc deficiency vary depending upon which organ systems are undergoing rapid development at the time the zinc deficient diet is administered. Such gross abnormalities as hydrocephalus, anencephalus, hydranencephalus, and exencephalus were observed in rat fetuses which were zinc deficient during days 0-21 of gestation (Hurley, Gowan, & Swenerton, 1971). Rats made zinc deficient during gestation gave birth to a very high percentage of congenitally malformed young. These malformations were observed in all organ systems (Hurley & Swenerton, 1966; Hurley, 1969; Hurley & Mutch, 1973). Zinc deficiency also has an adverse effect on postnatal development in the rat brain, altering normal nucleic acid and protein synthesis (Sandstead et al., 1972). Newborn mice deprived of colostrum and nursed by foster mothers have developed the symptoms of zinc deficiency (Nishimura, 1953).

Colostrum has a higher concentration of zinc than true milk and is essential in the early neonatal development of the mouse. The zinc concentration of colostrum is 3-5 times greater than true milk in cattle, pigs, and humans (reported in Underwood, 1971).

Zinc deficiency has stressful effects on parturition in the rat (Apgar, 1968a; 1968b; 1972). Rats placed on a zinc deficient diet beginning day 1 of gestation had difficult labor of long duration marked by excessive bleeding. Zinc deficient dams appeared listless, lethargic, and spent much time motionless. At birth zinc deficient dams failed to retrieve their pups and exhibited no specific nesting pattern, leaving pups randomly strewn about the cage. Caldwell and Oberleas (1969) have also observed this lack of normal maternal behavior.

Caldwell and Oberleas (1969) conducted the first study of the effects of zinc deficiency on learning in the rat. Ss were fed a diet containing 10-14 ppm zinc in which the zinc was made unavailable by soy protein with phytate added. The diet was administered 10 weeks before breeding and continued through gestation and lactation until day 21 postpartum. The pups were then fed a diet containing 70 ppm zinc. At 45 days the pups were subjected to behavioral testing. The zinc deficient Ss showed poorer performance than control Ss which had received adequate amounts of zinc (70 ppm). The zinc deficient Ss had longer latencies in the Lashley III water maze (a test for diffuse brain damage in the rat). In a one-way conditioned avoidance task the zinc deficient Ss showed significantly longer response latencies ($p<0.05$) and made significantly fewer conditioned avoidance responses ($p<0.001$). Zinc deficient Ss exhibited more emotional behavior in the open field test with significantly lower ambulation scores ($p<0.05$).

Experiments were also conducted in the above study to test the effects of post-weanling zinc deficiency on behavior. Ss were made zinc deficient on a diet containing 8 ppm zinc from days 30-78 and compared to controls receiving 70 ppm zinc. Zinc deficient Ss had significantly longer latencies in the Lashley III water maze ($p<0.025$). In the one-way avoidance learning task zinc deficient Ss had significantly longer response latencies ($p<0.001$) and made significantly fewer conditioned avoidance responses ($p<0.001$). Again, zinc deficient Ss exhibited more emotional behavior in the open field, with significantly lower ambulation scores ($p<0.001$).

Another post-weanling study (Caldwell, Oberleas, Clancy, & Prasad, 1970) in which male rats were made zinc deficient (8 ppm zinc) from days 30-78, and throughout 2 weeks of behavioral testing, again showed poorer performance by the zinc deficient Ss. Zinc deficient Ss made significantly more cul-de-sac and retrace errors in the Lashley III water maze ($p<0.001$) and had significantly longer latencies ($p<0.005$). In the one-way avoidance task zinc deficient Ss exhibited longer response latencies although the difference did not reach the 0.05 level of significance ($0.1>p>0.05$). Caldwell et al., separately analyzed latencies on trials 1-2 and this comparison showed a significant difference ($0.05>p>0.025$), with zinc deficient Ss exhibiting longer latencies. Zinc deficient Ss also made significantly fewer conditioned avoidance responses ($p<0.001$). It is unclear whether poorer performance by the zinc deficient Ss was due to a motivational deficit or an innate learning deficit because the animals were zinc deficient at the time of testing and were quite lethargic. Open field testing again showed zinc deficient Ss to be more emotional with significantly lower ambulation scores ($p<0.05$). This difference in emotionality was highly significant on day 1 op open field testing

($0.01 > p > 0.001$).

In a study of prenatal and postnatal deficiency by Caldwell, Oberleas, and Prasad (1973) the offspring of three generations of chronic mildly zinc deficient dams were subjected to behavioral testing. The dams were placed on a zinc deficient diet containing 15 ppm zinc beginning at age 3.5 weeks which was continued through breeding at 14 weeks and then through three successive generations of pups. The offspring were fed adequate amounts of zinc after weaning. The offspring of the zinc deficient dams showed no significant differences in performance in the Lashley III water maze when compared to control animals. The zinc deficient Ss had significantly longer response latencies in the one-way avoidance conditioning task ($p < 0.005$), and made fewer conditioned responses, although the difference was not significant.

Studies by Lokken, (1973) and Lokken, Halas, and Sandstead (1973) of both prenatal and postnatal zinc deficiency again showed poorer performance by zinc deficient Ss. In the prenatal experiment dams were made deficient (less than 1 ppm zinc) from days 14 through 19 of gestation and then rehabilitated with adequate amounts of zinc. The male offspring were tested on the alley Tolman-Honzik maze at age 50 days. Compared to a pair-fed and ad libitum control group, the zinc deficient Ss made more full body and total errors ($p=0.06$). There was no significant difference between the zinc deficient Ss and controls in traversing time.

In the postnatal experiment from the above study Ss were made zinc deficient (less than 1 ppm zinc) from birth through day 21 and then rehabilitated with a diet containing adequate zinc. The zinc deficient male Ss were tested at age 44 days in an elevated Tolman-Honzik maze and compared to a pair-fed and ad libitum control groups. The previously

zinc deficient Ss made significantly more full-body errors ($p<0.01$), and significantly more total errors ($p<0.01$), than the two control groups. The zinc deficient Ss had longer traversing times but this difference was not statistically significant.

Another study of prenatal deficiency (Rowe, 1974) in which dams were made zinc deficient days 14-19 of gestation, showed zinc deficient Ss to be inferior on physiological measures but not on two learning tasks. Zinc deficient female Ss exhibited better performance in the alley Tolman-Honzik maze and in a two-way avoidance conditioning task. These differences, however, were not statistically significant when compared to a pair-fed and ad libitum control group.

A prenatal deficiency study by Halas and Sandstead (1974) has shown that zinc deficient male rats may have an innate deficit in stress tolerance. Their Ss were made zinc deficient (less than 1 ppm zinc) from days 15-20 of gestation and then rehabilitated. The pups were tested at age 60 days in a two-way avoidance conditioning task. The previously zinc deficient Ss made significantly fewer conditioned avoidance responses than either a pair-fed or an ad libitum control group ($p<0.001$). The zinc deficient Ss exhibited a response decrement in which they made fewer conditioned responses over repeated trials after an initial peak in responding. This was interpreted as a lack of stress tolerance in the zinc deficient Ss. Zinc deficient Ss exhibited quicker extinction of conditioned avoidance responding than either of the two control groups ($p<0.001$). The zinc deficient Ss had significantly longer response latencies ($p<0.001$), and were less active than controls, exhibiting fewer intertrial responses ($p<0.001$).

A prenatal study by Halas, Rowe, Johnson, McKenzie, and Sandstead (1974) has shown that the behavior of males may be more adversely affected

than the behavior of females. Sex comparisons in a two-way avoidance task were made on male and female littermates. The dams of the experimental offspring were made zinc deficient from days 14 to 20 of gestation and then rehabilited. An analysis of the females' performance in the avoidance task showed nonsignificant differences among the zinc deficient, pair-fed, and ad libitum groups with respect to number of CRs, ITRs, and response latencies. The zinc deficient male littermates, however, showed impaired performance. An analysis of variance showed a significant difference among the three nutrition treatment groups with respect to number of CRs ($p<0.05$). The zinc deficient males exhibited significantly fewer ($p<0.001$) CRs than their pair-fed controls. There were nonsignicant differences between the zinc deficient and ad libitum groups and between the pair-fed and ad libitum groups. The zinc deficient males showed slower response latencies, however, this difference was not significant. The ad libitum group exhibited more ITRs but the difference among the three nutrition treatment groups was not significant. Male Ss were run on the Tolman-Honzik maze, but no significant differences were observed between the nutrition groups.

Statistical comparisons of males and females within the nutrition treatment groups in the above study showed that the zinc deficient males made significantly fewer CRs ($p<0.001$) and significantly fewer ITRs than their female littermates. The difference in response latencies within the zinc deficient group was not significant, but the males showed slower responding. Sex differences within the pair-fed and ad libitum groups were not significant with respect to CRs, ITRs, or response latencies.

## PART II: LITERATURE SURVEY ON KRYPTOPYRROLE

2,4-dimethyl-3-ethylpyrrole (kryptopyrrole) is one of the many substances which have been detected in the urine of psychiatric patients (Weil-Malherbe & Szara, 1971). In 1961 a urine factor was discovered in some schizophrenics which reacted with Ehrlich's reagent to produce a pink-mauve color (Irvine, 1961). This urine factor was subsequently named the "mauve factor," and its presence aroused much controversy. Some went so far as to designate a new psychiatric disease to those who excreted the mauve factor (Hoffer & Osmond, 1963). The mauve factor was later identified as kryptopyrrole, and was present in the urine of normal volunteers who had ingested LSD (Irvine, Bayne, Miyashiata, & Majer, 1969). The finding that the mauve factor was indeed kryptopyrrole was confirmed by Sohler, Beck, and Noval (1970).

Kryptopyrrole is a substance related to bile pigments and other porphyrins. The metabolic origin of kryptopyrrole is unknown. Russell (1972) has speculated that kryptopyrrole may be a product of abnormal porphyrin metabolism. Huszak, Durko, and Karsai (1972) have also speculated that urinary kryptopyrrole may result from abnormal porphyrin metabolism. Irvine and Wetterberg (1972) have reported a "kryptopyrrole-like" substance in the urine of patients with acute intermittent porphyria and suggest that it may be a causal factor in the neuropsychiatric symptoms associated with the disease. Ellman, Jones, and Rychert (1968) have suggested that kryptopyrrole may be a phenothiazine metabolite, since many patients who excrete kryptopyrrole also receive phenothiazine treatment. It has also been suggested that urinary kryptopyrrole may be derived from bile pigments through the action of intestinal bacteria (Weil-Malherbe & Szara, 1971).

Kryptopyrrole (mauve factor) has been found in a high percentage of psychotic patients. Incidence as high as 66% in schizophrenics has been reported (Ellman et al., 1968), in contrast to 9% of other mentally ill, and 8% of normals in a sample of 91 Ss. A study by O'Reilly, Ernest, and Hughes (1965) sampled 850 consecutive hospital admissions and found a positive mauve spot in 52% of schizophrenics, 47% of affective disorders, 37% of alcoholics, 32% of neurotics, 29% of organic states, 27% of character disorders, 25% of disturbed children, 12% of normal children and adults, and 50% of cancer patients!

Whatever the relationship of kryptopyrrole to psychosis, it does seem to be a behavorally active and toxic substance. Many pyrrolic substances have depressant effects on the central nervous system of rats and mice (Moffett, 1968). Depressant effects of kryptopyrrole on the rabbit brain have been observed after intravenous injection (Sohler et al., 1970). The intraperitoneal administration of 10-25 microliters/kg kryptopyrrole to rats induced catalepsy, ataxia, locomotor depression, and extreme startle reactions (Walker, 1974). Rats with chronically implanted electrodes in the Walker study showed EEG alterations including voltage reductions, periods of high voltage hypersynchronous waves, and "spike and dome" activity. Unusual postures and long periods of freezing behavior were also noted.

Kryptopyrrole has general toxic, behavioral, hypnotic, and hypothermic effects in mice (Wetterberg, 1973). At doses of 0.5 mmole/kg all Ss exhibited ataxia within 30 minutes and behavioral sleep within 120 minutes after intraperitoneal injection. Ss receiving 2-4 mmole/kg did not respond to high intensity stimulation such as tail pinching 120 minutes after injection. Kryptopyrrole decreased body temperature as much as 6 degrees centigrade within 60 minutes after injection of 2

mmole/kg. Kryptopyrrole may also have analgesic effects. Escape responses from a hot plate apparatus were reduced in number after injection of the substance. However, it is unclear whether this was due to ataxia or analgesic effects. Following the Wetterberg experiments 50% of the Ss injected with 1.0 mmole/kg and all Ss injected with higher doses were dead within 24 hours.

The present study will focus on the effects of postnatal zinc deficiency and rehabilitation on two behavioral measures--the open field test of emotionality and a two-way avoidance learning task. The first experiment deals with the effects of zinc deficiency on open field behavior. Possible zinc deficiency-kryptopyrrole interactions are studied with the avoidance learning task (experiment II), and more long term interaction effects of these variables are studied using the open field in experiment III.

# CHAPTER II

## METHODOLOGY

Sixteen timed pregnant rats of the Sprague-Dawley strain were received from Thorp Industries of White Bear Lake, Minnesota. The twelve dams that gave birth were randomly assigned to three nutrition treatment groups with four dams per group. The litter size of each dam was reduced to nine pups on the second day postpartum. Beginning on the day of birth and continuing until day 20 all Ss were fed a sprayed egg white diet (Luecke, Olman, & Baltzer, 1968) which contained less than 1 ppm zinc (Table 1). An ad libitum and pair-fed group received the experimental diet with zinc supplemented water (50 ppm in the form of zinc chloride). The zinc deficient group received the experimental diet and glass distilled water only. The pair-fed group were fed an equivalent amount of food consumed by the zinc deficient group on the previous day. On day 21 all three groups were taken off the experimental diet and fed Purina Lab Chow and tap water in unlimited quantities.

The lactating rat usually produces milk high in zinc (13-17 micrograms/ml). The zinc content of deficient dams' milk has been measured at 8.4 micrograms/ml on day 14 of lactation (Mutch & Hurley, 1974). Mutch and Hurley report that zinc deficient pups receive about 1/2 the total amount of milk zinc compared to controls on the basis of body weight.

The dams were housed in plastic cages with plastic bottoms. These

TABLE I

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 (Kl) | 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 |

[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.

were kept in a room with constant temperature (76°F) and automatic 12 hour on-off lighting. On day 24 the pups were weaned and placed in plastic cages, and on day 40 were segregated by sex and kept in stainless steel cages. The dams' weights were recorded every four days from birth through day 20. Pup weights were recorded every four days from birth through day 40.

## Experiment I

### Open field

The open field apparatus consisted of a white wooden box 30 inches square and 23½ inches deep. The bottom was marked off in 25-six inch squares with black borders. Nine squares were defined as central and 16 defined as peripheral. Light was provided by a 40 watt bulb suspended 31½ inches from the surface of the open field. At 54-55 days of age 12 male pups were randomly selected from each nutrition treatment group. The open field experiment consisted of 5 consecutive daily 5 minute trials per animal. The number of central and peripheral squares entered and the number of defecations were recorded for each trial. Animals were run in random order at approximately the same time each day.

## Experiment II

### Avoidance conditioning

Apparatus.--The Ss received avoidance conditioning trials in two automatic two-way platform avoidance conditioning chambers (Rat Toggle Floor Shuttle Cage model number 146-04 LeHigh Valley Electronics, 1972). The conditioning chambers were placed inside wooden boxes (116cm x 57cm x 73cm) which were lined with foam rubber sound insulation and ventilated with intake and exhaust fans. The boxes provided a light-proof and

sound-proof environment which kept extraneous stimuli at a minimum.

The conditioned stimulus consisted of 2900 Hz four pulse per second tone (Sonalert model SC628) and a 28 volt-.1 amp light which were presented simultaneously. One light bulb was located on each end of the conditioning chamber. A BRS Foringer 5-digit counter recorded the number of conditioned responses, the latency of response, and the number of intertrial responses.

Injection condition.--The 12 Ss within each nutrition group were matched by weight and 6 Ss of each group received an intraperitoneal injection of either 2,4-dimethyl-3-ethylpyrrole (Kryptopyrrole)[1] or an equivalent amount of 0.85% normal saline. Injections were made with a 50 micro-liter syringe (Hamilton Gas-tight #1305-LT). The experimenter wore rubber gloves when making injections. The dosages were administered 60 minutes before each conditioning trial. Table 2 shows the dosage used per body weight of the Ss. All dosages were increased by 1 microliter on days 4 and 8 because of habituation effects.

Conditioning parameters.--Each conditioning trial consisted of the presentation of the conditioned stimulus (CS) with the light turned on in the side of the conditioning chamber which received the unconditioned stimulus (UCS). The UCS was a grid shock of 0.8 milliamperes of scrambled direct current. The CS was presented 5 seconds prior to the UCS and was automatically discontinued if the Ss made a conditioned avoidance response (CR) or an escape response. If the S failed to avoid or escape the UCS was terminated after 15 seconds. A conditioned response consisted of an avoidance of the UCS by jumping over an 8cm high barrier to the end of the chamber. The barrier was electrified with a load of 45 volts

[1]Obtained from Aldrich Chemical CO., Inc., Milwaukee, Wisconsin

TABLE 2

INJECTION CONDITIONS

| Body weight (in grams) | Dosage (in microliter) | | |
|---|---|---|---|
| Conditioning days | 1-3 | 4-7 | 8-16 |
| 125-200 | 1.5 | 2.0 | 2.5 |
| 201-250 | 2.0 | 2.5 | 3.0 |
| 251-300 | 2.5 | 3.0 | 3.5 |
| 301-350 | 3.0 | 3.5 | 4.0 |
| 351-400 | 3.5 | 4.0 | 4.5 |
| 401-450 | 4.0 | 4.5 | 5.0 |

(A.C.) beginning on day 2 of avoidance acquisition to eliminate unauthorized escape behavior. An intertrial interval of 45 seconds separated the termination of either the CS or UCS and the succeeding CS presentation. Ss were counterbalanced in the two conditioning chambers; kryptopyrrole injected Ss and saline injected Ss were run concurrently.

Avoidance conditioning procedures

CS Habituation.--At the age of 59-60 days the Ss were given 3 consecutive days of CS habituation consisting of 25 trials per day.

Avoidance Acquisition.--Following CS habituation the Ss were given 5 consecutive days of avoidance conditioning training consisting of 25 trials per day.

Extinction.--Ss were run for 3 consecutive days of extinction consisting of 25 trials per day.

Reacquisition.--Ss were given 5 more consecutive days of avoidance conditioning training consisting of 25 trials per day.

## Experiment III

Immediately following the 16 day avoidance conditioning experiment, at the age of 75-76 days, the Ss were again exposed to the open field. Using the same apparatus as in experiment I, the Ss were again given 5 consecutive days of one 5 minute trial per day. Although the Ss were now differentiated with respect to injection condition, they received no injections in experiment III. This was done in order to study long-term behavioral effects of the kryptopyrrole, if any.

Interexperimental conditions

Between the avoidance conditioning and open field experiments animals were housed singly in stainless steel cages. All Ss received un-

limited Purina Lab Chow and tap water except during the 60 minute interval between injections and the avoidance conditioning trials.

## Statistical Methods

In experiment I and III (open-field) complex analyses of variance with repeated measures over trials were computed for the number of total, central, and peripheral squares entered. An additional analysis was used to test for significant differences in proportions of central to peripheral square entries between the three nutrition groups. A standard error of the differences in proportions was determined using the method presented in Guilford (1965). This statistical test was used to determine if the nutrition treatment groups differed significantly with respect to the proportion of central square entries in the open-field. $X^2$ tests were computed to test for significant differences in the number of defecation responses among the groups.

Experiment II latency, CR, and ITR data were analyzed with complex analyses of variance with repeated measures over trials. Internal comparisons of significant F ratios in experiments I, II, and III were done using the Newman-Keuls procedure (Winer, 1971).

# CHAPTER III

# RESULTS

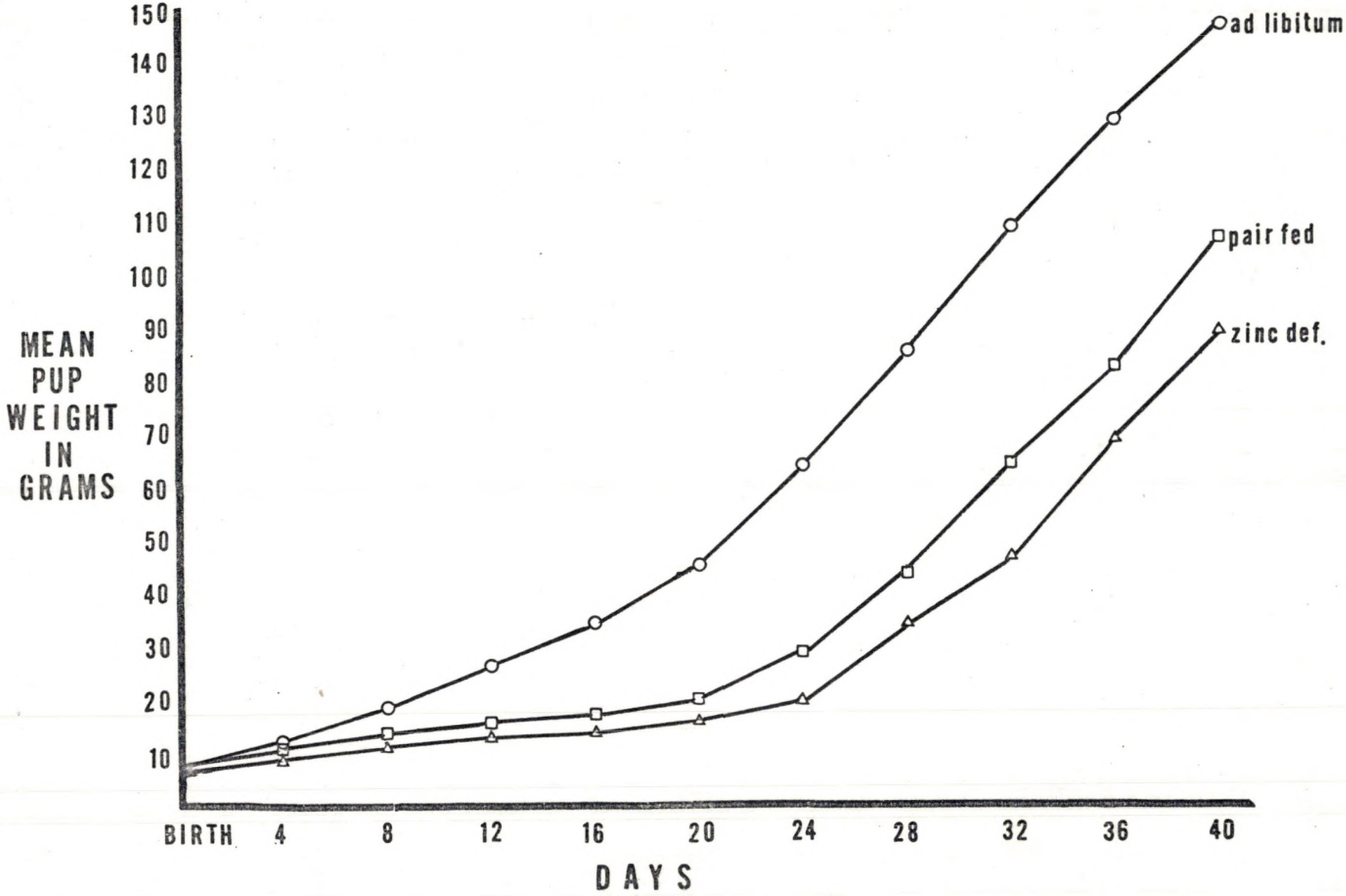
## Subject data

The zinc deficient pups had consistently lower mean body weights than either the pair-fed or ad libitum pups. The experimental zinc deficiency had an adverse effect on pup growth rate, but when the zinc deficient diet was stopped the pups exhibited a growth rate similar to the two control groups (Figure 1). At age 40 days the zinc deficient pups had a mean weight of 89.50g in contrast to 106.93g for the pair-fed group and 146.75g for the ad libitum group (Table 3). Dam weights and litter sizes are shown in table 23 of the appendix.

## Behavioral Results

The behavioral results are presented in two parts; open-field results (experiment I, III) and avoidance conditioning results (experiment II). The open-field data were of two types; number of defecations and number of squares (total, central, and peripheral) entered. Data analyzed in avoidance conditioning included number of conditioned responses, response latencies, and number of intertrial responses. Since experiments I and III both deal with the open field they will be presented together.

Experiment I.--There were highly significant differences among the


MEAN PUP WEIGHT IN GRAMS
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
BIRTH
4
8
12
16
20
24
28
32
36
40
DAYS
ad libitum
pair fed
zinc def.

nutrition groups in the number of defecation responses in the open-field (Figure 2). An analysis of day 1 of open-field testing showed a highly significant overall $X^2$ for the difference among nutrition groups ($X^2$=18.85, $p<0.001$, d.f.=2). The ad libitum group ranked first, making the most defecation responses, followed by the zinc deficient and pair-fed groups, respectively. These rankings remained the same over the total five days of open-field testing. A $X^2$ of the total number of defecation responses for the five days was highly significant ($X^2$=26.93, $p<0.001$. d.f.=2).

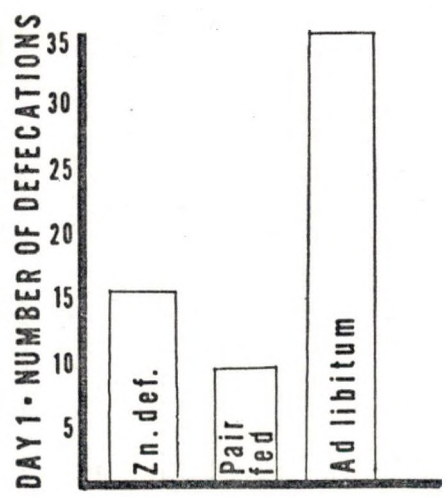
A two way analysis of variance (nutrition and repeated measures over trials) for number of squares entered showed no significant differences between nutrition groups over the five trials. This was true for number of total (Table 4), central (Table 5), and peripheral (Table 6), square entries (Figure 3).

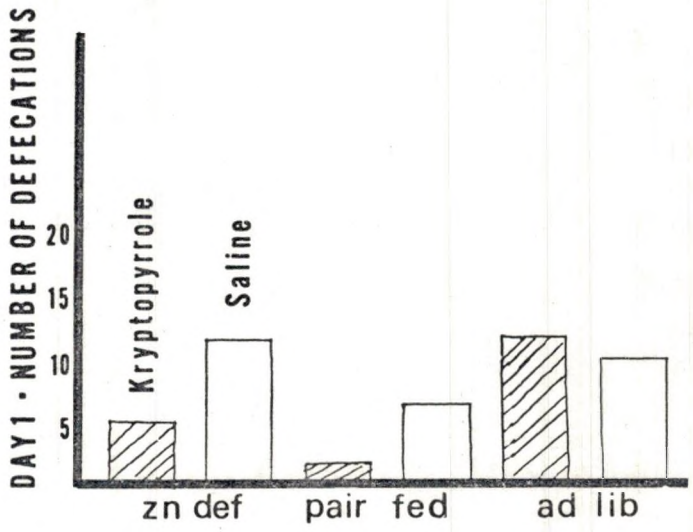
TABLE 4

ANALYSIS OF VARIANCE FOR TOTAL NUMBER OF SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT I)

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 1638.12 | <1.00 |
| Error between | 33 | 2487.23 | |
| Within Ss | | | |
| Trials | 4 | 3375.47 | 4.90* |
| Trials by nutrition | 8 | 476.09 | <1.00 |
| Error within | 132 | 689.00 | |

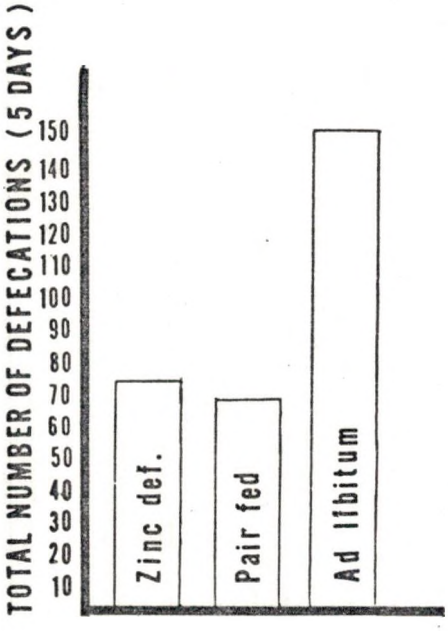
*$p<0.001$




NUMBER OF DEFECATIONS
EXPERIMENT ONE

NUMBER OF DEFECATIONS
EXPERIMENT THREE



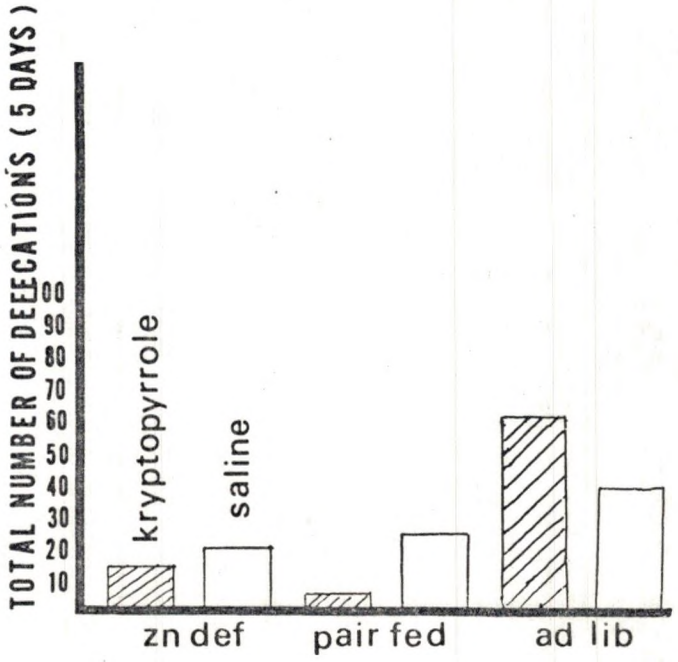



TABLE 5

ANALYSIS OF VARIANCE FOR NUMBER OF CENTER SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT I)

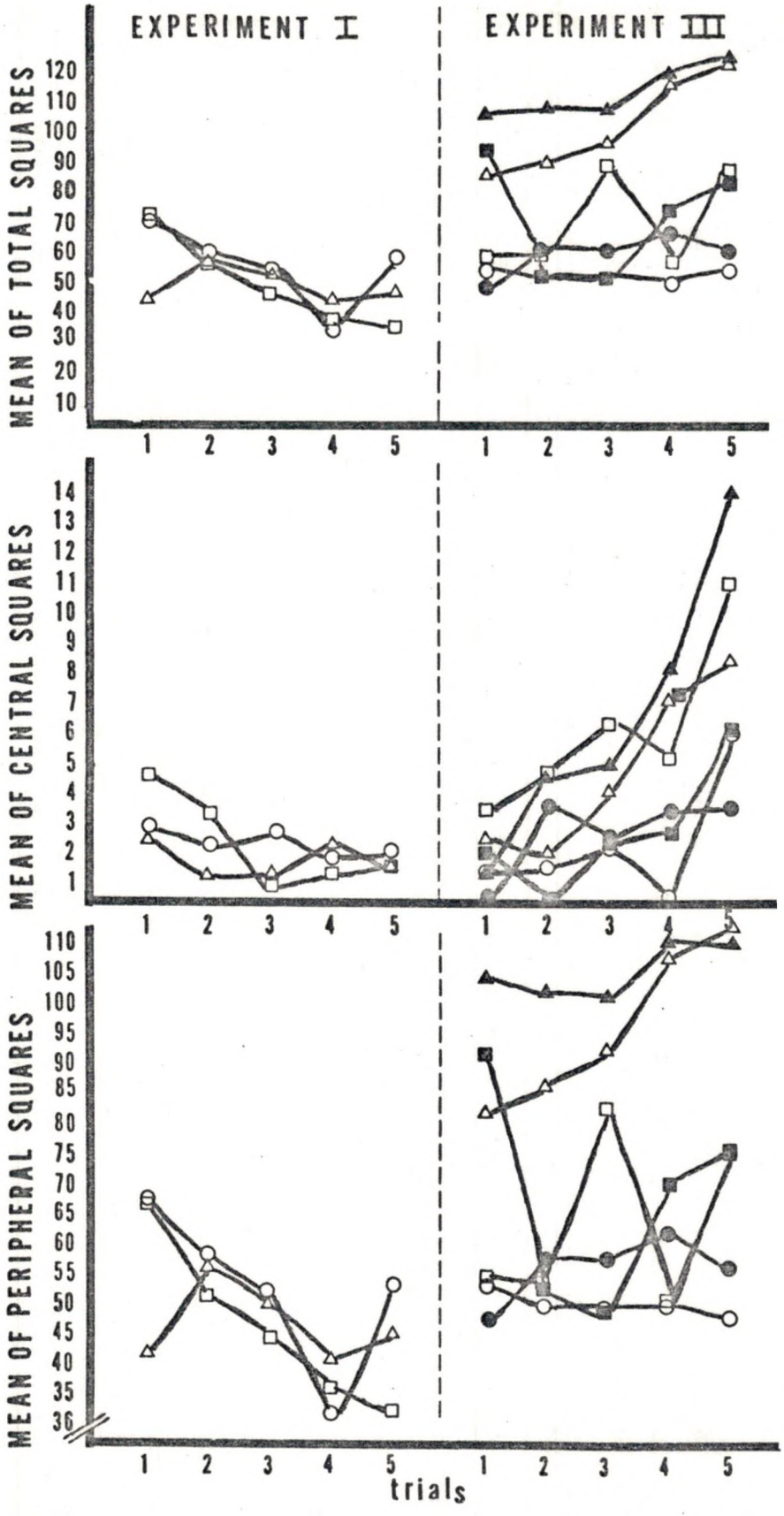
| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 5.71 | 1.28 |
| Error between | 33 | 4.48 | |
| Within Ss | | | |
| Trials | 4 | 18.02 | 2.18 |
| Trials by nutrition | 8 | 10.07 | 1.22 |
| Error within | 132 | 8.26 | |

TABLE 6

ANALYSIS OF VARIANCE FOR NUMBER OF PERIPHERAL SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT I)

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 547.07 | <1.00 |
| Error between | 33 | 2327.35 | |
| Within Ss | | | |
| Trials | 4 | 2995.20 | 5.10* |
| Trials by nutrition | 8 | 998.21 | <1.00 |
| Error within | 132 | | |

*$p < 0.001$


EXPERIMENT I
EXPERIMENT III
MEAN OF TOTAL SQUARES
MEAN OF CENTRAL SQUARES
MEAN OF PERIPHERAL SQUARES
trials
SQUARES ENTERED IN THE OPEN FIELD
fig. 3a
fig. 3b
fig. 3c
zn def
pair fed
ad lib
krypt
zn def
pair fed
ad lib
saline

The trials main effect for total and peripheral square entries was highly significant ($p<0.001$, Tables 4 & 6). t-test comparisons of the mean number of total entries on day 1 of open field testing showed the zinc deficient group made fewer total entries than either the pair-fed or the ad libitum group. Day 1 activity is thought to be a better measure of emotion than later trials (Caldwell et al., 1970). However, these differences did not reach the 0.05 level of significance ($0.1>p>0.05$, Table 7).

TABLE 7

t-TEST COMPARISONS FOR MEANS OF TOTAL NUMBER OF SQUARE ENTRIES IN THE OPEN FIELD: DAY ONE

| | zinc deficient n=12 | pair-fed n=12 | ad libitum n=12 |
|---|---|---|---|
| $\bar{X}$ | 43.17 | 71.17 | 68.67 |
| S | 31.14 | 43.19 | 32.69 |

| | | |
|---|---|---|
| Zinc deficient vs pair-fed | t = 1.745* | d.f. = 22 |
| Zinc deficient vs ad libitum | t = 1.786* | d.f. = 22 |
| Pair-fed vs ad libitum | t = 0.153 | d.f. = 22 |

*$0.1>p>0.05$

The total proportions (over five days) of central square entries to peripheral square entries for the nutrition treatment groups were as follows: zinc deficient--.033, pair-fed--.045, ad libitum--.040. The difference between proportions of the zinc deficient and the pair-fed groups was significant ($z=2.40$, $p<0.02$), with the zinc deficient Ss exhibiting fewer central square entries. The difference between proportions of the zinc deficient and ad libitum groups was not significant. The difference between proportions of the pair-fed and ad libitum groups

was also not significant.

Experiment III.--Again there were highly significant differences in the number of defecation responses on the open field. A 2x3 chi square (injection by nutrition) showed highly significant differences ($X^2$=23.79, $p<0.001$, d.f.=2). However, the injection treatments contributed little to this large difference since a one-way chi square of injection treatments was not significant, and a one-way chi square of the nutrition treatments was highly significant ($X^2$=76.74, $p<0.001$, d.f.=2, Figure 2).

A three-way analysis of variance (nutrition by injection with repeated measures over trials) showed nonsignificant differences with respect to both the nutrition and injection main effect in the number of total squares entered in the open field (Table 8, Figure 3a). The analysis of variance for number of central squares entered showed nonsignificant

TABLE 8

ANALYSIS OF VARIANCE FOR TOTAL NUMBER OF SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT III)

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 41550.00 | 2.66 |
| Injection conditions | 1 | 16955.61 | 1.09 |
| Nutrition by injection | 2 | 4862.82 | <1.00 |
| Error between | 30 | 15613.81 | |
| Within Ss | | | |
| Trials | 4 | 7721.55 | 2.29 |
| Trials by nutrition | 8 | 3999.90 | 1.19 |
| Trials by injection | 4 | 1379.47 | <1.00 |
| Trials by nutrition by injection | 8 | 2432.51 | <1.00 |
| Error within | 120 | 3371.99 | |

differences among both nutrition and injection main effects. There was a significant difference in number of central squares entered over trials ($p<0.001$, Table 9, Figure 3b).

TABLE 9

ANALYSIS OF VARIANCE FOR NUMBER OF CENTER SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT III)

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 164.77 | 1.60 |
| Injection conditions | 1 | 6.81 | <1.00 |
| Nutrition by injections | 2 | 108.57 | 1.06 |
| Error between | 30 | 102.80 | |
| Within Ss | | | |
| Trials | 4 | 218.68 | 11.66* |
| Trials by nutrition | 8 | 25.43 | 1.36 |
| Trials by injection | 4 | 4.21 | <1.00 |
| Trials by nutrition by injection | 8 | 17.60 | <1.00 |
| Error within | 120 | 18.76 | |

*$p<0.001$

The analysis of variance for number of peripheral squares entered showed a highly significant F ratio for the difference among nutrition treatments ($p<0.001$, Table 10, Figure 3c). A Newman-Keuls internal comparison test (Winer, 1971) showed the zinc deficient group made significantly more peripheral square entries than either the pair-fed group or the ad libitum group ($p<0.01$). The difference between the pair-fed and the ad libitum groups was not significant.

TABLE 10

ANALYSIS OF VARIANCE FOR NUMBER OF PERIPHERAL SQUARES ENTERED IN THE OPEN FIELD (EXPERIMENT III)

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 38182.81 | 9.58* |
| Injection conditions | 1 | 2046.94 | <1.00 |
| Nutrition by injection | 2 | 114.02 | <1.00 |
| Error between | 30 | 3987.67 | |
| Within Ss | | | |
| Trials | 4 | 776.54 | 1.56 |
| Trials by nutrition | 8 | 601.16 | 1.21 |
| Trials by injection | 4 | 702.09 | 1.41 |
| Trials by nutrition by injection | 8 | 938.15 | 1.89 |
| Error within | 120 | 496.59 | |

*$p < 0.001$

The total proportions (over five days) of central to peripheral squares entries for nutrition treatment groups were as follows: zinc deficient--.053, pair-fed--.061, ad libitum--.042. The difference between proportions of the zinc deficient and pair-fed groups was not significant. The difference between proportions of the zinc deficient and the ad libitum groups was significant ($z=2.37$, $p<0.02$). The difference between proportions of the pair-fed and ad libitum groups was highly significant ($z=3.63$, $p<0.0004$).

Experiment II.--A three-way analysis of variance (three nutrition classifications, two injection classifications, and repeated measures over trials) was used in data analysis. The number of conditioned responses (CRs) and the response latencies were blocked into two groups of ten

trials per day. Intertrial responses (ITRs) were blocked into daily groups consisting of 20 trials. The data of the first five trials of each day were discarded because of warm-up effects, such as random "shuttling" (ITR) behavior at the beginning of the first trial block.

Number of Conditioned Responses

CS Habituation.--The three-way analysis of variance showed nonsignificant differences among both nutrition and injection main effects. There was a significant trials effect ($p<0.05$, Table 11, Figure 4). This significant difference was probably due to a higher number of flight responses from the novel CS in the earlier CS habituation trials.

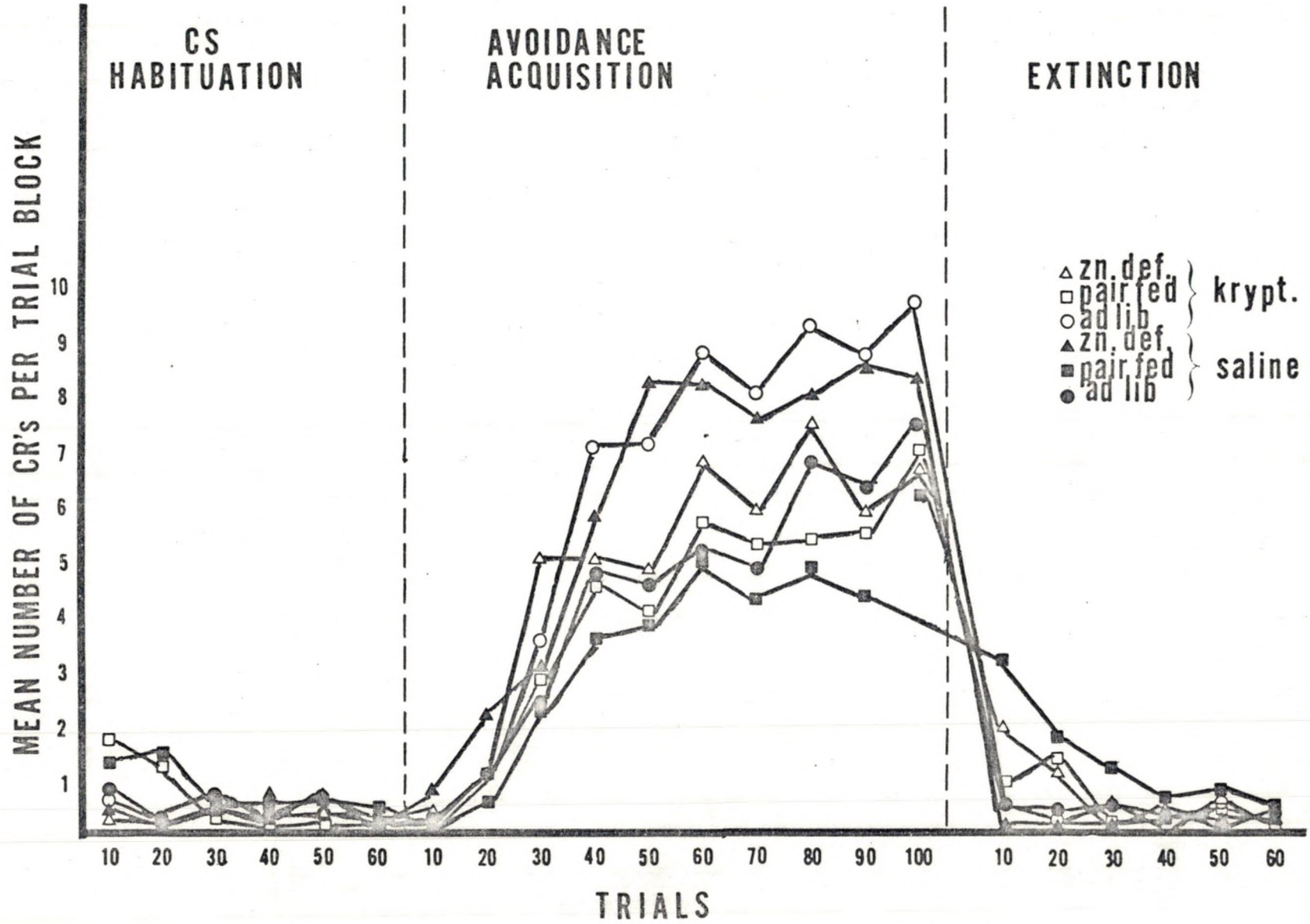
TABLE 11

ANALYSIS OF VARIANCE FOR NUMBER OF CR'S IN CS HABITUATION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 0.51 | <1.00 |
| Injection conditions | 1 | 0.02 | <1.00 |
| Nutrition by injection | 2 | 3.92 | 2.97 |
| Error between | 30 | 1.32 | |
| Within Ss | | | |
| Trials | 5 | 1.97 | 2.49* |
| Trials by nutrition | 10 | 0.58 | <1.00 |
| Trials by injection | 5 | 0.15 | <1.00 |
| Trials by nutrition by injection | 10 | 0.99 | 1.25 |
| Error within | 150 | 0.79 | |

*$p<0.05$

Avoidance Acquisition.--The analysis of variance showed no significant differences with respect to both the nutrition and injection main effects


CS
HABITUATION
AVOIDANCE
ACQUISITION
EXTINCTION
MEAN NUMBER OF CR's PER TRIAL BLOCK
1
2
3
4
5
6
7
8
9
10
zn. def.
pair fed
ad lib
krypt.
zn. def.
pair fed
ad lib
saline
10 20 30 40 50 60 10 20 30 40 50 60 70 80 90 100 10 20 30 40 50 60
TRIALS

in the number of CRs (Table 12, Figure 4). The trials main effect was highly significant ($p<0.001$, Table 12).

TABLE 12

ANALYSIS OF VARIANCE FOR NUMBER OF CR'S IN AVOIDANCE ACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 100.41 | 1.48 |
| Injection conditions | 1 | 65.02 | <1.00 |
| Nutrition by injection | 2 | 59.43 | <1.00 |
| Error between | 30 | 67.64 | |
| Within Ss | | | |
| Trials | 9 | 222.85 | 51.35* |
| Trials by nutrition | 18 | 3.62 | <1.00 |
| Trials by injection | 9 | 4.11 | <1.00 |
| Trials by nutrition by injection | 18 | 3.21 | <1.00 |
| Error within | 270 | 4.34 | |

*$p<0.001$

Extinction.--The analysis of variance showed a significant difference among nutrition conditions in the number of CRs exhibited in extinction ($p<0.01$, Table 13, Figure 4). A Newman-Keuls internal comparison test showed the zinc deficient group made significantly more CRs than either the pair-fed or the ad libitum groups ($p<0.01$). The difference between the pair-fed and ad libitum groups was not significant. The trials effect was highly significant ($p<0.001$). The trial by nutrition interaction was also highly significant ($p<0.001$, Table 13, Figure 5b). The trial by nutrition interaction appears to be due to a "floor" effect with the zinc deficient and ad libitum groups exhibiting a relatively consistent

low rate of CRs and the pair-fed showing a sharp decrease in CRs over trials.

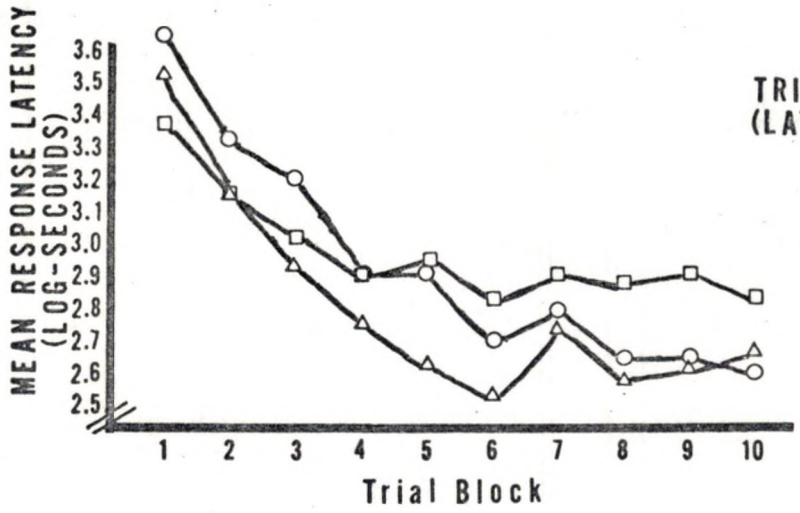
TABLE 13

ANALYSIS OF VARIANCE FOR NUMBER OF CR'S IN EXTINCTION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 50.17 | 7.88* |
| Injection conditions | 1 | 5.67 | <1.00 |
| Nutrition by injection | 2 | 11.70 | 1.84 |
| Error between | 30 | 6.37 | |
| Within Ss | | | |
| Trials | 5 | 17.12 | 10.78** |
| Trials by nutrition | 10 | 8.46 | 5.32** |
| Trials by injection | 5 | 1.40 | <1.00 |
| Trials by nutrition by injection | 10 | 1.85 | 1.16 |
| Error within | 150 | 1.59 | |

*$p < 0.01$
**$p < 0.001$

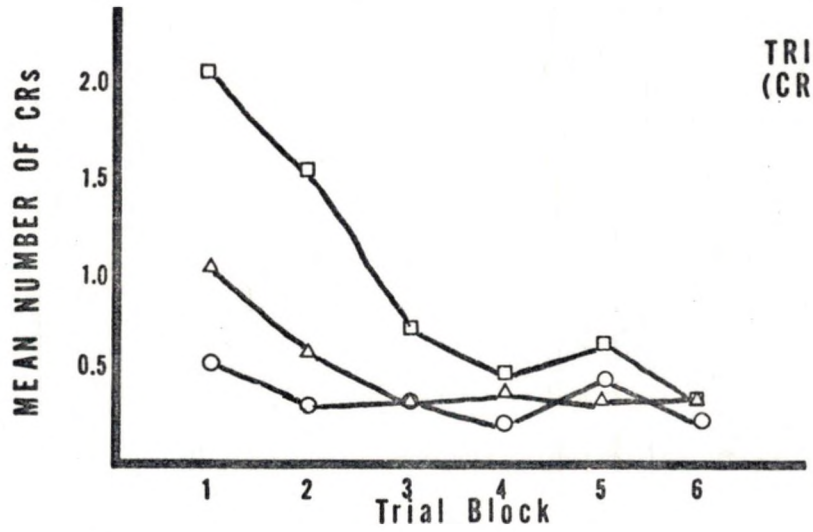
Reacquisition.--The analysis of variance showed no significant differences with respect to either the nutrition or injection main effects in the number of CRs (Table 14, Figure 6a). The trials effect was significant ($p < 0.05$).




TRIALS X NUTRITION
(LATENCIES IN AVOIDANCE)
ACQUISITION)

$p < 0.05$

fig.5a

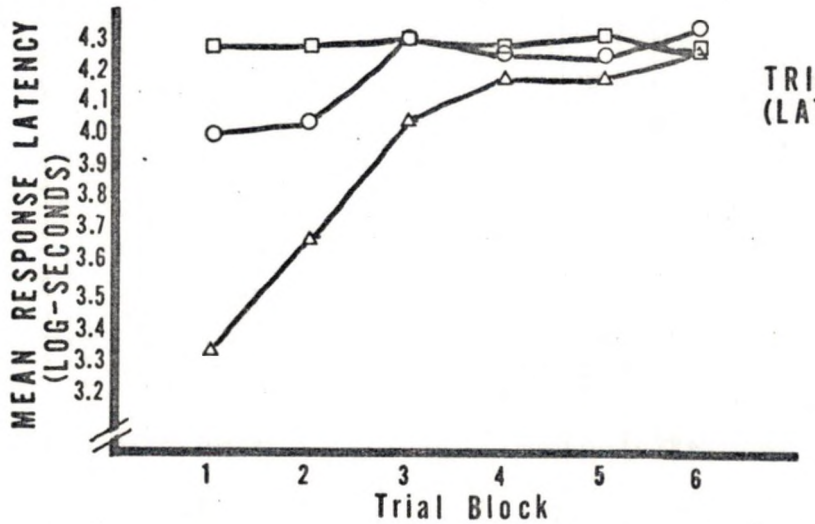



TRIALS X NUTRITION
(CRs IN EXTINCTION)

△zn def
□pair fed
○ad lib

$p < 0.001$

fig.5b




TRIALS X NUTRITION
(LATENCIES IN EXTINCTION)

$p < 0.001$

fig.5c

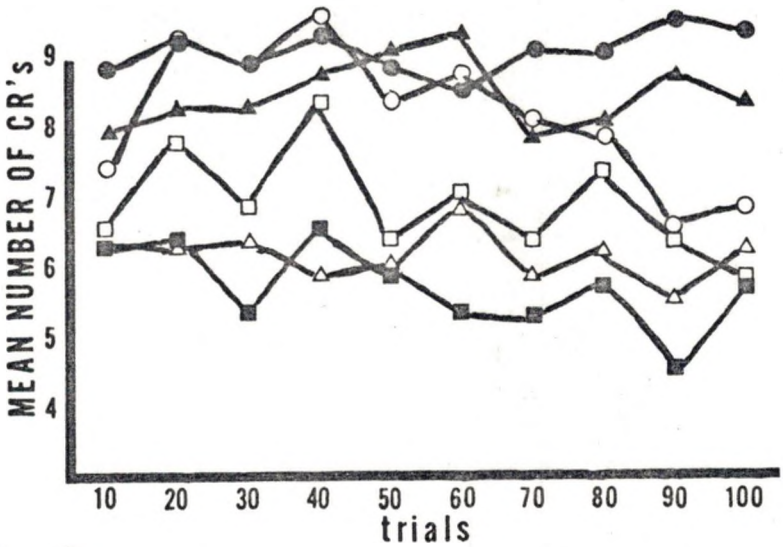



Fig. 5a

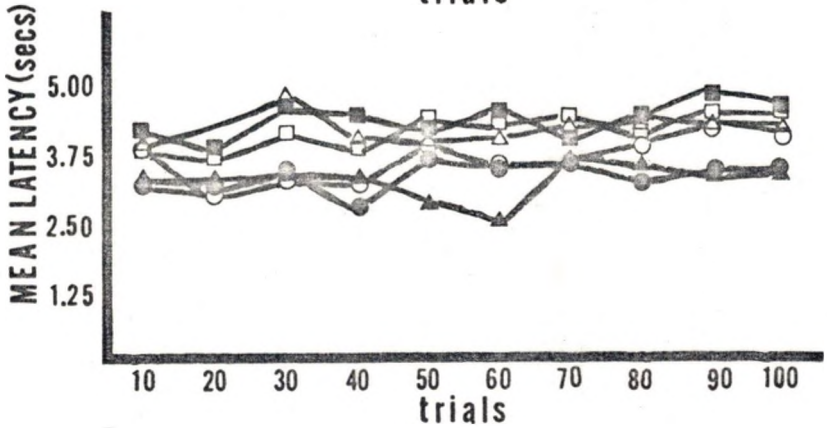



Fig. 5b

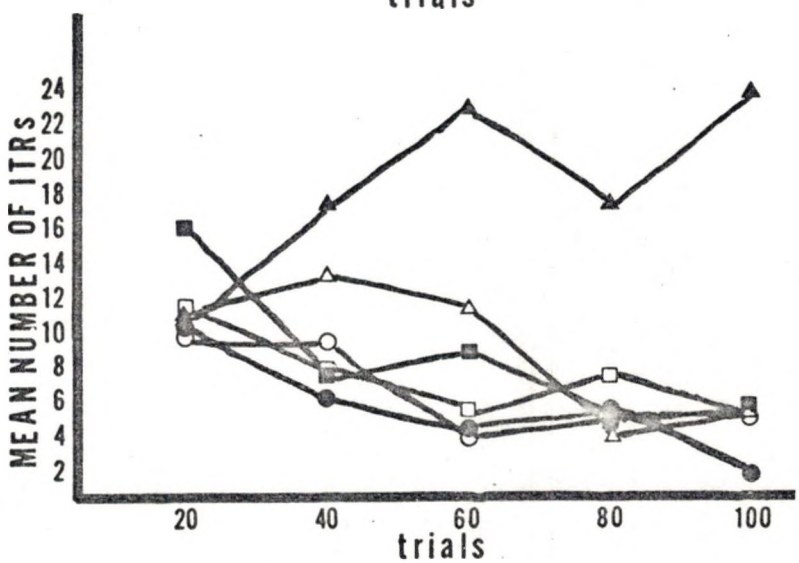



Fig. 5c

REACQUISITION

TABLE 14

ANALYSIS OF VARIANCE FOR NUMBER OF CR'S IN AVOIDANCE REACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 158.31 | 2.12 |
| Injection conditions | 1 | 41.34 | <1.00 |
| Nutrition by injection | 2 | 92.67 | 1.24 |
| Error between | 30 | 74.70 | |
| Within Ss | | | |
| Trials | 9 | 4.70 | 2.17* |
| Trials by nutrition | 18 | 1.72 | <1.00 |
| Trials by injection | 9 | 2.36 | 1.09 |
| Trials by nutrition by injection | 18 | 1.67 | <1.00 |
| Error within | 270 | 2.17 | |

*$p < 0.05$

Response Latencies

Because of extreme skewness, all the response latency data were normalized with a natural log transformation.

CS Habituation.--The analysis of variance for response latencies showed nonsignificant differences among the nutrition and between the main effects. The trials effect was significant ($p < 0.01$, Table 15). Again, this probably reflected flight responses from the novel CS presentations in the early trials.

TABLE 15

ANALYSIS OF VARIANCE FOR RESPONSE LATENCIES IN CS HABITUATION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 0.020 | <1.00 |
| Injection conditions | 1 | 0.041 | <1.00 |
| Nutrition by injection | 2 | 0.056 | <1.00 |
| Error between | 30 | 0.069 | |
| Within Ss | | | |
| Trials | 5 | 0.086 | 3.41* |
| Trials by nutrition | 10 | 0.016 | <1.00 |
| Trials by injection | 5 | 0.009 | <1.00 |
| Trials by nutrition by injection | 10 | 0.019 | <1.00 |
| Error within | 150 | 0.025 | |

*$p < 0.01$

Avoidance acquisition.--There were nonsignificant differences among both the nutrition and injection main effects with respect to response latency. The trials effect was highly significant ($p < 0.001$, Table 16, Figure 7). The trial by nutrition interaction was significant ($p < 0.05$, Figure 5a), with the pair-fed group beginning with shorter response latencies in the first three blocks and then finishing the later trials with longer latencies relative to the zinc deficient and ad libitum groups.

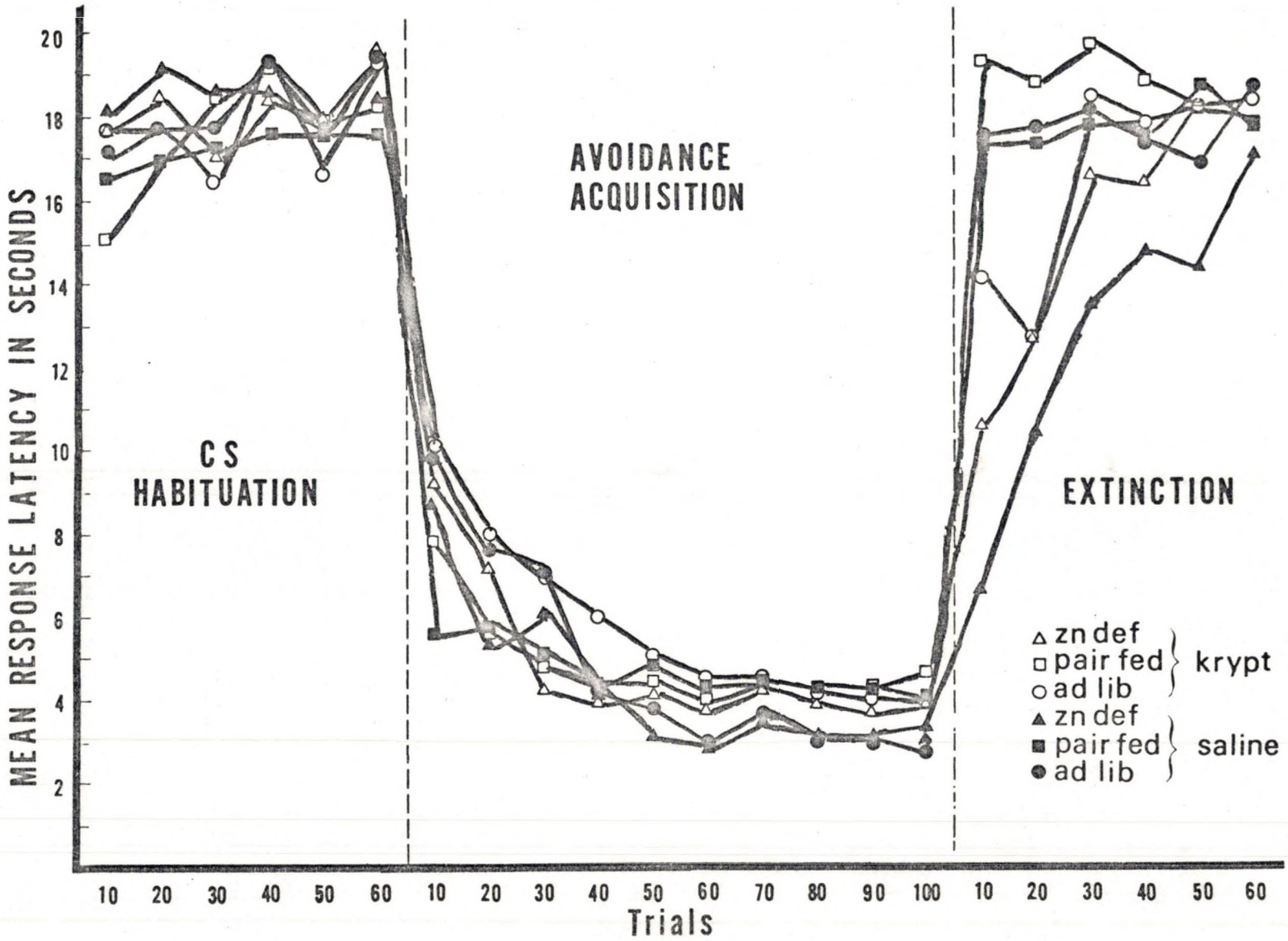

MEAN RESPONSE LATENCY IN SECONDS
20
18
16
14
12
10
8
6
4
2
CS
HABITUATION
AVOIDANCE
ACQUISITION
EXTINCTION
zn def
pair fed
ad lib
krypt
zn def
pair fed
ad lib
saline
10 20 30 40 50 60 10 20 30 40 50 60 70 80 90 100 10 20 30 40 50 60
Trials

TABLE 16

ANALYSIS OF VARIANCE FOR RESPONSE LATENCIES IN AVOIDANCE ACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 0.863 | 1.21 |
| Injection conditions | 1 | 1.372 | 1.93 |
| Nutrition by injection | 2 | 0.398 | <1.00 |
| Error between | 30 | 0.711 | |
| Within Ss | | | |
| Trials | 9 | 2.730 | 41.36* |
| Trials by nutrition | 18 | 0.141 | 2.14** |
| Trials by injection | 9 | 0.063 | <1.00 |
| Trials by nutrition by injection | 18 | 0.056 | <1.00 |
| Error within | 270 | 0.066 | |

*$p < 0.05$
**$p < 0.001$

Extinction.--The analysis of variance showed significant differences between the nutrition conditions ($p < 0.01$, Table 17, Figure 7). A Newman-Keuls internal comparison test showed the zinc deficient group had significantly shorter response latencies than the pair-fed ($p < 0.01$) and the ad libitum groups ($p < 0.05$). The difference between the pair-fed and ad libitum groups was not statistically significant. The trials effect and the trial by nutrition interaction were significant beyond the 0.001 level (Table 17, Figures 7 & 5c). The trial by nutrition interaction showed that the zinc deficient group exhibited short latencies during the first three trial blocks. This is in contrast to the pair-fed and ad libitum groups which exhibited relatively longer latencies over all the trial blocks in extinction.

TABLE 17

ANALYSIS OF VARIANCE FOR RESPONSE LATENCIES IN EXTINCTION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 2.348 | 6.35* |
| Injection conditions | 1 | 0.337 | <1.00 |
| Nutrition by injection | 2 | 0.454 | 1.23 |
| Error between | 30 | 0.370 | |
| Within Ss | | | |
| Trials | 5 | 1.044 | 14.11** |
| Trials by nutrition | 10 | 0.413 | 5.58** |
| Trials by injection | 5 | 0.053 | <1.00 |
| Trials by nutrition by injection | 10 | 0.084 | 1.10 |
| Error within | 150 | 0.074 | |

*$p < 0.01$
**$p < 0.001$

Avoidance reacquisition.--The analysis of variance showed nonsignificant differences among both the nutrition and injection main effect with respect to response latency (Table 18, Figure 7). There was a significant trials effect ($p < 0.01$, Table 18).

TABLE 18

ANALYSIS OF VARIANCE FOR RESPONSE LATENCIES IN AVOIDANCE REACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 1.091 | 2.10 |
| Injection conditions | 1 | 0.643 | 1.24 |
| Nutrition by injection | 2 | 0.354 | <1.00 |
| Error between | 30 | 0.519 | |
| Within Ss | | | |
| Trials | 9 | 0.097 | 3.03* |
| Trials by nutrition | 18 | 0.055 | 1.72 |
| Trials by injection | 9 | 0.008 | <1.00 |
| Trials by nutrition by injection | 18 | 0.036 | 1.13 |
| Error within | 270 | 0.032 | |

*$p < 0.01$

Intertrial Responses

CS Habituation.--The analysis of variance showed no significant differences among both the nutrition and injection main effect in the number of ITRs (Table 19, Figure 8).

TABLE 19

ANALYSIS OF VARIANCE FOR INTERTRIAL RESPONSES IN CS HABITUATION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 175.26 | 1.45 |
| Injection conditions | 1 | 144.68 | 1.20 |
| Nutrition by injection | 2 | 138.26 | 1.14 |
| Error between | 30 | 121.06 | |
| Within Ss | | | |
| Trials | 2 | 3.23 | <1.00 |
| Trials by nutrition | 4 | 57.72 | 1.13 |
| Trials by injection | 2 | 5.45 | <1.00 |
| Trials by nutrition by injection | 4 | 7.08 | <1.00 |
| Error within | | | |

Avoidance Acquisition.--The analysis of variance for number of ITRs showed significant differences among the nutrition treatment groups ($p<0.05$, Table 20, Figure 8). A Newman-Keuls internal comparison test showed the zinc deficient group made significantly more ITRs than the pair-fed group ($p<0.05$). However, the difference between the zinc deficient and the ad libitum groups failed to reach the 0.05 level of significance ($0.1>p>0.05$). The difference between the pair-fed and ad libitum groups was not significant.

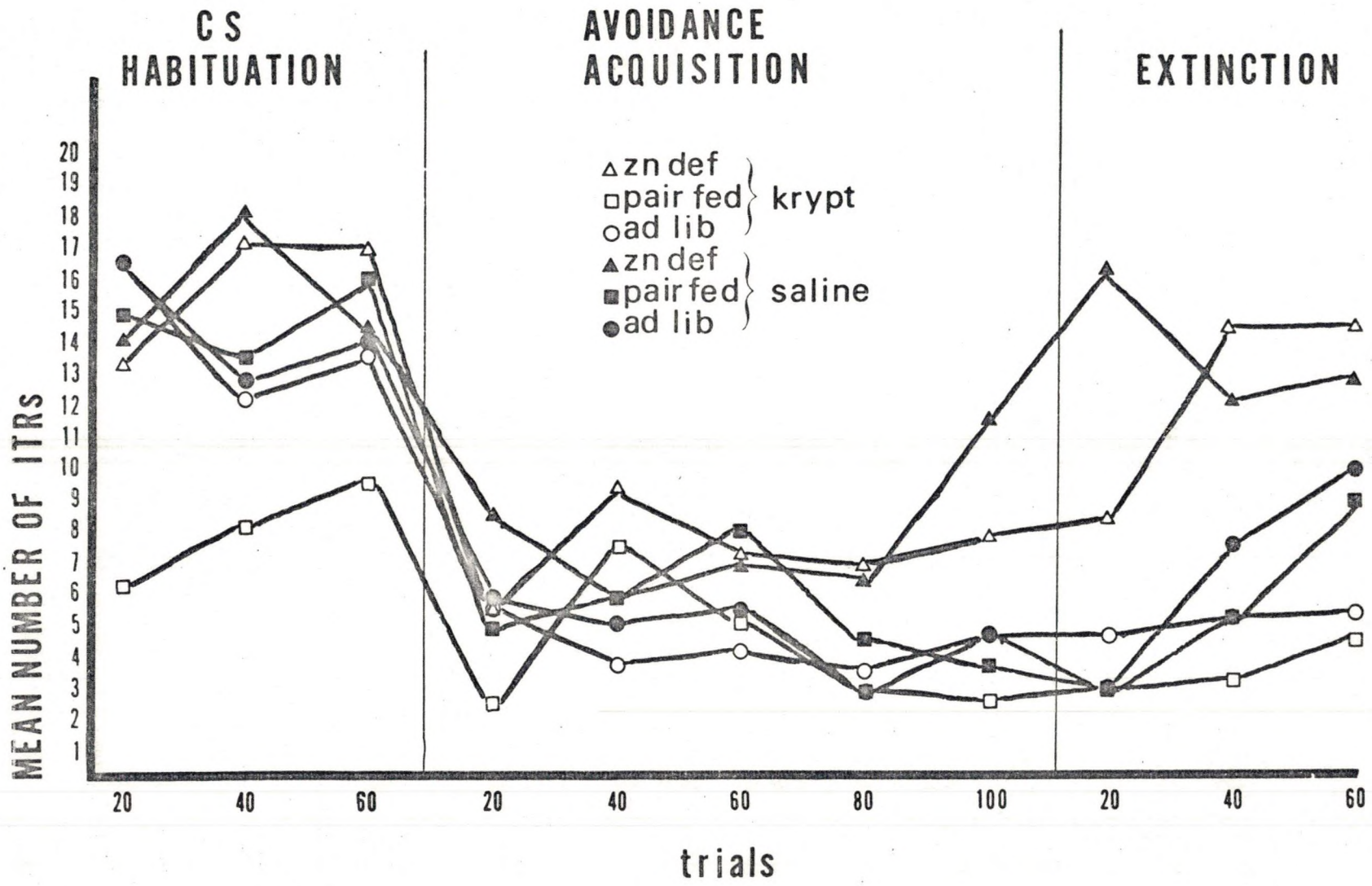

CS
HABITUATION
AVOIDANCE
ACQUISITION
EXTINCTION
zn def
pair fed
ad lib
krypt
zn def
pair fed
ad lib
saline
MEAN NUMBER OF ITRs
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
20
40
60
20
40
60
80
100
20
40
60
trials

TABLE 20

ANALYSIS OF VARIANCE FOR NUMBER OF INTERTRIAL RESPONSES IN AVOIDANCE ACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 175.54 | 3.79* |
| Injection conditions | 1 | 31.25 | <1.00 |
| Nutrition by injection | 2 | 2.92 | <1.00 |
| Error between | 30 | 46.34 | |
| Within Ss | | | |
| Trials | 4 | 18.11 | <1.00 |
| Trials by nutrition | 8 | 21.10 | 1.09 |
| Trials by injection | 4 | 15.56 | <1.00 |
| Trials by nutrition by injection | 8 | 10.16 | <1.00 |
| Error within | 120 | 19.29 | |

*$p < 0.05$

Extinction.--The analysis of variance for number of ITRs in extinction showed significant differences among nutrition treatment groups ($p < 0.01$, Table 21, Figure 8). A Newman-Keuls internal comparison test showed the zinc deficient group made significantly more ITRs than either the pair-fed or the ad libitum groups ($p < 0.01$). The pair-fed group and the ad libitum group were not significantly different. There was a significant trials effect ($p < 0.01$, Table 21, Figure 8). Figure 9 is a graphic representation of the significant trial by nutrition by injection interaction. There was a general increase of ITRs during extinction for both control groups, but the saline injected groups showed a rate which increased more rapidly over trials than the kryptopyrrole injected groups. This relationship did not hold for the zinc deficient group. The

zinc deficient-kryptopyrrole injected group exhibited increasing ITRs, while the zinc deficient-saline injected group exhibited decreasing ITRs over trials.

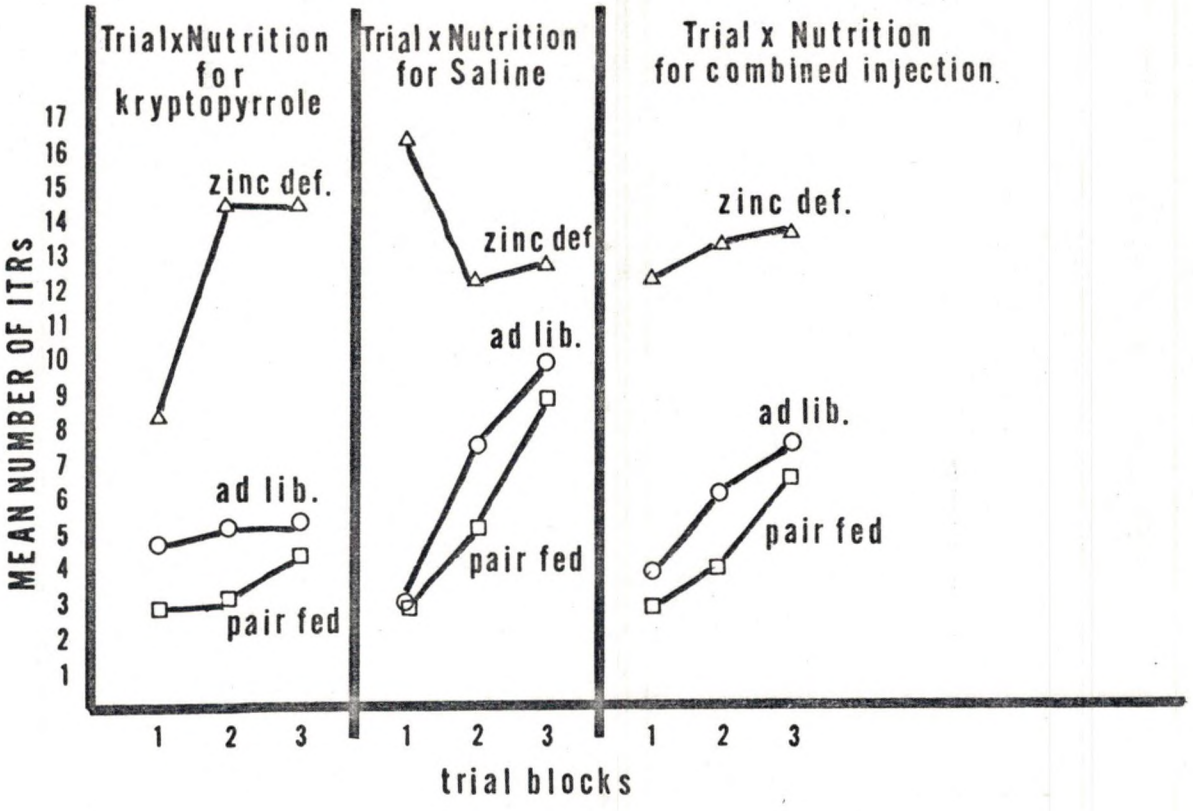
TABLE 21

ANALYSIS OF VARIANCE FOR INTERTRIAL RESPONSES IN EXTINCTION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 753.69 | 7.60* |
| Injection conditions | 1 | 85.33 | <1.00 |
| Nutrition by injection | 2 | 1.58 | <1.00 |
| Error between | 30 | | |
| Within Ss | | | |
| Trials | 2 | 81.08 | 5.33* |
| Trials by nutrition | 4 | 7.82 | <1.00 |
| Trials by injection | 2 | 8.58 | <1.00 |
| Trials by nutrition by injection | 4 | 68.04 | 4.47* |
| Error within | 60 | 15.21 | |

*$p < 0.01$

Reacquisition.--The analysis of variance showed a significant difference among nutrition conditions in the number of ITRs ($p < 0.01$, Table 22, Figure 6c). A Newman-Keuls internal comparison test showed the zinc deficient group made significantly more ITRs than the pair-fed and ad libitum groups ($p < 0.01$). The difference between the pair-fed and ad libitum groups was not significant.


Trial x Nutrition for kryptopyrrole
Trial x Nutrition for Saline
Trial x Nutrition for combined injection
zinc def.
ad lib.
pair fed
MEAN NUMBER OF ITRs
trial blocks

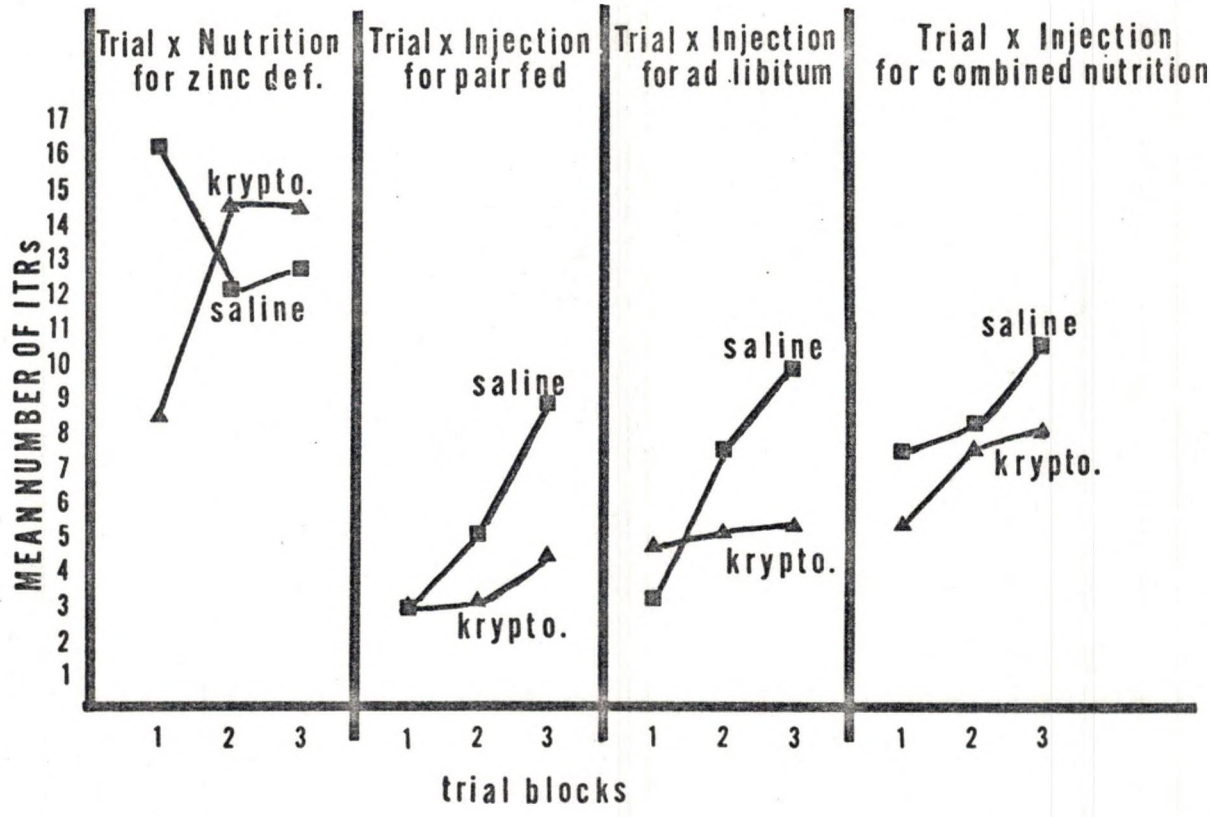

Trial x Nutrition for zinc def.
Trial x Injection for pair fed
Trial x Injection for ad libitum
Trial x Injection for combined nutrition
krypto.
saline
MEAN NUMBER OF ITRs
trial blocks

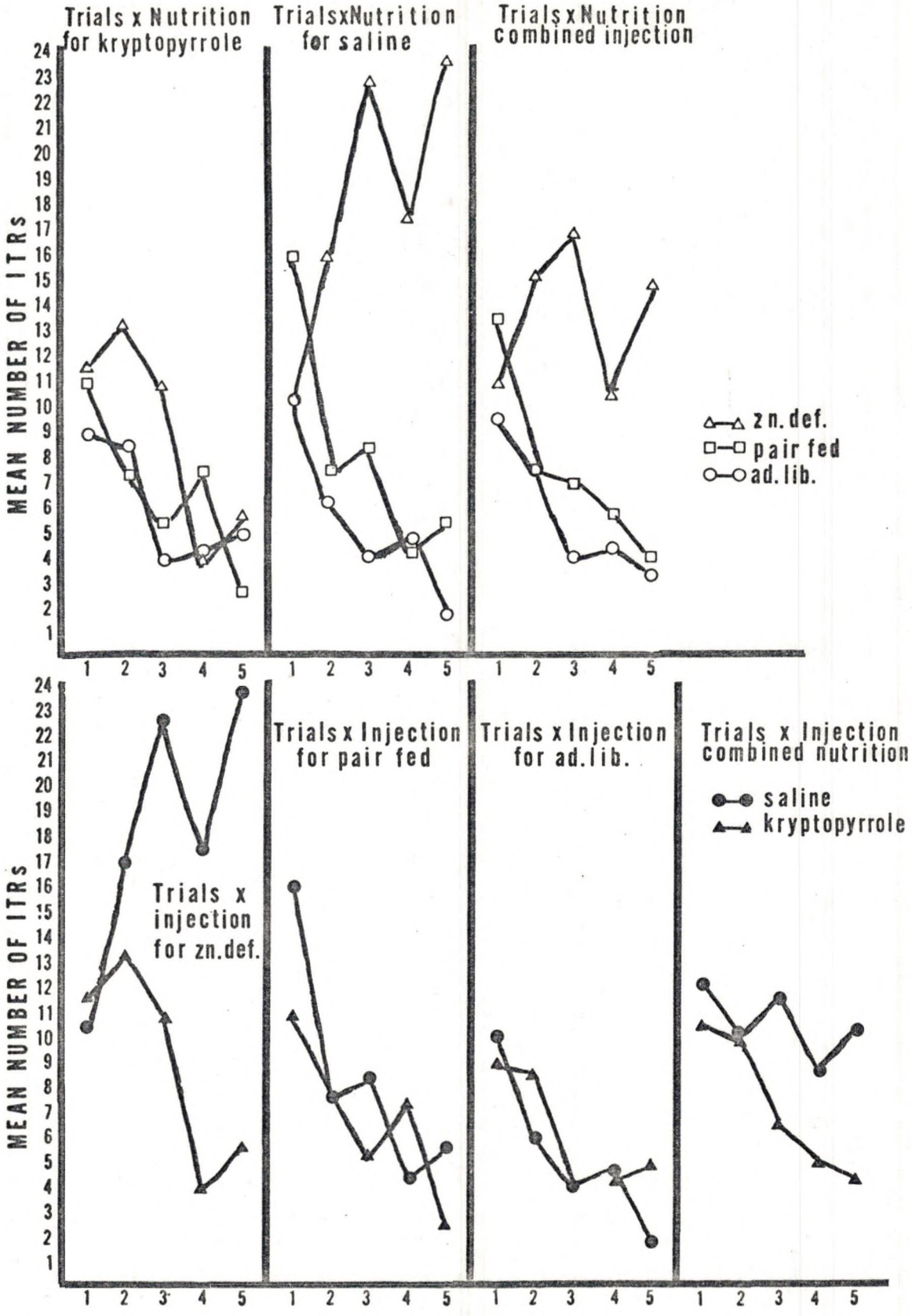
TABLE 22

ANALYSIS OF VARIANCE FOR INTERTRIAL RESPONSES IN AVOIDANCE REACQUISITION

| Source | d.f. | M.S. | F ratio |
|---|---|---|---|
| Between Ss | | | |
| Nutrition conditions | 2 | 1014.65 | 8.09* |
| Injection conditions | 1 | 493.36 | 3.94 |
| Nutrition by injection | 2 | 401.77 | 3.20 |
| Error between | 30 | 125.37 | |
| Within Ss | | | |
| Trials | 4 | 118.43 | 4.13* |
| Trials by nutrition | 8 | 101.37 | 3.53* |
| Trials by injection | 4 | 47.23 | 1.65 |
| Trials by nutrition by injection | 8 | 86.40 | 3.01* |
| Error within | 120 | 28.67 | |

*$p < 0.01$

In addition to a significant trials effect ($p < 0.01$), the analysis of variance showed a significant trials by nutrition interaction ($p < 0.01$, Table 22). The trials by nutrition interaction is shown graphically in Figure 10 (top panel, part C.) which shows that the zinc deficient groups maintained a high rate of intertrial responding over trials, while the pair fed and ad libitum groups exhibited a general decrease over trials. The significant trials by nutrition by injection interaction ($p < 0.01$, Table 22), graphically represented in Figure 10, shows that the zinc deficient-saline group maintained a relatively high rate of ITRs. There was a general decrease in intertrial responding in all other treatment groups. This is a reversal of the trials by nutrition by injection interaction found in extinction (Figure 8), in which the zinc deficient-saline group exhibited decreasing ITRs over trials.


Trials x Nutrition for kryptopyrrole
Trials x Nutrition for saline
Trials x Nutrition combined injection
MEAN NUMBER OF ITRs
zn. def.
pair fed
ad. lib.
Trials x injection for zn.def.
Trials x Injection for pair fed
Trials x Injection for ad.lib.
Trials x Injection combined nutrition
saline
kryptopyrrole

# CHAPTER IV

## DISCUSSION

The evaluation of the results obtained in experiments I and III will be made with reference to the following representative papers pertaining to open field behavior in the rat.

1. Defecation responses in the open field are highly correlated with emotion (discussed in Gray, 1971).
2. Open field ambulation scores are positively correlated with a factor called exploration and negatively correlated with a factor called fear (Whimbey & Denenberg, 1967). Fear inhibits exploratory behavior (Hayes, 1960).
3. Handling of Ss produces an increase in ambulation score, i.e., exploratory activity (Denenberg, 1963; Williams & Russell, 1972).
4. Age is an important variable in open field behavior. Valle (1971) has found younger Ss (90 days and less) to show a general increase in ambulatory activity over repeated trials, while older Ss (150 days or more) tend to show a decrease in activity with repeated exposure. Bronstein (1972) has found Ss 40 days or younger to exhibit increments in activity with repeated trials, while Ss 70 days or older show no increase. Furthermore, Bronstein suggests a possible age-handling interaction, i.e., handling Ss under 70 days is

associated with activity increase, but handling Ss older than this is not associated with increase.

5. Open-field activity on trial 1 has been suggested as a better measure of fear than the activity of later trials (Caldwell et al., 1970), in which exploratory activity begins. Ambulation scores should not be used as the sole criterion of fear, but in conjunction with the number of defecation responses (Whimbey & Denenberg, 1967).
6. Emotionally reactive rats exhibit "freezing" behavior (low ambulation scores) and tend to stay away from the middle of the field, keeping near the periphery (Denenberg, 1963).

The analysis of variance for the open field (experiment I) data showed no differences between the nutrition groups in ambulation scores which exceeded the 0.05 level of significance. Day 1 ambulation scores showed the zinc deficient group had lower activity ($0.1 > p > 0.05$) which is consistent with studies by Caldwell and Oberleas (1969), Caldwell et al., (1970), anc Caldwell et al., (1973). Although this finding is not highly significant it does point to perhaps increased emotionality in the zinc deficient Ss.

The proportion of central to peripheral square entries in experiment I was significantly lower in the zinc deficient group than in the pair-fed group. This may have been due to "fear running" in which the zinc deficient Ss stayed close to the periphery of the field. This is supported by the significant differences between proportions of central to peripheral entries in the zinc deficient and pair-fed groups, but not by the lack of significance between the zinc deficient and the ad libitum groups.

The zinc deficient Ss also showed a significantly higher rate of peripheral entries in experiment III, which may be interpreted as "fear running". In experiment III the differences between square entry proportions were significant between the zinc deficient group and both control groups. The difference between square entry proportions of the control groups was also highly significant with the pair-fed group making more central entries. This highly significant difference is important since it shows that postnatal starvation had a significant effect on open field behavior.

The findings of increased emotionality are not supported by the fact that the ad libitum group made more defecation responses than the zinc deficient group in both experiments I and III. The difference between total ambulation scores in experiments I and III is probably due to handling, the age difference between experiments, or a combination of these variables. These variables do not account for the differentially large increase in activity of the zinc deficient group, however. The zinc deficient group increased their activity at a relatively high rate in comparison with the control groups. The evidence seems to suggest that the zinc deficient group was more fearful in experiment I but probably not in experiment III.

With repeated exposures on the open field during experiment III when the tendency of fear response was probably at a minimum, the zinc deficient Ss were hyperactive. Technicians in our laboratory working with zinc deficient animals have voiced the opinion that the zinc deficient animals appear "hyperactive and nervous". Three technicians have independently made these comments over a period of three years and without questioning by the researchers.

The injection variable seemed to have no clearly observable effect on the observed open field behavior. The kryptopyrrole injected Ss of both the zinc deficient and ad libitum groups showed lower ambulation scores than their saline injected counterparts. This difference may have been due to the sedative effects of kryptopyrrole, however, the differences were not significant.

Although the difference between nutrition treatments failed to exceed the 0.05 level of significance, the zinc deficient group performed relatively well in the two-way avoidance learning task. The zinc deficient groups as a whole exhibited more CRs and shorter response latencies which is in opposition to studies by Caldwell and Oberleas (1969), Caldwell et al., (1970), and Caldwell et al., (1973). These studies are perhaps not directly comparable to the present study because they used a one-way avoidance task, different conditioning parameters, and different periods of zinc deficiency. The present study is probably more comparable to the Caldwell et al., (1970) study which used male Ss than the Caldwell and Oberleas (1969) and Caldwell et al., (1973) studies which used mixed sex samples.

The results of the present study are also in opposition to the Halas and Sandstead (1974) and Halas, Rowe, Johnson, McKenzie, and Sandstead (1974) studies. These studies are perhaps more directly comparable to the present study because they used a two-way avoidance task and the same sexed Ss. However, there are important differences in experimental conditions. The Halas and Sandstead study used a 28 volt light CS and a UCS (shock) intensity of 1.0mA, while the present study used a 28 volt light-2900 Hz tone CS complex and a UCS intensity of 0.8mA. The Halas et al., study used a 12 volt light CS and a UCS intensity of o.8mA. It

should be noted that the Caldwell and Oberleas, and the two Caldwell et al., studies also used a visual conditioned stimulus, and the shock intensity used was 1.3mA. When interpreted in view of the Halas and Sandstead (1974) study, the zinc deficient Ss in these studies may have shown an avoidance learning deficit because of their lower stress tolerance due to the relatively strong 1.3mA shock. If zinc deficient rats have lowered stress tolerance, the 0.5mA shock intensity difference between these studies and the present study may account for the different experimental results. The difference between the conditioning parameters, especially the conditioned stimuli, warrant further investigation. It is conceivable that the CS light-tone complex facilitates avoidance learning to a greater extent than the light only CS in zinc deficient Ss.

Another important difference was the time period of experimental zinc deficiency. While the Halas and Sandstead and the Halas et al., studies used a prenatal (days 15-20 and days 14-20, respectively) deficiency, the present study used a postnatal (days 1-20) deficiency. There may have been a more adverse effect on avoidance behavior from the shorter prenatal period than the longer postnatal period. Hypothesizing, this difference in deficiency periods could have produced differential deficits in learning depending on the CS modalities used. Transitory periods of zinc deficiency may have variable effects on behavior depending upon the state of development of the central nervous system or the particular sensory system. Rowe (1974) urged that different CS's be compared in order to determine if zinc deficiency has a differential effect on different sensory modalities. The brain undergoes rapid development during infancy and zinc deficiency may impair this development, depending upon the period of deficiency. The above results may lend support to

such a concept, and should be studied to see if variations exist with respect to the period of zinc deficiency.

The zinc deficient group exhibited significantly more intertrial responses in avoidance acquistion, extinction, and reacquisition. This is in direct opposition to the Halas and Sandstead study in which zinc deficient Ss exhibited significantly fewer ITRs. It appears that the zinc deficient Ss exhibited a large amount of responding that was not under direct CS control, and while the two control groups in general decreased the amount of intertrial responding over trials, the zinc deficient group increased or maintained a high level of these responses. The high ITR activity parallels somewhat the activity of the zinc deficient Ss in the open field in experiment III, with increases over trials. This is inconsistent with a number of reports of behavioral lethargy associated with zinc deficiency. It should be pointed out, however, that the Ss were not receiving the zinc deficient diet at the time of testing.

The kryptopyrrole injected-zinc deficient animals increased their rate of ITRs in extinction while the saline injected-zinc deficient animals tended to decrease. In reacquisition the reverse occurred with the zinc deficient saline animals exhibiting an increasing number of ITRs over trials and the kryptopyrrole injected zinc deficient animals showed a decrease. It is unclear what caused this differential increase in ITRs over trials with the zinc saline group. Repeated kryptopyrrole injection may have had a differentially adverse effect on the high ITR activity level of the zinc deficient group. The metabolism of kryptopyrrole by the previously zinc deficient group may have been different than that of the two control groups. The postnatal zinc deficiency may

have "metabolically programmed" the deficient pups to metabolize kryptopyrrole in a different manner than the controls. This specific metabolic response to kryptopyrrole may be a factor in the reduction of the high ITR activity over repeated trials. Relative to their controls, the zinc deficient animals may have different metabolic responses to other substances. Perhaps future research should be conducted to gain information about these possible relationships.

A factor which may have contributed to the lack of significant results in avoidance learning is that two deviant Ss in the pair fed-saline group and one deviant S in the pair fed-kryptopyrrole group showed nearly 0% avoidance even on the last day of acquisition training. Therefore, 25% of the total pair fed group never learned avoidance. Perhaps if a larger n size per cell was used these deviant scores would have been more evenly distributed among the groups. If the deviant pair fed Ss would have been dropped from the analysis the pair fed group would probably have performed as well or better than the zinc deficient group.

In summary, the male zinc deficient animals seemed to show no learning deficit and exhibited more exploration of the open field when zinc deficiency is induced postnatally (days 1-20). The kryptopyrrole appeared to have no clearcut effect on the dependent variables studied with the possible exception of the sharp reduction of ITR activity seen in the zinc deficient Ss. A larger but not lethal dosage of the substance would probably result in more clear-cut behavioral effects.

# APPENDIX

## RAW DATA

TABLE 23

DAM'S WEIGHTS IN GRAMS

| Day | Zinc deficient | | Pair fed | | Ad libitum | |
|---|---|---|---|---|---|---|
| | #739 | #741 | #745 | #746 | #747 | #750 |
| | #742 | #743 | #748 | #749 | #752 | #753 |
| 13 | 249.4 | 249.3 | 262.1 | 247.9 | 262.5 | 247.8 |
| | 237.4 | 265.7 | 250.3 | 237.2 | 262.1 | 235.8 |
| 18 | 310.4 | 304.5 | 319.5 | 277.8 | 309.5 | 318.9 |
| | 279.5 | 319.5 | 295.1 | 290.3 | 314.8 | 296.5 |
| Birth | 273.0 | 267.5 | 269.8 | 235.2 | 294.0 | 282.1 |
| | 250.0 | 285.5 | 271.3 | 265.2 | 278.5 | 260.5 |
| 4 | 254.4 | 222.1 | 247.8 | 197.0 | 290.3 | 271.7 |
| | 209.0 | 235.4 | 262.8 | 240.0 | 283.8 | 250.5 |
| 8 | 221.2 | 213.3 | 214.4 | 177.9 | 285.3 | 292.9 |
| | 186.5 | 213.4 | 230.2 | 210.2 | 262.2 | 243.5 |
| 12 | 197.8 | 188.5* | 191.9 | 170.0 | 298.1 | 285.0 |
| | 174.1 | 198.1 | 208.0 | 197.7 | 270.5 | 255.3 |
| 16 | 193.0 | | 208.3 | 166.7 | 295.0 | 286.5 |
| | 170.6 | 193.0 | 210.3 | 202.5 | 278.1 | 242.3 |
| 20 | 185.1 | | 190.1 | 168.2 | 278.5 | 263.6 |
| | 162.7 | 189.5 | 189.6 | 187.0 | 261.2 | 231.4 |
| 24 | 231.6 | | 255.1 | 220.5 | 298.9 | 245.8 |
| | 224.5 | 240.0 | 246.4 | 240.7 | 278.7 | 247.1 |

*All pups (9) died--dam was sacrificed

NUMBER OF PUPS PER DAM AT BIRTH

| Zinc deficient | Pair fed | Ad libitum |
|---|---|---|
| #739 - 11 | #745 - 13 | #747 - 8 |
| #741 - 12 | #746 - 11 | #750 - 12 |
| #742 - 10 | #748 - 6 | #752 - 9 |
| #743 - 12 | #749 - 10 | #753 - 13 |

TABLE 24

NUMBER OF DEFECATIONS PER ANIMAL IN THE OPEN FIELD (EXPERIMENT I, III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | I | III | I | III | I | III | I | III | I | III |
| Ss | | | | | | | | | | |
| Zinc Deficient | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| 01 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| 06 | 7 | 0 | 1 | 0 | 2 | 0 | 4 | 0 | 1 | 0 |
| 08 | 1 | 0 | 0 | 1 | 2 | 1 | 3 | 0 | 3 | 0 |
| 09 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 1 | 1 |
| 00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Saline | | | | | | | | | | |
| 02 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| 03 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| 04 | 0 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 |
| 05 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 07 | 1 | 3 | 3 | 0 | 4 | 0 | 3 | 0 | 1 | 0 |
| 36 | 3 | 4 | 4 | 1 | 1 | 1 | 5 | 3 | 9 | 1 |

TABLE 25

NUMBER OF DEFECATIONS PER ANIMAL IN THE OPEN FIELD (EXPERIMENT I, III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | I | III | I | III | I | III | I | III | I | III |
| Ss | | | | | | | | | | |
| Pair Fed | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| 12 | 1 | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 3 | 0 |
| 16 | 1 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 3 | 0 |
| 18 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 34 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Saline | | | | | | | | | | |
| 11 | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 2 | 0 |
| 14 | 1 | 0 | 2 | 1 | 2 | 1 | 2 | 0 | 2 | 1 |
| 15 | 0 | 1 | 2 | 3 | 3 | 2 | 2 | 0 | 5 | 1 |
| 17 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 2 | 0 |

TABLE 26

NUMBER OF DEFECATIONS PER ANIMAL IN THE OPEN FIELD (EXPERIMENT I, III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | I | III | I | III | I | III | I | III | I | III |
| Ss | | | | | | | | | | |
| Ad libitum | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| 21 | 0 | 2 | 2 | 3 | 0 | 3 | 1 | 0 | 3 | 0 |
| 22 | 3 | 1 | 2 | 1 | 0 | 2 | 6 | 1 | 3 | 5 |
| 23 | 2 | 0 | 3 | 1 | 1 | 0 | 4 | 0 | 0 | 0 |
| 25 | 8 | 2 | 0 | 0 | 3 | 2 | 0 | 3 | 0 | 0 |
| 29 | 6 | 4 | 4 | 4 | 1 | 3 | 2 | 4 | 4 | 7 |
| 30 | 2 | 3 | 2 | 5 | 6 | 3 | 4 | 1 | 1 | 0 |
| Saline | | | | | | | | | | |
| 24 | 3 | 2 | 3 | 0 | 3 | 6 | 6 | 4 | 5 | 1 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| 27 | 6 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 5 | 3 | 9 | 2 | 2 | 3 | 3 | 1 | 4 | 0 |
| 31 | 0 | 0 | 4 | 0 | 3 | 0 | 0 | 0 | 4 | 0 |
| 32 | 0 | 2 | 0 | 2 | 7 | 3 | 3 | 3 | 5 | 2 |

TABLE 27

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT I)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Zinc deficient | | | | | | | | | | |
| #01 | 7 | 0 | 26 | 1 | 69 | 0 | 3 | 0 | 3 | 0 |
| #02 | 64 | 1 | 82 | 3 | 11 | 0 | 7 | 0 | 3 | 0 |
| #03 | 12 | 1 | 23 | 0 | 19 | 0 | 19 | 0 | 11 | 0 |
| #04 | 71 | 0 | 71 | 0 | 51 | 0 | 3 | 0 | 37 | 0 |
| #05 | 99 | 8 | 114 | 4 | 109 | 1 | 58 | 1 | 101 | 6 |
| #06 | 26 | 3 | 28 | 3 | 37 | 0 | 61 | 0 | 64 | 3 |
| #07 | 20 | 1 | 12 | 1 | 59 | 2 | 82 | 9 | 33 | 0 |
| #08 | 18 | 2 | 89 | 0 | 59 | 0 | 62 | 7 | 66 | 4 |
| #09 | 5 | 0 | 43 | 1 | 5 | 0 | 3 | 0 | 33 | 0 |
| #10 | 54 | 9 | 51 | 0 | 43 | 0 | 29 | 0 | 50 | 1 |
| #00 | 41 | 1 | 40 | 0 | 17 | 0 | 51 | 0 | 73 | 0 |
| #36 | 73 | 2 | 83 | 0 | 112 | 11 | 106 | 8 | 59 | 1 |

*P Peripheral
**C Central

TABLE 28

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT I)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Pair fed | | | | | | | | | | |
| #11 | 7 | 3 | 17 | 0 | 51 | 1 | 23 | 0 | 15 | 0 |
| #12 | 96 | 0 | 33 | 0 | 15 | 0 | 4 | 2 | 3 | 0 |
| #13 | 66 | 13 | 65 | 6 | 81 | 1 | 30 | 1 | 31 | 0 |
| #14 | 24 | 2 | 9 | 0 | 13 | 0 | 37 | 0 | 41 | 11 |
| #15 | 10 | 1 | 37 | 0 | 63 | 0 | 35 | 0 | 48 | 1 |
| #16 | 82 | 5 | 31 | 0 | 5 | 0 | 7 | 0 | 57 | 3 |
| #17 | 33 | 1 | 91 | 2 | 46 | 0 | 56 | 1 | 39 | 0 |
| #18 | 53 | 0 | 19 | 0 | 17 | 0 | 19 | 2 | 15 | 0 |
| #19 | 128 | 4 | 107 | 3 | 71 | 0 | 91 | 4 | 37 | 0 |
| #20 | 139 | 8 | 35 | 0 | 79 | 0 | 92 | 5 | 39 | 0 |
| #33 | 88 | 12 | 74 | 14 | 26 | 3 | 3 | 0 | 3 | 0 |
| #34 | 74 | 5 | 90 | 13 | 56 | 2 | 19 | 0 | 52 | 0 |

*P Peripheral
**C Central

TABLE 29

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT I)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Ad libitum | | | | | | | | | | |
| #21 | 118 | 4 | 92 | 0 | 94 | 4 | 21 | 3 | 131 | 6 |
| #22 | 45 | 0 | 49 | 0 | 51 | 0 | 15 | 0 | 19 | 0 |
| #23 | 106 | 2 | 52 | 0 | 48 | 3 | 24 | 2 | 31 | 0 |
| #24 | 50 | 0 | 69 | 1 | 54 | 2 | 30 | 9 | 39 | 2 |
| #25 | 15 | 0 | 36 | 2 | 28 | 0 | 19 | 0 | 16 | 2 |
| #26 | 78 | 8 | 65 | 2 | 73 | 15 | 41 | 3 | 21 | 7 |
| #27 | 45 | 0 | 36 | 4 | 66 | 3 | 43 | 0 | 55 | 0 |
| #28 | 32 | 3 | 13 | 0 | 31 | 0 | 39 | 0 | 32 | 0 |
| #29 | 65 | 6 | 39 | 4 | 7 | 0 | 3 | 0 | 103 | 3 |
| #30 | 69 | 1 | 70 | 10 | 77 | 3 | 28 | 3 | 67 | 0 |
| #31 | 115 | 4 | 85 | 0 | 31 | 0 | 55 | 0 | 78 | 2 |
| #32 | 56 | 2 | 71 | 1 | 41 | 0 | 37 | 0 | 37 | 0 |

*P Peripheral
**C Central

TABLE 30

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Zinc deficient | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| #01 | 71 | 0 | 92 | 2 | 70 | 0 | 141 | 1 | 138 | 7 |
| #06 | 111 | 9 | 94 | 4 | 118 | 7 | 132 | 15 | 145 | 7 |
| #08 | 113 | 5 | 113 | 5 | 119 | 2 | 108 | 12 | 99 | 9 |
| #09 | 57 | 0 | 19 | 0 | 19 | 0 | 35 | 0 | 54 | 0 |
| #10 | 55 | 0 | 93 | 0 | 91 | 9 | 88 | 3 | 91 | 8 |
| #00 | 80 | 0 | 105 | 0 | 132 | 5 | 140 | 11 | 145 | 19 |
| Saline | | | | | | | | | | |
| #02 | 131 | 0 | 82 | 2 | 109 | 3 | 118 | 13 | 98 | 5 |
| #03 | 51 | 0 | 85 | 0 | 56 | 2 | 100 | 7 | 103 | 14 |
| #04 | 109 | 0 | 113 | 4 | 113 | 2 | 108 | 8 | 88 | 8 |
| #05 | 109 | 5 | 157 | 21 | 139 | 21 | 193 | 19 | 188 | 32 |
| #07 | 113 | 2 | 86 | 0 | 109 | 1 | 72 | 0 | 76 | 1 |
| #36 | 112 | 0 | 91 | 0 | 83 | 0 | 70 | 2 | 105 | 24 |

*P Peripheral
**C Central

TABLE 31

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Pair fed | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| #12 | 3 | 0 | 3 | 0 | 19 | 0 | 3 | 0 | 11 | 0 |
| #16 | 77 | 0 | 66 | 1 | 72 | 2 | 31 | 0 | 35 | 0 |
| #18 | 27 | 0 | 37 | 0 | 81 | 0 | 50 | 2 | 93 | 10 |
| #19 | 32 | 0 | 29 | 0 | 87 | 8 | 41 | 0 | 38 | 3 |
| #33 | 87 | 15 | 109 | 25 | 100 | 9 | 83 | 25 | 123 | 19 |
| #34 | 98 | 4 | 72 | 1 | 130 | 17 | 88 | 3 | 149 | 33 |
| Saline | | | | | | | | | | |
| #11 | 98 | 2 | 11 | 0 | 3 | 0 | 103 | 5 | 134 | 17 |
| #13 | 67 | 2 | 104 | 0 | 139 | 13 | 104 | 4 | 94 | 12 |
| #14 | 41 | 2 | 7 | 0 | 3 | 0 | 39 | 5 | 29 | 0 |
| #15 | 87 | 0 | 47 | 0 | 59 | 0 | 51 | 0 | 49 | 0 |
| #17 | 87 | 0 | 47 | 0 | 21 | 0 | 67 | 1 | 61 | 1 |
| #20 | 166 | 5 | 91 | 1 | 59 | 0 | 53 | 0 | 84 | 6 |

*P Peripheral
**C Central

TABLE 32

NUMBER OF SQUARES ENTERED IN THE OPEN FIELD
(EXPERIMENT III)

| Days | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Squares | P | C | P | C | P | C | P | C | P* | C** |
| Ss | | | | | | | | | | |
| Ad libitum | | | | | | | | | | |
| Kryptopyrrole | | | | | | | | | | |
| #21 | 7 | 0 | 7 | 0 | 31 | 0 | 21 | 0 | 3 | 0 |
| #22 | 74 | 3 | 74 | 4 | 41 | 2 | 33 | 0 | 47 | 6 |
| #23 | 21 | 0 | 19 | 0 | 27 | 2 | 19 | 0 | 42 | 8 |
| #25 | 77 | 3 | 105 | 4 | 91 | 4 | 119 | 1 | 81 | 9 |
| #29 | 59 | 0 | 23 | 0 | 50 | 4 | 71 | 1 | 45 | 0 |
| #30 | 76 | 1 | 64 | 0 | 49 | 0 | 29 | 0 | 66 | 12 |
| Saline | | | | | | | | | | |
| #24 | 61 | 0 | 61 | 1 | 67 | 2 | 71 | 3 | 59 | 4 |
| #26 | 13 | 0 | 73 | 15 | 65 | 4 | 64 | 11 | 25 | 3 |
| #27 | 54 | 1 | 50 | 1 | 24 | 1 | 56 | 1 | 51 | 3 |
| #28 | 37 | 0 | 51 | 3 | 25 | 0 | 55 | 1 | 49 | 3 |
| #31 | 45 | 0 | 69 | 0 | 75 | 5 | 62 | 3 | 84 | 5 |
| #32 | 67 | 0 | 39 | 0 | 83 | 2 | 57 | 0 | 62 | 2 |

*P Peripheral
**C Central

TABLE 33

MEAN NUMBER OF CR'S PER ANIMAL IN CS HABITUATION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 0.3 | 1.7 | 0.7 | 0.5 | 1.3 | 0.5 |
| 11-20 | 0.2 | 1.2 | 0.2 | 0.3 | 1.5 | 0.2 |
| 21-30 | 0.5 | 0.3 | 0.7 | 0.3 | 0.5 | 0.5 |
| 31-40 | 0.3 | 0.2 | 0.2 | 0.8 | 0.5 | 0.0 |
| 41-50 | 0.5 | 0.2 | 0.7 | 0.7 | 0.7 | 0.2 |
| 51-60 | 0.0 | 0.2 | 0.0 | 0.2 | 0.5 | 0.0 |

TABLE 34

MEAN NUMBER OF CR'S PER ANIMAL IN AVOIDANCE ACQUISTION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 0.5 | 0.0 | 0.0 | 0.8 | 0.0 | 0.2 |
| 11-20 | 1.2 | 1.2 | 1.2 | 2.2 | 0.7 | 2.2 |
| 21-30 | 5.0 | 2.7 | 2.5 | 3.0 | 2.3 | 3.5 |
| 31-40 | 5.0 | 4.5 | 4.8 | 5.8 | 3.7 | 7.0 |
| 41-50 | 4.7 | 4.0 | 4.5 | 8.2 | 3.8 | 7.2 |
| 51-60 | 6.8 | 6.7 | 5.2 | 8.2 | 5.0 | 8.7 |
| 61-70 | 5.8 | 5.2 | 4.8 | 7.5 | 4.3 | 8.0 |
| 71-80 | 7.5 | 5.3 | 6.8 | 8.0 | 4.8 | 9.3 |
| 81-90 | 5.8 | 5.5 | 6.3 | 8.5 | 4.3 | 8.7 |
| 91-100 | 6.7 | 7.0 | 7.5 | 8.3 | 6.2 | 9.8 |

TABLE 35

MEAN NUMBER OF CR'S PER ANIMAL IN EXTINCTION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 1.9 | 0.9 | 0.5 | 0.1 | 3.1 | 0.5 |
| 11-20 | 1.1 | 1.3 | 0.4 | 0.0 | 1.7 | 0.2 |
| 21-30 | 0.5 | 0.2 | 0.5 | 0.1 | 1.2 | 0.2 |
| 31-40 | 0.5 | 0.3 | 0.3 | 0.2 | 0.6 | 0.1 |
| 41-50 | 0.3 | 0.4 | 0.2 | 0.3 | 0.8 | 0.6 |
| 51-60 | 0.3 | 0.1 | 0.3 | 0.3 | 0.5 | 0.1 |

TABLE 36

MEAN NUMBER OF CR'S PER ANIMAL IN REACQUISITION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 6.2 | 6.5 | 7.3 | 7.8 | 6.2 | 8.7 |
| 11-20 | 6.2 | 7.7 | 9.2 | 8.2 | 6.3 | 9.2 |
| 21-30 | 6.5 | 6.8 | 8.8 | 8.2 | 5.3 | 8.8 |
| 31-40 | 5.8 | 8.3 | 9.5 | 8.7 | 6.5 | 9.3 |
| 41-50 | 6.0 | 6.3 | 8.3 | 9.0 | 5.8 | 8.8 |
| 51-60 | 6.8 | 7.0 | 8.7 | 9.3 | 5.3 | 8.5 |
| 61-70 | 5.8 | 6.3 | 8.0 | 7.8 | 5.2 | 9.0 |
| 71-80 | 6.2 | 7.3 | 7.8 | 8.0 | 5.7 | 9.0 |
| 81-90 | 5.5 | 6.3 | 6.5 | 8.7 | 4.5 | 9.5 |
| 91-100 | 6.2 | 5.8 | 6.8 | 8.3 | 5.7 | 9.3 |

TABLE 37

MEAN RESPONSE LATENCY PER ANIMAL IN CS HABITUATION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 70.5 | 60.5 | 70.7 | 72.3 | 66.2 | 68.6 |
| 11-20 | 74.5 | 68.0 | 70.6 | 76.2 | 67.7 | 71.6 |
| 21-30 | 68.3 | 74.0 | 65.8 | 74.4 | 69.6 | 71.7 |
| 31-40 | 73.9 | 77.4 | 76.3 | 74.2 | 70.3 | 69.4 |
| 41-50 | 72.5 | 71.7 | 66.9 | 69.8 | 70.3 | 69.4 |
| 51-60 | 78.5 | 73.4 | 77.3 | 74.1 | 73.7 | 78.6 |

TABLE 38

MEAN RESPONSE LATENCY IN QUARTER SECONDS PER ANIMAL IN AVOIDANCE ACQUISITION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 36.9 | 31.4 | 40.5 | 34.8 | 21.8 | 39.7 |
| 11-20 | 28.1 | 22.3 | 32.1 | 21.6 | 23.2 | 30.6 |
| 21-30 | 16.7 | 19.7 | 27.8 | 24.4 | 20.4 | 28.4 |
| 31-40 | 15.9 | 18.2 | 24.0 | 17.5 | 18.4 | 16.7 |
| 41-50 | 16.8 | 18.6 | 20.5 | 12.3 | 19.3 | 15.4 |
| 51-60 | 14.9 | 16.1 | 18.2 | 11.6 | 17.7 | 12.2 |
| 61-70 | 17.2 | 18.3 | 18.4 | ;4.2 | 18.1 | 14.6 |
| 71-80 | 14.3 | 17.5 | 16.6 | 12.6 | 17.8 | 11.9 |
| 81-90 | 15.4 | 17.9 | 16.5 | 12.9 | 17.7 | 12.3 |
| 91-100 | 15.5 | 19.2 | 15.7 | 13.6 | 16.4 | 11.5 |

TABLE 39

MEAN RESPONSE LATENCY PER ANIMAL IN EXTINCTION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 42.0 | 77.1 | 56.5 | 27.5 | 69.5 | 70.4 |
| 11-20 | 51.2 | 75.7 | 50.6 | 41.9 | 69.5 | 71.0 |
| 21-30 | 66.6 | 78.8 | 74.3 | 54.2 | 71.2 | 73.1 |
| 31-40 | 66.1 | 75.4 | 71.7 | 59.9 | 70.0 | 69.6 |
| 41-50 | 74.1 | 72.9 | 73.3 | 58.5 | 75.3 | 67.7 |
| 51-60 | 70.8 | 71.9 | 74.1 | 68.6 | 71.1 | 75.4 |

TABLE 40

MEAN RESPONSE LATENCY IN QUARTER SECONDS PER ANIMAL IN REACQUISITION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-10 | 15.1 | 15.0 | 15.3 | 13.1 | .6.3 | 12.9 |
| 11-20 | 17.0 | 14.6 | 12.2 | 13.1 | .5.2 | 12.1 |
| 21-30 | 19.0 | 16.1 | 13.5 | 13.6 | 17.8 | 12.8 |
| 31-40 | 15.9 | 15.0 | 13.0 | 13.0 | 17.2 | 11.0 |
| 41-50 | 15.5 | 17.0 | 15.4 | 11.6 | 16.6 | 14.7 |
| 51-60 | 15.3 | 16.9 | 14.2 | 10.0 | 18.2 | 14.3 |
| 61-70 | 16.5 | 17.3 | 13.9 | 14.6 | 15.7 | 14.5 |
| 71-80 | 17.3 | 16.2 | 15.3 | 14.0 | 16.9 | 12.7 |
| 81-90 | 17.1 | 17.7 | 16.9 | 13.3 | 19.1 | 13.5 |
| 91-100 | 16.7 | 17.6 | 16.2 | 13.6 | 17.9 | 13.3 |

TABLE 41

MEAN NUMBER OF INTERTRIAL RESPONSES PER ANIMAL IN CS HABITUATION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-20 | 13.0 | 6.0 | 16.3 | 13.8 | 14.7 | 16.2 |
| 21-40 | 17.0 | 7.8 | 12.0 | 18.0 | 13.2 | 12.5 |
| 41-60 | 16.7 | 9.3 | 13.3 | 14.3 | 15.8 | 13.8 |

TABLE 42

MEAN NUMBER OF INTERTRIAL RESPONSES PER ANIMAL IN AVOIDANCE ACQUISITION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-20 | 5.0 | 2.2 | 5.2 | 8.3 | 4.7 | 5.7 |
| 21-40 | 9.2 | 7.2 | 3.5 | 6.0 | 5.7 | 4.8 |
| 41-60 | 7.0 | 4.8 | 4.0 | 6.7 | 7.8 | 5.3 |
| 61-80 | 6.7 | 2.8 | 3.3 | 6.2 | 4.3 | 2.7 |
| 81-100 | 7.5 | 2.3 | 4.5 | 11.2 | 3.5 | 4.5 |

TABLE 43

MEAN NUMBER OF INTERTRIAL RESPONSES PER ANIMAL IN EXTINCTION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-20 | 8.2 | 2.7 | 4.5 | 16.2 | 2.7 | 3.0 |
| 21-40 | 14.3 | 3.0 | 5.0 | 12.0 | 5.0 | 7.3 |
| 41-60 | 14.3 | 4.3 | 5.2 | 12.7 | 8.8 | 9.8 |

TABLE 44

MEAN NUMBER OF INTERTRIAL RESPONSES PER ANIMAL IN REACQUISITION

| | Kryptopyrrole injected | | | Saline injected | | |
|---|---|---|---|---|---|---|
| Trials | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 | Zinc deficient n=6 | Pair fed n=6 | Ad libitum n=6 |
| 1-20 | 11.3 | 10.8 | 8.8 | 10.2 | 15.8 | 10.0 |
| 21-40 | 13.2 | 7.5 | 8.3 | 16.8 | 7.3 | 6.0 |
| 41-60 | 10.7 | 5.2 | 3.8 | 22.5 | 8.3 | 4.0 |
| 61-80 | 3.8 | 7.2 | 4.2 | 17.2 | 4.2 | 4.5 |
| 81-100 | 5.5 | 2.5 | 4.8 | 23.5 | 5.3 | 1.7 |

# REFERENCES

Apgar, J. Comparison of the effects of copper, manganese, and zinc deficiencies on parturition in the rat. American Journal of Physiology, 1968, 215, 1478-1481. (a)

Apgar, J. Effects of zinc deficiency on parturition in the rat. American Journal of Physiology, 1968, 215, 160-163. (b)

Apgar, J. Effects of zinc deprivation from day 12, 15, or 18 of gestation on parturition in the rat. Journal of Nutrition, 1972, 102, 343-348.

Bronstein, P.M. Open field behavior of the rat as a function of age: Cross-sectional and longitudinal investigations. Journal of Comparative and Physiological Psychology, 1972, 80, 335-341.

Caldwell, D.F., & Oberleas, D. Effects of protein and zinc nutrition on behavior in the rat. Pan American Health Organization Scientific Publication, 1969, 185, 2-8.

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.

Caldwell, D.F., Oberleas, D., & Prasad, A.S. Reproductive performance of chronic mildly zinc deficient rats and the effects on behavior of their offspring. Nutrition Reports International, 1973, 7, 309-319.

Chesters, J.K., & Quarterman, J. Effects of zinc deficiency on food intake and feeding patterns of rats. British Journal of Nutrition, 1970, 24, 1061-1069.

Day, H.G., & McCollum, E.V. Effects of acute dietary zinc deficiency in the rat. Proceedings of the Society for Experimental Biology and Medicine, 1940, 45, 282-284.

Day, H.G. & Skidmore, B.E. Some effects of dietary zinc deficiency in the mouse. Journal of Nutrition, 1947, 33, 27-38.

Denenberg, V.H. Early experience and emotional development. Scientific American, 1963, 208, 138-146.

Ellman, G.L., Jones, R.T., & Rychert, R.C. Mauve spot and schizophrenia. American Journal of Psychiatry, 1968, 125, 849-851.

Follis, R.H., Day, H.G., & McCollum, E.V. Histological studies of rats fed a diet extremely low in zinc. Journal of Nutrition, 1941, 22, 223-238.

Gray, J.A. The Psychology of Fear and Stress. McGraw-Hill, 1971.

Guilford, J.P. Fundamental Statistics in Psychology and Education. McGraw-Hill, 1965, 185.

Halas, E.S., Rowe, M.C., Johnson, O.R., McKenzie, J.M., & Sandstead, H.H. Effects of intra-uterine zinc deficiency on subsequent behavior. International Symposium on Trace Elements in Human Health and Disease (in press).

Halas, E.S., & Sandstead, H.H. Some effects of prenatal zinc deficiency on behavior of the adult rat. Pediatric Research (in press).

Hayes, K.J. Exploration and fear. Psychological Reports, 1960, 6, 91-93.

Hoffer, A., & Osmond, H. Malvaria: A new psychiatric disease. Acta Psychiatrica Scandinavica, 1963, 39, 335-366.

Hurley, L.S. Zinc deficiency in the developing rat. American Journal of Clinical Nutrition, 1969, 22, 1332-1339.

Hurley, L.S., Gowan, J., & Swenerton, H. Teratogenic effects of short-term zinc deficiency in rats. Teratology, 1971, 4, 199-204.

Hurley, L.S., & Mutch, P.B. Prenatal and Postnatal development after transitory gestational zinc deficiency in rats. Journal of Nutrition, 1973, 103, 649-656.

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

Huszak, I., Durko, I., & Darsai, K. Experimental data to the pathogensis of cryptopyrrole excretion in schizophrenia. Acta Physiologica Scientiarum Hungaricae, 1972, 42, 79-86.

Irvine, D.G. Apparently non-indolic Ehrlich-positive substances related to mental illnesses. Journal of Neuropsychiarty, 1961, 2, 292-305.

Irvine, D.G., Bayne, W., Miyashita, H., & Majer, J.R. Identification of kryptopyrrole in human urine and its relation to psychosis. Nature, 1969, 224, 811-813.

Irvine, D.G., & Wetterberg, L. Kryptopyrrole-like substance in acute intermittent popphyria. Lancet, 1972, 2, 1201.

Kienholz, E.W., Turk, D.E., Sande, M.L., & Hoekstra, W.G. Effects of zinc deficiency in the diet of hens. Journal of Nutrition, 1961, 75, 211-221.

Lokken, P.M. Maze acquisition in the zinc deficient rat. Unpublished master's thesis, University of North Dakota, 1973.

Lokken, P.M., Halas, E.S., & Sandstead, H.H. Influence of zinc deficiency on behavior. Proceedings of the Society for Experimental Biology and Medicine, 1973, 144, 680-682.

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.

Miller, J.K., & Miller, W. Experimental zinc deficiency and recovery of calves. Journal of Nutrition, 1962, 76, 467-474.

Moffett, R.B. Central nervous system depressants. VIII. Pyrroles. Journal of Medicinal Chemistry, 1968, 11, 1251-1252.

Mutch, P.B., & Hurley, L.S. Effect of zinc deficiency during lactation on postnatal growth and development of rats. Journal of Nutrition, 1974, 104, 828-842.

O'Dell, B.L., Newberne, P.M., & Savage, J.E. Significance of dietary zinc for the growing chicken. Journal of Nutrition, 1958, 65, 503-524.

O'Reilly, P.O., Ernest, M., & Hughes, G. The incidence of malvaria. British Journal of Psychiatry, 1965, 111, 741-744.

Ott, E.A., Smith, W.H., Stob, M., & Beeson, W.M. Zinc deficiency syndrome in the young lamb. Journal of Nutrition, 1964, 82, 41-50.

Prasad, A.S. A century of research on the metabolic role of zinc. Journal of Clinical Nutrition, 1969, 22, 1215-1221.

Prasad, A.S., Halsted, J.A., & Nadimi, M. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism, and geophagia. American Journal of Medicine, 1961, 31, 532-546.

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, 1963, 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, 1963, 111, 407-428.

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

Prasad, A.S., Oberleas, D., Wolf, P., & Horwitz, J.P. Studies on zinc deficiency: Changes in trace elements and enzyme activities in tissues of zinc deficient rats. Journal of Clinical Investigation, 1967, 46, 549-557.

Ronaghy, H., Spivey Fox, M.R., Garn, S.M., Israel, H., Harp, A., Moe, P.G., & Halsted, J.A. Controlled zinc supplementation for malnourished school boys: A pilot experiment. American Journal of Clinical Nutrition, 1969, 22, 1279-1289.

Rowe, M.C. Physiological and behavioral effects of zinc deficiency in rats. Unpublished master's thesis, University of North Dakota 1974.

Russell, C.S. Biosynthesis of porphyrins and the origin of the "Mauve factor." Journal of Theoretical Biology, 1972, 35, 277-283.

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

Sandstead, H.H., Prasad, A.S., Farid, Z., Miale, A., Bassilly, S., & 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, 81-84.

Schneider, E., & Price, C.A. Decreased ribonucleic acid levels: A possible cause of growth inhibition in zinc deficiency. Biochimica et Biophysica ACTA, 1962, 55, 406-407.

Sever, L.E., & Emanuel, I. Is there a connection between maternal zinc deficiency and congenital malformations of the central nervous system in man? Teratology, 1973, 7, 117-118.

Sohler, A., Beck, R., & Noval, J. Mauve factor reidentified as 2,4-dimethyl-3-ethylpyrrole and its sedative effects on the CNS. Nature, 1970, 288, 1318-1320.

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.

Tucker, H.F., & Salmon, W.D. Parakeratosis or zinc deficiency disease in the pig. Proceedings of the Society for Experimental Biology and Medicine, 1955, 88, 613-616.

Valle, F.P. Rats' performance on repeated tests in the open field as a function of age. Psychonomic Science, 1971, 23, 333-335.

Underwood, E.J. Trace Elements in Human and Animal Nutrition. Academic Press, 1971, 208-252.

Walker, J.L. Neurological and behavioral toxicity of kryptopyrrole in the rat. Pharmacology, Biochemistry, & Behavior, 1974, (in press).

Weil-Malherbe, H., & Szara, S.I. The Biochemistry of Functional and Experimental Psychoses. Charles C. Thomas, Pub., 1971, 163-173.

Wetterberg, L. Pharmacological and toxic effects of kryptopyrrole in mice. Upsala Journal of Medical Science, 1973, 78, 78-80.

Whimbey, A.E., & Denenberg, V.H. Two independent behavioral dimensions in open field performance. Journal of Comparative and Physiological Psychology, 1967, 63, 500-504.

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

Williams, D.I., & Russell, P.A. Open-field behavior in rats: Effects of handling, sex, and repeated testing. British Journal of Psychology, 1972, 63, 593-596.

Williams, R.B., & Chesters, J.K. The effects of early zinc deficiency on DNA and protein synthesis in the rat. British Journal of Nutrition, 1970, 24, 1053-1059.

Winder, F., & Denneny, J.M. Effects of iron and zinc on nucleic acid and protein synthesis in mycobacterium smegmatis. Nature, 1959, 184, 742-743.

Winer, B.J. Statistical Principles in Experimental Design. McGraw-Hill, 1971.