

Behavioral Impairment in Adult Rats Following Acute Zinc Deficiency¹ (34703)

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Zinc deficiency is classically characterized by growth retardation, anorexia, seborrhea, hyperkeratosis, loss of hair, testicular atrophy, and parakeratosis (1, 2). Less well described manifestations are the more subtle, but equally characteristic, behavioral changes which occur following prolonged periods of zinc insufficiency in man and animal (3, 4). Notable among these is the marked lethargy or slowing of behavior which accompanies a chronic, severe zinc deficiency. In the adult rat, we have observed a state similar to the "nutritional catatonia" described by Spitz and Wolff (5) as part of the syndrome of kwashiorkor, a disease attributed to the failure to achieve an adequate protein level in the diet (6). The present investigation was designed to determine whether the effects of an acute zinc deficiency in rats could be discerned in measures of learning ability and emotionality.

Materials and Method. Twenty-four male Harlan Wistar strain² 21-day-old rats served as subjects. All animals were individually caged in air-conditioned quarters and placed on a standard laboratory regimen of Purina Rat Chow and water *ad libitum* for a 9-day adaptation period prior to initiation of the experiment. At 30 days of age, the animals were paired by weight and given a zinc deficient diet *ad libitum* or a control diet adequately supplemented with zinc. The intake of the control diet was limited to the level of intake of the paired deficient animal during

preceding 24 hr. Treatment assignment within pairs was random.

The zinc deficient diet (viz., basal diet) contained the following ingredients computed (g/kg): glucose monohydrate (Cerelese), 623.5; corn oil, 100; soybean assay protein (EDTA washed), 200; minerals, 50; calcium carbonate, 20; phytic acid, 6.5; vitamins and methionine.³ This diet was calculated to contain 16.0% crude protein (18% by analysis), 1.6 % calcium, 0.6% inorganic phosphorus and 1.0% phytate. By analysis the zinc content was 8.0 mg/kg. The control diet was identical in composition to the basal diet except the protein was not washed with EDTA and 55 mg/kg of zinc as the carbonate was added (70 mg/kg of total zinc). This latter diet was the same as was used previously (7).

Animals were maintained in stainless steel cages and given distilled water in vinyl stoppered glass bottles with stainless steel drinking tubes. Subjects were weighed at weekly intervals and testing was started after 48 days of dietary treatment (viz., 78 days of age) and remained on their respective diets

³The following minerals were supplied (g/kg of diet): CaCO₃, 10.6; CaHPO₄·2H₂O, 16.5; MgCO₃, 1.0; MgSO₄·7H₂O, 1.2; NaCl, 5.0; KCl, 0.8; FePO₄ (soluble), 1.6; KH₂PO₄, 12.5; MnSO₄·H₂O, 0.8; CuSO₄·5H₂O, 0.06; AlK(SO₄)₂·12H₂O, 0.01; KIO₃, 0.03; CoCl₂·6H₂O, 0.002; NaF, 0.04. Vitamins were supplied at the following levels per kilogram of diet: vitamin A, 20,000 IU; vitamin D, 3000 IU; menadione, 10 mg; α-tocopheryl acetate, 30 mg; thiamine HCl, 16 mg; riboflavin, 16 mg; pyridoxine HCl, 16 mg; Ca pantothenate, 40 mg; biotin, 0.2 mg; folacin, 5 mg; cyanocobalamin, 0.05 mg; choline chloride, 1000 mg; methionine, 2.0 g. The antioxidant, butylated hydroxyanisole, was supplied at 100 mg/kg.

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²Harlan Industries, Cumberland, Indiana.

throughout the behavior testing period of approximately 2 weeks. Subjects received one 5 min trial on the open field test of emotionality (8-11) for each of 3 consecutive days. This test measured the amount of motor activity, an indication of emotionality in the rat, in a walled enclosure measuring 4×4 ft with a 5-W incandescent bulb centered 2 ft above the floor. The floor was calibrated in 6-in. squares and subject entries constituted the recorded data. Second, each animal was given 50 continuous trials on the "platform box," a totally automated test of one-way avoidance learning (12). Parameters for this test were: conditioned stimulus = 4-W light of 10-sec duration; unconditioned stimulus = 1.3-mA electric shock of 30-sec duration delivered through stainless steel grids by a scrambler circuit; and an intertrial interval of 40 sec. Response latency and number of conditioned avoidance responses were recorded. An eight-blind water maze designed to detect diffuse brain damage in rats (modified Lashley III maze (13), was the third test in

the series. Animals received three 5-min trials (total allowable time) in a single test session and latencies, cul-de-sac entries and alley retraces were measured. All animals were tested on the same performance measure before beginning the next test in the series.

Results and Discussion. Mean growth rate for a period of 8 weeks was 12.3 ± 3.1 (SD) g/week for the zinc-deficient group, while the pair-fed control subjects gained an average of 16.7 ± 2.8 g/week ($F = 3.61$; degrees of freedom = 1 and 22; $p < .005$). At time of testing (viz., 78 days), nonsupplemented animals exhibited a moderately severe zinc deficiency, but were not morbid.

Reduced emotionality levels (viz., increased motor activity) were observed for zinc-supplemented subjects for the 3 days of testing in the open-field apparatus (see Fig. 1, mean = 82.52 and 144.58 squares traversed/day for 3 consecutive days for zinc-deficient and supplemented groups, respectively; $F = 4.62$; degrees of freedom = 1 and 22; $p < .05$). Group differences for the first test

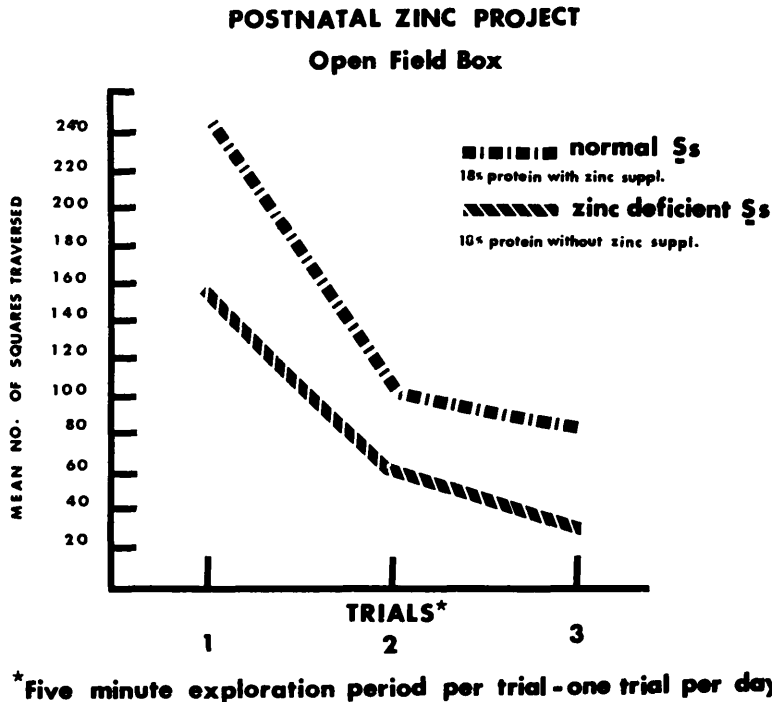


FIG. 1. Mean number of squares traversed for each of three daily 5-min test periods on the open-field test for subjects receiving either a zinc-supplemented ($N = 12$) or nonsupplemented ($N = 12$) soy protein diet for 48 days commencing at time of weaning.

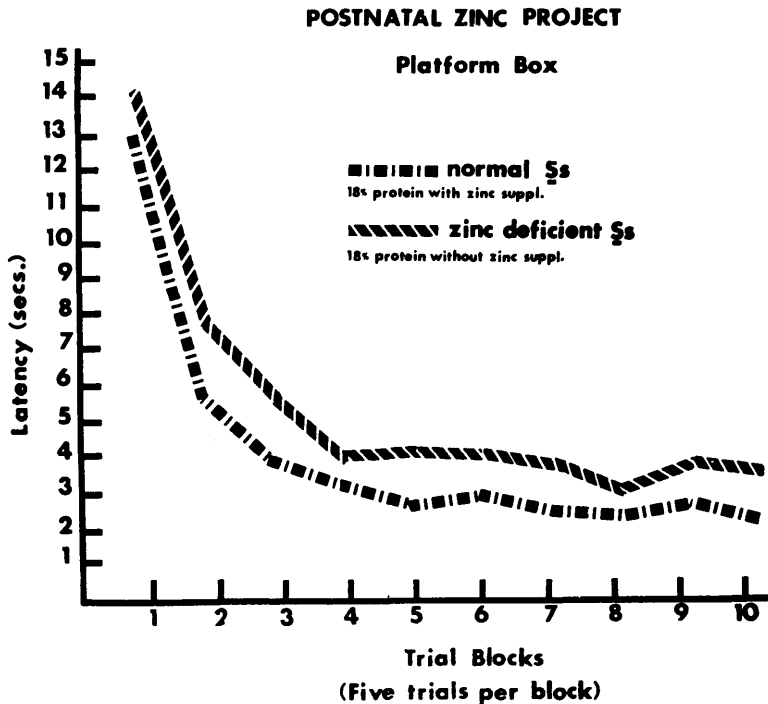


FIG. 2. Mean response latency on the one-way conditioned avoidance test for subjects receiving either a zinc-supplemented ($N = 12$) or nonsupplemented ($N = 12$) 18% soy protein diet for 48 days commencing at time of weaning.

day, when emotionality might be expected to be greatest, revealed greater exploration by supplemented subjects (mean = 248.92 squares) than for zinc-deficient animals (mean = 157.58 squares; $t = 3.25$; $.01 > p > .001$)

Figure 2 depicts the mean response latency for both treatment groups on the conditioned avoidance test. Although zinc-supplemented subjects were superior in performance when compared to nonsupplemented animals, group differences were not significant (mean for total 100 trials = 4.06 and 5.19 sec, respectively; $F = 3.95$; $df = 1$ and 22 ; $.10 > p > 0.05$). Figure 2 reveals maximum rate of learning occurred for both groups during the first and second trial blocks (*viz.*, trials 1–10). An analysis of this period revealed the zinc-supplemented subjects performed significantly faster than nonsupplemented animals (mean for trials 1–10 = 9.2 and 10.7 sec, respectively; $F = 4.82$; $df = 1$ and 22 ; $.05 > p > .025$). A significantly larger proportion of conditioned responses were recorded for

zinc-supplemented animals ($X^2 = 21.04$; $p < .001$) computed for all trial blocks. Mean response latencies for performance on the Lashley III water maze for zinc-deficient and control subjects are shown in Fig. 3. Zinc-deficient animals were notably slower in traversing the maze than supplemented subjects (mean = 161.22 versus 102.72 sec, respectively; $F = 10.92$; $df = 1$ and 22 ; $p < .005$) and made significantly greater numbers of both cul-de-sac and retrace errors between the first and third maze trials compared to zinc-supplemented subjects (mean = 9.00 and 3.33 errors for zinc-supplemented subjects first versus third trial; $t = 4.90$; $p < .001$; and mean = 9.58 and 9.75 errors for zinc-deficient subjects first versus third trial).

Marked lethargy was observed in all zinc-deficient animals by the time testing was begun. This fact lead us to predict that response latencies would be greater in all tests for zinc-deficient subjects and the data confirm this point. However, it is difficult to assess how much of the performance decre-

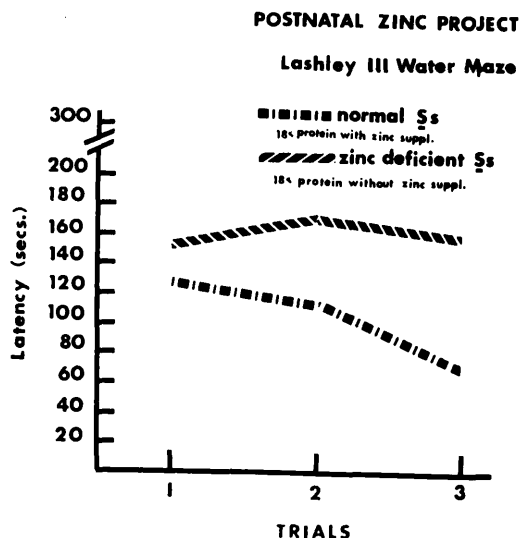


FIG. 3. Mean latency to traverse the Lashley III water maze on each of 3 trials for 3 consecutive test days. Subjects received either a zinc-supplemented ($N = 12$) or nonsupplemented ($N = 12$) 18% soy protein diet for 48 days commencing at time of weaning.

ment of zinc-deficient animals was the result of a reduced level of motivation or a reduction in *intrinsic* learning ability since both factors are inextricably related. Performance for both treatment groups improved on the conditioned avoidance test, although the significantly greater proportion of avoidance responses for the zinc-supplemented group indicates superior learning ability for these subjects. However, both latency and errors data for the Lashley III maze indicated superior learning for zinc-supplemented subjects throughout the duration of testing when compared to zinc-deficient animals.

This investigation has clearly demonstrated that change in behavior is discernible between animals administered either a low or adequate level of zinc in their diet and, furthermore, for those behaviors measured in this investigation, performance of zinc-deficient animals were generally impaired. The mechanism(s) whereby an acute zinc deficiency is detrimental to behavior is beyond the present scope of this investigation. Research has shown a reduction in synthesis and/or utilization of protein following zinc insufficiency (7). In light of this finding, it is

noteworthy that impaired performance has been observed in animals maintained on low protein diets to which zinc was not supplemented (14, 15). The recent discovery of the zinc complexing property of phytate (16), a naturally occurring substance generally found in greatest concentration in plant seeds, and in some roots and tubers, poses the question whether exclusive plant protein diets (*e.g.*, soy protein) without zinc supplementation may contribute to the development of maladaptive behavior in the organism. The syndrome of lethargy and reduced learning capacity consequent to an inadequate zinc level clearly warrants further investigation.

Summary and Conclusions. Behavioral measures were obtained for rats administered a zinc deficient diet, (8.0 mg/kg) and adequate in all other constituents, for 48 days beginning at 30 days of age. Pair-fed controls received a zinc-supplemented diet (70 mg/kg of total zinc). Lethargy and reduced weight gain were characteristic of all zinc-deficient subjects. Performance on two measures of learning ability (*viz.*, a one-way conditioned avoidance test and an eight-blind water maze) and an test of activity-emotionality (*viz.*, the open-field test) revealed zinc-deficient animals to be inferior when compared to zinc-supplemented subjects. The relationship between zinc and protein utilization was posited as a possible mechanism explaining the effects of zinc insufficiency.

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