

Influence of Zinc Deficiency on Behavior¹ (37661)

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The protein-calorie malnutrition (PCM) syndrome results from multiple deficiencies. One of the deficiencies which may occur is zinc deficiency. The significance of zinc deficiency in patients with PCM is ill-defined. It is known, however, from animal studies that the element is essential for growth of the brain via its role in nucleic acid and protein synthesis (1-3).

Studies in animals to assess the adverse effects of PCM on behavior have primarily been focused on protein and calories and have not been addressed to the effects of deficiencies of other nutrients which are often deficient in PCM and which are necessary for utilization of dietary protein. We therefore have studied the effects of deficiency of one such nutrient, zinc, on behavioral development of the rat.

Methods and Materials. Six pregnant Sprague-Dawley rats were divided at delivery into 3 groups (zinc-deficient, pair-fed, and *ad libitum*-fed), housed in plastic cages with perforated bottoms, and fed a zinc-deficient diet containing < 1.0 ppm zinc (3) along with glass-distilled water from the day of delivery. Zinc chloride was added to the drinking water (50 μ g of Zn/ml) of the pair-fed and *ad libitum*-fed dams. Each pair-fed dam was fed an amount of diet equivalent to what her counterpart in the zinc-deficient group ate on the previous day. As a consequence of this procedure, the pups of the pair-fed dams experienced starvation during the first 21 days of life and showed growth retardation which was of similar magnitude to

that which occurred in the pups nursed by zinc-deficient dams (Fig. 1A). The *ad libitum*-fed dams were allowed unlimited food. Accordingly, growth of their pups was much greater than that of pups nursed by either pair-fed or zinc-deficient dams. The zinc-deficient and pair-fed dams nursed from 6-9 pups each.

All pups were weaned at 21 days of life, fed Purina chow *ad libitum*, and given tap water. Nutritional rehabilitation was continued until 44 days of age at which time the rats appeared healthy and behavioral studies were begun on groups of rats selected from the respective litters. There were ten rats each in the previously zinc-deficient and pair-fed groups and four in the *ad libitum*-fed group. This was in sharp contrast to the procedure used by Caldwell *et al.* (4) who studied the adverse effects of zinc deficiency on maze acquisition of 78-day-old rats while they were being fed a zinc-deficient diet. Caldwell was unable to determine whether the inferior maze performance of the zinc-deficient rats was due to illness and associated poor motivation or whether the rats had an intrinsic learning disability.

In the study reported here, the nutritionally rehabilitated animals and the controls underwent a training period during which they ran a 6-ft elevated runway 4 trials a day for 3 days after being deprived of food for 23 hr. Food in the form of a wet mash was placed at the end of the runway and each rat was allowed to eat for 15 sec; then it was placed at the start of the runway for the next trial. When each rat finished its 4 trials, it was fed for 45 min in a separate cage and then returned to its home cage. At the completion of this preliminary runway training,

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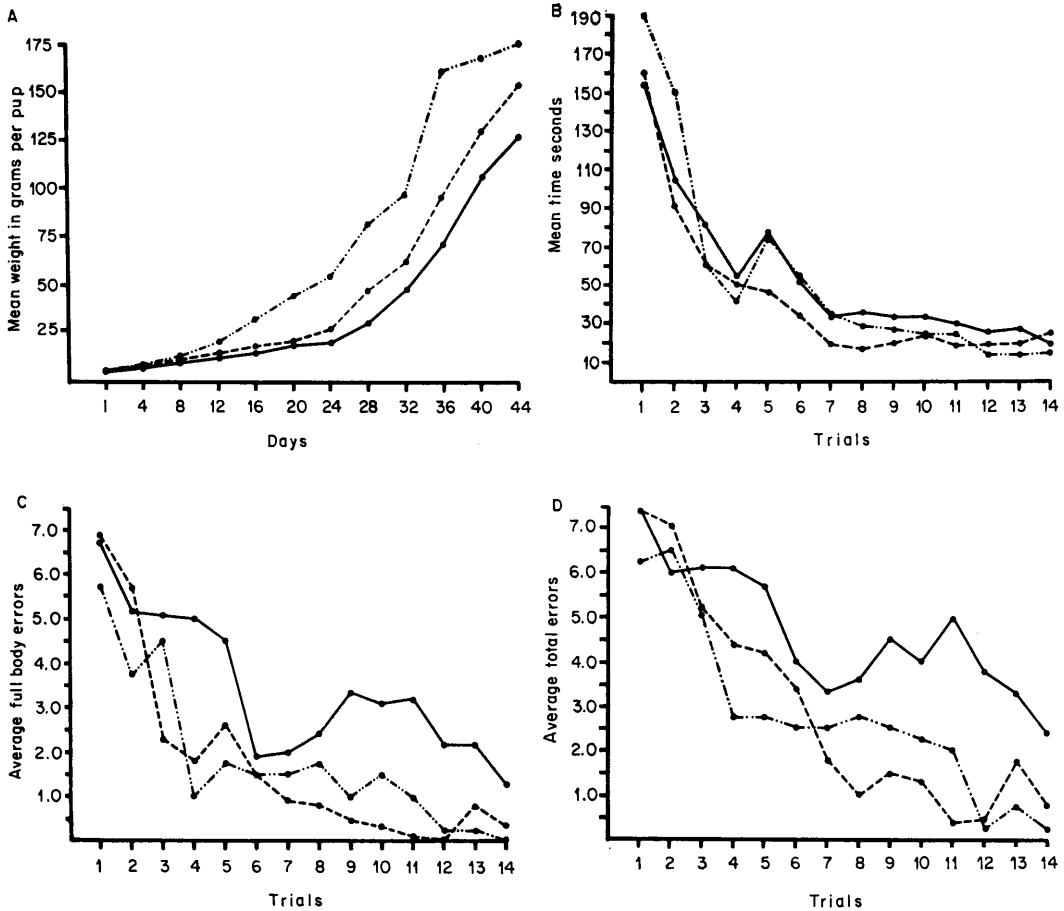


FIG. 1A. The weight gains of the 3 groups until the start of the behavioral training. Note the similarity of weight gain for the zinc-deficient and pair-fed groups during the first 20 days of life. The similarity of running time shown in B suggests that the level of motivation was the same for all 3 groups. C shows the average number of full body errors made by the 3 groups, and D shows the average total number of errors made by the 3 groups. (●—●) Zinc-deficient Group, (●---●) Pair-fed Control Group, (●····●) *Ad Libitum* Control Group.

the rat began training on the 14-choice point, elevated Tolman-Honzik maze. Each unit of the maze was 2 ft long, 2 in. wide, and 18 in. high. The cul-de-sacs were 6 in. deep. The units were painted flat black. After each day's trials, the maze was disassembled and wiped clean. The maze was reassembled the next day prior to the training trials. The elevated 6-ft runway noted above was constructed of 3 units of the maze. The rats were given 1 trial per day for 14 consecutive days and were deprived of food for 23 hr prior to the start of each trial. The elapsed time from the start of the trial to the moment the rat touched the food at the end of the maze

was recorded. The rat was allowed to eat for 15 sec and then placed in a separate cage for 45 min with food before being returned to its home cage. Five types of errors were recorded: hesitation error, the rat paused before choosing the correct turn; full body error, the rat entered the cul-de-sac completely; $\frac{3}{4}$ body error, the rat entered the cul-de-sac as far as the rear legs; $\frac{1}{2}$ body error, the rat entered the cul-de-sac as far as the waist; and $\frac{1}{4}$ body error, the head and front feet were in the cul-de-sac. This again is in contrast to methods used by others as reported in abstract (5) in which latency was the only measure used to investigate the effects of zinc

deficiency on maze learning of rehabilitated rats. In general, error scores are regarded by most investigators as the best measure of maze learning (6). In the study reported here, a 2-way analysis of variance was computed for running time, total errors, and full body errors.

Results. The results show that rats made zinc deficient during the critical period for brain growth did not learn the maze as quickly as those that had been starved or adequately fed during the first 20 days of life. The animals that had experienced zinc deficiency made more full body errors and total errors than either of the 2 control groups (Fig. 1C, D). For full body errors, the slope of the regression line for the zinc deficient group was significantly different from that for the pair-fed group ($F = 10.55$, $df = 1$, 216; $p < 0.01$) and for the *ad libitum* control group ($F = 15.79$, $df = 1$, 151; $p < 0.01$). There was no significant difference between the slope of the regression lines for the pair-fed and *ad libitum* control groups. For total errors, the slope of the regression line for the zinc-deficient group was significantly different from that for the pair-fed group ($F = 11.26$, $df = 1$, 216; $p < 0.01$) and for the *ad libitum* control group ($F = 17.31$, $df = 1$, 151; $p < 0.01$). The F -ratio between the pair-fed and *ad libitum* controls was again not significant. Although the zinc-deficient group was somewhat slower than either of the 2 control groups (Fig. 1B), the time differences were not statistically significant. The slower running time of the zinc-deficient group can be readily attributed to the greater number of errors.

Discussion. The findings were consistent with the concept that zinc deficiency during the critical period for brain growth results in a residual impairment in behavior. This

was in striking contrast to the effect of starvation which was sufficient to obviously retard growth. It cannot be stated with certainty that the effect of the deficiency was on learning because of the complex factors which may influence the performance of a rat on a maze. Motivation and emotional stability may influence the results of an experiment such as we are reporting. Because of the known effects of zinc deficiency on nucleic acid and protein synthesis, and upon brain maturation as assessed by total lipid concentration (1-3), it seems reasonable to presume that brain maturation was retarded in the zinc-deficient rats and that this retardation had adverse effects on their ability to acquire the maze.

Summary. Acquisition of an elevated Tolman-Honzik maze was tested in rats that had been subjected to zinc deficiency from birth through 21 days of age and then adequately fed for 23 days. Their maze acquisition was impaired compared with that of rats that had been nursed by pair-fed dams or by *ad libitum*-fed dams.

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