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BRIEF COMMUNICATION

Postweaning Copper Restriction and Behavior in the Long-Evans Rat[1]

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THORNE, B. M., K.-N. LIN, M. L. WEAVER, B. N. WU AND D. M. MEDEIROS. *Postweaning copper restriction and behavior in the Long-Evans rat.* PHARMACOL BIOCHEM BEHAV **19**(6) 1041–1044, 1983.—A variety of behaviors was assessed in Long-Evans male rats placed on either a low copper diet, a marginal copper diet, or an adequate copper diet at weaning. Rats in the low copper group had slightly, but significantly, enlarged hearts and gained less weight than rats fed diets containing higher copper levels. Treatment effects were not detected in measurements of muricide, open-field activity, water intake, shock sensitivity, and shock avoidance and memory.

Copper deficiency Muricide Open-field activity Shock avoidance learning Weight gain Water intake

DESPITE the importance of dietary copper for the growth and development of mammals, reports of the behavioral effects of dietary copper manipulation are virtually nonexistent. Of possible relevance are reports of altered copper levels in certain psychiatric and/or behavior disorders. For example, Tyrer, Delves and Weller [14] found that schizophrenics had lower cerebrospinal fluid levels of copper than controls. Of course, there is little evidence to support the idea of a causative relationship between copper levels in diet, CSF, or blood and any of the disorders in which reduced levels have been seen.

Of possible relevance to the issue of the behavioral effects of dietary copper restriction are studies in which rats have been treated with D-penicillamine (D-pen), a substance promoting the excretion of copper. For example, Zawilich [15] fed adult male rats diets including one containing 1% D-pen and another combining both copper deficiency and 1% D-pen. The D-pen rats were anemic, lost weight and showed increased intake of NaCl, KCl, and NH_4Cl. The taste preference changes were not caused by a change in sensitivity.

In another investigation of the effects of D-pen on taste in rats, Ito [4] fed weanling males D-pen in several concentrations. Animals fed the highest concentration (1%) had a retarded growth rate, increased water intake (expressed as ml/g body weight), and a significant decrease in serum copper level. No changes in taste preferences were seen, and there were no differences in chorda tympani nerve responses to taste stimuli.

Dietary copper deficiency can have an effect on brain and body growth and on certain neurotransmitter levels. Prohaska and Smith [10] studied the offspring of rat and mouse dams maintained throughout gestation on reduced copper levels. A large growth deficit was found in addition to neural changes such as impaired brain growth, low brain copper levels, and decreased norepinephrine. No change in dopamine level was found and behavioral effects were not examined.

The purpose of the present study was to investigate the longitudinal effects of dietary copper deficiency on a variety of behaviors in the rat. Thus, rats maintained from weaning on purified diets containing either low copper, marginal copper, or adequate copper were assessed on muricidal behavior, open-field activity, water intake, shock sensitivity and a learning and memory task. In addition, weekly weight measurements were taken to assess growth in the animals.

METHOD

Subjects

The subjects were 74 Long-Evans male rats. At approximately 21 days of age, the litters were weaned, each animal was weighed, and the rats were randomly assigned to one of three different treatment groups. There were no significant differences between groups in weight at weaning. From weaning until sacrifice, all rats were kept in stainless steel cages measuring 24.5×17.8×18 cm in a room with a 12:12 light timing cycle.

Animals were fed a purified diet with either adequate copper (8 mg Cu/kg feed, Group AC), marginal copper (3 mg Cu/kg feed, Group MC), or low copper (Group LC). The

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concentrations of copper in Groups AC and LC were identical to those used by Allen, Hassel and Lei [1]. Analysis of the diets by atomic absorption spectrophotometry (AAS) revealed that they averaged 0.7 ppm, 3.9 ppm, and 9.1 ppm Cu for the LC, MC, and AC diets, respectively [11].

Apparatus

Activity was measured in a box measuring 76.2×76.2×25.4 cm. The box was painted flat black, with white lines dividing the floor into 25 equal squares. Wire mesh covered the top.

Shock sensitivity was assessed in a Plexiglas box with a grid floor and interior dimensions of 28.9×8.3×12.5 cm. Shock was delivered by a shock generating device (Lafayette Instruments) calibrated in milliamperes.

Avoidance training was performed in a Thompson-Bryant box without choice chamber, leaving a startbox and goalbox. For further details of the training apparatus, see Thorne, Wallace and Danzig [13].

Procedure

All measurements were made during the light portion of the L-D cycle. Each animal was weighed on Friday throughout the study. In addition, the rats' open-field activity was measured at 3-week intervals on Fridays beginning at approximately 42 days of age. Each animal was placed into the center of the open-field box, and the number of squares crossed in a 1-min period was recorded. Elevation of the front half of the body (rearing) was also counted.

The rats' water intake was measured for 5 consecutive day periods at 4-week intervals beginning at approximately 8 weeks old. A daily average was computed for each animal after the 5-day period.

A muricidal test was given at approximately 10 weeks of age and consisted of placing an adult albino mouse into each rat's home cage. Continuous observation was conducted for the first 20 min of the test with killing latency noted during this period. If the rat failed to kill during the observation period, a check was made after 24 hr and any live mice or the remains of dead ones were removed. A 24 hr latency was given to kills after the observation period.

The shock sensitivity test, modified from Lints and Harvey [6], was given to 26 rats (Groups LC and MC, 9 each; Group AC, 8) approximately 10 days after the muricidal test. In it, each rat was placed into the apparatus and 4 series of brief shocks were delivered in alternating ascending and descending order beginning with an ascending series. The magnitudes presented were 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 mA. There was a 30-sec intershock interval with a 2-min interval between series.

The responses recorded were either no response, a flinch response (startle, hind feet remain on grid), or a jump response (both hind feet leave the grid). Flinch and jump thresholds were determined by computing the average shock magnitude producing either the first response on an ascending series or the last response on a descending series.

Approximately one month after the shock sensitivity test, avoidance training began for the animals tested for shock sensitivity. Prior to training, the minimum current producing a jump response was determined in the startbox. This current was used for both preliminary training to push aside a gray card to gain access to the goal box and for training on the black-positive, white-negative discrimination. For further details of the avoidance training procedure, see Thorne *et al.* [13].

With the exception of one Group LC rat which died, all subjects were tested for retention two weeks after original learning. The retention test required relearning the original discrimination and a savings score (SS=OL−RL/OL × 100) was computed for each animal.

Following retention testing, animals were sacrificed; hearts, livers, and brains were removed for copper content analysis; and blood samples were taken by cardiac puncture to test for anemia. Tissue and blood samples were not available for two subjects in Group LC. Brain, heart, and liver tissues were analyzed for copper by AAS [8].

RESULTS

Organ Weight and Copper Content Analysis

An analysis of variance performed on the liver concentrations of copper was not significant although the means were in the right direction (Group LC, N=7, mean=3.38 μg/g wet weight; Group MC, N=9, mean=4.15; Group AC, N=8, mean=4.32). When liver weights were expressed as a function of body weight, the groups were not found to differ.

When heart weights were expressed as a function of body weight, the groups were found to differ, $F(2,21)=4.6$, $p<0.05$. Individual comparisons using the protected t-test [3] revealed that both Groups MC and AC differed from Group LC, LC vs. MC, $t_p(21)=2.29$, $p<0.05$; LC vs. AC, $t_p(21)=3.05$, $p<0.01$, but not from each other. The group averages were as follows: Group LC, 0.0034; Group MC, 0.0028; Group AC, 0.0027.

Although Group LC had the smallest mean, the groups did not differ significantly with respect to blood hematocrit levels (Group LC, 36.7%; Group MC, 42.4%; Group AC, 42.7%). The lack of significance was probably due to the small sample sizes available for this comparison (LC=3; MC and AC, 6 each).

The groups did not differ in brain concentration of copper, and the averages in μg/g wet weight were as follows: Group LC, 3.42; Group MC, 4.41; Group AC, 3.74.

Weight Gain

Figure 1 shows the average weekly weights for each group. Study of the graph reveals that the weekly averages were virtually identical at weaning and at the first weight period but were clearly separate throughout the rest of the study with Group LC having the smallest weight gain followed by Groups MC and AC, in that order. A 1-way ANOVA at each weight period revealed significant differences for weeks 9 and 10, $F(2,69)=3.47$, $p<0.05$; $F(2,69)=4.45$, $p<0.05$, respectively. A posteriori tests (Tukey LSD) following the significant F-tests showed that for week 9 there was a significant difference between Groups LC and AC. For week 10 both Groups MC and AC differed from Group LC but not from each other.

Behavioral Tests

There were no significant differences between groups on any of the following behavioral tests: open-field activity, squares crossed or rearing; water intake; muricide; shock sensitivity; learning and memory.

Incidental Observations

Although no quantification is available, some observations are worthy of note. First, there appeared to be much greater food spillage by animals in Groups LC and MC than

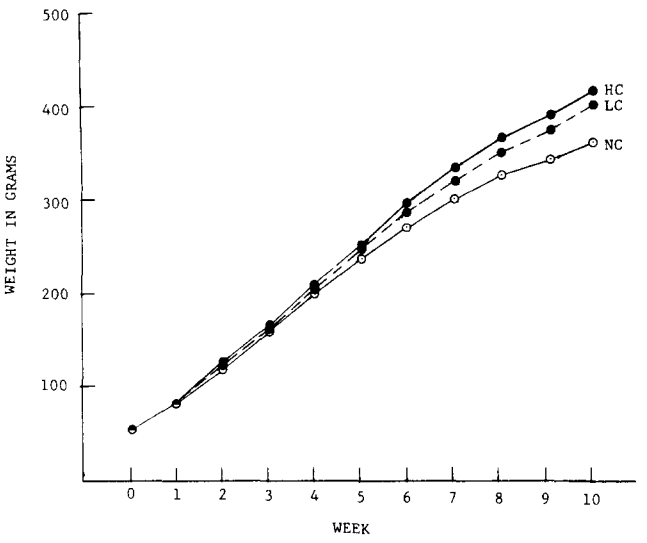

FIG. 1. The average weight in grams by week of the study. Week 0 was the time of weaning.

by animals in Group AC. This observation is probably related to the reduced weight gain noted particularly in Group LC. In addition, after several weeks on the LC diet, a few animals reduced their food intake precipitiously and either failed to gain further weight or actually began to show slight losses.

Rats in Groups LC and MC seemed to have much less pigmentation in their hair than did animals in Group AC. This was particularly true for Group LC subjects who often appeared gray relative to animals on the adequate copper diet.

DISCUSSION

With the exception of weight gain, there was no evidence for behavioral change as a result of the copper deficient diets used in the present study. The LC diet was marginally effective in inducing physiological changes, and Group LC animals were found to have slightly, but significantly, enlarged hearts. Cardiac enlargement has been noted in a previous study of copper deficiency in rats [5].

Liver concentration was not significantly different between groups although Group LC had the lowest mean followed by Groups MC and AC, respectively. Blood hematocrit levels were lower in Group LC also, but the difference was not significant probably due to the small sample size for this analysis. In addition, although we were unable to quantify it, several of the animals in Groups LC and MC seemed lighter in color. Loss of pigmentation is a frequently observed symptom of copper deficiency [7].

The failure to gain weight normally with lowered copper levels has been noted by other investigators [4, 12, 15]. Although the group average for LC animals clearly began to lag behind that for the other groups by the second week of the study, because of the variability in scores the differences were not significant until the last two observation periods.

Although no attempt at quantification was made, we did observe an increase in food spillage by rats on the copper deficient diets. This might reflect either unpalatability of the diets, a change in taste sensitivity or preference in the rats, anorexia, or some combination of factors. The studies by Ito [4] and Zawilich [15] would seem to rule out changes in either taste sensitivity or taste preferences in the present study. In addition, if the copper deficient diets were extremely unpalatable, it would seem that they would have been rejected very early in the study, i.e., upon first exposure. It was our impression that the spillage became much more noticeable after the animals had been on the diets for several weeks. Thus, there remains the possibility that the copper deficient animals became more anorexic with continued exposure to the diets.

Although Ito [4] observed an increase in water intake when expressed as a function of body weight in animals with lowered serum copper, no treatment differences in water intake were seen in the present experiment. This discrepancy is quite possibly due to methodological differences between the experiments since Ito used D-pen to lower copper levels while our rats became copper deficient following dietary restriction. That is, it is quite possible that one of the side effects of D-pen administration is an increase in thirst quite apart from its effect of lowering serum copper; e.g., D-pen can induce a zinc deficiency which may influence taste and/or water consumption.

It is possible that we failed to observe behavioral changes with long term copper deficiency because the weanling rat brain is sufficiently developed to be resistant to the deleterious effects of the diet. In addition, our diets did not induce the magnitude of physiological change observed by others [2, 5, 9]. Prohaska and Smith [10] studied the offspring of animals maintained throughout gestation on reduced copper levels and found a variety of neural changes including impaired brain growth, low copper levels in the brain, and decreased norepinephrine. Thus, one possibility for future study is to assess the behavior of rats subjected to reduced copper intake prior to weaning.

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