Zinc Deficiency: Effects on Brain and Behavior of Rats and Rhesus Monkeys

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The importance of adequate zinc nutrure for normal growth of the rat fetus was suggested by work of Newell and McCollum in '33, but was not established until 33 years later by Hurley et al., '66. Subsequent work has confirmed and extended her observations (Swenerton et al., '69; Cox et al., '69; Apgar, '68; Warkany and Petering, '72; Apgar, '72; Hurley and Shrader, '72; McKenzie et al., '75; Halas and Sandstead, '75; Halas et al., '75). While all tissues of the body were affected by zinc deficiency, the adverse effects on the nervous system (Hurley and Shrader, '72) were particularly intriguing to us, and prompted some of the experiments described in this paper.

In part, because of the effects of zinc deprivation on fetal rats, the requirement for zinc in human pregnancy was examined and found to be substantial (as much as 750 µg retained daily during the latter half of gestation) (fig. 1) (Sandstead, '73). It thus became apparent that some women in the United States (and probably elsewhere) might not consume sufficient, readily available dietary zinc to meet the needs of pregnancy (Anonymous, '73; White, '75).

Based on the finding of zinc deficiency in adolescents in the Middle East (Sandstead et al., '67; Halsted et al., '72), it was suggested by Sever and Emmanuel ('72) that some of the human teratology which occurs in that region might be due to zinc deficiency. In support of this hypothesis are reports of decreased concentrations of zinc in plasma in pregnancy (Henkin et al., '71; Metcalf et al., '76; Jameson, '76), an apparent relationship between decreased concentrations of zinc in amniotic fluid and decreased fetal size (Pavier et al., '72), and the finding that zinc nutrure is apparently one of the factors which influence the outcome of pregnancy (Metcalf et al., '76; Jameson, '76). According to Jameson ('76) there is a strong association between complications in pregnancy and low levels of plasma zinc during the first trimester, and there seems to be a higher incidence of congenital malformations in the infants of women who have low concentrations of plasma zinc during the first trimester. Observations by Metcalf et al. ('76) suggest that maternal zinc nutrure influences fetal size.

We, therefore, decided to study the adverse effects of zinc deprivation, limited to the latter third of pregnancy, on the brain and subsequent brain function. Observations made on rats (McKenzie et al., '75; Halas and Sandstead, '75; Halas et al., '75) and non-human primates (Sandstead et al., '76) are the subject of this report.

METHODS

To assess consequences of zinc deprivation in pregnant rats during the last 7 days of pregnancy (McKenzie et al., '75), a 20% sprayed-egg-white, biotin-enriched diet containing <1 µg of zinc/g was fed. Fetuses were taken for chemical analysis on the 21st day of gestation and brains were analyzed for DNA, RNA, and protein.

For behavioral studies (Halas and Sandstead, '75; Halas et al., '75) the zinc deficient diet was started on the fourteenth day and commercial feed was resumed on the twentieth day. The litters were culled to the eight most viable pups. In all experiments, three groups were studied. The zinc-deficient group (ZD) was given the diet ad libitum and glass distilled/deionized water. Rats in the second group, pair fed (PF), were individually paired with animals in the first group. Each PF animal was given an amount of diet equiva lent to that eaten by its pair mate the previous day. Also, each PF animal was given 25 µg
of zinc/ml of drinking water. The third group (AL) was given the diet ad libitum and 25 μg of Zn/ml of drinking water.

Behavior was assessed in rat offspring after they reached young adulthood. Male and female offspring were tested for active avoidance (McKenzie et al., '75; Halas et al., '76) in a shuttle box housed in a light and soundproof box. The data were collected electronically. Aggression was also studied. Pairs of 75-day-old offspring were placed in a plastic cage with metal grid floor through which random, short, low-level shocks were given (Halas et al., '75). The responses were recorded on videotape and, subsequently, viewed by a panel of three judges to ascertain whether aggression had occurred. As the momentary aggressions during the shocks were obvious, the judges agreed 97% of the time.

The studies on non-human primates were done on 12 Rhesus mothers and their infants (Sandstead et al., '76). A diet containing appropriate levels of vitamins and minerals and otherwise similar to the ZD rat diet was fed from the 110th to 150th day of pregnancy. Four PF and four AL dams were each given 50 μg of zinc/ml of drinking water. The maternal-infant interactions were recorded during 22 separate 10-minute observation sessions through a one-way mirror at intervals throughout the nursing period. The data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

Zinc deprivation in rats resulted in anorexia and weight loss (McKenzie et al., '75). Effects on the PF dams were less severe than those on the ZD dams. The differences in food consumption and weight loss were significant among the groups. ZD resulted in a marked decrease in maternal serum zinc and an increase in maternal hematocrit, which presum-
FETAL WEIGHT

Fig. 2 Weight of 21-day old rat fetuses delivered by cesarean from zinc-deficient, pair fed, and ad libitum fed dams (McKenzie et al., ’75).

BRAIN WEIGHT

Fig. 3 Weight of brains of fetuses of figure 2.

PROTEIN/DNA

Fig. 4 DNA content of brains of fetuses of figures 2 and 3.

Fig. 5 Protein/DNA ratios of brains of fetuses of figures 2, 3 and 4.
ably reflected a decrease in plasma volume. Fetal weight (fig. 2) was decreased by ZD; intra-uterine undernutrition due to PF also decreased fetal weight, but to a lesser degree. Fetal brain weight (fig. 3) was also decreased by ZD; pair feeding had an intermediate effect.

Analysis of brains for total DNA (fig. 4) revealed a decrease in the pups of ZD dams, but not in pups of PF dams. Total RNA and protein were not significantly affected, but the ratio of protein to DNA (fig. 5) was increased in the brains of ZD pups. These studies show that zinc deprivation during the latter third of pregnancy has adverse effects on rats which impair cell division during the period of maximal macroneuronal replication.

At birth, pups of the ZD dams were smaller than pups of the PF or AL fed dams. As the subsequent growth patterns were similar, ZD pups did not catch up with the other two groups.

Active avoidance was impaired in ZD males exposed to 1 mA shock. ZD males also displayed an apparent increased sensitivity to shock which resulted in a decline in learning (fig. 6) (Halas and Sandstead, '75). When exposed to 0.8 mA shock, the ZD males again displayed impaired avoidance (Halas et al., '76). At this level of shock, the offspring of the PF dams displayed avoidance superior to that of the AL offspring. When ZD female litter mates were tested, they displayed normal avoidance, and were superior to their male litter mates (fig. 7). Aggression was higher in 75-day-old ZD female offspring than in either of the two con-

Fig. 6 Avoidance conditioning of male offspring of rat dams deprived of zinc or pair fed or ad libitum fed during the latter third of pregnancy (Halas and Sandstead, '75).

Fig. 7 Avoidance conditioning of male and female litter mate offspring deprived of zinc during the latter third of pregnancy (Halas et al., '76).
Fig. 8 Aggression of adult (75-day-old) female offspring of rat dams deprived of zinc or pair fed or ad libitum fed during the latter third of pregnancy (Halas et al., '75).

PLASMA ZINC

Fig. 9 Mean plasma zinc concentrations of pregnant Rhesus monkeys exposed to zinc deprivation from day 110 to 150 of pregnancy compared to plasma zinc concentrations of four ad libitum fed controls (Sandstead et al., '76).

trol groups (fig. 8). Increasing the level of shock caused an increase in aggression in the offspring of the pair-fed dams.

These observations have been replicated and extended to include 105-day-old female and male offspring. In the replicate study similar findings were observed in 75-day-old females. At 105 days of age, the level of aggression in the ZD females was higher than at 75 days. In ZD males, increased aggression was not observed at 75 days of age; however, at 105 days, a significant (P < 0.05) increase in aggression occurred when 1.3 mA shock was given.

These studies have revealed a sex difference in response to intra-uterine zinc deprivation. Males displayed impaired active avoidance but females did not. On the other hand, females displayed increased aggressiveness at both test ages, but males displayed increased aggressiveness only at 105 days.

Zinc deprivation in pregnant Rhesus monkeys resulted in the appearance of a rash, loss of hair and a decrease in plasma zinc to very low levels (fig. 9) (Sandstead et al., '76). The PF mothers tolerated the experiment poorly. Two aborted and one had an early neonatal death. One ZD mother refused her infant. That infant was cross fostered to the PF mother whose new born infant died the same day.

After giving birth, the AL mothers displayed a greater weight loss than did the ZD mothers (P < 0.05). Weight gain of the ZD
infants was more rapid than that of the AL infants (P<0.01). Presumably, this finding resulted from more nursing by the ZD mothers.

By analysis of variance the ZD infants were found to associate with their mother a significantly greater percent of the time that did the AL infants (P = 0.044). They also appeared to be in contact with the mother more (P = 0.160) and to nurse a greater percent of the time (P = 0.280) than the AL infants. Their play frequency was significantly less (P = 0.021) and they displayed significantly less exploratory behavior (P = 0.045) than the AL infants.

From these studies on experimental animals we hesitate to suggest that marginal or mildly deficient zinc nutriture during pregnancy would have adverse effects on the brain and subsequent behavior of human infants. Though we produced abnormalities in the primate offspring, the dams were subjected to severe zinc deficiency. Studies have not been done as yet on effects of marginal zinc deprivation on behavior. Reports consistent with marginal to deficient dietary intakes of zinc in some pregnant women have been cited (Sandstead, ’73; Anonymous, ’73; White, ’75) however, and a hypothesis (Sever and Emanuel, ’72) relating adverse zinc nutriture in Middle Eastern populations to increased levels of teratology, and supporting literature were noted in the introduction (Sandstead et al., ’67; Halsted et al., ’72; Henkin et al., ’71; Metcalf et al., ’76; Jameson, ’76; Favier et al., ’72). Though we have reservations about extrapolating animal experiments to man, we believe it prudent for women to consume levels of zinc during pregnancy sufficient to meet the World Health Organization guidelines (Anonymous, ’73).