Plasma Zinc and Leukocyte Changes in Weanling and Pregnant Rats during Zinc Deficiency* (32971)

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Previous work (1,2) has shown that a relatively short period of dietary zinc deficiency in pergnant rats leads to a high incidence of congenital malformations in the offspring. In addition, it was demonstrated that growth ceases in weanling rats within a few days after the beginning of a zinc-deficient regime (3). The rapidity with which these effects were induced suggested that a physiological state of zinc deficiency occurred in rats before there was any significant depletion of the body zinc deposits.

In the present investigation, plasma zinc levels were studied in an attempt to establish the rate at which zinc becomes limiting in rats deprived of this element. In addition, since zinc is reported to occur in particular abundance in the white cells (4) an investigation was made into the effect of zinc deficiency upon the concentration of zinc in the leukocytes of adult rats and the total and differential leukocyte counts.

Methods. Virgin female (210 ± 10 gm) and weanling male (50± 5 gm) rats of the Sprague-Dawley strain were purchased from a commercial source. The weanling males were given the experimental diets on arrival, whereas the adult females were fed the zinc-supplemented (control) diet for a few days while mating was effected. All animals were housed individually in stainless steel cages and stringent precautions were taken to eliminate zinc contamination from the environment (3).

The basal zinc-deficient diet consisted of, in percent, soybean protein,[1] 30.0; sucrose, 57.3; corn oil, 8.0; salt mix,[2] 4.0; and DL-methionine, 0.7. The soybean protein was treated with ethylenediaminetetraacetic acid (EDTA) to lower its zinc content (3,5), and the entire ration was assayed by atomic absorption spectroscopy and found to contain 0.36 ± 0.05 ppm of zinc. Crystalline vitamins[3] were given separately. The control animals received the same diet supplemented with 100 ppm of zinc as zinc carbonate.

Pregnant adult rats were given the experimental diets on day zero of gestation so that the number of days of pregnancy corresponded to the number of days on the experimental regime. Matings were carefully timed by observation of sperm in the daily vaginal smear; the day of finding sperm was considered day zero of gestation.

Pair-fed (control) animals received daily amounts of the zinc-supplemented diet that were equal to the food intake of their paired, zinc-deficient counterparts for the previous day.

Paired-weight (control) weanlings received amounts of the zinc-supplemented diet that were adjusted daily in order to ensure that their body weight changes paralleled those of their paired, zinc-deficient counterparts. This group was included in order to provide inanition controls more comparable to the anorexic, deficient animals than were the still growing pair-fed controls.

Blood samples (0.5 ml) for leukocyte counts were withdrawn from the retro-orbital venous sinus. Because of the small volume of blood taken, it was possible to study the

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[1] ADM C-1 Assay Protein, Archer-Daniels-Midland Company, Cincinnati, Ohio.

[2] Composition of basal salt mix in grams: $CaCO_3$, 600; $Ca(H_2PO_4)_2 \cdot H_2O$, 220; K_2HPO_4, 650; NaCl, 336; $MgSO_4 \cdot 7H_2O$, 250; $FeSO_4 \cdot 7H_2O$, 50; $MnSO_4 \cdot H_2O$, 4.6; KI, 1.6; $CuSO_4 \cdot 5H_2O$, 0.6.

[3] A mixture of crystalline vitamins in glucose was given three times each week in amounts to provide the following intake, in micrograms, per day; Ca-pantothenate, 500; *p*-aminobenzoic acid and riboflavin, each 100; thiamine • HCl, pyridoxine, and nicotinic acid, each 300; menadione, 250; folic acid, 6; biotin, 2.5; vitamin B_{12}, 0.3; choline chloride, 10 mg; inositol, 5 mg; α-tocopherol and ascorbic acid, each 1 mg; vitamin A, 150 IU and vitamin D, 15 IU. During pregnancy the vitamin supplement was doubled.

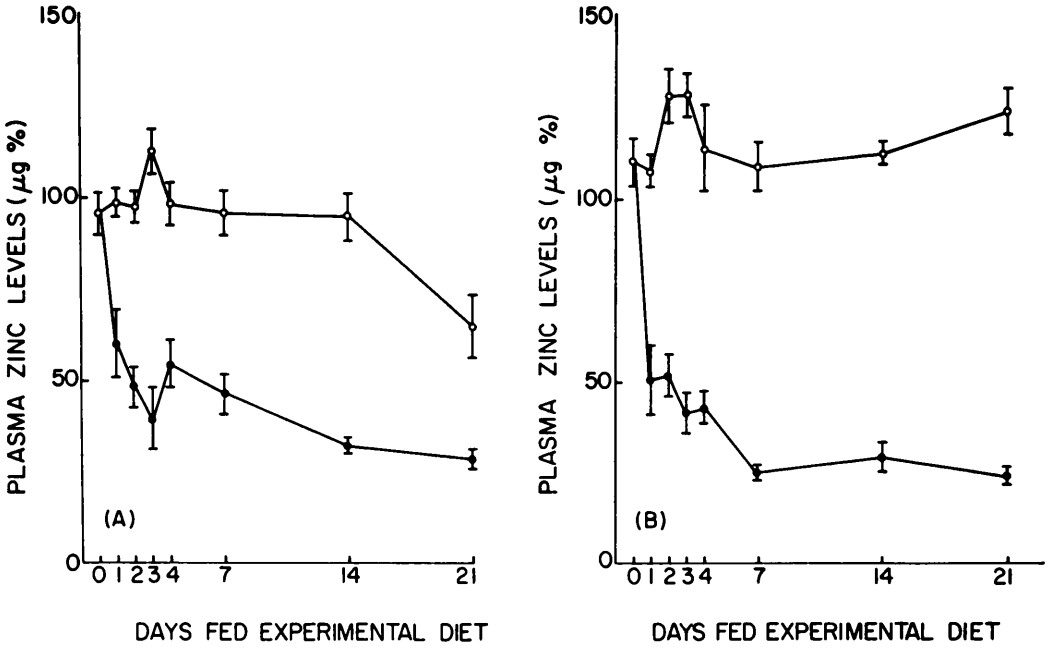

FIG. 1. Plasma zinc levels in (A) pregnant adult and (B) male weanling rats; open circles, zinc-supplemented controls; closed circles, zinc-deficient rats. Points represent the mean ± SE of five animals in each group.

leukocyte picture of the same animals at weekly intervals.

Blood samples (5 ml) for plasma zinc analyses were withdrawn directly from the heart following Nembutal anesthesia. Clotting was avoided by the additon of 40 IU heparin (zinc-free)/ml of blood.

Leukocytes were separated as the "buffy coat" following sedimentation in an International centrifuge (model PR 2) at 1000*g* for 20 min. The "buffy coat" was resuspended in a small volume of physiological saline (0.5 ml) and recentrifuged in a fine-bore hematocrit tube under the conditions described above. The middle portion (0.1 ml) of the evenly-packed leukocyte layer was withdrawn and used for subsequent analysis. Microscopic examination of this layer confirmed that it was composed of leukocytes. Dried leukocyte samples were wet ashed with 0.1 ml of concentrated sulfuric acid and 0.1 ml of concentrated nitric acid, and zinc was assayed by atomic absorption spectroscopy using a Perkin-Elmer (model 303) atomic absorption spectrophotometer.

Plasma zinc levels were determined by atomic absorption spectroscopy on diluted (1:5) plasma samples. Values obtained in this way were found to be of the same order as those obtained from samples that had been subjected to a preliminary "wet digestion" step.

Total leukocyte counts were made on a Max Levy hemocytometer. Differential leukocyte counts were made on blood smears prepared with Jenner-May Grunwald-Giemsa stain and the results are expressed as the percentage of polymorphonuclear (PMN) leukocytes and the percentage of lymphocytes.

Results. The plasma zinc levels of pregnant female and weanling male rats after increasing periods on the experimental diets are summarized in Fig. 1. In both groups, plasma zinc levels fell rapidly. After 1 day on the zinc-deficient diet, plasma zinc decreased by 38% in the pregnant animals and by 55% in the young males. Thereafter the decrease became progressively less marked and reached a plateau by about 7 days.

The effects of zinc deficiency and of general food restriction on the leukocyte counts of adult and weanling rats are summarized in

TABLE I. Plasma Zinc, Leukocyte Zinc, and Leukocyte Counts in Pregnant and Nonpregnant Adult Rats as Affected by Zinc Deficiency.[a]

Treatment	Days on diet	Plasma zinc[b] (μg/100 ml)	Leukocyte zinc[b] (μg/mg of dry wt.)	No. of animals	Total white cell count	Polymorphonuclear leukocytes (%)	Lymphocytes (%)	Polymorphonuclear:lymphocyte ratio
Nonpregnant zinc-supplemented control, *ad libitum*	0			12		16 ± 1	81 ± 1	0.20 ± 0.01
	7			6		16 ± 1	81 ± 1	0.20 ± 0.02
	14			6		18 ± 2	77 ± 3	0.25 ± 0.04
	21	86.8 ± 6.3 (6)	0.027 ± 0.002 (6)	12		17 ± 2	80 ± 2	0.22 ± 0.04
Pregnant zinc-supplemented control, *ad libitum*	0			12	10,654	16 ± 1	81 ± 2	0.20 ± 0.02
	7			6	9292	14 ± 1	84 ± 1	0.16 ± 0.00
	14			6	9233	17 ± 2	78 ± 2	0.22 ± 0.03
	21	50.6 ± 3.6[c](10)	0.028 ± 0.003 (6)	14	9404	18 ± 1	79 ± 1	0.23 ± 0.02
Pregnant zinc-supplemented control, pair-fed	21	48.8 ± 5.0[c](10)		10	7450	46 ± 4[c]	52 ± 4[c]	0.97 ± 0.14[c]
Nonpregnant zinc-depleted	0			16		14 ± 1	84 ± 1	0.17 ± 0.01
	7			6		17 ± 2	81 ± 2	0.21 ± 0.02
	14			6		12 ± 1	86 ± 1	0.15 ± 0.02
	21	21.0 ± 2.4[d](10)	0.025 ± 0.001 (10)	16		20 ± 2	76 ± 2	0.28 ± 0.04
Pregnant zinc-depleted	0			18	12,317	17 ± 1	80 ± 2	0.22 ± 0.03
	7			8	10,775	15 ± 1	83 ± 1	0.18 ± 0.02
	14			8	10,681	16 ± 2	82 ± 2	0.19 ± 0.02
	21	19.6 ± 2.8[d](16)	0.021 ± 0.002 (10)	21	8912	53 ± 5[ce]	44 ± 5[ce]	2.39 ± 0.73[ce]

[a] Means ± SE.

[b] Levels were determined at the end of the experiment on the number of animals indicated in parentheses.

[c] $p < 0.01$ as compared with nonpregnant zinc-supplemented controls, *ad libitum*.

[d] $p < 0.001$ as compared with pregnant zinc-supplemented controls, *ad libitum*.

[e] $p < 0.01$ as compared with nonpregnant zinc-deficient animals.

Tables I and II. Leukocyte zinc of adult rats is also shown in Table II. No significant change was observed in the total leukocyte count or in the zinc concentration of these cells, in either the zinc-deficient or the restricted-intake controls, either in adults or in weanlings. However, in the zinc-deficient adults, as well as in the zinc-deficient weanling rats, a marked increase was noted in the percentage of PMN leukocytes after 21 days. At the same time, there was a decrease in the percentage of lymphocytes, so that the PMN leukocyte to lymphocyte (P:L) ratio changed by a factor of 10 (from 0.22 to 2.39 in pregnant rats and from 0.23 to 2.16 in weanlings).

Food restriction had no effect on the P:L ratio in either the pair-fed or the paired-weight weanlings, whereas a significant change (from 0.22 to 0.97) occurred in the pregnant pair-fed group. This change, however, was not as great as in the deficient groups.

Discussion. The present investigation with rats indicates that plasma zinc levels fall immediately upon removal of the dietary supply of this element. This suggests that body deposits of zinc are not mobilized to meet increased demand. These findings may account, at least in part, for the rapid cessation of growth in weanling rats given a zinc-deficient diet, and for the rapid effect of zinc deficiency during pregnancy in producing congenital malformations (1,2,3).

It has previously been observed in humans that plasma zinc levels fell during pregnancy (6,7). In the present investigation, pregnant rats receiving the zinc-supplemented (control) diet showed little change in the concentration of plasma zinc until the last week of gestation, whereupon the levels fell by about 32%. Whether this decline in plasma zinc was due to an hormonal effect or to an increased fetal requirement is not known, but it is clearly a phenomenon that became pronounced during the last week of pregnancy in this species.

Despite the current uncertainty regarding the value of plasma zinc analyses as an index of zinc status in animals (8,9), the present data demonstrate the sensitivity of the plasma zinc level to a deficiency regime. However, it must be stressed that the reliability of the method depends largely upon the elimination of all adventitious traces of zinc and avoidance of hemolysis of the zinc-containing erythrocytes during collection of the plasma samples.

In weanling rats, zinc deficiency but not starvation produced an increase in the P:L ratio. In adult rats, on the other hand, restricted food intake coupled with pregnancy elicited a similar response. The increased number of PMN leukocytes in these animals cannot be attributed to a compensatory mechanism arising from a lowered level of zinc in the white blood cells; no difference was demonstrated between the levels of zinc in the leukocytes of the control animals and those showing altered P:L ratios. Clearly these changes are unlikely to provide an effective tool for the diagnosis of zinc deficiency in animals owing to the general nature and comparative slowness of the response, but they are nevertheless of interest as a further manifestation of zinc deficiency in mammalian physiology.

A comparison of the individual plasma zinc levels and their corresponding P:L ratios indicated that although increased PMN leukocyte counts were always accompanied by reduced plasma zinc levels, the highest P:L ratios were not always associated with the lowest plasma zinc concentrations. In fact, in the nonpregnant zinc-deficient animals, low plasma zinc levels were not associated at all with increased PMN leukocyte counts. Thus, the plasma zinc level cannot be considered a causative agent for the changes observed in leukocytes.

The present data confirm and extend the recent findings of Macapinlac *et al.* (10) that zinc deficiency leads to a decrease in the percentage of lymphocytes in rats.

Summary. Dietary zinc deficiency was shown to result in a rapid fall in the plasma zinc levels of pregnant female and weanling male rats. In both groups, plasma zinc fell to 45–60% of its original level after only one day on the deficiency regime. Longer periods of zinc depletion (21 days) were found to increase the ratio of PMN leukocytes to

TABLE II. Plasma Zinc and Leukocyte Counts in Male Weanling Rats as Affected by Zinc Deficiency.[a]

Treatment	Days on diet	Plasma zinc[b] (μg/100 ml)	No. of animals	Total white cell count	Polymorphonuclear leukocytes (%)	Lymphocytes (%)	Polymorphonuclear leukocyte : lymphocyte ratio
Zinc-supplemented control, *ad libitum*	7		6	9853	23 ± 3	74 ± 4	0.32 ± 0.02
	14		6	9058	20 ± 2	77 ± 2	0.26 ± 0.03
	21		27	11,778	19 ± 1	79 ± 1	0.25 ± 0.02
	28	107.2 ± 4.4 (6)	29	10,865	18 ± 1	80 ± 1	0.23 ± 0.01
Zinc-supplemented control, pair-fed	21		8	7381	20 ± 3	79 ± 3	0.27 ± 0.05
	28		7	9857	17 ± 1	80 ± 1	0.22 ± 0.02
Zinc-supplemented control, paired-weight	7		6	7558	16 ± 1	82 ± 1	0.20 ± 0.02
	14		5	6340	15 ± 1	84 ± 0	0.18 ± 0.01
	21		6	6692	12 ± 2	87 ± 2	0.15 ± 0.03
	28	89.9 ± 4.4[c] (5)	6	6075	13 ± 1	85 ± 2	0.16 ± 0.02
Zinc-deficient	7		6	8408	18 ± 1	80 ± 1	0.23 ± 0.02
	14		6	7825	24 ± 3	74 ± 3	0.34 ± 0.05
	21		30	12,513	53 ± 3	46 ± 3	1.48 ± 0.19[d]
	28	26.4 ± 3.3[d] (4)	25	11,666	60 ± 3	38 ± 3	2.16 ± 0.38[d]

[a] Means ± SE.

[b] Plasma zinc levels were determined at the end of the experiment on the number of animals indicated in parentheses.

[c] $p < 0.05$ as compared with zinc-supplemented controls, *ad libitum*.

[d] $p < 0.001$ as compared with zinc-supplemented controls (paired-weight).

lymphocytes in the blood by a factor of 10. General food restriction elicited a similar response in pregnant females but not in young males. The zinc deficiency regime had no effect upon the total leukocyte count or upon the concentration of zinc in the leukocytes.

Addendum. Since preparing this manuscript a recent publication by Mills, C. F., Dalgarno, A. C., Williams, R. G., and Quarterman, J., has come to our attention (Br. J. Nutr. **21**, 751, 1967). These workers have reported a rapid fall in the plasma zinc levels of zinc-deficient calves and lambs similar to our own findings in rats.

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Transitory Impairment of Interferon Production in Serotonin Treated Mice* (32972)

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Interferon production appears to represent an important defense mechanism against viral infections. While studies have shown the influence of steroid hormones both on interferon production (1) and on susceptibility to infection (2), reports are limited which relate vasoactive amines to such responses (3). Based on the observations that certain amines, e.g., epinephrine, histamine, and serotonin, are secreted in response to various stimuli and that alterations in interferon production (4) and in susceptibility to viral infections (5) have also been noted in response to noxious stimuli, a study was undertaken to determine if a correlation between these observations could be demonstrated. This report describes the influence of serotonin (5-hydroxytryptamine; 5-HT) on interferon production.

Materials and Methods. Animals. Both male and female, 7 to 12-week-old Swiss-Webster BRVS mice were used. Sex and age were held constant within each experiment.

Serotonin. Mice were injected i.p. with 1-mg doses of 5-HT as the creatinine sulfate salt suspended in 0.25 ml of saline. Control mice were injected with 0.25 ml of saline.

Interferon stimulation and assay. At intervals from 30 min before to 8 hours after 5-HT injections, groups of 7 mice were injected i.v. with 0.2 ml of either a 1:5 dilution of egg allantoic fluid containing about 5.6×10^7 plaque forming units (pfu) of Newcastle disease virus (NDV), or with 100 μg of *E. coli* lipopolysaccharide endotox-

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