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Bone Mineralization during a Developing Zinc Deficiency[1,2] (40023)

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Hurley and Swenerton (1) reported that pregnant adult rats were unable to release sufficient quantities of bone zinc to meet the demands of the developing fetus during a 21-day period of dietary zinc deficiency and concluded that in the mature skeleton "zinc in bone is not available to the rat even in the face of demonstrated need." However, more recently Harland *et al.* (2) suggested that bone may store zinc consumed in excess of the requirement and that this zinc may be available for utilization during a subsequent period of zinc deprivation. The studies used young, immature Japanese quail. The purpose of the reported experiment was to monitor bone and zinc status and to determine if the net zinc content of bone was altered during the development of a zinc deficiency in the young growing rat.

Materials and methods. One hundred male weanling (45-50 g) Sprague-Dawley rats[5] were housed in individual stainless-steel cages free of solder. Deionized water (< 0.05 ppm zinc) was provided *ad libitum.* The animals were divided into three experimental groups of comparable body weight and fed the following diets: zinc sufficient, *ad libitum;* zinc deficient, *ad libitum;* and zinc sufficient, pair-fed by restricting the average daily intake to that of the zinc-deficient group.

The composition of the basal diet has been described elsewhere (3). The basal diet (1.5 μg of Zn/g of diet) was supplemented with $ZnCO_3$ to give a total of 93 mg of Zn/kg for the zinc-sufficient diet.

Upon arrival, six rats were sacrificed to provide baseline values. Four rats in the zinc-sufficient, *ad libitum* group were sacrificed on Days 9 and 13, while three were sacrificed on Day 23. Four rats in the pair-fed zinc-sufficient group were sacrificed twice weekly. Six rats in the zinc-deficient, *ad libitum* group were sacrificed on Days, 1, 2, and 3 of the experiment and, thereafter, twice weekly.

Animals were sacrificed under light ether anesthesia. Blood was collected in polyethylene syringes with stainless-steel needles and polypropylene hubs. Two drops of a 30% sodium citrate solution were added to each sample as an anticoagulant. Any hemolyzed samples were discarded. The right femur was removed, stripped of tissue, and dried to a constant dry weight in a vacuum oven at 70°.

Plasma and bone for all animals were analyzed for zinc using atomic absorption spectrophotometry after acid digestion using a mixture of $HClO_4$ and H_2O_2 (3). Bone from animals sacrificed at 0, 9, 16, and 23 days of the experiment was analyzed for calcium by atomic absorption spectrophotometry[6] and phosphorus by the colorimetric method of Fiske and Subbarow (4).

Results. Figure 1 shows the weight gain of all three groups of rats. After the second day of treatment the zinc-deficient group displayed a retarded growth rate, and after 3 days there was little increase in weight for the remainder of the experiment. The zinc-sufficient pair-fed group grew at a rate intermediate to the other groups.

[1] Some of these data were presented at the Fifty-Ninth Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 13-18, 1975.

[2] The animals were maintained in animal care facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

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[6] "Analytical Methods for Atomic Absorption Spectrophotometry." Perkin Elmer Corp., Norwalk, Connecticut (1973).

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Plasma zinc (Fig. 2) for the zinc-deficient group dropped 55% (107 to 48 μg/ml, $P < 0.001$) after 1 day of dietary deficiency. The plasma zinc decreased an additional 16% (to 31 μg/ml, $P < 0.001$) by Day 3, for a total of 71% (107 to 31 μg/ml, $P < 0.001$), and then remained relatively constant for the rest of the experiment. The plasma zinc of the zinc-sufficient and pair-fed controls remained in the normal range throughout the experimental period.

Femur concentration of zinc in the zinc-deficient group decreased steadily until Day 13 (Fig. 3), when the mean level was 61% (150 to 58 μg/g dry weight, $P < 0.001$) less than baseline. In contrast, the sufficient and pair-fed groups increased their femur zinc concentrations from the baseline. The mean total zinc content of the femurs from the zinc-deficient rats decreased 21% (11.7 to 9.2 μg/femur, $P < 0.05$) by Day 3 and then decreased more gradually to an overall decrease of 37% (11.7 to 7.4 μg/femur, $P < 0.01$) on Day 23. The zinc-sufficient rats showed a steady increase in the total zinc content of their femurs. The pair-fed group also increased its total femur zinc, though at a lesser rate. As the experiment progressed, the femurs increased in weight for all groups, including the zinc-deficient rats (Fig. 4). However the increase was least for the zinc-deficient group, while the pair-fed group had an intermediate gain in femur weight.

Table I shows that although the total and concentration of calcium and phosphorus increased in the femurs, regardless of treatment, the calcium to phosphorus ratio remained relatively constant over the dietary period. All three groups had radios ranging between 1.80 and 2.03. However, the calcium and phosphorus accumulation in fe-

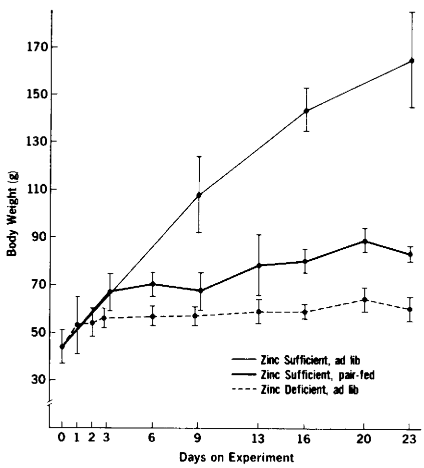

FIG. 1. Body weight during the development of a zinc deficiency.

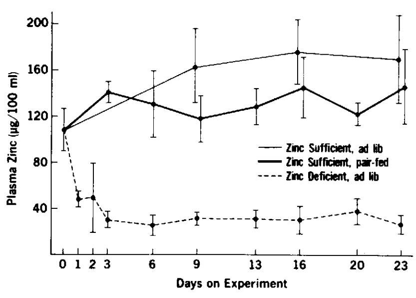

FIG. 2. Plasma zinc during the development of a zinc deficiency. Hemolyzed samples were discarded. Each point represents four to six samples, except for three samples on Day 23 of zinc-sufficient, *ad libitum* diet.

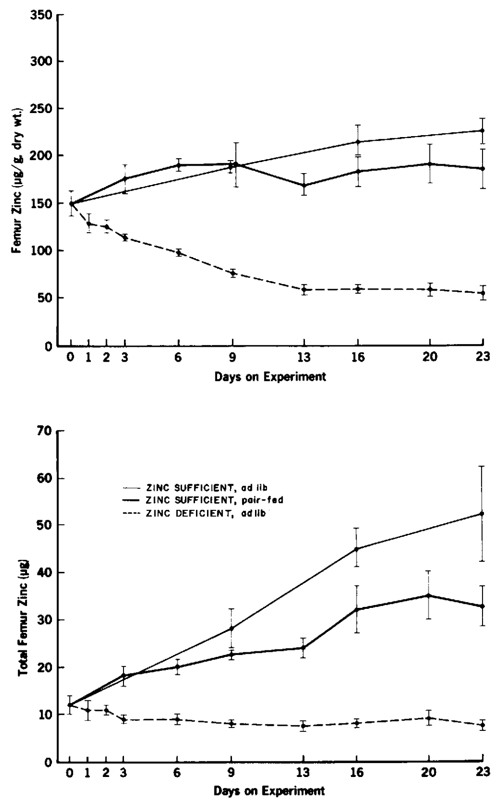

FIG. 3. Femur zinc concentration and total femur zinc during the development of a zinc deficiency.

murs in the zinc-deficient group was significantly less ($P < 0.05$) than that in the other groups by the end of the experimental period.

Discussion. The present data are in agreement with previous reports that during the course of an acute zinc deficiency, plasma zinc decreases drastically over the first 24 hr and continues to decline for the next 2 days (6–8). Furthermore, in this experiment there was little body weight gain after 3 days. Femur zinc concentration declined steadily until the 13th day of the deficiency. These three parameters, body weight, plasma, and bone concentration, are commonly used to assess zinc status and clearly indicate a zinc-deficiency state by the end of the experimental period. However, the time course of the decline was different for each parameter. Plasma zinc responded quickly to the dietary zinc inadequacy, whereas femur zinc concentration decreased slowly. This may indicate most accurately the gradual deterioration of the overall zinc status of the animal.

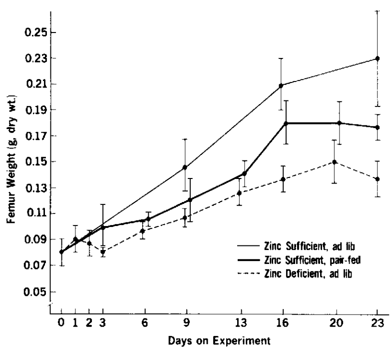

FIG. 4. Femur weight during the development of a zinc deficiency.

The total femur zinc of the zinc-deficient group decreased 37% over the experimental period. Extrapolation of the femur data to the whole skeleton suggests that an appreciable net amount of zinc was lost or released during the developing zinc deficiency in these young growing rats. However, the released zinc was not available and/or insufficient to prevent the eventual zinc deficiency. The total net quantity of skeletal zinc released can not be estimated from our data since the different types of skeletal bone may have zinc concentrations differing from that of the femur. Also, zinc may be released at different rates for different bones.

Our data indicate that some zinc in bone is released during a zinc deficiency. This supports the results of Harland *et al.* (2). Both investigations used immature animals, whereas Hurley *et al.* (1) concluded that zinc was not released from the skeleton of

TABLE I. FEMUR CALCIUM AND PHOSPHORUS DURING THE DEVELOPMENT OF ZINC DEFICIENCY.

	Number of samples	Calcium		Phosphorus		
		(mg/femur)	(mg/g dry wt)	(mg/femur)	(mg/g dry wt)	Ca:P
Baseline, Day 0	6	12.4 ± 2.7*	159 ± 20	6.5 ± 1.6	83 ± 13	1.92 ± 0.11
Day 9						
ZnS,[a] *ad libitum*	4	29.1 ± 3.7[a]	197 ± 10[a]	15.6 ± 2.6[a]	105 ± 5[a]	1.88 ± 0.18[a]
ZnS, pair-fed	4	23.3 ± 2.8[b]	193 ± 12[a]	12.1 ± 1.5[b]	101 ± 9[a]	1.92 ± 0.07[a]
ZnD, *ad libitum*	6	20.1 ± 1.1[b]	188 ± 14[a]	10.3 ± 0.7[b]	97 ± 8[a]	1.95 ± 0.15[a]
Day 16						
ZnS, *ad libitum*	4	43.4 ± 4.5[a]	207 ± 7[a]	21.7 ± 1.8[a]	104 ± 1[a]	2.00 ± 0.06[a]
ZnS, pair-fed	4	34.4 ± 3.6[b]	195 ± 3[b]	17.5 ± 2.1[b]	99 ± 4[a]	1.97 ± 0.08[a]
ZnD, *ad libitum*	6	25.5 ± 1.6[c]	188 ± 9[b]	12.6 ± 1.1[c]	93 ± 4[b]	2.03 ± 0.09[a]
Day 23**						
ZnS, *ad libitum*	3	52.8 ± 8.3[a]	228 ± 9[a]	28.1 ± 3.9[a]	121 ± 4[a]	1.88 ± 0.04[a]
ZnS, pair-fed	4	38.8 ± 3.8[b]	221 ± 11[a]	21.5 ± 1.9[b]	123 ± 5[a]	1.80 ± 0.03[a]
ZnD, *ad libitum*	6	27.6 ± 2.8[c]	199 ± 9[b]	14.9 ± 1.4[c]	107 ± 5[b]	1.86 ± 0.05[a]

[a] ZnS = zinc sufficient; ZnD = zinc deficient.

* Mean ± standard deviation; means within the same treatment day with different superscripts are significantly different, $P < 0.05$. The data were subjected to one-way analysis of variance and the means compared using Duncan's multiple range test (5).

** On Day 23 all calcium and phosphorus parameters were significantly different from their respective baseline values ($P < 0.05$). The Ca:P ratios were not significantly different from the baseline.

older pregnant rats. Thus, a major factor in the release of bone zinc during periods of deprivation appears to be the stage of skeletal maturity. The kinetics and mechanisms of the zinc release still need to be defined.

Although the total femur zinc decreased 37%, the femur zinc *concentration* decreased by 61%. This discrepancy can be partially explained by changes in the femur weight. The femur continued to increase in weight during zinc deficiency, although at a decreased rate compared to the control groups. Thus, the large decrease in bone zinc concentration could be, in part, due to a "dilution" resulting from the increased femur weight. In addition, the decrease in total zinc contributed to the decreased concentration. The femurs increased in weight during a period of a decrease in body weight as the deficiency developed. We did not measure femur length and thus were unable to determine if linear growth contributed to this weight gain of the femurs.

The femur weight gain for the zinc-deficient group appears to be due to an increase in bone mineralization. Both the total and concentration of calcium and phosphorus increased as the deficiency developed while, concomitantly, zinc (total and concentration) was decreased. Furthermore, the Ca:P ratio remained relatively constant. Therefore, the decrease in zinc appears to be independent from calcium and phosphorus in the bone. Thus, zinc may not be necessary in a precise ratio to calcium or phosphorus, but rather as a cofactor or catalyst in the mineralization process.

Summary. Alterations in bone mineralization were examined during the progression of a zinc deficiency in rats. One hundred rats were divided into three groups and fed the following diets: zinc sufficient, *ad libitum;* zinc deficient, *ad libitum;* and zinc sufficient, pair-fed the daily intake of the deficient group. Plasma zinc concentration of the zinc-deficient group decreased dramatically during the first 24 hr of deficient diet and continued to decline over the next 2 days. Body weight gain essentially stopped after 1 week. During the experimental period of 23 days, femur zinc concentration decreased 61%, while total femur zinc decreased 37%. Yet, both the total and concentration of calcium and phosphorus increased and the Ca:P ratio remained relatively constant. The present data indicate that zinc can be released from bone during periods of dietary zinc deficiency. Furthermore, the decrease in zinc appears to be independent from calcium and phosphorus in the bone.

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