Changes in Plasma Proteins in Zinc-Deficient Rats[1] (35218)

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Swenerton and Hurley reported a severe and specific zinc deficiency in rats by the use of a purified diet containing isolated soybean protein and by careful elimination of environmental contamination (1). Rats fed the zinc-deficient diet showed symptoms of deficiency including extreme growth retardation, alopecia, and dermal lesions. In all animal species studied, a major and rapid effect of zinc deficiency is impairment of growth (1–5). These findings suggested that zinc plays a role in protein metabolism.

Plasma or serum proteins have been investigated in zinc-deficient animals. A decrease in total plasma or serum protein content has been reported in zinc-deficient rats (6) and chicks (7). An increase in serum γ-globulin with compensating decrease in serum albumin has been shown in zinc-deficient swine (8) and lambs (3). Lower plasma protein levels and changes in the electrophoretic patterns were observed in zinc-deficient quail after 24 and 48 hr of fasting (9, 10). However, blood protein fractionation in zinc-deficient rats has not been previously reported. The present paper describes the effect of zinc deficiency on protein metabolism in rats including both total plasma protein levels and their electophoretic patterns.

Methods. Male weanling rats of the Sprague-Dawley strain (body wt, 50 ± 5 g) were purchased from a commercial source and were fed either a basal zinc-deficient diet, containing 0.3 to 0.4 ppm of zinc,[2] or a zinc-supplemented control diet. The zinc-supplemented control diet was the same as the basal zinc-deficient diet except that $ZnCO_3$ was added to the salt mix to provide 100 ppm of dietary zinc. Since a marked reduction in food consumption occurs in zinc-deficient animals, pair-fed and paired-weight groups were included in the experiment to serve as inanition controls. Pair-fed control animals were fed the zinc-supplemented control diet daily in amounts equal to that consumed by their zinc-deficient pairs the previous day. Paired-weight control animals were given even less food daily so that their weight was equal to that of their zinc-deficient pairs.

The basal zinc-deficient diet contained (%): isolated soybean protein,[3] 30.0; sucrose, 57.3; corn oil, 8.0; salt mix,[4] 4.0; and DL-methionine, 0.7. The soybean was treated with Na_4EDTA[5] to lower its zinc content. Crystalline vitamins[6] were given separately. Extreme precautions were taken in diet preparation and animal care to eliminate zinc contamination from the environment. Details of the dietary procedures and experimental methods have been described previously (1).

After 28 days of the experimental regime, blood samples were obtained by heart puncture after sodium pentobarbital anesthesia. Plasma was separated by centrifugation in an International centrifuge (Model PR 2) at 1000*g* for 20 min, using heparin as anticoagulant. Total plasma protein was determined by the Lowry *et al.* method (11) at 650 mμ using bovine serum albumin as a standard.

[1] Supported in part by Grant No. HD-01743 from the National Institute of Health.

[2] Determined by atomic absorption spectroscopy.

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

[4] Composition of basal salt mix (g): $CaCO_3$, 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.

[5] Tetrasodium salt of ethylenediaminetetraacetic acid.

[6] A mixture of crystalline vitamins in glucose was given three times each week in amounts to provide the following daily intake (μg): Ca-pantothenate, 500; *p*-aminobenzoic acid and riboflavin, each 100; thiamine·HCl, pyridoxine·HCl 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-tocopheryl acetate and ascorbic acid, each 1 mg; vitamin A, 150 IU and vitamin D, 15 IU.

TABLE I. Total Plasma Protein Content of Zinc-Deficient and Zinc-Supplemented Rats.[a]

Treatment	No. of rats	Body wt (g)	Total plasma protein (g/100 ml)
Zn-supplemented, *ad libitum*	14	230	7.67 ± 0.12
pair-fed	4	81	7.34 ± 0.42
paired-weight	13	67	7.23 ± 0.18
Zn-deficient, *ad libitum*	11	59	5.95 ± 0.20[b,c,d]

[a] Means ± SE.

[b] $p < 0.001$ as compared to Zn-supplemented *ad libitum* controls.

[c] $p < 0.001$ as compared to Zn-supplemented paired-weight controls.

[d] $p < 0.02$ as compared to Zn-supplemented pair-fed controls.

Plasma proteins of the same sample were fractionated in 7.5% polyacrylamide gel by disc electrophoresis following the method described by Ornstein (12) and Davis (13). Fifty μl of sample solution containing 0.01 ml of plasma was applied to each gel tube and was separated under a current of 3 mA/tube and 150 V for 40 min. Bromphenol blue was added in the upper buffer to serve as reference dye. Protein bands were stained with buffalo black solution (0.05% in 15% acetic acid) immediately after electrophoresis. Gels were stored in 7% acetic acid solution after destaining.

Results. The total plasma protein levels of zinc-deficient and zinc-supplemented rats after 4 weeks on the experimental diet are shown in Table I. There was a significant decrease in the total plasma protein contents of zinc-deficient rats as compared with any of the three zinc-supplemented controls. Rats fed restricted amounts of the zinc-supplemented diet did not show this change.

The electrophoretic patterns of the plasma from both the zinc-deficient and control animals are shown in Fig. 1. Since the protein bands were not further characterized, arbitrary numbers were given for comparison (Fig. 2). Seventeen bands were usually observed in the control plasma. Two bands were sometimes observed in the band 1 region. There was a general decrease in concentration in most of the protein bands of plasma from zinc-deficient rats (Fig. 1). These results confirmed the findings of the lower plasma protein levels obtained by chemical analysis. In the zinc-deficient rats, but not in the pair-fed or paired-weight controls, band 7 was markedly decreased and sometimes showed reduced mobility (Fig. 1). The electrophoretic patterns of plasma proteins from rats fed the zinc-supplemented diet in restricted amounts were similar to those of rats fed the zinc-supplemented diet *ad libitum*.

Discussion. In contrast to the results of Miller *et al.* (14) in the baby pig, total plasma proteins in the present experiment were decreased in zinc-deficient rats. Disc gel electophoresis studies also demonstrated a general decline in plasma protein content. These results are in agreement with the earlier findings by Hove *et al.* (6) and Rahman *et al.* (7), in zinc-deficient rats and chicks.

Fox and Harrison (9, 10) have reported profound changes in the plasma protein patterns of zinc-deficient quail during fasting. After 48 hr of fasting, all the deficient birds showed loss of a major protein component and in some birds this protein band was entirely missing. Marked protein loss was shown in the present experiment without fasting, although none of the deficient rats showed a missing band in their plasma protein patterns.

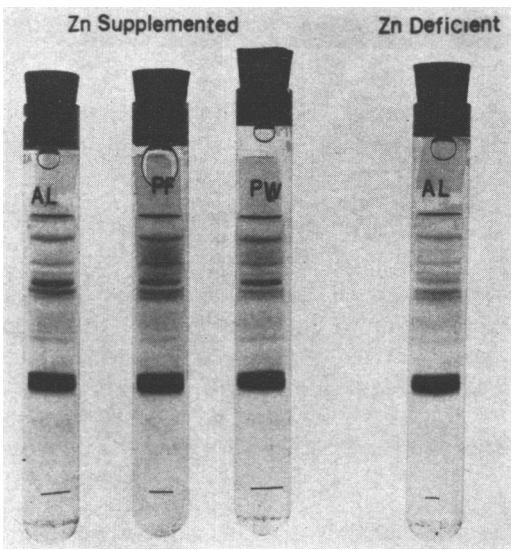

FIG. 1. Effect of zinc deficiency on the plasma protein patterns in rats: AL, *ad libitum*; PF, pair-fed; PW, paired-weight.

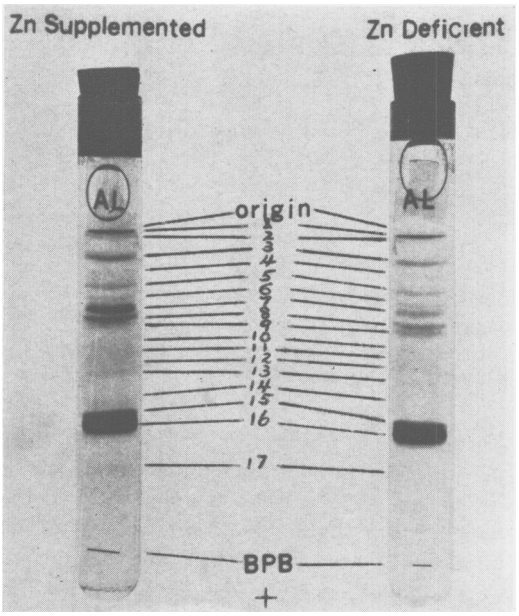

FIG. 2. Plasma protein patterns of zinc-supplemented and zinc-deficient rats after 4 weeks on the experimental diets: AL, *ad libitum;* BPB, bromphenol blue.

The alterations in plasma protein of zinc-deficient animals suggest either an impairment in protein synthesis or an increase in protein breakdown (or both). A possible role of zinc in protein synthesis has been suggested by studies primarily on microorganisms and plants (15–20); however, few reports of experiments on animals have appeared in the literature. Macapinlac *et al.* (21) found a normal incorporation of ^{14}C-leucine and ^{14}C-adenine into protein and RNA, respectively, in the testes of zinc-deficient rats. They postulated that zinc deficiency resulted in an increase in the catabolism of protein and RNA rather than impaired synthesis. The studies of Theuer and Hoekstra (22) showed that an accelerated oxidation rate of some ^{14}C-labeled amino acids to $^{14}CO_2$ occurred in zinc-deficient rats.

In the present investigation, decreased total plasma proteins and altered eltctrophoretic patterns indicate changes in protein metabolism. Whether this reflects a specific role of zinc in the synthesis or in the breakdown of protein or proteins has not been established.

Summary. A reduction in total plasma proteins was observed in young zinc-deficient rats after 4 weeks on the experimental diet. Zinc deficiency also resulted in alterations in plasma protein patterns obtained by disc gel electrophoresis. These results were due to zinc deficiency, *per se,* and not due to inanition since neither pair-fed nor paired-weight controls showed these changes.

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