

Homeostatic Regulation of Zinc Absorption in the Rat¹ (37400)

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(Introduced by H. H. Sandstead)

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The intestinal absorption of zinc is influenced by a variety of biological and dietary factors (1), one of which is the adequacy of tissue zinc levels. For example, when ruminants are fed a diet containing sub-optimal zinc levels, absorption of the element increases as the body stores become depleted (2-5). In addition, Cotzias, Borg and Selleck (6) observed that zinc absorption in mice varies inversely with the body's enrichment with zinc. These observations suggest that zinc balance is maintained, in part, by homeostatic mechanisms which regulate zinc absorption in proportion to bodily requirements. Attempting to describe some of the factors involved in maintaining zinc homeostasis, we have examined zinc absorption in both normal and zinc-deficient rats.

Methods. Male Sprague-Dawley rats age 70-90 days were used in all experiments. The animals were housed individually in stainless steel cages and were given free access to food and water. All animals were fasted for 18 hr prior to being used in experiments.

To produce zinc deficiency, animals were fed the zinc-deficient diet described by Luecke, Oلمان and Baltzer (7) as modified in our laboratory. The modified diet contained inositol at 1.0 g/kg and dextrose at 630 g/kg; chlortetracycline was omitted. The zinc content of this diet was less than 1.0 $\mu\text{g Zn/g}$. Animals fed the zinc-deficient diet were also maintained on resin-deionized water.

For experiments reported here, five groups of six animals each were used: (a) animals maintained on Purina Laboratory Chow²

and tap water; (b) animals fed the zinc-deficient diet described above but supplemented with zinc by the addition of 50 $\mu\text{g Zn/ml}$ to the drinking water; (c) animals maintained on the zinc-deficient diet for 13 days; and (e) animals maintained on the zinc-deficient diet for 13 days and injected intraperitoneally with 200 $\mu\text{g Zn}$ in the form of ZnCl_2 24 hr before zinc absorption was measured.

Zinc absorption was measured by diluting 1.0 μCi carrier-free zinc-65 (0.122 ng Zn^{2+}) in 1.0 ml 0.85% NaCl solution and introducing the solution into the stomach by a gastric tube. One hour after administration of the isotope, the animals were decapitated, blood was collected in a heparinized tube and the stomach and entire intestinal tract were removed. The radioactivity in the carcass was monitored in a whole body counter and the results are expressed as the percentage of the dose administered. We analyzed zinc-65 absorption during a 1-hr period in an attempt to minimize the complications of zinc secretion back into the intestine. Moreover, since the carcass was monitored for radioactivity with the gastrointestinal tract removed, the experimental results represent absorption and net retention of zinc-65 by the whole body.

The small intestine was prepared for analysis by removing a 5-cm segment, beginning at the pylorus, and washing the segment thoroughly with 0.25 M sucrose. Thereafter, the mucosal cells were scraped from the

¹ Supported in part by USDA Cooperative Agreement 12-14-100-11, 178 (61), Amend. 1.

² Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product by the U.S. Department of Agriculture.

serosal tissue by using a glass slide; the mucosa was suspended in 10 ml of 0.25 *M* sucrose, and the suspension was homogenized in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. A measured volume of the homogenate was removed for analysis of radioactivity and protein content, and the remainder of the homogenate was used for determining zinc concentration of the intestine.

Zinc concentration of the intestinal homogenate was analyzed by atomic absorption spectrophotometry after the samples were dry ashed at 600° and extracted into 2 *N* HCl. Plasma zinc concentration was determined by atomic absorption after dilution of 1.0 ml of plasma to 4.0 ml with glass distilled water. Radioactivity of the intestinal homogenate was measured in a gamma-well counter, and the protein concentration of the homogenate was determined with biuret reagent.

Results and Discussion. Figure 1 illustrates the effect of zinc deficiency on intestinal zinc concentration, intestinal zinc-65 binding and zinc-65 absorption. In animals consuming the zinc-deficient diet for 7 days, zinc concentration of the intestine was significantly less ($p < 0.01$) than that of control animals, whereas both intestinal uptake of zinc-65 and zinc-65 absorption were significantly greater ($p < 0.01$) than that of the control animals. In animals consuming the zinc-deficient diet for 13 days, the zinc concentration of the intestine was significantly less ($p < 0.01$) than that of the 7-day deficient animals, and both intestinal uptake of zinc-65 and zinc-65 absorption were significantly greater ($p < 0.01$) than that of the 7-day deficient animals. When rats were injected intraperitoneally with zinc after consuming the zinc-deficient diet for 13 days, the intestinal zinc concentration increased and was significantly greater ($p < 0.01$) than that of the noninjected 13-day deficient animals. Moreover, in the animals injected with zinc, both intestinal uptake of zinc-65 and zinc-65 absorption were significantly less ($p < 0.01$) than that of the noninjected 13-day deficient rats. These results suggest that the intestinal uptake of zinc and subsequent zinc absorption are regulated, in part, by the zinc con-

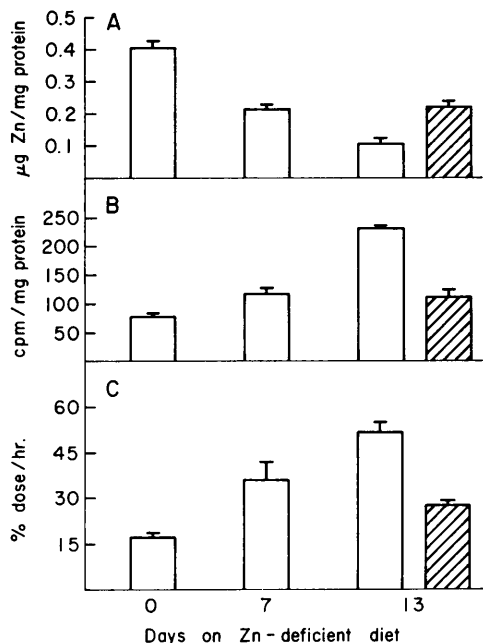


FIG. 1. The effect of zinc deficiency on intestinal zinc concentration, intestinal uptake of zinc-65, and zinc-65 absorption. (A). Intestinal zinc concentration. (B). Zinc-65 content of the intestine 1 hr after oral administration of the isotope. (C). Absorption and net retention of zinc-65 by the carcass 1 hr after oral administration of the isotope. The values listed for zero days on the zinc-deficient diet represent the mean \pm SD of 12 animals, 6 of which were fed the stock diet and 6 of which were fed the zinc-deficient diet supplemented with zinc as described in the text. The values listed for the experimental animals at 7 and 13 days represent the mean \pm SD of 6 animals. The crosshatched bars represent the mean \pm SD of 6 animals which had been consuming the zinc-deficient diet for 13 days and were injected intraperitoneally with 200 μ g Zn^{2+} 24 hr before oral administration of zinc-65.

centration of the intestinal mucosa.

As shown in Table I, plasma zinc concentration was significantly decreased in zinc-deficient rats at both 7 and 13 days. In the 13-day deficient animals injected intraperitoneally with zinc, the plasma zinc concentration was significantly greater ($p < 0.01$) than that of the noninjected deficient animals. These results demonstrate that zinc injections partially restore the zinc content of tissue and blood in zinc-deficient animals.

TABLE I. Effect of Zinc Deficiency on Plasma Zinc Concentration.

Experimental group ^a	Plasma zinc ($\mu\text{g}/100\text{ ml}$)
Zinc supplemented	115 \pm 10
7 days zinc deficient	60 \pm 5
13 days zinc deficient	54 \pm 6
13 days zinc deficient + 200 μg Zn ²⁺ ip	98 \pm 8

^a The experimental groups are identical to those described in Fig. 1.

Furthermore, as described above, the intestinal zinc concentration of zinc-deficient rats was increased following zinc injection which suggests that blood plasma transports zinc to the intestinal mucosa.

Cotzias, Borg and Selleck (6) first suggested that zinc absorption is regulated by a negative feedback system. The experiments outlined here substantiate that hypothesis and further describe the homeostatic mechanisms which regulate zinc absorption. The soft tissues of the body exchange zinc with the zinc-binding fractions of blood and thereby regulate the zinc content of the blood. The blood, in turn, transports zinc to the intestinal mucosa where the metal becomes bound to specific cellular components. Thus, since the number of available binding sites in the intestinal mucosa influences zinc uptake and subsequent absorption, zinc is absorbed in proportion to bodily requirements.

Summary. Zinc concentration of the intestine, zinc-65 uptake by the intestine and zinc-65 absorption were measured in control

and zinc-deficient rats. As zinc deficiency progressed, zinc concentration of the intestinal mucosa decreased whereas both the amount of zinc-65 taken up by the mucosa and the absorption of the isotope increased. In addition, zinc concentration of the plasma was decreased in zinc-deficient animals. When zinc-deficient rats were injected with ZnCl₂ prior to oral administration of zinc-65, the zinc concentration of both the plasma and the intestine increased and there was a concomitant decrease in both zinc-65 uptake by the intestine and absorption of the isotope. The results of these experiments suggest that zinc absorption is regulated, in part, by the zinc content of the intestinal mucosa which, in turn, is regulated by the zinc content of the plasma.

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Received Mar. 1, 1973. P.S.E.B.M., 1973, Vol. 143.