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Effect of Acute Infection and Endotoxemia on Zinc Absorption in the Rat (39119)

R. S. PEKAREK AND G. W. EVANS

United States Department of Agriculture, Agricultural Research Service, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201

Acute and chronic infectious diseases (1–3), endotoxemia (4), and other inflammatory stresses (3, 5, 6) have been shown to produce significant alterations in zinc metabolism in humans and experimental animals. Initially there is a rapid redistribution of zinc within the body, which is characterized by hypozincemia, hypozincuria, and an increased hepatic uptake of zinc (7, 8). Later in the disease process and in early convalescence hypozincemia may be influenced by a decreased dietary intake and body losses due to sweating, diarrhea, or hyperzincuria (8, 9).

While a lessening of dietary zinc intake may occur as a result of stress-induced anorexia, little is known concerning the influence of acute inflammation on zinc absorption. Recent studies of normal and zinc-deficient rats have indicated that zinc may be absorbed across a concentration gradient into the cells of the intestinal mucosa via a low molecular weight ligand to binding sites on the basolateral plasma membrane where it is removed by metal-free albumin (10, 11). Thus the amount of zinc absorbed would be determined by the quantity of metal-free albumin or possibly other plasma transport proteins available at the basolateral membrane. In the case of acute inflammatory stress where serum zinc concentrations are depressed, one might hypothesize that more zinc-free plasma transport proteins might be available which, in turn, should enhance the absorption of zinc. Therefore, the present study was done to see whether acute infection and endotoxemia would, in fact, cause an increase in the intestinal absorption of zinc.

Materials and methods. Male Sprague–Dawley rats, weighing 200–250 g were used in the study. The animals were housed in stainless steel cages and were maintained on Purina Laboratory Chow[1] and tap water. *Francisella tularensis*, live vaccine strain (LVS), (National Drug Company)[2] and endotoxin (lipopolysaccharide) from *Escherichia coli* 0127:B8 (Difco Laboratories) were employed as the inflammatory agents.

To ensure that these two agents would produce the characteristic alterations in zinc metabolism in this particular strain of rats, rats were divided into two groups and intraperitoneally (ip) administered 10^7 LVS organisms and 100 μg of endotoxin, respectively. At 8 hr (endotoxin group) and 24 hr (LVS-infected group) postadministration, the rats were bled and then killed by cervical dislocation. The livers were perfused with saline, freeze-dried and wet-ashed for analysis. Serum and liver zinc concentrations were determined by atomic absorption spectrophotometry. The results from these two groups were compared with those from a third group of shaminoculated (saline) rats.

In the first absorption study, rats were infected IP with 10^7 LVS organisms and fasted immediately thereafter. Eighteen-hr post-infection zinc absorption was studied by diluting carrier-free ^{65}Zn (International Chemical and Nuclear Corp.) with cold $ZnCl_2$ in 0.85% NaCl solution to yield a final concentration of 1.3 μg Zn^{2+}/ml. A 0.5-ml dose was administered to both infected and control rats by gastric intubation. One hour after administration of the isotope, the rats were decapitated, blood was col-

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Obtained from the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland.

lected in heparinized tubes, and the entire gastrointestinal tracts were removed. The radioactivity in the carcasses, the stomachs, and the remaining intestinal tracts was measured in a whole-body ARMAC Scintillation Detector (Model 446, Packard Instrument Co., Inc.) by an Auto Gamma Spectrometer (Model 2001, Packard Instrument Co., Inc.). The 1-hr pulse was employed initially to minimize the secretion of zinc back into the intestine. With the gastrointestinal tract removed the experimental data would represent both absorption and net retention of the ^{65}Zn and should be expressed as the percentage of the dose administered. However, marked fluid retention was observed in the stomachs of the infected rats as compared to the controls. Therefore, the amount of isotope absorbed was expressed as the percent of that which passed from the stomach into the intestine as follows:

$$\% \text{ absorbed} = \frac{\text{carcass } ^{65}Zn}{\text{carcass} + \text{intestine}}$$

In an attempt to circumvent the problem of fluid (isotope) retention in the stomach a longer pulse was employed. Rats were fasted 12 hr prior to receiving the ip administration of either 10^7 LVS organisms, 100 μg endotoxin, or sterile saline. Three hours after the administration of the different agents, all rats were given a dose of ^{65}Zn as described above. After an additional 24 hr the rats were decapitated, blood collected, and the entire gastrointestinal tracts were removed.

TABLE I. The Effect of LVS Infection and Endotoxin on Serum and Liver Zn Concentrations in the Rat

Treatment of rat (ip)	Serum Zn (μg/100 ml)[a]	Liver Zn (μg/g dry wt)[a]
Saline control	136 ± 5	84 ± 7
10^7 LVS	71 ± 8[b, c]	149 ± 13[b, c]
100 μg endotoxin	30 ± 2[b, d]	121 ± 5[b, d]

[a] Mean of a minimum of six animals ± SE.
[b] Significantly different from the controls ($P < 0.01$).
[c] Measured at 24 hr postinfection.
[d] Measured at 8 hr postadministration.

TABLE II. Effect of LVS Infection on the Absorption of ^{65}Zn in the Rat[a]

Treatment (ip)	Carcass (percentage absorbed[b, c]
Saline control	22.1 ± 3
10^7 LVS	42.8 ± 4[d]

[a] 18-hr infection plus a 1-hr pulse.
[b] Percent of dose reaching intestine.
[c] Mean of a minimum of six animals ± SE.
[d] Significantly different from the controls ($P < 0.01$).

Again radioactivity in the carcasses, the stomachs, and the remaining intestinal tracts was measured. Since no activity remained in the stomachs, absorption was expressed as the percentage of the dose administered. In addition, the livers were removed, freeze-dried, and analyzed for radioactivity in a gamma-well counter (Nuclear-Chicago), and serum zinc concentrations were determined by atomic absorption spectrophotometry.

Results. The results in Table I show the effects of acute LVS infection and endotoxin intoxication on serum and liver zinc concentrations. Both inflammatory stresses produced significant ($P < 0.01$) decreases in serum concentrations with concomitant hepatic increases of zinc in this strain of rats. These data are compatible with those previously reported in other strains (4, 6, 12).

Table II shows the effect of the LVS infection (18 hr) on ^{65}Zn absorption following a 1 hr pulse. Based on the amount of isotope that reached the intestine from the stomach, ^{65}Zn absorption was significantly ($P < 0.01$) higher in the infected animals than in the saline-treated controls.

Similar results were obtained when ^{65}Zn was given 3 hr after infection or endotoxin intoxication, and the isotope measured after an additional 24 hr. As shown in Table III, both acute infection and endotoxemia produced significant ($P < 0.01$) increases in the percentage of ^{65}Zn absorbed. It was observed that the values (including those for controls) in this experiment were similar to those where a 1-hr pulse was allowed, indicating that the zinc absorbed was not readily lost through excretion. Furthermore, when the livers of the LVS-infected rats were assayed

TABLE III. THE EFFECT OF LVS INFECTION AND ENDOTOXIN ON ZINC ABSORPTION AND DISTRIBUTION IN THE RAT

Group	Carcass (percentage of dose absorbed[a, b]	Liver ^{65}Zn (cpm/g dry wt)[b]	Serum Zn (μg/100 ml)[b]
10^7 LVS	47.8	1022	91
	+4.0[c]	±102[c]	±8[c]
Control	26.6	290	140
	±4.6	±36	±9
100 μg endotoxin	51.9	—	37
	±6.1[c]		±3[c]
Control	27.7	—	144
	±2.8		±3

[a] Percent of total dose after 24-hr pulse.

[b] Minimum of six animals ± SE.

[b] Significantly different from the controls ($P < 0.01$).

for radioactivity, they demonstrated over a threefold increase in ^{65}Zn as compared to controls (Table III). In addition, the cold serum zinc was found to be depressed in both stress groups.

Discussion. Alterations in zinc metabolism during acute infection, endotoxemia, and other inflammatory stresses have been well documented (1–8) and include a redistribution of zinc as shown in Table I. However, relatively little has been known concerning the effects of acute inflammation on the intestinal absorption of zinc. Turk and Stephens (13) reported that chickens suffering from coccidiosis caused by the intestinal protozoan *Emeria necatrix* demonstrated increased ^{65}Zn absorption with mild intestinal mucosa cell damage and inflammation. With severe cellular damage and hemorrhaging, ^{65}Zn absorption was decreased or even prevented.

The present investigation was designed to study the influence of acute systematic infection or inflammation on zinc absorption where direct mucosa damage was not involved. As shown from our study, both LVS infection and endotoxin intoxication produced significant increases in ^{65}Zn absorption during the first 24 hr of these two stresses in the rat.

Initially a 1-hr pulse was employed following an 18-hr LVS infection to minimize the possibility of zinc secretion back into the intestine. While an increase in absorption was observed (Table II) with this procedure, some retention of the ^{65}Zn dose (fluid) was noted in the infected rats. It is possible that during this infection, intestinal mobility was altered, thus hindering the passage of the dose from the stomach to the intestine. Consequently, a longer time lapse was employed. While the longer pulse may allow for the secretion of zinc back into the intestine, this phenomenon appeared negligible owing to the facts that values obtained were similar, whether the 1-hr or the 24-hr time lapse was employed. Furthermore, the livers in both the LVS-infected and endotoxin-intoxicated rats displayed increased concentrations of the ^{65}Zn, which would suggest not only increased absorption but also increased retention of zinc. If the zinc absorbed is rapidly diverted and sequestered in the liver, then less zinc would be in the serum for secretion back into the intestine. In support of this, serum zinc concentrations were depressed in the stressed rats at necropsy (Table III). Significant losses ot ^{65}Zn via the urine also seems unlikely, since it has been shown that during acute infection hypozincuria develops (8). Thus, acute inflammation appears to produce both increased absorption and retention of zinc.

The increased absorption of zinc during acute inflammation may result in part from an alteration in serum zinc concentration. Evans *et al.* (11) have suggested that the amount of zinc-free albumin available at the site of intestinal absorption may regulate the quantity of zinc transported to the body. Preliminary observations indicate that another plasma protein may also be involved in the transport process (unpublished observations, G. W. Evans). Thus, since acute inflammation results in a marked decrease in serum zinc levels, the quantity of zinc-free transport proteins available at the intestinal cell is probably increased; this results in a concomitant increase in zinc absorption from the intestine. The observations described in this paper may be another example of the homeostatic regulation of zinc absorption in mammalian systems.

While the mechanism for altered zinc absorption during these stresses remains uncertain, recent studies (7, 14, 15) have shown that the infection-induced alterations in zinc metabolism may be mediated by a low molecular weight protein released by polymorphonuclear leukocytes. When administered to a normal rat, this substance, called leukocytic endogenous mediator (LEM), produces a decrease in serum zinc concentrations and an increased hepatic uptake of the metal. This substance was detected in the set of patients with a variety of acute infections (16, 17), and in experimentally infected laboratory animals (14). Studies are currently in progress to see if LEM may be responsible for the increased intestinal absorption and liver retention of zinc observed in the present study.

Summary. Intestinal zinc absorption was found to be significantly increased during acute bacterial infection and endotoxemia in the rat. Although serum zinc concentrations were depressed, there was a significant accumulation of ^{65}Zn in the livers of the stressed animals. This study demonstrates that acute inflammation produces a redistribution of zinc within the host, which results in both increased zinc absorption and retention.

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