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Effect of Leukocytic Endogenous Mediator (LEM) on Zinc Absorption in the Rat (39443)

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Hypozincemia, hypozincuria, and the increased hepatic uptake of zinc are characteristic responses of the mammalian host during many infections, endotoxemia, and other inflammatory stresses (1-4). Recently, it was postulated that leukocytic endogenous mediator (LEM), a heat labile, low molecular weight protein released by polymorphonuclear (PMN) leukocytes, may be a key intermediate in stimulating the above alterations in zinc metabolism during inflammation (5-7).

More recently, Pekarek and Evans (8) demonstrated that acute infection and endotoxemia in rats enhanced the intestinal absorption of zinc with a significant accumulation of zinc in the livers of the stressed animals. While the mechanism for altered zinc absorption during inflammation remains uncertain, the present investigation was undertaken to determine whether LEM could be responsible, in part, for the increased intestinal absorption and hepatic uptake and retention of zinc observed during acute inflammatory stresses.

Materials and methods. Healthy adult male New Zealand white rabbits, weighing 2-3 kg, and male Sprague-Dawley rats, weighing 150-175 g, were used in our study. The LEM was prepared by infusing rabbits intraperitoneally (ip) with approximately 300 ml of a sterile pyrogen-free normal saline solution containing 0.2% shellfish glycogen (Mann Research Laboratories).[1] Peritoneal exudates were collected aseptically 14 hr after infusion, and the PMN leukocytes were obtained by centrifugation. The harvested cells were washed twice with cold saline; resuspended at 1×10^8 cell/ml in sterile pyrogen-free normal saline which contained 10 U/ml of heparin, 150,000 U/liter of penicillin G, and 0.4 g/liter of streptomycin; and incubated at 37° for 2 hr with gentle shaking (5, 6). Following incubation, the cells were removed by centrifugation; the supernatant represented the crude LEM preparation.

The effect of LEM on zinc metabolism was tested by injecting each of a group of rats ip with 1 ml of LEM preparation. Each of another group of rats was injected ip with an equal aliquot of the LEM preparation that was heat inactivated (90° for 30 min). This was done as a control to insure that the observed changes in zinc metabolism were not due to any contaminating heat-stable endotoxins. Each rat of a third group received an equal volume of sterile pyrogen-free normal saline ip and the group served as an additional set of controls.

Six hours after the administration of the above materials, zinc absorption was studied by diluting carrier-free ^{65}Zn (International Chemical and Nuclear Corp.)[1] with cold $ZnCl_2$ in normal saline to give a final concentration of 1.3 μg Zn^{2+}/ml. All rats in each test group received a 0.5-ml dose of the radiolabeled Zn by gastric intubation. One hour following the administration of the isotope, the rats were decapitated, blood was collected in heparinized tubes, and the entire gastrointestinal tracts were removed. The radioactivity in the carcasses, the stomachs and remaining intestinal tracts were measured in a whole-body ARMAC Scintillation Detector (Model 446, Packard Instrument Co., Inc.)[1] by an Auto Gamma Spectrometer (Model 2001, Packard Instrument Co., Inc.)[1]. Zinc absorption was measured 1 hr after the oral administration of the isotope in order to minimize the secretion of zinc back into the intestine (8). The

[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.

amount of isotope absorbed into the body was calculated as follows:

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

In addition, the livers were removed and the amount of isotope taken up and retained by the liver was expressed in a similar manner:

$$\% \text{ uptake} = \frac{\text{liver } ^{65}\text{Zn}}{\text{carcass} + \text{liver}}.$$

Plasma zinc concentrations were determined by atomic absorption spectrophotometry.

Results. Based on the amount of isotope that reached the intestine from the stomach, ^{65}Zn absorption was significantly ($P < .005$) higher in the LEM-treated rats than in either the saline or heat inactivated LEM-treated controls (Table I). In addition, LEM produced a significant increase in the hepatic uptake of ^{65}Zn and decrease in plasma zinc concentrations. While the LEM preparation was highly active, the heat treated (90° for 30 min) LEM failed to produce any changes in zinc metabolism in the rat; thus, showing that the LEM preparation used in this study was apparently free of any contaminating heat-stable endotoxins.

Discussion. Evans *et al.* (9, 10) have shown that zinc absorption is inversely proportional to the intestinal and plasma zinc concentrations. More specifically, Richards and Cousins (11) have demonstrated that zinc absorption is in part regulated by the concentration of metallothionein, a zinc binding protein, in the intestinal mucosa. metallothionein synthesis, in turn, is regulated by the amount of zinc present in the intestinal cells. Thus, when the plasma zinc content is low, less zinc enters the intestinal cells from the lamina propria resulting in a concomitant reduction in metallothionein synthesis. In turn, more zinc is transported across 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 binding plasma proteins (12).

In this regard, acute infection and endotoxemia have been shown to produce significant alterations in normal zinc metabolism, resulting in a rapid flux of zinc into the liver, a lowering of the plasma zinc concentration and an increase in the intestinal absorption of this metal (8). Thus, the lowering of plasma zinc due to the rapid redistribution of this metal to the liver and possibly to other tissues would explain the increase in zinc absorption observed during the inflammatory process according to our present knowledge of zinc homeostasis.

The data from the present study demonstrate that a factor released from PMN leukocytes also initiates these changes in zinc metabolism when PMN leukocytes are administered to a normal rat. This factor has been shown to be present in the plasma of patients with a variety of acute infections (13, 14) and in experimentally infected laboratory animals (5). While LEM may be a key intermediate in altering zinc homeostasis during the inflammatory process, its mechanism of action remains somewhat obscure. However, LEM also has been shown to induce the hepatic uptake of plasma free amino acids (15) and the increase synthesis and/or release of various acute phase glycoproteins (16, 17). Since zinc has been shown to be essential for RNA, DNA and certain aspects of protein synthesis (18–20), there may be a greater requirement for this metal in the liver during inflammation. While the functional role of zinc in the liver and the mechanism by which LEM acts to increase the hepatic zinc content during inflammation are still unknown, the fact remains that

TABLE I. EFFECT OF LEM ON ZINC ABSORPTION AND DISTRIBUTION IN THE RAT

Treatment of rat (1 ml ip)	Carcass Zn (% absorbed)[a]	Liver Zn (% absorbed)[a]	Plasma Zn (μg/100 ml)[a]
LEM	38.1 ± 1.9[b]	52.1 ± 2.4[b]	36 ± 2[b]
Heat inactivated LEM	22.2 ± 3.1	38.8 ± 2.8	124 ± 5
Saline control	22.9 ± 1.8	42.3 ± 0.9	125 ± 5

[a] Minimum of six animals ± SE.
[b] Significantly different from control groups ($P < .005$).

the inflammatory process causes a redistribution of zinc within the tissue of the body with a concomitant increase in the intestinal absorption of this metal.

Summary. Leukocytic endogenous mediator (LEM) was shown to produce a significant increase in the intestinal absorption of zinc within 7 hr after its administration to normal rats. Consistent with earlier studies, LEM also produced a significant decrease in the plasma zinc concentration and an increased hepatic uptake of this metal which further demonstrates that LEM may be a key intermediate in altering zinc homeostasis during inflammation.

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