The Effect of Leukocytic Endogenous Mediator (LEM) on the Tissue Distribution of Zinc and Iron[1] (36531)

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It is well known that acute and chronic infectious diseases (1–4) endotoxemia (5–7), and a variety of other stresses (5, 8, 9) can induce alterations in zinc and iron metabolism in man and experimental animals. Recent studies by Kampschmidt and Upchurch (10, 11) and Pekarek and Beisel (12) have shown that the lowering of serum zinc and iron concentrations during infection and endotoxemia are mediated by a common endogenous humoral factor released, in part, by polymorphonuclear (PMN) leukocytes.

This leukocytic endogenous mediator (LEM) has been characterized as a heat-labile, low molecular-weight protein, and it appears within the plasma of the rat within 2 hr after initiating an experimental infection or endotoxemia. The administration of LEM prepared from rat or rabbit peritoneal leukocytes will significantly reduce serum zinc and iron concentrations within 6 hr in a normal recipient rat (12). The rapidity in which these alterations in zinc and iron metabolism occur during an inflammatory process in both man and the experimental animal suggest that these changes are not due to decreased gastrointestinal absorption or increased excretion, but rather to a redistribution of these two metals within the tissues of the host.

The present investigation demonstrated the effect of LEM on the tissue distribution of zinc and iron; thus giving a further understanding of host metabolism during the inflammatory process.

Materials and Methods. Male Dunning–Fisher rats (Microbiological Associates), weighing 175–200 g, were used in this study. LEM was prepared from rat peritoneal PMN leukocytes by a previously described method (12). The protein concentration of the LEM preparation was determined by the method of Lowry *et al.* (13). Its activity was assayed by administering 150 μg of LEM (protein) in 1 ml ip to a group of rats and measuring the depression of serum zinc and iron concentrations 6 hr later. To insure that changes in the metabolism of these two metals were not due to contaminating heat-stabile endotoxins, a portion of the LEM preparation was heated at 90° for 30 min before being administered to another group of rats. The results from these two groups were compared to serum zinc and iron concentrations in a third group of sham-inoculated (saline) rats. Serum zinc concentrations were determined by an atomic absorption spectrophotometric method described by Pekarek and Beisel (7) and serum iron concentrations by the colorimetric method of Levy and Vitacca (14).

To test the effect of LEM on the tissue distribution of zinc and iron, 20 rats were pulse-labeled intraperitoneally (ip) with 5 $\mu Ci/100$ g body weight of $^{65}ZnCl_2$ (New England Nuclear) and another 20 rats with 2.5 $\mu Ci/100$ g body wt of $^{59}FeCl_3$ (New England Nuclear). After 18 hr 10 rats in each of the two isotope groups were administered 150 μg LEM protein ip; the remaining 10 rats in each group were given 1 ml of sterile pyrogen-free saline ip and served as controls. After an additional 6 hr, all rats were bled and

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

TABLE I. The Effect of LEM and Heated LEM Preparations on Serum Zn and Fe Concentrations in the Rat.

Treatment of rat (ip)	Serum Zn (μg/100 ml)[a]	Serum Fe (μg/100 ml)[a]
Saline control	134 ± 5	144 ± 3
150 μg LEM	77 ± 3[b,c]	69 ± 4[b,c]
150 μg LEM, heated 90° for 30 min	127 ± 3[c]	146 ± 5[c]

[a] Mean of a minimum of six animals ± SE.
[b] $p < .01$, compared to controls.
[c] Measured at 6 hr postadministration.

then killed by cervical dislocation. The liver, spleen, heart, lungs, kidneys, pancreas, and gastrointestinal tract of each rat were removed, weighed, placed in a whole-body ARMAC Scintillation Detector (Model 440, Packard Instrument Co., Inc.) and the respective isotope counted by an Auto Gamma Spectrometer (Series 410A, Packard Instrument Co., Inc.). The remaining carcass, which included bone, muscle, skin, and hair also was weighed and counted along with samples of plasma.

Results. As shown in Table I, the LEM obtained from rat peritoneal PMN leukocytes produced significant depressions in both serum zinc and iron concentrations in the rat when measured 6 hr postadministration. When LEM was heated at 90° for 30 min, its serum zinc- and iron-depressing effects were lost. Thus, the unheated LEM preparation was highly active and appeared to be free of contaminating endotoxins, which are heat stabile.

Figure 1 illustrates the effect of LEM on the distribution of ^{65}Zn within the various tissues and organs of the rat. This experiment clearly demonstrated that within 6 hr, the LEM caused a significant accumulation of ^{65}Zn in the liver, while significant decreases in ^{65}Zn concentrations were observed in the kidney, carcass, and plasma.

Similarly, Fig. 2 shows that there was a significant increase of ^{59}Fe within the liver of the rat after the administration of LEM. However, no significant changes in the values for ^{59}Fe were observed in any of the other tissues.

Discussion. Alterations in the metabolism of zinc and iron during infection, endotoxemia, and other inflammatory stresses have been well documented and include a redistribution of iron (15–17) and zinc (18). Until recently, little has been known concerning the pathophysiologic mechanism, or mechanisms, leading to these alterations. This study not only confirms the observation of Kampschmidt and Upchurch (10, 11) and Pekarek and Beisel (12) by showing that LEM produces significant depressions in serum zinc and iron concentrations, but it also adds a further mechanistic insight, by demonstrating that this endogenous intermediate of the inflammatory process produces a rapid redistribution of these two metals within the tis-

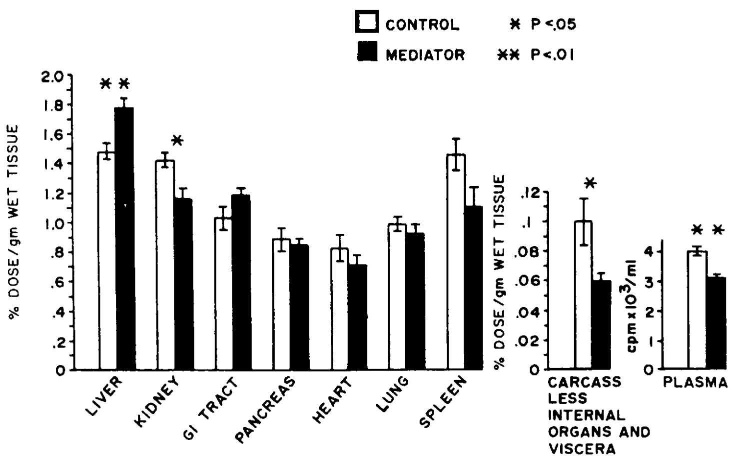

FIG. 1. Effect of LEM on the distribution of ^{65}Zn in the rat.

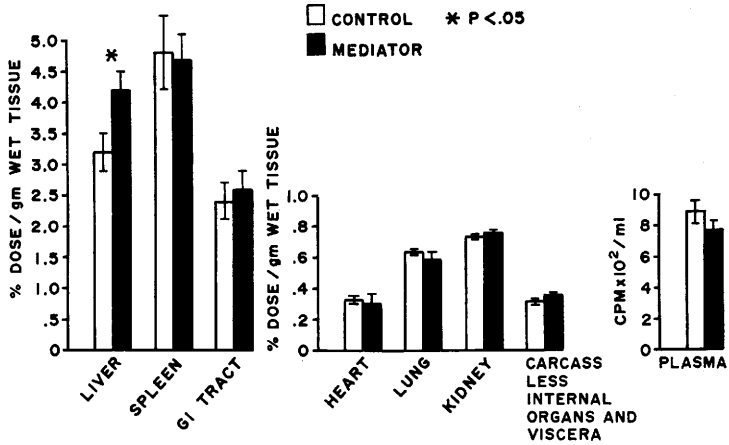

FIG. 2. Effect of LEM on the distribution of ^{59}Fe in the rat.

sues of the host.

Since LEM is prepared from PMN leukocytes by a procedure similar to that used for obtaining endogenous pyrogen (EP), it is only natural that crude preparations would contain both factors. Although LEM shares similar characteristics to EP, it is still not known whether they are the same or different substances. Only the further purification of these mediators will resolve this question.

Besides being pyrogenic and responsible for alterations in zinc and iron metabolism, Wannemacher *et al.* (19) recently have shown that either active infection or the experimental administration of LEM will induce an increased flux of amino acids into the liver of the rat. Similarly, Eddington *et al.* (20) have demonstrated that extracts from PMN leukocytes stimulated an increase in the level of α_1-acute phase (AP) globulin within 48 hr and the appearance and synthesis of a new protein designated α_2-AP globulin within 10 hr in the rat. Further, Pekarek (21) showed that LEM produced a significant increase in serum copper concentrations and its carrier protein ceruloplasmin. Therefore, with infection, endotoxemia or the administration of LEM a definite sequence of alterations is observed in the rat model. There is a rapid increased flux of amino acids into the liver, which is followed by a significant liver uptake of zinc and iron: both metals being co-factors for a variety of enzymes and important for protein synthesis and for maintaining the integrity of the ribosomes (22, 23). Thus, LEM or a group of such mediators, by inducing a flux of amino acids and necessary co-factors (zinc and iron) into the liver, may provide the necessary metabolic precursors and steps leading to the synthesis of specific serum proteins, such as the α_1 and α_2-AP globulins and ceruloplasmin. The synthesis of these serum proteins lends further support to the role of liver during the inflammatory process. It appears that LEM, or a group of mediators, may play an important role in the stimulation of these nonspecific host defense mechanisms.

Summary. Leukocytic endogenous mediator (LEM) was shown to induce significant alterations in zinc and iron metabolism. Isotopic studies, employing ^{65}Zn and ^{59}Fe demonstrated that LEM stimulated a rapid redistribution of zinc and iron within the tissues of the host, with a significant uptake of these two metals by the liver.

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