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Effect of Endotoxin on Serum Zinc Concentrations in the Rat

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Serum zinc concentrations decreased significantly in a dose-dependent response after endotoxin administration in the rat. The reproducibility and sensitivity of the biological response offer a potential bioassay of endotoxin.

Lipopolysaccharides from various gram-negative bacteria produce a rapid, significant, and dose-dependent hypoferremic response when injected into normal mice and rats (3, 6, 7), and, as in studies on endotoxin pyrogenicity and fever (1), the development of tolerance to the induced hypoferremic state has been demonstrated (7). Although alterations of iron metabolism within the host have been studied extensively during endotoxemia and infection, a possible effect of endotoxin on zinc metabolism has not been reported. Significant decreases in serum zinc concentrations in patients with various infections and malignancies (2, 10) indicate that serum zinc may respond rapidly in a manner analagous to the alterations found in iron metabolism during endotoxemia. This investigation demonstrates that serum zinc concentrations, like those of iron, decrease significantly in a dose-dependent manner after endotoxin administration.

MATERIALS AND METHODS

Male Dunning-Fisher rats weighing 175 to 200 g, obtained from Microbiological Associates, Walkersville, Md., and lipopolysaccharide from *Escherichia coli* 0127:B8, obtained from Difco (catalog no. 3880), were used in this investigation. The rats were divided into five dose-response groups of 0.01, 0.1, 1.0, 10, and 100 μg of endotoxin given intraperitoneally in 0.5 ml of normal saline at 8:30 AM. Ten rats from each group were anesthetized with 0.1 ml of a 5% sodium thiopenthol solution via the dorsal penal vein at 3, 6, 9, 12, and 24 hr after endotoxin administration, and blood was collected from an axillary-fold pouch of individual rats after severing the brachial artery. An equal number of control animals receiving 0.5 ml of pyrogen-free normal saline intraperitoneally were bled at each time period in order to eliminate any variability in test values caused by diurnal periodicity. To minimize zinc contamination from exogenous sources, zinc-free polyethylene pipettes and test tubes were used in the collecting and handling of the blood and serum throughout the study.

Serum zinc concentrations were determined on a Perkin-Elmer atomic absorption spectrophotometer (model 303) equipped with an Intensitron hollow-cathode zinc lamp, a three-slot Boling burner head, and an automatic Null recorder readout. The samples were diluted manually with triple-distilled water free from detectable zinc and were placed in a Technicon Sampler II from which they were automatically presented to the flame. All samples were run in replicate and never varied by more than 5% of the total zinc concentration.

RESULTS AND DISCUSSION

A diurnal periodicity of serum zinc was confirmed by our measurements in control animals. This amounted to a 19% difference between peak control values occurring at noon and lowest values occurring at midnight. When experimental groups were compared with their respective controls, significant decreases in serum zinc concentrations were observed in four of the dose-response groups as early as 3 hr after endotoxin administration, with all five groups showing significant maximal reductions between 6 and 9 hr (Fig. 1). Thus, after correction for diurnal changes, the degree and duration of reduction in serum zinc values appeared to be dependent upon the dose of endotoxin administered. Figure 2 illustrates the linear relationship between the reduction in serum zinc concentration and the logarithm of the dose of endotoxin within the range of 0.01 to 100 μg.

The observed decreases in serum zinc concentrations closely corresponded, in magnitude and duration, to endotoxin-induced hypoferremias found in mice and rats (3, 7). The only notable difference was in the time that maximal decreases occurred. Maximal hypoferremias were reported to occur at 12 hr after endotoxin administration, whereas maximal serum zinc reductions occurred between 6 and 9 hr. The difference in the timing may result from differences in

mechanisms and kinetics involving the dearrangement of the two metals or from a lack of simultaneously bled controls necessary for an evaluation of the effects of the known diurnal, afternoon depression of serum iron.

Although the mechanism or mechanisms involved in the reductions of serum zinc and iron are unknown in endotoxemia and infection, it appears that the observed alterations in the metabolism of both metals may be related to a common mechanism of host response. Illness-related decreases in serum zinc values, like iron, do not appear to be the result of increased zinc excretion, but rather to a redistribution of the metal to cells of the reticuloendothelial system (4, 9). Consequently, the alterations in both zinc and iron metabolism during endotoxemia may be mediated through a common mechanism involving the well-documented changes in reticuloendothelial system activity (8).

The superiority of the hypoferremia bioassay over other commonly used bioassay methods for endotoxin (5) are discussed by Baker and Wilson (3). However, we feel that the measurement of serum zinc reductions by the atomic absorption method offers an even more sensitive and accurate bioassay of endotoxin for the following reasons. Preliminary studies on control serum zinc and serum iron concentrations revealed that

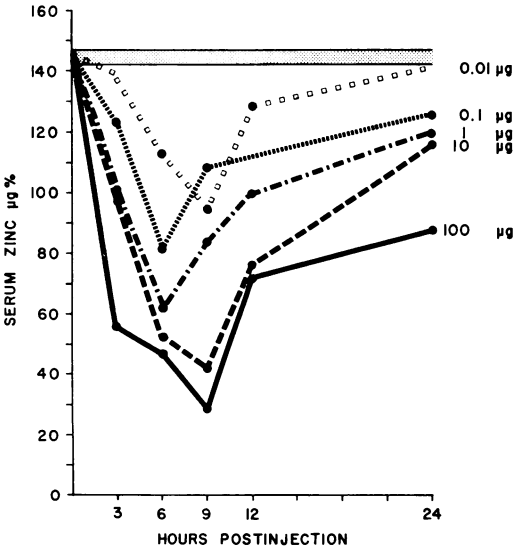

FIG. 1. *Response of serum zinc to five different doses of endotoxin. Shaded horizontal band represents the average of all control values ± SE. Solid circles represent values significantly different ($P < 0.01$) from control.*

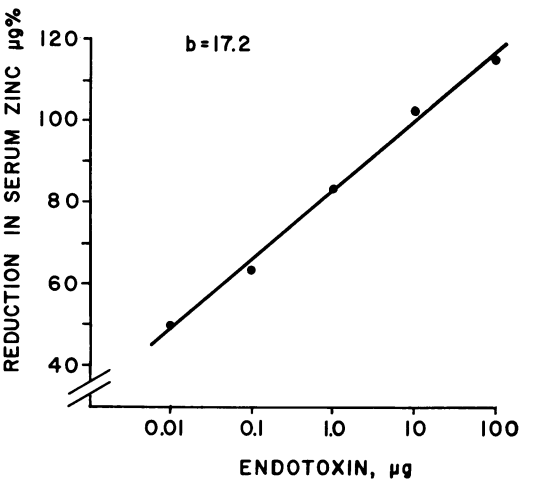

FIG. 2. *Reduction in serum zinc in rats given five different doses of endotoxin.*

there is a narrower range of variability in serum zinc values than in serum iron within the same species and strain of test animals. Further, the atomic absorption method requires only 0.3 ml of serum in comparison to 1 ml or more for colorimetric methods. This reduced amount of serum is more than adequate to run individual samples in duplicate, and it eliminates the pooling of serum from two or more animals. Finally, in contrast to colorimetric methods, values determined by the atomic absorption method are not affected by increased lipemia in serum samples, which has a tendency to obscure colorimetric readings. (Atomic absorption spectrophotometry has become an important analytical technique in the study of trace metal metabolism. With the increased application of atomic absorption spectrophotometry in biological sciences, relatively low priced instruments of acceptable sensitivity have become available.)

ACKNOWLEDGMENT

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