The Effect of Endogenous Pyrogen on the Plasma Zinc Concentration of the Rat (34962)

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Recently Pekarek and Beisel (1) found that serum zinc concentration decreased significantly in a dose-dependent response after endotoxin administration in the rat. The serum zinc concentration reached a minimum at 6 to 9 hr after injecting from 0.01 to 100 μg of endotoxin. Our previous investigations had shown that endotoxin would decrease plasma iron concentration in the rat reaching a minimum 8 to 12 hr after injection (2, 3). Repeated injections led to the development of tolerance (2, 4) and the major reason for the decrease in plasma iron after the injection of endotoxin was found to be an inhibited re-utilization of iron from recently destroyed erythrocytes (3, 5). The lowering of plasma iron concentration after injections of endotoxin was shown to be due to a protein intermediate produced by the leukocytes (6). This intermediate appears to be similar to endogenous pyrogen (7).

Plasma zinc, like plasma iron, has been shown to be decreased in concentration during acute infection, leukemia, cirrhosis, and various malignant diseases (8–11). It seemed possible that plasma zinc lowering after endotoxin may also be acting through a protein intermediate. We, therefore, investigated the effect of endogenous pyrogen on the plasma zinc concentration of rats and the effect of daily injections of either endotoxin or endogenous pyrogen.

Materials and Methods. Measurements of plasma zinc concentration were in inbred, male Fischer rats, weighing approximately 200 g. Blood was collected from the heart of anesthetized rats with a heparinized syringe and the plasma was separated by centrifugation. The plasma zinc was measured using zincon reagent by Auerbach's modification (12) of the procedure of Zak *et al.* (13).

The endotoxin was *Escherichia coli* 055:B5, lot 460830 from Difco Laboratories, Detroit, Michigan. Tolerance to endotoxin was produced by giving 1 μg ip for 7 daily injections.

Endogenous pyrogen was prepared from rabbit peritoneal leukocytes by the method of Kaiser and Wood (14). The techniques and special precautions which we have used have been described previously (7, 15).

Results. The effect of endogenous pyrogen, prepared from rabbit peritoneal leukocytes, on plasma zinc concentration is shown in Fig.

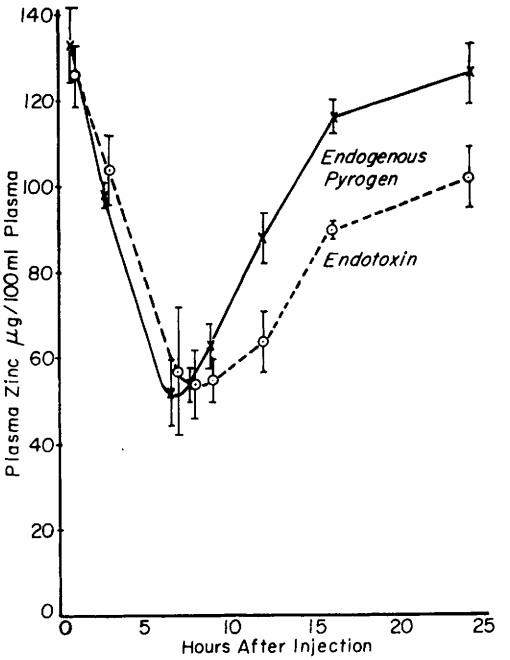

FIG. 1. Plasma zinc concentration in male Fischer rats at various times after an ip injection of either 5 μg of *E. coli* endotoxin or endogenous pyrogen obtained from 2×10^8 rabbit peritoneal leukocytes. Each point is an average value for a group of 3–9 rats with the brackets indicating the SE.

TABLE I. Effect of Endogenous Pyrogen on Plasma Zinc Concentration in Normal Rats and Rats Tolerant to *E. coli* Endotoxin.

Recipient rat	Material injected	No. of trials	Plasma zinc[a] (μg/100 ml of plasma)
Normal	None	21	129 ± 5
	1 μg endotoxin	12	66 ± 7
	Endogenous pyrogen from 1 × 10^8 PMN[b]	18	71 ± 6
	Endogenous pyrogen from 2 × 10^8 PMN	18	54 ± 5
Tolerant[c]	1 μg endotoxin	12	122 ± 6
	Endogenous pyrogen from 1 × 10^8 PMN	12	74 ± 5
	Endogenous pyrogen from 2 × 10^8 PMN	12	52 ± 5

[a] Plasma zinc concentration was determined 8 hr after the last injection and the values are mean ± SE.

[b] Polymorphonuclear leukocytes from the peritoneal cavity of rabbits.

[c] Tolerance was produced by 7 daily injections of 1 μg of endotoxin.

1. The amount of lowering produced by the supernatant from 2 × 10^8 leukocytes was comparable to injecting 5 μg of *E. coli* endotoxin. The endogenous protein seemed to cause an earlier decrease and a more rapid return of the plasma zinc to normal levels in the rat than that produced by injections of endotoxin. In confirmation of the investigations of Pekarek and Beisel (1), the plasma zinc reached a minimum at 6 to 9 hr after injecting endotoxin and the decrease was dose-dependent.

The effect of endogenous pyrogen on plasma zinc in endotoxin-tolerant rats is shown in Table I. Seven daily injections of 1 μg of endotoxin produced a tolerance, and at 8 hr after the last injection the plasma zinc was within the normal range. Endogenous pyrogen was equally effective in decreasing plasma zinc concentration in normal rats and in those tolerant to endotoxin. A group of 8 animals was given 7 daily injections of endogenous pyrogen from 1 × 10^8 leukocytes, and the plasma zinc 8 hr after the last injection was 70 ± 6. This would indicate that the endogenous pyrogen not only was effective in endotoxin-tolerant rats but does not cause the development of tolerance.

Discussion. Previous investigations had shown that endogenous pyrogen in addition to producing fever would also lower plasma iron concentration (6) and that a similar protein was apparently responsible for both activities (7). There has been some evidence that a small portion of the plasma zinc may be transported by transferrin (16). The marked lowering of plasma zinc after injections of endotoxin or endogenous protein would suggest that more than the transferrin-bound zinc was involved.

Animals with acute infection or inflammation show a number of typical alterations in their metabolism. Three of these changes, fever (14), lowering of plasma iron concentration (7), and lowering of plasma zinc concentration, have now been demonstrated after injection of low molecular weight proteins produced by leukocytes.

A number of different conditions have been shown to cause leukocyte activation and the release of the endogenous protein (17, 18). If this endogenous protein will cause several different alterations when injected, the occasional production of one effect without the others must be due to the responsiveness of the host's target tissue. More information is also needed on leukocytic activation and release of these proteins during the chronic conditions, such as malignant diseases.

Summary. Plasma zinc concentration was depressed in rats following the injection of endogenous pyrogen. The endogenous pyrogen, obtained from peritoneal leukocytes, was active in endotoxin-tolerant animals and repeated injections of this protein did not produce tolerance.

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