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Role of Zinc in the Abatement of Hepatocellular Damage and Mortality Incidence in Endotoxemic Rats

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Intraperitoneal administration of zinc (ZnIP) as zinc chloride prior to or simultaneously with a lethal quantity of intraperitoneally administered *Salmonella typhimurium* endotoxin significantly protected rats against toxin-induced mortality and hepatocellular damage. Pretreatment with amounts of zinc chloride ranging from 0.4 to 2.0 mg/100 g of body weight resulted in 80 to 100% survival compared with 10% survival in untreated control rats at 24 h after endotoxin treatment. Zinc chloride treatment in excess of 2.0 mg/100 g of body weight appeared to be toxic and provided diminished protection. In contrast with the protection obtained with ZnIP, intravenously administered zinc did not provide protection. The effectiveness of ZnIP to enhance survival if it was given after endotoxin was greatly diminished as a function of time after endotoxin. The extent of hepatocellular damage was assessed at various times after endotoxin administration in ZnIP-treated and untreated rats by measurement of plasma ornithine carbamoyltransferase activity and histological examination of liver sections. Endotoxin absorption from the peritoneal cavity and hepatic uptake were studied by using ^{51}Cr-labeled endotoxin. ZnIP pretreatment significantly reduced ^{51}Cr-labeled endotoxin content of blood and liver when compared to untreated controls, and effectively prevented endotoxin-induced elevations in plasma ornithine carbamoyltransferase activity and hepatic tissue necrosis. These data indicate that protection afforded by ZnIP treatment results as a consequence of the ability of zinc to diminish absorption of the toxin from the peritoneal cavity and subsequent hepatic uptake.

A recent study performed by Snyder and Walker (8) has demonstrated that pretreatment of mice with zinc chloride significantly enhances survival incidence after intraperitoneal (i.p.) challenge with a lethal dose of *Salmonella typhosa* endotoxin. In addition, we have recently reported preliminary results (Fed. Proc. 35:360, 1976) that a similar protective phenomenon occurs in zinc-pretreated rats exposed to lethal quantities of *Salmonella typhimurium* endotoxin. It has been proposed (8) as one possibility that zinc may exert its protective effect through its documented (3) role in the stabilization of biological membranes: in particular, lysosomal membranes, with subsequent prevention of the lethal event(s) attributed to endotoxin-induced release of lysosomal hydrolases.

Initially, the present study was performed with rats to evaluate further and characterize the efficacy of zinc treatment in experimental endotoxemia and to investigate the possibility that protection of the liver against endotoxin-induced cellular damage may be a contributing factor to survival. The latter aspect of this study was prompted by our observation (Fed. Proc. 35:360, 1976) that zinc, when administered i.p., effectively prevented endotoxin-induced hyperaminoacidemia and the elevation of plasma transaminase levels. Additional experiments were performed to determine whether zinc in some manner alters the absorption and hepatic uptake of the toxin.

MATERIALS AND METHODS

Animals. Male Fisher-Dunning rats (Microbiological Associates, Inc., Walkersville, Md.), weighing 175 to 225 g, were housed 10 per cage and used 1 week after acclimation in a room maintained at 22 to 24°C and lighted from 6 a.m. to 6 p.m. Water and Wayne Lab-Blox (Allied Mills Inc., Chicago, Ill.) were fed ad libitum.

Endotoxin. *S. typhimurium* (Lipopolysaccharide B, Difco Laboratories, Detroit, Mich.) endotoxin was administered i.p. as a saline suspension, 1.0 mg/100 g of body weight. This amount of endotoxin was approximately a lethal dose for 90% of the rats used

in these experiments. Zinc, as zinc chloride in physiological saline, was also administered i.p. at various doses on a body-weight basis. In one series of experiments, zinc was administered via the penile vein. Control rats received an equivalent volume of physiological saline, 1.0 ml/100 g of body weight, in place of either endotoxin or zinc chloride. ^{51}Cr-labeled *S. typhimurium* endotoxin was prepared as described by Zlydaszyk and Moon (10). Labeled endotoxin was administered i.p. and intravenously (i.v.) on a body weight basis, 50×10^3 cpm/100 g.

Tissue sampling. Rats were killed, at times specified in figures and tables, after endotoxin administration by exsanguination after halothane anesthesia. Blood samples were obtained by cutting the vena cava and then collecting samples in the pleural cavity. Heparin (10 IU/ml) was used to prevent clotting. Plasma samples were stored at −20°C for no longer than 2 weeks prior to analyses. In separate experiments, the entire liver was extirpated at the time of blood collection and either perfused with saline until free of visible blood for radioactivity determinations or immediately placed in neutral buffered 10% formalin for subsquent histological procedures. Tissues were embedded in paraffin, sectioned at 4 to 6 μm, and stained with hematoxylin and eosin.

Analytical procedures. Plasma zinc concentration was determined by atomic absorption spectrophotometry (6). Plasma samples obtained from zinc-treated and untreated endotoxemic rats were assayed for ornithine carbamoyltransferase (OCT) (EC 2.1.3.3.) activity by an automated procedure (2) that measures the citrulline generated from the condensation of ornithine and carbamoylphosphate. ^{51}Cr radioactivity was determined in blood and liver samples by counting in a Nuclear Chicago model 1185 gamma counter. Efficiency of counting was 60%.

Statistics. The Student *t* test was used to determine the significance of the difference between group means at each time period studied.

RESULTS

The effect of i.p. administration of various amounts of zinc chloride, 1 h prior to i.p. challenge with *S. typhimurium* endotoxin, on mortality at 24 h is shown in Fig. 1. Injected amounts of zinc chloride ranging from approximately 0.4 to 2 mg/100 g of body weight provided optimum protection and 80 to 100% survival; diminished protection was obtained with an amount greater than 2 mg/100 g. A zinc chloride dose of 1.6 mg/100 g was selected for subsequent experiments, since it provided an optimum protective effect and was comparable to the dose used in mice (8). This pretreatment dose of zinc chloride produced a fivefold increase in plasma-zinc levels (966 ± 62 μg/100 ml, mean ± standard error of the mean, $n = 5$) at 1 h (the time of endotoxin administration). In marked contrast with the protection obtained

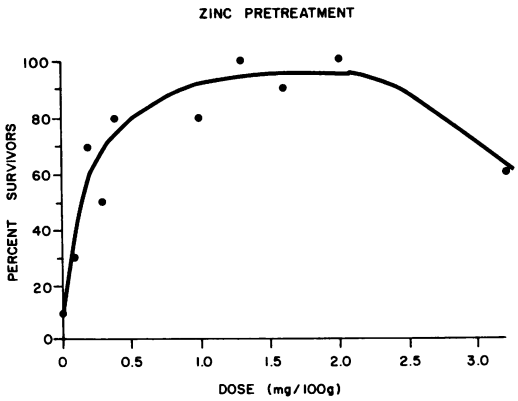

FIG. 1. *Effect of pretreatments with various amounts of zinc chloride on survival at 24 h after endotoxin challenge. Rats, 10 per group, were injected i.p. with S. typhimurium endotoxin (1.0 mg/100 g of body weight) 1 h after i.p. injection of zinc, as zinc chloride, or an equivalent volume of physiological saline.*

with i.p. administration of zinc, the i.v. administration of this amount of zinc 1 h prior to endotoxin challenge did not provide protection.

Table 1 shows the ^{51}Cr activity for blood and liver 5 h after either i.p. or i.v. administration of ^{51}Cr-labeled endotoxin in zinc-treated and untreated rats. Zinc pretreatment significantly reduced the blood and hepatic concentration of ^{51}Cr-labeled endotoxin by 5 h after endotoxin administration as compared with untreated controls. In contrast, zinc pretreatment had no apparent effect on the amount of radioactivity in blood and liver when ^{51}Cr-labeled endotoxin was administered i.v.

The effect of timing of zinc chloride administration on mortality, relative to *S. typhimurium* endotoxin challenge, is shown in Fig. 2. One-hour pretreatment with zinc or simultaneous administration of zinc with endotoxin provided significant protection against the lethal aspects of endotoxemia. If zinc was given subsequent to endotoxin, it only altered the time until death and not the ultimate percentage of mortality.

In other experiments (Fed. Proc. 35:360, 1976), we have observed that zinc pretreatment effectively diminished the elevation in plasma β-glucuronidase and glutamic pyruvic transaminase activities induced by endotoxin administration. However, since it is difficult to identify the cellular source of these enzymes, a series of experiments was conducted to measure plasma OCT activity, an enzyme considered to be a specific indicator of hepatic injury because of its normally high concentration in liver (7).

TABLE 1. *^{51}Cr radioactivity in blood and liver 5 h after either i.p. or i.v. injection of ^{51}Cr-labeled endotoxin in zinc-treated and untreated rats*

Treatment	Radioactivity	
	Blood (cpm/ml)[a]	Liver (cpm/g)[a]
Intraperitoneal[b]		
Zinc (10)	314 ± 58[c]	427 ± 109[c]
Saline (10)	1,137 ± 131	2,547 ± 360
Intravenous		
Zinc (2)	716 (612–820)	3,716 (3,849–3,582)
Saline (2)	688 (584–792)	3,795 (2,739–4,851)

[a] Value shown is the mean ± standard error of the mean or the mean and range (shown in parentheses) for the number of animals.

[b] Either zinc chloride, 1.6 mg/100 g of body weight, or an equivalent volume of saline was intraperitoneally administered 1 h prior to ^{51}Cr-labeled endotoxin (50×10^3 cpm/100 g of body weight).

[c] $P < 0.001$ versus zinc-untreated controls.

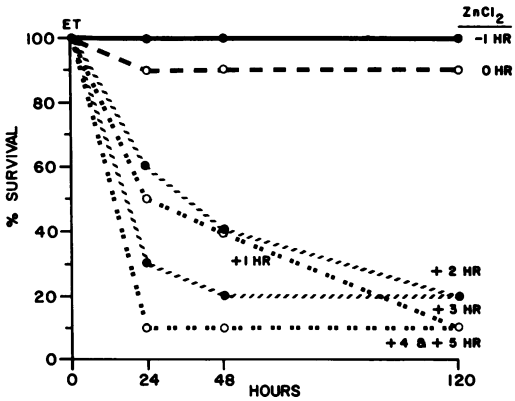

FIG. 2. *Effect of timing of zinc chloride administration relative to endotoxin challenge on survival. S. typhimurium endotoxin (1.0 mg/100 g of body weight) was administered i.p. at zero time. Zinc chloride was injected i.p. at −1, 0, 1, 2, 3, 4, and 5 h. Ten rats were used at each time period.*

The alteration in plasma OCT activity at various times after *S. typhimurium* endotoxin administration in zinc-treated and untreated rats is shown in Table 2. Zinc pretreatment significantly ($P < 0.01$) inhibited the plasma OCT elevation induced by endotoxin at all times studied.

Zinc chloride administered alone commonly produced fibrinous deposits on the liver capsule, often containing intact and degenerating neutrophils and lymphocytes, together with a subcapsular, unicellular zone of necrosis (Fig. 3). The severity of frequency of these lesions did not appear to be time dependent.

Not all lobes of livers exposed to endotoxin were equally affected. Figure 4 shows primarily midzonal foci of peracute hepatocellular degeneration 5 h after endotoxin administration. These foci contained hyperchromatic nuclei and increased cytoplasm eosinophilia. At 10 h after endotoxin administration, multiple foci of midzonal necrosis containing pyknotic hepatic nuclei, loss of cell detail, and neutrophilic infiltrate with minimal sinusoidal congestion were present (Fig. 5). Phosphotungstic acid-hematoxylin staining of these sections did not demonstrate the presence of fibrin.

Zinc chloride followed by endotoxin administration elicited deposits of fibrinous plaques on the liver capsule. In addition, pretreatment with zinc chloride did not completely eliminate the hepatic degenerative changes or the occurrence of small foci of midzonal necrosis in all rats given endotoxin. However, the hepatocellular damage as estimated by plasma OCT elevation was considerably reduced. For example, only one zinc-pretreated rat of five studied at 10 h had elevated plasma OCT activity (20.1 nmol/ml per min). Figure 6 shows a section of liver obtained from this animal, and it illustrates the fibrinous capsulitis and a subcapsular, unicellular zone of necrosis associated with zinc treatment together with a small focal area of hepatocellular necrosis. The presence of fibrinous deposits on the liver capsule and a unicellular zone of hepatocellular necrosis, evidence of exudative inflammatory reaction, may be related to the well-known irritant property of zinc chloride.

DISCUSSION

Experimental evidence obtained in this study demonstrates that i.p. zinc pretreatment of rats provides protection from endotoxin-induced mortality similar to the protection reported in mice (8). However, the protective effect of lower amounts of zinc chloride appears to be greater in rats than that observed in mice (8). Zinc

TABLE 2. *Plasma OCT activity at various times after endotoxin administration in zinc-treated and untreated rats*

Time (h)	OCT (nmol/ml per min)[a]		
	$ZnCl_2$	$ZnCl_2$ + endotoxin[b]	Saline + endotoxin[b]
3	7.1 ± 0.3 (5)	15.1 ± 3.5 (5)[c]	38.7 ± 5.9 (5)
5	5.5 ± 0.8 (5)	15.0 ± 6.3 (5)[c]	72.2 ± 17.2 (5)
10	7.2 ± 1.1 (5)	10.6 ± 2.4 (5)[c]	296.2 ± 102.9 (4)

[a] Value shown is the mean ± standard error of the mean for the number of animals shown in parentheses.

[b] *S. typhimurium* endotoxin (1.0 mg/100 g of body weight) was administered 1 h after treatment with either zinc chloride (1.6 mg/100 g of body weight) or an equivalent volume of physiological saline. All substances were administered i.p.

[c] $P < 0.01$ versus zinc-untreated controls.

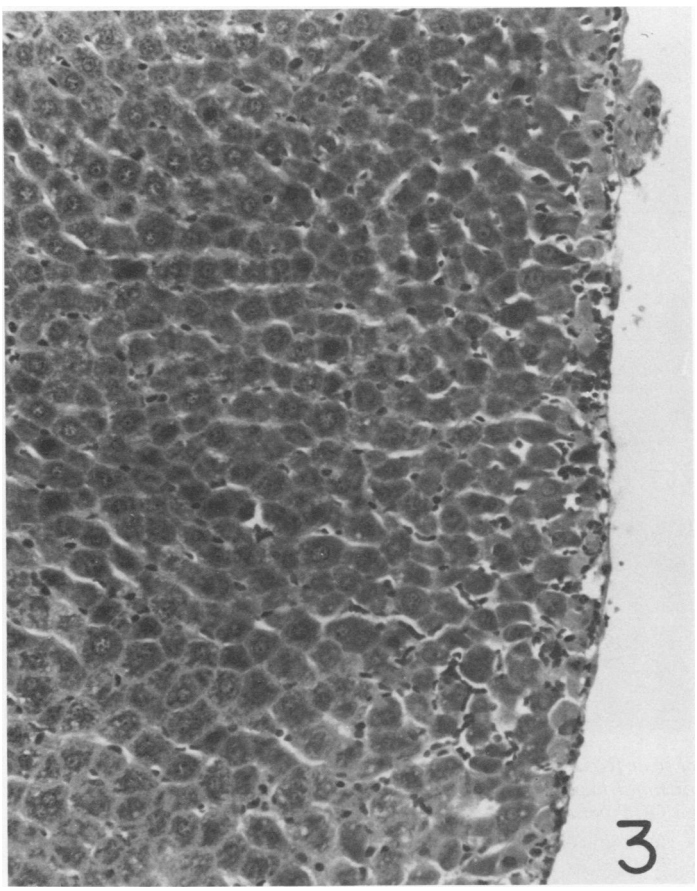

FIG. 3. *Section of liver from rat 10 h after treatment with zinc chloride (1.6 mg/100 g of body weight) showing fibrinous capsular deposit and subcapsular, unicellular zone of necrotic hepatocytes. Hematoxylin and eosin, ×184. Plasma OCT value, 6.7 nmol/ml per min.*

chloride pretreatments in excess of 2.0 mg/100 g of body weight produced diminished protection. This finding may be related to the toxicity of zinc alone (9).

This study provides the first demonstration that i.p. zinc pretreatment in rats can protect the liver from the necrotic lesions induced by endotoxin (1, 4). Additionally, our results suggest that zinc, when i.p. administered, has prophylactic rather than therapeutic value against normally lethal quantities of endotoxin administered i.p. Since we could not demonstrate that

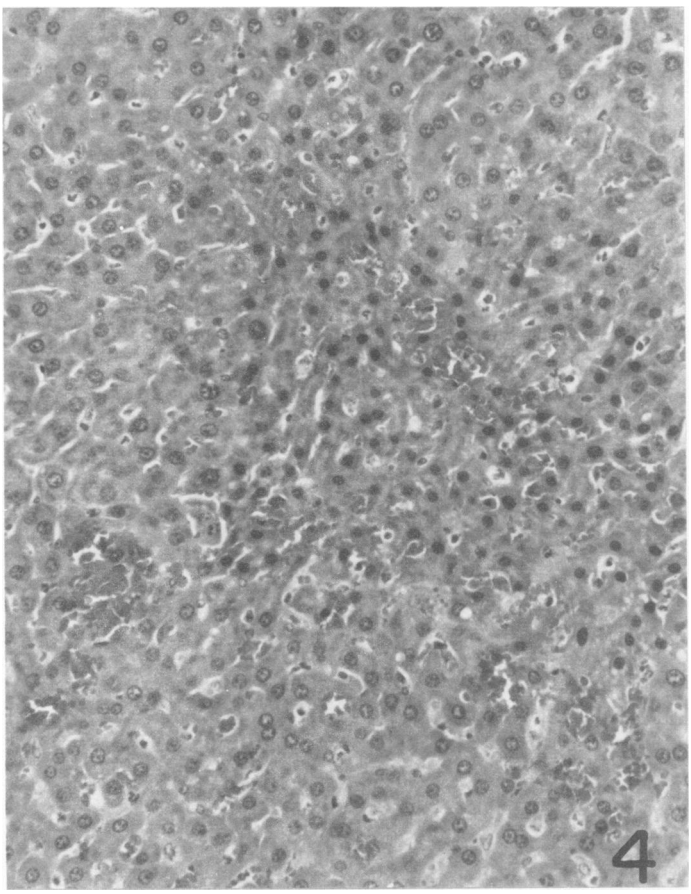

FIG. 4. *Section of liver from rat 5 h after S. typhimurium endotoxin administration (1.0 mg/100 g of body weight) showing midzonal hepatocellular degeneration containing hyperchromatic nuclei. Hematoxylin and eosin, ×184. Plasma OCT value, 102.6 nmol/ml per min.*

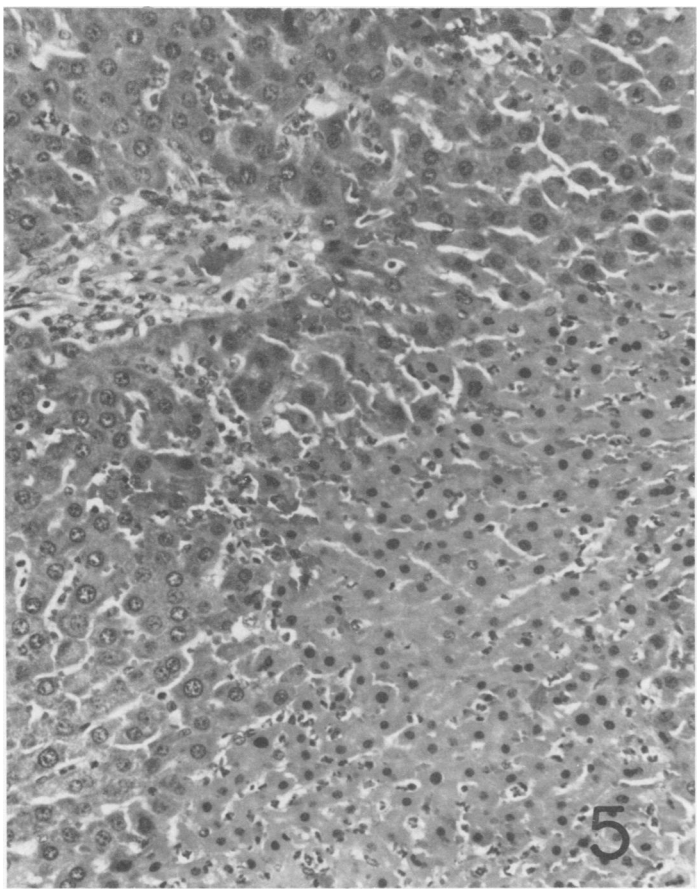

FIG. 5. *Section of liver from rat 10 h after S. typhimurium endotoxin administration (1.0 mg/100 g of body weight) showing focus of midzonal necrosis containing pyknotic nuclei, loss of cell detail, and neutrophilic infiltrate with minimal sinusoidal congestion. Hematoxylin and eosin, ×184. Plasma OCT value, 327.3 nmol/ml per min.*

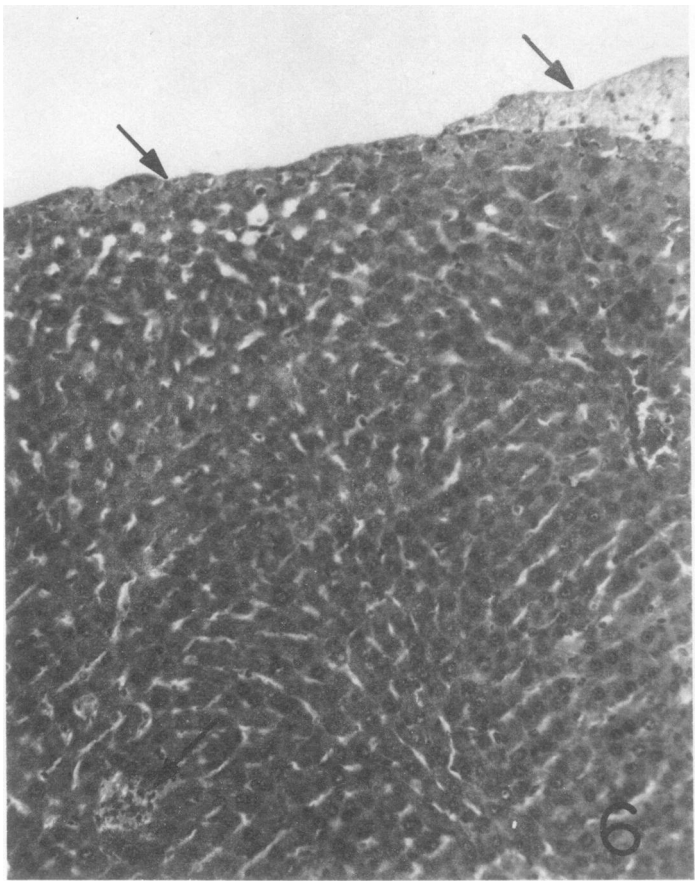

FIG. 6. *Section of liver from zinc chloride (1.6 mg/100 g of body weight)-pretreated rat 10 h after S. typhimurium endotoxin (1.0 mg/100 g of body weight) showing fibrinous capsulites (upper right), focal hepatocellular necrosis (lower left), and subcapsular, unicellular zone of necrosis (upper left). Hematoxylin and eosin, ×160. Plasma OCT value, 20.1 nmol/ml per min.*

i.p. zinc pretreatment is effective in reducing hepatic uptake of i.v. administered ^{51}Cr-labeled endotoxin, it appears unlikely that zinc directly effects hepatic uptake of endotoxin. At least one possible explanation for hepatic protection involves the ability of zinc, in some as yet unidentified manner, to decrease the absorption of endotoxin from the pertioneal cavity and subsequent hepatic uptake.

At least in rats, protection against hepatocellular damage may in part contribute to the enhanced survival after endotoxin challenge, since there is evidence that endotoxin is a hepatotoxin causing cell injury and death (5). The possibility that zinc in some manner contributes to the inactivation of endotoxin appears unlikely, since we could not demonstrate any protective effect with i.v.-administered zinc. In preliminary experiments (unpublished data), we could not demonstrate any significant differences between zinc-treated and untreated rats in either the depression of peripheral leukocytes or depletion of hepatic glycogen induced by endotoxin 5 h after administration. Apparently, sufficient endotoxin is available to promote these biological responses, but not mortality. Results obtained in this study suggest that a reduction in the circulating levels of endotoxin by a factor of approximately 4 is sufficient to protect against the lethal aspects of endotoxin. This finding is consistent with and of the same order of magnitude as the dose reduction factor of 2.5 reported by Snyder and Walker (8) and the factor of 3 found in our own studies (data not shown).

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LITERATURE CITED

1. **Cameron, G. R., M. E. Delafield, and J. Eilson.** 1940. Pathological changes produced in rats and mice by the toxic fraction derived from Bact typhimurium. J. Pathol. Bacteriol. 51:223-233.
2. **Ceriotti, G.** 1974. Ornithine carbamoyltransferase: determination with automatic analysers, p. 695-698. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, vol. 2. Academic Press Inc., New York.
3. **Chvapil, M.** 1973. New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. Life Sci. 13:1041-1049.
4. **Levy, E., R. J. Slusser, and B. H. Ruebner.** 1968. Hepatic changes produced by a single dose of endotoxin in the mouse. Am. J. Pathol. 52:477-502.
5. **Nolan, J. P.** 1975. The role of endotoxin in liver injury. Gastroenterology 69:1346-1356.
6. **Pekarek, R. S., W. R. Beisel, P. J. Bartelloni, and K. A. Bostian.** 1972. Determination of serum zinc concentrations in normal adult subjects by atomic absorption spectrophotometry. Am. J. Clin. Pathol. 57:506-510.
7. **Reichard, H.** 1957. A new specific liver test: determination of ornithine-carbamyl transferase in human serum. Scand. J. Clin. Lab. Invest. 9:103-104.
8. **Snyder, S. L., and R. I. Walker.** 1976. Inhibition of lethality in endotoxin-challenged mice treated with zinc chloride. Infect. Immun. 13:998-1000.
9. **Van Reen, R.** 1966. Zinc toxicity in man and experimental species, p. 411-426. *In* A. S. Prasad (ed.), Zinc metabolism. Charles C Thomas, Publisher, Springfield, Ill.
10. **Zlydaszyk, J. C., and R. J. Moon.** 1976. Fate of ^{51}Cr-labeled lipopolysaccharide in tissue culture cells and livers of normal mice. Infect. Immun. 14:100-105.