HEPATIC METALLOTHIONEIN INDUCTION IN INFLAMMATION*†

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Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

HEPATIC METALLOTHIONEIN INDUCTION IN INFLAMMATION*†

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INTRODUCTION

Hypozincemia as a consequence of the movement of endogenous zinc from plasma to liver during inflammatory stress is well known.[1] The redistributed zinc is sequestered in the liver,[2-5] by a unique pleomorphic metalloprotein, metallothionein.[6] The mechanism(s) involved in the *de novo* synthesis of metallothionein has been the subject of extensive studies since it appears to be intimately involved in zinc homeostasis in normal,[7] pathophysiologic,[2-4] and stress[8] conditions. Since hepatic accumulation of metallothionein occurs during the early phase of inflammatory stresses, such as bacterial infection[2] and endotoxemia,[9-11] it appears that it can be appropriately classified as an acute phase alteration.[12]

The stimulus which triggers hepatic metallothionein synthesis in these diverse conditions remains unknown. Its synthesis and accumulation apparently are not in every instance dependent on the presence of inflammatory stress since hepatic accumulation has been reported during other stresses such as exposure to cold environment[13] and exercise.[8] Identification of a common *endogenous mediator* for hepatic metallothionein synthesis has not been conclusively demonstrated. In contrast, existence of a common endogenous mediator influencing hepatic synthesis of certain plasma acute-phase proteins appears to be established.[14] The latter mediator has been termed leukocytic endogenous mediator (LEM) although its exact chemical characterization remains elusive and controversial.[15,16] Experimental evidence indicating that LEM mediates hepatic synthesis of the intracellular protein, metallothionein, is lacking,[17] although it induces hypozincemia[18] and a cascade of other acute-phase alterations[1] when administered to rats.

Recently, Etzel and Cousins[13] have reported that glucocorticoids and glucagon may be involved in the regulation of hepatic metallothionein in a synergistic manner. Results of recent studies from our laboratory, however, have not supported a role for pituitary[5] or adrenal hormones[19] in the induction of hepatic metallothionein during inflammatory stress. The potential role of glucagon in stress-induced hepatic metallothionein accumulation has not been evaluated. The present preliminary study was performed to examine further the possibility that glucagon may be a common mediator of stress-induced hepatic synthesis of

*In conducting the research described in this report, the investigation adhered to the *Guide for the Care and Use of Laboratory Animals* as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

†The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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metallothionein. Turpentine administration and experimental endotoxemia in the rat were selected as models to evaluate this possibility. Results support the concept that glucagon may be a common mediator in hepatic metallothionein induction during these stresses.

Materials and Methods

Animals

Male Fisher-Dunning rats ranging in weight from 200–250 g (Microbiological Associates) and male mice of the C3H/HeJ and C3Heb/FeJ strains (Jackson Laboratory) weighing 32–37 g were used after being housed for at least one week under controlled environmental conditions.[2] Animals were fed standard laboratory chow and provided water *ad libitum* except that food was removed after endotoxin or turpentine administration, as described below.

Treatments

Lipopolysaccharide W, *E. coli* 0127:B8 and lipopolysaccharide B, *S. typhimurium* (Difco Laboratories) were suspended in physiological saline just prior to use. Endotoxin was administered intraperitoneally on a 100 g body weight basis at the doses specified in legends to figures and tables. Control animals were given an equivalent volume of saline. In certain experiments turpentine (1 ml) was injected subcutaneously to initiate an inflammatory response. Additionally, Actinomycin D (Calbiochem) pretreatment of rats was used to inhibit metallothionein synthesis[20] and was administered subcutaneously (0.08 mg/100 g body weight) four hours prior to administration of turpentine. Control rats were given an equivalent volume of 50% (V/V) propylene glycol in physiological saline used to solubilize the Actinomycin D.

Tissue Sampling and Analyses

Animals were killed, at times specified in the text, by exsanquination under halothane anesthesia. Plasma and liver samples were obtained and processed as previously described[2] for the determination of plasma glucose,[21] glucagon,[21] zinc,[2] insulin,[21] and hepatic metallothionein-Zn[2] and glycogen.[22] Trasylol (FBA Pharmaceuticals) was added to whole blood samples (1,000 U/ml) to prevent glucagon degradation.

Statistical Analyses

Significance of differences between group means was performed by analysis of variance with a $p \leq 0.05$ considered significant.

Results

Administration of a single dose (100 μg/100 g body weight) of endotoxin to rats produced a significant decrease in plasma zinc concentration (Figure 1) and an

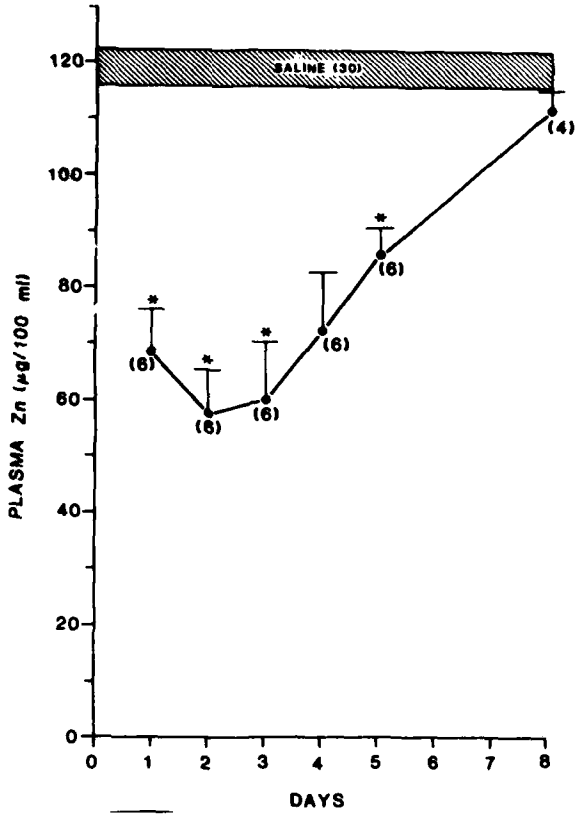

FIGURE 1. Alterations in plasma zinc concentrations at various times after daily intraperitoneal injections of *S. typhimurium* endotoxin, 100 μg/100 g body weight. Horizontal bar represents mean ± SEM of control values. Number of animals is shown in parentheses. Rats were killed 5 h after endotoxin administration. Injections were started on day 1. *Values significantly different from controls, $p \leq 0.05$.

increase in hepatic metallothionein-Zn concentration (FIGURE 2). Repeated daily injections of equal amounts (100 μg) resulted in sustained hypozincemia over a period of four days with a progressive increase in normal plasma zinc values by day 8 (FIGURE 1). Thus, tolerance to the plasma zinc depressing activity of endotoxin was evident by day eight. Accumulation of metallothionein-Zn increased to an apparent "steady-state" concentration by day four (FIGURE 2), which represents approximately a tenfold increase in metallothionein-Zn compared to controls.

The data presented in FIGURE 3 demonstrate that the amount of accumulated metallothionein is rapidly reduced when daily injections of endotoxin are omitted. Additionally, the metallothionein-Zn responses to single challenge doses of endotoxin (100 μg) in rats previously rendered tolerant are identical to that illicited in nontolerant rats. Previously tolerant rats became nontolerant to the plasma zinc depressing activity of endotoxin within seven days (FIGURE 4) after discontinuance of daily endotoxin injections.

The effect of administration of either of two different endotoxins on plasma zinc concentrations in mice of the endotoxin-resistant, C3H/HeJ, and -responsive, C3HeB/FeJ, strains is shown in TABLE 1. Both endotoxins induced significant

decreases in plasma zinc concentrations by 5 hr in both strains of mice when compared to saline-injected controls. No significant difference was observed in the extent of hypozincemia induced by either *S. typhimurium* or *E. coli* endotoxin in C3HeB/FeJ mice. In contrast, *S. typhimurium* endotoxin produced a significantly greater hypozincemia in C3H/HeJ mice when compared to that induced by *E. coli* endotoxin. In addition to hypozincemia, *S. typhimurium* endotoxin induced approximately an eight-fold increase in accumulation of hepatic metallothionein-Zn in both strains of mice when compared to controls (TABLE 2). Only a small amount of metallothionein-Zn was found in saline-injected controls. The effect of *E. coli* endotoxin on hepatic metallothionein-Zn accumulation was not evaluated.

In addition to the alterations in zinc homeostasis, endotoxin induced hyperglucagonemia in rats when administered in doses of 100 μg (TABLE 3) and 1000 μg (FIGURE 5). Further, the data shown in TABLE 1 demonstrate that tolerance to the toxin-induced hyperglucagonemia can be achieved after daily administration of single doses of endotoxin (100 μg), i.e., in a manner similar to that used to achieve tolerance to the zinc depressing activity of endotoxin described above.

The results presented in FIGURE 5 provide evidence that an increase in peripheral plasma glucagon concentration occurs within 1 hr following endotoxin (1000 μg) administration. Significant hyperglucagonemia persisted during the

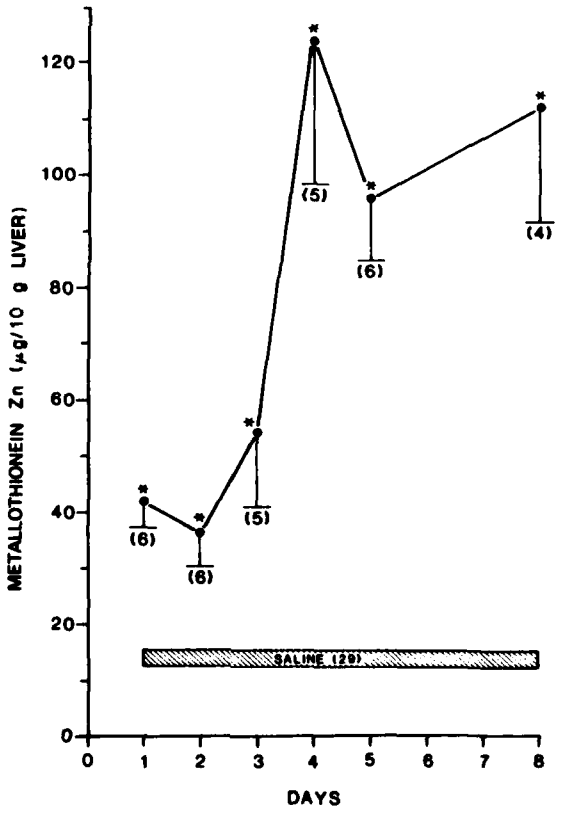

FIGURE 2. Hepatic metallothionein-Zn concentrations at various times after daily intraperitoneal injections of *S. typhimurium* endotoxin, 100 μg/100 g body weight. *Values significantly different from controls, $p \leq 0.05$.

remainder of the 5-hr experimental period. Concomitant with the increase in plasma glucagon at 1 hr, a significant elevation in plasma glucose concentration (FIGURE 5) occurred; however, plasma glucose was significantly decreased at 5 hr when values for both time periods were compared to glucose concentrations in control rats. Hepatic glycogen in endotoxemic rats was significantly decreased at 5 hr (0.27 ± 0.03 mg/g wet liver weight, N = 10) when compared to control values (18.60 ± 2.20, N = 10). The initial increase in plasma glucose concentration was followed by a significant transient increase in insulin concentration (FIGURE 5) at

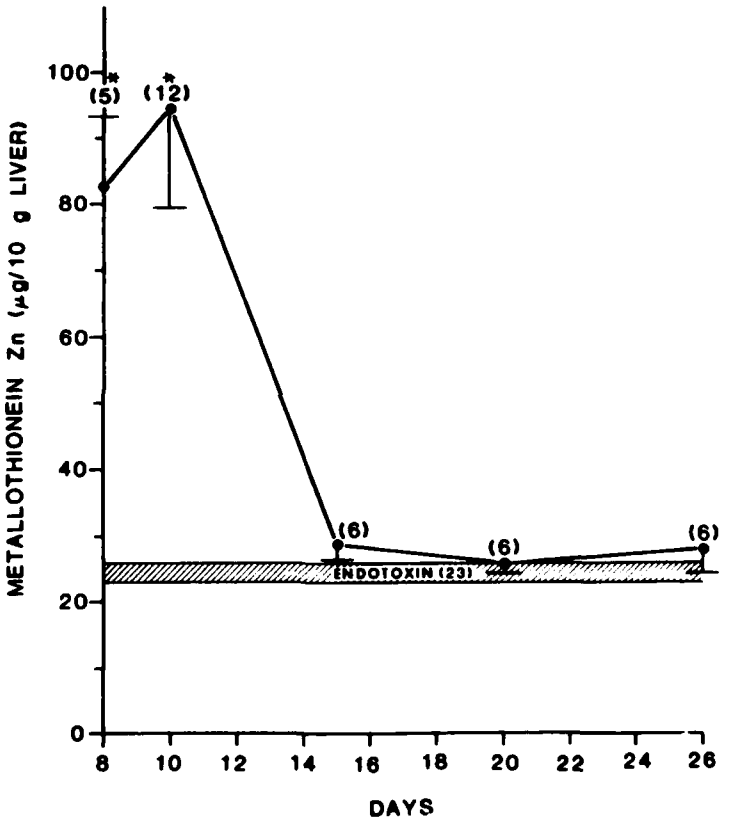

FIGURE 3. Effect of endotoxin administration on hepatic metallothionein-Zn concentrations at various times after establishment of tolerance. Rats were injected intraperitoneally with a single challenge dose of *S. typhimurium* endotoxin, 100 μg/100 g body weight. Rats were killed 5 hr after endotoxin administration. Number of animals is shown in parentheses. Horizontal bar represents mean ± SEM for endotoxin-injected nontolerant controls. *Values significantly different from controls, $p \leq 0.05$.

1.5 hr with an apparent maximum at 2 hr and a return to basal levels at 3 hr. No further increase in insulin was observed for the remainder of the experimental period.

Since Actinomycin D-treated animals are highly susceptible to the lethal aspects of endotoxemia,[28] turpentine-injected rats were used to determine whether this drug inhibited inflammation-induced hypozincemia and the accumulation of metallothionein-Zn. The data shown in TABLE 4 indicates that

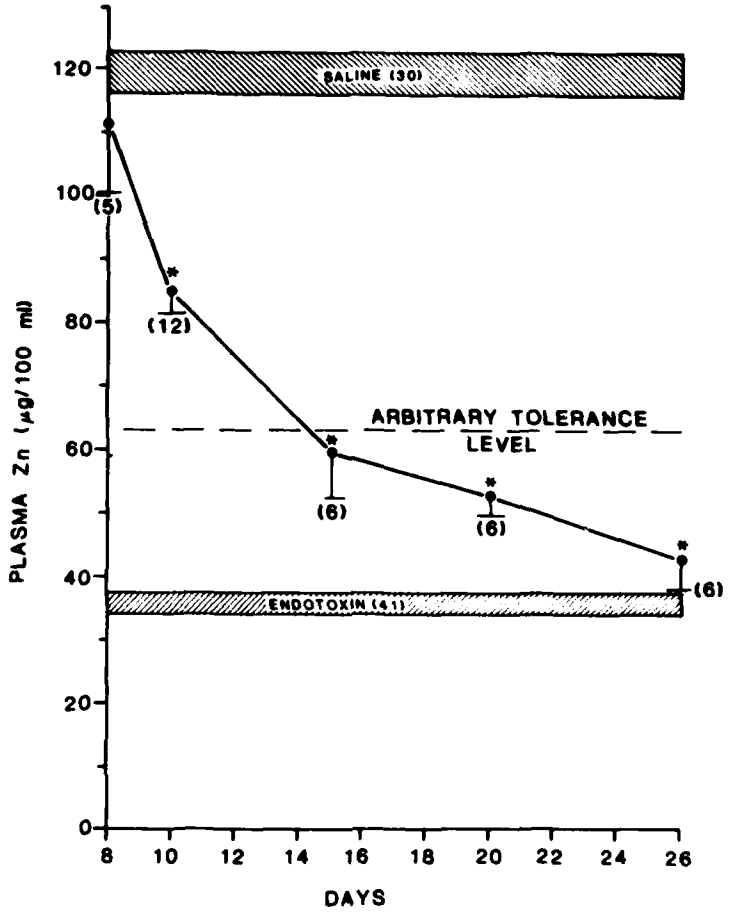

FIGURE 4. Effect of endotoxin administration on plasma zinc concentrations at various times after establishment of tolerance. Rats were injected intraperitoneally with a single challenge dose of *S. typhimurium* endotoxin, 100 μg/100 g body weight. Rats were killed 5 hr after endotoxin administration. Number of animals is shown in parentheses. Horizontal bars represent mean ± SEM for saline and endotoxin-injected nontolerant controls. *Values significantly different from saline-injected controls, $p \leq 0.05$.

TABLE 1

EFFECT OF ENDOTOXIN (LPS) ON PLASMA ZINC CONCENTRATION IN C3H/HeJ AND C3HeB/FeJ STRAINS OF MICE

		Strain (n)	
Group	Treatment*	C3H/HeJ (Zn, μg/100 ml)†	C3HeB/FeJ (Zn, μg/100 ml)†
1	*S. typhimurium* LPS	28.8 ± 2.5 (5)‡	36.0 ± 1.6 (5)‡
	Saline	79.5 ± 2.0 (4)	78.9 ± 2.2 (5)‡
2	*E. coli* LPS	41.3 ± 3.4 (9)‡§¶	28.9 ± 2.4 (1)‡
	Saline	78.5 ± 2.3 (5)	84.0 ± 3.2 (5)

*Mice were injected ip with LPS (1 mg/100 g body weight) or an equivalent volume of physiological saline (1 ml/100 g) and killed 5 hr later.

†Values are means ± SEM.

‡Significantly different ($p \leq 0.05$) compared to saline control.

§Significantly different ($p \leq 0.05$) compared to C3HeB/FeJ strain.

¶Significantly different ($p \leq 0.05$) compared to C3H/HeJ administered *S. typhimurium* LPS.

TABLE 2

EFFECT OF ENDOTOXIN (LPS) ON HEPATIC METALLOTHIONEIN-ZINC ACCUMULATION IN C3H/HeJ AND C3HeB/FeJ STRAINS OF MICE

	Strain	
Treatment*	C3H/HeJ (Zn, μg/10 g wet liver weight)†	C3HeB/FeJ (Zn, μg/10 g wet liver weight)†
S. typhimurium LPS	110	105
Saline	14	14

*Mice were injected ip with LPS (1 mg/100 g body weight) or an equivalent volume of physiological saline (1 ml/100 g) and killed 5 hr later.

†Value obtained from pooled livers (6–7 g) of 5 mice and represents the average of duplicate determinations that did not differ by more than 10 percent.

although pretreatment of rats with Actinomycin D did abolish inflammation-induced hypozincemia, the treatment produced only an approximate 40% reduction in hepatic metallothionein-Zn accumulation when compared to propylene glycol-injected controls. Further, pretreatment of rats with Actinomycin D alone produced significant hyperzincemia and decreased hepatic metallothionein-Zn when compared to propylene glycol-injected controls. Propylene glycol injection induced a significant increase in metallothionein-Zn but had no significant effect on plasma zinc concentrations. All treatments, with the exception of saline injection alone, produced significant hyperglucagonemia (TABLE 4).

DISCUSSION

Results obtained in this study further document the role of metallothionein in the sequestration of endogenous zinc in the liver during inflammatory stress. Our initial observations that endotoxin induced an hepatic zinc-binding protein,[9] identified as metallothionein,[11] has subsequently been confirmed by Suzuki and Yamamura.[10] The present study further characterizes the toxin-induced metallothionein response in rats and provides evidence that its accumulation is a transient phenomenon, i.e., once the initiating exogenous stimulus (endotoxin) is

TABLE 3

EFFECT OF ENDOTOXIN (LPS) CHALLENGE ON PLASMA GLUCAGON CONCENTRATIONS IN LPS-TOLERANT AND NONTOLERANT RATS

Group (N)	Pretreatment* dose (μg)	Challenge* dose (μg)	Glucagon† (pg/ml)
1 (5)	100	100	328 ± 17‡
2 (5)	100	None	343 ± 15‡
3 (5)	None	100	674 ± 69

*S. typhimurium endotoxin was suspended in physiological saline and administered intraperitoneally on a 100 g body weight basis. Pretreatment was performed for 7 consecutive days with challenge on day 8. All rats were killed 5 hr after time of challenge.

†Values are means ± SEM.

‡Significant (group 1, 2 vs 3), $p \leq 0.05$.

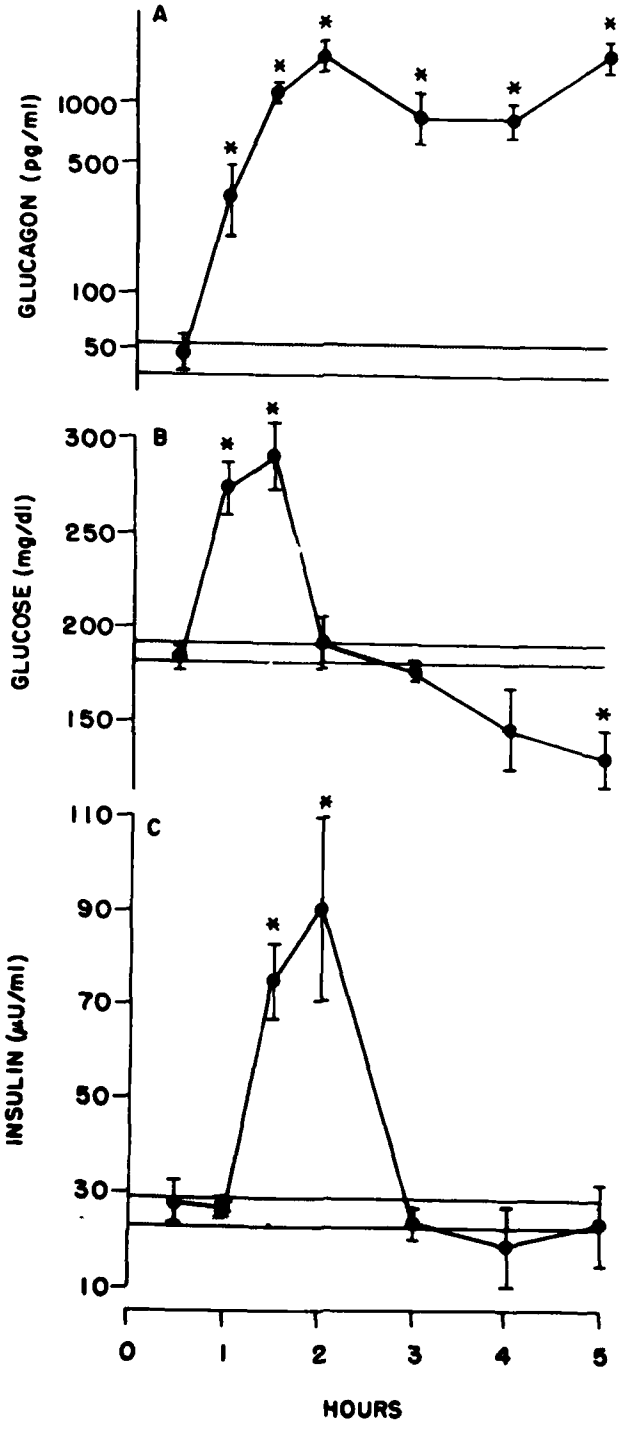

FIGURE 5. Alterations in plasma glucagon (A), glucose (B) and insulin (C) concentrations at various times after intraperitoneal administration of *S. typhimurium* endotoxin, 1.0 mg/100 g body weight. Horizontal bars represent mean ± SEM of saline-injected controls. * Values significantly different from controls, $p \leq 0.05$ (N = 5).

withdrawn the accumulated metallothionein is rapidly degraded. This finding is consistent with the relatively short half-life of Zn-thionein[2] and further suggests that endotoxin does not irreversibly effect metallothionein degradation. The relative contribution of rates of synthesis and degradation to hepatic accumulation of metallothionein during endotoxemia remains to be determined.

The redistribution of endogenous zinc from plasma to liver constitutes only one of a series of nonspecific metabolic alterations which occur during endotoxemia and other inflammatory stresses.[1] The search for a common mediator of these metabolic responses has focused for many years on a substance obtained from stimulated phagocytic cells, termed leukocytic endogenous mediator (LEM). When administered either peripherally or centrally to rats, LEM induces many, if not all, of the metabolic alterations observed during various inflammatory stresses.[1] At the present time, however, it is not known conclusively whether LEM is a single chemical entity or a group of closely related molecules acting on

TABLE 4

CONCENTRATIONS OF PLASMA ZINC, GLUCAGON AND HEPATIC METALLOTHIONEIN-ZN IN TURPENTINE-TREATED RATS WITH AND WITHOUT ACTINOMYCIN D PRETREATMENT

		Plasma†‡		Liver†‡
Group	Treatment*	Zn (μg/100 ml)	Glucagon (pg/ml)	Metallothionein-Zn (μg/10 g wet liver weight)
1	Turpentine, ACT-D	173.2 ± 7.6 (5)§¶	2088 ± 151 (4)§	73.5 ± 11.1 (5)§¶
2	Turpentine, PG	77.2 ± 5.9 (5)¶	1050 ± 50 (3)	130.6 ± 11.4 (5)¶
3	Saline, ACT-D	317.6 ± 7.0 (5)§	3180 ± 508 (5)§	8.5 ± 1.8 (5)§
4	Saline, PG	115.2 ± 3.5 (5)	1076 ± 74 (5)	37.5 ± 0.7 (5)

*Rats were injected subcutaneously with 1 ml of turpentine or an equivalent amount of saline (control) 4 hr after pretreatment with Actinomycin D (ACT-D), or propylene glycol (PG, control). Animals were killed 20 hr after turpentine administration.

†Number of rats is shown in parentheses.

‡Values are means ± SEM.

§Significantly different ($p \leq 0.05$) from control (group 1 vs 2 and group 3 vs 4).

¶Significantly different ($p \leq 0.05$) from saline control.

directly or indirectly single or multiple target cells.[1] It seems logical to assume that certain metabolic alterations attributed to LEM action occur through secondary mechanisms involving known hormonal regulators. For example, as shown in this study endotoxin administration (which presumably stimulates LEM production[18]) results in hyperglucagonemia, glycogenolysis, transient hyperglycemia, and transient hyperinsulinemia. This series of metabolic responses is consistent with the known roles of glucagon and insulin in carbohydrate metabolism. LEM's role, if any, as a mediator in this instance must involve events leading directly or indirectly to the hyperglucagonemic state. It is not known with certainty if LEM directly affects the endocrine pancreas.

In a similar manner, the role of LEM and possibly other hormones in hepatic metallothionein induction is as yet undefined. LEM does not apparently act directly on hepatocytes to induce metallothionein synthesis.[17] Its role as a common mediator in hepatic metallothionein induction is further in doubt since noninflammatory stress results in metallothionein accumulation and the production of LEM during such stresses has not been established.

Evidence obtained in this study clearly indicates that administration of large amounts of endotoxin to mice induces marked hypozincemia and hepatic accumulation of metallothionein. Previous work has established that metallothionein is inducible in mice[24] and rats[20] exposed to various salts of heavy metals whereas the induction of metallothionein during endotoxemia or other inflammatory stresses has only been reported in studies with rats. In addition, the findings presented indicate that C3H/HeJ mice, although uniquely resistant to lethal and certain immunological[25-27] effects of endotoxin, respond to the plasma zinc depressing activity of endotoxin in a manner nearly identical to that exhibited by endotoxin-susceptible C3Heb/FeJ mice. These results are in marked contrast to those reported by Kampschmidt *et al.*[28] that C3H/HeJ mice injected ip with very low doses of endotoxin (0.1 and 1 μg) do not respond to several endotoxin-induced metabolic effects. These included plasma iron depression, an effect together with plasma zinc depression believed to be mediated by LEM. These workers also reported that C3H/HeJ mice responded with plasma iron depression following LEM administration. The conceptual implication in these studies reported by Kampschmidt *et al.*[28] is that phagocytic cells in the C3H/HeJ strain do not "release" LEM. If this is true, then the results presented in our study suggest that LEM is not a mediator of hepatic metallothionein induction. One possible explanation for these divergent results is that markedly different doses of endotoxin were employed in their and our studies. It is conceivable that small doses of endotoxin may be rapidly detoxified by the augmented peritoneal inflammatory response which occurs in C3H/HeJ compared to C3Heb/FeJ mice.[29] Studies by Walker *et al.*[30] as well as Moeller *et al.*[29] have demonstrated that induction of a peritoneal inflammatory response may be related to resistance against lethal aspects of ip administered endotoxin. Since peritoneal phagocytes detoxify endotoxin,[31] higher doses of endotoxin, such as were employed in the present study, may well overwhelm this mechanism of resistance. It should be pointed out that in studies concerning endotoxin-induced increases in acute-phase serum protein AA (SAA) conducted by McAdam and Sipe,[32] the C3H/HeJ strain responded to ip administered endotoxin only when large doses (*E. coli* LPS, 3000 μg; *S. typhosa* LPS, 1000 μg) were given. It seems apparent that additional studies are needed to evaluate further the usefulness of this mouse model in establishing the role of LEM as a common mediator in various metabolic responses following endotoxin administration.

Recently, Etzel and Cousins[13] reported that glucagon and certain glucocorticoids regulate hepatic metallothionein-Zn in an independent and synergistic manner. This report is of particular interest in the present study since endotoxin induces hyperglucagonemia in monkeys[33] and as shown in this study, in rats. Furthermore, endotoxin induces a rapid early increase in adrenal corticol output in rats.[34] Etzel and Cousins[13] have suggested that alterations in hepatic metallothionein during various stresses involves synergistic effects of these endocrine hormones. From their data, it appears that the effect of glucocorticoids (dexamethasone), but not glucagon, on metallothionein induction requires *de novo* synthesis of mRNA since only the dexamethasone effect could be blocked by Actinomycin D. Results from the present study indicate that only a portion of the hepatic metallothionein-Zn accumulated during turpentine-induced inflammation is prevented by prior treatment of rats with Actinomycin D. Similar results with Actinomycin D have been reported by this laboratory in studies concerning hepatic metallothionein accumulation in hypersensitivity reactions.[5] These findings seemingly support a requirement for *de novo* mRNA synthesis in metallothionein induction during inflammatory stresses. However, in recent work from

this laboratory, we have not been able to confirm a major requirement for glucocorticoids in the metallothionein response which is induced following turpentine injection.[19] This finding suggests that *de novo* synthesis of mRNA is stimulated by some other factor(s). It is conceivable that zinc itself may fulfill this role through metal-stimulated mechanisms involving translational and transcriptional processes.[35] In addition to its effect on mRNA synthesis, Actinomycin D effectively inhibits the redistribution of zinc from plasma to liver during inflammatory stress as shown in this and other studies.[3,36]

Our present preliminary findings appear to support a role, although limited, for glucagon in the hepatic metallothionein response which accompanies inflammatory stress. However, since all treatments used in our initial experimental design resulted in hyperglucagonemia (TABLE 4), it is not possible to clearly delineate its role. Further, our data suggests that hyperglucagonemia can increase hepatic metallothionein accumulation above basal levels without concomitant hypozincemia. This finding is consistent with the observations made by Etzel and Cousins[13] that exogenous glucagon administration induces metallothionein but does not alter plasma zinc concentration. Additionally, our data indicates that despite Actinomycin D pretreatment, a certain amount of plasma zinc is redistributed to the liver during inflammatory stress. Oh *et al.*[8] have clearly demonstrated the importance of available zinc in the metallothionein response. They observed that zinc deficiency effectively prevented induction of hepatic metallothionein during CCl_4-induced inflammation in rats.

In conclusion, it appears from the available information concerning hepatic metallothionein induction during inflammatory stress that the phenomenon depends on the following: 1) redistribution of endogenous zinc from plasma to liver, and 2) mRNA-dependent and -independent mechanisms. The latter may involve independent and synergistic actions of endocrine hormones that include glucocorticoids, glucagon, and other hormone-like substances. Clearly, more work is required to conclusively establish the involvement of these substances in metallothionein induction which occurs in inflammatory as well as noninflammatory stresses.

SUMMARY

Numerous chemically distinct phlogistic substances have been shown to induce hepatic metallothionein-Zn (MT) accumulation when administered to rats. These findings suggest that induction of this cysteine-rich metalloprotein occurs through the action of some common mediator(s). Possible mediators include substances such as leukocytic endogenous mediator (LEM) and/or hormones known to influence hepatic protein synthesis. Studies were performed to examine further the mechanism(s) and potential mediators involved in endotoxin-induced MT accumulation. Additionally, the studies were performed to determine the possible involvement of genetic factors, which reportedly influence LEM production, in the induced MT response. Endotoxin (ET) was administered ip to rats and to ET-resistant, C3H/HeJ, and susceptible, C3Heb/FeJ, strains of mice. ET induced hypozincemia, hyperglucagonemia, and increased MT concentrations in rats. ET induced hypozincemia and MT accumulation to the same extent in both strains of mice. The induction of tolerance in rats to Zn depressing activity of ET also prevented hyperglucagonemia and additional accumulation of MT. Results suggest that glucagon, but not LEM, may be a common mediator in MT response during inflammatory stress.

ACKNOWLEDGMENTS

The excellent technical assistant of L. Harris, J. Simila, K. Bostian, E. Hauer, and secretarial aid of C. Brown are appreciated. The authors are grateful to Dr. Richard I. Walker, U. S. Navy Medical Research Institute, Bethesda, Maryland, for his assistance in performing the studies with mice described in this report.

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DISCUSSION

C. B. LAURELL: It was most interesting to learn that metallothionein in the hepatocytes had such a short half-life. How do you measure the half-life of an intracellular protein? Is it the *zinc* you have measured or have you really measured the half-life of the protein? My concern is that conclusions drawn about the effect of actinomycin may not apply to actual rate of synthesis if they are based solely on zinc turnover studies.

P. Z. SOBOCINSKI: We measured half life by ^{65}Zinc.

LAURELL: So you have not studied the protein directly.

SOBOCINSKI: Correct; however, the literature does indicate that you get the same results whether one uses incorporation of labelled amino acids or ^{65}Zn turnover. Such data suggest that ^{65}Zn is a valid method for looking at the turnover of this particular protein.

K. P. W. J. MCADAM: I wonder if you could tell me what happened in those experiments with the C3H/HeJ mice; what dose of endotoxin you were using and what type of endotoxin?

SOBOCINSKI: We used two. We used *E. coli* endotoxin and *S. typhimurium*. The doses we initially used were the same doses we used in rats: 1 milligram per hundred gram body weight, which far exceeds the dose Dr. Kampschmidt reported.

MCADAM: My only comment about the C3H/HeJ is some reluctance to base much on endotoxin that hasn't been very strictly stripped of its protein. Unless the protein is stripped off the LPS one does get response in nonresponder mice responding to the associated protein.

SOBOCINSKI: Then the question arises: is the protein inducing the LEM? I believe the concept here is that they will not make LEM to endotoxin. Do the data indicate that they do make LEM in response to the protein contamination of endotoxin?

MCADAM: Yes.

S. DEMCZUK (*Oklahoma University Health Sciences Center*): Can you elaborate more on the role of messenger RNA when glucagon causes an increase of this protein?

SOBOCINSKI: Cousins and colleagues at Rutgers administered glucagon to rats. I believe the dose was in milligrams and got hepatic metallothionein induction not inhibitable by actinomycin-D. While not conclusive it's well known during inflammatory stress glucagon does go up. Our data do suggest that we cannot,

using actinomycin-D, completely inhibit the hepatic metallothionein accumulation in the liver, leaving the door open for more work as far as glucagon's role in mediating the response.

LAURELL: This is a most exciting protein for all clinicians. Everybody should like to have a method for its estimation in blood. You suggested you found small amounts in plasma. Most people have had great difficulties inducing anti-sera against metallothionein because it's so alike in different species. Have you been successful and could you tell us how to produce an anti-serum against metallothionein for radioimmunoassay?

SOBOCINSKI: Methods have been published. I can't quote them right now but there are some tricks involved in getting antibody to metallothionein. We have not measured plasma metallothionein levels because we didn't have the antibody. I don't believe metallothionein is a plasma protein and if it is detected in the plasma I think one is looking at cellular destruction and leakage. In my opinion it's not primarily an export protein.