Hypoglycemia and Depressed Hepatic Gluconeogenesis During Endotoxicosis in Lead-sensitized Rats[1] (37143)

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In order to elucidate the essential pathophysiological alterations in endotoxin shock, experimental models based on induction of hypersensitivity to endotoxin have been widely employed. These have included such diverse treatments as BCG (1, 2), zymosan (3), glucan (4, 5) carbon tetrachloride (6) or adrenalectomy (2, 7, 8). As pointed out by Shands, Miller and Martin (2), a common denominator in endointoxicated, hyperactive animals is a profound and often lethal hypoglycemia due primarily to an inability to conduct adequate gluconeogenesis subsequent to depletion of hepatic and other tissue glycogen stores (9–11).

Recently, lead salts have been shown to induce a profound sensitization to endotoxicosis and shock pathogenesis (12, 13); however, no defects in either the vascular clearance or the hepatic detoxification of endotoxin were manifest after lead treatment (14, 15). The current study therefore evaluated the potential role of altered carbohydrate homeostasis as a factor in endointoxicated, lead-treated rats. Specifically, blood glucose and lactate levels, hepatic glycogen stores, and isolated hepatocyte gluconeogenesis were evaluated in rats sensitized to endotoxin by the concomitant injection of lead acetate.

Materials and Methods. Animals and treatments. Male rats of the Holtzman strain (Holtzman Co., Madison, WI) of 280–300 g body weight were used. Experimental rats for the measurements of blood glucose, blood lactate and liver glycogen were fed Purina chow and water *ad libitum* in animal quarters maintained at 76–78°F and at an automatically regulated 12 hr light–dark cycle. For the *in vitro* gluconeogenesis studies the rats were fasted with access to water for 48 hr prior to use.

Endotoxin was purchased from Difco Laboratories, Detroit, MI as the Boivin lipopolysaccharide of *Salmonella enteritidis*. Endotoxin solutions were prepared daily in 0.9% sodium chloride and were injected via the dorsal vein of the penis under light, ether anesthesia. Lead acetate was purchased as the trihydrate salt from Mallinckrodt Chemical Works, Inc., St. Louis, MO and was prepared daily by dissolving the salt in distilled, deionized water. Lead solutions were also injected iv. For control groups sodium acetate was prepared in water and injected iv.

Hepatocyte isolation and the in vitro *measurement of gluconeogenesis.* Hepatocytes were isolated according to a modification of the method of Berry and Friend (16). Male rats were anesthetized via the dorsal vein of the penis with sodium pentobarbital at a dose of 30 mg/kg. Midline laparotomy was performed, the abdominal vena cava and portal vein were isolated, and 500 units of heparin were injected iv. After ligating the abdominal vena cava, a PE 280 polyethylene catheter was inserted and secured in the portal vein. The liver was rapidly flushed using 15–20 ml of Ca^{2+}-free, glucose-free Hanks' solution. The thorax was opened and a PE 280 polyethylene catheter was inserted into the thoracic vena cava via penetration of the right atrium. The liver was excised and placed in a modified Miller isolated liver perfusion apparatus as described previously (17). The portal cannula was rapidly connected and perfusion was initiated. The elapsed time from ligation of the abdominal vena cava un-

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til portal perfusion was maintained at less than 3 min. The perfusion media consisted of 0.05% collagenase and 0.10% hyaluronidase in Ca^{2+}-free, glucose-free Hanks' solution; the enzymes were purchased from the Sigma Chemical Co., St. Louis, MO. The perfusion media was oxygenated by aeration with 100% O_2. Liver temperature was maintained at 37° as measured by a thermistor wedged between the lobes. The perfusion was conducted at 20–30 ml/min and at a hydrostatic pressure of 10–12 cm H_2O. After 15–20 min of perfusion the liver exhibited marked breaks in the capsular surface; the liver was then removed and rapidly cooled in 50 ml of iced perfusion media. The liver was gently dispersed by pressing with a spatula. The liver cell suspension was incubated at 37° in a Dubnoff metabolic shaker bath for 15 min at 100 oscillations/min in order to disperse the remaining tissue aggregates. The hepatocytes were filtered through 100 mesh silk, sedimented by centrifugation at 250*g* at + 10°, washed three times, and suspended in glucose-free, Krebs–Ringer bicarbonate buffer. Cell counts and viability measurements were made in 0.05% trypan blue. The cells were rejected if the viability was less than 85%. Cell aliquots were analyzed for protein using the Oyama and Eagle modification (18) of the Lowry method. The hepatocytes were incubated at a concentration of 4×10^6 per ml as 3 ml samples in 25 ml Erlenmeyer flasks. The flasks were gassed with 95% O_2–5% CO_2 and incubated for 120 min at 37° and 100 oscillations/min in a Dubnoff metabolic shaker bath. For measurements of gluconeogenesis the cells were incubated with either 10 m*M* alanine, pyruvate, lactate, or fructose. The incubations were terminated by rapid freezing to —15°. Upon thawing the samples were deproteinized with 1 ml barium hydroxide and 1 ml zinc sulfate, centrifuged at 1000*g*, and 1 ml samples of the supernatant were analyzed for glucose using the glucose oxidase method (Worthington Biochemicals Corp., Freehold, NJ). Gluconeogenic glucose production was calculated as micromoles produced per gram of cell protein per 120 min.

Blood glucose, lactate and liver glycogen measurements. Blood glucose levels were determined using the glucose oxidase method. Blood lactate levels were determined by the Barker and Summerson method (19). Hepatic glycogen was determined using the extraction, precipitation, and hydrolysis technique of Good, Kramer and Somagyi (20); glucose concentrations of the hydrolysates were measured via the glucose oxidase method.

Data analysis. All data were compared for statistical significance at the 95% confidence level using Student's unpaired *t* test. Tabulated data are expressed as the mean ± standard error.

Results. Liver glycogen and blood glucose

TABLE I. Effect of Lead Acetate and Endotoxin on Blood Glucose, Blood Lactate, and Liver Glycogen Levels.[a]

Experimental treatment	Blood		Liver glycogen % (wet wt)
	Glucose (mg/100 ml)	Lactate (mg/100 ml)	
Control (5 mg sodium acetate)	109 ± 3.3 (8)	12.4 ± 1.2 (8)	4.08 ± 0.25 (10)
Lead (5 mg lead acetate)	99 ± 2.6 (18)	12.6 ± 1.1 (8)	1.52 ± 0.18[b] (16)
Endotoxin control (100 μg + 5 mg sodium acetate)	117 ± 1.0 (22)	17.1 ± 1.8 (8)	1.00 ± 0.13[b] (6)
Endotoxin + lead (100 μg + 5 mg lead acetate)	36 ± 4.0[b] (28)	30.0 ± 2.4[b] (7)	0.06 ± 0.01[b] (12)

[a] All materials were injected iv 4–6 hr prior to blood sampling and sacrifice. Values are expressed as the mean ± standard error. Parentheses enclose number of rats per group.

[b] $p < .05$ compared to control treatment group.

TABLE II. Effect of Lead Acetate and Endotoxin on Gluconeogenesis in Isolated Hepatocytes.[a]

Hepatocyte donor rats	Krebs-Ringer bicarbonate (KRB)	Gluconeogenesis (μmoles of glucose/g protein/120 min) KRB + 10 mM lactate	KRB + 10 mM pyruvate	KRB + 10 mM alanine	KRB + 10 mM fructose
Control (5 mg sodium acetate)	13 ± 1 (8)	159 ± 13 (8)	194 ± 22 (8)	132 ± 12 (8)	431 ± 52 (4)
Lead (5 mg lead acetate)	18 ± 1 (3)	81 ± 12[b] (3)	84 ± 14[b] (3)	60 ± 5[b] (3)	488 ± 16 (3)
Endotoxin (100 μg + 5 mg sodium acetate)	19 ± 2 (3)	146 ± 16 (3)	189 ± 21 (3)	160 ± 11 (3)	530 ± 22 (3)
Endotoxin + lead (100 μg + 5 mg lead acetate)	19 ± 2 (3)	57 ± 12[b] (3)	49 ± 10[b] (3)	65 ± 8[b] (3)	478 ± 39 (3)

[a] All materials were injected iv 5 hr prior to sacrifice. Donor rats were fasted 48 hr prior to use. Values are expressed as a mean ± standard error. Parentheses enclose number of hepatocyte preparations.

[b] $p < .05$ compared to control substrate group.

and lactate changes after lead and endotoxin treatments. As indicated in Table I, the iv administration of lead acetate at a dose of 5 mg resulted in no significant alteration in blood glucose or lactate levels as compared to control injected rats which received 5 mg of sodium acetate iv. Lead treatment, however, resulted in a decrease in liver glycogen stores. Endotoxin at a dose of 100 μg iv produced no changes in blood glucose or lactate levels but also produced a decrease in liver glycogen. It should be noted that endotoxin at this dose is almost innocuous to non-lead-sensitized animals. In contrast, the administration of endotoxin with lead results in marked sensitization to endotoxin shock (12, 13). As shown in Table I the combined treatment of lead acetate with endotoxin produced a profound hypoglycemia, marked lactacidemia, and a drastic depletion of liver glycogen.

Hepatic gluconeogenesis after lead and endotoxin treatments. The ability of isolated hepatocytes to conduct gluconeogenesis is depicted in Table II. In the control donor rats treated with 5 mg of sodium acetate, only slight glucose production was measured in samples incubated in Krebs–Ringer bicarbonate buffer. In confirmation of Berry and Friend (16) the addition of the gluconeogenic precursors—10 mM lactate, pyruvate, alanine, and fructose—to the isolated hepatocytes resulted in augmented gluconeogenesis. Lead acetate treatment of donor rats depressed gluconeogenesis from lactate, pyruvate and alanine but not from fructose. Endotoxin at a low dose of 100 μg in non-lead-treated rats produced no alterations in hepatocyte gluconeogenesis. The combination of endotoxin plus lead resulted in depressed gluconeogenesis from lactate, pyruvate, and alanine, but not from fructose.

Discussion. Among the manifold effects of shock in general (21–23) and endotoxic shock in particular (11, 24) are marked alterations in carbohydrate metabolism. While the initial responses to stress are generally associated with hyperglycemia, the later stages of progressive shock are often characterized by profound hypoglycemia. Recent studies in a number of experimental shock models have provided the unifying insight that hypersensitivity to endotoxin shock is often associated with impaired glucose homeostasis and death in the throes of hypoglycemia (2). Our findings in the lead-sensitized rat support the notion that animals rendered sensitive to endotoxin develop marked hypoglycemia. Since the hypoglycemia is associated with almost complete depletion of liver glycogen, the hypoglycemic state is definitely not due to impaired mobilization of hepatic

glycogen stores. Indeed the ability of either lead or endotoxin to partially deplete hepatic glycogen may indicate that these agents share the ability to sensitize the liver to the glycogenolytic effects of sympathetic activation. While the interactions of endotoxin with norepinephrine are well known (25), no similar effects of lead have been reported.

The mechanisms underlying the hypoglycemia incident to endotoxicosis are thought to reflect an inability to conduct adequate gluconeogenesis in the face of both the depletion of carbohydrate stores and the augmented glucose needs of ischemic tissues (9–11). The marked lactacidemia in the lead-treated, endointoxicated rat supports the existence of tissue ischemia and a block in Cori cycle gluconeogenic activity. The ability of lead to depress gluconeogenesis in isolated hepatocytes is a key finding since it appears likely that the mechanism of lead sensitization to endotoxin resides in this capability to undermine carbohydrate metabolic adjustments during endotoxicosis. Among the probable mechanisms of lead depression of gluconeogenesis are (a) an effect on mitochondrial function, *viz,* oxidative phosphorylation; (b) an inhibition of gluconeogenic enzyme activity; (c) a sensitization of the hepatocyte to insulin; or (d) a depressed responsiveness of the hepatocyte to glucocorticoids. The inability of lead to depress gluconeogenesis from fructose is suggestive of a mitochondrial locus of action since fructose interactions in gluconeogenesis require only cytosol reactions. Since lead also sensitized rats to other forms of experimental shock (15), the role and mechanism of gluconeogenic depression in these states are currently being evaluated.

Summary. The administration of *Salmonella enteritidis* endotoxin to male rats rendered hypersensitive by lead acetate resulted in profound hypoglycemia, lactacidemia, and depletion of liver glycogen. Lead acetate depressed gluconeogenesis as studied in isolated hepatocytes. Possible mechanisms for lead's action on gluconeogenesis and the significance of this metabolic lesion in lead hypersensitivity to shock are discussed.

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