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Depression of Hepatic Gluconeogenesis by Acute Lead Administration[1] (38344)

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Previous investigators (1–4) have long identified the liver as a primary site of toxicity following acute lead administration. However, except for a slight hyperglycemia noted after acute lead ingestion in man (1), lead was until recently generally considered to have no significant effects on the liver's vital physiological role in regulating blood glucose levels. Recent studies have revealed that nonlethal parenteral doses of lead to experimental animals (i) depleted hepatic glycogen levels (5, 6), (ii) inhibited fasting-induced increases in the activity of a hepatic gluconeogenic enzyme (6), (iii) inhibited hepatocyte gluconeogenesis (5), and (iv) produced significant lethality in adrenalectomized rats (7). Furthermore depression of hepatic glucoregulatory function may contribute to the markedly enhanced shock lethality to bacterial endotoxins and other stress of experimental animals pretreated with lead (8, 9) or other hepatotoxins (10). The purpose of the present study was to investigate the effect of acute intravenous lead administration in rats on hepatic gluconeogenesis as evaluated both *in vivo* and *in vitro*. In view of the fact that lead is thought to have a mitochondrial locus of action within the liver (11–14), attention was focused on mitochondrial steps as the possible site of lead inhibition of hepatic gluconeogenesis.

Materials and Methods. Animals and treatments. Male rats of the Holtzman strain (Holtzman Co., Madison, WI) of 280–320 g body wt were used. Animals were fed Purina chow and water *ad libitum* in animal quarters maintained at 24–26° and at a regulated 12 hr light–dark cycle. Experimental rats were injected with 5 mg of lead acetate (Mallinckrodt Chemical Works, St. Louis, MO) and control rats with 5 mg sodium·acetate in 1 ml of distilled water via the dorsal vein of the penis under ether anesthesia. Injections were performed at 4 PM, the rats were fasted overnight, and experiments commenced at 10 AM the next morning. This period without access to food reduced liver glycogen from $6.21 \pm 0.27\%$ ($n=16$) to $0.32 \pm 0.14\%$ ($n=6$).

Measurements of gluconeogenesis in vivo. Whole rat gluconeogenesis was evaluated as described previously (15). In essence, fasted male rats under pentobarbital anesthesia were injected iv with either 1 mmole of L-alanine—with or without 4 μCi L-alanine-U-^{14}C, or 0.33 mmole of sodium pyruvate—with or without 4 μCi of pyruvate-U-^{14}C. Blood samples were removed at 0 min, *i.e.*, prior to alanine or pyruvate injection, and at 30 min postinjection. The hyperglycemic response *per se* and the radiotracer incorporation into blood glucose were evaluated as indices of gluconeogenesis. Glucose determinations were performed on aliquots of heparinized tail blood using the glucose oxidase micromethod in Tris buffer (Worthington Biochemicals Corp., Freehold, NJ). ^{14}C-Alanine and pyruvate were purchased from Amersham-Searle, Arlington Heights, IL. Radioactivity measurements were performed in a Searle-Analytic Isocap 300 System using efficiency calculations based on quench correction factors determined via the sample channels ratio method.

Measurements of hepatic gluconeogenesis in vitro. Rat hepatocytes were isolated from overnight fasted rats and gluconeogenesis was evaluated as described in detail previously (16). In essence, the liver was rapidly extirpated, per-

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fused with 0.05% collagenase and 0.10% hyaluronidase, and dispersed to yield the hepatocytes. After washing, the cells were incubated in a balanced salt buffer with either 10 m*M* L-alanine, lactate, pyruvate, or oxaloacetate or 1 m*M* glycerol or fructose. Substrates were purchased from the Sigma Chemical Co., St. Louis, MO. The gluconeogenic production of glucose was measured via the glucose oxidase method. Cell protein was measured by the Lowry *et al.* (17) method and the gluconeogenic rate was calculated as μmoles of glucose/g of protein/min.

Hepatocyte metabolism was also evaluated by adding either 0.25 μCi L-alanine-U-^{14}C tracer in 10 m*M* L-alanine carrier or 0.25 μCi pyruvate-U-^{14}C tracer in 10 m*M* pyruvate carrier to 3 ml of the isolated cells. The hepatocytes were incubated for 120 min at 37° and then deproteinized with 1 ml each of $Ba(OH)_2$ and $ZnSO_4$. An aliquot of the deproteinized supernatant was resin extracted to remove labeled alanine or pyruvate using 300 mg of Dowex 50X8-200 and 600 mg of Dowex 1X8-200 resins in 15 ml plastic centrifuge tubes. After vortex mixing for 15 min and centrifugation, duplicate aliquots of the resin supernatant were counted in order to determine the extent of incorporation of radiotracer into ^{14}C-glucose. In addition, hepatocytes together with 10 m*M* L-alanine-U-^{14}C, 10 m*M* pyruvate-U-^{14}C, or 5 m*M* glucose-U-^{14}C were incubated in metabolic reaction flasks (Kontes Co., Vineland, NJ). The formation of ^{14}C-carbon dioxide from these precursors was determined by counting the radioactivity trapped on alkali-moistened filter paper in the side arm vial of the reaction flasks (18). Gluconeogenesis and carbon dioxide formation from the radiotracers were calculated as the percentage of the total available radioactivity.

Data analysis. All data were analyzed for statistical significance at the 95% confidence level using Student's paired or unpaired *t* test as indicated. Tabulated data are expressed as the mean plus or minus the standard error.

Results. Influence of lead on in vivo *gluconeogenesis.* The ability of acute lead administration to alter gluconeogenesis induced by an overnight fast was evaluated both by the hyperglycemic response to a substrate load and by the conversion of either ^{14}C-alanine or pyruvate to blood ^{14}C-glucose. As indicated in Table I, control rats receiving sodium acetate displayed significant hyperglycemic responses 30 min after the 1.0 mmole alanine and 0.33 mmole pyruvate loads, *i.e.*, 17.0 and 14.2 mg of glucose/dl blood, respectively. In contrast, lead-treated rats did not display a significant hyperglycemic response at 30 min to either of the substrate loads. Similarly, the ability of lead-treated rats to incorporate both ^{14}C-alanine and ^{14}C-pyruvate into blood ^{14}C-glucose was significantly depressed, *i.e.*, 26.0 and 17.0%, respectively, as compared to control rats.

Effect of lead treatment on gluconeogenesis in isolated hepatocytes. Hepatocytes isolated from rats treated with sodium acetate and fasted overnight displayed gluconeogenesis from alanine, lactate, pyruvate, oxaloacetate, glycerol and fructose (Table II). Lead treatment, however, caused a significant depression of *in vitro* gluconeogenesis from all substrates except glycerol. The percent decreases in gluconeogenesis by hepatocytes of lead-treated rats compared to control values were greater for alanine, pyruvate and lactate—all substrates with obligatory mitochondrial steps. In contrast, the addition of lead acetate to hepatocytes *in vitro* in concentrations of 0.01–1.0 mg/3 ml of cells had no significant effects on gluconeogenesis (Table III).

The effect of *in vivo* lead administration on isolated hepatocyte metabolism was also evaluated utilizing ^{14}C-alanine and pyruvate. As indicated in Table IV, gluconeogenesis as determined by the incorporation of ^{14}C-alanine and ^{14}C-pyruvate into ^{14}C-glucose was significantly depressed from 21 to 39%, respectively, in hepatocytes of lead-treated rats as compared to control rats. In addition, carbon dioxide formation from ^{14}C-alanine, ^{14}C-pyruvate and ^{14}C-glucose was also significantly depressed in hepatocytes of lead-treated rats as compared to control rats (Table IV).

Discussion. The specific toxic effects of lead depend for the most part on the site of localization of this ubiquitous natural substance. Early investigators including Flury (3) and Cantarrow and Trumper (1) recognized that the liver possessed a high concentration of lead in acute lead poisoning. The understanding that the toxic effect of lead on the liver could be evidenced by a decreased resistance to infection (1) was followed later by the finding that lead treatment of experimental animals increased by 1000-fold the lethality to bacterial endotoxins (8, 9).

TABLE I. Effect of *in Vivo* Lead on *in Vivo* Gluconeogenesis.*

Substrate (dose)	Sampling time (min)	Blood Glucose (mg/dl) Sodium acetate	Lead acetate
Alanine	0	83.7 ± 2.4	87.0 ± 2.7
(1.0 mmole)	30	100.7 ± 3.2[a]	88.2 ± 2.4
Pyruvate	0	84.8 ± 1.9	88.4 ± 2.8
(0.33 mmole)	30	99.0 ± 2.4[a]	93.9 ± 2.0
		Blood ^{14}C-glucose (dpm × 10^4/ml)	
		Sodium Acetate	Lead acetate
^{14}C-alanine (1.0 mmole)	30	1.47 ± 0.05	1.08 ± 0.04[b]
^{14}C-pyruvate (0.33 mmole)	30	1.23 ± 0.03	1.02 ± 0.03[b]

* Data are expressed as means ± SE. Rats (6–12 per group) were injected iv with 5 mg of either sodium acetate or lead acetate and then fasted overnight. Alanine (1.0 mmole) or pyruvate (0.33 mmole) with or without radiotracer were administered iv the next morning. *In vivo* gluconeogenesis was evaluated by the hyperglycemic response or radiotracer incorporation into blood ^{14}C-glucose.

[a] Significantly ($P < 0.05$) different by paired t test compared to respective 0 time blood glucose level.

[b] Significantly ($P < 0.05$) different by unpaired t test to respective sodium acetate control group.

Goyer and Rhyne (13) stated that parenterally administered lead salts attached primarily to erythrocyte membranes with the remainder precipitating rapidly to form colloidal particles. In this regard, early studies of the distribution of intravenously administered colloidal lead revealed that the immediate storage of lead paralleled the distribution of reticuloendothelial cells with the largest quantity of lead in the liver and smaller amounts in the spleen and bone marrow (1, 3, 19). Furthermore, the observation was made that colloidal lead, although removed originally from the blood by hepatic Kupffer cells was subsequently passed on to the hepatic parenchymal cells (1). Recently Evdokimoff and Wagner (20) explained the 40-fold increase in toxicity of indium when administered to mice in the form of colloidal indium rather than the ionic

TABLE II. Effect of *in Vivo* Lead on *in Vitro* Hepatocyte Gluconeogenesis.*

Substrate (mm)	Gluconeogenesis μmoles lucose/g protein/min Sodium acetate	Lead acetate	% Depression
Buffer	0.18 ± 0.01	0.08 ± 0.01[a]	56
Alanine (10)	0.98 ± 0.04	0.60 ± 0.05[a]	39
Lactate (10)	1.38 ± 0.04	0.56 ± 0.04[a]	59
Pyruvate (0)	1.84 ± 0.05	0.96 ± 0.08[a]	48
Oxaloacetate (10)	1.32 ± 0.04	1.04 ± 0.05[a]	21
Glycerol (1)	0.81 ± 0.05	0.72 ± 0.07	11
Fructose (1)	1.46 ± 0.06	1.28 ± 0.06[a]	12

*Data are expressed as means ± standard error. Rats (7 per group) were injected iv with either 5 mg sodium acetate or lead acetate and then fasted overnight. The increment in rate of gluconeogenesis by isolated hepatocytes with substrates is corrected for the endogenous rate in buffer.

[a] Significantly ($P < 0.05$) different by unpaired t test compared to respective sodium acetate control group.

TABLE III. Effect of *in Vitro* Lead on *in Vitro* Hepatocyte Gluconeogenesis.[a]

Experimental treatment	Concentration (mg/3 ml cells)	Gluconeogenesis (μmoles glucose/g protein/min)				
		Buffer	Alanine (10 m*M*)	Lactate (10 m*M*)	Pyruvate (10 m*M*)	Fructose (1 m*M*)
Control	0	0.24 ± 0.01	1.08 ± 0.06	1.72 ± 0.13	1.90 ± 0.11	1.45 ± 0.12
Lead acetate	0.01	0.26 ± 0.02	0.94 ± 0.05	1.56 ± 0.20	1.72 ± 0.13	1.44 ± 0.12
Lead acetate	0.1	0.22 ± 0.01	0.99 ± 0.10	1.54 ± 0.24	1.84 ± 0.12	1.49 ± 0.13
Lead acetate	1.0	0.22 ± 0.01	1.16 ± 0.04	1.69 ± 0.17	1.88 ± 0.12	1.52 ± 0.13

[a] Data are expressed as means ± SE. Lead acetate was added to 3 ml hepatocytes at doses indicated just prior to incubation. Donor rats (four per group) were fasted overnight. The increment in rate of gluconeogenesis by isolated hepatocytes with substrates is corrected for the endogenous rate in buffer. No significant ($P < 0.05$) differences by paired *t* test compared to respective control substrate groups were noted.

form as due to the fact that hepatic phagocytosis enhanced indium toxicity by concentrating indium in the Kupffer cells from which it subsequently leaked out to damage the hepatocytes. Further support for the possible transfer of toxic lead from cell to cell in the liver is that removal of the spleen and thus this organ's contribution to colloidal lead clearance markedly increased hepatocellular damage (1).

Recent studies by DiLuzio and co-workers (21, 22) utilizing precisely the same protocol as the present investigation revealed that ultrastructural damage to the liver was greater for Kupffer cells than for hepatocytes; however, derangements of hepatic parenchymal cell function including increased plasma retention of bromsulphalein, elevated plasma unconjugated bilirubin, elevated serum alkaline phosphatase, and elevated glutamic oxaloacetic transaminase were predominant functional defects (2). Impairment of hepatocyte mixed function oxidases and drug metabolizing activities have also been

TABLE IV. Effect of *in Vivo* Lead on *in Vitro* Hepatocyte Metabolism.*

Subtrate (m*M*)	Gluconeogenesis percent incorporation into ^{14}C-glucose		
	Sodium acetate	Lead acetate	% Depression
^{14}C-Alanine (10)	9.26 ± 0.26	7.30 ± 0.54[a]	21
^{14}C-Pyruvate (10)	24.9 ± 1.05	15.3 ± 0.88[a]	39
	Carbon dioxide formation percent incorporation into $^{14}CO_2$		
	Sodium acetate	Lead acetate	% Depression
^{14}C-alanine (10)	2.03 ± 0.16	0.88 ± 0.18[a]	57
^{14}C-pyruvate (10)	14.5 ± 1.50	6.89 ± 0.52[a]	52
^{14}C-glucose (5)	0.32 ± 0.02	0.18 ± 0.01[a]	44

* Data are expressed as means ± SE. Rats (five per group) were injected iv with 5 mg of either sodium acetate or lead acetate and then fasted overnight. Gluconeogenesis by isolated hepatocytes is calculated as the percent of radiotracer incorporated into ^{14}C-glucose during 120 min of incubation. Carbon dioxide formation by isolated hepatocytes is calculated as the percent of radiotracer incorporated into $^{14}CO_2$.

[a] Significantly ($P < 0.05$) different by unpaired *t* test compared to respective sodium acetate control group.

reported following intravenous administration of lead salts (23, 24).

The hepatocyte's role in the regulation of blood glucose levels by glycogenolysis and gluconeogenesis is without a doubt the most vital short-term function of the liver (25, 26). In the present study, hepatic gluconeogenesis evaluated both *in vivo* and *in vitro* from a variety of gluconeogenic substrates was depressed following acute lead administration to rats. In agreement with these findings, Rippe and Berry (6) reported that acute lead treatment of mice inhibited fasting-induced stimulation of phosphoenolpyruvate carboxykinase—a key gluconeogenic enzyme (26). Furthermore, increased lethality to acute lead administration following adrenalectomy in rats was found by Erve and Schumer (7) to be reversed by glucocorticoid therapy which enhances hepatic gluconeogenesis (25). The fact that the intravenous injection of 5 mg of lead acetate was not lethal to intact rats in the present study was reflected by the finding that *in vivo* hepatic gluconeogenesis from pyruvate and alanine loads was depressed only 17.0–26.0% compared to control rats (Table I). Moreover, the reduction of hepatocyte gluconeogenic function has been proposed by this laboratory as the possible stress factor explaining the marked 1000-fold increase in lethality to bacterial endotoxins in lead-treated rats (5).

In vitro gluconeogenesis by isolated hepatocytes of lead-treated rats was depressed relatively more from alanine, pyruvate, and lactate — *i.e.*, from 39 to 59%—compared to control rats than was *in vivo* gluconeogenesis. However, the addition of lead acetate in very high concentrations—up to 1 mg/3 ml of cells—to normal hepatocytes prior to incubation did not decrease glucose production from these same gluconeogenic substrates. This was however most likely due to the immediate precipitation of lead with phosphate present in both the cells and buffer medium as suggested by Cardona *et al.* (12). Since alanine, pyruvate, and lactate are all gluconeogenic substrates with obligatory mitochondrial steps in rat hepatocytes (26), the greater depression of *in vitro* gluconeogenesis by hepatocytes of lead-treated rats from these three precursors compared to glycerol, oxaloacetate, and fructose is suggestive evidence that lead may have a mitochondrial locus of gluconeogenic inhibition. The findings in the present study that both glucose and carbon dioxide formation from radiotracer precursors by hepatocytes from lead-treated rats were depressed further support this hypothesis. A mitochondrial locus of lead action has been previously reported in morphological (11, 14) as well as functional (13, 17) investigations of acute lead toxicity.

Summary. Male rats were administered either 5 mg lead acetate or sodium acetate iv and then fasted overnight. Gluconeogenesis was assessed both *in vivo* employing radiotracer as well as chemical conversions of alanine to glucose, and *in vitro* using isolated hepatocytes. Lead-treated rats had depressions in both ^{14}C-alanine incorporation into blood glucose and the hyperglycemic response to an alanine load. Isolated hepatocyte gluconeogenesis from either 10 m*M* alanine, lactate or pyruvate was depressed from 40 to 60% for lead-treated rats as compared to controls; the addition of lead acetate to normal hepatocytes *in vitro* had no effect on gluconeogenesis. Glucose synthesis from three precursors which do not require a mitochondrial step for conversion to glucose—*i.e.*, fructose, glycerol, and oxaloacetate—was not markedly depressed in hepatocytes from lead-treated rats. Incorporation of ^{14}C-alanine into both glucose and carbon dioxide were also depressed following lead treatment. These data on hepatic gluconeogenesis support a mitochondrial locus of lead action and suggest that defects in hepatic glucoregulation may play a role in the toxicity of acute lead poisoning.

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