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Acute Lead-Induced Increase in Serum Calcium in the Rat Without Increased Secretion of Calcitonin[1] (40400)

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It is well known that increased secretion of calcitonin occurs in mammals in response to hypercalcemia induced by parathyroid hormone, vitamin D or calcium salts (1). However, it remains to be established whether endogenous calcitonin is released in response to hypercalcemia induced by other agents.

Recently, it has been shown that the acute administration of lead acetate to rats produces an increase in the serum calcium concentration (2, 3). To gain insight into the possible response of endogenous calcitonin to lead-induced hypercalcemia we further investigated the mechanisms responsible for the increased serum calcium following the acute administration of lead acetate.

Materials and methods. Animals and lead solution. Sprague Dawley albino rats obtained from commercial suppliers were maintained on Wayne Laboratory Blox (Granville Milling Co., Creedmoor, N.C.) and tap water *ad lib.* Young rats (~75 g) were used in the dose-response study. Since the hypercalcemic response in males and females was not different, the data were pooled. For the time-course study and subsequent experiments, relatively older male rats (~200 g) were used because larger volumes of blood could be collected for analyses. Lead solution was prepared by dissolving lead acetate in deionized water. To obtain a clear solution, 1–2% (v/v) of glacial acetic acid was added. The doses are expressed in terms of elemental lead. A solution of sodium acetate equimolar with the lead acetate solution was given to controls. A constant volume of 0.25 ml/100 g body wt was given by iv injection into a tail vein.

Blood collection and analysis. Blood was obtained routinely under ether anesthesia by tail bleeding or by cardiac puncture. It was allowed to clot, and serum was separated by centrifugation within 1 hr. The collection was performed between 10 AM and 12 noon to minimize changes due to the known diurnal variation of serum calcium (4, 5). The concentration of total serum calcium and dialyzable calcium was determined by automated methods (6, 7). Protein bound calcium was calculated by subtracting total ultrafilterable calcium from total calcium. In several experiments serial blood samples were collected from the tail, and calcium concentration was determined by semi-automatic fluorometric titration using a Calcette (Precision Systems, Inc., Sudbury, MA.) (8). Total plasma proteins were measured by the biuret reaction (9) and plasma albumin by a dye method (10). Plasma proteins other than albumin were calculated by subtracting albumin from total proteins. The serum levels of calcitonin were measured by radioimmunoassay as described by Cooper *et al.* (11). For the analysis of whole blood pH, blood was drawn from the abdominal aorta into heparinized syringes, care being taken not to expose the samples to air. The samples were analyzed within 5–10 min of collection using a Radiometer capillary blood electrode.

Statistical analysis. Experimental data were subjected to analysis of variance. Standard errors were calculated from the residual error term of the analysis of variance. The significance of differences between mean values was evaluated either by the F ratio or by a two-tailed t test.

Results. The results shown in Fig. 1 illustrate that the hypercalcemic effect observed 90 minutes after a single iv injection of lead was dose-related. At 1.67 and 5 mg/kg the serum calcium concentration was not different from controls, but a significant increase in the level of serum calcium ($P < .001$) was observed with 15 mg/kg and a further increase in serum calcium concentration occurred with 45 mg/kg. Animals receiving

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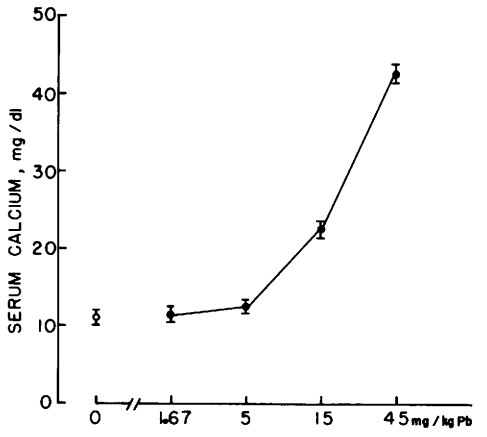

FIG. 1. Hypercalcemic effect of lead. Rats were bled 90 min after iv injection of lead acetate. Numbers of rats varied from 5–15 per group. The open circle represents the mean serum calcium value in control rats. The solid circles represent mean serum calcium values from separate groups of rats injected with different doses of lead. The vertical lines indicate the SE.

doses of 15 mg Pb/kg displayed no mortality during the period of the experiments despite severe hypercalcemia (~20 mg Ca/dl). However, one third (4 of 12) of the animals died 1–2 min after the injection of a dose of 45 mg/kg.

The results presented in Fig. 2 show that the hypercalcemia induced in rats by a single injection of lead (15 mg/kg) was both rapid in onset and long lasting. By 30 minutes after injection, the serum calcium concentration had increased from the level of 11.1–18.1 mg/dl, and the hypercalcemia was still evident at 6 hr.

Results presented in Fig. 3, Experiment A, show that at 30 min following a single iv injection of lead (15 mg/kg), the level of serum calcium increased significantly ($P < .001$). Despite an increment of 3.6 mg/dl in serum calcium, no resulting increase in the level of circulating calcitonin was apparent. In a separate experiment (Fig. 3, Experiment B) an even more severe hypercalcemia (>20 mg Ca/dl) was observed 90 min after iv injection of a larger dose of lead (22.5 mg/kg). Despite an increment of 12.3 mg/dl of serum calcium, again no apparent increase in the circulating calcitonin was found. These findings were in contrast to the results shown in Fig. 4 in which hypercalcemia was induced by iv administration of calcium ($CaCl_2$). In this experiment a smaller but significant ($P < .001$) increase in the concentration of serum calcium was accompanied by a concomitant increase in the level of circulating calcitonin in each rat.

The results shown in Table I illustrate that rats given a single iv injection of lead (22.5 mg/kg) 90 min earlier developed a significant ($P < .001$) increase in total calcium that was due solely to calcium found in the non-dialyzable fraction. In the ultrafilterable fraction, which includes ionic calcium, there was no increase; in fact, a slight but significant ($P < .01$) decrease in calcium concentration was observed. Table II shows that, in a separate experiment, the concentration of total plasma proteins was slightly increased ($P < .02$) and blood pH was not altered by lead.

Discussion. Our study shows that the increased concentration of calcium in serum produced by acute administration of lead acetate was prompt and long-lasting (Figs. 1 and 2). The possibility that lead may interfere with the analytic measurement of serum calcium was excluded because we found that addition of lead directly to rat serum at concentrations up to 100 mg/dl did not interfere with the analysis. Furthermore, the same change in serum calcium concentration after

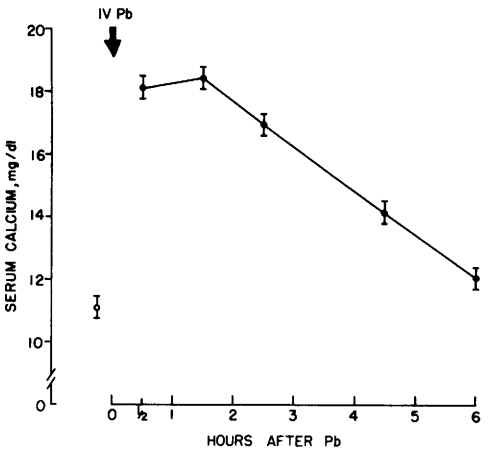

FIG. 2. Onset and duration of hypercalcemia. Rats were bled serially at different intervals as indicated on the horizontal scale. Lead was given at a dose of 15 mg/kg. Each point represents the mean value from 10 rats. The vertical lines indicate the SE. The open circle represents the mean serum calcium value before lead injection. The solid circles represent mean serum calcium values at different intervals after the injection of lead.

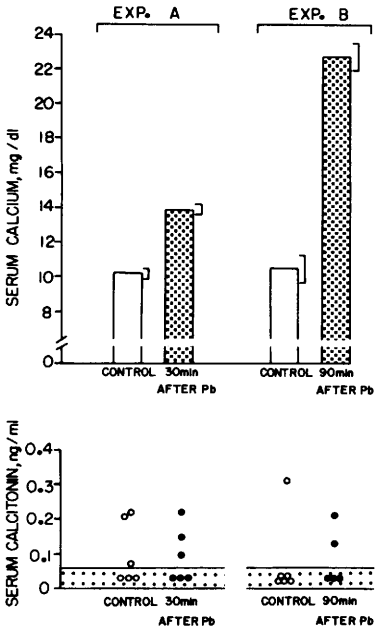

FIG. 3. Lead-induced hypercalcemia without increase in circulating calcitonin. Experiment A (Upper and lower panels on the left): Rats were bled 30 minutes after iv injection of lead (15 mg/kg). There were six rats per group. Mean serum calcium values are shown on the upper left panel by the height of each bar. The brackets show the SE. The individual values for serum calcitonin are shown on the lower left panel. The open circle represents controls and the solid circles show lead injected rats. The horizontal line shows the lower limit of detectability of the radioimmunoassay for calcitonin. Levels below 0.06 ng/ml were not detectable. Experiment B (Upper and lower panels on the right): Rats were bled 90 min after iv injection of lead (22.5 mg/kg). Number of rats varied from five to six per group.

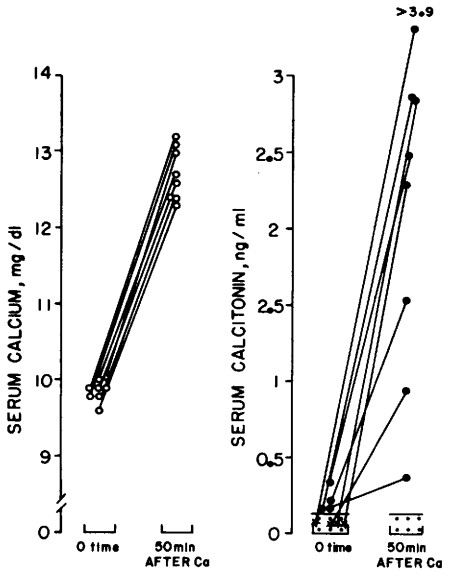

FIG. 4. $CaCl_2$-induced hypercalcemia associated with increase in circulating calcitonin. Rats were bled 50 min after iv injection of 0.1 *M* $CaCl_2$ solution (0.4 ml/100 g bw). There were eight rats per group. The panel on the left shows the change in serum calcium in each rat after injection of $CaCl_2$ and that on the right shows the increase in serum calcitonin in each animal. The horizontal line represents the lower limit of detectability of the radioimmunoassay for calcitonin. Levels below 0.12 ng/ml were not detectable.

the administration of lead acetate was observed using fluorometric, colorimetric, and atomic absorption flame photometric methods, three methods based on entirely different physicochemical properties (unpublished data).

The difference in calcitonin response to the administration of calcium as opposed to lead (Figs. 3 and 4) can not be ascribed to an interference between lead and the calcitonin assay since concentrations of lead as high as 100 mg/dl did not interfere with the calcitonin assay (unpublished data).

Our inability to stimulate calcitonin release with lead-induced hypercalcemia prompted us to evaluate further the mechanisms involved in the production of these elevated

TABLE I. EFFECT OF LEAD ON DIFFERENT FRACTIONS OF SERUM CALCIUM.[a]

	Control	Lead	*P*
Calcium (mg/dl)			
Total	10.9 ± .70	18.4 ± .70	<.001
Ultrafilterable	6.5 ± .09	6.0 ± .09	<.01
Nondialyzable	4.4 ± .69	12.3 ± .69	<.001

[a] Values are shown as mean ± SE. There were six rats per group. Blood was collected 90 min after iv injection of lead (22.5 mg/kg).

TABLE II. EFFECT OF LEAD ON PLASMA CALCIUM AND PROTEINS AND ON BLOOD pH.[a]

	Control	Lead	*P*
Total Calcium (mg/dl)	9.7 ± .30	20.1 ± .30	<.001
Proteins (g/dl)			
Total	4.7 ± .10	5.1 ± .10	<.02
Albumin	2.6 ± .06	2.7 ± .06	NS
Other than albumin	2.1 ± .12	2.4 ± .12	NS
Blood pH	7.39 ± .03	7.44 ± .03	NS

[a] Values are shown as mean ± SE. There were six rats per group. Blood was collected 60 min after iv injection of lead (15 mg/kg).

levels of total serum calcium. Since the calcium concentration in the ultrafilterable fraction, which includes the ionic calcium moiety, was not increased (Table I), the absence of a rise in calcitonin release is not surprising since the secretion of calcitonin is known to be regulated by the concentration of ionic calcium in plasma (1). It should be cautioned, however, that the lack of an acute effect of lead on calcitonin release is distinct from the chronic action of lead intoxication on thyroidal C-cells, since we have found in a separate study (12) that hypersecretion of calcitonin and C-cell hyperplasia occur in rats chronically exposed to lead for one year. Additionally, the absence of an increase in ultrafilterable calcium provides an explanation for the absence of mortality in animals with total calcium values around 20 mg/dl. The significance of the increase in total plasma proteins is not known (Table II).

This study also provides an explanation for the mechanism underlying lead-induced hypercalcemia. It is known that most calcium can be removed from plasma by shaking it with lead phosphate (13). Lead acetate has minimal solubility in plasma so it is not surprising that the intravenous administration of lead acetate would result in the formation of a nondialyzable, calcium-containing material. Furthermore, Talmage and associates have observed that the addition of lead acetate to plasma *in vitro* is associated with the formation of a calcium phosphate complex that can be removed by high speed centrifugation (personal communication). Thus, it is reasonable to conclude that the acute effect of lead administration on the calcium content of serum involves the trapping of calcium in a non-dialyzable form in serum as a consequence of the physico-chemical properties of lead in plasma. Another agent, 1-hydroxyethylidene-1-1-diphosphonic acid (HEDP) produces hypercalcemia in dogs apparently by the same mechanism as lead, i.e., by elevation of the nondialyzable plasma calcium fraction (14).

Summary. An acute marked increase in total serum calcium was observed after a single iv injection of lead. The response was prompt, serum calcium rising above 20 mg/dl 60–90 min after doses ≥ 15 mg/kg. The hypercalcemia was due entirely to a rise in the non-dialyzable calcium fraction. In contrast, the ultrafilterable fraction which includes ionic calcium was actually diminished. This explains the absence of an increase in serum calcitonin despite severe hypercalcemia. A slight rise in total plasma proteins also was observed after lead injection but the significance of this observation remains to be determined. These acute effects of lead on calcium homeostasis are apparently different from its chronic effects since we have found, in a separate study, that rats exposed to lead for 1 year developed hyperplasia of C-cells and an increase in the levels of calcitonin in both the blood and the thyroid glands.

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