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Maternal Lead Exposure Inhibits Intestinal Calcium Absorption in Rat Pups[1]

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Maternal Lead Exposure Inhibits Intestinal Calcium Absorption in Rat Pups. TORAASON, M. A., BARBE, J. S., AND KNECHT, E. A. (1981). *Toxicol. Appl. Pharmacol.* **60**, 62–65. Female rats were fed a diet containing 0.5% lead acetate for 5 weeks prior to breeding, during gestation, and through Day 17 of lactation. The effect of this exposure on the maturation of duodenal calcium absorption in 7- to 30-day-old rat pups was temporary. At Days 7 and 16, *in vitro* calcium uptake into duodenal tissue was low and there was no significant difference between control and exposed pups. Between Days 16 and 24, calcium absorption increased fourfold, which indicates the initiation of an active process for the uptake of calcium. Although calcium absorption increased markedly in both groups, calcium accumulation was significantly reduced in 20-day-old pups maternally exposed to lead. By Day 24, calcium absorption in lead-exposed pups was still decreased, but not significantly. Because only the mothers were fed lead, the exposure to pups ended at weaning. As a result, blood lead concentration in pups dropped to control levels at Day 30 and there was essentially no difference in calcium absorption between the groups.

Just before rats are weaned, a dramatic change takes place in the handling of calcium by the small intestine (Batt and Schachter, 1969). In the newborn, the duodenum exhibits little ability to absorb calcium actively. By 18 days of age, there are clear indications of an active transport process. Saturation kinetics can be demonstrated and metabolic inhibitors abolish the ability of duodenal tissue to concentrate calcium. In rats 20 to 25 days old, the concentrating ability, now several times greater than at birth, levels off and begins a gradual decline as animals age.

If low doses of lead are given orally to 35-day-old rats, the duodenal transport of calcium will be inhibited (Gruden *et al.*, 1973). But because lead crosses the placenta (Kelman and Walter, 1980), and also passes from mother to nursing young (Kostial and Momcilovic, 1974) exposure can begin at conception. To determine if such an early access to lead would retard the maturation of calcium absorption, we examined the effect of maternal lead exposure on the development of duodenal calcium absorption in rat pups 7 to 30 days of age.

METHODS

Female Sprague–Dawley rats weighing 250 g were housed individually in wire-mesh cages at 24 ± 1°C with a 12-hr light–12-hr dark photoperiod. Powdered commercial rat chow, held in feeder cups, and tap water were provided *ad libitum*. Dams receiving lead had 0.5% lead acetate by weight mixed thoroughly

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TABLE 1

BODY WEIGHTS AND BLOOD LEAD LEVELS OF CONTROL AND LEAD-EXPOSED MOTHERS

	Body weight (g)		Blood lead (μg/100 ml)	
	Control	Exposed	Control	Exposed
Prior to breeding	294 ± 6	292 ± 9	5	76 ± 4[a]
Midgestation	384 ± 9	390 ± 9	—	—
Midlactation	353 ± 8	343 ± 9	5	142 ± 16[a]
Weaning	355 ± 15	355 ± 9	5	50 ± 2[a]

Note. Rats were exposed to lead by adding 0.5% lead acetate by weight to their food for 5 weeks prior to breeding, during gestation, and through Day 17 of lactation. Values are mean ± SE for three determinations.

[a] Significantly different from control, $p < 0.001$.

with their food, using a motorized mixer. The food was not analyzed for uniformity, but previous experience showed that this mixture would maintain blood lead concentration in nonpregnant animals between 60 and 80 μg per 100 ml of blood. After 5 weeks on this diet, females were placed in cages with proven breeders from our colony. On Day 17 of lactation, lead-laden rat chow was replaced with the uncontaminated control chow to insure that rat pups, who at this time begin eating solid food in addition to taking their mother's milk, would not have access to lead other than via maternal exposure.

Blood lead concentration in dams was determined prior to cohabitation, at midlactation and at weaning. Blood was drawn into microtubules from a nick at the end of the tail. The concentration of lead in the blood was determined by anodic stripping voltometry, utilizing an Environmental Sciences Associates Trace Metal Analyzer.

Litters were reduced at birth to 10 pups. Pups were weaned at Day 21 of lactation. Two rats from each litter were examined on Days 7, 16, 20, 24, and 30 for body weight, blood lead concentration, and intestinal calcium absorption. In all, 30 pups from both the control and exposed groups were tested.

Calcium absorption into isolated intestinal segments was determined by a method similar to that used by Batt and Schachter (1969). Rats were killed by decapitation and 100 μl of blood was taken for lead determination. The 4 cm of intestine immediately posterior to the stomach was considered the duodenum and removed. Segments were cut into 1-cm rings, and transferred to 10 ml of a solution containing 0.8 μCi of ^{45}Ca (New England Nuclear) per milliliter, 0.25 mM $CaCl_2$, 125 mM NaCl, 25 mM fructose, and 30 mM Tris buffer (Sigma) adjusted to a pH of 7.4 with HCl and NaOH. The solution was continually gassed with 95% O_2 in 5% CO_2. After 1 hr of incubation at 37°C, rings were removed from the incubation medium, blotted on absorbent paper, rinsed in a Ringer's solution similar to the above but containing no radiocalcium, blotted again, and weighed. Tissue concentration of ^{45}Ca was determined by digesting the tissue in 1 ml of Protosal (New England Nuclear), cleansing the sample with 100 μl of H_2O_2 and scintillating with 10 ml of Econofluor (New England Nuclear). All samples were counted twice on a Beckman LS 8100 scintillation spectrometer utilizing the "H#" to monitor quench for calculation of counting efficiency. Calcium accumulation by duodenal tissue is expressed as micromoles of calcium per gram of tissue.

Rats exposed to lead were compared with controls using Student's t test. Data are shown as mean ± SE.

RESULTS

Exposure of females to the 0.5% lead acetate diet for 5 weeks produced blood lead levels between 69 and 84 μg/100 ml (Table 1). This exposure to lead did not affect gross appearance or body weight. During lactation, blood lead concentration increased further in these dams, but body weight was still unaffected.

In contrast, the body weights of pups maternally exposed to lead were significantly reduced at 7 and 16 days of age (Table 2). Intestinal weight was also reduced in lead exposed pups on Days 7 through 30, but the reduction was never significant.

Blood lead concentration in pups was maximum at day 16 of lactation (Fig. 1). On Day 17, the lead diet was removed from the mothers. The lack of access to lead was evident. Blood lead levels decreased by two-thirds in 20-day-old pups, and declined further to about 7 μg/100 ml by Day 30. Lead levels in controls were always less than 5 μg/100 ml.

Calcium absorption in controls remained relatively constant from Day 7 through Day 16 (Fig. 1). Between Days 16 and 24, calcium uptake rose markedly and then leveled off as is evident by values obtained on Day 30. Calcium absorption in lead-exposed pups followed a similar pattern, but at Day 20 lagged significantly behind control values. Absorption was also reduced at Day 24, but not significantly. At Day 30, calcium ab-

sorption of control and exposed rats was essentially the same.

DISCUSSION

Gruden *et al.* (1973) observed reduced *in vitro* calcium transport in 5-week-old rats exposed to lead. The effect occurred at a low lead dose and increasing the concentration 200-fold did not produce further inhibition of calcium transport. They speculated that lead binds irreversibly with the duodenal wall, and that the binding component becomes saturated at low levels of lead and prevents a further effect from additional lead. It was conjectured that the bound lead reduced the channels for passive transport of calcium ions. This was supported by later work showing no effect of similar lead doses on the active transport ratios of calcium from everted duodena (Gruden, 1975), and the observation that lead and calcium both compete for binding sites on two separate protein fractions of intestinal mucosa (Barton *et al.*, 1978).

In 7- and 16-day-old rat pups from the present investigation, calcium uptake into duodenal tissue was minimal and primarily passive. Blood lead concentration from maternal exposure peaked at this time, yet there was no significant difference in calcium uptake between control and exposed rat pups. After Day 16, there was a well-defined increase in calcium absorption in both groups which indicates the initiation of an active transport process (Batt and Schachter, 1969). Despite blood lead levels being one-third what they were at Day 16, calcium uptake in 20-day-old exposed rats was significantly depressed. The inhibition, though not significant, was still apparent at Day 24, but was virtually gone by Day 30. This suggests that the presence of lead affects some aspect of calcium transport that is enhanced during maturation.

Calcium absorption is stimulated by the most active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Frolik and DeLuca, 1971). In addition to enhancing both active and passive processes of calcium transport (Corradino, 1978) 1,25-

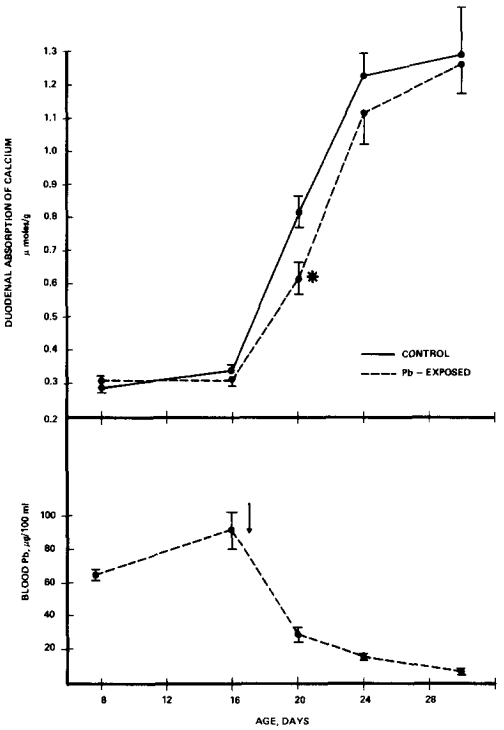

FIG. 1. Duodenal calcium absorption and blood lead concentration of control and maternally lead-exposed pups. Arrow indicates time of removal of lead from mother's diet. Values are mean ± SE of five or six determinations. Asterisk indicates significantly different from control, $p < 0.05$.

TABLE 2

BODY AND INTESTINAL WEIGHT OF CONTROL AND MATERNALLY LEAD-EXPOSED RAT PUPS

Age (days)	Body weight (g)		Intestinal weight (mg/cm)	
	Control	Exposed	Control	Exposed
7	19.6 ± 0.4	15.8 ± 0.2[a]	15.5 ± 2.3	12.2 ± 1.1
16	31.2 ± 0.8	28.2 ± 0.4[b]	21.4 ± 1.9	19.2 ± 1.3
20	43.2 ± 2.1	42.6 ± 0.9	40.3 ± 1.6	38.3 ± 2.3
24	59.3 ± 1.6	60.1 ± 0.9	68.0 ± 3.7	61.8 ± 2.6
30	100.2 ± 6.2	94.8 ± 5.9	80.2 ± 5.2	77.4 ± 2.9

Note. Rat pups were exposed to lead *in utero* and during lactation via the mother's milk. Intestinal weight is the wet weight of the duodenal segments used for the *in vitro* determination of calcium absorption. Values are mean ± SE for five or six determinations.

[a] Significantly different from control, $p < 0.05$.

[b] Significantly different from control, $p < 0.01$.

$(OH)_2D_3$ stimulates production of calcium-binding protein (Spencer *et al.*, 1978). The marked increase in calcium absorption that occurs in 16- to 24-day-old rats appears to be due, at least in part, to $1,25(OH)_2D_3$ (Ueng *et al.*, 1979; Halloran *et al.*, 1979; Halloran and DeLuca, 1980a, b).

Recently, elevated blood lead in children has been found to reduce circulating $1,25-(OH)_2D_3$ and to depress serum calcium concentration (Rosen *et al.*, 1980). Presumably, the hypocalcemia was due to decreased intestinal absorption of calcium. Hence, retarded calcium uptake in lead-exposed pups could be due to repression of $1,25(OH)_2D_3$.

This does not rule out the hindrance of calcium ion movement by the irreversible bonding of lead to duodenal tissue. However, the present findings indicate the constituent of the mucosa, possibly calcium-binding protein (Barton *et al.*, 1978; Spencer *et al.*, 1978), that would serve as a binding site for lead in the inhibition of calcium absorption becomes available during the onset of the active transport mechanism for calcium that occurs 16 to 24 days postpartum.

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