Intestinal Lead and Calcium Absorption: Effect of 1,25-Dihydroxycholecalciferol and Lead Status (43088)

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Abstract. **This study was designed to investigate, in some detail, the relative effects of the hormonal form of vitamin D (1,25-dihydroxycholecalciferol) on duodenal Pb and Ca absorption as a function of dietary Pb level. When cholecalciferol-deficient chicks were chronically repleted with physiologic levels of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), as the sole source of the vitamin, ^{203}Pb and ^{47}Ca absorption were enhanced over 4- and 8-fold, respectively. Ingestion of Pb during the repletion period had no significant effect on the intestinal Ca absorption response to $1,25\text{-}(OH)_2D_3$ even at a very high dietary Pb level. The efficiency of intestinal ^{203}Pb absorption was, however, significantly diminished by dietary Pb, in an apparent dose-dependent fashion. The results indicate that the extent to which systemic Ca homeostatic mechanisms influence intestinal Pb absorption is dependent, in large part, on Pb status.**

[P.S.E.B.M. 1990, Vol 194]

Lead toxicity remains a significant health hazard today despite efforts to reduce environmental exposure. It is currently the most common preventable pediatric illness in the United States (1, 2), with an estimated 17.2% of all preschool children at risk (3).

Lead enters the body primarily through the gastrointestinal tract (4, 5) by a mechanism which is not well understood but proposed to involve both passive diffusion (6, 7) and active (carrier-mediated) transport (8). It has been recognized for some time that a number of nutritional factors, including Ca, P_i, and vitamin D status (9–11), can significantly influence the body retention of Pb.

The common thread connecting these factors is now known to be the cholecalciferol (vitamin D) endocrine system, which functions to regulate systemic Ca homeostasis in response to Ca need and intake. Parathyroid hormone levels, elevated in response to diminished plasma Ca concentration, enhance the renal synthesis of $1,25(OH)_2D_3$ which, in turn, stimulates intestinal Ca absorption (12). Associated with this response is the synthesis of the cholecalciferol-dependent intestinal calcium-binding protein, calbindin D (13), and an increase in alkaline phosphatase activity (14). The observations that dietary Ca deficiency (15, 16) cholecalciferol repletion (17, 18), and exogenous $1,25(OH)_2D_3$ (18–20) also stimulate intestinal Pb absorption in a similar fashion establish a link between Pb and Ca metabolism. The importance of this relationship was initially revealed by the studies of Six and Goyer (15) in which the clinical severity of Pb toxicity was shown to be dramatically augmented by dietary Ca deficiency.

Indications that Pb-Ca interactions may be somewhat more complex arose from clinical studies on Pb-intoxicated children which demonstrated significantly reduced serum $1,25(OH)_2D_3$ levels associated with diminished Ca intake and high blood Pb concentrations (21, 22). These observations were subsequently confirmed experimentally in rats (23) and chicks (20), indicating that dietary Pb can diminish both the synthesis and circulating levels of the hormone in the Ca-deficient state. In rats (23), intestinal Ca absorption was also reduced, in the normal dietary Ca situation, and totally blocked, in the low Ca situation, by 0.82% dietary Pb. In the latter case, exogenous $1,25(OH)_2D_3$ administration did not reverse the inhibition, suggesting an additional effect of Pb, directly on the intestinal cell (23). These converging lines of evidence indicate that

Received September 7, 1989. [P.S.E.B.M. 1990, Vol 194]
Accepted March 5, 1990.

0037-9727/90/1943-0258$2.00/0

intestinal Pb absorption, as well as body Pb retention, are significantly influenced by systemic Ca homeostasis which, in turn, may be significantly influenced by Pb status. The effect of Pb status on intestinal Pb absorption remains unknown. The present studies were designed to compare and contrast the effects of dietary Pb on intestinal Pb and Ca absorption and other relevent indices of Ca and Pb metabolism. By administering $1,25(OH)_2D_3$ as the sole source of vitamin D, it was possible to limit the effects to post-renal events.

Materials and Methods

Hatchling White Leghorn cockerels (ISA-Babcock Industries, Inc., Ithaca, NY) were fed a semisynthetic vitamin D-deficient diet (20) for 2 weeks. They were then divided into three groups and fed the same diet containing 0, 0.2, or 0.8% Pb (as $PbCl_2$) for an additional 2 weeks. During this experimental period, one-half of the chicks in each group were injected (subcutaneously) daily with 0.1 μg of $1,25(OH)_2D_3$ in 10% ethanol in propylene glycol. At the end of the experimental period, chicks were fasted from food, but not water, for 16 hr. Each group was further subdivided for determination of intestinal ^{47}Ca and ^{203}Pb absorption by the *in situ* ligated duodenal segment procedure (24).

For the ^{47}Ca absorption studies, the intraluminal dosing solution (1.0 ml) contained 20 m*M* $CaCl_2$, 0.1 μCi of 47 Ca, and 10 m*M* Hepes in saline (0.9% NaCl), pH 7.0. The absorption period was 20 min, after which, the chicks were bled by cardiac puncture and killed. The intact duodenal segment was removed, placed in a counting vial containing ice-cold saline, and counted (Gamma-300; Beckman Instruments) for 1 min to determine total radioactivity remaining in the segment. The segment was then drained, rinsed with cold saline (20 ml), and counted again, as before, for radioactivity associated with the tissue. The segment was then slit lengthwise, blotted, and the mucosal tissue scraped from the muscle layers on a cold glass stage.

The mucosal tissue was homogenized in 4 volumes (w/v) of cold Tris buffer (pH 7.4) and samples were removed for determination of alkaline phosphatase activity (25) and homogenate total protein (26). The remaining homogenates were then centrifuged at 38,000*g* for 30 min and the supernatants retained for determination of intestinal calbindin D levels (27) and supernatant total protein (26).

One tibia was also removed, stripped free of soft tissue, and counted for 1 min for ^{47}Ca. Blood plasma was analyzed for total Ca by atomic absorption spectrophotometry (model 360; Perkin-Elmer, Norwalk, CT) and inorganic phosphate, according to Kraml (28). Carrier-free ^{47}Ca, in saline, was obtained from Amersham (Arlington Heights, IL).

^{203}Pb absorption studies were performed similarly to the ^{47}Ca absorption studies. The intraluminal dosing solution (1.0 ml) contained 0.01 m*M* Pb acetate, 0.1 μCi of 203 Pb, and 1.0 m*M* sodium acetate in saline (pH 6.5). At the end of the absorption period (60 min), the chicks were killed and the intact and rinsed segments counted, as before, for ^{203}Pb. One tibia was removed, stripped free of soft tissue, and counted for 1 min for ^{203}Pb. Carrier-free ^{203}Pb was obtained from the DuPont Co. (New England Nuclear Research Products, Boston, MA).

From identically treated chicks, not subjected to absorption studies, the mucosal tissue was harvested, as were the kidneys, extracted with 4 volumes (w/v) of a mixture of 20% trichloroacetic acid, 5% lanthanum oxide, 25% HCl, and analyzed for stable Pb and Ca by atomic absorption spectrophotometry. In addition, one tibia from each chick was removed and stripped free of soft tissue for determination of total bone ash Pb and Ca by atomic absorption spectrophotometry following ashing.

The following terms were used to define the various steps in the overall absorption process: (i) absorption refers to the percentage of administered dose (isotope) which left the intact segment and is computed as (100 − % dose retained), (ii) tissue retention is the percentage of administered dose remaining associated with the segment after rinsing, and; (iii) luminal efflux is the percentage of administered dose which left the lumen and is equivalent to the sum of absorbed and tissue retention. Statistical analyses were performed using one-way analysis of variance and Duncan's multiple range test (29).

Results

The effects of cholecalciferol deficiency and $1,25(OH)_2D_3$ repletion on body weight, plasma Ca, and plasma P_i are shown in Table I. Body weight gain, over the 2-week experimental period, was significantly increased by $1,25(OH)_2D_3$ repletion at 0 and 0.2%, but not at 0.8% dietary Pb. Plasma Ca levels, as expected, were elevated to normal by repletion. However, cholecalciferol-deficient chicks fed 0.8% Pb were normocalcemic and there was no additional increase due to repletion. Plasma P_i levels were lower in the deficient

Table I. Effect of Dietary Pb and $1,25(OH)_2D_3$ Status on Body Weight, Plasma Ca, and Plasma P_i

Treatment	Body weight (g)	Plasma Ca (mg/100 ml)	Plasma P_i (mg/100 ml)
-D	150 ± 3a,c	7.5 ± 0.4a	3.0 ± 0.4a
$1,25\text{-}D_3$	237 ± 10b	10.7 ± 0.2b	5.9 ± 0.3b
-D + 0.2% Pb	148 ± 9a	7.9 ± 0.3a	1.6 ± 0.1c
$1,25\text{-}D_3$ + 0.2% Pb	241 ± 7b	10.0 ± 0.1b	3.6 ± 0.2a
-D + 0.8% Pb	141 ± 9a	10.0 ± 0.1b	2.1 ± 0.4a,c
$1,25\text{-}D_3$ + 0.8% Pb	175 ± 11c	10.4 ± 0.1b	5.0 ± 0.3b

Note. Values are mean ± SE for five chicks. Mean bearing a common letter within a column are not significantly different ($P > 0.05$).

than in the repleted chicks, and there was an additional significant reduction at 0.2% dietary Pb.

Repletion with $1,25(OH)_2D_3$ significantly enhanced intestinal absorption (over 8-fold) and diminished mucosal tissue uptake (3 to 4-fold) of ^{47}Ca, resulting in a substantial increase in luminal efflux (Fig. 1). There was no significant influence of dietary Pb, even at the very high level of 0.8%, on the intestinal Ca transport response to $1,25(OH)_2D_3$.

Intestinal ^{203}Pb absorption was also significantly enhanced (4-fold) by $1,25(OH)_2D_3$ repletion in Pb-naive chicks (Fig. 1). Tissue uptake of ^{203}Pb, while extensive, was not affected by repletion and, therefore, the difference in luminal efflux was attributed solely to absorption. Dietary Pb had no effect on intestinal ^{203}Pb absorption, in cholecalciferol-deficient chicks, but did reduce tissue uptake and this was reflected in the luminal efflux. For the case of the $1,25(OH)_2D_3$-replete chicks, dietary Pb diminished intestinal ^{203}Pb absorption, in an apparent dose-dependent fashion, so that at 0.8% Pb, there was no difference due to repletion. Tissue uptake of ^{203}Pb was also somewhat less at the 0.8% Pb level, resulting in an even greater difference in luminal efflux.

Results essentially identical to the absorption studies were obtained from measurements of the tibial uptake of ^{47}Ca and ^{203}Pb (Fig. 2). Again, repletion with $1,25(OH)_2D_3$ markedly increased ^{47}Ca incorporation into bone, regardless of dietary Pb status. The increase in ^{203}Pb incorporation into bone of Pb-naive chicks in

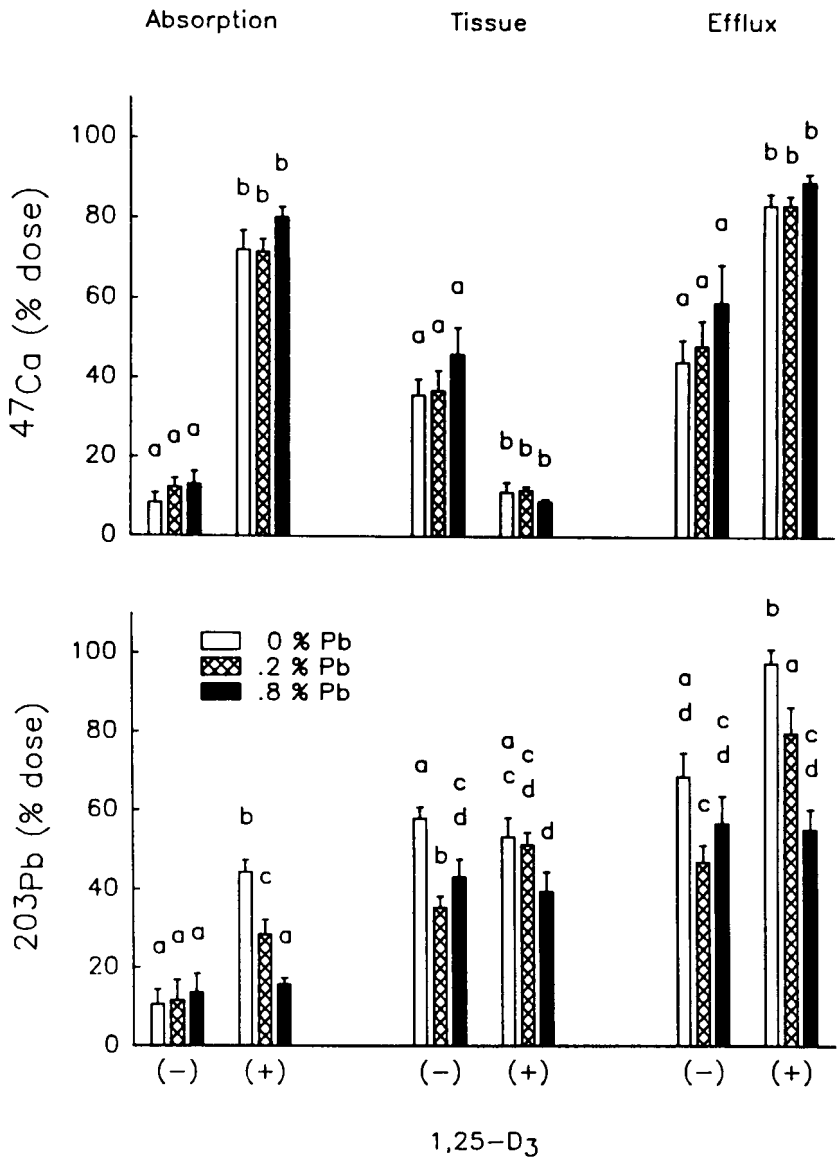

Figure 1. Intestinal absorption, tissue retention, and luminal efflux of ^{47}Ca and ^{203}Pb in cholecalciferol-deficient (−) and $1,25(OH)_2D_3$-replete (+) chicks fed the indicated levels of Pb. Each point represents the mean ± SE for five chicks. Means bearing a common superscript are not significantly different ($P > 0.05$) for each measured parameter.

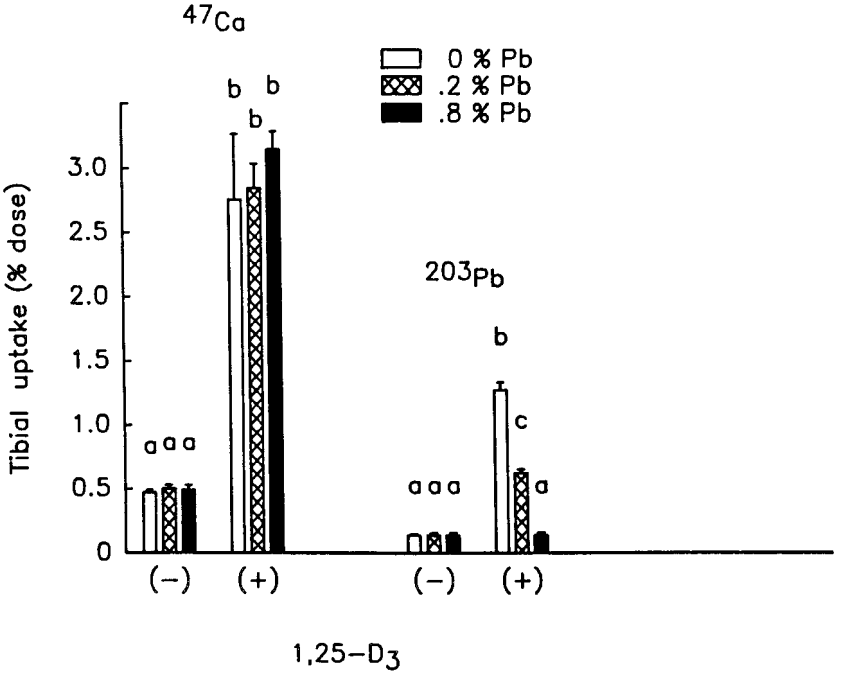

Figure 2. Tibial uptake of ^{47}Ca and ^{203}Pb in cholecalciferol-deficient (−) and $1,25(OH)_2D_3$-replete (+) chicks fed the indicated levels of Pb. Each point represents the mean ± SE for five chicks. Means bearing a common superscript are not significantly different ($P > 0.05$) for each ion.

response to $1,25(OH)_2D_3$ was partially reversed by 0.2% and completely inhibited by 0.8% dietary Pb. These results indicate that, at least during the absorption period, tibial uptakes of Ca and Pb are entirely dependent on the extent of intestinal absorption, rather than on local factors.

The synthesis of intestinal calbindin D in response to $1,25(OH)_2D_3$ was unaffected by dietary Pb status (Fig. 3) and the usual increase in alkaline phosphatase activity may well have been augmented by Pb. These results, in combination with those for ^{47}Ca absorption, indicate that the overall intestinal Ca transport response to $1,25(OH)_2D_3$ is not influenced by dietary Pb.

Additional pertinent indices of Pb and Ca status are shown in Table II. Kidney total Ca level was unaffected by dietary Pb in the cholecalciferol-deficient chicks, but was somewhat elevated by the combination of $1,25(OH)_2D_3$ repletion and Pb. Kidney total Pb levels were significantly increased as a function of dietary Pb in both the deficient and replete groups, although the $1,25(OH)_2D_3$-replete chicks retained considerably more.

The total content of Ca in the intestinal mucosa (Table II) was reduced by $1,25(OH)_2D_3$ repletion, at all levels of dietary Pb. These data agree with ^{47}Ca results and confirm the importance of cholecalciferol in the transepithelial transport (as opposed to tissue uptake) of Ca. The total mucosal Pb content increased with increasing dietary Pb level in the deficient, but not the repleted chicks.

Tibial Ca content (as % ash) was significantly elevated by $1,25(OH)_2D_3$, but unaffected by dietary Pb level, again indicating that Ca metabolism was not compromised by Pb.

Tibial Pb content (Table II) was increased dramat-

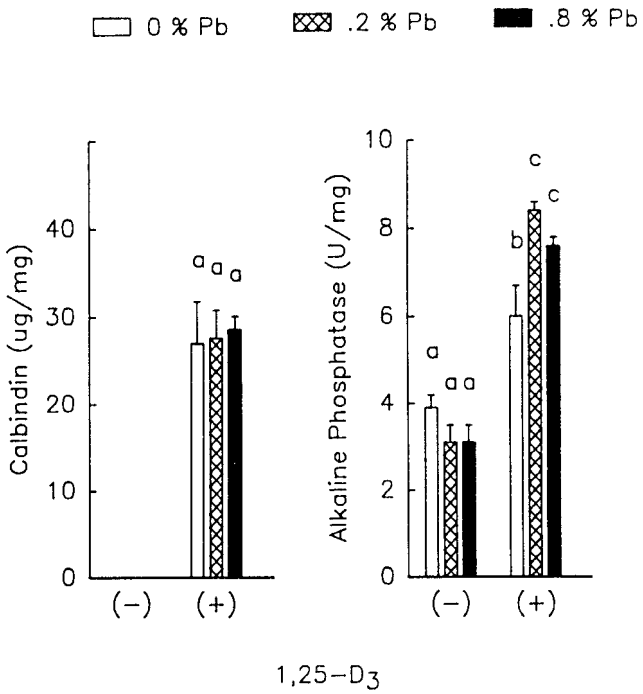

Figure 3. Intestinal calbindin D and alkaline phosphatase levels in cholecalciferol-deficient (−) and $1,25(OH)_2D_3$-replete (+) chicks fed the indicated levels of Pb. Each point represents the mean ± SE for five chicks. Means bearing a common superscript are not significantly different ($P > 0.05$).

Table II. Kidney, Gut, and Bone Ca and Pb Levels as a Function of $1,25(OH)_2D_3$ and Dietary Pb Status

Treatment	Kidney Ca (ppm)	Kidney Pb (ppm)	Gut Ca (ppm)	Gut Pb (ppm)	Tibial Ca (% ash)	Tibial Pb (ppm ash)
-D	69.0 ± 8.8a,c	0a	116.8 ± 14.6a	0a	33.2 ± 0.4a	23.4 ± 14.3a
$1,25\text{-}D_3$	64.1 ± 1.5a	0a	74.6 ± 4.3b	0a	36.0 ± 0.2b	10.9 ± 6.7a
-D + 0.2% Pb	56.6 ± 3.8a	4.8 ± 0.5b	87.0 ± 3.4c	3.8 ± 0.4b	33.5 ± 0.3a	133.1 ± 24.1b
$1,25\text{-}D_3$ + 0.2% Pb	80.2 ± 13.4a,c	13.7 ± 2.4c	69.6 ± 2.0b	3.0 ± 0.0b	35.5 ± 0.2b,c	335.1 ± 15.8c
-D + 0.8% Pb	62.4 ± 2.4a	9.2 ± 0.9c	104.4 ± 8.0a,c	7.0 ± 1.0c	32.8 ± 0.2a	299.8 ± 4.8c
$1,25\text{-}D_3$ + 0.8% Pb	90.1 ± 7.6d	32.4 ± 7.6d	69.5 ± 2.3b	4.2 ± 1.0b	34.7 ± 0.4c	1008.8 ± 71.2d

Note. Values are mean ± SE for five chicks. Means bearing a common letter within a column are not significantly different ($P > 0.05$).

ically by both dietary Pb level and by $1,25(OH)_2D_3$ repletion. These results are not entirely in agreement with the ^{203}Pb absorption data. At the 0.2% dietary Pb level, elevated tibial Pb content can be accounted for by increased Pb absorption due to repletion. At the 0.8% Pb level, however, there was no substantial difference in ^{203}Pb absorption (due to repletion) and, yet, over three times as much Pb was incorporated into the tibia of repleted chicks.

Discussion

The present studies compare and contrast the effects of dietary Pb intoxication on the duodenal Ca and Pb transport responses to the hormonal form of cholecalciferol. By supplying $1,25(OH)_2D_3$ as the only source of cholecalciferol, it is experimentally possible to assess the effects of Pb on processes other than formation of the hormone. This is important, since there is evidence that Pb can both inhibit (20, 23) and enhance (30) the renal conversion of 25-hydroxycholecalciferol to $1,25(OH)_2D_3$. However, since it is possible that Pb may also influence the biologic half-life of the circulating levels of hormone (23), such an approach does not necessarily pinpoint the intestine as a potential site of Pb action. To this end, ^{203}Pb and ^{47}Ca absorption experiments were conducted in parallel.

Chronic repletion of cholecalciferol-deficient, Pb-naive chicks with $1,25(OH)_2D_3$ markedly increased duodenal ^{203}Pb as well as ^{47}Ca absorption. These results confirm, in general, previous observations in rats (19) and chicks (18, 20) employing both acute (18, 19) and chronic (20) administration of $1,25(OH)_2D_3$. Although the responses of ^{203}Pb and ^{47}Ca to repletion were qualitatively similar, there were differences in detail. Enhanced ^{47}Ca absorption, in response to $1,25(OH)_2D_3$, involved the movement of Ca from the lumen to blood (transepithelial), but also from the tissue to the blood (transcytosolic). The response for ^{203}Pb involved only transepithelial transport, with no change in tissue ^{203}Pb.

Smith *et al.* (23) reported a dose-dependent inhibition of *in vitro* Ca transport by dietary Pb in cholecalciferol-deficient (normal dietary Ca) rats chronically repleted with cholecalciferol. Cholecalciferol-mediated duodenal (everted sac) Ca transport was completely blocked by 0.82% dietary Pb. Chronic repletion with $1,25(OH)_2D_3$ partially, but not completely reversed this inhibition, suggesting dietary Pb exerted some influence at the level of the intestine (23).

The present *in situ* results are not in agreement with this suggestion. Since the intestinal response to exogenous $1,25(OH)_2D_3$ measured either directly as ^{47}Ca absorption or the associated molecular events (calbindin D synthesis and alkaline phosphatase activity) was unaltered by dietary Pb status, it is concluded that under the present conditions, dietary Pb does not significantly influence the biologic half-life of the hormone, its entry into the intestinal epithelial cell, or interaction with receptor and/or subsequent genomic and nongenomic events.

Unlike intestinal ^{47}Ca absorption, ^{203}Pb absorption was significantly inhibited by increasing levels of dietary Pb. at 0.8% dietary Pb, enhanced intestinal ^{203}Pb absorption in response to $1,25(OH)_2D_3$, was completely inhibited. For the most part, the site of inhibition appears to be the entry of Pb into the epithelial cells, i.e., a reduction in luminal efflux. The effect is apparently related specifically to $1,25(OH)_2D_3$-mediated Pb transport, since there was no influence of dietary Pb on ^{203}Pb absorption in the cholecalciferol-deficient chicks.

An additional point of interest is that, while absorption and tibial uptake of ^{203}Pb in the cholecalciferol-deficient and $1,25(OH)_2D_3$-replete chicks fed 0.8% Pb were similar, tibial and renal accumulations of dietary Pb were markedly increased by chronic repletion with the hormone. Such results might suggest an effect of $1,25(OH)_2D_3$ on bone and kidney Pb retention, independent of any influence of $1,25(OH)_2D_3$ on Pb absorption. Dietary Ca deficiency, known to elevate $1,25(OH)_2D_3$ levels, also increases bone and kidney Pb retention in some fashion(s) which may not necessarily be related to enhanced Pb absorption (30–32).

Another explanation would be the influence of temporal factors, perhaps related to body Pb burden. It must be emphasized that values for tibial (and renal) Pb content are cumulative, whereas ^{203}Pb absorption uptake data result from point measurements and indicate duodenal capacity to absorb Pb only at the end of the 2-week experimental period. Therefore, at the si-

multaneous onset of $1,25(OH)_2D_3$ repletion and Pb intoxication, the initial response may be enhanced Pb (and Ca) absorption at all levels of dietary Pb, similar to the situation observed in Pb-naive animals (18). Such an initial event might result in body Pb burden (and tibial Pb accumulation) proportional to Pb intake. A secondary response, triggered by increased Pb burden would then reverse, and ultimately inhibit, the intestinal Pb absorption response to $1,25(OH)_2D_3$. Clearly, it is necessary to conduct thorough time studies of Ca and Pb absorption and body Pb retention related to $1,25(OH)_2D_3$ administration and Pb intoxication.

The location of the inhibitory effect of dietary Pb on Pb absorption is not known. It is difficult to envision a negative feedback mechanism from bone or kidney to intestine which would be independent of the cholecalciferol endocrine system and would not influence Ca absorption as well. The intestinal epithelial cell is a likely choice. Competition between Pb and Ca for potential transport components has been suggested and experimental evidence exists both for (30) and against (16) such a possibility. Intestinal Pb accumulation does not appear to be involved, since the mucosal Pb content in $1,25(OH)_2D_3$-replete chicks fed 0.8% Pb was significantly less than the cholecalciferol-deficient counterparts, but not different from either groups of chicks fed 0.2% Pb. The localization of this Pb in the intestinal cell, however, is not known.

In order to fully elucidate the mechanism of intestinal Pb absorption, it is necessary to understand not only those elements common to Pb and Ca transport, but also those which are not. The present results demonstrate that differences exist between Pb-naive and Pb-intoxicated animals and these should be given consideration in future studies.

This work was supported by National Institutes of Health Grant ES-04072. The efforts of Mary Brindak and Norma Jayne are gratefully acknowledged. The 1,25-dihydroxyvitamin D_3 was a generous gift of Dr. M. R. Uskokovic' and Hoffmann-LaRoche, Inc. of Nutley, NJ.

1. Centers for Disease Control. Preventing lead poisoning in young children (U.S. Department of Health Human Services, Atlanta, GA), CDC 99-2230, 1985.
2. Mahaffey KR, Annest JL, Roberts J, Murphy R. National estimates of blood lead levels: United States, 1976–1980. N Engl J Med **307**:573–579, 1982.
3. Agency for Toxic Substances and Disease Registry. The nature and extent of lead poisoning in children in the United States: A report to Congress (U.S. Department of Health Human Services, Washington, DC), 1988.
4. Falk HL. Conclusions of the Committee on Human Health Consequences of Lead Exposure from Automobile Emissions. Environ Health Perspect **19**:243–246, 1977.
5. Mahaffey KR. Quantities of lead producing health effects in humans: Sources and bioavailability. Environ Health Perspect **19**:285–295, 1977.
6. Blair JA, Coleman IPL, Hilburn ME. The transport of the lead cation across the intestinal membrane. J Physiol **286**:343–350, 1979.
7. Hilburn ME, Coleman IPL, Blair JA. Factors influencing the transport of lead across the small intestine of the rat. Environ Res **23**: 301–308, 1980.
8. Aungst BJ, Fung H-L. Kinetic characterization of in vitro lead transport across the rat small intestine. Toxicol Appl Pharmacol **61**:39–47, 1981.
9. Sobel AE, Yuska H, Peters DD, Kramer B. The biochemical behavior of lead. 1. Influence of calcium, phosphorus, and vitamin D on lead in blood and bone. J Biol Chem **132**:239–265, 1940.
10. Lederer LG, Bing FC. Effect of calcium and phosphorus on retention of lead by growing organisms. J Am Med Assoc **114**:2457–2461, 1940.
11. Shields JB, Mitchell HH. The effect of calcium and phosphorus on the metabolism of lead. J Nutr **21**:541–552, 1941.
12. DeLuca HF, Schnoes HK. Vitamin D: Recent advances. Annu Rev Biochem **52**:411–440, 1983.
13. Wasserman RH, Fullmer CS. Calcium transport proteins, calcium absorption and vitamin D. Annu Rev Physiol **45**:375–390, 1983.
14. Haussler MR, Nagode LA, Rasmussen H. Induction of intestinal brush border alkaline phosphatase by vitamin D and identity with CaATPase. Nature **228**:1199–1201, 1970.
15. Six KM, Goyer RA. Experimental enhancement of lead toxicity by low dietary Ca. J Lab Clin Med **76**:933–942, 1970.
16. Mykkanen HM, Wasserman RH. Gastrointestinal absorption of lead (^{203}Pb) in chicks: influence of lead, calcium and age. J Nutr **111**:1757–1765, 1981.
17. Smith CM, DeLuca HF, Tanaka Y, Mahaffey KR. Stimulation of lead absorption by vitamin D administration. J Nutr **108**:843–847, 1978.
18. Mykkanen HM, Wasserman RH. Effect of vitamin D on the intestinal absorption of ^{203}Pb and ^{47}Ca in chicks. J Nutr **112**:520–527, 1982.
19. Mahaffey KR, Smith C, Tanaka Y, DeLuca HF. Stimulation of gastrointestinal lead absorption by 1,25-dihydroxyvitamin D_3. Fed Proc **38**:825, 1979.
20. Edelstein S, Fullmer CS, Wasserman RH. Gastrointestinal absorption of lead in chicks: Involvement of the cholecalciferol endocrine system. J Nutr **114**:692–700, 1984.
21. Sorrell M, Rosen JF, Toginsky M. Interactions of lead, calcium, vitamin D and nutrition in lead-burdened children. Arch Environ Health **32**:160–164, 1977.
22. Rosen JF, Chesney RW, Hamstra A, DeLuca HF, Mahaffey KR. Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. N Engl J Med **302**:1128–1131, 1980.
23. Smith CM, DeLuca HF, Tanaka Y, Mahaffey KR. Effect of lead ingestion on functions of vitamin D and its metabolites. J Nutr **111**:1321–1329, 1981.
24. Morrissey RL, Wasserman RH. Calcium absorption and calcium-binding protein in chicks on differing calcium and phosphorus intakes. Am J Physiol **220**:1509–1515, 1971.
25. Morgenstern S, Kessler G, Auerbach J, Flor RV, Klein B. An automated p-nitrophenylphosphate serum alkaline phosphatase procedure for the autoanalyzer. Clin Chem **11**:876–888, 1965.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
27. Corradino RA, Wasserman RH. Vitamin D: Induction of calcium-binding protein in embryonic chick intestine in vitro. Science **172**:731–733, 1971.
28. Kraml M. A semi-automated determination of phospholipids. Clin Chim Acta **16**:442–448, 1966.

29. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York: McGraw-Hill, 1960.
30. Baksi SN, Kenny A. Vitamin D mechanism in Japanese quail: Effects of lead exposure and dietary calcium. Toxicol Appl Pharmacol **51**:489–495, 1979.
31. Barton JC, Conrad ME, Harrison L, Nuby S. Effects of calcium on the absorption and retention of lead. J Lab Clin Med **91**:366–376, 1978.
32. Mahaffey KR. Nutrient-lead interactions. In: Singhal R, Thomas J, Eds. Lead Toxicity. Baltimore: Urban & Schwarzenberg, 1980.