MASTER

MOLECULAR MECHANISMS OF THE EPITHELIAL
TRANSPORT OF TOXIC METAL IONS

PROGRESS REPORT

(January 1, 1980 - December 31, 1980)

Prepared for the
DEPARTMENT OF ENERGY

R. H. Wasserman

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MOLECULAR MECHANISMS OF THE EPITHELIAL TRANSPORT OF TOXIC METAL IONS,
PARTICULARLY MERCURY, CADMIUM, LEAD, ARSENIC, ZINC AND COPPER

Progress Report for Period

January 1, 1980 - December 31, 1980

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Summary Statement

Investigations were continued to elucidate the mode of transepithelial transport of toxic metal ions across the gastrointestinal tract, as well as their interactions with biological processes and other metal ions. All experimental details that are either published, submitted for publication or in press during this report period are included in the Appendix.

Primary attention for this report has been given to the intestinal absorption of lead and its interaction with other biological moieties. Details of these experiments are attached in the enclosed Technical Progress Report and highlights are presented here, as follows.

1. Comparisons of the intestinal absorption of lead and calcium established that:

- a) Both metals are rapidly sequestered by the mucosal tissue.

Calcium is rapidly released serosally to the circulation, whereas lead is transferred slowly.

- b) The absorption of lead and calcium show similar responses to various experimental conditions (low calcium intake, age of animal, pH of dosing solution). However, increasing luminal stable lead concentration significantly reduced the percentage of radiolead absorbed, but did not inhibit the absorption of radiocalcium.

- c) Increasing luminal calcium concentration did not significantly affect radiolead absorption.

These data imply that, despite similarities in the response of of lead and calcium absorptive processes to various treatments, there is no apparent direct interaction between these cations in the intestine of the chick.

2. Studies of the effect of cholecalciferol on intestinal lead absorption provided the following information:

- a) Cholecalciferol administration to rachitic chicks resulted in a dose dependent elevation of intestinal transepithelial transport of lead. This effect is regarded as a direct action of cholecalciferol on the capacity of the intestine to transport lead and not an indirect action, such as alteration of transit time.

- b) The transfer of lead in cholecalciferol-deficient chicks was greatest in the distal intestinal segments and decreased proximally. Administration of cholecalciferol enhanced lead transfer in all

segments and eliminated differences observed among segments. Therefore, the effect of cholecalciferol on lead transfer was greatest in the duodenum, followed by the jejunum and ileum, respectively.

c) Luminal efflux of lead was not significantly affected by cholecalciferol, whereas the transfer of lead to body was clearly enhanced.

d) Lead transfer to body was significantly elevated 8 hr. after the administration of cholecalciferol, followed by further enhancement to 48 hr. This time-dependent increase was accompanied by a decline in the amount of radiolead retained by the intestinal tissue, resulting in no net change in the luminal efflux of radiolead. These results imply that cholecalciferol affects the facilitation of lead transfer from the mucosal tissue to the body rather than by increasing luminal efflux.

e) Luminal efflux of lead was significantly increased by $1,25(\text{OH})_2$ cholecalciferol and was maximal 8 hrs post-dosing. At 32 hr post-dosing, luminal efflux returned to control levels. $1,25(\text{OH})_2$ cholecalciferol did not significantly affect the retention of radiolead by the intestinal mucosal tissue.

f) While cholecalciferol repletion increased the back flux of ^{47}Ca , it had no influence on the amount of ^{203}Pb transferred from plasma to luminal fluid.

3. Intestinal absorption of lead was influenced by phosphorus, as follows:

a) Phosphate reduced the intestinal uptake of radiolead by the intestine primarily via precipitation of ^{203}Pb phosphate. However, the reduction in transfer of radiolead to body could not be accounted

for completely by precipitation. Competition between phosphate and lead for some intestinal component involved in lead transport is suggested as a possibility.

b) Low dietary phosphate enhanced the transfer of mucosal radiolead to the body. This indicates that altered metabolic status may affect the intestinal absorption of lead. It is also possible that increased synthesis of $1,25(\text{OH})_2$ cholecalciferol as a result of low dietary levels of phosphorus may have resulted in the enhanced transfer of lead.

4. Studies on the effect of lanthanum on intestinal lead absorption showed the following:

a) The absorption of both lead and calcium was inhibited by lanthanum, suggesting the presence of a non-cytoplasmic path across the epithelial membrane through which these cations move. This effect of lanthanum on lead absorption, unlike the case for calcium absorption, was not dependent on cholecalciferol. While these studies provide evidence for an extracellular shunt pathway for the (presumably) diffusional movement of lead, the possibility of lanthanum-lead interaction at other sites was not eliminated.

Gastrointestinal Absorption of Lead (^{203}Pb) in Chicks, With a
Special Reference to the Possible Interaction of Calcium With
Lead During the Absorption Process

Abstract

The present study was designed to investigate, in more detail, the mechanism of lead transport by the gastrointestinal tract and particularly the similarities or dissimilarities between lead and calcium in this process. The absorption of these metals was determined in 3-week old White Leghorn cockerels, raised on a commercial diet or special diets, using an in vivo ligated loop procedure. The dose administered into the loop usually contained 0.5 μCi ^{203}Pb (and/or 0.1 μCi ^{47}Ca), 0.01 mM lead acetate (and/or 1 mM CaCl_2) in 0.5 ml of 0.15 M NaCl, pH 6.5. It was shown that lead is rapidly taken up by the intestinal tissue, and only slowly transferred into the circulation whereas calcium, also accumulated rapidly by the tissue, is rapidly released from the tissue in the serosal direction. The absorption processes of these cation show similar responses to various experimental conditions (low calcium intake, age of the animal, pH of the dosing solution). However, increasing luminal stable lead concentration from 0.01 to 1 mM Pb, significantly reduced the percentage of radiolead absorbed, but did not inhibit the absorption of radiocalcium. Also, luminal Ca (0-25 mM) did not significantly affect the absorption of ^{203}Pb . These data imply that, in spite of the similarities in the response of the lead and calcium absorptive processes to various treatments, there is no direct interaction between these cations in the intestine of the chick.

Introduction

Lead is a well-known environmental hazard and its toxicity depends upon its concentration in various sensitive tissues and organs. Many dietary and physiological factors affect the toxicity of lead (1), including those factors that determine the amount of ingested lead that is absorbed from the gastrointestinal tract and the rate of loss of lead from the body. One of the more significant determinants of lead toxicity is the level of dietary calcium. Sobel and colleagues (2) showed several decades ago that a low dietary calcium intake enhances tissue retention of lead in experimental animals. Their observation was verified by others and, in addition, it was shown that a high calcium intake decreased the retention of lead (3-8). Given the evidence that dietary calcium does not affect the retention of lead administered intraperitoneally (6,9), an interaction between calcium and lead at the intestinal level is indicated.

The mechanism of lead transport across the intestinal membrane is not fully understood, but proposed to be by passive diffusion (10,11). A recent observation demonstrated that vitamin D stimulates the absorption of lead from the gastrointestinal tract of rats (12). Calcium is absorbed by both an active process and by passive diffusion, and both of these processes are affected by vitamin D (13-16). It might be speculated that these two metal ions might share a common diffusional transport system that is affected by vitamin D.

The present studies were designed to investigate in more detail the mechanism of lead transport by the gastrointestinal tract of chicks with an emphasis on the particular similarities or dissimilarities of the behavior of lead and calcium.

Methods

Chicks used for absorption studies were 3-week old White Leghorn cockerels (Babcock Industries, Inc., Ithaca, NY) raised after hatching either on a commercial diet (Chick Starter Diet, Agway, Inc., Ithaca, NY) or on experimental diets specified subsequently. The absorption of ^{203}Pb was determined by the ligated loop procedure, as described by Morrissey and Wasserman (17).

The following indices of absorption were obtained: 1) "duodenal absorption" refers to the amount of ^{203}Pb (% dose) that left the lumen during the absorption period and is calculated as $100 - (\% ^{203}\text{Pb in the intestinal loop} - \% ^{203}\text{Pb in the intestinal tissue})$; 2) "intestinal retention" which is the amount of ^{203}Pb (% dose) remaining in the intestinal tissue; 3) "transfer to body" refers to the amount of ^{203}Pb (% dose) in the whole body without the intestinal loop, and is determined by counting the carcass in a whole body counter or from the equation $100 - (\% ^{203}\text{Pb retained in the intestinal loop})$; 4) "tibia uptake" which is the amount of ^{203}Pb (% dose) accumulated by the tibia.

The effects of the following factors on ^{203}Pb absorption were assessed: length of absorption period, luminal concentration of stable lead or stable calcium, pH of the dosing solution, site of absorption, body burden of lead, age, and dietary calcium levels. In some of these experiments, the absorption of calcium was determined, using ^{47}Ca as tracer.

Expt. 1. Absorption of ^{203}Pb and ^{47}Ca as a function of time.

Chicks raised on the commercial diet were fasted overnight and injected intraduodenally with 0.5 ml solution containing 0.01 mM lead acetate, 1 mM CaCl_2 , 1 mM sodium acetate, 0.15 M NaCl , $0.5 \mu\text{Ci } ^{203}\text{Pb}$ and $0.1 \mu\text{Ci } ^{47}\text{Ca}$, pH 6.5. At intervals of 5, 15, 30 and 60 minutes after

the administration of the dose, six chicks at each time period were killed and the degree of ^{203}Pb and ^{47}Ca absorption was determined.

Expt. 2. Effect of varying concentrations of stable lead in the dosing solution on ^{203}Pb absorption. Prefasted chicks raised on the commercial diet were divided into 5 groups of 6 chicks each. One group was injected intraduodenally with 1.0 ml of a solution containing 0.5 μCi ^{203}Pb in 0.01mM lead acetate, 0.15 M NaCl and 0.1 M sodium acetate, pH 6.5, and the other groups the same basal solution containing one of the following concentrations of stable lead: 0.03, 0.10, 0.30, or 1.00 mM lead (as acetate). To determine whether sodium acetate affects the absorption of ^{203}Pb , another group was injected with a dose containing 0.5 μCi ^{203}Pb , 0.01 mM lead acetate, 0.15 M NaCl and 1 mM sodium acetate, pH 6.5. Sixty minutes after dosing, the chicks were killed and the degree of ^{203}Pb absorption determined.

Expt. 3. Effect of luminal lead concentration on the duodenal absorption of ^{47}Ca . Two groups of prefasted chicks raised on the commercial diet were dosed with 0.15 ml of solution containing 0.1 μCi ^{47}Ca , either 0.01 or 25 mM CaCl_2 , 0.01 mM lead acetate, 0.15 M NaCl and 0.1 M sodium acetate, pH 6.5. The other groups were injected with the same basal solutions but containing either 0.10 or 1.00 mM lead (as acetate). Fifteen minutes after dosing, the chicks were killed and the degree of ^{47}Ca absorption was assessed.

Expt. 4. Effect of luminal calcium concentration on the duodenal absorption of ^{203}Pb . Chicks raised on the commercial diet and prefasted were dosed with the 0.5 ml of a solution containing 0.5 μCi ^{203}Pb , 0.01mM lead acetate, 1 mM sodium acetate and 0.15 M NaCl, pH 6.5. The other groups were dosed with the same solution but contained 1.0 or 25 mM CaCl_2 . The

degree of absorption of ^{203}Pb was determined, as above, 60 min. after dosing.

Expt. 5. Effect of various concentrations of dietary lead on the absorption of ^{203}Pb . One-day old cockerels were divided into 5 groups of 6 birds each. The groups were fed the commercial diet containing either 0, 3, 10, 100 or 1000 ppm lead (as acetate). After receiving the appropriate diets for 3 weeks, the chicks were pre-fasted and the absorption of ^{203}Pb from in situ duodenal loops was determined as above.

Expt. 6. Effect of the pH of the dosing solution on the absorption of radiolead. The degree of ^{203}Pb absorption from solutions adjusted to different pH values was determined in 3-week old chicks raised on the commercial diet as outlined above. The dose (1 ml) contained 0.01 mM lead acetate, 1 mM sodium acetate, 0.15 M NaCl and 0.5 μCi ^{203}Pb adjusted with NaOH or HCl to one of the following pH values: 4, 6 or 7. The absorption period was 60 minutes.

Expt. 7. Absorption of ^{203}Pb from different segments of the small intestine. The absorption of ^{203}Pb from the different segments of the small intestine was determined in the manner described above. The dose (0.5 ml) contained 0.5 μCi ^{203}Pb , 0.01 mM lead acetate, 1 mM sodium acetate and 0.15 M NaCl, pH 6.5. The absorption period was 60 min. The jejunum was indentified as the segment proximal to the yolk sac and distal to the duodenum, and the ileum was localized distal to the yolk sac and proximal to the ileocaecal junction.

Expt. 8. The effect of age on lead absorption. Chicks were fed the commercial diet and the absorption of ^{203}Pb was determined by the ligated duodenal loop procedure at 1, 3, or 5 weeks of age; the dosing solution was as in Expt. 7.

Expt. 9. Effect of low calcium diets on the duodenal absorption of ^{203}Pb . Two-week old cockerels raised on the commercial diets were assigned to one of the following dietary groups of 6 birds each: 1) Low Ca (containing 0.03% Ca and 1.0% P); and 2) Normal Ca (containing 1.2% Ca and 1.0% P). The experimental diets were prepared by adding calcium and phosphorus as calcium carbonate and mono-basic potassium phosphate to the semisynthetic basal diet. The composition of the basal diet is given in Table 1 with calcium and phosphorus omitted. Vitamin D_3 was also added to yield a concentration of 1200 IU cholecalciferol per kg. After maintaining the chicks on the appropriate diets for one week, the absorption of ^{203}Pb was determined by the procedure previously outlined.

For measurement of calcium-binding protein (CaBP), the mucosal tissue was scraped from the segment and homogenized (1:4 w/v) in Tris buffer { (0.0137 M tris (hydroxy-methyl) aminomethane, 0.12 M NaCl, 4.74 mM KCL adjusted to pH 7.4 with HCL) }. The homogenate was centrifuged at 38,000 x g for 20 min in a refrigerated centrifuge. The CaBP content of the supernatant fluid was determined by the radial immunodiffusion method (18). The supernatant protein was measured according to the method of Lowry et al (19).

The ^{203}Pb and ^{47}Ca content of the samples were determined by their gamma emissions, using a well-type gamma counter (Beckman 300, Beckman Instruments, Inc., Irvine, CA); the counts were expressed as % of the initial dose. The radioactivity content of the carcass (minus the duodenal loop) was determined by the use of a whole body counter (Tobor, Nuclear Chicago, Des Plaines, IL).

Carrier-free ^{203}Pb (as acetate) in 0.1 M acetic acid was obtained from New England Nuclear (Boston, MA), and high specific activity ^{47}Ca

(as chloride) in 1N HCl was from Union Carbide Corp. (Oak Ridge, TN).

The significance of all treatment differences was determined by Duncan's Multiple Range Test (20) or by Student's t-test.

Results

The time course of the absorption of ^{203}Pb and ^{47}Ca in 3-week old chicks raised on commercial diet is shown in Fig. 1. Both of the tracers were rapidly taken up by the intestinal tissue, with more than 50% of the ^{203}Pb dose bound to the tissue within 5 min after administering the dose. Tissue ^{203}Pb concentrations remained virtually unchanged over the period of observation, whereas tissue ^{47}Ca was rapidly released from the mucosal cell. The transfer of calcium to body was considerably more rapid than that for lead, indicating that the exit mechanism of these metals from the intestinal cell to the lamina propria are quantitatively and perhaps qualitatively different.

The absorption of ^{203}Pb decreased as the concentration of carrier lead in the dosing solution increased (Fig. 2). The percentage of ^{203}Pb transferred to body similarly decreased. A hundred-fold increase in acetate concentration (from 1 mM to 100 mM) did not affect the percentage of lead absorbed.

The absorption of ^{47}Ca , on the other hand, was unaffected by the lead concentration in the dosing solution (Table 2). This also pertained to the ^{47}Ca accumulated by the intestinal tissue and that transferred to body, although the highest ratio of Pb to Ca in the experiment was 100. The percentage of ^{47}Ca absorbed and transferred to body was less in those chicks receiving 25 mM Ca compared to the 0.01 mM group; the reverse held for the uptake of ^{47}Ca by the intestinal tissue.

Calcium administered together with radiolead did not significantly

effect the duodenal absorption of ^{203}Pb , the amount transferred to body and that retained by the intestinal tissue (Table 3).

Data in Table 4 show that feeding high concentrations of lead in the diet for 3 weeks did not appear to affect the duodenal absorption of this metal. Only the highest concentration of Pb used in this experiment (1000 ppm) significantly reduced the growth of the birds and caused a reduction in the intestinal retention of ^{203}Pb , accompanied by an increase in the amount of ^{203}Pb accumulated by the tibia.

The studies on lead absorption are hampered by the low solubility of lead salts under physiological conditions and dosing solutions of abnormally low Ph have been used in some previous experiments (21). Present results indicate that the pH of the dosing solution does not significantly affect the absorption or intestinal retention of ^{203}Pb in the chick duodenum (Table 5).

The rate of calcium absorption is greatest in the duodenum and decreases towards the distal parts of the small intestine (22). Unlike calcium absorption lead absorption appears to be similar in all segments (Table 6).

In accordance with early observations in rats (23,24), the absorption of lead, like that of calcium and other minerals decreases, with the increasing age of the chick (Fig. 3).

The data in Table 7 confirm the previous results from this laboratory that alterations in the dietary content of calcium influence the efficiency of calcium absorption (17). Furthermore, it was seen that the increase in calcium absorption was accompanied by an increase in mucosal calcium-binding protein and a decrease in retention of ^{47}Ca by the intestinal tissue. Similarly, the transfer of ^{203}Pb to the body was increased by a calcium deficiency, whereas the treatment had no effect on the amount of

radiolead retained by the intestinal tissue (Table 7).

Discussion

The present studies show that the gastrointestinal absorption of lead in chicks occurs in two distinct phases. First, there is a rapid uptake by the intestinal tissue, with more than 50% of the dose bound by the tissue within 5 min after dosage. The nature of this interaction is not known, but may be due to binding of lead to some components of the external surface of the mucosal membrane. A predominantly covalent bonding of lead by phosphate has been suggested by Blair and coworkers (11). The next step, transfer of mucosal lead to body, progresses slowly, indicating that this might be the rate limiting step in the intestinal transport of lead. These results are in accordance with those reported by Conrad and Barton (25), using *in vivo* ligated loop procedure with rats. Since the intestinal mucosa apparently serves as a significant barrier to the total transfer of lead from the lumen to the lamina propria, the exact localization of ^{203}Pb in intestinal tissue (extracellular or intracellular) would be of considerable interest. Prominent features of this barrier could be high affinity binding of ^{203}Pb to the external surface of the intestinal brush border and sequestration by intracellular proteins and organelles. The interaction of calcium with these intestinal components does not apparently represent a significant deterrent to its transmural movement since ^{47}Ca , after being rapidly taken up by the intestinal tissue, is also rapidly released from the mucosal cell with time (Fig. 1).

Interestingly, the absorption of lead shows characteristics of a saturable process (Fig. 2), suggesting the presence of a carrier-system for lead. On the other hand, this observation could be explained simply

by precipitation of lead in the lumen. Lead salts are known to be quite insoluble at physiologic conditions. In fact Blair and coworkers (11) have reported that the passage of lead into the serosal side of an everted gut sac preparation does not display saturation kinetics, but follows the passive movement of water. The stable lead concentrations in their study ranged from 10^{-7} to 10^{-5} M. In the present study, the in vivo ligated loop procedure was used and the luminal lead concentration varied from 10^{-5} to 10^{-3} M. This difference might explain the discrepancy in results.

The chronic intake of dietary lead by the chick could affect the intestinal absorption of lead by increasing luminal and/or mucosal-bound lead via a feedback mechanism or by toxic response. The present study showed clearly that feeding various amounts of lead in the diet for three weeks prior to the absorption experiment did not appreciably affect the intestinal absorption or tissue retention of radiolead. This is in agreement with the results in rats reported by Conrad and Barton (25) and clearly indicates that there is no feedback mechanism for lead absorption similar to that reported for the absorption of calcium (26). It is interesting to note that doses of lead acetate greater than 200 μ g per day decreased the transfer of calcium across the duodenal wall in rats (27) and that intakes of lead considerably greater than 200 μ g per day had no inhibitory effect on ^{203}Pb absorption.

The ingestion of a low calcium diet increased lead absorption by increasing the transfer of lead into the body (Table 7). These observations and those by Lederer and Bing (9) and Quarterman and Morrison (6) appear to be in conflict with the conclusions of Barton and coworkers (21). The latter investigators contend that calcium deficient diets do not affect the absorption of lead, but cause a decrease in the excretion of lead,

thus resulting in increased tissue retention of lead as observed by Sobel and others (2,3,6). The enhanced transfer of ^{203}Pb to the body due to calcium deficiency was associated with an increase in the mucosal calcium-binding protein (CaBP). This vitamin D-induced protein is known to bind many cations, including lead (21), and has been implicated in the intestinal absorption of calcium (28). Since vitamin D increases the absorption of lead (12), it is conceivable that CaBP is involved in the absorption of both actions. Recent information (29) suggests an intracellular localization for CaBP and, therefore, this protein could have a role in the transfer of calcium and possibly of lead through and out of the intestinal cell.

It has been proposed that lead and calcium share a common transport system in the gastrointestinal tract. The present study shows some similar responses of the absorption processes of these cations under various experimental conditions. However, luminal Pb at concentrations that clearly reduced the absorption of ^{203}Pb did not inhibit the absorption of ^{47}Ca (Fig. 2 and Table 2), and luminal Ca did not significantly affect the absorption of ^{203}Pb (Table 3). These data imply that there is no direct interaction between lead and calcium at the limiting step in the absorptive process, at least under the experimental conditions described here. Previously it has been observed, in rats and under different conditions, that luminal calcium may affect the absorption of lead (7,21). Further studies are needed to resolve this discrepancy.

The present results, therefore, document similarities in the response of the lead and calcium absorptive processes to various dietary treatments, and differences in their rate of absorption and accumulation

by the intestinal tissue. It was not possible to demonstrate a direct, competitive interaction between these cations in the intestine of the chick.

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TABLE 1

Composition of the Basal Diet

<u>Ingredient</u>	<u>%</u>
Isolated soy protein	25.00
DL-methionine	.60
Glycine	.40
Corn oil	4.00
Cerelose (Dextrose)	60.27
Cellulose (Solka floc)	3.00
Choline chloride	.20
Vitamin premix ¹	1.00
Mineral premix ²	5.53

¹ Supplies the following (mg/kg of the diet): thiamine HCl 15; riboflavin 15; nicotinic acid 50; folic acid 6; pyridoxine HCl 6; biotin 0.6; vitamin B₁₂ 0.02; Ca-pantothenate 20; menadione sodium bisulfite 1.5; BHT 100; vitamin E 50 IU/kg; vitamin A 4500 IU/kg, with 9.74 g glucose/kg.

² Supplies the following (g/kg of the diet): CaHPO₄ 16.2; CaCO₃ 18.0; KH₂PO₄ 10.0; KCl 1.0; NaCl 6.0; MnSO₄·H₂O 0.35; FeSO₄·7 H₂O 0.5; MgSO₄ 3.0; KIO₃ 0.01; CuSO₄·5 H₂O 0.03; ZnCO₃ 0.15; CoCl₂ 0.0017; NaMoO₄·2 H₂O 0.0083; Na₂SeO₄ 0.002.

TABLE 2

Effect of Luminal Lead Concentration on the Duodenal Absorption of ^{47}Ca
in Normal Chicks

Luminal Pb (mM)	Duodenal absorption	Intestinal retention ^{47}Ca (% dose)	Transfer to body
A. <u>Stable Ca 0.01 mM</u>			
0.01	62.62 $\pm$ 3.17 ^a	12.21 $\pm$ 1.17 ^a	44.58 $\pm$ 3.12 ^a
0.10	60.44 $\pm$ 1.58 ^a	12.64 $\pm$ 0.29 ^a	44.07 $\pm$ 1.52 ^a
1.00	59.89 $\pm$ 2.16 ^a	12.17 $\pm$ 0.84 ^a	43.58 $\pm$ 2.41 ^a
B. <u>Stable Ca 25 mM</u>			
0.01	57.77 $\pm$ 5.07 ^b	15.87 $\pm$ 3.55 ^a	31.60 $\pm$ 2.92 ^b
0.10	49.43 $\pm$ 2.48 ^b	13.71 $\pm$ 0.61 ^a	31.20 $\pm$ 2.70 ^b
1.00	53.00 $\pm$ 2.96 ^{a,b}	15.30 $\pm$ 1.02 ^a	28.67 $\pm$ 1.89 ^b

All values are mean $\pm$ SEM of 5-6 chicks. Values in the same column having a common superscript are not significantly different ($P < 0.05$).

TABLE 3

Effect of Luminal Calcium Concentration on the Duodenal Absorption
of ^{203}Pb in Normal Chicks

Luminal Ca (mM)	Duodenal absorption	Intestinal retention ^{203}Pb (% dose)	Tibia uptake
0	58.80 $\pm$ 2.21 ^a	45.31 $\pm$ 1.53 ^a	0.42 $\pm$ 0.05 ^a
1	62.57 $\pm$ 1.55 ^a	52.59 $\pm$ 1.50 ^b	0.51 $\pm$ 0.06 ^a
25	50.92 $\pm$ 4.86 ^a	40.28 $\pm$ 3.69 ^a	0.50 $\pm$ 0.06 ^a

All values are mean $\pm$ SEM of 6 chicks. The values in the same column having a common superscript are not significantly different ($P < 0.05$).

TABLE 4

Effect of Feeding Diets Containing Lead on the Duodenal Absorption of
 ^{203}Pb in Chick

Lead in the diet (ppm)	Body weight (g)	Duodenal absorption ^{203}Pb (% dose)	Intestinal retention	Tibia uptake
0	181 ± 10^a	77.56 ± 0.49^a	54.23 ± 1.87^a	0.62 ± 0.07^a
3	177 ± 8^a	74.60 ± 1.89^a	53.86 ± 1.67^a	0.53 ± 0.09^a
10	183 ± 9^a	76.93 ± 3.03^a	54.38 ± 3.20^a	0.57 ± 0.06^a
100	186 ± 5^a	72.42 ± 1.91^a	52.56 ± 2.64^a	0.53 ± 0.04^a
1000	138 ± 3^b	70.25 ± 2.57^a	40.83 ± 0.99^b	0.93 ± 0.09^b

All values are mean $\pm$ SEM of 6 chicks. Values in the same column having
a common superscript are not significantly different ($P < 0.05$).

TABLE 5

Effect of the pH of the Dosing Solution on the Duodenal Absorption
of ^{203}Pb in the Normal Chick

pH	Duodenal absorption	Intestinal retention ^{203}Pb (% dose)	Transfer to body
4	56.13 ± 5.01	46.29 ± 4.04	9.84 ± 1.25
6	59.16 ± 5.35	47.84 ± 4.15	11.31 ± 1.76
7	59.45 ± 3.72	48.97 ± 3.05	10.47 ± 1.95

All values are mean $\pm$ SEM of 6 chicks. There were no significant differences between the treatments ($P < 0.05$).

TABLE 6

The Absorption of ^{203}Pb from Different Segments of the Small Intestine
in Normal Chicks

	Absorption	Intestinal retention	Transfer to body
	^{203}Pb (% dose/mg of tissue dry weight)		
Duodenum	0.19 ± 0.02	0.13 ± 0.02	0.05 ± 0.01
Jejunum	0.22 ± 0.02	0.17 ± 0.02	0.06 ± 0.005
Ileum	0.22 ± 0.03	0.16 ± 0.02	0.06 ± 0.007

All values are mean $\pm$ SEM of 6 chicks. No significant ($P < 0.05$)
differences were observed.

TABLE 7

Effect of Deficient Dietary Calcium on the Duodenal Absorption of
 ^{203}Pb and ^{47}Ca in Chicks

	Transfer to body		Intestinal retention		Mucosal CaBP
	^{203}Pb	^{47}Ca	^{203}Pb	^{47}Ca	
	(% dose)		(% dose)		($\mu\text{g}/\text{mg}$ protein)
Low Ca	33.10 ± 3.84^a	96.0 ± 0.80^a	40.60 ± 1.64^a	0.46 ± 0.08^a	40.60 ± 3.27^a
Normal Ca	11.03 ± 3.31^b	82.95 ± 4.62^b	42.61 ± 2.13^a	3.05 ± 0.52^b	14.13 ± 1.68^c

All values are mean $\pm$ SEM of 6 chicks. The values in the same column having a common superscript are not significantly different ($P < 0.05$).

Figure Legends

Figure 1. Duodenal absorption of ^{203}Pb and ^{47}Ca as a function of time. Each point represents the mean $\pm$ SEM of 6 birds.

Figure 2. Duodenal absorption of ^{203}Pb as affected by varying levels of stable lead in the dosing solution. Each point represents the mean $\pm$ SEM of 6 birds.

Figure 3. Duodenal absorption of ^{203}Pb as affected by the age of the animal. Each point represents the mean $\pm$ SEM of 6 birds.

Figure 1

Figure 2

Figure 3

Effect of Vitamin D on the Intestinal Absorption
of ^{203}Pb and ^{47}Ca in Chicks

Earlier work by Sobel and colleagues (1) showed that the retention of lead, and therefore its toxicity, is governed in part by the vitamin D status of the animal. Vitamin D is known to be intimately involved in the gastrointestinal absorption of calcium (2-4) and it might be speculated that a significant aspect of the mechanism of action of vitamin D on lead metabolism is to increase the transport of this metal across the intestinal membrane. Indeed, in a recent report, Smith and coworkers (5) showed that vitamin D stimulates the absorption of lead in rats. However, the mechanism whereby vitamin D affects the intestinal transport of lead remains unresolved. The data by Barltrop and Khoo (6) suggest that this effect on lead is not due to a direct action of the vitamin on the gut. Quarterman and Morrison (7), after studying the effect of low calcium and low phosphorus diets on the absorption of lead in rats, proposed that the increase in lead absorption by these treatments is caused by changes in the concentration of the vitamin D-induced calcium-binding protein in the intestinal mucosa. The synthesis of this protein in intestines of several species is known to be dependent on the supply of vitamin D (8). On the other hand, Barton et al (9) suggest that the absorption of lead is mediated primarily by another mucosal protein of higher molecular weight than CaBP and not dependent on vitamin D. The same group later proposed that any action of vitamin D on the intestinal transport of lead was to prolong the gastrointestinal transit time (10). Clearly, the information cited above indicates that additional studies are required in order to define the precise role of this vitamin in the intestinal absorption of lead.

The present investigation was designed to examine aspects of the action of vitamin D on lead absorption in the chick. In addition, the

comparative effects of this vitamin and its metabolite, 1,25-dihydroxy-cholecalciferol, on the absorption of lead and calcium in chicks were investigated.

Materials and Methods

Chicks used for the absorption studies were 3-week old White Leghorn cockerels (Babcock Industries, Inc., Ithaca, NY) raised after hatching on a rachitogenic (cholecalciferol-deficient) chick diet (Table 1). The absorption of ^{203}Pb and ^{47}Ca was determined by the in situ ligated loop procedure, described in detail by Wasserman and Taylor (11). The following parameters were used to describe the various steps in the absorption process: 1) "luminal efflux" which refers to the amount of ^{203}Pb or ^{47}Ca (% dose) that left the lumen during the absorption period and was calculated as $100 - (\% ^{203}\text{Pb} \text{ or } \% ^{47}\text{Ca} \text{ in the intestinal loop} - \% ^{203}\text{Pb} \text{ or } \% ^{47}\text{Ca} \text{ in the intestinal wall})$; 2) "intestinal retention", the amount of the isotope (% dose) in the intestinal tissue after flushing the luminal contents with ice-cold saline; 3) "transfer to body" (absorption) which refers to the amount of the isotope retained in the whole body minus the intestinal loop, and determined either by the use of a whole body counter or by calculation from the equation: $100 - (\% ^{203}\text{Pb} \text{ or } \% ^{47}\text{Ca} \text{ retained in the duodenal loop})$; 4) "tibia uptake", the amount of ^{203}Pb or ^{47}Ca (% dose) accumulated by a tibia.

The following factors were assessed: level of dietary cholecalciferol; the parenteral administration of cholecalciferol (dose, time); parenteral administration of 1,25-dihydroxycholecalciferol (time); transfer of plasma ^{203}Pb and ^{47}Ca into the intestinal lumen of rachitic and vitamin D-repleted chicks, and the absorption of ^{203}Pb from different segments of the small

intestine in rachitic and vitamin D-repleted chicks.

Expt. 1. Comparative absorption of ^{203}Pb from different segments of the small intestine in rachitic and vitamin D-replete chicks.

At three weeks of age, half of the vitamin D-deficient chicks received 500 IU cholecalciferol in 0.2 ml of propylene glycol by intramuscular injection 72 hrs prior to the absorption study. The chicks, fasted overnight, were anesthetized with ether and 0.5 ml of a solution containing 0.5 μCi ^{203}Pb , 0.01 mM lead acetate, 1 mM sodium acetate and 150 mM NaCl, pH 6.5, injected into the lumen of the ligated segment. Sixty minutes later, the chicks were killed with an overdose of pentobarbital and the degree of ^{203}Pb absorption determined. The jejunum was identified as the segment proximal to the yolk sac and distal to the duodenum, and the ileum was localized distal to the yolk sac and proximal to the ileocaecal junction.

Expt. 2. Effect of dietary cholecalciferol on the duodenal absorption of lead.

Thirty one-day old chicks were divided into 5 groups of 6 chicks each. The groups were fed the rachitogenic diet supplemented with 0, 150, 600 or 1200 I.U. cholecalciferol per kg of the diet for 3 weeks at which time the absorption of ^{203}Pb and ^{47}Ca was determined. The prefasted chicks were dosed with 0.5 ml of dosing solution containing 0.5 μCi ^{203}Pb , 0.1 μCi ^{47}Ca , 0.01 mM lead acetate, 1 mM CaCl_2 , 1 mM sodium acetate and 150 mM NaCl, pH 6.5, using the ligated loop technique. Twenty minutes after dosing, the degree of absorption of ^{203}Pb and ^{47}Ca was determined.

Expt. 3. Effect of parenteral administration of cholecalciferol on the absorption of ^{203}Pb and ^{47}Ca .

In the first experiment, the 3-week old chicks raised on rachitogenic diet, received an intramuscular injection of a solution containing either

0, 10, 100, 1000 or 5000 I.U. cholecalciferol in 0.2 ml of propylene glycol, and the duodenal absorption of ^{203}Pb and ^{47}Ca was determined 24 hr later. In another experiment, the absorption of ^{203}Pb at different time intervals after the administration of a single dose of cholecalciferol (100 I.U. in 0.2 ml of propylene glycol, intramuscularly) was determined.

Expt. 4. Effect of 1,25-dihydroxycholecalciferol on the duodenal absorption of ^{203}Pb and ^{47}Ca .

At various intervals before the absorption study, 6 rachitic chicks at each time period were injected intravenously with 1 μg of $1,25(\text{OH})_2$ cholecalciferol (gift of Dr. Uskoković, Hoffmann-LaRoche, Nutley, NJ) in 0.1 ml of propylene glycol. The duodenal absorption of ^{203}Pb and ^{47}Ca was determined as previously outlined. The absorption period was 60 min.

Expt. 5. Transfer of plasma ^{203}Pb and ^{47}Ca to the duodenal lumen.

The transfer of plasma ^{203}Pb and ^{47}Ca into a ligated duodenal loop (backflux) was determined, as follows: Prefasted rachitic or vitamin D-repleted chicks were injected intravenously with 0.1 ml of a dosing solution containing 1.0 μCi ^{203}Pb , 0.5 μCi ^{47}Ca , 0.01 mM lead acetate, 1 mM CaCl_2 , and 150 mM NaCl, pH 7.0. Before the intravenous administration of the isotopes, the duodenum was ligated and 0.5 ml of the unlabeled mixture containing 0.01 mM lead acetate, 1 mM CaCl_2 and 150 mM NaCl (pH 6.5) was injected into the duodenal lumen. Two and one-half minutes after the intravenous injection, blood samples were taken by cardiac puncture for analysis of ^{47}Ca and stable calcium, and the chicks were killed with an overdose of pentobarbital. The duodenal segments were rinsed with 2 x 20 ml of ice-cold saline into the counting vials for determination of ^{203}Pb and ^{47}Ca activities in the luminal fluid. The rinsed intestinal segment

and one tibia from each animal were also counted for ^{203}Pb and ^{47}Ca .

Analyses and Materials

Blood samples were collected by cardiac puncture and the serum assayed for calcium concentration by atomic absorption spectrophotometry (Perkin-Elmer Atomic Absorption Spectrophotometer 360, Perkin-Elmer, Co., Norwalk, CT). For measurement of calcium-binding protein (CaBP), the mucosal tissue was scraped from the segment and homogenized (1:4 w/v) in Tris buffer [0.0137 M Tris (hydroxymethyl) aminomethane, 0.12 M NaCl, 4.74 mM KCl adjusted to pH 7.4 with HCl]. The homogenate was centrifuged at $38,000 \times g$ for 30 min. in a refrigerated centrifuge. The CaBP content of the supernatant fluid was determined by the radial immunodiffusion method (12). The supernatant protein was measured according to the method of Lowry et al (13), using bovine serum albumin as the reference protein.

Radioactivities of ^{203}Pb and ^{47}Ca in the samples were counted, using a well-type gamma counter (Beckman γ -300 Beckman Instruments, Inc., Irvine, CA), and the counts were expressed as % of the administered dose. The radioactivity in the whole body without the loop was determined, using a whole body counter (Tobor, Nuclear Chicago, Des Plaines, IL).

Carrier-free ^{203}Pb (as acetate) in 0.1 M acetic acid was obtained from New England Nuclear (Boston, MA), and ^{47}Ca (as chloride), with the specific activity ranging from 1574 to 2557 mCi/g in 1 N NaCl, was from Union Carbide Corp. (Oak Ridge, TN).

The significance of all treatment differences was determined by Duncan's Multiple Range Test (14) or by Student's t-test.

Results

The rate of calcium absorption is greatest in the duodenum and decreases

towards the distal parts of the small intestine (15). In contrast, the transfer of lead to body in vitamin D-repleted rachitic chicks was similar in all of the segments tested when the data are expressed on a dry weight basis (Table 2). Similar results were obtained with chicks fed the commercial chick starter diet adequate in vitamin D (Mykkänen and Wasserman, submitted for publication). In rachitic chicks, however, the absorption of lead appeared to be greater in the more distal segments. Because of this, the enhancing effect of vitamin D on lead transport was greatest in the duodenal segment.

The chronic intake of cholecalciferol increased the transfer of ^{203}Pb to body although this was not reflected by significant changes in the luminal efflux or intestinal retention of that radionuclide (Table 3). The latter is due to the relatively small amount of ^{203}Pb actually transferred to body and the over-all variability of the data. Vitamin D intake, as expected, resulted in an increase in the luminal efflux and transfer of ^{47}Ca to body. The vitamin also reduced the residual amount of ^{47}Ca in the intestinal tissue, reflecting an increase in release of ^{47}Ca from that tissue, as noted previously (16,17). CaBP was synthesized by the mucosal tissue in response to vitamin D and the levels of CaBP were independent of the amount of vitamin D contained in the diet under the present conditions.

Fig. 1 shows that the transfer of radiolead to body was enhanced in a dose-dependent fashion by the intramuscular injection of increasing amounts of cholecalciferol into rachitic chicks. A similar pattern was observed in the transfer of radiocalcium. The percentage of ^{47}Ca absorbed was, however, about 4-5 times that of ^{203}Pb , whereas more ^{203}Pb was bound to the intestinal tissue than ^{47}Ca , as already observed in the previous experiment.

The results of the time-response experiment are shown in Fig. 2.

There was a significant increase in the amount of ^{203}Pb transferred to body 8 hours after the administration of the vitamin, followed by a further enhancement up to 48 hrs after dosing. This increase was accompanied by a decrease in the amount of radiolead retained by the intestinal tissue, resulting in no net change in the percentage of ^{203}Pb leaving the intestinal lumen. These data would imply that one effect of vitamin D on the process of lead absorption is to facilitate the transfer of this metal from intestinal mucosa to body, i.e., in the serosal direction.

The luminal efflux of both ^{203}Pb and ^{47}Ca was significantly increased by the intravenous administration of 1,25-dihydroxycholecalciferol to rachitic chicks (Fig. 3). The maximal percentage of ^{203}Pb efflux was observed 8 hrs after the administration of $1,25(\text{OH})_2\text{CC}$, whereas the peak for ^{47}Ca efflux was seen at 16 hrs. Moreover, 32 hrs after the intravenous injection of this metabolite, the luminal efflux of radiocalcium was still significantly higher than in the control group whereas at that time, the efflux of radiolead was not different from the untreated control group. The same respective patterns were seen in the values of the transfer of ^{203}Pb and ^{47}Ca to body. The $1,25(\text{OH})_2$ cholecalciferol did not affect the amount of ^{203}Pb retained by the intestinal tissue but, in contrast, did significantly reduce the amount of ^{47}Ca retained by the tissue. As expected there was a significant increase in the mucosal calcium-binding protein (CaBP) in chicks receiving this metabolite. As noted previously, the degree of absorption of ^{47}Ca decreased more rapidly at later time periods before any change in CaBP concentrations was observed (18). The correlation between CaBP levels and ^{203}Pb absorption with time was even less than that for ^{47}Ca .

In agreement with the previous results from this laboratory (19), it was seen that a significantly greater amount of plasma ^{47}Ca penetrates the duodenal membrane in cholecalciferol-repleted chicks as compared to the vitamin D-deficient chicks (Table 4). However, cholecalciferol did not appear to influence the amount of ^{203}Pb transferred to the luminal fluid.

Discussion

The administration of cholecalciferol to rachitic chicks resulted in an increase in the transepithelial transport of lead (^{203}Pb) by the intestine. This verified, in general, the observations of others (5,10). Barton et al (10), however, explained their results as due to an effect of the vitamin on intestinal transit time and not to a modification of the transport capacity of the gut. The method used in the present study, i.e., the in situ ligated loop procedure, discounts the proposal of Barton et al (loc cit) since the test dose of ^{203}Pb (and ^{47}Ca) is confined to the defined segment by the ligatures. We conclude, therefore, that the effect of cholecalciferol is directly on the capacity of the intestine to transport lead and not due to any indirect action, such as alteration of transit time.

Of interest was the response of various segments of the intestine to the steroid. First of all, the transfer of lead to body in the rachitic chick appeared to increase in the order: ileum > jejunum > duodenum, whereas the order for calcium absorption is: duodenum > jejunum > ileum (20). Vitamin D increased the transfer of lead to body in all segments and no differences among segments were now observed (Table 2). The absorption of calcium in vitamin D-repleted chicks (20) yielded the same order as previously noted for the rachitic chick (duodenum > jejunum > ileum). When the effect of cholecalciferol on lead absorption is calculated by

difference, the largest response was in the duodenum, followed by the jejunum and then the ileum (Table 2).

The over-all transfer of lead and calcium by the duodenum was also increased in chicks consuming the cholecalciferol-supplemented diet as compared to those fed the rachitogenic diet (Table 3). The luminal efflux of lead was not significantly affected by cholecalciferol although a slight, non-significant increase was noted. As before (Table 2), most of the lead that left the intestinal lumen was associated with the intestinal tissue and only a fraction of this accumulated lead was transferred to body. Calcium efflux from the lumen, in contrast, was significantly increased by the cholecalciferol diet, and most of this calcium was transferred to body, i.e., a relatively small amount retained by the intestinal tissue.

Similar patterns were observed after various amounts of cholecalciferol were given as a single oral dose (Fig. 1). Both of these experiments (Table 2 and Fig. 1) document another difference between calcium and lead metabolism in the intestine. In contrast to calcium, lead that is accumulated by the intestinal tissue is only slowly released therefrom in the serosal direction, as was noted previously (unpublished observation).

Vitamin D, from earlier studies, affects both the uptake and release of ^{47}Ca by the intestinal epithelium (22). There is no information from the present studies that allows any statement concerning an effect of vitamin D on the accumulation of ^{203}Pb by the tissue. However, the transfer step of ^{203}Pb to body is clearly enhanced by cholecalciferol. The molecular nature of the release of ^{203}Pb (and ^{47}Ca) from the enterocyte is not precisely known although various models have been proposed for calcium. These have been recently summarized (23) and include exocytosis

of lysosomal sequestered mineral (24,25), the extrusion of the cation by a pump ATPase (25,27), or extrusion by a Na^+ exchange mechanism (28-30). It has been reported, in this context, that absorbed lead localized in microvilli, the terminal web and the apical cytoplasm of the rat duodenal epithelial cell at 1 min. after exposure and later (10 min) in mitochondria, nuclei and basal cytoplasm (31).

These differences in lead and calcium transport were also observed in the study in which $1,25\text{-(OH)}_2$ cholecalciferol was used. For example, the retention of lead by the tissue was unaffected by the metabolite, whereas the ^{47}Ca retained by the tissue was decreased. In addition, the time patterns of luminal efflux and transfer to body of lead and calcium differed. Firstly, peak values for lead absorption occurred before that of calcium and, secondly, there was a considerably longer response of the calcium absorptive system to the acute dose of $1,25\text{-(OH)}_2$ cholecalciferol than that of the lead absorptive system. An obvious interpretation of these observations is that $1,25\text{-(OH)}_2$ cholecalciferol affects the lead and calcium transport systems in different ways, separable by their temporal response to the hormone. In this acute experiment, there is a lack of correlation between changes in CaBP levels with time and that of ^{203}Pb transport. As noted here and previously (18), the same lack of correlation also pertains to calcium absorption.

The backflux of ^{47}Ca from blood to lumen was shown, as before (19), to be cholecalciferol-dependent but no difference in the backflux of ^{203}Pb was noted, thus representing another difference between the behavior of the two divalent cations.

It is apparent from the present and previous studies that factors

affecting calcium transport, such as vitamin D status and level of calcium intake, also influence lead absorption (1, 5-7, 9, 10, 21). Yet, differences in their detailed behavior were noted. This makes difficult a proposal that the transcellular path used by calcium is identical to that for lead. However, the similarities give support to the notion that at least part of the changes due to vitamin D are shared by both cations.

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Table 1. Composition of the rachitogenic (cholecalciferol-free) chick diet

<u>Ingredient</u>	<u>%</u>
Isolated soy protein	25.00
DL-methionine	.60
Glycine	.40
Corn oil	4.00
Cerelose (Dextrose)	60.27
Cellulose (Solka floc)	3.00
Choline chloride	.20
Vitamin premix ¹⁾	1.00
Mineral premix ²⁾	5.53

1) Supplies the following (mg/kg of the diet): thiamine HCl 15; riboflavin 15; nicotinic acid 50; folic acid 6; pyridoxine HCl 6; biotin 0.6; vitamin B₁₂ 0.02; Ca-pantothenate 20; menadione sodium bisulfite 1.5; BHT 100; vitamin E 50 IU/kg; vitamin A 4500 IU/kg, with 9.74 g glucose/kg.

2) Supplies the following (g/kg of the diet): CaHPO₄ 16.2; CaCO₃ 18.0; KH₂PO₄ 10.0; KCl 1.0; NaCl 6.0; MnSO₄·H₂O 0.35; FeSO₄·7 H₂O 0.5; MgSO₄ 3.0; KIO₃ 0.01; CuSO₄·5 H₂O 0.03; ZnCO₃ 0.15; CoCl₂ 0.0017; NaMoO₄·2 H₂O 0.0083; Na₂SeO₄ 0.002.

Table 2

Absorption of ^{203}Pb from different segments of the small intestine in rachitic and cholecalciferol-repleted chicks¹⁾

	Luminal efflux	Intestinal retention ^{203}Pb	Transfer to body (% dose)	Vitamin D-dependent transfer ²⁾
Rachitic				
Duodenum	50.67 ± 2.96^a	43.19 ± 3.56^a	7.48 ± 1.26^a	-
Jejunum	63.60 ± 6.13^b	51.76 ± 4.67^a	11.84 ± 3.25^a	-
Ileum	63.45 ± 9.80^b	$46.64 \pm 7.24^{a,b}$	$16.83 \pm 5.21^{a,c}$	-
D-treated ³⁾				
Duodenum	63.32 ± 3.16^b	33.72 ± 1.47^b	29.69 ± 3.14^b	22.21
Jejunum	69.50 ± 4.25^b	45.18 ± 3.38^a	24.32 ± 3.73^b	12.48
Ileum	67.85 ± 10.70^b	$43.17 \pm 7.08^{a,b}$	$24.67 \pm 3.94^{b,c}$	7.84
^{203}Pb (% dose/mg of dry weight)				
Rachitic				
Duodenum	0.19 ± 0.01^a	0.16 ± 0.01^a	0.03 ± 0.004^a	-
Jejunum	0.33 ± 0.04^b	0.27 ± 0.04^b	$0.06 \pm 0.02^{a,b}$	-
Ileum	0.39 ± 0.07^b	0.28 ± 0.05^b	$0.11 \pm 0.04^{b,c}$	-
D-treated ³⁾				
Duodenum	0.26 ± 0.01^c	0.14 ± 0.003^a	0.12 ± 0.01^c	0.09
Jejunum	0.35 ± 0.03^b	$0.22 \pm 0.01^{a,b}$	0.13 ± 0.02^c	0.07
Ileum	0.45 ± 0.08^b	0.28 ± 0.05^b	0.17 ± 0.03^c	0.06

All values are mean $\pm$ SEM of 5-6 chicks. The values in the same column having a common superscript are significantly different ($P < 0.05$).

1) The dose contained 0.5 μCi ^{203}Pb in 0.5 ml of 150 mM NaCl, 0.01 mM lead acetate and 1 mM sodium acetate, pH 6.5.

2) Difference between "Transfer to body" values of vitamin D-treated and rachitic chicks for each segment.

3) 500 IU cholecalciferol in 0.2 ml of propylene glycol (i.m.) 72 hrs. before the absorption study.

Table 3

Effects of varying levels of dietary cholecalciferol on the
duodenal absorption of ^{203}Pb and ^{47}Ca in the chick

Cholecal- ciferol in the diet (IU/kg)	Luminal efflux		Intestinal retention		Transfer to body		Mucosal
			(% dose)				CaBP
	²⁰³ Pb	⁴⁷ Ca	²⁰³ Pb	⁴⁷ Ca	²⁰³ Pb	⁴⁷ Ca	(µg/mg protein)
0	60.12±4.77 ^a	47.34±3.56 ^a	56.99±5.02 ^a	26.44±2.53 ^a	3.13±1.25 ^a	20.91±2.39 ^a	0 ^a
150	70.81±4.71 ^a	74.24±5.43 ^b	61.79±4.06 ^a	17.03±1.64 ^b	9.02±1.78 ^b	57.21±6.60 ^b	7.73±1.23 ^b
600	70.02±5.37 ^a	78.38±6.36 ^b	62.27±4.62 ^a	18.00±2.18 ^b	7.75±1.45 ^b	60.38±7.53 ^b	7.87±1.50 ^b
1200	64.56±3.96 ^a	78.16±4.30 ^b	56.34±1.98	18.31±2.74	8.22±1.99 ^b	59.85±6.93 ^b	7.51±1.69 ^b

All values are mean $\pm$ SEM of 6 chicks. The values in the same column having a common superscript are not significantly different ($P < .05$).

The dose contained 0.5 μCi ^{203}Pb and 0.1 μCi ^{47}Ca in 0.5 ml of 150 mM NaCl, 0.01 mM lead acetate and 1 mM CaCl_2 , pH 6.5. The length of the absorption period was 20 min.

Table 4

Effect of vitamin D status on the transfer of plasma ^{203}Pb
and ^{47}Ca across the chick duodenum

	Rachitic	Vitamin D-treated ¹⁾	
Luminal (% dose)			
^{203}Pb	0.17 ± 0.01	0.16 ± 0.01	N.S.
^{47}Ca	0.19 ± 0.02	0.40 ± 0.05	P < 0.01
Tissue retention (% dose)			
^{203}Pb	2.66 ± 0.21	2.06 ± 0.25	N.S.
^{47}Ca	0.96 ± 0.10	1.12 ± 0.05	N.S.
Tibia Uptake (% dose)			
^{203}Pb	2.36 ± 0.25	2.08 ± 0.16	N.S.
^{47}Ca	2.55 ± 0.25	2.49 ± 0.09	N.S.
Plasma calcium			
^{47}Ca (%/ml)	1.51 ± 0.18	1.17 ± 0.08	N.S.
Ca (mM)	1.93 ± 0.10	2.67 ± 0.06	P < 0.001
SA (% ^{47}Ca /mmole Ca)	789 ± 101	449 ± 37	P < 0.001

All values are mean $\pm$ SEM of 6 chicks. The chicks were sacrificed 2.5 min after intravenous injection of the radioisotopes. The dose (0.01 ml) injected contained 1.0 μCi ^{203}Pb and 0.5 μCi ^{47}Ca , 0.01 mM lead acetate, 1 mM CaCl_2 , and 150 mM NaCl, pH 7.0. A nonlabeled solution, 0.5 ml, containing 0.01 mM lead acetate, 1 mM CaCl_2 and 150 mM NaCl, pH 6.5, was injected into the ligated duodenal loop prior to the intravenous dosing of the tracers. Statistical analysis by Student's t-test. N.S.= not significantly different.

1) 500 IU cholecalciferol in 0.2 ml of propylene glycol (i.m.) 72 hrs. prior to the study.

Figure Legends

- Figure 1. Effect of varying amounts of vitamin D₃, administered as a single dose, on the transfer to body and intestinal retention of ⁴⁷Ca and ²⁰³Pb.
- Figure 2. Effect of 100 IU vitamin D₃ on various parameters of ²⁰³Pb absorption as a function of time after dosing.
- Figure 3. Effect of a single dose of 1,25-dihydroxycholecalciferol (1 µg) on various parameters of ²⁰³Pb and ⁴⁷Ca absorption as a function of time after dosing.

Figure 1

Figure 2

Figure 3

Effect of Lanthanum on Lead (^{203}Pb) Absorption in Chicks

Lead is a non-essential heavy metal with well-recognized adverse effects on human health. The mechanism by which lead is absorbed from the gastrointestinal tract is not known, but appears to be regulated by nutritional and physiological factors similar to those that affect the absorption of calcium (1-5). In 1975 Wasserman et al (6) reported that La^{3+} depressed ^{47}Ca absorption in the chick duodenum and that Ca^{2+} inhibited

La absorption. These cross-inhibitory effects were observed in vitamin D-replete chicks but not in rachitic animals, suggesting that the site of interaction between La^{3+} and Ca^{2+} was vitamin D-dependent. Since La^{3+} presumably does not enter cells (or only slightly), the observation that La^{3+} is absorbed constituted evidence of the existence of a paracellular path (7).

The gastrointestinal absorption of lead, like that of calcium and lanthanum, is affected by vitamin D (3-6, 8), it can be postulated that lanthanum might exert an effect on the intestinal absorption of lead similar to its effect on the absorption of calcium. The results of the present study show that the absorption of ^{203}Pb from ligated duodenal loops of vitamin D-replete chicks is reduced by 50% in the presence of 5-10 mM La^{3+} , whereas the inhibition of lead absorption in rachitic chicks is considerably less at 10 mM La^{3+} .

Methods

Chicks used for the experiments were three-week-old White Leghorn cockerels (Babcock Industries, Inc., Ithaca, NY) weighing 150-200 g. The chicks, after hatching, were fed a commercial diet (Chick Starter Diet, Agway, Inc., Ithaca, NY) or a rachitogenic diet prepared as in Table 1 with phosphorus and calcium omitted. The absorption of ^{203}Pb

and ^{47}Ca was determined from in situ ligated duodenal loops of prefasted chicks as described by Wasserman and Taylor (8). The following indices of absorption were obtained: 1) "Luminal efflux", which refers to the amount of ^{203}Pb (or ^{47}Ca) that left the lumen during the absorption period and is calculated as $100 - (\% ^{203}\text{Pb}$ or ^{47}Ca in the intestinal loop - $\% ^{203}\text{Pb}$ or ^{47}Ca in the intestinal tissue); 2) "intestinal retention", which is the amount of ^{203}Pb or ^{47}Ca remaining in the intestinal tissue after rinsing the luminal contents with ice-cold saline, and 3) "transfer to body", which is the amount of ^{203}Pb or ^{47}Ca in the whole body without the intestinal loop.

Expt. 1. Effect of various concentrations of stable lanthanum in the dosing solution on the duodenal absorption of ^{203}Pb and ^{47}Ca in normal chicks.

Chicks raised on the commercial diet were fasted overnight and dosed intraduodenally with 0.5 ml of solution containing 0.5 μCi ^{203}Pb , 0.1 μCi ^{47}Ca , 0.01 mM lead acetate, 0.01 mM CaCl_2 , 1 mM sodium acetate, 150 mM NaCl , and 0, 1, 5 or 10 mM LaCl_3 ; pH 6.5. Sixty minutes after dosing the animals were killed with an overdose of sodium pentobarbital and the degree of ^{203}Pb and ^{47}Ca absorption determined.

Expt. 2. Effect of stable lanthanum on the duodenal absorption of ^{203}Pb and ^{47}Ca in rachitic and vitamin D-replete chicks.

Forty-eight chicks were fed the semi-synthetic cholecalciferol-free diet (Table 1) for three weeks after hatching. Twenty-four of the chicks were dosed (i.m.) with 500 IU D_3 in 0.2 ml of propylene glycol 72 hours prior to the absorption study. The absorption of ^{203}Pb was determined in 12 rachitic and 12 vitamin D-replete birds by dosing the anesthetized animals intraduodenally with 0.5 ml of a solution containing 0.5 μCi

^{203}Pb , 0.01 mM Pb-acetate, 1 mM sodium acetate, 150 mM NaCl, and 0 or 10 mM LaCl_3 , pH 6.5. The absorption period was 60 min. The remaining animals were dosed with 0.5 ml of a solution containing 0.1 μCi ^{47}Ca , 0.01 mM CaCl_2 , 150 mM NaCl and 0 or 10 mM LaCl_3 , pH 6.5, and the degree of ^{47}Ca absorption was determined after 30 min and 15 min in rachitic and vitamin D-replete animals, respectively. The ^{203}Pb and ^{47}Ca content of the samples were determined by γ -spectrometry, using a well-type gamma counter (Beckman γ -300, Beckman Instruments, Inc., Irvine, CA). The radioactivity content of the carcass without the duodenal loop was determined by the use of a whole body counter (Tobor, Nuclear Chicago, Des Plaines, IL). Counts were expressed as % of the initial dose in order to correct for decay. Carrier-free ^{203}Pb (as acetate) in 0.1 M acetic acid was obtained from New England Nuclear (Boston, MA), and high specific activity ^{47}Ca (as chloride) in 1 N NaCl was from Union Carbide (Oak Ridge, TN).

The significance of all treatment differences was determined by Duncan's Multiple Range Test or by Student's t-test (9).

Results

As shown in Table 2, 5 mM stable lanthanum in the dosing solution significantly depressed the duodenal absorption of ^{203}Pb , and there was a 40-50% inhibition of the transfer of mucosal ^{203}Pb to body by 5 and 10 mM La^{3+} . In contrast to lead absorption, the intestinal transport of ^{47}Ca was reduced, but not significantly, by the highest lanthanum concentration used in these experiments. Retention of both ^{203}Pb and ^{47}Ca by the intestinal tissue appeared to be enhanced by increasing luminal La^{3+} concentration (Table 2).

In accordance with the results of Wasserman et al (6), the present

data indicate that lanthanum inhibits intestinal ^{47}Ca transport only in the vitamin D-replete chicks (Fig. 1). Unlike calcium transport, the transfer of ^{203}Pb to body was significantly reduced by 10 mM La^{3+} in both vitamin D-replete and rachitic animals. Furthermore, it was observed in the present experiments that the inhibition of both ^{203}Pb and ^{47}Ca transfer in the vitamin D-replete chicks was accompanied by an enhancement in the tissue retention of the isotopes (Fig. 1).

Discussion

Present data indicate clearly that the intestinal absorption of both lead (^{203}Pb) and calcium (^{47}Ca) is inhibited by lanthanum. If lanthanum is used to indicate the presence of a non-cytoplasmic path across the epithelial membrane, then these results would suggest that both lead and calcium are absorbed, at least partly, by the extracellular route. Furthermore, it appears that more lead than calcium is absorbed via this route, since the transport of ^{203}Pb was inhibited by La^{3+} to a greater degree and with a lower concentration than the transport of ^{47}Ca (Table 2).

It has been suggested that the hypothetical extracellular path would pertain only to the diffusional component of the calcium absorptive process (6). This suggestion and the observation that there are similarities in the absorption process of lead and the diffusional absorption of calcium (5), appear to support the contentions that intestinal lead absorption occurs by diffusion (10,11), and one of the avenues available for lead is the extracellular pathway via the tight junctions and the intercellular space. However, it should be emphasized that this interpretation of the present results depends solely on the assumptions that La^{3+} does not enter the epithelial cell (7), and that there is no direct interaction between

these ions within the lumen prior to absorption, such as precipitation of ^{203}Pb with sparingly soluble lanthanum salts. The latter postulation stems from the observation that the percentage of soluble ^{203}Pb in the luminal contents is reduced by excess stable lanthanum (unpublished data).

The inhibitory effect of lanthanum on ^{47}Ca absorption is clearly enhanced by vitamin D-treatment, there was a significant decrease by 10 mM La^{3+} in the percentage of ^{47}Ca transferred to body in vitamin D-treated chicks (Fig. 1), a non-significant decrease in the normal chicks (Table 2) and no change in the rachitic animals (Fig. 1). Thus the present results like the data of Wasserman et al (6) clearly indicate that the movement of Ca^{2+} ions via the extra-cellular shunt pathway is dependent on vitamin D. The mechanism of this effect is not known at present, but it should be noted that vitamin D is known to enhance the bidirectional permeability of the intestinal mucosa to calcium (12).

In contrast, the inhibitory effect of lanthanum on lead absorption does not appear to be dependent on vitamin D. The transfer of ^{203}Pb was significantly reduced in both vitamin D-replete and rachitic animals. Ten mM La^{3+} reduced the transfer in both groups by about 50%, indicating that the percentage of ^{203}Pb available for absorption was reduced, probably by co-precipitation of ^{203}Pb with lanthanum prior to absorption. Both lanthanum and lead ions are known to form insoluble salts with hydroxide, phosphate and bicarbonate at physiologic pH. It remains to be shown whether the effect of La^{3+} on lead absorption is totally accounted for by precipitation. If that is true and lead is not transported via the extracellular path in competition with lanthanum, this study provides another evidence for the differences between lead and calcium absorptive systems.

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Table 1. Composition of the rachitogenic (cholecalciferol-free) chick diet

<u>Ingredient</u>	<u>%</u>
Isolated soy protein	25.00
DL-methionine	.60
Glycine	.40
Corn oil	4.00
Cerelose (Dextrose)	60.27
Cellulose (Solka floc)	3.00
Choline chloride	.20
Vitamin premix ¹⁾	1.00
Mineral premix ²⁾	5.53

- 1) Supplies the following (mg/kg of the diet): thiamine HCl 15; riboflavin 15; nicotinic acid 50; folic acid 6; pyridoxine HCl 6; biotin 0.6; vitamin B₁₂ 0.02; Ca-pantothenate 20; menadione sodium bisulfite 1.5; BHT 100; vitamin E 50 IU/kg; vitamin A 4500 IU/kg, with 9.74 g glucose/kg.
- 2) Supplies the following (g/kg of the diet): CaHPO₄ 16.2; CaCO₃ 18.0; KH₂PO₄ 10.0; KCl 1.0; NaCl 6.0; MnSO₄·H₂O 0.35; FeSO₄·7 H₂O 0.5; MgSO₄ 3.0; KIO₃ 0.01; CuSO₄·5 H₂O 0.03; ZnCO₃ 0.15; CoCl₂ 0.0017; NaMoO₄·2 H₂O 0.0083; Na₂SeO₄ 0.002.

Table 2

Effect of stable lanthanum (La^{3+}) on the duodenal absorption of ^{203}Pb and ^{47}Ca in normal chicks

Lanthanum in the dosing solution (mM)	Duodenal absorption		Intestinal retention		Transfer to body	
	^{203}Pb	^{47}Ca	^{203}Pb	^{47}Ca (% dose)	^{203}Pb	^{47}Ca
0	66.89 $\pm$ 4.60 ^{a,c}	89.79 $\pm$ 3.85 ^{a,c}	46.01 $\pm$ 3.53 ^{a,c}	2.15 $\pm$ 0.37 ^a	13.15 $\pm$ 2.30 ^a	78.94 $\pm$ 3.93 ^{a,c}
1	56.23 $\pm$ 1.97 ^{b,c}	93.90 $\pm$ 0.92 ^a	42.59 $\pm$ 1.18 ^a	2.00 $\pm$ 0.26 ^a	10.20 $\pm$ 0.93 ^{a,b}	87.46 $\pm$ 1.18 ^a
5	55.37 $\pm$ 4.35 ^b	87.21 $\pm$ 2.10 ^{a,b}	45.44 $\pm$ 3.30 ^{a,c}	4.01 $\pm$ 0.51 ^{a,b}	6.92 $\pm$ 1.46 ^{b,c}	80.37 $\pm$ 2.89 ^{a,c}
10	61.71 $\pm$ 4.09 ^{a,b}	84.47 $\pm$ 1.02 ^{b,c}	53.43 $\pm$ 3.71 ^c	5.17 $\pm$ 0.60 ^b	7.59 $\pm$ 1.12 ^{b,c}	75.92 $\pm$ 1.15 ^c

Each value represents mean $\pm$ SEM of 5-6 birds. The values in the same column having a common superscript are not significantly different ($P < 0.05$).

Figure Legend

Figure 1. Effect of luminal La^{3+} on the duodenal transport of ^{47}Ca and ^{203}Pb in rachitic (R) and vitamin D-replete (D) chicks.

Figure 1

Figure 1. Effect of luminal La^{3+} on the duodenal transport of ^{47}Ca and ^{203}Pb in rachitic (R) and vitamin D-replete (D) in chicks.

Effect of Phosphate on the Intestinal
Absorption of Lead (^{203}Pb) in Chicks

Introduction

Although considerable attention has been given to the effects of dietary calcium and phosphorus on the metabolism and toxicity of lead, (1-5) only a few studies have attempted to specify the role that these minerals play in the metabolism of lead. In addition, the conclusions reported on the effect of dietary phosphorus appear somewhat contradictory. Feeding experiments by Shelling (1) and Quarterman et al (6) provided evidence that increased dietary phosphorus reduces the uptake and toxicity of lead in the diet. To the contrary, Lederer & Bing (2) reported that the addition of phosphate to the diet produced no significant effect on lead retention in rats, and a similar conclusion can be drawn from the data of Morrison et al (7) in sheep.

Feeding studies cannot provide information on the site of interaction between dietary constituents, since several processes can contribute to the degree of interaction, such as tissue distribution, retention and excretion, particularly in the long-term experiments. The site of interaction between phosphorus and lead has been proposed to be the gastrointestinal tract, since dietary phosphorus does not affect the retention of lead administered intraperitoneally (2-5). Using a more direct approach to measure absorption, Barltrop & Khoo (8) demonstrated in rats that the uptake of lead (^{203}Pb) incorporated in a semi-synthetic diet was increased when phosphorus was omitted from the diet, and decreased when phosphorus was increased three-to four-fold the recommended amount. The short period of exposure (48 hrs) used in their study suggested, to them, that the observed effect was due to a direct action of phosphorus on lead absorption from the gut. However, adaptation of animals to low

calcium or low phosphorus diets can occur within that time frame (9).

Quarterman and Morrison (5), using a longer feeding period, suggested that the enhanced retention of lead from animals on a phosphorus-deficient diet may be at the intestinal level and possibly due to changes in the calcium-binding protein (CaBP) content of the intestinal mucosa. Although mucosal CaBP concentrations were not measured in their study, there is evidence that the synthesis of this protein is enhanced by low dietary levels of phosphorus (9).

The above reports suggest that dietary phosphorus might affect intestinal lead absorption both directly, by interacting with lead in the gut, and indirectly, by changing the metabolic status of the animal.

The present studies were undertaken to examine, in more detail, these hypotheses using the in situ ligated loop absorption technique in chicks.

Methods

Chicks used for the experiments were 3-week old White Leghorn cockerels (Babcock Industries, Inc., Ithaca, NY) raised after hatching on either a commercial diet (Chick Starter Diet, Agway, Inc., Ithaca, NY) or on experimental diets specified subsequently. The absorption of lead (^{203}Pb) was determined from in situ ligated duodenal loops of prefasted chicks as described by Wasserman and Taylor (10). The following indices of absorption were obtained: 1) "luminal efflux", which refers to the amount of ^{203}Pb (% dose) exiting the lumen during the absorption period and calculated as $100 - (\% ^{203}\text{Pb})$ in the intestinal loop - $\% ^{203}\text{Pb}$ in the intestinal tissue); 2) "intestinal retention", which is the amount of ^{203}Pb remaining in the intestinal tissue after rinsing the luminal contents with ice-cold

saline, and 3) "transfer to body", which refers to the amount of ^{203}Pb in the whole body without the intestinal loop.

Expt. 1. Effect of various concentrations of inorganic phosphate in the solution on the duodenal absorption of ^{203}Pb in normal chicks.

Chicks raised on the commercial diet were fasted overnight and dosed intraduodenally with 0.5 ml of solution containing 0.5 μCi ^{203}Pb , 0.01 mM lead acetate, 1 mM sodium acetate, 150 mM NaCl and 0, 0.01, 0.1, 1 or 2 mM phosphate (as Na_2HPO_4), pH 6.5. Sixty minutes after dosing the animals were killed with an overdose of sodium pentobarbital and the degree of ^{203}Pb absorption was determined.

Expt. 2. Effect of inorganic phosphate on the duodenal absorption of ^{203}Pb in rachitic and vitamin D-replete chicks.

Thirty chicks were fed a semi-synthetic cholecalciferol-free diet (Table 1) for three weeks after hatching. Fifteen of the chicks were dosed (i.m.) with 500 IU D_3 in 0.2 ml of propylene glycol 72 hours prior to determination of ^{203}Pb absorption. The dose (0.5 ml) contained 0.5 μCi ^{203}Pb , 0.01 mM lead acetate, 1 mM sodium acetate and 150 mM NaCl, pH 6.5, and 0, .003 or .10 mM phosphate (as Na_2HPO_4). The soluble ^{203}Pb in the luminal fluid was determined by centrifuging the luminal fluid at 5000 x g for 10 min in a refrigerated centrifuge and counting an aliquot of the supernate.

Expt. 3. Effect of dietary phosphate on the duodenal absorption of ^{203}Pb .

Two-week old cockerels raised on the commercial diet were assigned to one of the following dietary groups of 6 birds each: 1) No supplemental phosphorus (containing 1.22% Ca and 0.16% P), 2) low phosphorus (1.22% Ca, 0.33% P), 3) normal phosphorus (1.22% Ca, 1.06%), 4) high phosphorus

(1.22% Ca, 2.12% P). The experimental diets were prepared by adding calcium and phosphorus as CaCO_3 and K_2HPO_4 respectively, to the semi-synthetic basal diet. Vitamin D_3 was also added to yield a concentration of 1200 IU cholecalciferol per kg of diet. After maintaining the chicks on the appropriate diets for one week, the absorption of ^{203}Pb was determined as previously outlined. For measurement of calcium-binding protein (CaBP), the mucosal tissue was scraped from the segment and homogenized (1:4 w/v) in Tris buffer [0.0137 M Tris(hydroxymethyl) amine, 0.12 M NaCl, 4.74 mM KCl, adjusted to pH 7.4 with HCl]. The homogenate was centrifuged at $38,000 \times g$ for 20 min in a refrigerated centrifuge. The CaBP content of the supernatant fluid was determined by the radial immunodiffusion method (11). The supernatant protein was measured according to the method of Lowry et al (12).

The residual ^{203}Pb activity in the samples was determined by γ spectrometry, using a well-type gamma counter (Beckman γ -300, Beckman Instruments, Inc., Irvine, CA). The radioactivity content of the carcass (minus the duodenal loop) was determined by the use of a whole body counter (Tobor, Nuclear Chicago, Des Plaines, IL). The counts were expressed as % of the initial dose. Carrier-free ^{203}Pb (as acetate) in 0.1 M acetic acid was obtained from New England Nuclear (Boston, MA).

The significance of all treatment differences was determined by Duncan's Multiple Range Test or Student's t-test (13).

Results

The data in Fig. 1 show that inorganic phosphate in concentrations of 0.01 to 2 mM, significantly reduced the absorption of ^{203}Pb from the in situ ligated duodenal loops of chicks. This effect was not unexpected

even at the lowest P:Pb ratio (1:1), since lead phosphate is sparingly soluble in water. However, it should be pointed out that no precipitation of lead phosphate was observed by visual inspection of any of the dosing solutions prior to the intraduodenal injection. There was a slight increase in the intestinal retention and transfer of ^{203}Pb to body in the two highest phosphate groups (1 and 2 mM) as compared to the groups receiving 0.01 or 0.10 mM phosphate.

The second experiment shows that the parameters of lead absorption in both rachitic and vitamin D-replete chicks were significantly affected by 0.1 mM phosphate in the dosing solution (Fig. 2). The data in Table 2 also indicate that the decrease in ^{203}Pb absorption was associated with a decrease in the soluble ^{203}Pb from the luminal fluid. Furthermore, the calculations show that the reduction in the intestinal uptake of ^{203}Pb by phosphate is due to the precipitation of luminal lead as ^{203}Pb -phosphate, whereas the reduction in the transfer of ^{203}Pb to body by phosphate cannot be accounted by precipitation alone. Therefore, a competition between phosphorus and lead for some intestinal component required in the transfer of mucosal ^{203}Pb to the serosal side is suggested. Another possibility is that ^{203}Pb , although not precipitated, might exist as a nonionized complex with phosphate or some other constituent(s) of the luminal fluid, and therefore, still not be available for absorption.

Table 3 shows that the low phosphorus diet significantly enhanced the transfer of ^{203}Pb to body, and this was accompanied by a reduction in the amount of ^{203}Pb retained by the intestinal wall, resulting in no net change in the total luminal efflux. The high phosphorus diet also tended to increase the transfer of ^{203}Pb , but not significantly as compared

to the normal phosphorus group. Concentrations of calcium-binding protein in the intestinal mucosa appeared to correlate with the transfer of ^{203}Pb to body values, the low phosphorus group had the highest CaBP content followed by (in decreasing order) high phosphorus, normal phosphorus. The very low phosphorus group (no added P) had the lowest concentration of CaBP and absorbed less ^{203}Pb than the other groups due to the severity of the phosphorus deficiency.

Discussion

The rationale of phosphate therapy in lead intoxication is based on the fact that lead forms a highly insoluble salt with phosphate which is unavailable for absorption and is therefore excreted in the feces (1). Thus the observations that low dietary phosphorus enhances uptake and retention of orally administered lead, and conversely, that a high phosphorus intake reduces lead retention can be explained by precipitation of lead phosphate in the gut (3, 14, 8, 5, 6). The results of the present study, in which the absorption of lead (^{203}Pb) was determined directly from in situ ligated duodenal loops, appear to support the contention that precipitation of lead phosphate is a major factor in the reduction of intestinal lead absorption by phosphate. An additional effect of phosphate is indicated by the calculations in Table 2. The transfer of ^{203}Pb to body was clearly inhibited by 0.1 mM phosphorus in the dosing solution even though the precipitation effect was eliminated by expressing the results as % of soluble ^{203}Pb . It is possible, then that lead and phosphorus exhibit competition for the same intestinal component(s) required for the translocation of these ions from the mucosal cell into the body. A more exact characterization of this phosphate effect remains for future experiments.

As already discussed, formation of sparingly soluble lead phosphate is likely when lead is given along with food. This reaction is obviously dependent on the amount of phosphorus in the diet. However, when lead is given in the diet, it is not possible to separate the metabolic effects of phosphorus on lead absorption from the direct action of phosphorus on the gut. In the present study, the absorption of lead (^{203}Pb) was determined directly (in situ) from ligated duodenal loops of prefasted chicks, thereby creating conditions in which the luminal phosphate concentrations were presumably not affected by various amounts of phosphorus in the diet (Expt. 4). The results show that a low phosphorus intake for one week prior to the absorption study clearly enhanced the transfer of mucosal ^{203}Pb into the body (Table 3). Thus it appears that an altered metabolic status of the animal affects lead absorption from the intestine. Deficient phosphorus intake is known to increase the formation of $1,25-(\text{OH})_2\text{D}_3$ and evidence for this in the present study is the increased synthesis of the vitamin D-dependent calcium-binding protein (9). This protein binds lead (15), and thus may function in the transport of ^{203}Pb through and out of the mucosal cell. On the other hand, this association may be coincidental.

In summary, the present study demonstrates that precipitation of lead phosphate is the major effect of phosphate on intestinal lead absorption. Other direct effects by phosphate on the transport of lead are suggested but need to be proven in future experiments. Changes in vitamin D metabolism of the animal most likely explains the indirect effect of a low P intake on lead absorption.

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Table 1. Composition of the rachitogenic (cholecalciferol-free) chick diet

<u>Ingredient</u>	<u>%</u>
Isolated soy protein	25.00
DL-methionine	.60
Glycine	.40
Corn oil	4.00
Cerelose (Dextrose)	60.27
Cellulose (Solka floc)	3.00
Choline chloride	.20
Vitamin premix ¹⁾	1.00
Mineral premix ²⁾	5.53

1) Supplies the following (mg/kg of the diet): thiamine HCl 15; riboflavin 15; nicotinic acid 50; folic acid 6; pyridoxine HCl 6; biotin 0.6; vitamin B₁₂ 0.02; Ca-pantothenate 20; menadione sodium bisulfite 1.5; BHT 100; vitamin E 50 IU/kg; vitamin A 4500 IU/kg, with 9.74 g glucose/kg.

2) Supplies the following (g/kg of the diet): CaHPO₄ 16.2; CaCO₃ 18.0; KH₂PO₄ 10.0; KCl 1.0; NaCl 6.0; MnSO₄·H₂O 0.35; FeSO₄·7 H₂O 0.5; MgSO₄ 3.0; KIO₃ 0.01; CuSO₄·5 H₂O 0.03; ZnCO₃ 0.15; CoCl₂ 0.0017; NaMoO₄·2 H₂O 0.0083; Na₂SeO₄ 0.002.

Table 2

Soluble ^{203}Pb in the luminal fluid and the intestinal retention and transfer of soluble ^{203}Pb as a function of the stable phosphate concentration (Expt. 2).

Phosphate in the dosing solution (mM)		Soluble ^{203}Pb in the luminal fluid ¹⁾ (%)	Intestinal retention ^{203}Pb (% of soluble ^{203}Pb) ²⁾	Transfer to body
Rachitic	0	75.08 ± 6.94^a	68.17	6.98
	0.003	61.49 ± 6.61^a	72.84	9.82
	0.100	8.11 ± 1.01^b	238.92	2.71
D-treated ³⁾	0	65.53 ± 2.67^a	42.98	54.19
	0.003	71.98 ± 3.90^a	40.98	58.41
	0.100	14.31 ± 4.22^b	50.56	13.41

¹⁾The fraction of ^{203}Pb in the supernatant fluid of the luminal contents after centrifuging at $5000 \times g$ for 10 min in a refrigerated centrifuge.

²⁾Values are mean $\pm$ SEM of 5 birds. The values with a common superscript are not significantly different ($P < 0.05$).

³⁾Based on calculations using the % of dose-values, the initial dose was $0.01 \text{ mM Pb}/0.5 \text{ ml} = 5 \text{ nmoles Pb}$.

500 IU D (i.m.) 72 hours prior to the absorption study.

Table 3

Effect of dietary phosphorus on the duodenal absorption of ^{203}Pb in chicks (Expt. 4).

Phosphorus in the diet (%)	Duodenal absorption	Intestinal retention ^{203}Pb (% dose)	Transfer to body	Mucosal CaBP $\mu\text{g}/\text{mg}$ protein
0.16	50.48 ± 4.03^a	45.41 ± 2.54^a	6.67 ± 1.47^a	5.08 ± 0.95^a
0.33	$57.75 \pm 3.96^{a,b}$	32.48 ± 2.83^b	22.27 ± 2.78^b	27.69 ± 4.85^b
1.06	63.54 ± 5.72^b	43.51 ± 1.34^a	$11.83 \pm 2.24^{a,c}$	17.95 ± 2.78^c
2.12	64.17 ± 4.41^b	40.28 ± 0.97^a	$17.72 \pm 1.62^{b,c}$	$19.97 \pm 1.77^{b,c}$

All values are mean $\pm$ SEM of 6 chicks. The values in the same column having a common superscript are not significantly different ($P < 0.05$).

Figure Legends

Figure 1. Effect of luminal phosphate concentration on the duodenal absorption, intestinal retention and transfer to body of ^{203}Pb in normal chicks.

Figure 2. Effect of luminal phosphate concentration on the transfer to body and intestinal retention of ^{203}Pb in rachitic (R) and vitamin D-replete (D) chicks.

Figure 1

Figure 2