The Effect of Calcium on Lead Absorption in Rats

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The effects of Ca^{2+} on lead absorption as PbCl_2 and $^{203}\text{PbCl}_2$ were studied in rats.

1. Doubling of dietary calcium with $\text{Ca}_3(\text{PO}_4)_2$ significantly decreased lead absorption as assessed by ^{203}Pb retention, tissue lead concentration, urinary excretion of δ -aminolaevulinic acid and increased activities of δ -aminolaevulinic acid dehydratase and ferrochelatase. 2. Similar effects on lead absorption were shown by the Ca^{2+} salts, Cl^- , CO_3^{2-} , PO_4^{3-} , SO_4^{2-} , gluconate and glycerophosphate. 3. CaCl_2 and calcium glycerophosphate were found to be most effective in decreasing lead absorption when administered immediately before lead dosage. 4. A negative exponential relationship was found between CaCl_2 concentration and ^{203}Pb absorption at 120 h. The results suggest that, above 4 mmol of administered calcium, residual lead absorption is unaffected by increasing gastrointestinal calcium concentrations. 5. Increased systemic calcium had no effect on lead retention. 6. Calcium in the concentrations found in domestic hard-water supplies significantly decreased absorption of a solution of ^{203}Pb dissolved in it compared with absorption of ^{203}Pb dissolved in soft or distilled water. 7. Milk and skimmed milk were found to have no effect on ^{203}Pb absorption in rats.

There has been renewed controversy over the role of milk as a prophylactic agent in lead exposure, with the suggestion that milk facilitates the absorption of lead in rats (Kello & Kostial, 1973). The principal mineral in milk is calcium, and studies since the turn of the century have considered the effects of calcium on lead absorption. Shelling (1932) produced evidence to suggest that diets high in calcium render lead more toxic than do diets high in phosphorus, and concluded that the introduction of large amounts of calcium without phosphate into rats merely diverts the available phosphate to rid the body of excess calcium as phosphate and thus interferes with the formation of relatively inert lead phosphate. Studies with mice (Tompsett, 1939; Tompsett & Chalmers, 1939) have shown that a low-calcium diet favoured high contents of lead in the soft tissues and a high calcium intake resulted in a preferential deposition of lead in the skeleton. Lederer & Bing (1940) suggested that the influence of calcium on lead assimilation is dependent mainly or entirely on events occurring in the digestive tract. After intraperitoneal administration of lead, lead retention was independent of the amounts of calcium and phosphorus in the diet. Lederer & Bing (1940) also suggested that lead incorporated in the diet was retained in much greater amounts in bone and kidney, but not in liver, when the calcium content of the diet was low than when it was high. Further evidence provided by Sobel *et al.* (1940) suggested that the addition of either calcium or phosphorus to a low-calcium low-phosphorus diet containing added lead will decrease lead retention in rats.

A contradictory note was introduced by the studies of Shields & Mitchell (1941). They considered it important to investigate the relationship between dietary calcium, phosphorus and lead at lead concentrations likely to be encountered environmentally, unlike previous work that had used comparatively high dietary lead. They concluded that a diet low in calcium or phosphorus or both, induces a high retention of lead in comparison with diets with higher mineral contents. In fact, lead storage in adult rats only occurred when the calcium content of the diet was inadequate or at borderline values. Finally, Shields & Mitchell (1941) noted that, when dietary content of calcium and phosphorus were raised to excess, there was no appreciable protection against the assimilation of lead by the body compared with the required concentrations of these dietary constituents.

There has been renewed interest in the interaction of dietary calcium with lead. Mahaffey *et al.* (1973) have reported increased retention of orally ingested lead in rats fed on a diet deficient in calcium, and concluded that significant increases in tissue lead storage and pathological effects of lead occur when the diets contain less than the rat's physiological requirement for calcium. The influence of high dietary calcium has also received further attention (Hsu *et al.*, 1975). When dietary calcium was raised from its normal content of 0.7% to 1.1% in weanling pigs, the clinical symptoms of lead toxicity were lessened, suggesting that high dietary calcium has a protective effect against the adverse effects of dietary lead.

Despite the large numbers of studies of the effects of diet and, in particular, calcium on lead retention, many aspects remain unresolved. The present investigation was designed specifically to evaluate fully the effects of increased dietary calcium on lead retention in rats.

Experimental and Results

Materials

$^{203}\text{PbCl}_2$ was kindly supplied by the MRC Cyclotron Unit, Hammersmith Hospital, London W.12, U.K. Calcium salts were supplied as follows: CaCl_2 , CaCO_3 , $\text{Ca}_3(\text{PO}_4)_2$ by BDH Chemicals, Poole, Dorset, U.K.; CaSO_4 , calcium gluconate by Hopkin and Williams, Chadwell Heath, Essex, U.K.; calcium glycerophosphate by Merck, Darmstadt, Germany. The diet used throughout was diet 41B (Oxoid, London S.E.1, U.K.). Animals used were male Sprague-Dawley rats maintained in an animal house at 25°C . Results were analysed statistically by Student's *t* test and expressed as means $\pm$ s.d. Curve fitting was carried out by least-squares non-linear regression analysis on Northumbrian Universities multiple-access computer (NUMAC) by using Numerical Analysis Group (NAG) routines.

Effects on lead retention of a diet supplemented with $\text{Ca}_3(\text{PO}_4)_2$

For this, 24 rats weighing 200 g were divided into two groups of 12 each. One group received standard diet 41B and the other group received the same diet supplemented with calcium to raise the content from 175 to 350 mmol of Ca^{2+} /kg of diet. This was done by using $\text{Ca}_3(\text{PO}_4)_2$, which maintained the calcium/phosphate molar ratio at 1.5:1, which is optimal for calcium absorption. Both groups of animals were given tap water *ad lib*. for 50 days.

After 21 days on the appropriate diets the 12 animals in each group were given a single oral dose of $^{203}\text{PbCl}_2$ ($50\ \mu\text{Ci}$, 48 pmol of Pb^{2+} in 1 ml of water) by stomach tube. Radioactivity was measured immediately after dosage and then at 24, 72, 144, 192 and 240 h after dosage. The rats were placed in plastic containers and radioactivity was counted in an IDL/Echo double-probe counter against a 'phantom' (i.e. a container with $^{203}\text{PbCl}_2$ -soaked cottonwool to the same volume as a rat). The retention of ^{203}Pb (Fig. 1) was significantly decreased ($P < 0.001$) in the calcium-supplemented group compared with the control group at 24, 72, 144, 192 and 240 h after dosage. Retention of ^{203}Pb in the calcium-supplemented group at 240 h was $0.30 \pm 0.05\%$ as compared with $0.85 \pm 0.10\%$ in the control group.

After a further 20 days on their respective diets, the 12 animals in each group were given equal doses

Fig. 1. Effect of calcium supplementation on retention of orally administered ^{203}Pb

A group of 12 rats receiving calcium-supplemented diet (Δ) and 12 rats receiving normal diet ($\circ$) were given a single oral dose of ^{203}Pb , and whole-body ^{203}Pb retention was monitored for 240 h.

of $^{203}\text{PbCl}_2$ ($50\ \mu\text{Ci}$, 48 pmol of Pb^{2+} in 1 ml of water) intraperitoneally. Radioactivity was followed over 240 h (Fig. 2). Retention of ^{203}Pb administered in this manner was similar for both groups, there being no significant difference between the two groups at any time during the 10-day period of study.

At 50 days after the start of the experiment the two groups of animals were further subdivided into four groups of six each. Six control and six calcium-supplemented animals were given lead in their drinking water at a concentration of $24.2\ \mu\text{mol/litre}$, and the six remaining animals in each of the original groups continued to be given tap water. These groups were supplied the appropriate diet for a further 80 days.

Food and water consumption were measured for each group over the final 6 days of the experiment (Table 1), there being no significant difference between the groups for each of these parameters. There was no significant difference in weight gain between the four groups throughout the experimental period, and, after the animals had been killed and their livers and kidneys excised, there was found to be no significant difference in these organ weights between the four groups (Table 1).

Over the final 6 days of the experiment, urinary excretion of δ -aminolaevulinate per group was

determined by the method of Mauzerall & Granick (1956). The animals were killed by cervical dislocation and exsanguination, the livers, kidneys and femurs

excised and their lead contents determined. Liver and kidney lead contents were determined by direct flameless atomic-absorption spectrophotometry [Perkin-Elmer model 306 fitted with heated graphite atomizer (HGA) 72] and those in femur were determined polarographically (Shandon-Southern-Davis A1660 polarograph) after dry ashing in a muffle furnace at 350°C for 12h and wet digestion of bone ash in conc. HNO₃/conc. H₂SO₄. Hepatic δ-aminolaevulinate dehydratase (EC 4.2.1.24) activity was measured by the method of Berlin & Schaller (1974). Ferrochelatase (EC 4.99.1.1) activity in liver was measured by the incorporation of ⁵⁹Fe into protoporphyrin to form haem by the method of Goldberg *et al.* (1956), the results being expressed as percentage uptake of ⁵⁹Fe. The findings for each of the parameters studied was similar (Table 2), there being a significant decrease in δ-aminolaevulinate dehydratase and ferrochelatase activities in both lead-treated groups when compared with the control groups, and a significant increase in tissue and femur lead contents and urinary excretion of δ-aminolaevulinate in both lead-treated groups when compared with control groups. In addition, and more impor-

Fig. 2. Effect of calcium supplementation on retention of a single intraperitoneal dose of ²⁰³Pb
A group of 12 rats receiving calcium-supplemented diet (Δ) and 12 rats receiving normal diet (○) were given a single intraperitoneal dose of ²⁰³Pb, and whole-body ²⁰³Pb retention was monitored for 240h.

Table 1. Group weight gains, daily food and water consumption, liver and kidney wet weights

Groups of rats were fed on either normal diet with and without Pb²⁺ (24 μmol/l) in their drinking water or on a diet with the Ca²⁺ content doubled to 350mmol/kg by addition of Ca₃(PO₄)₂ with and without Pb²⁺ (24 μmol/l) in their drinking water for 80 days. Each result represents the mean ± s.d. of six animals.

Group	Weight gain (g)	Daily diet consumption (g)	Daily water consumption (ml)	Liver wet wt. (g)	Kidney wet wt. (g)
Control	176 ± 18	134 ± 16	180 ± 36	11.3 ± 1.2	1.05 ± 0.07
Calcium	185 ± 19	129 ± 20	198 ± 47	12.5 ± 0.6	1.13 ± 0.11
Lead	168 ± 23	128 ± 24	160 ± 30	10.3 ± 0.7	1.00 ± 0.06
Lead and calcium	172 ± 18	124 ± 18	172 ± 36	10.5 ± 0.9	1.07 ± 0.10

Table 2. Effect of dietary Pb²⁺ and Ca²⁺ supplementation on the activity of two haem-biosynthetic enzymes, tissue Pb²⁺ and δ-aminolaevulinate excretion

Groups of rats were given combinations of diets containing Pb²⁺ (24 μmol/l of drinking water) and/or Ca²⁺ (350mmol/kg of diet) or normal diet 41B and tap water for 80 days. Each result represents the mean ± s.d. of six animals. * P < 0.001 with respect to control; † P < 0.01 with respect to lead.

Group	Hepatic δ-amino-laevulinate dehydratase (nmol of δ-amino-laevulinate/min per g wet wt. of tissue)	Hepatic ferrochelatase (% ⁵⁹ Fe incorporation into haem)	Kidney Pb (nmol of Pb/g wet wt. of tissue)	Liver Pb (nmol of Pb/g wet wt. of tissue)	Femur Pb (nmol of Pb/g of bone ash)	Urinary δ-amino-laevulinate (μmol of δ-amino-laevulinate excreted/24h per group)
Control	49.9 ± 7.5	52.6 ± 4.2	4.93 ± 0.92	0.68 ± 0.34	6.63 ± 1.64	0.96 ± 0.12
Calcium	47.6 ± 6.1	54.0 ± 3.1	4.59 ± 1.69	0.77 ± 0.34	5.70 ± 1.26	0.98 ± 0.13
Lead	26.2 ± 3.7*	37.8 ± 6.1*	14.3 ± 2.17*	2.75 ± 0.72*	15.7 ± 2.07*	1.43 ± 0.22*
Lead and calcium	36.6 ± 2.8*†	49.4 ± 4.8†	10.6 ± 2.51*†	1.88 ± 0.53*†	11.1 ± 2.27*†	1.21 ± 0.18*†

tantly, the enzyme activities were significantly higher in the group treated with lead, whose diet was supplemented with calcium, than in the group on normal diet treated with lead. Hepatic, renal and femur lead contents, together with urinary excretion of δ -aminolaevulinate, were significantly lower in the calcium-plus-lead group than in the lead-alone group.

Effect of different calcium salts on retention of orally administered ^{203}Pb

Seven groups, each of six rats (350 g), were deprived of food, but given water *ad lib.*, overnight. The six animals in each group were intubated with a 5 ml solution/suspension containing 1.25 mmol of calcium as one of the following calcium salts: CaCl_2 , $\text{Ca}_3(\text{PO}_4)_2$, CaCO_3 , CaSO_4 , calcium gluconate and calcium glycerophosphate. A control group of six animals were intubated with 5 ml of distilled water. At 20 min after the first intubation, each animal was intubated with $^{203}\text{PbCl}_2$ (25 μCi , 50 μmol of Pb^{2+} in 1 ml of water). Whole-body retention of ^{203}Pb was followed over 120 h as outlined above.

At 120 h after ^{203}Pb administration there was a highly significant ($P < 0.001$) decrease in ^{203}Pb retention in the calcium-treated groups when compared with the control group, but there was no significant difference between any of the calcium-treated groups (Table 3).

Effect on ^{203}Pb retention of calcium given orally at various times before oral ^{203}Pb administration

Two groups of 24 male rats (350 g), which had been deprived of food overnight, but given water *ad lib.*, were intubated with 1.5 mmol of calcium in 5 ml of solution/suspension as either CaCl_2 or calcium glycerophosphate. $^{203}\text{PbCl}_2$ (25 μCi , 5.0 μmol of Pb^{2+} in 1 ml of water) was then administered orally at

either 8, 35, 55 or 100 min after administration of calcium, six animals from each group being used at each time interval. A control of six animals was intubated with 5 ml of distilled water and then 8 min later with ^{203}Pb . Whole-body retention of ^{203}Pb was monitored for 120 h.

Both calcium salts administered 8 min before lead significantly decreased retention of ^{203}Pb at 120 h (Table 4). As the time of administration of the calcium before lead ^{203}Pb was increased, ^{203}Pb retention increased. This occurred to a smaller extent in the group given calcium glycerophosphate than in the group given CaCl_2 .

Effect of concentration of calcium on retention of orally administered ^{203}Pb

Six groups of six rats (370 g) were deprived of food overnight, but given water *ad lib.* The animals were intubated with a 5 ml solution containing 0, 0.25, 0.5, 1.25, 2.5 or 5.0 mmol of CaCl_2 . Then 10 min later each animal was intubated with $^{203}\text{PbCl}_2$ (25 μCi , 5.0 μmol of Pb^{2+} in 1 ml of water). Whole-body retention of ^{203}Pb was monitored over 120 h.

Increasing concentrations of calcium resulted in progressively lower retention of ^{203}Pb at 120 h after administration. The retention of ^{203}Pb decreased exponentially as calcium concentration increased. This relationship had the form ($r = 0.97$; $P < 0.001$) (Fig. 3):

$$^{203}\text{Pb retention at 120 h} = (1.40 \pm 0.08)e^{(-1.0 \pm 0.15[\text{Ca}^{2+}])} + (0.85 \pm 0.07)$$

Table 3. Retention of a single oral dose of ^{203}Pb after oral administration of appropriate calcium salt
 $^{203}\text{PbCl}_2$ (5 μmol) was given orally 20 min after administration of 1.25 mmol of Ca^{2+} as each of the salts shown. Whole body retention of ^{203}Pb was followed for 120 h. Each result represents the mean $\pm$ S.D. of six animals.

Group	Whole-body ^{203}Pb retention at 120 h (% of dose)
Control (no calcium)	2.03 $\pm$ 0.52
Calcium chloride	1.10 $\pm$ 0.11
Calcium sulphate	1.20 $\pm$ 0.21
Calcium carbonate	1.26 $\pm$ 0.18
Calcium orthophosphate	1.24 $\pm$ 0.29
Calcium glycerophosphate	1.18 $\pm$ 0.25
Calcium gluconate	1.15 $\pm$ 0.20

Table 4. Effect of ^{203}Pb retention of calcium salts given orally at various times before oral lead administration
Animals were intubated with Ca^{2+} (1.5 mmol) as either CaCl_2 or calcium glycerophosphate, at the times shown before oral $^{203}\text{PbCl}_2$ (5 μmol) administration. Whole-body retention of ^{203}Pb was monitored up to 120 h. ** $P < 0.001$; * $P < 0.005$; † $P < 0.05$ (all with respect to control).

Time of administration of appropriate calcium salt before oral ^{203}Pb administration (min)	Whole-body ^{203}Pb retention at 120 h (% of dose)
CaCl_2	
8	0.89 $\pm$ 0.20**
35	1.18 $\pm$ 0.25*
55	1.44 $\pm$ 0.37†
100	1.89 $\pm$ 0.25
Calcium glycerophosphate	
8	0.86 $\pm$ 0.22**
35	0.90 $\pm$ 0.18**
55	0.98 $\pm$ 0.08**
100	1.52 $\pm$ 0.21†
Control (no calcium)	2.08 $\pm$ 0.52

Effect of systemic calcium on ^{203}Pb retention

A group of 12 rats weighing 370 g were injected intraperitoneally daily for 10 days with $1.25\text{ }\mu\text{mol}$ of calcium gluconate, and 12 control animals were injected daily with 0.9% NaCl. After 10 days six animals in each group were given an oral dose of

Fig. 3. Effect of calcium concentration on retention of orally administered ^{203}Pb

Six groups of six rats each were intubated with various amounts of calcium, followed 10 min later by an oral dose of ^{203}Pb , and whole-body retention of ^{203}Pb was monitored for 120 h. Each point represents the mean ($\pm$ S.D.) retention of ^{203}Pb for the appropriate groups at 120 h.

$^{203}\text{PbCl}_2$ ($50\text{ }\mu\text{Ci}$, $5.0\text{ }\mu\text{mol}$ of Pb^{2+} in 1 ml of water), and the remaining six animals in each group were injected intraperitoneally with the same dose in 0.9% NaCl. Whole-body retention of ^{203}Pb was monitored for 120 h in the orally dosed groups and for 168 h in the intraperitoneally administered groups. After ^{203}Pb retention had been monitored for the appropriate length of time, the animals were killed by cervical dislocation and exsanguination and the livers, left kidneys and femurs were excised. ^{203}Pb content of these tissues was measured by counting in a γ -counter (Wallac DCEM series). Counts were related to a 'phantom' and results expressed as a percentage of whole-body ^{203}Pb in the appropriate organ.

In the animals given lead orally, there was no significant difference in ^{203}Pb retention or tissue distribution after 120 h between the calcium-treated and the control group (Table 5). Similarly in animals given lead intraperitoneally, there was no significant difference in ^{203}Pb retention or tissue distribution 168 h after dosage (Table 5).

Effect of calcium in hard water on oral ^{203}Pb retention

Three groups of six rats weighing 350 g were deprived of food overnight, but given water *ad lib*. The animals were then intubated with an 8 ml solution of $^{203}\text{PbCl}_2$ ($25\text{ }\mu\text{Ci}$, $5.0\text{ }\mu\text{mol}$ of Pb^{2+}) in distilled water (pH 6.2), 3 mM- CaCl_2 in distilled water (pH 6.2), water from a hard-water area (pH 6.3; containing 3 mM- Ca^{2+} and 0.23 mM- Mg^{2+}) or soft water (pH 6.8; containing 0.01 mM- Ca^{2+} and 0.04 mM- Mg^{2+}).

Whole-body retention of ^{203}Pb was monitored over 120 h. There was a significant decrease ($P < 0.05$) in the retention of ^{203}Pb at 120 h in the groups of animals given lead in calcium solution or in hardened water compared with the control group given lead in

Table 5. Effect of systemic calcium on ^{203}Pb retention and distribution

Two groups of 12 animals were injected intraperitoneally daily for 10 days with either $1.25\text{ }\mu\text{mol}$ of calcium or 0.9% NaCl. The groups were then subdivided into two groups, one receiving oral ^{203}Pb and the other intraperitoneal ^{203}Pb . Whole-body retention and tissue distribution of ^{203}Pb were determined at 120 h and 168 h in the two intraperitoneal groups. Each value represents the mean $\pm$ S.D. for six animals.

Group	Whole-body ^{203}Pb retention at 120 h (oral) or 168 h (intraperitoneal) (% of dose)	Percentage of whole-body ^{203}Pb in:		
		Liver	Kidney	Femur
Oral lead				
Ca^{2+}	1.99 ± 0.42	10.7 ± 1.0	7.5 ± 1.0	2.3 ± 0.6
Control	1.96 ± 0.35	10.8 ± 1.5	7.3 ± 1.1	2.1 ± 0.2
Intraperitoneal lead				
Ca^{2+}	36.3 ± 3.7	8.3 ± 1.7	8.1 ± 0.6	4.2 ± 0.5
Control	35.8 ± 1.7	8.2 ± 0.5	8.3 ± 0.7	4.5 ± 0.1

distilled water (Table 6). The retention of ^{203}Pb in the animals given lead in hard water was less than that in the animals administered lead in a solution containing a similar concentration of calcium, although this was not statistically significant.

Effect of milk and skimmed milk on oral ^{203}Pb retention

Three groups of six rats weighing 350 g were deprived of food overnight, but given water *ad lib*. Each animal was intubated with 8 ml of distilled water (control), cow's milk or skimmed cow's milk (Marvel; Cadburys, Bournville, U.K.). At 10 min after the first intubation, each animal was intubated with $^{203}\text{PbCl}_2$ (25 μCi , 5.0 μmol of Pb^{2+} in 1 ml of distilled water). Whole-body retention of ^{203}Pb was monitored over 120 h.

There was no significant difference in ^{203}Pb retention at 120 h between any of the three groups (Table 7). It is of interest to note that retention in the group given skimmed milk was higher than that in the control group, and retention in the group given cow's milk was higher still, although this did not attain statistical significance.

Table 6. *Effect of calcium in hard water on retention of orally administered ^{203}Pb*

Four groups of six animals each were intubated with ^{203}Pb in distilled water, calcium in distilled water, domestic water from a hard-water area or soft water. Retention of ^{203}Pb was monitored for 120 h after administration. * $P < 0.05$ as compared with control.

Group	Calcium concn. (mmol/l)	Whole-body ^{203}Pb retention at 120 h (% of dose)
Control (distilled water)	0	2.38 ± 0.80
Calcium in distilled water	3.0	$1.85 \pm 0.47^*$
Hard water	3.0	$1.63 \pm 0.47^*$
Soft water	0.01	2.22 ± 0.43

Table 7. *Effect of milk and skimmed milk on retention of orally administered ^{203}Pb*

Three groups of six animals each were intubated with 8 ml of distilled water (control), skimmed milk or cow's milk, followed 10 min later by 1 ml of ^{203}Pb . Whole-body ^{203}Pb was monitored for 120 h.

Group	Whole-body ^{203}Pb retention at 120 h (% of dose)
Control	1.96 ± 0.50
Skimmed milk	2.04 ± 0.66
Cow's milk	2.21 ± 0.50

Discussion

The interaction of calcium and lead has been the subject of many investigations from the viewpoint of both raised and diminished calcium concentrations. The mechanisms of such interaction remain largely unresolved. The present study demonstrates that the doubling of dietary calcium with $\text{Ca}_3(\text{PO}_4)_2$ limits the absorption of lead in rats. This was shown both by the oral absorption of ^{203}Pb and by the decreased body burden of stable lead as assessed by enzyme activities and tissue lead contents. Retention of intraperitoneal ^{203}Pb was unaffected by calcium supplementation of the diet, suggesting that decreased lead absorption by calcium orthophosphate was mediated through the gastrointestinal tract. The similar decrease in oral ^{203}Pb absorption caused by other calcium salts suggests that the decreased lead absorption is associated solely with the calcium supplementation and not with raised phosphorus contents in the diet. This finding is contrary to those by Shelling (1932) and Sobel *et al.* (1940), who showed that the addition of either calcium or phosphorus to rats fed on a low-calcium or a low-phosphorus diet containing added lead will decrease lead retention. The likelihood is that the effect that they described is associated with lowered calcium and phosphorus tissue contents and not with a gastrointestinal effect as observed in the present study.

The prolonged decrease in absorption of orally administered ^{203}Pb by calcium glycerophosphate compared with CaCl_2 is probably associated with the relative insolubility of the former calcium salt, increasing its stasis within the gastrointestinal tract.

Further evidence that decreased lead absorption caused by calcium salts is mediated through a gastrointestinal effect is provided by the effects of systemic calcium on lead absorption. Whole-body and tissue distribution of ^{203}Pb was unaffected by intraperitoneal calcium supplementation, this being so for ^{203}Pb administered both orally and intraperitoneally.

The exponential relationship demonstrated between calcium and oral ^{203}Pb retention shows that increasing oral doses of calcium decrease the retention of orally administered ^{203}Pb . Doses of calcium greater than 4 mmol do not limit lead retention further, leaving a residual fraction of ^{203}Pb absorption, which is unaffected by the concentration of calcium within the gut. This suggests that there are two mechanisms for transport of lead from the lumen of the gastrointestinal tract across the gut wall.

The calcium content of hard water is sufficiently large to produce a significant decrease in retention of an oral dose of ^{203}Pb when compared with distilled water. Thus in soft-water areas, where it has been previously shown that plumbosolvency is greater than in hard-water areas (Moore, 1973), lead uptake may be increased not only by increased availability

but also by enhanced absorption in subjects drinking such water.

The findings of the present study confirm the dietary studies by Tompsett (1939) and Hsu *et al.* (1975) and the studies in gut loops by Barltrop & Khoo (1975a) that the decrease in lead absorption by calcium is mediated solely through decreased gastrointestinal absorption. This contrasts with the effects of calcium depletion in the diet of rats (Mahaffey *et al.*, 1973; Quarterman *et al.*, 1974), which enhances lead absorption, but unlike calcium supplementation of the diet is likely to be the result of a series of complex interactions involving the gastrointestinal tract, kidney, parathyroid, blood and bone.

Calcium supplementation has been shown to modify the absorption of both magnesium (Morris & O'Dell, 1963) and strontium (McDonald *et al.*, 1955). It is therefore possible that the effects of added calcium on lead retention are due to the existence of a common pathway for the absorption of calcium and other minerals, including lead, from the gut. Indeed, it is noteworthy that lead acetate decreased the transfer of both calcium and strontium across the duodenal wall of rats and that this transfer was further decreased by increasing doses of lead (Gruden *et al.*, 1974). Such a common pathway for absorption may also explain the effects of other nutritional factors on lead absorption. High-protein diets have been shown to enhance lead uptake (Barltrop & Khoo, 1975b) and decrease calcium uptake, whereas diets high in fats are known to decrease the availability of calcium (Calverley & Kennedy, 1949) and increase lead absorption (Barltrop & Khoo, 1975b). Such interactions may play an important role in the effects of milk on lead absorption. The lack of effect of milk on lead absorption demonstrated in the present paper and others (Weyrauch & Necke, 1932; Gerber & Wei, 1974) suggests that the nutritional constituents of milk, such as protein and fat, may interact with any effect that the raised calcium concentrations in milk may have in decreasing lead absorption. The findings of Kello & Kostial (1973) that milk increased lead absorption may well be associated with such interactions. The animals in that study did not receive diets of nutritional equivalence; thus animals fed partly or wholly on milk received less calcium and iron than did the controls and considerably more protein and fat.

Our results have demonstrated that oral calcium supplementation can limit lead absorption in rats. This decrease has been shown to be associated with the mechanisms of lead absorption from the gastrointestinal tract. It is always difficult to extrapolate such findings to human populations. However, the possibility that calcium may have a prophylactic application in industrial and environmental lead exposure is of obvious importance and warrants further investigation.

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