Influence of Dietary Factors on the Gastrointestinal Absorption of Lead

BRAD T. GARBER AND EDDIE WEI

University of California, School of Public Health, Berkeley, California 94720

Received June 7, 1973; accepted August 10, 1973

Influence of Dietary Factors on the Gastrointestinal Absorption of Lead. GARBER, B. T. AND WEI, E. (1974). Toxicol. Appl. Pharmacol. 27, 685-691. Gastrointestinal absorption of lead was investigated in mice after oral administration of lead acetate labeled with $^{210}$Pb. When doses of 0.2, 2 and 20 mg of Pb/kg were given, the magnitude of the dose did not appear to affect significantly the percent absorbed. The presence of food in the gastrointestinal tract reduced lead absorption when a tracer dose was administered but did not affect absorption after 2 mg of Pb/kg po. The chelators nitrilotriacetic acid and sodium citrate increased absorption of lead, as did orange juice, a source of citric acid. Milk and the chelating agents, ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid, did not affect significantly lead absorption.

A major route by which lead enters the body is through gastrointestinal absorption (National Academy of Sciences, 1972). In evaluating the hazard from exposure to lead, it is therefore important to understand the extent to which dietary constituents influence absorption. Previous studies involving gastrointestinal absorption of lead have mainly emphasized the interaction of lead with calcium metabolism. For example, Sobel et al. (1955) showed that dietary levels of calcium, phosphorus and vitamin D influence tissue levels of lead administered orally to rats. Shields and Mitchell (1941) demonstrated that dietary levels of calcium and phosphorus affect body levels of lead when only small amounts of lead are ingested, and Lederer and Bing (1940) concluded that the effect of calcium in the diet on the amount of lead found in the body was due to changes in absorption of lead from the intestine. More recently, Six and Goyer (1970) reported that dietary calcium not only affects tissue concentrations of lead but also lead toxicity. The purpose of this investigation was to determine, under conditions of normal calcium metabolism, the effects of certain food chemicals on the gastrointestinal absorption of lead.

METHODS

Male Swiss-Webster mice, 6–7 weeks old, were used in all experiments. Treated and control mice were housed in the same cage before the experiments and were randomly assigned to experimental groups on the day of the experiment. The animal room was

1 This investigation was supported in part by General Research Support Grant 5 S01 RR 05441 from the NIH; Environmental Health Training Grant, 5 T01 OH 00020 from the NIOSH; and by U.S. Atomic Energy Commission Contract W7405-ENG-48.
maintained on a 12-hr cycle of light and dark. All 4-hr absorption studies were started approximately 3 hr after the beginning of the light cycle. Unless stated otherwise, mice received food and water ad libitum before administration of lead, but no food or water was available during the absorption period. Except in the time course study, mice were housed 8–10 animals per cage after the administration of lead. Cages were provided with screen bottoms in order to avoid surface contamination of mice with urine and feces.

Lead solutions were prepared using lead acetate. \(^{210}\text{Pb}\) acetate, at a specific activity of 15.1 mCi/mg Pb, was obtained from International Nuclear and Chemical Corporation. Solutions were administered po using a curved, ball-tipped 20-gauge needle. Injection volumes were held constant at 0.1 ml per mouse. In all experiments each mouse received approximately 1 \(\mu\)Ci of \(^{210}\text{Pb}\).

\textit{Measurement of }\(^{210}\text{Pb}\). Concentrations of lead in the gastrointestinal (GI) tract, carcass and excreta were determined by analyzing for the 47 kev gamma emissions from \(^{210}\text{Pb}\) using a small-animal whole-body counter with 2 opposed 12-cm diameter by 3-mm thick sodium iodide detectors, which were coupled to a 400-channel pulse-height analyzer. The sodium iodide detectors were positioned 20 cm apart, and the sample was placed midway between them. To determine the extent of the counting error caused by tissue absorption, 0.1 ml of water containing 1 \(\mu\)Ci of \(^{210}\text{Pb}\) was diluted to volumes of 0.5, 1.0, 3.0, 5.0, 7.5, 10 and 15 ml in a 2-cm diameter cylindrical plastic vial. The number of counts for the different dilutions did not differ by more than 3\% from the counts obtained with the 0.1 ml volume. A more thorough discussion of the principles of counting low energy gammas in mice has been presented previously by Wright (1972). The counter described by Wright is the same instrument used in our experiments.

\textit{Determination of lead absorption from the gastrointestinal tract}. Mice were sacrificed by cervical dislocation. After clamping the esophagus with a hemostat to prevent leakage of stomach contents, the entire GI tract and the lower part of the esophagus were removed. Concentrations of lead in the GI tract, carcass, and excreta were then analyzed for \(^{210}\text{Pb}\). The percent of lead absorbed, reported as the mean \pm SE was calculated by multiplying by 100 the amount of \(^{210}\text{Pb}\) remaining in the body after removal of the GI tract divided by the amount of \(^{210}\text{Pb}\) in the GI tract plus the carcass. Suzuki \textit{et al.} (1969) have used a similar experimental procedure for measuring cadmium absorption from the GI tract.

The calculation of lead absorption does not take into account possible resecretion of the absorbed lead back into the intestine or the excretion of lead via the urine or feces. The following experimental results indicated that these sources of error were small under the 4-hr time period chosen for study: (1) Four hours after im injection of 8 mice with 2 mg of Pb/kg, 14.43 \(\pm\) (SE) 1.87\% of the amount absorbed was found in the GI tract and 11.36 \(\pm\) 4.40\% in the urine and feces. (The amount absorbed was taken to be the amount in the body after removal of the leg containing the injection site.) (2) Four hours after oral administration of 2 mg of Pb/kg to 8 mice, the average amount passing through the GI tract and excreted in the feces was less than 5\%.

Because lead is so poorly absorbed from the GI tract, even a small amount of contamination of the carcass by the oral administration procedure could cause considerable error in the determination of the percent absorption. It was therefore necessary to determine the magnitude of this source of error. We conducted an experiment, using
10 mice, in which we followed the entire procedure used for administering 2 mg of Pb/kg po but without depressing the syringe plunger. Four hours later the mice were sacrificed. Any $^{210}$Pb found in the body was then most likely due to small amounts of liquid adhering to the outer surface of the syringe needle. Of the $^{210}$Pb received by the mice, 85.51 ± 2.50% was found in the GI tract. This indicated that most of the small amounts of lead which contaminated the mouth and esophagus moved into the GI tract. The amount of $^{210}$Pb found in the carcass represented less than 5% of the amount absorbed in any of our 4-hr absorption experiments. Therefore, surface contamination of the carcass by the method utilized for lead administration does not appear to contribute a significant source of error.

Experimental variables. For the dose-response studies, lead was administered to mice at doses of 0.2, 2 and 20 mg of Pb/kg po and 4 hr later the animals were sacrificed and the carcass and GI tract analyzed for $^{210}$Pb. The time-course of lead absorption was measured by determining the amount of lead in the carcass at 4, 8, 16 and 24 hr after 2 mg of Pb/kg po. Mice were housed separately in cages designed to collect the urine and feces. The 8-, 16- and 24-hr groups received food and water before and throughout the entire experiment. Mice in the 4-hr group were also fed ad libitum before the experiment but received no food or water during the absorption period. To assess the effect of food on lead absorption, food pellets were removed from cages 16 hr prior to the administration of lead. Control mice received food ad libitum during this 16-hr period. Starved mice and controls received a tracer dose of lead (0.003 mg of Pb/kg po) in 1 experiment and 2 mg of Pb/kg po in another experiment.

The chelators, DTPA (diethylenetriaminepentaacetic acid, pentasodium salt), EDTA (ethylenediaminetetraacetic acid, disodium salt), NTA (nitrilotriacetic acid, disodium salt) and d-penicillamine, were orally administered, in equimolar amounts with 2 mg of Pb/kg. Pb, 2 mg/kg, was also given in 0.1 N HCl, 5% sodium citrate, orange juice or milk. Injection volumes were kept constant at 0.1 ml/mouse. The orange juice was prepared by diluting 1 vol of Co-op brand frozen concentrated orange juice with 3 vol of water, and the milk was prepared by diluting 1 vol of Co-op brand evaporated milk with 1 vol of water. Controls received 2 mg of Pb/kg in water.

Differences in lead absorption were statistically compared using the rank-sum test (Dixon and Massey, 1969). Statistical hypotheses were tested at the 5% level of significance.

RESULTS

The percent of lead absorbed appeared to be independent of dose at dose levels of 0.2 to 20 mg of Pb/kg (Fig. 1). Blaxter (1950) has reported similar results in sheep. The time-course for lead absorption and excretion after oral administration is shown in Table 1. Lead appears to move relatively quickly through the GI tract. After 24 hr, 96.25 ± 0.79% of the lead had been excreted (Table 1). Analysis of intestinal segments of 8 mice, 4 hr after oral administration of 2 mg of Pb/kg, showed that of the lead remaining in the GI tract, 9.65 ± (SE) 2.45% was in the stomach, 3.92 ± 0.49% in the proximal half of the small intestine, 8.92 ± 1.74% in the distal half of the small intestine and 77.51 ± 3.28% in the large intestine.

When given a tracer dose of lead, mice starved for 16 hr showed a significantly increased absorption as compared to controls (Fig. 2). No observed difference between
starved and control mice was evident when 2 mg of Pb/kg was administered (Fig. 2). These results indicate that when mice are starved, the dose of lead ingested may influence the percent absorbed.

![Graph showing the effect of dose upon the gastrointestinal absorption of lead. Lead was administered p.o. to mice 4 hr before sacrifice. Each point represents the datum from 1 mouse. The bars indicate mean ± SE.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>% Absorbed</th>
<th>% Excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.67 ± 0.43</td>
<td>4.65 ± 2.50</td>
</tr>
<tr>
<td>8</td>
<td>5.69 ± 2.01</td>
<td>32.54 ± 7.48</td>
</tr>
<tr>
<td>16</td>
<td>3.63 ± 0.50</td>
<td>93.90 ± 0.79</td>
</tr>
<tr>
<td>24</td>
<td>2.08 ± 0.28</td>
<td>96.25 ± 0.79</td>
</tr>
</tbody>
</table>

*The percent absorbed (±SE) represent the portion of the injected dose found in the carcass after removal of the GI tract.*

The average lead absorption values for different control groups receiving 2 mg of Pb/kg, 4 hr prior to sacrifice, ranged from 2.65 to 5.83% (Fig. 3). Most of this variability can be attributed to 1 or 2 mice which absorbed large amounts of lead. It is unknown what factors contributed to these individual differences or to the differences between control groups. Individual variability in man has also been noted. Hursh and Suomela (1968) estimated that 3 human subjects receiving approximately equal oral doses of $^{210}$Pb absorbed 1.3, 8.1 and 16%, respectively.

Neither EDTA nor DTPA, despite being strong chelators of lead, significantly changed the amount of lead absorbed from the GI tract (Fig. 3). Castellino and Aloj (1965), in a study using rats, also reported that EDTA did not modify absorption of lead. Nitrilotriacetic acid (NTA), a chelator which has been considered for use as a
Fig. 2. Gastrointestinal absorption of lead in fed and starved mice. Lead was administered po to mice 4 hr before sacrifice. Each point represents the datum from 1 mouse. The bars indicate mean ± SE. * indicates $p < 0.05$ vs controls.

Partial replacement for sodium tripolyphosphate in detergents (Tjalve, 1972), increased the absorption of lead (Fig. 3). D-Penicillamine also appeared to increase the absorption of lead but the results were not significant at $p < 0.05$ (Fig. 3).

Fig. 3. The effects of dietary factors on the gastrointestinal absorption of lead. Two mg of Pb/kg was administered po with each of the substances shown in the figure. Mice were sacrificed 4 hr later. Treated groups are shown in gray. For NTA, 5% sodium citrate and orange juice, lead absorption was significantly different from controls ($p < 0.05$). Each point represents the datum from 1 mouse. The bars indicate mean ± SE.
When lead was administered with the chelator sodium citrate, GI absorption was increased (Fig. 3). Orange juice, which contains citric acid [0.56–0.98 g/100 ml in juices analyzed by Clements (1964)], also increased absorption (Fig. 3). When lead was given in 0.1 N HCl to determine if the acidity of the citrate solution affected absorption, no increase was observed (Fig. 3). Milk did not significantly affect absorption of lead from the GI tract (Fig. 3).

DISCUSSION

Chelating agents are frequently used as food additives and may also occur as natural food constituents (Furia, 1968). From the results of this investigation it appears that some chelating agents will facilitate the intestinal transport of lead. The ability of chelating agents to increase lead absorption may be correlated with the ease with which the chelating agent crosses the intestinal barrier. For example, CaNa₂EDTA and CaNa₃-DTPA, are poorly absorbed from the intestine (Goodman and Gilman, 1965) and lead chelates of EDTA and DTPA also appeared to be poorly absorbed. Nitrilotriacetic acid, which significantly increased the absorption of lead, is well absorbed from the intestines of rats, but is poorly absorbed in certain other species (Michael and Wakim, 1971). It would be of interest to determine whether the absorbability of the NTA-lead chelate parallels the species differences in NTA absorption.

The results of the experiment in which lead was given with D-penicillamine are difficult to interpret since the increase in absorption was not statistically significant. Since the mean percent absorption for mice receiving lead with D-penicillamine was higher than any of the 8 control groups, it is possible that the D-penicillamine effect would have been statistically significant if a larger sample size had been studied. It is interesting to note that oral administration of D-penicillamine has been suggested for the treatment of lead poisoning (Goldberg et al., 1963).

Sodium citrate and citric acid are added to many kinds of foods for a variety of reasons (Furia, 1968). Citrate also occurs naturally in foods and plays a role in several biochemical systems. In this investigation, it was found that both sodium citrate and orange juice, a source of citric acid, increased GI absorption of lead. It is common knowledge that lead poisoning can occur as a result of storing acidic beverages in pottery coated with lead-containing glazes (Klein et al., 1970). The enhancement of lead absorption by sodium citrate and orange juice should be distinguished from the ability of acidic solutions to leach lead from lead-containing pottery glazes. Both factors may contribute to the health hazards associated with the storage of acidic fruit juices in improperly glazed vessels.

Administration of a daily ration of milk to workers occupationally exposed to lead has been used as a preventive measure in the control of lead poisoning (Fowler, 1965). Our results indicate that milk does not influence the GI absorption of lead. Aub et al. in 1926 also found that retention of lead was not affected by milk. Milk as a source of calcium, however, may alter the pattern of lead toxicity if dietary calcium levels are low (Six and Goyer, 1970).

In summary, this investigation demonstrates that lead absorption from the GI tract depends on factors such as the chemical composition of the food containing the lead and the amount of food present in the GI tract. It would appear desirable that dietary factors
be considered in current deliberations over an acceptable level of lead in food (King, 1971).

ACKNOWLEDGMENTS

The skilled assistance of Mr. William Hemphill and the support of Dr. Howard Parker are gratefully acknowledged.

REFERENCES


