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Effects of Ethanol on the Absorption and Retention of Lead (40317)

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The frequent clinical association of plumbism and increased alcohol intake has suggested that ethanol may augment lead absorption and toxicity. This investigation was undertaken to determine the effects of acute and chronic ethanol administration on lead absorption and excretion.

Materials and methods. Male albino rats of a pathogen-free Wistar strain weighing 200 to 250 g at the time of absorption measurements or intravenous lead injection were used in all experiments. The principles of laboratory animal care as promulgated by the National Research Council were observed. All animals were housed in polypropylene cages containing absorbent bedding in a room provided with automatically controlled temperature and lighting. The rats were given a standard pelleted laboratory chow (Wayne Lab-Blox, Allied Mills, Inc.) fed *ad libitum.* Demineralized deionized water was supplied to all animals except in some experiments in which 10% ethanol (v/v) was substituted for water.

Lead absorption studies were performed by measurement of total body radioactivity in a small animal whole-body liquid scintillation detector (Packard-ARMAC). The radioisotopes utilized were obtained from New England Nuclear as ^{203}Pb acetate (sp act 10–50 mCi/mg of lead) or ^{210}Pb nitrate (sp act 10 mCi/mg of lead). Because of its half-life of 22 years, ^{210}Pb was selected for use in excretion studies only; ^{203}Pb, having a half-life of 2.2 days, was used for all other experiments. All measurements of radioactivity were corrected for radiodecay by comparison to an appropriate standard after subtraction of background radioactivity. Lead absorption experiments were performed in rats fasted overnight from food but not fluids. Under ip pentobarbital anesthesia (4 mg/100 g), the urethra was tied with a silk suture to prevent urinary loss of absorbed lead. A laparotomy was performed, the small intestine was isolated proximally and distally with umbilical tape, and the bile duct was ligated with silk suture. One milliliter of radiolabeled lead-containing test solution was injected into the isolated intestinal segment. Injections were accomplished by entering the gut lumen proximal to the proximal ligature with a 21-gauge hypodermic needle, passing it intraluminally through the ligature loop, tightening the ligature, and then injecting the test dose into the isolated segment with subsequent withdrawal of the needle and tying of the ligature. In one experiment, animals were administered test doses through an oroesophageal tube following laparotomy. The abdomen was then closed with stainless-steel clips and the rats were placed in 1-quart vented cardboard ice cream containers. Total body radioactivity was measured in a whole-body detector and compared to a 250-ml water-filled plastic bottle containing a test dose equal to that injected into the animals. Four hours after administration of the test dose, each animal was killed by cervical dislocation. Isolated intestinal segments were excised from the carcass and whole-body radioactivity was again quantified and compared to the original whole-body radioactivity.

To assess the effects of chronic ethanol ingestion on lead absorption, a group of eight animals was given 10% ethanol (v/v) as their exclusive source of fluids for 3 weeks while eight controls received water. The rats took food and fluids readily. While animal weights in experimental and control groups were initially the same (140 ± 5 and 141 ± 7 g, respectively), weight gain in ethanol-treated animals was less than that of controls (62 ± 5 vs 84 ± 15 g, $p < 0.05$); isocaloric pair feeding also results in a similar diminution in weight gain in animals receiving ethanol (1). Animals were given a test dose of 1 μg of Pb and absorption was determined. Specimens of duodenum and liver were taken from ad-

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ditional similarly prepared experimental and control animals for light and electron microscopic studies. The influence of acute alcohol ingestion was studied by the quantification of lead absorption in groups of animals receiving 1 ml of the following solutions in 50% ethanol: (1) 1 μg of Pb; (2) 10 μg of Pb; (3) 100 μg of Pb; and (4) 1 mg of Pb. Controls received the same quantities of lead in aqueous solutions. Segments of duodenum from similarly prepared experimental and control rats were examined by light and electron microscopy.

To determine whether the diminished lead absorption from ethanol solutions was due to a direct effect on the intestine, 16 rats were given 1 μg of Pb in the isolated intestinal segment. Half the animals simultaneously received 1 ml of 50% ethanol above the pyloric ligature by oroesophageal intubation. Control animals received 1 ml of saline. In an additional experiment, intestinal loops with open distal ends were injected with 50% ethanol followed after 15 min by lavage with 0.5 ml of air and 1 ml of saline and subsequent tying of the cecal ligature. Intestinal loops in controls were pretreated with saline followed by similar washing. Lead absorption experiments were then performed using 1 μg of Pb. In a final study to assess the role of the site of absorption in lead absorption, six groups of eight animals were subjected to laparotomy with bile duct and cecal ligation. The rats in each group received 1 μg in water or 50% ethanol (pH 4) by the following means: (1) aqueous and (2) alcoholic lead via oroesophageal tube, the solution confined to the stomach by a pyloric ligature; (3) aqueous and (4) alcoholic lead via oroesophageal tube without pyloric ligation; (5) aqueous and (6) alcoholic lead in isolated gut loop. Lead absorption was then quantified as previously described.

To study the effects of aqueous and alcoholic solutions on lead solubility, 100 ml of each of the following solutions were prepared as controls: (1) 1 μg of Pb/ml; (2) 10 μg of Pb/ml; (3) 100 μg of Pb/ml; and (4) 1 mg of Pb/ml. Similar solutions of lead in 50% ethanol were also prepared. One microCurie of ^{203}Pb was added to each 100-ml solution and the pH was adjusted to 2.0. After mixing, 1 ml of each of the resulting solutions was removed as a standard. Each solution was titrated against 0.1 *N* NaOH to pH 10.0 with 2-ml samples being removed at each integral pH value. Similar samples were taken upon returning to pH 2 with 0.1 *N* HCl. Aliquots taken from each titration were centrifuged at 3000 rpm × 30 min and 1 ml of supernatant was removed from each for quantification of radioactivity in a Packard auto-gamma spectrometer. Solubility of aqueous and alcoholic lead was expressed as percentage of standard as a function of pH. Supernatants from ethanol solutions varying from aqueous controls by more than 5% were applied to Sephadex G-25 columns equilibrated with 50% ethanol at the appropriate pH. Column fractions were counted successively to detect peaks of radioactivity which would suggest the presence of soluble lead-containing macromolecules.

In lead excretion experiments, experimental animals were given 10% ethanol for drinking for 3 weeks prior to injection and controls received water. Ethanol was continued in the experimental group throughout excretion measurements. While similar weights for experimental and control rats at the start of fluid conditioning (143 ± 7 g, 141 ± 5 g) were again noted, ethanol-treated animals weighed less at the time of injection (205 ± 11 vs 229 ± 9 g, $p < 0.05$). Each animal was given ip sodium pentobarbital anesthesia (4 mg/100 g) to facilitate injection of 1.0 μCi of ^{210}Pb in 0.5 ml of 0.9% NaCl (pH 7.4) into the dorsal vein of the penis. Whole-body counts were obtained immediately after dosing and at intervals thereafter. Body retention of radiolead was calculated by comparison to initial counts with correction for decay by comparison to a standard. At the termination of excretion studies 4 weeks after dosing, a final whole-body radioactivity was measured. Tissues for electron microscopic studies were fixed in 2% glutaraldehyde, postfixed in 1% osmic acid, and embedded in Araldite. Sections 150 to 200 Å thick were stained with saturated uranyl acetate and lead citrate and examined using an RCA EMU4 electron microscope. Thick sections (1 μm) were stained with toluidine blue. Additional specimens for light microscopy were fixed in 10% formalin, paraffin embedded, and stained with hematoxylin and eosin.

In all absorption and excretion experiments groups of eight animals were used. Except as noted above for animals receiving ethanol chronically, there were no differences in mean animal weights among the various groups compared in this study. For absorption studies, all rats received 1 ml of a lead-containing solution adjusted to pH 4.0, which simulates the pH of gastric contents and maintains lead solubility. All lead quantities are expressed as grams of elemental lead as $PbCl_2$; 1 μCi of ^{203}Pb or ^{210}Pb was used as a radioisotopic label for each rat. Data are expressed as means and standard errors of the mean. Statistical comparisons were made using Student's two-tailed t test for unpaired data.

Results. Chronic ethanol ingestion significantly reduced the absorption of a single dose of aqueous lead. While animals receiving water as a fluid source for 3 weeks absorbed 21.7 ± 1.6% of a test dose of 1 μg of Pb, those prepared with 10% ethanol for 3 weeks absorbed only 15.2 ± 2.9% ($p < 0.05$). While livers from animals receiving alcohol for 3 weeks showed moderate fatty change, no light microscopic or ultrastructural changes were noted in duodenal mucosa of the same animals. As illustrated in Fig. 1, the absorption of lead from aqueous solutions was significantly greater than that from ethanol solutions at concentrations of 1 and 10 μg of Pb/ml ($p < 0.005$, $p < 0.005$). At lead concentrations of 100 and 1 mg of Pb/ml, absorption from alcoholic solutions appeared slightly greater than from controls but the differences were not significant ($p = 0.20$, $p = 0.25$). Duodenal mucosa from animals receiving 50% ethanol acutely with or without lead showed disruption of villous tips, pyknotic nuclei, and increased villous invasion by mononuclear cells. By electron microscopy, destruction of microvilli, mitochondrial swelling, and irregularity of mitochondrial size were noted in addition. No abnormalities were noted in animals given saline or aqueous lead solutions. The solubility of ^{203}Pb in aqueous solutions is shown in the upper half of Fig. 2. Lead is more soluble in acid solutions and increasing amounts are precipitated as pH increases. As illustrated in the lower half of Fig. 2, little change in radiolead solubility occurs when 50% ethanol is used as a carrier. Application of supernatants obtained in these experiments to Sephadex G-25 columns revealed no evidence of lead-containing macromolecules.

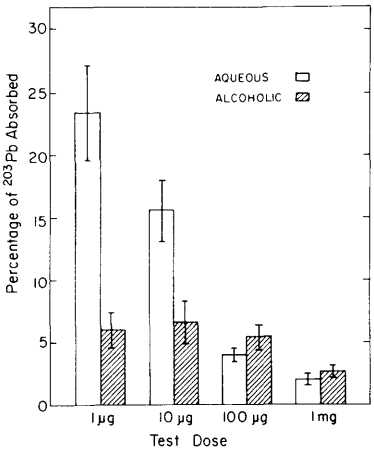

FIG. 1. The acute effects of ethanol administration on the absorption of a single dose of lead chloride.

Since animals receiving ethanol on both an acute and chronic basis appeared to have diminished lead absorption unattributable to reduced lead solubility or macromolecule formation in the presence of alcohol, additional experiments were performed to determine whether at least part of this inhibitory effect was due to a direct effect of ethanol on intestinal mucosa. Rats with a pyloric ligature simultaneously administered 1 μg of Pb in the intestinal loop and 50% ethanol in the stomach showed lead absorption (Fig. 3) which did not significantly vary from that observed in control animals. Absorption of lead in animals with ethanol-pretreated intestinal loops, however, was significantly less than that seen in rats with saline-pretreated gut loops (3.9 ± 0.5 vs 13.5 ± 1.5% control, $p < 0.0005$). As shown in Table I, only small quantities of aqueous or alcoholic lead were absorbed by the stomach (2.5 ± 0.7 and 2.1 ± 0.4%, respectively). The absorption of lead in aqueous solution by the intestine (30.6 ± 1.5%) was significantly higher than that of lead in alcoholic solution (8.2 ± 0.8%, $p < 0.005$) and is similar to findings shown in Fig. 1. When alcoholic lead solutions were given via oroesophageal intubation such that both stomach and intestine could act as absorptive sites, lead absorption increased to 22.4 ± 3.2%. This value was less, however, than lead

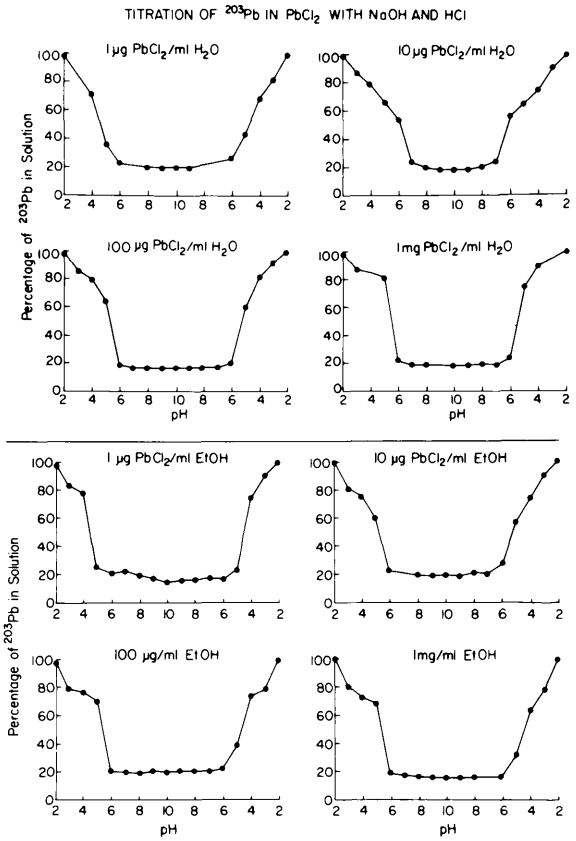

FIG. 2. The solubility of ^{203}Pb in $PbCl_2$ as affected by pH. Upper diagrams indicate solubility in aqueous solutions; lower diagrams indicate solubility in 50% ethanol.

uptake from aqueous solutions (28.6 ± 1.7% $p < 0.05$). The findings suggest that a gastric factor, perhaps ethanol-stimulated gastric acid, may act to modify lead absorption although in these experiments alcoholic lead uptake remained significantly less than controls.

As seen in Fig. 4, the excretion of lead in animals chronically receiving 10% ethanol did not significantly vary from control animals at any time during the experiment. Both groups showed an initial rapid phase of lead elimination during the first week after injection, in which time about one-half of the administered dose was excreted. This was followed by a slower phase of lead loss from the body. By using a best fit slope derived from mean-square analysis plotted on semilogarithmic graph, the half excretion time for lead remaining after Day 7 was approximately the same for each group, about 160 days.

Discussion. A variety of clinical reports of lead poisoning in heavy consumers of alcohol (2–4) has suggested that ethanol may enhance lead accumulation and potentiate its toxic manifestations. While Mahaffey *et al.* (1) concluded that there was little synergism of ethanol and lead as measured by morphologic and biochemical parameters of lead toxicity, no studies to date have directly measured the effects of alcohol on lead absorption or retention. The results of this study indicate that: (1) the acute and chronic administration of ethanol inhibits the ability of the rat small intestine to absorb lead; (2) the effect does not seem attributable to diminished lead solubility in alcohol; (3) the inhibitory effect may be related, in part, to the direct toxicity of ethanol on intestinal mucosa; and (4) chronic ethanol ingestion does not appear to alter the excretion of lead given as a single intravenous dose. While the mechanism of lead absorption is unknown, Krawitt (5, 6)

concluded that acute or chronic ethanol administration inhibited calcium transport in everted rat gut sacs and that this effect was associated with direct mucosal toxicity. Since evidence exists that one or more intestinal proteins important in calcium mucosal binding and transfer may participate in lead absorption (7), a similar direct toxic effect on intestinal mucosa may be responsible for the diminished lead absorption found in these experiments. Whether the anatomic damage of duodenal mucosa observed in these experiments after acute ethanol administration is responsible for the diminished lead absorption after acute or chronic ethanol administration cannot be determined at present. The lack of obvious mucosal damage in rats chronically fed ethanol suggests that acute and chronic alcohol exposure may diminish lead absorption by different mechanisms. Other workers, however, have noted ultrastructural changes in small intestinal mucosa following more prolonged low-level ethanol exposure (8). While there is evidence that a gastric factor may modify the absorption of alcoholic lead from stomach and intestine, the lead absorption does not exceed that observed in aqueous lead control animals.

Factors enhancing the susceptibility to lead poisoning have been reviewed (9). Several dietary deficiencies common among heavy alcohol users have been established as capable of potentiating the manifestations of lead toxicity. While protein deficiency reduces lead absorption (11), it produces greater susceptibility to lead toxicity (12, 13). Dietary calcium deficiency increases lead retention (14, 15) and potentiates morphological and biochemical parameters of lead poisoning (16) but does not alter lead absorption (7). Iron deficiency both enhances lead toxicity (17) and increases lead absorption (10, 11). The effects of ascorbic acid, pyridoxine, and other micronutrients on lead metabolism and toxicity are not known with certainty (9).

Since these experiments indicate that acute or chronic ethanol exposure does not increase lead absorption, particularly at concentrations commonly seen in lead-containing "moonshine" whiskey (1–10 μg of Pb/ml) (18), the apparent synergism of lead and ethanol reported in alcoholics may be related to increased lead exposure (lead-contaminated illicit whiskey or industrial environments) and/or nutritional deficiencies as previously concluded (1). These studies suggest that chronic ethanol ingestion does not alter

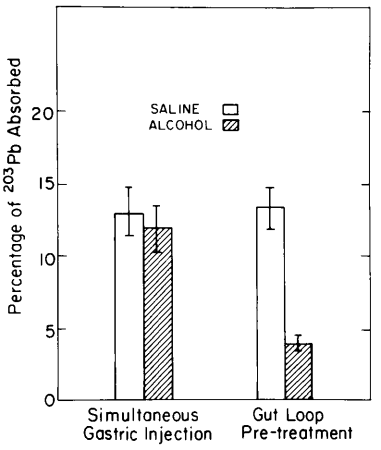

FIG. 3. The intestinal absorption of a single dose of lead chloride in rats without prior ethanol exposure as influenced by gastric injection of saline or 50% ethanol (left) and by pretreatment of the intestinal loop by saline or 50% ethanol (right).

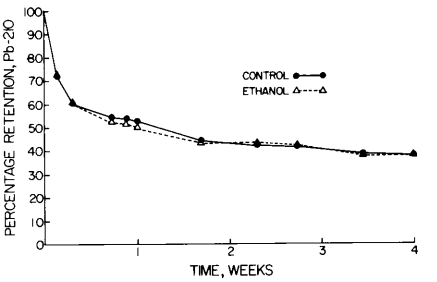

FIG. 4. The whole-body retention of lead following a single intravenous dose of lead-210 in rats chronically ingesting 10% ethanol.

TABLE I. EFFECT OF ABSORPTIVE SITE ON ABSORPTION OF LEAD FROM AQUEOUS AND ALCOHOLIC LEAD SOLUTIONS.

	Site of absorption					
	Stomach		Stomach and intestine		Intestine	
Lead solution	Aqueous	Alcoholic	Aqueous	Alcoholic	Aqueous	Alcoholic
Lead absorption (%)	2.5 ± 0.7	2.1 ± 0.4	28.6 ± 1.7	22.4 ± 3.2	30.6 ± 1.5	8.2 ± 0.8

the elimination of small quantities of lead administered as a single intravenous dose. Although there are no previously published reports of the effects of ethanol on lead excretion, the variety of renal lesions seen in plumbism and the known augmentation of lead-induced renal abnormalities by alcohol (1) suggest that diminished excretion may be of significance only when large quantities of lead are involved.

Summary. To determine the effects of acute and chronic ethanol ingestion on the absorption of lead, experiments were performed using an *in vivo* isolated gut loop technique. Acute administration of 50% ethanol significantly reduced the absorption of lead at concentrations of 1 and 10 μg of Pb/ml. This effect appears to be independent of lead solubility in alcohol and is associated with structural changes in intestinal mucosa, suggesting toxicity. Absorption of a single dose of lead was also diminished in animals chronically exposed to ethanol. Elimination of a single intravenous dose of lead was not affected by chronic alcohol ingestion. These findings suggest that the clinically reported synergism of lead toxicity and ethanol is related not to increased lead absorption or diminished lead excretion but to nutritional deficiencies and increased lead exposure among some alcoholics.

1. Mahaffey, K. R., Goyer, R. A., and Wilson, M. H., Arch. Environ. Hlth. **28,** 217 (1974).
2. Gilfillan, S. C., J. Occup. Med. **7,** 53 (1965).
3. Owen, C., Dodson, W. H., and Hammack, W. J., S. Med. J. **60,** 44 (1967).
4. Cramer, K., Acta Med. Scand. Suppl. **445,** 56 (1966).
5. Krawitt, E. L., J. Lab. Clin. Med. **85,** 665 (1975).
6. Krawitt, E. L Proc. Soc. Exp. Biol. Med. **146,** 406 (1974).
7. Barton, J. C., Conrad, M. E., Harrison, L., and Nuby, S., J. Lab. Clin. Med. **91,** 366 (1978).
8. Rubin, E., Rybak, B. J., Lindenbaum, J., Gerson, C. D., Walker, G., and Lieber, C. S., Gastroenterology **63,** 801 (1972).
9. Goyer, R. A., and Mahaffey, K. R., Environ. Hlth. Perspect. **2** (1962).
10. Ragan, H. A., J. Lab. Clin. Med. **90,** 700 (1977).
11. Conrad, M. E., and Barton, J. C., Gastroenterology **74,** 731 (1978).
12. Baernstein, H. D., and Grand, J. A., J. Pharmacol. Exp. Ther. **74,** 18 (1942).
13. Gontzea, I., *et al.*, Arch. Sci. Physiol. **18,** 211 (1964).
14. Lederer, L. G., and Bing, F. C., J.A.M.A. **114,** 2457 (1940).
15. Shields, J. B., and Mitchell, N. H., J. Nutr. **21,** 541 (1941).
16. Six, K. M., and Goyer, R. A., J. Lab. Clin. Med. **76,** 933 (1970).
17. Six, K. M., and Goyer, R. A., J. Lab. Clin. Med. **79,** 128 (1972).
18. Morris, C. E., Heyman, A., and Pozefaky, T., Neurology **14,** 493 (1964).

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