Acute Effects of Lead on the Renal Handling of Zinc in Dogs

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Acute Effects of Lead on the Renal Handling of Zinc in Dogs. VICTERY, W., SOIFER, N. E., WEISS, J. S., AND VANDER, A. J. (1981). *Toxicol. Appl. Pharmacol.* **61**, 358–367. Urinary zinc excretion was monitored in anesthetized dogs before and during 4 hr after acute exposure to lead (doses: 0.3 mg Pb (as acetate)/kg as iv prime, followed by infusion of 0.057 mg Pb/min; 3mg Pb/kg, followed by 0.57 mg Pb/min as infusion; or equimolar sodium acetate in the control group). Zinc excretion in time controls was relatively constant over the 4 hr, but it rose above baseline values an average of 140 ng/min in 0.3 mg Pb/kg injected animals, and an average of 300 ng/min in 3 mg Pb/kg injected animals. Other indices of renal function, including excretion of protein, Na, K, and Mg, were relatively constant. Plasma Zn concentration was stable in time control and low Pb-administered animals, but rose significantly after the higher Pb dose. Clearance experiments using ^{65}Zn and *in vitro* ultrafiltration of plasma were performed in another series of dogs under antidiuretic conditions. Zn excretion (monitored by ^{65}Zn) was sevenfold higher in Pb-treated dogs; plasma Zn concentration was slightly, but not significantly, elevated. Ultrafiltrable Zn concentration was 2.5-fold higher and fractional Zn excretion was three times higher in Pb-treated dogs. The stop-flow pattern for Zn after Pb treatment showed no change in the distal tubular handling of Zn, but revealed prominent net proximal tubular secretion of Zn in all animals, a frequency statistically different from that observed in control animals. Thus, acute Pb treatment in dogs produced an increase in urinary Zn excretion which was related both to an increase in ultrafilterable plasma Zn and a change in renal tubular Zn transport. The plasma concentrations of insulin and glucagon were not altered by lead.

The purpose of this study was to determine whether lead can alter the renal excretion of the essential trace element, zinc. A number of studies have documented that the chronic administration to rats of large quantities of zinc (either in the diet or drinking water) can reduce the concentrations of lead in blood and other tissues (Cerklewski and Forbes, 1976; Thawley *et al.*, 1977; El-Gazzar *et al.*, 1978; Cerklewski, 1979). These changes were associated with a protective effect against the effects of lead on heme synthesis (Cerklewski and Forbes, 1976; Thawley *et al.*, 1977). This protective effect is very likely due not only to the diminished body burden of lead, but to a direct interaction between lead and zinc on certain enzymes, e.g., amino levulinic acid dehydratase (Meredith *et al.*, 1977; Cantrell *et al.*, 1977; Davis and Avram, 1978; Mauras and Allain, 1979). Paradoxically, the chronic administration of extremely large amounts of zinc to pigs (Hsu *et al.*, 1975) and horses (Hsu *et al.*, 1975) produced an increase in blood and soft-tissue lead, with either an increase in lead toxicity (Hsu *et al.*, 1975) or a decrease (Willoughby *et al.*, 1972).

To our knowledge there have been only two studies of the reverse relationship, i.e.,

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the effects of lead on zinc metabolism. In a study of mice, Seth et al. (1976) found no effect of lead administration on the concentrations of zinc in kidney and liver; El-Gazzar et al. (1978), in a larger study with rats, found that the addition of 100 ppm Pb to drinking water caused a decrease in plasma, liver, and bone zinc concentrations. In neither study was urinary excretion of zinc measured.

We have previously reported that both lead (Vander et al., 1977; Victery et al., 1979a,b) and zinc (Victery et al., 1981) undergo glomerular filtration and tubular reabsorption, and probably also tubular secretion. The reabsorption sites for these two elements are coextensive, raising the possibility that lead and zinc might compete for tubular transport pathways. It is also possible that some competition might exist between them for the low-molecular-weight ligands in plasma that permit their ultrafilterability. These facts, taken together with the Pb–Zn interactions described above, suggest the hypothesis that lead would alter the renal excretion of zinc. To test this hypothesis, and to obtain information concerning the mechanism of any observed change, we utilized renal clearance and stop-flow techniques in anesthetized dogs. The plasma concentrations of glucagon and insulin were also studied, since we have previously shown that glucagon can enhance zinc excretion (Victery et al., 1980); a rise in the plasma concentrations of these hormones might be expected since the hepatic clearance of another protein hormone, renin, is reduced under the conditions of these experiments (Goldman et al., 1980).

METHODS

Series A. Studies were performed on 15 nonfasted male dogs weighing 15–21 kg. The dogs were anesthetized with pentobarbital (30 mg/kg iv, with supplements given as required) and were prepared surgically for renal clearance experiments. Both cephalic veins, a femoral vein, and an artery were catheterized, as was the right ureter via a small flank incision. After completion of surgery, each dog received an iv prime of p-aminohippurate (PAH) and creatinine (0.25 and 0.6 g, respectively), dissolved in 20 ml saline, followed by an infusion of approximately 6 mg/min of each substance in a total of 0.21 ml isotonic saline/min. Following a 1-hr equilibration period, sequential 15-min urine collections were obtained from the ureteral catheter during the next 5 hr; each clearance value to be reported is the average of two consecutive 15-min collections. Twenty-milliliter arterial blood samples (replaced with an equal volume of isotonic saline) were obtained 45 min into each hr. All blood samples were immediately chilled and centrifuged at 4°C, and the plasma was frozen.

The first of the five hourly periods was a baseline period for all dogs. Following this, five dogs (high-Pb) received, over 10 min, 3 mg Pb (as Pb acetate)/kg iv as a prime dissolved in 10 ml 5% dextrose, followed by an infusion of 0.57 mg Pb (as acetate)/min in 0.21 ml of 5% dextrose. Five dogs (low-Pb) received one-tenth the dose of lead (prime 0.3 mg Pb/kg, followed by infusion of 0.057 mg Pb/min), with sodium acetate added to obtain a solution equimolar in acetate to the high-Pb dose. The five remaining dogs served as time controls and received a sodium-acetate prime and infusion, equimolar in acetate to the high-Pb dose. Throughout the experiment, mean arterial pressure, respiratory rate, hematocrit, and rectal temperatures were recorded.

Series B. In another group of four dogs, ^{65}Zn was used to permit the performance of stop-flow analysis (Victery et al., 1979b) and the determination of ultrafilterable zinc after the administration of Pb (3 mg/ kg). As described in detail elsewhere (Victery et al., 1980, 1981), the use of ^{65}Zn permits measurement of the very small concentrations of urinary zinc present during the high rates of urine flow characteristic of stop-flow analysis; it also avoids zinc contamination of in vitro filtration materials and facilitates measurement of the very small quantity of plasma zinc which is ultrafilterable (Victery et al., 1980, 1981). Animals were prepared surgically as described for series A, except that one additional venous catheter was placed in a saphenous vein for infusion of ^{65}Zn, and inulin rather than PAH was administered. ^{65}Zn infusion was given in isotonic saline at 0.21 ml/min; it contained carrier Zn at 10 μg Zn/kg/min and 0.30 mCi ^{65}Zn/hr. The ^{65}Zn infusion was begun 1.5–2 hr following the Pb dose and 1 hr prior to the beginning of clearance collections. After this hour, two 15-min urine collections were obtained while the animals were in the same antidiuretic state as the animals of series A. An infusion of 15% mannitol in isotonic saline at 0.75 ml/min/kg was then begun, and after stabilization of urine flow at 7–10 ml/min (30–40 min after starting the mannitol), two 5-min urine collections were obtained. During both the antidiuretic and mannitol clearance periods, two 15-ml vacutainers of nonanticoagulated blood were obtained and spun im-

mediately for separation of plasma. Ultrafiltrates of plasma were prepared (Vander *et al.*, 1977) using Amicon CF-50A (molecular weight cutoff 50,000) Centriflo membranes under 240g centrifugation. All filtrates were checked for protein (dipstick method) and values discarded if they exceeded 1 + (<20 mg/dl). Samples of blood, plasma, filtrate, and urine were placed in tared tubes for ^{65}Zn counting. Urinary ^{65}Zn excretion and percentage of plasma ^{65}Zn which was ultrafilterable were determined from these counts; the increments in both "cold" Zn excretion and the plasma and ultrafilterable concentrations of "cold" zinc produced by the carrier zinc infusion were calculated from the specific activity of the dose. No attempt was made to measure endogenous Zn concentrations in these experiments.

After completion of the mannitol clearances, a standard stop-flow procedure was performed (Malvin and Wilde, 1973): two or three urine samples ("free-flow" samples) were collected over 30 sec, and immediately thereafter the ureteral catheter was completely occluded for 5 min; following release of the occlusion, urine was collected in 30–50 sequential aliquots, each approximately 0.5 ml ("stop flow" [SF] samples). The concentration of ^{65}Zn in each stop-flow samples was divided by the [^{65}Zn] in the free-flow (FF) samples preceding the occlusion, and this ratio, [Zn]$_{SF}$/[Zn]$_{FF}$, was divided by the analogous ratio for inulin, [In]$_{SF}$/[In]$_{FF}$. The values obtained by this double ratio calculation reflect sequential changes in [Zn]$_{SF}$ relative to free-flow after elimination of any concentration changes due solely to water reabsorption. A value of this double ratio greater than unity signifies net zinc secretion into that sample during ureteral occlusion; a value less than unity signifies the occurrence of net Zn reabsorption from that sample.

The timing and protocols of both the clearances and stop-flow procedures of series B were set to mimic precisely a large series (14 dogs) of time control experiments performed simultaneously in our laboratory in association with other studies (Victery *et al.*, 1980, 1981); these 14 dogs had received precisely the same surgical preparation and infusions, and had clearances and stop-flow analysis performed at exactly the same length of time after the onset of the infusion of ^{65}Zn and inulin. Accordingly, this group of animals has been used as the control group to compare the effects of lead on the present dogs of series B. Such a control group is necessary since stop-flow analysis cannot be performed both before and after lead exposure, i.e., the Pb-exposed dogs cannot act as their own controls.

Analytical methods. Urinary and plasma concentrations of zinc were measured by atomic absorption (Varian Instruments, Model 375, 214 nm, slit = 0.5); urinary magnesium concentration was also measured by atomic absorption (202.5 nm, slit = 0.5). Urine sodium and potassium concentrations were determined by flame photometry. Urinary and/or plasma concentrations of

protein, creatinine, PAH, and inulin were determined by standard methods described previously (Victery *et al.*, 1979a). Plasma concentrations of glucagon and insulin were measured by radioimmunoassay (Hayaski *et al.*, 1977; Pek *et al.*, 1972).

The values for excretion of zinc, magnesium, protein, sodium, and potassium, and for glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were averaged for each 0.5-hr period (two consecutive 15-min collections) and treated as a single value.

All grouped data are presented as the mean ± one standard error (SE). Statistical comparisons in the Series A experiments are between experimental and time control groups, using regression analysis with Scheffé allowances for multiple comparisons. In series B, differences between Pb-treated and control animals were compared using Student's t test for group mean, or χ^2 for frequency data.

RESULTS

Series A

Figure 1 depicts the pattern of urinary zinc excretion over time in each of the three
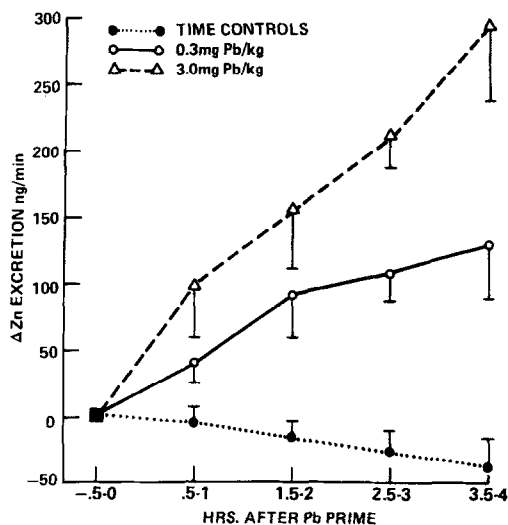

FIG. 1. Changes in urinary zinc excretion for the three groups of dogs are plotted against time after administration of the Pb or acetate prime. Excretion in the baseline half-hour clearance period (−0.5–0 hr) has been set at zero, with successive time points plotted as the mean change from this baseline value (control dogs = 88.9 ± 30.9, low-Pb = 46.5 ± 7.0 and high-Pb = 49.5 ± 3.8). See text for statistical analysis of data.

experimental groups of Series A. For clarity, excretion during the baseline clearance period (−0.5–0 hr) has been set at zero; values of subsequent collections are the mean change (±1 SE) in Zn excretion for each group. Zn excretion, in nanograms per minute, for the baseline clearances for control dogs averaged 88.9 ± 30.9, low-Pb animals = 46.5 ± 7.0, and high-Pb 49.5 ± 3.8. There were no significant differences between these baseline group means; the larger mean for the control animals was attributable primarily to one animal whose initial zinc excretion was 200 ng/min. Over the remaining 4-hr time course of the experiment, zinc excretion in control animals tended to decrease; the mean decrease at 4 hr was 36 ng/min. In contrast, zinc excretion in both Pb-treated groups rose dramatically and continuously beginning 0.5 hr after lead exposure. Four hours after the high-Pb dose, zinc excretion was 294.3 ± 56.0 ng/min above the baseline clearance value (a sevenfold increase). Four hours after the low-Pb dose, zinc excretion was 138.8 ± 51.4 ng/min greater than before Pb exposure (a fourfold increase). Comparison of the slopes of the curves for treated animals to that of controls yielded a *p* value for the low dose of <0.01, and <0.0002 for the high dose.

Figure 2 presents the changes in plasma zinc concentrations; again, all values have been normalized with respect to the first (baseline) values; there were no differences between the baseline group means (control = 65.0 ± 4.5 μg/100 ml; low dose = 70.6 ± 7.4; high dose = 67.6 ± 14.5). Plasma zinc in control animals fell slowly throughout the experiment, and at 4 hr was about 20 μg/100 ml lower than the baseline value. We have consistently observed this pattern in control animals; the cause is unknown but may relate to the trauma of anesthesia and surgery. Plasma zinc in animals which received the low dose of Pb tended to remain quite stable over time, and this pattern was not significantly different from that for the time controls. The larger dose of Pb pro-
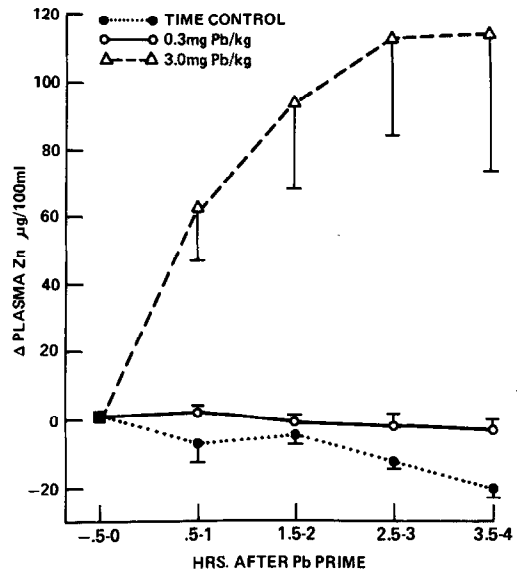

FIG. 2. Changes in plasma zinc concentration are plotted against time after Pb or acetate prime. Values for the baseline blood collection (−0.5–0 hr) have been normalized to zero, with subsequent values plotted as the change from this baseline value (control dogs = 65.0 ± 4.5 μg/100 ml, low Pb = 70.6 ± 7.4, high Pb = 67.6 ± 14.5). See text for statistical analysis of data.

duced a marked, significant (*p* < 0.01) rise in plasma zinc concentration over time; by 1 hr, there was a 64 μg/100 ml increase, and by 4 hr, the increase was 113 μg/100 ml (a two- to threefold increase).

The increase in plasma zinc might be due to displacement of zinc from erythrocytes to plasma. To test this possibility, a small sample of whole anticoagulated blood was removed from a dog before Pb treatment. To one aliquot of the blood, sufficient Pb acetate was added *in vitro* to increase Pb concentration by 400 μg/100 ml (the estimated blood concentration of lead present 2 hr after the lead prime). Both aliquots were incubated at 37°C for 1 hr, then centrifuged to separate the plasma. There was no difference in plasma zinc concentrations between the two aliquots.

Other renal function data obtained in these experiments are summarized in Table 1. There were no significant effects of Pb on the excretion of three other cations—mag-

TABLE 1

SUMMARY OF RENAL FUNCTION PARAMETERS AFTER EXPOSURE TO AN ACUTE Pb DOSE

Variable	Pb dose (mg/kg)	Time after Pb prime, hr.				
		−0.5–0	0.5–1	1.5–2	2.5–3.0	3.5–4.0
GFR (ml/min)	0	45.2 ± 2.3	48.1 ± 2.6	50.4 ± 3.0	53.7 ± 2.8	54.6 ± 2.6
	0.3	40.6 ± 2.9	44.5 ± 2.5	42.7 ± 2.4	40.7 ± 3.7	42.4 ± 4.4
	3.0	41.4 ± 8.4	36.6 ± 6.2	35.1 ± 6.1	28.1 ± 4.5*	27.2 ± 4.5*
ERPF (ml/min)	0	84.9 ± 1.8	88.3 ± 5.1	93.2 ± 5.8	97.7 ± 5.1	98.7 ± 5.5
	0.3	82.9 ± 4.8	92.2 ± 8.3	85.2 ± 5.7	80.8 ± 4.8	87.8 ± 6.3
	3.0	97.7 ± 25.6	88.6 ± 19.0	79.9 ± 13.5	62.4 ± 8.9*	60.4 ± 11.8*
$U_{Na}V$ (µEq/min)	0	12.6 ± 9.9	18.6 ± 14.7	21.4 ± 14.8	18.7 ± 11.6	20.3 ± 12.5
	0.3	15.1 ± 4.7	14.6 ± 2.8	13.1 ± 3.1	14.2 ± 4.3	15.7 ± 6.8
	3.0	9.9 ± 3.2	7.4 ± 2.8	11.4 ± 7.2	10.8 ± 4.6	7.1 ± 2.4
$U_{K}V$ (µEq/min)	0	22.8 ± 9.4	24.2 ± 7.7	27.8 ± 7.0	30.3 ± 6.6	29.1 ± 7.2
	0.3	27.2 ± 6.3	24.4 ± 5.5	23.4 ± 5.5	23.5 ± 4.8	24.1 ± 5.2
	3.0	24.5 ± 3.8	23.2 ± 5.3	18.7 ± 1.8	20.5 ± 4.2	17.0 ± 2.2
Urine flow (ml/min)	0	0.141 ± 0.030	0.168 ± 0.036	0.177 ± 0.031	0.182 ± 0.025	0.177 ± 0.012
	0.3	0.169 ± 0.021	0.150 ± 0.013	0.149 ± 0.010	0.169 ± 0.019	0.194 ± 0.047
	3.0	0.129 ± 0.018	0.170 ± 0.040	0.195 ± 0.044	0.400 ± 0.177	0.294 ± 0.073
$U_{Mg}V$ (µg/min)	0	10.3 ± 2.9	9.6 ± 3.4	8.1 ± 5.1	5.9 ± 0.8	5.7 ± 1.3
	0.3	9.5 ± 6.4	15.7 ± 5.6	9.1 ± 3.5	7.4 ± 3.1	5.7 ± 2.5
	3.0	14.8 ± 5.0	17.0 ± 6.9	6.6 ± 2.0	6.6 ± 1.7	7.3 ± 1.6
$U_{protein}V$ (mg/min)	0	1.7 ± 0.1	1.8 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	2.0 ± 0.12
	0.3	2.3 ± 0.3	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2
	3.0	2.4 ± 0.4	2.2 ± 0.3	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.2

* $p < 0.05$, compared to control animals at the same time.

nesium, sodium, and potassium; if anything, there was a slight (but not significant) tendency for sodium excretion to decline in the last collection period after high Pb dose. Since most plasma zinc is protein bound, increased excretion of protein could contribute to increased urinary zinc levels; however, urinary protein excretion was similar in the three groups of animals (Table 1).

GFR was stable in the control and low-dose animals, but fell significantly in high-dose animals by 3 to 4 hr after Pb exposure. A similar decrease was observed for ERPF at 3 to 4 hr. There were no differences in urine flow rates between the treatment groups; the tendency for urine flow to be elevated at 3 and 4 hr in the high-Pb group was due to very high values observed in one animal at those times. Throughout the experiment there were no changes within or between groups in mean arterial pressure, respiratory rate, hematocrit, or rectal temperature.

The data for the plasma concentrations of glucagon and insulin are summarized in Fig. 3. For the time controls, plasma glucagon increased slightly in the last 2 hr, whereas insulin generally decreased. There was a tendency for plasma glucagon to increase over time in both Pb-treated groups (the increase over baseline was approximately 50% by hours 3 and 4), but the observed differences between this pattern and that of the time control group were not statistically significant, either by regression analysis of the entire curves or by analysis of variance at each time. The jump in glucagon at 4 hr in the high-Pb group was due to a very large increase in one of the five animals. Plasma insulin concentrations remained stable throughout the experiment in the Pb-treated groups and were not significantly different from those of the time control groups.

Series B

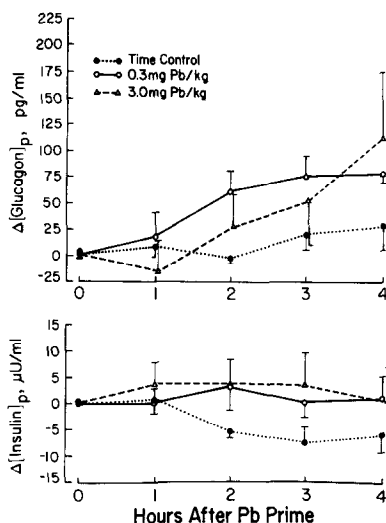Clearance data obtained for antidiuretic dogs using ^{65}Zn are summarized in Fig. 4;

FIG. 3. Changes in plasma glucagon concentration (upper panel) and plasma insulin concentration (lower panel) for the three groups of animals. All values are presented as the change from the value during baseline collection period at 0 hr after Pb prime (baseline insulin values for control dogs = 21.1 ± 4.5, low-Pb = 19.1 ± 6.0, and high-Pb = 19.0 ± 4.5; baseline glucagon values for control dogs = 133.5 pm 47.1, low-Pb = 205.0 ± 39.3, and high-Pb = 215.2 ± 15.9). See text for statistical analysis of data.

each panel compares values obtained from control dogs with those from dogs 3 hr after Pb exposure. Zn excretion (Fig. 4A) was 7.6-fold higher in the Pb-treated dogs; this increment is comparable to the rise in endogenous zinc excretion seen in Series A dogs. It should be reemphasized that all data for Series B dogs represent only the increment in Zn excretion caused by infusion of carrier Zn along with the ^{65}Zn. The increment in plasma zinc (Fig. 4B) caused by the Zn infusion was not statistically significant. The increment in ultrafilterable zinc concentration (Fig. 4C) produced by the zinc infusion was two and one-half times higher in Pb-exposed animals ($p < 0.01$); the percentage of the increment in plasma zinc which was ultrafilterable was not different between the two groups. The clearance of ultrafilterable ^{65}Zn was 1.67 ± 0.30 ml/min in control dogs and 4.75 ± 1.37 after Pb exposure; this difference was significant ($p < 0.05$). Frac-
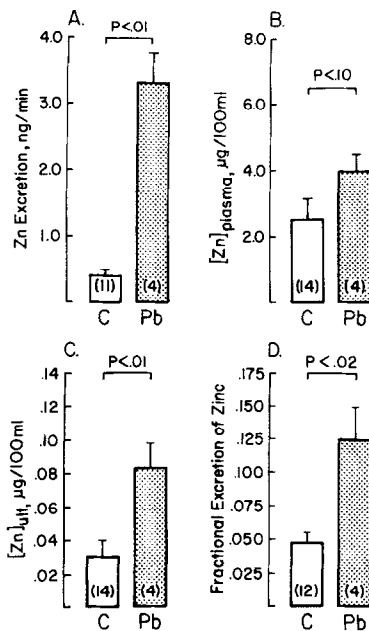
FIG. 4. Clearance data obtained in control dogs (shown in open bars) and in Pb-treated dogs (hatched bars) using ^{65}Zn infusion with 10 μg carrier Zn/kg/min. All values are presented as the average increment (± 1 SE) in Zn values calculated from the specific activity of the infused solution. See text for details. (A) Increment in urinary Zn excretion; (B) increment in plasma Zn concentration; (C) increment in ultrafilterable Zn concentration; (D) fractional excretion of Zn ($C_{ult\text{-}Zn}/C_{cr}$).

FIG. 5. The mean fractional excretion of Zn (+1 SE) for control and Pb-treated animals during antidiuresis and osmotic diuresis (mannitol). Statistical comparison is discussed in the text.

tional zinc excretion (the ratio of C_{ultZn} to C_{cr}), illustrated in Fig. 4D, was threefold higher ($p < 0.02$) in the Pb-exposed dogs than in the controls. The effects of mannitol diuresis on fractional zinc excretion in control and Pb-exposed dogs are summarized in Fig. 5. In control dogs, mannitol (an osmotic diuretic) increased fractional excretion from 0.044 $\pm$ 0.008 to 0.100 $\pm$ 0.020 ($p < 0.02$); in Pb-exposed dogs, fractional excretion was already elevated (0.123 $\pm$ 0.025) and mannitol produced only a small further (nonsignificant) elevation to 0.142 $\pm$ 0.022. Ultrafilterable zinc concentrations in both control and Pb-exposed animals were not altered by mannitol.

Stop-flow analysis permits crude localization of sites of tubular reabsorption or secretion of transported substances. In pre-
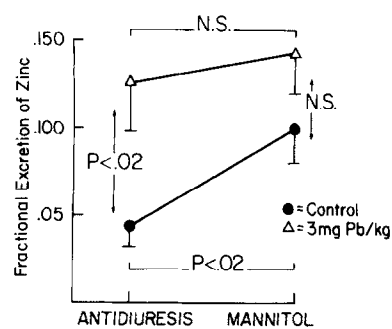
vious studies (Victery *et al.*, 1981), we have reported that control stop-flow patterns for Zn invariably showed net reabsorption in the distal nephron but not the proximal nephron; proximal reabsorption was seen only during infusion of citrate or histidine. The stop-flow pattern dipicted in Fig. 6 is typical of those obtained after Pb exposure in all four dogs. Prominent net Zn reabsorption occurred in the distal nephron segment corresponding to that for the distal minimum for sodium; for the control and Pb-treated groups, the mean "double ratios" (Zn/In) at the nadir of the Zn reabsorptive dip averaged 0.24 $\pm$ 0.07 and 0.31 $\pm$ 0.04, respectively, values which are not significantly different from each other. The absolute concentrations of the carrier Zn administered with ^{65}Zn at the nadirs were 0.062 $\pm$ 0.019 vs 0.283 $\pm$ 0.130 ng/ml, also not significantly different. The results, therefore, fail to demonstrate Pb-induced inhibition of distal Zn reabsorption. The stop-flow pattern for Zn shown in Fig. 6 also reveals prominent net proximal nephron secretion of Zn; the double ratio in this experiment reached a maximum value of 1.75 and then returned to preocclusive free-flow values. After Pb exposure, definite net secretion of Zn by the proximal nephron was observed in all four animals (double ratio = 1.94, 1.75, 1.6, and 1.32). In contrast, definite net secretion occurred in only 4 of 14 control animals. This difference in
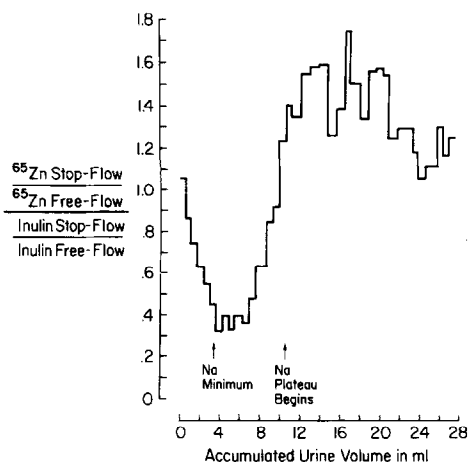
FIG. 6. A typical stop-flow pattern obtained 3 hr after Pb treatment (approximately 40 min after initiation of mannitol diuresis). See text for description of Zn pattern. The sodium concentration pattern (not plotted here) provides coarse localization of nephron segments in that the minimal sodium concentration (about 3.5 ml in Fig. 6), ie., the point of maximal reabsorption, corresponds to fluid trapped during ureteral occlusion in distal portions of the nephron (probably including collecting duct, distal tubule, and the ascending loop of Henle); the beginning of the plateau in sodium concentration (approximately 11–12 ml in Fig. 6) at its preocclusive values is presumed to represent fluid trapped only in the proximal portions of nephrons during occlusion.

frequency of occurrence of net proximal secretion is highly significant ($P < 0.01$ by χ^2 analysis).

DISCUSSION

These experiments demonstrate that the acute iv administration of lead to anesthetized dogs causes a dose-dependent, marked increase in urinary excretion of zinc. In this study, the effect was specific, in that there were no associated changes in urine flow or in the excretion of another divalent cation (magnesium) or of two monovalent cations (sodium and potassium). We have previously reported (Mouw et al., 1978) that, under similar experimental conditions, 3 mg Pb/ kg produced significant increases in the urinary excretion of potassium and calcium and an increased sodium excretion of borderline

significance (magnesium was not previously studied). We have no explanation for the failure to observe these changes in the present experiments; however, it must be emphasized that the previously observed changes in the other cations were, proportionally, of much lesser magnitude (approximately twofold) than the present changes in zinc excretion (seven- to eightfold) at the same dose of lead.

What are the mechanisms for the Pb-induced changes in zinc excretion? We have previously shown that the urinary excretion of zinc is the result of glomerular filtration, tubular reabsorption, and, possibly, tubular secretion (Victery et al., 1981). Thus, the effect(s) could, theoretically, be on GFR, ultrafilterable zinc concentration, or the tubular transport pathways. An increase in GFR can be ruled out since GFR either remained unchanged or, at the high dose, decreased. Changes in the filtration of protein (carrying bound zinc) also seem very unlikely since protein excretion was not altered by lead.

Plasma zinc concentration did rise markedly at the higher dose of lead and this raised the strong possibility that ultrafilterable zinc, the important variable, might also have risen. To test this possibility, we performed the ^{65}Zn experiments. The rationale for this approach has been described in detail elsewhere (Victery et al., 1980); the crucial point validating its use is that Pb increased urinary excretion of acutely administered ^{65}Zn to essentially the same degree that it increased urinary excretion of endogenous zinc. This documents that the ^{65}Zn had equilibrated adequately with whatever pools of zinc were contributing to Pb-alterable zinc excretion. The ultrafilterable data indicated that, at least at the higher dose, Pb did, indeed, elevate ultrafilterable zinc concentration, a phenomenon which should contribute to increased excretion. However, even at the higher dose of lead, the increase in ultrafilterable zinc was proportionally much less than the rise in zinc excretion; the

resulting large increase in fractional excretion of zinc is a strong indication that a change in tubular transport also plays a role in the induced elevation of zinc excretion. The source of the increased plasma and ultrafilterable zinc induced by lead is unknown but is unlikely to be the erythrocytes, as demonstrated by the *in vitro* experiment described in the methods section.

Stop-flow analysis was used to evaluate the possible tubular site influenced by lead and whether reabsorption was being inhibited or secretion stimulated. The major effect was clearly on the proximal nephron; the stop-flow patterns document the existence of prominent proximal secretion of zinc in all Pb-treated dogs, in contrast to the much lower frequency of such secretion seen in control dogs. However, this finding does not conclusively prove that increased proximal secretion is the effect produced by lead; decreased proximal reabsorption is just as likely a possibility. If bidirectional transport normally exists for zinc in the proximal nephron (Victery *et al.*, 1981), inhibition of reabsorption would permit secretion, unopposed, to become manifest. Accordingly, the present data do not permit a distinction between Pb-induced stimulation of secretion or inhibition of reabsorption. Theoretically, the latter would seem the more likely, assuming that lead and zinc compete for a transport pathway. Indirect evidence also favoring inhibition of proximal reabsorption is the fact that lead had relatively little further enhancing effect on zinc excretion when the latter had already been raised by mannitol diuresis.

Whether lead influences proximal zinc transport indirectly or via a direct renal effect is not answered by the present experiments. They do, however, rule out the possibility that increased plasma glucagon or decreased plasma insulin is responsible, since these hormones showed little if any, alteration during Pb exposure. This failure to increase is, however, of considerable interest in another context; it suggests that the abil-ity of lead to block the hepatic clearance of renin (Goldman *et al.*, 1980), another protein hormone, may be a highly specific effect.

Finally, the most important question for future investigation raised by these experiments is whether chronic exposure to low doses of lead might similarly elevate zinc excretion. Such an effect would be in addition to any Pb-induced inhibition of gastrointestinal zinc absorption and might importantly contribute to the development of zinc deficiency.

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