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Mobilization and Redistribution of Lead Over the Course of Calcium Disodium Ethylenediamine Tetraacetate Chelation Therapy[1]

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ABSTRACT

After its successful application to the treatment of acute Pb poisoning, Ca disodium EDTA came into routine clinical use for diagnosis and treatment of subacute and chronic Pb poisoning. Despite widespread use, few definitive conclusions have emerged about the sources of Pb mobilized by Ca disodium EDTA. Furthermore, the possibility that mobilized Pb may be redistributed has been suggested. The current studies indicate that the standard therapeutic protocol for Ca disodium EDTA has little impact on critical organs such as brain and liver and moreover, that diagnostic Ca disodium EDTA chelation may even increase the concentration of Pb in these tissues. After a 3 to 4 month exposure to Pb acetate in drinking water, different groups of rats received daily i.p. injections of saline (control), 75 or 150 mg/kg of Ca disodium EDTA for either 1, 2, 3, 4 or 5 days and were then sacrificed 24 hr after the final injection. Tissue analyses indicated that Pb was mobilized from bone and kidney and redistributed initially to both brain and liver. Levels in both brain and liver declined with subsequent Ca disodium EDTA injections, but no net loss from either tissue occurred over the 5-day treatment period despite a decline in blood Pb levels and a marked enhancement of urinary Pb excretion. These findings stress the need for further investigation of Ca disodium EDTAs effects and for parallel evaluation of alternate chelating agents, and suggest that a re-evaluation of both the diagnostic and therapeutic roles of Ca disodium EDTA may be advisable.

After the introduction in 1950 of Ca disodium EDTA, a nonspecific metal-binding agent, the mortality rate for acute Pb encephalopathy in children dropped from about 60% to 20 to 30% (Foreman, 1961). The efficacy of Ca disodium EDTA for occupational Pb poisoning was established as well (Reiders *et al.*, 1955). Parenteral administration of Ca disodium EDTA, sometimes accompanied by administration of British anti-Lewisite (*i.e.*, 2,3-dimercaptopropanol), remains the predominant treatment for elevated Pb burden. Another important role for Ca disodium EDTA is as a diagnostic tool. In the Ca disodium EDTA mobilization test, elevation of urinary Pb excretion within a specified time after the administration of one (*e.g.*, Teisinger and Srbova, 1959; Chisolm, 1971) or sometimes two (*e.g.*, Markowitz and Rosen, 1984) injections of Ca disodium EDTA, is presumed to reflect an elevated body burden and to warrant additional treatment with Ca disodium EDTA, usually a 5-day course of therapy. More recently, Ca disodium EDTA chelation has been promoted for conditions ranging from "heart disease to gangrene to senility" (Walker and Gordon, 1982).

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The source of the body stores of Pb mobilized in response to Ca disodium EDTA is the subject of considerable dispute. Hammond and colleagues (Hammond *et al.*, 1967; Hammond, 1971) assessed changes in tissue Pb burden after i.v. Ca disodium EDTA administration. These reports concluded that Ca disodium EDTA mainly released Pb deposited in bone, although a direct, independent, effect on soft tissue was postulated as well. Bone depletion of Pb occurred during the period of Ca disodium EDTA infusion, whereas soft tissue mobilization generally followed the infusion period. The delay in onset of soft tissue mobilization was ascribed to the formation of Pb-EDTA ternary complexes. In contrast, Castellino and Aloj (1965) reported that i.v. Ca disodium EDTA did not mobilize Pb from bone, but from soft tissues such as liver, kidney, lung, spleen and heart. Hammond (1971) cited differences in skeletal tissue sampling procedures as one potential source of the discrepancy between these findings.

The studies above administered Pb i.v., but the predominant problems of Pb toxicity today arise from chronic low-level exposure to sources such as street dust. Intelligence test scores and other psychometric variables in children have been associated with such environmental exposures in several studies (Needleman *et al.*, 1979; Yule *et al.*, 1981; Winneke *et al.*, 1983;

ABBREVIATIONS: ZPP, zinc protoporphyrin; AAS, atomic absorption spectrophotometry.

Bellinger *et al.*, 1987). On the basis of a continually declining definition of a safe blood Pb level, some groups currently recommend the Ca disodium EDTA mobilization test for children whose values meet or exceed 30 μg/dl (Saenger *et al.*, 1982), with possible subsequent chelation therapy.

Information on the pattern of tissue Pb mobilization produced by Ca disodium EDTA after such chronic low-level exposures is both limited and discrepant. Goyer and Cherian (1979) injected 400 mg/kg of Ca disodium EDTA i.p. to rats for 5 consecutive days after a 10-week exposure to 1000 ppm of Pb acetate in drinking water. The animals were sacrificed at the end of the treatment period. Subsequent analyses revealed a loss of Pb from bone, liver and kidney, but not from brain. When Ca disodium EDTA was administered i.p. in another study for 3 days every 2 weeks over 12 weeks, only kidney Pb levels declined significantly, whereas brain, bone, liver and blood Pb levels were unchanged (Goyer *et al.*, 1978). Bankowska and Hine (1985) also administered 50 mg/kg of Ca disodium EDTA i.p. to rats 3 times weekly for 6 weeks, after a 4-week exposure to a 350 ppm Pb acetate drinking solution. This regimen reduced brain, liver and calvarium (flat bone) Pb, but did not affect kidney or femur burden.

In all three of the above studies, tissue Pb levels were measured after chelation treatment ended. The conclusions from such a protocol, however, could be confounded by the possibility that Pb mobilized during repeated Ca disodium EDTA administrations may be redistributed. Hammond *et al.* (1967) found direct evidence for such an effect, reporting that muscle, lung and genitalia served as temporary repositories for Pb during and after the chelation process.

The study reported here examined the pattern and sources of tissue Pb mobilization over the course of five injections of Ca disodium EDTA (a standard therapeutic protocol) after prolonged low level Pb exposure. To evaluate possible redistribution of mobilized Pb, different groups of rats received a daily i.p. injection of saline or Ca disodium EDTA for 1, 2, 3, 4 or 5 days. Urinary Pb excretion and tissue Pb burdens were determined 24 hr after the final injection.

Methods

Animals and Pb exposure. Long-Evans hooded rats (105 male), 21 days of age, obtained from Blue Spruce Farms (Altamont, NY) were divided into 15 groups of 7 rats each of approximately equal average weight. They then started on an exposure regimen of 50 ppm of Pb acetate in drinking water. This exposure concentration was chosen because of extensive information obtained from previous experiments (Cory-Slechta and Thompson, 1979; Cory-Slechta *et al.*, 1983) on its behavioral effects and resulting tissue Pb concentrations. Animals were housed individually in a colony room maintained at 22 ± 2°C with a 12-hr light-dark cycle and fed the U.S. Biochemical Corp. (Cleveland, OH) semipurified mouse-rat diet (American Institute of Nutrition recommended diet for rats: basically a casein high nitrogen, cornstarch, sucrose, fiber-celufil, corn oil diet that maintains vitamin and mineral levels at required rather than excess levels). The rats were allowed free access to food until body weights reached 300 g, the value at which they were maintained *via* restricted food access for the remainder of the experiment.

This feeding regimen was designed to produce animals comparable in size and nutritional history to those assigned to concurrent behavioral experiments (D. A. Cory-Slechta, in preparation). After 3 to 4 months, depending on the availability of metabolic cages, Pb exposure ended and the animals were moved to Nalgene metabolic cages. Ca disodium EDTA administration began after 24-hr urine and blood samples were collected. Different groups of rats were then injected daily with saline, 75 or 150 mg/kg of Ca disodium EDTA i.p. for either 1, 2, 3, 4 or 5 days. A 24-hr urine sample was collected after the final injection and the animals then sacrificed by ether. Liver, brain, femur, kidney and blood were collected for Pb determinations; blood also was used for ZPP determination. All rats within a specified injection group were subjected to these procedures at the same time.

To evaluate further the effects of a single injection of Ca disodium EDTA on brain Pb levels, additional groups of rats were exposed to either 25 or 500 ppm of Pb acetate under conditions identical to those described above. Blood was collected before a single injection of saline, 75 or 150 mg/kg of Ca disodium EDTA. A 24-hr urine sample and blood and brain were harvested 24 hr after the injection.

Tissue Pb determination. Blood Pb (analyzed without digestion of the sample) was measured by anodic stripping voltammetry (Environmental Science Associates, Bedford, MA, model 3010 Trace Metals Analyzer) according to the method described by Morell and Geridhar (1976). The linear range of this method is reported to be 5 to 700 μg/dl with a detection limit of 5 μg/dl. ZPP determinations were made using the AVIV ZPP Hematoflourometer. Analyses were carried out in the analytical core unit of the Environmental Health Sciences Center, which participates in both the Centers for Disease Control and the State of Pennsylvania Department of Health blood Pb and erythrocyte protoporphyrin proficiency testing programs. Relationships between values determined in the core facility and testing program values, calculated over 1-year periods, have ranged from $r^2 = 0.96$ to 1.00 over the period 1984 to 1986.

For brain, liver and kidney, wet tissue weights were recorded. After digestion with a 1:1 mixture of nitric and perchloric acids, samples were brought to a constant volume, and determination of tissue Pb content performed by graphite AAS (Hitachi 170-70). For each analysis, controls (blanks) and standards were carried in 0.7% nitric acid and calibration curves determined at the beginning and end of a sample run. Because extra tissue samples were not available, recoveries were based on standards carried through the digestion procedure, and resulting values were: 96 ± 9% for liver, 105 ± 5% for kidney and 110 ± 10% for brain.

Bone (femur) was scraped of all adhering tissue and ashed in a muffle furnace at 450°C for a minimum of 48 hr. Samples were then ground with an agate and mortar pestle. A portion of this ash (approximately 20 mg) was dissolved in 500 μl of concentrated nitric acid and brought to a constant volume for AAS analysis. Standards were prepared as above. Recoveries (119 ± 18%) were determined by adding a known amount of Pb to a pooled bone ash sample.

Urine analysis involved a modification of procedures described by Leung and Henderson (1983). Samples were centrifuged and an aliquot taken for further dilution with 0.7% nitric acid. Analysis was performed by graphite furnace AAS (Perkin-Elmer 3030) using a L'vov platform and a matrix modifier (ammonium phosphate and magnesium nitrate). Triton X-100 was added to each sample for easier pipetting. Recoveries, based on a known amount of Pb added to pooled samples, were 101 ± 9% and the limit of detection for this method is 0.5 ng/ml.

Statistical Evaluation

Tissue Pb levels over five Ca disodium EDTA injections. After log transformation of the data to stabilize variability, a two-way analysis of variance (Ca disodium EDTA dose × number of injections) was used to evaluate the differences in tissue Pb levels resulting from chelation. In some cases, from one to three (in one group) values were missing from a group data set. To achieve a balanced analysis of variance, the mean of the observations in the group was substituted for each missing value. When significant main effects or interactions were found, the nature of the effect was evaluated by the method of contrasts with a single degree of freedom. These contrasts compare means of the various treatment and injection combinations.

The main-effect contrasts included: pairwise comparisons of the two dose groups with each other and with saline (collapsing each dose group across injections) and evaluation of linear, cubic, quadratic and quartic

trends across the series of injections (collapsing across Ca disodium EDTA dose). The latter contrasts describe the trends in the various tissue Pb levels with successive Ca disodium EDTA injections. Trend contrasts were examined if the analysis of variance indicated no statistically significant interaction between Ca disodium EDTA dose and number of injections.

When significant interactions were present, each Ca disodium EDTA dose group was compared to the saline group in each specific injection group (*e.g.*, injection group, saline *vs.* 75 mg/kg; injection group, saline *vs.* 75 mg/kg, etc.). In addition, doses of Ca disodium EDTA and saline were compared for differences in trends across the series of injections. Contrasts were compared only if the corresponding analysis of variance F test was significant (with the exception of bone, see "Discussion"). In this way, overall control of error rates was achieved in the analysis of data from each tissue. This analysis included the examination of residuals as a check for outliers and departures from assumptions.

Brain Pb values after a single Ca disodium EDTA injection. Brain Pb values in the groups that received a single injection of Ca disodium EDTA after 25 or 500 ppm of Pb acetate exposure were analyzed by one-way analysis of variance. Subsequent post-hoc analyses used a nonpooled two-sample *t* test that assumes that the S.D.s of the populations are unequal and adjusts the degrees of freedom appropriately, providing a more conservative approach. These analyses were based on the MINITAB algorithms (Ryan *et al.*, 1985). In all cases, statistical significance was defined as $P \leq .05$.

Results

The effects of Ca disodium EDTA on two of the standard clinical indicators of chelation efficacy, blood and urine Pb concentrations, are presented in figure 1 and table 1 (blood Pb analysis). Ca disodium EDTA administration decreased the level of Pb in blood (table 1, lines 1, 6 and 7) although values never declined to the range of nonexposed controls (<5 μg/dl, Cory-Slechta *et al.*, 1983, 1985). This decline in blood Pb was evident even after the first Ca disodium EDTA injection and the difference between saline-control and Ca disodium EDTA-injected groups remained uniform across the series of injections (table 1, lines 2 and 9). The lack of an interaction between Ca disodium EDTA dose and number of injections (table 1, line 3) confirmed that no additional reduction in blood Pb level was produced after the first injection, and indicated parallelism of the three dose curves. The two doses of Ca disodium EDTA did not differ in effect (table 1, line 8). Main effect trend contrasts revealed a significant cubic component (table 1, line 11) in the saline and Ca disodium EDTA curves. As indicated in figure 1, the cubic component derives from the decline in blood Pb from injection 1 to 2, the increase from injection 2 to 4 and a slight drop after the final injection. This oscillation in blood Pb values may be a response to the termination of Pb exposure, which happened simultaneously in chelated animals and saline-injected controls, as well as reflecting individual differences and restricted sample size.

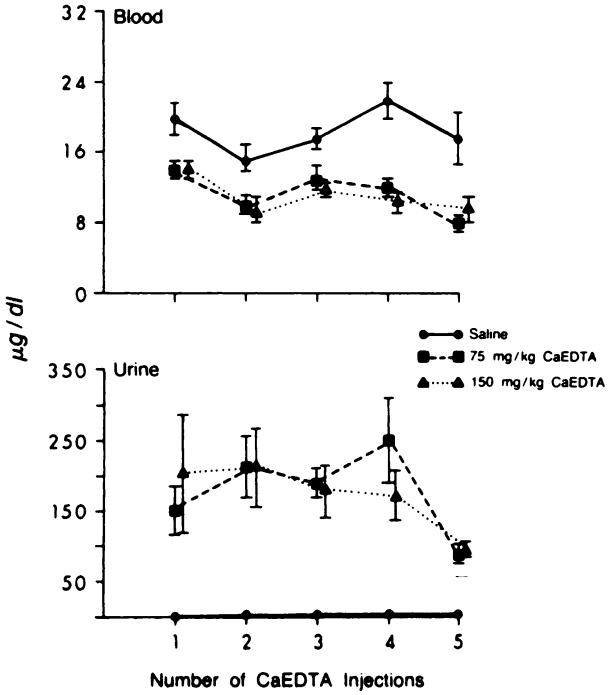

Fig. 1. Concentrations of Pb in blood (top panel) and urine (bottom panel) as a function of the number of Ca disodium EDTA injections. Different groups of animals (n = 7 per group) received a daily i.p. injection of saline, 75 or 150 mg/kg of Ca disodium EDTA for 1, 2, 3, 4 or 5 days and were sacrificed 24 hr after the final injection. Each data point represents the resulting group × ± S.E.

As expected, urinary Pb excretion increased substantially after Ca disodium EDTA injections, as compared to the minimal values of saline-injected controls. As plotted in figure 1 (bottom panel), little evidence for a differential effect of Ca disodium EDTA dose was apparent. Urinary excretion appeared to decline after the fifth Ca disodium EDTA injection relative to the concentrations measured after four or fewer injections of the chelating agent.

Pb was mobilized from bone in response to Ca disodium EDTA (fig. 2, top panel; table 2, lines 1, 6 and 7) although not in a dose-related manner (table 2, line 8). No significant interaction was found between the Ca disodium EDTA dose and the number of injections (table 2, line 3) indicating that the difference between saline-control and Ca disodium EDTA groups in bone Pb remained uniform over the series of injections. Also, the oscillation of bone Pb values seen in figure 2 resulted in a significant cubic and quadratic trend across injections in the three dose groups (table 2, lines 10 and 11).

Kidney Pb levels declined markedly in response to both doses of Ca disodium EDTA (fig. 2, middle panel; table 3, lines 1, 6 and 7) with the effect achieving statistical significance after the second injection of each dose (table 3, lines 11–13 and 15–18). In this case, the difference between the 75- and 150-mg/kg doses was significant (table 3, line 8), confirming a dose-effect relationship. Kidney Pb levels declined further during the series of injections (fig. 2; table 3, line 2). There was a significant linear trend to this decrease for each dose group of Ca disodium EDTA as compared with saline-control (table 3, lines 19 and 20), confirming an additional removal of kidney Pb burden by successive injections of Ca disodium EDTA, whereas little, if any, net loss occurred as a result of termination of Pb exposure alone (saline group).

Ca disodium EDTA treatment produced an initial redistribution of Pb into liver (fig. 2, bottom panel; table 4). This elevation of Pb content was observed in the liver after the first injection of the higher dose of Ca disodium EDTA and after two injections of the lower dose, suggesting a type of dose-effect relationship (table 4, lines 14 and 10). Subsequent mobilization of Pb from the liver was minimal (fig. 2), attaining statistical significance only for the highest Ca disodium EDTA dose after five injections (table 4, line 18). There was a significant linear decline in Pb concentration over the course of Ca disodium EDTA injections, but no such trend in the saline-injected group (fig. 2; table 4, lines 19 and 20). This difference probably reflects

TABLE 1

Effects of Ca disodium EDTA chelation on blood Pb: analysis of variance

Line	Source of Variation	SS	dF	F	P	Significant
1	Dose (D)	4.06	2	13.65	.0001	Yes
2	No. of injections (I)	1.58	4	2.66	.0379	Yes
3	Interaction (D × I)	1.15	8	0.97	.464	
4	Error	13.37	90			
5	Corrected total	20.17	104			
			Coefficient of determination 34%			
Line	**Contrasts of Main Effect**	**SS**	**dF**	**F**	**P**	**Significant**
6	(P)[a] Saline *vs.* D = 75 mg/kg	3.045	1	20.49	.0001	Yes
7	(P) Saline *vs.* D = 150 mg/kg	3.041	1	20.47	.0001	Yes
8	(P) D = 75 mg/kg *vs.* D = 150 mg/kg	0.000	1	0.00	.9981	
9	(R)[b] Linear	0.088	1	0.59	.4430	
10	(R) Quadratic	0.413	1	2.78	.0988	
11	(R) Cubic	1.059	1	7.13	.0090	Yes
12	(R) Quartic	0.018	1	0.12	.7303	

[a] P, pairwise contrasts of doses, collapsed across the series of injections.
[b] R, regression or trend contrasts for the number of injections averaged over all Ca disodium EDTA doses.

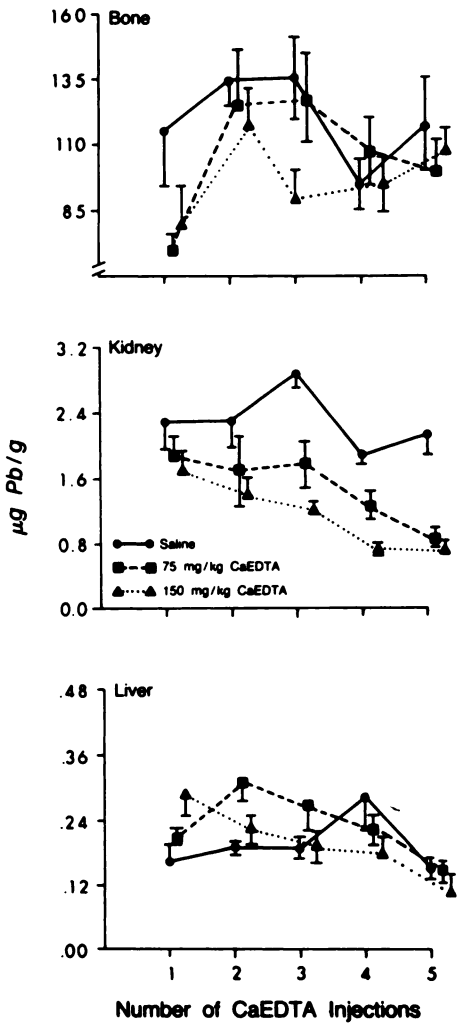

Fig. 2. Concentrations of Pb in bone (top panel; dry weight), kidneys (middle; wet weight) and liver (bottom; wet weight) in micrograms of Pb per gram, as a function of the number of Ca disodium EDTA injections. As in figure 1, different groups of animals received a daily i.p. injection of saline, 75 or 150 mg/kg of Ca disodium EDTA for 1, 2, 3, 4 or 5 days and were sacrificed 24 hr after the final injection. Each data point represents a group × ± S.E.

the initial redistribution of Pb into liver at the start of chelation. The initial increase, followed by a decrease in liver Pb content as a response to Ca disodium EDTA may also explain the absence of a significant effect of dose (table 4, line 1): in the analysis of variance each dose group was averaged across the 5 days of treatment and compared, thereby minimizing dose-related differences. This dependence of Pb levels on the duration of Ca disodium EDTA treatment was documented by the statistically significant influence of number of injections on Pb levels (table 4, lines 2 and 3).

A single injection of 150 mg/kg of Ca disodium EDTA doubled the concentration of Pb in the brain (fig. 3, right panel; table 5, line 14). After two additional injections, brain Pb declined below control levels (table 5, line 16). After the fourth and fifth injections, however, brain Pb rose again, so that by the end of the standard 5-day course of treatment, brain Pb burden of chelated rats did not differ from nonchelated controls (fig. 3; table 5, line 18). That is, no net loss of Pb from brain occurred. This was confirmed by the absence of an effect of dosing with Ca disodium EDTA (table 5, line 1) and by the observation that individual Ca disodium EDTA doses did not differ either from each other or from saline controls when effects were averaged across the full course of treatment (table 5, lines 6–8). On the other hand, the sequence of injections of Ca disodium EDTA had a significant influence on brain Pb levels (table 5, lines 2 and 3). A similar pattern of effects was observed at the lower dose of Ca disodium EDTA (fig. 3, left panel), although the resulting Pb levels did not differ significantly either from those of saline-treated controls or of animals treated with the higher Ca disodium EDTA dose (table 5, lines 6, 8 and 9–13). Statistical analysis of the trend in Pb concentrations across the series of injections yielded ambiguous results (table 5, lines 19 and 20). The test examined linear trend, whereas the actual sequence of changes oscillated: an initial rise above control level, followed by a drop below, and then a return to control level (fig. 3).

Additional groups of rats exposed to 25 or 500 ppm of Pb acetate were used to evaluate further the effects of a single injection of Ca disodium EDTA on brain Pb levels. The results are shown in figure 4 (data for 50 ppm from fig. 3 included for comparison). A one-way analysis of variance based on Ca disodium EDTA dose indicated a significant elevation of brain Pb in the 25 ppm group ($F = 4.23$, dF = 2,18, $P < .05$). Although

TABLE 2
Effects of Ca disodium EDTA chelation on bone Pb: analysis of variance

Line		Source of Variation	SS	dF	F	P	Significant
1		Dose (D)	1.046	2	0.523	.0071	Yes
2		No. of injections (I)	1.827	4	4.57	.0021	Yes
3		Interaction (D × I)	1.141	8	1.43	.1965	
4		Error	8.999	90			
5		Corrected total	13.013	104			
				Coefficient of Determination = 31%			
Line		**Contrasts of Main Effect**	**SS**	**dF**	**F**	**P**	**Significant**
6	(P)[a]	Saline *vs.* D = 75 mg/kg	0.547	1	5.47	.0215	Yes
7	(P)	Saline *vs.* D = 150 mg/kg	0.963	1	9.63	.0026	Yes
8	(P)	D = 75 mg/kg *vs.* D = 150 mg/kg	0.058	1	0.58	.4466	
9	(R)[b]	Linear	0.0336	1	0.34	.5636	
10	(R)	Quadratic	1.0330	1	10.33	.0018	Yes
11	(R)	Cubic	0.7506	1	7.51	.0074	Yes
12	(R)	Quartic	0.0092	1	0.09	.7615	
Line		**Interaction Contrasts**	**SS**	**dF**	**F**	**P**	**Significant**
13	(P)	Saline *vs.* 150 mg/kg, injection 1	0.5195	1	5.20	.0250	Yes
14	(P)	Saline *vs.* 150 mg/kg, injection 2	0.1089	1	1.09	.2995	
15	(P)	Saline *vs.* 150 mg/kg, injection 3	0.6806	1	6.81	.0106	Yes
16	(P)	Saline *vs.* 150 mg/kg, injection 4	0.0000	1	0.00	.9780	
17	(P)	Saline *vs.* 150 mg/kg, injection 5	0.0962	1	0.96	.3292	

[a] P, pairwise comparison contrasts of doses.
[b] R, trend contrasts across the series of injections.

TABLE 3
Effects of Ca disodium EDTA chelation on kidney Pb: analysis of variance

Line		Source of Variation	SS	dF	F	P	Significant
1		Dose (D)	10.026	2	49.58	.0001	Yes
2		Injection (I)	5.478	4	13.55	.0001	Yes
3		Interaction (D × I)	2.092	8	2.59	.0137	Yes
4		Error	9.099	90			
5		Corrected total	26.695	104			
				Coefficient of determination = 66%			
Line		**Contrasts of Main Effect**	**SS**	**dF**	**F**	**P**	**Significant**
6	(P)[a]	Saline *vs.* D = 75 mg/kg	4.423	1	43.75	.0001	Yes
7	(P)	Saline *vs.* D = 150 mg/kg	9.619	1	95.14	.0001	Yes
8	(P)	D = 75 mg/kg *vs.* D = 150 mg/kg	0.997	1	9.86	.0023	Yes
Line		**Interaction Contrasts**	**SS**	**dF**	**F**	**P**	**Significant**
9	(P)	Saline *vs.* 75 mg/kg, injection 1	0.094	1	0.93	.3369	
10	(P)	Saline *vs.* 75 mg/kg, injection 2	0.665	1	6.57	.0120	Yes
11	(P)	Saline *vs.* 75 mg/kg, injection 3	1.045	1	10.34	.0018	Yes
12	(P)	Saline *vs.* 75 mg/kg, injection 4	0.695	1	6.87	.0103	Yes
13	(P)	Saline *vs.* 75 mg/kg, injection 5	2.974	1	29.41	.0001	Yes
14	(P)	Saline *vs.* 150 mg/kg, injection 1	0.279	1	2.76	.1001	
15	(P)	Saline *vs.* 150 mg/kg, injection 2	0.839	1	8.30	.0049	Yes
16	(P)	Saline *vs.* 150 mg/kg, injection 3	2.765	1	27.35	.0001	Yes
17	(P)	Saline *vs.* 150 mg/kg, injection 4	3.105	1	30.71	.0001	Yes
18	(P)	Saline *vs.* 150 mg/kg, injection 5	4.267	1	42.20	.0001	Yes
19	(R)[b]	Linear-saline × linear-75 mg/kg	1.537	1	15.20	.0002	Yes
20	(R)	Linear-saline × linear-150 mg/kg	0.814	1	8.05	.0056	Yes

[a] P, pairwise comparison contrasts of doses.
[b] R, trend contrasts across the series of injections.

a similar pattern was observed in the 500 ppm group, the effects were more variable and not statistically significant ($F = 2.21$, dF = 2,16, $P > .05$). Subsequent post hoc analysis in the 25 ppm group by two-sample t tests indicated that the increase in brain Pb content was attributable to the 150 mg/kg of Ca disodium EDTA dose ($t = -2.72$, dF = 11.4, $P = .02$), confirming our findings with 50 ppm (fig. 3; table 5, lines 6, 7 and 9–18). The mobilization of Pb into brain after a single Ca disodium EDTA injection occurred despite a dramatic enhancement of urinary Pb excretion as shown in figure 5, and a consistent decline in blood Pb concentration, as indicated in table 6.

An indirect assessment of the predictive validity of the Ca disodium EDTA mobilization test as an indicator of elevated Pb body burden was carried out by examining the correlations between urinary Pb excretion (micrograms/24 hr) and the concentrations of Pb in various tissues, and with ZPP levels in blood, in 50 ppm Pb-exposed animals that received a single injection of either 75 or 150 mg/kg of Ca disodium EDTA. Final analysis was based on a sample of 10 (3 of the 14 urine sample volumes were insufficient for analysis; one additional urine sample was excessively high in Pb, affecting the resulting correlations significantly, as indicated by a large standardized residual and was excluded from the data set). The resulting

TABLE 4
Effects of Ca disodium EDTA chelation on liver Pb: analysis of variance

Line		Source of Variation	SS	dF	F	P	Significant
1		Dose (D)	0.542	2	1.49	.2299	
2		Injection (I)	7.149	4	9.86	.0001	Yes
3		Interaction (D × I)	3.363	8	2.50	.0167	Yes
4		Error	11.324	90			
5		Corrected total	16.316	104			
				Coefficient of determination = 41%			
Line		**Contrasts of Main Effect**	**SS**	**dF**	**F**	**P**	**Significant**
6	(P)[a]	Saline *vs.* D = 75 mg/kg	0.216	1	1.19	.2784	
7	(P)	Saline *vs.* D = 150 mg/kg	0.069	1	0.38	.5391	
8	(P)	D = 75 mg/kg *vs.* D = 150 mg/kg	0.528	1	2.91	.0912	
Line		**Interaction Contrasts**	**SS**	**dF**	**F**	**P**	**Significant**
9	(P)	Saline *vs.* 75 mg/kg, injection 1	0.180	1	1.00	.3212	
10	(P)	Saline *vs.* 75 mg/kg, injection 2	0.877	1	4.84	.0304	Yes
11	(P)	Saline *vs.* 75 mg/kg, injection 3	0.248	1	1.37	.2456	
12	(P)	Saline *vs.* 75 mg/kg, injection 4	0.089	1	0.49	.4857	
13	(P)	Saline *vs.* 75 mg/kg, injection 5	0.273	1	1.51	.2230	
14	(P)	Saline *vs.* 150 mg/kg, injection 1	1.096	1	6.05	.0159	Yes
15	(P)	Saline *vs.* 150 mg/kg, injection 2	0.059	1	0.32	.5705	
16	(P)	Saline *vs.* 150 mg/kg, injection 3	0.006	1	0.03	.8552	
17	(P)	Saline *vs.* 150 mg/kg, injection 4	0.442	1	2.44	.1218	
18	(P)	Saline *vs.* 150 mg/kg, injection 5	1.284	1	7.09	.0092	Yes
19	(R)[b]	Linear-saline × linear-75 mg/kg	2.775	1	15.31	.0002	Yes
20	(R)	Linear-saline × linear-150 mg/kg	0.979	1	5.40	.0224	Yes

[a] P, pairwise comparison contrasts of doses.
[b] R, trend contrasts across the series of injections.

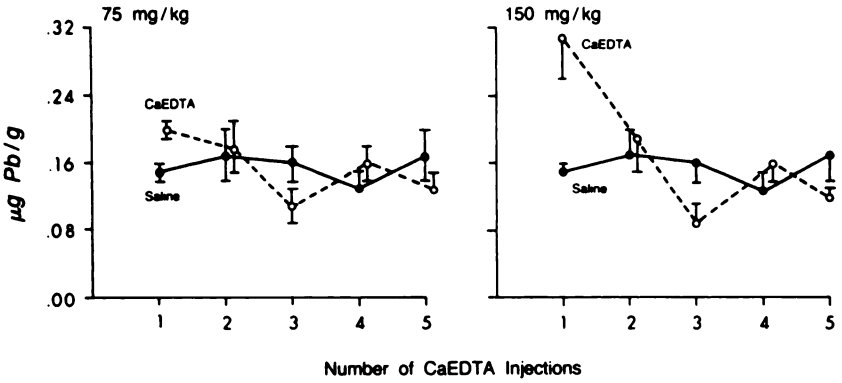

Fig. 3. Brain Pb concentrations (micrograms of Pb per gram of wet weight) as a function of the number of 75 (left panel) or 150 mg/kg of Ca disodium EDTA injections (right panel). Plotted as in figure 1, except that each panel compares one dose level to the saline-injected control group.

scatterplots are presented in figure 6. Pb excretion in urine correlated significantly with bone Pb and marginally ($0.10 > P > .05$) with postchelation blood Pb. The correlations with Pb in liver, kidneys, prechelation blood Pb (not shown, $r = 0.60$) and ZPP were positive, but not statistically significant. It should be noted that the exclusion of the excessively high urinary Pb value markedly altered the results for bone (increase from $r = 0.56$, $P > .05$ to $r = 0.75$, $P \leq .02$), prechelation blood Pb ($r = 0.90$, $P < .05$ to $r = 0.60$, $P > .05$), postchelation blood Pb ($r = 0.77$, $P < .05$ to $r = 0.59$, $0.10 > P > .05$) and liver Pb ($r = 0.79$, $P < .05$ to $r = 0.48$, $P > .05$).

Discussion

Chelation with Ca disodium EDTA was widely adopted as a treatment for Pb poisoning after it was demonstrated to relieve the clinical signs of intoxication and reverse some of the hematopoietic toxicity of Pb (Reiders *et al.*, 1955; Teisinger and Srbova, 1959; Chisolm, 1968; Chisolm *et al.*, 1976). Further support for Ca disodium EDTA therapy came from studies indicating marked elevations in urinary Pb and decreases in blood Pb after administration to both pediatric and occupationally exposed populations. This body of data was accepted as implicit evidence that Ca disodium EDTA reduced Pb concentrations in all tissues. The data reported here suggest that this assumption may not be tenable. Ca disodium EDTA chelation raised Pb concentrations in the brain and the liver (fig. 2, 3 and 4), two target organs for Pb toxicity. Evidence for redistribution of mobilized Pb was reported previously by Hammond *et al.* (1967) who found that muscle, lung and genitalia served as temporary repositories for Pb during and after the Ca disodium EDTA chelation process.

A single injection of 150 mg/kg of Ca disodium EDTA elevated brain Pb content markedly (figs. 3 and 4), an effect of special significance because the single injection protocol parallels the clinical diagnostic procedure termed the Ca disodium EDTA mobilization test. In this procedure, a single injection of Ca disodium EDTA is administered and subsequent 8 hr (*e.g.*, Markowitz and Rosen, 1984) or, more commonly, 24 hr urinary Pb excretion is measured. If the total Pb content exceeds a specified value, further chelation therapy with Ca disodium EDTA is deemed necessary, based on the presumption that total urinary Pb excretion is proportional to either the body burden of Pb or the amount in soft tissue. Our data

TABLE 5

Effects of Ca disodium EDTA chelation on brain Pb: analysis of variance

Line		Source of Variation	SS	dF	F	P	Significant
1		Dose (D)	0.041	2	0.12	.8845	
2		Injection (I)	4.092	4	6.13	.0002	Yes
3		Interaction (D × I)	3.647	8	2.73	.0096	Yes
4		Error	15.009	90			
5		Corrected total	22.788	104			
				Coefficient of determination = 34%			
Line		**Contrasts of Main Effect**	**SS**	**dF**	**F**	**P**	**Significant**
6	(P)[a]	Saline *vs.* D = 75 mg/kg	0.041	1	0.25	.6213	
7	(P)	Saline *vs.* D = 150 mg/kg	0.010	1	0.06	.8061	
8	(P)	D = 75 mg/kg *vs.* D = 150 mg/kg	0.010	1	0.06	.8035	
Line		**Interaction Contrasts**	**SS**	**dF**	**F**	**P**	**Significant**
9	(P)	Saline *vs.* 75 mg/kg, injection 1	0.338	1	2.03	.1577	
10	(P)	Saline *vs.* 75 mg/kg, injection 2	0.013	1	0.08	.7801	
11	(P)	Saline *vs.* 75 mg/kg, injection 3	0.186	1	1.11	.2943	
12	(P)	Saline *vs.* 75 mg/kg, injection 4	0.398	1	2.38	.1261	
13	(P)	Saline *vs.* 75 mg/kg, injection 5	0.196	1	1.18	.2806	
14	(P)	Saline *vs.* 150 mg/kg, injection 1	1.678	1	10.06	.0021	Yes
15	(P)	Saline *vs.* 150 mg/kg, injection 2	0.015	1	0.09	.7661	
16	(P)	Saline *vs.* 150 mg/kg, injection 3	1.230	1	7.38	.0079	Yes
17	(P)	Saline *vs.* 150 mg/kg, injection 4	0.192	1	1.15	.2856	
18	(P)	Saline *vs.* 150 mg/kg, injection 5	0.272	1	1.63	.2045	
19	(R)[b]	Linear-saline × linear-75 mg/kg	1.101	1	6.60	.0118	Yes
20	(R)	Linear-saline × linear-150 mg/kg	0.235	1	1.41	.2381	

[a] P, pairwise comparison contrasts of doses.
[b] R, trend contrasts across the series of injections.

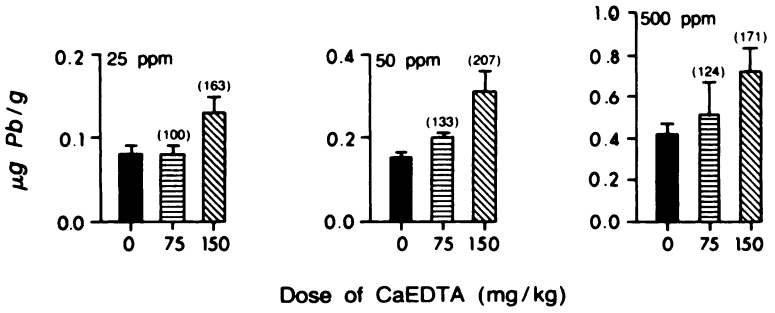

Fig. 4. Change in brain Pb levels (micrograms of Pb per gram of wet weight) after a single administration of saline (0 mg/kg), 75 or 150 mg/kg of Ca disodium EDTA to rats after a 3 to 4 month exposure to 25 (left panel), 50 (middle panel; data from fig. 3) or 500 ppm (right panel) of Pb acetate in drinking water. Each bar represents a mean ± S.E. for groups of $n = 5$ to 7 animals. The numbers above the 75 and 150 mg/kg bars indicate the percentage of change from the 0 mg/kg control group.

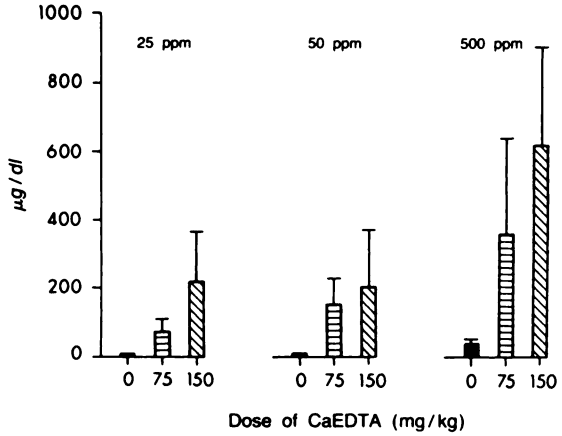

Fig. 5. Twenty-four hour urinary Pb excretion (micrograms per deciliter) after a single administration of saline (0 mg/kg), 75 or 150 mg/kg of Ca disodium EDTA to rats after a 3 to 4 month exposure to 25 (left panel), 50 (middle panel) or 500 ppm (right panel) of Pb acetate in drinking water. Each bar represents a mean ±S.E. for groups of $n = 5$ to 7 animals.

TABLE 6

Blood Pb levels before and 24 hr after a single injection of Ca disodium EDTA

Mean ± S.D.

	Dose of Ca disodium EDTA			
	75 mg/kg		150 mg/kg	
	Pre	Post	Pre	Post
ppm Pb acetate exposure				
25	10.7 ± 4.8	5.0 ± 2.7*	15.4 ± 4.2	7.4 ± 2.5*
50	24.3 ± 4.8	13.1 ± 4.5*	25.6 ± 13.2	12.7 ± 5.8*
500	38.4 ± 4.4	28.9 ± 3.2*	42 ± 6.8	29.9 ± 9.4*

* Significantly different from preblood Pb level at P ≤ .05 by matched pair *t* test.

suggest that the safety of the Ca disodium EDTA mobilization test should be re-evaluated.

The rise in brain Pb content in response to a single injection of 150 mg/kg of Ca disodium EDTA was observed in rats exposed to 25 and 50 ppm of Pb acetate. Based on positive findings at these two lower Pb exposure concentrations, a two-sample *t* test comparing just the 150-mg/kg dose to saline-injected controls was undertaken in the 500 ppm group even though the analysis of variance failed to yield a significant *F*.

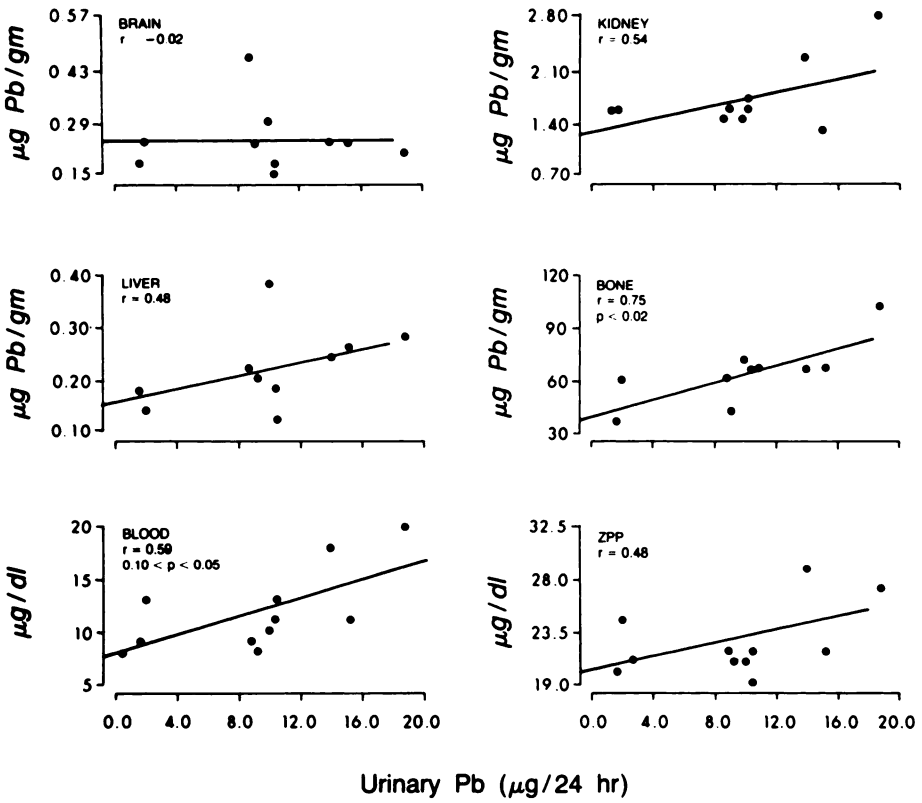

Fig. 6. Tissue Pb concentrations (as identified in each panel) plotted against the urinary rate of Pb excretion (micrograms of Pb per 24 hr). Bottom right panel shows blood levels of ZPP. Each data point represents a value for an animal that received a single injection of either 75 or 150 mg/kg of Ca disodium EDTA. Lines of best fit are shown, together with the computed correlation values (*r*) and significance levels.

It revealed a significant elevation of Pb burden in the chelated group ($t = -2.61$, dF = 8.3, P = .031).

The failure of Ca disodium EDTA to produce any net loss of Pb from brain over the course of the 5-day treatment arouses further concern (fig. 3). Figure 7 summarizes the effects of the high dose of Ca disodium EDTA (150 mg/kg) and plots Pb levels in tissue as a percentage of corresponding levels in saline-injected controls over the course of five injections. Goyer and Cherian (1979) also subjected rats to a 5-day dosing regimen of Ca disodium EDTA after chronic Pb exposure and similarly reported no decline in brain Pb; only the more extended treatment used by Bankowska and Hine (1985) yielded a loss. It should be noted that a 5-day course of treatment also is the one most often used clinically to treat elevated Pb burden.

Redistribution of mobilized Pb to the liver was dose-related; it was produced by a single injection of 150 mg/kg and by two injections of 75 mg/kg (fig. 2 and 7). Goyer *et al.* (1978) similarly noted an increase, although not statistically significant, in liver Pb content. As with brain, there was little evidence for any net loss of Pb from liver over the 5-day course of Ca disodium EDTA. These findings disagree with several others which have consistently observed a loss of Pb from liver (*e.g.*, Goyer and Cherian, 1979; Bankowska and Hine, 1985; Castellino and Aloj, 1965; Hammond *et al.*, 1967). The reasons for this discrepancy are unclear, but may be related to dose. The current study used a lower total dose of Ca disodium EDTA than did most previous studies, suggesting that higher doses or more prolonged chelation may be required to decrease liver Pb burden. Goyer *et al.* (1978), however, using a dose of Ca disodium EDTA equivalent to those in other studies, also failed to show a loss of Pb from liver, reporting instead a slight, although nonsignificant increase. These data, along with the findings of the current study, suggest that liver Pb burden may fluctuate with repeated Ca

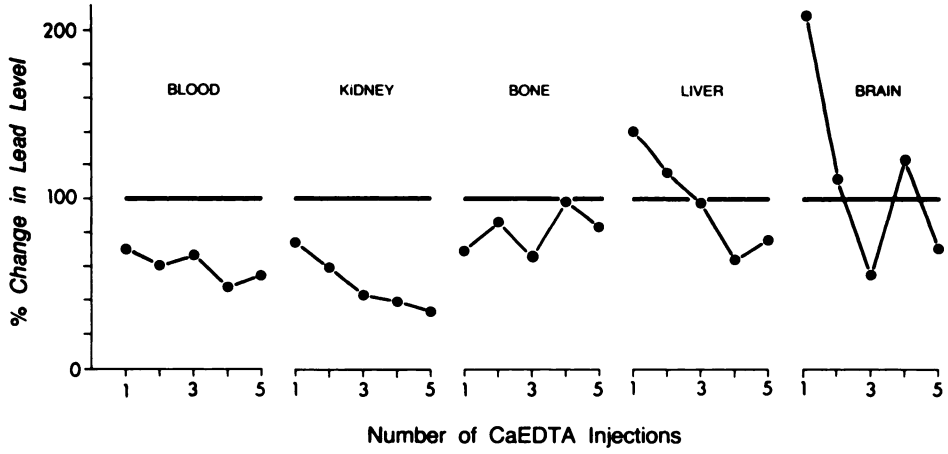

Fig. 7. Mean changes in tissue Pb levels over the course of the five injections of 150 mg/kg of Ca disodium EDTA. Data for each Ca disodium EDTA injection group were plotted as a percentage of the corresponding saline-control value for that particular injection.

disodium EDTA administration as a result of alternate redistribution and mobilization.

Data from this study support the contention that the primary source of Pb mobilized by Ca disodium EDTA is bone, with an additional contribution from kidney. Rapid mobilization of Pb from bone, as observed by Hammond *et al.* (1967) and Hammond (1971), and in this study, could temporarily spike blood Pb levels with subsequent transport to and deposition in liver and brain, a process which would not be seen with the 24 hr latency used here. Such a spike was observed in humans given Ca disodium EDTA, not in whole blood Pb, but in plasma Pb, by Araki *et al.* (1984) and by Ishihara *et al.* (1984). In both of those studies, substantial increases in plasma Pb concentration occurred within 1 hr after the onset of Ca disodium EDTA infusions, whereas whole blood Pb concentrations either declined or remained unchanged. These data are consistent with the report by Foreman and Trujillo (1954) showing a 1-hr turnover time for Ca disodium EDTA in humans.

Previous studies have reported discrepant findings in mobilization of Pb from bone in response to Ca disodium EDTA administration. Results from this study agree with those of Goyer and Cherian (1979), who reported a reduction in bone Pb content after Ca disodium EDTA was administered to rats after a 10-week exposure to 1000 ppm of Pb acetate in drinking water. The current findings are also consistent with those reported by Hammond and colleagues (Hammond *et al.*, 1967; Hammond, 1971) who reported a decline in skeletal Pb content after a single infusion of Ca disodium EDTA after acute i.v. Pb exposure.

They conflict, however, with those of Castellino and Aloj (1965), Goyer *et al.* (1978) and Bankowska and Hine (1985), all of which reported no loss of Pb from femur. The discrepancy among studies may arise from what appears to be a dynamic exchange process produced by repeated injections of Ca disodium EDTA. Statistical analysis of Pb concentrations in bone (table 2) indicated no interaction between number of Ca disodium EDTA injections and dose, *i.e.*, that the loss of bone Pb in chelated animals remained uniform over the course of injections. Figure 2, however, suggests oscillation of bone Pb values in response to successive 150-mg/kg Ca disodium EDTA injections. The possibility that mobilization of Pb from bone might not be consistent over the treatment period was explored as shown in table 2, lines 13 to 17. This analysis indicated that significant mobilization of Pb from bone occurred only in response to the first and third injections of 150 mg/kg of Ca disodium EDTA.

Thus, one possible explanation for the pattern of results observed with Ca disodium EDTA is that Pb mobilized from bone, blood and kidney may be recirculated back to bone during continued treatment. Our data support this hypothesis. A mean drop of 36 μg of Pb per g of bone ash occurred in response to the first 150-mg/kg Ca disodium EDTA injection. Based on a presumed total of 9 g of bone ash for a 300-g adult male rat (Weikel *et al.*, 1955), a total mobilization of 36 μg/g $\times$ 9 g or 324 μg of Pb occurred after the first Ca disodium EDTA injection. This amount is far greater than the total urinary excretion observed after one Ca disodium EDTA injection, which generally ranged up to 20 μg (fig. 6) and, similarly, could not be completely accounted for by the quantities redistributed to liver and brain. Thus, some of the Pb mobilized from bone may be deposited in liver and brain, some may be excreted in urine and some redeposited in bone. This would account for the increase in bone Pb from the first to the second injection of 150 mg/kg of Ca disodium EDTA (fig. 2). Hammond *et al.* (1967) also noted that a decline in bone Pb content was followed by a subsequent rise from the 48th to the 72nd hr after a single infusion of Ca disodium EDTA. Consequently, what appear to be discrepant findings among the various studies may simply reflect differences in number of Ca disodium EDTA injections and timing of subsequent sampling of bone.

A progressive dose-related decline in kidney Pb content occurred in response to successive Ca disodium EDTA administrations. With the exception of Bankowska and Hine (1985), other investigators have also noted a reduction in kidney Pb burden (Hammond *et al.*, 1967; Hammond, 1971; Castellino and Aloj, 1965; Goyer *et al.*, 1978; Goyer and Cherian, 1979). In this study, a statistically significant loss of Pb from the kidney occurred only after the second Ca disodium EDTA injection, indicating that mobilization from kidney was slightly delayed compared to bone, whose Pb content fell substantially after the first Ca disodium EDTA injection. These effects concur with Hammond *et al.* (1967) and Hammond (1971), who observed that depletion of soft tissue Pb levels occurred after bone Pb mobilization. Pb could be removed from kidneys directly into urine or could be removed for redistribution to bone as a result of altered concentration gradients (as suggested by Hammond *et al.*, 1967). Such secondary redistribution, however, is not supported by the continued depletion of kidney Pb whereas bone Pb levels appear to oscillate (figs. 2 and 7).

Interestingly, mobilization and redistribution as produced by Ca disodium EDTA chelation, does not appear to be restricted to Pb. A recent study by Domingo *et al.* (1986) reported a dramatic increase in brain, as well as cardiac and muscle aluminum content with a corresponding decline in bone, spleen and liver aluminum levels after Ca disodium EDTA administration. Moreover, these effects were accompanied by a striking increase in lethality.

The amount of Pb excreted in response to a single injection of Ca disodium EDTA (Ca disodium EDTA mobilization test) should reflect, by definition, the transfer of Pb from the total body pool. It is rather surprising, given the extensive use of this diagnostic test, how little is known about the relationship between the hypothetical pool and actual concentrations in various tissues. Hammond (1971) found no consistent relationship between the amount of Pb in soft tissues and the amount of Pb excreted in response to Ca disodium EDTA, as indicated by a decline in the EDTA-sensitive pool of Pb over a 60-day period after Pb administration. In conformity with the clinical findings (*e.g.*, Araki *et al.*, 1986), we observed a correlation between blood Pb values and urinary Pb excretion after chelation (fig. 6). Correlations between urinary Pb excretion and tissue Pb burdens observed in the present study were somewhat tenuous, however. Significant relationships were noted only between urinary Pb excretion and the concentrations in bone and liver. Even there, however, correlations depended on a single data point. However, sample sizes were small in the current study (n = 10 or 11), and the range of tissue Pb values was narrow. Moreover, our assessment of validity was necessarily indirect, because it was based on postchelation, rather than prechelation tissue Pb concentrations. Further work is needed to examine the implications of the Ca disodium EDTA mobilization test.

The current findings pose diagnostic, therapeutic and toxicological issues for species extrapolation. The metabolism of

Ca disodium EDTA seems comparable in rats and humans (*cf.* Foreman and Trujillo, 1954; Foreman *et al.*, 1953) and, although the two species exhibit some kinetic differences, tissue Pb distributions in bone and soft tissue are quite similar. There is no doubt that Ca disodium EDTA can prevent lethality associated with acute Pb encephalopathy. However, chelation treatment may not be as useful for lower Pb burdens.

Our current knowledge of equivalent doses of Ca disodium EDTA for rat and human is insufficient to permit direct comparisons of changes in tissue Pb content in the two species in response to chelation. Although the doses of Ca disodium EDTA used in the present study are generally higher on a body-weight basis than those administered clinically, information on equivalent doses for rat and human is not available, If the two-thirds power of body weight (basal metabolic equivalent), rather than body weight, is used as the basis for comparing doses (Freireich *et al.*, 1966), then the rat/human ratio lies close to unity for the doses in this study and Chisolm's (1976) dose of 25 mg/kg. The current findings stress the need for additional investigations of effects of Ca disodium EDTA on the dose of Pb to target organs, and suggest that a re-evaluation of the protocols for the use of Ca disodium EDTA chelation in diagnosis and treatment may be advisable.

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