

Metabolism of Meso-2,3-Dimercaptosuccinic Acid in Lead-Poisoned Children and Normal Adults

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Meso-2,3-dimercaptosuccinic acid (DMSA, or succimer) is an oral chelating agent for heavy-metal poisoning. While studying the urinary elimination of unaltered DMSA, altered DMSA (i.e., its mixed disulfides), and lead in children with lead poisoning, we observed a pattern of urinary drug elimination after meals suggestive of enterohepatic circulation. The excretion of lead in urine patterned the elimination of altered DMSA rather than the parent molecule. In addition, the half-life of elimination of DMSA via the kidney was positively associated with blood lead concentration. Two additional cross-over studies of DMSA kinetics were conducted in normal adults to confirm the presence of enterohepatic circulation of DMSA after meals. In one, increases in plasma total DMSA concentration were observed after meals in all six subjects; these increases were prevented by cholestyramine administration 4, 8, and 12 hr after DMSA. In the second, the administration of neomycin also prevented increases in DMSA after meals. These studies indicate that 1) a metabolite(s) of DMSA undergoes enterohepatic circulation and that microflora are required for DMSA reentry; 2) in children, moderate lead exposure impairs renal tubular drug elimination; and 3) a metabolite of DMSA appears to be an active chelator. *Key words:* chelation, enterohepatic circulation, lead, meso-2,3-dimercaptosuccinic acid, pharmacokinetics, succimer. *Environ Health Perspect* 103:734-739 (1995)

Meso-2,3-dimercaptosuccinic acid (DMSA, or succimer) is an orally active chelating agent recently approved by the U.S. Food and Drug Administration for the treatment of childhood lead poisoning (1). Aside from its specified use in children (2,3), the drug has been reported to be clinically effective in the treatment of occupational plumbism (4-6), mercury vapor exposure (7) and acute arsenic poisoning (8). Increases in urinary heavy-metal elimination have been consistently reported in these studies, with little effect on essential mineral excretion (2-5).

Research concerning the pharmacokinetics and disposition of DMSA in humans includes a phase I [¹⁴C]DMSA study (9) and several single-dose HPLC studies (10-12). Collectively, these studies of normal male volunteers have led to the conclusion that about 20% of an administered dose is eliminated in the urine; the remainder has been presumed to be unabsorbed.

In addition, DMSA appears to be extensively metabolized in that 89% of the drug recovered in urine was in the form of mixed disulfides with the amino acid cysteine (10); the major metabolite contained two cysteine residues, while a minor metabolite contained one.

We conducted a controlled study of the pharmacokinetics of DMSA in children with elevated blood lead (BPb) concentrations, a subject about which little is known (13). Because lead poisoning is believed to inhibit renal uric acid secretion (14) and is known to impair hepatic cytochrome P450-mediated drug metabolism (15), we set out to test the hypothesis that lead poisoning might alter the kinetics and disposition of DMSA. To our surprise, we observed a pattern of postprandial (after meals) peaks in urinary DMSA elimination, which was highly suggestive of enterohepatic circulation. We therefore conducted two additional studies (in adults) to determine whether the presumed enterohepatic circulation could be blocked by the administration of cholestyramine and whether gut microflora are required for DMSA reentry. During the course of our work, Dart and co-workers evaluated the pharmacokinetics of DMSA in three children and three adults with lead poisoning and reported that renal DMSA clearance was decreased in comparison to that in healthy adult males (16).

Materials and Methods

The clinical studies were approved by the Columbia-Presbyterian Medical Center Institutional Review Board. Informed consent was obtained for each participant.

Drugs and chemicals were obtained from the following sources: DMSA (Chemet), in 100 mg capsules, was a gift from McNeil Consumer Products Company (Fort Washington, Pennsylvania); cholestyramine (Questran Light) was a gift from Bristol Myers Company (Evansville, Indiana); dimercaptopropane sulfonate (DMPS), dithiothreitol (DTT), and tetrabutylammonium bromide (TBAB) were purchased from Sigma (St. Louis, Missouri); monobromobimane (MBB) came from Calbiochem-Behring (La Jolla, California); HPLC-grade acetonitrile came from Baxter (Muskegon, Michigan); HPLC-grade methanol and methylene chloride, sodium acetate trihydrate, sodium hydroxide, hydrochloric acid, acetic acid,

and ammonium bicarbonate was from Fisher Scientific (Springfield, New Jersey); lead nitrate atomic absorption spectrometry standard solution was from Alfa Products (Danvers, Massachusetts); and Ultrex HNO₃ came from J.T. Baker (Phillipsburg, New Jersey).

Study 1: Pharmacokinetics in Children

This study aimed to determine 1) whether environmental lead exposure, as assessed by blood lead (BPb) levels, influences the pharmacokinetics of DMSA in children, and if saturable processes are involved in the drug's absorption, metabolism, and/or elimination.

This was a randomized, single-dose, open-label non-cross-over study. Ten children with BPb levels of 25-53 µg/dL were recruited; at the time, elevated BPb was defined by the Centers for Disease Control as 25 µg/dL or greater (17). An unpublished study in dogs had suggested that renal clearance of DMSA might be saturable. Therefore, to determine if saturable processes were involved in absorption, metabolism, and/or elimination, half of the children received a single oral dose of 350 mg/m² DMSA (i.e., the recommended individual dose) and the other half received a dose of 700 mg/m². Children whose initial BPb was 50 µg/dL or more were immediately given a full 5-day course of DMSA (3) at the completion of this 56-hr study.

On admission to our Pediatric Clinical Research Center, each child underwent baseline laboratory testing and a complete physical examination. At 0600 hr the next morning, a single oral dose of 350 or 700 mg/m² of DMSA was administered. Breakfast was withheld until 0800 hr to prevent possible interference of food with drug absorption. During the ensuing 56 hr, oral intake of fluids was encouraged and each urine void was collected and analyzed for unaltered and altered DMSA as

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described below. In children who were not toilet trained, urine was collected via a fitted disposable plastic collection bag. To analyze for unaltered DMSA, portions of each urine sample were immediately derivatized with monobromobimane which prevents the oxidation of the sulfhydryl groups of DMSA and imparts fluorescence to it.

Study 2: Influence of Cholestyramine

This study aimed to determine whether postprandial increases in plasma DMSA could be observed. In addition, we sought to determine whether the suspected enterohepatic circulation of DMSA could be interrupted *in vivo* by cholestyramine, a nonabsorbable, nonspecific anionic resin.

We first conducted a pilot study to determine whether cholestyramine could bind DMSA *in vitro*. Standard solutions of 20 mM, 10 mM, 4 mM, and 2 mM DMSA were prepared from a 40-mM stock solution using either deionized water or deionized water containing cholestyramine. The DMSA solutions were electrolytically reduced, derivatized with MBB (to give final DMSA concentrations of 50 μ M, 25 μ M, 10 μ M, and 5 μ M) and then analyzed by HPLC; DMPS served as an internal standard in the HPLC analyses (see "Analysis" below). On average, there was a 98.5% decrease in the ratios of DMSA peak area to DMPS peak area when the DMSA standard solutions were prepared from the stock solution using cholestyramine instead of deionized water.

Three male and three female normal adult volunteers were recruited as subjects for the study: two were Hispanic, two were African-American, and two were Caucasian. Normal physical exams and baseline laboratory tests (complete blood cell count and blood chemistries) were required for enrollment into the study.

This was a single dose, randomized, cross-over study. Each subject was admitted twice to the Clinical Research Center. After an overnight fast, three subjects received 10 mg/kg DMSA orally without cholestyramine, and three received DMSA followed by cholestyramine orally. Standardized high-fat meals were given at 5 and 10 hr after administration of DMSA. When cholestyramine was given, 8 g were administered three times at 4, 8, and 12 hr after DMSA. One week later, the subjects were crossed over. Eighteen urine samples were collected over the next 15 hr and analyzed for unaltered and total DMSA. In addition, 29 blood samples were obtained at specified intervals over the 15-hr period for measurement of total DMSA. Plasma was immediately separated and frozen at -20°C .

Study 3: Influence of Neomycin

Drug metabolites excreted in bile as polar conjugates often require hydrolysis by gut microflora to reform the more absorbable parent drug molecule (18). The objective of this study was to determine whether gut microflora are required for the enterohepatic circulation of DMSA.

Although these were not the same subjects as those in study 2, their gender and ethnic distributions were the same. Normal physical exams and baseline laboratory tests were required for participation in this study. Each subject was then admitted twice to the Clinical Research Center for a single dose, randomized, cross-over study. After an overnight fast, three subjects received 10 mg/kg of DMSA orally without neomycin, and three received neomycin orally the day before the study (1 g at 0600, 1200, 1800, and 2400 hr) and DMSA followed by neomycin on the day of the study. The subjects received 1 g neomycin twice, at 4 and 10 hr after DMSA. One week later, the subjects were crossed over. The protocol for blood sampling and the schedule and composition of meals was the same as for study 2.

Analysis

Measurements of unaltered and altered DMSA in urine. We used a published assay (19) based on the HPLC separation of a highly fluorescent and stable derivative of DMSA. The derivative is prepared by the reaction of the sulfhydryl groups of DMSA with the methylene bromide moiety of MBB in aqueous solution at pH 8.3. We modified the method by adding the internal standard, DMPS, before the electrolytic reduction step. To assure that our assay was consistent with that of Maiorino et al. (19), portions of approximately 300 urine samples from study 1 were sent to Maiorino and Aposhian for analysis at the University of Arizona. There was excellent agreement between our laboratories.

Briefly, 500 μ L of each urine sample were derivatized immediately after voiding with MBB and these were analyzed to determine the concentration of unaltered DMSA. Separate 20-mL aliquots of each sample were frozen until analyzed, then electrolytically reduced and derivatized with MBB. [Electrolytic reduction of DMSA was carried out using custom synthesized mercury pool electrolytic cells connected in series (20)]. HPLC analyses of the latter samples provided total DMSA concentrations. The concentration of altered DMSA was obtained as the difference between the total and free DMSA concentrations. Quantification was accomplished by adding DMSA standards to "blank" urine samples that were collected before drug administration and treating

them in the same manner as the post-drug urine samples (19–21).

We put the remaining volume of each urine sample in 100-ml sterile polypropylene containers and froze them; aliquots of these samples were used to determine urine lead concentrations.

HPLC conditions. A Perkin-Elmer LC-600 autosampler, LC-250 pump, LS 40 fluorescence detector, and model 914 reporting integrator were used. The fluorescence detector was set at an excitation wavelength of 391 nm and an emission wavelength of 483 nm. Separation was accomplished by ion-interaction chromatography with a reverse-phase Spherisorb S5 ODS2 column (25 \times 4.6 mm; 5 μ m particle size; Rainin, Woburn, Massachusetts). Mobile phase A consisted of 0.02 M TBAB and 0.01 M acetic acid in methanol; mobile phase B contained the same concentrations of TBAB and acetate in water (pH 4.1). Initial conditions consisted of isocratic elution of 52% A:48% B for 10 min. Mobile phase A was then increased to 90% over a 2-min linear gradient and maintained for 5 min, after which it was returned to 52%, again over a 2-min linear gradient. Isocratic elution at 52% A:48% B continued for an additional 11 min to re-equilibrate the column. A flow rate of 1 mL/min was maintained throughout the 31-min run.

Urine and BPb analyses. Urine and BPb concentrations were determined by graphite furnace atomic absorption spectrometry using a modified version of the method of Fernandez and Hilligoss (22). Our laboratory is certified for BPb analysis by the U.S. Occupational Safety and Health Administration. During this study, there was an intraclass correlation coefficient of 0.97 for the agreement of our BPb determinations with the actual values for OSHA's unknowns.

A Perkin-Elmer Model 3030 Zeeman atomic absorption spectrophotometer equipped with an HGA-600 graphite furnace, PR-100 printer, AS-60 autosampler, and L'vov platform was used for quantitating urine Pb and BPb. Urine samples were first diluted with 0.7% Ultrex HNO₃ in a 1:1 ratio and then further diluted with Triton X-100 in a 1:1 ratio, instead of the 1:4 ratio used for whole blood. The graphite furnace temperature program was as follows: 130°C for 5 sec, 200°C for 20 sec, ashing at 600°C for 45 sec, atomization at 1700°C for 6 sec, and cool down at 20°C for 10 sec. The first drying stage was ramped for 10 sec and both the second drying and the ashing stages were ramped for 15 sec. The detection limit of both the urine and blood assays was 1.0 μ g/dL (0.05 μ M).

Measurement of DMSA in plasma. Plasma was analyzed for total DMSA using a modification of the method described by

Maiorino et al. (11). We modified the method by adding the internal standard, DMPS, before reduction with DTT. Quantification was accomplished by adding DMSA standards to “blank” plasma samples collected before drug administration and treating them in the same manner as the plasma samples collected after DMSA administration.

The method described for preparing plasma DMSA standards (11) was also modified. A 50 mM DMSA in 0.1 M NaOH (pH 5.3) stock solution was used to prepare all standards. A 2 mM DMSA solution was prepared by diluting the stock solution with distilled deionized water and intermediate standards of 10, 20, 40, 80, 200, 400, and 800 μM DMSA were prepared by further serial dilution. Buffered DMPS (6 μM in 0.1 M NH_4HCO_3) was used as the internal standard.

For reduction, 50 μL of the DMSA standard and 150 μL of the blank plasma were added to a tube containing 50 μL of 100 mM DTT and 1.75 mL of internal standard buffer (pH 8.2). After purging with nitrogen for 10 sec and vigorously mixing for 1 min, the contents were incubated in the dark for 30 min at room temperature. The solution was then ultrafiltered in 2 mL Centricon 30 microconcentrators by centrifugation (Sorvall RC-5B) at 6000 rpm for 1 hr at 23°C. The filtrate was transferred to a glass tube and 200 μL of 80 mM MBB was added. After purging with nitrogen and mixing, the contents were incubated for 10 min in the dark at room temperature. The solution was then extracted twice with 4 mL dichloromethane. After centrifugation at 2500 rpm for 2 min, the aqueous phase was transferred into a polypropylene tube, and 17 μL of 6M HCl was added to adjust the pH to 6–7 before HPLC analysis. The ratios of the DMSA peak areas to the DMPS peak

area were plotted against the corresponding DMSA standard concentration. The detection limit of the assay was 0.25 μM and within-run variation and the day-to-day variation was approximately 3–5%.

The method for determining total DMSA in the plasma samples was similar to the one used in preparing the DMSA standards except that 200 μL of the subject's plasma was used in place of the 50 μL of DMSA standard and 150 μL of blank plasma.

Pharmacokinetic analyses. The area under the curve (AUC) was calculated by the trapezoidal method for the interval between 5 hr (i.e., 1 hr after the first dose of cholestyramine was given) and 15 hr for the cholestyramine study and 0–15 hr for the neomycin study. *A priori*, absence of the anticipated postprandial peaks and/or a decrease in AUC when cholestyramine (or neomycin) was administered was taken as support for the hypothesis that DMSA undergoes enterohepatic circulation.

Results

Study 1: Pharmacokinetics in Children

The subjects in the two dose groups were comparable in terms of mean BPb, mean age, race, and gender (Table 1). The mean BPbs of the 350 and 700 mg/m^2 groups were 38 and 39 $\mu\text{g}/\text{dL}$, respectively, and their mean ages were 3.3 and 4.1 years. The study population consisted primarily of ethnic minorities; this is typical of the pattern of lead poisoning in the United States. Overall, 5.7 ± 2.6 (SD) and $3.8 \pm 2.5\%$ of the administered dose of DMSA was recovered in the urine (as DMSA or altered DMSA) in the 350 and 700 mg/m^2 groups, respectively.

Urinary excretion of lead and unaltered and altered DMSA were plotted against time for each child, and a consistent pattern

was observed. Figure 1 illustrates data from two typical children. Unaltered DMSA excretion peaked around 4 hr and then fell and remained at baseline. Altered DMSA excretion peaked slightly later, returned almost to the baseline, and then subsequently rose slightly after each meal. Urinary lead elimination also rose after meals.

We used urine drug measurements to obtain crude estimates of the early DMSA half-life of elimination. The early half-lives ranged from 1.7 to 3.8 hr, with an overall mean of 2.6 hr. In the two groups that received 350 mg/m^2 and 700 mg/m^2 , the mean half-lives of 2.8 ± 1 hr and 2.5 ± 0.6 hr, respectively, were not significantly different.

There was a significant positive correlation between the initial BPb and DMSA half-life ($r = 0.668$, $p = 0.049$). In other words, high BPbs were associated with longer half-lives of elimination of DMSA. This finding supports the hypothesis that

Table 1. Characteristics of the pediatric study population

	DMSA dose	
	350 mg/m^2	700 mg/m^2
N	5	5
Mean BPb ($\mu\text{g}/\text{dL}$)	38.1 ± 9.7	39.3 ± 8.4
BPb range ($\mu\text{g}/\text{dL}$)	21.8–46.6	33.1–53.3
Mean age (years)	3.3 ± 0.9	4.1 ± 3.3
Race/ethnicity		
African-American	2	2
Caucasian	1	1
Hispanic	1	2
Indian	1	0
Gender		
Male	1	3
Female	4	2
% of dose recovered in urine	5.7 ± 2.6	3.8 ± 2.5
Mean drug half-life (hr)	2.8 ± 1.0	2.5 ± 0.6

Abbreviations: DMSA, meso-2,3-dimercaptosuccinic acid; BPb, blood lead.

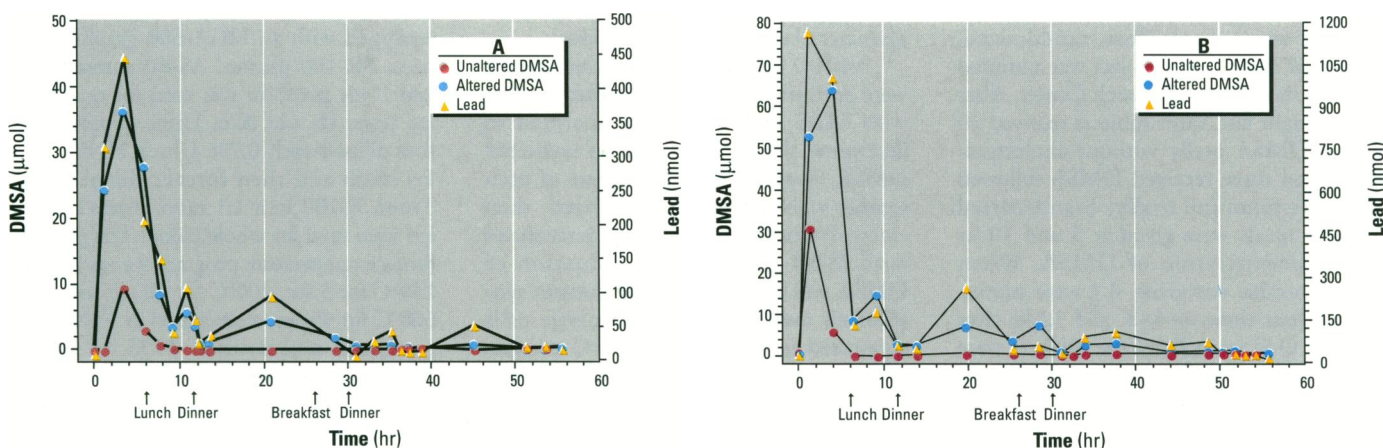


Figure 1. Urinary excretion of lead and unaltered and altered meso-2,3-dimercaptosuccinic acid (DMSA) in typical subjects who received either (A) 350 mg/m^2 or (B) 700 mg/m^2 . Unaltered DMSA peaked around 4 hr and then fell to the baseline and did not reappear. Both altered DMSA and lead peaked slightly after 4 hr, returned almost to the baseline, and then subsequently peaked after meals. DMSA was given at 0600 hr, lunch 6 hr later, dinner at 12 hr later, and breakfast the next day at 26 hr.

elevated BPb is associated with slower DMSA elimination.

On average, of the DMSA recovered in urine, $15.8 \pm 14.7\%$ was excreted unaltered, with an extremely wide range of 1.3–46.9%. The percentage excreted as unaltered DMSA was not influenced by gender (15.0% for boys, 16.3% for girls) or dose (16.5% and 15.0% for the 350 and 700 mg/m² groups, respectively). Furthermore, regression analyses revealed no associations between the percentage of unaltered DMSA and either age ($r = -0.08$, $p = 0.84$) or BPb ($r = 0.32$, $p = 0.37$). There was a hint of a relationship between the percentage of unaltered DMSA and ethnicity: two Caucasians and one Indian child eliminated only 1–2% unaltered DMSA, while four African-Americans eliminated 10–20%, and three Hispanics eliminated 10, 33, and 44% unaltered DMSA, respectively. However, ethnicity was not seen to have any influence in the subsequent studies with adults (*vide infra*).

Study 2: Influence of Cholestyramine

Total DMSA in plasma was measured at intervals from 0 to 15 hr after the administration of a single dose of 10 mg/kg (roughly equivalent to 350 mg/m²) in six normal adult subjects with a mean age of 29 ± 10 years and a mean BPb of 4.4 ± 1.8 µg/dL. Three were male and three were female. By design, two were African-American, two Hispanic, and two Caucasian. Note that the plasma DMSA assay measures total DMSA and cannot distinguish between the altered and unaltered forms (see Methods).

In all six subjects, when DMSA was administered alone, postprandial peaks in plasma DMSA were obvious, particularly after dinner, 10 hr after DMSA administration; data from two typical subjects are illustrated in Figure 2. Cholestyramine administration at 4, 8, and 12 hr abolished

all evidence of DMSA reentry (Fig. 2). On average, cholestyramine significantly decreased the DMSA versus time AUC(5–15 hr) by 20% ($t = 13.643$, $p < 0.001$; paired t -test). Cholestyramine did not significantly affect C_{\max} (54 ± 10 µM versus 55 ± 10 µM), t_{\max} (2.5 ± 0.4 hr versus 2.4 ± 0.6 hr) or the initial DMSA half-life (0–5 hr) (4.1 ± 1.4 hr versus 3.3 ± 1.4 hr). Overall, these findings were taken as support for the hypothesis that DMSA undergoes enterohepatic circulation and that this recycling of drug can be interrupted by cholestyramine.

On average, $18.3 \pm 4.9\%$ of the administered dose was recovered in urine in 15 hr when DMSA was administered alone; the range was 11.0–26.3%. Of the drug eliminated in the urine, the percentage eliminated in the unaltered form varied from 11 to 25% across all subjects.

Study 3: Influence of Neomycin

The six subjects had a mean age of 24 ± 5 years and a mean BPb of 2.9 ± 1.1 µg/dL. When DMSA was administered alone, postprandial peaks in plasma DMSA were again obvious (Fig. 3). Neomycin administration before and after DMSA abolished all evidence of DMSA reentry (Fig. 3). On average, neomycin significantly decreased C_{\max} by 33% (43 ± 14 µM versus 29 ± 13 µM, $p < 0.001$, paired t -test) and reduced the DMSA versus time AUC (0–15 hr) by 32% ($p < 0.001$). Neomycin had no significant effect on either t_{\max} (2.4 ± 0.5 hr versus 2.5 ± 0.5 hr) or the initial DMSA half-life (0–5 hr) (3.4 ± 1.0 hr versus 3.1 ± 1.1 hr).

On average, $10.1 \pm 3.5\%$ of the administered dose of DMSA was recovered in the urine in 15 hr when DMSA was administered alone; the range was 6.9–15.7%. Of the drug eliminated in the urine, the percentage eliminated in the unaltered form varied from 18 to 35% across all subjects.

Discussion

The cyclical pattern of excretion of altered DMSA in urine observed in children in study 1 (Fig. 1) is typical for drugs excreted in bile and undergoing enterohepatic circulation (18). These data, together with our documentation of postprandial reentry peaks of DMSA in plasma (Figs. 2 and 3), lead us to conclude that one or more metabolites of DMSA undergoes enterohepatic circulation. Because compounds excreted in human bile are typically highly polar and have molecular weights >400 daltons (18), we speculate that glucuronides of DMSA and/or its mixed disulfides may be secreted into bile.

Although neomycin can interfere with several small intestine functions, its administration has been used to demonstrate a role of microflora in the enterohepatic circulation of other drugs (23). In the current study, neomycin decreased AUC and abolished the postprandial peaks of DMSA in every subject. The decrease in AUC by neomycin was likely due to both impairment of absorption of the initial DMSA dose (as indicated by reduced C_{\max}) and interruption of enterohepatic circulation (indicated by abolition of postprandial peaks of DMSA); an unlikely but possible explanation is that neomycin bound DMSA and prevented its absorption. We conclude, however, that gut microflora hydrolyze poorly absorbable polar conjugates of DMSA, generating more readily absorbed unconjugated form(s), and are necessary for the enterohepatic circulation of DMSA. The finding that microflora are required for enterohepatic circulation implies that concomitant use of oral antibiotics may slightly reduce the efficacy of DMSA. For similar reasons, antibiotic use reportedly impairs the efficacy of oral contraceptives (24,25).

Urinary lead elimination paralleled the elimination of altered DMSA and not the

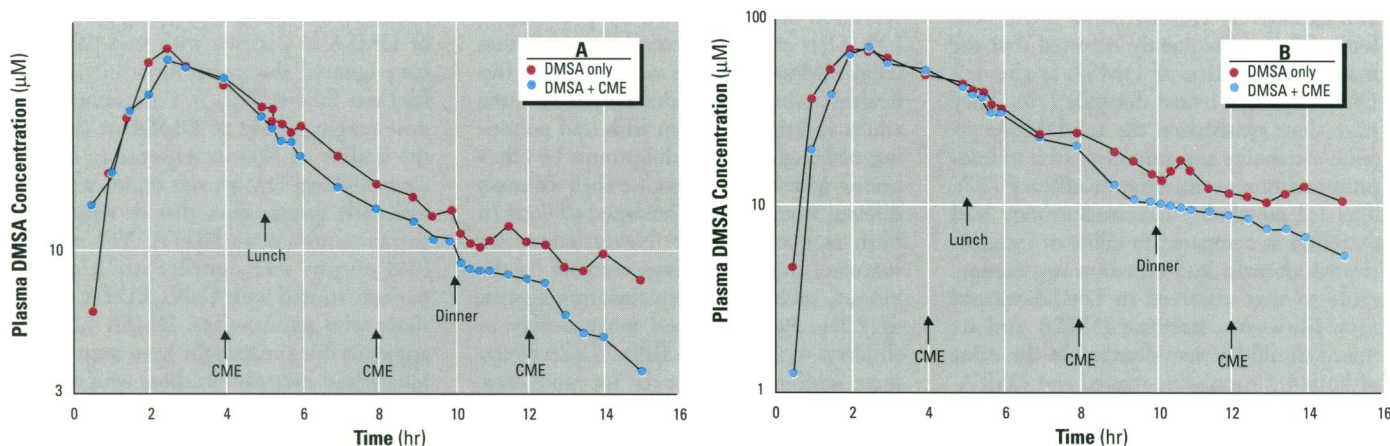


Figure 2. Semilog plots of plasma total meso-2,3-dimercaptosuccinic acid (DMSA) concentration versus time in two typical normal adults (panels A and B) following the administration of a single dose of 10 mg/kg DMSA at time zero. When DMSA was given alone, the plasma DMSA concentration peaked at 2.5 hr and then fell gradually; postprandial peaks were evident. When DMSA was given with cholestyramine (CME), the plasma DMSA concentration again peaked at 2.5 hr but fell gradually without any evidence of postprandial reentry.

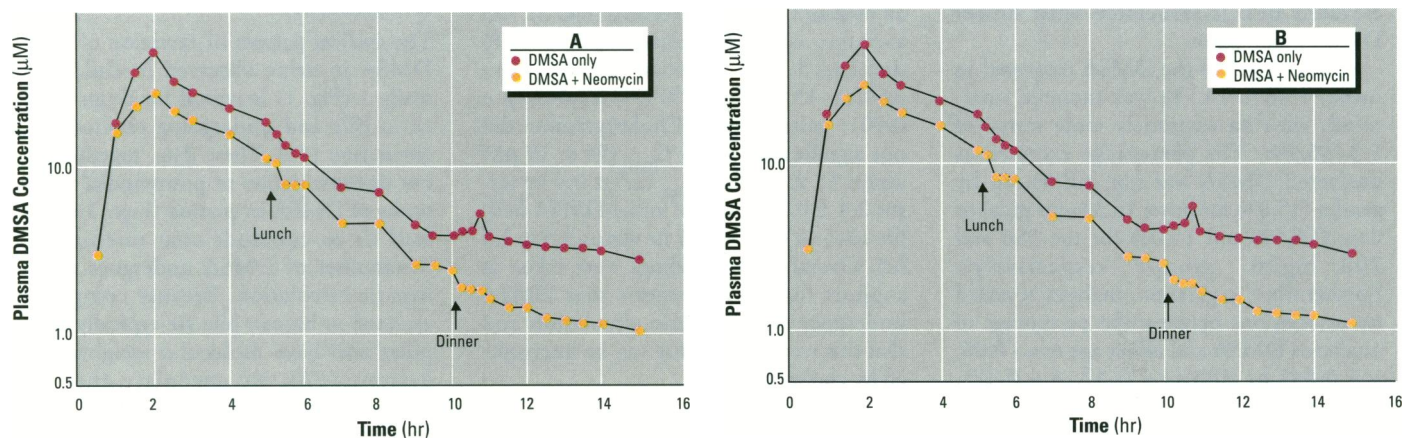


Figure 3. Semilog plots of plasma total meso-2,3-dimercaptosuccinic acid (DMSA) concentration versus time in two typical normal adults (panels A and B) following the administration of a single dose of 10 mg/kg DMSA at time zero. When DMSA was given alone, the plasma DMSA concentration peaked and then fell gradually; postprandial peaks were evident. When DMSA was given with neomycin, the plasma DMSA concentration peaked but fell gradually without any evidence of postprandial reentry.

parent DMSA molecule (Fig. 1). It therefore appears that a metabolite of DMSA is an active lead chelator, if not the active chelator. Indeed, if we assume that each urine sample found to have undetectable amounts of unaltered DMSA had a concentration of 0.01 μM DMSA (i.e., the detection limit of the assay), we calculate the average molar ratio of lead:DMSA for the subjects shown in Figure 1 to be 77. Thus, the parent DMSA molecule cannot be responsible for more than a minuscule portion of the observed postprandial lead excretion. However, lead is excreted in bile under basal conditions (26), and we cannot rule out the possibility that comparable postprandial lead excretion might have occurred following a placebo. Thus, the possibility remains that the postprandial peaks in the urinary lead observed in study 1 may not be causally related to the excretion of altered DMSA.

In 1989, Aposhian and co-workers (12) first speculated that DMSA may be a pro-drug after observing that DMSA was rapidly and extensively eliminated in the altered form. They subsequently reported that the major metabolite of DMSA is the 1:2 DMSA:cysteine mixed disulfide (10). They have since synthesized the 1:2 DMSA:cysteine metabolite and administered it to lead-poisoned rats to examine its efficacy (27). The 1:2 metabolite increased urinary lead excretion as compared to saline or cysteine-treated animals; lead excretion was comparable to that observed in DMSA-treated rats. However, because DMSA and its mixed disulfides were detected in the urine of both 1:2 metabolite-treated and DMSA-treated animals, the authors could not conclude that the 1:2 metabolite is the sole active compound. Another recent study, however, has clearly demonstrated that the 1:2 mixed disulfide and not DMSA can prevent the *in vitro* immunotoxicity of gallium

arsenide (28). Collectively, these studies imply that the 1:2 DMSA:cysteine metabolite plays a major role in ameliorating heavy-metal toxicity.

An initial hypothesis of this work was that the metabolism and/or elimination of DMSA may be saturable. This hypothesis, based on an unpublished study in dogs, was tested by estimating the mean half-life of elimination of DMSA in the children in the two dose groups. We conclude that there are no immediately saturable processes across the dose range of 350–700 mg/m².

A second hypothesis was that environmental lead exposure, as assessed by BPb, influences the pharmacokinetics of DMSA in children. We observed a significant positive association between BPb and DMSA half-life, suggesting that lead exposure may interfere with renal elimination of DMSA. Although many explanations are possible, it seems likely that lead may interfere with renal tubular secretion of DMSA. In rats, DMSA is eliminated by renal tubular secretion and is blocked by probenecid (29). Dart et al. (16) recently reported that renal DMSA clearance was greater in five healthy, white male adults than in three adults and three children with lead poisoning, although those findings must be interpreted with caution because their controls differed from the lead-poisoned subjects in both race and gender. Nevertheless, we speculate that other drugs excreted by the kidney, including other chelating agents, may also be eliminated more slowly in children with elevated BPbs. Collectively, these findings reveal a need for more careful assessment of renal tubular secretion and drug elimination in children with moderately elevated BPbs.

In a phase 1 study involving [¹⁴C] DMSA administration to normal male adult volunteers, 80% of the recovered

radioactivity was found in the feces, 1% in expired air, and 19% in the urine (9). HPLC studies in adults also estimated urinary drug recovery at 20% (12). We recovered only about 5% of the administered dose in urine in the children in study 1, an unexplained observation that requires further study; across the two subsequent studies in adults, we recovered an average of 14.2% of the administered dose of DMSA in urine in 15 hr. In the ¹⁴C study, it was assumed that fecal radioactivity represented unabsorbed drug (9). Our findings imply that fecal drug elimination is partially derived from bile. Also, while the initial $t_{1/2e}$ of [¹⁴C]DMSA was 2.1 hr, comparable to those observed in our studies, a terminal $t_{1/2e}$ of 2.1 days was also reported. We suspect that the long second phase of elimination may reflect storage of drug metabolites in the gallbladder.

The observation that DMSA and/or a metabolite undergoes enterohepatic circulation also implies that the drug may also induce biliary excretion of heavy metals. All previous studies concerning the efficacy of DMSA in patients with lead poisoning have ignored this possibility; only urinary lead was followed (2–5). For example, in a dose-ranging study of DMSA in children, the decline in BPb in response to a 5-day course of oral DMSA was found to be significantly greater than that in response to intravenous CaNa₂EDTA. Yet, urinary lead output was significantly higher in patients treated with CaNa₂EDTA than in those who received oral DMSA (2). This apparent dilemma might have been due to biliary lead excretion in those who received oral DMSA.

Reichl et al. (30) studied the effect of DMSA and other chelators on the excretion of arsenic in bile in guinea pigs. A dose-dependent rise in biliary arsenic was observed, along with a rise in the bile/blood

ratio of arsenic concentrations. In contrast, DMSA was not detected in bile after intravenous DMSA administration in the rat (31). It is possible that biliary DMSA excretion may only occur after oral drug administration; alternatively, the known species differences in drug biliary excretion may be responsible (18).

Finally, we note that enterohepatic recycling has both pharmacokinetic and pharmacodynamic implications. In the event of an accidental DMSA overdose, cholestyramine may prove to be therapeutically useful. Renowden et al. (32) successfully treated a case of warfarin overdose with cholestyramine. The latter is a far more palatable antidote than traditional activated charcoal.

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"Mechanisms and Prevention of Environmentally Caused Cancers", a symposium presented by The Lovelace Institutes, will be held October 21-25, 1995, in Santa Fe, New Mexico. The purpose of this symposium is to promote collaboration between scientists interested in the basic mechanisms of environmentally-caused cancer and investigators focusing on preventing cancer development with chemo-intervention strategies. Dr. Bruce Ames (University of California) will be the keynote speaker. Other speakers include Dr. Eric Stanbridge (UC Irvine), Dr. Stephen Friend (Harvard), and Dr. Gary Stoner (Ohio State University).

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