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MESO-2,3-DIMERCAPTOSUCCINIC ACID: Chemical, Pharmacological and Toxicological Properties of an Orally Effective Metal Chelating Agent

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INTRODUCTION

The Need

There is increasing concern for young children and pregnant women with above normal exposure to lead. The concern is due to the generally accepted results of epidemiologic studies indicating that low levels of lead may affect fetal and child development. As pointed out in a very thought-provoking article by Davis & Svendsgaard (1), "There can now be little doubt that exposure to lead, even at blood levels as low as 10–15 μg/dl and possibly lower, is linked with undesirable developmental outcomes in human fetuses and children. These effects include impaired neurobehavioral development . . . and other possible effects on early-development and growth." More specifically, such levels of Pb may lead to impairments of the central

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nervous system such as delayed cognitive development, reduced IQ scores, impaired hearing, and neurobehavioral deficiencies (1). These are only a few of the effects of lead exposure, of which some may not be reversible.

A recent report prepared for Congress (2) stated that 3 million children in the United States have blood lead levels above 15 μg/dl. In addition, in the absence of effective abatement of lead exposure, 4 million fetuses are estimated to be at risk for lead toxicity during the next 10 years in the United States (2).

If the child is moved from the lead exposure and if the blood lead levels are above normal, chelation therapy is usually considered. According to Centers for Disease Control (CDC) records from 1973–1978, 22,254 children received British Anti-Lewisite (BAL) or chelation therapy (4).

As the concern for and knowledge about lead toxicity has increased, the amount of lead in the blood of children considered to warrant medical attention has progressively decreased. At present, the blood-lead level believed to be indicative of toxic exposure according to the CDC is 25 μg Pb/dl and above (6); or 20 μg/dl according to WHO (7). The Clean Air Scientific Advisory Committee to the U.S. Environmental Protection Agency, on the other hand, regards a 10–15 μg Pb/dl blood and above (7) as a matter of concern. Thus, as the blood Pb level believed to be indicative of toxic exposure decreases, it would not be surprising for the use of chelating agents to lower elevated blood Pb values of children and pregnant women to increase. If this occurs, the need for a safe and effective chelating agent to reduce blood Pb levels becomes of increasing importance. An excellent evaluation by Chisholm (8) of the role of chelation therapy in the treatment of lead poisoning of children has appeared recently and is strongly recommended reading for those who would like an expert appraisal of the many problems in this field.

$CaNa_2EDTA$,D-penicillamine and BAL have been used in the past for treating the toxicity of lead as well as other metals (9). *meso*-Dimercaptosuccinic acid (DMSA) has used been extensively in the People's Republic of China, and 2,3-dimercapto-1-propanesulfonic acid, Na salt (DMPS) was developed in the Soviet Union. Since the 1950s, it has been an official drug, Unithiol, and has replaced BAL in the Soviet armamentarium. The early Chinese and Soviet contributions up to 1982 have been extensively reviewed previously (10).

The U.S. Food and Drug Administration has designated *meso*-DMSA as an Orphan Drug (11) and classified it as an Investigational New Drug. A New Drug Application was filed in August, 1989.

The purpose of this paper is to present the chemical, pharmacological, and toxicological properties of *meso*-DMSA, which appears to be the most promising chelating agent available for treatment of lead poisoning in hu-

mans. This will be done by reviewing its (*a*) chemical properties such as the chemical structures and formation constants of some of its chelates; (*b*) antidotal properties for heavy metal poisoning in humans (*c*) pharmacokinetics and biotransformation (d) properties as an antidote in experimental animals and (*e*) other properties of interest. We do not list the drugs of choice for the treatment of the toxicity of individual metals, nor is this an exhaustive, all-inclusive review. Most publications prior to 1982 are not even mentioned because they were covered and cited in the previous review published in 1983 (10). Other excellent reviews have appeared also (12, 13).

DMSA is Distributed Extracellularly

For some time, the belief that *meso*-DMSA cannot enter a cell has been attributed to the fact that its highly charged carboxyl groups prevent its passage through the cell membrane, although direct experimental evidence has been lacking. On the other hand, there has been evidence for DMPS entering the cell (14, 15). Recent experiments clearly support the extracellular, not intracellular, distribution of *meso*-DMSA (W. Zheng & H. V. Aposhian, paper submitted). DMSA was injected iv into rats and their bile was analyzed for it by bimane derivatization, high performance liquid chromatography (HPLC) separation, and fluorescence detection (16, 17). Under these conditions, neither DMSA nor its disulfide form was found in the bile. Although DMPS was present, it was quickly biotransformed to its disulfide form. N-(2,3-dimercaptopropyl)phthalamidic acid (DMPA) was found to be the most stable dithiol in the bile (W. Zheng & H. V. Aposhian, paper submitted). For one of these compounds to appear in the bile, it must pass through the cell membranes of hepatocytes. Our results demonstrate that *meso*-DMSA is distributed in an extracellular manner.

CHEMISTRY

Structure of Metal Chelates

meso-DMSA (Figure 1) is a weak acid with four ionizable hydrogens. The acid dissociation constants have been determined under a variety of conditions by a number of groups (18–22). Egorova (19) indicated that at 0.01M ionic strength, 20 C, 1.0×10^{-5}M *meso*-DMSA, the $pK_1=2.31$, $pK_2=3.69$, $pK_3=9.68$, and the $pK_4=11.14$. The pK_a values for the hydrogens of the carboxyl groups are the two lower pKs, whereas the two higher values are for the dissociation of the hydrogens of the thiols. The pK values for dl-DMSA have also been determined (18, 22). The differences between the values determined by various groups are not small. Such measurements are not easy to make because of the limitations of glass electrodes.

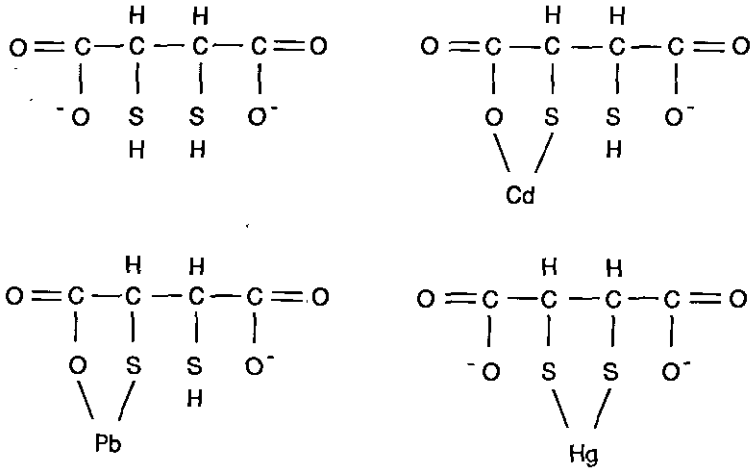

Figure 1 Metal chelate structures of *meso*-2,3-dimercapotosuccinic acid (23).

It has generally been assumed that the chelates of all dimercapto chelating agents are formed by coordination of both sulfur atoms with the metal or metalloid. In long-needed and elegant experiments using NMR and infrared spectrometry together with potentiometric titrations of DMSA suspensions, Rivera et al (23) showed that Pb^{2+} or Cd^{2+} coordinates with one of the sulfur and one of the oxygen atoms of *meso*-DMSA. On the other hand, Hg^{2+} or Ni^{2+} coordinate with each of the two sulfur atoms (Figure 1). The coordination sites on *meso*-DMSA depend on the type of metal ion being coordinated. Of course, the structure of any compound is determined positively only by using X-ray crystallography. Such experiments are in progress. More in-depth knowledge of the crystalline lattice structure of these chelates might lead to better chelating agents.

Rivera et al (23) also found that the solubilities of all the DMSA metal chelates examined were pH-dependent. The complexes of Cd^{2+} or Pb^{2+} are insoluble in the pH range of 1.0–7.1, but they are solubilized when the noncoordinated thiol and carboxylic acid groups are dissociated. The Hg^{2+} complex is insoluble in the pH range of 1.0–3.0. It dissolves when one of the noncoordinated carboxylic acid groups is dissociated.

The dimethyl ester of *meso*-DMSA was synthesized and its dissociation constants were found to be $pK_1=6.38$ and $pK_2=8.00$. Esterification of the carboxyl groups changed the coordination properties of *meso*-DMSA. The two sulfur atoms of the dimethyl ester of meso-DMSA coordinate with Cd^{2+}, Pb^{2+} or Hg^{2+}. Also, when it was given to rats three days after $CdCl_2$ administration, the dimethyl ester analog greatly increased the biliary excretion of Cd, whereas *meso*-DMSA was devoid of such activity (23).

In an extension of these careful and elegant studies by Mario Rivera, a graduate student in the laboratory of Professor Quintus Fernando of the Chemistry Department, University of Arizona, the lead chelates of racemic-DMSA were synthesized and characterized by potentiometric measurements and infrared spectroscopy (24). The results were quite unexpected. Two types of lead chelates for racemic-DMSA were isolated. One in which racemic-DMSA is coordinated to Pb^{2+} via one oxygen and one sulfur atom and the other chelate in which the Pb^{2+} is coordinated via the two sulfur atoms. Only one type of *meso*-DMSA-Pb chelate was found and that was shown previously to involve coordination via one oxygen and one sulfur atom. Rivera et al (24) also demonstrated that the *meso*- and racemic-DMSA have such distinct features in their infrared spectra that this type of spectroscopy can be used to detect and identify the presence of either diasteroisomer. It is surprising that no report seems to exist in the literature as to the activity of racemic-DMSA as a lead antidote.

It should be kept in mind that chelates made up of one Pb ion and one DMSA molecule may *not* be the structure of the lead chelate formed in vivo. The actual form of the chelate that occurs in vivo and under conditions of metal toxicity must await elucidation. Such chelates must be isolated from the urine or other in vivo sources and be chemically characterized.

The formation constants for DMSA-metal chelates (Table 1) are of particular interest in understanding the pharmacology of DMSA. As mentioned later, one of the major disadvantages of the treatment of plumbism with $CaNa_2$ EDTA is the large and often dangerous diuresis of Zn^{2+} that occurs. One reason why this may not occur with *meso*-DMSA can be seen by comparing the complex formation constants in Table 1. The K_{ML} for DMSA and Pb^{2+} are 50–100 times greater than that for Zn^{2+}. Unfortunately, the K_{ML_2} value for lead and *meso*-DMSA is not yet available. Formation constants for *meso*-DMSA and Pb^{2+}, Zn^{2+}, Cu^{2+} or Hg^{2+} determined in the same laboratory would be of great help in the theory of chelation of these metals with DMSA.

Matsuda (26) used the disodium salt of DMSA to study the stability constants with a number of metals. The metal salts used were $FeCl_3 \cdot 6H_2O$, $HgCl_2$, $NiSO_4 \cdot 6H_2O$, $Pb(NO_3)_2$, $CdCl_2 \cdot 2H_2O$, and $Zn(NO_3)_2$. He concluded from titration data that the stabilities of the metal chelates of the disodium salt of DMSA were in the order $Cd^{2+} > Pb^{2+} > Fe^{2+} > Hg^{2+} > Zn^{2+} > Ni^{2+}$. In vivo, however, these figures could be misleading since most of the Cd^{2+} in a cell is firmly bound to metallothionein. Unless the chelating agent can enter the cell and compete with metallothionein for the Cd^{2+}, its therapeutic use is limited regardless of how stable the chelate.

In a superb and sophisticated investigation using two-dimensional NMR, O'Connor et al (28) have synthesized and proven the structures of the DMSA

Table 1 Formation constants for some chelates of dimercaptosuccinic acid

Metal	DMSA	$K^{M}_{MH_2L}$	K^{M}_{MHL}	K_{ML}	K_{MLOH}	K_{ML_2}	K_{M_2L}	Reference
Pb^{+2}	meso-			2.9×10^{17}			1.6×10^{27}	19
	meso-						1.7×10^{27}	22
	d,1-						1.1×10^{28}	22
Zn^{+2}	meso-	6.3×10^{2}	4.0×10^{9}	6.6×10^{15}	5×10^{-13}	3.7×10^{3}	7.1×10^{3}	25
	meso-	6.3×10^{3}	2.0×10^{9}	2.6×10^{14}	8×10^{-11}	2.1×10^{3}	1.2×10^{4}	20
Ni^{+2}	meso-	7.9×10^{3}	3.2×10^{9}	4.9×10^{11}	7×10^{-12}	4.4×10^{2}		20
In^{+3}	meso-		1.8×10^{13}	4.8×10^{21}				21

M = Metal
H_2L = DMSA

adducts of phenyldichloroarsine or *trans*-2-chlorovinylarsine oxide. Five-membered rings containing As and the two sulfur atoms are formed.

Analytical Methods for DMSA Determination

Although a number of methods are available for the analytical determination of thiol compounds (29), most are not specific enough and are of questionable value for the analysis of biological fluids in which more than one type of thiol compound is present. This has necessitated the development of reliable methods for the determination of DMSA and its metabolites in urine and blood. In our laboratory, a method has been developed which uses monobromobimane (mBB) for the derivatization of the thiol groups (16, 17). This method is reasonably rapid and clearly quantitative. It can detect 10 pmol of a dithiol per 20μl injection and the precision is 7.4% at the 100 pmol level. The method has been extended by electrolytic or dithiothreitol reduction of oxidized dithiols.

The principle on which the determination of DMSA is based consists of converting DMSA to its highly fluorescent and stable bimane derivative, which is separated by using HPLC ion-interaction chromatography and fluorescence detection. This is accomplished by the reaction between the thiol groups of DMSA and the methylene bromide moiety of mBB in aqueous solution at pH 8.3. *Unaltered* DMSA is determined by the direct analysis of a urine sample by this method. *Total* DMSA (unaltered + altered) is determined by the same method, except that a sample of the urine first undergoes electrolytic reduction to reduce any oxidized DMSA (e.g. disulfides and/or polymeric DMSA). The sample is then immediately derivatized with mBB and continued through the analytical procedure. *Altered* DMSA is obtained by subtracting the amount of *unaltered* DMSA from the amount of *total* DMSA. *Altered* DMSA can be expected to represent DMSA disulfides and mixed disulfides of DMSA with cysteine, GSH, and other thiols, as well as any polymeric DMSA. Quantification is accomplished by adding DMSA standards to urine or other body fluid samples, which are collected before drug administration (blanks), and treating them in the same manner as the samples collected after DMSA administration.

Knudsen & McGown (30) have developed a gas chromatographic assay for DMSA in urine samples. In their procedure, electrochemical reduction is used to reduce any oxidized DMSA and the solution is then extracted with ethyl acetate. After evaporation of the ethyl acetate, the DMSA is derivatized by reaction with N,O-bis-(trimethylsilyl)-acetamide. The amount of the trimethylsilyl derivative of DMSA is determined by gas chromatography using a flame ionization detector. The detection limit is 1.9 nmol DMSA per 1μl aliquot of derivatized extract. Heptadecanoic acid was used as the internal standard but was added after the extraction step because of its insolubility.

The method has been used to quantitate *meso*-DMSA in the urine of rats. The paper also contains information of interest about the auto-oxidation of *meso*-DMSA in rat urine and in distilled water.

HUMAN STUDIES

Antidote

LEAD Friedheim et al (31) gave five lead-smelter workers with Pb poisoning *meso*-DMSA, p.o., for six days at levels from 8.4–12.7 mg/kg/day on the first day to 28.1–42.2 mg/kg/day on the final day. The mean Pb concentration of the bloods was 97±6 μg/dl before treatment. It decreased to about half that value after the course of treatment. *meso*-DMSA appeared to be without effect on the urinary excretion of Zn, Ca, Mg, or Fe. Although the urinary excretion of Cu increased, the amount was considered to be clinically unimportant. A limited comparison of *meso*-DMSA, given p.o., was made with $CaNa_2EDTA$, given i.v. The Pb excretion after $CaNa_2EDTA$ administration was greater than that found after *meso*-DMSA. The Zn-excretion after $CaNa_2EDTA$ was about 7 times greater than after *meso*-DMSA. The authors concluded that "DMSA seems to be safe and effective for the treatment of lead poisoning."

Graziano et al (32) continued the study of the safety and efficacy of orally administered *meso*-DMSA by a balanced, multiple-dose, single-blind, randomized study. In this first comprehensive, clinical evaluation of *meso*-DMSA, the subjects were eighteen men between the ages of 24–58 years, with occupationally associated Pb poisoning. DMSA was given at 10, 20, or 30 mg/kg/day in three divided daily doses for 5 days. The initial blood-Pb concentrations ranged from 44–88 ug/dl. They decreased to about 65, 40, and 28% of the pretreatment values after 5 days of treatment with 10, 20, or 30 mg DMSA/kg, respectively. When DMSA therapy was stopped, a rebound of blood-Pb concentration occurred, which is also seen with most other treatments of Pb intoxications. There was also a dose-dependent increase in the mean red cell d-aminolevulinic acid dehydratase activity. The 30 mg DMSA/kg regimen gave the greatest increase of mean urinary Pb. It increased to almost 7.4 mg Pb after one day of treatment. This was more than three times greater than that found with either of the two lower-dose regimens. Urinary excretion of d-aminolevulinic acid and coproporphyrin also decreased with DMSA treatment. Urinary excretion of other metals such as Cu, Zn, Fe, and Ca did not show any changes of clinical significance. The excretion of such metals is discussed further and in more detail in the section *Excretion of Other Metals*. A transient SGPT elevation was noted in two subjects and was the only adverse drug reaction observed. This suggests that serum enzyme determinations should accompany the future clinical uses of *meso*-DMSA. A careful and complete study of other blood and serum parameters was carried

out in these important clinical studies of DMSA safety and effectiveness by Graziano et al (32).

These DMSA studies have been extended further by Graziano et al (4) to include young children. Twenty-one children, 2–7 years old, with elevated blood Pb concentrations of 31–49 μg/dl and a positive $CaNa_2EDTA$ mobilization test were treated with three divided doses of *meso*-DMSA po per day or two divided doses of $CaNa_2EDTA$ i.v. per day for 5 days. The *meso*-DMSA dose of 350, 700, or 1050 mg/m^2/day is equivalent to 10, 20, or 30 mg/kg/day. The $CaNa_2EDTA$ dose was 1000 mg/m^2/day.

The highest dose of DMSA was significantly more effective than the conventional i.v. $CaNa_2EDTA$ treatment or the lower doses of DMSA in reducing blood-lead concentrations and restoring red cell d-aminolevulinic acid dehydratase activity. DMSA reduced blood Pb values to about 25–30% of the pretreatment value. Under the experimental conditions used, however, the increased urinary excretion of Pb was greater after $CaNa_2EDTA$ treatment than after DMSA. Again, DMSA did not significantly increase the urinary excretion of Zn, Cu, Fe, or Ca, whereas $CaNa_2EDTA$ did. The increase of urinary Zn after $CaNa_2EDTA$ was ten times greater than that seen after *meso*-DMSA. Increased urinary excretion of Zn always has been, and will continue to be, a problem with $CaNa_2EDTA$.

meso-DMSA appears to be safe, since all 15 children tolerated it well and no clinically significant abnormal blood counts, vital signs or clinical chemistry values occurred. The authors stated that "DMSA was well tolerated and appears extremely promising as a drug which will simplify the management of childhood lead poisoning" (4).

Chisholm has begun an extensive study of the efficacy of *meso*-DMSA in the treatment of young children with Pb poisoning. Results of initial studies (8) with four children given five 10-day courses of oral *meso*-DMSA have confirmed the effectiveness of this chelating agent in treating children with Pb intoxication. Therapy was initiated with three divided doses of 1050 mg DMSA/m^2/day for 5 days and diminished to 350 mg/m^2/day given in divided doses for the next 5-day course of treatment. According to Chisholm (8), "DMSA is a most promising new oral chelating agent for the treatment of low and moderate lead exposures. Early studies indicate that short courses are safe and efficacious." Both Graziano and Chisholm point out the need for further study of *meso*-DMSA safety and efficacy for long-term treatment.

Fournier et al (33) used the 5-day course of 30 mg *meso*-DMSA/kg/day to treat nine people with lead poisoning. Blood Pb concentrations at admission were from 43.5–710 μg/dl. The blood-lead concentrations were reduced by 35–81% and there was a 4.5–16.9-fold increase in mean urinary lead after treatment. One of these patients was given this daily dose of DMSA for 15 days because of her very elevated blood-lead levels. If encephalopathy or lead

colic was present initially, improvement was noted after DMSA treatment. No serious side effects of DMSA were found. They concluded with the statement (33) "Our data strongly indicate that oral DMSA is a positive alternative to EDTA therapy for lead poisoning in humans."

The need for newer and longer courses of therapy with *meso*-DMSA are apparent—one different from the customary 5-day $CaNa_2EDTA$ regimens. One must keep in mind that $CaNa_2EDTA$ has many definite disadvantages. It is administered parenterally, which causes pain; it reduces plasma Zn and causes a huge Zn diuresis, as well as an increased urinary excretion of Mn and Fe after its administration. Chisholm has suggested that much of the toxicity seen with the use of $CaNa_2EDTA$ may be connected with the mobilization of zinc (8). Chisholm, with many years experience in the treatment of children with lead poisoning, states that $CaNa_2EDTA$ "does not appear to be an ideal agent for the treatment of modest increases in body lead burden." He also points out the need for new biologic markers that must be developed to help assess the efficacy of DMSA and other chelating agents on the developing central nervous system of young children.

A major advantage of *meso*-DMSA is that in rats it decreases brain Pb. This does not appear to be the case with $CaNa_2EDTA$. Cory-Slechta et al gave $CANa_2EDTA$ for 5 days to rats that had been chronically exposed to low-lead levels (34). At a dose of 75 or 150 mg $CaNa_2EDTA$ per kg, the concentration of Pb in the brain equaled almost that of control animals even though the urine lead increased and lead levels in the blood, bone, and kidney decreased. Because the developing brain is the critical organ for lead in the fetus and young child (35), such results are troublesome, to say the least, when past and future therapy of children with $CaNa_2EDTA$ is considered. Cory-Slechta's contribution is significant and very important.

In a subsequent paper, Cory-Slechta (36) reported experiments in which rats, after 3–4 months exposure to lead, were given 50 mg DMSA/kg. The DMSA did not appear to cause a redistribution of lead. No decrease in the lead from the femur bone was detected. DMSA mobilized Pb only from the soft tissue.

Such lengthy chronic experiments are still not easy to accomplish, and therefore the importance of Cory-Slechta's contribution cannot be overemphasized. More of such chronic experiments using primates to confirm and extend her studies in the rat are highly desirable.

Cory-Slechta (36) further suggests that DMSA might be used as an adjunct to $CaNa_2EDTA$. The latter would release Pb from the bone and DMSA would be expected to prevent its redistribution. Although this is an interesting concept and certainly some kind of combination therapy might be quite useful in treating lead intoxication, any use of $CaNa_2EDTA$ is fraught with risk, for the reasons presented above.

Chisholm (8) points out that to decrease the possibility of the redistribution of bone lead to the brain and other tissues "once the mobilization of lead is started with chelating agents it must be sustained. Unfortunately, $CaNa_2EDTA$ cannot be safely given on a daily basis for more than five days." He goes on to call "for a reappraisal of the role of $CaNa_2EDTA$, particularly its diagnostic use." Such concerns of an outstanding pediatric investigator who has dealt for many years with the treatment of lead poisoning in children should not be ignored. His editorial (37) is highly recommended reading for both the clinician and basic science investigator.

BAL forms a toxic chelate with iron. Treatment with BAL may result in iron depletion. Because DMSA is a chemical analog of BAL, the question arose whether iron supplementation during DMSA therapy would produce any untoward effects. Haust et al (38) gave iron im as iron sorbitol (100 mg elemental iron every three days for a total of 400 mg) to a patient with a large body burden of lead during the fifth of ten long-term courses with DMSA. No untoward effects were noted.

Haust treated this patient from approximately July, 1986, to January, 1989. The shortest course was 20 and the longest was 66 consecutive days—the longest course of treatment with DMSA of which we are aware. The mean urinary Pb value was 468 μg/24hr for the 10 periods before DMSA was administered. The average daily urinary Pb after DMSA treatment was 3,027 μg/24hr. Therefore, there was a 6.5 fold increase in urinary Pb/day over the 340 days of DMSA treatment (H. L. Haust, personal communication). The publication of the details of the extensive treatment of this unusual patient with chronic lead intoxication will be awaited by those interested in treating lead intoxication.

A 1984 case report from France (39) in which the treatment of a 63-yr old man with $CaNa_2EDTA$ before and after DMSA treatment showed that, under the conditions used, DMSA was superior to $CaNa_2EDTA$ in increasing the urinary excretion of lead.

When given orally, *meso*-DMSA does not appear to increase the absorption of lead from the GI tract of rats (40). It is hoped that such experiments will be confirmed in higher animals, especially primates, since there appears to be species differences with *meso*-DMSA absorption as reported below.

ARSENIC AND MERCURY Fournier et al (33) reported the treatment of two patients with arsenic poisoning. Using 30 mg *meso*-DMSA/kg/day for 5 days, clinical improvement and arsenic excretion were accomplished. The plasma arsenic concentration of one of the patients was half the pretreatment level after 7 days.

Three patients with mercury intoxication were treated with 30 mg *meso*-DMSA/kg/day for 5 days (33). Hg exposure occurred after application of a

skin ointment in one case and by vapor inhalation in the others. Although the mercury intoxications were not severe and allergy to mercury was suggested, DMSA treatment increased daily Hg excretion 1.5–8.5-fold and returned kidney function to normal.

There is evidence that *meso*-DMSA, when given 73 days after exposure of humans to elemental mercury, increased the urinary excretion of mercury (A. J. Heath, personal communication). A spill of an estimated 300 tons of mercury in a chlorine plant exposed 50 workers to mercury vapor for up to 16 hrs. Seventy-three days later, eight of the exposed workers were given 30 mg *meso*-DMSA/kg/day for four days in hospital. The DMSA administration resulted in a three-fold increase in the urinary excretion of mercury. Since mercury has a half-life of 31–100 days in the body, the effectiveness of DMSA even when administered 73 days after exposure is promising for therapy of mercury intoxication in the human, especially when therapy is not or cannot be started immediately. Publication by the Heath group of the data resulting from *meso*-DMSA and other treatments of these workers exposed to mercury will be awaited with interest.

Excretion of Other Metals

In the studies of 18 men with significant body burdens of lead, Graziano et al (32) demonstrated that *meso*-DMSA had no significant effect on the urinary excretion of Fe, Ca, or Mg under the conditions of their studies. Of greater importance, the urinary excretion of Zn after the highest *meso*-DMSA dose regimen was less than a doubling. This is much less than the 1000% increase in urinary Zn excretion that occurs after $CaNa_2EDTA$ treatment (32).

Various groups disagree as to whether DMSA influences the urinary excretion of copper. In the study by Fournier et al (33), the median level and range of changes before and after DMSA treatment for copper, iron, calcium, and magnesium concentrations in the plasma were not significant. The changes in plasma Zn were significant.

As a tangential study while investigating the excretion and metabolism of *meso*-DMSA in normal subjects, the urinary excretion of 27 metals was also examined (41). Six normal, young volunteer men were fasted for 11 hr and *meso*-DMSA (10mg/kg), in hard gelatin capsules, was given p.o., followed by distilled water. There were statistically significant but only small increases in the excretion of Zn, Cu, and Pb. The urinary Cu, Pb, and Zn excretions were compared to the urinary excretion of total DMSA by linear regression analysis. The significant correlation coefficients for total DMSA vs Cu, Pb, and Zn were 0.942, 0.603, and 0.594, respectively. DMSA did not influence the urinary excretion of 27 other metals and elements.

Pharmacokinetics and Biotransformation Studies

PHARMACOKINETIC Until recently, very little was known about the metabolism and pharmacokinetics of *meso*-dimercaptosuccinic acid. With the development of the highly sensitive and accurate monobromobimane assay for DMSA by Maiorino et al (16, 17), it became possible for the first time to determine DMSA accurately in biological fluids of humans without the use of radioisotopes. In addition, the same assay has been recently modified and extended for the determination of DMSA and its metabolites in blood and plasma (R. M. Maiorino & H. V. Aposhian, in preparation).

Six normal, healthy, young men, between the ages of 22 and 31 years, were fasted for 11 hr and given *meso*-DMSA (10mg/kg) p.o., in hard gelatin capsules, followed by distilled water (41). The urine was collected at specified times over a 14 hr period. By 14 hr after DMSA administration, only 2.5% of the administered DMSA was excreted in the urine as unaltered DMSA; 18.1% of the dose was found in the urine as altered forms of DMSA. Of the total DMSA found in the urine, 12% was unaltered DMSA which reached a peak in the urine before the altered DMSA peaked. The altered, or biotransformed DMSA reached a peak in the urine between 2–4 hr after DMSA administration and made up 88% of the total DMSA found in the urine. Reductive electrolytic treatment of the urine resulted in an eightfold increase in urinary DMSA. This indicates that when DMSA is given orally to humans the majority of it is excreted in the form of a disulfide(s).

It is worthy of note that when ^{14}C-DMSA was given p.o. to four juvenile monkeys (Macaca mulatta) by McGowan's group (42), about 18% of the label was found in the urine, 65% in the feces, and 2% in the expired air. This suggests that the monkey and the human may have similar urinary profiles and may handle DMSA in the same way. This is not the case with the hamster (42), mouse (42), or rabbit (43). When McGowan's group gave DMSA po to hamsters, they recovered 81% of the radioactivity in the urine (42).

BIOTRANSFORMATION What are the structures of the altered DMSA? To find out, two fasted, normal, young men were given 10 mg *meso*-DMSA/kg, po. Urines were collected over a 14 hr period and analyzed for DMSA and other metabolites before and after electrolytic reduction. Metabolites were isolated by ion-pairing extraction, ion-exchange extraction and TLC (44). The major form (90%) of altered DMSA in the urine 2–4 hr after DMSA administration was DMSA in disulfide linkage with L-cysteine. This has been called *DMSA mixed disulfide*. It consists of DMSA in disulfide linkages with two molecules of L-cysteine. One molecule of cysteine is attached to each of the sulfur atoms of DMSA. The remaining 10% of the altered DMSA was in the

form of cyclic disulfides of DMSA. In urine collected 4–6 hr after DMSA administration, 97% of the mixed disulfides consisted of two cysteine residues per DMSA molecule and 3% consisted of one cysteine per DMSA. These are very novel DMSA structures. The urinary excretion of altered DMSA and altered L-cysteine after *meso*-DMSA administration had a correlation coefficient of 0.952 with a $p<0.003$. Thus, it appears that when *meso*-DMSA is given orally to humans, it forms mixed disulfides with L-cysteine in preference to forming cyclic disulfides of DMSA. In addition, L-cysteine after DMSA administration forms this mixed disulfide in preference to forming L-cystine. It appears that a thiol-disulfide exchange between L-cystine and DMSA or a direct reaction between DMSA and L-cysteine takes place.

There appears to be a species difference as to the biotransformation of *meso*-DMSA. In rabbits given ^{14}C-*meso*-DMSA im, 73% of the radioactivity was unaltered DMSA and 7% was mercaptosuccinic acid. None of the mixed disulfide of DMSA and cysteine could be detected (43). This was true even when the route of administration and dose level in the rabbit was changed to be equivalent to that given to man (K. Blaha, R. M. Maiorino & H. V. Aposhian, unpublished data).

The assay for DMSA in human blood after administration of *meso*-DMSA, clearly shows that very little, if any, free unaltered DMSA occurs in the blood. The DMSA in the blood is found in disulfide linkage with plasma protein. No DMSA in any form is found in the red blood cells (R. M. Maiorino & H. V. Aposhian, unpublished data).

The biotransformation pathway of *meso*-DMSA, in which two molecules of cysteine attach to it in disulfide linkage, warrants the study of DMSA as a treatment for cystinuria, such as in L-cystine nephrolithiasis. Past treatments of cystinuria have been less efficient and more toxic. Penicillamine or other monothiols result in the urinary excretion of only one molecule of cysteine per molecule of thiol compound. DMSA would be expected to bring two molecules of cysteine with each DMSA molecule excreted.

STUDIES IN EXPERIMENTAL ANIMALS

Space limitations prevent us from discussing in detail the signs and symptoms of metal intoxication as well as occupational exposure. Instead we recommend with great enthusiasm the monograph *Biological Monitoring of Toxic Metals* (45), in our opinion the best book on metal toxicology currently available.

Aluminum, Arsenic, Bismuth, Cadmium, Cobalt, and Copper

ALUMINUM The efficacy of a number of chelating agents for the treatment of acute aluminum toxicity has been investigated (46). Aluminum nitrate, 3.1

mmol/kg, was administered ip to mice and immediately followed with an ip injection of the chelating agent to be tested. The dose of the chelating agent was one third of its LD50. *meso*-DMSA reduced Al in the liver, spleen, and kidney to concentrations less than those of the untreated controls. For these tissues, DMSA was the most efficacious of the chelating agents examined. The Al level in the brain after DMSA treatment was so low that it could not be detected, unlike the other seven chelating agents studied. But DMSA did not increase the urinary and fecal excretion of Al. What happened to the Al is unclear since it was not deposited in the tissues examined nor was it excreted in the urine and feces. Of all the chelating agents tested by these methods, citric acid appeared to be the most efficacious in causing the increase in the excretion of Al. The activity of DMSA needs to be studied for the removal of aluminum which has been deposited under conditions of chronic exposure.

ARSENIC Arsenic poisoning continues to be seen because it is used in herbicides, pesticides, mining, and industrial processes. Aposhian et al (47) have used a variety of in vitro and in vivo procedures to compare DL- and *meso*-DMSA as arsenic antidotes. The two forms of DMSA were found to be equally effective in mobilizing tissue ^{74}As of rabbits. The activity of DL- and *meso*-DMSA in preventing the lethal effects of arsenic in mice seems to be equal as shown by their ED50 values under the same experimental conditions. There appears to be no advantage in using DL-DMSA instead of *meso*-DMSA as an arsenic antidote. For the mouse s.c. the LD50 of the meso form is 13.73 mmol/kg and for DL-DMSA, it is 10.84 mmol/kg. In vitro assays dealing with overcoming the arsenic inhibition of the pyruvate dehydrogenase enzymes are discussed under *Enzyme Studies*. Much of the Aposhian group's work on arsenic intoxication, DMSA, and other dithiol chelating agents has been summarized in a full-length symposium paper (48).

Such experiments have been extended by Maehashi & Murata (49). Mice were given arsenic trioxide (5 mg As/kg) sc, followed immediately with DMSA or DMPS (100 mg/kg). Arsenic excretion in the urine and feces were determined by atomic absorption. After 48 hr, animals given DMSA excreted about 71–81% of injected dose of arsenic in the urine and about 18% in the feces. When DMPS was given after the arsenic, only about 34% of the arsenic dose was detected in the urine by 48 hr; whereas 62% was found in the feces. This suggests that DMPS entered the hepatocytes and promoted excretion of As through the bile and the feces. Zheng et al (paper submitted) have shown that within 30 min after the i.v.-injection of DMPS into the rat, it can be detected in the bile. When *meso*-DMSA is injected, it is not detected in the bile. This demonstrates the extracellular, not intracellular, distribution of DMSA and explains some of the arsenic excretion experimental results of Maehashi & Murata (49).

D-penicillamine has been advocated for some time for the treatment of arsenic poisoning with little if any experimental evidence to support its use. Kreppel et al (50) have evaluated and written an excellent critique of the literature dealing with the use of D-penicillamine for the treatment of As intoxication. In addition, they have compared it experimentally with other chelating agents including DMSA. D-penicillamine was ineffective against arsenic trioxide toxicity in mice and guinea pigs using a variety of criteria. The potency of DMSA, DMPS, BAL, and D-penicillamine was compared in decreasing the arsenic content of guinea pig and mouse tissues of animals given As_2O_3. DMPS was the most effective agent, with DMSA equal or extremely close to it as an arsenic antidote when tissue levels of arsenic were used as the criteria.

An excellent article by Szinicz & Forth (51) dealing with the mechanisms of arsenic toxicity is highly recommended reading even though it does not deal with DMSA.

In vitro dialysis and in vivo extracorporeal hemodialysis with dogs indicated that even without any added DMSA or any other chelating agents, hemodialysis alone resulted in efficient removal of arsenic that had been given to dogs in the form of As_2O_3 (52). DMSA, however, forms disulfides with plasma proteins (R. M. Maiorino & H. V. Aposhian, in preparation) and such protein-DMSA complexes would not be expected to pass through the dialysis membrane.

BISMUTH In humans, Bi preparations used for gastrointestinal disorders can cause an encephalopathy. When $Bi(NO_3)_3 \cdot H_2O$ (125mg/kg) was given i.p. to male mice, none of the 10 animals survived. Survival was 100% when the Bi injection was followed 20 min later with i.p. *meso*-DMSA. A mole ratio of 10 *meso*-DMSA to $1Bi^{+3}$ was used. DMPS was as effective (53). Bismuth was found to accumulate in the liver and kidney of animals not treated with antidote but no Bi was detected in the liver or kidneys of mice given *meso*-DMSA. Unfortunately, it is not clear at what time the livers and kidneys were removed and analyzed.

CADMIUM One of the most difficult problems in metal antidote research is the design of effective chelating agents that will mobilize tissue stores of Cd for excretion and, in addition, meet the other requirements of a therapeutically useful agent. Obviously, a fresh approach is required. Most of the cadmium administered to an animal becomes bound to metallothionein after about 24 hrs (54). One primary requirement of an agent effective as an antidote for chronic cadmium toxicity is that it must enter the cell and compete with metallothionein for cadmium which it has firmly bound.

The dimethyl-, diethyl-, di-*n*-propyl-, diisopropyl, and di-*n*-butyl esters of

meso-DMSA have been synthesized (55). The esters are low-melting, waxy solids and were administered in peanut oil. All the esters except the dimethyl- were found to be more effective than BAL in reducing the whole-body levels of cadmium-109 in male mice. The diisopropyl ester of DMSA was the most effective. It reduced the body ^{109}Cd to 41% of the control values whereas BAL reduced it to only 82% of the control. Although all except the dimethyl ester were better than BAL in reducing the liver Cd, none of the DMSA esters was superior to BAL for decreasing the Cd in the kidney, a critical organ for Cd. The esters increased fecal excretion of ^{109}Cd. Another report (56) on the synthesis of the butyl and amyl esters of *meso*-DMSA presents an excellent review and comparison of the mobilizing efficacy of a number of chelating agents in mobilizing cadmium in the mouse or rat.

It should be remembered that *meso*-DMSA does not appear to enter hepatocytes (W. Zheng et al, paper submitted) and therefore should not be expected to increase the biliary excretion of cadmium. These less-polar esters of DMSA would be expected to enter the cell and increase the biliary excretion of Cd. Rivera et al (23) have shown this to be so for the dimethyl ester. Some of these ester analogs of *meso*-DMSA warrant further study because their administration did not increase brain Cd. The esters, however, did not appear to be superior to sodium N-benzyl-D-glucamine dithiocarbamate in mobilizing Cd.

Eybl et al (57) compared a number of chelating agents as to acute toxicity of $CdCl_2$, whole-body retention and tissue distribution of Cd after i.v.-injection of $^{115m}CdCl_2$. DMSA was the most effective. The combined use of two chelating agents was not more effective than the individual chelating agents. It should be remembered that in such acute studies the involvement of intracellular metallothionein is at a minimum.

COBALT The ED50 of *meso*-DMSA was 1.50 mmol/kg in preventing the lethal effects of 1.18 mmol $CoCl_2$/kg given i.p. DMSA was given i.p. immediately after the cobalt compound. Under the same conditions, $Na_3CaDTPA$ and $Na_2CaEDTA$ were more effective with ED50 values of 0.61 and 0.66 mmol/kg, respectively (58). Eleven other chelating agents were compared as antidotes for acute Co^{+2} toxicity.

COPPER DMSA and DMPS have been found to be effective for the treatment of Wilson's disease (59, 60). To determine the relative effectiveness of a number of chelating agents as cupruretic agents, Aposhian et al (41) gave each one to normal rats at the level of 1 mmol/kp po twice a day for 4 days. Urine was collected for each 24 hr period and analyzed. The mean 24 hr urinary excretion of copper was 45.4 μg after *D*-penicillamine, 32.8 after DMPS, 17.9 after DMSA and 17.7 μg after DMPA. Again, it should be emphasized that these experiments were performed in normal, not copper-

loaded, animals. Interestingly, the cupruretic action of DMPS is one instance where this chelating agent appears to be more potent than *meso*-DMSA.

Tandon et al (61) gave normal male albino rats daily i.p. injections of 0.5 mmol DMSA/kg, 0.5 mmol diethyldithiocarbamate sodium/kg or 0.25 mmol DL-penicillamine/kg. Liver zinc increased and renal copper decreased after administration of any of the three agents. Only penicillamine, however, decreased liver copper.

Aaseth et al (62) used human red cells to investigate copper toxicity and its prevention by a number of chelating agents and scavengers. DMSA (0.3mM) decreased from about 15 to 3% the hemolysis induced by an equimolar amount of copper sulfate. DMPS, on the other hand, increased the hemolysis. DMSA retained its protective activity even when its concentration was reduced to 0.1 mM. Aaseth et al (62) suggested that the DMPS-increased copper hemolytic effect may be due to DMPS inhibiting superoxide dismutase (SOD). The effects of this chelating agent, as well as others, on SOD would be received with great interest because of the growing concern about superoxide radicals.

Gold, Manganese, Mercury, Platinum, Polonium-210 and Vanadium

GOLD One of the major treatments or rheumatoid arthritis is the administration of gold compounds. Their use may be accompanied by tachycardia, thrombocytopenia, dermatitis, bone marrow damage, nephropathy, and other toxic reactions.

When male rats were given Na aurothiolate (SATM) (5 mg Au/kg) s.c. and treated for two weeks with 50 mg DMSA/kg/day i.p., the Au-concentration in the kidney decreased 50% and the copper concentration, which had increased after SATM administration, decreased about 36% (63).

Jones' group (64) at Vanderbilt University gave mice i.p. a >LD99 dose of gold sodium thiosulfate (GST), followed 20 min later with one of a number of possible antidotes ip. The antidote:GST mole ratio was 3:1. None of the controls (no antidote) (n=10) survived, whereas 18/20 mice treated with *meso*-DMSA and 16/20 mice given DMPS survived. A statistical analysis of the results was not presented. *meso*-DMSA or DMPS gave strikingly greater survival rates as compared to D-penicillamine, N-acetyl-D,L-penicillamine and N-acetyl-L-cysteine. In addition, when the mice were challenged with a lower dose of GST (140 mg/kg), *meso*-DMSA was found to decrease the kidney and liver concentration of gold by about 5- and 2-fold, respectively. DMSA, though, did not reverse the renal histopathological effects of GST under the experimental conditions employed.

MANGANESE DMSA, 0.5 mmol/kg, did not influence the excretion or the tissue concentration of ^{54}Mn in rats. Since other thiol chelating agents were

without significant activity in this respect, Tandon & Khandelwal (65) concluded that Mn has a poor affinity for thiols groups.

MERCURY A study by Planas-Bohne (66) is of great interest regarding DMSA and DMPS activity in the treatment of methylmercury intoxication. Male rats were injected with ^{203}Hg-methylmercuric chloride. The decorporation effectiveness was DMSA+DMPS>DMSA>DMPS>N-acetyl-dl-penicillamine. The brain was different in that DMSA+DMPS was not superior to DMSA alone. In the kidney, DMPS removed more Hg than did DMSA. The separate determination of ^{203}Hg-methylmercuric chloride and inorganic $^{203}Hg^{++}$ indicated that DMSA removed more of the *organic* Hg whereas DMPS removed more of the *inorganic* mercury from the liver and kidney. We think that more studies of this kind, in which the speciation of the metal is determined in a number of tissues, would be of value.

Extracorporeal complexing hemodialysis containing DMSA has been found to be an effective method for removing methylmercury from dogs. Female dogs were injected i.v. with ^{203}Hg-methylmercury (2.5mg Hg/kg) (67). DMSA was mixed with arterial blood before the blood entered the hemodialyzer. When both urinary and dialyzer output were taken into account, the mean whole body half-time of ^{203}Hg in untreated dogs was 137 days. When extracorporeal complexing hemodialysis with DMSA was used, the half-time decreased to 0.25 days.

Liang et al (71) at the Shanghai Institute of Materia Medica injected rats with ^{203}Hg. After treatment with *meso*-DMSA, the Hg bound to protein in the serum and tissues decreased and urinary excretion of diffusible complexes increased. They proposed the mechanism of action of DMSA in the treatment of Hg poisoning to involve the transfer of mercury from high molecular protein complexes "to low molecular weight Hg-complexes by competitive binding of DMSA."

In our laboratory, Dr. Karel Blaha, on leave from the Institute of Hygiene and Epidemiology in Prague, has given $^{203}HgCl_2$ i.v. to rabbits followed by *meso*-DMSA and collected urine samples over a six-hour period. A major radioactive fraction containing ^{203}Hg and DMSA has been isolated by purification on Sephadex G-75 and G-15 and is in the process of being characterized. The complex does not appear to consist of 1 molecule of DMSA:1 molecule of Hg, which would have a molecular weight (MW) of about 385. Instead, it has a molecular weight of approximately 800–1000. Further characterization by mass spectroscopy and other methods are in progress (K. Blaha et al, paper in preparation).

PLATINUM The cancer chemotherapeutic agent, *cis*-dichlorodiammine platinum II (CP), is effective in the treatment of carcinomas of the neck, testis, and other organs. The dose and effectiveness of this compound are

limited by its dose-related renal toxicity and the overexpression of metallothionein that it can induce (72). If these could be controlled, CP could probably be a more effective cancer chemotherapy agent. Two groups have investigated whether DMSA of DMPS influences the accumulation of Pt in the kidney. Graziano et al (73) gave rats 6 mg CP/kg, i.v., followed by two divided doses of 100 or 200 mg DMSA/kg/day for 8 days. The first dose was given 3 hrs after CP. This regimen reduced the renal Pt-concentration by 50%, after only four days of DMSA therapy. It did not reduce renal toxicity as measured by renal histology, urinary excretion of N-acetyl-β-glucosaminidase, or serum creatinine concentrations. Planas-Bohne et al (74) gave 4 or 6.5 mg CP/kg i.v. and 24 hrs later gave DMSA or DMPS at the 1mmol/kg level, i.p., for either one day or daily for 4 days. They essentially confirmed Graziano et al (69), showing that DMSA reduced kidney Pt in rats. The magnitude of the reduction was not impressive. Both groups indicated that DMSA and similar compounds do not appear to be useful for reducing CP toxicity seen clinically.

POLONIUM-210 Exposures to this metal, although rare, have occurred as a result of accidents in nuclear industries (see 77). This alpha-emitting radioactive element is extremely hazardous (75). Hematopoietic depression and functional impairment of the liver have been reported after exposure. Like other heavy metals, it is believed to bind thiols and thiol-containing proteins in vivo. The interesting metabolism and biological effects of polonium-210 have been reviewed by Moroz & Parfenov (76).

Male rats given 40 μCi^{210}Po/kg i.p. had a median survival time (mst) of 39 days (77). The mst was increased to 106 days when DMSA, DMPA, or DMPS was administered s.c. Decorporation studies were performed by giving rats 0.4 μCi polonium-210, s.c., followed by a series of dithiol injections beginning one hr later. After 21 days of treatment, kidney levels of polonium-210 in rats given DMPA were only 28% of those of the untreated controls and significantly lower than those of rats receiving DMSA, DMPS, N-acetyl-L-cysteine, or WR2721 (77). Further studies in which rats were given ^{210}Po i.p. or i.v. indicated that DMPA is a true decorporating agent, since it increased the urinary, biliary and fecal excretion of polonium-210 in rats (G. Bogdan & H. V. Aposhian, manuscript submitted).

VANADIUM Eighteen chelating agents have been compared in mice as antidotes for ip-administered sodium vanadate (VO_3^{-3}) or vanadyl sulfate (VO^{+2}). When DMSA was given i.p. 20 min after the vandyl sulfate, only 1 of 5 mice survived. When DMSA was administered after sodium vanadate, 9 of 10 mice survived. This was interpreted to mean that when vanadate was the toxicant, the antidotal action of DMSA was due to its reducing properties and not its chelating properties (78).

Other Properties of Interest

COMPARATIVE STUDIES WITH OTHER CHELATING AGENTS An important study by Cantilena & Klaassen (79) compared the activity of a number of chelating agents in increasing the excretion of endogenous metals in normal Swiss Webster mice. The chelating agent, at a dose equal to 25% of its LD50 value, was given i.p. once daily for 3 consecutive days or twice daily for 7 consecutive days. Although seven chelating agents were studied, only the results for DMSA, $CaNa_2EDTA$ and BAL are discussed here. No pathological lesions of the heart, liver, skeletal muscle, small intestine, and kidney were visible by light microscopy. But mice given $CaNa_2EDTA$ twice daily for seven days showed a marked loss in body weight. The administration of $CaNa_2EDTA$ for 3 consecutive days resulted in the increase of urinary Fe, Zn (10- to 15-fold each day as compared to controls) and Mn (40–60-fold). DMSA or BAL treatment resulted in an increase of urinary Cu. DMSA or BAL did not increase urinary Fe, Zn, or Mn. However, in this interesting study, equitoxic, not equimolar, amounts of the chelating agents were used.

The Klaassen group, continuing its contributions to an understanding of the role of metallothionein in metal toxicity, also has studied its induction in mouse livers by a number of different chelating agents (80). The dose of the chelating agent administered was equal to one fourth of its LD50. Twenty-four hr after *meso*-DMSA, D-penicillamine, DTPA or EDTA administration i.p. the liver metallothionein concentrations increased fourfold. The authors suggested a number of mechanisms to explain this induction by chelating agents and pointed out that after prolonged use, some chelating agents may enhance Cd toxicity.

DEVELOPMENTAL TOXICITY With the increasing concern about lead exposure of pregnant women and their possible treatment with *meso*-DMSA, the study of the developmental toxicology of *meso*-DMSA by Domingo & Llobet at the University of Barcelona (81) is of importance and interest. Pregnant Swiss mice were given 410, 820, or 1640 mg *meso*-DMSA/kg/day s.c. on days 6–15 of gestation. At the 1640 mg/kg/day dose level, a pronounced inhibition of maternal growth as indicated by body weight was noted, suggesting that DMSA has maternal toxicity at this dose level. In addition, at this higher dose there were increases in both early and late resorptions. The level at which no teratogenic effects were observed was 410 mg/kg/day. At the 820 and 1640 mg/kg/day level, reductions in fetal body weight and fetal body length were observed. By other criteria, they concluded that for mice, DMSA was embryotoxic at 1640 mg/kg/day level and fetotoxic at 820 mg/kg/day. It should be kept in mind that in mice the LD50 of *meso*-DMSA when given s.c. was 8,192 mg/kg (81) and that the therapeutic dose in humans is usually about

30 mg/kg (4). The studies suggest caution if this chelating agent is given to pregnant women.

ENZYME STUDIES Biochemical studies using *meso*-DMSA as a research tool have been limited. The introduction of EDTA into biochemistry by Racker (82) allowed the biochemist to begin innovative experiments that were difficult to do previously, such as studying the role of metals in enzymatic reactions. The introduction of BAL by Peters and coworkers (83) made another chelating agent available for use in exploring metal requiring enzyme reactions. BAL, however, is a lipid soluble oil. DMPS or DMSA are water-soluble solids. The latter two dithiols although available in the West for the last nine years have been used very little by the biochemist. Studies with a compound as pure as *meso*-DMSA, instead of BAL, might yield less ambiguous results.

DL- and *meso*-DMSA are equally effective in preventing or reversing, in vitro, the arsenite inhibition of the activity of mouse kidney pyruvate dehydrogenase (47). An excellent method for testing the reversal of arsenite inhibition by chelating agents is to give arsenite to mice followed at a suitable time by the putative chelating agent. At various times the kidneys are removed from the mice, a homogenate prepared and assayed for pyruvate dehydrogenase (47).

DMSA reverses in vitro the inhibition by arsenic of gluconeogenesis from pyruvate in rat kidney tubules (85).

MISCELLANEOUS

Peele et al have studied the behavioral consequences of the administration of chelating agents in acute cadmium toxicity in male rats (86). They used the conditioned-flavor aversion paradigm to test the acute toxicity of cadmium and the effect of BAL or DMSA on the response. The results indicated that DMSA is superior to BAL in reducing the neurobehavioral toxicity of cadmium. Such tests, although more difficult for the uninitiated to understand and especially to reproduce, are certainly worth following and doing. They add another important and original dimension to investigating the interactions between metals and chelating agents. The same group has studied and found that DMSA blocks the behavior effects of lead conditioned-flavor aversion (87).

^{99m}Tc-dimercaptosuccinic acid scans are useful in detecting renal defects. Their usefulness has been compared with other upper renal tract imaging techniques (88).

A side effect of anti-tumor drugs like cyclophosphamide is a hemorrhagic cystitis as a result of the release of its biotransformant, acrolein, within the

bladder. The concurrent administration of *meso*-DMSA with cyclophosphamide to rats protected against this cystitis (89). Prior administration was not protective.

DMSA did not inhibit galactosamine-induced hepatic necrosis (90). Cysteamine, penicillamine, and N-acetyl-cysteine were able to inhibit the necrosis.

UNANSWERED QUESTIONS

Considerable progress has been made in understanding the pharmacology and toxicology of *meso*-DMSA. Its expected introduction into the official armamentarium will be a major step forward for the treatment of lead intoxication. Many other questions about DMSA and metal toxicity remain.

What are the structures of the metal chelates of DMSA found in vivo? This question has received very little study and deserves more. Recent results from experiments in which rabbits were given $^{203}HgCl_2$ and DMSA indicate that there is a huge excess of *meso*-DMSA in the urine compared to the amount of chelated metal (K. Blaha & H. V. Aposhian, manuscript in preparation). Does such an excess mean that the excreted chelates consist of two (or more) molecules of DMSA per metal ion rather than one molecule as often hypothesized?

Is *meso*-DMSA a pro-drug in the human? Since it is biotransformed to the mixed disulfide of DMSA and cysteine, perhaps the mixed disulfide is the active chelating agent in vivo. Obviously, to answer this question, chemical synthesis of the mixed disulfides is necessary to make adequate amounts available for research. This is being attempted in our laboratories, but with the involvement of 4 carboxyl groups, 4 thiol groups and 2 amino groups, the synthesis is far from easy.

SUMMARY

The primary purpose of this article is to summarize the recent investigations dealing with the pharmacology and toxicology of *meso*-2,3-dimercaptosuccinic acid, an orally effective chelating agent. The need for a better chelating agent for treating young children and pregnant women with lead intoxication has been apparent for some time. Preclinical and clinical evidence now indicate that *meso*-2,3-dimercaptosuccinic acid, an Orphan Drug, shows the most promise for being effective in this regard. It has an extracellular distribution that may be responsible for its low toxicity compared to other dithiols. No attempt has been made to compare it in a rigorous and thorough manner with other chelating agents. That has not been the purpose

of this review. The advantages of *meso*-DMSA, however, compared to $CaNa_2EDTA$ for the treatment of lead intoxication, have been outlined.

Significant advances have been made recently in elucidating the structures of the metal chelates of DMSA. There is a striking difference between the structures of the lead chelate of *meso*-DMSA and those of racemic-DMSA. The former chelates by coordination of one sulfur and one oxygen atom with Pb. The latter can form chelates via the two sulfur atoms or via one oxygen and one sulfur atom. Solubility of the lead chelates depends on the ionization of the noncoordinated thiol and carboxylic acid groups. Bimane derivatization, HPLC, and fluorescence, as well as gas chromatography can be used for analysis of DMSA in biological fluids. The acid dissociation constants for meso- and recemic-DMSA have been summarized from the literature as have the formation constants of some of the DMSA chelates.

DMSA is biotransformed to a mixed disulfide in humans. By 14 hr after DMSA administration (10 mg/kg), only 2.5% of the administered DMSA is excreted in the urine as unaltered DMSA and 18.1% of the dose is found in the urine as altered forms of DMSA. Most altered DMSA in the urine is in the form of a mixed disulfide. It consists of DMSA in disulfide linkages with two molecules of L-cysteine. One molecule of cysteine is attached to each of the sulfur atoms of DMSA. The remaining 10% of the altered DMSA was in the form of cyclic disulfides of DMSA. So far, the mixed disulfide has been found in human but not in rabbit, mouse, or rat urine. Apparently there are species differences in how organisms metabolize *meso*-DMSA.

Animal studies using *meso*-DMSA as an antidote for intoxication with aluminum, arsenic, bismuth, cadmium, cobalt, copper, gold, mercury, platinum, manganese, polonium-210, and vanadium are summarized as are other properties of this dithiol chelating agent.

The question still remains whether *meso*-DMSA is a prodrug.

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