

Determination and Metabolism of Dithiol Chelating Agents

VII. Biliary Excretion of Dithiols and Their Interactions with Cadmium and Metallothionein¹

WEI ZHENG,* RICHARD M. MAIORINO,† KLAUS BRENDEL,*‡
AND H. VASKEN APOSHIAN†‡

*Department of Pharmacology and Toxicology. †University Department of Molecular and Cellular Biology, and ‡Department of Pharmacology, University of Arizona, Tucson, Arizona 85721

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Determination and Metabolism of Dithiol Chelating Agents. VII. Biliary Excretion of Dithiols and Their Interactions with Cadmium and Metallothionein. ZHENG, W., MAIORINO, R. M., BRENDEL, K., AND APOSHIAN, H. V. (1990). *Fundam. Appl. Toxicol.* 14, 598-607. *N*-(2,3-Dimercaptopropyl) phthalamidic acid (DMPA), *meso*-dimercaptosuccinic acid (DMSA), and 2,3-dimercapto-1-propanesulfonic acid (DMPS) are dithiol chelating agents with antidotal activity for lead, mercury, arsenic, and other heavy metals. The biliary excretion of these compounds was studied in male Sprague-Dawley rats. After iv administration of DMPA, 72% of the injected dose was recovered in the bile. Half of the recovered DMPA was in the unaltered form (parent compound) and the other half was in the altered form (parent compound recovered after chemical reduction by DTT). An altered, presumably disulfide, form of DMPS was found in the bile. Neither unaltered nor altered DMSA was detected in the bile. DMPA (0.10 mmol/kg), given to rats 3 days after exposure to Cd, elicited within 30 min a 20-fold increase in biliary Cd excretion. The increase of biliary Cd by DMPA was dose-related and not due to an increase of bile flow rate. DMSA and DMPS did not significantly affect the biliary excretion of Cd. Incubation of DMPA or DMSA with Cd-saturated metallothionein (MT) resulted in the removal of Cd from MT. DMPA was more active than DMSA in this respect. The evidence strongly supports the mechanism that the increase of biliary cadmium following DMPA administration is the result of DMPA entering cells and mobilizing and removing the cadmium from MT. The removal of cadmium from metallothionein by dithiol chelating agents provides another dimension to their mechanisms of action and may provide an important new tool for the study of cadmium as well as metallothionein. © 1990 Society of Toxicology.

N-(2,3-Dimercaptopropyl) phthalamidic acid (DMPA),² *meso*-dimercaptosuccinic acid (DMSA), and 2,3-dimercapto-1-propanesulfonic acid sodium salt (DMPS) are chemical analogs (Fig. 1) of the well-known chelating agent 2,3-dimercaptopropanol

(British Anti-Lewisite, BAL). They are antidotes for arsenic, lead, mercury, and other heavy metals (for reviews see Aposhian and Aposhian, 1990; Aposhian, 1983; Aaseth, 1983). DMPA is also an excellent decorporating agent for polonium-210 in rats (Aposhian *et al.*, 1987). Although these dithiol chelators have been studied extensively as heavy metal antidotes, the experimental evidence as to whether DMPA, DMSA, and DMPS are intracellular and/or extracellular in their *in vivo* distribution is limited. DMPA contains a benzene ring that should endow it with

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² Abbreviations used: DMPA, *N*-(2,3-dimercaptopropyl) phthalamidic acid; DMSA, *meso*-dimercaptosuccinic acid; DMPS, 2,3-dimercapto-1-propanesulfonic acid; DDC, diethyldithiocarbamate; DTT, dithiothreitol; mBBR, monobromobimane; MT, metallothionein.

greater lipid solubility and thus might allow it to penetrate cellular membranes more readily than DMSA or DMPS.

Effective chelation therapy for chronic metal intoxication is sometimes dependent upon the accessibility of the chelating agent to the sites where the metal is deposited and bound. For example, the treatment of chronic Cd intoxication has not been overwhelmingly successful. This appears to be due to the strong binding of Cd to the intracellular ligand, metallothionein (MT) (Cherian, 1977), and in some cases due to the extracellular, rather than intracellular, distribution of the chelating agent (Klaassen *et al.*, 1984). Thus, whether a chelating agent can enter the cells becomes crucial in the treatment of chronic Cd intoxication.

Excretion into bile is a major route for the elimination of many metals including lead, manganese, mercury, copper, zinc, and cadmium (Klaassen, 1976). Compounds that mobilize toxic metals into the bile would be expected to enter hepatocytes. Correspondingly, biliary appearance of a parent compound could be used as an indicator of whether the compound enters cells and decorporates hepatic metals. Since almost all of the hepatic Cd is eventually bound to MT (Webb and Cain, 1982), the compound that increases biliary Cd in chronic Cd intoxication would be expected to remove Cd from MT.

The aims of the present paper are to determine whether DMPA, DMSA, or DMPS, when injected into rats, is present in the bile, to determine whether the presence or absence of the chelating agents in the bile correlates with the biliary excretion of Cd in chronic Cd poisoning, and to determine whether these compounds remove Cd from MT *in vitro*. The evidence indicates that DMPA enters the cell and remains there in its biologically active, reduced form for a longer period of time than DMPS. Consequently, when DMPA is given to rats 3 days after the administration of cadmium-109, the biliary excretion of Cd increases 20-fold. Under similar *in vivo* experimental conditions, DMSA is not detect-

able in the bile. *In vitro*, however, DMPA as well as DMSA removes Cd from MT.

MATERIALS AND METHODS

Chemicals. Chemicals were obtained from the following sources: DMPA from EISAI (Tokyo, Japan); DMPS from Heyl (Berlin, FRG); DMSA from Johnson and Johnson, Baby Products Co. (Skillman, NJ); cadmium-109 (sp act, 124.5 mCi/mg) from Du Pont (Claremont, CA); cadmium chloride from J. T. Baker (Phillipsburg, NJ); diethyldithiocarbamate (DDC), glutathione, and monobromobimane (mBBr) from Calbiochem-Behring (La Jolla, CA); dithiothreitol (DTT), fluorodinitrobenzene (FDNB), and tris[hydroxymethyl]aminomethane (Trizma base) from Sigma Chemical Co. (St. Louis, MO); tetrabutylammonium hydroxide (TBAOH) and urethane from Aldrich (Milwaukee, WI); methanol and methylene chloride from Burdick and Jackson (Muskegon, MI); Sephadex G-15, 50, and 75 from Pharmacia (Piscataway, NJ). All reagents used in this experiment were of analytical grade, HPLC grade, or the best available pharmaceutical grade.

Animals All rats (male, Sprague-Dawley) at the time they were used in the experiments were 7-8 weeks old (200-220 g). They were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). Animals were quarantined for 1 week after arrival and kept in a temperature-controlled, 12-hr light/dark cycle facility. They were fed *ad libitum* with a Teklad rat diet purchased from Teklad (Madison, WI). Animals were anesthetized with 1.5 g urethane/kg, ip.

Administration of chelating agents. All compounds that were injected iv were dissolved in 5% NaHCO₃-0.9% saline solution (pH 7.4) and administered via the jugular vein at a rate of 0.1 ml/min.

Assay for dithiols. The dithiols were assayed by published, well-established procedures (Maiorino *et al.*, 1986; 1987; 1988). In brief, they were derivatized with mBBr before and after DTT reduction, separated, and quantitated using high-performance liquid chromatography (HPLC) and fluorescence detection. Methodology pertinent to the present experiments is detailed below. Bile samples were collected at 10-min intervals. The samples were collected directly into a vial containing 0.1 ml of 80 mM mBBr and 1.8 ml of 0.1 M ammonium bicarbonate buffer (pH 8.4). The vials were wrapped with aluminum foil to avoid exposure to light. After collection, the bile sample was allowed to react for 10 min at room temperature in the dark. The volume of each bile sample was measured and the samples were extracted with 4 ml of methylene chloride to remove excess mBBr. The aqueous phase was then diluted 10-fold and an aliquot analyzed by HPLC. Another aliquot of the mBBr-treated sample underwent DTT reduction as described below.

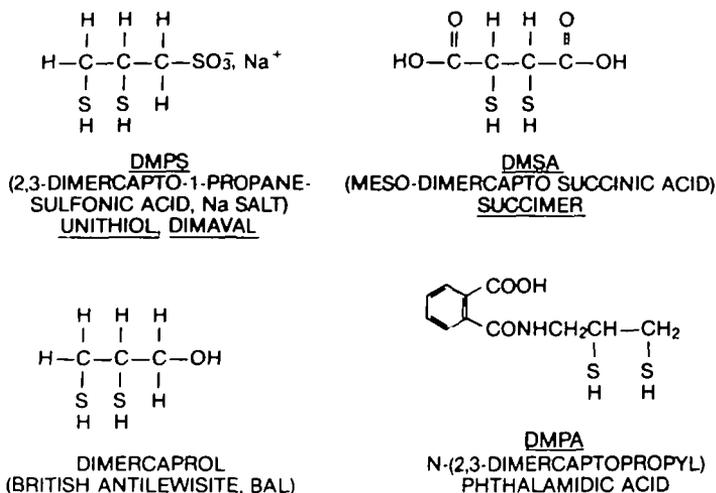


FIG. 1. Structures of dimercapto chelating agents.

Unaltered dithiols³ were determined by the direct analysis of a bile sample by this method. Total dithiols³ (unaltered + altered) were determined by the same method except that the sample first underwent DTT reduction and then was immediately derivatized with mBBBr. Altered dithiols³ were obtained by subtracting unaltered dithiols from total dithiols.

Reduction procedure. Reduction of disulfides was carried out by adding 20 μl of 50 mM dithiothreitol (DTT) to the methylene chloride-extracted samples, and incubating them for 30 min at room temperature. The reduction was terminated by adding another 50 μl of 80 mM mBBBr and incubating the samples for 10 min in the dark. The rest of the treatment was the same as the above.

HPLC analysis. The HPLC analysis of dithiol-bimane derivatives was performed using the method of Maiorino *et al.* (1986). Samples were fractionated on a 250 \times 4.6-mm ultrasphere IP C18 reversed-phase column, using a Beckman Model 157 fluorescence detector with 356 nm excitation and 450 \pm 20 nm emission filters. Separations were performed at room temperature and at a rate of 1 ml/min. The mobile gradient system was described in the procedure reported by Maiorino and Aposhian (1988). Mobile phase A was 20 mM acetic acid and 20 mM TBAOH in methanol. Mobile phase B was 20 mM acetic acid in methanol:water (25:75, v/v). A typical HPLC profile is shown in Fig. 2.

³ Unaltered dithiol is the amount of dithiol determined before reduction of the sample. Total dithiol is the amount of dithiol determined after reduction of the sample with DTT. Altered dithiol is the difference between total dithiol and unaltered dithiol.

Biliary excretion of cadmium. Rats (200–220 g) were injected ip with 1 mg Cd, 25 μCi ¹⁰⁹Cd/kg (as CdCl₂). Three days later, the rats were anesthetized with 1.5 g urethane/kg, ip. The bile duct and jugular vein were cannulated with PE-50 polyethylene tubing (Clay Adams). The tubing was inserted into the bile duct close to the side of its joining the duodenum and deeply enough (about 1–1.5 cm) to avoid contamination with any exocrine excretion from the pancreas. During the experiment, the rats were infused with saline via the jugular vein at a rate of 1.5 ml/hr. After a steady bile flow rate was established, bile samples were collected at 30-min intervals for 6.5 hr. Rats were kept on an electrical heating pad so that their core temperature was maintained at 36 \pm 0.5°C during bile collection. The bile samples were counted in a LKB type-1282 CompuGamma Counter.

Preparation of Cd-saturated MT. Rats (200–220 g) were injected ip with 1.0 mg Cd/kg daily for a period of 3 days. The last injection contained 15 μCi ¹⁰⁹Cd. After rats were terminated on Day 4, the liver was excised and homogenized in 10 mM Tris-HCl buffer, pH 8.6 (1:2 w/v), in the cold. The homogenate was centrifuged at 37,000g for 30 min at 4°C. The supernatant was removed and heated in a water bath at 80°C for 2 min and then centrifuged at 180,000g at 4°C for 12 hr. An aliquot of heat-treated supernatant (7 ml) was applied to a Sephadex G-75 column (1.4 \times 90 cm) and eluted with 10 mM Tris-HCl buffer, pH 8.6, at a flow rate of 50 ml/hr at 4°C. Fractions (2.65 ml) were collected and UV absorption at 254 and 280 nm and radioactivity were determined. The fractions ($V_f/V_0 = 1.9\text{--}2.2$) of the radioactive peak having a high optical density at 254 nm and a low at 280 nm were pooled and used as MT (Lehman and Klaassen, 1986).

The pooled fractions (14 ml) were saturated with Cd by incubating with 0.5 mg Cd, 1.5 μCi ^{109}Cd (as CdCl_2) at 37°C for 1 hr. In order to get rid of excess Cd, the solution was applied to a Sephadex G-15 column (2.5 \times 15 cm) and washed with 10 mM Tris-HCl buffer, pH 8.6, at a flow rate of 156 ml/hr at 4°C. The peak radioactivity fractions ($V_e/V_0 = 0.9-1.1$) with a high optical density at 254 nm were pooled and analyzed for Cd concentration by atomic absorption spectrophotometry (Instrumentation Lab Video 12), using an air-acetylene flame. The concentration of MT was estimated by assuming that 7 g-atoms of Cd are bound per mole of MT. The molecular weight 6500 was used in the calculation (Kagi and Kojima, 1987).

In vitro Cd-MT-chelator binding studies. A 1.5-ml aliquot of Cd-saturated radioactive MT (42 μg MT/ml, 0.045 μmol Cd/ml) was incubated in a capped glass tube at 37°C for 10 min. DMPA or DMSA was added to a final concentration of 0.45 μmol /ml and the reaction mixture was then incubated for 1 hr with constant stirring. At the end of the incubation, the solution was quickly frozen and stored at -75°C in a Revco freezer. For controls, an equal volume of 5% NaHCO_3 -0.9% saline (vehicle for chelating agents) replaced the chelating agents in the reaction mixture. An aliquot of the reaction solution (0.6 ml) was then fractionated on a Sephadex G-50 column (0.9 \times 85 cm) using 10 mM Tris-HCl buffer, pH 8.6, at a flow rate of 10 ml/hr at 4°C (Fig. 6). The fractions of the radioactive peak(s) were pooled and stored at -75°C. The V_e/V_0 of peak No. 1 was 1.3-1.5 and of peak No. 2 was 1.8-2.0.

Ultrafiltration studies. A 1.0-ml aliquot of the pooled peak No. 1 or No. 2 was centrifuged in a Centricon-3 microconcentrator (YM membrane, MW cutoff 3000, Amicon) at 5000g for 2 hr at 25°C. The solution passing through the membrane was called the filtrate and collected in the filtrate cup. The retentate (that which did not pass through the membrane) was recovered in a retentate cup by inversion of filter unit and centrifugation at 1000g for 5 min at 25°C. The radioactivity in the filtrate cup, retentate cup, and membrane was counted in a LKB type-1282 gamma counter. For the DMPA-treated group, an aliquot of filtrate was treated with DTT, derivatized with mBBR, and fractionated on HPLC in order to analyze for DMPA in the filtrate.

Statistics. The statistical analyses were carried out by paired *t*-testing for single comparison, or by analysis of variance (ANOVA) where multiple comparison was required. If ANOVA revealed an overall treatment effect, contrast analysis was performed at individual time points using one-way ANOVA.

RESULTS

Are the dithiols, DMPA, DMPS and DMSA excreted in the bile? Dithiols are labile

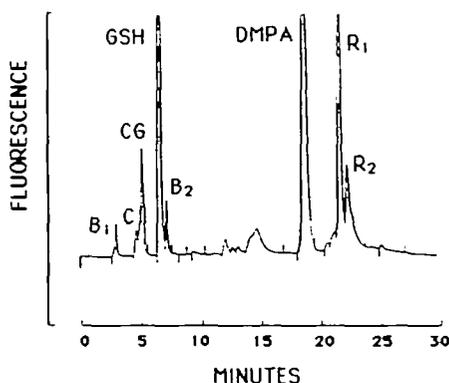


FIG. 2. HPLC profile of bile sample from a rat given DMPA. Rats were anesthetized ip with 1.0 g urethane/kg. DMPA (0.20 mmol/kg) was administered via the jugular vein. The bile sample was collected into a vial containing bromobimane for 30 min after injection of DMPA. Peaks were identified as follows: B₁ and B₂, mBBR hydrolysis products; C, cysteine; CG, cysteinylglycine; GSH, glutathione; DMPA; R₁ and R₂, peaks related to DMPA.

compounds. DMPA, DMPS, and DMSA can be easily oxidized to form disulfides with themselves or mixed disulfides with other thiol compounds such as cysteine or proteins (Maiorino *et al.*, 1988a,b). After iv injection, the recovery of total DMPA³ (unaltered + altered) in the bile was 72.1% of the injected dose. About half (36.1%) of this was in the unaltered parent form³ (Fig. 2), and the other half (36%) in the altered form.³ Most (93%) of unaltered DMPA found in the bile appeared within 40 min (Fig. 3). Altered DMPA slowly appeared in the bile.

In contrast, most of DMPS (92%) excreted in bile was in its altered form (Fig. 4). The recovery of total DMPS in bile was 40% of administered dose; 37% was in altered form and only 3% in the unaltered form. Neither unaltered nor altered DMSA could be detected in the bile (Fig. 4).

DMPA increases biliary excretion of cadmium. DMPA (0.10 mmol/kg), when given iv to rats that had received ^{109}Cd 3 days previously, had a striking effect on the biliary excretion of ^{109}Cd (Fig. 5). Within 30 min after DMPA administration, the biliary excretion

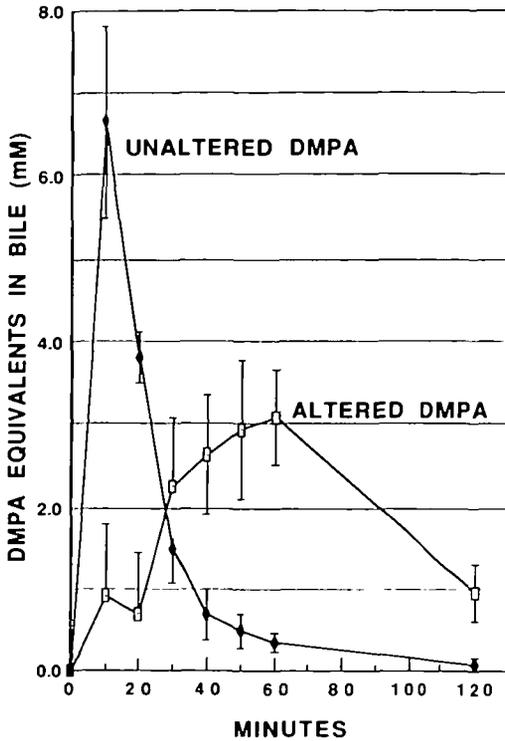


FIG. 3. Unaltered (parent form) and altered (parent form recovered after DTT reduction) DMPA are excreted in the bile. See Fig. 2 legend for method. DMPA was administered at zero time. Altered DMPA was determined by DTT reduction as described in the text. There were three rats in each group. In this and subsequent figures, error bars indicate standard error of the mean.

of Cd increased 20-fold. A second injection of DMPA (0.1 mmol/kg), 3 hr after the first, elicited a 14-fold increase in biliary Cd excretion within 30 min. The maximum effect of DMPA occurred within 2 hr after DMPA administration. This corresponded to the time course of the biliary excretion of most of the DMPA (unaltered + altered) (Fig. 3). The DMPA effect was dose-related. Doubling the amount of DMPA injected doubled the biliary excretion of Cd (data not shown). The action of DMPA in increasing the biliary excretion of Cd was not due to an increase of bile flow rate (Table 1).

DMPS and DMSA did not affect biliary excretion of Cd. According to two-way ANOVA

analysis, DMPS or DMSA in amounts equimolar with DMPA (0.10 mmol/kg) did not significantly influence the biliary excretion of Cd (Fig. 5). Neither did they increase or decrease the bile flow rate during the period of bile collection (data not shown). In order to determine whether the action of DMPA on biliary Cd was unique to DMPA, the effects of other common chelating agents or thiols on biliary excretion of Cd were tested. The other chelating or thiol agents did not affect biliary excretion of Cd as shown by two-way ANOVA (Fig. 5).

Removal of Cd from Cd-MT by DMPA and DMSA. When $^{109}\text{Cd-MT}$ was incubated for 1 hr at 37°C and then placed on a Sephadex G-50 column, the radioactivity was eluted as a single peak ($V_e/V_0 = 1.4$) (Fig. 6). After an identical preparation of $^{109}\text{Cd-MT}$

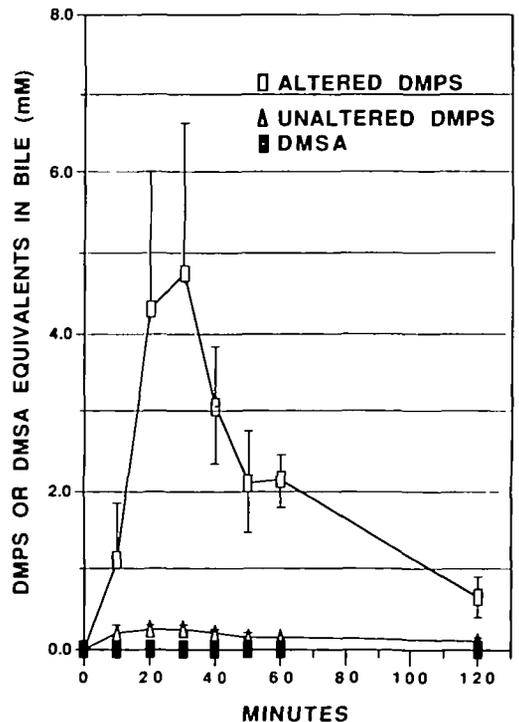


FIG. 4. Determination of DMPS and DMSA in the bile. DMPS and DMSA (0.20 mmol/kg) were administered via the jugular vein at zero time. The method used was the same as that in Fig. 2. There were three rats in each group.

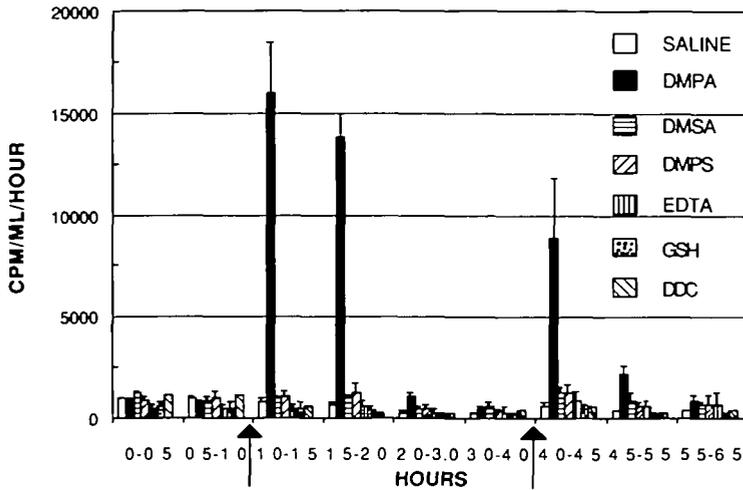


FIG. 5. DMPA increases biliary excretion of cadmium-109. Rats were injected ip with 1 mg Cd ($25 \mu\text{Ci } ^{109}\text{Cd}$)/kg. Three days later, the bile duct and jugular vein were cannulated. Chelating agents (0.10 mmol/kg) or saline was administered via the jugular vein as indicated by the arrows. There were four rats in each of the saline, DMPA, or DMSA groups; three in DMPS, DDC, EDTA, or GSH groups. The data of 1.0–1.5 and 1.5–2.0 hr and data of 4.0–4.5 and 4.5–5.5 hr in the DMPA-treated animals were significantly different from those of saline-treated animals ($p < 0.001$, $p < 0.01$, respectively).

was incubated with DMPA and fractionated on the same column, not only was the radioactivity in peak No. 1 decreased, but a new peak (No. 2) was eluted with a V_c/V_0 of 1.9, suggesting that a lower molecular weight material had been formed (Fig. 6). Whereas 100% of the radioactivity eluted in one peak in the case of the control, when the ^{109}Cd -MT preparation that had been incubated

with DMPA was chromatographed, two peaks were found. The second peak contained about 62% of total amount of radioactivity eluted from the column. In other words, DMPA removed 62% of Cd from MT.

Ultrafiltration (MW cutoff 3000) of the peak fractions revealed that 85% of the radioactivity in the Cd-MT fraction of the control was recovered in the retentate and only 8% in

TABLE I
INCREASE OF THE BILIARY EXCRETION OF Cd BY DMPA IS NOT DUE TO DMPA
INCREASING THE RATE OF BILE FLOW

	Response from 30 min before to the time of DMPA injection	Response from the time of DMPA injection to 30 min after	Percentage increase
Bile flow rate (ml/30 min)	0.454 ± 0.081	0.523 ± 0.105	15
Biliary cadmium (cpm/ml/30 min)	480 ± 68	$9556 \pm 3191^*$	1891

Note. The bile samples from male rats ($n = 3$) were collected 3 days after administration of 1 mg CdCl_2 ($90 \mu\text{Ci } ^{109}\text{Cd}$)/kg ip. DMPA (0.20 mmol/kg) was injected via the jugular vein. Data represent the mean \pm SE.

* $p < 0.01$ (compared with that before DMPA injection).

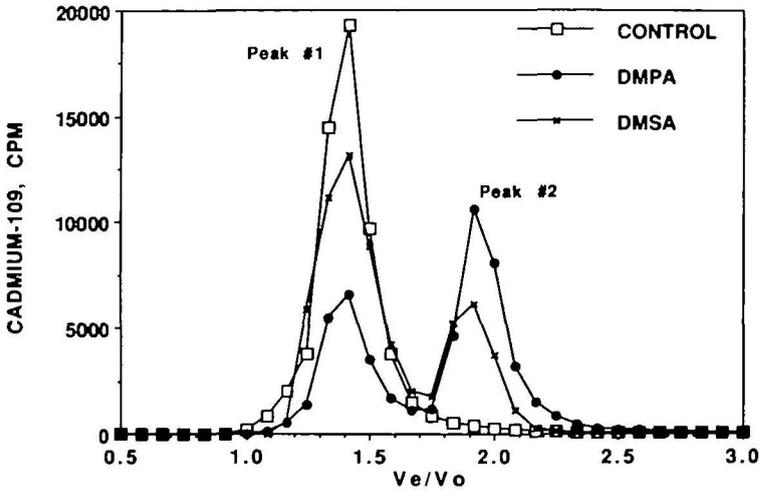


FIG. 6. DMPA or DMSA removes Cd from MT, *in vitro*. DMPA, DMSA, or for the control 5% NaHCO₃-0.9% NaCl was incubated with Cd-saturated MT for 1 hr. After incubation, 0.6 ml of reaction mixture was fractionated on a Sephadex G-50 column (0.9 × 85 cm) at 4°C using 10 mM Tris·HCl, pH 8.6, with a flow rate of 10 ml/hr.

the filtrate (Fig. 7). When peak No. 2 from Fig. 6 was filtered through another membrane with a 3000 MW cutoff as above, only

6% of the radioactivity was found in the retentate, while 61% appeared in the filtrate (Fig. 7). Further analysis of the filtrate of peak

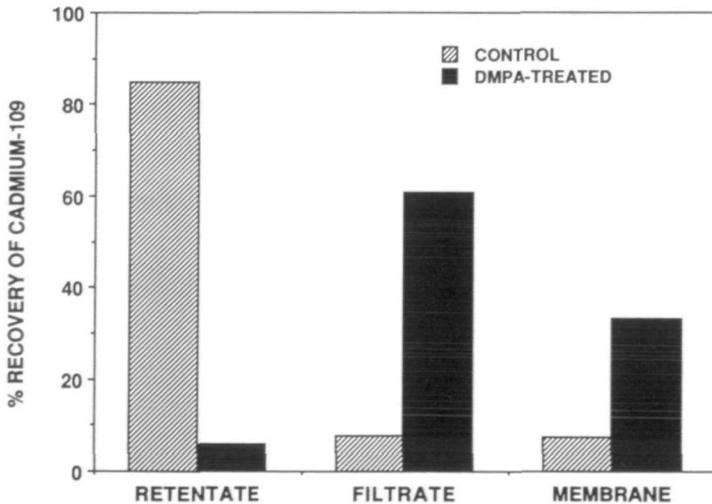


FIG. 7. Cadmium-109 of metallothionein after incubation with DMPA passes through a 3000 MW cutoff membrane. An aliquot of the pooled peak No. 1 of the control MT of Fig. 6 ($V_e/V_0 = 1.3-1.5$) or a peak No. 2 of the DMPA-treated MT ($V_e/V_0 = 1.8-2.0$) was centrifuged in a Centricon-3 microconcentrator (MW cutoff = 3000) at 5000g for 2 hr at 25°C to obtain the filtrate which passed through the membrane. The retentate (which did not pass through the membrane) was recovered by inversion of the filter unit and centrifugation at 1000g for 5 min. The ordinate axis expresses the ¹⁰⁹Cd found in the filtrate, retentate, and membrane as the percentage of total recovered ¹⁰⁹Cd after ultrafiltration.

No. 2 using DTT reduction followed by mBBr derivatization and HPLC demonstrated the presence of DMPA in the filtrate. It is evident that DMPA, when incubated with Cd-saturated MT, *in vitro*, removes Cd from MT and forms a low-molecular-weight complex with Cd. Incubation of DMSA, at the same concentration as DMPA, also resulted in the formation of a second peak in gel filtration chromatography (Fig. 6). The area under the peak No. 2, however, was only 27% of total peak area. DMSA, thus, seems less potent than DMPA in decorporating Cd from MT. It should be noted that these experiments were performed *in vitro*.

DISCUSSION

DMPA but not DMSA is found in the bile after injection. Excretion of foreign compounds by the liver involves their uptake from the blood by parenchymal and/or non-parenchymal cells, intracellular storage and transport and finally excretion into blood, lymph, or bile (Reichen and Paumgartner, 1980). Thus, the biliary appearance of a compound can indicate that it penetrates the cell membrane and has an intracellular distribution. DMPA can pass through the cell membrane of hepatocytes, since 36% of the administered DMPA was found in the bile in an unaltered form (Fig. 3). The intracellular presence of a compound is important for it to be useful for the treatment of chronic Cd poisoning, since most of the Cd is firmly bound to intracellular metallothionein, even within 24 hr after exposure (Cherian, 1977). Thus, not only is the decorporating agent required to have the functional groups for Cd chelation, but it also must get into the cells. DMPA meets both these criteria. Thus, it can compete for and mobilize Cd deposited inside of cells.

In contrast, DMSA, which forms a Cd chelate *in vitro* (Rivera *et al.*, 1989), was not excreted in the bile (Fig. 4). Neither unaltered nor altered DMSA was detected. The two

charged carboxyl groups of DMSA would be expected to limit its ability to penetrate cell membranes. Whole-body autoradiography of mice given [^{14}C]DMSA *iv* has shown that most of the radioactivity was localized in the extracellular fluids and spaces (Liang *et al.*, 1986). Also, erythrocytes do not appear to take up DMSA (Planas-Bohne and Olinger, 1981; Aaseth *et al.*, 1981).

DMPS was detected in the bile, but most (92%) of it was in an altered form(s)³ (Fig. 4). The altered DMPS consists of a mixture of disulfides since it was converted to unaltered DMPS by DTT reduction. The rapid oxidation of DMPS has also been found in the rabbit (Maiorino *et al.*, 1988b). After *im* administration of DMPS, 84% of the total DMPS found in rabbit urine was identified as acyclic and cyclic polymeric disulfides of DMPS. Wildenauer *et al.* (1982) have proposed that DMPS enters cells by using the anion transport mechanism. Their experimental evidence is based on the *in vitro* fate of [^{14}C]DMPS and the use of inhibitors of the anion transport mechanism.

DMPA increases biliary excretion of cadmium. Difficulties in the therapy of chronic Cd intoxication can be attributed in part to the intracellular distribution of cadmium, tightly bound to metallothionein, and the extracellular distribution of many chelators (Planas-Bohne and Lehmann, 1983; Klaassen *et al.*, 1984). The results of the present experiments show that DMPA, when given *iv* 3 days after exposure to Cd, rapidly increased the elimination of Cd via bile (Fig. 5) and therefore from the liver, the organ in which the greatest amount of Cd is deposited (Lucis *et al.*, 1969). Bile flow rate is an important consideration in the study of the biliary excretion of metals. Yonaga and Morita (1981) reported that DMPA enhances the bile flow (19%) and the biliary excretion of mercury in mice. We found that DMPA increased the bile flow rate 15% in rats, but it increased the concentration of Cd in the bile almost 1900% (Table 1). Therefore, the increase by DMPA

of the biliary excretion of Cd can not be due to the enhanced bile flow alone.

DMPA removes cadmium from metallothionein. The *in vitro* binding studies clearly demonstrate that DMPA removed 62% of Cd from MT. Peak No. 2 was shown to contain both ^{109}Cd and DMPA (Figs. 6 and 7). The evidence strongly supports the mechanism that the increase of biliary cadmium following DMPA administration (Fig. 5) is the result of DMPA entering the hepatocytes and mobilizing and removing the cadmium from metallothionein.

DMSA can chelate Cd *in vitro* (Rivera *et al.*, 1989) and remove Cd from MT *in vitro* (Fig. 6). But since DMSA after injection was not found in the bile (Fig. 4) and did not increase biliary Cd (Fig. 5), it is reasonable to conclude that the failure of DMSA to mobilize Cd *in vivo* is due mainly to its inability to enter cells. Gale *et al.* (1983) found that Cd levels were not reduced in any organ after DMSA injection thrice per week for 4 weeks.

Although DMPS was found in the bile (Fig. 4), it did not affect biliary excretion of Cd (Fig. 5). This may be due to the rapid oxidative transformation of DMPS (Fig. 4) to metabolites which are less likely to chelate Cd in the hepatocytes.

The removal of cadmium from metallothionein by dithiol chelating agents provides another dimension to their mechanisms of action and may provide an important new tool for the study of cadmium as well as metallothionein.

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