Biochemical Pharmacology, Vol. 30, No. 17, pp. 2427–2433, 1981.
Printed in Great Britain.

0006-2952/81/172427-07 $02.00/0

EFFECT OF DIMERCAPTOSUCCINATE ON THE ACCUMULATION AND DISTRIBUTION OF CADMIUM IN THE LIVER AND KIDNEY OF THE RAT

RICHARD MASON

Toxicology Research Unit, Medical Research Council of New Zealand, University of Otago Medical School, Dunedin, New Zealand

(*Received* 28 *January* 1981; *accepted* 20 *March* 1981)

Abstract—The effect of dimercaptosuccinic acid (DMSA) on the tissue distribution, renal and hepatic subcellular localization of Cd^{2+}, Zn^{2+} and Cu^{2+} in Cd^{2+}-pretreated male rats and on the tissue distribution of Zn^{2+} and Cu^{2+} in the normal rat was studied. Cd^{2+}-pretreated rats which received 3×1 mg Cd^{2+}/kg body wt s.c. at 48 hr intervals followed after 7 days by DMSA (50 mg/kg body wt i.p.) daily for 17 days had total hepatic Cd^{2+} concentrations 25 per cent lower than Cd^{2+}-pretreated controls ($P < 0.01$). DMSA did not influence the concentration or distribution of Cd^{2+} in the liver cytosol whereas in the mitochondrial-lysosomal and nuclei + cell debris fractions the Cd^{2+} concentration was reduced by 54 and 48 per cent respectively. Total renal and hepatic Cu^{2+} concentrations were increased by Cd^{2+} treatment and reduced by treatment of the Cd^{2+}-exposed animal with DMSA. In the liver cytosol Cu^{2+}, Zn^{2+} and Cd^{2+} accumulated in the metallothionein fraction and none was mobilized from the cytosol by DMSA. In the kidney cytosol Cu^{2+} accumulated in fractions in addition to metallothionein and was eliminated from each of these fractions following treatment with DMSA. It is concluded that the high affinity of metallothionein for these cations prevented their elimination from the cytosol and that the interaction of Cd^{2+} and Cu^{2+} with DMSA occurred in the particulate fraction and therefore delayed the response to DMSA treatment. It is suggested that long term treatment with DMSA, although ineffective in mobilizing Cd^{2+} from the kidney may provide a useful therapeutic measure to reduce the liver burden of Cd^{2+} and the high renal Cu^{2+} concentrations in the Cd^{2+}-exposed animal.

Meso-2,3-dimercaptosuccinic acid (DMSA), a water soluble chelating derivative of 2,3-dimercaptopropanol (BAL) is nontoxic and effective in decreasing the toxicity and body burden of Hg in mice, guinea pigs [1], rabbits [2] and rats [3]. In the rat the decrease in body burden following DMSA administration is paralleled by a decrease in the Hg content of the kidney [3] which is the critical organ in Hg^{2+} and also in Cd^{2+} poisoning.

The long biological half-life of the toxic Cd^{2+} ion is a function of its high affinity for metallothionein [4] a low mol. wt, cysteine-rich protein the synthesis of which is induced in the liver, kidney and intestinal mucosa of the Cd^{2+}-treated animal. In male rats Hg^{2+} displaces Cd^{2+} from renal metallothionein as well as inducing its synthesis in both male and female rats [5] and in rats treated regularly with Hg^{2+}, metallothionein-bound Hg^{2+} accounts for a significant proportion of the total renal Hg^{2+} concentration [6]. These data suggest that the mobilization of metallothionein-bound Hg^{2+} by DMSA may not be necessary for the elimination of Hg^{2+} from the kidney and that similarly the binding of Cd^{2+} by metallothionein may not preclude the possibility that DMSA might chelate and eliminate the Cd^{2+} ion from other renal and/or hepatic binding sites.

Although DMSA *per se* is relatively non toxic [1] its interaction with essential trace elements in the Cd^{2+}-exposed animal may modify its toxicity particularly if the chelant is administered for several weeks. In long-term dietary treatment of experimental animals with Cd^{2+} for example, Zn^{2+} concentration in the liver and Cu^{2+} concentration in the kidney is increased and the altered distribution of these trace elements is correlated with increased liver and kidney content of metallothionein-bound Zn^{2+} and Cu^{2+} respectively [7]. Administration of a chelating agent may in turn further influence the distribution and toxicity of these cations by altering the composition and turnover of metallothionein [8, 9]. Metal ions function as cofactors or inhibitory regulators in many enzymes. It is possible that competition between chelant and Zn^{2+} and/or Cu^{2+}-dependent enzymes for these (and other) functional cations could also mediate toxic effects of the chelating agent. DMSA, like serum amino acids, might also compete with serum albumin for Zn^{2+} and Cu^{2+} possibly resulting in enhanced transport of these cations from the serum and increased cellular uptake [10]. In the present work DMSA was administered to normal and Cd^{2+}-pretreated rats. Its effects on the tissue distribution, renal and hepatic subcellular localization of Cd^{2+}, Zn^{2+} and Cu^{2+} in the Cd^{2+}-pretreated rat and on the tissue distribution of Zn^{2+} and Cu^{2+} in the normal animal are summarized in this report.

MATERIALS AND METHODS

Animal experiments. Random bred male Wistar rats (140–160 g body wt) were fed ICI pelleted diet (Dunedin, New Zealand) and tap water *ad lib.* throughout the experiments. Rats were treated with three (and in some experiments, five) doses of Cd^{2+}

(1 mg/kg body wt s.c.) as $CdCl_2$ at 48 hr intervals. Seven days after the last injection of Cd^{2+} daily treatment (excluding Saturday and Sunday) with DMSA (50 mg/kg body wt i.p.) was commenced and continued for 17 days. Each animal received 13 doses of DMSA. Control animals which had also been treated with Cd^{2+} received saline instead of DMSA.

In other experiments normal (untreated) animals were given daily injections of saline or DMSA (50 mg/kg body wt i.p.) using the same dosage schedule as described above.

Rats were killed by decapitation 24 hr after the final injection of saline or DMSA. Liver, kidneys, muscle, spleen, heart and intestinal tissue was removed, liver and kidneys weighed and samples of each tissue analysed for Zn^{2+}, Cu^{2+} and Cd^{2+}.

The effect of DMSA treatment on the distribution of Cd^{2+} following a single i.v. injection of the cation was determined. Male rats were injected with Cd^{2+} (0.5 mg/kg body wt i.v.) as $CdCl_2$ followed after 30 min by saline (controls) or DMSA (100 mg/kg body wt i.p.). Both groups were killed 60 min after the Cd^{2+} injection, whole liver removed, weighed and sampled for metal analysis.

Subcellular fractionation procedure. Portions of fresh liver or whole kidneys from each animal in a group were pooled, minced and homogenized in 3 vol. 0.25 M sucrose in 5 mM Tris–HCl buffer pH 8.0 using a Sorvall Omnimixer. The homogenate was fractionated by differential centrifugation to yield a 'nuclear + cell debris' fraction (480 *g*, 10 min), a mitochondrial-lysosomal fraction (12,000 *g*, 7 min), a microsomal pellet (100,000 *g*, 60 min) and the post microsomal supernatant (cytosol). Portions of the cytosol and resuspended mitochondrial-lysosomal and microsomal fractions were analysed for Zn^{2+}, Cu^{2+} and Cd^{2+}. The cytosol (equivalent to 0.75–1.00 *g* wet wt tissue) was fractionated by gel filtration on a Sephadex G-75 column (80 × 1.5 cm) with 10 mM Tris–HCl buffer pH 8.0 at a flow rate of 15–18 ml/hr as eluant.

Analytical methods. DMSA (Purum grade) was obtained from Fluka AG, Buchs, Switzerland. All other reagents were obtained from BDH Chemicals Ltd, Poole, England and were of Aristar grade. Solutions were prepared using distilled-deionized H_2O which was also used for all normal laboratory procedures. Samples of whole tissue, subcellular fractions and cytosol were digested to dryness with 0.5 ml conc. HNO_3 and then with 0.25 ml H_2O_2. Samples were analysed in 5 ml 5 per cent HCl by atomic absorption spectrometry. Column eluate fractions were analysed without digestion.

RESULTS

Cadmium accumulated mainly in the liver and kidney of rats treated parenterally with Cd^{2+}. Several other tissues also accumulated Cd^{2+} although in much lower concentrations (Table 1). As in long term feeding experiments, accumulation of Cd^{2+} was accompanied by increased concentration of Cu^{2+} in the kidney [7] and liver. Cu^{2+} concentrations in muscle, spleen, heart and intestinal mucosa and Zn^{2+} concentrations in these tissues and in the liver and kidney were unaffected by Cd^{2+} treatment (Table

Table 1. Effect of dimercaptosuccinate on the tissue distribution of cadmium, zinc and copper in the male rat

Tissue	Normals		Cd-pretreated controls			Cd-pretreated + DMSA		
	Zn^{2+}	Cu^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}
	μg/g wet wt tissue ± S.E.M.							
Liver	33.87 ± 2.08	5.28 ± 0.45	36.52 ± 7.00	8.35 ± 0.47	34.48 ± 1.64	39.33 ± 3.34	5.58 ± 0.37	25.82 ± 0.91
Kidney	32.87 ± 0.86	9.73 ± 0.95	34.34 ± 2.41	22.76 ± 2.65	25.14 ± 0.96	31.95 ± 1.63	14.76 ± 0.50	22.80 ± 0.32
Muscle	17.48 ± 1.35	1.72 ± 0.62	15.55 ± 0.52	1.81 ± 0.10	0.36 ± 0.07	14.16 ± 1.97	1.80 ± 0.16	0.38 ± 0.14
Spleen	24.64 ± 1.57	3.00 ± 1.29	18.04 ± 1.47	2.69 ± 0.15	3.10 ± 0.47	20.19 ± 1.37	2.36 ± 0.08	2.85 ± 0.19
Heart	18.95 ± 1.14	5.24 ± 0.85	17.25 ± 0.47	6.47 ± 0.23	1.08 ± 0.11	17.10 ± 2.48	8.05 ± 0.69	1.16 ± 0.11
Intestine	24.23 ± 0.76	2.07 ± 0.49	25.57 ± 6.77	2.51 ± 0.07	0.77 ± 0.08	19.53 ± 1.12	2.43 ± 0.28	0.84 ± 0.29

Male rats (140–160 g) received three s.c. injections of Cd^{2+} (1 mg/kg body wt) at 48 hr intervals. Seven days after the final injection of Cd^{2+} daily treatment with saline (Cd^{2+}-pretreated controls) or DMSA (50 mg/kg i.p.) (Cd^{2+}-pretreated + DMSA) commenced and continued for 17 days. Normal animals were treated with saline as described above but did not receive Cd^{2+}. Three animals were used in each group. The terminal body wt of the Cd^{2+}-pretreated control group (259 ± 13 g, mean ± S.D.), was not significantly different from that of the Cd^{2+}-pretreated + DMSA group (257 ± 13 g, mean ± S.D.), $P > 0.90$.

Table 2. Effect of dimercaptosuccinate on the tissue distribution of zinc and copper in the normal rat

Tissue	Controls		DMSA-treated	
	Zn^{2+}	Cu^{2+}	Zn^{2+}	Cu^{2+}
	μg/g wet wt tissue ± S.E.M.			
Liver	33.27 ± 2.30	4.81 ± 0.14	31.55 ± 0.82	4.76 ± 0.30
Kidney	30.58 ± 2.11	8.98 ± 1.57	27.65 ± 0.06	6.15 ± 0.34
Muscle	15.18 ± 0.43	1.12 ± 0.15	15.51 ± 0.25	1.24 ± 0.16
Spleen	23.81 ± 0.13	2.40 ± 0.54	22.61 ± 0.93	2.24 ± 0.48
Heart	19.81 ± 0.24	5.23 ± 0.16	19.62 ± 0.38	5.25 ± 0.38
Intestine	24.96 ± 0.79	2.14 ± 0.29	23.12 ± 0.47	3.33 ± 1.62

Normal male rats (3 in each group) were treated i.p. with saline or DMSA (50 mg/kg body wt) daily for 17 days and killed 24 hr after the last injection.

1). In rats which received five doses of Cd^{2+} however hepatic Zn^{2+} concentration in the Cd^{2+}-pretreated animals (50.04 ± 3.67 μg Zn^{2+}/g wet wt liver) was significantly greater than in normal animals (31.63 ± 2.00 μg Zn^{2+}/g wet wt liver) ($P < 0.001$).

Cd^{2+}-pretreated rats which received daily treatment with DMSA had total hepatic Cd^{2+} concentrations significantly lower than that in the Cd^{2+}-pretreated controls ($P < 0.01$). The terminal body weight of the Cd^{2+}-pretreated control group was not significantly different from that of the Cd^{2+} + DMSA-treated group (Table 1). Thus the lower total hepatic Cd^{2+} concentration in the DMSA-treated group was not a function of altered body weight gain. Total renal Cd^{2+} concentration as well as Cd^{2+} concentrations in muscle, spleen, heart and intestinal mucosa were not affected by DMSA treatment (Table 1). In Cd^{2+}-pretreated animals DMSA had no effect on the Zn^{2+} concentration in kidney, muscle, spleen, heart and intestinal mucosa and did not reduce the Zn^{2+} concentration which was possibly elevated in the liver following Cd^{2+} pretreatment. Total renal and hepatic Cu^{2+} concentrations which were significantly increased by Cd^{2+} pretreatment ($P < 0.01$) were reduced in DMSA treated animals ($P < 0.05$ and $P < 0.01$ respectively) though not in the kidney to normal levels. DMSA was ineffective in mobilizing Cu^{2+} from muscle, spleen, heart and intestinal mucosa (Table 1). DMSA was also ineffective in altering the total concentration of Zn^{2+} and Cu^{2+} in the liver, kidney, muscle, spleen, heart and intestinal mucosa of the normal animal (Table 2).

Table 3 shows that in the liver of the Cd^{2+}-pretreated controls the mitochondrial-lysosomal and microsomal fractions contained only a small proportion (3 per cent) of the total Cd^{2+} concentration. It is inferred that most of the hepatic Cd^{2+} was associated with the nuclei + cell debris fraction and with cytosolic metallothionein [Fig. 1(B)].

In the liver of the normal animal Zn^{2+} was present in the mitochondrial-lysosomal, microsomal, cytosolic and nuclei + cell debris fractions which contained 4, 8, 45 and, by difference, 43 per cent respectively of the total hepatic Zn^{2+} concentration (Table 3). Figure 1(A) shows that most of the cytosolic Zn^{2+} was associated with a high mol. wt fraction ($Ve/V_0 = 1.0$) and a fraction of intermediate mol. wt ($Ve/V_0 = 1.45$) with a small proportion bound to metallothionein ($Ve/V_0 = 2.0$) or present as a low

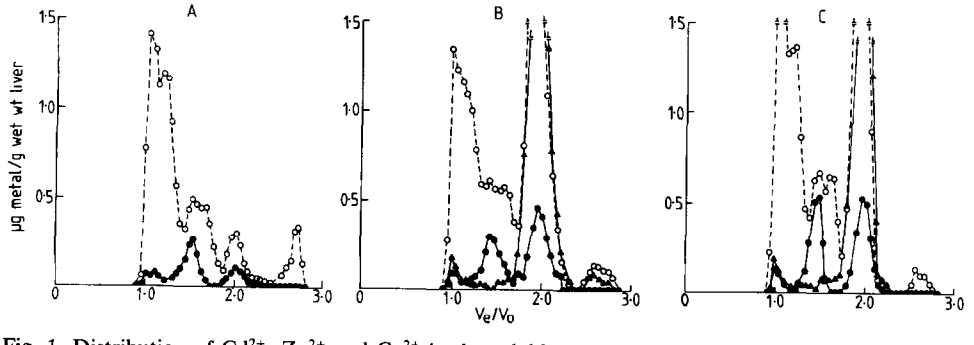

Fig. 1. Distribution of Cd^{2+}, Zn^{2+} and Cu^{2+} in the soluble fraction of the liver in normal (A), Cd^{2+}-treated (B), and Cd^{2+} + DMSA-treated (C) male rats. For experimental details see Methods. Soluble fractions were prepared by centrifugation of tissue homogenates obtained by pooling tissue from three animals in each group and fractionating on a column (85 × 1.5 cm) of Sephadex G-75 using 10mM Tris–HCl buffer, pH 8.0 as eluant. O---O, Zn^{2+}; ●——●, Cu^{2+}; ▲——▲, Cd^{2+}.

Table 3. Effect of dimercaptosuccinate on the subcellular distribution of cadmium, zinc and copper in rat liver

	Normals		Cd^{2+}-pretreated controls			Cd^{2+}-pretreated + DMSA		
	Zn^{2+}	Cu^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}
				μg/g wet wt tissue ± S.E.M.				
Total	32.84 ± 4.03	5.06 ± 0.45	36.52 ± 7.00	8.35 ± 0.47	34.48 ± 1.64	39.33 ± 3.34	5.58 ± 0.37	25.82 ± 0.91
Particulate	18.19 ± 2.91	3.19 ± 0.52	12.69	4.37	20.15	13.60	1.38	10.78
M-L	1.39 ± 0.28	0.79 ± 0.39	1.91	0.47	0.72	2.44	0.37	0.33
Micr	2.54 ± 0.89	0.70 ± 0.35	2.22	0.78	0.38	2.53	0.32	0.51
Cytosol	14.65 ± 1.14	1.87 ± 0.14	23.83	3.98	14.33	25.73	4.20	15.04
HMW	12.13 ± 1.33	0.29 ± 0.10	11.68	0.28	0.57	13.64	0.29	0.60
Int MW		1.15 ± 0.15		1.51			1.85	
MT	1.46 ± 0.24	0.43 ± 0.21	11.44	2.19	13.47	11.58	2 06	14.14
LMW	1.06 ± 0.21		0.71		0.29	0.51		0.30

For experimental details see Table 1. Mitochondrial-lysosomal (M-L), microsomal (Micr) and cytosolic fractions were prepared from tissue homogenates by differential centrifugation as described in Methods. Cytosol was separated into high (HMW), intermediate (Int MW), metallothionein (MT) and low mol. wt (LMW) fractions by gel filtration (Fig. 1) using a column (85 × 1.5 cm) of Sephadex G-75 and 10 mM Tris–HCl buffer pH 8.0 as eluant. Three animals were used in each group. Mean values for the normal group were obtained after three separate experiments and for the Cd^{2+}-pretreated and Cd^{2+}-pretreated + DMSA groups from one experiment.

mol. wt species. The cytosolic Zn^{2+} concentration was increased in the Cd^{2+}-pretreated rat with all of the additional Zn^{2+} being bound to metallothionein [Fig. 1(B), Table 3].

In the liver of the normal animal Cu^{2+} was also present in the mitochondrial-lysosomal, microsomal, cytosolic and nuclei + cell debris fractions which contained 16, 14, 37 and 33 per cent respectively of the total hepatic Cu^{2+} concentration (Table 3). In contrast with Zn^{2+} most of the Cu^{2+} in the cytosol was associated with the fraction of intermediate mol. wt with smaller proportions bound to metallothionein or to a high mol. wt fraction [Fig. 1(A), Table 3]. In the Cd^{2+}-pretreated controls the total hepatic Cu^{2+} concentration was increased with most of the additional Cu^{2+} present as metallothionein [Fig. 1(B), Table 3].

Treatment of Cd^{2+}-pretreated rats with daily doses of DMSA had no effect on the concentration or distribution of Zn^{2+}, Cu^{2+} and Cd^{2+} in the hepatic cytosol [Table 3, Fig. 1(C)]. Thus the fall in total hepatic concentration of Cd^{2+} and Cu^{2+} can be associated with elimination of these cations from the particulate fraction, particularly from the nuclei + cell debris fraction and from the mitochondrial-lysosomal (Cd^{2+}) and microsomal (Cu^{2+}) fractions (Table 3).

In the kidney of Cd^{2+}-pretreated animals Cd^{2+} was located in the mitochondrial-lysosomal, microsomal, cytosolic and nuclei + cell debris fractions which contained 1, 1, 34 and 64 per cent respectively of the total renal Cd^{2+} concentration (Table 4). In contrast with the distribution of Cd^{2+} in the hepatic cytosol, a significant proportion (25 per cent) was associated with a high mol. wt fraction, the remainder being bound to metallothionein [Fig. 2(B)].

In the kidney of the normal animal Zn^{2+} was distributed similarly to that in the liver (Table 4). In the renal cytosol more Zn^{2+} was bound to metallothionein and less associated with the high molecular weight fraction [Fig. 2(A) and see Tables 3 and 4]. Cd^{2+} pretreatment did not cause any significant change in the renal distribution of Zn^{2+} [Table 4, Fig. 2(B)].

Significant proportions of the total renal Cu^{2+} concentration in normal animals were associated with the mitochondrial-lysosomal (8 per cent) and microsomal (13 per cent) fractions with most of the cation distributed between the nuclei + cell debris and cytosolic fractions (Table 4). In the renal cytosol of the normal animal in which the Cu^{2+} concentration was higher than in the hepatic cytosol the major binding site was metallothionein [Table 4, Fig. 2(A)]. In the Cd^{2+}-pretreated animal the Cu^{2+} concentration in both particulate and cytosolic fractions was increased (Table 4). Most of the additional Cu^{2+} in the cytosol was associated with metallothionein [Table 4, Fig. 2(B)]. In the particulate fraction Cu^{2+} appeared to be redistributed from the mitochondrial-lysosomal and microsomal fractions to the nuclei + cell debris and/or cytosolic fractions (Table 4).

Treatment of Cd^{2+}-pretreated rats with daily injections of DMSA had no effect on either the concentration or distribution of Cd^{2+} in the renal cytosol [Fig. 2(C), Table 4]. Although the total kidney con-

Table 4. Effect of dimercaptosuccinate on the subcellular distribution of cadmium, zinc and copper in rat kidney

	Normals		Cd^{2+}-pretreated controls			Cd^{2+}-pretreated + DMSA		
	Zn^{2+}	Cu^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}
	$\mu g/g$ wet wt tissue ± S.E.M.							
Total	32.87 ± 0.64	9.73 ± 0.95	34.34 ± 2.41	22.76 ± 2.65	25.14 ± 0.96	31.95 ± 1.65	14.76 ± 0.50	22.80 ± 0.32
Particulate	19.31 ± 0.92	6.44 ± 1.21	21.57	13.30	16.50	14.61	9.28	14.30
M-L	2.08 ± 0.22	0.80 ± 0.43	1.65	0.62	0.34	5.22	0.44	0.30
Micr	3.24 ± 0.63	1.30 ± 0.86	1.95	0.49	0.29	2.61	0.80	0.15
Cytosol	13.56 ± 0.94	3.29 ± 0.55	12.77	9.46	8.64	17.34	5.48	8.50
HMW	9.55 ± 0.80	0.35 ± 0.11	9.19	0.94	2.16	10.41	0.71	1.45
Int MW		0.68 ± 0.14		1.14			0.77	
MT	2.84 ± 0.23	2.26 ± 0.44	2.69	7.38	6.48	5.72	4.00	6.89
LMW	1.17 ± 0.09		0.89			1.21		0.16

For experimental details see Table 3.

centration of Zn^{2+}, like Cd^{2+}, was unaffected by DMSA treatment, the Zn^{2+} concentration in the cytosol was increased. All of the additional Zn^{2+} was bound to metallothionein [Fig. 2(C)] and accompanied by a concomitant fall in particulate Zn^{2+} concentration (Table 4). The fall in total renal Cu^{2+} concentration (35 per cent) produced by DMSA treatment was associated with a similar proportional decrease in Cu^{2+} concentration in the particulate (30 per cent) and cytosolic (42 per cent) fractions (Table 4). DMSA treatment however did not influence the distribution of Cu^{2+} in the cytosol which was therefore significantly different from that observed in the normal animal [Fig. 2 (A–C)].

Table 5 shows that 60 min after a single i.v. injection of Cd^{2+} a large proportion (68 per cent) of the dose was accumulated by the liver and distributed equally between particulate components and the cytosol. Most of the cytosolic Cd^{2+} (61 per cent) as anticipated was associated with the high mol. wt fraction the remainder being bound to metallothionein (30 per cent), presumably by displacement of Zn^{2+} and/or Cu^{2+} (unpublished data), and a low mol. wt species (9 per cent). Treatment with DMSA (100 mg/kg i.p.) 30 min after Cd^{2+} and 30 min before sacrifice did not appear to significantly influence the concentration, content or distribution of Cd^{2+} in the liver (Table 5).

DISCUSSION

The ability of a chelating agent to mobilize Cd^{2+} and the toxicity of the chelant depends on the competing side reactions between the chelant and endogenous hydrogen and metal ions and other Cd^{2+}-binding ligands and on the pharmacokinetics of the chelating agent. Thus if the stability of complexes formed between Cd^{2+} and endogenous binding molecules is greater than the affinity of DMSA for Cd^{2+} ions no mobilization would be expected. The present results show that DMSA does not mobilize Cd^{2+} from binding sites in the cytosol of the liver and kidney of the rat. In the liver and kidney of the Cd^{2+}-pretreated animal the greater proportion of cytosolic Cd^{2+} was bound to metallothionein which has a very high affinity for the cation [4]. Following an acute i.v. injection of Cd^{2+} much of the Cd^{2+} in the hepatic cytosol was associated with the high mol. wt fraction from which it would have been subsequently transferred to metallothionein [11]. When DMSA was administered 30 min after Cd^{2+} however the chelating agent had no effect on the Cd^{2+} concentration in the high mol. wt fraction of the liver cytosol. These results suggest that, in addition to its strong binding to metallothionein, Cd^{2+} also forms a co-ordination complex with an endogenous high molecular weight species to which it is also strongly bound and exchanged only slowly.

DMSA may have a physiological distribution different from that of Cd^{2+}, Zn^{2+} and Cu^{2+} and mobilization would only occur when the chelating agent and metal(s) share the same distribution space. In long term treatment with DMSA, Cd^{2+} and Cu^{2+} were lost from the particulate fraction of the hepatocyte. Neither acute administration of DMSA nor

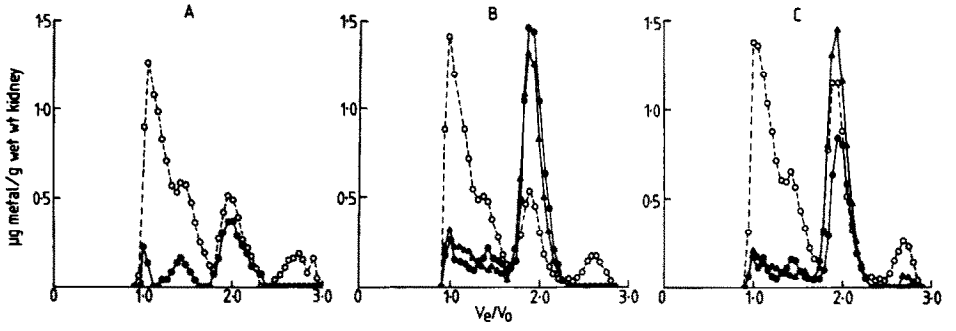

Fig. 2. Distribution of Cd^{2+}, Zn^{2+} and Cu^{2+} in the soluble fraction of the kidney in normal (A), Cd^{2+}-treated (B), and Cd^{2+} + DMSA-treated (C) male rats. For experimental details see Methods. Soluble fractions were prepared and separated as described in Fig. 1. O---O, Zn^{2+}; ●——●, Cu^{2+}; ▲——▲, Cd^{2+}.

repeated dosing with DMSA over 24 hr to Cd^{2+}-pretreated rats (unpublished data) had any effect on the concentration of Cd^{2+} or Cu^{2+} in the particulate fraction of the liver. Thus the time course of response to DMSA treatment is consistent with delayed compartmentalization of the chelating agent to nuclear, mitochondrial, lysosomal and/or microsomal sites.

In the Cd^{2+}-pretreated rat renal Cu^{2+} distribution was particularly modified with increased Cu^{2+} concentration in both particulate and cytosolic fractions. Predictably Cu^{2+} was subsequently chelated and eliminated from these sites. In the kidney cytosol of the Cd^{2+}-treated animal Cu^{2+} was accumulated not only by metallothionein but also by the high and intermediate mol. wt fractions from which it was eliminated following DMSA treatment. In the liver on the other hand Cu^{2+} which in the cytosol accumulated only in metallothionein was eliminated only from the particulate fraction. Cd^{2+} treatment increased total Zn^{2+} concentration in the liver with essentially all of the additional Zn^{2+} being associated with metallothionein. DMSA treatment had no effect either upon Zn^{2+} concentration or distribution in the liver of the Cd^{2+}-pretreated rat. These results support the view that the sites of chelation of Cd^{2+} and Cu^{2+} by dimercaptosuccinate are located in particulate components of the cell and the high affinity of metallothionein for these cations prevents their elimination from the cytosol particularly when all excess metal in the cytosol is bound to this protein.

The degree of loss of Cd^{2+} from the liver is dependent upon the concentration of Cd^{2+} in the distribution volume of DMSA. The present results show that whilst the total hepatic Cd^{2+} concentration is reduced by 25 per cent the fall in Cd^{2+} concentration in the particulate, mitochondrial-lysosomal and nuclei + cell debris fractions were 47, 54 and 48 per cent respectively and represents a significant mobilization of the cation from sites of potential cellular toxicity. The mobilized Cd^{2+} is presumably eliminated from the body, possibly in the bile [12], since there is no concomitant increase in Cd^{2+} concentration in other tissues and Cd^{2+} is not accumulated by redistribution to the kidney.

The present data suggest that the subcellular toxicity of DMSA is unlikely to result from chelant induced alterations in trace element distribution. Treatment of normal rats with DMSA for example had no effect on the tissue distribution of Zn^{2+} and Cu^{2+} and whereas treatment with Cd^{2+} significantly increased the concentration of Cu^{2+} in the functional components (i.e. the particulate fraction and high

Table 5. Effect of dimercaptosuccinate on the hepatic distribution of cadmium 1 hr after i.v. administration of Cd^{2+} to male rats

Treatment	Whole liver	Particulate fraction	Cytosol		
			HMW fraction	MT-bound	LMW
	μg/g wet wt tissue ± S.E.M. (μg/liver)				
Cd^{2+} + saline	7.47 ± 0.99 (49.82 ± 7.35)	3.75	2.27	1.12	0.33
Cd^{2+} + DMSA	7.34 ± 1.46 (45.23 ± 8.95)	3.26	2.12	1.55	0.41

Male rats received Cd^{2+} (0.5 mg/kg i.v.) followed after 0.5 hr by saline or DMSA (100 mg/kg i.p.). Animals were killed 1 hr after Cd^{2+} injection, liver from each animal sampled for Cd^{2+} analysis and portions of tissue from each animal in the group pooled, homogenized and fractionated into particulate and cytosolic fractions as described in Methods. Three animals were used in each group.

and intermediate molecular weight fractions of the cytosol) of the kidney subsequent treatment with DMSA advantageously decreased the Cu^{2+} concentration in a similar proportion to the fall in total renal Cu^{2+} concentration.

Since the liver burden of Cd^{2+} may be an important factor in the renotoxicity of Cd^{2+} [13], DMSA should provide a useful therapeutic measure to reduce the hepatic concentration of Cd^{2+} as well as to reduce the renal Cu^{2+} concentration which is elevated in the Cd^{2+}-exposed animal.

REFERENCES

1. E. Friedheim and C. Corvi, *J. Pharm. Pharmac.* **37**, 624 (1975).
2. Shi Jue-min, Ting Kuang-sheng, Chen Li-xin, Yu Yue-gui, Liang Yu-i and Chen Yin-jiu, *Kexue Tongbao* **17**, 409 (1966).
3. L. Magos, *Br. J. Pharmac.* **56**, 479 (1976).
4. J. H. R. Kagi and B. L. Vallee, *J. biol. Chem.* **236**, 2435 (1961).
5. M. Webb and L. Magos, *Chem.–Biol. Interact.* **14**, 357 (1976).
6. J. K. Piotrowski, B. Trojanowska, J. M. Wisniewka-Knypl and W. Bolanowska, *Toxic. appl. Pharmac.* **27**, 11 (1974).
7. M. D. Stonard and M. Webb, *Chem.–Biol. Interact.* **15**, 349 (1976).
8. K. Cain and D. Holt, *Chem.–Biol. Inter.* **28**, 91 (1979).
9. Z. A. Shaikh and J. C. Smith, *Chem.–Biol. Interact.* **15**, 327 (1976).
10. M. Webb, in *Clinical Chemistry and Chemical Toxicology of Metals* (Ed. S. S. Brown), p 51. Elsevier, Amsterdam (1977).
11. M. Cempel and M. Webb, *Biochem. Pharmac.* **25**, 2067 (1976).
12. M. G. Cherian, *J. Toxicol. Environ. Health* **6**, 393 (1980).
13. K. Cain and B. Griffiths, *Biochem. Pharmac.* **29**, 1852 (1980).