BioMetals 1992, **5**, 17–22

Increased urinary excretion of zinc and copper by mercuric chloride injection in rats

Xiaoyan Liu, Gunnar F. Nordberg & Taiyi Jin

Department of Environmental Medicine, University of Umeå, Umeå, Sweden

Received 23 May 1991

The effects of $HgCl_2$ on urinary excretion of Zn, Cu and metallothionein at different time intervals were observed in male Wistar rats. The rats were given a daily intraperitoneal injection of $^{203}HgCl_2$ (0.5 or 1.0 mg Hg kg^{-1}) for 2 days. ^{203}Hg, Zn, Cu and metallothionein in urine, kidney and liver were analyzed. Significant increases in urinary Zn and Cu concentrations were found in $HgCl_2$-dosed groups. Elevated urinary Zn and Cu concentrations were accompanied by an increased metallothionein excretion in urine at different time periods. Zn concentration in urine remained elevated during the entire observation period of 7 days. There were also increased concentrations of Cu and Zn in the renal cortex in one of the two exposed groups. The results indicate that urinary Cu and Zn are related to the manifestation of renal toxicity and/or the synthesis of metallothionein in kidney induced by mercury.

Keywords: mercury, zinc, copper, metallothionein

Introduction

Toxic effects of metals and their compounds on animals or human beings can be potentiated or prevented depending on the metabolic status of essential elements; on the other hand, the metabolism of essential elements can also be affected by exposure to metals. It may be of interest to hypothesize that the disturbance in the homeostasis of essential elements might contribute to one of the mechanisms of toxicity of metals. In recent years, many studies have indicated the existence of interactions among metals either *in vivo* or *in vitro* (Nordberg 1978, Holt *et al.* 1980, Day *et al.* 1984, Funk *et al.* 1987). When animals were exposed to inorganic mercury, a significant increase in kidney Cu concentration was found (Szymanska & Zelazowski 1979). In rat kidneys, metallothionein (MT) synthesis induced by Hg is accompanied by changes in endogenous Cu (Suzuki & Yamamura 1979, Tandon *et al.* 1980, Brzeznicka & Chmielnicka 1985). Hg also increases the retention of Zn in rat liver and kidney (Magos & Webb 1976, Lee *et al.* 1983). One previous study has shown an increased urinary excretion of endogenous Zn and Cu at 1 day after administration of inorganic Hg and/or sodium selenite (Chmielnicka *et al.* 1986). Bogden *et al.* (1980) suggested that the classical nephrotoxic effects of inorganic Hg may be due, in part, to the associated elevated Cu levels.

However, the results reported above mainly concerned the changes in metal concentrations in tissues; little information is available about the excretion of urinary Zn and Cu at different time periods when animals have been exposed to $HgCl_2$. The purpose of this study was to investigate the effects of $HgCl_2$ exposure on the urinary excretion of Zn and Cu, as well as its relationship to MT turnover induced by $HgCl_2$.

Materials and methods

Animals

Eighteen male Wistar rats weighing 150–190 g were obtained from ALAB (Stockholm, Sweden). After conditioning for 1 week in a temperature/light-controlled room, they were divided into three groups and housed individually in metabolic cages. Food (Ewos-ALAB rat/mouse food powder) and tap water were provided *ad libitum*. After an adaptation period of 24 h in metabolic cages and

Address for correspondence: X. Liu, Department of Environmental Medicine, University of Umeå, S-901 87 Umeå, Sweden. Fax: 4690 119630.

collection of the urine for a baseline value, the control group (group A) received intraperitoneally 0.9% normal saline (1.0 mg kg^{-1} body mass). Group B was given a daily intraperitoneal dose of 0.5 mg Hg kg^{-1} body mass as $HgCl_2$ labeled with ^{203}Hg (0.2 μCi ^{203}Hg/1.0 mg Hg) for 2 days. Group C was given the radiolabeled $HgCl_2$ 1.0 mg Hg kg^{-1} by the same route and with the same 2-day schedule. Urine samples were collected at the following intervals after the first $HgCl_2$ injection: 4, 8, 12, 16, 24, 28, 32, 36, 48 h and 3, 4, 5, 6 and 7 days.

Analysis of samples

Samples of urine (1–5 ml) were used for measuring ^{203}Hg with a standard and blank by using a Packard auto-gamma scintillation spectrometer. One whole kidney and one piece of liver were removed for the measurement. For metal analysis urine was digested with an equal volume of concentrated nitric acid; 100–250 mg renal cortex and liver was dried at 105 °C for 16 h and then ashed at 450 °C in an automatically controlled furnace for 24 h. The ashed samples were dissolved in 1.0 M nitric acid. Zn and Cu concentrations were measured by atomic absorption spectrophotometry in the flame mode with deuterium background correction (AA 875 series, Varian). For quality control purposes, bovine liver from the National Bureau of Standards (SRM 1577a) was analyzed. The recovery of Zn and Cu were 102% and 93.5% of the values specified for SRM 1577a, respectively. MT in urine and tissue homogenate were measured by the Cd/hemoglobin affinity assay method (Eaton & Toal 1982). Renal cortex and liver tissue thus were homogenized in 0.01 M Tris pH 8.0 and ultracentrifuged. Supernatant was taken for MT measurement. Creatinine determination was performed as described by Hare (1950). The difference between groups of metal and MT concentrations were compared by using the rank-sum test performed by the statistical package of Status 11 from Foresco AB (1988).

Results

Urinary Zn, Cu, Hg and MT excretion after injections of $HgCl_2$ labeled with ^{203}Hg are shown in Figures 1 & 2 at different time points. As seen in Figure 1, following the injection of $HgCl_2$, the urinary excretion of Zn and Cu increased in both groups B and C compared with group A. The urinary Zn concentration in Group C started to increase significantly between 8 and 12 h, and the urinary Zn levels in both groups remained higher during the whole experimental period. A significant increase in urinary Cu excretion was also demonstrated in group C. The increased Cu excretion in urine appeared as early as 8 h after $HgCl_2$ injection in group C and recovered to the control level after 36 h.

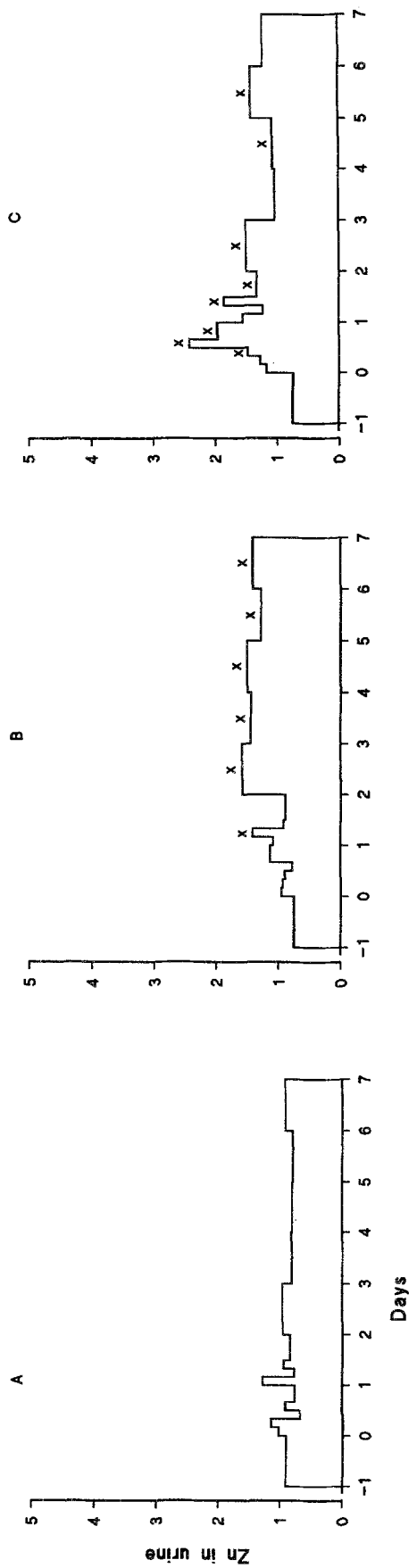
For purposes of comparison the MT and Hg concentrations in urine (previously reported in relation to Ca, Mg excretion by Liu *et al.* 1991) are given in Figure 2; the highest peak of MT in urine did not occur at the same time as that for Zn or Cu.

Urinary concentration of Hg in group C was much higher than that group B. Following the increased level of urinary Hg, a significantly increased excretion of total urinary protein was observed in group C (data not shown) concomitantly with the increase in MT excretion; statistically significant increase of total protein in urine was not observed in group B except for a marginal effect at the time interval 2–3 days which may be a chance observation (Liu *et al.* 1991).

When these rats were sacrificed 7 days later, the concentrations of Zn and Cu in renal cortex was found to be significantly increased in group B, while the corresponding values in group C were not significantly increased (Table 1). No changes were found for Zn and Cu in liver of rats exposed to $HgCl_2$. There were significant increases of Hg and MT in both renal cortex and liver of rats injected with $HgCl_2$ (Table 2). The concentrations of Hg in kidney and MT in renal cortex were much higher than that in liver in both groups B and C.

Discussion

The most important observation in this study is the illustration of the time relationships between increased urinary excretion of Zn and Cu and the excretion of MT and Hg. Such relationships have not previously been reported in rats injected with $HgCl_2$. It is known that both Cd and Hg, which are nephrotoxic, can produce an increase of the renal Cu concentration (Szymanska & Zelazowski 1979, Bogden *et al.* 1980). Also, kidney Zn concentration was increased after $HgCl_2$ exposure (Bogden *et al.* 1980). In the present study, an increased excretion of urinary Zn and Cu and, in addition, increased renal Cu and Zn concentrations in rats injected with $HgCl_2$ were observed. These changes may be related to the increased renal MT synthesis induced by Hg as suggested also by other authors (Chmielnicka *et al.* 1986). This explanation appears to be plausible since mercury itself has been shown to induce the synthesis of MT in the kidney of rat (Piotrowski *et al.* 1974, Cherian & Clarkson 1976) and rabbits (Nordberg *et al.* 1974). MT identified in rat liver and kidney with exposure to cadmium is known to be rich in Zn and Cu (Webb 1982). This is similar to the observation in the present study that an increased renal Hg level in kidneys was parallel to increased Cu and MT concentrations in the same organ.



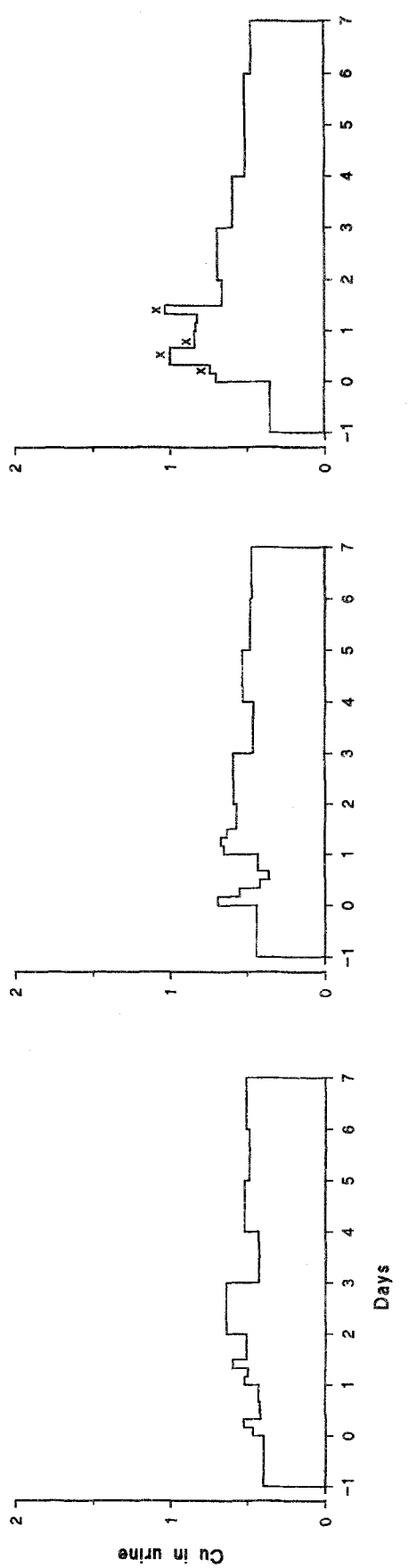


Figure 1. Excretion of urinary Zn (top panel) and Cu (bottom panel) [μg mg creatinine^{-1}] for groups A–C. Values are presented as geometric means; (×) $P < 0.05$ (rank-sum test), as compared with group A.

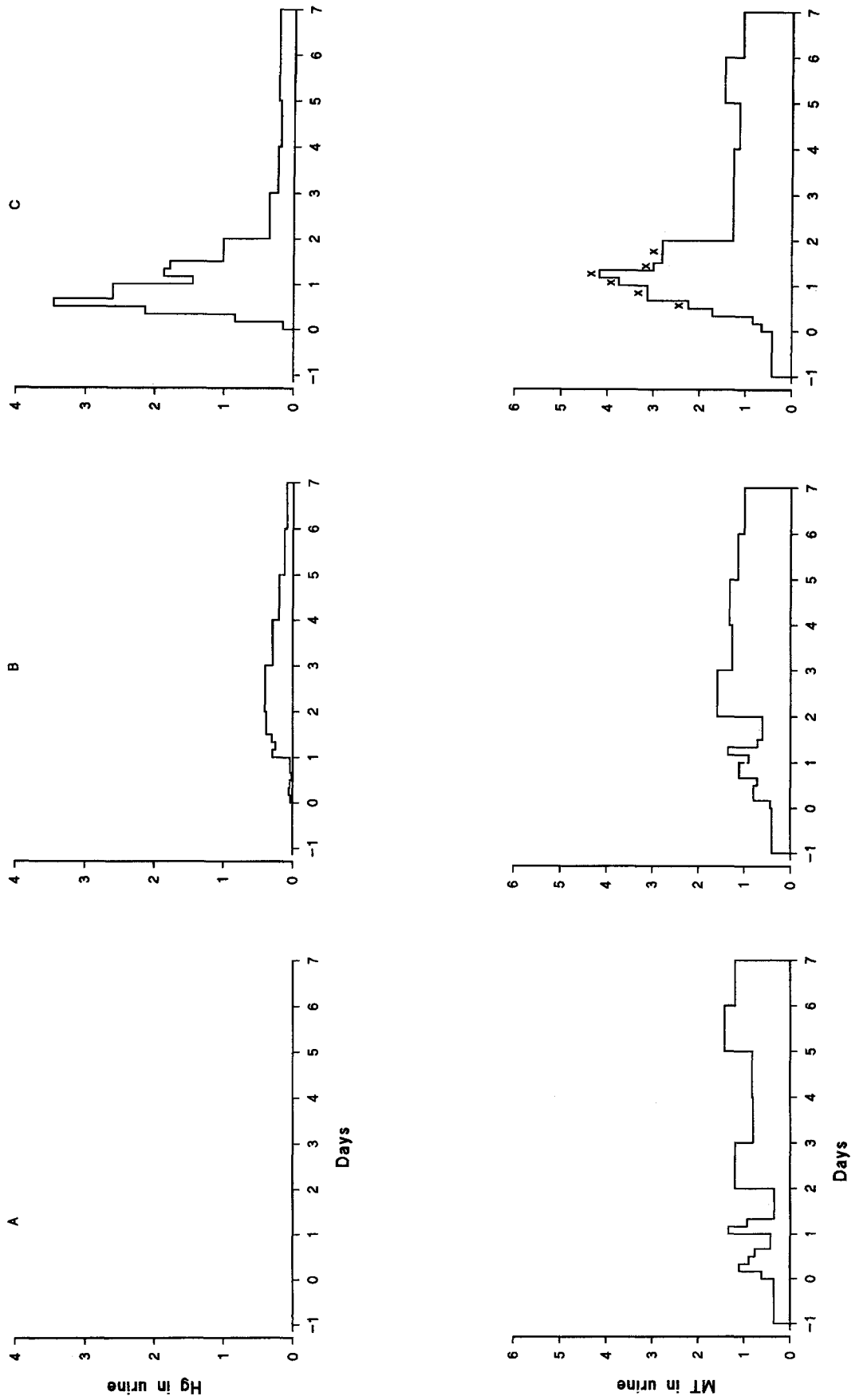


Figure 2. Excretion of urinary Hg (top panel) and MT (bottom panel) [μg mg creatinine^{-1}] for groups A–C. Values are presented as geometric means; (×) $P < 0.05$ (rank-sum test), as compared with group A.

Table 1. Renal cortex and liver concentrations of Zn and Cu in rats injected with $HgCl_2$

Group	Concentration (mean ± SEM μg/g wet mass)			
	Renal cortex		Liver	
	Zn	Cu	Zn	Cu
A	43.5 ± 0.85	13.5 ± 3.23	43.5 ± 1.88	4.4 ± 0.69
B	48.2 ± 1.84*	27.3 ± 4.86*	44.7 ± 2.2	5.1 ± 0.73
C	52.2 ± 3.92	19.9 ± 3.59	45.3 ± 4.86	4.9 ± 0.94

Group A received two daily intraperitoneal (i.p.) saline injections of 1.0 ml kg^{-1}; group B was injected i.p. with $^{203}HgCl_2$ at 0.5 mg Hg kg^{-1} body mass on two consecutive days; group C were injected i.p. with $^{203}HgCl_2$ at 1.0 mg Hg kg^{-1} body mass on two consecutive days. All rats were killed on the 7th day.
*$P < 0.05$ compared to group A (rank-sum test).

The peak increases of Zn (12–16 h) and Cu (8–12 h) in urine occurred before the peak excretion of urinary MT (24–32 h). This is similar to the situation for excretion of Ca and Mg (Liu *et al.* 1991). Whanger & Deagen (1983) found that, although not as effective as Cd, Hg could cause an accumulation of Zn in MT in the kidney by dietary Hg exposure in rats. In another study, with two successive intraperitoneal injections of 1.3 mg kg^{-1} in rats, little Zn was induced in urinary MT (Sato *et al.* 1989). Thus, the increased urinary and renal Zn concentrations could be partly due to the non-MT-bound components with an acute exposure such as in the present study. The increased urinary Zn excretion did not completely follow the pattern of urinary MT excretion in the present study. An increase of the Cu content of renal MT by daily subcutaneous injection in rats has been reported (Szymanska & Zelazowski 1979). It is known that, in Cd-exposed animals, the content of Cu in renal MT depends upon the species of animal (Suzuki 1979). Rats and guinea-pigs have high Cu content in renal MT while many other animals, e.g. mice and hamsters, have low Cu after exposure to Cd.

The importance of increased urinary Zn and Cu excretion might be a reflection of a disturbance in the homeostasis of essential elements that might, in turn, be a mechanism for $HgCl_2$ nephrotoxicity. Vogel (1960) reported necrosis of the renal proximal tubular epithelium in mice given high doses of Cu. Mercury-poisoned chicks on Cu-deficient diets had a lower mortality rate than those with normal dietary Cu intake (Hill *et al.* 1964). Possibly, Cu may be released from MT by mercury and exert a toxic effect on the nephron (Bogden *et al.* 1980). The transitory necrotic damage of the proximal tubules caused during repeated injections of Cd was reported to be accompanied by a rapid decrease of the Cu content in the kidney MT (Suzuki 1984). Another possibility is that the increased urinary excretion of Cu may be partly due to the increased urinary excretion of copper-binding MT caused by renal tubular damage similar to that observed in humans with Cd-induced tubular damage (Nogawa *et al.* 1984). This might also explain the observation in the present study that a statistically significant increase of Cu in renal cortex was observed only in the low-dose group (Table 1). The total amount of Cu excreted via urine during the period of 0–36 h after the first injection of $HgCl_2$ was 19.6 μg in group C and 7.5 μg in group B.

The urinary Zn excretion in the present study increased significantly at about 12 h and reached the highest level at 16 h and, moreover, remained high even on the 7th day. Thus, in inorganic Hg exposure, an increased excretion of Zn and/or Cu

Table 2. Renal cortex and liver concentrations of Hg and MT in rats injected with $HgCl_2$

Group	Concentration (μg g wet mass^{-1})							
	Hg				MT			
	Kidney		Liver		Renal cortex		Liver	
	GM	Range	GM	Range	GM	Range	GM	Range
A	–	–	–	–	0.8	0.1–6.2	1.8	1.0–3.9
B	15.6	3.1–25.1	0.2	0.1–0.5	19.0	2.9–103.8*	5.8	1.3–27.2*
C	14.5	7.2–53.6	0.8	0.1–2.0*	100.8	52.8–161.1*	6.5	2.8–22.3*

GM = geometric mean value. Group A received two daily intraperitoneal (i.p.) saline injections of 1.0 ml kg^{-1}; group B was injected i.p. with $^{203}HgCl_2$ at 0.5 mg Hg kg^{-1} body mass on two consecutive days; group C was injected i.p. with $^{203}HgCl_2$ at 1.0 mg Hg kg^{-1} body mass on two consecutive days. All rats were killed on the 7th day.
*$P < 0.05$ (rank-sum test) as compared to group A (MT) or group B (Hg).

might be an indicator of nephrotoxicity. However, further studies of this possibility in animals and human beings seem warranted.

Acknowledgments

This study was performed by X.L. while she was a WHO research fellow at the Department of Environmental Medicine, University of Umeå. Grants to G.F.N. from the Swedish Environmental Protection Agency (SNV 611-152-89-Uf) and from the Swedish Work Environment Fund (89-0592) are gratefully acknowledged.

References

Bogden JD, Kemp FW, Troiano RA, *et al.* 1980 Effect of mercuric chloride and methylmercury chloride exposure on tissue concentrations of six essential minerals. *Environ Res* **21**, 350–359.

Brzeznicka EA, Chmielnicka J. 1985 Interaction of alkylmercuric compounds with sodium selenite. III Biotransformation on the levels of metallothionein-like proteins and endogenous copper in some tissues of rats exposed to methyl or ethylmercuric chloride with and without sodium selenite. *Environ Health Perspect* **60**, 423–431.

Cherian MG, Clarkson TW. 1976 Biochemical changes in rat kidney on exposure to elemental mercury vapor: effect on biosynthesis of metallothionein. *Chem-Biol Interact* **12**, 109–120.

Chmielnicka J, Brzeznicka E, Sniady A. 1986 Kidney concentrations and urinary excretion of mercury, zinc and copper following the administration of mercuric chloride and sodium selenite to rats. *Arch Toxicol* **59**, 16–20.

Day FA, Funk AE, Brady FO. 1984 *In vivo* and *ex vivo* displacement of zinc from metallothionein by cadmium and by mercury. *Chem-Biol Interact* **50**, 159–174.

Eaton DL, Toal BF. 1982 Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. *Toxicol Appl Pharmacol* **66**, 134–142.

Funk AE, Day FA, Brady FO. 1987 Displacement of zinc and copper from copper-induced metallothionein by cadmium and by mercury: *in vivo* and *ex vivo* studies. *Comp Biochem Physiol* **86C**, 1–6.

Hare RS. 1950 Endogenous creatinine in serum and urine. *Proc Soc Exp Biol* **74**, 148–151.

Hill CH, Starcher B, Matrone G. 1964 Mercury and silver interrelationships with copper. *J Nutr* **83**, 107–110.

Holt D, Magos L, Webb M. 1980 The interaction of cadmium-induced rat renal metallothionein with bivalent mercury *in vitro*. *Chem-Biol Interact* **32**, 125–135.

Lee YH, Shaikh ZA, Tohyama C. 1983 Urinary metallothionein and tissue metal levels of rats injected with cadmium, mercury, lead, copper or zinc. *Toxicology* **27**, 317–345.

Liu XY, Jin TY, Nordberg GF. 1991 Increased calcium and magnesium excretion in rats injected with mercuric chloride. *Pharmacol Toxicol* **68**, 254–259.

Magos L, Webb M. 1976 The interaction between cadmium, mercury and zinc administered subcutaneously in a single injection. *Arch Toxicol* **36**, 53–61.

Nogawa K, Yamada Y, Honda R, *et al.* 1984 Copper and zinc levels in serum and urine of cadmium-exposed people with special reference to renal tubular damage. *Environ Res* **33**, 29–38.

Nordberg GF. 1978 Factors influencing metabolism and toxicity of metals. A consensus report by the task group on metal interaction. *Environ Health Perspect* **25**, 3–41.

Nordberg M, Trojanowska B, Nordberg GF. 1974 Studies on metal-binding proteins of low molecular weight from renal tissue of rabbits exposed to cadmium or mercury. *Environ Physiol Biochem* **4**, 149–158.

Piotrowski JK, Trojanowska B, Sapota A. 1974 Binding of cadmium and mercury by metallothionein in the kidneys and liver of rats following repeated administration. *Arch Toxicol* **32**, 351–360.

Sato M, Nagai Y, Bremner I. 1989 Urinary excretion of metallothionein-I and its degradation product in rats treated with cadmium, copper, zinc or mercury. *Toxicology* **56**, 32–33.

Suzuki KT. 1979 Copper content in cadmium-exposed animal kidney metallothioneins. *Arch Environ Contam Toxicol* **8**, 255–268.

Suzuki KT. 1984 Studies of cadmium uptake and metabolism by the kidney. *Environ Health Perspect* **54**, 21–30.

Suzuki KT, Yamamura M. 1979 Dose-dependent formation of zinc-thionein in livers and kidneys of rats and mice by zinc injection. *Biochem Pharmacol* **28**, 2852–2854.

Szymanska JA, Zelazowski JK. 1979 Effect of cadmium, mercury and bismuth on the copper content in rat tissues. *Environ Res* **19**, 121–126.

Tandon SK, Magos L, Cabral JRP. 1980 Protection against mercuric chloride by nephrotoxic agents which do not induce thionein. *Toxicol Appl Pharmacol* **52**, 227–236.

Vogel FS. 1960 Nephrotoxic properties of copper under experimental conditions in mice. *Am J Pathol* **36**, 699–712.

Webb M. 1982 Role of metallothionein and other binding proteins in the renal handling and toxicity of metals. In: Bach PH, Bonner FW, Bridges JW, Lock FA, eds. *Nephrotoxicity*. UK: St Edmundsbury Press; 296–309.

Whanger PD, Deagen JT. 1983 Effects of dietary mercury level and cadmium on rat tissue metallothionein: mercury binding and influence on zinc. *Environ Res* **30**, 372–380.