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Studies on Poisonous Metals. XIX.¹⁾ Comparative Effects of Chelating Agents on Distribution and Excretion of Inorganic Mercury in Rats

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The effects of various chelating agents, sodium *N*-benzyl-D-glucamine dithiocarbamate (NBG-DTC), 2,3-dimercaptopropanol (BAL), D-penicillamine (D-PEN), and sodium *N*-methyl-D-glucamine dithiocarbamate (NMG-DTC), on the distribution and excretion of inorganic mercury were compared in rats exposed to HgCl_2 . Rats were injected i.p. with $^{203}\text{HgCl}_2$ (300 μg of Hg and 2 μCi of $^{203}\text{Hg}/\text{kg}$) and 30 min or 24 h later, were injected with a chelating agent (400 $\mu\text{mol}/\text{kg}$). NBG-DTC and BAL mainly promoted the biliary excretion of mercury, while D-PEN and NMG-DTC significantly promoted the urinary excretion of mercury. In rats pretreated with HgCl_2 30 min earlier, the injection of NBG-DTC reduced the content of mercury in the pancreas. BAL significantly reduced the contents of the metal in the liver, kidney, and pancreas. D-PEN reduced the contents of mercury in the pancreas, lung, and heart. In rats pretreated with HgCl_2 24 h earlier, NBG-DTC reduced the content of mercury in the kidney. BAL reduced the contents of mercury in the liver and kidney, although BAL caused the redistribution of mercury to the lung, heart, and brain. In rats injected with NBG-DTC at 30 min after pretreatment with HgCl_2 , the biliary excretion of mercury increased with increasing dose of NBG-DTC. In rats injected with NBG-DTC at 24 h after pretreatment with HgCl_2 , the biliary excretion of mercury increased with increasing dose of NBG-DTC at the doses of 400—1200 $\mu\text{mol}/\text{kg}$ and at the doses above 1200 $\mu\text{mol}/\text{kg}$ the urinary excretion of this metal increased remarkably. A long time interval between the administrations of mercury and NBG-DTC resulted in the decreased biliary excretion of mercury. The reduced stimulatory effect of NBG-DTC on the biliary excretion of mercury with time after the pretreatment with mercury may result from the decrease of tissue mercury concentration and labile mercury content in the rat body.

Keywords—inorganic mercury; tissue distribution; excretion; *N*-benzyl-D-glucamine dithiocarbamate; 2,3-dimercaptopropanol; D-penicillamine; *N*-methyl-D-glucamine dithiocarbamate; chelate effect

Mercury has been recognized as a highly toxic metal to man for many years. This substance is accumulated in the kidney, liver, myocardium, mucosa of the intestines, upper respiratory tract, mouth, interstitial tissue of the testes, and skin, among other organs.²⁾ Inorganic mercury is especially accumulated in the renal cortex, and affects the morphology and function of the proximal tubules.^{3–5)} Slow elimination and considerable retention of mercury were found in some parts of the brain, in the interstitial tissue of the testes, in the skin, in the buccal mucosa, and in the kidney. No satisfactory method for enhancing mercury mobilization from deposits in organs has yet been found. The most potent mercury detoxicating agents are SH-containing compounds, of which D-penicillamine (D-PEN) and 2,3-dimercaptopropanol (BAL) are used in clinical medicine. The practical usefulness of BAL, however, is limited by a low therapeutic index.⁶⁾ The search for less toxic antidotes has led to the synthesis of chelating agents, such as *N*-acetyl-DL-penicillamine,⁷⁾ 2,3-dimercaptopropane-1-sulfonate,⁸⁾ 2,3-dimercaptosuccinic acid,^{9–11)} and *N*-(2,3-dimercaptopropyl)phthalamidic

acid.^{12,13)} It has been reported that diethyldithiocarbamate and its N,N-disubstituted analogs can promote mobilization and excretion of cadmium from many organs.¹⁴⁻²⁰⁾ In addition, we have reported that the injection of sodium *N*-benzyl-D-glucamine dithiocarbamate (NBG-DTC), which was newly synthesized by us, into rats pretreated with cadmium was more effective in decreasing the cadmium concentration in liver and kidney than injection of BAL and *N*-methyl-D-glucamine dithiocarbamate (NMG-DTC).²¹⁾ The purpose of this study was to evaluate the relative effectiveness of NBG-DTC, BAL, D-PEN, and NMG-DTC in mobilizing inorganic mercury from its principal sites of accumulation and promoting its excretion when given at various time intervals after injection of HgCl₂.

Experimental

Materials—²⁰³HgCl₂ (specific activity, 4.85 mCi/mg) was obtained from New England Nuclear (Boston, Mass). Mercuric chloride was obtained from Wako Pure Chemical Ind. (Osaka). BAL was obtained from Nakarai Chemicals Ltd. (Kyoto). NMG-DTC was prepared according to the procedure of Shinobu *et al.*¹⁶⁾ NBG-DTC was prepared by the method reported in our previous paper.²¹⁾ All other chemicals were of reagent grade.

In Situ Rat Biliary Excretion Experiments—Male Wistar rats, weighing 190–240 g, were anesthetized with urethane (1 g/kg, i.p.) and the bile duct was cannulated with polyethylene tubing (PE 10) as reported previously.²²⁾ Thereafter, the rats were injected i.p. with ²⁰³HgCl₂ (300 μg Hg and 2 μCi of ²⁰³Hg/kg) and, 30 min or 24 h later they were injected i.p. with saline, NBG-DTC (400 μmol/kg), BAL (400 μmol/kg), D-PEN (400 μmol/kg), or NMG-DTC (400 μmol/kg). Each chelating agent was dissolved in 0.5 ml of saline. Bile samples were collected for an experimental period of 5 h. Then the rats were killed and various tissues were collected. The urine in the bladder was collected into an injection syringe and combined with the urine excreted. The levels of ²⁰³Hg radioactivity in bile, urine, and tissues were determined by using an Aloka auto well gamma scintillation counter (model ARC 300).

Gel Filtration of Kidney Soluble Fraction—The kidneys from rats after the *in situ* biliary and urinary excretion experiment were homogenized in 4 vol of chilled Tris buffer [0.1 M tris (hydroxymethyl)aminomethane-HCl, 0.1 M NaCl, pH 7.4], with a glass-Teflon homogenizer. The homogenate was centrifuged at 102000 *g* for 1 h at 4 °C. An aliquot (1 ml) of the supernatant fraction was applied to a Sephadex G-75 column (1.5 × 46 cm). The column was eluted with Tris buffer at a flow rate of 6 ml/h at room temperature, and the effluent was collected in 2.5 ml fractions. The ²⁰³Hg radioactivity of each fraction was determined. The Sephadex G-75 column was calibrated by using the following proteins of known molecular weight: ovalbumin (45000), chymotrypsin (25000), and cytochrome c (12500).

Partition Coefficient of Chelating Agent–Mercury Complex—A solution (1 ml) of each chelating agent (200 μM NBG-DTC, 100 μM BAL, 100 μM D-PEN or 200 μM NMG-DTC) was added to 1.0 ml of a solution of HgCl₂ (50 μM) containing 1 μCi of ²⁰³HgCl₂ and 0.1 M Tris buffer (pH 7.4). After addition of 2.0 ml of *n*-octanol to each tube, the mixtures were shaken vigorously at 37 °C for 10 min. After separation of the two phases by standing for 45 min, 1 ml of each phase was collected and counted in the gamma counter. The partition coefficients were expressed as log₁₀ (cpm of ²⁰³Hg in the *n*-octanol phase/cpm of ²⁰³Hg in the aqueous phase).

Statistical Analysis—Data were compared by analysis of variance. When the analysis indicated a significant difference, the treated groups were compared with controls by using Student's *t* test.

Results

Figure 1A shows the biliary and urinary excretions of mercury when each chelating agent was injected at 30 min after pretreatment with HgCl₂. The analysis of excreta for ²⁰³Hg radioactivity showed that the major route of excretion of mercury after treatment with NBG-DTC, BAL, and NMG-DTC in rats exposed to HgCl₂ was in the bile and that of mercury after treatment with D-PEN was in the urine. The cumulative biliary excretion of mercury in a 5 h period after injection of NBG-DTC was significantly smaller than that with BAL but larger than that with D-PEN or NMG-DTC. In addition, the injection of D-PEN showed a greater stimulatory effect on the urinary excretion of mercury than that of NBG-DTC, BAL, or NMG-DTC.

Furthermore, we investigated the comparative effects of these four chelating agents on the biliary and urinary excretions of mercury in rats at 24 h after the pretreatment with HgCl₂ (Fig. 1B). The biliary excretion of mercury was significantly enhanced by treatment with BAL

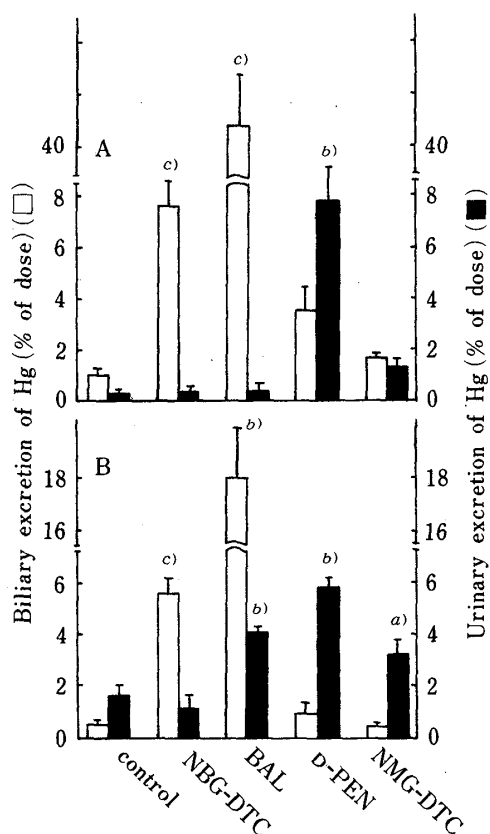


Fig. 1. Effects of Chelating Agents on Biliary and Urinary Excretions of Mercury in Rats

Time of Hg treatment: A, 30 min; B, 24 h.

Significantly different from control: a) $p < 0.05$; b) $p < 0.01$; c) $p < 0.001$.

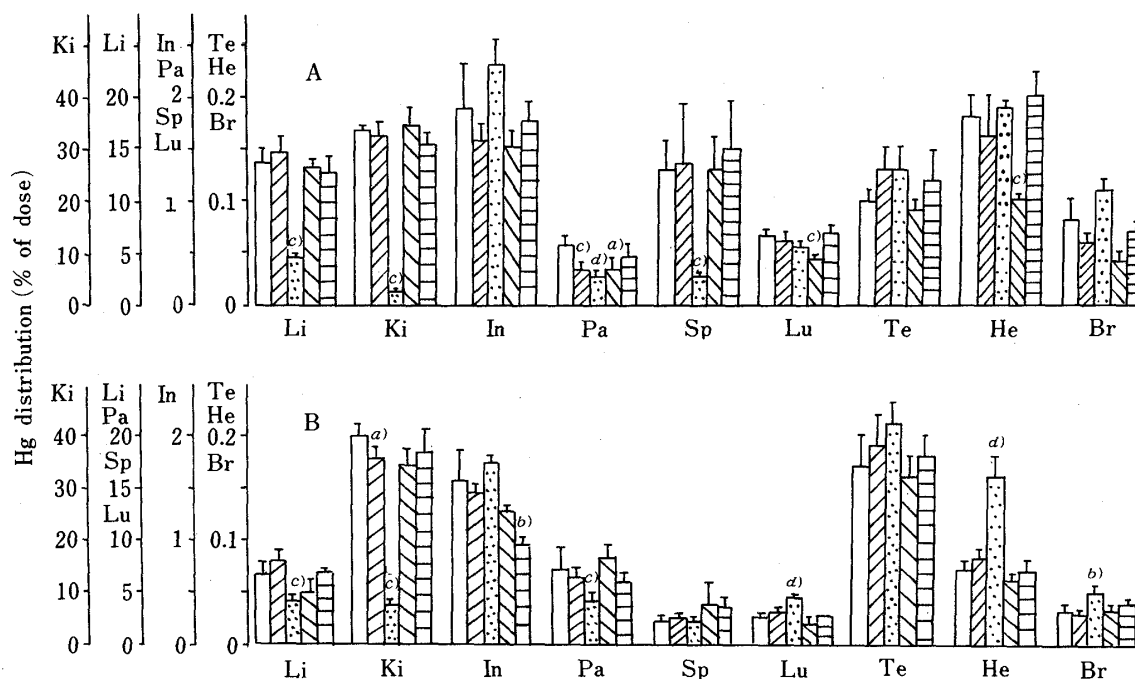


Fig. 2. Effects of Chelating Agents on Tissue Distribution of Mercury in Rats Treated with HgCl_2

Li, liver; Ki, kidney; In, intestine; Pa, pancreas; Sp, spleen; Lu, lung; Te, testes; He, heart; Br, brain. Time of Hg treatment: A, 30 min; B, 24 h. □, control; ▨, NBG-DTC; ▩, BAL; ▧, D-PEN; ▦, NMG-DTC. Significantly different from control, a) $p < 0.05$; b) $p < 0.02$; c) $p < 0.01$; d) $p < 0.001$.

and NBG-DTC. However, the delayed treatment with these chelating agents was less effective on the biliary excretion of mercury than that at 30 min after the pretreatment with HgCl_2 . The urinary excretion of mercury was markedly enhanced by administration of BAL, D-PEN, and NMG-DTC. In particular, the delayed treatment with BAL and NMG-DTC showed greater effectiveness on the urinary excretion of mercury than that at 30 min after the pretreatment with HgCl_2 . On the other hand, no difference between the increased urinary excretion caused by the administration of D-PEN at 30 min and 24 h after mercury was observed.

Figure 2A shows the tissue distribution of mercury at 5 h after a single injection of the chelating agents into rats pretreated with HgCl_2 , 30 min earlier. The injection of BAL significantly reduced the contents of mercury in the liver, kidney, pancreas, and spleen. NBG-DTC reduced the content of mercury in the pancreas, but the distribution of mercury in the liver and kidney after treatment with NBG-DTC did not change as compared with that of the control. D-PEN reduced the contents of mercury in the pancreas, lung, and heart. The contents of mercury in other organs did not change after treatment with the chelating agents. Figure 2B shows the tissue distribution of mercury at 5 h after the injection of the chelating agents into rats pretreated with HgCl_2 24 h earlier. NBG-DTC reduced the content of mercury in the kidney. BAL reduced the contents of mercury in the liver and kidney. Among the four chelating agents tested, BAL showed the greatest effectiveness in the removal of

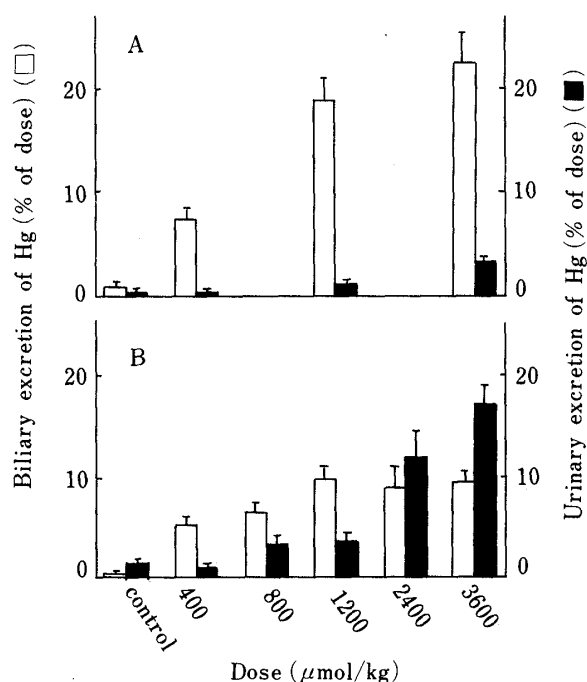


Fig. 3. Effects of Doses of NBG-DTC on Biliary and Urinary Excretion of Mercury in Rats
Time after Hg treatment: A, 30 min; B, 24 h.

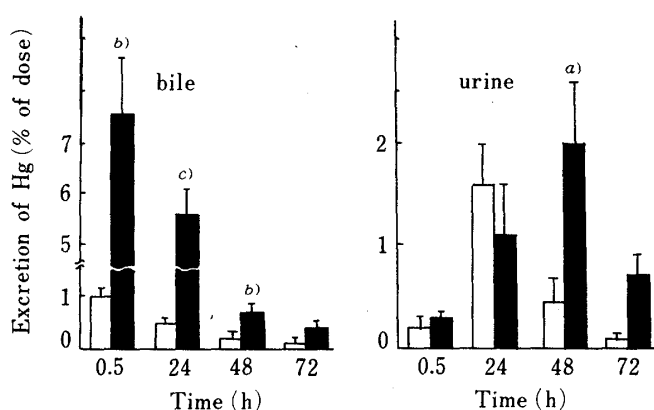


Fig. 4. Effects of Time Interval between Administration of HgCl_2 and NBG-DTC on Biliary and Urinary Excretions of Mercury in Rats

The rats were injected i.p. with $^{203}\text{HgCl}_2$ (300 μg Hg and 2 μCi of $^{203}\text{Hg/kg}$) and 0.5, 24, 48 or 72 h later they were given i.p. saline or NBG-DTC (400 μmol/kg). The bile and urine samples were collected for 5 h.

□, control; ■, NBG-DTC.
Significantly different from control: a) $p < 0.05$; b) $p < 0.01$; c) $p < 0.001$.

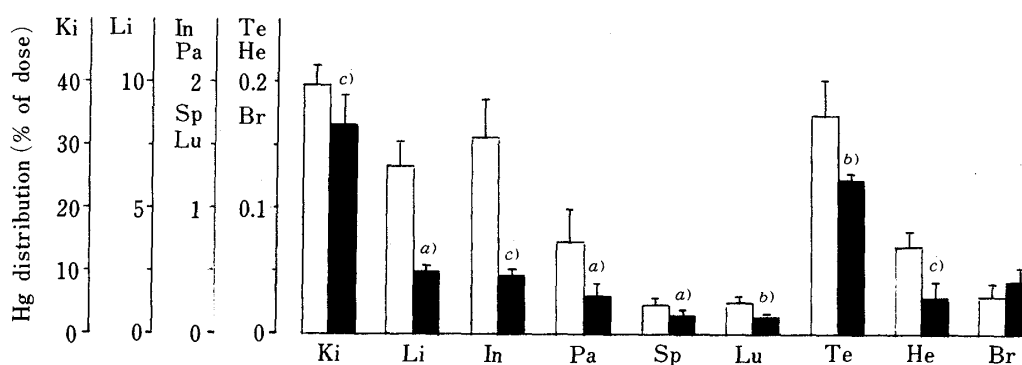


Fig. 5. Tissue Distribution of Mercury at 24 and 72 h after Pretreatment of Rats with HgCl_2

Li, liver; Ki, kidney; In, intestine; Pa, pancreas; Sp, spleen; Lu, lung; Te, testes; He, heart; Br, brain. \square , 24 h; \blacksquare , 72 h. Significantly different from 24 h: a), $p < 0.05$; b), $p < 0.02$; c), $p < 0.01$.

TABLE I. Mercury Distribution between Supernatant and Precipitate in Centrifugal Fractions of Kidney^{a)}

Treatment time with HgCl_2		Distribution of mercury ^{b)} ($\mu\text{g Hg/g wet tissue}$)	
		Supernatant	Precipitate
24 h	HgCl_2 alone	12.62 ± 2.33 (79)	2.77 ± 0.25 (21)
	HgCl_2 + NBG-DTC	9.23 ± 0.70 (80)	2.33 ± 0.07 (20)
72 h	HgCl_2 alone	11.61 ± 0.56 (80)	2.82 ± 0.16 (20)
	HgCl_2 + NBG-DTC	9.70 ± 0.89 (78)	2.67 ± 0.16 (22)

Numbers in parentheses represent percent distribution. a) The kidneys from rats after the *in situ* excretion experiment were homogenized in 4 vol of chilled Tris buffer with a glass Teflon homogenizer. The homogenate was centrifuged at $102000g$ for 1 h at 4°C . b) Each value is the mean \pm standard deviation for 3 to 4 animals.

mercury from rats pretreated with HgCl_2 . However, BAL caused the redistribution of mercury to the lung, heart, and brain.

The effects of NBG-DTC at higher doses (800–3600 $\mu\text{mol/kg}$) on the biliary and urinary excretions of mercury were further investigated (Fig. 3). The injection of NBG-DTC at higher doses (800–1200 $\mu\text{mol/kg}$) at 30 min or 24 h after the pretreatment with HgCl_2 increased the biliary excretion of mercury. However, at the doses above 1200 $\mu\text{mol/kg}$ of NBG-DTC to rats pretreated with HgCl_2 24 h earlier, no further increase in the biliary excretion of mercury was observed, although the urinary excretion of this metal increased remarkably.

In addition, a long time interval between the administrations of mercury and NBG-DTC resulted in decreased biliary excretion of mercury, although the administration of NBG-DTC 48 h after the pretreatment with HgCl_2 resulted in maximal urinary excretion of mercury (Fig. 4). The decreased biliary excretion of mercury caused by the long time interval between the administrations of mercury and NBG-DTC was further investigated. Figure 5 shows the tissue distributions of mercury in rats at 24 and 72 h after pretreatment with HgCl_2 . The distribution of mercury in most tissues at 24 h after the pretreatment with HgCl_2 was somewhat greater than that at 72 h after the mercury pretreatment (Fig. 5). Since about 39% of inorganic mercury injected into rats was distributed in the kidney and 80% of the kidney

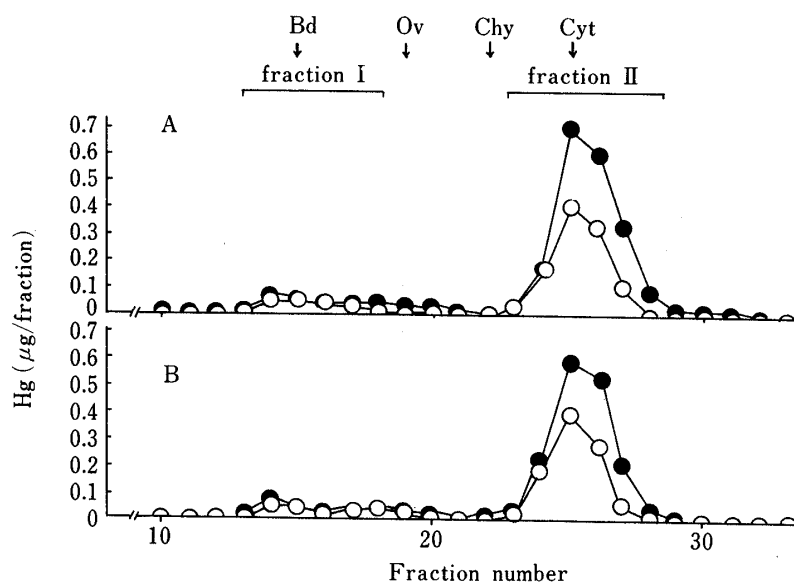


Fig. 6. Sephadex G-75 Chromatographic Profiles of Kidney Soluble Fraction

The markers used to calibrate the column (1.5 × 45 cm), were Bd (blue dextran, 2000000), Ov (ovalbumin, 45000), Chy (chymotrypsin, 25000), and Cyt (cytochrome c, 12500). Time of Hg treatment. A, 24 h; B, 72 h. ●, control; ○, NBG-DTC.

TABLE II. Partition Coefficients of Mercuric Ion and Mercury Complexes with Chelating agents in an *n*-Octanol/Aqueous System

	Partition coefficient ^{a)}	
	0.9% NaCl	0.1 M Tris buffer
HgCl ₂ only	-0.273 ± 0.002	-1.076 ± 0.006
HgCl ₂ + NBG-DTC	2.084 ± 0.040	2.068 ± 0.026
HgCl ₂ + BAL	1.229 ± 0.084	0.352 ± 0.127
HgCl ₂ + D-PEN	-1.865 ± 0.029	-2.388 ± 0.041
HgCl ₂ + NMG-DTC	-1.066 ± 0.004	-1.245 ± 0.003

^{a)} Expressed as log₁₀ (cpm in *n*-octanol phase/cpm in aqueous phase) at 37°C. Each value is the mean ± standard deviation for 3 experiments.

mercury was observed in the supernatant (Table I), the soluble fraction of kidney in rats treated with NBG-DTC or 0.9% NaCl at 24 and 72 h after the pretreatment with HgCl₂ was chromatographed on a Sephadex G-75 column (Fig. 6). Mercury-binding protein with a low molecular weight (fraction II), corresponding to metallothionein, is present in organs of mercury-treated rats.^{23,24)} Most of the mercury in the kidney soluble fraction at 24 and 72 h after injection of mercury was bound to metallothionein. The decrease in the amount of mercury bound to metallothionein caused by the administration of NBG-DTC was almost the same at 24 and 72 h after pretreatment with HgCl₂.

Results of the partition coefficient determinations of each complex of Hg²⁺ with NBG-DTC, BAL, D-PEN, and NMG-DTC are shown in Table II. NBG-DTC and BAL yielded lipid-soluble complexes with Hg²⁺, and the complexes of NMG-DTC and D-PEN with the metal were lipophobic.

Discussion

The purpose of this study was to evaluate the effectiveness of NBG-DTC, BAL, D-PEN,

and NMG-DTC for removal of inorganic mercury from rats exposed to HgCl_2 30 min or 24 h earlier. Mercury has a high affinity for the intracellular metallothionein^{23,24)} and the excretion of mercury from the body is rather slow. The present results showed that the injection of NBG-DTC or BAL enhanced mainly the biliary excretion of mercury, and D-PEN enhanced mainly the urinary excretion of the metal (Fig. 1). However, Gabard⁸⁾ reported that the urinary excretion of mercury after the injection of BAL was significantly larger than the biliary excretion in rats. In addition, Yonaga and Morita¹³⁾ reported that the fecal excretion of mercury after the injection of DL-PEN (50 mg/kg) for 5 d was significantly larger than the urinary excretion in mice. Such different effects of these chelating agents on the biliary and urinary excretions of mercury may be attributable to a species difference or to the different doses of chelating agents. The increased biliary excretion of mercury caused by the injection of NBG-DTC and BAL at 24 h after the pretreatment with HgCl_2 was smaller than that at 30 min after the mercury pretreatment. However, the increased urinary excretion of mercury caused by the injection of NBG-DTC, BAL or NMG-DTC at 24 h after the pretreatment with HgCl_2 was greater than that at 30 min after the mercury pretreatment.

Among the chelating agents used here, only BAL significantly reduced the mercury deposited in the liver, kidney and pancreas of rats pretreated with HgCl_2 30 min or 24 h earlier (Fig. 2). However, the injection of BAL at 24 h after the mercury pretreatment caused the redistribution of mercury to the lung, heart, and brain, resulting in an increase of the mercury load of these tissues. The Lipophilic property of the BAL-mercury complex seems to cause the redistribution of mercury to these tissues (Table II). Gabard⁸⁾ reported that BAL caused the redistribution of inorganic mercury to the kidney. However, the mobilization of mercury from the kidney by NBG-DTC did not promote the redistribution of mercury to the lung, heart, and brain in spite of the higher lipophilicity of the NBG-DTC-mercury complex.

The LD_{50} values of NBG-DTC, BAL and D-PEN have been reported to be 11.10,²⁵⁾ 0.85,²⁶⁾ and 2.53²⁶⁾ mmol/kg, respectively. Since the acute toxicity of NBG-DTC was much less than that of BAL and D-PEN, the effects of NBG-DTC at higher doses on the biliary and urinary excretions of mercury were investigated (Fig. 3). The injection of NBG-DTC at doses of 800–3600 $\mu\text{mol/kg}$ at 30 min after the pretreatment with HgCl_2 remarkably increased the biliary excretion of mercury. However, the injection of NBG-DTC at the same doses at 24 h after the pretreatment with HgCl_2 significantly enhanced both the biliary and urinary excretions of mercury. The total biliary and urinary excretions of mercury in rats injected with 3600 $\mu\text{mol/kg}$ of NBG-DTC (32.4% of LD_{50} value) at 30 min and 24 h after pretreatment with HgCl_2 were 27 and 26% of the dose, respectively. In addition, the undesirable redistribution of mercury to the lung, heart, and brain was not observed at these doses of NBG-DTC (data not shown). The administration of BAL at a dose of 800 $\mu\text{mol/kg}$ (94% of LD_{50} value) resulted in the death of rats (data not shown).

A long time interval between the administrations of HgCl_2 and NBG-DTC resulted in the decreased biliary excretion of mercury (Fig. 4). Therefore, the decreased effect of NBG-DTC on the excretion of mercury was investigated. Figure 5 shows that the amount of residual mercury in rats at 24 h after the pretreatment with HgCl_2 was somewhat larger than that at 72 h after the mercury. Sephadex G-75 chromatography of the soluble fraction of kidney in rats injected with NBG-DTC at 24 or 72 h after pretreatment with HgCl_2 showed that a larger part of the mercury was bound to metallothionein and that the administration of NBG-DTC at 24 or 72 h after pretreatment with HgCl_2 decreased the amount of mercury bound to metallothionein to the same extent (Fig. 6). These results suggest that the reduced stimulatory effect of NBG-DTC on the biliary excretion of mercury with increasing time after the pretreatment with HgCl_2 may result from a decrease of tissue mercury concentration and labile mercury content in the rat body.

In summary, our study reveals that the injection of NBG-DTC, which was newly

synthesized by us, into rats pretreated with HgCl_2 can effectively remove mercury from the body through fecal and urinary excretions without redistribution of mercury to other tissues, such as brain, testes, and heart. The effectiveness of NBG-DTC was less than that of BAL and similar to that of D-PEN. However, since the acute toxicity of NBG-DTC is much less than that of BAL and D-PEN, and the lower toxicity of NBG-DTC permits the use of a relatively high dose.

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