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EFFECT OF VARIOUS AMOUNTS OF SELENIUM ON THE METABOLISM OF MERCURIC CHLORIDE IN MICE

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Abstract—Male ddY mice were given one injection of (1) mercury (mercuric chloride) simultaneously with various doses of selenium (sodium selenite), (2) mercury alone, or (3) various doses of selenium alone. The interaction between mercury and selenium in the liver and kidneys at 1, 5, 24, 120, and 240 hr after administration was investigated. The concentrations of mercury in the liver of mice receiving mercury and selenium simultaneously were higher than those after administration of mercury alone, while the concentrations of mercury in the kidney decreased markedly over a 1–120 hr period after administration, depending on the dose of selenium administered simultaneously with mercury. Clearly, selenium had a different effect on the accumulation of mercury in the liver and kidneys. Subcellular distribution studies revealed that mercury and selenium which were administered simultaneously were incorporated into the crude nuclear and mitochondrial fractions as stable complexes. The transport of these complexes to the kidneys seems to be limited. In addition, gel filtration of supernatant fractions of liver and kidney through a Sephadex G-75 column indicated that the proportion of mercury bound to metallothionein fraction decreased depending on the dose of selenium administered simultaneously with the mercury. This reduction was attributed to the decreased synthesis of mercury-thionein due to a reduction in the activity of Hg^{2+} which results from binding between mercury and selenium in the cells.

It is known that selenium is an essential trace element playing an important role from a nutritional and physiological standpoint, but it is very toxic at higher concentrations [1–3]. On the other hand, selenium has been shown to reduce the toxicity of heavy metals such as mercury [4, 5]. According to Parizek and Ostadalova [6], in animal experiments, simultaneous administration of sodium selenite with mercuric chloride reduces the incidence of renal necrosis and mortality due to mercuric chloride. However, the mechanism of this detoxication has not yet been clarified. The presence of metallothionein has been related to detoxification of toxic heavy metals and to their transport in the body [7, 8]. When animals are experimentally treated with mercuric chloride, mercury-thionein is formed in the liver and kidney [9, 10]. A gel filtration of the soluble fraction of cytoplasma showed that mercury is eluted in a fraction that corresponds to proteins of an approximate molecular weight of 10,000, similar to the so-called metallothionein-like protein fraction [11–13]. In the present study, mice were treated once with mercuric chloride alone (Hg mice) or sodium selenite alone (Se mice) or with simultaneous doses of mercury and differing amounts of selenium (Hg–Se mice).† Concentrations of mercury and selenium in the liver and kidney were determined, and changes in the mercury-binding pattern were investigated by gel filtration on a Sephadex G-75 column at various periods after administration.

MATERIALS AND METHODS

Animals and chemicals. SPF male mice of the ddY strain, 30 days of age, were given free access to solid food (MF manufactured by Oriental Yeast Industries Co., Ltd.) and tap water in an environment at 25° for 10 days prior to the experiment. A total of 392 mice weighing 28–32 g were used.

Mercuric chloride and sodium selenite were used as sources of mercury and selenium. Mercuric chloride ($HgCl_2$; Wako Pure Chemical Ind. Co., Ltd.) and sodium selenite ($Na_2SeO_3 \cdot 5H_2O$; E. Merk A.G.) were of special grade. These chemicals were dissolved, respectively, in physiological saline solution to allow for single administrations of volume doses of 10 ml/kg. The doses used were 20 μmoles/kg of mercuric chloride and 5, 20, and 40 μmoles/kg of sodium selenite. Mercuric chloride was injected intraperitoneally and sodium selenite subcutaneously in the gluteal region. Mice treated with mercuric chloride alone were injected simultaneously with physiological saline solution subcutaneously in the gluteal region, while those treated with sodium selenite alone were injected intraperitoneally with physiological saline solution. Hg–Se mice received 20 μmoles/kg of mercuric chloride coupled with one of three doses of sodium selenite. Mice receiving the same treatment were divided into five subgroups and killed at 1, 5, 24, 120, and 240 hr after administration. Control mice were injected with only physiological saline solution intraperitoneally and subcutaneously in the gluteal region and killed 24 hr

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† Abbreviations: Hg–Se mice = mice receiving injections of mercury and selenium simultaneously; Hg mice = mice receiving injections of mercury alone; and Se mice = mice receiving injections of selenium alone.

later. A total of fifty-six subgroups of seven animals each were used. Mice were killed under ether anesthesia and exsanguinated. The liver and kidney were removed, rinsed with physiological saline solution, and stored at −25° until analysis.

Determination of mercury and selenium. The total mercury was determined using the method described by Deitz *et al.* [14]. The tissue sample was digested with a wet technique; mercury ions were reduced with hydroxylamine hydrochloride and stannous chloride; and the total mercury was measured at 253.7 nm in the flameless atomic absorption spectrometer (model HG-1, Hiranuma Industrial Co., Ltd.). Selenium was determined by spectrofluorometry as described by Watkinson [15]. The fluorescence intensity was measured on a fluorescent spectrophotometer (model RF-500, Shimadzu Co.) with excitation at 378 nm and emission at 520 nm.

Preparation of subcellular fractions of liver and kidney. Subcellular fractions of liver and kidneys were prepared using the method of Hogeboom and Schneider [16]. A 2 g portion of tissue was cut into pieces and homogenized with 5 vol. of 0.25 M sucrose solution at 4° for 5 min at a rate of 10 strokes/min in a Potter–Elvehjem homogenizer. The homogenate was fractionated into mainly crude nuclear and mitochondrial (10,000 *g* for 30 min), microsomal (105,000 *g* for 1 hr) and supernatant (cytosol) fractions at 4° in a refrigerated ultracentrifuge (model 65P-7, Hitachi Machine Co., Ltd.)

Gel filtration of supernatant fraction on Sephadex G-75 column. A 2-ml portion of the supernatant fraction obtained from each of the liver and kidney homogenates was applied to a 2.6 × 60 cm column of Sephadex G-75 (Pharmacia Fine Chemicals, Uppsala, Sweden) for gel filtration with 0.01 M Tris–HCl buffer (pH 7.4) at 4° at a rate of 19 ml/hr. The eluate was collected in fractions of 5.8 ml each. The u.v. absorption of each fraction was measured at 280 nm. Two fractions eluted successively were combined, and the mercury concentrations in each of the combined fractions were determined. The molecular weight of substances contained in each fraction was estimated using the standard substances cytochrome *c*, chymotrypsinogen A, ovalbumin, and bovine serum albumin.

RESULTS

Concentrations of mercury in the liver and kidney. The concentrations of mercury in the liver and kidney of Hg mice and Hg–Se mice are shown in Table 1. In Hg–Se mice, the concentrations of mercury in the liver were markedly higher than those in Hg mice except for the concentration 1 hr after administration of 5 μmoles/kg of selenium. In Hg mice and in those receiving mercury and 5 μmoles/kg of selenium simultaneously, the concentrations attained peaks at 24 hr after administration and then decreased with time. On the contrary, in mice receiving mercury simultaneously with 20 and 40 μmoles/kg selenium, the concentrations of mercury in the liver were virtually identical during the experimental period from 24 hr after administration onward and 7.7 times as high as the concentrations in Hg mice at 120 hr after administration.

Table 1. Mercury concentrations in liver and kidney after injection of mercuric chloride with or without sodium selenite*

	Mercury (μg/g wet tissue)									
	Liver					Kidney				
	Time after injection (hr)					Time after injection (hr)				
Treatment†	1	5	24	120	240	1	5	24	120	240
Hg	5.18 ± 0.12	7.81 ± 0.37	9.43 ± 0.30	5.16 ± 0.24	2.58 ± 0.13	57.28 ± 0.78	98.04 ± 2.11	49.86 ± 1.47	29.49 ± 0.90	6.24 ± 0.34
Hg–Se (5)	5.36 ± 0.15	9.87 ± 0.29‡	16.01 ± 0.31‡	8.58 ± 0.24‡	6.90 ± 0.31‡	31.16 ± 0.69‡	62.47 ± 1.84‡	44.00 ± 1.98‡	22.15 ± 0.64‡	7.06 ± 0.33‡
Hg–Se (20)	7.55 ± 1.16‡	20.20 ± 0.59‡	21.95 ± 0.63‡	22.83 ± 1.40‡	20.41 ± 1.25‡	6.45 ± 0.21‡	12.38 ± 0.63‡	19.89 ± 0.67‡	18.32 ± 0.45‡	9.45 ± 0.41‡
Hg–Se (40)	10.22 ± 0.48‡	15.41 ± 0.85‡	18.89 ± 0.90‡	21.04 ± 0.62‡	19.18 ± 0.34‡	5.93 ± 0.34‡	7.63 ± 0.66‡	11.75 ± 0.52‡	9.65 ± 0.50‡	7.95 ± 0.31‡

* Results are the mean ± S.D. of seven mice in each group.

† Male mice of the ddY strain (SPF, 28–30 g body wt) were used. Hg: mice were injected with mercuric chloride (20 μmoles/kg, i.p.) alone. Hg–Se: mice were injected with mercuric chloride and sodium selenite at the same time. Numbers in parentheses show doses of sodium selenite (5, 20 or 40 μmoles/kg, s.c., respectively).

‡ Significantly different from the group injected with mercuric chloride alone ($P < 0.01$).

Table 2. Selenium concentrations in liver and kidney after injection of sodium selenite with or without mercuric chloride*

	Selenium (μg/g wet tissue)									
	Liver					Kidney				
	Time after injection (hr)					Time after injection (hr)				
Treatment†	1	5	24	120	240	1	5	24	120	240
Control			1.13 ± 0.08					0.93 ± 0.02		
Se (5)	3.05 ± 0.25	1.84 ± 0.05	1.48 ± 0.06	1.22 ± 0.12	1.25 ± 0.12	1.88 ± 0.06	1.31 ± 0.05	1.19 ± 0.03	1.18 ± 0.05	1.18 ± 0.04
Se (20)	7.83 ± 0.53	4.68 ± 0.29	2.30 ± 0.09	1.45 ± 0.04	1.34 ± 0.03	3.51 ± 0.11	2.29 ± 0.10	1.46 ± 0.09	1.25 ± 0.06	1.18 ± 0.04
Se (40)	9.13 ± 0.69	6.71 ± 0.46	2.62 ± 0.20	1.62 ± 0.10	1.29 ± 0.08	6.15 ± 0.27	4.15 ± 0.19	1.68 ± 0.06	1.55 ± 0.05	1.27 ± 0.05
Hg–Se (5)	2.99 ± 0.12	3.80 ± 0.19‡	3.58 ± 0.14‡	2.49 ± 0.14‡	2.40 ± 0.15‡	2.77 ± 0.19‡	2.67 ± 0.12‡	2.62 ± 0.13‡	2.37 ± 0.11‡	1.87 ± 0.05‡
Hg–Se (20)	6.97 ± 0.25	8.33 ± 0.19‡	7.74 ± 0.26‡	7.49 ± 0.17‡	7.48 ± 0.34‡	4.82 ± 0.30‡	4.65 ± 0.21‡	3.08 ± 0.18‡	2.52 ± 0.08‡	2.01 ± 0.08‡
Hg–Se (40)	9.68 ± 0.49	8.96 ± 0.36‡	7.97 ± 0.40‡	7.59 ± 0.23‡	7.69 ± 0.30‡	7.07 ± 0.42‡	5.17 ± 0.27‡	3.45 ± 0.15‡	2.70 ± 0.12‡	2.13 ± 0.12‡

* Results are the mean ± S.D. of seven mice in each group.

† Control: 0.14 M NaCl alone. Se: mice were injected with sodium selenite alone. Numbers in parentheses show doses of sodium selenite (5, 20 or 40 μmoles/kg, s.c., respectively). Other treatments and abbreviations are the same as described in Table 1.

‡ Significantly different from the respective group injected with sodium selenite alone ($P < 0.01$).

In Hg mice, the concentrations of mercury in the kidney exceeded those in the liver and there was 12.6 times as much mercury in the kidney as that in the liver at 5 hr after administration. In Hg–Se mice, the concentrations of mercury in the kidney decreased with higher doses of selenium between 1 and 120 hr after administration; however, the concentration of mercury in the kidney increased at 240 hr after administration.

Concentrations of selenium in the liver and kidney. The concentrations of selenium in the liver and kidney of Se mice as well as Hg–Se mice are shown in Table 2. In Se mice, the concentrations of selenium in the liver peaked at 1 hr after administration. At 120 hr after administration, the concentrations were essentially the same regardless of the dose of selenium. In Hg–Se mice, the concentrations of selenium in the kidney over a period from 1 to 240 hr after administration were markedly higher than those of Se mice receiving a corresponding dose of selenium alone. As in the case of mercury incorporated into the liver after simultaneous administration of mercury and selenium, the concentrations of selenium in the liver of mice receiving mercury simultaneously with 20 and 40 μmoles/kg doses of selenium remained essentially unchanged without decrease over a period from 5 to 240 hr after administration.

In Se mice, the concentrations of selenium in the kidney showed peaks at 1 hr after administration, regardless of the dose of selenium; however, these peaks were lower than the corresponding selenium concentrations in the liver. The opposite occurred with mercury concentrations in Hg mice: peak concentrations in the kidney were higher than in the liver. On the other hand, in the Hg–Se mice the concentrations of selenium in the kidney were higher than the corresponding concentrations in mice receiving the same dose of selenium alone and tended to decrease more slowly than the selenium concentrations of Se mice.

Molar ratios of selenium to mercury in the liver and kidney. Table 3 presents molar ratios of Se/Hg based on concentrations of mercury and selenium in the liver and kidney of Hg–Se mice (Tables 1 and 2). When mercury and selenium were administered simultaneously, the Se/Hg ratios in the liver were about 1 in the case of doses of both 20 and 40 μmoles/kg of selenium at the hours between 24 and 240 after administration. The Se/Hg ratios in the kidney ranged from 0.54 to 0.68 at 240 hr after administration.

Subcellular distribution of mercury and selenium. Figure 1 shows subcellular distribution of mercury in the liver and kidney of Hg mice and Hg–Se mice. When mercury and selenium were administered at the same time, mercury was increasingly incorporated into the crude nuclear and mitochondrial fraction of the liver homogenate with time. Of the total mercury in the liver, about 91% was accumulated in this fraction at 120 hr after administration in mice receiving mercury and 20 or 40 μmoles/kg of selenium at the same time. In contrast, the proportions of mercury in the supernatant fraction of liver decreased when mercury and selenium were administered simultaneously. These results suggest

Table 3. Molar ratios of selenium to mercury in liver and kidney after simultaneous injection of mercuric chloride and sodium selenite*

	Molar ratios of selenium to mercury									
	Liver					Kidney				
	Time after injection (hr)					Time after injection (hr)				
Treatment	1	5	24	120	240	1	5	24	120	240
Hg–Se (5)	1.42	0.98	0.56	0.74	0.89	0.09	0.11	0.15	0.27	0.68
Hg–Se (20)	2.35	1.05	0.90	0.80	0.93	1.90	0.96	0.39	0.35	0.54
Hg–Se (40)	2.41	1.48	1.07	0.92	1.02	3.03	1.72	0.75	0.71	0.68

* Treatments and abbreviations are the same as described in Tables 1 and 2. Molar ratios of selenium to mercury were calculated on the value of mercury or selenium shown in Tables 1 and 2 respectively.

that selenium profoundly affected the subcellular distribution of mercury.

The subcellular distribution of mercury in the kidney of Hg–Se mice was similar to that in the liver over a period from 24 to 120 hr after administration, but the proportions hardly showed any change with time. Figure 2 represents the subcellular distribution of selenium. In Hg–Se mice, the proportions of selenium incorporated into the crude nuclear and mitochondrial fraction were increased markedly compared with the proportions in Se mice and, regardless of the dose of selenium, these proportions ranged

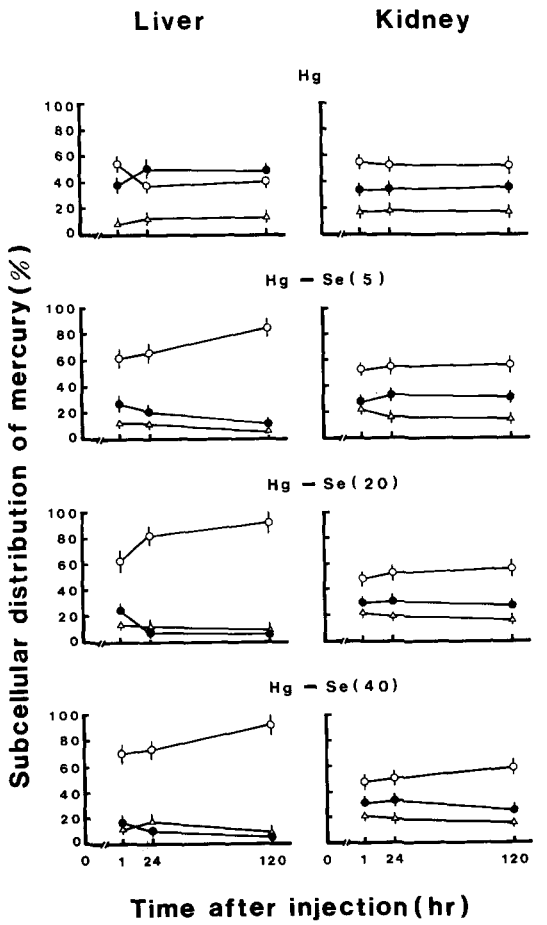

Fig. 1. Subcellular distribution of mercury in the liver and kidney at various times after injection of mercuric chloride with or without sodium selenite. The treatments and abbreviations are the same as described in Tables 1 and 2. Key: (○——○) crude nuclear and mitochondrial, 10,000 *g*; (△——△) microsomal, 105,000 *g*; and (●——●) supernatant. Each point represents the mean ± S.D. of seven mice in each group.

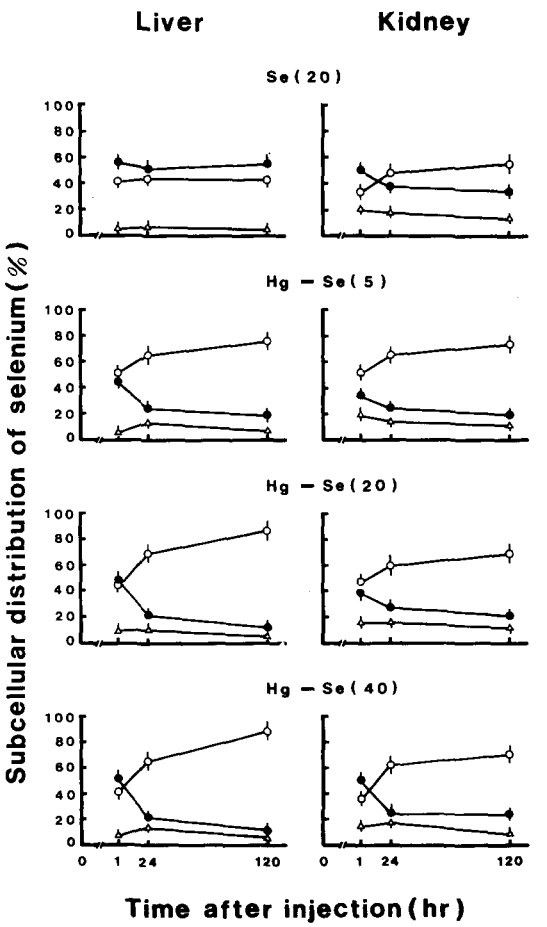

Fig. 2. Subcellular distribution of selenium in the liver and kidney at various times after injection of sodium selenite with or without mercuric chloride. The treatments and abbreviations are the same as described in Tables 1 and 2. Key: (○——○) crude nuclear and mitochondrial, 10,000 *g*; (△——△) microsomal, 105,000 *g*; (●——●) supernatant. Each point represents the mean ± S.D. of seven mice in each group.

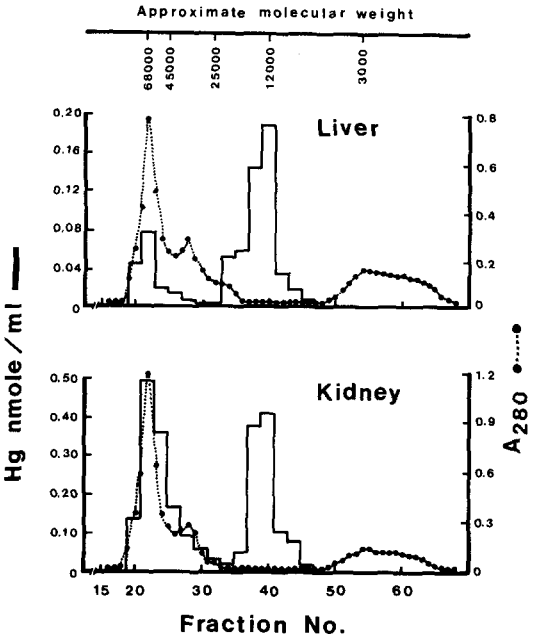

Fig. 3. Elution patterns of the liver and kidney supernatant fraction at 24 hr after injection of mercuric chloride (20 μmoles/kg, i.p.) on Sephadex G-75. Experimental conditions are described in Materials and Methods.

from 78 to 88% of the total selenium in the kidney at 120 hr after administration. The molar ratios of Se/Hg in the crude nuclear and mitochondrial fractions of the liver removed from mice at 120 hr after simultaneous administration of mercury and selenium were calculated from the results given in Tables 1 and 2 and Figs. 1 and 2. Respectively, these molar ratios were 0.71, 0.82 and 0.90 when 5, 20 and 40 μmoles/kg selenium were administered with mercury.

Gel filtration of supernatant fraction on Sephadex G-75 column. Figure 3 illustrates patterns of gel filtration, approximate molecular weight and u.v. absorption on supernatant fraction of liver and kidney of Hg mice at 24 hr after administration. The u.v. absorption of the eluate peaked at fractions 21–22, 28–29, and 54–55. Mercury was mainly associated with fractions 21–22 eluted at void volume and containing high-molecular-weight proteins and with fractions 40–41 containing proteins of molecular weight lower than those eluted in the preceding fractions.

Metallothionein consisting of thionein, approximate molecular weight of 10,000 obtained by gel filtration, and of mercury has been demonstrated in the supernatant fractions of the liver and kidney of rats receiving mercuric chloride [9, 10]. As fractions 40–41 of gel filtration in the present study correspond to proteins of approximate molecular weight 12,000 and were eluted at 2.02 of V_e/V_o, they were regarded as the metallothionein-like protein fraction described by Norheim and Steinnes [13].

Figure 4 shows incorporation of mercury into fractions from Sephadex G-75 gel filtration of liver supernatant of Hg mice as well as Hg–Se mice. When mice were treated with mercury alone, mercury was incorporated into fractions of high-molecular-weight proteins eluted at void volume and into metallothionein fractions. Mercury was demonstrated in the metallothionein fractions as early as 1 hr after administration, and the concentrations became highest at 24 hr. In Hg–Se mice, the amount of mercury incorporated into metallothionein fractions markedly decreased depending on the dose of selenium. Figure 5 shows mercury incorporation into fractions from gel filtration of kidney supernatant. The gel filtration patterns for kidney supernatant are similar to those for liver supernatant. Elution of u.v. absorption at 280 nm from the Sephadex G-75 column is not given in Figs. 4 and 5 but is essentially identical to those given in Fig. 3. The fractions of mercury incorporated into metallothionein are given in Fig. 6. In Hg–Se mice, mercury in metallothionein fraction decreased depending on the dose of selenium. The decrease became especially significant at 24 hr after administration. This suggests that the interaction of selenium with mercury may affect the proportion of mercury bound to metallothionein fraction.

DISCUSSION

The accumulation of mercury in the liver and kidney after administration of mercury alone was markedly different from that after simultaneous administration of mercury and selenium (Tables 1 and 2). The concentrations of mercury in the liver of Hg–Se mice were higher than those of Hg mice over the whole experimental period. The concentrations of mercury in the kidney of Hg–Se mice were lower than those of Hg mice, and the difference became more significant over a period from 1 to 120 hr after administration, depending on the dose of selenium. Accordingly, selenium affects the accumulation of mercury in the liver and kidney differently. The incorporation of selenium and mercury into the liver was similar, but their incorporation into the kidney was apparently different.

Moffit and Clary [17] demonstrated that the simultaneous administration of mercuric chloride and selenium elevates concentrations of mercury in the blood and liver and stated that the direct binding between mercury and selenium could reduce the toxicity of mercury. On the other hand, Burk *et al.* [18] speculated that protein-S-Se-Hg^+ is formed in the blood through the binding of selenium to SH-group in protein and followed by binding of mercury to the selenium. This was based on the results of an experiment in which $^{203}HgCl_2$ and $Na_2{}^{75}SeO_3$ were administered simultaneously to rats. Gasiewicz and Smith [19] and Komiya *et al.* [20], however, stated that mercuric chloride and selenium may exist in the blood as colloidal Hg–Se resulting from binding of mercury and selenium to protein. Komiya *et al.* [20] further reported that such reaction products as mercury and selenium are gradually transferred from the blood to the liver, kidneys, spleen and elsewhere, and accumulate in such organs. In the present study, in Hg–Se mice the molar ratio of selenium to mercury incorporated into the liver was approximately 1 in the cases at 24 and 240 hr after administrations of 20 and 40 μmoles/kg of selenium. This suggests a 1:1 binding between mercury and selenium. Although

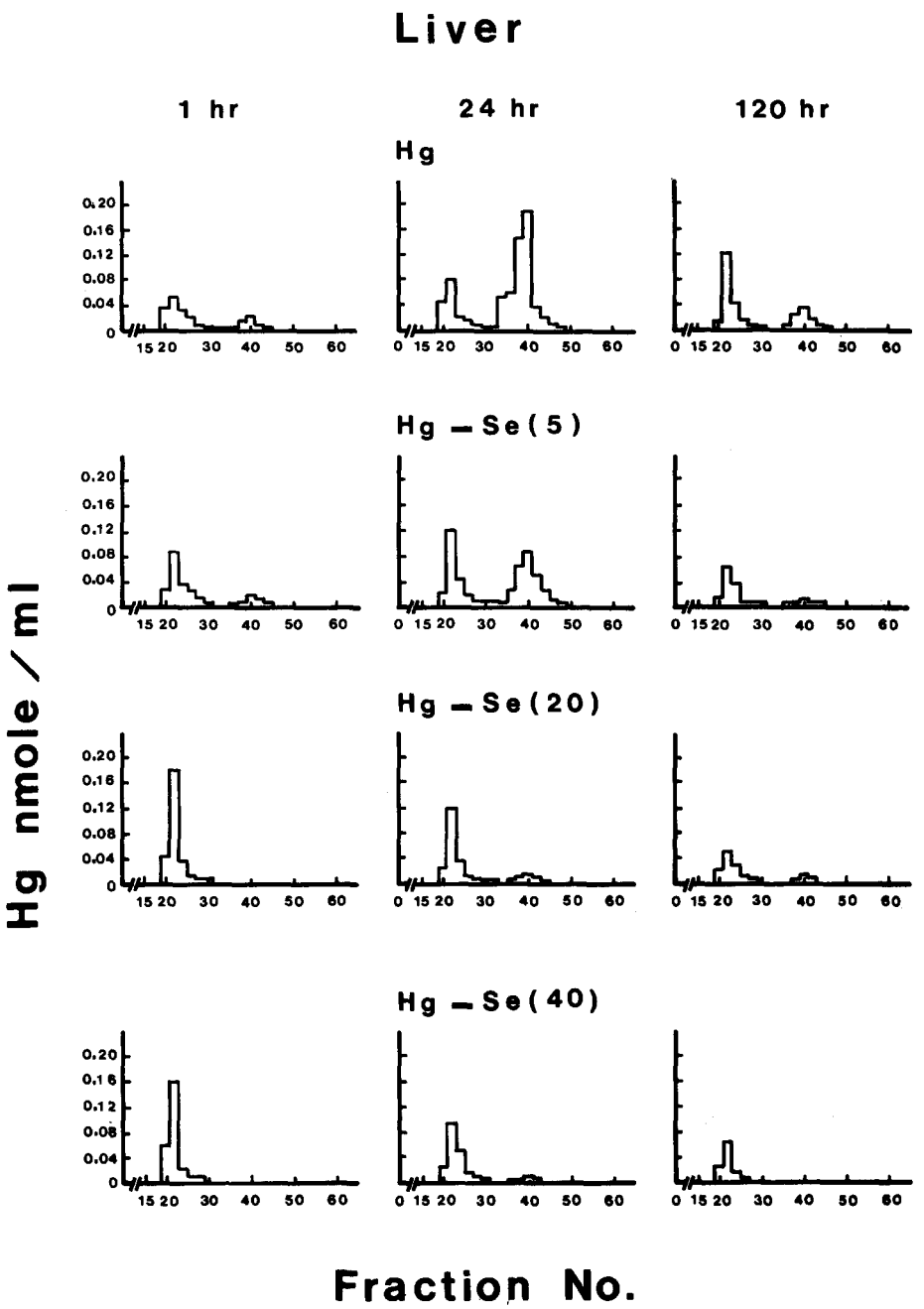

Fig. 4. Elution patterns of liver supernatant fraction on Sephadex G-75 at various times after injection of mercuric chloride with or without sodium selenite. The treatments and abbreviations are the same as described in Tables 1 and 2.

the bond form of mercury and selenium in the liver is not yet clarified, the compound consisting of mercury and selenium might be transferred from blood to the liver and accumulated there. On the other hand, we speculated that the product of mercury and selenium binding does not accumulate in the kidney. Consequently there is a decrease in the accumulation of mercury in the kidney of Hg–Se mice from 1 to 120 hr after administration of mercury and selenium at the same time.

When mercury and selenium were administered simultaneously to mice, the subcellular distributions of mercury and selenium were virtually identical in the liver (Figs. 1 and 2). In the liver, the proportions of mercury and selenium in the crude nuclear and mitochondrial fraction were greatly increased with time, while those in the supernatant fraction were decreased. In addition, molar ratios of selenium to mercury (Se/Hg) in the crude nuclear and mitochondrial fraction ranged from 0.71 to 0.90 at 120 hr

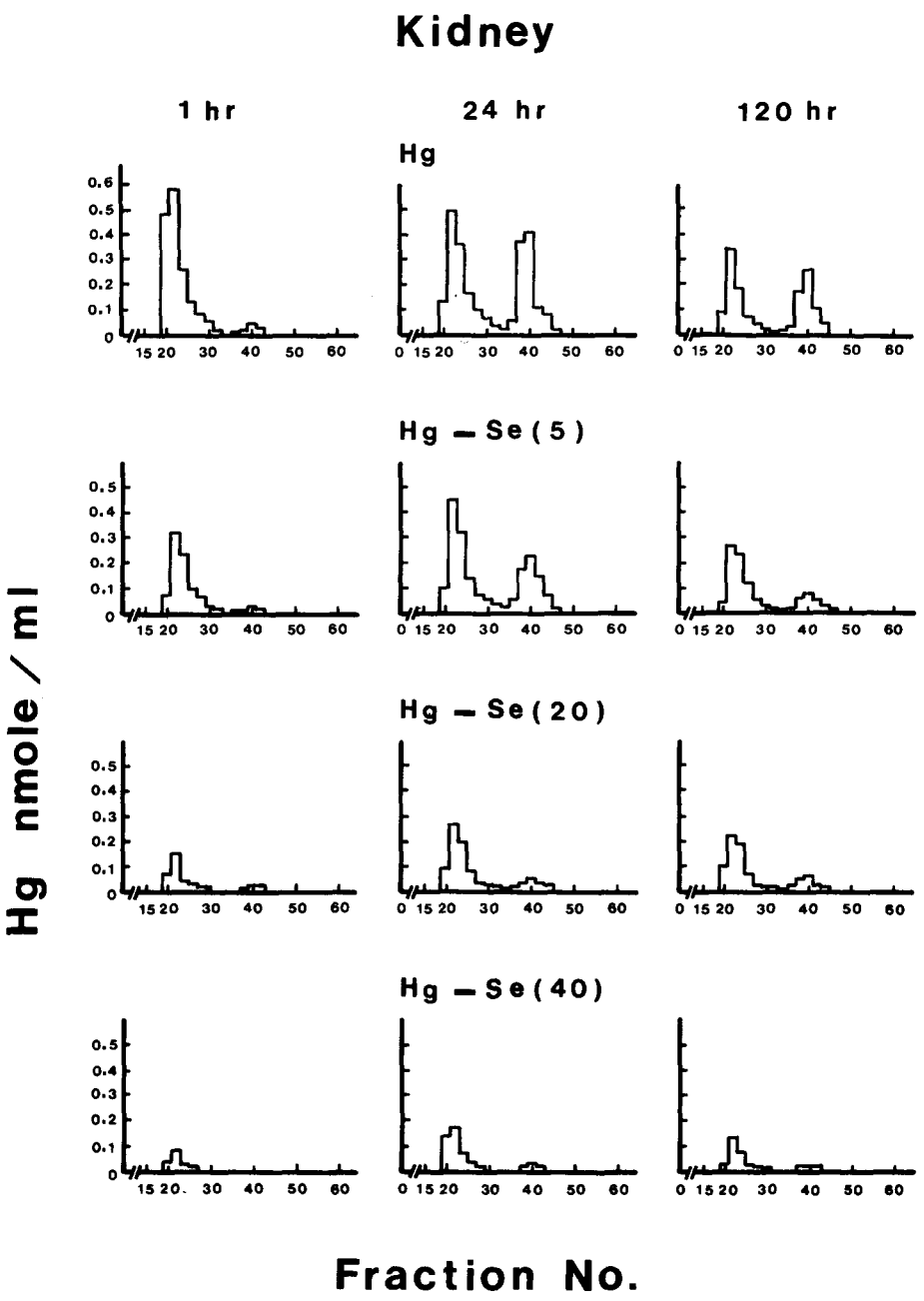

Fig. 5. Elution patterns of kidney supernatant fraction on Sephadex G-75 at various times after injection of mercuric chloride with or without sodium selenite. The treatments and abbreviations are the same as described in Tables 1 and 2.

after administration. These results suggest that, when mercury and selenium are administered simultaneously, mercury bound to selenium increasingly accumulates in the crude nuclear and mitochondrial fraction of liver. In the kidney of Hg–Se mice, however, changes in the crude nuclear and mitochondrial fraction of mercury with time were less remarkable than in the liver. Accordingly, it may be that the subcellular distribution behaviour of mercury in the kidney is different from that in the liver when mercury and selenium are administered at the same time.

It seems that metallothionein can prevent toxic effects of mercury through synthesis of mercurythionein in the body treated with mercuric chloride. In the present study, of the total mercury in supernatant fractions of liver and kidney, 65 and 47%, respectively, were incorporated into metallothionein fractions in Hg mice at 24 hr after administration (Fig. 6). In contrast, in Hg–Se mice, the proportions of mercury incorporated into metallothionein fractions of liver and kidney decreased with increasing doses of selenium. On the other hand, it has been pointed out that the patterns of gel filtration of the

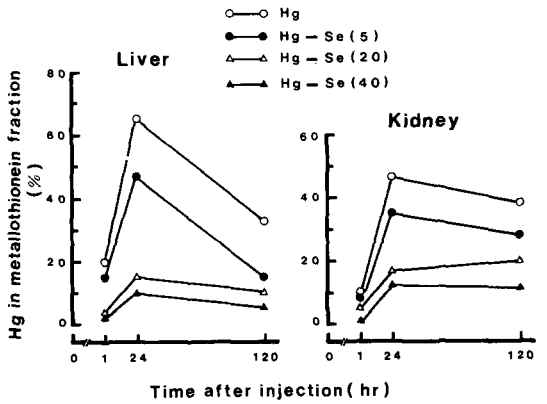

Fig. 6. Ratios of mercury in metallothionein-fraction (No. 36–45) on Sephadex G-75 to total mercury content of the liver and kidney supernatant fractions shown in Figs. 5 and 6. The treatments and abbreviations are the same as described in Tables 1 and 2.

liver supernatant in rats after simultaneous administration of mercuric chloride and sodium selenite are apparently different from those found when each element is given separately [21]. According to Chmielnicka and Brzeznicka [22], the concentration of thionein in kidney homogenate of rats treated simultaneously with mercuric chloride and selenium is apparently lower than that of rats treated with mercuric chloride alone. In mice, mercury that was administered simultaneously with selenium may exist by binding to selenium mainly in the crude nuclear and mitochondrial fraction of liver. The present study confirmed that the simultaneous administration of mercury and selenium reduced the percentage of mercury in metallothionein fraction by gel filtration of supernatant. Furthermore, increasing doses of selenium resulted in lower percentages of mercury. Therefore, it appears that synthesis of mercury-thionein is suppressed by a decrease in the concentration of Hg^{2+} resulting from binding to selenium.

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