Pharmacology Biochemistry & Behavior, Vol. 15, pp. 449–454, 1981, Printed in the U.S.A.

Properties of Mercury and Selenium in a High-Molecular Weight Substance in Rabbit Tissues Formed by Simultaneous Administration

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Received 19 February 1981

NAGANUMA, A AND N. IMURA. *Properties of mercury and selenium in a high-molecular weight substance in rabbit tissues formed by simultaneous administration.* PHARMAC. BIOCHEM. BEHAV. 15(3) 449–454, 1981.—The properties of mercury and selenium existing in high-molecular weight substance(s) (HMWS) obtained from plasma, stroma-free hemolysate, soluble and insoluble fractions of the liver of rabbits dosed with mercuric chloride and selenite were studied. Analytical procedures—gel filtration, ion exchange chromatography, and sucrose density gradient ultracentrifugation—showed that equimolar amounts of mercury and selenium were present in HMWS in the blood and the liver. Dialysis against mercaptoethanol suggested that selenium and mercury were tightly bound to HMWS in the liver soluble fraction. The formation of the HMWS may contribute to reducing the toxicity of both mercury and selenium. Since mercury and selenium in HMWS in the blood and the liver were present in smaller fragments after trypsin digestion, these HMWS appeared to be protein associated with mercury and selenium. However, after exhaustive digestion with Pronase, mercury and selenium in HMWS were rendered insoluble. The above process appears to take place in the body of rabbits after simultaneous administration of mercury and selenium, because the livers excised from these rabbits were found to contain iso-molar and sodium dodecyl sulfate-insoluble selenium and mercury which increased in amount with time.

Mercuric mercury Selenium Rabbit Plasma Stroma-free hemolysate Liver
Mercury-selenium complex Proteinase digestion

SINCE Parizek and Ostadalova [21] reported that selenite dramatically decreased acute renal toxicity of mercuric mercury in rats, many studies on the mutual modification of the toxicities in animals between inorganic mercury and selenium have been reported, demonstrating that a certain mercury-selenium interaction occurred in animals.

The primary interaction of mercuric mercury and selenite in animals may take place in the blood [17, 18, 19]. When mercuric mercury and selenite co-existed in the blood, the uptake of mercury and selenium by erythrocytes was markedly increased [7, 9, 17, 19], and most of these elements in the plasma [1, 3, 12, 19] or erythrocytes [9, 17, 19] were associated with high-molecular weight substance(s) (HMWS). These interactions in the blood decreased mercury transport to the kidney at an early stage of administration, probably because (1) mercury and selenium were retained in the erythrocytes in HMWS for a long period at high concentrations [9, 16, 19], and (2) HMWS in the plasma could not pass through the glomeruli to accumulate in the tubular cells [15,19]. It was thus possible to considerably depress the acute renal toxicity of mercuric mercury.

On the other hand, marked increase of mercury accumulation by simultaneous administration of selenite has been observed in the liver and the spleen [4, 5, 7, 14, 16, 19]. When mercuric mercury and selenium compound were chronically administered to rats, markedly high levels of mercury and selenium accumulated in the liver, while little accumulation was observed in other rats which received only one of these, but the histopathological lesions usually caused by administration of mercuric mercury alone were hardly observed in the case of simultaneous administration [2,6]. We have recently reported that most of the mercury and selenium in the soluble [19] and insoluble [20] fractions of rabbit liver co-existed in HMWS 24 hr after a single simultaneous administration of mercuric mercury and selenite. The existing state of mercury and selenium in the HMWS ac-

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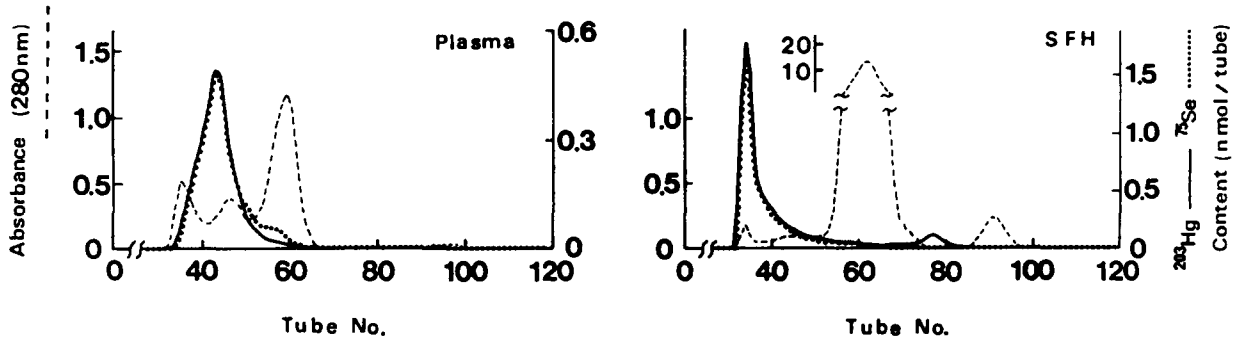

FIG. 1. Sephadex G-200 chromatogram of rabbit plasma and stroma-free hemolysate (SFH) 24 hr after IV injection of $^{203}HgCl_2$ and $Na_2\ ^{75}SeO_3$.

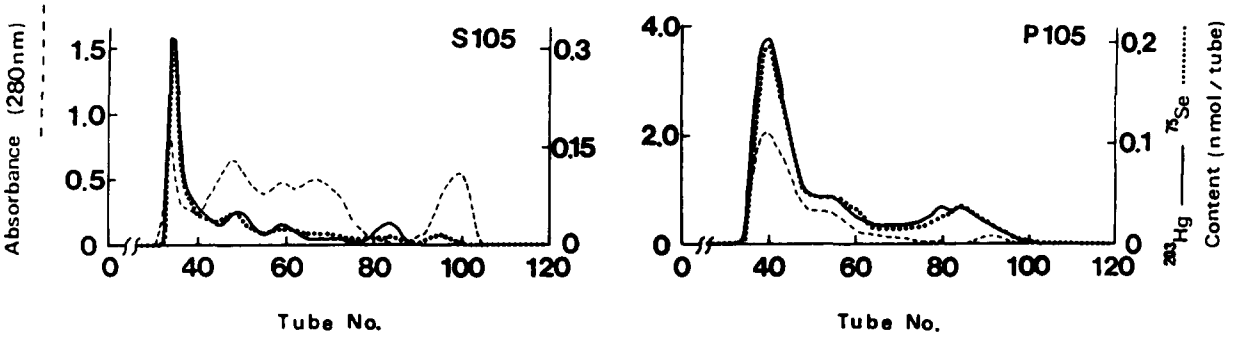

FIG. 2. Sephadex G-200 chromatogram of soluble (S105) and insoluble (P105) fractions of rabbit liver after IV injection of $^{203}HgCl_2$ and $Na_2\ ^{75}SeO_3$.

cumulated in the liver is expected to influence the organ retention and ultimate reduction of toxicities of both elements.

Thus, the present study was conducted in order to further clarify the properties of mercury and selenium in HMWS found in the rabbit liver and blood.

METHODS

Male rabbits weighing about 1 kg and fed solid feed (CR-1 type available from Nippon CLEA Co. Ltd., Tokyo) and water ad lib were used in the experiment. (^{203}Hg)-Mercuric chloride and (^{75}Se)-sodium selenite were purchased from Radiochemical Centre, Amersham.

(^{203}Hg)-Mercuric chloride (200 μCi/1.5 μmol/kg) and (^{75}Se)-sodium selenite (100 μCi/1.5 μmol/kg) were injected into the auricular veins of opposite ears of the rabbits at the same time. Twenty-four hr after administration, the blood and perfused liver were obtained from the rabbit by the method described previously [19]. The blood sample was separated into plasma and stroma-free hemolysate [9], and the liver was homogenized and separated by centrifugation at 4°C for 1 hr at 105,000 × G into soluble and insoluble fractions [19]. The insoluble fraction was solubilized with 2% sodium dodecyl sulfate (SDS) as reported previously [20].

Sephadex G-200 Chromatography

One and a half milliliters of plasma, 5.0 ml of stroma-free hemolysate, soluble fraction or insoluble fraction of liver was applied on a Sephadex G-200 column (26.4×920 mm). The column was eluted with 50 mM Tris-HCl buffer (pH 7.6), at a flow rate of about 28 ml/hr, and 5.0 ml of the eluate was pooled in a tube.

Ion Exchange Chromatography

HMWS fraction was applied to a column (10×150 mm) of DEAE-cellulose (DE52, Whatman, Inc.) equilibrated with 50 mM Tris-HCl buffer (pH 7.6). The column was washed with 100 ml of 50 mM Tris-HCl buffer with or without 0.1 M NaCl and then eluted with a linear gradient of 0–0.3 M or 0.1–0.5 M NaCl solution, respectively, in 50 mM Tris-HCl buffer at a flow rate of 30 ml/hr.

Sucrose Density Gradient Ultracentrifugation

HMWS fraction was concentrated by covering the dialysis tube with polyethylene glycol 20,000 [8] and sedimented through 50 mM Tris-HCl buffer (pH 7.6) containing 0.1% SDS with 5–30% sucrose density gradient at 24°C for 4 hr at 40,000 rpm in Spinco SW41 rotor.

SDS-Polyacrylamide Gel Electrophoresis

HMWS fraction concentrated by polyethylene glycol 20,000 was dialysed against a 100-fold volume of 62.5 mM Tris-HCl buffer (pH 6.8) at 4°C for 12 hr. The dialysed sample (40 μl) was added to 40 μl of 62.5 mM Tris-HCl buffer (pH 6.8) containing 4% SDS and 20% glycerol. The proteins in the sample were dissociated by immersing the samples for 1.5 min in boiling water. Electrophoresis was carried out on 5% polyacrylamide gel according to the method of Laemmli

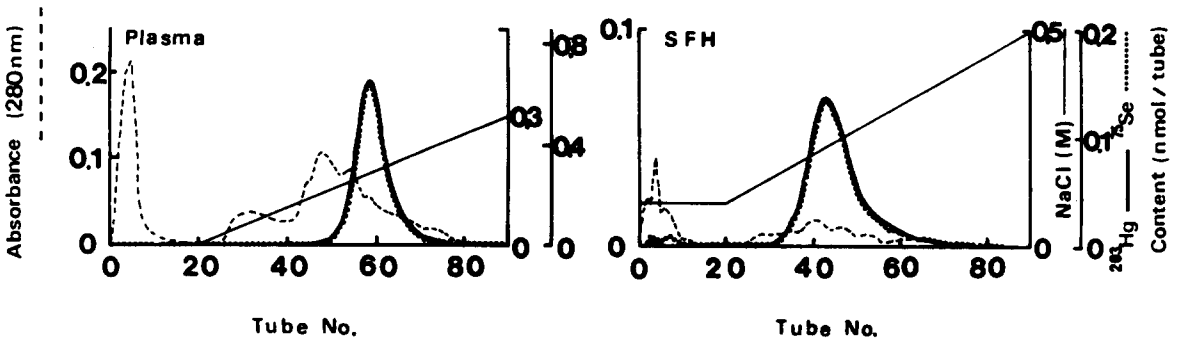

FIG. 3. DEAE-cellulose chromatogram of high-molecular weight substance containing mercury and selenium obtained from plasma and stroma-free hemolysate (SFH) of rabbits administered $^{203}HgCl_2$ and Na_2 $^{75}SeO_3$. The high-molecular weight substance was separated by gel filtration.

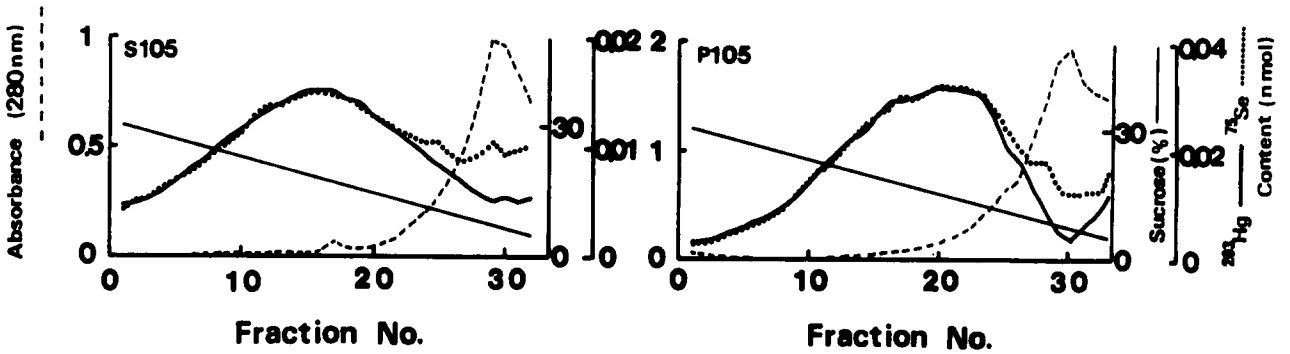

FIG. 4. Sucrose density gradient ultracentrifugation of a high-molecular weight substance containing mercury and selenium obtained from soluble (S105) and insoluble (P105) fractions of the liver of rabbits administered $^{203}HgCl_2$ and Na_2 $^{75}SeO_3$. The high-molecular weight substance was separated by gel filtration.

[13] in the absence of 2-mercaptoethanol. After electrophoresis, the gel was dried on filter paper and ^{203}Hg and ^{75}Se were visualized by autoradiography.

Evaluation of Affinity

The liver-soluble fractions of rabbit administered with (^{203}Hg)-mercuric chloride and/or (^{75}Se)-sodium selenite were dialysed against a 400-fold volume of 9.5 mM phosphate buffered saline (PBS, pH 7.4) for 12 hr and subsequently dialysed for 4 hr against a 100-fold volume of PBS containing various concentrations of 2-mercaptoethanol. After dialysis, the residual radioactivities of ^{203}Hg and ^{75}Se in a dialysis tube were measured.

Pronase or Trypsin Digestion

Pooled HMWS fraction obtained by gel filtration was concentrated by polyethylene glycol 20,000 and incubated with Pronase or trypsin at an enzyme-to-substrate ratio of 1:6.7 (w/w) for 12 hr at 37°C.

Determination of Radioactivities

The radioactivities of ^{203}Hg and ^{75}Se were calculated from the values measured at 0.28 MeV and 0.40 MeV with Aloka Auto well gamma system.

RESULTS

Most of the ^{203}Hg and ^{75}Se accumulating in the plasma, stroma-free hemolysate, soluble or insoluble fraction of the liver of rabbits injected with (^{203}Hg)-mercuric chloride and (^{75}Se)- sodium selenite were found in HMWS at a molar ratio of about 1:1 after gel filtration on Sephadex G-200 (Figs. 1 and 2).

By rechromatography on DEAE-cellulose, ^{203}Hg and ^{75}Se existing in the HMWS obtained from the plasma or stroma-free hemolysate were also eluted as a single peak with a molar ratio of about 1:1 (Fig. 3).

However, ^{203}Hg and ^{75}Se in the HMWS of the liver soluble fraction or insoluble fraction could not be eluted from the DEAE-cellulose column even when the molarity of NaCl was increased to 3 M. When the HMWS of the soluble or insoluble fraction of the liver was centrifuged on a sucrose density gradient, most of the ^{203}Hg and ^{75}Se sedimented in equal amounts and showed broad peaks (Fig. 4).

In order to evaluate the affinity of mercury and selenium for the HMWS, the soluble fraction of the liver prepared from rabbits dosed with (^{203}Hg)-mercuric chloride and/or (^{75}Se)- sodium selenite was dialysed against solutions of 2-mercaptoethanol of several concentrations. The rate of release to the outer solution of ^{203}Hg and ^{75}Se from the soluble fraction of liver of rabbits receiving both (^{203}Hg)- mercuric chloride and (^{75}Se)- sodium selenite was much less than that of rabbits which received only one of the compounds (Fig. 5).

Figure 6 shows the effects of trypsin digestion of the HMWS on the electrophoretogram on 5% polyacrylamide gel. Without trypsin treatment ^{203}Hg and ^{75}Se in the HMWS

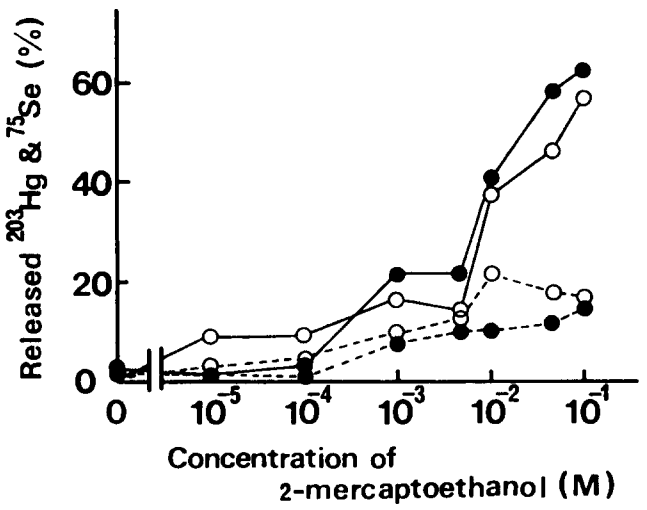

FIG. 5. Release of mercury and selenium from the soluble fraction of rabbit liver by dialysis against 2-mercaptoethanol solution. Liver soluble fractions were obtained from rabbits 24 hr after IV injection of $^{203}HgCl_2$ (●–●), $Na_2{}^{75}SeO_3$ (○–○), or $^{203}HgCl_2$ and $Na_2{}^{75}SeO_3$ simultaneously (●– –●,○– –○).

from the plasma were separated to two or more bands which contained both elements, but either ^{203}Hg or ^{75}Se in HMWS of the liver and stroma-free hemolysate did not move into the polyacrylamide gel on electrophoresis probably due to the size of the HMWS (Fig. 6A). After trypsin digestion, ^{203}Hg and ^{75}Se were found in the digested fragments which had been separated by electrophoresis (Fig. 6B). After Pronase digestion, however, most of ^{203}Hg and ^{75}Se in HMWS of the plasma, stroma-free hemolysate, and the liver were not moved into the gel at all (data not shown).

Table 1 shows the behavior of ^{203}Hg and ^{75}Se in HMWS on centrifugation after trypsin or Pronase digestion. The ^{203}Hg and ^{75}Se in HMWS were mostly precipitated by centrifugation at 800 × G after Pronase digestion but remained in the supernatant after trypsin digestion. As shown in Table 2, SDS-insoluble mercury and selenium were gradually formed in the liver of rabbits administered both compounds. Under the experimental conditions used, however, no SDS-insoluble mercury or selenium was detected in rabbit kidney (data not shown).

DISCUSSION

It has been observed that equimolar amounts of mercury and selenium co-existed in HMWS in the plasma after con-

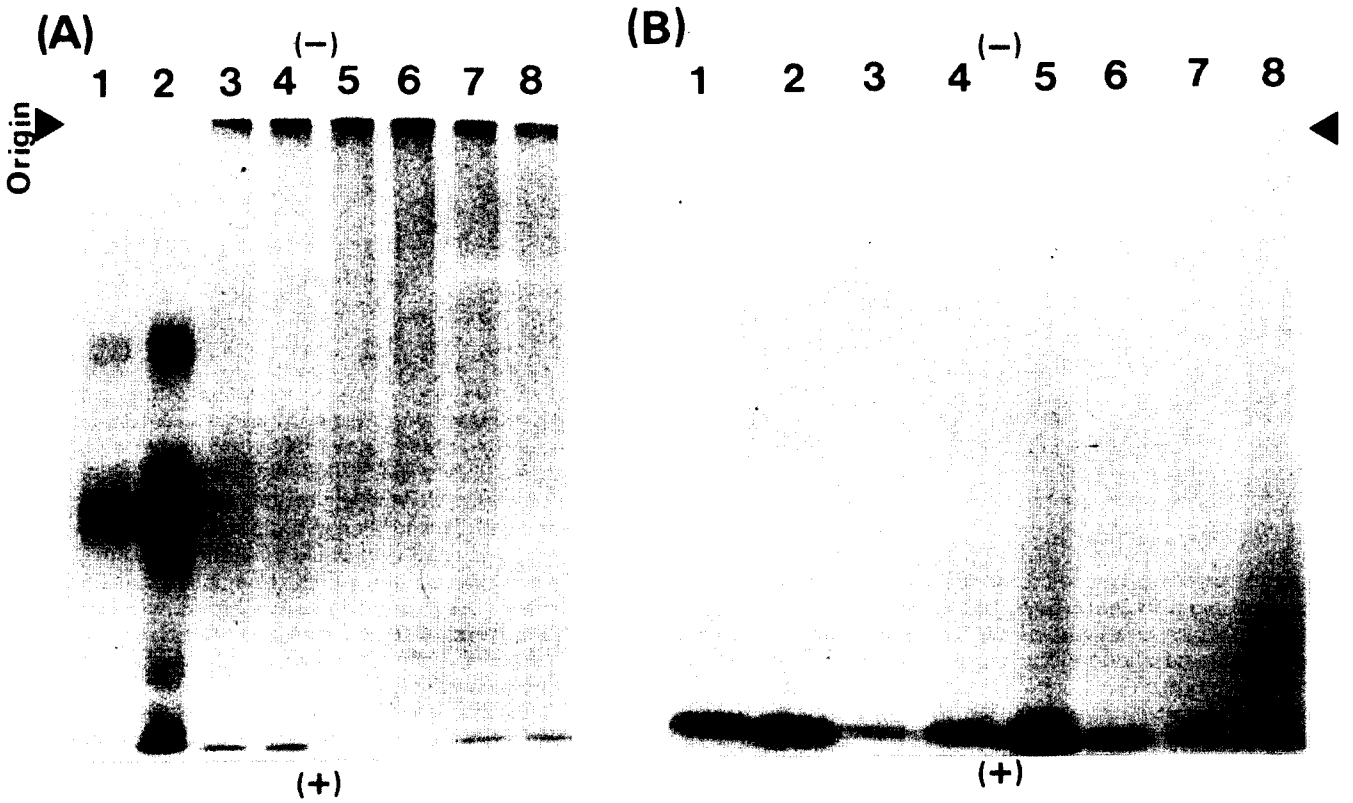

FIG. 6. SDS-polyacrylamide gel electophoresis of the mercury and selenium contained in high-molecular weight substances obtained from the blood and liver of the rabbit administered $^{203}HgCl_2$ with Na_2SeO_3, or $HgCl_2$ with $Na_2{}^{75}SeO_3$. ^{203}Hg-labelled (1, 3, 5, 7) or ^{75}Se-labelled (2, 4, 6, 8) high-molecular weight substances were separated by gel filtration from plasma (1, 2), stroma-free hemolysate (3, 4), liver soluble fraction (5, 6), or liver insoluble fraction (7, 8). These high-molecular weight substances were treated with (B) or without (A) trypsin.

TABLE 1

BEHAVIORS OF MERCURY AND SELENIUM ASSOCIATED TO HIGH-MOLECULAR WEIGHT SUBSTANCE ON TREATMENT WITH TRYPSIN OR PRONASE*

	Sources of high-molecular weight substance							
	Plasma		SFH†		Liver (S105)‡		Liver (P105)‡	
	Hg	Se	Hg	Se	Hg	Se	Hg	Se
—	1.8	3.3	8.7	7.9	1.5	2.0	2.2	2.5
Trypsin	3.6	4.5	9.5	9.7	5.0	5.6	2.4	1.7
Pronase	91.6	86.2	99.9	98.2	90.2	84.2	95.3	89.0

*Precipitated Hg and Se (%).
†Stroma-free hemolysate.
‡Soluble (S105) or insoluble (P105) fraction of liver.

TABLE 2

FORMATION OF SDS-INSOLUBLE COMPOUND CONTAINING MERCURY AND SELENIUM IN RABBIT LIVER AFTER IV INJECTION OF MERCURIC CHLORIDE AND SELENITE*

Fraction		Time after injection					
		1 hr		24 hr		4 days	
		Hg	Se	Hg	Se	Hg	Se
Homogenate		7.21	8.63	10.89	10.39	17.10	16.42
Soluble†		2.72	4.91	3.86	3.49	4.38	4.29
Insoluble†	SDS-soluble‡	4.40	3.62	6.75	6.60	9.28	8.90
	SDS-insoluble‡	0.09	0.10	0.28	0.30	3.44	3.23

*Values in the table were nmol Hg or Se/g liver.
†Separated from liver homogenate by centrifugation at 105,000 × G for 1 hr.
‡Separated from insoluble fraction of liver homogenate by centrifugation at 105,000 × G for 1 hr after solubilization with 2% SDS.

current administration of mercuric chloride and selenite to the rat [1,12]. Mercury and selenium in the erythrocytes and liver of rabbits injected inorganic mercury and selenite were found mainly in HMWS obtained by gel filtration at a molar ratio of 1:1 as in the case of plasma (Figs. 1 and 2). The behavior of both elements in these HMWS was almost identical regardless of the analytical procedure (Figs. 1, 2, 3, 4 and 6). These results suggest that an equimolar amount of mercury and selenium may exist in a complex form associated to the HMWS. However, properties of the HMWS obtained from the plasma, stroma-free hemolysate and liver appeared to be different.

On simultaneous administration of inorganic mercury and selenium to the rabbit, mercury and selenium in the soluble fraction of the liver were found mainly in the HMWS (Fig. 2) and were hardly released from the HMWS in dialysis against buffer containing 2-mercaptoethanol (Fig. 5). Contrary to this, the mercury or selenium present in the soluble fraction after administration of either mercuric mercury or selenite was readily dialysable (Fig. 5). These observations suggest that mercury and selenium were firmly bound to the HMWS when they were simultaneously administered. This will presumably make it difficult for these elements to move into other physiologically important milieu such as enzyme systems to inhibit their functions. We believe this is likely to be at least one of the detoxification mechanisms in effect when mercury and selenium are taken simultaneously.

Two different hypotheses regarding the state of mercury and selenium in HMWS have been presented based on studies in rat plasma. One is that selenium is attached to a sulfhydryl group of the high-molecular weight protein and mercury is attached to this selenium [1]. Another is that the HMWS is constituted by a high-molecular weight complex of colloidal mercuric selenide [12]. However, the second assumption is inconsistent with our finding that most of mercury and selenium in the HMWS in the blood and the liver of the rabbit were found in the smaller fragments after the digestion with trypsin (Fig. 6B). It may be also reasonable to assume that these HMWS are protein associated with mercury and selenium. However no experimental evidence was obtained in the present study which could corroborate the first hypothesis.

Pronase treatment of the HMWS, on the other hand, readily generates an insoluble compound, probably mercuric selenide (Table 1). Thus, mercury and selenium seem to remain as a soluble complex by the aid of the relatively large

peptides after trypsin treatment while rendered into an insoluble complex when the peptides are extensively digested with Pronase.

We demonstrated that formation of insoluble mercury and selenium can also take place in the rabbit liver when mercuric mercury and selenite are simultaneously administered (Table 2). This result suggests *in vivo* conversion of mercury and selenium in the HMWS to an insoluble form in the liver through such a proteolytic process as observed in the *in vitro* experiment described above. The insoluble mercury and selenium thus formed would not be readily diffusable but would rather accumulate in the tissues as an inert complex. In fact, high levels of mercury and selenium have been observed in the tissues of some animals exposed to mercury for a long period [10, 11, 22, 23]. On microscopic examination, Groth *et al.* [6] observed black particles containing equimolar amounts of mercury and selenium in the liver and the kidney of rats receiving mercuric mercury and selenium compound every day for 16–20 months. We previously reported, however, that 24 hr after simultaneous administration of mercuric mercury and selenite such a coexistence of mercury and selenium in HMWS of the rabbit kidney was not observed as it was in the liver [19,20]. The accumulation of the particles consisting of insoluble mercury and selenium in the kidney may occur in animals after chronic administration of mercury and selenium compound, but this obviously needs to be clarified by further study.

ACKNOWLEDGEMENT

The authors wish to thank Dr. G. Ohi for his helpful suggestions in the preparation of this manuscript.

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