CHANGES IN \textit{IN VITRO} INTERACTION PROFILES OF MERCURIC MERCURY AND SELENIUM IN RABBIT BLOOD UNDER VARIOUS REACTION CONDITIONS

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\textit{In vitro} interaction profiles of mercuric mercury and selenite in rabbit blood under various reaction conditions were investigated. The most remarkable changes in the incorporations into the erythrocytes and gel filtration profiles of mercury and selenium in blood were observed when $10^{-5}$M mercuric chloride and $10^{-5}$M selenite were added to rabbit blood. Under this condition, the mercury and selenium added always behaved with each other on gel filtration suggesting that most of mercury and selenium existed in a complex at a molar ratio of 1. When the concentration of mercuric chloride was $10^{-7}$M in blood, however, the incorporation of mercury into erythrocytes was also increased by the simultaneous addition of selenite, but $10^{-7}$M mercury did not stimulate the selenium incorporation. The behaviors of mercury and selenium in blood were altered by the order of addition of mercuric chloride and selenite to the blood. Compared to the simultaneous addition of both compounds, the final amount of mercury and selenium incorporated into erythrocytes were reduced by the addition of mercuric chloride prior to selenite, whereas the rates of incorporation of mercury and selenium were lowered by the addition of selenite prior to mercury. The gel filtration patterns of mercury and selenium in the plasma and stroma-free hemolysate of the blood preincubated with selenite before the addition of mercury were different from the case of simultaneous addition of both compounds or addition of mercury prior to selenite. The variety of interaction profiles of mercuric mercury and selenium in blood under different reaction conditions as observed in the present \textit{in vitro} study may reflect the complex modes of interaction actually occurs \textit{in vivo}.

\textbf{Keywords} — mercury; selenium; interaction; blood; distribution; gel filtration

INTRODUCTION

Selenium, a biologically essential trace element,\textsuperscript{1} is known to have ability to modify the toxicity of mercury compounds.\textsuperscript{2–5} Selenite causes rapid incorporation of methylmercury into erythrocytes without any change in the final content of methylmercury.\textsuperscript{9} On the other hand, co-existence of mercuric chloride and selenite \textit{in vitro}\textsuperscript{7,8} and \textit{in vivo}\textsuperscript{9,10} markedly increased uptake of both mercury and selenium by erythrocytes and most of both elements distributed in the plasma and erythrocytes associated with high-molecular weight substance(s) (HMWS) at a molar ratio of about 1.\textsuperscript{8,11} These interactions between mercuric mercury and selenite in blood seem to be an essential process for the modification of the toxicity of mercury by selenium.\textsuperscript{9,10} In the present study, to obtain further information on the mechanism of interaction between mercuric mercury and selenium in blood, effects of \textit{in vitro} co-existence of mercuric mercury and selenite under various conditions on their behaviors in rabbit blood were investigated.

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FIG. 1. *Incorporation of Mercury (A) or Selenium (B) into Rabbit Erythrocytes*

FIG. 2. *Effect of Concentration of Selenite or Mercuric Chloride on Respective Incorporation of Mercury (A) or Selenium (B) into Rabbit Erythrocytes*
MATERIALS AND METHODS

$^{203}\text{HgCl}_2$ (253 $\mu$Ci/μmol) and $\text{Na}_2^{75}\text{SeO}_3$ (410 $\mu$Ci/μmol) were purchased from Amersham, Int. Corp. and diluted with solutions of non-radioactive mercuric chloride or sodium selenite in 9.5 mM phosphate-buffered saline (PBS, pH 7.4). Blood was obtained from male rabbit and used immediately after the heparinization.7)

$^{203}\text{HgCl}_2$ (0.1 $\mu$Ci, 10 μl) and $\text{Na}_2^{75}\text{SeO}_3$ (0.05 μCi, 10 μl) were added into rabbit blood (1 ml) as follows; $^{203}\text{HgCl}_2$ and $\text{Na}_2^{75}\text{SeO}_3$ were added simultaneously, $^{203}\text{HgCl}_2$ added 30 min before the addition of $\text{Na}_2^{75}\text{SeO}_3$, or $^{203}\text{HgCl}_2$ added 30 min after the addition of $\text{Na}_2^{75}\text{SeO}_3$. In a part of the study, one of these two salts was used as non-radioactive solution. After 30 min incubation at 37°C, the distribution of $^{203}\text{Hg}$ and $^{75}\text{Se}$ between the plasma and erythrocytes was determined by the method described previously.7)

Samples for gel filtration were obtained by treating the blood (5 ml) with $^{203}\text{HgCl}_2$ (3.0 $\mu$Ci, 50 μl) and $\text{Na}_2^{75}\text{SeO}_3$ (1.5 $\mu$Ci, 50 μl) as described above. After the incubation, the plasma and stroma-free hemolysate were prepared from the blood samples.7,10) Then plasma (1.5 ml) or stroma-free hemolysate (5.0 ml) obtained 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/h, and 5 ml each of eluate was fractionated.

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

RESULTS

The incorporation rate of $^{203}\text{Hg}$ or $^{75}\text{Se}$ into

<table>
<thead>
<tr>
<th>Concentration (M) of added compounds</th>
<th>Distribution (nmol/ml blood)</th>
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<tbody>
<tr>
<td></td>
<td>Plasma</td>
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<tr>
<td></td>
<td>Hg</td>
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<tr>
<td>$^{10^{-6}}$ HgCl$_2$  $^{10^{-6}}$ Na$_2$SeO$_3$</td>
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<td>(95.0)</td>
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<tr>
<td></td>
<td>(92.4)</td>
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<tr>
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<td>(92.4)</td>
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<tr>
<td>$^{10^{-6}}$ HgCl$_2$  $^{10^{-6}}$ Na$_2$SeO$_3$</td>
<td>0.22</td>
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<td>(21.8)</td>
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<td>$^{10^{-5}}$ HgCl$_2$  $^{10^{-5}}$ Na$_2$SeO$_3$</td>
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<td>(74.6)</td>
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<td>$^{10^{-5}}$ HgCl$_2$  $^{10^{-5}}$ Na$_2$SeO$_3$</td>
<td>2.76</td>
</tr>
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<td>(27.6)</td>
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Rabbit blood was incubated with $^{10^{-6}}$ or $^{10^{-5}}$ $^{203}\text{HgCl}_2$ and/ or $\text{Na}_2^{75}\text{SeO}_3$ at 37°C for 30 min, and the contents of $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma and erythrocytes were measured. Values in parentheses are percentage of added Hg or Se.
the erythrocytes after 30 min incubation of rabbit blood with $^{203}$HgCl$_2$ or Na$_2$SeO$_3$ individually is shown in Fig. 1. More than 90% of added $^{203}$Hg was distributed in the plasma regardless of the concentration of mercury added. Mercuric chloride above $5 \times 10^{-4}$ M caused hemolysis. The $^{75}$Se incorporation rate into the erythrocytes was also less than 10% after incubation of blood with $10^{-6}$ to $10^{-8}$M Na$_2$SeO$_3$. However, when the concentration of Na$_2$SeO$_3$ was increased to $5 \times 10^{-6}$M or more, the incorporation of $^{75}$Se into the erythrocytes was greatly increased as reported by other investigators.

Addition of various concentrations of selenite to rabbit blood with $10^{-5}$, $10^{-6}$ or $10^{-7}$M $^{203}$HgCl$_2$ markedly increased $^{203}$Hg incorporation into the erythrocytes (Fig. 2A). When the blood was incubated with $10^{-5}$ or $10^{-6}$M $^{203}$HgCl$_2$, the maximum incorporation of $^{203}$Hg into the erythrocytes was observed by the addition of an equimolar amount of selenite to mercury, while two or more optimum selenite concentrations for $^{203}$Hg uptake were observed when the mercury concentration was reduced to $10^{-7}$M. On the other hand, $^{75}$Se incorporation into the erythrocytes was also increased by the addition of a certain concentration of $^{203}$HgCl$_2$ (Fig. 2B). In the presence of $10^{-5}$ or $10^{-6}$M Na$_2$SeO$_3$, 5-times as high concentration of mercury as that of selenite was required for the maximum incorporation of $^{75}$Se, but when the concentration of Na$_2$SeO$_3$ was $10^{-7}$M, no stimulating effect of mercury co-existence of the $^{75}$Se incorporation was observed. Any clear correlation between the amounts of mercury and selenium incorporated into the erythrocytes was not observed after the incubation of blood with $10^{-5}$ or $10^{-6}$M $^{203}$HgCl$_2$ and Na$_2$SeO$_3$ (Table I). When blood was incubated with $10^{-5}$M

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**Fig. 3.** Sephadex G-200 Chromatography of Plasma and Stroma-Free Hemolysate (S.F.H.) of Rabbit Blood incubated with $10^{-5}$M $^{203}$HgCl$_2$ plus $10^{-5}$M Na$_2$SeO$_3$ (A), or with $10^{-6}$M $^{203}$HgCl$_2$ plus $10^{-6}$M Na$_2$SeO$_3$ (B)
$^{203}\text{HgCl}_2$ and $10^{-5}\text{M Na}_2^{75}\text{SeO}_3$, the mol of $^{203}\text{Hg}$ and $^{75}\text{Se}$ incorporated into the erythrocytes were almost equal, however, when the concentration of both compounds was $10^{-6}\text{M}$, approximately 3-times as large amount of $^{203}\text{Hg}$ as that of $^{75}\text{Se}$ was incorporated into the erythrocytes.

To study the existing states of $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma or stroma-free hemolysate, the samples obtained under the conditions listed in the Table were gel filtered on Sephadex G-200. At $10^{-6}\text{M}$ for both compounds, $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma were found in the 3 different HMWS and those in the stroma-free hemolysate were found in a HMWS fraction eluted at the void volume of the column, and the molar ratios of $^{203}\text{Hg}$ and $^{75}\text{Se}$ found in those HMWS in the plasma and stroma-free hemolysate were about 1 (Fig. 3A). These gel filtration patterns of $^{203}\text{Hg}$ and $^{75}\text{Se}$ (Fig. 3A) were different from those found in the plasma and erythrocytes of the blood incubated with $10^{-5}\text{M}^{203}\text{HgCl}_2$ or $\text{Na}_2^{75}\text{SeO}_3$ individually as reported earlier, indicating an interaction of mercury and selenium in rabbit blood. The gel filtration patterns of $^{203}\text{Hg}$ or $^{75}\text{Se}$ in plasma and stroma-free hemolysate after the incubation of blood with $10^{-6}\text{M}^{203}\text{HgCl}_2$ or $10^{-6}\text{M} \text{Na}_2^{75}\text{SeO}_3$ alone (data not shown) were similar to those obtained from the blood incubated with $10^{-6}\text{M}^{203}\text{HgCl}_2$ or $10^{-5}\text{M} \text{Na}_2^{75}\text{SeO}_3$ alone. After the incubation of blood with both $10^{-6}\text{M}^{203}\text{HgCl}_2$ and $10^{-6}\text{M} \text{Na}_2^{75}\text{SeO}_3$ (Fig. 3B), $10^{-6}\text{M}^{203}\text{HgCl}_2$ and $10^{-5}\text{M} \text{Na}_2^{75}\text{SeO}_3$ (Fig. 4A), or $10^{-5}\text{M}^{203}\text{HgCl}_2$ and $10^{-6}\text{M} \text{Na}_2^{75}\text{SeO}_3$ (Fig. 4B), the excessively distributed element in the plasma or stroma-free hemolysate showed a similar elution pattern as that shown by its sole addition to the blood, however, the elution pattern of the other element was markedly different from that obtained.

FIG. 4. Sephadex G-200 Chromatography of Plasma and Stroma-free Hemolysate (S.F.H.) of Rabbit Blood incubated with $10^{-6}\text{M}^{203}\text{HgCl}_2$ plus $10^{-6}\text{M Na}_2^{75}\text{SeO}_3$ (A), or with $10^{-6}\text{M}^{203}\text{HgCl}_2$ plus $10^{-6}\text{M Na}_2^{75}\text{SeO}_3$ (B).
when it was added alone to the blood.

Figure 5 shows the time course of $^{203}$Hg and $^{78}$Se uptake into the erythrocytes during the incubation of rabbit blood added with the com-

FIG. 5. Time Courses of Mercury (●) and Selenium (○) Incorporations into Erythrocytes

Blood was incubated with $10^{-5} M$ $^{203}$HgCl$_2$ and $10^{-5} M$ Na$_2^{78}$SeO$_3$ (A, B and C), or $10^{-6} M$ $^{203}$HgCl$_2$ and $10^{-6} M$ Na$_2^{78}$SeO$_3$ (D, E and F).

A and D; $^{203}$HgCl$_2$ and Na$_2^{78}$SeO$_3$ were added to blood simultaneously.

B and E; Blood was added with Na$_2^{78}$SeO$_3$ after 30 min preincubation with $^{203}$HgCl$_2$.

C and F; Blood was added with $^{203}$HgCl$_2$ after 30 min preincubation with Na$_2^{78}$SeO$_3$. 
pounds as follows: $^{203}\text{HgCl}_2$ and Na$_2^{75}\text{SeO}_3$ simultaneously, $^{203}\text{HgCl}_2$ 30 min after preincubation of the blood with Na$_2^{78}\text{SeO}_3$, or Na$_2^{75}\text{SeO}_3$ 30 min after preincubation of the blood with $^{203}\text{HgCl}_2$. After simultaneous addition of $^{203}\text{HgCl}_2$ and Na$_2^{75}\text{SeO}_3$ to the blood, rapid incorporation of $^{203}\text{Hg}$ and $^{75}\text{Se}$ into erythrocytes were observed (Figs. 5A and 5D). The final amounts of $^{203}\text{Hg}$ and $^{75}\text{Se}$ incorporated were reduced by the addition of $^{203}\text{HgCl}_2$ prior to selenite (Figs. 5B and 5E), and the rates of incorporation of $^{203}\text{Hg}$ and $^{75}\text{Se}$ were lowered by the addition of Na$_2^{75}\text{SeO}_3$ prior to mercury (Figs. 5C and 5F).

The gel filtration patterns of $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma or stroma-free hemolysate of the blood preincubated with $^{203}\text{HgCl}_2$ ($10^{-5}\text{M}$) for 30 min before the addition of Na$_2^{75}\text{SeO}_3$ ($10^{-5}\text{M}$) were almost the same as that obtained by simultaneous addition of the two compounds as described in Fig. 3A (Fig. 6A). However, when Na$_2^{75}\text{SeO}_3$ was preincubated with the blood before the addition of $^{203}\text{HgCl}_2$, $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma and stroma-free hemolysate were eluted at the void volume of the column at the molar ratio of about 1 (Fig. 6B). The elution patterns of $^{203}\text{Hg}$ and $^{75}\text{Se}$ in the plasma (Fig. 6B) was not identical with those obtained by the simultaneous addition (Fig. 3A).

**DISCUSSION**

Interaction of inorganic mercury with coadministered selenite in the blood seems to increase the incorporation of mercury and selenium into the erythrocytes $^{9,10}$ resulting in the increase of mercury accumulation in the liver, spleen and blood, and decrease of the renal mercury deposit $^{9,10,16-22}$ These effects are remarkable when approximately equimolar doses of inorganic mercury and selenium compounds were used. $^{18}$

![FIG. 6. Sephadex G-200 Chromatography of Plasma and Stroma-free Hemolysate (S.F.H.) of Rabbit Blood added with $10^{-5}\text{M} \text{Na}_2^{75}\text{SeO}_3$ after 30 min Preincubation with $10^{-5}\text{M}^{203}\text{HgCl}_2$ (A), or added with $10^{-5}\text{M}^{203}\text{HgCl}_2$ after 30 min Preincubation with $10^{-5}\text{M} \text{Na}_2^{75}\text{SeO}_3$ (B)](image-url)
After simultaneous administration of inorganic mercury and selenite, most of mercury and selenium in the plasma, erythrocytes and liver are found in a HMWS at a molar ratio of 1. Burk et al. reported that the molar ratio of mercury and selenium in the HMWS of the plasma was constantly about 1 despite the molar ratio of mercury and selenium dosed. These observations suggest that equimolar amounts of inorganic mercury and selenium interact with each other in animals to cause the mutual modification of their toxicities.

However, in the present in vitro study, a maximum incorporation of $^{203}$Hg and $^{75}$Se into the erythrocytes did not always occur when equimolar amounts of $^{203}$HgCl$_2$ and Na$_2$$^{75}$SeO$_3$ were added to rabbit blood (Fig. 2 and Table 1). Satoh et al. reported that the ratio of administered mercuric mercury and selenite which caused the maximum alteration in the tissue distribution of mercury in mice was not necessarily one.

The most remarkable in vitro interaction in blood was observed when $10^{-5}\text{M}$ $^{203}$HgCl$_2$ and $10^{-5}\text{M}$ Na$_2$$^{75}$SeO$_3$ were added to rabbit blood. Under this condition, the $^{203}$Hg and $^{75}$Se added was always observed with each other suggesting that most of the $^{203}$Hg and $^{75}$Se in the blood formed a complex at a molar ratio of 1 (Table 1 and Fig. 3). However, when blood was incubated with $10^{-6}\text{M}$ $^{203}$HgCl$_2$ and $10^{-6}\text{M}$ Na$_2$$^{75}$SeO$_3$, the amount of $^{203}$Hg incorporated into the erythrocytes was about 3-times as high as that of $^{75}$Se and a substantial part of $^{203}$Hg incorporated into the erythrocytes found in the low-molecular weight fraction on gel filtration (Fig. 3B), in which most of the mercury was eluted when the blood was incubated with $^{203}$HgCl$_2$ alone. Furthermore, when the concentration of $^{203}$HgCl$_2$ was $10^{-7}\text{M}$ in rabbit blood, incorporation of $^{203}$Hg into erythrocytes was increased by the co-existence of selenite (Fig. 2A), but, $10^{-7}\text{M}$ mercury did not stimulate the $^{75}$Se incorporation (Fig. 2B). From these results the increase of mercury incorporation into erythrocytes induced by the co-existence of selenite seems to be not due to the formation of HMWS in erythrocytes.

In the present study the behaviors of $^{203}$Hg and $^{75}$Se in rabbit blood were altered by the order of addition of $^{203}$HgCl$_2$ and Na$_2$$^{75}$SeO$_3$ to rabbit blood (Figs. 5 and 6). Gasiwcz and Smith reported that the in vitro interaction of cadmium and selenite in rat blood occurred by the simultaneous addition of both elements or by preincubation of blood with cadmium before addition of selenite, but not by preincubation with selenite before the addition of cadmium. Contrary to the result of Gasiwcz and Smith, substantial extent of interaction between mercuric mercury and selenium was observed when $^{203}$HgCl$_2$ was incubated with the rabbit blood pretreated with Na$_2$$^{75}$SeO$_3$, but the interaction profiles observed were different from the case of simultaneous addition of both the compounds (Figs. 5 and 6). Selenite selenium is known to be metabolized in blood by a glutathione dependent system to selenide, the selenium of which can bind to plasma proteins. It is possible, therefore, that the selenium which is once bound to blood proteins by preincubation interacts with mercuric mercury in a different mode of action from that of the selenium simultaneously administered with mercuric mercury as selenite. The difference between our present result and that of Gasiwcz and Smith may be due to the species differences and/or metal specificities.

The variety of interaction profiles of mercuric mercury and selenite in blood under different reaction conditions as demonstrated in the present in vitro experiment, may reflect the complex modes of interaction which actually occurs in vivo. These results of our present experiment suggest that some further detailed examinations concerning the relationship between the molar ratio of administered mercury and selenium and the mode of their interaction in animals are needed to understand the mechanism actually involved between these elements ingested in relatively low doses from the foods.

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