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Binding of Simultaneously Administered Inorganic Selenium and Mercury to a Rat Plasma Protein[1] (37894)

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(Introduced by J. P. Hannon)

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Selenium has been shown to alter mercury metabolism and to decrease mercury toxicity. Parizek and his colleagues (1, 2) demonstrated that injection of selenite markedly diminished the toxicity of simultaneously administered mercuric chloride while paradoxically appearing to cause increased retention of the mercury. Ganther *et al.* (3) reported that chronic methylmercury toxicity was decreased by feeding 0.5 ppm selenium. Recently Potter and Matrone (4) confirmed the findings of Ganther *et al.* using a dietary selenium concentration of 5 ppm and showed that this amount of selenium also decreases chronic inorganic mercury toxicity.

Little is known of the mechanism by which selenium decreases mercury toxicity. Parizek *et al.* (2) found that selenium administration altered ^{203}Hg distribution after $^{203}HgCl_2$ injection. It caused a much higher blood ^{203}Hg concentration which might be related to the diminished acute toxicity. This report is concerned with the nature of that increased blood ^{203}Hg.

Materials and Methods. Six male Holtzman rats[3] (av wt 249 g) which had been fed a selenium-deficient torula yeast diet (5) ad lib. since weaning were studied. Injections of $^{203}HgCl_2$ and $^{75}SeO_3^{2-}$ (New England Nuclear, Boston, MA) were administered in 0.65–0.76 ml of 0.9% NaCl. The injected solutions contained approximately 200 μCi of the appropriate isotope and 5 or 10 μmoles of the corresponding element in the same chemical form/kg body weight. The selenium solutions were injected intraperitoneally and the mercury solutions subcutaneously at the same time as indicated in Table I. The animals were exsanguinated under ether anesthesia 20 hr later and EDTA (2 mg/ml) was added to the blood to prevent coagulation. Plasma was separated in a refrigerated centrifuge.

Gel filtration was done using Sephadex G200 (particle size 40–120 μm) in a 2.6 cm diameter column. Void volume was 57 ml and total bed volume was 178 ml. Sample size was 1 ml. Buffer was 0.1 *M* NaCl, 0.1 *M* Tris–HCl, pH 7.6. Samples in 20% sucrose were layered on a sample applicator and constant downward flow was maintained with a pump. Ion exchange chromatography was performed with Whatman

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[3] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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TABLE I. Doses of Se and Hg Administered and Molar Ratios of Se to Hg in Se–Hg-containing Protein Peak from Gel Filtration.

Rat	Dose (μmoles/kg) SeO_3^{2-}	Dose (μmoles/kg) $HgCl_2$	Molar ratio[a] Se/Hg
1	0	5	—
2	5	0	—
3	5	5	1.15[b]
4[c]	10	5	—
5	5	10	0.98
6	10	10	0.91

[a] Calculated using cpm in fraction at top of Se–Hg containing protein peak on gel filtration chromatogram.

[b] Molar ratio was also calculated on the ion exchange chromatography of this peak (Fig. 2) and was 1.02.

[c] Died (see text).

DE52 resin. Bed dimensions were 1.6 × 38 cm. The primary buffer was 0.05 *M* Tris–HCl (pH 8.8) and the secondary buffer was the same with 0.4 *M* NaCl. ^{75}Se and ^{203}Hg were determined by the channel ratio technique using the 0.40 MeV ^{75}Se photopeak and the 0.28 MeV ^{203}Hg photopeak.

Duplicate 0.5 ml samples from the gel filtration experiment were dialyzed in stirred 1 liter baths at 4° for 24 hr using dialysis tubing with a pore radius permeability of 24 Å. An undialyzed sample was used for calculating ^{75}Se and ^{203}Hg loss.

Results. When 5 μmoles/kg $HgCl_2$ and 200 μCi $^{203}HgCl_2$ were injected, the plasma gel filtration chromatogram seen in Fig. 1A was obtained. The ^{203}Hg bound to plasma protein in all regions of the chromatogram. When similar amounts of SeO_3^{2-} and $^{75}SeO_3^{2-}$ were injected, a single well-defined peak of protein-bound ^{75}Se occurred on the chromatogram at about 100 ml after sample application (Fig. 1B). In marked contrast to the protein binding of the elements when administered alone, Fig. 1C demonstrates the occurrence of both ^{203}Hg and ^{75}Se in a single peak 68 ml after sample application when the elements were administered simultaneously. Also the great increase in ^{203}Hg and ^{75}Se present due to simultaneous administration should be noted. In that experiment selenium administration caused a 15-

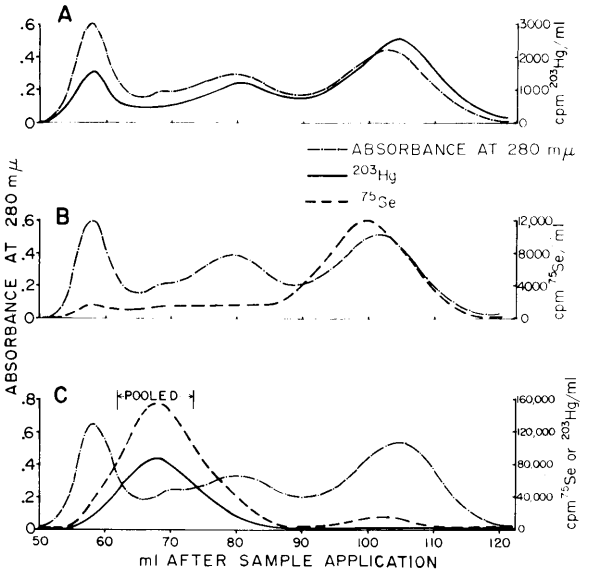

FIG. 1. Gel filtration of plasma from rats receiving: (A) 5 μmoles of $HgCl_2$/kg and 200 μCi $^{203}HgCl_2$; (B) 5 μmoles of SeO_3^{2-}/kg and 200 μCi $^{75}SeO_3^{2-}$; and (C) both of the above simultaneously. Column conditions were the same for each experiment. Counts per minute is graphed per 1 ml fraction.

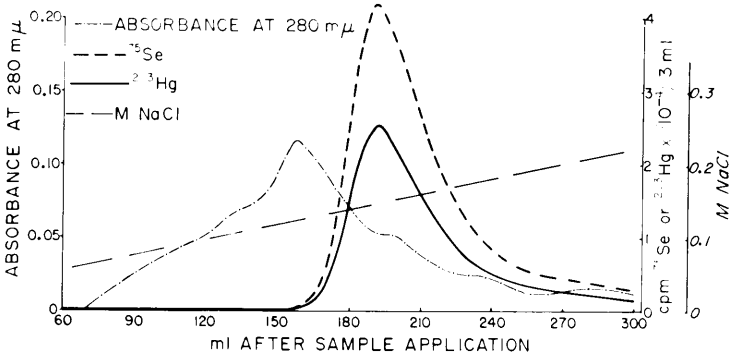

FIG. 2. Ion exchange of pooled ^{75}Se–^{203}Hg peak indicated in Fig. 1C. Counts per minute is graphed per 3 ml fraction.

fold increase in plasma ^{203}Hg. Assuming plasma volume was 9 ml, 23% of the injected ^{203}Hg was present in the plasma when selenium was also injected.

Figure 2 is an ion exchange chromatogram of the pooled ^{75}Se–^{203}Hg peak in Fig. 1C. The ^{203}Hg and ^{75}Se are still present in one peak strongly suggesting that both isotopes are bound to a single protein.

Varying doses of $HgCl_2$ and SeO_3^{2-} were given to study the effect this would have on the molar ratio of selenium to mercury in the selenium–mercury-containing protein peak. Results are seen in Table I. Rat 4, which received 10 μmoles of SeO_3^{2-} and 5 μmoles $HgCl_2$/kg, died several hours after injection and had hemorrhagic lungs at necropsy. No plasma was obtained from it. The molar ratios in the other rats were all close to 1 suggesting that a fixed relationship of one selenium atom to one mercury atom exists in the protein.

The results of dialysis studies of the selenium–mercury-containing protein peak are shown in Table II. ^{203}Hg but not ^{75}Se was removed by dialysis against $HgCl_2$. Substantial amounts of ^{75}Se and ^{203}Hg were removed by mercaptoethanol and smaller, possibly significant, quantities of each were removed by Na_2SO_3. Very little ^{75}Se or ^{203}Hg was removed by alkaline dialysis.

Discussion. The results of this study strongly suggest that selenium and mercury bind to a single plasma protein when both inorganic elements are administered simultaneously. Plasma protein binding of each element administered alone (Fig. 1 A and B) was found to be as previously described (6, 7). Parizek *et al.* (8) noted that increased ^{75}Se and ^{203}Hg were present in the macromolecular fractions of plasma under conditions of simultaneous administration, but they did not further characterize these fractions.

The limited data on molar ratios of selenium to mercury (Table I) suggest that a stoichiometric ratio of one atom of selenium to one of mercury exists in the protein. However, it must be pointed out that calculation of these molar ratios is based on the assumption that endogenous selenium and mercury did not significantly change the specific activity of the injected material. A very liberal estimate of whole-body selenium

TABLE II. Dialysis of Pooled Peak Indicated in Fig. 1C.

Bath	Percentage lost during dialysis[a]	
	^{75}Se	^{203}Hg
0.9% NaCl, pH 6.6	8.1	4.3
1 m*M* EDTA, pH 4.6	12.1	8.3
50 m*M* Na_2SO_3, pH 9.5	16.8	11.5
0.5 *M* mercaptoethanol, pH 4.8	32.2	52.9
1.8 m*M* $HgCl_2$, pH 4.9	10.8	66.2
1 m*M* Na_2SeO_3, pH 7.1	11.5	7.7
8 *M* urea, pH 8.7	9.5	3.2
0.5 *M* NaOH, pH 11.7	11.4	10.4

[a] Average of duplicate determinations.

in these rats based on liver selenium concentrations after only 4 wk on this diet (9) would be 15 μg. Of this almost certainly less than a third would be exchangeable. Thus, since at least 98 μg of selenium was administered to each rat, a dilution of less than 5% of the injected selenium probably occurred. No such data are available for the mercury, but no mercury was knowingly administered before the injections.

The dialysis data in Table II show that ^{203}Hg could be released by dialysis against $HgCl_2$ without release of ^{75}Se, but that when ^{75}Se was released by mercaptoethanol and sodium sulfite, ^{203}Hg was also released. This is compatible with attachment of selenium to the protein through a sulfhydryl group and mercury attachment to the selenium. Preliminary experiments in which injection times of both elements and plasma collection times were varied show a time lag after $^{75}SeO_3^{2-}$ injection before protein binding can be detected, suggesting that the $^{75}SeO_3^{2-}$ must be metabolized to another form before this binding can take place. The molar ratio observed suggests that only one mercury atom attaches for each selenium atom present.

This type of selenium binding to protein is quite unlike that observed when selenium is administered alone. In that case it cannot be removed with disulfide-reducing agents but can by alkaline dialysis (6).

The relationship of this binding to the amelioration of mercury toxicity by selenium is not clear. It could be directly related to relief of acute inorganic mercury toxicity since up to 23% of the administered mercury is sequestered in this form 20 hr after injection.

Summary. Simultaneous administration of 5 μmoles of $HgCl_2$ and of SeO_3^{2-}/kg along with traces of $^{203}HgCl_2$ and $^{75}SeO_3^{2-}$ to a rat radically alters plasma protein binding of ^{75}Se and ^{203}Hg from that found when each element is given alone. After simultaneous administration both elements are present in the plasma in much greater quantities due to their binding to a single plasma protein. When the doses of the elements are varied the molar ratio of selenium to mercury in the protein remains close to 1. Dialysis data suggest that selenium is attached to a sulfhydryl group of the protein and that mercury is attached to selenium. This protein may play a role in preventing acute inorganic mercury toxicity by preventing a large part of the mercury dose from reaching target tissues.

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