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Selenium Metabolite Levels in Human Urine after Dosing Selenium in Different Chemical Forms

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It has been well known that selenium in marine fish such as tuna and swordfish protects the toxicity of methylmercury in vivo (Ganther et al. 1972: Ohi et al. 1976: Friedman et al. 1978). The protective potency might depend on the chemical forms of selenium in the meat of marine fish sebastes and sperm whale (Ohi et al. 1980).

Little has been revealed, however, on the chemical forms of selenium in the meat of these animals or the selenium metabolites in urine, because the amount of the element is very scarce.

Urine is the major excretory route for selenium (Levander et al. 1981, Swanson et al. 1983). The chemical forms of urinary selenium may reflect the metabolism of the element.

We have developed methodology for analysis of selenium-containing components in human urine (Hasunuma et al. 1993). Using this method, we have observed the time courses of excretory levels of urinary selenium components after a single dose of selenium as selenious acid, selenomethionine, trimethylselenonium ion or tuna meat.

MATERIALS AND METHODS

Selenium standard solution (1000 ppm as selenious acid, for atomic absorption spectrometry) was from Wako (Osaka, Japan). Trimethylselenonium (TMSe) iodide (> 99.999%) was a product of Tri Chemical Lab. (Kanagawa, Japan). An L-Selenomethionine (SeMet) source, "Yeast free organic selenium supplement" as tablets, was from Cantassium Co. (London, U.K.). By silica gel sintered TLC (Hasunuma et al. 1991), we found that the tablet (250 mg) contained 36 μg as selenium (S.D.=5, n=3) in a form of SeMet. Tuna (Parathunnus sibi) was obtained from Tokyo Metropolitan wholesale market (Tokyo, Japan) in frozen state. The meat contained 0.51 mg of selenium per kg (S.D.=0.09, n=3).

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Healthy volunteers (5 males of the ages ranging 22 to 58 and 4 females of 21 years old) were dosed with various selenium sources orally. Dose experiments with different chemical forms of selenium were carried out on the same volunteer groups at least one month after the preceding experiment. Volunteers took 4 ml of selenious acid solution (1000 ppm selenium), 15 SeMet-containing tablets, or 1 kg of tuna meat. The amounts of selenium were 4, 0.54, or 0.51 mg, respectively. TMSe was also dosed as a selenium source (4 mg), to estimate a rate of "direct excretion" of the compound. During the first 24 h after the dose, all the urine was collected with recordings of intervals and volumes of urination by volunteers. During the next 4 days, single-void urine was collected once a day.

The urine sample (10 mL), adjusted to pH 2.2 to 2.4 with 6M hydrochloric acid, was applied to a Dowex 50W-X4 (200 to 400 mesh, H^+ form) column (17.5 mL) and eluted stepwise with 70 mL each of water and 0.05, 0.1, 0.5 and 1 M hydrochloric acid and finally 100 mL of 4 M hydrochloric acid. Ten mL portions of the eluate were collected. The void fractions (10 mL) adjusted to pH 7 with 6 M sodium hydroxide were applied to an AG1-X8 (200 to 400 mesh, H^+ form) column (17.5 mL) and eluted stepwise with 70 mL each of water and 0.1, 1 and 4 M hydrochloric acid. Ten mL portions of the eluate were collected.

Urinary selenium level was represented as μg selenium per g creatinine (Se/CT) (Hojo 1982) or as μg selenium excretion per hour (Se/h), which was calculated from the selenium concentration and volume of the urine and interval of the urinations (Tsuda and Kawanishi 1990). Selenium was determined fluorometrically using 2,3-diaminonaphthalene (DAN), according to the method described previously (Hasunuma et al. 1982, Hasunuma et al. 1990). Creatinine levels in the urine were determined by the method of Folin and Wu (Bonsnes and Taussky 1945).

RESULTS AND DISCUSSION

Typical time courses of urinary selenium excretion are presented

Table 1.
Urinary excretion of selenium after dosing of selenium from the different sources.

Selenium source	n[a]	Max. excretion time (h)		Recovery in 24h (%)	
		Mean	S.D.	Mean	S.D.
Selenious acid	6	8.3	2.9	32.0	10.1
SeMet	6	3.6	0.7	26.0	13.6
TMSe	4	1.5	0.3	63.5	2.6
Tuna meat	6	8.5	2.3	29.5	3.4

a: The number of volunteers.

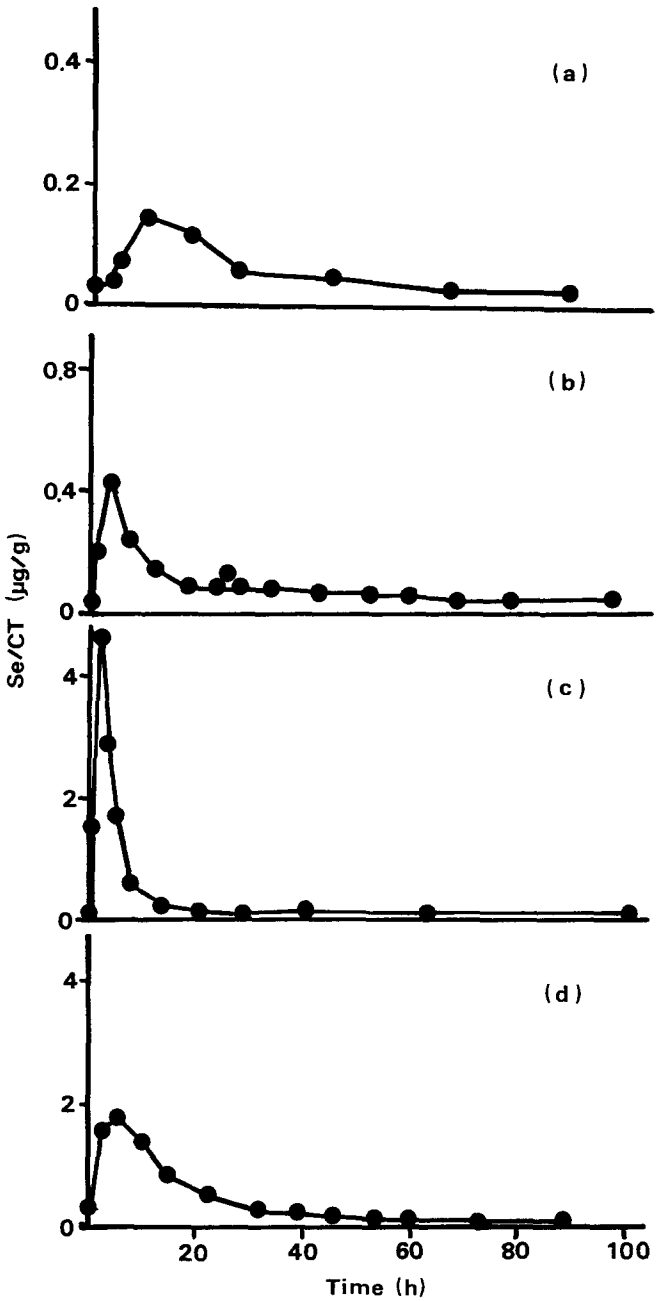

Figure 1. Changes in the rate of urinary excretion of selenium following a single oral dose of tuna meat (a), selenomethionine (b), trimethylselenonium iodide (c) and selenious acid (d).

in Figure 1. As shown in Table 1, the urinary selenium concentration reached a peak after 1.5, 4, 8 and 9 h from respective

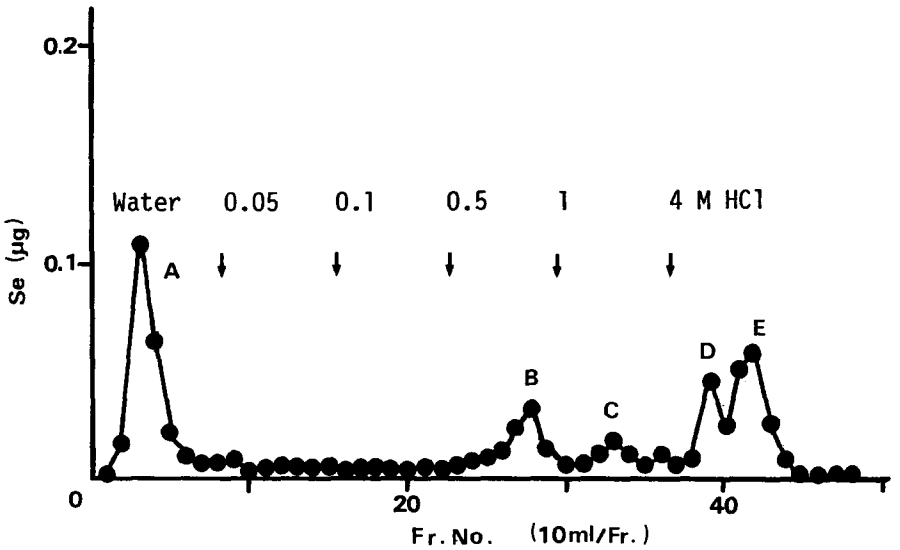

Figure 2. Separation of urinary selenium components by Dowex 50W-X4 column chromatography. Arrows indicate changes of eluents. The eluents are water, 0.05, 0.1, 0.5, 1 and 4 M hydrochloric acid in the elution order.

dosing of TMSe, SeMet, selenious acid and tuna meat. Recoveries of selenium in 24 h urine were 64, 26, 32 and 30%, respectively. The urinary excretion after the dose of TMSe was distinguished from those of other selenium sources. Very rapid and sharp appearance of selenium in urine suggested "direct excretion" of selenium without metabolic processing.

Urine samples obtained from volunteers were analyzed by ion exchange chromatography described in the section of materials and methods. Selenium was found to be distributed among five major peaks as shown in Figure 2. Similar elution patterns were obtained previously with the normal urine sample. The five peaks were named A, B, C, D and E, according to the elution order. Peak C was identified as TMSe by Dowex 50W-X4 column chromatography and silica gel sintered TLC with authentic TMSe (Hasunuma et al. 1991: Hasunuma et al. 1993). Peaks A, B, D, and E have not been identified yet. However, at least none of these peaks corresponds with a known biomaterial, SeMet, besides selenotaurine and selenocystine, on paper and silica gel sintered thin layer chromatograms.

Figure 3 shows time courses of these selenium-containing components excreted following a single oral dose of selenious acid. The rate of selenium excretion for each selenium component reached to the maximum within 10 h after the dose.

Figure 4 shows the result of SeMet dose experiment. It must be noted that the rate of selenium excretion reaches the maximum within a period as short as about 4 h, in all the selenium components except D.

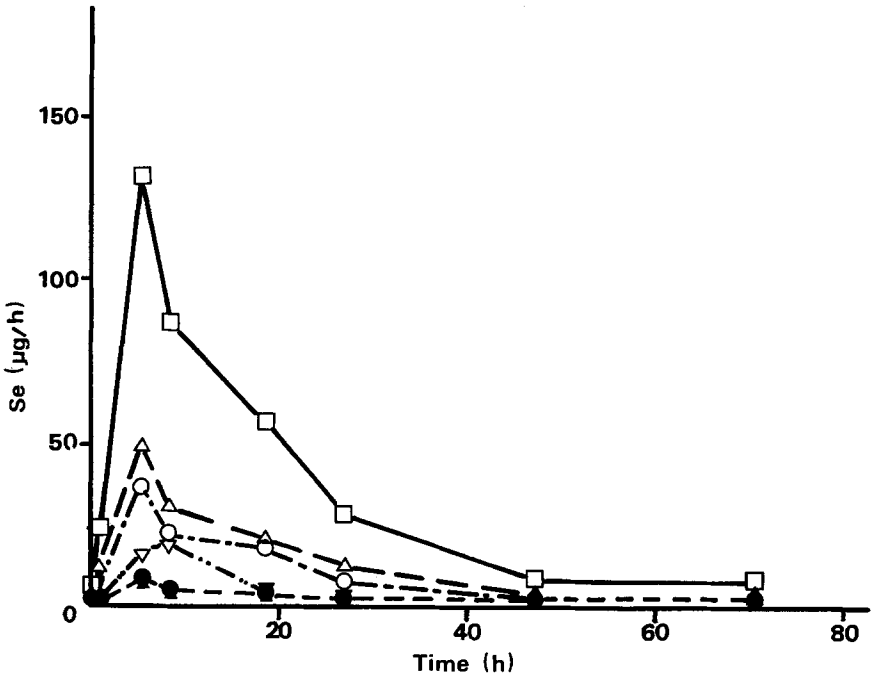

Figure 3. Changes in the rate of urinary excretion of selenium components following a single oral dose of 4 mg selenium as selenious acid. Symbols correspond to the peaks indicated in Figure 2: △,A ; ●,B ; ○,C ; ▽,D ; ▲,E. Symbol □ represents the amount of total selenium.

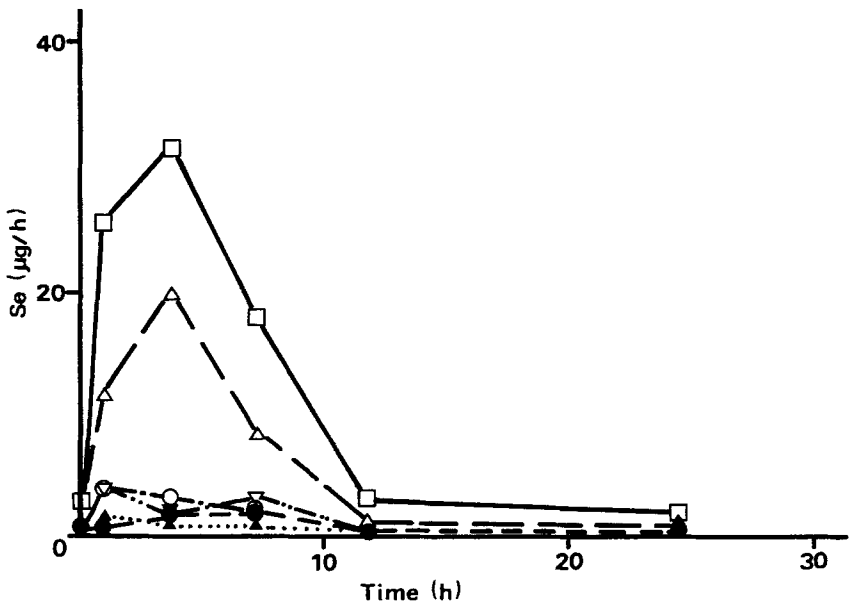

Figure 4. Changes in the rate of urinary excretion of selenium components following a single oral dose of 0.54 mg selenium as SeMet. All symbols represent the same components appeared in Figure 3.

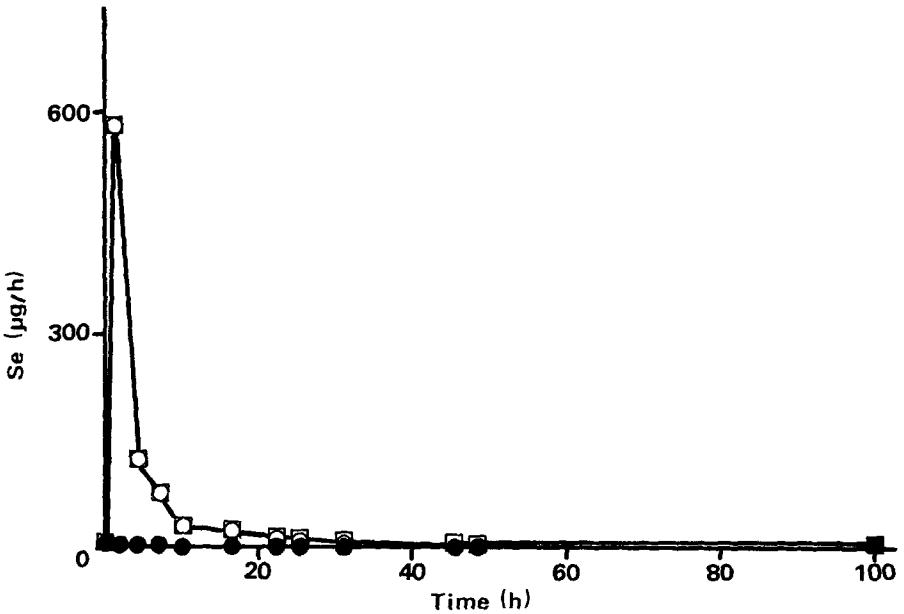

Figure 5. Changes in the rate of urinary excretion of selenium components following a single oral dose of 4 mg selenium as TMSe. All symbols represent the same components appeared in Figure 3.

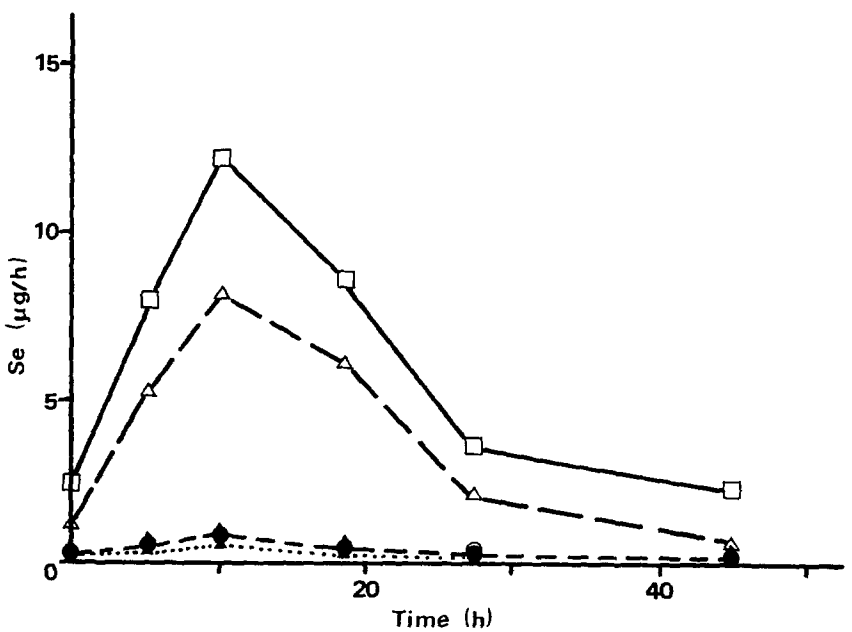

Figure 6. Changes in the rate of urinary excretion of selenium components following a single oral dose of 0.51 mg selenium as tuna muscle. All symbols represent the same components appeared in Figure 3.

When TMSe was dosed, the compound itself was excreted in urine as shown in Figure 5. About 50% of TMSe were excreted in urine within 10 h after dose.

Time courses after dosing of tuna muscle are shown in Figure 6. As can be seen from Table 1, the feature of total selenium excretion could hardly be distinguished between selenious acid and tuna meat. Comparing Figures 3 and 6, the difference between the cases of selenious acid and other materials could be clearly recognized. While peak A constitutes the majority of urinary selenium components in tuna meat, selenious acid gave comparable quantities of other components, suggesting involvement in more complex metabolic pathway.

In this experiment, peak A, the void fraction from a Dowex 50W-X4 column, was a major component except the case of TMSe. The peak was separated further on an AG1-X8 column into an unknown selenium fraction and selenite which constituted a few percent of the total urinary selenium (Hasunuma et al. 1993).

It should be noted that excess dose of selenious acid or SeMet did not result in the increasing of urinary excretion of the dosed substance. Although excess amounts of selenium seemed to have eventually promote methylation of selenium for the detoxication (Ganther et al. 1987), peak A was a primarily increased component in all the experiments.

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