

Biliary excretion of essential trace elements in rats under oxidative stress caused by selenium deficiency

Kosuke Yamasaki · Yasunobu Sakuma · Junya Sasaki · Ken-ichiro Matsumoto · Kazunori Anzai · Keisuke Matsuoka · Chikako Honda · Masamichi Tsukada · Kazutoyo Endo · Shuichi Enomoto

Received: 16 March 2011 / Revised: 8 August 2011 / Accepted: 11 August 2011 / Published online: 26 August 2011
© Springer-Verlag 2011

Abstract The excretion of essential trace elements, namely, Se, Sr, As, Mn, Co, V, Fe, and Zn into the bile of Se-deficient (SeD) Wistar male rats was studied using the multitracer (MT) technique, and instrumental neutron activation analysis (INAA). Normal and Se-control (SeC) rat groups were used as reference groups to compare the effects of Se levels on the behaviors of the essential trace elements. The excretion (% dose) of Se, Sr, As, Mn, Co, and V increased with Se levels in the liver. The biliary excretion of Mn and As dramatically enhanced for SeC rats compared with SeD rats, while that of V

accelerated a little for SeC rats. The radioactivity levels of ^{59}Fe and ^{65}Zn in the MT tracer solution were insufficient to measure their excretion into bile. The role of glutathione and bilirubin for biliary excretion of the metals was discussed in relation to Se levels in rat liver.

Keywords Essential trace element · Oxidative stress · Glutathione peroxidase · Glutathione · Bile · Biliary excretion · INAA · Multitracer

K. Yamasaki · M. Tsukada
School of Agriculture, Meiji University,
1-1-1 Higashi-Mita, Tama-ku,
Kawasaki, Kanagawa 214-8571, Japan

Y. Sakuma · J. Sasaki · K. Matsuoka · C. Honda · K. Endo (✉)
Department of Physical Chemistry,
Showa Pharmaceutical University,
3-3165 Higashi-Tamagawagakuen,
Machida, Tokyo 194-8543, Japan
e-mail: kazutoyo@ac.shoyaku.ac.jp

K.-i. Matsumoto · K. Anzai
Research Center for Charged Particle Therapy,
National Institute of Radiological Science,
4-9-1 Anagawa, Inage-ku Chiba-shi,
Chiba 263-8555, Japan

S. Enomoto
Radioisotope Technology Division, Cyclotron Center,
The Institute of Physical and Chemical Research (RIKEN),
Wako, Saitama 351-0106, Japan

Present Address:

S. Enomoto
Department of Analytical Chemistry, School of Pharmacy,
Okayama University,
1-1-1 Tsushima-Naka,
Okayama 700-8530, Japan

Introduction

Biliary excretion is an important route for a number of essential and toxic metals. In the past, many investigators studied and reviewed the distribution of metals in the excretory organs, as well as the biliary and fecal excretions of metals [1–5]. Studies on biliary excretion have been concerned with the dependence of the processes on dose and time. Metals such as Mn, Cu, Cd, Ag, and As excreted into bile are characterized by large bile/plasma concentration ratios, and their rates of transport into bile are major factors that determine their biological half-lives. In contrast, Fe, Zn, Co, Au, Ni, Cr, inorganic Hg, and Sn showed low bile/plasma concentration ratios, and biliary excretion is less important for their elimination [5]. The effects of bile flow on the secretion of trace elements have been systematically evaluated for normal rats [6].

We have so far investigated alteration of mineral balance in the liver of Se-deficient (SeD) rats in relation to the oxidative stress caused by Se deficiency [7–11]. Liver metabolism in liver plays important roles for the homeostasis of trace elements in animals. It is known that distributions of essential elements in organs of aerobic animals change to maintain mineral balance, when the

organs are exposed to oxidative stress under Se-deficient conditions [12]. Complex antioxidant defense systems for the animals consist of antioxidant enzymes, glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), heme oxygenase, metal-binding proteins (transferrin, ceruloplasmin), and other low molecular weight compounds such as glutathione (GSH) and vitamins C and E. Selenium has also important health effects particularly in relation to anti-inflammatory response and cancer prevention, and these studies have been extensively reviewed [13, 14].

In this study, in order to clarify the effects of Se levels in rat liver, we examined the excretion of trace elements (V, Mn, Fe, Co, Zn, As, Sr, and Se) in three different types of rats, Se-deficient (SeD), normal, and Se-control (SeC) rats for rather short time intervals (i.e., less than 120 min) after administration via the multi-tracer (MT) technique. For comparison, the contents of Fe, Co, Zn, and Se were determined by using instrumental neutron activation analysis (INAA). The MT technique provides information on the dynamics of radioactive tracers into the bile after MT injection, whereas INAA provides information on bile metal contents. INAA is the analytical technique without chemical treatment, and the MT technique is that using a variety of radioisotopes simultaneously under the same chemical condition. The combined use of the two nuclear methods revealed clearly difference in the excretion rates of essential trace elements under oxidative stress mediated by Se deficiency.

Materials and methods

Materials

SeD diet was purchased from Oriental Yeast Co. LTD (Tokyo, Japan). The Se content of SeD diet was reported in our previous paper as 0.017 mg/kg [9]. Deionized water prepared with a Milli-Q system was used for all experiments and as drinking water for rats.

Animals

Female Wistar rats (15th day after pregnancy, purchased from Japan Laboratory Animals, Inc.) were fed on a SeD diet (Oriental Yeast Co., Ltd.) and given Milli-Q-treated water. Sawdust was used in order to relax nervous pregnant rats from stress in a rearing cage. Newly born rats were kept with their own mother rat for 4 weeks. Four weeks after birth, young male rats were weaned, and then fed SeD diet and Milli-Q-treated water until the experiments began. At 8 weeks, the male rats were used for experimentation in the SeD group. SeC rats were fed on SeD diet with Milli-Q-treated water containing a total of 2 mg/kg Se (seleno-L-methionin, sodium selenite, sodium selenate=8:1:1). Normal male Wistar rats of the same age as the rats of the same age in the SeD group were purchased from the same vendor and used as the normal control group. The animal experiments were carried out in compliance with the Guidelines for Animal Care and Use at Showa Pharmaceutical University (2004), and approved by the Ethical Committee for Animal Care and Use of the university.

Table 1 shows the contents of Se, Fe, Zn, and Co in Se-deficient and normal diets, as well as sawdust. The normal diet used in this work was CE-2 (CLEAR Japan, Inc., Tokyo, Japan). Selenium was found in the normal diet, but not in the SeD diet and sawdust under the present conditions (less than 0.005 mg/kg). The Fe and Co contents in the SeD diet were approximately 1/10 and 1/70 those in the normal diet, respectively.

GSH-Px activity in liver

Three rats from SeD group and seven rats from normal group were food deprived for 24 h before the experiment and were sacrificed by decapitation. The liver was perfused with ice-cooled physiological saline (0.9% NaCl). The liver was removed and homogenized with a 4-fold volume of Milli-Q-treated water. The liver homogenate was then diluted with a 250–1,250-fold volume of water. GSH-Px activity in the liver homogenate was measured using the method described by Paglia and Valentine with some modifications [15]. The

Table 1 Contents of elements in diet and sawdust

	Se	Fe	Co	Zn
Se-deficient diet	–	47.0±4.1*	0.03±0.002*	42.7±1.5*
Normal diet ^a	0.86±0.13	482±6	0.90±0.02	62.0±1.8
Sawdust	–	1.5±0.1	0.02±0.02	6.3±0.5

Values are means±S.D. mg/kg in dried powder sample

* $p < 0.001$, significant difference between Se-deficient and normal diets

^a CLEA Japan, Inc. Tokyo

absorbance of NADPH was measured at 340 nm using a spectrophotometer (Hitachi U4100), and the activity was estimated by a decrease in the absorbance per unit time. GSH-Px activity was standardized with protein concentration and expressed as U/mg protein.

GSH concentration

GSH content was assayed following the procedure described by Griffith [16], and GSH concentration was normalized by the amount of amount. The sample solution was measured at 412 nm with the spectrophotometer, and the GSH concentration was calculated from the change in the absorbance.

Multitracer

The MT solutions were prepared from silver foil irradiated with N-14 beams of 140 MeV/nucleon at the RIKEN Ring Cyclotron. After the chemical separation of Ag^+ , a hydrochloric acid solution containing the multitracer was prepared [17–19]. The solution was then evaporated to dryness and the MT was dissolved in 2 mL of a 0.9% NaCl solution for administration to rats. The biliary duct was cannulated with PE-10 polyethylene tubing under Nembutal anesthesia (intraperitoneal injection of 50 mg/kg body weight (b.w)). After intravenous (i.v.) injection of a saline solution (200 μL) containing MT to the tail, the bile was collected in polyethylene tubing every 10 min and the γ -ray spectra of each fraction were measured. The elements in the MT solution may consist of the following: VO_3^- , Mn^{2+} , Fe^{3+} , Co^{2+} , Zn^{2+} , AsO_4^{3-} , SeO_4^{2-} , and Sr^{2+} due their salt form [17].

Sample preparation for INAA

a) Liver sample

Rats were anesthetized with a 50 mg/kg b.w. intraperitoneal injection of Nembutal (Dinabot). Entire blood

was drawn from the abdominal aorta. The entire body was then perfused by ice-cooled saline (0.9% NaCl), and the liver was removed. Subsequently, the liver was wiped with KimWipes (Nippon Paper Crexia Co. Ltd) and weighed. Livers from three rats of the same group were mixed and homogenized with a 4-fold volume of Milli-Q-treated water. The homogenate was kept at $-30\text{ }^\circ\text{C}$ until it was frozen, after which the frozen samples were lyophilized and ground into powder. An aliquot (approximately 100 mg) of the powdered sample was weighed precisely and sealed in a quartz tube with oxygen burner. Standard Reference Material 1577b (bovine liver) obtained from the National Institute of Standards and Technology (Bethesda, MD) was used to quantify the inorganic elements. Three tubes were prepared for each sample and subjected for irradiation in order to test the precision of analyses.

b) Bile sample

Rats were food deprived for 24 h (with drinking water being available) before collecting bile. SeD, normal, and SeC rats were given i. p. injections of Nembutal (Dianabot Co. Ltd., Osaka, Japan) at a dose of 50 mg/kg b.w. The bile duct was cannulated with 50-cm segment of PE-10 tubing, connected to an Eppendorf tube for 2 h. Collection started at about 2 p.m. to reduce the variation in bile flow due to rhythmus [6]. The collected bile (100 μL) was added to 100 mg of Si powder (purity of 99.9%, Wako Pure Chemicals) in a polyethylene tube. After weighting, the sample was kept at $-30\text{ }^\circ\text{C}$, and the freeze-dried sample was weighted again. The net dry weight of the bile residue was estimated. The dried sample was powdered in an agate mortar and was subjected to INAA. The bile was freeze-dried, and the powdered samples were weighed accurately (~100 mg per sample) and put into quartz tubes with powdered silicon. The contents were then freeze-dried and the quartz tube was sealed.

Neutron activation

Neutron irradiation was carried out for 1 h at the D-pipe (thermal neutron flux; 4.3×10^{13} n/cm²/s) of the JRR-4 nuclear reactor at the Japan Atomic Energy Research Agency. The irradiated samples were cooled for at least 1 week. The γ -ray spectra of irradiated samples were measured with a high-purity germanium semiconductor detector equipped with a multichannel analyzer (Seiko EG&G Co., Ltd. Matsudo, Japan). γ -Ray spectra were analyzed with a least-square fitting program of Gaussian functions, and the criterion of photo-peak identification was above the 3σ of the base line counts. The radioactive isotopes (half-life and γ -ray energy) analyzed in the MT experiments were listed in Table 2.

Table 2 The radioactive isotopes analyzed in the MT experiments

Radioisotope	Half-life (day)	γ -ray energy (keV)
⁴⁸ V	15.97	983.5
⁵⁴ Mn	312.12	834.83
⁵⁸ Co	70.92	810.77
⁵⁹ Fe	44.50	1,099.25
⁶⁵ Zn	243.90	1,115.55
⁷⁴ As	17.78	595.93
⁷⁵ Se	119.77	264.66
⁸⁵ Sr	64.84	514.15

Results

GSH-Px activity and GSH levels

Cytosolic GSH-Px activities and GSH levels in liver homogenates of SeD, normal, and SeC rats are listed in Table 3. The cytosolic GSH-Px activity in the liver homogenate of normal and SeC rats was consistent with those reported for similarly aged rats [7, 11], while remarkably low cytosolic GSH-Px activity was observed in the SeD rats. Significantly low GSH levels were obtained for SeD rats. The GSH content in liver homogenates appears to be correlated with the Se contents in liver.

Contents of Se, Fe, Co, and Zn in liver

The contents of Se, Fe, Co, and Zn in the livers of SeD, normal, and SeC rats are listed in Table 4. The Se content in the livers of SeD rats was below the detection limit under the present experimental conditions. The SeD and SeC rats were fed the same SeD diet, i.e., the former rats were fed with drinking water, and the latter rats were fed with drinking water containing Se compounds. Therefore, Fe, Co, and Zn levels were compared between the SeD and SeC rat. The Fe level in the livers of SeD rats was higher than that of SeC rats, which is consistent with our previous results [7, 9, 11]. However, the Fe level in the livers of SeD rats was lower than that of normal rats in the present study. The Fe contents in the SeD diet were arranged to approximately 1/10 of that in the normal diet, in order to avoid a condition of Fe overlord due to Se deficiency. The Co contents were lower in SeD and SeC rats in normal rats. This can be attributed to the low content of Co in the SeD diet. The Zn level in the livers of SeD rats was somewhat higher than that of SeC rats, although the difference was not significant ($p < 0.06$).

Contents of Se, Fe, Co, and Zn in bile

The contents of Se, Fe, Co, and Zn in bile are listed in Table 5. No Se was detected in the bile of SeD and normal rats whereas Se was excreted into bile when SeC rats were fed with drinking water containing 2 ppm Se. Biliary excretion

of Se depends on the status of Se in the liver. In the normal and SeD rats, Se was not measured. However, a content of 1.06 ± 0.12 mg/kg was measured in the bile of SeC rats (Table 5). It has been reported that this level is not a lethal dose and that it does not alter body weight [20]. In the same report, it was reported that biochemical parameters concerning lipid status as well as hepatic function were altered at Se levels greater than 2.0 ppm. In this study, the Se level was selected in order to clearly show the effect of Se on the excretion of metals independent of toxic effect. The contents of Fe and Co in bile were found to be regulated irrespective of different Se levels in the normal and SeD diets. The Zn level in the bile of SeD rats was found to be higher than that of normal or SeC rats, although SeD and SeC rats were fed the same SeD diet as stated above.

Bile flow rate

Bile flow rate is plotted against time in Fig. 1, in which the quantity of bile collected for every 10 min is shown. The cumulative flow rate for 120 min was not significantly different between SeD and SeC rats ($p < 0.06$), or between normal and SeC rats ($p < 0.5$), although a small decrease was seen at 90–120 min after starting collection.

Multitracer in bile

In this study, we set the bile collection time to every 10 min from the end of MT injection up to 2 h post injection. We used a measure of cumulative excretion (% dose) value in order to evaluate more reliable data instead of the value for each fraction. Therefore, the counting error is small, and the standard deviation of the measurements is below the mark in logarithmic scale in Figs. 2, 3, and 4.

Cumulative excretion(% dose)

$$= \frac{\text{radioactivity(cps) in bile}(t \text{ min})}{\text{radioactivity(cps) injected in a rat}} \times 100(\%)$$

Cumulative excretions for V, Mn, Co, As, Sr, and Se into bile of SeD, normal, and SeC rats are shown in Figs. 2, 3,

Table 3 GSH-Px activity and concentration of rat liver

Rat group	<i>n</i>	GSH-Px (U/mg protein)	GSH (mmol/mg protein)
SeD	4	21 ± 7*	0.007 ± 0.003*
SeC	4	337 ± 18**	0.025 ± 0.02**
Normal	4	246 ± 35	0.016 ± 0.004

Values are expressed as means ± S.D.

n number of rats

* $p < 0.001$, significant difference between SeD and normal rats; ** $p < 0.05$, significant difference between SeC and normal rats

Table 4 Contents of elements in liver of 8-week-old rats

	<i>n</i>	Se	Fe	Co	Zn
SeD	3	–	329±15*	0.03±0.03*	102±10*
SeC	3	13.5±0.4**	163±15**	0.05±0.01**	60±27**
Normal	3	4.9±0.7	557±7	0.58±0.06	133±18

Values are means±S.D. expressed as mg/kg

n number of rats

p*<0.001, significant difference between SeD and SeC rats; *p*<0.001, significant difference between SeC and normal rats

and 4, respectively. The radioactivity of ⁵⁹Fe and ⁶⁵Zn in every bile fraction of the three rat groups were not sufficient to calculate the Fe and Zn excretion under the present condition. Biliary excretion of Fe and Zn which was markedly dependent on the dosage has been reported [1]. Using MT technique in the present study, the radioactivity of each nuclide corresponding to an element is 1/10 of that used in the earlier study. Hence, the dosage of an element is estimated to be low.

The cumulative excretion curves of the essential trace elements obtained in the bile of SeD, normal, and SeC rats showed characteristic features. The cumulative excretion of each radioactive tracer in the bile of SeD rats (Fig. 2) is lower than that of normal and SeC rats, although the ratio of the fastest cumulative excretion to the lowest at 20 and 120 min after administration varied across two orders of magnitude. That is, for SeD rats, the fastest and slowest rates were found to be 0.002% dose for Mn, 0.0002% dose for As at 20 min after administration, 15.16% dose for Sr, and 0.17% dose for V at 120 min after administration.

In the case of normal rats, the fastest and slowest excretions were found to be 0.14% dose for As, 0.02% dose for V at 20 min after administration, and 8.5% dose for Mn and 1.2% dose for V at 120 min. As shown in Fig. 3, the ratios of the fastest to lowest cumulative excretions were within an order of magnitude.

In the case of SeC rats Fig. 4, the fastest and slowest excretions were 3.51% dose for As, 0.08% dose for V at 20 min after administration, 28.5% dose for As, and 1.50% dose for V at 120 min. The excretion of all elements tested was accelerated for SeC rats, as compared with that of normal and SeD rats.

Table 5 Contents of elements in bile

	<i>n</i>	Se	Fe	Co	Zn
SeD	7		19.5±0.9	0.038±0.002	0.53±0.05
SeC	8	1.06±0.12	20.5±6.3	0.04±0.01	0.41±0.05
Normal	7		18.5±2.7	0.034±0.005	0.42±0.06

Values are expressed as mg/kg and means±S.D.

n number of rats

The biliary excretion of all elements decreased for SeD rats, and there were large fluctuations in the excretion. In other words, the difference between the fast and the slow rates was large for SeD rats, whereas it was small for normal and SeC rats.

The cumulative excretions of the essential trace elements at 120 min are depicted for all groups in Fig. 5, which plots only the data at 120 min. Clearly, the cumulative excretions of all elements are fastest for SeC rats, intermediate for the normal, and slowest for SeD rats, respectively.

Discussion

Bile consists of biosynthesized materials in liver, bile acid salts, cholesterols, phospholipids, bile dyes, electrolytes, proteins, and mostly water. Bile owes its functions mainly to bile salts, which serve to emulsify and solubilize lipids with their surface-active actions and which play a role in the digestion and absorption of lipids. In addition, bile acts as a regulator of intestinal putrefaction, excretion of bile dyes, hormone, drugs, and heavy metals.

In the present study, the excretion of V, Mn, Co, As, Sr, and Se into bile was examined with MT and INAA techniques. Sr accumulated in bone marrow after administration and was not distributed in other tissues and/or organs of both normal and SeD rats, and nearly 70% of Sr was excreted into feces within 1 day [20, 21]. Therefore, we suggest that most of the Sr was transferred through the blood flow after administration, and that the fractions other than those accumulated in bone were rapidly excreted into bile. As Sr is not directly related to Se deficiency, the reduction in its excretion rate is likely to be a result of the functional decline of the liver due to oxidative stress.

The extent of Mn excretion into bile was second only to As for SeC rats, and the difference among the three rat groups was large (Fig. 5). It has been reported that in normal rats, more than 95% of Mn is rapidly excreted into bile via the liver, and ultimately into feces [22–25]. It has also been reported that metals excreted selectively into bile are those which have a large ability for complexation with glutathione (i.e., with the thiol group), and that Mn is classified into such a category of

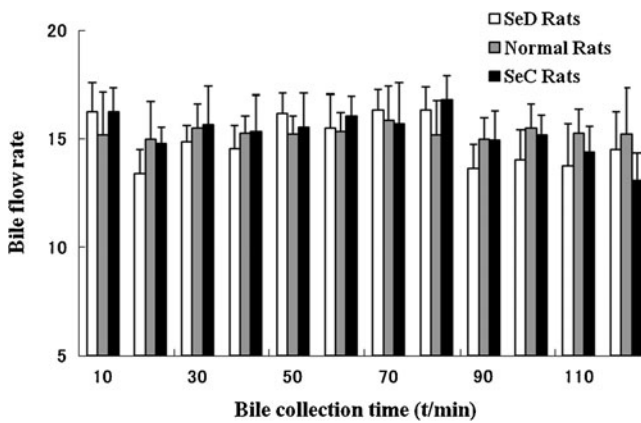


Fig. 1 Bile flow rate of Se-deficient (SeD), normal, and Se-control (SeC) rats. Values are means±S.D. for three rats in each group respectively

metals [5]. GSH concentrations increased in the liver and bile of SeC rats (Table 3) to favor the excretion of Mn into bile, whereas they decreased for normal and SeD rats.

Mutual detoxification effects have been reported between Se and As in animals and humans in many systems [26–29]. Uptake of one of the elements affects the distribution and metabolism of other elements [30–32]. Selenium deficiency decreases the rate of As methylation and/or the rate of As in bile excretion [33]. The As^{5+}/As^{3+} redox reaction and As methylation are linked to the detoxification of As. Normally, inorganic As^{5+} and As^{3+} occur as AsO_4^{3-} (arsenate) and AsO_3^{3-} (arsenite) in aqueous solutions. It has also been demonstrated in biochemical studies that the reductive metabolism of As and Se is highly related to the availability

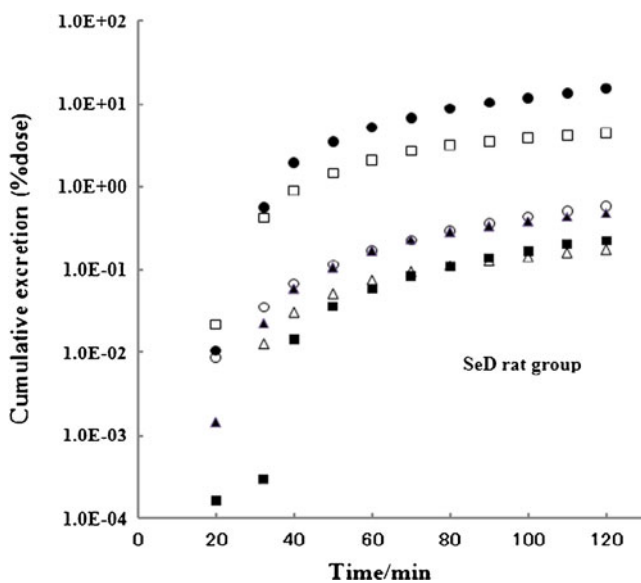


Fig. 2 Cumulative excretion (% dose) of Sr (filled circle), Mn (square), Se (circle), Co (filled triangle), As (filled square), and V (triangle) into the bile of Se-deficient rats after i.v. administration of multitracer. Values are means±S.D. for the three rats of the Se-deficient (SeD) group

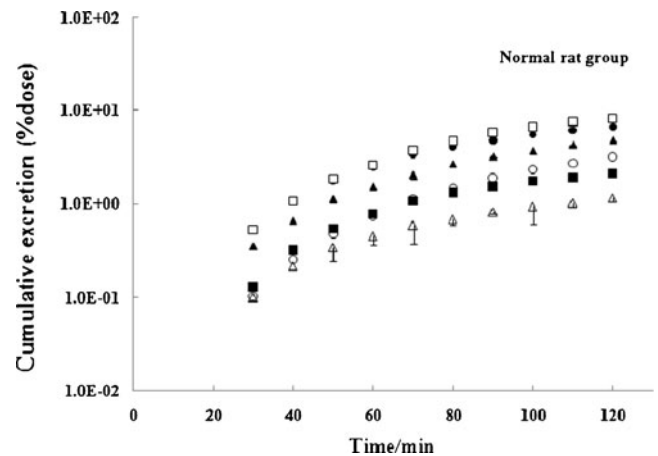


Fig. 3 Cumulative excretion (% dose) of Sr (filled circle), Mn (square), Se (circle), Co (filled triangle), As (filled square), and V (triangle) into bile of normal rats after i.v. administration of multitracer. Values are means±S.D. for the three rats of the normal rat group

of GSH [34, 35]. In fact, the GSH concentration in liver homogenates of SeD rats decreased (Table 2). The decrease in GSH concentration reduced the reduction rate of As^{5+} and led to the impairment of As methylation. Consequently, biliary excretion of As was slow for SeD rats and was enhanced for SeC rats.

It has been reported that V regulates the metabolism of sugar and lipids, and it plays a role in hematogenesis [36]. Similar to iron, which is the main component of erythrocytes, V is stored mainly in the liver. The insulin-like properties of V for the treatment of diabetes have been

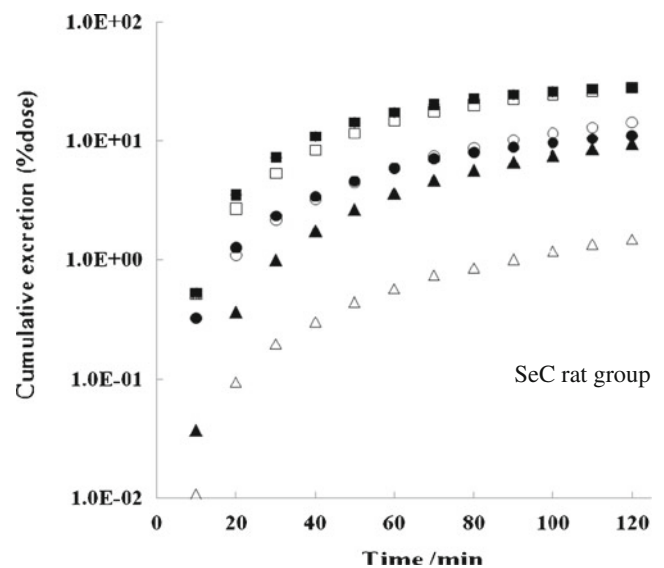


Fig. 4 Cumulative excretion (% dose) of Sr (filled circle), Mn (square), Se (circle), Co (filled triangle), As (filled square), and V (triangle) into bile of control rats after i.v. administration of multitracer. Values are means±S.D. for the three rats of the control rat (SeC) group respectively

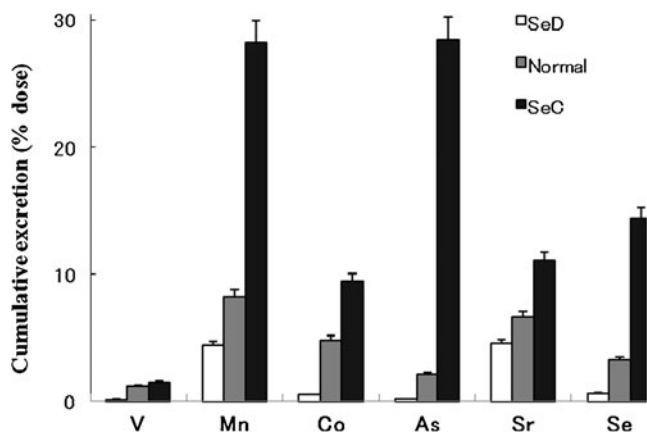


Fig. 5 Cumulative excretion (% dose) of V, Mn, Co, As, Sr, and Se into bile of SeD, normal, and SeC rats 120 min after i.v. administration of multitracer. Values are means \pm S.D. for the three rats in each respective group

studied by Sakurai and co-workers [37]. Earlier work had shown that the main route of V excretion is via urine, with lesser amounts lost via the feces [38]. Under decreased GSH concentrations, the reduction of V^{5+} to V^{3+} is decreased by a mechanism similar to that in the reduction of As^{5+} [39, 40]. Vanadium occurs in aqueous solutions as a tetravalent vanadyl ion (VO^{2+}) under reducing conditions and as a pentavalent vanadate anion (VO_3^- or VO_4^{3-}) under oxidizing conditions. In the body, the most common state in extracellular fluid is vanadate, while the predominant state in intracellular fluid is vanadyl ions. The present findings of decreased biliary excretion of V in liver of SeD rats support the notion that the reduction rate of $V(V)$ to $V(IV)$ is decreased via an intercellular GSH-mediated reduction mechanism, due to the reduced condition of GSH.

The MT data indicated that the rate of Co uptake into liver increased in order to supplement the insufficient contents of Co in the SeD diet [7]. It has also been reported that transcriptional induction of heme oxygenase by Coprotoporphyrin is induced [41]. Cobalt has been suggested to play a role in the oxidative stress mediated by Se deficiency [42]. In the present study, however, the Co contents in the liver of SeD and SeC rats were very low (0.052–0.086 mg/kg) as compared with that of normal rats (Table 3). Therefore, under the present conditions, the contribution of Co to heme oxygenase is likely to be small for the oxidative stress of SeD rats.

It is known that Fe^{3+} binds to transferrin in blood and that the resulting complexes are transferred via blood circulation to the liver, spleen, and/or bone marrow, where Fe is stored as ferritin, or hemocytidine. In our previous report [43], the composition of lipids did not change in the bile of SeD and SeC rats. However, the concentration of bilirubin (the major metabolite of heme) decreased significantly in SeD rats, compared with SeC rats, in the reduction of heme oxygenase.

Consequently, oxidative degradation of heme is reduced, and hence Fe levels increase in the liver under Se-deficient condition. Heme-iron is metabolized in the liver, and the iron-ions are excreted into bile and reabsorbed at the duodenum. Heme metabolism is an important metabolic process for iron because many proteins contain heme as a prosthetic group. When these hemoproteins turn over, heme is not recovered, but degraded, and new heme is synthesized for replacements. This explains why newly administered ^{59}Fe (i.e., radioactive iron) was not observed in bile within the time range after i.v. administration examined in this study.

Zn is an active center in many enzymes in organisms and is involved in the regulation of metabolism and growth, as well as in the activation of hormones. The synthesis of GSH is impaired in the liver of SeD rats. Heavy metals such as Cu, Zn, and Cd induce metallothionein where GSH is sufficiently present. GSH concentration in the liver of SeD rat was impaired, less than that of normal and SeC rats (Table 2). Thus, the induction of metallothionein is reduced in the liver of SeD rats. Metallothionein is an essential protein which contributes to detoxification and homeostasis of heavy metal ions. In this study, drinking water containing a mixture of seleno-L-methionin, Na_2SeO_3 , and Na_2SeO_4 was used as a supplement for SeC rats. Among them, reducible SeO_4^{2-} can release Zn ions from Zn-metallothionein through the oxidation of thiol groups [44]. A portion of the released Zn will be excreted into bile. Consequently, it may be recognized in chronic conditions that the Zn level in the liver of SeC rats is lower than that in SeD rats, despite the fact that SeD and SeC rats are fed the same diet (Table 3). This is supported by our previous results on Se supplemented with Na_2SeO_4 for control [11]. In this study, ^{65}Zn observed in the bile of three rat groups within 2 h of administration was not statistically robust. These comparisons suggest that newly administered ^{65}Zn is stored and/or used for synthesizing Zn finger proteins as well as Zn-metallothionein in the liver. In addition to the biochemical reasons described above, the radioactivity levels of ^{59}Fe and ^{65}Zn injected into a rat were an order of magnitude smaller than those used in an earlier study [6], and the biliary excretion of the two elements was dose dependent. Therefore, ^{59}Fe and ^{65}Zn were not detected in the present study.

Conclusions

In this study, the contents of Fe, Co, Zn and Se in the liver and bile of SeD, normal, SeC rats were analyzed with INAA. In addition, V, Mn, Co, As, Sr, and Se in the bile of the three rat groups were analyzed using the MT technique. Under Se-deficient conditions, the excretion of all six elements decreased compared with that for normal rats. The radioactivity of ^{59}Fe and ^{65}Zn in the MT solution was

insufficient to analyze their behaviors. The decline in the biliary excretion rate of Mn and As was explained by the depleted complexation ability of the metal ions with GSH in SeD rats, due to the reduced biosynthesis of GSH. In contrast, under Se-adequate conditions in the liver of SeC rats, biliary excretion of Mn and As was enhanced. The finding supports the notion that complexation of Mn with GSH contributed to the acceleration of excretion. The reduction of As⁵⁺ to As³⁺ favored to promote of methylation with GSH under Se-adequate conditions in SeC rats, which accelerated the biliary excretion of As. Similarly, biliary excretion of V in rats of three different Se statuses was explained by the GSH-mediated reduction mechanism.

Acknowledgments We are grateful to the staff of the RIKEN Ring Cyclotron for their help with heavy-ion irradiation, and also the staff of the Open Laboratory, The University of Tokyo, for the use of the JRR-4 nuclear reactor at the Japan Atomic Energy Agency (Tokai, Japan). The work was supported by a grant-in-aid for the Scientific Research C from the Japan Society for promotion of Science.

References

- Gregus Z, Klassen CD (1986) *Toxicol Appl Pharm* 85:24–38
- Ishihara N, Matsushiro T (1986) *Arch Environ Health* 41:324–330
- Dijkstra M, Kuopers F, Smit EP, Havinga R, Vonk RJ, Lab L (1993) *Clin Med* 121:751–758
- Sternlieb I (1982) Pathobiology of metals. In: Arias I, Poper H, Schachter D, Shafriz DA (eds) *The liver: Biology and Pathology*. Raven Press, New York, pp 385–392
- Balloatori N (1991) *8/4 Prog Pharmacol Clin Pharmacol* 283–306
- Marjan D, Folkert K, Smit K, Egbert P, Vries DE, Jeanette J, Rick H, Roel V (1991) *J Hepatol* 13:12–19
- Matsumoto K, Inagaki T, Hirunuma R, Enomoto S, Endo K (2001) *Anal Sci* 17:587–591
- Matsumoto K, Kawahigashi H, Hirunuma R, Enomoto S, Endo K (2002) *Yakugaku Zasshi* 122:277–282
- Matsumoto K, Yamazaki M, Satoh K, Ushio F, Endo K (2002) *Yakugaku Zasshi* 122:283–290
- Matsumoto K, Hirunuma R, Enomoto S, Endo K (2005) *Biol Pharm Bull* 28:2029–2034
- Matsumoto K, Ariyoshi M, Terada S, Okajo A, Urata H, Sakuma Y, Satoh K, Ushio F, Tsukada M, Endo K (2006) *J Health Sci* 52:694–702
- Halliwel B (1987) *FASEB J* 1:358–364
- Mckenzie RC, Rafferty TS, Beckett TS (1998) *Trends Immunol Today* 19:342–345
- Rayman MP (2000) *Lancet* 356:233–241
- Paglia DE, Valentine WN (1967) *J Lab Clin Med* 70:158–169
- Griffith WO (1980) *Anal Biochem* 106:207–212
- Yanaga M, Enomoto S, Hirunuma R, Furuta R, Endo K, Tanaka A, Ambe S, Tozawa M, Ambe F (1996) *Appl Radiat Isot* 47:235–240
- Sotogaku N, Endo K, Hirunuma R, Enomoto S, Ambe S, Ambe F (1999) *J Trace Elem Med Biol* 13:1–6
- Hirunuma R, Endo K, Yanaga M, Enomoto S, Ambe S, Tanaka A, Tozawa M, Ambe F (1997) *Appl Radiat Isot* 48:727–733
- Crespo A, Maria N, Jean P, Ruy E (1993) *Biol Trace Elem Res* 38:139–147
- Papworth DG, Patrick G (1994) *J Physiol* 210:999–1020
- Archibald FS, Tyree C (1987) *Arch Biochem Biophys* 256:638–650
- Hancock RG, Evans DJR, Fritze K (1973) *Bochim Biophys Acta (G)* 320:486–493
- Scheuhammer AM, Cherian MG (1985) *Bochim Biophys Acta* 840:163–169
- Papavasiliou PS, Miller ST, Cotzias GC (1966) *Am J Physiol* 211:211–216
- Glatte E, Mravcova A, Lener J, Vobecky M, Egertova E, Myslivcckova M (1995) *Biol Trace Elem Res* 49:177–186
- Zeng H, Uthus EO, Combs GF Jr (2005) *J Inorg Biochem* 99:1269–1274
- Lavender OA (1977) *Health Perspect* 19:159–164
- Zakharyan RA, Aposhian HV (1999) *Toxicol Appl Pharmacol* 154:287–291
- Zeng H (2001) *Biol Trace Elem Res* 83:1–15
- Walton FS, Waters SB, Jolley SL, LeCluyse EL, Thomas DJ, Styblo M (2003) *Chem Res Toxicol* 16:261–265
- Csanaky I, Gregus Z (2002) *Comp Biochem Physiol C131:355–365*
- Gregus Z, Nemeti B (2007) *Toxicol Sci* 100:44–53
- Gailer J (2007) *Coord Chem Rev* 251:234–254
- Balazs N, Zoltan G (2007) *Toxicol Sci* 100:36–43
- Meyerovitch J, Farfel Z, Sack J, Shechter Y (1987) *J Biol Chem* 262:6658–6662
- Sakurai H (2007) *Biomed Res Elem* 18:241–248
- Wiegmann TB, Day HD, Patak RV (1982) *J Toxicol Environ Health* 10:233–245
- Scibior A, Zaporowska H (2007) *J Toxicol Environ Health Part A* 70:696–704
- Kobayashi K, Himeno S, Satoh M, Kuroda J, Shibata N, Seko Y, Hasegawa T (2006) *Toxicology* 228:162–170
- Mitani K, Fujita H, Fukuda Y, Kappas A, Sassa S (1993) *Biochem J* 290:819–825
- Okuno T, Yasaka Y, Okitsu K, Hasegawa G, Ido R, Ueno H, Tanaka M, Nakamuro K (2003) *Biomed Res Trace Elem* 14:47–56
- Sakuma Y, Sasaki J, Futami A, Yamasaki K, Matsuoka K, Honda C, Endo K, Tsukada M (2007) *Chem Phys Lipids* 148:70–76
- Chen Y, Maret W (2001) *Antioxid Redox Signal* 3:651–656