

# Feeding Period of Selenium-Deficient Diet and Response of Redox Relating Minerals

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Dynamics of redox relating biotrace elements, selenium (Se), iron (Fe), and zinc (Zn) in liver, kidney, and spleen of selenium deficient Wistar male rats in a series of feeding period (from 0 to 8 weeks) were studied using instrumental neutron activation analysis (INAA). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN) for the plasma fraction of the rat bloods and the concentration of vitamin C and vitamin E in the liver homogenates were measured. The initial purpose of this study was to find Fe and Zn as sensitive indices of the tissue oxidative stress levels. However, the relationships among the biotrace elements and the oxidative stress/injury were much complicated. Control group, which was fed Se-deficient diet with Na<sub>2</sub>SeO<sub>4</sub> (0.1 mg selenium/l) in drinking water, showed strange response of Se and Zn contents in the kidney and showed high BUN. Supplementation of inorganic Se by biased Se source may serve as another source of a stress especially in the kidney. The Fe and Zn contents in the liver and kidney look sensitive to the Se-deficiency and/or relating oxidative stresses. Short term exposure to the Se-deficiency appeared to consume Fe and Zn in the liver and kidney. In contrast, long term or chronic exposure to Se-deficiency appeared to accumulate Fe and Zn in liver and kidney.

**Key words** — instrumental neutron activation analysis, bio-trace element, mineral, vitamin, oxidative stress

## INTRODUCTION

The relationship between oxidative stresses and the dynamics of minerals such as selenium (Se), iron (Fe), and zinc (Zn), have been studied using a Se-deficient (SeD) rat model, which had been fed torula yeast base SeD diet from pregnant mother.<sup>1,2)</sup> Se, which has important roles of the redox regulation system in our body, is an essential trace element. Se-deficiency causes a malfunction of glutathione peroxidase (GSH-Px). Consequently, Se-deficiency caused oxidative stress due to increasing hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>3)</sup> The oxidative stress caused in SeD rat, however, is not a lethal stress contrarily to the marked inactivation of the GSH-Px. The SeD model rat can live more than 50 weeks, although they have almost no GSH-Px throughout their life.<sup>4)</sup> Ultimately the GSH-Px is collaborating with other antioxidative enzymes and/or antioxidants to buffer severe oxidative stresses.

Under the condition of lacking GSH-Px activity, H<sub>2</sub>O<sub>2</sub> levels increased when severe oxidative stress was added. Inactivity of GSH-Px can enhance the effect of oxidative stress. In other words, inactivity of GSH-Px itself does not give any oxidative stresses. Therefore, the level of oxidative stress caused in the SeD rat model may depend on how the rat was exposed to the stress. Basic metabolism can synthesize reactive oxygen species (ROS), such as superoxide (O<sub>2</sub><sup>•-</sup>), which is dismutated to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> by SOD. Therefore, the Se-deficiency can give a weak chronic oxidative stress due to such basic metabolisms, while H<sub>2</sub>O<sub>2</sub> generation in SeD condi-

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tion may be basted by an additional severe oxidative stress.

Due to the buffering mechanism of oxidative stress in organs, relatively weak oxidative stress may not appear to be visible signal. However, a severe oxidative stress can be a lethal for tissue cells and can show direct visible signal of emergency in such an unbalanced redox status. In addition, the weak chronic oxidative stress may be accumulated in the unbalanced redox system gradually and may finally cause aging, cancer, or other lethal diseases. Therefore, finding those hiding sources of oxidative stress can provide a possible prevention of spontaneous diseases.

Variation of Fe and Zn contents in several organs of SeD and vitamin E deficient rats was reported.<sup>2)</sup> It was considered that variation of Fe and Zn in the liver of SeD rats can be caused as a results of sensitive response to the oxidative stress. Contents of Fe and Zn in the tissues may be related to each other in the *in vivo* redox system.

Several rat groups of different oxidative stress levels were prepared by various feeding period of SeD diet before 8 weeks of age. Different feeding period of SeD diet causes various H<sub>2</sub>O<sub>2</sub> level in the bile.<sup>3,5)</sup> The effect of feeding period of SeD diet, *i.e.* several oxidative stress levels in the body, to the contents of Fe and Zn was tested among several rats groups. The Se, Fe, and Zn contents in liver, kidney, and spleen were measured simultaneously by the instrumental neutron activation analysis (INAA). Biological data, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN) for the plasma fraction of the

rat bloods, and the concentration of vitamin C and E in the liver homogenates were measured and compared with the mineral contents. The initial purpose of this study was to test Fe and Zn as an index depending on the *in vivo* oxidative stress level. The current opinion of nutritional interactions of minerals, period of stressing, and relation of oxidative stress were described.

## MATERIALS AND METHODS

**Animals** ——— Wistar rats which were on 15th day after pregnancy were fed on torula yeast based SeD diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and ultra pure water. Table 1 summarizes analytical values of contents of minerals and vitamins in normal and SeD diet used in this paper. Newly born rats were kept with own mother rat for four weeks. Four weeks after birth, young rats were weaned, and then fed on SeD diet and ultra pure water until experiments. The male rats were used for experiment at 8 weeks of their age as SeD groups. Se-control (SeC) rats were bred under identical condition of SeD rats except that Na<sub>2</sub>SeO<sub>4</sub> (0.1 mg selenium/l) was added to their ultra pure water. Normal 3, 4, 5, 6, and 7 week old male Wistar rats were purchased and fed by SeD diet for 5, 4, 3, 2, and 1 weeks, respectively. Until purchasing, those rats had been fed on CE-2 (CLEA Japan, Inc., Tokyo), which we regard as normal diet in this paper. The rats are used as SeD'3-8, SeD'4-8, SeD'5-8, SeD'6-8, and SeD'7-8 group at 8 weeks of their age. Normal 8 week old male Wistar rats were purchased and used as the control for SeD'7-

**Table 1.** Contents of Several Elements and Vitamins in Diets

	Method	Se-deficient diet	Normal diet
Se (mg/kg)	INAA	0.017 ± 0.002 <sup>b)</sup>	0.86 ± 0.13 <sup>a)</sup>
Mn (mg/kg)	INAA	105 ± 4.4 <sup>a)</sup>	516 ± 25 <sup>a)</sup>
Fe (mg/kg)	INAA	591 ± 64.6 <sup>b)</sup>	482 ± 5.6 <sup>a)</sup>
Co (mg/kg)	INAA	0.029 ± 0.002 <sup>b)</sup>	0.9 ± 0.02 <sup>a)</sup>
Cu (mg/kg)	AAS <sup>c)</sup>	8.36 ± 1.11 (2)	16.99 ± 0.18 (2)
Zn (mg/kg)	INAA	64.5 ± 3.87 <sup>b)</sup>	62 ± 1.8 <sup>a)</sup>
VC (mg/kg)	PC-HPLC UV	30.27 ± 0.00 (2)	111.67 ± 5.77 (3)
	<i>Values from the Catalog</i>	<i>50 mg/kg</i>	<i>190 mg/kg</i>
VE (mg/kg)	HPLC UV	103.05 ± 6.22 (2)	39.2 ± 5.05 <sup>b)</sup>
	<i>Values from the Catalog</i>	<i>102 mg/kg</i>	<i>70 mg/kg</i>

The Se-deficient diet is torula yeast based Se-deficient diet (Oriental Yeast Co., Ltd., Tokyo, Japan). The normal diet is CE-2 (CLEA Japan, Inc., Tokyo, Japan). Values indicated as mean ± S.D. Numbers in parenthesis are number of samples. Values marked by *a)* and *b)* are referred from our previous reports.<sup>1,2)</sup> Values in *italic* are calculated from printed value or the printed value in the catalog. *c)* Atomic absorption spectrophotometry.

8, SeD'6-8, DeD'5-8, SeD'4-8, and SeD'3-8 groups. The animal experiments were carried out in compliance with the Guidelines for Animal Care and Use at Showa Pharmaceutical University (2001), and approved by the Ethical Committee for Animal Care and Use of Showa Pharmaceutical University.

**Sample Preparation** — The rats were anesthetized by 50 mg/kg b.w. intraperitoneally injection of Nembutal (Dinabot, Osaka, Japan). Whole blood was drawn from abdominal aorta and collected in a test tube. Then whole body was perfused by ice cooled saline (0.9 % NaCl). Liver, kidney, and spleen were removed. After perfused solution was spoiled by paper, each organ was weighed. The organs were mixed within each group ( $n = 5$ ) and homogenized with a 4-fold volume of ultra pure water ( $\times 5$  homogenate) and kept in  $-30^{\circ}\text{C}$  until frozen. The frozen samples were lyophilized and ground into powder. An aliquot (approximately 100 mg) of the powdered sample was weighed and sealed in quartz tube. The Standard Reference Material 1577b (bovine liver) obtained from the National Institute of Standards and Technology was used to quantify the inorganic elements.

**Neutron Activation and Data Analysis** — Neutron irradiation was carried out for 1 hr in a JRR-4 nuclear reactor D-pipe (flux of thermal neutron =  $4.3 \times 10^{13}$  n/cm<sup>2</sup>s) at the Japan Atomic Energy Research Institute (Tokai, Ibaraki, Japan). The irradiated samples were cooled down at least for 1 week. The  $\gamma$ -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, Chiba, Japan). <sup>75</sup>Se, <sup>59</sup>Fe, and <sup>65</sup>Zn were analyzed by the photopeaks at 264.6, 1098.6, and 1115.4 keV respectively.<sup>1,2)</sup>

**Copper in the Diet** — The chemical reagents, 68% HNO<sub>3</sub>, HClO<sub>4</sub>, used for sample digestion were TAMAPURE-AA-10, (ultra-pure grade reagents for chemical analysis). Working curve was made using standard solutions for atomic absorption (Wako Pure Chemicals, Tokyo). A sample of about 0.3–1.0 g was weighted accurately, and was decomposed in 10 ml of HNO<sub>3</sub> with a microwave digestion system (MDS-2000, CEM Corporation, Matthews, NC, U.S.A.). Then the solution was transferred to a Teflon beaker, and heated in a 5 ml mixed acid solution of HClO<sub>4</sub> and HNO<sub>3</sub> (1 : 4) on a hot plate. The procedure was repeated to complete dissolution when it was necessary. After being heated to dryness, the sample was dissolved in 1% HNO<sub>3</sub> solution, and subjected for analysis. Copper was assayed with an

atomic absorption spectrophotometer (AA-62501, Shimadzu, Kyoto, Japan).

**Liver GSH-Px Activity** — Five rats from each group were fastened for 24 hr before the experiment and sacrificed by decapitation. The liver was perfused with ice-cold physiological saline (0.9% NaCl) until the blood was sufficiently removed. The liver was removed and homogenized with a 4-fold volume of ultra pure water. The liver homogenate was diluted by water to 1/125–1/1250. GSH-Px activity in the liver homogenate was measured based on the method described by Paglia and Valentine<sup>6)</sup> with some modifications. 200  $\mu\text{l}$  of PBS (pH 7.4), 50  $\mu\text{l}$  20 mM NaN<sub>3</sub>, 50  $\mu\text{l}$  of 40 mM glutathione, 50  $\mu\text{l}$  of 20 U/ml glutathione reductase, and 50  $\mu\text{l}$  of 4 mM NADPH were added in a microtube. A portion (500  $\mu\text{l}$ ) of the diluted liver homogenate was added into the reaction mixture. The reaction was started by adding 100  $\mu\text{l}$  of 1.0 mM H<sub>2</sub>O<sub>2</sub>. Time course of the absorption at 340 nm (NADPH) of the reaction mixture was measured. The GSH-Px activity was calculated from slope of the plot of absorption with time. Consumption of 1  $\mu\text{mol}$  NADPH per minute was converted as 1 U of GSH-Px activity. The GSH-Px activity were standardized with protein concentrations and expressed as U/mg protein. The protein concentration of the liver homogenate was measured using Bio-Rad Protein Assay Kit (Bio-Rad laboratories, Hercules, CA).

**ALT, AST, and BUN in Plasma** — The collected blood was centrifuged at  $1000 \times g$  for 10 min. An aliquot of the plasma fraction was tested for ALT, AST, and BUN, performed by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo).

**Vitamin E** — Vitamin E ( $\alpha$ -tocopherol) concentration in diets and liver homogenates were determined by HPLC-based methods using 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC) as the internal standard.<sup>7)</sup> A mixture of 400  $\mu\text{l}$  of  $\times 50$  diluted homogenates of diet samples or  $\times 5$  homogenates of liver sample and 1 ml of 6%(w/v) pyrogallol ethanol solution was stored at  $-20^{\circ}\text{C}$  until of analysis of vitamin E. Then, 200  $\mu\text{l}$  of 60%(w/v) KOH solution and 1 ml of ethanol solution containing 0.3  $\mu\text{g}$  PMC was added and the mixture was saponified with occasional shaking at  $70^{\circ}\text{C}$  for 30 min. After cooling in ice water, 4.5 ml of 1%(w/v) NaCl solution was added to the saponified medium. The medium was shaking vigorously with 3 ml of 10% ethylacetate in n-hexane for 1 min. The mixture was centrifuged at  $2500 \times g$  for 5 min, and 2 ml of upper layer was evaporated under nitrogen flow at  $35^{\circ}\text{C}$ .

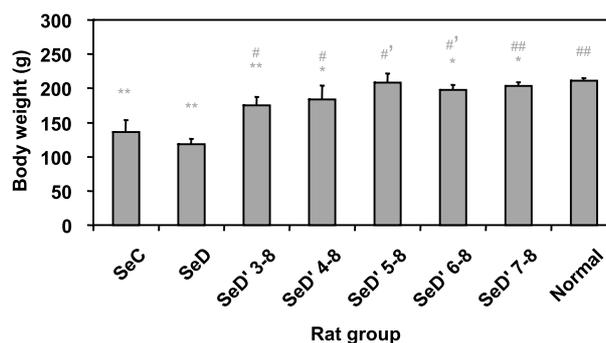
The residue was dissolved in 300  $\mu$ l of n-hexane and analyzed by HPLC. Conditions of HPLC were follows; the basic HPLC system was Model CCPM-II Series (Tosoh, Tokyo), column was Cosmosil 5NH-MS (4.6 mm i.d.  $\times$  250 mm length, Nacalai Tesque Inc., Kyoto, Japan), mobile phase was n-hexane-isopropyl alcohol (97 : 3 v/v) flow rate was 1.0 ml/min, detector was FS-8010 fluorescence detector (Tosoh), the detection wavelength was set at 298 nm for excitation, and at 325 nm for emission respectively.

**Vitamin C** — Vitamin C (ascorbic acid) concentration in diets and liver homogenates was determined by post-column HPLC (PC-HPLC) methods.<sup>8,9)</sup> A mixture of 100  $\mu$ l of 5-fold diluted homogenates of samples and 100  $\mu$ l 5% metaphosphoric acid was stored at  $-80^{\circ}\text{C}$  until the analysis of Vitamin C. Then, these samples were mixed with 200  $\mu$ l of 5% metaphosphoric acid and centrifuged at 12000  $\times$  g at  $4^{\circ}\text{C}$  for 5 min. 50  $\mu$ l of the supernatants obtained was subjected to the PC-HPLC. Conditions of HPLC were follows; column was Shimpac SCR-101N (7.8 mm i.d.  $\times$  300 mm length, Shimadzu, Kyoto, Japan), mobile phase was 10 mM oxalic acid including 1 mM EDTA/Na, flow rate was 1.0 ml/min, reaction coil (type-J piping kit, Shimadzu) was worked at  $35^{\circ}\text{C}$  controlled by AS-8020 column oven (Tosoh), post-column reagent was 50 mM sodium borohydride ( $\text{NaBH}_4$ ) in 100 mM NaOH, flow rate was 0.5 ml/min, detector was UV-8020 (Tosoh) worked at 300 nm wavelength.

**Statistical Test** — The statistical differences were estimated with alternative Student's or Welch's *t*-test. The suitable test for the data was automatically selected according to variance of the data. Grades of significance were estimated by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ .

## RESULTS

Figure 1 shows body weight of each rat group. Body weights of rats fed SeD diet decreased. All groups except for SeD'5-8 had significance with normal group. However, no significant difference was obtained between SeD and SeC. All other rats were significantly heavier than SeC and SeD groups. Growth of rat was varied with the starting age of SeD diet. Consumption rates of the diets by 8 week old (56–62 day old) normal and SeD rats were  $0.11 \pm 0.01$  ( $n = 4$ ) and  $0.12 \pm 0.03$  ( $n = 10$ ) g/g b.w./day, respectively. Consumption rates of drinking water



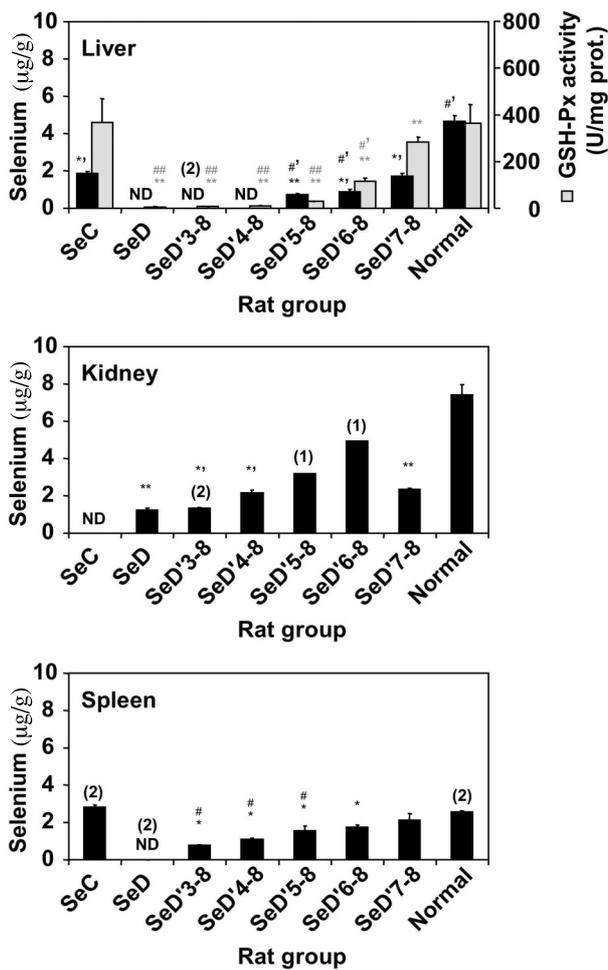
**Fig. 1.** Body Weight of Rat

Values are indicated as mean  $\pm$  S.D. Number of rats was 5 for all groups except number of rats in SeC group was 4. \* and \*\* indicate significance compared with the normal rat by  $p < 0.05$  and  $p < 0.001$ , respectively. #, #\* and ## indicate significance compared with SeC rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

(ultra pure water) were  $0.17 \pm 0.04$  for normal rat and  $0.18 \pm 0.02$  ml/g b.w./day for SeD rat. No marked difference of appetite was observed between 8 week old normal and SeD rats.

Figure 2 shows Se contents in liver, kidney, and spleen of each rat group. Gray column in A indicate GSH-Px activity in the rat livers. The liver GSH-Px activities showed a similar profile as liver Se contents. The liver Se contents of the SeC group was 2/3 of the normal group, while the GSH-Px activity in the SeC rat liver was recovered to the same level shown in the normal group. Se contents in the kidney decreased with period of feeding SeD diet except that 1/3 of normal level, which was same as SeD'4-8 group, was obtained by one week feeding from 7 to 8 week old. Although SeD group still showed Se in the kidney, no Se content was detected in SeC rat kidney. Se in spleen also decreased with feeding period of SeD diet. However, rate of decreasing seemed to be smaller. No Se was detected in the SeD rat spleen. SeC rat showed similar Se level as normal group.

Figure 3 shows Fe contents in liver, kidney, and spleen of each rat group. Fe contents in the liver decreased compared with normal level at 8 week old when SeD feeding started from 4 week old or later. While Fe contents in the liver increased when SeD feeding started from 3 week old or earlier. SeC group showed similar Fe level as normal group in the liver. The Fe contents in the kidney were relatively smaller compared with liver and spleen. Fe contents in the kidney of SeC and SeD'7-8 groups were lower than the normal level. SeD group showed higher Fe level than the normal group. Fe contents in spleen de-

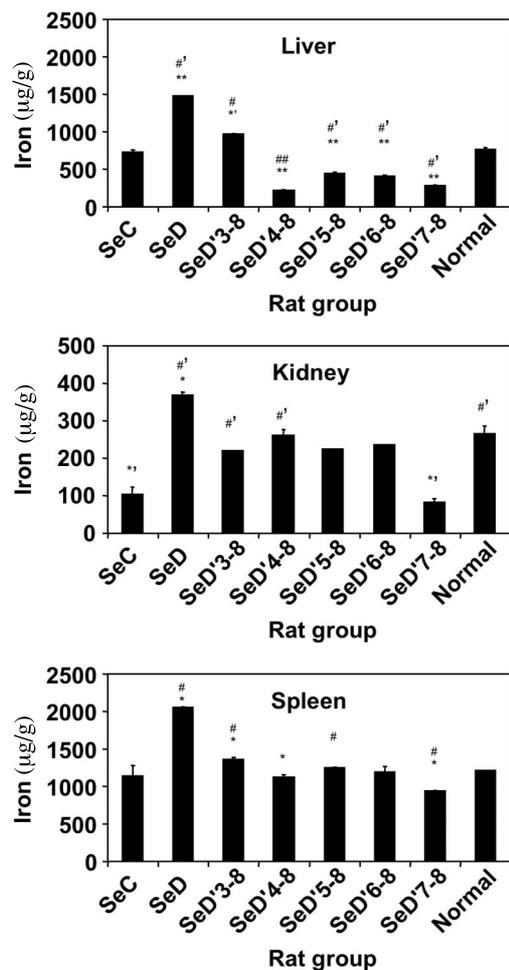


**Fig. 2.** Se Contents in Liver, Kidney, and Spleen of Each Rat Group

Contents of Se in (A) liver, (B) kidney, and (C) spleen were measured by INAA. The values are indicated by mean ± S.D. of 3 samples. Numbers in parenthesis indicate number of sample when the number of sample was not 3 by some reasons. ND indicates “not detected.” \*, \*’ and \*\* indicate significance compared with the normal rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. #, #’ and ## indicate significance compared with SeC rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. Gray column in A indicate GSH-Px activity in the rat livers. Values of GSH-Px activity were indicated as mean ± S.D. of 5 rats except that number of rats were 4 for SeC and normal groups.

creased when SeD feeding start from 7 week old or from 4 week old, while Fe contents increased when SeD feeding start from 3 week old or younger. Fe contents in spleen of SeC group were the same level of normal group.

Figure 4 shows Zn contents in liver, kidney, and spleen of each rat group. Zn contents in the liver showed similar pattern as shown in Fe in the liver. When SeD feeding started from 4 week old or later, Zn contents in the liver decreased compared with normal level. While Zn contents in the liver increased when SeD feeding started from 3 week old or ear-

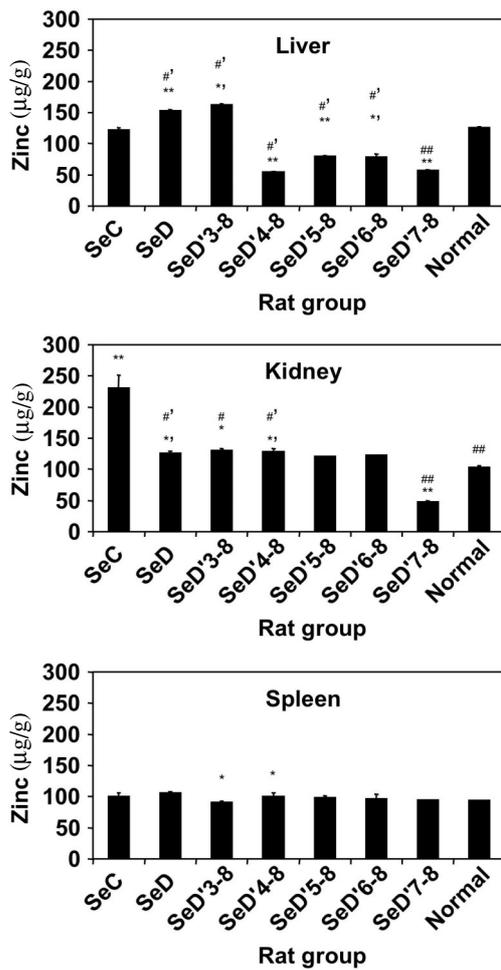


**Fig. 3.** Fe Contents in Liver, Kidney, and Spleen of Each Rat Group

Contents of Se in (A) liver, (B) kidney, and (C) spleen were measured by INAA. The values are indicated by mean ± S.D. of 3 samples. Numbers of samples were the same as Fig. 1. \*, \*’ and \*\* indicate significance compared with the normal rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. #, #’ and ## indicate significance compared with SeC rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

lier. Zn contents in kidney decreased in SeD’7-8 group, while other groups showed increase compared with the normal group. Increase on the kidney Zn in SeC group was markedly larger — almost double of the normal group. Zn contents in spleen did not affected by feeding period of the SeD diet.

Figure 5 shows AST, ALT, and BUN levels in the blood plasma of each rat group. AST of the SeC group showed almost same level with the normal group. Other groups showed slightly higher level than the normal group, but no significance was obtained. ALT of SeC, SeD’7-8, and SeD’6-8 groups showed smaller values than the normal group, while no significance was obtained. Other groups showed higher values than the normal group, while no sig-

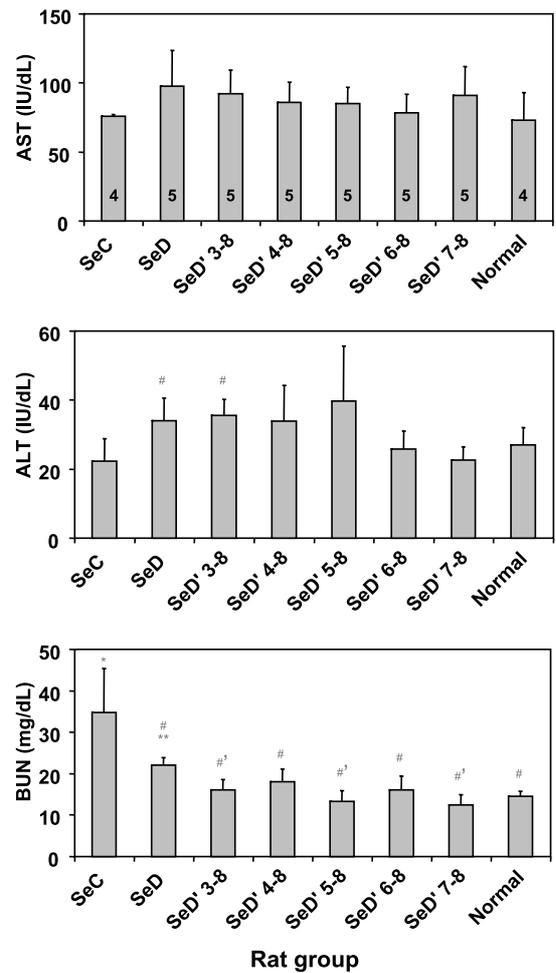


**Fig. 4.** Zn contents in Liver, Kidney, and Spleen of Each Rat Group

Contents of Se in (A) liver, (B) kidney, and (C) spleen were measured by INAA. The values are indicated by mean ± S.D. of 3 samples. Numbers of samples were same as Fig. 1. \*, \*' and \*\* indicate significance compared with the normal rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. #, #' and ## indicate significance compared with SeC rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

nificance was obtained with the normal group. SeD and SeD'3-8 groups had a significance with the SeC group. However, damages in the liver caused by Se-deficiency were small. BUN seemed to be increasing with period of feeding SeD diet. SeC and SeD groups showed significantly higher values compared with the normal group.

Figure 6 shows vitamin C and E contents in the liver of each rat group. Liver vitamin C levels of SeC and SeD groups were significantly lower than the normal level. Although the vitamin C levels of other groups were slightly lower than the normal level, no significance was obtained compared with the normal level. Liver vitamin E levels of SeC, SeD,



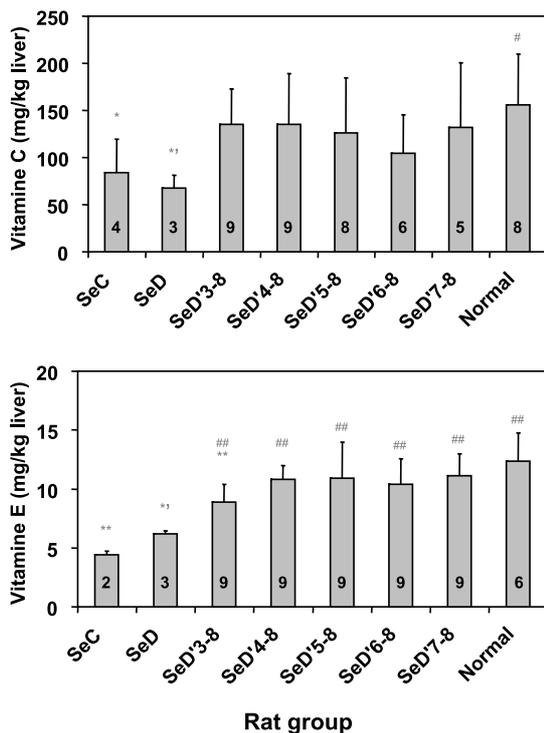
**Fig. 5.** AST, ALT, and BUN Levels in Blood Plasma of Each Rat Group

(A) AST, (B) ALT, and (C) BUN. The values are indicated by mean ± S.D. Numbers of samples were indicated on bottom of each bar. \*\* indicates significance compared with the normal rat by  $p < 0.01$ . # and #' indicate significance compared with SeC rat by  $p < 0.05$ , and  $p < 0.01$ , respectively.

and SeD'3-8 groups were significantly lower than the normal level. The vitamin E levels of other groups were also slightly lower than normal level, while there was no significance compared with the normal level.

## DISCUSSION

Initial purpose of this study was to find Fe and/or Zn contents in the tissue/organ as an index of *in vivo* oxidative stress levels. Therefore, liver, kidney, and spleen, which were able to be a target of biopsy test in the clinic, were tested in this paper. However, any simple relation was not found between the feed-



**Fig. 6.** Vitamin C and E Contents in Liver of Each Rat Group (A) vitamin C and (B) vitamin E. The values are indicated by mean  $\pm$  S.D. Numbers of samples were indicated on bottom of each bar. \*, \*\*, and \*\* indicate significance compared with the normal rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. #, #\*, and ## indicate significance compared with SeC rat by  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

ing periods, *i.e.* oxidative stress levels, and the mineral contents. The relationships among the bio-trace elements and the oxidative stress/injury were much complicated.

The SeD diet used in this experiment is an assembled diet based on tolura yeast with several additional nutrients (Table 1). The nutritional status of SeD diet may be lower than the normal diet. Contents of Co, Mn, and Cu in the SeD diet were 3, 20, and 50% the normal diet respectively. The SeD diet was considered as relatively a low nutritional status. However, contents of those minerals, such as Co, Mn, and Cu, in the diet were probably enough or excessive compared with the minimum recommended level. Role of Co is known only in terms of the effect of vitamin B<sub>12</sub>, and vitamin B<sub>12</sub> is added to the SeD diet at the recommended level (0.017 mg/kg diet was calculated from the value printed in the catalog, Oriental Yeast Co., Ltd., Tokyo). The Mn contents in the diets were 17–60 times higher than the Mn contents in the dried rat liver samples.<sup>1)</sup> Although Cu contents in the dried rat tissue samples have not been tested in our model, predicted Cu con-

tents in dried tissues (0.7–1.8 mg/kg) from a previous report<sup>10)</sup> is much less than contents in Cu in the diet (8–16 mg/kg).

The contents of vitamins in the normal diet might be decreased during keeping the diet at room temperature. The normal diet was fed from a bucket holding an aliquot of the normal diet in the animal room at room temperature, while the SeD diet was kept at 4°C until feeding. However, the levels of both vitamins were lower in the SeD rat liver compared to those of the normal rat liver. Other SeD' groups were kept both vitamin levels similar as the normal rat group, except that vitamin E in SeD'3-8 group was significantly lower than the normal group. Those results suggest that the levels of vitamin C and E were highly maintained, while the vitamin E level was slightly sensitive to the chronic oxidative stress.

The body weight of 8 week old SeC group showed no significant difference from SeD of same age. The body weights of both SeC and SeD groups were significantly smaller than the normal group. This result suggested that the low growth rate is due to low nutritional status of SeD diet rather than Se-deficiency. In addition, SeC group showed somewhat strange response of Se and Zn contents in the kidney. It can be predicted from high BUN value of SeC group that the kidney of SeC group is highly damaged. This result is supported by a fact that the markedly high acetoacetate level of the same 8 weeks old SeC model.<sup>11)</sup> Addition of inorganic Se to the SeD diet may make another stress and/or toxicity to the kidney. This toxicity may be due to ion form of Se in the blood, because Se did not detect in the kidney of SeC group. This experiment measured intracellular mineral contents, because of that the organs were enough perfused before removing and contribution from the blood was quite low.

In this study, the SeC group was administered selenate in the drinking water. The oxidized form of inorganic selenium, *i.e.* selenate, was expected to have a higher absorption when it was orally administered.<sup>12–14)</sup> However, protocol for the control to the SeD diet, *i.e.* Se supplement with SeD diet, is in progress. The chemical forms of minerals and/or vitamins may be important when considering its nutritional functions in the experimental animal model. Unfortunately INAA technique employed in this paper can quantitatively detect only amounts of nuclides (consequently elements) in samples. To investigate the mechanisms of increasing Zn, decreasing Se and Fe during the renal damaging process in the SeC group, information of chemical forms of

the elements, distribution of elements in the micro-environment in tissue cells, and detail time-course of damaging tissue and contents of elements is necessary.

Renal toxicity of selenium has been known. For example, sodium selenite administration was associated with increased incidences of renal papillary regeneration in rats.<sup>15)</sup> In addition, it is reported that extracellular selenite may affect to ion channels of cardiac muscles and change ion currents.<sup>16–18)</sup> Selenite is more toxic than selenate in general. The SeC model in this experiment was chronically exposed to selenate in drinking water with 0.1 mg selenium/l from the pregnant of their mother. However, we can not say which form of selenium is causing renal damages from the results in this paper. The selenate was reduced to selenite or vice versa *in vivo*, and reaction may be equilibrated in the SeC rat.

Combination of Cu, Zn, Fe, and Se has been widely used as indices of biological function against several stresses. Redistribution of Cu and Zn to the liver and Se to the kidney following burn injury was reported.<sup>19)</sup> Related metallo-enzyme (SOD for Cu and Zn, catalase for Fe, and GSH-Px for Se) activities also widely used to investigate redox status and/or oxidative stress. Catalase and GSH-Px activities in lymphocytes were increased after maximal exercise, but SOD activities reduced.<sup>20)</sup> However, simultaneous measurement of Cu with other elements by INAA method is technically difficult due to absence of suitable nuclide.<sup>1)</sup> Cu was measured only for the diets by atomic absorption spectrophotometry in this paper. Interaction of enzymatic Se and Cu is in progress. From result in this paper, an indirect relation between enzymatic Se, Fe, and Zn was considered.

Dynamics of Fe and Zn in the liver and kidney appear to have some relation with Se-deficiency. However, contents of Fe and Zn in the spleen looked more stable. Short term exposing to the Se-deficiency, *i.e.* starting SeD diet 4 week old or later, appeared to consume Fe and Zn in the liver. Long term exposing to Se-deficiency, *i.e.* starting SeD diet 3 week old or earlier, appeared to accumulate Fe and Zn in liver. In addition, 1 week exposing to Se-deficiency markedly decrease Fe and Zn in the kidney. However, starting SeD diet 6 week or earlier showed Fe levels in kidney slightly lower than the normal level, except Fe in the kidney of the SeD group was increased. Starting SeD diet 6 week old or earlier kept Zn levels slightly higher than the normal level. The different period of feeding SeD diet showed

several unexpected effects. Oxidative stress may consume Fe and Zn in earlier stage, and then accumulate them in later stage. Such variations of Fe and Zn may be due to regulation and/or buffering of oxidative stresses, and liver may be damaged relatively low.

In conclusions, age of starting SeD diet feeding can affect later growth of rats. SeC group showed somewhat strange response of Se and Zn contents in the kidney and showed high BUN. Therefore, supplementation of inorganic Se by the biased Se source may give another stress to the kidney. The Fe and Zn contents in the liver and kidney look sensitive to the Se-deficiency and/or relating oxidative stresses. Short term exposing to the Se-deficiency appeared to consume Fe and Zn in the liver and kidney. Long term exposing to Se-deficiency appeared to accumulate Fe and Zn in liver and kidney.

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## REFERENCES

- 1) Matsumoto, K., Inagaki, T., Hirunuma, R., Enomoto, S. and Endo, K. (2001) Contents and uptake rates of Mn, Fe, Co, Zn, and Se in Se-deficient rat liver cell fractions. *Anal. Sci.*, **17**, 587–591.
- 2) Matsumoto, K., Yamazaki, M., Satoh, K., Ushio, F. and Endo, K. (2002) Measurement of contents in organs of selenium- or vitamin E-deficient rat using instrumental neutron activation analysis. *Yakugaku Zasshi*, **122**, 283–290.
- 3) Ueda, Y., Matsumoto, K. and Endo, K. (2000) Evidence of hepatic endogenous hydrogen peroxide in bile of selenium-deficient rats. *Biochem. Biophys. Res. Commun.*, **271**, 699–702.
- 4) Matsumoto, K., Terada, S., Ariyoshi, M., Okajo, A., Hisamatsu, A., Ui, I. and Endo, K., (unpublished result).
- 5) Matsumoto, K., Ui, I., Mochida, T., Sakamoto, R. and Endo, K. (2002) Relation of bile hydrogen peroxide level and liver superoxide dismutase activity in selenium deficient rats. *Yakugaku Zasshi*, **122**, 407–411.

- 6) Paglia, D. E. and Valentine, W. N. (1967) Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. *J. Lab. Clin. Med.*, **70**, 158–169.
- 7) Ueda, T., Ichikawa, H. and Igarashi, O. (1993) Detection of  $\alpha$ -tocopherol stereoisomers in biological specimens using chiral phase highperformance liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **39**, 207–219.
- 8) Ito, T., Murata, H., Yasui, Y., Matsui, M., Sakai, T. and Yamauchi, K. (1995) Simultaneous determination of ascorbic acid and dehydroascorbic acid in fish tissues by high-performance liquid chromatography. *J. Chromatogr. B: Biomed. Appl.*, **667**, 355–357.
- 9) Ushio, F., Yokoyama, T., Ueta, T. and Ichikawa, H. (1997) Determination of vitamin C in foodstuff by HPLC method with post-column derivatization. *Toukyo Eiken Nenpou*, **48**, 190–193.
- 10) Chareonpong-Kawamoto, N. and Yasumoto, K. (1995) Histological study of iron deposits in selenium-deficient rats. *Biosci., Biotechnol., Biochem.*, **59**, 1913–1920.
- 11) Matsumoto, K., Takuwa, A., Terashi, A., Ui, I., Okajo, A. and Endo, K. (2005) Correlation between keton body level in selenium-deficient rats and oxidative damages. *Biol. Pharm. Bull.*, **28**, 1142–1147.
- 12) Thomson, C. D., Stewart, R. D. and Robinson, M. F. (1975) Metabolic studies in rates of [ $^{75}\text{Se}$ ]selenomethionine and of  $^{75}\text{Se}$  incorporated in vivo into rabbit kidney. *Br. J. Nutr.*, **33**, 45–54.
- 13) Wolffram, S., Arduser, F. and Scharrer, E. (1985) In vivo intestinal absorption of selenate and selenite by rats. *J. Nutr.*, **115**, 454–459.
- 14) Arduser, F., Wolffram, S. and Scharrer, E. (1985) Active absorption of selenate by rat ileum. *J. Nutr.*, **115**, 1203–1208.
- 15) No authors listed (1994) NTP toxicity studies of sodium selenate and sodium selenite (CAS Nos. 13410-01-0 and 10102-18-8) administered in drinking water to F344/N rat and B6C3F1 mice. *Toxic. Rep. Ser.*, **38**, E1–E5.
- 16) Ugur, M., Ayaz, M., Ozdemir, S., and Turan, B. (2002) Toxic concentrations of selenite shortens repolarization phase of action potential in rat papillary muscle. *Biol. Trace Elem. Res.*, **89**, 227–238.
- 17) Ayaz, M., Ozdemir, S., Ugur, M., Vassort, G. and Turan, B. (2004) Effects of selenium on altered mechanical and electroical cardiac activities of diabetic rat. *Arch. Biochem. Biophys.*, **426**, 83–90.
- 18) Ayaz, M., Ozdemir, S., Yaras, N., Vassort, G. and Turan, B. (2005) Selenim-induced alteration in ionic currents of rat cardiomyocytes. *Biochem. Biophys. Res. Commun.*, **327**, 163–173.
- 19) Agay, D., Anderson, R. A., Sandre, C., Bryden, N. A., Alonso, A., Roussel, A. M. and Chancerelle, Y. (2005) Alterations of antioxidant trace elements (Zn, Se, Cu) and related metallo-enzymes in plasma and tissues following burn injury in rats. *Burns*, **31**, 366–371.
- 20) Tauler, P., Aguilo, A., Gimeno, I., Guix, P., Tur, J. A. and Pons, A. (2004) Different effects of exercise tests on the antioxidant enzyme activities in lymphocytes and neutrophils. *J. Nutr. Biochem.*, **15**, 479–484.