Comparison of selenium level and glutathione peroxidase activity in tissues of vitamin B_6-deficient rats fed sodium selenite or DL-selenomethionine

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The effect of vitamin B_6 on the levels of tissue selenium (Se) and glutathione peroxidase (GSH-Px) was studied. Male Wistar 4-week-old rats were fed a vitamin B_6-Se-deficient basal diet for 2 weeks, then divided into 10 groups of five or six rats and fed their respective diets for 4 weeks. The experimental design was a $2\times2\times2$ factorial with two levels of vitamin B_6, two forms of Se, and two levels of Se, plus two extra groups (vitamin B_6-supplemented and deficient without Se). Vitamin B_6 was 0 and 250 μg pyridoxine·HCl/100 g of diet; Se forms were Na_2SeO_3 and DL-selenomethionine; Se levels were 0.5 and 5.0 mg Se/kg of diet. Regardless of form or level of Se, vitamin B_6-deficient rats had lower body weights and organ weights than vitamin B_6-supplemented rats. At 5.0 mg Se/kg of diet, Na_2SeO_3 caused a further depression. Vitamin B_6 deficiency resulted in a higher Se level and GSH-Px activity in plasma of rats fed selenomethionine. However, Se content and GSH-Px activity in erythrocytes were significantly elevated in vitamin B_6-supplemented rats compared with vitamin B_6-deficient rats. Se levels in muscle and heart were significantly lower in vitamin B_6-deficient groups fed Na_2SeO_3 than in vitamin B_6-supplemented groups. Vitamin B_6-deficient rats fed selenomethionine had higher Se levels in muscle, heart, spleen, liver, and kidneys than vitamin B_6-supplemented rats. Activity of GSH-Px in muscle, heart, and spleen was significantly lower in vitamin B_6-deficient groups than in vitamin B_6-supplemented groups, regardless of form of Se. A significant decrease of GSH-Px in liver was observed in vitamin B_6-deficient rats fed selenomethionine compared with vitamin B_6-supplemented rats, whereas no significant decrease was observed in those fed Na_2SeO_3. These results suggest that vitamin B_6 is involved in the distribution and transportation of Se in body and the metabolism of selenomethionine in liver.

Keywords: selenium; selenite; DL-selenomethionine; glutathione peroxidase; vitamin B_6; rats

Introduction

Glutathione peroxidase (GSH-Px) is the only selenoprotein[1] in animals that has a crucial role in catalyzing the reduction of hydroperoxides and protecting cells from oxidative damage.[2] Selenium (Se) in the enzyme is reported to be present as selenocysteine (SeCys) derived from serine mediated by seryl-tRNA,[3,4] regardless of the form in which the element is fed. The incorporation of Se into the polypeptide of GSH-Px is postulated to be mediated through a pyridoxal phosphate-dependent modification.[5] There have been conflicting reports on the effect of dietary vitamin B_6 on Se metabolism in rats. An earlier study showed that the biopotency of selenomethionine (SeMet) for increasing GSH-Px activity in the rat liver was decreased by vitamin B_6-deficiency.[5] In the study of Sunde et al,[6]

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this effect could not be confirmed. In another experiment that used a lower dietary level of Se (0.20mg Se/kg),[7] the levels of Se and GSH-Px in erythrocytes were lower in vitamin B_6-deficient rats than in vitamin B_6-supplemented rats.

Vitamin B_6 is frequently identified as a nutrient with a high prevalence of inadequate dietary intakes[8-11] in the population relative to the recommended dietary allowance (RDA) for vitamin B_6 issued in 1989.[12] This suggests that a large subset of the population may be at risk of poor vitamin B_6 nutritional status.

The results mentioned above indicate that vitamin B_6 must be taken into consideration in the study of Se metabolism and function. In the present study, we compared the effect of dietary vitamin B_6 deficiency on the biopotency of Se when given at normal or toxic levels, as Na_2SeO_3 or SeMet, for maintaining tissue level and GSH-Px activity in rats.

Materials and methods

Fifty-four male 4-wk-old rats (Wistar SD Donryu, Tokyo Laboratory Animals, Ltd., Tokyo, Japan) weighing 80 ± 5 g (mean ± SD) were kept individually under standardized laboratory conditions in hanging stainless-steel mesh cages with a 12-hr light/dark cycle in an air-conditioned animal room at 20–22° C. The rats were weighed at the start of the test period and at weekly intervals. Food and deionized water were provided ad libitum. The basal diet was prepared according to the original formulation of the AIN-76 purified diet based on 20% casein (vitamin-free casein, Oriental Yeast, Tokyo, Japan) and 50% sucrose.[13] Pyridoxine·HCl in the vitamin mixture and Na_2SeO_3 in the mineral mixture were omitted. Because methionine in particular increases the vitamin B_6 requirement of rats,[14] and the methionine content calculated in our casein-based diet was near the minimum requirement for rats (0.62%),[15] the 0.3% methionine added in the original formula was not included. The basal diet contained 0.03 mg Se/kg as determined by the fluorometric analysis.[16]

All rats were fed the basal diet containing no added vitamin B_6 or Se for 2 weeks to induce a mild vitamin B_6-Se depletion, and then divided by weight into 10 groups of five or six rats. The rats were used in a 2×2×2 factorial experiment involving two levels of vitamin B_6, two forms of Se, and two levels of Se, plus two extra groups (vitamin B_6-supplemented and deficient without Se). Vitamin B_6 was 0 and 250 μg/100 g of diet as pyridoxine·HCl (WAKO Pure Chemical Industries, Ltd., Osaka, Japan); Se forms were Na_2SeO_3 (WAKO) and DL-selenomethionine (Sigma Chemical Company, St. Louis, MO USA); Se levels were 0.5 and 5.0 mg Se/kg of diet. The low SE diet (0.5mg Se/kg compared with 5.0mg Se/kg) represents a nutritionally generous but nontoxic amount of Se. Each group was fed its respective diet for 4 weeks. Feed consumption was carefully measured. The diets were provided ad libitum.

Rats were anesthetized with ether and blood was drawn from the abdominal aorta using a heparinized syringe. Plasma was separated from erythrocytes by centrifugation at room temperature and the erythrocytes were washed three times with 0.85% NaCl. Muscle (from left hind leg), heart, spleen, liver, and kidneys were removed. Approximately 0.5 g of tissue was homogenized with an ultra high speed homogenizer in 5 volumes of 0.25 M sucrose containing 0.25 mmol/L EDTA in 0.1 M potassium phosphate buffer, pH 6.8. Homogenates were centrifuged (40,000g) for 20 min at 4° C. The supernatants were analyzed for enzyme and protein.

Tissue Se was analyzed by a fluorometric method.[14] For the measurement of GSH-Px activity in tissues, the coupled enzyme procedure[17] using H_2O_2 as the substrate[2] was applied. For the assay of vitamin B_6-dependent enzymes, plasma alanine aminotransferase (ALT, EC 2.6.1.2), plasma, and liver aspartate aminotransferase (AST, EC 2.6.1.1.) were assayed using prepared enzymatic kits (WAKO). Liver cystathionase (EC 4.4.1.1) was assayed by the Matsuo and Greenberg method[18]; a unit of cystathionase activity was defined as the amount that yields 1 μmol of α-ketobutyric acid formed per min for cystathionase. Assays for liver aspartate aminotransferase and cystathionase were performed with and without the preincubation of 10 μmol of pyridoxal 5-phosphate (PLP, Sigma Chemical Company) per mL supernatant (30 min, 37° C). Percentage stimulation of enzyme activity by PLP was calculated. Protein was determined by the Lowry method.[19] The data were expressed as means ± SEM. Statistical analysis consisted of analysis of variance and the Student-Newman-Keuls' procedure.[20]

Results

Final body weight, heart and liver weights

Regardless of dietary level or chemical form of Se, the rats fed vitamin B_6-deficient diets gained less weight and had lower heart and liver weights than the rats fed the vitamin B_6-supplemented diets *(Table 1)*. This was due to a low feed efficiency (data not shown). Addition of 5.0 mg Se/kg as Na_2SeO_3 to the vitamin B_6-deficient diet further depressed growth and heart weight.

Vitamin B_6-dependent enzymes

When assayed without preincubation of PLP, plasma alanine aminotransferase, aspartate aminotransferase, and liver aspartate aminotransferase and cystathionase activities were significantly lower in vitamin B_6-deficient rats than in vitamin B_6-supplemented rats regardless of level and form of Se. Vitamin B_6-supplemented rats in higher Se groups tended to have higher plasma alanine, aspartate aminotransferase, and cystathionase activities than those in low Se groups *(Table 2)*. Whether this is related to the mechanism of Se toxicity under these conditions is not known. There was no significant difference in the activity of hepatic aspartate aminotransferase and cystathionase between the vitamin B_6-deficient and -supplemented rats when incubated with PLP (data not shown); whereas percentage stimulation of both these enzymes by PLP was significantly higher for the vitamin B_6-deficient rats. The percentage stimulation (± SEM) of liver aspartate aminotransferase by PLP for vitamin B_6-deficient and -supplemented groups were, respectively, basal, 94±3 and 77±3; L-SeL, 81±2 and 75±2; L-SeMet, 85±2 and 75±1; H-SeL, 85±3 and 78±2; H-SeMet, 86±1 and 71±4; and that of liver cystathionase by PLP for vitamin B_6-deficient and -supplemented groups were, respectively, basal, 94±1 and 79±3; L-SeL, 94±1 and 81±2; L-SeMet, 94±1 and 83±2; H-SeL, 93±1 and 77±1; H-SeMet, 89±2 and 74±2.

Table 1 The effect of dietary vitamin B_6 on final body, heart, and liver weight of rats fed sodium selenite or DL-selenomethionine*

Diets	Final weight		Heart weight		Liver weight	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
			(g)			
Basal	185 ± 9^{ax}	291 ± 11^{aby}	0.59 ± 0.04^{ax}	0.88 ± 0.03^{ay}	5.3 ± 0.2^{ax}	9.0 ± 0.4^{aby}
0.5 mg Se/kg						
L-SeL	194 ± 7^{ax}	286 ± 7^{aby}	0.62 ± 0.04^{ax}	0.87 ± 0.02^{ay}	5.7 ± 0.2^{abx}	8.5 ± 0.3^{by}
L-SeMet	202 ± 12^{ax}	302 ± 5^{by}	0.63 ± 0.07^{ax}	0.93 ± 0.03^{ay}	6.3 ± 0.5^{bx}	8.7 ± 0.3^{bcy}
5.0 mg Se/kg						
H-SeL	158 ± 8^{bx}	258 ± 13^{cy}	0.49 ± 0.03^{bx}	0.80 ± 0.02^{by}	5.4 ± 0.4^{abx}	9.7 ± 0.6^{acy}
H-SeMet	183 ± 6^{ax}	281 ± 8^{acy}	0.59 ± 0.02^{ax}	0.90 ± 0.04^{ay}	6.2 ± 0.4^{bx}	10.4 ± 0.6^{ay}

*Rats fed a vitamin B_6-Se–deficient basal diet for 2 wk were then either depleted further (Basal), or fed the diet supplemented with 250 μg vitamin B_6/100 g as pyridoxine·HCl (Basal + B_6), or 0.5 or 5.0 mg Se/kg as Na_2SeO_3 (L-SeL or H-SeL), or DL-selenomethionine (L-SeMet or H-SeMet), or Se and vitamin B_6 (L-SeL + B_6 or H-SeL + B_6, and L-SeMet + B_6 or H-SeMet + B_6) for 4 wk. The results are means ± SEM of five or six rats per group. Means in the same column with different superscripts a-e are significantly different as a result of chemical forms and dietary levels of Se at the $P < 0.05$ level. Means within a horizontal row with different superscripts x,y are significantly different as a result of vitamin B_6 status at the $P < 0.05$ level.

Table 2 Effect of dietary vitamin B_6 on activity of vitamin B_6-dependent enzymes in rats*

Diets	Plasma				Liver			
	ALT†		AST‡		AST‡		Cystathionase	
	IU/mg protein						U/mg protein§	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	5 ± 1^{ax}	10 ± 3^{ay}	27 ± 3^{ax}	76 ± 7^{ay}	0.03 ± 0.02^{ax}	0.13 ± 0.03^{ay}	7 ± 1^{ax}	22 ± 2^{ay}
0.5 mg Se/kg								
L-SeL	5 ± 1^{ax}	9 ± 2^{ay}	27 ± 5^{ax}	65 ± 7^{ay}	0.09 ± 0.02^{bx}	0.13 ± 0.01^{ay}	7 ± 1^{ax}	20 ± 2^{ay}
L-SeMet	5 ± 0^{ax}	9 ± 4^{ay}	34 ± 6^{ax}	60 ± 7^{ay}	0.07 ± 0.01^{bx}	0.12 ± 0.01^{ay}	7 ± 0^{ax}	21 ± 1^{ay}
5.0 mg Se/kg								
H-SeL	5 ± 1^{ax}	17 ± 5^{ay}	34 ± 5^{ax}	106 ± 13^{by}	0.07 ± 0.02^{bx}	0.11 ± 0.01^{ay}	8 ± 1^{ax}	25 ± 1^{by}
H-SeMet	5 ± 2^{ax}	13 ± 3^{ay}	26 ± 3^{ax}	88 ± 18^{aby}	0.07 ± 0.01^{bx}	0.14 ± 0.02^{ay}	9 ± 2^{ax}	28 ± 1^{by}

*See *Table 1* for explanation of each treatment.
†ALT, alanine aminotransferase.
‡AST, aspartate aminotransferase.
§A unit of cystathionase activity is defined as the amount that yields 1 μmol of α-ketobutyric acid formed per min for cystathionase.

Selenium levels in tissues

Se contents of plasma, erythrocytes, muscle, heart, spleen, liver, and kidneys were higher in rats fed the organic form of Se than in those fed the inorganic form *(Tables 3–5)*. The differences due to Se forms were increased in the higher Se groups. Furthermore, Se levels in tissues (except the erythrocytes) of vitamin B_6-deficient rats fed SeMet were further elevated. For example, feeding 0.5 or 5.0 mg Se/kg of diet as SeMet to vitamin B_6-supplemented animals caused 2.2- and 10-fold increase in muscle Se retention compared with animals fed SeL. In vitamin B_6-deficient animals however, muscle Se levels were 2.8- and 13.6-fold greater as a result of feeding the organic form of Se compared with the inorganic form. This was due more to lower Se levels in the H-SeL group than higher levels in the H-SeMet group. In plasma, heart, spleen, liver, and kidneys, the same effects were obtained as seen in muscle.

Vitamin B_6-deficient rats had a higher plasma Se concentration than vitamin B_6-supplemented rats, and significant differences were observed in rats fed SeMet. On the contrary, vitamin B_6-supplemented rats had significantly higher Se concentrations in erythrocytes than vitamin B_6-deficient rats *(Table 3)*.

In muscle and heart, there was little effect of increasing dietary Se level from 0.5–5.0 mg Se/kg on Se levels when the rats were fed SeL. On the other hand, the rats fed higher level of SeMet had significantly higher Se concentrations in muscle and heart than those fed lower Se level of SeMet *(Table 4)*. Se content of muscle and heart was lower in vitamin B_6-deficient rats fed SeL than in vitamin B_6-supplemented rats. Se levels of both tissues were elevated in vitamin B_6-deficient groups fed L-SeMet and H-SeMet compared with their supplemented counterparts. Cardiac muscle was consistently higher in the Se content than skeletal muscle, and this effect became more significant in the vitamin B_6-deficient rats.

The Se concentrations of spleen, liver, and kidneys in the basal + B_6 group were significantly lower than

Table 3 Effect of dietary vitamin B_6 on selenium content in rat plasma and erythrocytes*,†

Diets	Plasma		Erythrocytes	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	51.9 ± 3.7^{ax}	43.5 ± 2.5^{ay}	5.5 ± 0.2^{ax}	5.4 ± 0.4^{ax}
0.5 mg Se/kg				
L-SeL	116.2 ± 13.8^{bx}	94.5 ± 5.7^{bx}	6.6 ± 0.5^{bx}	14.3 ± 0.8^{by}
L-SeMet	122.8 ± 5.9^{bx}	106.6 ± 4.4^{by}	12.4 ± 0.8^{cx}	17.2 ± 0.7^{cy}
5.0 mg Se/kg				
H-SeL	126.1 ± 8.3^{bx}	130.1 ± 3.0^{cx}	74.0 ± 8.7^{dx}	115.4 ± 5.0^{dy}
H-SeMet	262.5 ± 11.3^{cx}	210.3 ± 19.5^{dy}	82.2 ± 12.4^{dx}	127.5 ± 5.4^{dy}

*See *Table 1* for explanation of each treatment.
†Se content was expressed as μmol Se/L per mg protein (plasma) or hemoglobin (erythrocytes).

Table 4 Effect of dietary vitamin B_6 on selenium content in muscle and heart*,†

Diets	Muscle		Heart	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	1.0 ± 0.1^{ax}	0.9 ± 0.0^{ax}	2.3 ± 0.1^{ax}	2.0 ± 0.1^{ay}
0.5 mg Se/kg				
L-SeL	1.4 ± 0.2^{bx}	1.8 ± 0.1^{by}	3.8 ± 0.2^{bx}	4.5 ± 0.1^{by}
L-SeMet	4.3 ± 0.2^{cx}	4.0 ± 0.1^{cx}	6.9 ± 0.2^{cx}	5.9 ± 0.1^{cy}
5.0 mg Se/kg				
H-SeL	2.1 ± 0.1^{dx}	2.7 ± 0.1^{dy}	6.8 ± 0.5^{cx}	7.8 ± 0.6^{dx}
H-SeMet	29.3 ± 1.1^{ex}	26.6 ± 1.2^{ex}	35.4 ± 2.3^{dx}	30.0 ± 1.5^{ey}

*See *Table 1* for explanation of each treatment.
†Values were expressed as nmol Se/g tissue (wet weight).

Table 5 Effect of dietary vitamin B_6 on selenium content in rat organs*,†

Diets	Spleen		Liver		Kidneys	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	4.3 ± 0.2^{ax}	3.5 ± 0.2^{ay}	4.4 ± 0.2^{ax}	2.4 ± 0.2^{ay}	11.6 ± 0.5^{ax}	9.6 ± 0.3^{ay}
0.5 mg Se/kg						
L-SeL	6.1 ± 0.3^{bx}	5.7 ± 0.2^{bx}	13.5 ± 0.4^{bx}	13.8 ± 0.5^{bx}	18.6 ± 0.2^{bx}	18.2 ± 0.2^{bx}
L-SeMet	8.5 ± 0.1^{cx}	6.6 ± 0.1^{cy}	19.6 ± 0.6^{cx}	15.6 ± 0.4^{cy}	29.2 ± 0.9^{cx}	22.1 ± 0.5^{cy}
5.0 mg Se/kg						
H-SeL	20.4 ± 1.1^{dx}	17.7 ± 0.8^{dy}	39.2 ± 5.5^{dx}	35.8 ± 3.1^{dx}	69.1 ± 14.9^{dx}	58.3 ± 6.6^{dx}
H-SeMet	37.9 ± 2.0^{ex}	32.8 ± 1.8^{ey}	82.4 ± 4.1^{ex}	68.7 ± 3.8^{ey}	130.6 ± 8.6^{ex}	98.7 ± 6.8^{ey}

*See *Table 1* for explanation of each treatment.
†Values were expressed as nmol Se/g tissue (wet weight).

those in the basal group due to growth. There was little effect of vitamin B_6 on Se levels in these tissues when the rats were fed SeL. However, vitamin B_6-deficient rats fed SeMet all had significantly higher Se levels than vitamin B_6-supplemented rats *(Table 5)*. Se levels in kidneys, liver, heart, spleen, and plasma were decreased 24, 17, 15, 14, and 20%, respectively, as a result of vitamin B_6 supplementation in rats fed the diets supplemented with 0.5 mg Se/kg as SeMet.

Glutathione peroxidase activities in tissues

Vitamin B_6-deficient diets resulted in higher plasma GSH-Px activity than vitamin B_6-supplemented diets, which was associated with the plasma Se level. However, the GSH-Px activity in erythrocytes was significantly higher in rats fed vitamin B_6-supplemented diets than in those fed vitamin B_6-deficient diets *(Table 6)*, which was well correlated with the magnitude of difference in erythrocyte Se level.

GSH-Px activities in muscle, heart, and spleen were significantly lower in vitamin B_6-deficient rats fed SeL or SeMet than in vitamin B_6-supplemented rats *(Table 7)*. Activity of GSH-Px in these tissues was poorly associated with their Se levels. In liver and kidneys, GSH-Px in the vitamin B_6-supplemented group was significantly lower than in the vitamin B_6-deficient basal group due to growth. Liver GSH-Px in vitamin B_6-

Table 6 Effect of dietary vitamin B_6 on glutathione peroxidase activity in rat plasma and erythrocytes*,†

Diets	Plasma		Erythrocytes	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	14 ± 2^{ax}	11 ± 2^{ax}	90 ± 4^{ax}	102 ± 6^{ax}
0.5 mg Se/kg				
L-SeL	29 ± 4^{bcx}	23 ± 5^{bcx}	97 ± 7^{abx}	234 ± 10^{by}
L-SeMet	41 ± 5^{cx}	30 ± 4^{cx}	82 ± 8^{ax}	210 ± 6^{cy}
5.0 mg Se/kg				
H-SeL	26 ± 6^{bx}	20 ± 4^{bx}	130 ± 9^{cx}	283 ± 13^{dy}
H-SeMet	28 ± 7^{bcx}	23 ± 2^{bcx}	112 ± 9^{bcx}	302 ± 20^{dy}

[1]See *Table 1* for explanation of each treatment.
[2]Activity was expressed as nmol NADPH oxidized/min per mg protein (plasma) or per mg hemoglobin (erythrocytes) with H_2O_2 as substrate.

Table 7 Effect of dietary vitamin B_6 on activity of glutathione peroxidase in rat muscle, heart, spleen, liver, and kidneys*,†

Diets	Muscle		Heart		Spleen		Liver		Kidneys	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	7 ± 1^{ax}	7 ± 0^{ax}	90 ± 8^{ax}	113 ± 6^{ay}	193 ± 8^{ax}	208 ± 6^{ax}	116 ± 9^{ax}	47 ± 2^{ay}	133 ± 12^{ax}	98 ± 5^{ay}
0.5 mg Se/kg										
L-SeL	10 ± 1^{abx}	48 ± 6^{by}	213 ± 16^{bx}	317 ± 36^{by}	231 ± 11^{bx}	309 ± 7^{by}	650 ± 8^{bx}	655 ± 32^{bx}	401 ± 25^{bx}	406 ± 12^{bx}
L-SeMet	16 ± 3^{bx}	41 ± 8^{by}	183 ± 13^{bx}	315 ± 28^{by}	229 ± 7^{bx}	336 ± 11^{cy}	557 ± 33^{cx}	660 ± 12^{by}	406 ± 12^{bx}	366 ± 6^{cy}
5.0 mg Se/kg										
H-SeL	25 ± 3^{cx}	43 ± 6^{by}	207 ± 18^{bx}	304 ± 32^{by}	229 ± 13^{bx}	339 ± 11^{cy}	483 ± 24^{dx}	487 ± 36^{cx}	401 ± 20^{bx}	427 ± 23^{bx}
H-SeMet	14 ± 3^{bx}	40 ± 6^{by}	212 ± 17^{bx}	328 ± 15^{by}	195 ± 9^{ax}	353 ± 12^{cy}	475 ± 24^{dx}	548 ± 32^{cy}	397 ± 17^{bx}	385 ± 8^{bx}

[1]See *Table 1* for explanation of each treatment.
[2]Activity was expressed as nmol NADPH oxidized/min per mg protein with H_2O_2 as substrate.

deficient rats fed SeL was similar to that in vitamin B_6-supplemented rats; whereas a signficant decrease was observed in the vitamin B_6-deficient group fed SeMet compared with the vitamin B_6-supplemented group. In the kidneys, opposite results were observed in the vitamin B_6-deficient group fed SeMet compared with the vitamin B_6-supplemented group.

Discussion

As shown by the lower body, heart, and liver weights *(Table 1)*, the rats fed this vitamin B_6-deficient diet were satisfactory for demonstrating any effect of dietary vitamin B_6 deficiency on the biopotency of Se for maintaining tissue level and GSH-Px activity. This was further supported by the significantly lower plasma alanine, aspartate aminotransferase, liver aspartate aminotransferase, and cystathionase activities in the vitamin B_6-deficient rats *(Table 2)*.

Although inorganic Se has been used for supplements in some human diseases such as Keshan[21] and Kashin-Beck diseases[22] in China, the organic form of Se, SeMet, was shown to be the better form to be used in the intervention of human Se deficiency.[23] In agreement, Se levels of all tissues in the present study were significantly increased by feeding SeMet compared with feeding SeL. This indicates that Se from SeMet can act as a storage form.[24] This was further supported by the lack of difference in GSH-Px activity in these tissues between SeL and SeMet groups. Because SeL-exchangeable components of body Se had a higher turnover than SeMet-containing components,[24] a large amount of the Se absorbed from SeL was lost via urine and feces.[25]

Vitamin B_6-deficient rats tended to have higher Se level in plasma compared with vitamin B_6-supplemented rats, even though erythrocyte Se level was significantly lower in vitamin B_6-deficient rats than in vitamin B_6-supplemented rats *(Table 3)*. This indicates that vitamin B_6 status affects the distribution of Se in the tissues rather than the absorption of Se via the intestine. The mechanism involved in the utilization of plasma Se by erythrocytes is not so clear. This might arise from the transport of Se to these tissues from plasma. Or the forms of Se in plasma or the conjugation or release of Se in the plasma protein may be changed due to vitamin B_6 deficiency. It is commonly assumed that a Se storage and transport system should exist to handle the distribution of Se in tissues by adjusting the plasma Se at various levels to supply Se to tissues such as the testes, liver, and kidney. Additional research on the aspect of different Se compounds on erythrocyte function is needed.

The tissues often involved in Se deficiency were muscle and heart, for example, muscular dystrophy in chicks,[26] white muscle disease in lambs and calves,[27] cardiomyopathy in Keshan disease,[21] and muscle pain and tenderness in long-term total parenteral nutrition[28,29] in humans. From our study, we found that Se levels in these tissues of vitamin B_6-deficient rats fed SeL

were significantly lower than those of corresponding vitamin B_6-supplemented rats; whereas an increased trend was observed in vitamin B_6-deficient rats fed SeMet, and a significant difference was observed in the heart *(Table 4)*. This was attributed to the fact that Se from SeMet is generally incorporated into protein as substituent for methionine.[30] It is believed that SeMet metabolism in animals is similar to that of Met[31] and is a PLP-dependent enzyme process. Vitamin B_6 deficiency would be postulated to affect the conversion and accumulation of metabolic intermediates of Se from SeMet in the tissues.

In previous reports,[5-7] the study of the effect of vitamin B_6 on Se levels was related mostly to the erythrocytes and liver. The present one has extended it to include plasma, muscle, heart, spleen, and kidneys. From the data presented here, the vitamin B_6 status appears to have different effects in various tissues. For example, Se contents in spleen, liver, and kidney were affected little by vitamin B_6 status when the rats were fed SeL; however, vitamin B_6 deficiency significantly increased Se retention in plasma, muscle, heart, liver, and kidneys in rats fed SeMet. The greater retention of Se was shown in vitamin B_6-deficient rats fed 5.0 mg Se/kg of diet as SeMet *(Table 5)*. Feeding vitamin B_6 caused a significant decrease in Se retention in rats fed 5.0 mg Se/kg of diet as SeMet: kidney Se levels were decreased by 24%; plasma, 20%; liver, 17%; heart, 15%; spleen, 14%; and muscle, 10% *(Tables 3–5)*. These results indicate that the distribution of Se in these tissues may not be influenced, but the metabolic conversion of SeMet to the biologically active form would be inhibited, by vitamin B_6 deficiency.

The biopotency of Se for GSH-Px activity in erythrocytes, muscle, heart, and spleen was dependent on vitamin B_6 status no matter which form of Se was used. For example, vitamin B_6-deficient rats all had significantly lower GSH-Px activities in these tissues than vitamin B_6-supplemented rats *(Tables 6,7)*. However, Se levels in these tissues (except erythrocytes) as mentioned above were not as significantly affected by vitamin B_6 status. These results indicate that the utilization of Se for GSH-Px may be restricted in vitamin B_6-deficient rats, at least in heart and spleen. This is not consistent with the results obtained by other investigators using rats.[5] In that study, no significant effect of vitamin B_6 was found in erythrocytes and liver with SeL. The mechanism by which vitamin B_6 affected the incorporation process of Se to GSH-Px in these tissues is not known in detail.

Figure 1 is a diagram of SeMet, SeCys, selenate, and selenite metabolism in mammalian tissues. Selenate is reduced to selenite, presumably by the same pathway as sulfate.[32] The reduction of selenite to selenide was via the GSH/glutathione reductase/NADPH-dependent pathway.[33,34] For the incorporation of selenide into GSH-Px or other selenoproteins, a seryl-tRNA and serine kinase may be required to form SeCys-tRNA.[4] Activity of GSH-Px in liver and kidney of vitamin B_6-deficient animals fed SeL was not significantly lower than that of vitamin B_6-supplemented

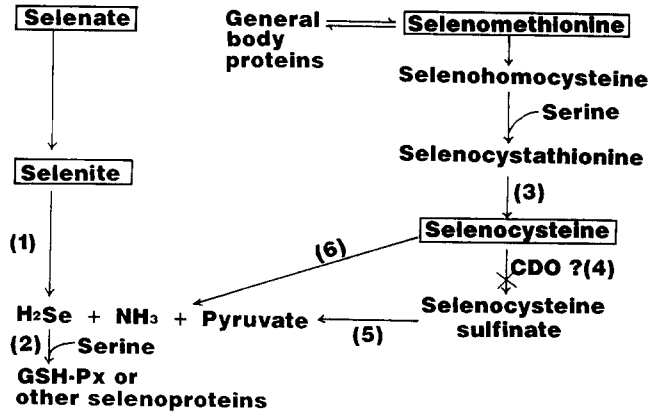

Figure 1 Pathways of selenomethionine, selenocysteine, selenate, and selenite metabolism in mammalian tissues. (1) glutathione reductase; (2) seryl-tRNA, serine kinase; (3) cystathionase; (4) CDO: cysteine dioxygenase; (5) transaminase or desulfinicase; (6) cysteine desulphhydrase.

animals. This indicates that vitamin B_6 status seems to be involved to a lesser extent in the conversion of selenite to selenide. However, the metabolism of free SeMet from diet or from protein turnover has two pathways, either incorporation into protein or degradation *(Figure 1)*. Incorporation of SeMet into general body proteins in place of Met is mediated by $tRNA^{Met}$.[35] SeMet would be converted by transulfuration pathway to selenocysteine catabolized by cystathionase.[36] Selenocysteine may be further degraded via selenocysteine sulfinate by transaminase or desulfinicase to pyruvate, NH_3, and selenide. However, cysteine dioxygenase [CDO, EC 1,12,11,20] catabolized the oxygenation of cysteine to cysteine sulfinic acid but could not use selenocysteine as substrate (unpublished from K. Yamaguchi), this metabolic route of selenocysteine was denied. It appears that selenocysteine is metabolized by cysteine desulphhydrase to pyruvate, NH_3, and selenide. Both cystathionase *(Table 2)* and cysteine desulphhydrase were pyridoxal phosphate-dependent enzymes involved in this pathway. In the present study, vitamin B_6-deficiency depressed the metabolic conversion of Se to selenide and then the incorporation of Se into GSH-Px or other selenocysteine-containing proteins in rats fed the SeMet. This resulted in a lower GSH-Px activity *(Tables 6 and 7)* and a higher Se retention in tissues *(Tables 4 and 5)*. The findings about the decrease in hepatic GSH-Px in vitamin B_6-deficient rats fed SeMet *(Table 7)* could be interpreted on the basis of a dietary interrelationship between SeMet and vitamin B_6.[5] Our data support the concept that vitamin B_6 is involved in the metabolism of SeMet. Why the vitamin B_6-supplemented rats fed SeMet had a low activity of GSH-Px in the kidneys was not clear.

The active transport for Se in tissues may involve vitamin B_6-dependent processes. Thus, a low intake of pyridoxine could impair Se utilization so that available Se is decreased. That would lead to an increase in the Se requirement. From the present studies, we conclude

that dietary vitamin B_6 is important for Se metabolism in tissues. Further experiments are under study.

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