Effect of Dietary Vitamin B_6 on Blood Selenium Level and Glutathione Peroxidase Activity in Rats

Shi-an YIN[1], Ikuo SATO[2], Shiro NIIZEKI[2], Hitomi TOJO[2], Yu HOSOKAWA[2], and Kenji YAMAGUCHI[3]

[1]*Institute of Nutrition and Food Hygiene Research Fellow of Human Science of Japan* *,

[2]*The National institute of Health and Nutrition* **,

[3]*Tokyo Kasei University* ***

ABSTRACT

Effect of dietary vitamin B_6 on blood selenium level and glutathione peroxidase activity was carried out. Male Wistar 12-week-old rats were fed a vitamin B_6-selenium-deficient basal diet for three weeks, and then the rats were divided into six groups. One group was fed the basal diet, the others were fed the diet supplemented with 250 μg vitamin B_6 / 100g as pyridoxine・HCl, or with 0.25mg selenium / kg as Na_2SeO_3 or DL-selenomethionine, or with vitamin B_6 and selenium for 12weeks. vitamin B_6 status had not significant effect on plasma selenium and glutathione peroxidase levels, however, selenium content and gultathione peroxidase activity in erythrocytes were significantly higher in vitamin B_6-supplemented groups than in vitamin B_6-deficient groups, regardless of the form of selenium. These results suggest that the utilization of selenium for erythrocytes was dependent vitamin B_6 status.

INTRODUCTION

Selenium (Se) requirements in animals and humans have been reported following the establishment of its essentiality[1-3]. However, the requirements are influenced by other nutrient status. Previously we reported that the rats fed vitamin B_6-deficient diets had significantly lower Se concentration and glutathione peroxidase(GSH-Px) activity in erythrocytes than the rats fed vitamin B_6-supplemented diets[4] even though the vitamin B_6-deficient rats had higher levels of Se and GSH-Px in plasma than the vitamin B_6-supplemented rats. This suggested that this vitamin may be involved in the transport or delivery of Se in plasma to the other tissues. In the present study, the effect of dietary vitamin B_6 on blood Se level and GSH-Px activity was further investigated with adult rats.

*所在地：北京市宣武区南緯路29号（100050）
**所在地：東京都新宿区戸山１－23－１（〒162）
***所在地：東京板橋区加賀１－18－１（〒173）

MATERIALS AND METHODS

Forty-two male 12-week-old Wistar rats purchased from Clea Japan (Tokyo) were housed individually in suspended mesh, stainless steel cages with a 12-h light / dark cycle in a room at 20～22℃, Rats were weighed weekly. Food and deionized water were provided ad libitum. The basal diet was a 20% vitamin-free casein and 50% sucrose-based diet, pyridoxine・HCl in vitamin mixture, Na_2SeO_3 in mineral mixture and 0.3% methionine were omitted, as previously described[4]. The basal diet was shown by analysis to contain 0.03mg Se / kg.

Rats weighing 370 ± 13g (mean ± SD) were fed the basal diet for three weeks, and then the rats were randomized by weight into six groups (seven rats per group). One group was fed the basal diet (Basal, $-B_6$), the remainders were fed the diet supplemented with 250 μg vitamin B_6 / 100g as pyridoxine・HCl (Basal, $+B_6$), or with 0.25mg Se / kg as Na_2SeO_3 (SeL, $-B_6$) or DL-selenomethionine (SeMet, $-B_6$), or with 0.25mg Se / kg and 250 μg vitamin B_6 / 100g (SeL, $+B_6$ or SeMet, $+B_6$). At 0, 1, 2, 3, and 7, 11, 13, and 15 weeks after depletion and repletion, Se and GSH-Px in the tail vein blood were assayed in all animals. Each groups of rats was fed its respective diet for 12 weeks.

Rats were anesthetized with ether, blood was drawn from the abdominal aorta using a heparinized syringe. Plasma was obtained by centrifuging at 1,800 × g, and then plasma was rapidly deproteinized by mixing it vigorously with 0.5 volume of 10% 5-sulfosalicylic acid, followed by centrifugation at 10,000 × g for 2 min. The supernatant liquid was assayed for non-protein-bound Se. Erythrocytes for Se and GSH-Px assays were washed three times with 0.85% NaCl.

Se content was determined with fluorometric method[5]. GSH-Px was assayed by the coupled enzyme method[6] using H_2O_2 as the substrate[7]. Plasma aspartate aminotransferase and alanine aminotransferase were assayed using prepared enzymatic kits (Wako, Code 275-34101 and 271-34201, respectively). Protein was determined by the Lowry method[8]. The data were given as means ± SEM and performed using analysis of variance and tested using the Student-Newman-Keuls procedure[9].

RESULTS AND DISCUSSION

Final body weight and vitamin B_6-deficient enzymes. Table 1 showed that regardless of dietary level or chemical form of Se, the rats fed vitamin B_6-deficient diets gained less significantly final body weight than the rats fed vitamin B_6-supplemented diets. There were no significant differences among vitamin B_6-supplemented groups. Activities of vitamin B_6-dependent enzymes, plasma aspartate aminotransferase and alanine aminotransferase, were significantly lower in vitamin B_6-deficient groups than in vitamin B_6-supplemented groups, regardless of Se treatment, suggested that vitamin B_6 deficiency was produced in the animals fed this vitamin B_6-deficient diets. The differences observed from our study can be contributed to vitamin B_6 deficiency.

The levels of Se and GSH-Px in whole blood during the periods of depletion and repletion.

Table 1. Rat growth and plasma aminotransferase activities[1]

Treatment	Final Weight		PALT[2]		PAST[3]	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
	(g)		IU / mg protein			
Basal	377 ± 9^{ax}	473 ± 9^{ay}	3 ± 1^{ax}	11 ± 1^{ay}	33 ± 4^{ax}	77 ± 13^{ay}
SeL	380 ± 3^{ax}	488 ± 12^{ay}	3 ± 0^{ax}	11 ± 1^{ay}	31 ± 4^{ax}	66 ± 8^{ay}
SeMet	380 ± 8^{ax}	470 ± 7^{ay}	3 ± 1^{ax}	12 ± 1^{ay}	33 ± 7^{ax}	65 ± 1^{ay}

[1]Rats fed a vitamin B_6-Se-dificient diet for 3-wk were then either depleted further (Basal), or substituted with the diet supplemented with 250 μg vitamin B_6 / 100g as pyridoxine · HCl (Basal + B_6), or with 0.25mg Se / kg as Na_2SeO_3 (SeL) or DL-selenomethionine (SeMet), or with Se and vitamin B_6 (SeL + B_6 or SeMet + B_6) for 12-wk. The results are means ± SEM of seven rats per group. Means in the same column with different superscripts a-c are significantly different as a result of chemical form and dietary level 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. [2]PALT : Plasma alanine aminotransferase. [3]PAST : Plasma aspartate aminotransferase.

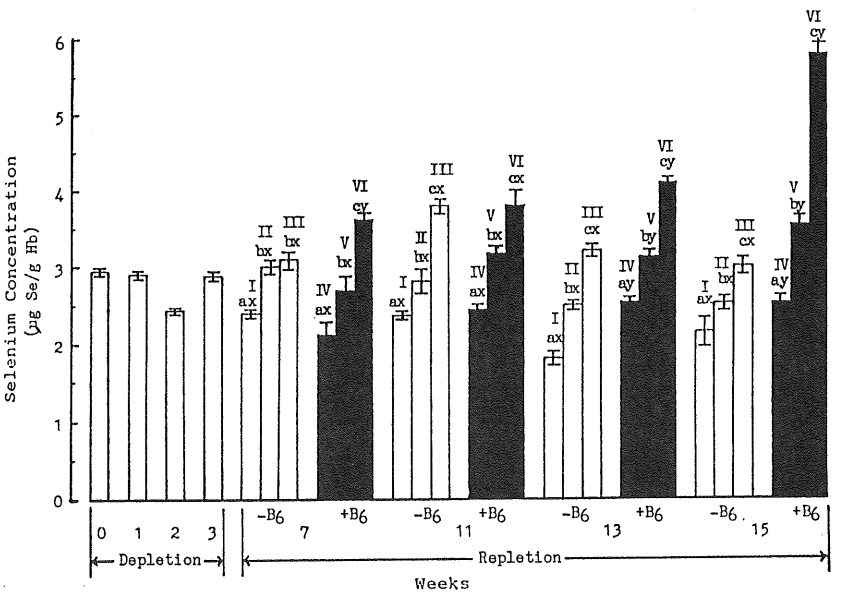

Fig. 1. Selenium (Se) concentration in whole blood from rat tail during the period of vitamin B_6-Se depletion and repletion. Each column represents the means ± SEM of 42 rats for depletion and 7 rats for repletion. The open bars represent vitamin B_6-deficient rats (Ⅰ, Basal; Ⅱ, SeL; Ⅲ, SeMet) and the solid bars represent vitamin B_6-supplemented rats (Ⅳ, Basal + B_6; Ⅴ, SeL + B_6; Ⅵ, SeMet + B_6). Significant differences at vitamin B_6-deficient or supplemented groups are indicated by different letters a-c at the $p < 0.05$ level. Significant differences between vitamin B_6-deficient and supplemented groups (Ⅰ and Ⅳ, Ⅱ and Ⅴ, Ⅲ and Ⅵ) are indicated by different letters x, y at the $p < 0.05$ level. Selenium concentration is expressed as μg Se / g Hb.

Fig. 1 showed that Se levels in whole blood were not significant differences between vitamin B_6-deficient and supplemented groups until 13 weeks, corresponding to the turnover period of erythrocytes. From 13 weeks, Se contents in vitamin B_6-supplemented three groups were significantly higher than those in vitamin B_6-deficient three groups ($p < 0.05$). GSH-Px activity in whole blood almost kept a relatively steady level in vitamin B_6-supplemented rats, but the levels of GSH-Px in vitamin B_6-deficient groups were gradually reduced and significantly lower than those in vitamin B_6-supplemented groups (Fig.2).

Se levels in plasma and erythrocytes. SeMet caused a large amount of Se to be retained in erythrocytes compared with SeL, but no differences could be observed in plasma (Table 2). Vitamin B_6 status had no significant effect on plasma Se concentrations. The contents of nonprotein-bound Se in

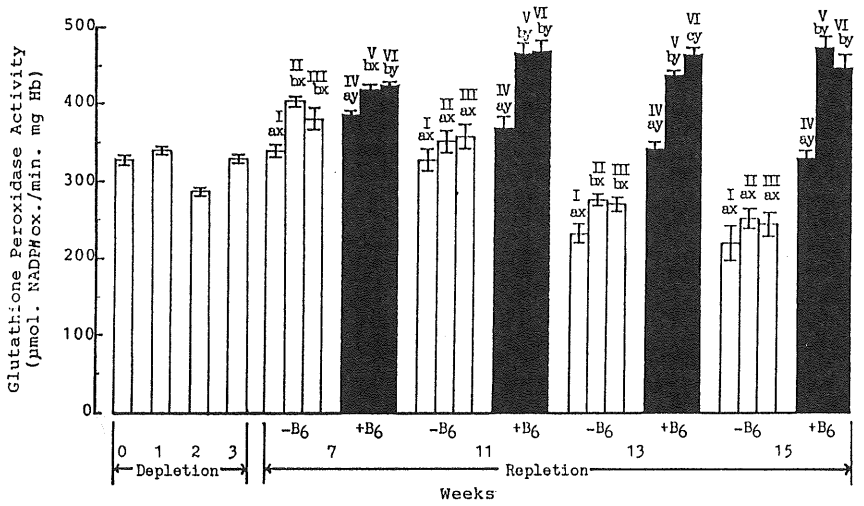

Fig. 2. Glutathione peroxidase activity in whole blood from rat tail during the period of vitamin B_6-Se depletion and repletion. See Fig. 1 for the detailed explanation of each treatment. Activity is expressed as μmol NADPH ox. / min per mg Hb.

Table 2. Effect of dietary vitamin B_6 deficiency on selenium levels in plasma and erythrocytes of rats[1]

Treatment	Plasma Se[2]		Plasma NPB Se[3]		Erythrocyte Se[2]	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	5.0 ± 0.0^{ax}	4.6 ± 0.2^{ax}	13.0 ± 0.6^{ax}	15.6 ± 1.4^{ax}	0.8 ± 0.1^{ax}	1.6 ± 0.1^{ay}
SeL	6.2 ± 0.3^{bx}	6.1 ± 0.2^{bx}	18.0 ± 1.6^{bx}	18.7 ± 1.0^{bx}	1.1 ± 0.1^{bx}	2.4 ± 0.1^{by}
SeMet	6.3 ± 0.2^{bx}	6.2 ± 0.2^{bx}	19.1 ± 0.8^{bx}	20.4 ± 0.5^{bx}	1.7 ± 0.1^{cx}	4.1 ± 0.1^{cy}

[1]See Table 1 for explanation of each treatment. [2]Se contents are expressed as μg Se / mg protein (Plasma) or per mg Hb (erythrocytes). [3]Plasma NPB Se: Plasma Se as nonprotein-bound; expressed as the percentage of total Se in plasma soluble in 0.5 volume of 10% 5-sulfosalicylic acid.

plasma (data not shown) were significantly higher in vitamin B_6-supplemented groups than in vitamin B_6-deficient groups, however, the percentage of nonprotein-bound Se to total Se contents in plasma was slightly higher in vitamin B_6-supplemented groups than in vitamin B_6-deficient groups. Beilstein and Whanger[10] showed that the retention of ^{75}Se in plasma was higher in vitamin B_6-deficient rats than vitamin B_6-supplemented rats. Therefore, Se levels in plasma did not bear to intracellular concentrations of Se[11], thus the biological effect of Se would not be related to Se level in plasma.

Se levels in erythrocytes were significantly higher in vitamin B_6-supplemented rats than in vitamin B_6-deficient rats. Beilstein and Whanger[10] found a similar reduction in erythrocyte Se level in vitamin B_6-deficient rats for all forms of Se (SeL, selenocystine and SeMet). The present data suggest that this vitamin might be involved in the distribution or transport of Se to erythrocytes from plasma.

GSH-Px activities in plasma and erythrocytes. Vitamin B_6 had little effect on GSH-Px activity in plasma. However, the activity in erythrocytes was significantly higher in vitamin B_6-supplemented groups than in vitamin B_6-deficient groups (Table 3), which was well correlated with the magnitude of differences in Se levels of erythrocytes. From the present experiment, we found that the biopotency of Se for GSH-Px activities in erythrocytes was dependent on dietary vitamin B_6 status, which suggests that the active transport mechanisms for Se are probably present in tissues in which vitamin B_6 would be involved.

Table 3. Effect of dietary vitamin B_6 deficiency on activity of glutathione peroxidase in plasma and erythrocytes of rats[1]

Treatment	Glutathione Peroxidase activity[2]			
	Plasma		erythrocytes	
	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Basal	56 ± 2^{ax}	53 ± 4^{ax}	220 ± 22^{ax}	430 ± 11^{ay}
SeL	74 ± 3^{bx}	71 ± 3^{bx}	298 ± 10^{bx}	619 ± 10^{by}
SeMet	66 ± 2^{cx}	62 ± 3^{cx}	294 ± 33^{bx}	607 ± 20^{by}

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

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References

1. SCHWARZ,K., J. G. BIERI, G. M. BRIGGS and M. L. SCOOT (1975) Proc. Soc. Exp. Biol. Med. 95 : 621-625
2. THOMPSON, J. N. and M. L. SCOTT (1969) J. Nutr. 97 : 335-342
3. YANG, G., K. GE, J. CHEN and X. CHEN (1988) Wld. Rev. Nutr. Diet. 55 : 98-152

4．YIN, S., I. SATO, Y. HOSOKAWA, S. NIIZEKI, H. TOJO and K. YAMAGUCHI (1990) 微量栄養素研究 7：103-108

5．WATKINSON, I. H (1966) Anal. Chem. 38：92-96

6．WENDEL, A. (1981) Methods in Enzymology, 77：325-332

7．LAWRENCE, R. A. and R. F. BURK (1976) Biochem. Biophys. Res. Commun. 71：952-958

8．LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL (1951) J. Biol. Chem. 193：265-275

9．STEEL, R. G. D. and J. H. TORRIE (1960) Principles and procedures of statistics, McGraw-Hill, New York

10．BEILSTEIN, M. A. and P. D. WHANGER (1989) J. Nutr. 119：1962-1972

11．JANGHORBANI, M., R. F. MARTIN, L. J. KASPER, X. F. SUN and V. R. YONG (1990) Am. J. Clin. Nutr. 51：670-677