ELSEVIER

Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of the rat

Y. Sun, P-C. Ha,* J.A. Butler, B-R Ou, J-Y Yeh, and P. Whanger**

Department of Agricultural Chemistry, Oregon State University, Corvallis, OR USA

The influence of deficient (0.004 μg/g), adequate (0.1 μg/g), and excessive (4.0 μg/g) levels of dietary selenium (Se) on the selenoprotein W (Se-W) content and glutathione peroxidase (GPX) activity was investigated in 28 tissues of the rat. GPX activity was found in all 28 tissues examined, and dietary selenium resulted in increased activities in all tissues, except for the spinal cord. Except for the brain, 0.1 μg Se per g diet resulted in significantly greater GPX activity in all tissues as compared with rats fed the deficient diet. When 4.0 μg Se per g diet was fed, however, this resulted in significantly greater activity in the brain as compared with the rats fed the deficient diet. Se-W was nondetectable in liver, thyroid, pancreas, pituitary, and eyes regardless of the level of Se fed. Se-W was not detected in heart, lungs, prostate, esophagus, small intestine, tongue, skin, diaphragm, and skeletal muscle from Se-deficient rats, but was present in these tissues when the two higher levels of Se were fed. In other tissues such as the kidney and seminal vesicles Se-W was detected only in rats fed 4.0 μg Se per g diet. These results indicate that the distribution of Se-W among rat tissues is more widespread than thought, and suggest that the regulation of Se-W by Se is markedly different between various tissues. (J. Nutr. Biochem. 9: 23–27, 1998) © *Elsevier Science Inc. 1998*

Keywords: selenoprotein W; Western blots; glutathione peroxidase; rat tissues

Introduction

It has now been established that the essential effects of selenium (Se) in mammals are the result of several biologically active Se compounds. They include the family of glutathione peroxidases (GPX), which are the classical GPX,[1] a plasma GPX,[2] a GPX present predominantly in the gastrointestinal tract,[3] and the monomeric phospholipid hydroperoxide GPX.[4] A second important enzymatic function of Se was identified when types I,[5] II,[6] and III[7] iodothyronine deiodinases were identified as selenoenzymes. The most recent selenoenzyme identified was thioredoxin reductase, which was isolated from human lung adenocarcinoma.[8] A few other selenoproteins have been identified but their biological functions have not yet been identified. They include selenoprotein P, the main Se compound in plasma[9] and selenoprotein W (Se-W) originally isolated from muscle.[10] Se-W is a selenoprotein with a molecular weight slightly less than 10 kDa.

The results of several tracer experiments indicate that many other Se-containing compounds exist in addition to the selenoproteins already identified.[11–14] After in vivo labeling of rats with ^{75}Se and separation of the tissue proteins by SDS-polyacrylamide gel electrophoresis and autoradiography of the labeled compounds, 13 Se-containing proteins were found.[13] In a subsequent study, the distribution of ^{75}Se was investigated in 27 tissues from rats.[15] In a number of these tissues Se was found in 10 kDa proteins. In a previous study from our laboratory, Se-W was found only in muscle, brain, spleen, and testis of the tissues examined from rats.[16] Thus, the purpose of the present experiment was to re-examine the tissue distribution of Se-W to determine if it is more widespread than we had initially anticipated. Levels of 0.004, 0.1, and 4 μg dietary

This paper was published with the approval of the Oregon State Agricultural Experiment Station as Technical Paper No. 11,171. This research was supported by Public Health Research Grant No. DK 38306 from the National Institute of Diabetes and Digestive and Kidney Diseases.
*Present address: Chinese Academy of Preventive Medicine, Institute of Nutrition and Food Hygiene, 29 Nan Wei Road, Beijing 100050, China.
**Present address: Department of Animal Sciences, Tunghai University, Taichung, Taiwan, China.
Address correspondence and reprint requests to Dr. P.D. Whanger, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331 USA.
Received June 6, 1997; accepted September 30, 1997.

655 Avenue of the Americas, New York, NY 10010
0955-2863/98/$19.00
PII S0955-2863(97)00162-9

Se per g diet were used. The purpose of these experiments was twofold. One was to investigate the distribution of Se-W in a wide range of tissues and second to determine the influence of deficient, adequate, and excessive levels of dietary Se on Se-W in various tissues. For comparative purposes, the activity of GPX was also determined in these tissues.

Methods and materials

Animals

Twelve male weanling rats were divided into three groups of four each and fed either the Se-deficient diet or this diet with either 0.1 or 4.0 μg Se as sodium selenate per g for 8 weeks. To evaluate Se-W content and GPX activity in reproductive organs from female rats, four weanling female rats were fed the diet with 0.1 μg Se per g also for 8 weeks. The basal Se deficient diet was shown by analysis to contain 4 ng Se per g diet. The composition of this diet is described elsewhere[17] but briefly it contained (in g/kg) 300 torula yeast (Rhinelander, WI USA), 510 sucrose, 90 purified cellulose (Solka Floc, Brown Co., Berlin, NH USA) 50 corn oil, 35 AIN-93M mineral mix without Se, 10 AIN-76 vitamin mix,[18] 3 DL-methionine and 2 choline citrate. At the end of the experiment, all animals were anesthetized with sodium pentobarbital (80 mg/kg, I. P.) and blood was taken via cardiac puncture. The tissues were removed, frozen immediately in liquid nitrogen and stored at −80°C. Se-W content and classical GPX activity were measured. This research with animals was reviewed and approved by the animal care committee at Oregon State University.

Western blot analysis

Tissues were homogenized and the protein content measured in the supernatants by the dye-binding assay using bovine serum albumin (Bio-Rad, Richmond, CA) as a standard. Samples (200 μg protein) were separated electrophoretically on SDS-polyacrylamide 7.5 to 15% gradient gels as described.[19] Proteins were transferred onto nitrocellulose membranes (0.2 μm, S & S, Keene, NH) in transfer buffer as described by Towbin et al.[20] After transfer, membranes were blocked and subsequently incubated with rabbit anti-selenoprotein W polyclonal antibody.[16] After three washes, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Bio-Rad) and the specific bonding of anti-Se-W antibody onto membrane detected by ECL detection system (Amersham, Arlington, IL USA). The membrane was then exposed to Kodak X-OMAT film (Eastman Kodak Co., Rochester, NY USA). Developed films were scanned with a Personal Densitometer SI analyzed by the ImageQuaaNT program (Molecular Dynamics, Sunnyvale, CA USA).

Glutathione peroxidase activity

The cellular GPX activity was measured by a coupled enzyme method using hydrogen peroxide as the substrate[21] with a DU Series 64 spectrophotometer (Beckman Instruments, Fullerton, CA USA).

Statistical analysis

The multiple *t* test was used to determine differences between the dietary groups. A significance level of 5% was adopted for all comparisons.

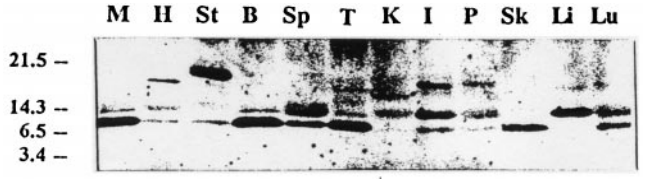

Figure 1 Tissue distribution of selenoprotein W (Se-W) in various tissues from rats fed 0.1 μg Se per g diet. The Se-W band is the one located between 6.5 and 14.3 kDa. The symbols for the various tissues are M, muscle; H, heart; St, stomach; B, brain; Sp, spleen; T, testis; K, kidney; I, intestine; P, pancreas; Sk, skin; Li, liver; and Lu, lung. Western blots were conducted as described in the methods and materials section.

Results

There were no differences in the growth of the rats fed the various diets. As expected, the final body weights of the male rats were significantly greater than the females (280 ± 15 vs 180 ± 12 g fed the diet with 0.1 μg Se per g).

A Western blot of tissue preparations from rats fed the diet with 0.1 μg Se per g is shown as an example in *Figure 1*. Of the tissues depicted in this figure, bands corresponding to Se-W were found in muscle, brain, spleen, testes, skin, and lungs. The bands were the darkest for muscle, brain, and testis.

The Se-W content and GPX activities in internal organs from the rats fed the three levels of Se are shown in *Table 1*. GPX activity increased with each increase of dietary Se in kidney, thymus and pancreas. However, no further increase of GPX activity occurred when 4.0 μg Se per g were fed as compared with 0.1 μg Se per g in heart, liver, lung, spleen, and thyroid. No Se-W was detected in liver, thyroid, or pancreas regardless of the amount of Se included in the diet and Se-W was detected only in the kidney from rats fed the highest amount of Se. Se-W was nondetectable in lungs from Se-deficient rats but was present when Se was added to the diet. Se-W levels in the lungs from rats fed the diet with 4.0 μg Se per g was significantly higher than in this organ from the rats fed the next lowest level of Se. The heart is similar to the lungs in that no Se-W was detected in this organ from Se-deficient rats, but was present when Se was added to the deficient diet. However, in contrast to the lungs, the Se-W level was not significantly different in this organ from rats fed diets with the two levels of Se. The response in thymus and spleen is similar in that Se-W was higher in rats fed the diet with 0.1 μg per g than in these organs from the deficient rats, but no further increase was found with the highest level of Se fed as compared to 0.1 μg Se per g. The adrenals showed a different pattern in that no difference was found between deficient rats and those fed diet with 0.1 μg Se per g, but an increase occurred when 4.0 μg Se per g was fed.

GPX activity was found in all of the reproductive tissues (*Table 2*). In all of the male reproductive tissues, 0.1 μg Se per g resulted in a significant increase of GPX activity as compared with these tissues from the deficient rats with no further increase of activity when the highest level of Se was fed. Se-W was undetectable in prostate and seminal vesicles from deficient animals and in seminal vesicles from rats fed the diet with 0.1 μg Se per g, but was detectable in this

Table 1 Selenoprotein W and glutathione peroxidase in internal organs of rats fed three dietary levels of selenium

Tissues	Dietary selenium		
	Deficient	0.1 μg/g	4 μg/g
Adrenals			
GPX	455 ± 114—a	1163 ± 165—b	1502 ± 44—b
Se-W	585 ± 58—a	646 ± 265—a	929 ± 87—b
Heart			
GPX	38 ± 5—a	610 ± 49—b	533 ± 62—b
Se-W	ND	25 ± 15—a	66 ± 25—a
Kidney			
GPX	24 ± 5—a	869 ± 109—b	1146 ± 209—c
Se-W	ND	ND	673 ± 25
Liver			
GPX	8 ± 2—a	992 ± 113—b	1224 ± 400—b
Se-W	ND	ND	ND
Lung			
GPX	21 ± 2—a	383 ± 41—b	295 ± 169—b
Se-W	ND	37 ± 25—a	136 ± 62—b
Spleen			
GPX	132 ± 31—a	589 ± 198—b	669 ± 112—b
Se-W	320 ± 68—a	1410 ± 13—b	1156 ± 191—b
Thymus			
GPX	49 ± 7—a	275 ± 33—b	371 ± 83—c
Se-W	148 ± 20—a	250 ± 19—b	283 ± 40—b
Thyroid*			
GPX	22	98	85
Se-W	ND	ND	ND
Pancreas			
GPX	17 ± 2—a	55 ± 2—b	133 ± 38—c
Se-W	ND	ND	ND

GPX activity is expressed as nm NADPH ox/min/mg protein; Se-W is expressed in scan units.
Values are means ± SE for four rats; ND, not detectable. Values in a horizontal row with different letters—a, b, c—are significantly different ($P < 0.05$).
*Single determination on pooled samples from four rats.

Table 2 Selenoprotein W and glutathione peroxidase in reproductive organs of rats fed three dietary levels of selenium

Tissues	Dietary selenium		
	Deficient	0.1 μg/g	4.0 μg/g
Epididimus			
GPX	32 ± 9—a	168 ± 26—b	182 ± 10—b
Se-W	444 ± 64—a	798 ± 211—ab	1099 ± 194—b
Prostate			
GPX	16 ± 3—a	149 ± 33—b	182 ± 60—b
Se-W	ND	1489 ± 9—a	1509 ± 13—a
Seminal Vesicles			
GPX	65 ± 8—a	123 ± 18—b	159 ± 37—b
Se-W	ND	ND	419 ± 16
Testis			
GPX	40 ± 1—a	115 ± 6—b	131 ± 19—b
Se-W	1688 ± 99—a	3020 ± 307—b	3147 ± 184—b
Female			
Uterus			
GPX		423 ± 39	
Se-W		266 ± 111	
Ovary			
GPX		210 ± 45	
Se-W		ND	

Values are means ± SE of four animals. Values in a horizontal row with different letters—a, b, c—are significantly different ($P < 0.05$).
GPX activity is expressed as nm NADPH oxidized/min/mg protein.
Se-W is expressed as scan units.

tissue with the highest amount of Se fed. The epididymis and testis showed similar patterns in that Se-W was significantly higher from rats fed the diet with 0.1 μg Se per g than from the deficient rats, but no further increase occurred with the higher level of Se fed. GPX was present in the uterus and ovary of the female rats but Se-W was detectable only in the uterus.

The eyes and diaphragm are the only organs where there was a significant increase of GPX activity with each increase of dietary selenium (*Table 3*). Se status had no effect on the activity of this selenoenzyme in the spinal cord. In tissues like the cerebellum and cortex (and presumably the pituitary) there was no difference in GPX activity between deficient rats and those given 0.1 μg Se per g, but a significant increase when 4.0 μg Se per g was included in the diet. This is in contrast to all the other tissues (brown adipose, esophagus, stomach, small intestine, tongue, skin, and skeletal muscle) where there was a significant increase of GPX activity with 0.1 μg Se per g diet as compared with the deficient animals, but no further increase with the highest level of Se fed. Regardless of the level of Se fed, Se-W was not detected in pituitary and eyes, and was undetectable in brown adipose, esophagus, stomach, small intestine, tongue, skin, skeletal muscle, and diaphragm from Se-deficient animals. Se status had no effect on Se-W in cerebellum or cortex. In other tissues such as brown adipose, esophagus, stomach, small intestine, tongue, skin, and skeletal muscle Se-W was undetectable in deficient animals and the levels were not different in rats fed diets with 0.1 μg Se versus 4.0 μg Se per g. Diaphragm showed a different pattern in that Se-W was not detectable in deficient rats, but was present in this tissue at higher levels in rats fed the diet with 4.0 μg Se per g than those fed the diet with 0.1 μg Se per g. The spinal cord showed another pattern in that no difference was found between the deficient rats and those fed the diet with 0.1 μg Se per g, but this organ from rats fed 4.0 μg Se per g contained significantly higher levels.

Discussion

These results indicate that the tissue distribution of Se-W in rats is more widespread than we originally thought. Of the tissues examined previously, Se-W was found only in muscle, brain, spleen, and testis.[16] Se-W was not detected in liver, thyroid, pancreas (*Table 1*), eyes or pituitary (*Table 3*) regardless of the Se status of the animal. This selenoprotein was found in all of the other tissues examined when Se was included in the diet (*Tables 1–3*). Se status had a different effect on Se-W content in the various tissues. In most tissues where Se-W was undetectable in deficient animals, it was present when 0.1 μg Se per g was present in the diet, but in some tissues like the kidney (*Table 1*) and seminal vesicles (*Table 2*) it was detected only in rats fed the diet with 4.0 μg Se per g. Further research is needed to determine what level of dietary Se is required to reach saturation of Se-W and GPX in these tissues because the present results should not be taken to imply that 4 μg Se per g diet is required to obtain maximum responses in these tissues.

Table 3 Selenoprotein W and glutathione peroxidase in brain, digestive tract and other tissues of rats fed three dietary levels of selenium

Tissues	Dietary selenium		
	Deficient	0.1 μg/g	4.0 μg/g
Brown Adipose			
GPX	26 ± 9—a	130 ± 8—b	168 ± 43—b
Se-W	ND	1215 ± 848—a	1525 ± 1212—a
Cerebellum			
GPX	61 ± 9—a	69 ± 16—a	122—b
Se-W	456 ± 143—a	569 ± 222—a	685 ± 114—a
Cortex			
GPX	57 ± 13—a	63 ± 14—a	83 ± 10—b
Se-W	428 ± 228—a	685 ± 127—a	704 ± 115—a
Pituitary*			
GPX	120	104	361
Se-W	ND	ND	ND
Esophagus			
GPX	21 ± 6—a	333 ± 54—b	277 ± 98—b
Se-W	ND	268 ± 48—a	286 ± 41—a
Stomach			
GPX	17 ± 6—a	558 ± 138—b	624 ± 197—b
Se-W	ND	120 ± 43—a	145 ± 70—a
Small Intestine			
GPX	28 ± 8—a	183 ± 42—b	269 ± 68—b
Se-W	ND	316 ± 35—a	337 ± 48—a
Spinal Cord			
GPX	86 ± 16—a	67 ± 32—a	83 ± 58—a
Se-W	40 ± 6—a	58 ± 2—a	201 ± 83—b
Tongue			
GPX	26 ± 5—a	218 ± 3—b	268 ± 6—b
Se-W	ND	466 ± 38—a	732 ± 76—b
Skin			
GPX	14 ± 2—a	196 ± 52—b	202 ± 51—b
Se-W	ND	1591 ± 99—a	1516 ± 174—a
Skeletal Muscle			
GPX	4 ± 1—a	196 ± 52—b	202 ± 51—b
Se-W	ND	453 ± 54—a	712 ± 21—b
Eyes			
GPX	12 ± 1—a	32 ± 7—b	68 ± 10—c
Se-W	ND	ND	ND
Diaphragm			
GPX	31 ± 3—a	599 ± 163—b	925 ± 60—c
Se-W	ND	525 ± 83—a	1084 ± 8—b

Values are mean ± SE of four animals. Values in a horizontal row with different letters—a, b,c—are significantly different ($P < 0.05$).
GPX activity is defined as nm NADPH oxidized/min/mg protein.
Se-W is expressed as scan units.
ND, not detectable.
*Single determination on a pool from four animals.

Of 27 tissues examined by ^{75}Se labeling, a 10 kDa band was found in brain, diaphragm, epididymis, eye, kidney, lungs, pituitary, prostate, seminal vesicles, skeletal muscle, skin, small intestine, spleen, stomach, testis, thymus, thyroid, and tongue.[15] Se-W was found in all of these tissues except for pancreas, thyroid, and pituitary (*Tables 1 and 3*). Tissues where no 10 kDa bands were found[15] and no Se-W were detected include the eye, liver, and ovary (*Tables 1–3*). However, the tissues where no 10-kDa bands were found but Se-W was detected include the adrenals, brown adipose tissue, esophagus, heart, spinal cord, and the uterus. Therefore, these results suggest that there are low molecular weight Se-containing proteins in mammalian tissues that are not Se-W.

The disagreements between the two methods could be attributable to several factors. The rats used in the labeling study had been depleted of Se for six generations,[15] and as shown in *Tables 1–3* Se-W was undetectable in many tissues from Se-deficient animals. Second, the turnovers of the Se containing proteins are not likely to be similar. Even though the rats were killed 6 and 21 days after injection of radioactive Se, this may have not been sufficient for some of the Se-containing proteins. If a Se containing protein has a very slow turnover rate, then it would be missed using this method. This is not to indicate that this method has not been useful in Se research because it has been used to identify new selenoproteins such as Type I iodothyronine deiodinase[5] and the prostatic epithelial selenoprotein.[22]

GPX activity was found in all 28 tissues examined in this study (*Tables 1–3*). This is the first report on the investigation of GPX activity in such a wide range of tissues. Liver and blood have been the most common tissues used for GPX assays. Other workers have determined the GPX activity in plasma, liver, and muscle;[23] heart, liver, testis, lung, and kidney;[24] brain;[25,26] brain, and liver;[27] heart;[28] liver and kidney;[29,30] lung;[31] thyroid;[32,33] and pancreas.[34] The results in the present investigation indicate that GPX responds differently in various tissues. In most tissues a significant increase was found with 0.1 μg Se per g diet as compared with deficient rats with no further increase with higher Se intake. However in tissues such as the kidney and pancreas (*Table 1*) and eyes and diaphragm (*Table 3*) there was a significant increase of GPX activity with each increase of dietary Se. It is interesting that Se status had no influence on GPX activity in the spinal cord, but Se affected the Se-W levels in this organ (*Table 3*). This suggests that the regulation of GPX and Se-W by Se in this organ is markedly different.

With the limited data obtained so far, it is evident that there are some species differences in the tissue distribution of Se-W. Se-W is present in the heart of sheep[34] and primates (Gu et al., Oregon State University, unpublished work) at the same concentration as the muscle whereas in the rat it is very low even when Se is given[17] (*Table 1*). Se-W responds differently to Se in various tissues. For example, Se-W in the testis responded very rapidly to low levels of Se (0.01 μg Se/g) but that in the muscle did not respond until much higher levels of Se were used.[17] Consistent with the present data there is no correlation of GPX activity with Se-W content between the various tissues.[17,34]

Like selenoprotein P,[9] the metabolic function of Se-W is not known. Consistent with our previous data,[35] the levels of this selenoprotein respond to Se intake. The cDNA for Se-W has been sequenced and there is a UGA in the open reading frame corresponding to the insertion of selenocysteine.[35] Because many selenoenzymes are involved in antioxidant functions, it has been suggested that Se-W may play such a role. This was strengthened when glutathione was demonstrated to be bound to it.[36] The wide distribution of Se-W among tissues suggest an important metabolic role for this selenoprotein.

In summary, GPX activity was found in all 28 tissues examined in the rat. Se-W was not detected in five (liver, thyroid, pancreas, pituitary, and eyes) tissues regardless of the Se status, but it was detected in all other tissues when excess Se was given. This is not to imply that excess Se is

required to increase Se-W to the maximal levels in some tissues, but further research is needed to determine the dietary levels of this element needed to result in saturation for this selenoprotein. The distribution of Se-W in tissues from rats is much wider than once thought.

Acknowledgment

We are grateful to Dr. Harold Engel, School of Veterinary Medicine, Oregon State University, for assistance in the identification of some of the tissues.

References

1 Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179,** 588–590

2 Takahashi, K., Avissar, N., Whitin, J., and Cohen, H. (1987). Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. *Arch. Biochem. Biophys.* **256,** 677–686

3 Chu, F.F., Doroshow, J.H., and Esworth, R.S. (1993). Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J. Biol. Chem.* **268,** 2571–2576

4 Schuckelt, R., Brigelius-Flohe, R., Maiorino, M., Roveri, A., and Reumken, J. (1991). Phospholipid hydroperoxide glutathione peroxidase is a selenoenzyme distinct from the classical glutathione peroxidase as evident from cDNA and amino acid sequencing. *Free Radical Res. Comm.* **14,** 343–361

5 Behne, D., Kyriakopoulos, A., Meinhold, H., and Kohrle, J. (1990). Identification of type I iodothyronine 5′-deiodinase as a selenoenzyme. *Biochem. Biophys. Res. Commun.* **173,** 1143–1149

6 Davey, J.C., Becker, K.B., Schneider, M.J., St. Bermain, D.L., and Galton, V.A. (1995). Cloning of a cDNA for the type II iodothyronine deiodinase. *J. Biol. Chem.* **270,** 26786–26789

7 Croteau, W., Whittemore, S.L., Schneider, M.J., and St. Bermain, D.L. (1995). Cloning and expression of cDNA for a mammalian type III iodothyronine deiodinase. *J. Biol. Chem.* **270,** 16569–16575

8 Tamura, T., and Stadtman, T.C. (1996). A new selenoprotein from human lung adenocarcinoma cells: purification, properties, and thioredoxin reductase activity. *Proc. Natl. Acad. Sci. USA* **93,** 1006–1011

9 Read, R., Bellow, T., Yang, J.G., Hill, K.E., Palmer, I.S., and Burk, R.F. (1990). Selenium and amino acid composition of selenoprotein P, the major selenoprotein in rat serum. *J. Biol. Chem.* **265,** 17899–17905

10 Vendeland, S.C., Beilstein, M.A., Chen, C.L., Jensen, O.N., Barofsky, E., and Whanger, P.D. (1993). Purification and properties of selenoprotein W from rat muscle. *J. Biol. Chem.* **268,** 17103–17107

11 Hawkes, W.C., Wilhelmsen, E.C., and Tappel, A.L. (1985). Subcellular distribution of selenium-containing proteins in the rat. *J. Inorg. Biochem.* **23,** 77–92

12 Danielson, K.G. and Medina, D. (1986). Distribution of selenoproteins in mouse mammary epithelial cells in vitro and in vivo. *Cancer Res.* **46,** 4582–4589

13 Behne, D., Hilmert, H., Scheid, S., Gessner, H., and Elger, W. (1988). Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochim. Biophys. Acta* **966,** 12–21

14 Evenson, J.K. and Sunde, R.A. (1988). Selenium incorporation into selenoproteins in the Se-adequate and Se-deficient rat. *Proc. Soc. Exptl. Biol. Med.* **187,** 169–180

15 Behne, D., Kyriakopoeulos, A., Weiss-Nowak, C., Kalcklosch, M., Westphal, C., and Gessner, H. (1996). Newly found selenium-containing proteins in the tissues of the rat. *Biol. Trace Elem. Res.* **55,** 99–110

16 Yeh, J-Y, Beilstein, M.A., Andrews, J.S., and Whanger, P.D. (1995). Tissue distribution and influence of selenium status on levels of selenoprotein W. *FASEB J.* **9,** 392–396

17 Yeh, J-Y, Vendeland, S.C., Gu, Q-P, Butler, J.A., Ou, B-R, and Whanger, P.D. (1997). Dietary selenium increases selenoprotein W levels in rat tissues. *J. Nutr.* **127,** 2165–2172

18 American Institute of Nutrition. (1977). Report of the AIN ad hoc committee on standards for nutritional studies. *J. Nutr.* **97,** 1340–1348

19 Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **227,** 680–685

20 Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354

21 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

22 Kalchlosch, M., Kyriakopoulos, A., Hammel, C., and Behne, D. (1995). A new selenoprotein found in the glandular epithelial cells of the rat prostate. *Biochem. Biophys. Res. Commun.* **217,** 162–170

23 Waschulewski, I.H. and Sunde, R.A. (1988). Effect of dietary methionine on utilization of tissue selenium from dietary selenomethionine for glutathione peroxidase in the rat. *J. Nutr.* **118,** 367–374

24 Lei, X.G., Evenson, J.K., Thompson, K.M., and Sunde, R.A. (1995). Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. *J. Nutr.* **125,** 1438–1446

25 Burk, R.F., Hill, K.E., Read, R., and Bellew, T. (1991). Response of rat selenoprotein P to selenium administration and fate of its selenium. *Amer. J. Physiol.* **261,** E26–E30

26 Huang, K., Lauridsen, E., and Clausen, J. (1994). The uptake of Na-selenite in rat brain. *Biol. Trace Elem. Res.* **46,** 91–102

27 Buckman, T.D., Sutphin, M.S., and Eckhert, C.D. (1993). A comparison of the effects of dietary selenium on selenoprotein expression in rat brain and liver. *Biochim. Biophys. Acta* **1163,** 176–184

28 Xia, Y., Hill, K.E., and Burk, R.F. (1985). Effect of selenium deficiency on hydroperoxide induced glutathione release from the isolated perfused rat heart. *J. Nutr.* **115,** 733–742

29 Burk, R.F., Hill, K.E., Awad, J.A., Morrow, J.D., and Lyons, P.R. (1995). Liver and kidney necrosis in selenium-deficient rats depleted of glutathione. *Lab. Invest.* **72,** 723–730

30 Viljoen, A.J., Motchnik, P.A., and Tappel, A.L. (1989). Selenium-containing proteins of rat kidney and liver microsomes. *J. Inorgan. Biochem.* **37,** 295–308

31 Jenkinson, S.G., Jordan, J.M., and Duncan, C.A. (1989). Effects of selenium deficiency on glutathione-induced protection from hyperoxia in rat. *Amer. J. Physiol.* **257,** L393–L398

32 Arthur, J.R., Nicol, F., Hutchinson, A.R., and Beckett, G.J. (1990). The effects of selenium depletion and repletion on the metabolism of thyroid hormones in the rat. *J. Inorgan. Biochem.* **39,** 101–108

33 Cammack, P.M., Zwahlen, B.A., and Christensen, M.J. (1995). Selenium deficiency alters thyroid hormone metabolism in guinea pigs. *J. Nutr.* **125,** 302–308

34 Yeh, J-Y, Gu, Q-P, Beilstein, M.A., Forsberg, N.E., and Whanger, P.D. (1997). Selenium influences tissues levels of selenoprotein W in sheep. *J. Nutr.* **127,** 394–402

35 Vendeland, S.C., Beilstein, M.A., Yeh, J-Y., Ream, R.W., and Whanger, P.D. (1995). Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium. *Proc. Natl. Acad. Sci. USA* **92,** 8749–8753

36 Beilstein, M.A., Vendeland, S.C., Barofsky, E., Jensen, O.N., and Whanger, P.D. (1996). Selenoprotein W of rat muscle binds glutathione and an unknown small molecular weight moiety. *J. Inorgan. Biochem.* **61,** 117–124