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Incorporation of Selenium into Liver Glutathione Peroxidase in the Se-Adequate and Se-Deficient Rat (40973)[1]

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Abstract. The biosynthesis of glutathione peroxidase was studied by measuring the rate of selenium (Se) incorporation into liver glutathione peroxidase in Se-adequate (0.2 ppm dietary Se as Na_2SeO_3) and Se-deficient rats (<0.02 ppm dietary Se). Rats were injected iv with [^{75}Se]selenite (1.29 μg Se/100 g rat) and sacrificed 0.5, 1, 3, 6, 12, 24, or 72 hr post-injection. Sephadex G-150 chromatography of liver supernatant was used to determine the level of ^{75}Se incorporation into glutathione peroxidase. Se-adequate rats incorporated ^{75}Se into glutathione peroxidase within 0.5 hr after ^{75}Se injection whereas Se-deficient rats had a lag of 2 to 3 hr before detectable ^{75}Se incorporation. After 72 hr, 70 and 50% of the supernatant ^{75}Se was incorporated into glutathione peroxidase in Se-adequate and Se-deficient rats, respectively. There were no ^{75}Se-labeled fractions in the chromatograms uniquely associated with rats of only one Se status, indicating that Se metabolism was similar in Se-adequate and Se-deficient rats. Cycloheximide injection 30 min prior to ^{75}Se injection completely blocked ^{75}Se incorporation into liver glutathione peroxidase in both Se-adequate and Se-deficient rats, demonstrating that protein synthesis was required for Se incorporation. These results suggest that large quantities of Se-free, glutathione peroxidase precursor do not accumulate in the liver; these results do not indicate, however, whether Se incorporation occurs during the translational or post-translational phase of protein synthesis.

The rate of selenium (Se) incorporation into glutathione peroxidase (glutathione:H_2O_2 oxidoreductase, EC 1.11.1.9) has not been studied extensively. Millar (1) followed ^{75}Se incorporation into rat liver, kidney, and plasma proteins before glutathione peroxidase (GSH-Px) was shown to be a selenoenzyme (2). Chromatography of liver and kidney supernatant and blood plasma, from rats injected 5 days prior with sodium [^{75}Se]selenite, revealed one major ^{75}Se-containing protein fraction in each tissue. At earlier times other ^{75}Se-labeled fractions were observed. Pierce and Tappel (3) found that selenite and selenomethionine significantly increased liver, kidney, and stomach GSH-Px activity 48 hr after oral Se administration. A number of workers have reported increases in tissue GSH-Px activity in several species after Se administration for longer preiods of time (4–9). These reports, however, do not reveal much information about the time course of Se incorporation and the biosynthesis of GSH-Px.

The present study investigated the time course of Se incorporation into liver GSH-Px in rats injected with ^{75}Se as sodium [^{75}Se]selenite, using gel filtration chromatography. The rate of ^{75}Se incorporation in Se-adequate rats was compared with the rate in Se-deficient rats, and the effect of cycloheximide treatment on ^{75}Se incorporation was determined.

Materials and methods. Male 21-day old weanling rats (Holtzman, Madison, Wisc.) were housed in individual hanging wire mesh cages and fed the 30% torula yeast-based diet of Schwarz (10) as modified by Hafeman and Hoekstra (11). The diet was supplemented with 0.3% DL-methionine (U.S. Biochemical Corp., Cleveland, Ohio) and 100 IU/kg of all-*rac*-α-tocopherol acetate (ICN Pharmaceuticals, Inc., Cleveland, Ohio). Half of the rats were fed the basal diet, which contained less than 0.02 ppm Se as determined by fluorometric analysis (5), and half were fed the diet

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supplemented with 0.2 ppm Se as Na_2SeO_3 (ICN K&K Laboratories, Cleveland, Ohio). Diet and water were provided *ad libitum*. The rats were fed these diets for at least 39 days before they were used for experimentation; the rats fed the basal diet were Se deficient as shown by an average liver GSH-Px activity of 0.015 EU/mg protein as compared to 0.765 EU/mg for the Se-supplemented rats. The Se-deficient rats weighed an average of 300 g as compared to 350 g for the Se-adequate rats.

Rats were anesthetized with ether and injected with 1.29 μg Se/100 g rat as sodium [^{75}Se]selenite (10.7 mCi/mg Se) (Amersham, Arlington Heights, Ill.) into the femoral vein. One Se-adequate and one Se-deficient rat were each sacrificed at 0.5, 1, 3, 6, 12, 24, and 72 hr after injection. The rats were anesthetized and blood was drawn by cardiac puncture with a heparinized syringe. The livers were perfused with ice-cold 0.15 *M* KCl to remove contaminating erythrocytes. The liver was homogenized in 3 vol of buffer (the buffer used for homogenization, dialysis, and chromatography was pH 6.85 buffer containing 0.05 *M* sodium phosphate, 0.5 m*M* GSH, and 0.25 m*M* EDTA) with a Potter–Elvehjem homogenizer, and centrifuged at 105,000*g* × 60 min to obtain the supernatant. A portion of the supernatant from rats sacrificed 12 hr or less postinjection was dialyzed against 400 vol of buffer for 18 hr to remove loosely bound ^{75}Se. Eight milliliters of supernatant or dialyzed supernatant were applied to a 2.2 × 88-cm column packed with Sephadex G-150, and eluted (20 ml/hr) with buffer; 5-ml fractions were collected. Supernatant was chromatographed sequentially, first from a Se-adequate rat and then from a Se-deficient rat, at each time point.

The GSH-Px activity of the homogenates, supernatants, and fractions was determined according to the procedure of Lawrence *et al.* (12) using H_2O_2 so only the Se-containing GSH-Px was assayed (13). ^{75}Se was measured with a Packard Model 5220 gamma counter with an efficiency of 40%. Protein concentration in the column fractions was estimated by the absorbance at 280 nm. Cytochrome *c*, α-chymotrypsinogen A, ovalbumin, bovine serum albumin, and human γ-globulin were used as standards for the molecular weight calibration of the Sephadex G-150 chromatograms (14).

Cycloheximide inhibition. A Se-adequate rat was injected ip with 5 mg/kg cycloheximide (Sigma, St. Louis, Mo.) 30 min prior to the ^{75}Se injection, and with 2.5 mg/kg cycloheximide 1.5 hr after the ^{75}Se injection; the rat was sacrificed 3 hr after the ^{75}Se injection. A Se-deficient rat was injected with 5 mg/kg cycloheximide 30 min prior to the ^{75}Se injection; an additional 1.5 mg/kg cycloheximide was administered at 2.5, 5.5, and 8.5 hr after the ^{75}Se injection. The rat was sacrificed 12 hr after the ^{75}Se injection. The livers were treated and analyzed exactly as the other livers. These levels of cycloheximide have been shown to block amino acid incorporation into rat plasma and liver protein (15) and the induction of metallothionein by zinc administration (16).

Results. The quantity of Se injected into these animals is seven times higher than the quantity of Se ingested daily by rats fed the basal diet, and half of the quantity consumed by the Se-supplemented rats (assuming daily feed consumption was 10% of the body weight), indicating that the quantity of Se injected was in the physiological range.

The percentage of the ^{75}Se dose in 1 ml of blood reached a maximum between 3 and 6 hr postinjection for both Se-adequate and Se-deficient rats. At each time point, 1 ml of blood from the Se-deficient rats contained a greater percentage of the dose as compared to the Se-adequate rats (data not shown).

The Se-adequate rats retained a greater percentage of the ^{75}Se in the liver than did the Se-deficient rats at 0.5 and 1 hr after ^{75}Se injection (Table I). However, 3 hr or later after injection, the Se-adequate rats retained less of the dose in the liver than did the Se-deficient rats. The percentage of liver ^{75}Se in the supernatant was approximately 45% at all times for the Se-adequate rats; the supernatant from the Se-deficient rats initially contained 18 to 33% of the liver ^{75}Se, but by 6 hr postinjection the percentage of liver ^{75}Se present in the supernatant

TABLE I. EFFECT OF TIME AFTER ADMINISTRATION ON THE RETENTION, DISTRIBUTION, AND RELEASE OF ^{75}Se IN RAT LIVER

Time after ^{75}Se administration (hr)	Percentage of ^{75}Se dose retained by the liver		Percentage of liver ^{75}Se in supernatant[a]		Percentage of supernatant ^{75}Se removed by dialysis[b]	
	+Se[c]	−Se[d]	+Se[c]	−Se[d]	+Se[c]	−Se[d]
0.5	36	28	46	25	77	32
1	54	37	47	18	83	20
3	22	30	47	33	83	47
6	18	22	44	43	53	15
12	6	11	34	44	22	6
24	6	13	43	44	—	—
72	5	6	47	40	—	—
3 + cycloheximide[e]	30	—	55	—	—	—
12 + cycloheximide[e]	—	16	—	33	—	—

[a] 105,000g × 60 min supernatant of the liver homogenate.
[b] This value is the percentage of supernatant ^{75}Se lost by dialysis against 400 vol of buffer for 18 hr.
[c] Se-adequate rats.
[d] Se-deficient rats.
[e] These rats were treated with cycloheximide 30 min prior to ^{75}Se administration and during the experiment, as described in the text.

was no longer less for the Se-deficient than for the Se-adequate rats. Dialysis of the supernatant from livers obtained 0.5 to 3 hr postinjection demonstrated that 80% of the ^{75}Se was released from Se-adequate liver supernatant and 20 to 50% from Se-deficient supernatant. The percentage of ^{75}Se released from the supernatant decreased for both Se-adequate and Se-deficient liver at 6 and 12 hr postinjection.

Sephadex G-150 chromatograms of liver supernatant from Se-adequate rats injected at 0.5, 3, 12, and 72 hr before sacrifice are shown in Fig. 1. As early as 0.5 hr postinjection, a small peak of ^{75}Se coeluted with the GSH-Px activity peak. Three additional peaks of >200,000, 20,000 to 30,000, and <5000 daltons were also present in this chromatogram. At 3 hr postinjection, the ^{75}Se peak coeluting with the GSH-Px activity peak was the largest peak except for the <5000-dalton peak; at 12, 24, and 72 hr postinjection the GSH-Px peak contained the major ^{75}Se peak (Table II). The <5000-dalton peak contained the majority of the ^{75}Se from 0.5 to 6 hr postinjection. After 6 hr, the GSH-Px peak was the major ^{75}Se-containing peak in the liver supernatant from Se-adequate rats.

The Sephadex G-150 chromatograms of liver supernatant from Se-deficient rats are shown in Fig. 2. Because of the severe Se deficiency, no GSH-Px activity was detected in any of the column fractions; however, the elution position for GSH-Px was between fractions 33 and 35 as determined by chromatography of supernatant from Se-adequate rats. At 0.5 hr the ^{75}Se peak near this region appeared to elute before fractions 33–35 and coincided with a major protein (A_{280}) peak; a discrete peak coeluting with GSH-Px was not detectable. At 3 hr postinjection a small ^{75}Se peak eluted where GSH-Px eluted. At 12, 24, and 72 hr postinjection the GSH-Px and the >200,000-dalton peak were the major ^{75}Se-containing peaks (Table II). In the Se-deficient rats an approximately 120,000-dalton ^{75}Se peak was observed at 0.5, 1, 3, and 12 hr postinjection. The <5000-dalton peak was a major ^{75}Se-containing peak only at 3 hr postinjection in the Se-deficient rats.

Dialysis experiments. Dialysis and subsequent Sephadex G-150 chromatography of the 0.5-hr postinjection supernatant from a Se-adequate rat resulted in loss of the <5000-dalton ^{75}Se peak, decreases in each of the other ^{75}Se peaks except GSH-Px, and the appearance of an approximately 120,000-dalton peak (Fig. 3). The ^{75}Se-

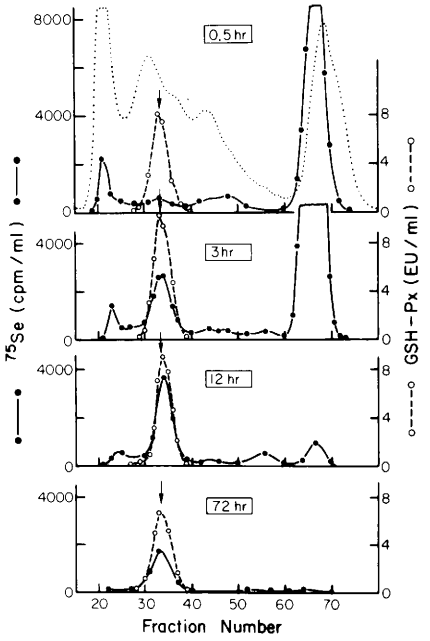

FIG. 1. Sephadex G-150 chromotograms of liver supernatant from Se-adequate rats injected with ^{75}Se as $Na_2{}^{75}SeO_3$ 0.5, 3, 12, or 72 hr prior to sacrifice, as described in the text. The GSH-Px peaks are indicated with arrows. The A_{280} profile, similar for all chromatograms, is shown in the 0.5-hr chromatogram.

labeled GSH-Px peak appeared as a shoulder of the 120,000-dalton peak. Dialysis of the 3-hr postinjection supernatant from the Se-adequate rat did not affect the ^{75}Se-labeled GSH-Px peak; all other peaks were reduced substantially by dialysis.

Dialysis of the supernatant from a Se-deficient rat injected 0.5 hr before sacrifice reduced the quantity of ^{75}Se in each peak except the 120,000-dalton peak (Fig. 4). Chromatography of the dialyzed 3-hr postinjection supernatant from a Se-deficient rat showed a 120,000-dalton peak with only a shoulder where GSH-Px eluted, whereas the GSH-Px peak was the major ^{75}Se peak in the chromatogram of the dialyzed, 3-hr supernatant from a Se-adequate liver. Dialysis of the 6- and 12-hr postinjection supernatants did not affect the ^{75}Se-labeled GSH-Px peaks in either the Se-adequate or Se-deficient rat liver chromatograms (data not shown).

Cycloheximide inhibition. Cycloheximide treatment did not substantially alter the percentage of ^{75}Se retained by the liver or the percentage of liver ^{75}Se present in the supernatant (Table I). Cycloheximide injection eliminated coelution of a ^{75}Se peak

TABLE II. EFFECT OF TIME AFTER ADMINISTRATION ON THE RELATIVE DISTRIBUTION OF ^{75}Se IN THE SEPHADEX G-150 CHROMATOGRAMS OF LIVER SUPERNATANT[a]

Time after ^{75}Se administration (hr)	Chromatogram peak[b]											
	>200,000		120,000		GSH-Px		20,000–30,000		10,000		<5000	
	+Se[c]	−Se[d]	+Se	−Se	+Se	−Se	+Se	−Se	+Se	−Se	+Se	−Se
0.5	7	28	—	17	5	—	9	23	—	—	61	14
1	5	23	—	26	4	—	3	23	—	—	83	8
3	2	21	—	9	9	6	2	8	2	—	82	39
6	8	35	—	—	22	24	2	11	—	—	52	12
12	6	19	—	9	56	36	5	10	10	14	14	3
24	8	23	—	—	68	49	—	5	10	11	5	—
72	2	27	—	—	72	49	—	2	9	10	5	—
3 + cycloheximide[e]	3	—	—	—	—	—	2	—	—	—	93	—
12 + cycloheximide[e]	—	40	—	—	—	—	—	19	—	—	—	24

[a] Sephadex G-150 chromatography of 8 ml of liver supernatant, from rats injected with ^{75}Se as $Na_2{}^{75}SeO_3$ at various times before sacrifice, as described in the text, resulted in chromatograms with up to six separate ^{75}Se peaks. The percentage of the total chromatogram ^{75}Se present in each peak is shown.

[b] Molecular weight calibration of the Sephadex G-150 chromatograms demonstrated that the ^{75}Se peaks had apparent molecular weights of approximately >200,000 (21–24), 120,000 (29–31), 80–95,000 (GSH-Px, 33–35), 20–30,000 (40–50), 10,000 (55) and <5000 (60–70) (fraction numbers in parentheses).

[c] Se-adequate rats.

[d] Se-deficient rats.

[e] These rats were treated with cycloheximide 30 min prior to ^{75}Se administration and during the experiment, as described in the text.

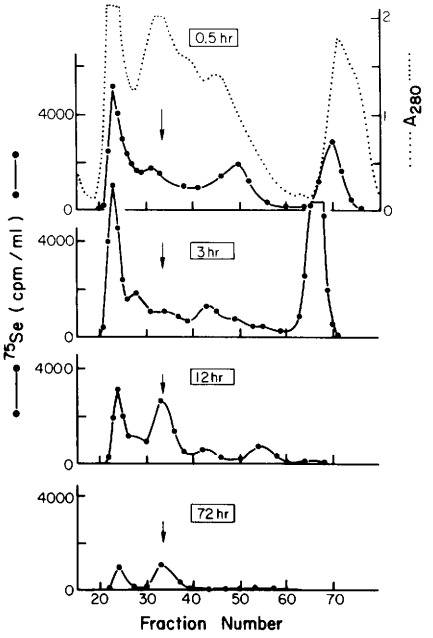

FIG. 2. Sephadex G-150 chromatograms of liver supernatant from Se-deficient rats injected with ^{75}Se as $Na_2{}^{75}SeO_3$ 0.5, 3, 12, or 72 hr prior to sacrifice, as described in the text. The arrows indicate where GSH-Px eluted when supernatant from Se-adequate rats was chromatographed. The A_{280} profile, similar for all chromatograms, is shown in the 0.5-hr chromatogram.

with the GSH-Px activity peak in the chromatogram of supernatant from a Se-adequate rat sacrificed 3 hr after the ^{75}Se injection (Fig. 5, Table II). Over 90% of the ^{75}Se was associated with the <5000-dalton

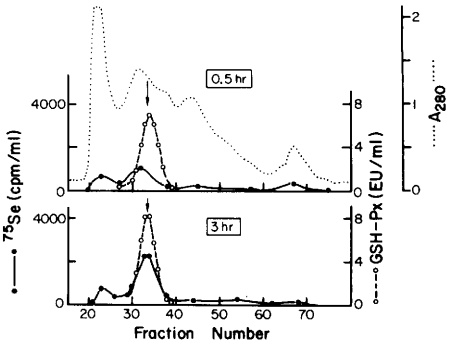

FIG. 3. Sephadex G-150 chromatograms of dialyzed liver supernatant from Se-adequate rats injected with ^{75}Se 0.5 or 3 hr prior to sacrifice, as described in the text. The GSH-Px peaks are indicated with arrows.

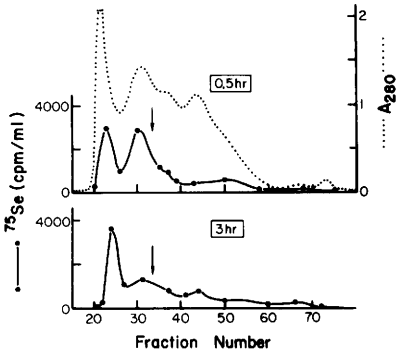

FIG. 4. Sephadex G-150 chromatograms of dialyzed liver supernatant from Se-deficient rats injected with ^{75}Se 0.5 or 3 hr prior to sacrifice, as described in the text. The arrows indicate where GSH-Px eluted when supernatant from Se-adequate rats was chromatographed.

peak. Similarly, no ^{75}Se eluted where GSH-Px eluted when supernatant from a Se-deficient rat, treated with cycloheximide and sacrificed 12 hr after the ^{75}Se injection, was chromatographed (Fig. 5, Table II). Peaks of >200,000, 33,000, and <5000 daltons were the major ^{75}Se peaks in this chromatogram.

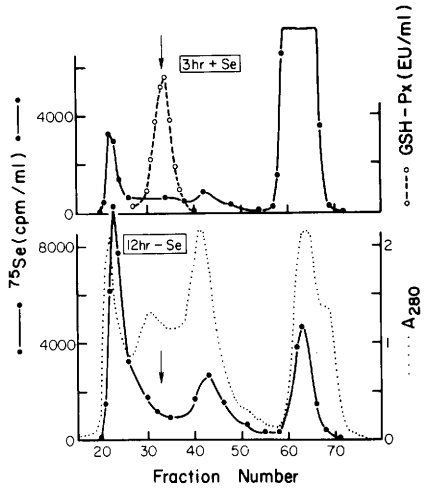

FIG. 5. Sephadex G-150 chromatograms of liver supernatant from cycloheximide-treated rats. The upper chromatogram is from a Se-adequate rat sacrificed 3 hr after the ^{75}Se injection and 3.5 hr after the first cycloheximide injection. The lower chromatogram is from a Se-deficient rat sacrificed 12 hr after the ^{75}Se injection and 12.5 hr after the first cycloheximide injection. A detailed explanation is in the text. The elution position for GSH-Px is indicated with arrows.

Discussion. Sephadex G-150 chromatography of liver supernatant from Se-adequate rats demonstrated coelution of GSH-Px activity and ^{75}Se when the rats were sacrificed only 30 min after ^{75}Se injection. In contrast, chromatograms of supernatant from Se-deficient rats showed elution of a detectable ^{75}Se peak where GSH-Px eluted only when the rats were sacrificed 3 hr or longer after ^{75}Se injection. A small amount of undetected ^{75}Se incorporation may have occurred in the Se-deficient livers prior to 3 hr, but it was quantitatively far less than that observed in the Se-adequate livers. A substantial ^{75}Se-labeled GSH-Px peak, which was the major ^{75}Se peak after dialysis, was observed in Se-adequate rats 3 hr after the ^{75}Se injection but not until 6 hr after injection in Se-deficient rats. The GSH-Px peak contained 70% of the supernatant ^{75}Se in Se-adequate rats and 50% of the supernatant ^{75}Se in Se-deficient rats sacrificed 24 or 72 hr after ^{75}Se injection; the difference was accounted for by the >200,000-dalton ^{75}Se peak which was much larger in the Se-deficient than in the Se-adequate chromatograms.

Millar (1) injected rats, fed a diet containing 0.07 ppm Se, with 0.7 μg Se as sodium [^{75}Se]selenite and found a ^{75}Se distribution similar to that observed in this study. The 3-day Sephadex G-200 chromatogram of liver supernatant had ^{75}Se peaks of 278,000, 101,000, 12,900, and 3600 daltons. The 5- and 14-day chromatograms consisted primarily of the 101,000-dalton ^{75}Se peak; this peak is most likely GSH-Px, as shown by the chromatograms of the present experiment, and because 70% of the cytosolic Se in rat liver can be accounted for by GSH-Px (unpublished data). The other peaks in the 3-day chromatogram of Millar correspond quite well with the major peaks observed in the present experiment. Black *et al.* (18) have also reported 90,000-, 30,000-, and 10,000-dalton ^{75}Se binding peaks in Sephadex G-150 chromatograms of liver cytosol from sheep injected 6 and 10 days prior to sacrifice with sodium [^{75}Se]-selenite. These workers reported that the Se status of the sheep had only a minor effect on the ^{75}Se distribution in the chromatograms.

The nature of the non-GSH-Px ^{75}Se-binding proteins observed in the chromatograms is unknown. One possibility is that the 20,000-dalton fraction might be subunits of GSH-Px; similarly, the larger molecular weight fractions might be oligomers of GSH-Px, or GSH-Px subunits binding to other proteins. The approximately 10,000-dalton protein may be related to the Se-binding protein observed in sheep tissues (18) or may be Se bound to metallothionein (19). However, dialysis increased the amount of ^{75}Se bound to the 120,000-dalton peak (at early time points) yet removed ^{75}Se from the other peaks, but not from GSH-Px. This suggests that the Se present in these other peaks may be nonspecifically bound Se rather than Se incorporated into selenoproteins.

We have previously shown, using CM-cellulose chromatography of pooled GSH-Px-containing fractions from the Sephadex G-150 chromatography, that ^{75}Se coeluting with GSH-Px under these conditions is specifically incorporated into GSH-Px and that Sephadex G-150 chromatography alone is sufficient to determine the level of ^{75}Se incorporation into rat liver GSH-Px (17). Pierce and Tappel (3) have reported significant increases in liver GSH-Px activity 48 hr after large single oral doses of Se (300 μg/90 g rat) were given to Se-deficient rats. Nominal increases in GSH-Px activity were observed as early as 3 hr after Se administration, indicating that substantial Se incorporation into GSH-Px can occur within the time periods examined in the present study.

Cycloheximide treatment of rats prior to injection of ^{75}Se blocked completely ^{75}Se incorporation into GSH-Px in either Se-adequate or Se-deficient rats. This suggests that large quantities of Se-free precursor, in which Se can be incorporated, are not present in Se-adequate or Se-deficient rat liver, and that protein synthesis is required for Se incorporation into GSH-Px.

The Se moiety in GSH-Px, at least in the fully reduced form of the enzyme, is thought to be selenocysteine (20, 21). Selenoproteins are apparently very rare and thus it seems unlikely that a specific codon exists for selenocysteine. Instead, post-translational modification of an already

synthesized or partially synthesized polypeptide (22) is a more likely mechanism for Se incorporation into GSH-Px. The 2- to 3-hr lag in ^{75}Se incorporation observed in the Se-deficient rats might result because several hours are required for the induction of GSH-Px-mRNA, and possibly for the induction of other enzymes necessary for post-translational incorporation of Se into GSH-Px.

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