Origin and turnover of mitochondrial glutathione

(buthionine sulfoximine/ γ -glutamylcysteine synthetase/ γ -glutamyl cycle)

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ABSTRACT Mitochondrial glutathione in liver does not arise by intramitochondrial synthesis, but rather from the cytoplasm, by a process characterized by slow net transport and more rapid exchange transport.

Glutathione (GSH) biosynthesis is specifically inhibited by buthionine sulfoximine, a mechanism-based inhibitor of γ -glutamylcysteine synthetase (1, 2). After administration of this compound, GSH is rapidly depleted from many cells and tissues because it continues to be used by various transport and metabolic processes. Previous experiments showed that the levels of GSH in the liver and kidney of mice given a single dose of buthionine sulfoximine decrease within 30–60 min to values that are 15–20% of those of untreated controls. Further decrease of the GSH levels occurs much more slowly even if additional buthionine sulfoximine is administered. In contrast, similar studies with higher homologs of buthionine sulfoximine (hexathionine sulfoximine and heptathionine sulfoximine) showed rapid and virtually complete disappearance of GSH from both liver and kidney (2).

The present work elucidates the biphasic disappearance of GSH after administration of buthionine sulfoximine. Previous findings suggested that a separate pool of GSH is sequestered in certain cell types or within cell organelles. The observation that mitochondrial GSH decreases much more slowly than total tissue GSH after buthionine sulfoximine administration (3) suggested that there is a separate mitochondrial pool of GSH. However, since injected buthionine sulfoximine does not readily penetrate into mitochondria (3), it is possible that mitochondrial GSH synthesis occurs but is protected from inhibition by this transport barrier. The present studies exclude this interpretation. We have also examined the rates of incorporation of radiolabel from [35 S]cyst(e)ine into cytoplasmic and mitochondrial GSH and have found that these rates are similar.

EXPERIMENTAL PROCEDURES

Materials. Reagents used for the isolation of mitochondria and for the assay of enzymes and GSH were obtained from Sigma. Monobromobimane (Thiolyte) was obtained from Calbiochem. Rockefeller strain NCS male mice and Sprague-Dawley rats, obtained from Taconic Farms (Germantown, NY), were maintained on standard Purina laboratory chow. L-Buthionine-SR-sulfoximine (L-(S-n-butyl)homocysteine-SR-sulfoximine), L-hexathionine-SR-sulfoximine (L-(S-n-hexyl)homocysteine-SR-sulfoximine) (2, 4), and L-2-oxothiazolidine-4-carboxylic acid (5) were prepared as described. L-[35 S]Cysteine was obtained from New England Nuclear.

Methods. *Isolation of cytoplasmic and mitochondrial fractions for enzyme studies.* Mitochondria were isolated from the livers of fed rats by the procedure of Greenawalt (6). The

isolation medium (200 mM D-mannitol/70 mM sucrose/2 mM K⁺ Hepes, pH 7.4) did not contain bovine serum albumin but was supplemented with 5 mM 5-oxo-L-proline for studies of 5-oxoprolinase. The livers of 2 rats (24–27 g of tissue) yielded $\approx$ 2 ml of mitochondrial pellet, which contained 50–60% of the citrate synthase activity (7) of the whole liver homogenate. The mitochondrial wash solutions contained no citrate synthase, indicating that matrix enzymes remained sequestered in the isolated mitochondria. Examination of the mitochondrial pellet by electron microscopy showed intact and generally well-preserved mitochondria (>90%) as well as some small vesicles and lysosomes. For enzyme studies, isolated mitochondria were suspended in 1 ml of isolation medium and sonicated (Branson model W185D, with cup, at 180 W) for two 3-min intervals. To remove membrane-bound ATPase activity, the crude sonicate was centrifuged at 145,000 $\times$ g for 3 hr, and the supernatant was assayed for γ -glutamylcysteine synthetase, GSH synthetase, and 5-oxo-L-prolinase. Other enzymes were assayed in the crude sonicate; all results are calculated to refer to the original sonicate.

Isolation of cytoplasmic and mitochondrial fractions for GSH determination. Mouse livers were removed immediately after sacrifice and a small portion (0.3–0.5 g) was weighed and homogenized in 5 vol of 1% (wt/vol) picric acid. After centrifugation, a portion of the supernatant was assayed for whole liver GSH. The remainder of the liver ($\approx$ 1.5 g) was rinsed with cold isolation medium (see above), minced in 2 vol of the medium, and homogenized. A small portion was assayed for protein, and mitochondria were isolated from the remaining homogenate as described above. The mitochondria ($\approx$ 100 mg) were mixed with 500 μ l of isolation medium and 300 μ l of the resulting suspension was added to 100 μ l of 5% (wt/vol) picric acid in ethanol. The mixture was homogenized and then centrifuged. The supernatant was assayed for mitochondrial GSH; the remaining suspension was assayed for protein.

Assay protocols. Total glutathione [GSH and glutathione disulfide (GSSG) in GSH equivalents] was determined by using a recycling enzymatic assay based on GSSG reductase (8, 9). The specific activity of G 35 SH was determined after HPLC fractionation of monobromobimane derivatives of tissue thiols (10). Peaks were identified and quantitated fluorimetrically by comparison with standards; radioactivity was determined in collected fractions by liquid scintillation counting. Cytoplasmic protein was determined by a modification of the method of Lowry (11, 12). Mitochondrial protein was determined by this method or by the biuret assay (13). Bovine serum albumin was used as standard.

γ -Glutamylcysteine synthetase was determined as the formation of 5-oxo-[14 C]proline from L-[14 C]glutamate in the presence of excess γ -glutamylcyclotransferase (14). The standard reaction mixture contained in a final vol of 0.5 ml, the following: 150 mM Tris-HCl buffer (pH 8.2), 100 mM KCl, 10 mM ATP, 10 mM phosphoenolpyruvate, 25 mM MgCl₂, 0.2 mM EDTA, 5 mM L-[14 C]glutamate, 10 mM L-

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Abbreviations: GSH, glutathione; GSSG, glutathione disulfide.

α -aminobutyrate (or L-cysteine), 2 international units (IU) of sheep brain γ -glutamylcyclotransferase, 10 IU of pyruvate kinase, and either mitochondrial or cytoplasmic homogenate (added last to initiate the reaction). The reaction mixtures were incubated at 30°C for up to 30 min and were stopped by placing in boiling water (2 min). The product, 5-oxo-L-[¹⁴C]proline, was determined as radiolabel not binding to small Dowex 50 (H⁺) columns. Since this chromatographic procedure does not distinguish 5-oxo-[¹⁴C]proline from α -keto[¹⁴C]glutarate, a product of glutamate transamination, some reaction mixtures included 1 mM aminooxyacetate, a transaminase inhibitor that does not inhibit rat kidney γ -glutamylcysteine synthetase. In some experiments, 2 mM L-buthionine-SR-sulfoximine or 2 mM cystamine, inhibitors of γ -glutamylcysteine synthetase (1, 2, 15), were added. In all experiments, independent ATPase assays established that ATP was not fully depleted during the synthetase assay.

GSH synthetase was determined at 37°C in reaction mixtures (final vol, 0.5 ml) containing 100 mM Tris-HCl buffer (pH 8.2), 50 mM KCl, 10 mM ATP, 5 mM phosphoenolpyruvate, 20 mM MgCl₂, 2 mM EDTA, 5 mM L- γ -glutamyl-L- α -aminobutyrate, 5 mM [¹⁴C]glycine, 10 IU of pyruvate kinase, and either mitochondrial or cytoplasmic supernatant (added last to initiate reaction). Formation of radiolabeled tripeptide was determined as described (16). In some experiments, L- γ -glutamyl-L- α -aminobutyrate was replaced by D- γ -glutamyl-L- α -aminobutyrate or L- γ -glutamyl-L-cysteine.

γ -Glutamyl transpeptidase was assayed at 37°C in reaction mixtures (final vol, 1.0 ml) containing 150 mM Tris-HCl buffer (pH 8.0), 100 mM KCl, 1.0 mM L- γ -glutamyl-p-nitroanilide, with or without 20 mM glycylglycine and cytoplasmic or mitochondrial enzyme. After 60 min, formation of p-nitroaniline was quantitated spectrophotometrically ($E_{405} = 9.9 \times 10^3$) (17). In control experiments, the enzyme was added at the end of the 60-min incubation period.

γ -Glutamylcyclotransferase was assayed at 37°C in reaction mixtures (final vol, 0.2 ml) containing 150 mM Tris-HCl buffer (pH 8.0), 100 mM KCl, 5 mM L- γ -[¹⁴C]glutamyl-L- α -aminobutyrate, and enzyme (added last). In some experiments, 10 mM 5-oxo-L-proline was added to minimize metabolism of product. After 30 min, the mixtures were stopped by placing in boiling water (2 min). The product, 5-oxo-L-[¹⁴C]proline was separated from reactant on small columns of Dowex 50 and was quantitated by liquid scintillation counting (18).

5-Oxo-L-prolinase was assayed at 37°C in reaction mixtures (final vol, 0.5 ml) containing 100 mM Tris-HCl buffer (pH 7.8), 150 mM KCl, 5 mM ATP, 5 mM phosphoenolpyruvate, 10 mM MgCl₂, 4 mM dithiothreitol, 0.1 mM Na EDTA, 4 mM L-methionine-SR-sulfoximine, 3 mM (cytoplasm) or 4 mM (mitochondria) 5-oxo-L-[¹⁴C]proline, 10 IU of pyruvate kinase, and cytoplasmic or mitochondrial homogenate. In some experiments, 1 mM aminooxyacetic acid or 20 mM L-2-imidazolidone-4-carboxylate (a 5-oxo-prolinase inhibitor) was added. After 10 to 60 min, the reactions were stopped by placing in boiling water (2 min). The product, L-[¹⁴C]glutamate, was separated from radiolabeled reactant on small columns of Dowex 50 and was quantitated by liquid scintillation counting (19).

RESULTS

Effect of a Single Dose of Buthionine Sulfoximine on GSH Levels. In the studies described in Table 1, the GSH levels of the separated cytoplasmic and mitochondrial fractions were determined after giving mice a single dose of buthionine sulfoximine. The GSH level of the cytoplasmic fraction was 35% of the untreated controls 3 hr after injection of buthionine sulfoximine, whereas there was no significant decline in mitochondrial GSH. Ten hours after injection of

Table 1. GSH levels of liver mitochondria after administration of sulfoximines to mice

Compound	Time after injection, hr	% of control GSH level	
		Cytoplasm	Mitochondria
Buthionine	3	35 $\pm$ 10	99 $\pm$ 7
sulfoximine	10	79 $\pm$ 12	95 $\pm$ 16
Hexathionine	3	41 $\pm$ 9	52 $\pm$ 4
sulfoximine	10	3 $\pm$ 2	8 $\pm$ 11*

Mice were injected intraperitoneally with the sulfoximines (3 mmol per kg of body weight) at a time chosen so that the mice could be sacrificed between 10 and 11 a.m. Control mice were injected with saline. The cytoplasmic and mitochondrial fractions were separated and analyzed for GSH. Data are given as means $\pm$ SD ($n = 8$). The GSH content of the cytoplasmic and mitochondrial fractions obtained from control mice is 31 $\pm$ 5 nmol per mg of protein and 7.0 $\pm$ 0.5 nmol per mg of protein, respectively.

*No GSH was detected in the mitochondrial fractions obtained from three mice of this group.

buthionine sulfoximine, the cytoplasmic GSH level was $\approx$ 79% of the control [in general agreement with previous observations (20)], whereas the mitochondrial level of GSH was not significantly decreased. In contrast, administration of hexathionine sulfoximine was followed by substantial decrease of both cytoplasmic and mitochondrial GSH levels.

Effect of Long-Term Administration of Buthionine Sulfoximine. A group of 10 mice was injected with buthionine sulfoximine and buthionine sulfoximine was added to the drinking water. The control mice were given equivolume injections of saline and drank tap water. After 5 days, the GSH content of the kidney was 8.5% that of the controls (Table 2); in short-term experiments, the residual GSH is higher (15–20% of the controls) (20). The liver GSH levels were within the range found in short-term experiments. In a similar study in which mice were treated with buthionine sulfoximine for 10 days, the kidney GSH levels decreased to $\approx$ 5% of the controls, and the liver GSH levels were also lower than found in short-term experiments—i.e., $\approx$ 10% of the controls. Notably, the mitochondrial GSH content declined to 24% of the controls after 10 days of buthionine sulfoximine treatment.

To determine whether administered buthionine sulfoximine enters liver mitochondria, several groups of animals were given intraperitoneal injections of buthionine sulfoximine (6 mmol/kg). The animals were sacrificed 1, 2, and 4 hr after injection, and the liver mitochondrial fractions were isolated and analyzed for buthionine sulfoximine on an amino acid analyzer. No buthionine sulfoximine was found, suggesting that it does not readily penetrate into the mitochondrial matrix.

Activity of γ -Glutamyl Cycle Enzymes in Mitochondria. The studies described above indicate the importance of determining whether mitochondria have significant capacity to synthesize GSH. Therefore, the activities of five γ -glutamyl cycle enzymes were determined in the cytoplasmic and mitochondrial fractions of liver (Table 3). We were unable to detect any mitochondrial γ -glutamylcysteine synthetase activity under conditions in which an activity of 0.1 nmol per hr per mg of protein could be detected. The mitochondrial activity is therefore $<0.1\%$ of that found in cytoplasm. Similarly, a very low level ($\approx 0.3\%$ of the cytoplasmic value) of GSH synthetase was found. γ -Glutamyltranspeptidase and 5-oxoprolinase activities were not detected in mitochondria. The activity of γ -glutamylcyclotransferase in the mitochondria was $\approx 0.2\%$ of that found in the cytoplasm.

Incorporation of [³⁵S]Cysteine into Cytoplasmic and Mitochondrial GSH. The studies described above indicate that there is very slow net flow of GSH from mitochondria to

Table 2. GSH levels after extended treatment with buthionine sulfoximine

Compound	Duration of treatment, days	Whole liver, $\mu\text{mol/g}$	GSH level	
			Liver mitochondria, nmol per mg of protein	Whole kidney, $\mu\text{mol/g}$
Saline (control)	5	10.8 $\pm$ 1.4 (100%)		4.6 $\pm$ 0.2 (100%)
Buthionine sulfoximine	5	2.6 $\pm$ 1.6 (24%)		0.39 $\pm$ 0.14 (8.5%)
Saline (control)	10	10.6 $\pm$ 1.2 (100%)	7.9 $\pm$ 1.4 (100%)	5.8 $\pm$ 0.4 (100%)
Buthionine sulfoximine	10	1.05 $\pm$ 0.08 (10%)	1.9 $\pm$ 0.6 (24%)	0.28 $\pm$ 0.04 (4.8%)

Mice were given either saline or buthionine sulfoximine (2 mmol per kg of body weight) by intraperitoneal injection at 9 a.m. and 5 p.m. Mice given buthionine sulfoximine were also given 20 mM buthionine sulfoximine in place of drinking water. At the times indicated, the animals were sacrificed and whole tissue or mitochondrial total GSH levels were determined.

cytoplasm. However, when mice were given [^{35}S]cyst(e)ine, the rate of incorporation of the radiolabel into mitochondrial GSH was very similar to the rate of its incorporation into cytoplasmic GSH (Table 4). Similarly, when mice previously

treated with [^{35}S]cyst(e)ine were given L-2-oxothiazolidine-4-carboxylate, an intracellular cysteine precursor (21), the rates at which the specific radioactivities of cytoplasmic and mitochondrial GSH declined were about the same.

DISCUSSION

The biphasic decline in the GSH levels of liver and kidney after administration of buthionine sulfoximine appears to be related in large measure to sequestration of GSH in the mitochondria. The fact that the mitochondrial level decreases much more slowly than that of the cytoplasm (3), a finding recently confirmed (22), could possibly be explained on the basis that buthionine sulfoximine does not enter mitochondria. However, since mitochondria do not have the enzyme activities required for GSH synthesis, the failure of buthionine sulfoximine to enter mitochondria readily is not relevant.

Although hexathionine sulfoximine is about as effective as buthionine sulfoximine as an inhibitor of γ -glutamylcysteine synthetase, injected hexathionine sulfoximine produces more extensive depletion of liver and kidney GSH than does buthionine sulfoximine. In contrast to buthionine sulfoximine, hexathionine sulfoximine causes substantial depletion of mitochondrial GSH. It is probable that hexathionine sulfoximine perturbs mitochondrial membranes in such a way as to facilitate loss of GSH. It seems significant that hexathionine sulfoximine produces substantial toxicity, whereas buthionine sulfoximine is relatively nontoxic and does not produce extensive loss of mitochondrial GSH. Modification of mitochondrial membranes, an apparently separate activity of hexathionine sulfoximine, seems to play a significant role in the toxicity produced by this compound.

The turnover of cellular GSH has been studied in a number of laboratories (see, for example, refs. 20 and 23–27), and there is evidence for at least two pools of intracellular GSH (20, 24, 27–30). Previous studies were interpreted to indicate that there is a separate pool of GSH in mitochondria, that mitochondrial GSH does not equilibrate with cytoplasmic GSH, that the mitochondrial membrane is impermeable to GSH, and that mitochondrial GSH is synthesized *in situ* (22, 27–30). In studies in which isolated hepatocytes were treated with diethyl maleate, no depletion of mitochondrial GSH was observed, whereas substantial depletion of cytoplasmic GSH was found, and it was concluded that the mitochondrial pool in the isolated hepatocyte is metabolically separate from that in the cytoplasm (27). Additional pulse-chase studies in which [^{35}S]methionine was used to label the cyst(e)ine and GSH pools of isolated hepatocytes indicated that the half-times for loss of radiolabel from cytoplasmic and mitochondrial GSH were $\approx$ 2 and $\approx$ 30 hr, respectively (27). The present and previous studies (20) using buthionine sulfoximine indicate the cytoplasmic pool of GSH is lost from mouse and rat liver *in vivo* with a half-time of $\approx$ 60 min. The studies reported in Table 2 suggest that the net transport of GSH from

Table 3. Activity of γ -glutamyl cycle enzymes in cytoplasmic and mitochondrial fractions

Enzyme	Enzyme activity, nmol/hr per mg of protein	
	Cytoplasm	Mitochondria
γ -Glutamylcysteine synthetase	83 $\pm$ 2	0
GSH synthetase	215 $\pm$ 16	0.7 $\pm$ 0.2
γ -Glutamyltranspeptidase	5.6	0
γ -Glutamylcyclotransferase	4470 $\pm$ 170	7.6 $\pm$ 0.4
5-Oxoprolinase	22.7 $\pm$ 1.3	0

γ -Glutamylcysteine synthetase was assayed in the presence of aminoxycetic acid; data are corrected for the amount of product formed in the presence of buthionine sulfoximine. With cytoplasmic enzyme, 3% and 7% of the apparent activity was not inhibited by buthionine sulfoximine and cystamine, respectively; replacement of L- α -aminobutyrate by L-cysteine decreased product formation by 75% (cysteine inhibits in long-term incubations). With mitochondrial supernatant (equivalent to 8 mg of crude sonicate), no product was formed in the presence of aminoxycetic acid (level of detection, <0.1 nmol/hr per mg). In the absence of aminoxycetic acid, radioactive product corresponding to 0.65 nmol/hr per mg of protein was formed, but its amount was not decreased by omission of aminobutyrate or by inclusion of buthionine sulfoximine; cystamine inhibited only 40%. This product is apparently α -ketoglutarate rather than 5-oxoprolinase. When L- α -aminobutyrate was replaced by L-cysteine, no product was observed. GSH synthetase was assayed by using cytoplasmic and mitochondrial extracts corresponding to 0.22 mg and 8.2 mg of protein, respectively. Cytoplasmic activity with L- γ -glutamyl-L- α -aminobutyrate and L- γ -glutamyl-L-cysteine were about equal; activity with D- γ -glutamyl-L-cysteine, which is not destroyed by cyclotransferase, was $\approx$ 29% less. Mitochondrial activity was observed only with L- γ -glutamyl-L- α -aminobutyrate; other substrates gave no activity above that observed in the absence of γ -glutamyl substrate. The apparent activity with L- γ -glutamyl-L- α -aminobutyrate is only 10% above the background. γ -Glutamyltranspeptidase was assayed in crude mitochondrial sonicate (32 mg of protein) or in a whole liver membrane fraction corresponding to 16.6 mg of crude homogenate protein. Cytoplasmic activity decreased 70% in the absence of glycylglycine; trace mitochondrial activity (<0.01 nmol/hr per mg) was unaffected by glycylglycine. γ -Glutamylcyclotransferase was assayed by using 8.0 mg of mitochondrial protein or 0.13 mg of cytoplasmic protein. The last mitochondrial wash showed no activity; the apparent mitochondrial activity is thus not due to easily dissociated cytoplasmic contamination. 5-Oxoprolinase was assayed by using cytoplasmic supernatant solution corresponding to 7.7 mg of crude homogenate or mitochondrial supernatant solution corresponding to 13.1 mg of crude mitochondrial protein. The cytoplasmic rate was not linear in the absence of aminoxycetic acid, and it was inhibited 87% by L-2-imidazolidone-4-carboxylate. Mitochondrial activity of 0.05 nmol/hr per mg was detected but was only 30% inhibited by L-2-imidazolidone-4-carboxylate.

Table 4. Incorporation of [³⁵S]cyst(e)ine into cytoplasmic and mitochondrial GSH

Compound	Time between injection and sacrifice, hr	Specific radioactivity of G ³⁵ SH (cpm/nmol)		
		Cytoplasm	Mitochondria	Cytoplasm/mitochondria ratio
L-[³⁵ S]Cyst(e)ine*	2	148 ± 18	152 ± 18	1.02 ± 0.01
	4	77 ± 17	87 ± 21	1.13 ± 0.04
	6	66 ± 10	78 ± 11	1.18 ± 0.01
L-2-Oxothiazolidone-4-carboxylate†	0	64.0 ± 10.7	61.5 ± 10.5	1.04 ± 0.19
	2	41.5 ± 2.1	44.8 ± 1.9	0.90 ± 0.07
	4	23.8 ± 4.3	27.8 ± 3.9	0.89 ± 0.05
	6	19.1 ± 0.6	19.2 ± 0.5	0.99 ± 0.08

The specific activity of G³⁵SH was determined as described. Each value is the mean ± SD of data from three to six mice. The reported ratio is the mean of the ratio values calculated for individual animals; in most cases, the animal-to-animal variation in the absolute specific activities shows much more variability than the ratio of cytoplasmic to mitochondrial specific activities.

*L-[³⁵S]Cyst(e)ine (2.7 μCi in 100 μl of 0.25 mM cystine; 1 Ci = 37 GBq) was given by intraperitoneal injection.

†Animals were given drinking water containing 0.25 mM L-[³⁵S]cystine for 4–5 days and were then given 150 mM L-2-oxothiazolidone-4-carboxylate (6 mmol per kg of body weight) by intraperitoneal injection.

mitochondria to cytoplasm proceeds with a $t_{1/2}$ of at least 30 hr.

In contrast to the slow net transport of GSH from mitochondria to cytoplasm, the studies reported in Table 4 indicate that radiolabeled GSH is fairly rapidly exchanged between cytoplasmic and mitochondrial pools. Thus, in separate 6-hr experiments, the specific activity of mitochondrial GSH was found to closely parallel both the increase in cytoplasmic GSH specific activity after administration of [³⁵S]cyst(e)ine, and the decrease in this specific activity after giving L-2-oxothiazolidone-4-carboxylate. These findings differ from those reported in studies with isolated rat liver hepatocytes in which methionine was used as a cysteine precursor (27); the reasons underlying the different observations are not yet apparent.

The scheme given in Fig. 1 accounts for the synthesis and transport of GSH in the liver. Although reversible conversion of GSH to GSSG occurs in both mitochondria and cytoplasm, synthesis of GSH occurs only in the latter compartment. We indicate GSH as the probable transport form in Fig. 1,

FIG. 1. Scheme for synthesis and transport of GSH in mitochondria and cytoplasm. GSH is synthesized only in the cytoplasm (Reactions 1 and 2). GSH is transported into mitochondria by a system characterized by slow net transport and more rapid exchange transport (Reaction 3). Reversible oxidation of GSH to GSSG occurs in both cytoplasm and mitochondria (Reaction 4). GSH is exported across the membranes of cells of the liver and other tissues (Reaction 5). Under conditions of oxidative stress, GSSG is transported out of the mitochondria and across cell membranes (Reaction 6).

because GSH rather than GSSG is the predominant form present, and it appears to be the major transport form in many cells under normal conditions. However, the possibility that GSSG may also be transported by this system cannot be excluded; such transport might function to protect mitochondria when they are under severe oxidative stress. Conceivably, two molecules of cytoplasmic GSH might exchange for one molecule of mitochondrial GSSG. The relatively rapid labeling of mitochondrial GSH observed here suggests that there is an exchange carrier accessible to both cytoplasmic and mitochondrial GSH. It will be of interest to examine the mechanism by which GSH is transported between cytoplasm and mitochondria.

The available data support the view that GSH is essential for mitochondrial function. That the net efflux of GSH from mitochondria is very slow suggests that this transport mechanism functions to conserve mitochondrial GSH during periods of cytoplasmic GSH depletion caused by nutritional and toxicological factors.

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- Griffith, O. W. & Meister, A. (1979) *J. Biol. Chem.* **254**, 7558–7560.
- Griffith, O. W. (1982) *J. Biol. Chem.* **257**, 13,704–13,712.
- Meister, A. & Griffith, O. W. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 2642.
- Griffith, O. W. (1981) *Methods Enzymol.* **77**, 59–64.
- Boettcher, B. & Meister, A. (1984) *Anal. Biochem.* **138**, 449–450.
- Greenawalt, J. W. (1974) *Methods Enzymol.* **31**, 310–323.
- Srere, P. A. (1969) *Methods Enzymol.* **13**, 3–11.
- Tietze, F. (1969) *Anal. Biochem.* **27**, 502–522.
- Griffith, O. W. (1980) *Anal. Biochem.* **106**, 207–212.
- Newton, G. L., Dorian, R. & Fahey, R. C. (1981) *Anal. Biochem.* **114**, 383–387.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
- Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427.
- Layne, F. (1957) *Methods Enzymol.* **3**, 450–451.
- Orlowski, M. & Meister, A. (1971) *Methods Enzymol.* **17B**, 495–500.
- Griffith, O. W., Larsson, A. & Meister, A. (1977) *Biochem. Biophys. Res. Commun.* **79**, 919–925.
- Oppenheimer, L., Wellner, V. P., Griffith, O. W. & Meister, A. (1979) *J. Biol. Chem.* **254**, 5184–5190.

17. Tate, S. S. & Meister, A. (1974) *J. Biol. Chem.* **249**, 7593-7602.
18. Taniguchi, N. & Meister, A. (1978) *J. Biol. Chem.* **253**, 1799-1806.
19. Van Der Werf, P., Orlowski, M. & Meister, A. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2982-2985.
20. Griffith, O. W. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5606-5610.
21. Williamson, J. M., Boettcher, B. & Meister, A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6246-6249.
22. Romero, F. J. & Sies, H. (1984) *Biochem. Biophys. Res. Commun.* **123**, 1116-1121.
23. Sekura, R. & Meister, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2969-2972.
24. Higashi, T., Tateishi, N., Naruse, A. & Sakamoto, Y. (1977) *J. Biochem. (Tokyo)* **82**, 117-124.
25. Lauterburg, B. H. & Mitchell, J. R. (1981) *J. Clin. Invest.* **67**, 1415-1424.
26. Hogberg, J. & Kristoferson, A. (1978) *Acta Pharmacol. Toxicol.* **42**, 271-274.
27. Meredith, M. J. & Reed, D. J. (1982) *J. Biol. Chem.* **257**, 3747-3753.
28. Jocelyn, P. C. (1975) *Biochim. Biophys. Acta* **369**, 427-436.
29. Jocelyn, P. C. & Kamminga, A. (1974) *Biochim. Biophys. Acta* **343**, 356-362.
30. Wahllander, A., Soboll, S. & Sies, H. (1979) *FEBS Lett.* **97**, 138-140.