Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2715-2719, June 1979
Biochemistry

Conversion of glutathione to glutathione disulfide by cell membrane-bound oxidase activity

(γ-glutamyl transpeptidase/γ-glutamyl cycle/amino acids/kidney/jejunum)

SURESH S. TATE, ELENA M. GRAU, AND ALTON MEISTER

Department of Biochemistry, Cornell University Medical College, 1300 York Avenue, New York, New York 10021

Contributed by Alton Meister, April 2, 1979

ABSTRACT **An apparently specific glutathione oxidase activity is present in renal cortex, epididymal caput, jejunal villus tip cells, choroid plexus, and retina (but not in liver). The activity is membrane-bound and is localized on the luminal surface of the brush border membranes of the kidney and jejunum. The distribution and localization of the oxidase are similar to those of γ-glutamyl transpeptidase, suggesting that there is a significant relationship among the translocation of intracellular glutathione, the extracellular oxidation of glutathione to glutathione disulfide, and the reactions of the γ-glutamyl cycle. Thus, both glutathione present in the blood plasma and intracellular glutathione translocated to the cell surface are accessible to oxidation and transpeptidation. Acceptor substrates of the transpeptidase (e.g., L amino acids) promote transpeptidation and decrease oxidation of glutathione. Conversion of glutathione to glutathione disulfide is followed by utilization of the latter compound by γ-glutamyl transpeptidase and dipeptidase. Although intracellular oxidation of glutathione to glutathione disulfide is readily reversed by the action of glutathione reductase, glutathione disulfide formed extracellularly cannot be reduced; instead, it undergoes hydrolytic and transpeptidation reactions leading to γ-glutamyl amino acid and amino acid products which may be recovered by being transported into the cell.**

Recent studies on the metabolism of glutathione have provided new evidence that the reactions of the γ-glutamyl cycle (1) occur *in vivo* (2-5) and that translocation of glutathione across renal cell membranes is a discrete step in the cycle (6, 7). The available data also indicate that intracellular glutathione is translocated out of other cells—e.g., liver (6, 8) and lymphoid cells (7). Cells that have relatively little γ-glutamyl transpeptidase activity translocate glutathione into the blood plasma whereas, in renal cells, intracellular glutathione is translocated to membrane-bound γ-glutamyl transpeptidase. Normally, little, if any, glutathione is found in the urine, and the blood plasma levels of glutathione are maintained at extremely low levels. However, after administration of a potent γ-glutamyl transpeptidase inhibitor to mice, the amount of glutathione excreted in the urine increased substantially and the blood plasma level of glutathione also increased appreciably; most of the glutathione found in the urine was in the form of glutathione disulfide (6). More than half of the glutathione of blood plasma is also present in the disulfide form. These observations are consistent with the function of a system or systems that mediate the oxidation of extracellular glutathione.

Some years ago, Elvehjem and collaborators (9, 10) found that mouse kidney homogenates catalyze oxygen-dependent conversion of glutathione to glutathione disulfide and that this reaction is inhibited by cyanide. These workers also found that kidney exhibits much higher glutathione oxidase activity than does liver. Recent studies by Orrenius and his colleagues (11, 12) on the metabolism of glutathione and of a glutathione conjugate by isolated rat kidney cells have confirmed these findings and have also provided evidence for stepwise breakdown of glutathione disulfide to its constituent amino acids, reactions that are presumably catalyzed by the combined actions of γ-glutamyl transpeptidase and dipeptidase. These workers found little or no activity in studies with suspension of liver cells, but they found that the reaction mediated by suspensions of kidney cells was inhibited by cyanide or by replacement of the atmosphere with nitrogen.

In the present work we have found that there is an apparently specific glutathione oxidase activity in kidney, epididymis, jejunum, choroid plexus, and other tissues.* The activity is membrane-bound and is associated with the brush border of the kidney and jejunum. Its distribution in tissues and in tissue fractions follows a pattern similar to that of γ-glutamyl transpeptidase; however, the oxidase was not found in two lymphoid cell lines that have moderate and high levels of γ-glutamyl transpeptidase activity. The distribution and localization of the oxidase suggest that there is a significant relationship among the function of the γ-glutamyl cycle, the translocation of glutathione, and the extracellular oxidation of glutathione to glutathione disulfide.

EXPERIMENTAL

Materials. Glutathione, *S*-methylglutathione, L-γ-glutamyl-*p*-nitroanilide, glutathione reductase (yeast), papain, NADPH, and concanavalin A-Sepharose were obtained from Sigma. L-Cysteinylglycine was prepared by incubating glutathione with purified rat kidney γ-glutamyl transpeptidase (14), followed by chromatography of the reaction products on a column of Dowex 1-acetate. L-γ-Glutamyl-(*o*-carboxy)-phenylhydrazide was synthesized in this laboratory by Owen W. Griffith (6).

Methods. Tissue homogenates were made in 4 vol (wt/vol) of 10 mM sodium phosphate/20 mM Hepes/0.15 M NaCl, pH 7.4 (designated here as Hepes/phosphate buffer). The subcellular fractionation of rat kidney to yield basal-lateral and brush border membranes and determination of marker enzymes have been described (15). γ-Glutamyl transpeptidase was assayed in the presence of 1 mM L-γ-glutamyl-*p*-nitroanilide, 20 mM Gly-Gly, and 0.1 M Tris·HCl (pH 8.0); its activity is expressed as μmol of *p*-nitroaniline released per min (units). Glutathione oxidase activity was assayed in reaction mixtures (final volume, 1 ml) containing Hepes/phosphate buffer, 2 mM glutathione, and the enzyme. Incubation was carried out with vigorous shaking on a reciprocating shaker at 37°C. Samples (0.2 ml) were withdrawn and mixed with 0.02 ml of either 50% sulfosalicylic acid or 2 M acetic acid. After deproteinization, aliquots were taken for free thiol determination with 5,5′-dithiobis(2-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

* A preliminary account of some of these findings has appeared (13).

Table 1. Activities of GSH oxidase and γ-glutamyl transpeptidase (GGTP) in rat tissues*

Tissue	GGTP, units/mg	Oxidase, units/mg	Ratio: GGTP/ oxidase
Kidney:			
Cortex	2.68	0.20	13
Medulla	2.15	0.15	14
Epididymis:			
Caput	0.64	0.056	11
Cauda	0.09	0.009	10
Jejunal epithelial cells:†			
Crypt cells	0.019	0.0021	9
Mid fractions	0.052	0.0072	7
Villus tip cells	0.075	0.0117	6
Liver	6.5×10^{-4}	ND	—

* ND, not detectable. For transpeptidase (GGTP), units are μmol of *p*-nitroaniline formed per min in tissue homogenates (assayed in presence of 1 mM L-γ-glutamyl-*p*-nitroanilide and 20 mM Gly-Gly). For oxidase, units are μmol of GSH oxidized per min (decrease in free thiol). Both are shown per mg of protein.

† Fractions of the jejunal epithelial cells were obtained by the micrometer planing technique of Imondi *et al.* (19).

nitrobenzoate) (16), for total glutathione (GSH + GSSG) determination with glutathione reductase by the method of Tietze (17), and for glutathione disulfide determination. Glutathione disulfide was determined by treating aliquots of the reaction mixture with 2-vinylpyridine to derivatize the glutathione present prior to application of the Tietze procedure (7). The glutathione oxidase activity is expressed either as the decrease in free thiols (μmol/min) or as μmol of glutathione disulfide formed per min (units). Controls containing no enzyme were run simultaneously. Hydrogen peroxide was determined by the horse radish peroxidase method (18).

RESULTS

Localization of Glutathione Oxidase in Rat Tissues. The data given in Table 1, which describe the distribution of glutathione oxidase activity in several rat tissues, indicate that the oxidase activity closely parallels that of γ-glutamyl transpeptidase. Thus, renal cortex had the highest transpeptidase activity and also exhibited high oxidase activity. In epididymis, the oxidase activity also paralleled transpeptidase activity, which is known to be more active in the caput than in the cauda (20). Of interest is the gradient of oxidase activity found in jejunal epithelial cells; these data resemble those found earlier for transpeptidase (19, 21) which exhibits highest activity in the differentiated villus tip cells. Under the conditions used, no oxidase activity could be detected in the liver.

In the studies described above, the glutathione oxidase activity was found in the particulate fractions of the homogenates, suggesting that the enzyme is membrane-bound. Subcellular fractionation of rat kidney carried out essentially as described (15) showed about an 18-fold enrichment of the oxidase activity in the brush border membrane fraction (Table 2; P_5B). This fraction also contained similarly enriched transpeptidase and alkaline phosphatase activities; these enzymes are considered to be brush border markers. The ratio of transpeptidase activity to oxidase activity remained constant during the fractionation procedure. The cytosolic fraction (which contained microsomes; 16,000 × *g* supernatant of the postnuclear fraction) contained 6–8% of the oxidase and transpeptidase activities of the homogenate.

Treatment of brush border vesicles with papain led to solubilization of about 65 and 60% of the oxidase and transpeptidase activities, respectively, indicating that a major portion of these activities is located on the outer or luminal surface of the cell membrane. Previous studies have shown that transpeptidase is located on the outer surface of kidney cells as well as of brush border vesicles (1, 15, 23). Consistent with this interpretation is the finding that both oxidase and transpeptidase activities were unaffected by treatment of brush border vesicles with detergents (e.g., Lubrol WX and sodium deoxycholate).

Data on the activities of transpeptidase and the oxidase in bovine tissues are given in Table 3. The ratios of the activities in kidney cortex, choroid plexus, and retina varied from 8 to 13. The ratio for kidney medulla was about 4, and no oxidase activity was detected in cerebral cortex. Studies were also carried out on two established human lymphoid cell lines, RPMI 8226 and RPMI 6237. These cells exhibit high and moderate γ-glutamyl transpeptidase activities, respectively (24); in the present studies, neither cell line was found to exhibit detectable glutathione oxidase activity.

Properties of Rat Kidney Glutathione Oxidase. Oxidation of glutathione to glutathione disulfide by preparations of rat kidney brush border membranes was found to be dependent upon molecular oxygen; when nitrogen was bubbled through the assay mixtures, less than 10% of the oxidase activity observed in air was found. No hydrogen peroxide was detected as a product of glutathione oxidation when this was examined by the *o*-dianisidine/horseradish peroxidase procedure (18). Although oxygen was the best electron acceptor, compounds such

Table 2. Enzyme activities in rat kidney homogenate and membrane fractions*

Membrane fraction	Protein, mg	GSH oxidase		Transpeptidase		Alkaline phosphatase, rel. act.	Na^+,K^+-ATPase, rel. act.
		Total units	Rel. act.	Total units	Rel. act.		
[Homogenate]	730	139	1†	1640	1†	1†	1†
Basal-lateral + brush border (P_5)	23.8	36.2	8	378	7	8	3
Basal-lateral (P_5A)	15.4	10.0	3.4	94	3	2	4
Brush border (P_5B)‡	5.4	18.1	18	198	17	13	2

* P_5; pre-free-flow electrophoresis fraction of Heidrich *et al.* (22) containing both the basal-lateral membranes (P_5A) and the brush border membranes (P_5B). The fractionation of P_5 into P_5A and P_5B was achieved as described (15). The activities were determined as stated in Table 1 and, for alkaline phosphatase and Na^+,K^+-ATPase, as reported (15).

† The specific activities (homogenates) were 0.19, 2.24, 0.70, and 2.3 units/mg of protein for the oxidase, transpeptidase, alkaline phosphatase, and ATPase, respectively.

‡ The brush border fraction (P_5B) did not exhibit glutathione peroxidase, glutathione reductase, or catalase activity.

Table 3. Activities of GSH oxidase and γ-glutamyl transpeptidase (GGTP) in bovine tissues*

Tissue	GGTP, units/mg	Oxidase, units/mg	Ratio: GGTP/ Oxidase
Kidney:			
Cortex	0.41	0.033	12
Medulla	0.13	0.030	4
Brain:			
Choroid plexus	0.014	0.0011	13
Cerebral cortex	0.0026	ND	—
Retina	0.0059	7.5×10^{-4}	8

* ND, not detectable; the activities were determined as in Table 1.

as cytochrome *c* and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride were about 2% as effective as oxygen. NAD^+ and $NADP^+$ did not serve as electron acceptors. Glutathione oxidase activity of brush border membranes as well as of Lubrol extracts of these membranes was strongly inhibited by EDTA, suggesting that a metal may be involved in the oxidation; further studies on this possibility are required.

The brush border oxidase appears to be specific for glutathione. *N*-Acetylcysteine and cysteinylglycine were not appreciably oxidized. Studies carried out at pH 6 (to avoid rapid nonenzymatic oxidation) with cysteine and dithiothreitol showed that these compounds are not substrates; at pH 6, the rate of oxidation of glutathione is about 42% of that which occurs at pH 7.4. The oxidation of glutathione was inhibited by *S*-substituted glutathione derivatives—e.g., *S*-methylglutathione and *S*-acetophenoneglutathione (Table 4). γ-Glutamyl amino acids which are substrates of transpeptidase, as well as inhibitors of transpeptidase—e.g., γ-glutamylhydrazone of pyruvate (25) and γ-glutamyl-(*o*-carboxy)phenylhydrazide (6)—had little or no effect on the oxidation of glutathione.

Under the conditions used, addition of L amino acids (e.g., methionine, serine, glutamate) or dipeptides (e.g., glycylglycine) to the reaction mixture decreased the oxidation of glutathione markedly. Such inhibitory effects were observed with membrane-bound as well as with detergent-solubilized enzyme preparations. Presumably, these compounds decrease oxidation of glutathione by promoting increased rates of transpeptidation. Although the hydrolysis of glutathione and of glutathione disulfide by transpeptidase, as judged by the rate of formation of free glutamate, occur at about the same rate, with glutathione the transpeptidase reaction is about 25 times more rapid than the hydrolytic reaction. Thus, under conditions similar to those previously used (25, 26), the formation of glutamate from glutathione and glutathione disulfide was 6.5 and 6.0 μmol/min per mg of purified rat kidney γ-glutamyl transpeptidase, respectively (14). The corresponding rates of transpeptidation (formation of γ-glutamylmethionine) were 154 and 34 μmol /min per mg. Under these conditions, there was additional disappearance of glutathione due to formation of γ-glutamylglutathione and di-γ-glutamylglutathione; the formation of these γ-glutamyl derivatives of glutathione was about 2- and 5-fold greater than the corresponding hydrolytic reactions with glutathione disulfide and glutathione, respectively. Although the effect of amino acids and dipeptides on glutathione oxidation may logically be ascribed to stimulation of transpeptidation, the possibility exists that these compounds exert a direct inhibitory effect on the oxidase. It might be expected that the glutathione binding site of the oxidase would resemble that of the transpeptidase. Thus, acceptor substrates of the transpeptidase (e.g., dipeptides, L amino acids) might also bind to the oxidase and thus inhibit the binding of glutathione. It seems notable that the apparent affinity of the oxidase activity for L amino acids and glycylglycine is substantially higher than that of the transpeptidase for these compounds (1).

The renal brush border glutathione oxidase appears to be a glycoprotein. The finding that the Lubrol-solubilized enzyme binds readily to concanavalin A-Sepharose and that it is eluted by α-methylglucoside is consistent with this possibility.

Relative Rates of Glutathione Oxidation and Utilization. Table 5 gives the results of studies in which the formation of glutathione disulfide and the disappearance of total glutathione (GSH + GSSG) [as determined by the glutathione reductase procedure (17)] were determined. The data indicate that oxidation and degradation (presumably catalyzed by transpeptidase and dipeptidase) proceed at similar rates. This suggests that the oxidation of glutathione by the renal brush border is a significant process in the overall metabolism of glutathione in the

Table 4. Effect of γ-glutamyl compounds, amino acids, peptides, and other compounds on GSH oxidase activity of rat renal brush border fraction (P_5B)

Addition	Activity, % of control*
None (control)	100
S-Methylglutathione (2 mM)	84
S-Methylglutathione (4 mM)	64
S-Acetophenoneglutathione (2 mM)	47
L-γ-Glutamyl-*S*-methyl-L-cysteine (2 mM)	100
L-γ-Glutamylhydrazone of pyruvate (2 mM)	100
L-γ-Glutamyl-(*o*-carboxy)phenylhydrazide (0.5 mM)	92
L-Methionine (2 mM)	22
D-Methionine (2 mM)	109
L-Serine (2 mM)	9
Glycine (2 mM)	25
L-Glutamate (2 mM)	20
Gly-Gly (2 mM)	8
L-Ala-Gly (2 mM)	20
EDTA (2 mM)	0
KCN (2 mM)	0

* Oxidase activity was determined as described in Table 1.

Table 5. Relative rates of GS oxidation and utilization by rat renal brush border membranes

Addition	GSSG formed, μmol/15 min	Disappearance of glutathione (GSH + GSSG), μmol/15 min
None (control)	0.45	0.55
EDTA (5 mM)	0.03	0.62
L-Methionine (5 mM)	0.08	0.83
Gly-Gly (5 mM)	0.02	1.59
L-γ-Glutamyl-(*o*-carboxy)-phenylhydrazide (0.5 mM)	0.39	0.14

Reaction mixtures (final volume, 1.0 ml) contained 2 mM GSH, renal brush border membranes (20 μg of P_5B protein), Hepes/phosphate pH 7.4 buffer, and other additions as shown. Incubations were at 37°C with aeration. Samples (0.2 ml) were withdrawn at 5-min intervals and mixed with 0.02 ml of 2 M acetic acid. Total glutathione (GSH + GSSG) and GSSG (after removal of GSH by reaction with 2-vinylpyridine) were determined enzymatically with glutathione reductase.

kidney. EDTA inhibited glutathione oxidation but had little effect on disappearance of total glutathione. L-Methionine and glycylglycine, excellent γ-glutamyl acceptor substrates of γ-glutamyl transpeptidase, increased glutathione disappearance and decreased formation of glutathione disulfide. L-γ-Glutamyl-(*o*-carboxy)phenylhydrazide inhibited the utilization of glutathione but had little effect on the formation of glutathione disulfide.

DISCUSSION

The data show an apparent parallelism between oxidase and γ-glutamyl transpeptidase activities. Both activities are membrane-bound and are evidently associated with the luminal surface of brush border membranes. In such a location, these enzymes would be accessible to glutathione present in the blood plasma and also to intracellular glutathione which is translocated to the cell surface. It seems possible then that the translocation of intracellular glutathione and its utilization by the transpeptidase (both before and after its oxidation) may be closely related and perhaps coordinated processes (Fig. 1). The data (Tables 3 and 4) show that glutathione disulfide is not an obligatory intermediate in the utilization of glutathione. In the studies reported here, L amino acids and dipeptides, which are good acceptor substrates of transpeptidase, produced inhibition of glutathione oxidation to glutathione disulfide. One may therefore reason that the presence of substantial concentrations of amino acids would increase transpeptidation and thus favor pathway 2 (Fig. 1). In the alternative pathway (pathway 1), in which glutathione is converted to glutathione disulfide, pathway 2′ would predominate and glutathione disulfide would be utilized by transpeptidation and by degradation catalyzed by the combined activities of transpeptidase and dipeptidase. The γ-glutamyl amino acids and free amino acids formed would be transported into the cell and reutilized for the synthesis of glutathione by the intracellular reactions of the γ-glutamyl cycle.

The mechanism by which intracellular glutathione is translocated out of the cell (Fig. 1, pathway 3) is not yet understood. Because the concentration of glutathione is far greater within the cell than in the extracellular fluid, one might think that translocation of glutathione could occur by simple diffusion. Administration of large amounts of L amino acids has been found to decrease the intracellular concentration of glutathione markedly, and this effect is greatly decreased by administration

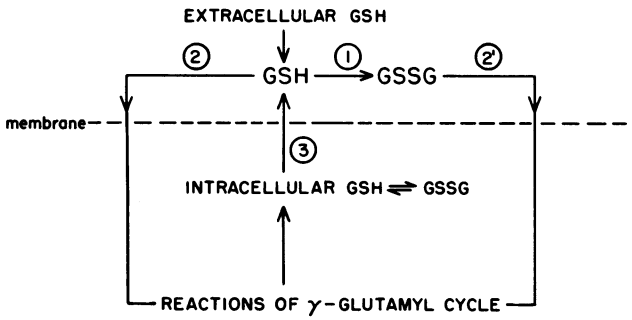

FIG. 1. General scheme for the metabolism of glutathione (GSH) (see text). Glutathione present extracellularly and intracellular glutathione translocated (pathway 3) to the cell surface are substrates of the membrane-bound activities γ-glutamyl transpeptidase (pathway 2) and glutathione oxidase (pathway 1). Acceptor substrates of the transpeptidase (e.g., L amino acids) promote metabolism by pathway 2. Oxidation of intracellular glutathione to glutathione disulfide (GSSG) may be reversed by the action of glutathione reductase. Glutathione disulfide formed extracellularly (pathway 1) is utilized by the combined actions of γ-glutamyl transpeptidase and dipeptidase to form products that are transported into the cell.

of inhibitors of γ-glutamyl transpeptidase (3, 27). Under these conditions, it seems likely that pathway 2 predominates. Thus, rapid utilization of extracellular glutathione by transpeptidation would lead to extensive translocation of glutathione and therefore to depletion of intracellular glutathione. Oxidation of glutathione to glutathione disulfide might also facilitate glutathione translocation by decreasing the extracellular concentration of glutathione. It is of significance that the *intracellular* oxidation of glutathione to glutathione disulfide may be readily reversed by the action of intracellular glutathione reductase. On the other hand, glutathione disulfide formed extracellularly cannot be reduced in this manner but instead appears to undergo degradation by hydrolytic and transpeptidation reactions leading to products that are transported into the cell.

The oxidase appears to be a glycoprotein and it may be a metalloenzyme. Its mechanism of action appears to differ significantly from a sulfhydryl oxidase that has been isolated from bovine milk (28); this enzyme, which is a metalloprotein, oxidizes glutathione to glutathione disulfide with formation of hydrogen peroxide, and it is active toward a number of sulfhydryl compounds including proteins.

The oxidase activity may be considered, at least in part, to have a metabolic role in the utilization and recovery of extracellular glutathione; glutathione disulfide cannot be reduced to glutathione extracellularly, as discussed above. It is possible that the physiological electron acceptor may not be molecular oxygen, and that the oxidase activity is part of a membrane electron transport assembly that functions in transport processes (29). It seems probable that the oxidase activity plays a significant role closely related to the process of glutathione translocation which, as discussed elsewhere (7), may serve in a protective function in relation to the cell membrane. Also to be considered is the possibility that the oxidase functions in another type of protective mechanism to remove excess glutathione from the cell surface, thus preventing reduction of the disulfide bonds of membrane proteins and the mucoproteins of the glycocalyx.

Note Added in Proof. The brush border fraction studied here exhibits both oxidase and transpeptidase activities. Thus far, efforts to separate these activities have not succeeded, and it has been observed that highly purified preparations of rat kidney γ-glutamyl transpeptidase (14) also exhibit glutathione oxidase activity. Each of the 12 isozymic forms of this enzyme previously demonstrated (30) exhibits glutathione oxidase activity; all of the isozymes have a constant ratio of transpeptidase to glutathione oxidase activities (31). These observations suggest that glutathione oxidase activity and γ-glutamyl transpeptidase activity may be associated with the same dimeric protein. Although suspensions of certain lymphoid cells exhibit substantial γ-glutamyl transpeptidase activity (24), they do not oxidize glutathione at a significant rate. However, recent studies on a partially purified preparation of γ-glutamyl transpeptidase from lymphoid cells (cell line RPMI 8226) showed that this preparation exhibits appreciable glutathione oxidase activity, suggesting that the oxidase activity may be masked in the intact membrane. In contrast, suspensions of kidney cells show substantial glutathione oxidase activity (9–12).

This research was supported in part by grants from the National Institutes of Health (GM 25152 and AM 12034) and from the American Cancer Society.

1. Meister, A. & Tate, S. S. (1976) *Annu. Rev. Biochem.* **45**, 559–604.
2. Van Der Werf, P., Stephani, R. A. & Meister, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1026–1029.
3. Griffith, O. W., Bridges, R. J. & Meister, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5405–5408.

4. Meister, A. (1978) in *Function of Glutathione in Liver and Kidney*, Proceedings in Life Sciences, eds. Sies, H. & Wendel, A. (Springer, Berlin), pp. 43–59.
5. Griffith, O. W., Anderson, M. E. & Meister, A. (1979) *J. Biol. Chem.* **254,** 1205–1210.
6. Griffith, O. W. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 268–272.
7. Griffith, O. W., Novogrodsky, A. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2249–2252.
8. Bartoli, G. M. & Sies, H. (1978) *FEBS Lett.* **86,** 89–91.
9. Ames, S. R. & Elvehjem, C. A. (1945) *J. Biol. Chem.* **159,** 549–562.
10. Ziegenhagen, A. J., Ames, S. R. & Elvehjem, C. A. (1947) *J. Biol. Chem.* **167,** 129–134.
11. Moldeus, P., Jones, D. P., Ormstad, K. & Orrenius, S. (1978) *Biochem. Biophys. Res. Commun.* **83,** 195–200.
12. Jones, D. P., Stead, A. H., Moldeus, P. & Orrenius, S. (1978) in *Functions of Glutathione in Liver and Kidney*, Proceedings in Life Sciences, eds. Sies, H. & Wendel, A. (Springer, Berlin), pp. 194–200.
13. Grau, E. M. & Tate, S. S. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 839.
14. Tate, S. S. & Meister, A. (1975) *J. Biol. Chem.* **250,** 4619–4624.
15. Grau, E. M., Marathe, G. V. & Tate, S. S. (1979) *FEBS Lett.* **98,** 91–94.
16. Ellman, G. L. (1959) *Arch. Biochem. Biophys.* **82,** 70–77.
17. Tietze, F. (1969) *Anal. Biochem.* **27,** 502–522.
18. Bernt, E. & Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis*, ed. Bergmeyer, H. U. (Verlag Chemie Weinheim, New York), Vol. 4, pp. 2246–2248.
19. Imondi, A. R., Balis, M. E. & Lipkin, M. (1969) *Exp. Cell. Res.* **58,** 323–330.
20. DeLap, L. W., Tate, S. S. & Meister, A. (1977) *Life Sci.* **20,** 673–680.
21. Cornell, J. S. & Meister, A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 420–422.
22. Heidrich, H. G., Kinne, R., Kinne-Saffran, E. & Hannig, K. (1972) *J. Cell Biol.* **54,** 232–245.
23. Kuhlenschmidt, T. & Curthoys, N. P. (1975) *Arch. Biochem. Biophys.* **167,** 519–524.
24. Novogrodsky, A., Tate, S. S. & Meister, A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2414–2418.
25. Tate, S. S. & Meister, A. (1974) *J. Biol. Chem.* **249,** 7593–7602.
26. Tate, S. S. & Meister, A. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3329–3333.
27. Palekar, A. G., Tate, S. S. & Meister, A. (1975) *Biochem. Biophys. Res. Commun.* **62,** 651–657.
28. Janolino, V. G. & Swaisgood, H. E. (1975) *J. Biol. Chem.* **250,** 2532–2538.
29. Low, H. & Crane, F. L. (1978) *Biochim. Biophys. Acta* **515,** 141–161.
30. Tate, S. S. & Meister, A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2599–2603.
31. Tate, S. S. & Orlando, J. (1979) *J. Biol. Chem.*, in press.