Glutathione Turnover in Human Erythrocytes

INHIBITION BY BUTORONINE SULFOXIMINE AND INCORPORATION OF GLYCINE BY EXCHANGE*

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Previous investigations have indicated that \( [^{14}C] \)glycine is incorporated into erythrocyte glutathione at a rate which exceeds by severalfold the rate of cysteine and glutamate incorporation. The discrepancy between the rates has generally been attributed to the known differences in the rates at which these amino acids are transported into erythrocytes. In the present investigation, human erythrocytes were incubated with \( [^{14}C] \)glutamate or \( [^{14}C] \)glycine, and the specific activities of the intracellular amino acid and glutathione pools were determined at intervals for 6 h. The experiments with \( [^{14}C] \)glycine indicate an apparent glutathione turnover time of about 1 day, whereas those with \( [^{14}C] \)glutamate indicate a turnover time of about 6 days. Since the calculations are based on the specific activities of the intracellular amino acid pools, the discrepancy between the two turnover rates cannot be attributed to differences in the rates of amino acid transport. It is concluded that the results with \( [^{14}C] \)glutamate reflect the true rate of glutathione biosynthesis, and that glycine is incorporated into erythrocyte glutathione primarily by an exchange reaction catalyzed by glutathione synthetase. In support of this conclusion, \textit{de novo} glutathione biosynthesis as measured with \( [^{14}C] \)glutamate or \( [^{35}S] \)cysteine is inhibited about 90% by buthionine sulfoximine, an inhibitor of \( \gamma \)-glutamylcysteine synthetase; glycine incorporation, in contrast, is inhibited <10% by buthionine sulfoximine. It is apparent that glutathione turnover cannot be meaningfully measured with radioactive glycine in erythrocytes or, by inference, in other tissues with high levels of glutathione synthetase or slow rates of glutathione biosynthesis.

The demonstrable importance of glutathione (GSH) to red blood cell stability and function has elicited numerous investigations of GSH metabolism in isolated erythrocytes. The role of GSH and glutathione peroxidase in the protection of hemoglobin, cell membranes, and other cell constituents from oxidative damage has been elucidated (1–5), and the role of glutathione reductase and NADPH in the reduction of the glutathione disulfide (GSSG) formed during peroxide and free radical quenching has been established (7). \textit{In vivo} the concentration of NADPH is normally sufficient to maintain over 98% of the total erythrocyte glutathione in the reduced form despite the high oxygen content of the cell and the continuing electron flux through the glutathione peroxidase pathway (8, 9). In addition to this oxidation-reduction turnover, GSH also turns over by \textit{de novo} biosynthesis. Thus, new GSH is synthesized from its constituent amino acids and existing GSH is either irreversibly utilized intracellularly (e.g. by glutathione-S-transferases (10)) or is secreted, possibly as GSSG (9, 11, 12). The rates of synthesis and loss are balanced so as to maintain a constant intracellular concentration of about 3 mM.

The rate of \textit{de novo} GSH synthesis in erythrocytes has been determined in several studies in which the incorporation of isotopically labeled precursor amino acids was monitored (3, 13–23). In general, the incorporation of radioactive glycine has been found to be much faster than the incorporation of radioactive glutamate or cysteine (17, 18, 20, 23). This apparent inconsistency is often attributed to the slow transport of glutamate or cysteine into erythrocytes (16, 18, 20, 22). In the present investigation, isolated human erythrocytes were incubated with radioactive precursor amino acids and the specific activities of both intracellular amino acid and GSH were determined as a function of time. In some studies, cells were incubated with radioactive glutamine or \( \alpha \)-ketoglutarate in place of glutamate; both compounds were converted to radioactive glutamate intracellularly. Buthionine sulfoximine, a potent and specific inhibitor of \( \gamma \)-glutamylcysteine synthetase (24), was used in some studies to prevent GSH biosynthesis. Use of the inhibitor allowed \textit{de novo} GSH synthesis to be distinguished from exchange reactions which might incorporate radioactive amino acids into pre-existing GSH.

**EXPERIMENTAL PROCEDURES**

\textit{Materials—}Whole blood was obtained in heparinized tubes from normal human volunteers and was centrifuged for 10 min at 700 \( \times \) g. The plasma and Buffy coat were removed, and the erythrocytes were washed twice with 2 volumes of 0.9% NaCl and then once with 2 volumes of modified Krebs-Ringer solution (see below). Following the washing procedure, the packed cells were combined into one pool to ensure homogeneity. DL-Buthionine-SR-sulfoximine was prepared as described (24, 25). Amino acids and \( \alpha \)-ketoglutarate were obtained from Sigma. Radioactive glutamine was obtained from Schwarz/Mann and was passed through Dowex 1 (acetate) before use to remove glutamate and 5-oxoproline. Other radioactive compounds were obtained from New England Nuclear.

\textit{Methods—}Erythrocytes were incubated at 37 \( ^\circ \)C in plastic tubes in a modified Krebs-Ringer solution (26) consisting of 95 mM NaCl, 4.74 mM KCl, 1.18 mM MgSO\(_4\), 1.18 mM KH\(_2\)PO\(_4\), 16.2 mM Na phosphate buffer, pH 7.4, 5.8 mM Na pyruvate, 6.2 mM Na fumarate, 4.74 mM NaHCO\(_3\), and 11.5 mM glucose; the final pH was 7.4. Other additions (e.g. amino acids and \( \alpha \)-ketoglutarate) are given in the figure legends. The incubation tubes were slowly rotated to maintain the cells in suspension. At the times indicated in the figures, three separate aliquots were removed from each incubation and analyzed as follows: (a) a 100-\( \mu \)l aliquot of the incubation mixture was added directly to 200 \( \mu \)l of 5% acetic acid and the resulting solution was quickly mixed. 5 s later, 200 \( \mu \)l of 10% 5'-sulfosalicylic acid was added to the hemolysate, and that mixture was mixed and centrifuged. An aliquot

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of the supernatant was analyzed for total glutathione by a modified 5,5′-dithiobis(2-nitrobenzoic acid)-glutathione reductase procedure (27, 28). A delay of more than 10–15 s in adding the 5′-sulfosalicylic acid to the hemolysate results in spuriously low glutathione values. (b) A 200-μl aliquot of the incubation mixture was added directly to 1.0 ml of 0.9% NaCl. The suspension was centrifuged 1 min (Beckman microfuge B), and the supernatant solution was removed. The packed cells were washed twice more with 1-ml portions of 0.9% NaCl and then 100 μl of 1.5% acetic acid containing 1.0 mM phenylalanine was added to the final pellet of packed cells. After mixing a few seconds, 200 μl of 10% 5′-sulfosalicylic acid was added and the suspension was centrifuged. An aliquot of the supernatant was chromatographed on a Durrum model 500 amino acid analyzer; the quantity of phenylalanine found allowed the actual volume of packed cells to be determined. The data were used to evaluate buthionine sulfoximine uptake by the cells. (c) A 2.0-ml aliquot of the incubation mixture was placed in an ice-cold tube and centrifuged 2 min at 1500 × g. An aliquot of the supernatant was saved and used to evaluate the specific activity of the amino acids in the medium; the bulk of the supernatant was aspirated. The packed cells were washed twice with 10 ml of ice-cold 0.9% NaCl (centrifuged as above). To the final pellet was added 4 ml of 2.5% acetic acid, and the suspension was vigorously mixed and transferred to a 15-ml Corex tube containing 1 ml of 4% picric acid in ethanol. After thorough mixing, the contents were centrifuged 5 min at 27,000 × g. The entire supernatant was applied to a small column (0.5 × 7 cm) of Dowex 1 (acetate). The column was washed with 4 ml of water, and then, sequentially, with 2-ml portions of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, and 1.8 mM acetic acid. The effluent from each portion was collected in a separate tube. Glycine and cystine are eluted together in the original flow-through and the water wash, while glutamate, GSH, and GSSG are eluted primarily by 0.6 M, 1.0 M, and 1.6 M acetic acid, respectively.

The specific activities of glutathione and its constituent amino acids were evaluated as follows: (a) The total glutathione concentration of the 1.0 M or 1.6 M acetic acid washes was determined as described (27, 28). Portions of 1.5 ml were submitted to liquid scintillation counting and the resulting data were used to calculate the specific activity. The specific activities of the GSH and GSSG fractions were identical when expressed as counts per min per μmol of GSH equivalent. (b) For determination of the glutamate specific activity, a 1.5-ml portion of the 0.6 M eluent was lyophilized and the residue redissolved in 120 μl of 5% 5′-sulfosalicylic acid. Aliquots of that solution were submitted to liquid scintillation counting and the amino acid analysis to determine the specific activity. (c) For determination of the glycine specific activity, the pooled water wash and load flow-through from the Dowex 1 chromatography was applied directly to a small column (0.5 × 5 cm) of Dowex 50 (H⁺). The column was eluted with 4 ml of water and then, sequentially, with 6-ml portions of 0.3 M pyridine adjusted to pH 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 with formic acid (i.e. 6 ml of pyridinium formate at pH 2.0, then 6 ml at pH 2.5 etc.). Glycine elutes at pH 4.5 or 5.0; a portion of the fraction of higher radioactivity was lyophilized and the specific activity of glycine was evaluated as described for glutamate. In control experiments it was shown that the Dowex 1 glutamate fraction does not contain glutamine, α-ketoglutarate, or GSH, and that at least 95% of the radioactivity of the Dowex 50 glycine fraction is, in fact, [14C]-glycine. In several experiments, aliquots of the 1.6 M acetic acid eluent from the Dowex 1 columns were reduced with dithiothreitol, derivatized with 2-vinylpyridine, and chromatographed on the amino acid analyzer. Fractions were collected from the analyzer every 2 min. All of the radioactivity eluted at a time corresponding to the 2-vinylpyridine derivative of GSH.

Calculations—The apparent rate of GSH turnover was calculated from the data presented in Figs. 1 to 3 as follows: For each hourly interval the average specific activity of the radioactive precursor amino acid was determined by mechanically integrating the area under the appropriate curve. Those values (in counts per min per μmol) were divided into the value of the net change of GSH specific activity occurring during the corresponding hourly interval (in counts per min per μmol per h). Those results, which represent apparent fractional synthesis of GSH per h, were averaged for each incubation. The average value was used to estimate the theoretical number of days necessary to synthesize the entire GSH pool (the apparent turnover time). The procedure is similar to that described by Zilverstat et al. (29). Since the specific activity of the GSH pool was always much less than that of the amino acid pool, the rate at which radioactive amino acid was lost from GSH was neglected.

RESULTS

Incorporation of Glycine, Glutamate, and Glutamate Precursors into Glutathione—The data presented in Fig. 1A demonstrate that the intracellular glycine pool of erythrocytes is rapidly labeled when the cells are suspended in about 0.4 mM [14C]glycine. The specific activity of intracellular glycine reaches a plateau in 2 to 3 h at a value equal to about 60% that of the original extracellular glycine pool. Glutamate is taken up more slowly by the erythrocytes; when suspended in [14C]glutamate, the intracellular specific activity does not begin to plateau until about 6 h. α-Ketoglutarate, which can form glutamate intracellularly by transamination, is taken up even more rapidly than glutamate; the specific activity of intracellular glutamate reaches a plateau in 3 to 4 h. With both glutamate and α-ketoglutarate the specific activity eventually attained intracellularly is 70 to 80% of that originally present externally. In contrast, glutamine, which is also converted to glutamate intracellularly, is not very efficient as a glutamate

![Fig. 1. Incorporation of glutamate, glutamate precursors, or glycine into glutathione. Four tubes containing 9 ml of washed, packed erythrocytes and 9 ml of modified Krebs-Ringer solution containing 0.25 mM L-cystine and 0.5 mM glycine were prepared. To tube A was added 90 μl of 100 mM Na+-l-glutamate and 100 μl of 0.60 mM [1,14C]glycine (1.2 × 10⁵ cpm/μmol). To tube B was added 90 μl of 100 mM L-[1,14C]glutamine (2.6 × 10⁵ cpm/μmol). To tube C was added 90 μl of 100 mM L-alanine and 90 μl of 100 mM Na+-α-keto[1,14C]glutamate (4.8 × 10⁴ cpm/μmol). To tube D was added 90 μl of 100 mM Na+-l-[1,14C]glutamate (2.4 × 10⁶ cpm/μmol). The tubes were incubated as described under "Methods." Curves A to D of IA show the specific activity of the intracellular glycine or glutamate pools as a function of incubation time. Curves A to D of IB show the corresponding specific activity of intracellular GSH. Aliquots of the incubation mixtures were also analyzed on a Coulter Counter; mean cell volume was found to be 90.3 ± 0.5 μm² with 4.1 × 10⁸ cells/ml. The hematocrits were 37.2 ± 0.5%.]
precurisor. As shown in Fig. 1A, the specific activity of the intracellular glutamate pool increases linearly with time when cells are suspended in [14C]glutamine; the specific activity of intracellular glutamate at 6 h is, however, only about 25% that of the extracellular glutamate.

The specific activity of erythrocyte GSH as a function of time is shown in Fig. 1B. Both glutamate (supplied originally as glutamate or a glutamate precursor) and glycine are incorporated into GSH, but glycine is incorporated substantially faster than glutamate. For example, at 4 h the specific activity of GSH is about 17% that of the glycine pool but is only about 2% that of the α-ketoglutarate-derived glutamate pool. The data in Fig. 1 can be used to calculate, on an hourly basis, the apparent rate of GSH synthesis (see "Experimental Procedures"); the results of those calculations are given in Table I; the turnover times shown are the averages of the times calculated for each of the six hourly intervals shown in the figure. The results indicate that if the erythrocyte glutamate pool is labeled by incubation with radioactive glutamate, glutamine, or α-ketoglutarate, GSH is synthesized with an apparent turnover time of about 6 days (i.e. about 0.7% of the GSH pool is synthesized per h). On the other hand, if glycine is the radioactive precursor, the apparent turnover time is only 0.8 day (about 5.2% of the GSH pool is synthesized per h).

Aliquots of the incubation mixtures described in Fig. 1 were assayed at hourly intervals for total glutathione. The cells were not separated from the medium, but it was shown in control experiments that neither GSH nor GSSG accumulates extracellularly to a level measurable by the procedure employed. The GSH content of the erythrocytes was found to be 290 to 300 nmol/10^8 cells (or about 3.3 μmol/ml cell volume). The GSH content did not change significantly in 6 h (e.g. 296 ± 11 nmol/10^8 cells initially compared to 289 ± 13 nmol/10^8 cells at 6 h). Since it is known that erythrocytes degrade GSH only very slowly, the finding that no significant net synthesis of GSH occurs in 6 h suggests that the glycine incorporation reported in Fig. 1 (implying a 31% increase in 6 h) does not represent true synthesis.

Effect of Buthionine Sulfoximine on the Incorporation of Glycine and Glutamate into Glutathione—To elucidate the mechanism by which glycine is incorporated into erythrocyte GSH, experiments similar to those reported in Fig. 1 were carried out in the presence of buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase. The results are shown in Fig. 2. With α-keto[14C]glutarate used as a source of intracellular [14C]glutamate, buthionine sulfoximine caused a marked inhibition of [14C]GSH formation (Fig. 2A). In contrast, buthionine sulfoximine had only a very small effect on the rate at which [14C]glycine was incorporated into glutathione (Fig. 2B). In both cases, the inhibitor had no significant effect on the labeling of the precursor amino acid pool.

Glutathione turnover times were calculated from the data in Fig. 2 and are presented in Table I. Based on the experiments with α-keto[14C]glutarate, buthionine sulfoximine increases the apparent turnover time of GSH from 6.3 days to 65.1 days (the rate of synthesis is decreased from 0.66%/h to only 0.064%/h, a drop of 90%). It is notable that the rate of GSH synthesis decreases as the intracellular buthionine sulfoximine concentration increases. Thus, for the six hourly intervals shown in Fig. 2A, the rates of synthesis (in per cent per h) were 0.245, 0.150, 0.098, 0.080, 0.068, and 0.060. In contrast, the hourly rates in the absence of inhibitor show no such trend (0.62, 0.61, 0.65, 0.69, 0.60, 0.57). With [14C]glycine as the radiolabel, the turnover times are 1.0 and 1.1 days in the absence and presence of buthionine sulfoximine, respectively.

Effect of Buthionine Sulfoximine on the Incorporation of Cysteine and Glycine into Glutathione—The results presented in Fig. 3 show that buthionine sulfoximine causes a

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**Table I**

Summary of apparent glutathione turnover times

| Figure no. | Radioactive precursor | Buthionine sulfoximine present | Turnover time |
|------------|-----------------------|-------------------------------|---------------|
| 1          | Glutamate             | -                             | 5.8 ± 0.5     |
| 1          | Glutamine             | -                             | 6.1 ± 0.7     |
| 1          | α-Ketoglutarate       | -                             | 6.1 ± 0.8     |
| 2          | α-Ketoglutarate       | +                             | 6.3 ± 1.0     |
| 2          | α-Ketoglutarate       | +                             | 65.1 ± 6.0    |
| 3          | Glycine               | -                             | 0.8 ± 0.1     |
| 3          | Glycine               | -                             | 1.0 ± 0.2     |
| 3          | Glycine               | -                             | 1.2 ± 0.1     |
| 3          | Glycine               | -                             | 1.3 ± 0.1     |

![Fig. 2A](http://www.jbc.org/)

**Fig. 2.** Effect of buthionine sulfoximine on the incorporation of glutamate or glycine into glutathione. Four tubes were prepared containing 9 ml of packed erythrocytes and 9 ml of modified Krebs-Ringer solution containing 0.25 mM L-cystine and 0.5 mM glycine. 2A, to tubes A (open symbols) and B (closed symbols) were added 90 μl of 100 mM L-alanine and 90 μl of 100 mM Na+ α-keto-[1-14C]glutarate (4.8 × 10^6 cpm/μmol); to tube C was also added 360 μl of 200 mM buthionine sulfoximine (BSO). 2B, to tubes C (open symbols) and D (closed symbols) were added 90 μl each of 100 mM L-alanine and 100 mM Na+ α-ketoglutarate and 100 μl of 600 mM [1-14C]glycine (1.2 × 10^6 cpm/μmol); to tube D was also added 360 μl of 200 mM buthionine sulfoximine. The figure shows the specific activities of intracellular glutamate, glycine, and GSH as a function of time in the absence of (□, △) and presence (○, ▲) of buthionine sulfoximine. Intracellular buthionine sulfoximine concentration (●) is also shown.

Mean cell volume was 94.2 ± 0.4 μm² with 3.6 × 10⁶ cells/ml; the hematocrits were 33.6 ± 0.2%.
marked (about 92%) decrease in the rate at which $[^3S]cysteine$ is incorporated into GSH, but again causes no significant diminution in the rate at which $[^1C]glucose$ is incorporated. Since the intracellular cysteine concentration is too small to determine accurately, turnover times for the $[^3S]cysteine$ experiments could not be calculated; the calculated turnover times for the $[^1C]glucose$ experiments are shown in Table I and are comparable to the values obtained from the experiments shown in Figs. 1 and 2.

The total glutathione content of the incubation mixtures described in Figs. 2 and 3 was determined at intervals. The results were similar to those given for the experiments shown in Fig. 1; no significant change in the total glutathione content was observed even when buthionine sulfoximine was included in the suspending medium.

**DISCUSSION**

The apparent discrepancy in the rates at which glucose and glutamate are incorporated into erythrocyte GSH is subject to several possible explanations. Since the specific activities of the intracellular amino acid pools were not determined in most previous studies, it was possible that glutamate is transported into erythrocytes more slowly than glucose. Extracellular $[^1C]glutamate$ would therefore be a poor precursor of GSH in consequence of being a poor precursor of intracellular $[^1C]glutamate$. This explanation, which is supported by studies of erythrocyte amino acid transport (18, 20, 32, 33), has been favored in several previous reports (16, 18, 20, 22). An alternative explanation for the relatively more rapid incorporation of $[^1C]glucose$ into erythrocyte GSH is suggested by the observation that purified glutathione synthetase catalyzes the exchange of radioactive glucose into GSH (34). Thus, the incorporation of radioactive glucose may not reflect de novo GSH synthesis. A third explanation for the more rapid incorporation of glucose would be possible if there were in the erythrocyte a source of $\gamma$-glutamylcysteine that is not derived directly from glutamate and cysteine. While there are early reports describing a widely distributed peptidase capable of hydrolyzing glucose from GSH (35, 36), more recent attempts to identify this activity in mouse liver, spleen, muscle, pancreas, and kidney have been unsuccessful (37). Although it is established that carboxypeptidase A will form bis-$\gamma$-glutamylcysteine from GSSG (38), there is no indication that the activity occurs in erythrocytes where the concentration of GSSG is, in any case, quite small. In the absence of continuing synthesis, the amount of $\gamma$-glutamylcysteine in the erythrocyte is too small (about 0.03 mM (39)) to account for more than a small fraction of the observed $[^1C]glucose$ incorporation.

The results given in Fig. 1A indicate that the slower incorporation into GSH of $[^1C]glutamate$ relative to $[^1C]glucose$ is, in fact, due in part to the relative rates of amino acid transport. Thus, the specific activity of intracellular glutamate does not reach a maximum for 6 to 7 h, whereas the specific activity of intracellular glucose is maximal within about 2 h. This difference in the rates of amino acid transport insures that during the first 6 to 7 h of incubation $[^1C]glucose$ will appear to be more effective than $[^1C]glutamate$ as a precursor of $[^1C]GSH$. The intracellular concentration of cyst(e)ine in erythrocytes is too low to allow an accurate determination of its specific activity to be made. However, the fact that the specific activity of GSH increased linearly from the 2nd h onward (Fig. 3A) suggests that the specific activity of intracellular $[^3S]cysteine$ had become constant by about 2 h. Thus, little, if any, of the apparent slowness at which $[^3S]cysteine$ is incorporated into GSH is attributable to a slower rate of cysteine transport.

The results given in Table I are based on the specific activities of the intracellular amino acid pools and are thus independent of the rates of amino acid transport. The rate of GSH synthesis indicated by the studies with $[^1C]glutamate$ is consistent with the observation that no net change in erythrocyte GSH content occurs after 6 h of incubation with buthionine sulfoximine. Assuming that inhibition of new synthesis was virtually complete and that GSH utilization proceeded at its normal rate, the $[^1C]glutamate$ studies suggest that the GSH content should drop only 4% in 6 h. That change is within the range of the experimental error of the GSH assay used. In contrast, the studies with $[^1C]glucose$ indicate that after 6 h of incubation with buthionine sulfoximine the intracellular GSH concentration should drop about 25%. A decrease of that size would have been easily detected.

In the studies described in Figs. 2 and 3, buthionine sulfoximine uptake by erythrocytes and the effect of buthionine sulfoximine on GSH synthesis were measured directly. It was found that buthionine sulfoximine is transported into erythrocytes very slowly; with an extracellular concentration of about 6 mM, the intracellular concentration reaches only 0.6 mM in 6 h. Fortunately buthionine sulfoximine is a powerful inhibitor of $\gamma$-glutamylcysteine synthetase; in vitro it causes virtually complete inhibition in 10 min when present at a
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concentration of 20 μM (24). With intact erythrocytes it was found that buthionine sulfoximine inhibits the incorporation of [14C]glutamate and [35S]cysteine into GSH by over 90%, but that it has little, if any, effect on the incorporation of [14C]glycine. Since there is in the erythrocyte no known source of γ-glutamylcysteine other than γ-glutamylcysteine synthetase, it is concluded that [14C]glycine incorporation is due to an exchange reaction and is thus not a measure of genuine GSH synthesis. The exchange reaction catalyzed by glutathione synthetase requires phosphate (or arsenate) and ADP, and involves a partial reversal of the overall enzymatic reaction to form enzyme-bound γ-glutamylcysteinyl phosphate and free glycine. Reaction of the enzyme-bound intermediate with [14C]glycine forms [14C]GSH (34, 40). No net synthesis of GSH occurs by this mechanism; only exchange of the glycine moiety is possible. Although the exchange reaction proceeds at only 10% of the rate of the overall reaction when studied in vitro (34), the exchange reaction predominates in the erythrocyte in vivo because γ-glutamylcysteine is produced so slowly that the maximal overall reaction cannot be expressed. Thus, erythrocytes have been shown to contain 150- to 800-fold more γ-glutamylcysteine synthetase and glutathione synthetase than is needed for the slow GSH turnover observed (1, 21, 41). Various control mechanisms, probably including cysteine availability (42) and feedback inhibition of γ-glutamylcysteine synthetase by GSH (1, 43, 44), prevent overall GSH biosynthesis from proceeding at more than a small fraction of its theoretically possible rate. The control mechanisms apparently do not significantly limit the rate of glycine exchange.

The present results clearly demonstrate that [14C]glycine is not a suitable tracer for use in the measurement of erythrocyte GSH turnover. It must be anticipated that [14C]glycine will be found to be similarly unsuitable in studies with some other tissues, particularly those in which GSH synthesis is slow but the activity of glutathione synthetase is relatively high. The present results also suggest that measurements of the rate of incorporation of [14C]glycine into erythrocyte GSH may be a valid and convenient assay for erythrocyte glutathione synthetase activity. Such an assay might be useful in connection with inherited deficiencies of glutathione synthetase. Patients with either a generalized deficiency of the enzyme (5-oxoprolinuria) (45, 46) or a deficiency limited to the erythrocyte (45-47) have low blood GSH levels; they are easily detected by use of simple, colorimetric GSH assays. Heterozygotes may have essentially normal erythrocyte GSH concentrations but do have reduced enzyme levels; measurements of the rate at which [14C]glycine is incorporated into erythrocyte GSH should prove useful for identification of the heterozygotes.

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