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Formation of 5-Oxoproline from Glutathione in Erythrocytes by the γ-Glutamyltranspeptidase-Cyclotransferase Pathway

(pyroglutamate/pyrrolidone carboxylate/γ-glutamyl cycle/γ-glutamyl cyclotransferase)

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ABSTRACT **γ-Glutamyltranspeptidase activity was demonstrated in the membrane fraction of rabbit erythrocytes. The activity observed (with glutathione and various amino-acid acceptors) was similar in magnitude to that of the γ-glutamylcyclotransferase and γ-glutamylcysteine synthetase activities found in the soluble fraction of the cell. No transpeptidase activity was observed with either γ-glutamyl *p*-nitroanilide or oxidized glutathione in contrast to the rabbit-kidney enzyme for which these compounds and glutathione serve as substrates. Erythrocyte suspensions and hemolysates formed 5-oxoproline (pyroglutamate; pyrrolidone carboxylate); the rate of 5-oxoproline formation from glutathione by hemolysates was increased by addition of methionine. The findings indicate that 5-oxoproline is an end-product of glutathione metabolism in erythrocytes, and that 5-oxoproline passes out of the erythrocyte and is metabolized in other tissues. The observed rate of 5-oxoproline formation is consistent with the conclusion that the γ-glutamyltranspeptidase-cyclotransferase pathway, together with the synthesis of glutathione from glycine, cysteine, and glutamate, account for a large fraction of the observed amino-acid turnover of erythrocyte glutathione.**

Although mature erythrocytes do not synthesize protein, they are significantly active in the synthesis of the tripeptide, glutathione. The functions of erythrocyte glutathione are not fully understood, but there is evidence that glutathione plays an important role in erythrocyte metabolism and in the protection of hemoglobin against oxidation (1, 2). Much attention has been given to the reversible oxidation of glutathione, and it has been suggested that glutathione may function in the regulation of the hexose monophosphate pathway (3, 4). Erythrocyte glutathione exhibits an amino-acid turnover that is relatively slow (several days) as compared, e.g., to that of the liver (5). The enzymes that catalyze the synthesis of glutathione have been found in and purified from erythrocytes (6–8), but little information is available about the metabolic fate of erythrocyte glutathione. It has been suggested that the observed turnover of glutathione in circulating erythrocytes is due to oxidation of glutathione to the corresponding disulfide followed by active transport of the oxidized form of glutathione out of the erythrocyte (9); thus, according to this interpretation glutathione is not degraded to its constituent amino acids within the erythrocyte.

In the present work, we have examined the possibility that erythrocyte glutathione is degraded by a pathway similar to that which takes place in several other cell types, i.e., enzymatic transpeptidation of glutathione with amino acids to form γ-glutamyl amino acids and cysteinylglycine (10). Cysteinylglycine is split by a peptidase to its constituent amino acids, and γ-glutamyl amino acids are converted by the action of γ-glutamylcyclotransferase (11-14) to the corresponding amino acids and 5-oxoproline*. Thus, glutathione is converted to cysteine, glycine, and 5-oxoproline. In kidney, liver, choroid plexus, ciliary body, and certain other mammalian tissues, 5-oxoproline is converted to glutamate in an ATP-dependent enzyme-catalyzed reaction (15–18). Glutamate, together with cysteine and glycine, are used for the resysthesis of glutathione. These six enzyme-catalyzed reactions have been termed the γ-glutamyl cycle; evidence has been obtained for the occurrence of this cycle in several mammalian tissues (15–22). The initial step in the degradation of glutathione by this pathway requires the utilization of free amino acids, which are regenerated in the reaction catalyzed by γ-glutamylcyclotransferase. These and other considerations have led to the proposal that the γ-glutamyl cycle functions in amino-acid transport. The findings reported here indicate that a substantial fraction of erythrocyte glutathione is degraded by the transpeptidase-cyclotransferase-catalyzed pathway.

EXPERIMENTAL

Materials. Glutathione, L-γ glutamyl-*p*-nitroanilide, glycylglycine, L-alanylglycine, sodium deoxycholate, and 5-oxo-L-proline, were obtained from Sigma. L-[*U*-^{14}C]Glutamic acid, L-[*U*-^{14}C]5-oxoproline, and L-[*U*-^{14}C]methionine were obtained from New England Nuclear Corp. [^{14}C]5-Oxoproline was freed of traces of [^{14}C]glutamate by passage through a column of Dowex 50 (H^+). The L-γ-glutamyl-L-amino acids were prepared by Dr. Ralph A. Stephani and Miss Charlene Michaud of this laboratory. White New Zealand rabbits (3–4 kg) were used.

Methods. Blood withdrawn from the marginal ear vein was collected in tubes containing heparin; after centrifugation the plasma and buffy coat were removed, and the erythrocytes were washed twice by centrifugation with 0.9% NaCl. The cells were suspended in an equal volume of 10 mM Tris·HCl buffer (pH 8.0) containing 80 mM $MgCl_2$, and hemolyzed by sonication for 15 sec with a Sonifier Cell Disruptor (model W185D, Heat Systems, Ultrasonics); the membrane fraction (pellet) was recovered by centrifugation at 17,000 × g for 20 min. The supernatant solution was used for the determination of γ-glutamylcysteine synthetase (EC 6.3.2.2) activity as described using [^{14}C]glutamate (23), and for determination of γ-glutamylcyclotransferase activity (see below). The pellet was suspended in 0.1 M Tris·HCl buffer (pH 8.0)

* L-pyroglutamic acid, L-2-pyrrolidone-5-carboxylic acid, L-5-oxopyrrolidine-2-carboxylic acid.

containing 1% sodium deoxycholate and homogenized in a Potter–Elvehjem homogenizer. After standing at 25° for 4 hr, the homogenates were centrifuged and the γ-glutamyltranspeptidase activity of the supernatant solution was determined in reaction mixtures (final volume, 0.2 ml) containing 0.1 M Tris·HCl buffer (pH 8.5), 5 mM glutathione (adjusted to pH 8.5 by addition of Tris), amino acids (40 mM), and deoxycholate extract. Controls in which enzyme or amino acid were separately omitted were performed. After incubation at 37° for 0–60 min, 0.1-ml samples were withdrawn and added to 0.9 ml of 2% sulfosalicylic acid; after centrifugation, 0.5 ml of the protein-free supernatant solution was used for determination of glutathione (24). Transpeptidation between glutathione and [^{14}C]methionine was determined in reaction mixtures (final volume, 0.2 ml) containing 50 mM Tris·HCl buffer (pH 8.5), 5 mM glutathione, 5 mM L-[*U*-^{14}C] methionine (7500 cpm/μmol), and the deoxycholate extract. Controls lacking either enzyme or glutathione were run. After incubation, the reaction mixtures were placed at 100° for 1 min and then cooled; the denatured protein was removed by centrifugation. The supernatant solutions were subjected to paper electrophoresis [10 V/cm; 50 mM sodium acetate (pH 5.0); 90 min] with methionine and γ-glutamylmethionine standards; the respective mobilities were +1 cm and +5 cm. The areas that contained methionine and γ-glutamylmethionine were cut out, and the radioactivity present was determined by liquid scintillation counting.

γ-Glutamylcyclotransferase was determined on hemolysates [dialyzed for 4 hr at 4° against 1000 volumes of Tris·HCl buffer (pH 8.0) containing 80 mM $MgCl_2$] with L-[^{14}C]-γ-glutamyl-α-aminobutyrate [prepared enzymatically (23)]. Formation of [^{14}C]5-oxoproline was determined in the deproteinized reaction mixtures by passing the sample through a small column of Dowex 50 (H^+); 5-oxoproline was eluted with water and the radioactivity present in the eluate was determined. γ-Glutamylcyclotransferase activity was also determined in a hemoglobin-free hemolysate fraction obtained as follows. The centrifuged hemolysate was passed through a column (2.5 × 10 cm) of carboxymethyl-cellulose (Whatman CM-52) equilibrated with 5 mM potassium phosphate (pH 6.1). The column effluent was treated with solid ammonium sulfate to achieve 50% of saturation and the precipitated protein was removed by centrifugation; additional solid ammonium sulfate was added to achieve 90% of saturation, and the resulting precipitate was collected by centrifugation and dissolved in 50 mM Tris·HCl buffer (pH 8.0). This solution was dialyzed against the same buffer for 12 hr at 0° and then assayed for γ-glutamylcyclotransferase activity (12).

Dipeptidase activity was determined in hemolysates prepared by suspending the cells in an equal volume of 25 mM potassium phosphate buffer (pH 7.7) followed by centrifugation; the supernatant solution was dialyzed against the same buffer for 12 hr at 4°. The reaction mixtures (final volume, 0.1 ml) contained 25 mM potassium phosphate (pH 7.7), 10 mM L-alanylglycine, and the dialyzed solution. After incubation at 37° for 30 min, 0.1 ml of 5% sulfosalicyclic acid was added and the precipitated protein was removed by centrifugation. Controls were done in which the substrate was added after addition of sulfosalicylic acid. Aliquots (50 μl) of the protein-free supernatant solutions were diluted with 0.5 ml of water, and samples were taken for determination of alanine and glycine with a Durrum model D-500 amino-acid analyzer.

Formation of 5-oxoproline by intact erythrocytes and hemolysates was determined as follows: After incubation, the reaction mixtures were treated with an equal volume of 10% trichloroacetic acid or placed at 100° for 1 min and then cooled; the denatured protein was removed by centrifugation. [^{14}C]5-Oxoproline (1 μl containing 0.5 nmol; 200,000 cpm) was added to an aliquot of the supernatant solution as carrier, and the solution was then applied to a Dowex 50 (H^+) column prepared in a Pasteur pipette. The supernatants from heat-treated samples were first mixed with 5 μl of 2 N HCl and then applied to the Dowex columns. Elution was done with water, and the eluate was evaporated to yield a residue that was dissolved in 0.1 ml of water and then subjected to descending paper chromatography (Whatman no. 3 MM) in solvent consisting of *n*-butanol–acetic acid–water; 4:1:1 (v/v) with [^{14}C]5-oxoproline as standard. The strips were scanned in a Nuclear Chicago Actigraph III Strip Counter, and areas corresponding to 5-oxoproline were cut out and eluted with water. The elutates were evaporated to dryness, and the residues were dissolved in 6 N HCl and placed at 100° for 4 hr. The hydrolysates were analyzed for glutamate with a Durrum analyzer. The identity of 5-oxoproline was further established by treating aliquots with a purified preparation of *Pseudomonas* 5-oxoprolinase (kindly provided by P. Van der Werf of this laboratory). The conditions were essentially the same as those described (18). The glutamate formed was identified and determined with a Durrum analyzer.

Erythrocyte suspensions and hemolysates were analyzed for amino acids and glutathione (oxidized plus reduced) after deproteinization with sulfosalicylic acid and trichloroacetic acid, respectively. The amino acids were determined with a Durrum analyzer; glutathione was kindly determined for us by Ronald Sekura of this laboratory by an automated column chromatographic technique.

RESULTS

The values for γ-glutamyltranspeptidase activity in Table 1 were obtained in studies with deoxycholate extracts of the membrane fraction. Much less γ-glutamyltranspeptidase activity was detected in assays done on hemolysates. Transpeptidation was determined by measurement of the rate of

TABLE 1. *Enzyme activities of erythrocyte hemolysates*

Enzyme	Activity* (units/ml)
γ-Glutamyltranspeptidase	100†[a], 172†[b], 151†[c]
γ-Glutamylcyclotransferase	192‡, 169§[a], 151§[b]
Dipeptidase	657
γ-Glutamylcysteine synthetase	220

* Expressed as units/ml of hemolysate; a unit is defined as the amount of enzyme that catalyzes the formation of 1 nmol of product per minute under the conditions of assay.

† Determined with glutathione and [a] glycylglycine; [b] L-alanine; and [c] L-methionine (24) using deoxycholate extracts of erythrocyte membranes. The formation of γ-glutamylmethionine was confirmed in parallel experiments in which L-[^{14}C]methionine was used. (The values are expressed as units per ml of hemolysate.)

‡ Determined on hemolysates with γ-[^{14}C]glutamyl α-aminobutyrate.

§ Determined on a fraction of the hemolysate obtained by ammonium sulfate precipitation with γ-glutamyl-γ-glutamyl-*p*-nitroanilide[a] or γ-glutamylmethionine[b] as substrates.

decrease in glutathione concentration in the presence of amino acids or glycylglycine, and the occurrence of the reaction was confirmed by demonstration of the formation of γ-glutamylmethionine using [^{14}C]methionine. The deoxycholate extract did not catalyze detectable transpeptidation in reaction mixtures containing γ-glutamyl *p*-nitroanilide [tested in the presence or absence of glycylglycine by the procedure described (25)]; under the conditions used, transpeptidation of γ-glutamyl *p*-nitroanilide equivalent to 0.04% of that observed with glutathione could have been detected. Furthermore, incubation (30 min; 37°) of γ-glutamyl *p*-nitroanilide with [^{14}C]methionine and either the deoxycholate extract of erythrocyte membranes or the dialyzed deoxycholate extract did not lead to the formation of γ-glutamylmethionine. Similar experiments with rabbit-kidney preparations for shorter periods of incubation led to formation of substantial amounts of γ-glutamylmethionine. Table 2 gives data on the amino-acid specificity of the glutathione transpeptidation reaction; values for kidney γ-glutamyltranspeptidase are included for comparison. The most active acceptors with the erythrocyte preparation were (in descending order of effectiveness): alanine, methionine, glutamine, and glycylglycine; in contrast, glycylglycine is more active than alanine with kidney preparations.

Transpeptidation was also determined with oxidized glutathione (5 mM) and L-[^{14}C]methionine (5 mM). No transpeptidation was observed with the erythrocyte membrane extract. However, oxidized glutathione was an efficient substrate of rabbit-kidney transpeptidase; the activity observed was 35% of that observed with glutathione and [^{14}C]-methionine.

The presence of γ-glutamylcyclotransferase was demonstrated in the soluble fraction of the erythrocytes in studies with γ-glutamylamino acids and with the model substrate γ-glutamyl-γ-glutamyl-*p*-nitroanilide. The presence of dipeptidase and γ-glutamylcysteine synthetase (in confirmation of earlier work) was also shown in the soluble fraction.

When a suspension of washed erythrocytes was shaken at 37° there was a significant increase in the concentration of 5-oxoproline (Table 3, Exp. 1). These studies showed that 5-oxoproline was present in the erythrocytes initially and that the concentration of 5-oxoproline increased linearly for 8 hr.

TABLE 2. *γ-Glutamyltranspeptidase activity of erythrocyte membrane fraction*

Acceptor	Activity* (units/ml)
None	18 (13)
Glycylglycine	100 (100)
L-Methionine	151 (45)
L-Alanine	172 (48)
Glycine	87 (19)
L-Lysine	64
L-Glutamine	101 (59)
L-Isoleucine	51 (20)
L-Asparagine	48
L-Arginine	45 (17)

* Transpeptidation between glutathione and various amino acids was determined as described in the *text*. The values in *parentheses* are the relative activities of rabbit-kidney γ-glutamyltranspeptidase (20).

TABLE 3. *Formation of 5-oxoproline by erythrocyte suspensions and hemloysates*

Exp. no.; incubation mixture	Incubation period (hr)	5-Oxoproline (nmol/ml)
1*a* Cell suspension	0	55
b Cell suspension	4	96
c Cell suspension	8	138
2*a* Hemolysate	0	45
b Hemolysate	6	61.5
c Hemolysate + glutathione	6	167
3*a* Dialyzed hemolysate	6	0
b Dialyzed hemolysate + L-methionine	6	0
c Dialyzed hemolysate + glutathione	6	63.6
d Dialyzed hemolysate + glutathione + L-methionine	6	125
e Glutathione	6	0

Exp. 1. Washed erythrocytes were suspended in an equal volume of Kreb's–Ringer phosphate solution containing 5 mM glucose; the suspensions were shaken at 37° and samples (1 ml) were removed for determination of 5-oxoproline at the intervals indicated. *Exp. 2.* Packed erythrocytes were hemolyzed by sonication in an equal volume of 10 mM Tris·HCl buffer (pH 8.0). The reaction mixtures (final volume, 1 ml) contained (*a*,*b*) 0.7 ml of hemolysate and 0.3 ml of water; and (*c*) 0.7 ml of hemolysate and 0.3 ml of glutathione (final concentration, 2 mM). *Exp. 3.* Erythrocyte hemolysate (see Exp. 2) was dialyzed against two changes of 1000 volumes each of 10 mM Tris·HCl buffer (pH 8.0) for 18 hours at 4°. Before dialysis the hemolysate contained 0.75 mM glutathione; no glutathione could be detected after dialysis. About 90% of the free amino acids were removed by dialysis. The reaction mixtures (final volume, 1 ml) contained 0.8 ml of hemolysate and L-methionine (2 mM) or glutathione (2 mM) as indicated. *Exp. 3e.* Glutathione (2 mM) in Tris·HCl buffer (pH 8.0).

Similar results were obtained with hemolysates (Table 3, Exp. 2), and formation of 5-oxoproline increased substantially when glutathione was added to hemolysates (Exp. 2*c*). Dialysis of hemolysates led to removal of more than 99% of the 5-oxoproline and glutathione and about 95% of the free amino acids. When such dialyzed hemolysates were incubated with glutathione, 5-oxoproline was formed (Exp. 3*c*), and the formation of 5-oxoproline was greatly increased when methionine was also added (Exp. 3*d*). No 5-oxoproline was formed in controls lacking glutathione (Exp. 3*a* and *b*) or hemolysate (Exp. 3*e*). These studies, and those that demonstrate the presence in erythrocytes of the transpeptidase and cyclotransferase activities, indicate that erythrocytes can degrade glutathione to 5-oxoproline by the transpeptidase-cyclotransferase pathway.

We also performed studies (essentially as described in ref. 26) in which whole blood was incubated with L-[^{14}C]glutamate L-cysteine, and glycine; after incubation, the formation of intracellular [^{14}C]glutathione was demonstrated, in agreement with the reported findings (26). In these experiments substantial amounts of [^{14}C]5-oxoproline were found in the erythrocytes and in the plasma; the rate of formation of [^{14}C]5-oxoproline in such experiments was similar to that found in the experiments on erythrocyte suspensions (Table 3, Exp. 1). Since plasma has virtually no γ-glutamylcyclotransferase activity, it may be concluded that 5-oxoproline,

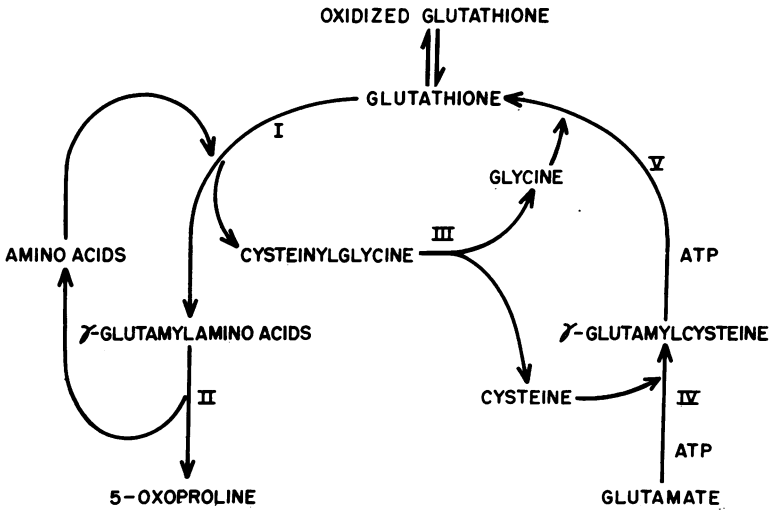

FIG. 1. Pathways of glutathione metabolism in erythrocytes. Enzymes: *I*, γ-glutamyltranspeptidase; *II*, γ-glutamylcyclotransferase; *III*, dipeptidase; *IV*, γ-glutamylcysteine synthetase; *V*, glutathione synthetase.

formed within the cells, readily passes out of the cell. In studies in which erythrocytes were suspended in Krebs–Ringer phosphate solution containing [^{14}C]5-oxoproline, there was rapid uptake of this compound into the cells. In experiments in which either washed cells or hemolysates were incubated with [^{14}C]5-oxoproline for 4 hr, no formation of $^{14}CO_2$, [^{14}C]glutamate, or [^{14}C]glutathione was detected. Assay of the hemolysates for 5-oxoprolinase (16) did not reveal this enzyme activity.

During these studies, reticulocytosis was induced in rabbits by subcutaneous administration of phenylhydrazine (a total of three injections of 15 mg/kg on alternate days). The percentage of reticulocytes was 6, 40, 60, 52, and 8%, respectively 2, 4, 6, 8, and 10 days after start of treatment. The respective γ-glutamyltranspeptidase activities observed (deoxycholate extract of pellet) were 6.6, 2.8, 4.1, 5.9, and 6.6 units/mg of protein. The γ-glutamylcysteine synthetase activity of the hemolysates was not affected by the phenylhydrazine treatment. The decrease in γ-glutamyltranspeptidase activity preceded the peak of reticulocytosis. Treatment of erythrocytes with phenylhydrazine (5 mM final concentration) *in vitro* for 18 hr at 37° had no significant effect on the γ-glutamyltranspeptidase activity of the cell membranes.

DISCUSSION

The findings demonstrate the presence of γ-glutamyltranspeptidase and γ-glutamylcyclotransferase activities in rabbit erythrocytes. Rouser *et al.* (27) reported the presence of γ-glutamyltranspeptidase in human erythrocytes, but the method of assay was not given. Jackson (28), using Ball's method (24) for determination of glutathione, obtained evidence for the presence of γ-glutamyltranspeptidase activity in hemolysates of human erythrocytes, but he observed values that were very low compared to those found for γ-glutamylcysteine and glutathione synthetases. In the present work, the occurrence of transpeptidation was established by demonstration of the γ-glutamylamino-acid product; it was also shown that the γ-glutamyltranspeptidase activity of erythrocytes is localized in the membrane fraction, as it is in other tissues. The membrane-associated γ-glutamyltranspeptidase activity of the erythrocyte was the same order of magnitude as those of γ-glutamylcyclotransferase and γ-glutamylcysteine synthetase. In our studies no activity was observed toward either γ-glutamyl *p*-nitroanilide or oxidized glutathione. While the use of γ-glutamyl *p*-nitroanilide and other chromogenic substrates has provided convenient assay procedures and effective tools for histochemical studies, the present findings suggest that it may be preferable to use the natural substrate, glutathione, for determination of the γ-glutamyltranspeptidase activity of certain cells, and also that the procedures involving use of chromogenic substrates may not detect certain transpeptidase activities. There is evidently a significant difference between the amino-acid specificity of the erythrocyte and kidney transpeptidases (Table 2). The clinical determination of serum γ-glutamyltranspeptidase involves the use of γ-glutamyl *p*-nitroanilide; our findings suggest that the activity measured in such determinations does not originate from erythrocytes.

Our studies have not revealed the presence of 5-oxoprolinase in erythrocytes. It is conceivable that the enzyme is actually present but was not detected by the procedures used; however, this interpretation appears unlikely since incubation of erythrocyte suspensions or hemolysates with [^{14}C]5-oxoproline did not show formation of labeled glutamate or glutathione. Furthermore, the 5-oxoproline content of erythrocytes increased linearly on incubation. Thus, it appears more likely that the 5-oxoproline produced by the transpeptidation-cyclotransferase pathway passes out of the erythrocyte and that it is transported to other tissues where it is metabolized. In this respect, erythrocytes seem to resemble the ocular lens; previous studies (22) did not show 5-oxoprolinase activity in lens. There is evidence that skin contains substantially higher concentrations of 5-oxoproline than do other tissues (29); this tissue may also be deficient in 5-oxoprolinase activity. That blood plasma contains a very low concentration of 5-oxoproline is consistent with its slow formation by erythrocytes and removal from plasma by other tissues.

Erythrocytes contain γ-glutamylcysteine and glutathione synthetases, and our findings on the degradation of glutathione indicate that synthesis of glutathione and its degradation by the transpeptidation-cyclotransferase pathway can account for a substantial fraction of erythrocyte glutathione turnover. The rate of 5-oxoproline formation observed with

erythrocyte suspensions (Table 3, Exp. 1) corresponds to a glutathione turnover time of about 3 days. If this estimate, derived from *in vitro* experiments, is valid *in vivo* it would seem that the transpeptidase-cyclotransferase pathway could account for most of the degradation of glutathione in erythrocytes. However, one cannot exclude the possibility (9) that some glutathione degradation occurs by oxidation followed by transport of oxidized glutathione out of the cell; nevertheless, it seems unlikely that this is a major quantitative pathway under normal conditions in which most of the glutathione is present in the reduced form.

Fig. 1 summarizes the pathways considered above. The findings indicate that 5-oxoproline is an end-product of erythrocyte metabolism and that the rate at which 5-oxoproline is formed is probably close to that of glutathione synthesis. However, since γ-glutamylcysteine is a substrate of γ-glutamylcyclotransferase, it is conceivable that some 5-oxoproline may arise from this dipeptide as well as from other γ-glutamylamino acids. It also seems possible [as suggested (23)] that the γ-glutamylcysteine formed by γ-glutamylcysteine synthetase is protected in some manner from the action of γ-glutamylcyclotransferase and is thus preferentially used by glutathione synthetase; such protection might be achieved by physical linkage between the two synthetases. Degradation of glutathione by the transpeptidase-cyclotransferase pathway involves uptake and release of free amino acids (Fig. 1); this phenomenon and the finding that the transpeptidase is associated with the cell membrane are consistent with the view expressed earlier in relation to other tissues (19, 20) that these reactions are involved in amino-acid transport. The details of such a relationship between amino-acid transport and glutathione metabolism in erythrocytes remain to be elucidated. Nevertheless, the idea that membrane-bound γ-glutamyltranspeptidase can interact with an amino acid outside the cell and also with intracellular glutathione to yield an intracellular γ-glutamylamino acid remains highly attractive. Studies on amino-acid transport in rabbit reticulocytes and erythrocytes suggest the presence of several amino-acid transport systems and indicate that the activities of some of these systems undergo regression associated with maturation and loss of ability to synthesize protein (30, 31). While the mature erythrocyte would not be expected to need amino acids for protein synthesis, it would seem to require glutamate, glycine, and cysteine for synthesis of glutathione. Apart from any other physiological function that the presence of amino acids in the mature erythrocyte may have, it would seem that transpeptidation between amino acids and glutathione is a quantitatively significant step in glutathione degradation in the erythrocyte.

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