Erythrocyte γ-Glutamyl Transpeptidase

To the Editor:

The presence of γ-glutamyl transpeptidase in erythrocyte membranes[1,2] has recently been disputed.[3] In view of the proposed role of γ-glutamyl transpeptidase in the catabolism of glutathione,[1] and the significance of glutathione in the economy of erythrocyte metabolism, it is of great importance that this controversy be resolved. We have now reinvestigated the presence of γ-glutamyl transpeptidase in human and sheep erythrocytes by a new technique that utilizes the proposed in vivo substrate of γ-glutamyl transpeptidase, reduced glutathione (GSH), and isolates the γ-glutamyl peptide reaction product by a simple ion-exchange chromatographic procedure.

Hemoglobin-free erythrocyte ghosts were prepared[4] from erythrocytes that had been either washed in 150 m*M* NaCl or filtered through cotton balls to remove leukocytes, and then washed in 150 m*M* NaCl. The ghosts were suspended in 0.1 *M* Tris-HCl, pH 8.0, containing 1% deoxycholate and kept at 4°C for 18 hr. γ-Glutamyl transpeptidase activity was determined in the ghost extracts by two separate methods. The first method utilized the synthetic substrate L-γ-glutamyl-*p*-nitroanilide. The release of *p*-nitroaniline was followed spectrophotometrically at 405 n*M* according to Sigma Technical Bulletin 415. In the second method, GSH was used as the γ-glutamyl donor and ^{14}C-L-alanine was used as the γ-glutamyl acceptor. The final reagent concentrations in the reaction mixture (0.5 ml) were as follows: Tris-HCl buffer, 0.2 *M*, pH 8.5; GSH 10 m*M*, ^{14}C-L-alanine 50 m*M*, 4μCi/m*M*, and ghost extract. The reaction was started by the addition of GSH, and after 60 min incubation at 37°C the reaction was stopped by heating at 95°C for 5 min. After centrifugation, 0.1 ml of the supernatant was applied to a 1.0-ml column of Dowex 1 formate, and the unincorporated ^{14}C-L-alanine was eluted with 40 ml of water. The final product, γ-L-glutamyl-^{14}C-alanine, was eluted with 2.5 ml of 4 *N* formic acid. The total formic acid eluate was collected, dissolved in Bray's solution, and counted in a liquid scintillation counter. Blanks were obtained by deleting GSH from the reaction mixture.

The data obtained from six normal laboratory personnel are presented in Table 1. It is quite evident that there is little if any γ-glutamyl transpeptidase activity in human erythrocyte membranes. The activity present in unfiltered samples can be attributed to leukocyte contamination. Similar results have been obtained utilizing sheep erythrocytes. These data clearly support the recent findings of Srivastava et al. (1976).[3]

Since γ-glutamyl transpeptidase is apparently not present in erythrocytes, it is no longer possible to explain the turnover of glutathione in erythrocytes on the basis of the "γ-glutamyl cycle." It has been suggested[3] that the oxidized glutathione (GSSG) transport system may account for glutathione turnover. However, the kinetic data available for the GSSG transport and GSSG reduction systems[5-7] suggest that GSSG is more likely to be reduced to GSH rather than be extruded by the transport system. Some alternate mechanism must therefore be sought to explain the turnover of glutathione in erythrocytes.

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Table 1. Activity of γ-glutamyl Transpeptidase in Human Erythrocytes

RBC	γ-Glutamyl Transpeptidase*	
	γ-Glutamyl-*p*-nitroanilide Substrate	Reduced Glutathione Substrate
Unfiltered	2.15 ± 0.195	2.12 ± 0.634
Filtered	0.05 ± 0.05	0.36 ± 0.144

*Mean of six samples ± SE. Units expressed as mμM/mg protein/min at 37°C.

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Absence of γ-Glutamyl Transpeptidase and the Role of GSSG Transport in the Turnover of GSH in Erythrocytes

To the Editor:

In 1964, Hochberg et al.[1] demonstrated the half-life of glutathione in red blood cells to be 2–4 days. Thus even before the confirmation in vivo of the synthesis of this compound[2] the problem of accounting for its rapid turnover had arisen. In the interim, three major hypotheses have been espoused to explain the rapid turnover of this unique tripeptide: (1) the formation of glutathione-protein mixed disulfides; (2) the catabolism of glutathione; and (3) the transport of oxidized glutathione (GSSG) from the red blood cell.

Mixed disulfides. Initially, it was proposed that glutathione may form mixed disulfides with hemoglobin and other proteins of the red blood cell. However, the turnover of glutathione could not be accounted for by this mechanism because glutathione reductase, in the presence of NADPH, was shown to cleave hemoglobin-S-S-G,[3] releasing GSH. Furthermore, even if glutathione reductase did not act in this manner, there was not enough protein in the red blood cell to account for the turnover rate.

Catabolism of glutathione. Glutathione contains a γ-peptide linkage between its glutamic acid and cysteine residues which normal intracellular peptidases, specific for an α-peptide linkage, cannot cleave. The only known enzyme that can cleave this γ-peptide linkage is γ-glutamyl transpeptidase (GGT or γ-glutamyl transferase, E. C. 2.3.2.2). This enzyme transfers the γ-glutamyl moiety of glutathione to an acceptor amino acid and has been proposed to play a role in amino acid transport.[4]

Several investigators have demonstrated the presence of low levels of GGT in red blood cells.[5–7] However, using the identical methods published by these investigators, as well as immunologic techniques, we were unable to demonstrate the presence of GGT or an antigen-like human kidney GGT in human or rabbit erythrocytes.[8,9] Although significant amounts of GGT were present in white blood cells, contamination of the red blood cell preparations by leukocytes could not explain the enzyme levels reported by the previous investigators. Other studies have confirmed the absence of this enzyme in rabbit[10] and now human red blood cells.[11] Thus the catabolism of glutathione by GGT neither accounts for the turnover of this compound nor the transport of amino acids in red blood cells.

Transport of GSSG. In 1969, we[12] demonstrated that GSSG is preferentially and actively transported from the red blood cell under oxidative stress. At that time, however, the substrate kinetics of the transport of GSSG could not be studied due to technical difficulties; therefore no definite conclusion was made as to the role of transport in the turnover of glutathione.

In subsequent investigations, we[13] used red cell ghosts to determine the kinetics of glutathione transport. These studies confirmed the requirement of energy in the form of ATP for the transport of GSSG. In addition, they demonstrated that the transport of GSSG from the red cell ghosts was substantially slower at lower concentrations of GSSG, those closer to physiologic in erythrocytes, as compared to