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Evidence against the Participation of the γ-Glutamyltransferase–γ-Glutamylcyclotransferase Pathway in Amino Acid Transport by Rabbit Erythrocytes

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The GSH concentration of rabbit erythrocytes was monitored under conditions of large net transport of alanine, phenylalanine and lysine in the absence of glucose. In no case was there an appreciable alteration in GSH concentration during amino acid uptake. It is suggested that the γ-glutamyltransferase–γ-glutamylcyclotransferase pathway does not participate in amino acid transport by these cells.

The initial step of GSH degradation may be enzymic γ-glutamyl transfer from GSH with amino acids to form γ-glutamyl-amino acids and cysteinylglycine (Hanes *et al.*, 1952). Cysteinylglycine is hydrolysed to its constituent amino acids by a peptidase, and γ-glutamyl-amino acids are converted into the corresponding free amino acids and 5-oxoproline by γ-glutamylcyclotransferase (Connell & Hanes, 1956; Orlowski *et al.*, 1969; Andamson *et al.*, 1971; Orlowski & Meister, 1973). 5-Oxoproline is converted into glutamate in an ATP-dependent reaction catalysed by 5-oxoprolinase (Van der Werf *et al.*, 1971*a*,*b*, 1973*a*,*b*). These enzymes, together with γ-glutamylcysteine synthetase and GSH synthetase (both ATP-dependent), form what has been termed the γ-glutamyl cycle. The reactions catalysed by γ-glutamyltransferase and γ-glutamylcyclotransferase involve the uptake and release of free amino acids. This and other considerations have led to the suggestion that these enzymes may participate in amino acid transport by a number of tissues, notably the kidney (Meister, 1973). The finding that both γ-glutamyltransferase and γ-glutamylcyclotransferase are present at high activity in the rabbit erythrocyte has prompted the proposal that GSH may also participate in amino acid transport by these cells (Palekar *et al.*, 1974). Indeed Agar *et al.* (1974) have suggested that this may represent a major role of GSH in the erythrocyte. An unattractive feature of this hypothesis is that it implies active transport, since ATP is required for the synthesis of GSH, whereas it is generally believed that mature mammalian erythrocytes do not show active amino acid transport (Winter & Christensen, 1964, 1965).

The hypothesis linking γ-glutamyltransferase and γ-glutamylcyclotransferase to erythrocyte amino acid transport predicts that 1 mol of GSH is degraded for each mol of amino acid entering the cell. To test this hypothesis we have monitored the intracellular GSH concentration of rabbit erythrocytes incubated in the absence of glucose, under conditions giving a large net transport of amino acids.

Materials and methods

Whole blood from adult New Zealand White rabbits was collected into heparinized tubes. The erythrocytes were washed three times with 20 vol. of a medium containing 106mM-$MgCl_2$ and 10mM-Tris–HCl buffer (pH 7.5 at 20°C) and once in incubation medium [135mM-NaCl, 5mM-KCl, 15mM-Tris–HCl buffer (pH 7.1 at 37°C), 3.1mM-$MgCl_2$ and 0.1mM-EDTA]. The buffy coat was discarded.

Amino acid uptake was measured by incubating the washed erythrocytes at 37°C (30% haematocrit) in incubation medium containing the appropriate U-^{14}C-labelled amino acid (The Radiochemical Centre, Amersham, Bucks., U.K.) at a concentration of 20mM (0.1 μCi/μmol). At predetermined time-intervals samples (0.3ml) were removed, and the cells were rapidly washed in 10vol. of ice-cold $MgCl_2$–Tris–HCl medium in an Eppendorf 3200 microcentrifuge (10s, 15000*g*). The packed cells were lysed in 0.5ml of 0.5% (v/v) Triton X-100 in water, and 0.5ml of 33% (w/v) trichloroacetic acid was added. The precipitate was removed by centrifugation (30s, 15000*g*), and a sample (0.9ml) of the supernatant was transferred to 7ml of Unisolve (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) and its radioactivity counted in a β-scintillation spectrometer with quench correction. Additional 0.1ml washed-cell samples (initial and final time-points) were lysed with 0.5ml of water, and 0.1ml of 35% (w/v) sulphosalicylic acid was added. Samples of these supernatants were analysed on a Locarte amino acid analyser.

Incubation samples were also assayed for GSH by methods using 5,5′-dithiobis-(2-nitrobenzoate) (Beutler *et al.*, 1963) and alloxan (Patterson & Lazarow, 1955) as chromogens. The former method measures total non-protein reduced thiol whereas the alloxan method is specific for GSH. In particular, alloxan distinguishes between GSH and γ-glutamylcysteine, cysteine and cysteinylglycine (Patterson & Lazarow, 1955).

Results and discussion

Fig. 1 shows the uptake of alanine, phenylalanine and lysine by rabbit erythrocytes. Net amino acid transport during the incubation period was confirmed by direct amino acid analyses (10.7, 13.0 and 4.7 mmol/l of cells for alanine, phenylalanine and lysine respectively). GSH analyses demonstrated that almost all the non-protein thiol in these cells was GSH. In no case was there an appreciable decline in GSH concentration or increase in non-GSH thiol concentration during amino acid uptake. In fact we find no evidence that amino acid transport by rabbit erythrocytes is associated with GSH degradation, and we suggest that γ-glutamyltransferase and γ-glutamylcyclotransferase play no part in amino acid transport by these cells.

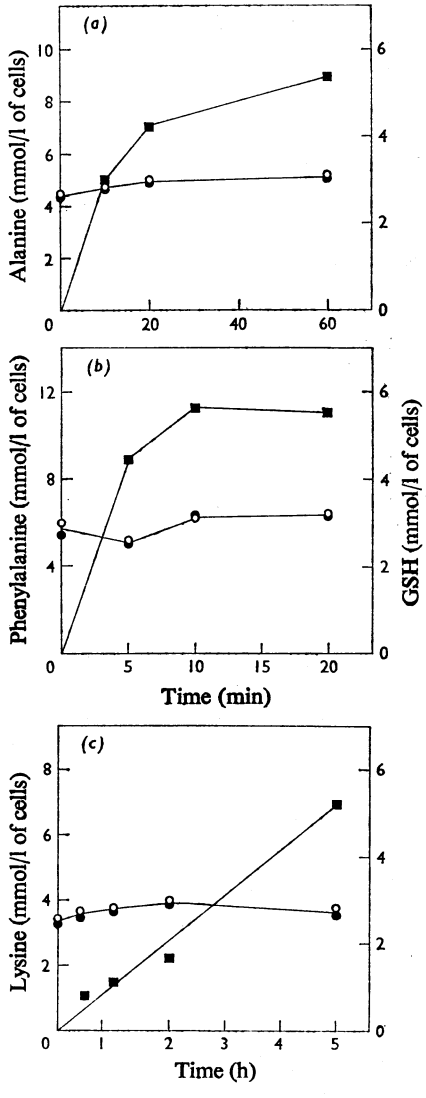

Fig. 1. *Concentration of GSH in rabbit erythrocytes during net alanine (a), phenylalanine (b) and lysine (c) transport*

GSH concentration was determined by the 5,5′-dithiobis-(2-nitrobenzoate) method (○) and by the alloxan method (●). Amino acid concentration was measured by a determination of radioactivity (■).

An inherited GSH deficiency in the erythrocytes of Finnish Landrace sheep is associated with high intracellular concentrations of certain amino acids, particularly ornithine and lysine (Tucker & Kilgour, 1970; Ellory *et al.*, 1972). It has been demonstrated that these cells show a markedly diminished permeability to alanine, cysteine, α-amino-*n*-butyrate, lysine and ornithine (Young *et al.*, 1975, 1976). It was concluded that the diminished amino acid transport of these GSH-deficient cells represented a membrane transport defect and was not a consequence of a low intracellular GSH concentration as predicted by the γ - glutamyltransferase – γ - glutamylcyclotransferase hypothesis, because a second type of GSH-deficient sheep erythrocyte (found in Tasmanian Merino sheep and associated with a diminished activity of the first enzyme of GSH biosynthesis) gave normal amino acid-uptake values (Tucker & Kilgour, 1972; Young *et al.*, 1975; Young & Nimmo, 1975). The low GSH concentration of GSH-deficient Finnish Landrace cells was attributed to a decreased availability of cysteine. The present results suggest there is no direct involvement of intracellular GSH in erythrocyte amino acid transport.

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