BBA 63308

Distribution of glutathione reductase and detection of glutathione–cystine transhydrogenase in rat tissues

A number of biochemical functions have been ascribed to glutathione, but the full extent of its involvement in metabolism is probably unknown Glutathione reductase (EC 1 6.4 2) catalyzes the reaction.

$$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+ \quad (1)$$

and may thus be responsible for maintaining glutathione in cells in its reduced form, since equilibrium lies far to the right There are a number of examples where evidence for the metabolic function of an enzyme has been obtained from the tissue distribution of enzyme activity (*e g* , arginase in ureogenesis, ATP citrate lyase in lipogenesis). It seemed possible that information about the metabolic role of glutathione reductase might be derived from a study of its activity in different tissues.

Tissues (ovaries excepted) were rapidly excised from adult male albino Wistar rats killed by decapitation, and washed in ice-cold buffer (0 1 M potassium phosphate, 2 mM EDTA, pH 7 5) and weighed. They were then extracted with the same buffer, either in a Potter–Elvehjem homogenizer (if less than half a gram) or in a Sorvall Omni-mixer The homogenates were centrifuged at 2000 × *g* for 10 min and the supernatants assayed for enzyme activity and protein concentration

Glutathione reductase activity was assayed in a Hilger–Gilford recording spectrophotometer by following the oxidation of NADPH at 340 mμ. The assay medium contained 3 mM GSSG, 50 μM NADPH, 2 mM EDTA and 0 1 M potassium phosphate, pH 7.5. Corrections were applied for NADPH oxidase activity by measurements made in the absence of GSSG. It was shown for each tissue that the concentrations of GSSG and NADPH used gave maximal velocity, and that the velocity was directly proportional to the amount of tissue extract present Protein concentrations in the extracts were measured by the method of LOWRY *et al.*[1], and glutathione reductase activities are quoted as units of enzyme (1 μmole of substrate transformed per min) per g protein. The activities in different tissues relative to one another were the same whether calculated on a wet weight or protein basis, except in adipose tissue, where activities were relatively lower when calculated on a weight basis (presumably because of the high content of lipid)

The distribution of glutathione reductase (expressed on a protein basis) in various tissues of the rat is shown in Table I. The activity of the enzyme is particularly high in kidney and small intestine, and low in cardiac muscle, skeletal muscle and testis Liver, a number of different glands (ovary, thyroid, pancreas, adrenal and pituitary), bladder, adipose tissue and brain show intermediate activities

One common feature of the majority of those tissues with high or intermediate glutathione reductase activity is active transport (small intestine and kidney) or secretion (small intestine, kidney and glands) Because of this possible correlation, glutathione reductase activities were compared in kidney cortex and medulla (separated from slices by dissection), and in epithelial cells (prepared by scraping the mucosal surface with a microscope slide) and the residual layers of the small intestine As shown in Table I, glutathione reductase activities in whole kidney, medulla and

TABLE I

GLUTATHIONE REDUCTASE ACTIVITY IN A NUMBER OF RAT TISSUES

Glutathione reductase activity in 2000 × *g* supernatants was assayed with saturating concentrations of substrates Results are expressed as mean ± S E

Tissue	*Glutathione reductase activity (units/g protein)*	*Number of rats*
Kidney	113 ± 2 7	6
Small intestine	90 ± 2 2	3
Liver	37 ± 1 5	6
Ovary	31 ± 1 5	4
Thyroid	29 ± 2 1	4
Pancreas	25 ± 1.3	4
Bladder	22 ± 3	2
Adrenal	21 ± 0 8	5
Pituitary	21	3 pooled
Epididymal adipose tissue	19 1 ± 0 64	6
Brain	11 3 ± 0 42	4
Testis	4 5 ± 0 4	5
Heart	4 4 ± 0 26	6
Gastrocnemius muscle	2 6 ± 0 28	4
Kidney whole	102 ± 6 9	3
medulla	92 ± 6 9	4
cortex	100 ± 4 2	4
Small intestine whole	85 ± 5 9	6
epithelial cells	87 ± 4 5	6
remaining layers	87 ± 8 4	6

cortex were the same In the small intestine, glutathione reductase activity was the same in the epithelial layer as in the remaining layers and in the whole tissue. This uniform distribution of the enzyme within these tissues does not suggest any direct link between active transport processes and high glutathione reductase activities

An alternative possibility for the role of glutathione reductase in small intestine and kidney would be as part of a system concerned with the reduction of substances during absorption or secretion In the small intestine, at least one reduction of quantitative importance is apparently necessary before absorbed compounds are utilized along known metabolic paths the reduction of disulphide bonds (originating in proteins) to free thiol groups Since most peptide bonds are hydrolyzed by proteolytic enzymes before the amino acids enter the intestinal cells, and since cystine may apparently be absorbed by the small intestine as such, it seemed reasonable to anticipate the occurrence of a reaction in which cystine is reduced to cysteine An enzyme catalyzing the following reaction was therefore sought

$$\text{Cystine} + 2\ \text{GSH} \rightarrow 2\ \text{cysteine} + \text{GSSG} \qquad (2)$$

The occurrence of Reaction 2 was followed by linking it with Reaction 1, the disappearance of NADPH being observed spectrophotometrically The cuvette contained cystine (220 μM), GSH (7 mM), NADPH (100 μM), glutathione reductase prepared from yeast and obtained from Boehringer (0 2 unit/ml), and tissue extract, in 0 1 M potassium phosphate, 2 mM EDTA, pH 6 5. Tissue extracts were prepared as for

the assay of glutathione reductase, except that they were dialyzed overnight against the extraction buffer to remove endogenous substrate Other reactions oxidizing NADPH were: (a) Reaction 2 occurring without enzymic catalysis; (b) reduction of cystine by tissue extract and NADPH in absence of GSH, (c) GSH oxidase activity of extract; (d) NADPH oxidase activity of extract The assay was carried out at pH 6 5 to lessen Reaction a, and Reactions a–d were corrected for by making appropriate omissions from the full system.

Extracts of all three tissues investigated (liver, kidney and small intestine) were found to catalyze Reaction 2. The observed activities of the enzyme (tentatively referred to as GSH–cystine transhydrogenase), in units/g tissue, were. liver 1.7, kidney 0 59, small intestine 2 4.

The activity of the transhydrogenase, expressed as a percentage of the glutathione reductase activity, is. liver 42%, kidney 6%, small intestine 40% Since the transhydrogenase activities measured may not represent maximal velocities, whereas glutathione reductase activities were measured at maximal velocity, these percentages may err on the low side The high activity of glutathione reductase in small intestine is thus compatible with a metabolic role in the reduction of absorbed cystine to cysteine The high glutathione reductase activity in kidney cannot, however, be so explained

A proper assessment of the role of glutathione–cystine transhydrogenase in the intestinal absorption of cystine awaits determination of the kinetic properties of the enzyme and the physiological concentrations of its substrates Information on the enzyme's substrate specificity is also needed to ascertain its relation to the one or more transhydrogenases, catalyzing the reduction of a variety of low molecular weight disulphides by GSH, that have been found in beef liver[3] and in yeast[4]

I should like to thank Professor P J. RANDLE for his advice and encouragement, and the Medical Research Council for a studentship.

Department of Biochemistry, P. L. WENDELL
University of Bristol,
Bristol (Great Britain)

1 O H LOWRY, N J ROSEBROUGH, A L FARR AND R J RANDALL, *J Biol Chem*, 193 (1951) 265
2 M W. NEIL, *Biochem J*, 71 (1959) 118
3 E RACKER, *J Biol Chem*, 217 (1955) 867
4 J F THOMPSON, S. BLACK AND B HUDSON, *Federation Proc*, 19 (1960) 33

Received December 27th, 1967