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DECREASED HEPATIC GLUCONEOGENESIS BY TREATMENT WITH SUBSTRATES OF THE GSH S-TRANSFERASES

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Glutathione (GSH) has been shown to play a major role in different cellular processes (1). However, little attention has been paid to the GSH content and GSH/GSSG status in the cell in relation with carbohydrate metabolism (2). GSH depletion can be achieved by treatment of the whole animal (3,4), or the isolated hepatocytes with substrates of the GSH S-transferases (5,6). Here we report that GSH depletion by these procedures promotes a decrease in gluconeogenic capacity without affecting other metabolic parameters. Under the experimental conditions used, we did not observe signs of cell damage, in agreement with previous reports (4-6).

Materials and Methods. 48 h starved Wistar rats (180-220 g body weight) were used. Hepatocytes were isolated according to Berry & Friend (7) by the simplified procedure previously described (8). Cells were incubated 30 min in 25 ml erlenmeyer flasks with Krebs-Henseleit saline equilibrated with O_2/CO_2 (19:1, v/v). GSH depletion was induced by either i.p. injection of 250 mg/kg of phorone (2,6-dimethyl-2,5-heptadiene-4-one, diluted in olive oil) (4), or of undiluted diethylmaleate (DEM) 0.6 ml/kg (3), or addition of both substrates to the incubation mixture (5,6). GSH, lactate dehydrogenase (LDH) activity, ATP (9), glucose (10), and malondialdehyde (MDA) (11) were measured as previously described.

Results and Discussion. Treatment of the rats with phorone or DEM 1 h prior to the normal isolation procedure led to a decrease in the rate of glucose formation when the isolated hepatocytes were incubated with lactate (10 mM) plus pyruvate (1 mM): 0.82 ± 0.08 for the controls; 0.41 ± 0.02 for rats pretreated with DEM; 0.12 ± 0.01 for rats pretreated with phorone (values are $\mu mol \times g^{-1} \times min^{-1}$, means $\pm$ S.D., n = 6), whereas the GSH content was undetectable in these cells.

Moreover, addition of the xenobiotics to cells isolated from normal rats, resulted in a decrease in the rate of gluconeogenesis from lactate plus pyruvate or glycerol. However, no significant decrease was observed in the rate of glucose formation from fructose (see Table). Parameters of cell viabili-

ty were not affected in cells incubated with DEM or phorone when compared with the controls. Leakage of LDH was: controls 1.46 ± 0.27; plus DEM 1.43 ± 0.13; plus phorone 2.07 ± 0.6 (values are I.U./ml of suspension medium, means ± S.D., n = 3). MDA accumulated in control samples was 173.8 ± 48.6; plus DEM 213.8 ± 62.4; and plus phorone 171.4 ± 41.7 (values are nmol/g, means ± S.D., n = 6). In all cases, more than 85% of the cells excluded trypan blue. ATP content was 1.8 ± 0.24 µmol/g (n = 5) in controls and was not significantly changed in cells incubated 30 min with DEM or phorone.

Interestingly, some enzymes involved in the gluconeogenic pathway contain thiol groups or disulphide bonds essential for their catalytic activity (12,13). Furthermore, we have observed that in freeze-clamped livers from GSH-depleted rats, the ratios of the concentrations of malate/phosphoenolpyruvate and glycerol-3-phosphate/dihydroxyacetone phosphate were higher than in controls, in agreement with the fact that the rate of gluconeogenesis decreases from lactate plus pyruvate or from glycerol but not from fructose (see Table). The possible role of GSH on hepatic gluconeogenesis is under current investigation in our laboratory.

Table. Rate of gluconeogenesis from different substrates in control and phorone- and DEM-treated hepatocytes

	Glucose formed (µmol x g^{-1} x min^{-1})		
Additions (mM)	Control	Phorone (0.5 mM)	DEM (1 mM)
Lactate (10) + pyruvate (1)	0.82±0.08 (6)	0.45±0.14§ (6)	0.56±0.07§ (6)
Lactate (10) + pyruvate (1) + oleate (1)	1.84±0.08 (4)	1.25±0.35* (4)	1.18±0.05§ (3)
Glycerol (10)	0.64±0.13 (8)	0.51±0.03* (5)	0.44±0.10* (9)
Fructose (10)	2.48±0.14 (8)	2.36±0.10 (5)	2.13±0.37 (4)

*$p<0.05$, §$p<0.005$. GSH concentrations (µmol/g) after 30 min incubation were 3.26±0.32 (10) for the controls; 1.11±0.64 (10) for phorone-treated cells; 0.26±0.25 (10) for DEM-treated cells. Results are Means ± S.D. with the number of observations in parentheses.

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