

Glutathione content of the small intestine: regulation and function

BY FRANK J. KELLY*

Department of Human Nutrition, University of Southampton, Southampton SO9 3TU

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In *ad lib.*-fed rats the epithelium of the small intestine, like the liver, contains large quantities of glutathione, 17.0 and 32.4 nmol/mg protein respectively. Following 24 h food restriction the glutathione content in both tissues fell 53 and 69% respectively. Unlike the liver, however, the glutathione content of the intestinal mucosa is not regulated to a diurnal rhythm, suggesting that the liver may provide glutathione or glutathione precursors to maintain intestinal glutathione levels. Intestinal epithelial cell preparations obtained from 24 h food-deprived rats had depleted glutathione stores (50%) and as a consequence were more susceptible to the oxidizing effects of cumene hydroperoxide. These results suggest that if glutathione plays a major role in the defence of the intestinal mucosa from ingested toxins then depletion of this defence during periods of food restriction could significantly increase the susceptibility of the individual to toxins present in the diet.

Glutathione: Intestinal mucosa: Liver: Dietary toxins: Oxidative stress: Rat

Glutathione, a tripeptide composed of γ -glutamic acid, cysteine and glycine, is the most important low-molecular-weight (non-protein) thiol in tissues (Kosower & Kosower, 1978; Meister, 1984). Glutathione plays an important role in detoxification reactions. It is a specific substrate for glutathione peroxidase (EC 1.11.1.9; Stadman, 1980) and glutathione S-transferases (EC 2.5.1.18; Kaplowitz, 1980) and participates in microsomal peroxidase and radical scavenging reactions (Reddy *et al.* 1981; Burk, 1983).

In comparison with a number of other tissues the liver has a particularly high content of glutathione (Kosower & Kosower, 1978). Liver glutathione plays a primary role in detoxification reactions. It conjugates with both endogenous and exogenous compounds to form products which can be subsequently eliminated from the body. Tissues other than liver are also involved in the defence of the body against potential toxins. For example, the gastrointestinal tract is exposed to a variety of xenobiotics, including food contaminants, drugs and, in some instances, peroxides (Ames, 1983; Golden & Ramdath, 1987). The majority of these insults normally go unnoticed as the intestine is protected against such contaminants by a variety of defences. These include mucus, the immune system and the glutathione-linked enzyme system (Glavind *et al.* 1971).

Following a short period of food deprivation hepatic glutathione levels fall appreciably (Leaf & Neuberger, 1947; Reister *et al.* 1959; Tateishi *et al.* 1977; Lauterburg *et al.* 1980; Jaeschke & Wendel, 1985). This response decreases the ability of the liver to withstand subsequent oxidative stress (Reed & Fariss, 1984). Glutathione status of the gastrointestinal tract is also sensitive to amino acid supply (Cho *et al.* 1981). It is not clear, however, if maintenance of intestinal glutathione status is dependent on amino acids obtained directly from the diet or indirectly from the liver via bile or blood.

In the present study the glutathione status of the intestinal mucosa has been examined

* Present address: Cardiovascular Research, The Rayne Institute, St Thomas's Hospital, London SE1 7EH.

and its sensitivity to amino acid availability and oxidative stress has been assessed: first, by determining if the glutathione content of the intestinal mucosa, like that of the liver, was regulated to the diet and, hence, followed a diurnal rhythm; second, by examining the total glutathione pool in liver, blood and intestinal mucosa in fed and food-restricted animals; third, by determining the ability of intestinal epithelial cell preparations to withstand oxidative stress following glutathione depletion.

METHODS

Animals. Male Wistar rats (120–135 g) obtained from the University of Southampton were randomly divided into four groups of six animals. All animals received food and water *ad lib.* and were killed at the following times over a 24 h period: group 1 10.00 hours, group 2 16.00 hours, group 3 22.00 hours, group 4 04.00 hours. In a second experiment a further sixteen male Wistar rats of similar body weight to Expt 1 were divided into four groups. All these animals were deprived of food but not water for 24 h before the beginning of the experiment. These animals were killed following the same schedule as described previously. In both experiments, following decapitation, a heparinized blood sample was collected, the peritoneum opened and the liver and small intestine removed. The liver was frozen in liquid N₂ and the jejunal mucosa was obtained as described previously (Kelly & Goldspink, 1982), the proximal 50% of the small intestine was removed, briefly flushed with cold saline (9 g NaCl/l), everted and the mucosa scraped off with a microscope slide before freezing.

Intestinal epithelial cell isolation. Intestinal mucosal scrapings were obtained from control or 24 h food-deprived animals as described previously. Cell suspensions were then obtained by incubating these mucosal preparations in Hanks medium in the presence of collagenase (*EC* 3.4.24.3; 1 g/l) and hyaluronidase (*EC* 3.2.1.35; 0.5 g/l) at 37° for 15 min (Grafstrom *et al.* 1979). The resulting cell suspension was diluted 3-fold with ice-cold Krebs–Hanseleit buffer, pH 7.8, supplemented with 5 mM-glucose and 30 IU heparin/ml, before filtering through gauze. All subsequent steps were performed at 4°. Isolated cells were recovered by centrifugation (800 g, 2 min). The supernatant fraction was removed by aspiration, the pellet quickly resuspended and the centrifugation step repeated. The final cell pellet was gently resuspended in Krebs medium to a concentration of approximately 2 × 10⁶ cells/ml. Cell visibility and concentration were assessed by the trypan blue exclusion test. Cell viability was usually greater than 90% and yield was approximately 40 × 10⁶ cells per preparation.

In vitro exposure to cumene hydroperoxide. Isolated cell preparations (2 × 10⁶/ml; final volume 2 ml; six animals per group) were incubated at 37° in a shaking water-bath with or without 10 μM-cumene hydroperoxide in the presence of O₂–CO₂ (95:5, v/v). Portions of cells were removed at 0, 30 and 60 min for the determination of cell viability and glutathione status. These experiments were performed in duplicate and the results averaged for each time-point.

Glutathione analysis. Whole blood (0.5 ml) was immediately mixed with 0.1 ml acetic acid (60 ml/l) and 0.4 ml sulphosalicylic acid (100 g/l) and centrifuged in a microfuge at 10000 g for 5 min. The supernatant fraction was retained and kept on ice for up to 1 h. Immediately before the assay a series of glutathione standards, 0.1–5.0 nmol/ml, were made with 40 g sulphosalicylic acid/l. For total glutathione analysis, i.e. reduced (GSH) and oxidized (GSSG), 0.2 ml of the sample supernatant fraction or the standard, in duplicate, were equilibrated with 0.1 ml 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.7 ml NADPH at 30° for 5 min. Glutathione reductase (0.9 U) was added to each sample, vortexed for 5 s, and the formation of nitrobenzoic acid monitored as an increase in absorbance at 412 nm over 1 min at 30°, pH 7.5, in an Ultraspec II spectrophotometer

(LKB, S. Croydon, Surrey). For the measurement of GSSG alone, the supernatant fractions were incubated with 0.01 ml 2-vinyl pyridine at room temperature for 1 h and then assayed as described for total glutathione. The GSH content of the samples was calculated by subtracting the GSSG content from the total glutathione content. Results were expressed as nmol/mg haemoglobin.

For analysis of tissue glutathione, separate samples (100 mg) of liver and intestinal mucosa were homogenized in 15 vol. perchloric acid (2 g/l) (Griffith, 1985). Following centrifugation the supernatant fractions were assayed for GSH and GSSG as described previously. Results were expressed as nmol/mg tissue protein.

Haemoglobin and protein assay. Haemoglobin concentration of lysates of whole blood (diluted 1:10000 in distilled water) was measured using the dithionite reduction method (Cross *et al.* 1978). Protein content of liver and intestinal mucosa samples was determined by the method of Smith *et al.* (1985).

Statistics. Results are expressed as mean values and standard deviations. Where appropriate, results were analysed using a three-way analysis of variance (ANOVA). Where significant interactions were indicated, statistical probability was determined using the two-tailed Student's *t* test. A probability value of less than 5% was taken as significant.

RESULTS

Liver glutathione content was consistently greater than intestinal glutathione content at all time-points considered (Fig. 1). This difference was maximal at 10.00 hours, when liver glutathione content was 1.5-fold greater than intestinal glutathione and 3.5-fold greater than blood glutathione content. Between 10.00 and 16.00 hours liver glutathione content fell 30% ($P < 0.05$). Thereafter, it gradually increased again reaching a maximum between 04.00 and 10.00 hours (Fig. 1). In contrast, the glutathione content of the intestinal mucosa did not exhibit a diurnal rhythm. A similar observation was made in blood which, although it had a lower concentration of glutathione than either the intestinal mucosa or liver, remained relatively constant over a 24 h period (Fig. 1).

As rats are active at night and sleep mostly during the day they consume the majority of their food during the night. It, therefore, appears that the hepatic diurnal glutathione rhythm is mainly a consequence of the supply of amino acid precursors. To test this hypothesis and examine the relationship between liver, blood and intestinal mucosa glutathione, a group of food-deprived animals was examined at similar times during a 24 h cycle. Following 24 h starvation hepatic glutathione concentration was 10 nmol/mg protein, a 65% reduction compared with fed controls. Liver glutathione concentration remained at this level in food-restricted animals over the next 18 h abolishing the normal diurnal rhythm. Food restriction (24 h) also decreased the glutathione concentration in blood (55%) and in the intestinal mucosa (53%). Extending the period of restriction for a further 18 h did not diminish either the blood or intestinal mucosa glutathione concentration (Fig. 1).

Close examination of the effects of food deprivation revealed that liver protein content was decreased 20%. In the remaining tissue, GSH concentration fell by 69% and GSSG by 73% (Table 1). As a result, even though the total glutathione content was substantially lowered, the proportion of glutathione present in the oxidized form remained unchanged. Food deprivation over 24 h also led to a fall (30%) in jejunal mucosa protein content. The remaining mucosa had 53% less GSH and 62% less GSSG (Table 1). Thus, as with liver, this period of food deprivation did not alter the GSH-GSSG balance. Blood GSH content fell (55%) in food-deprived animals (Table 1). This decrease was not associated with a change in the GSH-GSSG balance. No change in haemoglobin concentration was detected following 24 h food deprivation.

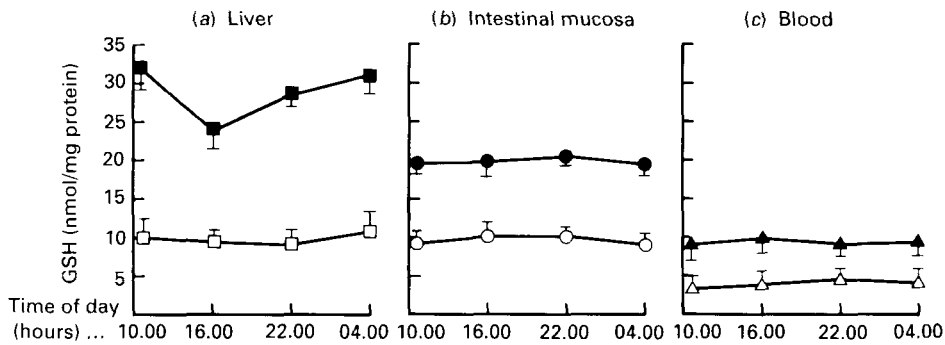


Fig. 1. Diurnal variation of liver, jejunal mucosa and blood glutathione (GSH) concentration in fed (■, ●, ▲) and 24 h fasted (□, ○, △) rats. Points are means and standard deviations represented by vertical bars for four to six animals. Tissue concentrations are expressed as nmol GSH/mg protein and blood concentration as nmol GSH/g haemoglobin. For details of procedures, see pp. 590–591.

Table 1. Effect of 24 h food deprivation on protein (mg) and glutathione status (nmol/mg protein) of the liver, intestinal mucosa and blood of rats†

(Mean values and standard deviations for four to six animals per group)

Treatment	Protein‡		Glutathione§			
			GSH		GSSG	
			Mean	SD	Mean	SD
Liver						
Control	598.4	21.0	32.41	2.7	1.26	0.12
Food-deprived	478.2*	31.4	10.04**	1.23	0.33**	0.11
	(–20%)		(–69%)		(–74%)	
Intestine						
Control	210.1	19.2	17.43	3.10	0.52	0.10
Food-deprived	147.2*	14.7	8.21**	1.11	0.20**	0.04
	(–30%)		(–53%)		(–62%)	
Blood						
Control	120.2	9.4	9.46	1.44	0.41	0.07
Food-deprived	131.0	8.6	4.30	0.58	0.15	0.03
	(+9%)		(–55%)		(–63%)	

Mean values were significantly different from control values: * $P < 0.05$, ** $P < 0.01$.

† For details of procedures, see pp. 590–591.

‡ Values for blood are expressed as mg haemoglobin/ml blood.

§ Values for blood are expressed as nmol/mg haemoglobin.

GSH, reduced glutathione; GSSG, oxidized glutathione.

The effect of intestinal GSH depletion was examined by determining the ability of intestinal epithelial cells to withstand oxidative stress. Intestinal epithelial cell preparations were prepared from control and 24 h food-deprived rats and incubated in the presence or absence of the organic hydroperoxide, cumene hydroperoxide. It should be noted that the initial glutathione concentration in these preparations, both control and food-restricted, was only approximately one-third of that observed in intact mucosal scrapings. It is assumed that marked losses of glutathione occur during the cell suspension preparative procedure.

Table 2. *Glutathione (GSH) status and viability of intestinal epithelial cell preparations from control and 24 h food-deprived rats following exposure to cumene hydroperoxide (10 μM)**

(Mean values and standard deviations for six rats per group)

Cumene hydroperoxide...	Incubation period (min)	GSH (nmol/mg protein)				Viability (%)			
		0		10 μM		0		10 μM	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fed	0	7.7 ^a	1.1	ND ^a		92 ^a	7	ND ^a	
	30	6.4	0.7	5.4 ^b	0.9	94	6	88	4
	60	5.2 ^{be}	0.9	3.6 ^{bf}	0.7	89 ^e	8	75 ^{bf}	5
Food-deprived	0	3.9 ^b	0.5	ND ^c		84	7	ND ^c	
	30	3.4 ^b	0.6	3.1 ^b	0.6	80 ^{be}	6	54 ^{bd}	6
	60	2.7 ^b	0.5	2.2 ^{bd}	0.5	76 ^{be}	5	42 ^{bd}	7

GSH content was related to presence of cumene hydroperoxide ($F_{12,24}$, $P < 0.002$, $df_{1,71}$), duration of exposure ($F_{60,2}$, $P < 0.001$, $df_{2,71}$) and nutritional status of donor animal ($F_{246,1}$, $P < 0.001$, $df_{1,71}$). Significant interactions between nutritional status and duration of exposure ($F_{8,54}$, $P < 0.001$, $df_{2,71}$) were noted. Cell viability was related to presence of cumene hydroperoxide ($F_{79,47}$, $P < 0.001$, $df_{1,71}$), duration of exposure ($F_{42,44}$, $P < 0.001$, $df_{2,71}$) and nutritional status of donor animal ($F_{147,53}$, $P < 0.001$, $df_{1,71}$). Significant interactions were noted between all variables.

^{a,b,c,d,e,f} Means with different superscript letters were significantly different ($P < 0.05$).

ND, not detected.

* For details of procedures, see pp. 590–591.

Using these cell preparations it was observed that, during a 60 min incubation, glutathione concentration in enterocytes isolated from control animals decreased 32% in the absence of peroxide and 47% in the presence of peroxide (Table 2). Glutathione concentration in enterocytes obtained from 24 h food-deprived animals, which initially was only 49% of control values, fell even further on incubation. In the absence of peroxide glutathione concentrations fell 65%, and in the presence of peroxide 71% compared with fed zero control preparations. Cell viability appeared to be closely linked to glutathione status. During a 60 min incubation period of control preparations in the absence of peroxide cell viability was unaltered. In the presence of peroxide, viability of similar preparations was reduced to a small extent. In contrast, viability of preparations from food-deprived animals fell markedly in the presence of peroxide (Table 2).

DISCUSSION

The night-orientated feeding behaviour of rodents was exploited to investigate the glutathione concentration in liver and intestinal mucosa in relation to fluctuations in amino acid availability. The results obtained confirm the existence of a diurnal glutathione rhythm in liver, supporting similar observations obtained by Beck *et al.* (1958), Brooks & Pong (1981) and Jaeschke & Wendel (1985). In this respect, liver glutathione is clearly sensitive to the progressive limitation of alimentary amino acid precursors during the day. The rate-limiting step for the increase of glutathione appears to be the intracellular supply of cysteine, the reduced form of cystine which is utilized for glutathione synthesis (Meister, 1983).

Substantial amounts of glutathione were found in the intestinal mucosa supporting the hypothesis that glutathione may play an important role in protecting the intestinal tract

and, hence, the rest of the body against potential toxins present in the diet. As in liver, the majority of glutathione was present as GSH, with less than 5% present as GSSG, consistent with the presence of an active glutathione reductase system. Unlike the liver, however, the intestinal mucosa did not exhibit a diurnal glutathione rhythm. Both intestinal mucosa and blood glutathione content remained relatively constant over the 24 h study period. The lack of a diurnal glutathione rhythm in intestinal mucosa strongly suggests that the glutathione status of the intestine is not subject to amino acid availability in the same manner as liver.

To test this finding further, the glutathione status of liver and intestinal mucosa was examined over a 24 h period following an initial period (24 h) of food restriction. The finding that 24 h food deprivation substantially decreased liver glutathione concentration and that further restriction eliminated the normal diurnal rhythm provided further support for the hypothesis that hepatic glutathione status is regulated by precursor supply. Following 24 h food deprivation the decrease in intestinal mucosal glutathione was, however, nearly as great as that seen in liver (53 and 69% respectively). This observation, in conjunction with the earlier finding that only the liver exhibits a diurnal rhythm, indicates that both tissues are sensitive to amino acid supply but suggests that regulation of GSH metabolism may differ between the liver and the intestine.

Adams *et al.* (1983) demonstrated that pharmacological depletion of hepatic glutathione was associated with a proportional decrease in blood glutathione. This finding and information from other studies have led to suggestions that the liver is a major source of circulating glutathione (Kaplowitz *et al.* 1985). The fall in blood glutathione following food deprivation (Table 1) can, therefore, be attributed to decreased export of glutathione from the liver and perhaps an increased clearance of glutathione from blood. Kaplowitz *et al.* (1985) suggested that the liver plays a central role in maintaining extra-hepatic tissue glutathione status. This could be achieved through the supply of glutathione precursors to these tissues (Lauterburg *et al.* 1984). Higashi *et al.* (1977) suggested the presence of two glutathione pools in liver; a cysteine reservoir utilized during periods of restricted food intake, and a more stable pool maintained as a source of liver glutathione.

At present, it is not clear to what extent the intestinal mucosa is dependent on a luminal supply, dietary- or bile acid-derived, or a plasma source of glutathione or glutathione precursors. The finding that intestinal mucosa glutathione levels fall in parallel with circulating and hepatic glutathione levels does not help differentiate between these sources. Decreased circulating glutathione, in accordance with the inter-organ homeostasis model proposed by Kaplowitz *et al.* (1985), will be of consequence to many extra-hepatic tissues. Indeed, similar findings to those reported here for the intestine have been reported for kidney, spleen, lung and muscle (Cho *et al.* 1981; Jaeschke & Wendel, 1985). The liver exports glutathione into both plasma and bile (Eberle *et al.* 1981; Deneke & Fanburg, 1989). The rate of appearance of glutathione in bile appears to be related to hepatic glutathione levels (Kaplowitz *et al.* 1983; Lauterburg *et al.* 1984). To date, the reason for this release of glutathione into bile is not known. However, as glutathione can be hydrolysed by γ -glutamyltransferase (EC 2.3.2.2) present in the intestine, this may represent a means for providing cysteine to the intestinal mucosa. This route may take on special significance during periods of food restriction. This statement is supported by kinetic studies on glutathione export in the isolated perfused liver which suggests that export into the bile can be maintained during periods of acute starvation (Inoue *et al.* 1983). This observation is partially supported by the present study in which the glutathione content of the intestinal mucosa did not fall to the same extent as liver glutathione following an acute period of food deprivation (Table 1).

A major observation of the present study is that intestinal mucosa glutathione content

fell markedly following a period of food restriction. The consequence of such a decrease in intestinal defences has potentially far-reaching effects. A fall in liver glutathione concentration has previously been shown to increase sensitivity to agents such as 1,1-dichloroethylene (Jaeger *et al.* 1973) and paracetamol (Schnell *et al.* 1983). Indeed, an inverse correlation between liver glutathione and the extent of paracetamol toxicity has been shown in the mouse (Schnell *et al.* 1983). In the present study it was found that enterocytes isolated from 24 h food-deprived animals had depleted GSH stores and as a consequence were more vulnerable to damage from oxidizing agents such as cumene hydroperoxide. These findings suggest that if the intestinal mucosa is dependent in part on glutathione for protection against toxins presented with food, then depletion of this defence during periods of food restriction could significantly increase the susceptibility of the individual to insult on refeeding. This is of particular relevance as it is in times of food shortage that spoiled foods are most likely to be consumed (Golden & Ramdath, 1987).

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