Glutathione replenishment capacity is lower in isolated perivenous than in periportal hepatocytes

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The zonal distribution of GSH metabolism was investigated by comparing hepatocytes obtained from the periportal (zone 1) or perivenous (zone 3) region by digitonin/collagenase perfusion. Freshly isolated periportal and perivenous cells had similar viability (dye exclusion, lactate dehydrogenase leakage and ATP content) and GSH content (2.4 and 2.7 $\mu\text{mol/g}$ respectively). During incubation, periportal cells slowly accumulated GSH (0.35 $\mu\text{mol/h per g}$), whereas in perivenous cells a decrease occurred ($-0.14 \mu\text{mol/h per g}$). Also, in the presence of either L-methionine or L-cysteine (0.5 mM) periportal hepatocytes accumulated GSH much faster (3.5 $\mu\text{mol/h per g}$) than did perivenous cells (1.9 $\mu\text{mol/h per g}$). These periportal–perivenous differences were also found in cells from fasted rats. Efflux of GSH was faster from perivenous cells than from periportal cells, but this difference only explained 10–20% of the periportal–perivenous difference in accumulation. Furthermore, periportal cells accumulated GSH to a plateau 26–40% higher than in perivenous cells. There was no significant difference in γ -glutamylcysteine synthetase or glutathione synthetase activity between the periportal and perivenous cell preparations. The periportal–perivenous difference in GSH accumulation was unaffected by inhibition of γ -glutamyl transpeptidase or by 5 mM-glutamate or -glutamine, but was slightly diminished by 2 mM-L-methionine. This suggests differences between periportal and perivenous cells in their metabolism and/or transport of (sulphur) amino acids. Our results suggest that a lower GSH replenishment capacity of the hepatocytes from the perivenous region may contribute to the greater vulnerability of this region to xenobiotic damage.

INTRODUCTION

There is mounting evidence that many metabolic processes are heterogeneously distributed within the liver acinus [1,2]. The metabolism of xenobiotics also appears to be zonally distributed [3,4]. The activation of numerous xenobiotics to potentially toxic metabolites predominates in the perivenous region of the acinus, owing to higher activities of the microsomal mixed-function oxidase enzymes as compared with the periportal region [4]. Histopathologically recognized regional differences in vulnerability to xenobiotic damage may, however, result from a local imbalance between the capacity of metabolic activation and subsequent detoxication of xenobiotics.

One major function of GSH is the protection of cells against attacks by reactive electrophiles and peroxidative agents [5,6]. The importance of maintaining a high intracellular content of GSH is demonstrated by the greater vulnerability of the GSH-depleted liver [7]. There is some [8,9], although not unequivocal [10,11], evidence that the content of GSH is lower in the perivenous region of the acinus. However, the rate of synthesis and turnover of GSH may in fact be more important than the steady-state amount of GSH in determining the cellular susceptibility to damage. In the present study we have isolated hepatocytes originating from the periportal or perivenous region by digitonin/collagenase perfusion [12,13] in order to study their GSH replenishment capacity. Our results suggest that cells from the peri-

venous region accumulate GSH more slowly, and to a lower final content, than do cells from the periportal region.

MATERIALS AND METHODS

Isolation of periportal and perivenous hepatocytes

Male rats of the Alko mixed strain weighing 200–330 g were provided with tap water and a standard R3 diet (Ewos Ab, Södertälje, Sweden). Hepatocytes from the periportal or the perivenous region were isolated under sodium pentobarbital anaesthesia (60 mg/kg, intraperitoneally) by using digitonin/collagenase perfusion [12]. This method is based on selective disruption of the hepatocytes of either the periportal zone or the perivenous zone by a brief infusion of digitonin via the portal vein or the hepatic veins, respectively, followed by conventional isolation with collagenase of the cells from the unaffected region. The following slight modifications were made to the original procedure. In the collagenase buffer [12] the concentrations of Hepes and NaCl were changed to 20 mM and 108 mM respectively. Hepatocytes from the digested liver were suspended in the incubation medium modified from Krebs/Henseleit buffer [14] by including 112.4 mM-NaCl, 2.5 mM- CaCl_2 , 2.5% (w/v) bovine serum albumin (fatty acid-free; from Boehringer, Mannheim, Germany) and normal plasma concentrations of physiological amino acids [15], except L-

Abbreviations used: GGT, γ -glutamyl transpeptidase; LOTC, (–)-2-oxo-4-thiazolidinecarboxylic acid.

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methionine and L-cyst(e)ine, and L-tyrosine, which was compensated by a double concentration of L-phenyl-alanine. After a 20 min equilibration in a rotatory shaker at ambient temperature, the cells were centrifuged twice at 25 $g_{\max}$ for 90 s and adjusted to a concentration of 30–45 mg wet wt./ml. The cell suspensions were kept continuously under O_2/CO_2 (19:1).

The cytosolic fraction was prepared by centrifugation of the sonicated hepatocytes at 20 000 g for 20 min and at 105 000 g for 70 min.

Incubation of hepatocytes

The hepatocytes were incubated at 37 °C in a shaking water bath in rubber-stoppered Erlenmeyer flasks. After 15 min of preincubation, 0.5 mM-L-methionine or L-cysteine (final concn.) was added. Cysteine was added every 30 min to ensure its presence in the reduced form [16,17]. The intracellular cysteine precursor (–)-2-oxo-4-thiazolidinecarboxylic acid (LOTC; Sigma, St. Louis, MO, U.S.A.) [5] was added to 1 mM final concn.

The specific γ -glutamyl transpeptidase (GGT) inhibitor Anthglutin [L- γ -glutamyl-(*o*-carboxy)phenylhydrazide] [18], kindly given to Y.K. by Dr. S. Minato (Bioscience Research Laboratories, Sankyo Co., Tokyo, Japan), was also added after the preincubation.

Samples from cell suspension, and from medium obtained after rapid centrifugation, were deproteinized with 1 vol. of ice-cold 1.23 M- $HClO_4$ /12 mM-EDTA. The intracellular content of GSH was calculated by subtracting the concentration in medium from that in the total suspension. With cells from fasted rats, GSH determined in the whole-cell suspension was taken to represent cellular GSH, since the contribution of extracellular GSH to total GSH is very small, owing to autoxidation of extracellular GSH. In some experiments, sampling for intracellular GSH and ATP was carried out by centrifugation of the cells through a layer of 1-bromodocane/dodecane mixture [19] as described elsewhere [20].

The rate of accumulation of intracellular GSH and of extracellular total glutathione (GSH+GSSG) was calculated from changes occurring during the first 60 min of incubation.

Analytical methods

Alanine aminotransferase, γ -glutamyl transpeptidase and glutamate dehydrogenase activities were assayed as previously cited [12]; lactate dehydrogenase was assayed as in ref. [21], and glutamine synthetase as in ref. [22]. GSH was determined fluorimetrically [23] with 2% (w/v) *o*-phthalaldehyde in methanol. Total glutathione was assayed as described in ref. [24], except that the final concentrations were (mM): NADPH 0.2, 5,5'-dithiobis-(2-nitrobenzoic acid) 0.1, EDTA 5, potassium phosphate 100 (pH 7.4), and 0.1 unit of glutathione reductase/ml. ATP was determined by the bioluminescence method with an ATP Monitoring Kit from LKB-Wallac, Turku, Finland.

γ -Glutamylcysteine synthetase activity was determined with L-glutamate and L- α -amino-n-butyric acid (from Sigma) [25], and glutathione synthetase activity with γ -glutamylalanine (Serva, Heidelberg, Germany) and glycine [26]. Both enzymes were determined in the cytosolic fraction, with substrates omitted in the blank reactions. P_i was measured as described in ref. [27], and protein by the fluorescamine method [28].

Statistics

The significances for the differences between periportal and perivenous hepatocytes were calculated by using Student's *t* test (two-tailed) for unpaired data.

RESULTS

Separation and characterization of periportal and perivenous hepatocytes

In the present study, the digitonin/collagenase perfusion technique [12,13] was routinely used for isolation of hepatocytes from either the periportal or the perivenous region of the liver acinus. Activities of the common acinar marker enzymes were determined; their activity ratios (periportal/perivenous) were calculated to be: alanine aminotransferase 1.8, LDH 1.4, GGT 2.3, glutamate dehydrogenase 0.86, and glutamine synthetase < 0.03. These ratios are in good agreement with previous results by us [12,29–31] and by others [1,13,32], and demonstrate that cell preparations highly enriched in hepatocytes from either the periportal or the perivenous region were obtained.

Both types of cells exhibited high viability as assessed by common criteria. Over 90% of the freshly isolated cells excluded eosin. During the incubation both types of cell preparations released lactate dehydrogenase into the medium at rates similar to those seen with conventionally isolated cells (Table 1). Also, the content of cellular ATP was comparable with that of conventionally isolated hepatocytes, and there was no difference between the two sets of cell preparations either at the start or at the end of the incubation (Table 1).

The content of GSH in freshly isolated hepatocytes was not significantly different in the periportal and the perivenous cell preparations, regardless of whether the cells were isolated from fed or fasted rats (Table 1).

Accumulation of cellular GSH

In the presence of plasma concentrations of physio-

Table 1. Characteristics of freshly isolated and of incubated periportal and perivenous hepatocytes

	Hepatocyte preparation	
	Periportal	Perivenous
Initial samples were taken after a 15 min preincubation of the freshly isolated hepatocytes at 37 °C. Samples drawn after 120 min of incubation were used for determination of the final cellular ATP and for the calculation of the rate of lactate dehydrogenase leakage. Means $\pm$ s.d. for the numbers of experiments in parentheses are given.		
Lactate dehydrogenase leakage (%/h)	3.8 $\pm$ 1.6 (16)	4.0 $\pm$ 2.0 (16)
Cellular ATP content (μ mol/g)		
Initial	2.6 $\pm$ 0.4 (13)	2.7 $\pm$ 0.4 (13)
Final	2.7 $\pm$ 0.5 (13)	2.8 $\pm$ 0.5 (13)
Initial cellular GSH content (μ mol/g)		
Fed rats	2.41 $\pm$ 0.48 (14)	2.68 $\pm$ 0.64 (15)
Fasted rats	1.87 $\pm$ 0.51 (4)	2.19 $\pm$ 0.04 (4)

Fig. 1. Replenishment of intracellular GSH and accumulation of extracellular total glutathione by periportal and perivenous hepatocytes

Freshly isolated cells were incubated (a) in the absence or (b) presence of 0.5 mM-L-methionine or (c) 0.5 mM-L-cysteine in a medium containing normal plasma concentrations of other physiological amino acids. L-Cysteine was added every 30 min. The cell concentration was 41.1 ± 6.4 mg/ml for periportal and 37.8 ± 4.0 mg/ml for perivenous hepatocytes ($n = 9$ for both). For intracellular GSH (O, ●) means $\pm$ S.D. are shown from six (control and methionine) or four (cysteine) experiments with periportal (O) and perivenous (●) cells. For extracellular total glutathione (Δ , $\blacktriangle$) means $\pm$ S.D. from six (control) or nine (methionine) experiments with periportal (Δ) and perivenous ($\blacktriangle$) cells are shown (except at 180 min, for only four experiments). * $P < 0.05$, ** $P < 0.02$ and *** $P < 0.01$ for the difference between periportal and perivenous cells.

logical amino acids except the GSH precursors L-methionine and L-cyst(e)ine, the periportal cells from fed rats formed GSH at a slow rate, but in the perivenous cells a slight decrease occurred (Fig. 1a). The efflux of GSH, estimated from accumulation of extracellular GSH+GSSG (Fig. 1a), was, on the other hand, faster from the perivenous than from the periportal hepatocytes (Table 2). Thus, whereas in suspensions of the periportal cells a net increment in the amount of GSH+GSSG occurred, in the perivenous-cell suspensions efflux of GSH from the cells roughly accounted for the net decrease in cellular GSH, and consequently there was no change in GSH+GSSG.

Hepatocytes from fasted animals accumulated GSH faster than did cells from fed animals, resulting in a net increase in GSH also in the perivenous cells (Table 2). However, the periportal-perivenous difference persisted, the periportal hepatocytes accumulating GSH at a rate about $0.5 \mu\text{mol/h per g}$ higher than that in the perivenous

cells regardless of whether the donors were fed or fasted (Table 2).

Supplementing the incubation medium with 0.5 mM-L-methionine or L-cysteine caused the cellular GSH to increase rapidly in both types of cells (Figs. 1b and 1c). The rates of GSH accumulation were comparable with those observed in isolated hepatocytes after partial depletion [16,33–36]. However, the periportal hepatocytes were observed to accumulate GSH at a rate $1.4\text{--}1.7 \mu\text{mol/h per g}$ faster than did perivenous cells (Table 2). Fasting further accelerated GSH formation by subsequently isolated hepatocytes, this effect being more pronounced in the perivenous cells. Consequently, although the periportal-perivenous difference persisted, the absolute difference was decreased to about $0.9 \mu\text{mol/h per g}$ (Table 2).

A maximal content of cellular GSH was reached after 120–180 min of incubation with methionine or cysteine (Figs. 1b and 1c). Cells from the periportal region were

Table 2. Rates of intracellular GSH increase and accumulation of extracellular total glutathione in periportal and perivenous hepatocytes

The cells were incubated as described in the legend to Fig. 1. The rates represent changes occurring during 60 min after addition of the precursor. Means $\pm$ s.d. are given, with the numbers of experiments in parentheses (N.D., not determined): * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ for the difference between periportal and perivenous hepatocytes.

Rats	GSH precursor	Rate of GSH increase in cells ($\mu\text{mol/h per g}$)		Rate of GSH + GSSG accumulation in medium ($\mu\text{mol of GSH equiv./h per g}$)	
		Periportal	Perivenous	Periportal	Perivenous
Fed	–	0.35 ± 0.18 (6)	$-0.14 \pm 0.10^{***}$ (6)	0.09 ± 0.03 (6)	$0.20 \pm 0.06^{**}$ (6)
	0.5 mM-L-Methionine	3.30 ± 0.32 (10)	$1.88 \pm 0.16^{***}$ (10)	0.16 ± 0.05 (9)	$0.30 \pm 0.12^{**}$ (9)
	0.5 mM-L-Cysteine	3.57 ± 0.33 (4)	$1.86 \pm 0.17^{***}$ (4)	N.D.	N.D.
Fasted	–	0.75 ± 0.26 (4)	$0.27 \pm 0.21^*$ (4)	0.11 ± 0.05 (4)	$0.24 \pm 0.06^*$ (4)
	0.5 mM-L-Methionine	4.13 ± 0.33 (4)	$3.20 \pm 0.36^{**}$ (4)	0.15 ± 0.06 (4)	$0.33 \pm 0.07^{**}$ (4)
	1.0 mM-LOTIC	1.53, 2.07 (2)	1.29, 1.33 (2)	N.D.	N.D.

capable of accumulating more GSH than were perivenous cells, and their maximal GSH content was 26–40 % (or 1.4–2.1 $\mu\text{mol/g}$) higher than in the perivenous hepatocytes.

Methionine and cysteine were equally efficient GSH precursors both in periportal and perivenous cells (Figs. 1b and 1c). In two pairs of experiments 1 mM-LOTIC was used as an alternative cysteine precursor, but was found to sustain GSH accumulation at only about 40 % of the rate observed with methionine (Table 2). As excess cysteine may be detrimental to cells [37], further experiments were carried out with methionine as the GSH precursor [16,17,33,38].

Glutathione efflux in the presence of methionine

In the presence of methionine, the rate of GSH + GSSG accumulation in the medium was about 50 % higher than without a GSH precursor (Table 2). The net rate of efflux from the perivenous hepatocytes was about twice that from the periportal cells, as found in the absence of GSH precursors (Table 2). The perivenous–periportal difference in the efflux rate (0.14 $\mu\text{mol/h per g}$) can, however, contribute only about 10 % to the corresponding difference in the rate of cellular GSH accumulation (1.42 $\mu\text{mol/h per g}$) (Table 2). During further incubation the rate of accumulation of GSH + GSSG in the medium from both types of cells increased to about 0.5 $\mu\text{mol of GSH/h per g}$ of cells, resulting in disappearance of the perivenous–periportal efflux difference (Fig. 1b). With cells from fasted rats, similar results were obtained irrespective of the GSH precursor supply.

As a consequence of the initial difference in the net efflux rate, the extracellular content of GSH + GSSG after 2 h was 0.39 $\mu\text{mol/g}$ higher in the perivenous than in the periportal cell suspensions when methionine was present (Fig. 1b). This was calculated to account for only 31 % of the corresponding difference in the cellular GSH content in those experiments.

Activities of the GSH-synthesizing enzymes

γ -Glutamylcysteine synthetase is generally considered to be the rate-limiting enzyme in GSH synthesis [6]. Therefore the cytosolic activities of this enzyme and of glutathione synthetase, the terminal enzyme in GSH synthesis, were determined in the periportal and the perivenous cell preparations. However, no significant periportal–perivenous difference was discerned with respect to either enzyme (Table 3).

Inhibition of γ -glutamyl transpeptidase

The plasma-membrane enzyme GGT catalyses the conjugation of the γ -glutamyl group of GSH with extracellular amino acids, with concomitant translocation of the reaction products across the plasma membrane [5]. It might thus facilitate the uptake of cysteine as such or as a constituent of GSH, or of other amino acids in the medium, to be used for GSH

Table 3. Activities of γ -glutamylcysteine synthetase and glutathione synthetase in isolated periportal and perivenous hepatocytes

The activities were determined in the cytosolic fraction by measuring the release of P_i . Means $\pm$ s.d. are given; N.S., not significant.

Hepatocytes ...	Enzyme activity (nmol of P_i /min per mg of cytosolic protein)		Periportal/ perivenous ratio
	Periportal (n = 8)	Perivenous (n = 6)	
γ -Glutamylcysteine synthetase	9.2 ± 0.9	8.8 ± 1.1	1.05 N.S.
Glutathione synthetase	1.2 ± 0.3	1.0 ± 0.3	1.20 N.S.

Table 4. Lack of effect of the γ -glutamyl transpeptidase inhibitor Anthglutin on the rate of GSH accumulation in isolated periportal and perivenous hepatocytes

The initial GSH content was $2.42 \pm 0.10 \mu\text{mol/g}$ in periportal and $2.66 \pm 0.49 \mu\text{mol/g}$ in perivenous hepatocytes (both $n = 4$). The cells were incubated in the presence of 0.6 mM-L-methionine, normal plasma concentrations of amino acids, and no or 1 mM-Anthglutin. Means $\pm$ S.D. are given; * $P < 0.02$, ** $P < 0.005$; N.S., not significant (paired t -test).

Addition...	Rate of GSH accumulation ($\mu\text{mol/h per g}$)	
	–	Anthglutin
Periportal	2.83 ± 0.07	2.86 ± 0.37 N.S.
Perivenous	1.71 ± 0.37	1.91 ± 0.33 N.S.
Periportal/perivenous ratio	1.65**	1.50*

synthesis. Since we have found the activity of GGT to be several-fold higher in isolated periportal hepatocytes than in perivenous cells [12], the possible contribution of this enzyme to the greater periportal GSH replenishment capacity was investigated. However, neither in periportal nor in perivenous cells did the presence of 1 mM-Anthglutin, a specific inhibitor of GGT, have any appreciable effect on GSH production (Table 4) or on the maximal amount of GSH (results not shown). This indicates a negligible role of GGT in the supply of GSH precursors, and supports a recent observation by Fernandez-Cleca *et al.* [39].

Availability of amino acids as GSH precursors

Apart from the feedback inhibition of γ -glutamyl-cysteine synthetase [25], synthesis of GSH is also dependent on the availability of cysteine [16,38]. To test whether other externally added amino acids are involved, the amino acid composition of the medium was varied. The accumulation of GSH was slightly decreased when the concentration of amino acids (except methionine) was decreased to one-tenth of the plasma concentration, but

this effect occurred in both periportal and perivenous cells (Table 5). On the other hand, increasing the concentration of methionine to 2 mM (over 40 times the plasma concentration) had no effect in periportal cells, but slightly increased both the rate and the final content of GSH in perivenous cells. However, this effect was too small to extinguish the periportal-perivenous difference. The rate of GSH efflux was not affected by the amino acid composition of the medium (Table 5).

Although the availability of glutamate usually is not considered as a rate-limiting factor in GSH synthesis, this possibility was also tested, in view of the distinct expression of the glutamate-utilizing enzyme glutamine synthetase in perivenous hepatocytes [40], as opposed to the periportal dominance of glutaminase [41]. Supplementation of the standard incubation medium with either 5 mM-L-glutamate or 5 mM-L-glutamine did not, however, influence the GSH replenishment or efflux in either type of cells, irrespective of the presence of 0.5 mM-methionine (results not shown).

DISCUSSION

This study shows that hepatocytes isolated from the perivenous region of the liver of either fed or fasted rats accumulate GSH not only more slowly, but also to a lower final concentration, than do cells from the periportal region. This suggests that there are constitutive differences between the cell types with respect to glutathione metabolism. As recently discussed by us [31], the periportal and perivenous preparations are taken to represent an average of the hepatocytes surrounding the afferent and efferent halves of the sinusoid, respectively, with the contamination from the opposite region being relatively small. Therefore, assuming linear sinusoidal gradients to exist for the functional parameters studied, differences between the most distal afferent and efferent hepatocytes may be much larger than those reported here.

Previous studies for establishing the local GSH content within the acinus, based mainly on histochemical methods, have produced conflicting results. Although some investigators have reported a lower content in the perivenous region [8,9,42], others have not observed any

Table 5. Effect of the concentration of methionine and the plasma amino acid mixture on GSH accumulation

L-Methionine (Met) was supplied to hepatocytes after 15 min preincubation in the presence of the sulphur-free mixture of amino acids (expressed as multiples of normal plasma concentration), as indicated in the Table. Means $\pm$ S.D. from three pairs of experiments, and the corresponding ratios between periportal and perivenous cell preparations (pp:pv), are shown. No statistically significant differences between the different incubation conditions were found; * $P < 0.05$, ** $P < 0.001$, *** $P < 0.005$ for the difference between the cell preparations.

Amino acids (times plasma concn.)	Met (mM)	Rate of intracellular accumulation ($\mu\text{mol/h per g}$)		Rate of efflux ($\mu\text{mol of GSH equiv. in}$ medium/h per g)		Maximal content at 120 min ($\mu\text{mol of GSH/g}$)	
		Periportal	Perivenous	Periportal	Perivenous	Periportal	Perivenous
0.1	0.5	2.60 ± 0.34	$1.94 \pm 0.19^*$	0.18 ± 0.04	$0.36 \pm 0.04^{**}$	5.84 ± 0.51	$4.67 \pm 0.42^*$
pp:pv			1.34		0.50		1.25
1	0.5	3.12 ± 0.35	$2.30 \pm 0.35^*$	0.21 ± 0.05	$0.42 \pm 0.03^{***}$	6.14 ± 0.42	5.02 ± 0.65
pp:pv			1.36		0.50		1.22
1	2.0	3.08 ± 0.19	2.67 ± 0.22	0.21 ± 0.10	0.36 ± 0.08	6.23 ± 0.60	5.31 ± 0.66
pp:pv			1.15		0.58		1.17

GSH gradient [10,11,43]. In the present study no significant difference between fresh periportal and perivenous cells was found. On the other hand, hepatocytes are known to lose their GSH partially during isolation [33,44]. Since the time required for preparation of hepatocytes by digitonin/collagenase perfusion is slightly longer, even greater losses may occur, so that the possible differences *in vivo* may have disappeared. The isolated periportal and perivenous hepatocytes are, however, metabolically equally as competent as conventionally isolated hepatocytes [13,29], and can therefore be used to study functional differences in glutathione metabolism.

The relative difference between the periportal and perivenous cell preparations in GSH accumulation was most conspicuous when cells were incubated in the absence of added GSH precursor. Whereas in periportal cells GSH was slowly formed, there was a gradual decrease of GSH in perivenous cells. Although prior fasting caused a net increase of GSH to occur also in the perivenous cells, the absolute periportal-perivenous difference in the GSH accumulation rate remained the same, i.e. about 0.5 $\mu\text{mol/h}$ per g. In the absence of an added GSH precursor, cysteine derived from protein degradation may be of importance. However, the rate of protein degradation, estimated as the rate of [^{14}C]valine release, is 3.1 and 2.9 %/h in periportal and perivenous hepatocytes respectively, in the presence of supra-physiological concentrations of plasma amino acids. This periportal-perivenous difference seems to be too small to give rise to a difference in the endogenous supply of cysteine, which could be responsible for the greater GSH production in the periportal cells.

The synthesis of GSH is considered to be controlled via feedback inhibition of γ -glutamylcysteine synthetase by GSH [6,25]. We were unable, however, to observe any significant difference between the periportal and perivenous cells in the activity of this enzyme or of glutathione synthetase. This indicates that the periportal-perivenous difference is unrelated to the acinar distribution of the GSH-synthesizing enzymes. Furthermore, since the periportal-perivenous differences persisted when cysteine was substituted for methionine as a GSH precursor, any difference in the cystathionine pathway [38] converting methionine into cysteine seems not to be involved.

The efflux of GSH has been shown to account substantially for the turnover of hepatic GSH [45,46]. We found that the perivenous hepatocytes released GSH about twice as fast as the periportal cells. However, this efflux difference can contribute only 10–20 % to the corresponding difference in the rate of GSH replenishment. Consequently, different efflux rates cannot explain the periportal-perivenous difference in cellular GSH accumulation.

Since neither the key enzyme activities of GSH synthesis nor the efflux or re-uptake of GSH seem to explain the periportal-perivenous difference in GSH replenishment, there may be unknown factors regulating the availability of precursors. Our results support previous notions [16,33,36,38] that availability of amino acids other than methionine or cysteine does not appreciably influence the capacity of the cells to produce GSH. On the other hand, increasing the concentration of methionine from 0.5 mM to 2.0 mM slightly increased both the accumulation rate and the maximal content of GSH in perivenous cells, but had no effect in periportal

cells. This implies that perivenous hepatocytes have a lower capacity to take up or utilize methionine. It appears that further characterization of the periportal and perivenous preparations with respect to the transport and metabolism of glutathione-related amino acids is needed.

Periportal cells were able to achieve a higher final intracellular content of GSH than were perivenous cells. At present it remains open whether this difference and the faster rate of GSH replenishment in periportal cells share common regulatory factors. Our result supports those previous histochemical observations [8,9] demonstrating a higher GSH content in the periportal region. We have recently shown that glutathione peroxidase activity is also higher in periportal hepatocytes [31]. It seems plausible that the periportal hepatocytes have adapted to the higher periportal oxygen partial pressure by more efficient protection mechanisms against spontaneous production of deleterious oxygen radicals. As the differences observed in the present study were obtained by incubating the periportal and perivenous hepatocytes under identical conditions, it must be emphasized that they may be further potentiated *in vivo* by sinusoidal gradients of oxygen, substrates and hormones [1,2].

Continuous availability of GSH is necessary for detoxication of electrophilic species via non-enzymic or glutathione *S*-transferase-mediated conjugation, and of peroxides via glutathione peroxidase [5,6]. Hepatocytes in the perivenous region contain relatively high activities of mixed-function oxidases, which catalyse the production of chemically activated species from various xenobiotics [4]. It has been shown that these cells have lower activity of glutathione peroxidase and of some other glutathione-coupled detoxifying enzymes (see [31]). Our present results indicate that the perivenous cells have also a lower capacity to replenish and accumulate GSH, which may make them less well equipped to cope with a challenge posed by peroxidative attack or by a GSH-conjugating agent [20]. It is therefore conceivable that under certain conditions the rate of production of reactive intermediates exceeds the capacity of perivenous hepatocytes for glutathione-mediated detoxication. The resulting imbalance may contribute to the greater vulnerability of the hepatic perivenous region to chemical damage.

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