The distribution, induction and isoenzyme profile of glutathione *S*-transferase and glutathione peroxidase in isolated rat liver parenchymal, Kupffer and endothelial cells

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The distribution and inducibility of cytosolic glutathione *S*-transferase (EC 2.5.1.18) and glutathione peroxidase (EC 1.11.1.19) activities in rat liver parenchymal, Kupffer and endothelial cells were studied. In untreated rats glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene and 4-hydroxynon-2-*trans*-enal as substrates was 1.7–2.2-fold higher in parenchymal cells than in Kupffer and endothelial cells, whereas total, selenium-dependent and non-selenium-dependent glutathione peroxidase activities were similar in all three cell types. Glutathione *S*-transferase isoenzymes in parenchymal and non-parenchymal cells isolated from untreated rats were separated by chromatofocusing in an f.p.l.c. system: all glutathione *S*-transferase isoenzymes observed in the sinusoidal lining cells were also detected in the parenchymal cells, whereas Kupffer and endothelial cells lacked several glutathione *S*-transferase isoenzymes present in parenchymal cells. At 5 days after administration of Aroclor 1254 glutathione *S*-transferase activity was only enhanced in parenchymal cells; furthermore, selenium-dependent glutathione peroxidase activity decreased in parenchymal and non-parenchymal cells. At 13 days after a single injection of Aroclor 1254 a strong induction of glutathione *S*-transferase had taken place in all three cell types, whereas selenium-dependent glutathione peroxidase activity remained unchanged (endothelial cells) or was depressed (parenchymal and Kupffer cells). Hence these results clearly establish that glutathione *S*-transferase and glutathione peroxidase are differentially regulated in rat liver parenchymal as well as non-parenchymal cells. The presence of glutathione peroxidase and several glutathione *S*-transferase isoenzymes capable of detoxifying a variety of compounds in Kupffer and endothelial cells might be crucial to protect the liver from damage by potentially hepatotoxic substances.

INTRODUCTION

The sinusoidal lining cells are the largest population of non-parenchymal cells in mammalian liver. They constitute about 30% of all liver cells, but they occupy only about 6% of total liver volume (Fabrikant, 1968; Greengard *et al.*, 1972). The sinusoidal lining cells include endothelial, Kupffer, fat-storing and pit cells. Kupffer and endothelial cells represent about 29% and 54% of sinusoidal cells respectively (Munthe-Kaas *et al.*, 1976; Blouin *et al.*, 1977; Blomhoff *et al.*, 1984). In previous studies (Steinberg *et al.*, 1987, 1988) it was shown that oxidative and post-oxidative drug-metabolizing enzymes are not restricted to parenchymal cells in rat liver: in Kupffer and endothelial cells the activities of oxidative enzymes (e.g. aminopyrine *N*-demethylase, ethoxyresorufin *O*-de-ethylase) are very low, whereas the post-oxidative enzyme activities (glutathione *S*-transferase, epoxide hydrolase) are relatively high.

The glutathione *S*-transferases comprise a group of isozymes that catalyse the conjugation of a wide variety of electrophilic compounds (endogenous substrates as well as xenobiotics) with glutathione (Jakoby *et al.*, 1976). Several glutathione *S*-transferase isozymes also possess the capacity to catalyse the reduction of organic

hydroperoxides but cannot reduce H₂O₂ (i.e. non-selenium-dependent glutathione peroxidase) (Mannervik & Jensson, 1982; Meyer *et al.*, 1984, 1985). In contrast, selenium-dependent glutathione peroxidase, a tetrameric selenium-containing enzyme, is able to reduce H₂O₂ to water (Flohé *et al.*, 1972) as well as organic hydroperoxides to the corresponding alcohols (Little *et al.*, 1970).

The aim of the present study was to examine the distribution and inducibility of glutathione *S*-transferase, non-selenium-dependent glutathione peroxidase and selenium-dependent glutathione peroxidase activities in liver parenchymal, Kupffer and endothelial cells. Therefore the above-mentioned enzyme activities were measured in the three cell types isolated from the livers of untreated rats or rats that had been treated 5 or 13 days previously with Aroclor 1254. Aroclor 1254 is a polychlorinated biphenyl mixture that not only exhibits both phenobarbital- and 3-methylcholanthrene-inducing properties of cytochrome *P*-450 (Alvares *et al.*, 1973) but also induces cytosolic glutathione *S*-transferase activity towards a wide range of substrates in the rat (Schramm *et al.*, 1985). In addition, a qualitative analysis of the individual glutathione *S*-transferase isoenzymes present in liver parenchymal and non-parenchymal cells from

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untreated rats was performed by chromatofocusing in an f.p.l.c. system and Western blotting.

MATERIALS AND METHODS

Materials

Pronase E and H_2O_2 were purchased from Merck (Darmstadt, Germany); collagenase was from Boehringer (Mannheim, Germany); Nycodenz was from Molter (Bammental, Germany); 1-chloro-2,4-dinitrobenzene, cumene hydroperoxide, GSH, SDS and *NN'*-bis-methyleneacrylamide were from Sigma (Deisenhofen, Germany); acrylamide was from Serva (Heidelberg, Germany); nitrocellulose sheets were from Schleicher und Schüll (Dassel, Germany); rabbit polyclonal antibodies against glutathione *S*-transferase subunits 1, 2, 3, 4 and 8 were from Bioprep (Dublin, Ireland); horseradish-peroxidase-conjugated anti-(rabbit immunoglobulin) antibodies were from Dakopats (Hamburg, Germany). Aroclor 1254 was generously given by Bayer A.G. (Leverkusen, Germany). 4-Hydroxynon-2-*trans*-enal was kindly provided by Professor H. Esterbauer (Graz, Austria). All other chemicals used were of the highest purity available. The f.p.l.c. equipment, Pharmalyte, Polybuffers 74 and 96, Sephadex G-25 (prepacked PD 10 columns) and Sephadex G-150 were purchased from Pharmacia (Uppsala, Sweden).

Animals and pretreatments

Male Sprague-Dawley rats (200–220 g body wt.) were purchased from Süddeutsche Versuchstierfarm (Tuttlingen, Germany), housed four per cage, and allowed free access to water and food. Aroclor 1254 in corn oil was administered as a single intraperitoneal dose (500 mg/kg body wt.) 5 or 13 days before the animals were killed. Control rats received appropriate volumes of corn oil.

Isolation of rat liver parenchymal, Kupffer and endothelial cells

Parenchymal and sinusoidal lining cells were isolated from different animals. The two sinusoidal lining cell types (Kupffer and endothelial cells) were isolated from the same livers.

Suspensions of total liver cells were prepared by a two-step collagenase perfusion method (Glatt *et al.*, 1981), and parenchymal cells were isolated from the suspension of total liver cells by differential centrifugation (Seglen, 1973). The preparation of non-parenchymal-cell suspensions and the purification of Kupffer and endothelial cells from these suspensions by centrifugal elutriation were performed as previously described (Steinberg *et al.*, 1987).

Eight isolations of Kupffer and endothelial cells from untreated and Aroclor 1254-pretreated rats were made; the cell fractions were frozen in liquid N_2 and stored at $-70^\circ C$ until used. For the enzyme assays the eight samples of Kupffer and endothelial cells from each group were pooled to give four samples ($n = 4$). All determinations were performed in cell homogenates obtained by sonicating the cells for 30 s at 60% duty cycle on a Branson cell disruptor (model B-15) with a microtip at a setting of 3.

Cell viability was assessed by the capacity of the cells to exclude 0.25% Trypan Blue. Cell counts were performed with a haemocytometer. Kupffer cells, endothelial cells and lymphocytes present in the elutriated cell

fractions were distinguished from each other by light-microscopy after they had been stained for peroxidase and non-specific esterase: in the rat peroxidase activity is only detected in Kupffer cells (Wisse, 1974), whereas, although esterase activity is found in all liver cells, lymphocytes do not exhibit this activity (Wisse & Knook, 1979). Fat-storing cells were identified by fluorescence microscopy (Blomhoff *et al.*, 1984).

Enzyme assays

In all assays activity was measured by product formation, which was linear with both time and protein concentration. Glutathione *S*-transferase activity was determined spectrophotometrically with 1-chloro-2,4-dinitrobenzene (Habig *et al.*, 1974) and 4-hydroxynon-2-*trans*-enal (Jensson *et al.*, 1986) as substrates. Glutathione peroxidase activity was measured by the coupled test procedure (Paglia & Valentine, 1967) with the reagents described by Wendel (1981): total glutathione peroxidase and selenium-dependent glutathione peroxidase activities were determined with cumene hydroperoxide and H_2O_2 respectively; the activity of non-selenium-dependent glutathione peroxidase was measured with cumene hydroperoxide as substrate after separation of this enzyme from selenium-dependent glutathione peroxidase by gel filtration (Lawrence & Burk, 1978). Proteins were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Glutathione *S*-transferase isoenzyme profile of the isolated rat liver cells

The glutathione *S*-transferase isoenzyme pattern of parenchymal and sinusoidal lining cells was determined in a pool of cells isolated from four untreated rats. Suspensions of parenchymal cells (40.71 mg of protein, 1.5 ml), Kupffer cells (11.58 mg of protein, 1.2 ml) and endothelial cells (6.73 mg of protein, 1.0 ml) in Gey's balanced salt solution (containing 140 mM-NaCl, 5 mM-KCl, 0.3 mM-MgSO₄, 1.1 mM-NaH₂PO₄, 1.5 mM-CaCl₂, 2.7 mM-NaHCO₃, 0.2 mM-KH₂PO₄, 1 mM-MgCl₂ and 5 mM-glucose, adjusted to pH 7.4) were homogenized by sonication. The volume of the cell homogenates was made up to 3 ml with Gey's balanced salt solution and centrifuged at 100 000 *g* for 60 min. The supernatants (the cytosolic fractions) were decanted over glass-wool; 1.3 ml portions of the cytosolic fraction were equilibrated with 75 mM-Tris/acetate buffer, pH 9.5, and 25 mM-Tris/HCl buffer, pH 7.5, with the use of Sephadex G-25 (prepacked PD 10 columns). The final volume of the desalted cytosolic fractions was 3.5 ml, 3.3 ml of which was filtered through a 0.22 μ m-pore-size Millipore filter and loaded on a Mono P HR 5/20 chromatofocusing column equilibrated with the corresponding Tris/acetate buffer, pH 9.5, or Tris/HCl buffer, pH 7.1. Elution was performed at a flow rate of 0.5 ml/min with 40 ml of 1:10-diluted Polybuffer 96 adjusted to pH 6.0 with 1 M-acetic acid or 1:10-diluted Polybuffer 74 adjusted to pH 4.0 with 1 M-iminodiacetic acid. Fractions (1 ml) were collected and glutathione *S*-transferase activity was measured in each of them with 1-chloro-2,4-dinitrobenzene as substrate (Habig *et al.*, 1974).

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed according to the procedure of Laemmli (1970) with the following modifications: the stacking gel was

5% (w/v), acrylamide and 0.1% (w/v) bisacrylamide in 0.058 M-Tris/phosphate buffer, pH 6.7, containing 0.1% (w/v) SDS, and the separating gel was 15% (w/v) acrylamide and 0.2% (w/v) bisacrylamide in 0.38 M-Tris/HCl buffer, pH 8.9, containing 0.1% (w/v) SDS. The transfer of proteins to nitrocellulose sheets was performed as described by Towbin *et al.* (1979). After blotting overnight, nitrocellulose sheets were blocked with 3% (w/v) bovine serum albumin and 0.5% (v/v) Tween 20 in Tris-buffered saline solution (10 mM-Tris/HCl buffer, pH 7.4, containing 0.9% NaCl) and incubated with the corresponding antiserum dilution in Tris-buffered saline solution containing 0.5% (w/v) elastin and 0.05% (v/v) Tween 20. The blots were washed with Tris-buffered saline solution, and the immunoreactive proteins were located with 4-chloronaphthol after incubation of the sheets with horseradish-peroxidase-conjugated anti-(rabbit immunoglobulin) antibodies.

Cross-reactivities of the antisera

Rat glutathione *S*-transferase can be grouped into families: subunits 1, 2 and 8 correspond to the Alpha class, and subunits 3 and 4 are aligned in the Mu class of glutathione *S*-transferases (Hayes & Mantle, 1986). The cross-reactivities of the antisera used in this study have been previously described (Hayes & Mantle, 1986). Briefly, there is no immunological cross-reactivity between classes, i.e. antiserum against subunit 1 does not cross-react with subunits 3 and 4, but shows very weak cross-reactivity with subunits 2 and 8. Furthermore, subunits 3 and 4 show strong immunological cross-reactivity, due to sequence homology (Ding *et al.*, 1986), but antisera against subunits 3 and 4 do not react with subunits 1, 2 and 8.

Statistics

Statistical analysis of the results was performed by using Dunnett's (1964) test for multiple comparisons with a control.

RESULTS

The yield, viability, purity and protein content of parenchymal and sinusoidal lining cells isolated from untreated rat livers are given in Table 1. The endothelial-cell preparations were found to contain $11 \pm 3\%$ lymphocytes and $3 \pm 2\%$ Kupffer cells. The Kupffer-cell preparations were contaminated with $9 \pm 3\%$ endothelial cells, $5 \pm 3\%$ fat-storing cells and $0.4 \pm 0.1\%$ parenchymal cells. These results show that the preparations of Kupffer

cells and endothelial cells were essentially free from parenchymal cells and therefore suitable for the biochemical analyses. Pretreatment of the animals with Aroclor 1254 did not affect the yield, viability and purity of all three isolated cell fractions; however, the mixed inducer increased the protein concentrations of parenchymal, Kupffer and endothelial cells by about 95, 40 and 30% respectively.

Glutathione *S*-transferase and glutathione peroxidase activities of parenchymal, Kupffer and endothelial cells isolated from untreated and induced rat livers are shown in Table 2. In the case of control animals glutathione *S*-transferase activity with the broad-spectrum substrate 1-chloro-2,4-dinitrobenzene was 1.7–2.7 times higher in parenchymal cells than in Kupffer and endothelial cells. Jensson *et al.* (1986) have shown that an acidic glutathione *S*-transferase (i.e. isoenzyme 8–8) conjugates 4-hydroxyalk-2-enals (toxic products of unsaturated fatty acid decomposition) with a high efficiency; parenchymal cells possessed a higher glutathione *S*-transferase activity with 4-hydroxynon-2-*trans*-enal as substrate than the two types of sinusoidal lining cells (1.7–2.2-fold differences). On the other hand, total, selenium-dependent and non-selenium-dependent glutathione peroxidase activities were similar in parenchymal, Kupffer and endothelial cells.

Schramm *et al.* (1985) have previously shown that during a 16-day time course following the administration of Aroclor 1254 a potent induction of glutathione *S*-transferase activity was seen; from day 8 to day 16 a significant decrease in glutathione peroxidase activity was observed. In order to test if this holds true for liver parenchymal as well as non-parenchymal cells, glutathione *S*-transferase and glutathione peroxidase activities were measured in parenchymal, Kupffer and endothelial cells isolated from rats treated with Aroclor 1254 5 or 13 days previously.

In parenchymal cells glutathione *S*-transferase activity was induced to a similar extent (about 4.2-fold) 5 or 13 days after the administration of Aroclor 1254 (Table 2). Although selenium-dependent glutathione peroxidase activity decreased by about 40% 13 days after the administration of Aroclor 1254, total glutathione peroxidase activity was not significantly altered: this was due to the strong induction of non-selenium-dependent glutathione peroxidase (2.6-fold).

In endothelial cells isolated from rats treated 5 days previously with Aroclor 1254 glutathione *S*-transferase activity was similar to that of untreated rats, and total, selenium-dependent and non-selenium-dependent glutathione peroxidase activities showed slight (although not

Table 1. Characteristics of liver parenchymal, endothelial and Kupffer cells isolated from untreated rats

Cells were isolated as described in the Materials and methods section. Values represent means $\pm$ S.D. for the numbers of rats given. Protein content is expressed as $\mu\text{g}/10^6$ cells present in each cell fraction.

Cell type	No. of rats	$10^{-6} \times$ Yield (cells/rat liver)	Viability (%)	Purity (%)	Protein content ($\mu\text{g}/10^6$ cells)
Parenchymal	4	342 ± 45	89 ± 4	92 ± 3	1760 ± 327
Endothelial	12	51 ± 6	94 ± 5	86 ± 5	76 ± 12
Kupffer	12	22 ± 3	95 ± 2	86 ± 3	129 ± 23

Table 2. Glutathione *S*-transferase and total, selenium-dependent and non-selenium-dependent glutathione peroxidase activities in liver parenchymal, endothelial and Kupffer cells from untreated and Aroclor 1254-pretreated rats

Enzyme assays were conducted in broken-cell preparations from untreated rats or rats pretreated 5 or 13 days previously with Aroclor 1254. The enzyme activities given for untreated rats correspond to liver cells isolated from rats treated 5 days previously with corn oil. Similar values are obtained if corn oil is administered 13 days before cell isolation. Values are expressed as means $\pm$ S.D. for four rats per treatment in the case of parenchymal cells, and eight samples of Kupffer and endothelial cells were split into four pairs ($n = 4$). Glutathione *S*-transferase activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) and 4-hydroxynon-2-*trans*-enal (4-HNE) as substrates. Total glutathione peroxidase and selenium-dependent glutathione peroxidase activities were determined with cumene hydroperoxide and H_2O_2 respectively. The activity of non-selenium-dependent glutathione peroxidase was measured with cumene hydroperoxide as substrate after separation of this enzyme from selenium-dependent glutathione peroxidase by gel filtration.

Enzyme activity	Cell type	Activity (nmol of product formed/min per mg of protein)		
		Untreated	Aroclor 1254-pretreated (5 days)	Aroclor 1254-pretreated (13 days)
Glutathione <i>S</i> -transferase (for CDNB)	Parenchymal	919 $\pm$ 238	3860 $\pm$ 1654*	3952 $\pm$ 1562*
	Endothelial	543 $\pm$ 167	588 $\pm$ 353†	1493 $\pm$ 620*†
	Kupffer	336 $\pm$ 125†	332 $\pm$ 74†	2044 $\pm$ 1176*
Glutathione <i>S</i> -transferase (for 4-HNE)	Parenchymal	286 $\pm$ 37	1472 $\pm$ 278*	1386 $\pm$ 419*
	Endothelial	165 $\pm$ 29†	193 $\pm$ 37†	597 $\pm$ 174*†
	Kupffer	125 $\pm$ 7†	144 $\pm$ 56†	671 $\pm$ 158*†
Total glutathione peroxidase	Parenchymal	393 $\pm$ 67	524 $\pm$ 164	475 $\pm$ 98
	Endothelial	285 $\pm$ 84	204 $\pm$ 45†	368 $\pm$ 83
	Kupffer	334 $\pm$ 74	223 $\pm$ 78†	203 $\pm$ 35*†
Selenium-dependent glutathione peroxidase	Parenchymal	206 $\pm$ 31	163 $\pm$ 43	125 $\pm$ 9*
	Endothelial	155 $\pm$ 48	116 $\pm$ 21	129 $\pm$ 16
	Kupffer	207 $\pm$ 66	179 $\pm$ 64	105 $\pm$ 19*
Non-selenium-dependent glutathione peroxidase	Parenchymal	195 $\pm$ 31	374 $\pm$ 122*	504 $\pm$ 130*
	Endothelial	154 $\pm$ 42	103 $\pm$ 34†	539 $\pm$ 208*
	Kupffer	139 $\pm$ 29	57 $\pm$ 15*†	272 $\pm$ 43*†

* Significantly different from the corresponding control value ($P < 0.05$, Dunnett's test).

† Significantly different from the corresponding parenchymal-cell activity ($P < 0.05$, Dunnett's test).

statistically significant) decreases (Table 2). At 13 days after the administration of Aroclor 1254 glutathione *S*-transferase and total glutathione peroxidase activities were induced 2.7-fold and 1.3-fold respectively; in the latter case the increase was due to the induction of non-selenium-dependent glutathione peroxidase.

In Kupffer cells glutathione *S*-transferase activity remained unchanged 5 days after the administration of Aroclor 1254, whereas total, selenium-dependent and non-selenium-dependent glutathione peroxidase activities were slightly decreased (Table 2). At 13 days after the administration of Aroclor 1254 selenium-dependent glutathione peroxidase activity decreased by about 45%; although glutathione *S*-transferase and non-selenium-dependent glutathione peroxidase activities were strongly enhanced, total glutathione peroxidase activity was decreased to about 65% of control values.

At 13 days after the administration of Aroclor 1254 glutathione *S*-transferase activity was induced in parenchymal, endothelial and Kupffer cells (Table 2). As shown by Western blotting, at that time point the expression of proteins immunochemically related to glutathione *S*-transferase subunits 1, 2, 3, 4 and 8 was strongly enhanced in all three cell types, when compared with controls (Fig. 1).

Glutathione *S*-transferase isoenzymes present in parenchymal, endothelial and Kupffer cells isolated from untreated rats were separated by chromatofocusing in an

f.p.l.c. system (Figs. 2a–2c). Four to six distinct peaks of glutathione *S*-transferase activity were observed in the pH gradient ranging from 9.50 to 7.20, and four to six neutral and acidic glutathione *S*-transferases with isoelectric points between 7.15 and 5.40 were detected. Individual glutathione *S*-transferase subunits in each peak fraction were identified by comparison of their isoelectric points and results of Western-blot analyses (with antibodies to glutathione *S*-transferase subunits 1, 2, 3, 4 and 8; Figs. 3a–3e) with data on cytosolic glutathione *S*-transferases from rat liver described previously in the literature. As summarized in Table 3, glutathione *S*-transferase isoenzymes with isoelectric points 8.10–8.15, 7.45–7.60, 6.45–6.55 and 5.40 were not observed in Kupffer cells, and glutathione *S*-transferase isoenzymes with isoelectric points 6.45–6.55 and 5.40 were absent from endothelial cells. Glutathione *S*-transferases with isoelectric points 7.00–7.15, 6.45–6.55 and 5.60–5.80 are acidic isoenzymes that do not correlate with any of the characterized forms.

DISCUSSION

It has been proposed that a major biological function of glutathione *S*-transferases is to protect cells against reactive electrophilic products of oxidative metabolism, such as epoxides, organic hydroperoxides and 4-hydroxyalk-2-enals (toxic aldehyde products of lipid peroxid-

Fig. 1. Immuno-blot analysis of the glutathione *S*-transferases in untreated and induced parenchymal, endothelial and Kupffer cells

Western blots of antibodies to glutathione *S*-transferase subunits 1 (a), 2 (b), 3 (c), 4 (d) and 8 (e) with endothelial cells (lanes 1), Kupffer cells (lanes 3) and parenchymal cells (lanes 5) from untreated rats and with endothelial cells (lanes 2), Kupffer cells (lanes 4) and parenchymal cells (lanes 6) from induced animals were performed as described in the Materials and methods section. Enzyme induction was achieved by administration of Aroclor 1254 13 days before the rats were killed. Each lane contains 60 μ g of total cell protein. Protein migration was from top to bottom.

ation) (Ålin *et al.*, 1985). The glutathione *S*-transferase isoenzyme with the highest specific activity towards 4-hydroxyalk-2-enals is glutathione *S*-transferase 8-8 (Jensson *et al.*, 1986). Furthermore, another acidic glutathione *S*-transferase isoenzyme with an isoelectric point of 5.6-5.8 has been isolated from rat liver cytosol and shown to possess a high specific activity towards 4-hydroxynon-2-*trans*-enal (I. Gath & H. Thomas, unpublished work). The two above-mentioned acidic glutathione *S*-transferase isoenzymes have now been detected and glutathione *S*-transferase activity towards 4-hydroxy- non-2-*trans*-enal and 1-chloro-2,4-dinitrobenzene has been measured in isolated rat liver parenchymal as well as Kupffer and endothelial cells. When a xenobiotic enters the hepatic circulation the first cells that it encounters are the sinusoidal lining cells; therefore the presence of several glutathione *S*-transferase isoenzymes capable of detoxifying a variety of compounds in Kupffer and endothelial cells might be crucial for

Fig. 2. Chromatofocusing of glutathione *S*-transferase isoenzymes from isolated rat liver cells

The glutathione *S*-transferase isoenzyme profile of parenchymal and sinusoidal lining cells was determined in a pool of cells isolated from four untreated rats. Separation of cytosolic glutathione *S*-transferases from rat liver parenchymal cells (a), endothelial cells (b) and Kupffer cells (c) in the pH ranges 9.5-6 and 7-4 (insets) by chromatofocusing in an f.p.l.c. system was performed as described in the Materials and methods section. Glutathione *S*-transferase activity (●) was measured with 1-chloro-2,4-dinitrobenzene as substrate; pH (▲) was monitored continuously.

protection of the liver from damage by potentially hepatotoxic substances.

The f.p.l.c. analyses revealed a certain cell-specific expression of glutathione *S*-transferase isoenzymes. All glutathione *S*-transferase isoenzymes observed in the sinusoidal lining cells were also detected in the parenchymal cells, but Kupffer and endothelial cells lacked

Fig. 3. Immuno-blot analyses of glutathione *S*-transferase isoenzymes present in liver parenchymal, Kupffer and endothelial cells isolated from untreated rats

The glutathione *S*-transferase isoenzymes present in parenchymal and non-parenchymal cells were separated by chromatofocusing in an f.p.l.c. system; portions of the peak fractions were subjected to SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose papers and incubated with antibodies to glutathione *S*-transferase subunits 1 (a), 2 (b), 3 (c), 4 (d) and 8 (e). Panels (a)–(d): lanes a–i correspond to peak fractions with isoelectric points of 9.00, 8.40, 8.15, 7.60, 7.30, 7.05, 7.00, 6.75 and 6.55 respectively from parenchymal cells; lanes j–n correspond to peak fractions with isoelectric points of 9.10, 8.55, 7.25, 7.00 and 6.70 respectively from Kupffer cells; lanes o–t correspond to peak fractions with isoelectric points of 9.10, 8.35, 7.45, 7.35, 7.15 and 6.70 respectively from endothelial cells. Panel (e): lanes a–d correspond to peak fractions with isoelectric points of 6.75, 6.55, 6.25 and 5.70 respectively from parenchymal cells; lanes e–h correspond to peak fractions with isoelectric points of 7.15, 6.70, 6.25 and 5.80 respectively from Kupffer cells; lanes i–l correspond to peak fractions with isoelectric points of 6.70, 6.45, 6.10 and 5.60 respectively from endothelial cells.

several of the glutathione *S*-transferase isoenzymes that were present in parenchymal cells.

Total glutathione peroxidase activity was similar in all three cell types. The selenium-dependent/non-selenium-dependent-glutathione peroxidase activity ratios ranged from 1.00 to 1.50. Similar activity ratios have previously been reported for rat liver cytosol (Lawrence & Burk, 1978; Schramm *et al.*, 1985). In the liver selenium-dependent glutathione peroxidase seems to be involved in the metabolism of H_2O_2 and in the prevention of lipid peroxidation (Flohé, 1982). The physiological role of non-selenium-dependent glutathione peroxidase is still uncertain. Under conditions of acute selenium deficiency, where selenium-dependent glutathione peroxidase activity is strongly depressed, non-selenium-dependent glutathione peroxidase activity is enhanced (Burk *et al.*, 1978; Lawrence *et al.*, 1978; Masukawa *et al.*, 1984;

Reiter & Wendel, 1984); under conditions of selenium sufficiency, however, the situation is less clear (Awasthi *et al.*, 1983).

It has previously been reported (Schramm *et al.*, 1985) that glutathione *S*-transferase and glutathione peroxidase activities are differentially regulated in the rat liver. At 5 to 13 days after a single injection of the commercial products Aroclor 1254 and fireMaster BP-6 a potent induction of glutathione *S*-transferase with a concomitant depression of selenium-dependent glutathione peroxidase activity was observed. In the present study it has been shown that 5 days after administration of Aroclor 1254 the induction of glutathione *S*-transferase was restricted to parenchymal cells in the rat liver. Furthermore, although selenium-dependent glutathione peroxidase activity decreased, total glutathione peroxidase activity was slightly enhanced. Thus the induction of

Table 3. Glutathione S-transferase subunits present in rat liver parenchymal, Kupffer and endothelial cells

Cytosolic glutathione S-transferases were separated by chromatofocusing in an f.p.l.c. system (Figs. 2a–2c). Individual glutathione S-transferase subunits in each peak fraction were identified by Western blotting (Figs. 3a–3e); it should be noted that the experimental procedures used in this study do not enable an identification of the glutathione S-transferase homo- and hetero-dimers present in each cell fraction. The glutathione S-transferases expected in each fraction are taken from data in the literature (Jakoby *et al.*, 1984; Mannervik, 1985; Jensson *et al.*, 1986).

Glutathione S-transferase activity maximum at isoelectric point	Glutathione S-transferase expected	Glutathione S-transferase subunit(s) detected		
		Parenchymal cells	Kupffer cells	Endothelial cells
9.00–9.10	1–1, 1–2, 2–2	1, 2	1, 2	1, 2
8.35–8.55	3–3	2, 3, 4	3, 4	3, 4
8.10–8.15	3–4	3, 4	—*	3, 4
7.80–7.90	4–6	N.D.†	N.D.†	N.D.†
7.45–7.60	3–?	1, 3, 4	—*	1, 2, 3, 4
7.25–7.35	5–5	N.R.‡	2	N.R.‡
7.00–7.15	N.I.§	N.R.‡	N.R.‡	N.R.‡
6.70–6.75	4–4	3, 4	N.R.‡	N.R.‡
6.45–6.55	N.I.§	4	—*	—*
6.10–6.25	8–8	8	8	8
5.60–5.80	N.I.§	8	N.R.‡	N.R.‡
5.40	6–6	N.R.‡	—*	—*

* No activity maximum at the isoelectric point mentioned.

† Not determined.

‡ No reaction with the five antisera tested.

§ Glutathione S-transferase isoenzyme(s) not described in the literature.

glutathione S-transferases bearing peroxidase activity could compensate, at least in part, for the loss of selenium-dependent glutathione peroxidase activity. However, one cannot speak of a total compensation, since non-selenium-dependent glutathione peroxidase does not utilize H_2O_2 as substrate (Lawrence & Burk, 1976) and its K_m values for organic hydroperoxides, such as cumene hydroperoxide and t-butyl hydroperoxide, are much higher than those of selenium-dependent glutathione peroxidase (Günzler *et al.*, 1972). In the case of Kupffer and endothelial cells, no induction of glutathione S-transferases had taken place 5 days after the administration of Aroclor 1254 and total glutathione peroxidase activities decreased to about 75% of control values. At 13 days after the administration of Aroclor 1254 a strong induction of glutathione S-transferases was observed in all three cell types, whereas selenium-dependent glutathione peroxidase activity remained unchanged (endothelial cells) or was depressed (parenchymal and Kupffer cells). However, total glutathione peroxidase activity in parenchymal and endothelial cells was enhanced, and this again might be ascribed to the increase of non-selenium-dependent glutathione peroxidase activity. This compensation was not observed in Kupffer cells. A depression of selenium-dependent glutathione peroxidase activity, particularly evident 13 days after the administration of Aroclor 1254, opens up the possibility that animals exposed to polyhalogenated biphenyls may be impaired in their ability to withstand lipid peroxidative processes or other oxidative stresses. The recent observation that selenium-dependent glutathione peroxidase activity was severely depressed following a lethal dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Stohs *et al.*, 1984) suggests that this biochemical response may be

generally involved in the toxicity of the polyhalogenated aromatic hydrocarbons.

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