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THE DISTRIBUTION OF UDP-GLUCURONOSYLTRANSFERASES IN RAT LIVER PARENCHYMAL AND NONPARENCHYMAL CELLS

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Abstract—Activities for the glucuronidation of 1-naphthol, morphine and bilirubin as well as for the sulfation of 2-naphthol have been determined in homogenates of parenchymal, Kupffer and endothelial cells isolated from livers of untreated and Aroclor 1254-pretreated rats. In addition, Western blot analyses using different polyclonal antibodies against UDP-glucuronosyltransferases (UDP-GTs) were performed with similar preparations. All enzymes under investigation were expressed at high levels in liver parenchymal cells. The constitutive expression and inducibility of UDP-GT isozyme(s) for 1-naphthol glucuronidation was also clearly demonstrated in Kupffer and endothelial cells. Furthermore, the presence of other UDP-GT isozymes was detected in preparations from these cells. No significant sulfation of 2-naphthol was detectable in Kupffer and endothelial cell homogenates. While the glucuronidation of 1-naphthol and morphine was significantly induced in all cell types by Aroclor 1254-pretreatment of the animals, the glucuronidation of bilirubin and the sulfation of 2-naphthol remained unchanged. Since the specific activity of conjugation reactions is much lower in liver nonparenchymal cells than in liver parenchymal cells, and nonparenchymal cells contribute only about 6% to the total liver protein, protection of the cells themselves rather than contribution to the overall metabolism of xenobiotics seems to be the significant role of these xenobiotic-metabolizing enzymes in the sinusoidal lining cells.

The UDP-glucuronosyltransferases (UDP-GTs§) (EC 2.4.1.17) and phenol sulfotransferases (EC 2.8.2.1) represent two families of isoenzymes that catalyse the conversion of primarily lipophilic compounds to water-soluble conjugates. Since the increase in water solubility facilitates the excretion of the compounds, these enzymes play a central role in the detoxication of exogenous and endogenous toxins. In a few cases, however, formation of reactive intermediates in the metabolism of carcinogens via sulfation has been reported [1–3].

In adult rat liver UDP-GT was reported to be exclusively localized in parenchymal cells [4]. However, Lafranconi *et al.* [5] were able to detect UDP-GT activity towards 1-naphthol in isolated rat nonparenchymal liver cells. Nonparenchymal cells, mainly Kupffer and endothelial cells that form the lining of the hepatic sinusoids, only constitute about 6% of the total cell mass but account for 30–35% of the total cell number in the liver [6].

The purpose of this study was to determine the distribution of selected UDP-GT and phenol sulfotransferase activities within liver parenchymal, Kupffer and endothelial cells isolated from untreated and Aroclor 1254-treated rats. Furthermore, a qualitative comparison of UDP-GT proteins present in the three cell types was performed by Western blotting.

MATERIALS AND METHODS

Chemicals. Digitonin, morphine hydrochloride, 1-naphthol and pronase E were purchased from Merck (Darmstadt, F.R.G.); 2-naphthol and collagenase were from Fluka (Buchs, Switzerland) and Boehringer Mannheim (Mannheim, F.R.G.), respectively. Aroclor 1254 was a gift from Bayer AG (Leverkusen, F.R.G.). Bilirubin, UDP-glucuronic acid, Brij 58, sodium dodecyl sulfate and 4-chloro-1-naphthol were obtained from the Sigma Chemical Co. (Deisenhofen, F.R.G.). Acrylamide, bis-methylene-acrylamide and *N,N,N′,N′*-tetraethylenediamine were from BioRad (Richmond, CA, U.S.A.). [1-^{3}H]Morphin and 1-[1-^{14}C]naphthol were purchased from Amersham Buchler (Braunschweig, F.R.G.). Adenosine 3′-phosphate 5′-phosphosulfate was synthesized and purified as described by Sekura [7]. Nycodenz and nitrocellulose sheets were from Molter (Bammental, F.R.G.) and Schleicher and Schuell (Dassel, F.R.G.), respectively. Anti-sheep IgG donkey serum as well as immunocomplexes consisting of horseradish peroxidase and anti-peroxidase IgG from sheep were gifts from the Scottish Antibody Production Unit (Glasgow, U.K.).

Animals and pretreatments. Male Sprague–Dawley

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§ Abbreviations: UDP-GT, UDP-glucuronosyltransferase; RAL, anti-rat liver UDP-GT antibody; RAK, anti-rat kidney UDP-GT antibody; IgG, immunoglobulin G.

rats (200–240 g body wt) were purchased from the Süddeutsche Versuchstierfarm (Tuttlingen, F.R.G.), housed four to a cage and allowed free access to both water and feed until used. Aroclor 1254 was dissolved in corn oil and administered as a single dose (500 mg/kg body wt, i.p.) 5 days before killing. Control animals received appropriate volumes of corn oil.

Isolation of rat liver parenchymal, Kupffer and endothelial cells. Parenchymal and nonparenchymal cells were isolated from different animals in order to obtain higher yields of the latter [8, 9]. The two nonparenchymal cell types were isolated from the same livers. Total liver cell suspensions were prepared using a collagenase perfusion method as described by Glatt *et al.* [10] and parenchymal cells were isolated from this preparation by differential centrifugation [11].

Nonparenchymal cell suspensions were prepared as described by de Leeuw *et al.* [8], Brouwer *et al.* [9] and Praaning van Dalen and Knook [12] by using pronase E, a mixture of proteolytic enzymes which destroys the parenchymal cells while leaving the nonparenchymal cells intact [13]. The separation of Kupffer and endothelial cells from the nonparenchymal cell suspension was performed by centrifugal elutriation [9].

From both untreated and Aroclor 1254-treated rats, 14 isolations of Kupffer and endothelial cells were performed. For the enzyme assays the preparations from three animals were pooled to yield four samples for each cell type and treatment. The remaining preparations (two individual samples for every cell type and treatment, respectively) were used for Western blotting. All determinations were performed in cell homogenates obtained by sonicating the cells for 30 sec at 60% duty cycle on a Branson cell disruptor (model B-15).

Characteristics of the isolated rat liver cells. Cell viability was estimated by assaying Trypan blue exclusion at a concentration of 0.25% and by the integrity of the cellular ultrastructure as observed by electron microscopy. The counting of the cells was performed with a hemocytometer. The distribution of Kupffer cells, endothelial cells and lymphocytes in the different fractions was determined by light microscopy after staining for peroxidase and esterase: in the rat liver, peroxidase is exclusively located in Kupffer cells [14] while lymphocytes, unlike liver cells, do not exhibit esterase activity [15]. Fat-storing cells were identified by fluorescence microscopy [16]. The percentages of lymphocytes, Kupffer, endothelial and fat-storing cells in the different fractions were confirmed by transmission electron microscopy as described previously [17].

Enzyme assays. Detergent-activated UDP-GT activity towards morphine, 1-naphthol and bilirubin was measured essentially as described by Del Villar *et al.* [18], Bock and White [19], and Heirwegh *et al.* [20], respectively. Phenol sulfotransferase activity towards 2-naphthol was determined at pH 5.5 to cover the isozymes III and IV (according to Sekura *et al.* [21]) as described previously [22]. The linear relationships between product formation and either incubation time or protein concentration were confirmed for each type of analysis using hepatocyte homogenates. The results were statistically evaluated using the procedure of Dunnett [23]. Protein content was determined by the method of Lowry *et al.* [24] using bovine serum albumin as standard.

Western blotting. SDS–PAGE was performed on 7.5% polyacrylamide gels as described by Laemmli [25]. Protein transfer from the gels to nitrocellulose sheets was carried out according to Towbin *et al.* [26]. The UDP-GT isoenzymes were then immunologically detected using the "sandwich" technique as described by Domin *et al.* [27]. Polyclonal antibodies against UDP-GT preparations from rat liver [28] and kidney [29] raised in sheep or goat were used as specific probes.

RESULTS

Characteristics of the isolated cells

The yield, viability, purity and protein content of parenchymal, Kupffer and endothelial cells isolated from untreated rats are shown in Table 1. On the basis of cell number the preparations of the parenchymal cells still contained 2 ± 1% endothelial cells, 2 ± 1% Kupffer cells and 6 ± 2% fat-storing cells. Nonparenchymal cell suspensions used for the separation of Kupffer and endothelial cells by centrifugal elutriation contained $103 \pm 24 \times 10^6$ cells/rat liver (53 ± 5% endothelial cells, 25 ± 3% Kupffer cells and 7 ± 1% fat-storing cells). In the intact liver, 25–40% of the sinusoidal lining cells are Kupffer cells and more than 50% are endothelial cells [30–32]; hence, the nonparenchymal cell suspensions reflected quite well the situation in the intact liver and were suitable for a further purification step of Kupffer and endothelial cells by centrifugal elutriation. The recovery of Kupffer and endothelial cells after centrifugal elutriation was 91 ± 3% and 87 ± 2%, respectively. The endothelial cell fractions included 11 ± 3% lymphocytes and 3 ± 1% Kupffer cells. They were essentially free of parenchymal cells. The Kupffer cell preparations were contaminated with 10 ± 3% endothelial cells, 4 ± 1% fat-storing cells and 0.2 ± 0.1% parenchymal cells.

Pretreatment of the animals with Aroclor 1254 did not alter the yield, viability and purity of the parenchymal, Kupffer and endothelial cell fractions. However, Aroclor 1254 increased the protein content of parenchymal, Kupffer and endothelial cells by about 85, 30 and 25%, respectively.

UDP-GT and phenol sulfotransferase activities in the different cell preparations

The activities for the glucuronidation of 1-naphthol, morphine and bilirubin as well as for the sulfation of 2-naphthol by the phenol sulfotransferase isoforms III and IV are shown in Fig. 1. While high activities for all the conjugation reactions were observed within the parenchymal cells prepared from the livers of untreated animals only the glucuronidation of 1-naphthol could be quantified in the corresponding Kupffer and endothelial cell preparations. Bilirubin glucuronidation was detectable in both nonparenchymal cell types but the sensitivity of the assay did not allow for valid quantification. The values given in Fig. 1 thus represent the closest estimate based on the data.

Table 1. Yield, viability, purity and protein content of liver parenchymal, Kupffer and endothelial cells isolated from untreated rats

Cell type	Number of rats	Yield ($\times 10^6$ cells/rat liver)	Viability (%)	Purity (%)	Protein content ($\mu g/10^6$ cells)
Parenchymal	4	310 ± 46	82 ± 5	90 ± 3	1369 ± 137
Endothelial	14	55 ± 13	94 ± 4	86 ± 4	74 ± 15
Kupffer	14	26 ± 8	95 ± 3	86 ± 6	115 ± 19

Values for yield, viability, purity and protein content are means ± SD.

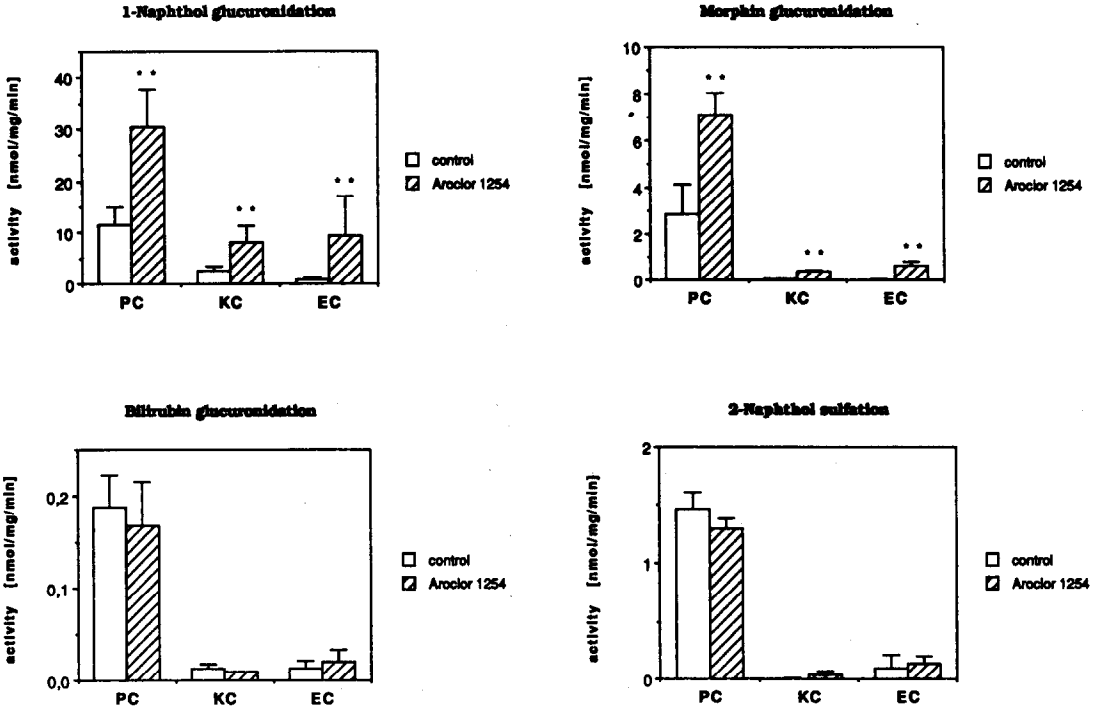

Fig. 1. Enzyme activities in the different liver cell types prepared from untreated or Aroclor 1254-pretreated rats. Values are expressed as means ± SD of four samples. In the case of nonparenchymal cells each sample contained the material from three rats. A double asterisk on top denotes statistically significant difference ($P > 0.99$) from the respective control as determined by Dunnett's test. PC, parenchymal cells; KC, Kupffer cells; EC, endothelial cells.

Morphine glucuronidation and 2-naphthol sulfation were at the border (sulfation in endothelial cells) or below the detection limit in the control preparations of these cell types. Administration of Aroclor 1254 to the animals resulted in a significant increase of 1-naphthol and morphine glucuronidation in the parenchymal cells and an even more pronounced increase in Kupffer and endothelial cells. Glucuronidation of bilirubin and sulfation of 2-naphthol were not significantly affected by the treatment in any of the cell types.

Western blot analysis of UDP-GT isozymes

The UDP-GT isozyme patterns of the different cell preparations were investigated by immunoblotting using two polyclonal antibody preparations of different specificity. RAL binds to a broad spectrum of isozymes including 4-nitrophenol UDP-GT, bilirubin UDP-GT, 3α-hydroxysteroid UDP-GT, and 17β-hydroxysteroid UDP-GT [28], while RAK only binds to 4-nitrophenol UDP-GT and bilirubin UDP-GT [29]. Figure 2 displays the result of the immunoblot analysis of the different cell types using RAK. To yield comparable immunosignal intensity a 10 times higher amount of the nonparenchymal cell protein as compared to the parenchymal cell protein was applied in the analysis. No signals for UDP-GTs were visible in any of the control preparations. In the case of Aroclor 1254 induction, the parenchymal cells displayed a double band on the blot of about 53–54 kDa while the nonparenchymal cells showed triple bands at the

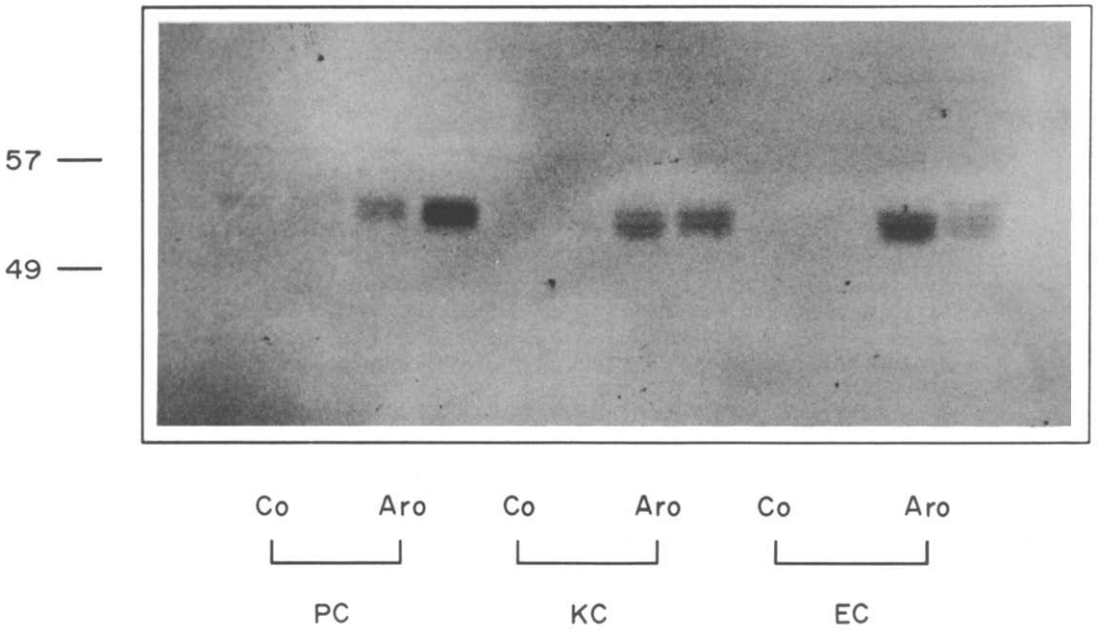

Fig. 2. Western blot analysis of the protein from different liver cell types isolated from untreated or Aroclor 1254-pretreated rats using RAK for the immunochemical detection. Separation of proteins was performed on a 7.5% polyacrylamide gel. The load was 20 μg of protein for parenchymal cell homogenates and 200 μg of protein for nonparenchymal cell homogenates. Two individual samples per cell type and treatment were subjected to the analysis. Immunochemical detection was carried out using a polyclonal antibody raised against purified rat kidney UDP-GT. Co, control; Aro, Aroclor 1254-pretreatment; PC, parenchymal cells; KC, Kupffer cells; EC, endothelial cells.

same position. In accordance with the enzyme activity data, the double band refers to 4-nitrophenol UDP-GT and probably another UDP-GT isozyme, with similar properties with respect to antigenicity and inducibility, that was first described by Bock and Lilienblum [33]. Recent studies of Bock *et al.* [34, 35] indicate that the latter species might be a differently processed (not glycosylated) form of the 4-nitrophenol UDP-GT. Up to the present time, the third band appearing in the nonparenchymal cell preparations has not been identified. Figure 3 shows the immunoblot of the different preparations using RAL for the detection of UDP-GT isozymes. Cells isolated from untreated animals show distinct isozyme patterns. The parenchymal cell preparations exhibit a dominant band with an apparent molecular weight of 50 kDa representing 17β-hydroxysteroid UDP-GT and a much weaker band at 52 kDa comprising 3α-hydroxysteroid UDP-GT. In contrast to the situation in the parenchymal cells, the signal intensity for those isozymes is similar within preparations from nonparenchymal cells. While the endothelial cells display stronger signals for the steroid UDP-GTs the Kupffer cells show two additional, faint bands at 53 and 54 kDa representing the above mentioned phenol UDP-GTs. After induction with Aroclor 1254 all cell types show similar patterns with increased signals for the steroid UDP-GTs and strong bands comprising the phenol UDP-GTs.

DISCUSSION

In a study using immunohistochemical methods, Roy Chowdhury *et al.* [4] have found UDP-GTs in rat liver to be localized exclusively in parenchymal cells [4]. However, in this paper we report the detection of UDP-GT activities as well as the presence of anti-UDP-GT IgG immunoreactive protein in Kupffer and endothelial cell preparations from the livers of untreated and Aroclor 1254-treated rats. To draw valid conclusions from these results it is important to make an estimate of the contribution of parenchymal cell protein to UDP-GTs in the nonparenchymal cell preparations. While the endothelial cell preparations were essentially free from contamination with parenchymal cells the Kupffer cell preparations contained up to 0.3% parenchymal cells, on the basis of cell number. Referred to the protein content this corresponds to a maximum contamination of 3.5%, which is not sufficient to explain the observed presence of UDP-GTs in the nonparenchymal cell preparations. However, since the parenchymal cells were destroyed by pronase E during the isolation of Kupffer and endothelial cells, it is not impossible that the nonparenchymal cells have simply taken up parenchymal cell debris. In order to inhibit phagocytosis Kupffer and endothelial cells have been isolated at 4° [12]. Even under the most pessimistic assumption that the morphine glucuronidation in Kupffer and endothelial cell stems completely from parenchymal cell constituent contamination, the presence of 1-naphthol glucuronidating form(s) of UDP-GT remains significant. Furthermore, the distinct UDP-GT isozyme pattern of the different cell types isolated from untreated rats as displayed on the

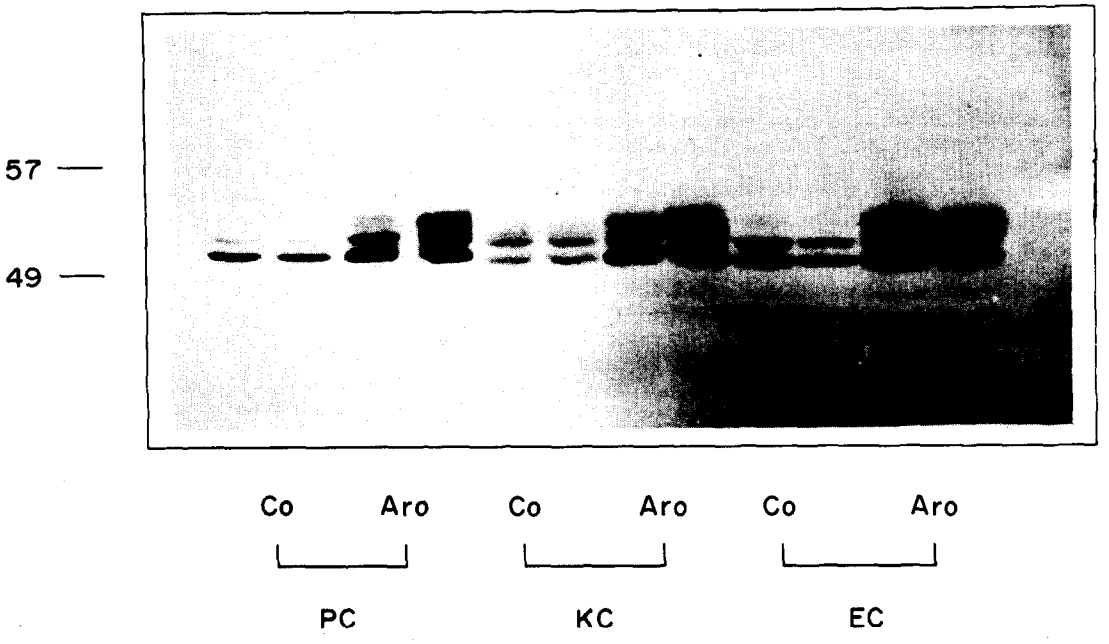

Fig. 3. Western blot analysis of the protein from different liver cell types isolated from untreated or Aroclor 1254-pretreated rats using RAL for the immunochemical detection. Separation of proteins was performed on a 7.5% polyacrylamide gel. The load was 20 μg of protein for parenchymal cell homogenates and 200 μg of protein for nonparenchymal cell homogenates. Two individual samples per cell type and treatment were subjected to the analysis. Immunochemical detection was carried out using a polyclonal antibody raised against purified rat liver UDP-GT. Co, control; Aro, Aroclor 1254-pretreatment; PC, parenchymal cells; KC, Kupffer cells; EC, endothelial cells.

immunoblot using RAL for detection (Fig. 3) cannot be explained by simple cross-contamination. We thus conclude from our data that different UDP-GT isozymes capable of glucuronidating phenols and steroids are present not only in parenchymal cells but also in Kupffer as well as endothelial cells from rat liver and may be efficiently induced by Aroclor 1254.

Of the preparations from untreated rats only Kupffer cells display an immunosignal that may be assigned to 4-nitrophenol glucuronosyltransferase(s) (Fig. 3), reflecting the low expression of this specific UDP-GT in livers of untreated rats [34]. The 1-naphthol glucuronidation observed in parenchymal and endothelial cells from untreated rats is therefore most likely carried out by the 17β-hydroxysteroid UDP-GT according to the findings of Falany and Tephly [36].

The activity of the phenol sulfotransferases III and IV, as measured by 2-naphthol sulfation at pH 5.5 [21], was just at the border of the detection limit in the endothelial cells and could not be detected in Kupffer cells. Only parenchymal cells displayed significant 2-naphthol sulfation which was not influenced by Aroclor 1254 administration. Those phenol sulfotransferase isozymes are of toxicological significance since it has been reported that phenol sulfotransferase IV is capable of sulfating *N*-hydroxy arylamines [37] thereby generating reactive species [38–40]. Their absence in non-parenchymal cells should render these cell types more resistant to the toxic effects of aromatic amines.

Since the investigated sinusoidal lining cells make up the first tissue within the liver to be exposed to all the xenobiotics passing through this organ these cells need mechanisms to protect themselves from the toxic effects of these substances. We have reported earlier the presence of cytochrome P450-dependent monooxygenase, epoxide hydrolase and glutathione-*S*-transferase activities within these nonparenchymal cells [41, 42]. The existence of phenol conjugating form(s) of UDP-GT and the presence of other isozymes of this enzyme family in these cell types, as shown in this study, completes the pattern of detoxifying enzymes expressed in these cells. Compared to the liver parenchymal cells the contribution of the nonparenchymal cells to the overall metabolism of xenobiotics in the body seems negligible since the parenchymal cells provide about 90% of the total liver protein and possess much higher specific activities for the detoxication reactions. The importance of detoxifying enzymes within the sinusoidal lining cells may therefore lie in defending the cells themselves from toxic substances during the passage of these compounds from the blood to the liver parenchymal cells.

Note added in proof. While this manuscript was in consideration Schrenk *et al.* [43] published observations on drug-metabolizing enzymes in hepatocytes, bile duct cells and cultured rat liver epithelial cells. In contrast to our present and previous [42] findings in the sinusoidal lining cells, both the bile duct and cultured rat liver epithelial cells showed no detectable amount of cytochrome P450

1A1 while many of the activities determined for conjugating enzymes (UDP-GT, glutathione-*S*-transferase, sulfotransferase) were equal to or higher than those observed in isolated hepatocytes but lacked inducibility by benz[*a*]anthracene. This metabolic phenotype has been discussed as supporting toxin resistance. It thus appears that at least three different patterns of metabolic competence for foreign compounds are existent in individual liver cell types.

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