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DIFFERENT DRUG METABOLIZING CAPACITIES IN CULTURED PERIPORTAL AND PERICENTRAL HEPATOCYTES

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Abstract—Isolated and cultured periportal (PP) and pericentral (PC) hepatocytes were used for studying the acinar distribution of several phase I and phase II drug metabolizing reactions and their induction by phenobarbital (PB) and 3-methylcholanthrene (MC) or a combination thereof. Ethoxycoumarin-*o*-deethylase (EC 1.14.14.1) (ECOD) activity was found to predominate in PC hepatocytes even after induction with MC and PB. Metabolism of biphenyl to a monosulfated product also predominated in PC hepatocytes as did the conversion of harmine to harmine glucuronide and sulfate. In contrast, metabolism of lonazolac was not zonated. The metabolism of all three substrates declined during cultivation for 24 hr and was differentially induced (biphenyl and harmine) or not affected (lonazolac) by MC or PB. Metabolic heterogeneity was best maintained by the combination of MC and PB indicating that the zonal differences are due to a specific balance of phase I and phase II reactions. Glutathione-*S*-transferase (GST) activities against 1-chloro-2, 4-dinitrobenzene (CDNB) were higher in PC hepatocytes and remained so after induction with PB. In contrast, GST activities against 1,2-dichloro-4-nitrobenzene (DCNB) were almost twice as high in PP cells but equilibrated due to a spontaneous increase during cultivation, particularly in PC hepatocytes. In the presence of PB or both, MC and PB, induction of the activity against DCNB occurred exclusively in the PP hepatocytes. The distribution of the GST subunits Ya and Yb_1 roughly corresponded to the pattern of GST activities against CDNB and DCNB, respectively. These results agree with earlier reports demonstrating that PP and PC hepatocytes show different patterns of phase I and phase II drug metabolizing enzymes which are maintained during short-term cultivation. *In vitro* induction of these activities does not result in equilibration but rather maintenance or even pronounciation of these zonal differences.

Key words: drug metabolism; glutathione-*S*-transferase; hepatocytes; 3-methylcholanthrene; phenobarbital; zonal differences

Liver parenchyma shows a remarkable heterogeneity of the hepatocytes along the porto-central axis with respect to enzyme content and metabolic activity [1, 2]. The different capacities of phase I and phase II drug metabolizing enzymes in PP† and PC hepatocytes [2–4] are responsible for a different balance between activating and conjugating reactions for each individual drug or xenobiotic and thus may contribute to the different hepatotoxic potential of such compounds in different zones of the liver acinus [2].

Hepatocytes isolated from the PP and PC region by the digitonin–collagenase technique [5, 6] have successfully been used for determining zonal differences in amino acid transport [7], intermediary metabolism [8–10] as well as some drug metabolizing enzymes [11–13]. In the present study we report on different capacities of phase I and phase II metabolism of xenobiotics in PC and PP hepatocytes.

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† Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; DMSO, dimethylsulfoxide; ECOD, ethoxycoumarin-*o*-deethylase (EC 1.14.14.1); GST, glutathione-*S*-transferase; HEPES, 4-(2-hydroxyethyl)-1-piperazinethan-sulfonic acid; MC, 3-methylcholanthrene; PB, phenobarbital; PC, pericentral; PP, periportal.

MATERIALS AND METHODS

Materials. Collagenase was purchased from Boehringer (Mannheim, Germany), digitonin was from Serva (Heidelberg, Germany) and Williams Medium E was from Flow Laboratories (Meckenheim, Germany). Antibodies against GST-Ya and GST-Yb_1 were purchased from Biotrin International (Dublin, Ireland). Biphenyl, harmine, CDNB and DCNB were obtained from Sigma (Munich, Germany). Lonazolac was a kind gift from Byk Gulden (Konstanz, Germany).

Isolation and cultivation of hepatocytes. Livers from adult male Sprague–Dawley rats (fed *ad lib.*) with body weights between 220 and 290 g were used. Hepatocytes from whole livers were isolated as described [14]. PP and PC hepatocyte subpopulations were isolated by the digitonin–collagenase perfusion technique [5, 6] modified according to Burger *et al.* [7]. Hepatocytes were suspended in Williams medium E containing 2 mM glutamine, penicillin (5 U/mL) and streptomycin (50 μg/mL) and seeded on collagen-coated [15] Petri dishes (35 mm) or six-well plates (Greiner, Nürtingen) at a cell density of 1.25×10^5 cells/cm^2. For some experiments uncoated dishes

were used [14] as indicated in the respective tables. Newborn calf serum (5%) was present only during the first 2 hr of cultivation. The culture medium was changed after 2 hr, and subsequently every 24 hr. Dexamethasone (10^{-7} M) was present throughout cultivation. In experiments designed to evaluate the influence of several inducers, culture media were supplemented with 1 μM MC (dissolved in DMSO, final concentration 1%), 2 mM PB or their combination after 2 hr as described [14]. No effect of 1% DMSO on the functions and reactions studied herein was found.

Metabolisms of biphenyl, harmine and lonazolac. At 2 and 24 hr after seeding cells were incubated with the model substrates biphenyl (0.1 mM) and harmine (0.1 mM) in bicarbonate-free Hanks buffer containing 10 mM HEPES and 5 mM glucose in a humidified incubator at 37°C for 30 min. Incubations with lonazolac (0.01 mM) were performed in a modified Krebs–Henseleit buffer containing bicarbonate [16] in a humidified atmosphere containing 5% CO_2 at 37° for 30 min. Afterwards the cells were harvested in the respective incubation buffer and homogenized with a Branson sonifier [17]. Aliquots of 200 μL were mixed with 150 μL acetonitrile, centrifuged and the supernatants analysed by HPLC. Separation of the metabolites of these compounds was carried out by reversed phase HPLC on a Sherisorb ODS 2 5 μm column. For biphenyl and harmine, a linear gradient from 20 to 100% acteonitrile in 2 mM sodium phosphate buffer over 23 min with a flow rate of 1.0 mL/min was used. Lonazolac metabolites were separated using a linear gradient from 10 to 100% acetonitrile in 10 mM sodium phosphate buffer over 23 min with a flow rate of 1.4 mL/min. Eluting peaks were detected by their fluorescence at excitation wavelengths of 275, 315 and 275 nm and emission wavelengths of 300, 420 and 350 nm for biphenyl, harmine and lonazolac, respectively.

Enzyme assays. For determination of ECOD (EC 1.14.14.1) activity the cells were scraped into a Tris buffer (100 mM, pH 7.4) and stored at −20°. ECOD activity was determined according to Aitio [18]. For determination of GST, activity cultures were scraped into potassium phosphate buffer (100 mM, pH 7.6) and measured using CDNB or DCNB as substrates as described [14]. The activities were related to cellular protein determined according to Lowry *et al.* [19].

Quantification of GST subunits. GST subunits Ya and Yb_1 were quantified in the 10,000 *g* supernatant of cell homogenates by a sensitive ELISA technique described elsewhere [14]. The primary antisera were diluted 1:5000 in phosphate-buffered saline. The amount of GST present was usually calculated from the 300 ng values using standard curves. Statistical evaluation was performed using Student's *t*-test.

RESULTS

ECOD

ECOD activity was found to be more than twice as high in PC hepatocytes as in PP hepatocytes (Fig. 1). Activities of total hepatocytes (0.86 ± 0.18 pmol/min/mg cell protein; N = 3) were well in between

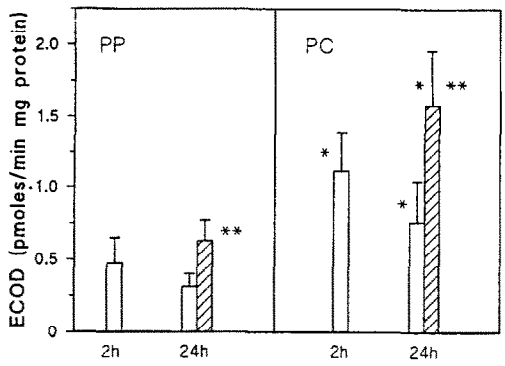

Fig. 1. Effect of cultivation on ECOD activity of PP and PC hepatocytes. Hepatocyte subpopulations were cultured for 24 hr in the absence (open bars) or presence (hatched bars) of MC and PB. ECOD activity was determined at the times indicated. Results represent means ± SD of 3–4 independent cultures. * $P < 0.01$ for difference between values of PP and PC hepatocytes after 2 and 24 hr. ** $P < 0.01$ for differences between treated and untreated values in each hepatocyte subpopulation after 24 hr.

the values of both subpopulations when determined after 2 hr of cultivation. During 24 hr of cultivation the enzyme activity declined in cultures of both subpopulations. In the presence of MC and PB a slight induction was observed (Fig. 1) which was mainly due to PB alone (not shown). The extent of this induction was similar in PP and PC hepatocytes (Fig. 1) and thus preserved the heterogeneity.

Phase I and phase II metabolism of model compounds

Several model substrates, namely biphenyl, harmine and lonazolac, were used to probe the metabolic capacity of total hepatocytes as well as of PP and PC subpopulations. For all compounds only the major phase I and phase II metabolites detected by HPLC which accounted for more than 85% of total metabolites were considered herein. With biphenyl, PC hepatocytes showed the highest and PP hepatocytes the lowest production of monosulfated biphenyl after 2 hr, while production rates of total hepatocytes were in between (Table 1). Formation of the 4-monosulfated product via conjugation of 4-OH-biphenyl was recently shown to represent the main metabolic fate of biphenyl in cultured rat hepatocytes [20]. After 24 hr all rates were reduced but zonal differences were still preserved. Incubation with MC led to an increased production of biphenyl sulfate in all cell preparations. Even under these conditions rates in PC hepatocytes were significantly higher than in PP cells (Table 1). PB, which alone was without effect, slightly enhanced the induction by MC. The hydroxylated intermediate could never be detected in significant amounts indicating that conjugation surmounted the capacity for phase I metabolism in all cell preparations.

In the case of harmine the heterogeneity of metabolism was more pronounced than with biphenyl (Table 1). Again PC hepatocytes showed the highest production of harmine glucuronide and harmine

Table 1. Metabolism of biphenyl, harmine and lonazolac in total, PP and PC hepatocytes cultured with and without MC and PB

Substrate Metabolite	Culture Time (hr)	Inducing agent	Production rate (pmol/min/mg cell protein)		
			Total hep.	PP	PC
Biphenyl:					
Biphenyl sulfate	2	—	133 ± 40	120 ± 23	186 ± 28*
	24	—	103 ± 31	92 ± 27	164 ± 39*
	24	MC	187 ± 29	165 ± 31	214 ± 22*
	24	PB	93 ± 14	92 ± 28	157 ± 29*
	24	MC/PB	211 ± 36	203 ± 32	285 ± 54*
Harmine:					
Harmine glucuronide	2	—	592 ± 157	258 ± 111	708 ± 129*
	24	—	249 ± 35	156 ± 63	342 ± 72*
	24	MC	271 ± 32	223 ± 41	364 ± 68*
	24	PB	526 ± 45	467 ± 88	644 ± 70*
	24	MC/PB	538 ± 42	591 ± 391	786 ± 55†
Harmine sulfate	2	—	1762 ± 234	1129 ± 225	2018 ± 626*
	24	—	1340 ± 347	1007 ± 288	1732 ± 782*
	24	MC	1478 ± 304	1979 ± 652	2081 ± 418
	24	PB	1367 ± 233	1127 ± 311	1775 ± 649†
	24	MC/PB	1456 ± 318	2185 ± 787	2828 ± 984†
Lonazolac:					
Lonazolac OH	2	—	131 ± 26	128 ± 24	155 ± 18
	24	—	144 ± 17	130 ± 22	162 ± 11
	24	MC/PB	149 ± 23	145 ± 27	167 ± 16
Lonazolac sulfate	2	—	24 ± 8	27 ± 5	31 ± 9
	24	—	21 ± 6	18 ± 4	23 ± 11
	24	MC/PB	22 ± 4	28 ± 8	21 ± 7

Total hep., total hepatocytes.
Values represent means ± SD of 3 to 13 independent cultures.
* $P < 0.01$, † $P < 0.05$ for difference between PP and PC hepatocytes.

sulfate. During cultivation in the absence of inducers harmine glucuronide production rate decayed stronger than that of harmine sulfate. While MC was almost without effect, PB acted as a strong inducer of harmine glucuronide, particularly in the PP hepatocytes. A synergistic effect of MC and PB was significant in periportal cells ($P < 0.01$). For both metabolites production rates were still slightly higher in the PC cells (Table 1). In the case of harmine sulfate, induction was inverse and MC acted as the main inducer, again in PP cells, while PB did not enhance the production rate. Similarly as before, both agents together further increased production of harmine sulfate preferentially in PC cells (Table 1).

In contrast, no significant zonal differences in phase I and phase II metabolism were found in the case of lonazolac. During cultivation neither the production of hydroxylated nor of sulfated lonazolac were reduced in the absence or induced in the presence of MC or PB (not shown) as well as the combination thereof (Table 1).

Heterogeneous distribution of GST

GST activities determined by CDNB as substrate were higher in PC than in PP hepatocytes, while those determined by DCNB were higher in PP cells (Table 2). In both cases, the activities measured in total hepatocyte cultures were in between those of the subpopulations indicating that true zonal differences were determined. During cultivation for 24 hr the activity against CDNB remained almost constant and could be induced by the combination of MC and PB, whereby PB acted as the main inducer. Induction did not affect the heterogeneity. In contrast, the activity against DCNB spontaneously rose during cultivation even in the absence of inducers. This increase was noted in total hepatocytes, PP cells and particularly in PC hepatocytes leading to very close values for all hepatocyte preparations after 24 hr (Table 2). Interestingly, during cultivation in the presence of PB or both MC and PB, significant induction was observed only in PP hepatocytes, thus maintaining the initial heterogeneous distribution.

Determination of two GST-subunits associated with these conjugation reactions, namely, subunit Ya (or 1) and Yb_1 (or 3), by a sensitive ELISA technique revealed corresponding differences (Table 3). Thus, GST-Ya predominated in PC cells, while GST-Yb_1, was slightly higher in PP hepatocytes. Also in this case the values determined for total hepatocytes ranged in between those of the subpopulations. During cultivation both subunits decreased and did not show significant differences after 24 hr, while they remained much higher in cultures of total hepatocytes. In the presence of PB, however, the initial heterogeneity was almost fully reestablished with the inducer acting on GST-Ya preferentially in PC cells and on Yb_1 mainly in PP cells (Table 3). MC alone or in combination with PB did not alter this pattern of induction.

Table 2. Induction of GST activities in PP and PC hepatocytes by MC and PB

Substrate	Culture time (hr)	Inducing agent	GST activity (mU/mg cell protein)		
			Total hep.	PP	PC
CDNB	2	—	298 ± 32	186 ± 45	285 ± 38†
	24	—	231 ± 42	165 ± 56	270 ± 49†
	24	MC	256 ± 37	268 ± 52	314 ± 46
	24	PB	288 ± 43	234 ± 39	402 ± 51†
	24	MC/PB	307 ± 61	301 ± 48	456 ± 104*
DCNB	2	—	6.1 ± 0.7	5.7 ± 0.9	3.4 ± 0.9†
	24	—	10.4 ± 1.8	11.6 ± 4.7	9.9 ± 2.3
	24	MC	11.8 ± 1.9	13.1 ± 3.6	9.1 ± 1.1
	24	PB	14.5 ± 2.2	19.7 ± 4.1	9.8 ± 1.4†
	24	MC/PB	13.7 ± 2.0	20.8 ± 3.2	9.8 ± 1.6†

Total hep., total hepatocytes.
Hepatocytes were cultured on uncoated Petri dishes (TC quality), see [14].
Values represent means ± SD of eight (total), four (PP) or three (PC) independent cultures.
* $P < 0.05$; † $P < 0.01$ for differences between PP and PC hepatocytes.

Table 3. Induction of two distinct GST subunits in PP and PC hepatocytes by MC and PB

GST-subunit	Culture time (hr)	Inducing agent	Specific amount (ng/mg soluble protein)		
			Total hep.	PP	PC
Ya	2	—	294 ± 48	215 ± 55	373 ± 59†
	24	—	206 ± 49	97 ± 37	144 ± 46
	24	MC	205 ± 46	123 ± 40	218 ± 39†
	24	PB	229 ± 47	108 ± 48	294 ± 42†
	24	MC/PB	239 ± 35	132 ± 33	349 ± 78†
Yb_1	2	—	148 ± 33	186 ± 38	124 ± 51*
	24	—	176 ± 57	143 ± 40	88 ± 32
	24	MC	182 ± 38	161 ± 44	96 ± 39*
	24	PB	218 ± 21	235 ± 57	104 ± 28†
	24	MC/PB	224 ± 41	217 ± 52	103 ± 46†

Total hep., total hepatocytes.
Hepatocytes were cultured on uncoated Petri dishes (TC quality), see [14].
Values represent means ± SD of eight (total), four (PP) or three (PC) independent cultures.
* $P < 0.05$; † $P < 0.01$ for difference between PP and PC hepatocytes.

DISCUSSION

The efficiency of the digitonin–collagenase technique for isolating PP and PC hepatocytes has been validated by several laboratories using a variety of different criteria [5–7, 11, 21, 22]. The enzymatic characterization of these subpopulations used in this study was in good accord with data published previously [7, 23]. In our laboratory these isolated subpopulations have been successfully used for characterizing zonal differences among hepatocytes with respect to metabolic capacities [24, R. Gebhardt, unpublished observations] as well as growth potential [22, 23].

The results of the present study add to the known fact that phase I and phase II drug metabolizing activities show considerable zonal differences. Usually, phase I reactions predominate in PC hepatocytes [1–4]. Such a pattern was found for ECOD with and without induction by MC in accordance with published data using similar [12] or different techniques [25–27], but at variance with other studies [28].

In order to obtain information about the integration of phase I and phase II reactions in the heterogeneity of hepatic drug metabolism, we have studied the metabolism and its induction of three substrates, namely lonazolac, biphenyl and harmine, with a different degree of zonation. These substrates mainly undergo a single phase I activation [20] followed by conjugation with sulfate and glucuronic acid. The lobular homogeneity of aryl hydroxylation and sulfation of lonazolac which was not affected by MC and/or PB may fit with reports on a uniform distribution of P450IIB1 in the uninduced rat [29, 30] and with the finding of Duffel *et al.* [31] that aryl sulfotransferase IV showing the broadest substrate spectrum of the sulfotransferases is present throughout the acinus.

The pronounced heterogeneity of biphenyl and

harmine metabolism, on the other hand, responded differently to cultivation in the absence or presence of inducers, but was never lost. Addition of MC or PB apparently favored either sulfation or glucuronidation presumably by inducing the respective sulfotransferases and UDP-glucuronosyl-transferases which in accord with findings of Ullrich *et al.* [32] and Conway *et al.* [33] predominate in the PC cells. The fact that a significant synergistic effect of both inducers was observed, particularly in the case of harmine, suggests that also phase I metabolism was induced under these conditions. Thus, zonal differences in drug metabolism result from the specific balance between the phase I and phase II reactions involved and have to be determined individually for each drug in question. In addition, zonal differences in co-factors for the conjugation pathways may influence the results.

The high degree of variability found for the distribution of phase II conjugating reactions is further exemplified by the conjugation with glutathione. In accordance with a previous report [11] we show herein that the GST activity against CDNB is predominantly PC. However, the preferential localization of GST activity against DCNB in PP hepatocytes demonstrates that the distribution pattern of GST activities cannot be generalized. This seems to be highly important when reasoning about the contribution of zonal imbalances of glutathione content and glutathione-dependent detoxification to the regiospecificity of many hepatotoxins [2, 4, 34, 35]. The opposite localization of the GST activities against CDNB and DCNB was further substantiated by the finding that the GST isoforms Ya and Yb_1 which are mainly responsible for these activities were localized similarly. For the GST subunit Yb_1 this is at variance with earlier findings based on immunohistochemistry [36, 37]. Whether this discrepancy is due to contaminated antibodies used in these old studies is not known.

Most interestingly, GST-Yb_1 was induced only in PP cells by PB, while a predominantly PC induction was observed for GST-Ya. This demonstrates that the cultured PP and PC hepatocytes maintain their zonal predisposition and do not simply equilibrate during short-term cultivation, despite the loss of functional activity in untreated cultures.

In conclusion, our results emphasize that the zonal heterogeneity of hepatocytes with respect to drug metabolism must be substantiated in every single case before conclusions about the zonation of the metabolism of individual drugs can be drawn. Primary cultures of PP and PC hepatocytes isolated by the digitonin–collagenase technique appear as a suitable model system for such studies, since specific differences in the enzymatic equipment of the hepatocyte subpopulations are well preserved during short-term cultivation and are pronounced rather than equilibrated by inducing compounds added to the culture medium.

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REFERENCES

1. Jungermann K and Katz N, Functional specialization of different hepatocyte populations. *Physiol Rev* **69**: 708–751, 1989.
2. Gebhardt R, Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol Ther* **53**: 275–354, 1992.
3. Anderson LM, Ward JM, Park SS and Rice JM, Immunohistochemical localization of cytochromes P450 with polyclonal and monoclonal antibodies. *Path Immunopath Res* **8**: 61–94, 1989.
4. Baron J, *In situ* sites for xenobiotic activation and detoxication: Implications for the differential susceptibility of cells to the toxic actions of environmental chemicals. In: *Progress in Histo- and Cytochemistry*, Vol. 23 (Eds. Graumann W and Drukker J), pp. 32–44. Fischer Verlag, Stuttgart, 1991.
5. Quistorff B, Gluconeogenesis in periportal and perivenous hepatocytes of rat liver, isolated by a new high-yield digitonin–collagenase perfusion technique. *Biochem J* **229**: 221–226, 1985.
6. Lindros KO and Penttilä KE, Digitonin–collagenase perfusion for efficient separation of periportal or perivenous hepatocytes. *Biochem J* **228**: 757–760, 1985.
7. Burger HJ, Gebhardt R, Mayer C and Mecke D, Different capacities for amino acid transport in periportal and perivenous hepatocytes isolated by digitonin/collagenase perfusion. *Hepatology* **9**: 22–28, 1989.
8. Chen KS and Katz J, Zonation of glycogen and glucose synthesis, but not glycolysis, in rat liver. *Biochem J* **255**: 99–104, 1988.
9. Tosh D, Alberti GM and Agius L, Glucagon regulation of gluconeogenesis and ketogenesis in periportal and perivenous hepatocytes. *Biochem J* **256**: 197–204, 1988.
10. Guzman M and Castro J, Zonation of fatty acid metabolism in rat liver. *Biochem J* **264**: 107–113, 1989.
11. Kera Y, Sippel HW, Penttilä KE and Lindros KO, Acinar distribution of glutathione-dependent detoxifying enzymes. Low glutathione peroxidase activity in perivenous hepatocytes. *Biochem Pharmacol* **36**: 2003–2006, 1987.
12. Suolinna EM, Penttilä KE, Winell BM, Sjoholm AC and Lindros KO, Drug metabolism by periportal and perivenous rat hepatocytes. Comparison of phase I and phase II reactions and their inducibility during culture. *Biochem Pharmacol* **38**: 1329–1334, 1989.
13. Gebhardt R, Histochemical approaches to the screening of carcinogens *in vitro*. In: *Progress in Histo- and Cytochemistry*, Vol. 23 (Eds. Graumann W and Drukker J), pp. 91–97 Fischer Verlag, Stuttgart, 1991.
14. Gebhardt R, Fitzke H, Fausel M, Eisenmann-Tappe I and Mecke D, Influence of hormones and drugs on glutathione-*S*-transferase levels in primary culture of adult rat hepatocytes. *Cell Biol Toxicol* **6**: 365–378, 1990.
15. Gebhardt R and Jung W, Biliary secretion of sodium fluorescein in primary cultures of adult rat hepatocytes and its stimulation by nicotinamide. *J Cell Sci* **56**: 233–244, 1982.
16. Gebhardt R and Williams GM, Amino acid transport in established adult rat liver epithelial cell lines. *Cell Biol Toxicol* **2**: 9–20, 1986.
17. Gebhardt R and Mecke D, The role of growth hormone, dexamethasone, and trijodothyronine in the regulation of glutamine synthetase in primary cultures of rat hepatocytes. *Eur J Biochem* **100**: 519–525, 1979.
18. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Anal Biochem* **85**: 488–491, 1980.
19. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.

20. Wegner H, Mecke D and Gebhardt R, The influence of inducing agents and co-cultivation on the stability of monooxygenase-activity in rat hepatocyte cultures. *Biol Chem (Hoppe-Seyler)* **371**: 753, 1990.
21. Gebhardt R, Lindros K, Lamers WH and Moorman AFM, Hepatocellular heterogeneity in ammonia-metabolism: demonstration of limited co-localization of carbamoylphosphate synthetase and glutamine synthetase. *Eur J Cell Biol* **56**: 464–467, 1991.
22. Gebhardt R, Different proliferative activity *in vitro* of periportal and perivenous hepatocytes. *Scand J Gastroenterol* **23**: (Suppl 151): 8–18, 1988.
23. Gebhardt R and Jonitza D, Different proliferative response of periportal and perivenous hepatocytes to EGF. *Biochem Biophys Res Commun* **181**: 1201–1207, 1991.
24. Ugele B, Kempen HJM, Gebhardt R, Meijer P, Burger HJ and Princen HMG, Heterogeneity of rat liver parenchyma in cholesterol 7 α-hydroxylase and bile acid synthesis. *Biochem J* **276**: 73–77, 1991.
25. Belinsky SA, Kauffman FC and Thurman RG, Reducing equivalents for mixed function oxidation in periportal and pericentral regions of the liver lobule in perfused livers from normal and phenobarbital-treated rats. *Mol Pharmacol* **28**: 371–376, 1985.
26. Väänänen H, The distribution of cytochrome P450 mediated drug oxidation and glutathione in periportal and perivenous rat hepatocytes after phenobarbital treatment. *J. Hepatol* **2**: 174–181, 1986.
27. Bengtsson G, Julkunen A, Penttilä KE and Lindros KO, Effect of phenobarbital on the distribution of drug metabolizing enzymes between periportal and perivenous rat hepatocytes prepared by digitonin–collagenase liver perfusion. *J Pharmacol Exp Ther* **240**: 663–667, 1987.
28. Gascon-Barre M, Benbrahim N and Tremblay C, Hepatic zonation of drug metabolizing enzymes. Studies on hepatocytes isolated from the periportal or perivenous region of the liver acinus. *Can J Physiol Pharmacol* **67**: 1015–1022, 1989.
29. Dees JH, Masters BSS, Muller-Eberhard U and Johnson EF, Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and phenobarbital on the occurrence and distribution of four cytochrome P450 isozymes in rabbit kidney, lung and liver. *Cancer Res* **42**: 1423–1432, 1982.
30. Schwarz M, Peres F, Buchmann A, Friedberg T, Waxmann DJ and Kunz W, Phenobarbital induction of cytochrome P450 in normal and preneoplastic rat liver: comparison of enzymes and mRNA expression as detected by immunohistochemistry and *in situ* hybridization. *Carcinogenesis* **8**: 1355–1357, 1987.
31. Duffel MW, Binder TP, Hosie L, Baden HA, Sanders JA, Knapp SA and Baron J, Purification, immunochemical localization of rat hepatic aryl sulfotransferase IV. *Mol Pharmacol* **40**: 36–44, 1991.
32. Ullrich D, Fischer G, Katz N and Bock KW, Intralobular distribution of UDP-glucuronosyl-transferase in livers of untreated, 3-methylcholanthrene- and phenobarbital-treated rats. *Chem Biol Interact* **48**: 181–190, 1984.
33. Conway JG, Kauffman FC, Tsukada T and Thurman RG, Glucuronidation of 7-hydroxycoumarin in periportal and pericentral regions of the lobule in livers from untreated and 3-methylcholanthrene-treated rats. *Mol Pharmacol* **25**: 487–493, 1984.
34. Harris C and Thurman RG, A new method to study glutathione adduct formation in periportal and pericentral regions of the liver lobule by micro-reflectance spectrophotometry. *Mol Pharmacol* **29**: 88–96, 1986.
35. Kera Y, Penttilä KE and Lindros KO, Glutathione replenishment capacity is lower in isolated perivenous than in periportal hepatocytes. *Biochem J* **254**: 411–417, 1988.
36. Redick JA, Jacoby WB and Baron J, Immuno-histochemical localization of glutathione-*S*-transferase in livers of untreated rats. *J Biol Chem* **257**: 15200–15203, 1982.
37. Wolf CR, Moll E, Friedberg T, Oesch F, Buchmann A, Kuhlmann WD and Kunz HW, Characterization, localization and regulation of a novel phenobarbital-inducible form of cytochrome P450, compared with three further P450-isozymes, NADPH P450-reductase, Glutathione transferases and microsomal epoxide hydrolase. *Carcinogenesis* **5**: 993–1001, 1984.