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CHARACTERIZATION OF DISTINCT FORMS OF CYTOCHROMES P-450, EPOXIDE METABOLIZING ENZYMES AND UDP-GLUCURONOSYLTRANSFERASES IN RAT SKIN

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Abstract—Study of drug metabolizing enzyme activity was undertaken in skin microsomal and cytosolic fractions of male and female rats. The presence of several isoforms was revealed from their activities towards selected substrates and from their cross immunoreactivity using antibodies raised against purified hepatic or renal cytochromes P-450, epoxide hydrolase and UDP-glucuronosyltransferases. Cytochrome P-450 content was precisely quantified by second derivative spectrophotometry, 23.1 and 16.5 pmol/mg protein in males and females, respectively. The monooxygenase activity associated to cytochromes P-450IIB1 and P-450IA1 was determined through O-dealkylation of ethoxy-; pentoxy- and benzoxy-resorufin. The activity ranged between 4 and 2 nmol/min/mg protein for male and female rats, respectively. These results and Western blot analysis indicated that rat skin microsomes contain both monooxygenase systems associated with cytochromes P-450IIB1 and P-450IA1. By contrast lauric acid hydroxylation, supported by cytochrome P-450IVA1, was not detectable. Activities of epoxide metabolizing enzymes (microsomal and cytosolic epoxide hydrolases; glutathione *S*-transferase) were also characterized in skin. Microsomes catalysed the hydratation of benzo(*a*)pyrene-4,5-oxide and *cis*-stilbene oxide at the same extent, whatever the sex, although the specific activity was 10 times lower than in liver. The hydratation of *trans*-stilbene oxide by soluble epoxide hydrolase was four times lower than in the liver. Conjugation of *cis*-stilbene oxide with glutathione in skin and liver proceeded at essentially similar rates, as the specific activity of glutathione *S*-transferase in skin was only two times less than that measured in hepatic cytosol. Glucuronidation of 1-naphthol, bilirubin but not of testosterone could be followed in the microsomal fraction. Revelation by Western blot indicated that both the isoforms involved in conjugation of phenols and bilirubin were present in skin microsomes. By contrast, the isoform catalysing the conjugation of testosterone was apparently missing. When immunoblotting was carried out using specific antibodies raised against the renal isoforms, the same result was obtained. In addition, an intense staining corresponding to a 57 kD-protein was observed.

Drugs and environmental pollutants can penetrate and concentrate into skin, which represents one of the body's largest organs. Although the liver is the major site for metabolization of these substances, extra-hepatic transformations, especially kidney or intestine, bring a non negligible contribution to their overall elimination from the body. In the liver, oxydation, epoxide hydration and glucuronidation of drugs or endogenous compounds, some of them potentially toxic such as bilirubin, are catalysed by distinct isozymes located both in the membrane and in the cytosol fraction. Each isoform can be differentiated by their substrate specificity [1], their immunoreactivity [2], or from their selective induction by phenobarbital, 3-methylcholanthrene or clofibrate [3]. The study of properties of such enzyme systems in skin has been limited by the lack of sensitive techniques of measurement and by the often poor performance of the subcellular fractionation. This present work was undertaken to characterize in term of substrate specificity and immunochemical reactivity the activity of monooxygenases cytochrome P-450IIB1- and IA1-dependent implicated in the O-dealkylation of drugs, of epoxide metabolizing enzymes (epoxide hydrolases, glutathione *S*-transferase), and of three distinct forms of UDP-glucuronosyltransferases measured with 1-naphthol, bilirubin and testosterone as substrates, in rat skin.

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MATERIALS AND METHODS

Animals treatment with inducers and preparation of the microsomal fraction. Male and female Sprague–Dawley rats from Iffa-Credo, (Saint-Germain l'Abresle, France), weighing 180–220 g were housed in an environmentally controlled room (12 hr light cycle, 22°). They were fed a rodent chow (UAR Alimentation, Villemoisson, France) and had tap water *ad lib*. For the immunochemical characterization of drug-metabolizing enzymes, male Sprague–Dawley rats were treated with phenobarbital and 3-methylcholanthrene. Phenobarbital (sodium salt, Fluka,

Buchs, Switzerland), previously dissolved in NaCl 0.9% w/v, was injected intraperitoneally for 5 days at the daily dose of 100 mg/kg body weight. 3-Methylcholanthrene (Sigma Chemical Co., St. Louis, MO), suspended in corn oil, was given intraperitoneally, once at the dose of 80 mg/kg body weight. Control animals received the vehicles, in the same conditions. The rats were killed by decapitation after 12 hr fasting on the sixth day.

Subcellular fractionation. Dorsal skin of rat was shaved, cut in small pieces and then homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA, 0.1 mM dithiothreitol, with an Ultra-Turrax apparatus (TP 8872, Junke and Kunkel, Staufen i Br., F.R.G.) in three strokes (1 min each) with 30 sec pauses in between. The homogenate was filtered through two layers of cheese-cloth and centrifuged at 1000 *g* for 10 min on a LKB Ultrospin 85 centrifuge (Bromma, Sweden). The corresponding supernatant was thereafter centrifuged for 20 min at 10,000 *g*. The pellet was discarded and the supernatant was centrifuged again for 60 min at 105,000 *g* to give the cytosolic and microsomal fractions. The microsome pellet was homogenized with a Dounce in 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol, 0.2 mM EDTA and 20% v/v glycerol. Hepatic microsomes and cytosol were prepared by the technique of Hogeboom [4] in 1 mM Tris–HCl buffer (pH 7.4), 250 mM sucrose. The subcellular fractions were stored at −20° and used within a week. The quality of the skin microsomal fraction was estimated from analysis by electron microscopy and measurement of marker enzymes such as NADH oxydase [5] (mitochondria) and NADPH cytochrome *c* reductase [6] (microsomes). The protein content was measured by the technique of Lowry *et al.* [7], with bovine serum albumin as standard.

Determination of drug-metabolizing enzymes. Cytochrome P-450 was quantitated from its second derivative spectrum after binding to carbon monoxide and reduction by dithionite on a Uvikon R-820 spectrophotometer (Kontron, Rotkreuz, Switzerland), as previously described [8]. The activities 7-ethoxy-, pentoxy- and benzoxyresorufin *O*-dealkylase were measured by the methods of Burke and Mayer [9] and that of Burke *et al.* [10]. The assays were carried out at 37° with 0.4 μM ethoxyresorufin or 3.5 μM pentoxy- and benzoxyresorufin; the reaction was initiated by 0.25 mM NADPH. The resorufin formed was detected on a SPF 500 spectrofluorimeter (Kontron, Rotkreuz, Switzerland). Hydroxylation of lauric acid was followed as Salaün *et al.* suggested [11]; the limit of detection of the assay was 0.03 nmol/min/mg protein. Microsomal epoxide hydrolase activity was monitored either with the fluorescent benzo(*a*)pyrene-4,5-oxide (IIT, Chicago, IL) according to the technique of Dansette *et al.* [12], or with tritiated *cis*-stilbene oxide [13]. The activity of the soluble epoxide hydrolase was determined in the cytosol with tritiated *trans*-stilbene oxide as substrate [13] in presence of 1 mM 1-chloro-2,4-dinitrobenzene (Sigma) to deplete glutathione. The activity of glutathione *S*-transferase was followed with tritiated *cis*-stilbene oxide and glutathione as co-substrates [13]. Activity of three forms of UDP-glucuronyltransferase was measured on fully activated microsomes with Triton X-100 (Prolabo, Paris, France) using [1-^{14}C]naphthol [14] and [^{3}H-1,2,6,7]testosterone (Amersham, Les Ullis, France) as substrates [15], and with digitonin (Sigma) for monitoring bilirubin glucuronidation [16]. The detection limit for testosterone glucuronidation was 0.09 nmol/min/mg protein.

Immunochemical characterization of drug-metabolizing enzymes. Microsomes were analysed by sodium dodecylsulfate polyacrylamide gel electrophoresis [17], using 10% w/v acrylamide in the separating gel and 5% w/v in the stacking gel. Proteins were transfered electrophoretically with a Trans-Blot system (Bio-Rad, Richmond, CA) at room temperature, for 90 min at 70 Volts, in 20 mM Tris–HCl buffer (pH 8.3) containing 20% v/v methanol and 150 mM glycine [18]. For the immunochemical detection, nitrocellulose sheets containing protein samples, after protein transfer, were incubated for 30 min, at room temperature in 150 mM sodium phosphate buffer (pH 7.2) containing 140 mM NaCl, 3 mM KCl, 10% v/v new born calf serum and 0.2% Triton X-100. IgG were purified from rabbit antibodies raised against purified hepatic cytochrome P-450IA1 (P-450 induced by 3-methylcholanthrene), P-450IIB1 (P-450 induced by phenobarbital) [19], and microsomal epoxide hydrolase (enzyme induced by phenobarbital) [20]. The IgG, which recognize several forms of rat liver UDP-glucuronosyltransferases (testosterone, androsterone, bilirubin, phenols) were obtained from sheep [21]. Antibodies raised against the renal isozymes conjugating bilirubin and phenols were also used. The IgG were added, after dilution, and the incubation was carried out for 1 hr at room temperature. The sheets were washed four times with the phosphate buffer and then incubated in a 1/500 dilution of peroxidase-conjugated sheep anti-rabbit IgG (Pasteur Institute, Paris, France) for 1 hr, followed by washing, as described above. Peroxidase activity was detected with 3-3′-diaminobenzidine (Sigma). The reaction was quenched after 5 min by immersing the nitrocellulose into water. The characterization of UDP-glucuronosyltransferase isozymes was monitored using the peroxidase-anti-peroxidase complex, and the peroxidase activity detected with 4-chloro 1-naphthol (Sigma) as substrate as previously described [22]. The alkaline phosphatase immunostaining of Western blots was also performed with nitroblue tetrazolium/5-bromo 4-chloro 3-indolyl phosphate as substrate, as described by the manufacturers (Promega Biotech).

RESULTS

Subcellular fractionation and activity of marker enzymes

Figure 1 shows a typical electron microscopy picture of skin microsomes. They were arranged as regular vesicles of similar diameter. Measurement of marker enzymes of the different subcellular fractions reveals, however, that some vesicles have been broken down during the fractionation procedure as 10% of the activity of the NADPH cytochrome *c* was also found in cytosol (Table 1). A weak con-

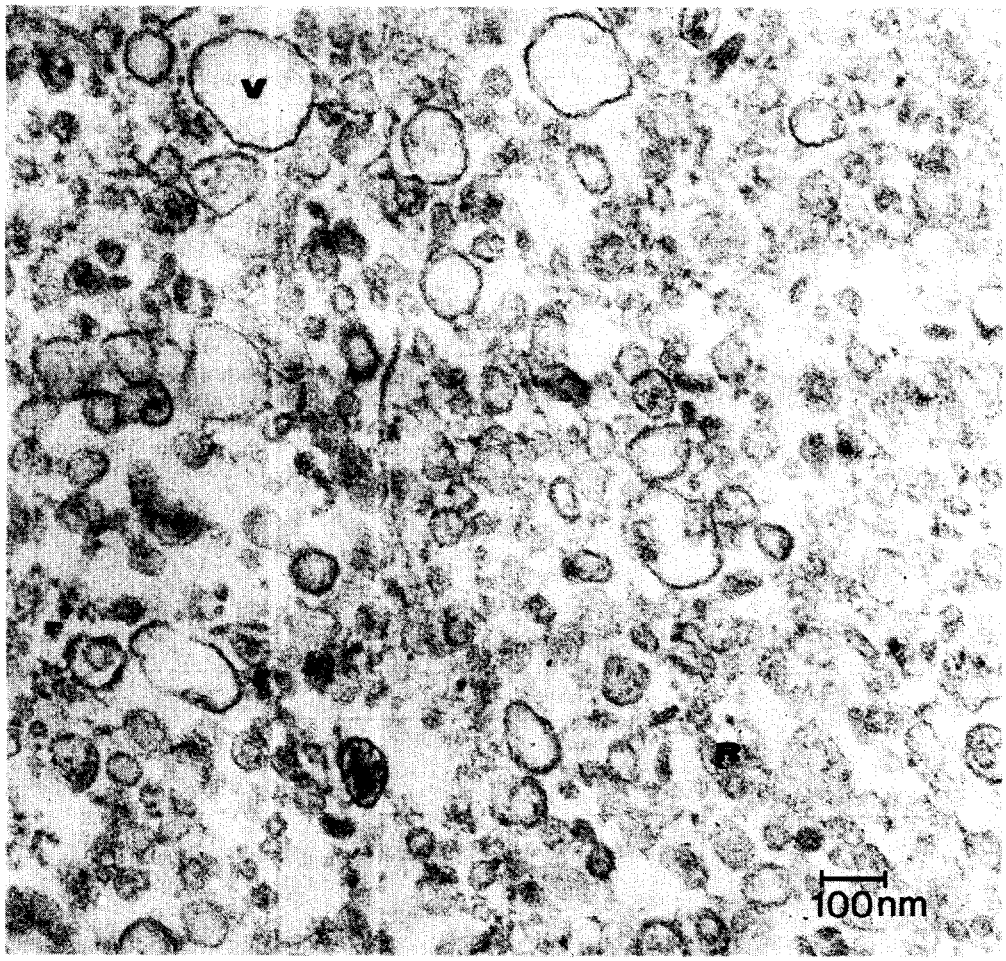

Fig. 1. Observation by electron microscopy of the microsomal fraction obtained from rat skin. V, microsomal vesicles; R, free ribosomes.

Table 1. Activity of marker enzymes in different subcellular fractions of rat skin

Subcellular fractions	NADPH cytochrome *c* reductase	NADH oxidase
Nuclei	2.3 ± 0.17 (10.3)	ND
Mitochondria	ND	6.4 ± 0.8 (100)
Microsomes	22.4 ± 3.2 (100)	0.6 ± 0.02 (9.4)
Cytosol	2.9 ± 0.4 (13.0)	ND

The activity of marker enzymes was expressed in nmol/min mg protein. The number between brackets corresponded to the activity percentage by comparison with the value found in the specific subcellular fraction. ND, not detectable. Values are the mean ± SD from, at least, four different subcellular fractionations.

tamination of microsomes by mitochondria could be observed on the basis of the NADH oxidase activity (Table 1). Expressed per gram of tissue, the protein contents in microsomes and cytosol from several fractionations were 20 and 56 mg in the liver, and 1 and 15 mg in skin, respectively.

Monooxygenases and epoxide metabolizing enzymes

Cytochrome P-450 was detected and precisely quantified in skin microsomes with the aid of second derivative spectrophotometry (Table 2). Like in liver, its content was lower in female than in male rats. However, whatever the sex, the concentration in skin was about 17 times smaller than in hepatic microsomes. The 7-pentoxyresorufin and 7-ethoxyresorufin *O*-dealkylase activity corresponding respectively to two forms of cytochromes P-450IIB1 and IA1 in skin microsomes also showed a difference

Table 2. Cytochrome P-450 content and monooxygenase activities in rat skin and liver microsomes

Organs	Cyt P-450	Hydroxylation	O-dealkylation		
		Lauric acid (IVA1)	POR (IIB1)	BOR (IIB1 + IA1)	EOR (IA1)
Skin					
Female	16.5 ± 2.1	ND	1.8 ± 0.1	2.1 ± 0.2	1.5 ± 0.2
Male	23.1 ± 1.8	ND	3.7 ± 1.3	4.4 ± 0.9	3.6 ± 0.3
Liver					
Female	287 ± 13	0.33 ± 0.07	8.9 ± 1.1	38.2 ± 3.5	19.7 ± 0.9
Male	383 ± 22	0.43 ± 0.01	13.8 ± 3.1	49.2 ± 1.3	23.4 ± 2

The content in cytochrome P-450 was expressed in pmol/mg protein. The activity of pentoxyresorufin- (POR), benzoxyresorufin- (BOR) and ethoxyresorufin- (EOR) *O*-dealkylases was expressed in pmol resorufin/min/mg prot. Values are the mean ± SD of, at least, three animals. ND, not detectable. The limit of detection of lauric acid hydroxylation was less than 0.03 nmol/min/mg protein. The form of cytochromes P-450 involved in monooxygenase reaction is indicated between brackets.

Table 3. Activities of epoxide metabolizing enzymes

Organs	Epoxide hydrolases			GST
	Microsomes		Cytosol	
	BP-4,5-oxide	CSO	TSO	CSO
Skin				
Female	0.16 ± 0.03	0.11 ± 0.01	0.027 ± 0.007	1.59 ± 0.10
Male	0.12 ± 0.05	0.16 ± 0.02	0.043 ± 0.010	1.65 ± 0.20
Liver				
Female	2.1 ± 0.2	1.02 ± 0.07	0.097 ± 0.008	3.25 ± 0.05
Male	3.8 ± 0.5	1.74 ± 0.10	0.180 ± 0.030	3.40 ± 0.14

The activity of microsomal epoxide hydrolase, cytosolic epoxide hydrolase and glutathione *S*-transferase (GST) was expressed in nmol/min/mg protein. The substrates were benzo(*a*)pyrene-4,5-oxide (BP-4,5-oxide), *cis*-stilbene oxide (CSO) and *trans*-stilbene oxide (TSO). Values are the mean ± SD of at least three animals.

according to the sex. The same result was observed with the 7-benzoxyresorufin *O*-dealkylase activity involving both cytochromes P-450 IIB1 and IA1. Compared to the liver, the rate of the reaction was 8 to 16 times less than in skin microsomes. Hydroxylation of lauric acid cytochrome P-IVA1 dependent was not detected in that fraction.

Hydratation of benzo(*a*)pyrene-4,5-oxide, *cis*-stilbene oxide and *trans*-stilbene oxide by the epoxide hydrolases also occurred in skin microsomes and cytosol (Table 3). No difference between sexes was observed in the case of the microsomal isoenzyme, while for the cytosolic form, the activity in male was higher than in female skin. The specific activity of cytosolic epoxide hydrolase in skin was 4 times lower than in liver microsomes. By contrast, conjugation of *cis*-stilbene oxide with glutathione was high in skin microsomes, since the activity was only two times lower than in hepatic microsomes (Table 3).

UDP-glucuronosyltransferases

Table 4 reports the specific activity of UDP-glucuronosyltransferases involved in the conjugation of 1-naphthol, testosterone and bilirubin. The optimal rate of 1-naphthol glucuronide formation in skin was 10 times less than that in liver microsomes. Bilirubin conjugation was even lower; no activity could be detected in skin or liver microsomes when Gunn rats were used (results not shown). Testosterone glucuronidation was absent in skin under the conditions of measurement used.

Immunoblotting

Figure 2 (A), which represents a typical Western blot experiment indicates that antibodies raised against rat liver cytochrome P-450IIB1 immunoreacted with cytochrome P-450 present in skin microsomes. Interestingly, from the migration of the protein within the gel, its apparent molecular weight

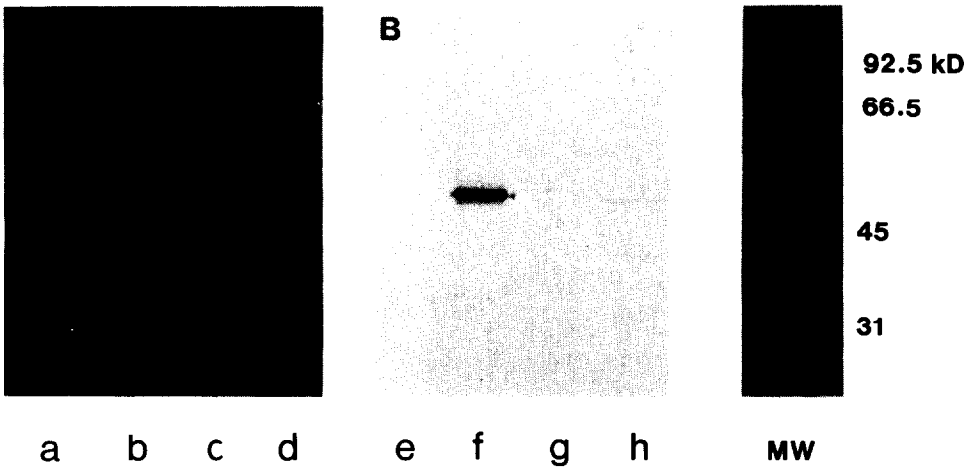

Fig. 2. Immunoblotting revelation of cytochrome P-450IIB1 (A) and cytochrome P-450IA1 (B) from cutaneous and hepatic microsomes. The doses of the inducers administered intraperitoneally were (mg/kg body weight), phenobarbital, 100 in saline, for 5 days; 3-methylcholanthrene, 80 once in corn oil. Lanes a, b, skin of rats non-treated and treated with phenobarbital, respectively; lanes c, d, liver of rats non-treated and treated with phenobarbital; lanes e, f, liver of rats non-treated and treated with 3-methylcholanthrene; lanes g, h, skin of rats non-treated and treated with 3-methylcholanthrene; MW, molecular weight standards.

Table 4. Activity of UDP-glucuronosyltransferases towards three substrates

	Substrates		
Organs	1-Naphthol	Bilirubin	Testosterone
Skin			
Female	2.5 ± 0.1	14.9 ± 2.1	ND
Male	4.3 ± 0.4	22.7 ± 3.0	ND
Liver			
Female	28.2 ± 1.3	432 ± 63	4.6 ± 0.2
Male	40.2 ± 4.9	880 ± 30	5.3 ± 0.4

The conjugation of 1-naphthol and testosterone was expressed in nmol/min/mg protein and that of bilirubin in pmol/min/mg protein. Values are the mean ± SD of at least three animals. ND, not detectable. The limit of detection of testosterone glucuronidation was less than 0.09 nmol/min/mg protein.

appeared lower than that of the corresponding liver protein. On the other hand, the native cytochrome P-450IA1 could not be detected in skin and was hardly seen in liver microsomes (Fig. 2B). Only a treatment with 3-methylcholanthrene, which induces selectively this form allowed the detection of the protein in the liver. Interestingly, a faint band could also be observed in skin microsomes of rats treated by the carcinogen (Fig. 2B), which suggests the presence of cytochrome P-450IA1 after induction.

When membrane epoxide hydrolase was considered, the bands corresponding to cutaneous and hepatic samples were located on the same molecular weight level (Fig. 3).

In the case of UDP-glucuronosyltransferase, the results of the immunoblotting obtained using sheep antibodies raised against purified renal isoforms involved in conjugation of bilirubin and phenols were shown in Fig. 4(A). This antibody recognized two bands strongly stained in non induced and phenobarbital-induced rat liver, which were also present in skin microsomes. In cutaneous microsomes, an additional band corresponding to a protein with a higher molecular weight (57 kD) could be seen (Fig. 4A). The immunostaining of the Western blot was also carried out using an antibody with broad specificity recognizing at least four hepatic UDP-glucuronosyltransferase isoforms active toward bilirubin (54 kD), phenols (53 kD), androsterone (52 kD), testosterone (50 kD) (Fig. 4B). In skin microsomes four bands were immunostained with molecular weights in the range of 50–52 kD. The lower band was not observed in skin microsomes of male rats.

DISCUSSION

The method used for preparation of the subcellular fractions gave a good reproducible separation and enrichment, as revealed by electron microscopy

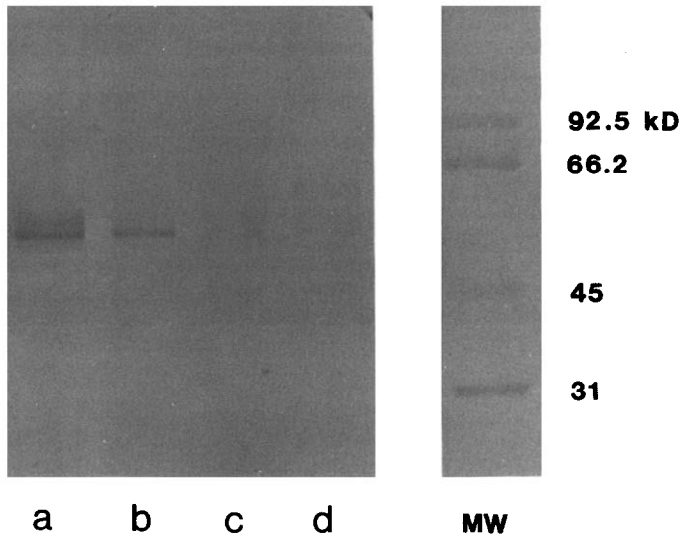

Fig. 3. Immunoblotting revelation of epoxide hydrolase from skin and liver microsomes. Lanes a, b, liver of rats treated and non-treated with phenobarbital, respectively; lanes c, d, skin of rats treated and non-treated with phenobarbital; MW, molecular weight standards. The dose of phenobarbital used is indicated in the legend of Fig. 2.

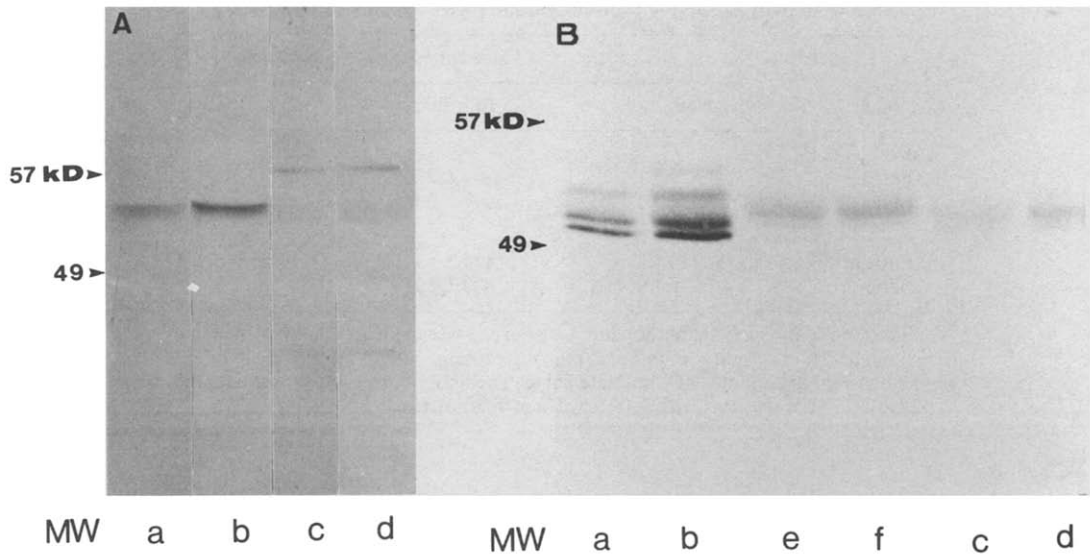

Fig. 4. Immunoblotting revelation of UDP-glucuronosyltransferase using IgG raised against purified rat kidney (A) or liver (B) enzyme. Lanes a, b, liver of rats non-treated and treated with phenobarbital, respectively; lanes c, d, skin of male rats non-treated and treated with phenobarbital; lanes e, f, skin of female rats not treated with phenobarbital; MW, molecular weight standards. The protein bands corresponded, from top to bottom, to the isozymes active toward bilirubin, phenols, androsterone and testosterone. The dose of phenobarbital used is indicated in the legend of Fig. 2.

observation and measurement of marker enzymes. The activity of NADPH cytochrome *c* reductase, which is representative of the endoplasmic reticulum, was also detected in cytosol. A possible explanation could be the release during the homogenization procedure from the membranes of the enzyme, since most of its hydrophilic active portion is in the cytosolic face [23], or, more likely, the presence of microsome fragments, which did not sediment with the experimental conditions used. A slight contamination of microsome pellets by mitochondria also occurred on the basis of NADH oxidase activity (9.4% of the value measured in mitochondria) detected in the microsomes. These contaminations were acceptable and consistent with the final yield in protein of the microsomes and cytosolic fraction, which allows measurements of the enzyme activity studied.

The existence of total cytochrome P-450 was routinely detected and quantified by second derivative spectrophotometry. This method was of adequate sensitivity to give reproducible results. The technique has proven to be useful to determine, without ambiguity, the presence of the haemoprotein at very low concentrations in tissues such as brain subcellular fractions [24]. The presence of cytochrome P-450 by spectral determination was reported for the first time in rat skin by Bickers *et al.* [25]. However, measurement of this pigment by conventional procedures did not give actual concentration, because of the presence of interfering haemoproteins. Some authors even failed to detect cytochrome P-450 in skin microsomes [26]. The presence of this pigment was corroborated in this work by measurement of the associated monooxygenase activities and that of NADPH cytochrome *c* reductase, which is known to catalyse the transfer of electrons from NADPH to iron, and finally from crossreactivity with antibodies raised against distinct forms of liver cytochromes P-450. Indeed measurement of monooxygenases that are representative of different isoforms of cytochromes and the determination of their immunoreactivity strongly suggested that, like in liver microsomes [19], at least two forms of cytochrome P-450 (IIB1 and IA1) should exist in skin. Dealkylation of 7-ethoxy- and pentoxyresorufin is mediated by cytochromes P-450IIB1 and P-450IA1, respectively, whereas dealkylation of benzoxyresorufin is cytochromes P-450IIB1 and IA1 dependent [27]. If cytochrome P-450IIB1 is a constitutive form in liver microsomes, cytochrome P-4501AI is a minor protein of the membranes under non-inducing conditions. This explains, probably why cytochrome P-450 IA1 was weakly stained after Western blot experiment when liver or skin microsomes of non-treated rats were used. However, after 3-methylcholanthrene given intraperitoneally, an increase of cytochrome P-450IA1 content, as revealed from the immunochemical characterization could be seen, even in skin. In a preliminary experiment we found a corresponding increase of about 60% of 7-ethoxyresorufin *O*-deethylase activity in skin microsomes. Such induction has been reported to occur also when carcinogens are applicated topically in rats and mice [28, 29].

By contrast, the presence of cytochrome P-450IVA1, estimated from hydroxylation of lauric acid, could not be detected. This protein also constitutes a minor part of the total cytochrome P-450, but its biosynthesis can be greatly enhanced by hypolipidaemic compounds such as clofibrate or other peroxisome proliferators [30]. We found that, when rats were treated intraperitoneally by clofibric acid (200 mg/kg/day for 5 days), cytochrome P-450IVA1 could be effectively induced in the liver but not in skin microsomes (data not shown). Furthermore the hypolipidaemic compound also failed to increase bilirubin glucuronidation in skin, whereas the corresponding activity in liver microsomes was two times enhanced [31]. These results suggest that the enzyme expression in skin differs from that in liver.

Glucuronidation of 1-naphthol, testosterone and bilirubin allowed to differentiate three UDP-glucuronosyltransferase isoforms, because these substrates are believed to be conjugated by separate proteins in the liver [32]. In skin, besides bilirubin, 1-naphthol glucuronidation was detected. Conjugation of 1-naphthol and other planar monohydroxylated substrates is catalysed by, at least, one transferase selectively enhanced by 3-methylcholanthrene [1]. These results could be favourably compared with those of Peters *et al.* [33], who localized by immunofluorescence an UDP-glucuronosyltransferase active toward 4-nitrophenol or bilurubin in the *stratum corneum* of human skin. However, under the measurement conditions used, testosterone glucuronidation could not be detected. The Western blot immunostained with two antibodies, with restricted and broad specificities, confirmed the existence of several isoforms of UDP-glucuronosyltransferase in skin. However the profile markedly differed between the two organs. The antibodies raised against phenol/bilirubin renal UDP-glucuronosyltransferase revealed an additional protein with a higher molecular weight in skin, and a marked band between 52–53 kD was much more stained in skin than in liver when the anti-liver UDP-glucuronosyltransferase antibody was used. Further investigations are required to ascertain whether these bands correspond to different isoenzymes or to same isoenzymes differently glycosylated.

Interestingly, like in liver microsomes, the enzyme exhibited latency. We have found that Triton X-100, at the optimal detergent–protein weight ratio of 0.4, activated the glucuronidation of 1-naphthol [34]. This result emphazised the role of the membrane lipids in the modulation of UDP-glucuronosyltransferase activity in skin.

Two forms of epoxide hydrolases and a glutathione *S*-transferase, which are involved in the detoxification of highly reactive chemical species [35] were present in skin. Values of specific activity for microsomal epoxide hydrolase obtained with benzo(*a*)pyrene 4,5-oxide and with *cis*-stilbene oxide in female and male skin rat were similar. Interestingly, in term of specific activity, hydratation of *trans*-stilbene oxide by cytosolic epoxide hydrolase and especially glutathione conjugation of *cis*-stilbene oxide proceeded at non negligible rates when compared with hepatic cytosol. Skin offers an efficient protection against potentially toxic epoxides, which can penetrate or are formed through it.

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REFERENCES

1. Okulicz-Kozaryn I, Schaefer M, Batt AM, Siest G and Loppinet V, Stereochemical heterogeneity of hepatic UDP-glucuronosyltransferase activity in rat liver microsomes. *Biochem Pharmacol* **12**: 1457–1461, 1981.

2. Thomas PE, Reik LM, Ryan DE, Bandiera S and Levin W, Polyclonal and monoclonal antibody probes of the structure and function of hepatic cytochrome P-450 isozymes. In: *Drug Metabolism, Molecular Approaches and Pharmacological Implications* (Ed. Siest G), pp. 131–139. Pergamon Press, Oxford, 1985.
3. Huang MT, West SB and Lu YH, Separation, purification and properties of multiple forms of cytochrome P-450 from the liver microsomes of phenobarbital treated-mice. *J Biol Chem* **15**: 4659–4665, 1976.
4. Hogeboom GH, Fractionation of cell component of animal tissues. General method for the isolation of liver cell components. In: *Methods in Enzymology* (Eds. Colonich SP and Kaplan NO), **1**: pp 16–19. Academic Press, New York, 1955.
5. Minakami S, Ringler RL and Singer TP, Studies of the respiratory chain-linked dihydrodiphosphopyridine nucleotide DH-I. Assay of the enzyme in particle and in soluble preparation. *J Biol Chem* **237**: 569–576, 1962.
6. Strobel HW and Dignam JD, Purification and properties of NADPH cytochrome P-450 reductase. In: *Methods in Enzymology* (Eds. Fleisher SE and Packer L) **52**: pp 89–98. Academic Press, London, 1978.
7. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
8. Ghersi-Egea JF, Walther B, Minn A and Siest G, Quantitative measurement of cerebral cytochrome P-450 by second derivative spectrophotometry. *J Neurosci Meth* **20**: 261–269, 1987.
9. Burke MD and Mayer RT, Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab Dispos* **2**: 583–588, 1974.
10. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, Pentoxy- and Benzoxyphenoxazone and homologous: a series of substrates to distinguish between different induced cytochrome P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
11. Salaün JP, Benveniste I, Reichhart D and Durst F, Induction and specificity of a cytochrome P-450 dependent laurate in-chain-hydroxylase from higher plant microsomes. *Eur J Biochem* **119**: 651–655, 1981.
12. Dansette PM, Dubois GC and Jerina DM, Continuous fluorimetric assay of epoxide hydrolase activity. *Anal Biochem* **97**: 340–345, 1979.
13. Wixtrom RN and Hammock BD, Membrane-bound and soluble-fraction epoxide hydrolases. In: *Methodological Aspects of Drug Metabolizing Enzymes*, pp. 4–93. Wiley, New York, 1985.
14. Bock KW and White INH, UDP-glucuronosyltransferase in perfused rat liver and in microsomes: influence of phenobarbital and 3-methylcholanthrene. *Eur J Biochem* **46**: 451–459, 1974.
15. Rao GS, Hauefer G, Rao ML and Breuer H, An improved assay for steroid glucuronosyltransferase in rat liver microsomes. *Anal Biochem* **74**: 35–40, 1976.
16. Heirwegh KPM, Van der Vijver M and Fevery J, Assay and properties of digitonin-activated bilirubin uridine diphosphate glucuronosyltransferase from rat liver. *Biochem J* **129**: 605–618, 1972.
17. Laemli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
18. Burnette WN, Western blotting: electrophoretic transfer of proteins from sodium dodecylsulfate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio iodinated protein. *Anal Biochem* **112**: 195–203, 1984.
19. Jayyosi Z, Totis M, Souhaili H, Goulon-Ginet C, Livertoux MH, Batt AM and Siest G, Induction of hepatic cytochrome P-450c-dependent monooxygenase activities by dantrolene in rat. *Biochem Pharmacol* **15**: 2481–2487, 1987.
20. Zhiri A, Muller J, Fournel S, Magdalou J, Wellman-Bednawska and Siest G, A new enzyme immunoassay of microsomal rat liver epoxide hydrolase. *Anal Biochem* **163**: 298–302, 1987.
21. Burchell B, Coughtrie MWH, Jackson MR, Shepherd SRP, Harding D and Hume R, Genetic deficiency of bilirubin glucuronidation in rats and humans. *Molec Aspects Med* **9**: 429–455, 1987.
22. Coughtrie MWH, Burchell B, Shepherd IM and Bend JR, Defective induction of phenol glucuronidation by 3-methylcholanthrene in Gunn rats is due to the absence of a specific UDP-glucuronosyltransferase isoenzyme. *Molec Pharmacol* **31**: 585–591, 1987.
23. Black SD and Coon MJ, Structural features of liver microsomal NADPH-cytochrome P-450 reductase. *J Biol Chem* **10**: 5929–5938, 1982.
24. Walther B, Ghersi-Egea JF, Minn A and Siest G, Subcellular distribution of cytochrome P-450 in the brain. *Brain Res* **375**: 338–344, 1986.
25. Bickers DR, Dutta-Choudhury T and Mukhtar H, Epidermis: a site of drug metabolism in neonatal rat skin. Studies of cytochrome P-450 content and mixed function oxidase and epoxide hydrolase activity. *Molec Pharmacol* **21**: 239–247, 1982.
26. Finnen MJ, Herdman ML and Shuster S, Distribution and sub-cellular localization of drug metabolizing enzymes in the skin. *Br J Dermatol* **113**: 713–721, 1985.
27. Wolf CR, Seilman S, Oesch F, Mayer RT and Burke MD, Multiple forms of cytochrome P-450 related to forms induced marginally by phenobarbital. Differences in structure and in the metabolism of alkoxyresorufins. *Biochem J* **240**: 27–33, 1986.
28. Asokan P, Das M, Rosenkranz HS, Bickers DR and Mukhtar H, Topically applied nitropyrenes are potent inducers of cutaneous and hepatic monooxygenases. *Biochem Biophys Res Commun* **129**: 134–140, 1985.
29. Lilienblum W, Irmscher G, Fusenig NE and Bock KW, Induction of UDP-glucuronosyltransferase and arylhydrocarbon hydroxylase activity in mouse skin and in normal and transformed skin cells in culture. *Biochem Pharmacol* **35**: 1517–1520, 1986.
30. Orton T and Parker GL, The effect of hypolipidemic agents on the hepatic microsomal drug-metabolizing enzyme system of the rat. *Drug Metab Dispos* **2**: 110–115, 1982.
31. Fournel S, Magdalou J, Thomassin J, Villoutreix J, Siest G, Caldwell J and André JC, Structure-dependent induction of bilirubin glucuronidation and lauric acid 12-hydroxylation by arylcarboxylic acids chemically related to clofibrate. *Biochim Biophys Acta* **842**: 202–213, 1985.
32. Siest G, Antoine B, Fournel S, Magdalou J and Thomassin J, The glucuronosyltransferases: what progress can pharmacologists expect from molecular biology and cellular enzymology? *Biochem Pharmacol* **36**: 983–989, 1987.
33. Peters WHM, Allebes WA, Jansen PLM, Poels LG and Capel PJA, Characterization and tissue specificity of a monoclonal antibody against human uridine 5′-diphosphate-glucuronosyltransferase. *Gastroenterology* **93**: 162–169, 1987.
34. Pham MA, Magdalou J, Fournel-Gigleux S and Siest G, La peau constitue-t-elle un modèle d'étude du métabolisme des médicaments? In: *Pharmacologie Clinique: Actualités et Perspectives II* (Eds. Strauch G and Morselli PL), **168**, pp. 193–200. Colloque INSERM, 1988.
35. Schladt L, Worner W, Setiabudi F and Oesch F, Distribution and inducibility of cytosolic epoxide hydrolase in male Sprague–Dawley rats. *Biochem Pharmacol* **19**: 3309–3316, 1986.