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IN VIVO ADMINISTRATION OF HYDROXYLATED PHENOBARBITAL METABOLITES: EFFECT ON RAT HEPATIC CYTOCHROMES P-450, EPOXIDE HYDROLASE AND UDP-GLUCURONOSYLTRANSFERASE

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Abstract—*p*-Hydroxyphenobarbital and *m*-hydroxyphenobarbital injected intraperitoneally to rats at the dose of 80 mg/kg induced a small increase of cytochrome P-450 in crude liver homogenate (expressed as nmoles/g liver) but a decrease of cytochrome P-450 concentration in isolated microsomes (expressed as nmoles/mg microsomal protein). A similar decrease of NADPH-cyt *c* reductase and epoxide hydrolase specific activities was observed. Pretreatment of animals with phenobarbital metabolites did not alter the native UDP-glucuronosyltransferase, but depressed the specific activity of digitonin-treated UDP-glucuronosyltransferase towards *p*-nitrophenol. Gel electrophoresis of microsomes showed that *p*-hydroxyphenobarbital and *m*-hydroxyphenobarbital did not induce a net biosynthesis of proteins with molecular weight near 50,000. Qualitative examination of monooxygenase activities indicated that the administration of hydroxylated phenobarbital did not modify the catalytic characteristics of microsomes, as compared with those of control microsomes. When phenobarbital and its *p*-hydroxyderivative were simultaneously injected to rats, specific enzyme activities of microsomes were increased as compared with controls but remained lower than with phenobarbital alone. The qualitative characteristics of the monooxygenase system were similar to those of microsomes from phenobarbital-induced rats. It may be concluded that phenobarbital produces both a proliferation of endoplasmic reticulum and an induction of drug-metabolizing activities, whereas its hydroxylated metabolites only retain the proliferative activity: thus, these two effects of phenobarbital might depend on two different molecular mechanisms.

The effects of phenobarbital on cytochrome P-450-dependent monooxygenase, epoxide hydrolase and UDP-glucuronosyltransferase activities are now well documented. Two different mechanisms appear to be involved: a liver hyperplasia [1] with a proliferation of smooth endoplasmic reticulum [1–4] is associated with a biosynthesis of cytochrome P-450 and related electron transfer enzymes [5–6], as well as epoxide hydrolase [7, 8] and UDP-glucuronosyltransferase [9–12]; thus, the metabolism and, as a consequence, the pharmacological effect of a drug may be altered by a prior or simultaneous phenobarbital administration. However, the mechanism of enzyme induction by phenobarbital remains unknown. Some indirect evidence indicates that phenobarbital may act before its binding to microsomes and metabolization [13–16]. *In vivo*, phenobarbital was shown to be metabolized mainly into *p*-hydroxyphenobarbital and excreted in urine as its glucuronide conjugate [17–20]. The purpose of the present study was to investigate the effect of *in vivo* administration of two hydroxylated metabolites of phenobarbital, *p*- and *m*-hydroxyphenobarbital, on cytochrome P-450 and related monooxygenase activities, epoxide hydrolase and UDP-glucuronosyltransferase in adult rat liver.

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¶ Abbreviations used: cyt P-450: cytochrome P-450; UDP-GT: UDP-glucuronosyltransferase (EC 2.4.1.17); EH: epoxide hydrolase (EC 3.3.2.3) (formerly epoxide hydratase, EC 4.2.1.64); PB: phenobarbital; *p*OHPB: *p*-hydroxyphenobarbital; *m*OHPB: *m*-hydroxyphenobarbital.

MATERIALS AND METHODS

Meta-hydroxyphenobarbital [5-ethyl, 5-(*m*-hydroxyphenyl) barbituric acid] was a gift from Rhone Poulenc Santé (Paris, France) and *para*-hydroxyphenobarbital [3-ethyl, 5-(*p*-hydroxyphenyl) barbituric acid] was synthetized by Dr V. Loppinet (Nancy, France). Sodium salts were prepared by addition of NaOH to free barbituric acids, freeze-dried and stored until use at −30°. Purity, checked by combined gas chromatography–mass spectrometry analysis [21] was > 99 per cent.

Male Sprague–Dawley rats (8-week-old animals weighing 200 g) were intraperitoneally injected daily for 4 days with barbiturates, 80 mg/kg in saline. When barbiturates were administered in association, animals received daily 80 mg of each barbiturate in a single injection. Controls received vehicle only. Animals were starved overnight and killed, 20 hr after the last injection, by a blow on the neck.

Livers were perfused with ice-cold isotonic NaCl, excised, weighed and homogenized in 0.25 M sucrose, 1mM EDTA, 1 mM Tris, pH 7.4, at a final concentration of 100 mg liver/ml.

An aliquot was preserved for cyt P-450-determination according to Greim [22]. Microsomes were prepared as previously described [23] and resuspended in 10 mM sodium phosphate, 20% glycerol buffer, pH 7.4. Proteins were determined according to Lowry *et al.* [24] and cyt P-450 by Omura and Sato's method [25].

Assays of glucose-6-phosphatase [26], NADPH-cyt *c* reductase [27], *p*-nitro-anisole-*O*-demethylase [28], aniline hydroxylase [27], 7-ethoxycoumarin-*O*-deethylase [29] and benzphetamine demethylation determined by the formation of formaldehyde [30], were carried out using 0.04–2 mg microsomal protein. Difference type I spectra of microsomes with barbiturates were recorded on a Beckman Acta M VI spectrophotometer; maximal binding and apparent K_s values were estimated by double reciprocal plots.

Epoxide hydrolase was assayed, using 20–40 μg microsomal protein, by hydration of ^{3}H-benzo[*a*]pyrene 4,5-oxide and separation of the diol by thin layer chromatography [31].

UDP-glucuronosyltransferase activity was determined with bilirubin and *p*-nitrophenol as substrate in native or digitonin-treated microsomes. The digitonin-treated microsomes (8 mg protein/ml) were obtained from untreated microsomal suspensions (16 mg/ml) which were diluted with an equal volume of 1.5% digitonin. Bilirubin UDP-GT activity was measured according to a micromodification of the method of Van Roy and Heirwegh [32] and Black *et al.* [33], i.e. a system using diazotized ethyl anthranilate and in which the excess of unconjugated bilirubin does not react [34]. The final concentrations in the incubation mixture (52 μl) were: 65 mM triethanolamine buffer, pH 7.4, 9.6 mM $MgCl_2$, 0.33 mM bilirubin, 0.12 mM human serum albumin, 2.7 mM UDP-glucuronic acid and microsomes (0.16 or 0.32 mg protein). Incubation was carried out at 37° for 15 min, a period during which linearity towards time kept constant. *p*-Nitrophenol conjugation was assayed by direct measurement of *p*-nitrophenol disappearance by a modification of the method of Gorski and Kasper [35]. Assays were carried out in cuvettes of 1 cm optical pathway containing 0.24 mM *p*-nitrophenol, 15 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.6, and microsomes (0.08 or 0.16 mg protein). The reaction was initiated, after preequilibration at 27°, with 5.5 mM UDP-glucuronic acid in the assay. The difference in absorbance between assay and corresponding blank was followed at 440 nm with a recording Perkin–Elmer double beam spectrophotometer. Electrophoresis was performed as described by Laemmli [36] on 10% polyacrylamide gels (15 cm length) in the presence of sodium dodecylsulfate.

Statistical significance of difference between groups was estimated by the Student's *t*-test.

RESULTS

The cytochrome P-450 concentration in total liver homogenate after barbiturate administration is

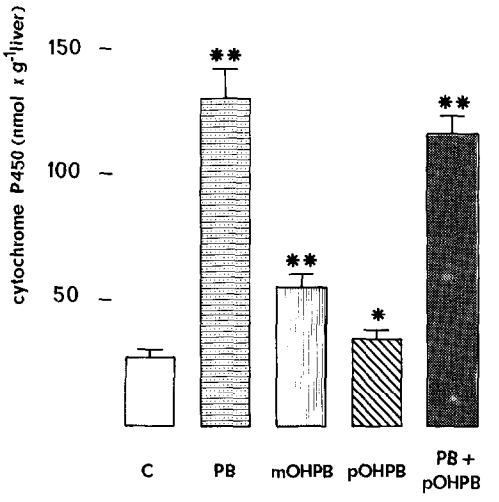

Fig. 1. Effect of *in vivo* barbiturate administration on cytochrome P-450 content of rat liver homogenates. Results are mean ± S.D. for 4–6 homogenates and expressed as nmoles cyt P-450 per g liver (wet wt). C is controls; PB, *m*OHPB, *p*OHPB, PB + *p*OHPB refer to animals pretreated, respectively, with 80 mg/kg of PB, *m*OHPB, *p*OHPB and the combination of PB and *p*OHPB for 4 days. *: $p < 0.05$; **: $p < 0.001$ as compared with controls.

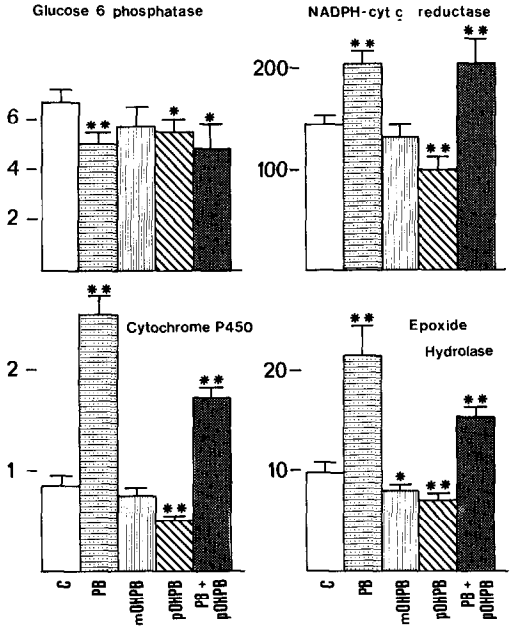

Fig. 2. Effect of *in vivo* barbiturate administration on some microsomal enzyme activities from adult rat liver. Results are mean ± S.D. for 4–6 microsomal preparations and expressed as μmoles Pi liberated in 20 min per mg protein for glucose-6-phosphatase; nmoles cyt P-450 per mg protein for cytochrome P-450; nmoles of ^{3}H-benzypyrene 4,5 diol formed per min per mg protein for epoxide hydrolase; nmoles cytochrome *c* reduced per min per mg protein for NADPH cyt *c* reductase. C: controls; PB, *m*OHPB, *p*OHPB, PB + *p*OHPB refer to animals pretreated, respectively, with 80 mg/kg *m*OHPB, *p*OHPB and the combination of PB and *p*OHPB for 4 days.

shown in Fig. 1. PB markedly increased the cyt P-450 concentration expressed as nmoles/ g liver (4.8-fold increase), in contrast with hydroxylated metabolites which provoked only a weak increase of cyt P-450 concentration: 2- and 1.25-fold with *m*- and *p*OHPB, respectively. When *p*OHPB was administered in association with PB, cyt P-450 concentration was comparable with that observed in PB-treated rats.

The effects of barbiturate administration on the specific activity of some microsomal enzymes are depicted in Fig 2. Two different responses were observed:

(a) glucose-6-phophatase was slightly decreased by all the barbiturates tested;

(b) cyt P-450, NADPH-cyt *c* reductase and EH activities exhibited a similar evolution: an important increase was observed after PB administration, in contrast with the significant decrease elicited by *p*OHPB, whereas *m*OHPB had no pronounced effect. When *p*OHPB and PB were injected in association, intermediate results were obtained, except for NADPH-cyt *c* reductase.

The glucose-6-phosphatase to cyt P-450 ratio indicates the relative importance of cyt P-450 in microsomal membranes. This ratio was lowered by PB (1.93 ± 0.05 vs 7.98 ± 0.74 for controls), unaffected by *m*OHPB (7.45 ± 1.25) and increased by *p*OHPB (9.82 ± 0.72). With the association PB + *p*OHPB, this ratio was lowered to 2.80 ± 0.75.

p-Nitroanisole demethylase is a non-specific monooxygenase activity: barbiturate administration did not change its molecular activity (expressed as nmoles of substrate transformed per min and per nmoles of cyt P-450) except *p*OHPB which increased it (Table 1). As a consequence, specific activities (expressed as nmoles of substrate transformed per min and per mg protein) followed roughly the microsomal cyt P-450 concentration.

Aniline is not a substrate preferentially metabolized by liver cyt P-450 from PB-treated animals [37]: however, both *m*OHPB and *p*OHPB increased its molecular activity, in contrast with PB which decreased it (Table 1). When the PB + *p*OHPB association was administered to animals, molecular activity was decreased, but less than with PB alone.

Benzphetamine-*N*-demethylase is mainly associated with cyt P-450 from PB-treated rats [37, 38]. Its molecular activity was highly increased by PB and the association PB + *p*OHPB, and to a smaller extent by *p*OHPB alone (Table 2). *m*OHPB was without any effect.

The molecular activity of 7-ethoxycoumarin deethylase was only slightly increased by *p*OHPB and more by *m*OHPB. However, only PB and PB + *p*OHPB association increased significantly the percentage of inhibition of ethoxycoumarin deethylase by 10^{-5}M metyrapone (Table 2).

In native microsomes, conjugation of both bilirubin and *p*-nitrophenol was not affected by a pre-

Table 1. Effect of *in vivo* barbiturate administration on *p*-nitroanisole demethylase and aniline hydroxylase activities from adult rat liver*

Pretreatment	*p*-Nitroanisole demethylase a	*p*-Nitroanisole demethylase b	Aniline hydroxylase a	Aniline hydroxylase b
None	4.62 ± 0.46	5.75 ± 1.00	0.437 ± 0.038	0.514 ± 0.033
PB	16.8 ± 2.7‡	6.58 ± 1.65	0.548 ± 0.052†	0.210 ± 0.011‡
*m*OHPB	4.67 ± 0.33	5.87 ± 0.86	0.442 ± 0.033	0.617 ± 0.050‡
*p*OHPB	4.76 ± 0.76	8.50 ± 1.10†	0.383 ± 0.034	0.705 ± 0.047‡
PB + *p*OHPB	10.2 ± 1.0‡	5.96 ± 0.71	0.482 ± 0.050	0.280 ± 0.019‡

* Results are mean ± S.D. for 4–6 preparations and expressed as: (a) nmoles substrate transformed per min per mg of microsomal protein; (b) nmoles substrate transformed per min per nmole cyt P-450.

† $P < 0.05$.

‡ $P < 0.001$ as compared with controls.

Table 2. Effect of *in vivo* barbiturate administration on benzphetamine demethylase and 7-ethoxycoumarin deethylase activities from adult rat liver*

Pretreatment	Benzphetamine demethylase a	Benzphetamine demethylase b	7-Ethoxycoumarin deethylase a	7-Ethoxycoumarin deethylase b	Inhibition by 10^{-5}M metyrapone (%)
None	3.77 ± 0.40	4.36 ± 0.31	0.82 ± 0.05	0.96 ± 0.08	28 ± 4
PB	38.2 ± 1.5†	13.75 ± 0.80‡	2.60 ± 0.19‡	1.05 ± 0.08	71 ± 2‡
*m*OHPB	3.32 ± 0.33	4.89 ± 0.36	1.06 ± 0.12†	1.40 ± 0.08‡	36 ± 5
*p*OHPB	3.40 ± 0.26	7.04 ± 0.78‡	0.65 ± 0.09†	1.19 ± 0.19†	27 ± 7
PB + *p*OHPB	23.3 ± 1.5†	13.63 ± 0.73‡	1.44 ± 0.17‡	0.82 ± 0.13	65 ± 3‡

* Results are mean ± S.D. for 4–6 preparations and expressed as: (a) nmoles substrate transformed per min per mg microsomal protein; (b) nmoles substrate transformed per min per nmole cyt P-450.

† $P < 0.05$.

‡ $P < 0.001$ as compared with controls.

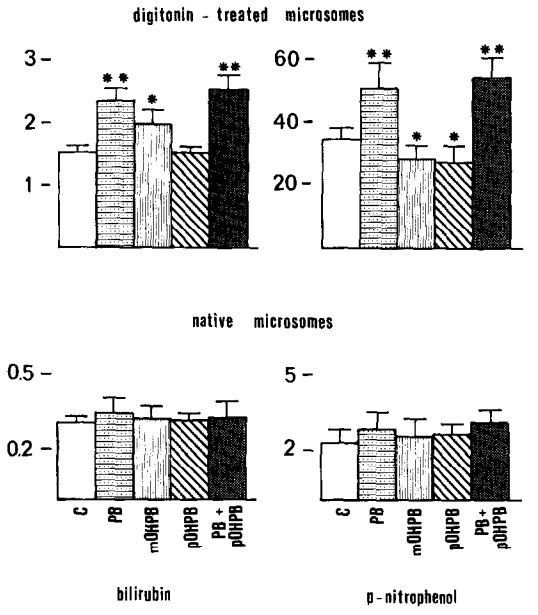

Fig. 3. Effect of *in vivo* barbiturate administration on UDP-glucuronosyltransferase activities from adult rat liver. Results are mean ± S.D. for 4–6 microsomal preparations and expressed as nmoles of bilirubin conjugated per min per mg microsomal protein (left rows) or *p*-nitrophenol (right rows). Incubations were performed with native microsomes (lower rows) or with digitonin-treated microsomes (upper rows). C: controls; PB, *m*OHPB, *p*OHPB, PB + *p*OHPB refer to animals pretreated, respectively, with 80 mg/kg PB, *m*OHPB, *p*OHPB and the combination of PB and *p*OHPB for 4 days.

treatment with barbiturates (Fig. 3). When microsomes were activated by digitonin treatment, significant differences due to induction were observed: pretreatment by PB or by PB + *p*OHPB resulted in an increased conjugation activity towards both bilirubin and *p*-nitrophenol. Both hydroxylated PB metabolites depressed the conjugation activity towards *p*-nitrophenol, whereas only *m*OHPB produced a slight increase of bilirubin conjugation. The activation of conjugation (activated to native activities ratio) due to digitonin treatment was higher in microsomes from PB- or PB + *p*OHPB- treated animals than in microsomes from animals pretreated by *m*OHPB or *p*OHPB alone.

The addition of *p*OHPB or *m*OHPB (1 mM final concentration) in incubation mixtures did not inhibit monooxygenase or conjugation activities when tested *in vitro*.

Gel electrophoresis of microsomes from the liver of barbiturate-treated rats are shown in Fig. 4. Electrophoretograms of microsomes from animals pretreated with *p*OHPB or *m*OHPB were similar to those from controls, whereas microsomes from PB-treated or PB + *p*OHPB-treated rats exhibited a large band with a molecular weight around 47,000 daltons. When rats were injected with the association PB + *p*OHPB, this band was less important than after pretreatment with PB only.

In order to estimate the binding capacity of control microsomes towards PB and its hydroxylated metabolites, spectral apparent K_S and the maximal binding were estimated by double reciprocal plots (Table 3). Spectral apparent K_S was slightly higher for hydroxylated metabolites than for PB; but maximal binding of *p*OHPB was half of PB binding or *m*OHPB binding on microsomes from control rats.

Table 3. Characteristics for binding of phenobarbital and its hydroxylated metabolites to liver microsomes from control rats*

Barbiturate	*A* max (375–412 nm) 10^{-3} × nmoles^{-1} cyt P-450	K_S (mM)
PB	25.5 ± 3.6	1.70 ± 0.30
*m*OHPB	28.5 ± 2.9	3.70 ± 1.10
*p*OHPB	14.8 ± 2.9	3.00 ± 0.30

* Maximal binding (expressed per nmole cyt P-450) and spectral apparent K_S of type I cytochrome P-450–barbiturate complex were calculated by double reciprocal plots. Results are mean ± S.D. for 5 different microsomal preparations.

DISCUSSION

Both endogenous and exogenous compounds are metabolized in liver by endoplasmic reticulum, a membrane which contains several drug-metabolizing enzymes such as monooxygenase, epoxide hydrolase and UDP-glucuronosyltransferase, but also enzymes of the intermediary metabolism such as glucose-6-phosphatase. A number of chemicals administered in a sufficient amount over several days may accelerate drug metabolism via an induction of drug-metabolizing enzymes.

When injected to adult animals, phenobarbital (PB) produces both a proliferation of smooth endoplasmic reticulum [1–4] and a net biosynthesis of cytochrome P-450 [5, 6], epoxide hydrolase [7, 8] and UDP-glucuronosyltranferase [9–12]. In rat foetus, PB has no inducing effect on total cyt P-450 content of liver cells [23, 39], but produces a premature transformation of endoplasmic reticulum, leading to an increase of monooxygenase specific activities [23, 40]. However, the mechanism which triggers those two events remains unknown.

In liver, PB is hydroxylated by a cyt P-450-dependent monooxygenase mainly into *p*OHPB which is conjugated with glucuronic acid and excreted in urine [17–20]. Thus, PB, *p*OHPB or its glucuronide may be involved in the initiation of drug-metabolizing enzyme induction and the proliferation of smooth endoplasmic reticulum, two phenomena which are not necessarily linked: 3-methylcholanthrene induces cyt P-448 biosynthesis but does not produce smooth endoplasmic reticulum proliferation [4, 6].

As already shown, PB increases both the liver concentration of cyt P-450 (when expressed as nmoles per g tissue, Fig. 1) and its 'specific content' in microsomes (when expressed as nmoles per mg microsomal protein, Fig. 2), and decreases the glucose-6-phosphatase to cyt P-450 ratio. *p*OHOB only moderately increases the hepatic concentration of cyt P-450 and markedly decreases the specific cyt P-450 content of microsomes. In addition, the glucose-6-phosphatase to cyt P-450 ratio was significantly increased by *p*OHPB administration. Such an obser-

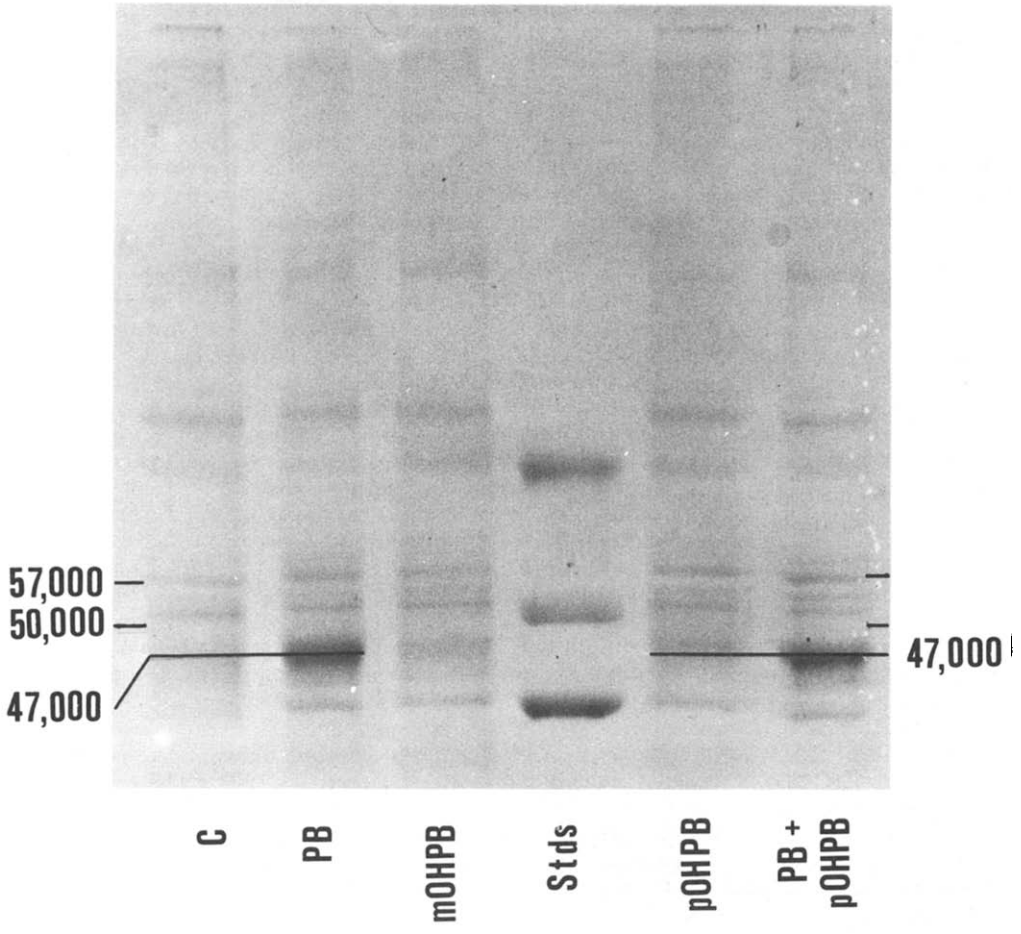

Fig. 4. SDS–polyacrylamide gel electrophoresis of liver microsomes from barbiturate-treated rats. Ten micrograms of protein were applied to the wells of 1 mm analytical gels. Standards (STDS) indicates molecular weight of 68,000, 50,000 and 41,000. Electrophoretic migration was from top to bottom. C: controls; PB, *m*OHPB, *p*OHPB, PB + *p*OHPB refer to animals pretreated, respectively, with 80 mg/kg PB, *m*OHPB, *p*OHPB and the combination of PB and *p*OHPB for 4 days.

vation may probably be explained by a proliferation of endoplasmic reticulum, without simultaneous biosynthesis of cyt P-450. Thus, PB may increase the insertion of cyt P-450 into endoplasmic reticulum membranes, whereas *p*OHPB may induce only the proliferation of endoplasmic membranes relatively devoid of cyt P-450.

The effect caused by a combination of PB and *p*OHPB is the sum of each individual effect. The increase of cyt P-450 concentration in crude liver homogenate was similar to that elicited by PB alone, but the increase of cyt P-450 content in isolated microsomes was smaller than for PB alone; the glucose-6-phosphatase to cyt P-450 ratio was less decreased than by PB pretreatment. Since cyt P-450 concentration in liver homogenate was similar for PB and PB + *p*OHPB administrations, the latter result is indicative of a higher proliferation of endoplasmic reticulum membranes than for PB pretreatment alone. A similar situation was observed with epoxide hydrolase which was increased in parallel with monooxygenases after PB pretreatment in adults [49] and, at least in part, with NADPH-cyt *c* reductase (Fig. 2).

The native UDP-GT was unaffected by animal pretreatment with barbiturates (Fig. 3), as previously observed with PB [9, 11, 12]. After pretreatment of microsomal membranes with digitonin, UDP-GT was activated and some differences were observed with respect to induction: PB enhanced the microsomal specific activity of both bilirubin and *p*-nitrophenol conjugations, whereas *p*OHPB administration depressed the specific activity of *p*-nitrophenol conjugation and was inefficient on bilirubin conjugation. Thus, as shown above for cyt P-450 and EH, the specific activity of the UDP-GT is increased by PB administration, but unaffected or decreased by pOHPB pretreatment. The association PB + *p*OHPB was as efficient as PB alone to enhance the maximal activity of both bilirubin and *p*-nitrophenol UDP-GT. This result might indicate different regulations of the mechanism of biosynthesis between UDP-GT on the one hand and cyt P-450 and EH on the other, as evoked by Burchell *et al.* [41].

The molecular weight of various purified, or partially purified, drug metabolizing enzymes was determined in rat liver: 47,000 (LM_2) or 53,000 (LM_4) for cytochromes P-450 [38], 49,000 for EH [42–44] and 47,000 for UDP-GT [45, 46]

In this study, a large band around 47,000, which may include different proteins with similar molecular weights, was markedly increased by PB or PB + *p*OHPB pretreatment, whereas the electrophoretograms of microsomes from *m*OHPB- or *p*OHPB-treated rats were identical to those from controls (Fig. 4). Thus, there is no apparent accumulation of cyt P-450 apoprotein in liver microsomes from hydroxylated PB-treated rats.

These data suggest that *p*OHPB has no quantitative inducing effect on the biosynthesis of drug-metabolizing enzymes. However, the biosynthesis of specific forms should be considered. The inhibition of ethoxycoumarin deethylase by metyrapone, which is mainly associated with PB-induced cyt P-450 [47, 48], was comparable in microsomes from controls and *p*OHPB-treated rats. In addition, this inhibition was quite similar between PB-treated and PB + *p*OHPB treated animals. The molecular catalytic activity of the specifically PB-induced benzphetamine demethylase [37] was slightly enhanced by *p*OHPB but did not reach the high molecular activity of PB-induced cyt P-450.

*m*OHPB, which is not an important biological metabolite of PB [20], also produced a proliferation of endoplasmic reticulum associated with a biosynthesis of cyt P-450. However, this synthesis was less than after PB pretreatment. The reason for this difference between the effects of *m*OHPB and *p*OHPB is unknown. Its biological importance must not be overestimated, in regard to the poor *meta*-hydroxylation of PB in the rat liver [19, 20].

This study raises a fundamental objection: does *p*OHPB, as PB [49, 50], really accumulate in liver cells in sufficient amounts to produce its full potential effects? In the present work, the intracellular concentration of *p*OHPB was not measured, but indirect answers may be proposed. The cellular concentration of *p*OHPB depends on (i) the permeability of liver plasma membrane towards this compound and (ii) the rate of its metabolization. The partition coefficients of PB and *p*OHPB between aqueous buffers and organic solvents and, as a consequence, their solubility in the plasma membrane are quite different. However, (a) Valerino *et al.* [15] concluded from their study on the inducing capacity of barbiturates that there is no relationship between partition coefficient and induction; (b) the intrahepatic *p*OHPB concentration was sufficient to trigger a proliferation of endoplasmic reticulum. An objection which may be raised is that the concentration of *p*OHPB was not sufficient to promote a net biosynthesis of drug-metabolizing enzymes. Thus, in another set of experiments, *p*OHPB was injected at a 5-fold higher concentration (400 mg/kg): a proliferation of endoplasmic reticulum membranes was observed but no cyt P-450 biosynthesis (data not shown).

The metabolizing capacity of normal rat liver towards *p*OHPB has been estimated to 0.40 μmoles of barbiturates conjugated per hr per g of liver [51]: less than one tenth of the dose administered daily (80 mg/kg) can be metabolized through this pathway by rat liver.

Valerino *et al.* [15] and Ioannides and Parke [16] explained the inducing capacity of PB partly by its low metabolization rate in hepatocytes. In a recent report, Tsyrlov *et al.* suggest that the activation of protein synthesis is affected by PB itself [52]. In this study, it was observed that the binding capacity of cyt P-450 from control microsomes towards *p*OHPB and *m*OHPB was not very different from that of PB; however, the hydroxylated derivatives were not potent inducers of drug-metabolizing enzymes. Thus, as generally accepted, the binding of PB to microsomal cyt P-450 is probably not the primary event which triggers enzyme induction.

The proliferation of endoplasmic reticulum, which is produced by hydroxylated PB metabolites as well as PB itself, should depend on a mechanism different from that leading to enzyme induction.

In conclusion, three different effects of exogenous compounds upon endoplasmic reticulum may be described:

(a) simple induction of drug-metabolizing activities without proliferation of membranes, as produced by polycyclic hydrocarbons, and proposed here as the first step of PB action;

(b) simultaneous induction of enzyme activities and proliferation of membranes as produced by the total sequence of events involving PB;

(c) proliferation of membranes, without enzyme induction, as produced by *p*OHPB. Further studies on the proliferative effects of *p*OHPB on endoplasmic reticulum are presently in progress.

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