Induction of Cytochrome P-450 and 5-Aminolevulininate Synthase Activities in Cultured Rat Hepatocytes

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ABSTRACT

Cytochromes P-450IIB1 and P-450IIB2 were recently shown to be inducible in rat hepatocyte cultures maintained on a reconstituted extracellular tumor matrix (Matrigel) as indicated by increases in P-450IIB1 and -IIB2 mRNAs and immunoreactive proteins (J. Cell. Physiol., 134: 309–323, 1988). Here we show that treatment of cultured rat hepatocytes with phenobarbital and other compounds known to induce P-450IIB1/2 in vivo increased spectral cytochrome P-450, immunoreactive proteins, and benzoyloxy- and pentoxy-resorufin dealkylases, activities known to be specific for cytochrome P-450IIB1/2. These increases were observed when cells were cultured on either Matrigel or collagen matrix in Williams E medium. Cytochrome P-450III was also increased by phenobarbital and dexamethasone on either matrix. Propoxyconuramin depropylase activity, which has been proposed as a specific activity catalyzed by cytochrome P-450III, was increased 3–4-fold more by treatment with 3-methylcholanthrene than by phenobarbital or dexamethasone. The activity catalyzed by P-450III could be distinguished from that catalyzed by other P-450 forms using the specific inhibitor triacetyloleandomycin. Benzoyloxysresorufin dealkylase was also increased in these cells by treatment with 2,4,5,2',4',5'-hexachlorobiphenyl, glutethimide, or mephénytoin. Treatment with phenobarbital or 2-allyl-2-isopropylacetamide slightly induced 5-aminolevulinate synthase activity. 5-Aminolevulinate synthase activity was slightly increased in cells treated with phenobarbital or 2-allyl-2-isopropylacetamide. Succinyl acetone also induced 5-aminolevulinate synthase activity and, in combination with either of the other two drugs, synergistically increased the enzyme activity regardless of whether cells were cultured on collagen or Matrigel. These results indicate that with simple and economical enzyme assays for holocytochrome P-450 and 5-aminolevulininate synthase, the rat hepatocyte culture system can be used for studies of the interrelationships between phenobarbital induction of cytochrome P-450 and heme metabolism.

INTRODUCTION

The cytochrome P-450s are a multigene family of hemoproteins that catalyze the oxidative metabolism of many endogenous and foreign substances. Many of these substances are also inducers of some of the forms of cytochrome P-450. For example, administration of phenobarbital to rats increases the amount of cytochrome P-450IIB1/2 in the liver (2). However, the mechanism of this adaptive response to phenobarbital and similar compounds has been difficult to characterize in vivo. Many workers have used cultured hepatocytes to study activation of hepatotoxins and carcinogens (see review in Ref. 3). Although such cultures express a wide array of liver-specific functions (4, 5), they fail to respond with induction of all the various forms of cytochrome P-450 that can be induced in the intact liver. In addition, rat hepatocytes in culture rapidly lose certain forms of cytochrome P-450 as indicated by spectral, enzymatic, and immunological studies (e.g., Refs. 6–9). For example, the content as well as the induction of the major PB*-induced forms, P-450IIB1 and P-450IIB2 (also known as b and e), and the MC-induced P-450I2A2(d) are reported to be greatly decreased or undetectable compared to the intact liver (7, 8, 10, 11). Although less well studied, the first enzyme of heme biosynthesis, ALA synthase, which is readily inducible in liver by chemicals that also induce P-450IIB1/2, has also been found to be poorly inducible in these cultures (12). In contrast, P-450III(p) and IA1(c) are highly inducible in rat hepatocyte cultures by phenobarbital and methylcholanthrene, respectively (8, 13). These results from many workers suggest that rat hepatocyte cultures are limited for studies of compounds such as carcinogens that require P-450-mediated activations.

The previous studies were made with cultures of hepatocytes maintained on plastic dishes precoated with a thin layer of collagen. Recently, Schuetz et al. (14) substituted for collagen a reconstituted biomatrix (Matrigel) extracted from a mouse sarcoma and found that when rat hepatocytes were exposed to PB in culture, P-450IIB1/2 mRNA and immunoreactive apoproteins accumulated. In this paper, we report the PB induction of cytochrome P-450IIB1/2 and III to near in vivo levels in rat hepatocyte cultures maintained in Williams E medium either on Matrigel or on other dish substrata. Increases in the active holoproteins of these cytochrome P-450 forms were shown by spectral and immunological techniques as well as by specific enzyme assays. Propoxyconuramin depropylase was investigated as a specific enzymatic indicator of P-450III induction. We also report that ALA synthase can be highly induced in rat cultures maintained in Williams E medium by PB when combined with succinyl acetone, an inhibitor of heme biosynthesis.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (180–200 g) were obtained from Charles River Laboratories (Wilmington, MA) and maintained in a controlled environment with 12-h light/dark cycles. Animals were fed rat chow and tap water ad libitum. For comparison of induced P-450s in culture and in vivo, a rat (180–200 g) was given an injection of PB (in saline), 100 mg/kg i.p., once daily for 3 days. After killing, the liver was perfused with saline and a 20% (w/v) homogenate was prepared in 20 mM Tris-HCl, pH 7.8. The homogenate was diluted to 2% (w/v) for assays.

Preparation of Hepatocytes. Hepatocytes were prepared from untreated male rats using modifications of the procedure of Bissell and Guzelian (15). Calcium-free [ethylenebis(oxoethylenenitrilo)tetraacetic acid] buffer was perfused through the vena cava, followed by 100 mg collagenase (Wako Chemical Co., Dallas, TX) in 200 ml 10 M 4-(2-hydroxyethyl)-1-piperazinethane sulfonic acid-20 mM CaCl2 buffer containing 10 mg trypsin soybean inhibitor (Sigma Chemical Co. St. Louis, MO) followed by 10 mg collagenase. Hepatocytes were washed with saline and resuspended in 20 ml of a 10% (w/v) collagenase-free gelatin solution in a 100 ml tissue culture flask. After 15 min, the hepatocytes were collected, washed with 3 ml of 3.8% (w/v) bovine serum albumin saline, centrifuged, and resuspended in 1 ml 20 mM Tris-HCl, pH 7.8, for a final concentration of 1×10⁷ cells/ml. The harvested cells were then counted in a hemocytometer.

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3 The PB-450 nomenclature used here corresponds to the family classification (1).
Louis, MO). The collagenase solution was aerated with 95% oxygen/5% carbon dioxide. The partially digested liver was removed to a flask containing 35 ml collagenase buffer and 6 mg collagenase. The liver was minced with scissors and incubated for 10 min at 37°C. The suspension was filtered through sterile gauze and washed twice by centrifugation at 50 x g with Williams E medium containing 1.7 mM insulin (porcine; Sigma) and penicillin G/streptomycin (100 units/ml each) (GIBCO Laboratories, Grand Island, NY). Cell yield was typically in excess of 250 million cells/liver, with viability above 85% as determined by trypan blue exclusion. Tissue culture dishes were precoated with Vitrogen (type 1 collagen; Collagen Corp., Palo Alto, CA) or with Matrigel. Matrigel was prepared from Engelbreth-Holm-Swarm tumor passages in mice, essentially as described (16), and stored frozen. Falcon Primaria dishes (Fisher Scientific, Boston, MA) were not further coated. Hepatocytes were seeded at approximately 3 million cells/6-cm dish in 3 ml Williams E medium containing 1.7 μM insulin plus 0.1 μM dexamethasone phosphate (injectable solution; ESI Pharmaceuticals, Cherry Hill, NJ), freshly dissolved ascorbic acid (25 μg/ml) and penicillin G/streptomycin as antibiotic. This concentration of dexamethasone phosphate, under our conditions, does not induce increase in cytochrome P-450III-immunoreactive protein (Fig. 2). Where used, human growth hormone (somatotropin) extracted from human pituitaries was from Sigma. The medium volume was 3 ml/dish and was changed every 24 h. The incubator temperature was 37°C and the atmosphere was 5% CO₂ in air. Inducing chemicals were added to cultures 48 h after inoculation as indicated in the figure legends. MC (puriss grade; Fluka, Ronkonkoma, NY) and DEX (Sigma) were added in DMSO (Fluka, puriss grade) (2 μl/ml culture medium). PB (Baker Chemical Co. Phillipsburg, NJ) and SA (U. S. Biochemical Corp., Cleveland, OH) were filter-sterilized aqueous solutions. Chemicals were added each day with each daily medium change as indicated in figure legends.

Enzyme Assays. Where indicated, Matrigel was separated from cells after harvesting. Cells were scraped in 2 ml 10 mM sodium EDTA-20 mMTris-HCl, pH 7.4 at 4°C (14). After 30 min on ice, cells were collected at 50 x g for 2 min and resuspended in 2 ml 20 mM Tris-HCl, pH 7.8.

Cells were sonicated for 6 s with a Heat Systems W140D sonicator equipped with a microtip. Alkoxynitroresorufin-O-dealkylase assays were performed on sonicates essentially as described (17). The assay for alkoxynitroresorufin and propoxyxycoumarin dealkylates contained 100 μl sonicate/assay and 10 μM dexamethor to inhibit diaphorase (18). The final concentrations of ethoxy-, pentoxy-, and benzyloxyresorufin (Mo

This procedure recovered greater than 80% of the total sonicate cytochrome P-450 and increased specific activity by 3–4-fold over that in the cell sonicate. Liver homogenate cytochrome P-450 was assayed as described (23).

Immunoblots. Samples were immunoblotted essentially as described (24). Samples were separated by electrophoresis in sodium dodecyl sulfate-10% polyacrylamide, transferred to nitrocellulose sheets electrophoretically at 100 V for 1 h, and the sheets were blocked by 2 h incubation in 0.3% Tween 20 in PBS. The sheets were rinsed and treated sequentially, with intermediate rinsing, with the H-8 mouse monoclonal anti-P-450IIB1/2 in 0.05% Tween 20 in PBS or with goat polyclonal anti-P-450III in 1% nonfat powdered milk in PBS containing 0.05% Tween 20. The sheets were then incubated with anti-mouse or anti-goat IgG with conjugated alkaline phosphatase (Bio-Rad, Richmond, CA) and the color was developed using 5-bromo-4-chloro-3-indolyloluidine phosphate and p-nitroblue tetrazolium chloride (Bio-Rad) according to the manufacturer's instructions. The monoclonal H-8 antibody against P-450IIB1/2 was a gift from Dr. M. Adesnick, New York University Medical Center, New York, NY.

Protein was determined with bovine serum albumin as standard by the method of Lowry et al. (25). To correct for Matrigel protein when not removed, the Matrigel substratum was harvested from a parallel set of dishes that were not inoculated with cells.

RESULTS AND DISCUSSION

Comparison of Different Substrata in Supporting Induction of Cytochrome P-450IIB1/2. Previously Schuetz et al. (14) reported that cells maintained on Matrigel were inducible by PB for cytochrome P-450IIB1/2, based on increases in mRNA and immunoreactive protein, whereas cells maintained on Vitrogen were inducible for these forms of cytochrome P-450. We chose two alkoxynitroresorufin dealkylase activities to follow induction of active P-450IIB1/2. PROD is an activity specifically catalyzed by P-450IIB1/2 and BZROD is a similar but much more sensitive reaction that readily detects induction of cytochrome P-450IIB1/2 by phenobarbital (26). Fig. 1 shows the effect of substratum on PB induction of these activities in cells maintained in Williams E medium. PB significantly induced BZROD (Fig. 1A) and PROD (Fig. 1B) activities regardless of whether cells were maintained on Matrigel, Vitrogen, or Primaria substrata. Northern blot analysis of RNA extracted from such cultures showed that P-450IIB1/2 mRNA was induced by PB in cells cultured on Matrigel to a greater extent when compared to cells cultured on Vitrogen (data not shown), a result consistent with our previously published work (14).

Fig. 2 shows that the proteins for P-450IIB1/2 and -III were induced in these cells by PB on both Matrigel and Vitrogen. Most basal forms of P-450 are lost during the 3 days before cells were treated with inducers. However, a low molecular weight protein (possibly P-450F) detected by the anti-IIB1/2 monoclonal antibody appears to be well retained and unresponsive to the inducers we tested, as observed previously (27). The responses for all inducers were somewhat better on Matrigel than on Vitrogen. There was more PB induction of BZROD activity in cells maintained on Matrigel than in the experiment shown in Fig. 1. The DEX is an inducer of P-450III mRNA and immunoreactive protein in rats and in rat hepatocytes in culture (14). Although DEX effectively induced total cytochrome P-450, Fig. 2 shows that DEX did not induce P-450IIB1/2 proteins and only weakly induced BZROD activity. MC induced the P-450III protein to a small extent but was a good inducer of P-450 1A1 as indicated by increased activity for ethoxyresorufin deethylase (see legend to Fig. 2).

The relative amounts of P-450IIB1/2 in the culture were compared to the amounts in liver homogenate from a rat treated...
with PB in a standard protocol. The amount of total spectrally determined P-450 induced by PB in the culture was 65%, that of BZROD was 40%, and that of PROD was about 30%, respectively, of the values obtained with liver from a PB-induced rat. These induced levels in the culture are the closest yet reported to induced levels in vivo. These results suggest that PB induces a higher proportion of forms of P-450 other than IIB1/2 and III in cultured hepatocytes than in the liver in vivo or, alternatively, that the induced P-450 was to some extent inactive.

Previously it was reported that Matrigel but not Vitrogen supported PB induction of P-450IIB1/2 (14). The finding reported here, that Matrigel and Vitrogen both supported PB induction of P-450IIB1/2 in rat hepatocytes, may have been due to the use of a modified Weymouth medium in the previous study rather than the Williams E used here. Under conditions used in the present study, PB induced BZROD in cells maintained in modified Weymouth medium on Matrigel to only 20–40% of the BZROD activity inducible in cells maintained in Williams E medium. Turner et al. (28) have noted that some media are relatively inhibitory to PB induction of P-450IIB1/2 in rat hepatocytes in culture. However, the induction of PROD reported by that group in their optimal medium (29) was much less than that observed here.

One possible factor in the lower induction of BZROD in the modified Weymouth medium was the relatively high phosphate content of Weymouth (6.5 mM) and some differences in amino acid content. Addition of this amount of phosphate to Williams E medium was highly inhibitory to PB induction of BZROD and total P-450 (data not shown). However, the ability of Weymouth medium to support induction of BZROD was not improved by adding CaCl2, pyruvate, alanine, arginine, leucine, biotin, and riboflavin to the concentrations found in Williams E medium to Weymouth medium prepared with the low phosphate content of Williams E (1.4 mM). Thus, rather than any single factor, there seem to be multiple components in Williams E medium responsible for the improved induction of BZROD. No further attempts were made to identify the combination of factors required to produce the dramatic difference observed.

Fig. 3 shows the effect of hormones on the induction of BZROD by PB. DEX and insulin alone increased the induced activity and the combination of both hormones produced an additive increase. Triiodothyronine had no effect beyond that of DEX alone. Human growth hormone was highly inhibitory for induction of BZROD activity agreeing with the recent report that this hormone inhibits PB-induced increases in P450-IIB1/2 mRNA in cultured rat hepatocytes (30). Fig. 4 shows the time course of induction by PB of BZROD under the optimal hormone supplemnetations. There was no induction for the first 2 days in culture. BZROD was highly induced during the third and fourth days in culture with a somewhat greater increase if the PB exposure was for 48 rather than for 24 h. The lack of response in the early period of the culture has been noted previously (10, 13) but has not been explained. In the fourth day of culture, the maximal induction of BZROD was at about 1 mM PB with no further increase at 2 mM. A 50% response occurred at 0.25 mM PB and there was no decrease at 2 mM (data not shown). A similar dose response was obtained for the PB-induced increase in spectrally detected P-450 (data not shown).

As shown in the immunoblot (Fig. 2), DEX strongly (and PB to a lesser extent) induced the protein moiety of P-450IIB1III, in agreement with previous results (13, 14). DEX did not induce cytochrome P-450IIB1II proteins or BZROD in cultured rat hepatocytes whereas PB induced both III and IIB1/2 proteins as shown previously for mRNA in hepatocytes on the Matrigel as well as in intact rat liver (14).

PCOD as an Enzyme Assay for P-450III. Recently, Yamazoe et al. (31) suggested that dealkylation of propoxycoumarin is a specific activity for the DEX-inducible forms of P-450. As shown in Fig. 5, DEX, PB, and MC all induced PCOD, with MC treatment being the most effective. We used inhibition by TAO to determine the contribution of P-450IIB1/2 to the enzymatic activities assayed, since TAO forms an inactive substrate enzyme complex specifically with P-450III (32). Fig. 5 shows the effect of TAO on PCOD in sonicates of cells treated with DEX, PB, or MC. At 10 μM in the reaction mixture, TAO inhibited DEX-induced PCOD by 80%, PB-induced PCOD by 50% and MC-induced PCOD by only 15%. These results indicate that PCOD activity can be used as a specific assay for P-450IIB1III only when the degree of TAO inhibition is also measured. Namkung et al. (33) have noted the need to check TAO inhibition when attributing BZROD activity to various P-450 forms.

The catalysis of PCOD by MC-induced P-450, presumably P-450IIB1A1, has not been reported previously. PB-induced BZROD and PROD were not inhibited by TAO (data not shown) indicating a minimal role of P-450IIB1/2 in catalyzing these activities. Most of the PCOD activity in cells treated with MC and about 50% of the activity induced by PB appear not to be catalyzed by P-450IIB1II. These results are compatible with induction by PB of both P-450IIB1/2, the PCOD activity catalyzed by P-450IIB1II being inhibitable by TAO, whereas that catalyzed by P-450IIB1II/2 was not.
Fig. 2. Induction of benzyloxyresorufin dealkylase and P-450IIB1/2 and -III immunoreactive proteins by phenobarbital, methylcholanthrene, and dexamethasone in hepatocytes maintained on Matrigel or Vitrogen. Cells were maintained on 10-cm dishes for 4 days as described in “Materials and Methods.” For the last 2 days they were treated with 2 mM PB, 3.7 μM MC, or 10 μM DEX. Cells were collected, the Matrigel was removed and cells were sonicated, and the 100,000 x g pellet was prepared. Assays of cytochrome P-450 and immunoblots were performed with the detergent-solubilized pellet, and enzyme activities were determined in the sonicates. The first four lanes contained 7.5 μg protein from cells maintained on Vitrogen; the last four lanes contained 7.5 μg protein from cells on Matrigel and the middle lane contained 1 μg hepatic microsomal protein from a PB-treated rat. BZROD activity in the latter was 2.6 nmol/min/mg protein. Values for cytochrome P-450 (pmol/mg protein) were as follows. Vitrogen: control, 142; PB, 285; DEX, 105; MC, 358. Matrigel: control 152; PB, 301; DEX, 617; MC, 352. Values for ethoxyresorufin deethylase (pmol resorufin/min/mg protein) were as follows. Vitrogen: control, 7 ± 3; PB, 17 ± 0; DEX, 69 ± 13; MC, 516 ± 37. Values represent means of 2 determinations and their ranges.

Fig. 3. Effect of dexamethasone, insulin and triiodothyronine (T3) on the induction of BZROD by PB. Cells were cultured on Matrigel for 24 h in Williams E medium containing 1.7 μM insulin. After 24 h, the medium was changed and the following hormones were added as indicated (1.7 μM insulin, 0.1 μM DEX, and 0.2 μM triiodothyronine). PB at 2 mM was present in the medium from the 48th to the 96th h of culture. BZROD was measured in cell sonicates as described in “Materials and methods.” Data are presented as means for duplicate dishes; bars, range.

Effects of Compounds Other Than Phenobarbital to Increase BZROD. 2,4,5,2′,4′,5′-Hexachlorobiphenyl, 2,4,2′,4′-tetrachlorobiphenyl, glutethimide, and mephenytoin induce cytochrome P-450IIB1/2 in rat liver and also induce a M, 50,000 P-450 in chick hepatocyte cultures maintained on plastic dishes without any special substratum (24). Fig. 6 shows that these compounds induced BZROD in the rat hepatocyte cultures. In a separate experiment, 1 μM mitotane [1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane] and 0.5 μM chlordane induced BZROD as effectively as did PB.

Fig. 6 also shows that AIA induced only a small increase in BZROD. AIA also decreased the amount of BZROD induced by PB, an effect seen with as little as 0.14 mM AIA (Fig. 6). This result is consistent with the action of AIA to degrade hepatic P-450 heme (34, 35). Furthermore, Srivastava et al. (36) reported that in rat liver, AIA increased mRNA for P-
Fig. 5. Effect of triacetyloleandomycin on propyloxycoumarin depropylase activity induced by dexamethasone, phenobarbital, and methylcholanthrene. Cells maintained on Matrigel or Vitrogen were removed as described in "Materials and methods." The enzyme activity was determined in sonicates in the presence (3) or absence (3) of 10 μM TAO. Values represent duplicate determinations with ranges (bars). CONT, control (untreated).

Fig. 6. Effect of various compounds to induce benzoyloresorufin dealkylase activity in hepatocytes maintained on Matrigel. Cells on 6-cm dishes were maintained in culture, during the last of which they were exposed to 2 μM PB, 0.7 μM AIA, 13 μM 2,4,5,2',4',5'-hexachlorobiphenyl (HCB), 15 μM 2,4,2',4'-tetrachlorobiphenyl (TCB), 46 μM glutethimide (Glut), or 138 μM mephenytoin (MepH). Data represent means and ranges (bars) for 2 separate dishes.

Fig. 7. Induction of 5-aminolevulinate synthase activity in hepatocytes maintained on Matrigel or Vitrogen. Cells were maintained in culture, during the last 2 days of which they were exposed to 2 μM PB, 0.7 μM AIA, 13 μM 2,4,5,2',4',5'-hexachlorobiphenyl (HCB), 15 μM 2,4,2',4'-tetrachlorobiphenyl (TCB), 46 μM glutethimide (Glut), or 138 μM mephenytoin (MepH). Data represent means and ranges (bars) for 2 separate dishes.

with SA plus either PB or AIA, there was a synergistic induction of ALA synthase to high levels of activity not previously attained in rat hepatocyte cultures. Such synergistic induction of ALA synthase activity caused by SA has been reported in chick hepatocyte cultures by others (37-40). The response was similar whether cells were maintained on Matrigel or Vitrogen. The data of Fig. 7 were obtained with cultures treated for 24 h from the third to the fourth days in culture. Similar activities were reached on the third day as early as 7 h after treatment. ALA synthase was not inducible by PB plus SA during the first 2 days in culture, a result similar to that for PB-induced BZROD. As with induction of BZROD by PB, the maximum inductions of ALA synthase by PB plus SA required both insulin and dexamethasone, the activity being doubled by the presence of both hormones compared to the effect of either alone. Furthermore, as reported for induction of ALA synthase mRNA (30), human growth hormone was highly inhibitory to the induction of ALA synthase activity. Doses of 0.1 to 0.25 mM SA maximally synergized the PB induction of ALA synthase.

SA, an inhibitor of ALA dehydrase (38), by itself induced ALA synthase activity quite effectively, an induction usually attributed to depletion of the free heme pool that represses ALA synthase transcription (40-42). SA alone also increases ALA synthase mRNA in vivo (36) and in rat hepatocyte cultures (30). However, in chick hepatocyte cultures, induction of ALA synthase mRNA appears to require other factors than changes in heme levels (43). Further work is required to determine whether the mechanism by which SA alone increases ALA synthase in the rat cultures is due to its inhibition of heme synthesis or to other effects of the compound.

Conclusions. This paper describes conditions for optimal induction of the major phenobarbital-inducible cytochrome P-450 in rat hepatocyte cultures. A major point is that with the use of Williams E medium, P-450IIIB1 and P-450IIIB2 are highly inducible regardless of the matrix on which cells are maintained. Benzoyloresorufin dealkylase is shown to be a sensitive fluorescent assay to follow induction of these P-450 forms and the level of the induced activity is closer to in vivo levels than previous reports. A major advantage of using BZROD is that this activity is not catalyzed by P-450III, unlike other assays such as benzphetamine and ethylmorphine demethylases (44). The dealkylation of propoxycoxumarin has also been reported by others to be a specific assay for P450III. However, we found this to be true only if the activity is checked with a specific inhibitor.

We also report for the first time that ALA synthase, the rate-limiting enzyme of heme biosynthesis, is highly inducible in rat cultures contrary to earlier reports of others. The high inducibility was due to our strategy of combining treatment with phenobarbital with an inhibitor of heme synthesis, succinyl acetone.

Together, these findings greatly extend the usefulness of rat hepatocyte cultures in duplicating the variety of responses of the intact liver in tissue culture. The simple and sensitive assays readily allow study of many variables (e.g., dose response and time course) in P-450II and P-450III responses with a relatively small amount of cells. Thus these cultures should now be of much greater use for studying the activation of carcinogens in intact liver cells by various forms of P-450, particularly the involvement of P-450IIIB1/2. Since many tumor promoters (including phenobarbital, 2,4,5,2',4',5'-hexachlorobiphenyl, and hexachlorobicyclohexane) are also inducers of P-450IIIB1/2 (45) the culture could also be used as a simple screen for testing chemicals as being potential promoters even though it would
not detect promoters such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and methanol which are not inducers of P-450IIIB1/2. The promoting activity of phenobarbital and similarly acting compounds is probably not related to the stimulation of P-450IIIB1/2 but rather to increases in liver weight and DNA synthesis (46).

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