Stimulation of *de novo* synthesis of cytochrome *P*-450 by phenobarbital in primary nonproliferating cultures of adult rat hepatocytes

(selenium/differentiation/induction/immunoprecipitation)

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ABSTRACT   Primary monolayer cultures of nonproliferating parenchymal cells prepared from adult rat liver and maintained in serum-free medium responded to additions of phenobarbital with concentration-dependent increases in synthesis and accumulation of a cytochrome *P*-450 protein immunochemically and catalytically indistinguishable from that found in the livers of adult rats treated with phenobarbital. Maximal stimulation of the rate of synthesis of this cytochrome protein by phenobarbital, as much as 20-fold higher than in control cultures (1.01% of the rate of synthesis of total cellular protein), could be achieved when the drug was first added to cultures no older than 24 hr and then was maintained in the medium for 96 hr. In addition to phenobarbital, chemicals classified as "phenobarbital-like" inducers *in vivo* (mephenytoin, mirex, 2,2',4,4',5,5'-hexabromobiphenyl) induced synthesis in culture of this same immunoreactive protein. Supplementation of the medium with 0.1 μM H$_2$SeO$_3$ plus phenobarbital produced an average 2-fold enhancement in the rate of synthesis of this inducible cytochrome protein as compared to that in cultures receiving phenobarbital alone. Inasmuch as there was a decline in selenium content and in the activity of the seleno-enzyme glutathione peroxidase in hepatocyte cultures maintained in standard culture medium for more than 24 hr, the added selenium appears to correct a spontaneously acquired cellular deficiency in selenium. Contrary to the concept that liver cells placed in culture promptly dedifferentiate with general loss of specialized functions such as cytochrome *P*-450, our data demonstrate that expression of the phenobarbital-inducible form of cytochrome *P*-450 is not extinguished in culture, but rather it is masked transiently and is attenuated as the cells adapt to the imperfect conditions of the culture environment.

"Cytochrome *P*-450" is a collective term for a group of isoenzymes located prominently in liver microsomes that catalyze the oxidation of a diverse array of environmental chemicals. Although it has been known for over a decade that the hepatic content of cytochrome *P*-450 increases in animals treated with such lipophilic chemicals as phenobarbital (PB) or 3-methylcholanthrene (1, 2), the mechanism of cytochrome *P*-450 induction is still poorly understood. Progress in this area has been hindered by the lack of practical methods to measure individual forms of cytochrome *P*-450 specifically and by the lack of *in vitro* systems of the liver that carry out drug-mediated induction of cytochrome *P*-450. Whereas many of the liver cell culture systems examined to date appear capable of responding to 3-methylcholanthrene with induction of forms of cytochrome *P*-450 that predominate in fetal liver (3), there has been no conclusive evidence that either maintenance cultures of hepatocytes or continuously dividing liver cell lines respond to PB with in-

creased *de novo* synthesis of the major form of cytochrome *P*-450 isolated from adult rats treated with PB (*P*450$_{PB}$) (4–8). A widely accepted interpretation of these findings is that hepatocytes in culture undergo embryonization, with loss of many differentiated functions of adult liver, including induction of *P*450$_{PB}$ (3, 5, 9, 10).

Recently, we challenged this concept by demonstrating that, when primary monolayer cultures of adult rat hepatocytes are exposed to a synthetic steroid, pregnenolone-16α-carbonitrile (PCN), there is a dramatic increase in *de novo* synthesis of a form of cytochrome *P*-450 protein immunochemically and electrophoretically identical to the *P*450$_{PCN}$ isolated from adult rats treated with PCN (11). In view of this finding, the apparent failure of PB to induce *P*450$_{PB}$ in cultured hepatocytes cannot be attributed to a generalized defect in genetic expression of the cytochromes *P*-450 found in mature rat liver. Therefore, we have reexamined the effects of PB in the same hepatocyte culture system, using a specific and quantitative immunochemical method for measuring the synthesis and degradation of *P*450$_{PB}$ and giving particular attention to the presence or absence of factor(s) that might affect expression of this cytochrome protein specifically. We now present unequivocal evidence that, when cultured hepatocytes are incubated under appropriate conditions and examined at an optimal age, exposure to PB elicits increased *de novo* synthesis and apparent accumulation of *P*450$_{PB}$. This phenomenon may have escaped previous notice because PB is relatively ineffective unless the serum-free culture medium is supplemented with selenium, a trace element.

MATERIALS AND METHODS

Materials. Male Sprague–Dawley rats (175–200 g) were purchased from Flow Laboratories (Dublin, VA) and were housed in wire-bottomed cages with free access to chow and water. [^{3}H]Leucine (120 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) was purchased from New England Nuclear, and sodium [^{75}Se]selenite (3.69 Ci/mmol) was from Amersham. Mephenytoin was a gift from Sandoz Pharmaceuticals, and 2,2',4,4',5,5'-hexabromobiphenyl was a gift from Steven Aust.

Hepatocyte Monolayer Cultures. Cultures were prepared as described (12). Freshly isolated hepatocytes were prepared by perfusion of the liver *in situ* with collagenase (0.036%) dissolved in complete culture medium consisting of a modification of Waymouth MB-752/1 medium (13) supplemented with 1 μM testosterone and 1 μM corticosterone; the cells were placed in 3 ml of culture medium in 60-mm plastic dishes coated with

Abbreviations: PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; *P*450$_{PB}$ and *P*450$_{PCN}$ refer to the major forms of hepatic cytochrome *P*-450 purified from rats treated with PB or PCN.
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rat tail collagen. In all experiments, media were renewed every 24 hr.

Synthesis of Cytochrome P-450 Protein in Hepatocyte Culture. Details of the immunoprecipitation assay have been described (11). Freshly isolated hepatocytes or monolayer cultures of various ages were pulse-radiolabeled for 4 hr with leucine-free culture medium to which [^{3}H]leucine had been added to give 3.3 μCi/ml. Then, the cells from three dishes were washed twice with iced phosphate-buffered saline (11), removed from the dishes by scraping into the same buffer, and centrifuged at 1,000 $\times$ g for 5 min. The cells were lysed by sonication and cytochrome P-450 was quantitatively extracted with detergents and immunoprecipitated with form-specific anti-P450$_{PB}$ IgG. The immunoprecipitate was subjected to electrophoresis on polyacrylamide slab gradient gels, and the radioactive material corresponding in mobility to purified P450$_{PB}$ protein was excised from the gel and quantitated by liquid scintillation spectrometry. Total radioactivity in this gel band minus that from a parallel control reaction using nonimmune IgG was divided by total radioactive trichloroacetic acid-precipitable protein in the sample and expressed as the rate of synthesis of P450$_{PB}$ protein relative to that of total cellular protein. We selected 4 hr as a pulse-labeling interval because this was the shortest time that gave acceptable net incorporation of isotope into P450$_{PB}$ (at least 100 dpm above background). The minimal rate of synthesis of P450$_{PB}$ detectable with the assay ranged between 0.02% and 0.06%, depending on the background value. Preliminary experiments provided evidence that incorporation of isotope into P450$_{PB}$ during this 4-hr interval largely reflects de novo synthesis. Thus, relative synthesis was the same with either a 4-hr or a 6-hr pulse interval. Furthermore, the half-life for P450$_{PB}$ protein in these cultures is more than 36 hr (data not shown).

Analytical Methods. Microsomes prepared by differential centrifugation of cell lysates as described (4) were used for measuring the concentrations of cytochrome P-450 (dithionite-reduced CO minus reduced difference spectrum) (14) and cytochrome b_5 (14) and the activities of NADPH-cytochrome c reductase (4) and benzphetamine demethylase (15) in the presence or absence of anti-P450$_{PB}$ IgG (20 mg of IgG per mg of microsomal protein). Glutathione peroxidase was measured in the cytosolic fraction of cell lysates with hydrogen peroxide as substrate (0.125 mM final concentration) (16). Tissue levels of selenium were determined in nitric acid-digested samples by atomic absorption spectrophotometry (Perkin-Elmer model 5000). Protein was determined according to the method of Schacterle and Pollack (17), using crystalline bovine serum albumin as standard. DNA was measured colorimetrically (18).

RESULTS

Freshly isolated hepatocytes prepared from a single liver were incubated for 24 hr in control medium to allow formation of a stable monolayer and then were incubated for 96 additional hours either in control medium or in medium containing 2 mM PB. Treatment of the cultures with PB increased the microsomal concentration of cytochrome P-450 (2 times) and the activities of NADPH-cytochrome c reductase (1.9 times) and benzphetamine demethylase (1.9 times) when compared to the values in control cultures (Table 1), but it had no effect on the concentration of cytochrome b_5 (data not shown). These results in culture are analogous to the effects of PB on rat liver in vivo (19). Furthermore, the accumulated benzphetamine demethylase activity in PB-treated cultured hepatocytes resembled that in hepatic microsomes from PB-treated rats (15) in being specifically inhibited by form-specific anti-P450$_{PB}$ IgG (Table 1). Nevertheless, because PB could produce these effects by nonspecifically stabilizing microsomal enzymes or by inducing fetal forms of cytochromes P-450 (3, 5, 9, 10), we measured de novo synthesis of P450$_{PB}$ specifically by quantitative immunoprecipitation with form-specific anti-P450$_{PB}$ IgG. Elsewhere, we present evidence for the specificity of this assay (11). When freshly isolated hepatocytes were incubated in the standard pulse-labeling culture medium during the first 4 hr in culture, the amount of [^{3}H]leucine incorporated into P450$_{PB}$ represented 0.36 $\pm$ 0.23% (SD, n = 3) of that incorporated into general cellular protein. However, during the next several hours in culture, the rate of synthesis of P450$_{PB}$ declined rapidly, becoming undetectable (less than 0.05%) within 24 hr and remaining undetectable for 120 hr thereafter (Fig. 1). When 24-hr-old cultures were exposed to PB for a subsequent 96 hr (cultures assayed at 120 hr of age), synthesis of P450$_{PB}$ protein increased to 0.42 $\pm$ 0.30% (SD, n = 14). The average increase in rate of synthesis produced by PB was more than 5-fold (Table 1). Stimulated de novo synthesis of the cytochrome was dose dependent, being maximal at 1–2 mM PB (Fig. 2). A similar curve was obtained when these data were expressed as isotope incorporated into immunoprecipitable cytochrome P-450 relative to total protein or DNA in the cell lysate.

As illustrated by the experiments in Fig. 1, the highest rate of synthesis of P450$_{PB}$ protein was observed in cells exposed to PB for 96 hr. Nevertheless, it should be noted that the age of the cells during their exposure to the inducer is crucial for peak stimulation of synthesis of this protein. The presence of PB in the culture medium for 96 hr starting with 48-hr-old cultures (48–144 hr of culture age) was less effective than drug treatment of equal duration from 24 to 120 hr in culture.

Table 1. Stimulation of de novo synthesis of cytochrome P-450 and accumulation of microsomal drug-metabolizing enzymes in cultured hepatocytes exposed to PB or mephenytoin for 96 hr

		% of control cultures			
				Benzphetamine demethylase activity	
Treatment	Relative synthesis of cytochrome P-450	Concentration of cytochrome P-450	NADPH-cytochrome c reductase activity	No IgG	With IgG
PB	>510 ± 174 (14)	219 ± 33 (3)	221, 163	187 ± 19 (3)	ND
Mephenytoin	418 ± 94 (3)	—	—	>600	ND

Cultures were incubated in the standard serum-free medium for 24 hr and then transferred to medium containing either PB (2.0 mM) or mephenytoin (0.09 mM) for an additional 96 hr. The cultures were then apportioned for measurement of de novo synthesis of P450$_{PB}$ protein or the concentrations of the indicated microsomal enzymes. Results are expressed as the mean ± SD (n) of the percent of the values in cultures of cells isolated from the same animal and incubated for 120 hr in standard medium. These control values were as follows: relative synthesis of P450$_{PB}$ protein, either 0.05–0.08% or undetectable; cytochrome P-450, 0.21 ± 0.06 nmol/mg microsomal protein [it should be noted that this value is significantly lower than that in freshly isolated hepatocytes, as reported previously (4)]; NADPH-cytochrome c reductase, 21.71 and 28.31 nmol of cytochrome c reduced per min per mg of microsomal protein; benzphetamine demethylase, 1.43 ± 0.43 nmol of HCHO formed per min per mg of microsomal protein. ND, not detectable (<0.75 nmol of HCHO formed per min per mg of microsomal protein); —, no measurement made.
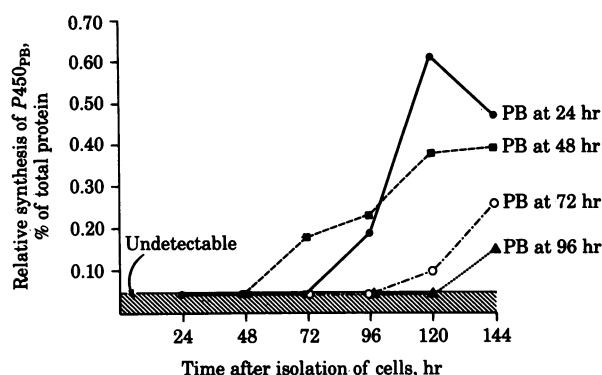
FIG. 1. Time course of induction of $P450_{PB}$ protein synthesis by PB in cultured hepatocytes. Cultures prepared from a single liver were incubated in the standard serum-free medium for 24 hr. Then, at the indicated times, PB (2 mM) was added to the culture medium and the cells were continuously exposed to the drug for the remainder of the experiment. At 24-hr intervals, some cultures from each group were pulse-labeled with [³H]leucine to measure the rate of $P450_{PB}$ protein synthesis relative to that of total cellular protein. The average of two experiments is shown.

Next, we examined the effects of several xenobiotics believed to be "PB-like inducers" of cytochrome P-450 in vivo (20–22). Addition of mephenytoin to the culture medium at a maximally effective dose (0.09 mM) 1/20th that of PB stimulated de novo synthesis of immunoreactive $P450_{PB}$ and increased the microsomal benzphetamine demethylase activity inhibitable (>90%) by form-specific anti-$P450_{PB}$ IgG (Table 1). Mirex also stimulated de novo synthesis of immunoreactive $P450_{PB}$ in culture (average 2.9 times in two experiments), as did 2,2',4,4',5,5'-hexabromobiphenyl (2.5 times). Gel electrophoresis and fluorography revealed that the immunoprecipitated radiolabeled proteins in cultures treated with PB, mephenytoin, or mirex migrated identically with purified $P450_{PB}$ (not shown).

Whereas addition of PB increased de novo synthesis of $P450_{PB}$ in each of the 14 preparations of cultured hepatocytes we have examined to date, the magnitude of the increase varied widely (see Figs. 1 and 2 and Table 1) and was less (maximum of 20-fold) than the reported 20- to 50-fold accumulation of this
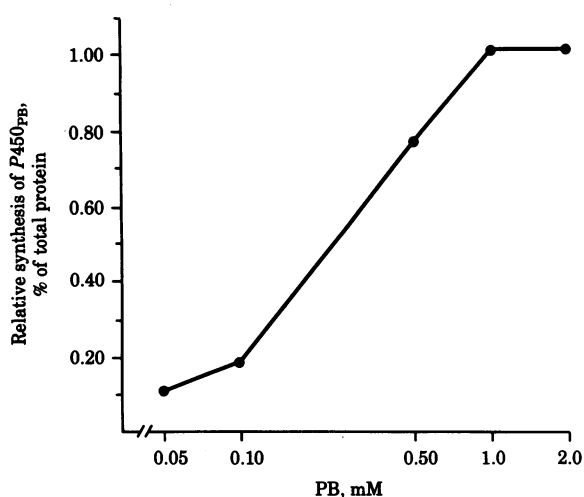

FIG. 2. Dose–response: Induction of $P450_{PB}$ synthesis by PB in cultured hepatocytes. Cultures prepared from a single liver were incubated in the standard serum-free medium for 24 hr and then transferred to medium containing PB (0.05–2 mM) for an additional 96 hr. At 120 hr in culture, the relative rate of synthesis of $P450_{PB}$ protein was determined.

cytochrome protein in liver microsomes of PB-treated rats (23, 24). On the assumption that the cultured cells might be deficient in some factor(s) needed for optimal induction of $P450_{PB}$, we supplemented our culture medium with serum or a variety of nutritional, hormonal, and metabolic factors reported to "maintain" cytochrome P-450 concentrations in hepatocyte culture (5, 7, 25). However, these complex media failed to augment the PB-stimulated synthesis of $P450_{PB}$. Prompted by reports that induction of cytochrome P-450 by PB is impaired in selenium-deficient rats (26, 27), we tested the effects of culture medium supplemented with 0.1 μM H_2SeO_3 and found that this trace element dramatically enhanced the PB-stimulated rate of synthesis of $P450_{PB}$ (Fig. 3). In 13 experiments, synthesis of $P450_{PB}$ in cultures exposed to selenium plus PB (average rate 0.68 ± 0.34, SD) was 4 to 21 times higher than the values in cultures incubated in standard medium or medium supplemented with selenium alone, 0.2 to 8 times higher (average 2.0 ± 1.8, SD) than the values in cultures incubated in standard medium plus PB, and 1.9 times higher than the initial rates in freshly isolated hepatocytes prepared from untreated rats (all significant differences, $P < 0.05$ by t test). Furthermore, the magnitude of the enhancement in synthesis of $P450_{PB}$ protein produced by selenium in the presence of PB was inversely proportional to the relative rate of synthesis of the protein in cells exposed to PB alone ($P < 0.025$ by t test of least-squares regression).

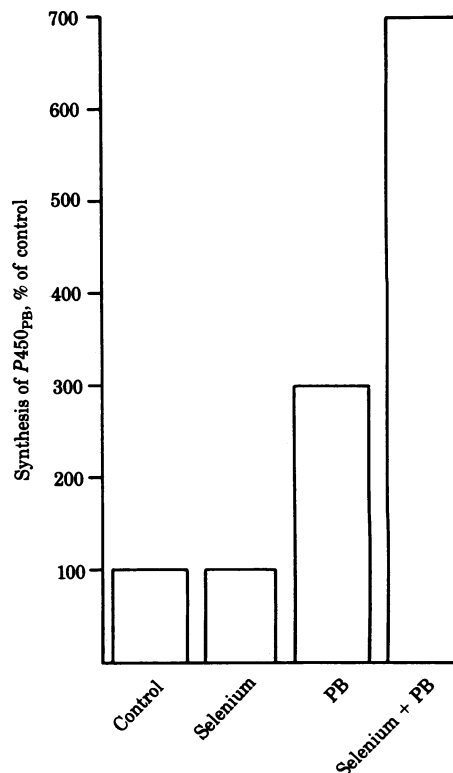Two additional observations suggested that hepatocytes become deficient in selenium when incubated under standard

FIG. 3. Effect of selenium on induction of cytochrome P-450 by phenobarbital in hepatocyte cultures. Half the freshly isolated hepatocytes prepared from a single liver were incubated in standard serum-free culture medium, the remainder in medium supplemented with 0.1 μM H_2SeO_3. After the first 24 hr of incubation, PB (2 mM) was added to half of the cultures in each group. All cultures were maintained with daily renewal of their medium for 96 hr thereafter. Then, the relative rate of synthesis of $P450_{PB}$ protein was determined. Results are expressed as the percent of the value in control cultures maintained in standard medium, which was <0.05%.

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culture conditions. The concentration of cell-associated selenium declined to less than 20% of initial levels within 72 hr in culture (Table 2) and remained at this low level thereafter. Furthermore, loss of total cellular selenium was accompanied by a 58% loss in the activity of the major hepatic seleno-enzyme, glutathione peroxidase (Table 2). The loss of glutathione peroxidase activity was not reversed when cultures were incubated for 120 hr in medium containing 0.1 μM H_2SeO_3 or Na_2SeO_3.

DISCUSSION

The present studies demonstrate that nonproliferating adult rat hepatocytes maintained under standard conditions for primary monolayer culture are competent to respond to PB with *de novo* synthesis of $P450_{PB}$ protein accompanied by its apparent accumulation. Resembling events *in vivo*, induction in hepatocyte culture involves *de novo* synthesis of a protein that is immunochemically and electrophoretically indistinguishable from $P450_{PB}$ (28, 29). The process in culture is dependent upon the concentration of PB and can be stimulated by agents other than PB (20–22). Failure of previous studies of liver cell cultures (5–8) [including our own (4)] to conclusively demonstrate inducible synthesis of $P450_{PB}$ may be attributed to use of nonspecific assay methods to test cultures of inopportune ages, or to omission of specific factor(s) essential for maximal induction by PB. Our results illustrate that culturing hepatocytes on floating collagen gels (10, 30) or on an infralayer of fibroblasts (31) is not essential for induction of cytochrome P-450 by PB. We conclude that induction of $P450_{PB}$, an example of a specialized function of differentiated adult liver, is not extinguished in cultured hepatocytes but merely is temporarily masked by adaptive responses to the conditions of cell culture.

The timing of the addition of PB to the cultures, and not simply the length of time the cultures are exposed to the drug (32), appears to be critical for eliciting a measurable response. Thus, little or no induction was detected within 24 or 48 hr when PB was added to 24-hr-old cultures, whereas a significant response was observed when the drug was added to cultures older than 72 hr (Fig. 1). This transient loss of responsiveness is not restricted to induction of $P450_{PB}$ inasmuch as there is a similar period of impaired induction of $P450_{PCN}$ in 24- to 48-hr-old cul-

tures exposed to PCN (ref. 11; unpublished observations) and also in the accumulation of aryl hydrocarbon hydroxylase activity in cultures exposed to dioxin (33). Nevertheless, maximal induction of $P450_{PB}$ required the presence of PB in cultures during the apparent unresponsive period of 24 to 48 hr of age (Fig. 1). This suggests that one or more of the many PB-responsive transcriptional or translational steps in the induction of this cytochrome may be retained by the cells during this period (19). Hence, the present culture system may provide a unique opportunity to examine the genetic expression of $P450_{PB}$ in cells from the same liver during both responsive and unresponsive states separated only by a brief period in culture.

Selenium and other trace elements have been mentioned previously as factors that support in some manner the survival of replicating cell lines maintained in culture in serum-free medium (34). In our system of nonproliferating hepatocyte cultures, selenium enhances induction of cytochrome P-450 by PB, apparently by correcting a state of acquired cellular deficiency in selenium. The cells spontaneously lose selenium; and this loss is accompanied by a decline in the activity of the seleno-enzyme glutathione peroxidase. Because the amount of cellular selenium lost in culture was variable among different preparations (Table 2), it is possible that the extent of impairment of selenium-dependent functions might also be variable. This may explain the lack of consistency in the magnitude of the effect of selenium in enhancing induction of cytochrome P-450 by PB. For example, cultures marginally deficient in selenium would be expected to be almost fully responsive to PB alone with little effect of added selenium, whereas the reverse would be true for cultures severely deficient in selenium. As predicted from this hypothesis, there was a significant inverse correlation between the magnitude of the selenium effect and the rate of synthesis of $P450_{PB}$ in cultures receiving PB alone.

Primary hepatocyte cultures resemble the intact liver of rats maintained on a selenium-deficient diet in several respects. Treatment of such rats with selenium for 6 hr restores the inducibility of cytochrome P-450 by PB without affecting the low activity of glutathione peroxidase (35). Likewise, with addition of selenium to the culture medium, enhanced induction of $P450_{PB}$ is apparent without a change in glutathione peroxidase activity. Furthermore, selenium appears to affect selectively the induction of $P450_{PB}$ because induction of cytochrome P-450 by 3-methylcholanthrene is unimpaired in the selenium-deficient rat and because induction of $P450_{PCN}$ in cultured hepatocytes exposed to PCN is unaffected by the presence or absence of selenium supplements to the culture medium (data not shown). These observations, coupled with the lack of involvement of glutathione peroxidase, suggest that selenium is a specific factor needed for synthesis of $P450_{PB}$, unrelated to any role this trace element may play in preventing lipid peroxidation or other nonspecific cytotoxic effects (36).

Correia and Burk (35) concluded that impaired induction of cytochrome P-450 in the selenium-deficient rat was due to a block in the assembly of heme with its apocytochrome in the microsomes. However, it should be noted that in those studies, measurements of the synthesis of $P450_{PB}$ protein involved electrophoretically separating the proteins contained in the radiolabeled microsomal fraction on polyacrylamide gels. It is open to question whether such a technique offers sufficient specificity to resolve the many proteins migrating in the 50,000 molecular weight region occupied by the cytochromes P-450. In contrast, utilizing hepatocyte cultures that permit the newly synthesized $P450_{PB}$ protein to be intensely radiolabeled, and measuring this protein specifically by using an immunochemical assay, we found that selenium was important for induction of the $P450_{PB}$ protein itself. The present culture system should be

Table 2. Selenium content and glutathione peroxidase activity in cultured hepatocytes

Age of culture, hr	Selenium content,* % of initial value	Glutathione peroxidase,† % of initial value
0	100	100
24	105 ± 25	102 ± 55
48	40 ± 45	82
72	23 ± 16	—
96	14 ± 10	—
120	—	42 ± 7

At the indicated times, freshly isolated hepatocytes (0 hr) or monolayer cultures of these cells were harvested for analysis of selenium content or glutathione peroxidase activity. —, no measurement made.
* For determination of selenium content, cells washed in selenium-free buffers were disrupted by brief sonication in 0.5 ml of selenium-free distilled water. Replicate portions of the lysate were digested in nitric acid in acid-washed glass digestion tubes, and the concentration of selenium was measured with a Perkin–Elmer 5000 atomic absorption spectrophotometer, using the AS40 autosampler. Selenium was undetectable in culture medium incubated without cells. Results are expressed as percent of the value in freshly isolated hepatocytes (±SD), which was 0.241 ± 0.031 ng of selenium per μg of cell lysate DNA, $n = 3$.
† Glutathione peroxidase activity is expressed as percent of the initial value in freshly isolated hepatocytes (±SD), which was 166.56 ± 60.99 μmol of NADPH oxidized per min per mg of protein, $n = 4$.

1. Remmer, H. (1972) *Eur. J. Clin. Pharmacol.* 5, 116–136.
2. Snyder, R. & Remmer, H. (1979) *Pharmacol. Ther.* 7, 203–244.
3. Owens, I. S. & Nebert, D. W. (1975) *Mol. Pharmacol.* 11, 94–104.
4. Guzelian, P. S., Bissell, D. M. & Meyer, U. A. (1977) *Gastroenterology* 72, 1232–1239.
5. Fahl, W. E., Michalopoulos, G., Sattler, G. L., Jefcoate, C. R. & Pitot, H. C. (1979) *Arch. Biochem. Biophys.* 192, 61–72.
6. Fry, J. R., Wiebkin, P. & Bridges, J. W. (1980) *Biochem. Pharmacol.* 29, 577–581.
7. Stenberg, A. & Gustafsson, J. A. (1978) *Biochim. Biophys. Acta* 540, 402–407.
8. Michalopoulos, G., Sattler, C. A., Sattler, G. L. & Pitot, H. C. (1976) *Science* 193, 907–909.
9. Bissell, D. M. (1976) *Prog. Liver Dis.* 5, 69–82.
10. Sirica, A. E., Richards, W., Tsukada, Y., Sattler, C. A. & Pitot, H. C. (1979) *Proc. Natl. Acad. Sci. USA* 76, 283–287.
11. Elshourbagy, N. A., Barwick, J. L. & Guzelian, P. S. (1981) *J. Biol. Chem.* 256, 6060–6068.
12. Bissell, D. M. & Guzelian, P. S. (1980) *Ann. N.Y. Acad. Sci.* 349, 85–98.
13. Guzelian, P. S. & Bissell, D. M. (1976) *J. Biol. Chem.* 251, 4421–4427.
14. Omura, T. & Sato, R. (1964) *J. Biol. Chem.* 239, 2379–2385.
15. Elshourbagy, N. A. & Guzelian, P. S. (1980) *J. Biol. Chem.* 255, 1279–1285.
16. Lawrence, R. A. & Burk, R. F. (1976) *Biochem. Biophys. Res. Commun.* 71, 952–958.
17. Schacterle, G. R. & Pollack, R. L. (1973) *Anal. Biochem.* 51, 654–655.
18. Richards, G. M. (1974) *Anal. Biochem.* 57, 369–376.
19. Rees, D. E. (1979) *Gen. Pharmacol.* 10, 341–350.
20. Poland, A., Mak, I., Glover, E., Boatman, R. J., Ebetind, F. H. & Kende, A. S. (1980) *Mol. Pharmacol.* 18, 571–580.
21. Moore, R. W., Sleight, S. D. & Aust, S. D. (1978) *Toxicol. Appl. Pharmacol.* 44, 309–321.
22. Remmer, H. (1962) in *Ciba Foundation Symposium on Enzymes and Drug Action*, eds. Mongar, J. L. & DeReuck, A. V. S. (Little Brown, Boston), pp. 276–298.
23. Thomas, P. E., Reik, L. M., Ryan, D. E. & Levin, W. (1981) *J. Biol. Chem.* 256, 1044–1052.
24. Harada, N. & Omura, T. (1981) *J. Biochem.* 89, 237–248.
25. Decad, G. M., Hsieh, D. P. H. & Byard, J. L. (1977) *Biochem. Biophys. Res. Commun.* 78, 279–287.
26. Burk, R. F., Mackinnon, A. M. & Simon, F. (1974) *Biochem. Biophys. Res. Commun.* 56, 431–436.
27. Burk, R. F. & Masters, B. S. S. (1975) *Arch. Biochem. Biophys.* 170, 124–131.
28. Kumar, A. & Padmanaban, G. (1980) *J. Biol. Chem.* 255, 522–525.
29. Bhat, K. S. & Padmanaban, G. (1978) *FEBS Lett.* 89, 337–340.
30. Michalopoulos, G., Sattler, G. L. & Pitot, H. C. (1976) *Life Sci.* 18, 1139–1144.
31. Michalopoulos, G., Russell, F. & Biles, C. (1979) *In Vitro* 15, 796–806.
32. Bridges, J. W. & Fry, J. R. (1979) in *The Induction of Drug Metabolism*, eds. Estabrook, R. W. & Lindenlaub, E. (Schattauer, Stuttgart, Federal Republic of Germany), pp. 343–354.
33. Steward, A. R. & Byard, J. L. (1981) *Toxicol. Appl. Pharmacol.* 59, 603–616.
34. Ham, R. G. & McKeehan, W. L. (1978) *In Vitro* 14, 11–22.
35. Correia, M. A. & Burk, R. F. (1978) *J. Biol. Chem.* 253, 6203–6210.
36. Hoekstra, W. G. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 34, 2083–2089.
37. Newman, S. L., Elshourbagy, N. A. & Guzelian, P. S. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 40, 879 (abstr.).