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HUMAN ADULT HEPATOCYTES IN PRIMARY MONOLAYER CULTURE

MAINTENANCE OF MIXED FUNCTION OXIDASE AND CONJUGATION PATHWAYS OF DRUG METABOLISM

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Abstract—The stabilities of several drug oxidation and conjugation pathways in human adult hepatocytes have been investigated during 72 hr culture. Cytochrome P-450-dependent mixed function oxidase was measured by the O-dealkylations of ethoxyresorufin (EROD), pentoxyresorufin (PROD) and benzyloxyresorufin (BROD), which are probes for different isozymes of cytochrome P-450 in the rat. EROD declined to 64% of initial fresh cell values after 72 hr in culture, whereas PROD increased to 162% and BROD remained relatively constant. Addition of phenobarbitone to the culture medium selectively increased PROD to a greater extent than EROD and did not affect BROD. NADPH-cytochrome *c* reductase and NADH-cytochrome b_5 reductase were markedly labile during culture, declining to 32% and 22% of fresh cell values respectively. Epoxide hydrolase (EH) showed a large transient increase (2–5-fold) in enzyme activity 24 hr after culture, declining to fresh cell values by 48 hr. UDP-glucuronyltransferase (GT) activity towards phenolphthalein and 1-naphthol also increased (2–3-fold) during the 72 hr of culture, the greater and more rapid increase being observed with phenolphthalein glucuronidation. Sulphotransferase activity declined rapidly within 24 hr of culture, whereas reduced glutathione (GSH) levels and GSH conjugation were maintained at fresh cell values for 72 hr.

Many carcinogens and cytotoxic xenobiotics are activated and detoxified by cytochrome P-450-dependent mixed function oxidation (MFO)§ and conjugation with glucuronic acid, sulphate and reduced glutathione (GSH). The extent of carcinogenicity and cytotoxicity depends on the balance between these activation and detoxification processes. The information available for the carcinogenic and cytotoxic potential of xenobiotics in man is usually obtained through extrapolation from animal studies. However, there is increasing evidence that the metabolism of xenobiotics in human tissue is different from that in animals [1], so it is essential to develop systems that can be used to measure the metabolism and to predict the toxicity of xenobiotics in man. Cell cultures from human tissues provide a system for studying human xenobiotic metabolism and mechanisms of cytotoxicity and carcinogenesis under defined conditions *in vitro*.

Primary cultures of hepatic parenchymal cells isolated from experimental animals have become an established method for such studies [2, 3]. However, a major problem inherent with cultured hepatocytes is the instability of a number of specific functions [4]. For example MFO activity declines to low levels within the first 24–48 hr of culture [3, 5]. Primary cultures of human hepatocytes have been reported to maintain the concentration of cytochrome P-450 more effectively than cultures of rodent hepatocytes [6], but there is little information available on the maintenance of the enzymic functions of cytochrome P-450 or on the activity of conjugation reactions during culture. In this paper the activities of three reactions catalysed by cytochrome P-450 and the activities of three conjugation reactions are described during primary culture of human adult hepatocytes.

MFO activity was measured using three homologous substrates, ethoxy- and pentoxy- and benzyloxy- resorufin, which identify the MFO activity of different isozymes of rat liver cytochrome P-450 [7]. UDP-Glucuronyltransferase (GT) activity was measured using 1-naphthol and phenolphthalein, which are preferential substrates for the 3-methylcholanthrene-inducible and phenobarbitone-inducible transferases respectively in rats [8]. Glutathione conjugation was investigated using 1-chloro-2, 4-dinitrobenzene (CDNB), which is a substrate for at least three different isozymes of cytosolic glutathione-S-transferase in human liver [9].

§ Abbreviations used: EROD, ethoxyresorufin O-deethylation; PROD, pentoxyresorufin O-dealkylation; BROD, benzyloxyresorufin O-dealkylation; CDNB, 1-chloro-2,4-dinitrobenzene; MFO, mixed function oxidase; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; GSH, reduced glutathione; GT, UDP-glucuronyltransferase; EH, epoxide hydrolase.

MATERIALS

Hydrocortisone-21-succinate, 5-aminolaevulinic acid, NADH, NADPH, isocitric acid, isocitrate

dehydrogenase, cytochrome *c*, Lubrol PX, bovine serum albumin (Fraction V) and reduced glutathione were all obtained from Sigma. Foetal calf serum was from Flow Laboratories and collagenase from Boehringer Mannheim. Resorufin, ethoxy-, pentoxy- and benzyloxy-resorufin were synthesised as described by Burke and Mayer [10]. Phenanthrene-9, 10-oxide and phenanthrene-9, 10-dihydrodiol were prepared as previously described [11].

METHODS

1. *Preparation of hepatocytes.* Liver samples were obtained from renal transplant donors. Immediately after removal of the kidneys the liver was excised into ice-cold 0.9% NaCl and within 30–45 min of removal from the body a section (approx. 50 g) was cut off, such that it had only one cut surface. The section was perfused at 37° via four cannulae of differing external diameter inserted into suitable vessels, with Hank's balanced salt solution (HBSS) containing 1% w/v bovine serum albumin, 0.6 mM EGTA and 12.5 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES). One litre of this solution was perfused at 20 ml/min without recirculation. A second solution of HBSS containing 12.5 mM HEPES, 1.25 g/l collagenase and 4 mM calcium chloride was perfused and recirculated for 45 min. The sample was transferred into a Petri dish and the cells were dispersed into HBSS containing 12.5 mM HEPES. The suspension was filtered, allowed to sediment on ice under $95\%O_2/5\%CO_2$, and washed twice by sedimentation in the same HBSS buffer. Viability was assessed by Trypan Blue exclusion.

2. *Culture of hepatocytes.* Cells were seeded in 10 cm Falcon Petri dishes at a concentration of 3×10^6 viable cells in 10 ml of modified Earle's medium [12] supplemented with 1U/l insulin, 10^{-6} M hydrocortisone-21-succinate, 10^{-6} M 5-aminolaevulinic acid, 5% (v/v) foetal calf serum, 2.5 μg/ml fungizone, 100 U/ml penicillin and 100 μg/ml streptomycin. The culture medium was changed 4–6 hr after seeding, and then daily. When sodium phenobarbitone (2 mM) was added to the medium, it was added daily after 24 hr in culture, and the medium was renewed every 24 hr.

3. *Analytical methods.* The measurements of cytochromes P-450 and b_5, EROD, PROD, BROD, EH and the cytochrome reductases in freshly isolated and cultured cells were carried out using whole cell homogenates, prepared as previously described for rat hepatocytes [13]. Homogenates were stored at −80° for up to one month until analysis. The concentration of cytochrome P-450 was measured spectrophotometrically [14] by the difference spectrum between carbon monoxide-treated, dithionite-reduced homogenate and carbon monoxide-treated homogenate [15] using a Varian Cary 219 spectrophotometer. Cytochrome b_5 content and the activities of NADH-cytochrome b_5 reductase and NADPH-cytochrome *c* reductase were measured as described by Falzon and co-workers [16]. The EROD, PROD and BROD reactions were measured using 5 μM substrate concentrations in 0.1 M sodium phosphate buffer, pH 7.6, as previously described [13].

EH was measured in cell homogenates using 20 μM phenanthrene-9, 10-oxide and enzyme activity was quantified by direct fluorimetric detection of the phenanthrene-9, 10-dihydrodiol formed [17]. Reactions containing 0.5 ml cell homogenate were carried out in 15 mM Tris buffer, pH 8.7, in air in round-bottomed tubes at 37° under yellow light. The fluorescence intensity of the reaction mixture was read at timed intervals at an excitation wavelength of 270 nm and an emission wavelength of 320 nm, and calibrated with authentic phenanthrene-9,10-dihydrodiol.

GT and glutathione-S-transferase activities were measured at timed intervals using whole cells. Incubations were carried out at 37° in HBSS, pH 7.4, containing 12.5 mM HEPES, in 25 ml conical flasks with suspensions of fresh cells (10^6 viable cells/ml) or in the Petri dishes with cultured cells. GT was measured using 100 μM 1-naphthol or phenolphthalein and the reactions terminated by the addition of 2 vol. of ice-cold methanol. Cell proteins were removed by centrifugation and the glucuronic acid conjugates formed were separated from the parent drugs by reversed phase ion-pair high pressure liquid chromatography and quantified by u.v. absorbance [18]. The glutathione conjugation of 50 μM CDNB was measured by direct spectrophotometry and quantified using a molar extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ at 340 nm [9]. Reactions were terminated by rapid centrifugation (10,000 *g* for 2–3 min) and the absorbance of the supernatant read immediately. Reduced glutathione (GSH) concentrations were measured fluorimetrically by the method of Hissin and Hilf [19] and cell protein was determined as described by Lowry and co-workers [20].

RESULTS

The information available on the kidney donor patients is shown in Table 1.

Cell yield varied greatly between livers (Table 2) and the viability of the preparations improved from 65 to 93% with experience. Attachment of cells to the plastic Petri dishes was between 37 and 69% after 24 hr in culture. Between 24 and 48 hr all the cultures showed a slight loss of protein (30% maximum loss), but, thereafter the culture system appeared to stabilise and very few cells were lost during medium changes.

Table 3 shows the activity of the MFO system in liver samples A to D during the first 72 hr in culture. Cells from sample E were used to compare control and phenobarbitone-treated cultures and where the number of cultured cells available was limited not all procedures were carried out on every sample. EROD was the most active reaction studied, but appeared to be the least stable in culture. The greatest inter-individual difference in fresh cell activities was seen with PROD (10-fold variation), largely due to sample D having a 5–10-fold higher PROD activity than the other three samples. It should be noted that (i) patient D was female, whereas A, B and C were

Table 1. Histories of kidney donor patients from whom livers were obtained

Liver sample	Patient age	Sex	Drug treatment	Cause of death	Other relevant information
A	25	M	Pancuronium Heparin	Subarachnoid haemorrhage	No further details known
B	19	M	Heparin Pancuronium	Road traffic accident	Known to be a non-smoker, did not drink alcohol
C	51	M	Dopamine Pancuronium	Subarachnoid haemorrhage	Occasional smoker
D	49	F	Heparin Dopamine Alcuronium	Head injury	Therapeutic blood concentrations of amitriptyline and trifluoperazine on admission
E	29	F	Heparin Mannitol Betamethasone Methylprednisolone Nalbuphine Droperidol	Road traffic accident	No further details known

All the patients had normal hepatic and renal function on admission, making previous heavy alcohol intake unlikely. Drugs named in the "Drug treatment" column were administered in the hospital prior to transplantation surgery. Only patient D was known to be receiving drugs (amitriptyline 100 mg/day and trifluoperazine 15 mg/day) immediately prior to admission, as indicated under "other relevant information".

Table 2. Experimental parameters for freshly isolated human liver cells

Liver sample	A	B	C	D	E
Yield ($\times 10^6$ cells)	630	333	138	493	331
Viability (% excluding Trypan Blue)	65	72	75	93	92
mg protein/10^6 cells	1.16	1.54	1.51	1.52	1.31
Cytochrome P-450 content (nmol/mg protein)	0.09	0.12	0.08	0.05	0.13
Attachment in primary culture after 24 hr (%)	47	69	37	60	54

The characteristics of five human hepatocyte preparations A–E are shown.
Yield: Total number of hepatocytes recovered freshly from each liver.
Viability: percentage of each sample of freshly isolated hepatocytes that excluded Trypan blue.
Protein: Total protein content of homogenates of 10^6 hepatocytes per ml.
Cytochrome P-450: Cytochrome P-450 content per mg total hepatocyte homogenate protein.
Attachment: percentage of each sample of freshly isolated hepatocytes that attached to culture plates.

male, and (ii) this was the only patient known to be receiving chronic drug therapy (amitriptyline and trifluoperazine) prior to hospital admission. An effect of these drugs on hepatic drug metabolism cannot be discounted. Since EROD and PROD are selectively induced in rats *in vivo* by 3-methylcholanthrene and phenobarbitone respectively [7], the ratio of EROD/PROD was calculated for each preparation as an index of equivalent changes in cytochrome P-450 occurring during culture. There was a 10-fold difference in the EROD/PROD ratio between different fresh cell preparations (Table 3). In samples A–C the ratio fell during culture due to both a decrease in EROD and an increase in PROD. The EROD/BROD ratio, however, was relatively constant between individual fresh cell preparations (3.6 to 1.9) and remained constant during culture.

Table 4 shows that, in contrast to the MFO activities, NADPH-cytochrome *c*- and NADH-cytochrome b_5 reductase activities dropped to approx. 25% of fresh cell values by 72 hr, while cytochrome b_5 content declined to approx. 50% of fresh cell values within 48 hr of culture. Cytochrome P-450 levels in fresh cells (Table 2) were within the

Table 3. Mixed function oxidase activities of hepatocytes during culture

(i) Ethoxyresorufin O-deethylation activity (EROD)				
Liver sample	A	B	C	D
Fresh cells	(27.19)	(20.90)	(16.46)	(28.26)
24 hr culture	35%	76%	86%	71%
48 hr culture	65%	65%	70%	66%
72 hr culture	84%	40%	69%	N.D.
(ii) Benzyloxyresorufin O-debenzylation activity (BROD)				
Liver sample	A	B	C	D
Fresh cells	(7.64)	(9.98)	(8.58)	(10.84)
24 hr culture	71%	108%	117%	145%
48 hr culture	97%	84%	110%	82%
72 hr culture	105%	88%	136%	N.D.
(iii) Pentoxyresorufin O-dependentylation activity (PROD)				
Liver sample	A	B	C	D
Fresh cells	(0.33)	(0.22)	(0.71)	(3.56)
24 hr culture	67%	136%	75%	88%
48 hr culture	161%	127%	173%	N.D.
72 hr culture	132%	182%	173%	N.D.
(iv) The ratio of EROD:PROD activities				
Liver sample	A	B	C	D
Fresh cells	82.4	95.4	23.2	7.94
24 hr culture	43.3	53.3	26.8	6.42
48 hr culture	33.2	45.3	9.3	N.D.
72 hr culture	52.3	21.2	9.3	N.D.

The values are means of duplicate measurements with hepatocytes from liver samples A–D. Variation between duplicates was less than 5%. Fresh cell values in parentheses are enzyme activities (pmol resorufin produced/min/mg total cell homogenate protein), whereas values for 24–72 hr cultures are percentages of the fresh cell activities. N.D.—not determined. Hepatocyte cultures and enzyme activity measurements were carried out as described in the Methods.

Table 4. Cytochrome reductase activities and cytochrome b_5 content of hepatocytes during culture

(i) NADPH-cytochrome *c* reductase (nmol/min/mg protein)				
Liver sample	A	B	C	D
Fresh cells	(23.28)	(24.90)	(22.66)	(15.14)
24 hr culture	35%	16%	30%	28%
48 hr culture	32%	23%	24%	18%
72 hr culture	50%	30%	16%	N.D.
(ii) NADH-cytochrome b_5 reductase (μmol/min/mg protein)				
Liver sample	A	B	C	D
Fresh cells	(0.54)	(0.16)	(0.17)	(0.42)
24 hr culture	18%	44%	35%	26%
48 hr culture	4%	37%	37%	19%
72 hr culture	17%	30%	18%	N.D.
(iii) Cytochrome b_5 content (nmol/mg protein)				
Liver sample	A	B	C	D
Fresh cells	(0.24)	(0.30)	(0.26)	N.D.
24 hr culture	56%	39%	63%	N.D.
48 hr culture	67%	42%	58%	N.D.

Values are means of duplicate measurements with hepatocytes from liver samples A–D. Variation between duplicates was less than 10%. Fresh cell values in parentheses are enzyme activities or cytochrome content (per mg total cell homogenate protein), whereas values for 24–72 hr cultures are percentages of the fresh cell activities (or cytochrome content). N.D.—not determined. Hepatocyte cultures and enzyme measurements were as described in the Methods.

Table 5. Epoxide hydrolase activity of hepatocytes during culture

Liver sample	A	B	C	D
Fresh cells	(67.16)	(40.30)	(48.80)	(137.36)
24 hr culture	524%	197%	238%	627%
48 hr culture	100%	96%	N.D.	114%
72 hr culture	N.D.	104%	120%	N.D.

Values are means of duplicate measurements with hepatocytes from liver samples A–D. Variation between duplicates was less than 5%. Fresh cell values in parentheses are enzyme activies (pmol/min/mg total cell homogenate protein), whereas values for 24–72 hr cultures are percentages of the fresh cell activities. N.D.—not determined. Hepatocyte cultures and enzyme activity measurements were as described in the Methods.

range reported for human hepatocytes: 32–166 pmol/mg cell protein [21]. The total number of hepatocytes obtained from each liver was insufficient to permit cytochrome P-450 measurements in cultured hepatocytes [13].

EH activity increased between 2- and 6-fold after 24 hr in culture (Table 5), but the effect was transient in all the cultures and the activity returned to fresh cell levels by 48 hr in culture.

Table 6 shows that the activity of GT increased with time in culture, particularly with respect to the glucuronidation of phenolphthalein. 1-Naphthol was also converted to its sulphate conjugate in fresh cells (4-fold less sulphation than glucuronidation in all preparations), but this reaction was not detectable in cultured hepatocytes. Phenolphthalein was not sulphated to a significant extent even in fresh cells. Intracellular GSH content and the GSH conjugation of CDNB were stable during the first 3 days of culture (Table 7).

Phenobarbitone (2 mM) was added to hepatocytes from a fifth liver sample (E) after 24 hr in culture and the activity of the MFO system was investigated in control and phenobarbitone treated cultures. After 96 hr in culture (72 hr of phenobarbitone exposure), the results indicated that both PROD and EROD were increased by the phenobarbitone (2.2- and 1.6-fold respectively), whereas there was no effect on BROD (Fig. 1). There was a 5-fold increase in EH activity in this sample during 96 hr culture under control conditions, which was partially prevented by the presence of phenobarbitone.

DISCUSSION

The availability of stable cultures of human adult hepatocytes would permit the characterisation of human liver drug metabolism and toxicity profiles to be carried out *in vitro*, before administering drugs to man. Most of the published work on the stability of enzymes in cultured hepatocytes has been carried out using rat hepatocytes. Compared with cultures from rodent liver, human adult hepatocytes have

Table 6. UDP-Glucuronyltransferase activity of hepatocytes during culture

Liver sample	A	B	C	D
(i) Phenolphthalein glucuronyltransferase				
Fresh cells	(0.12)	(0.20)	(0.33)	N.D.
24 hr culture	300%	135%	154%	N.D.
48 hr culture	392%	240%	133%	N.D.
72 hr culture	N.D.	295%	133%	N.D.
(ii) 1-Naphthol glucuronyltransferase				
Fresh cells	(0.44)	(0.85)	(0.71)	(1.09)
24 hr culture	52%	66%	120%	125%
48 hr culture	102%	58%	104%	41%
72 hr culture	116%	146%	230%	N.D.

Values are means of duplicate measurements with hepatocytes from liver samples A–D. Variation between duplicates was less than 5%. Fresh cell values in parentheses are enzyme activities (nmol/min/mg total cell homogenate protein), whereas values for 24–72 hr cultures are percentages of the fresh cell activities. N.D.—not determined. Hepatocyte cultures and enzyme activity measurements were as described in the Methods.

Table 7. Reduced glutathione content and glutathione transferase activity of hepatocytes during culture

(i) GSH content (nmol/mg protein)				
Liver sample	A	B	C	D
Fresh cells	(25.66)	(27.18)	(28.06)	N.D.
24 hr culture	71%	129%	93%	N.D.
48 hr culture	94%	107%	92%	N.D.
72 hr culture	116%	89%	N.D.	N.D.
(ii) Glutathione transferase activity with CDNB (nmol/min/mg protein)				
Liver sample	A	B	C	D
Fresh cells	(0.33)	(0.41)	N.D.	(0.30)
24 hr culture	119%	130%	N.D.	80%
48 hr culture	154%	141%	N.D.	90%
72 hr culture	128%	119%	N.D.	100%

Values are means of duplicate measurements with hepatocytes from liver samples A–D. Variation between duplicates was less than 5%. Fresh cell values in parentheses are enzyme activities or GSH content (per mg total cell homogenate protein), whereas values for 24–72 hr cultures are percentages of the fresh cell activities or GSH content. N.D.—not determined. Hepatocyte cultures and determination of enzyme activity and GSH content were as described in the Methods. CDNB, chlorodinitrobenzene.

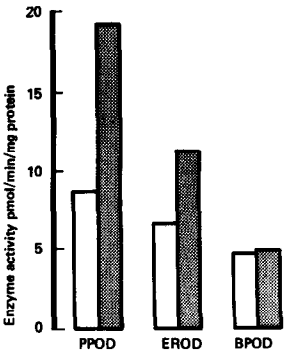

Fig. 1. Apparent phenobarbitone induction of alkoxyresorufin O-dealkylase activities during hepatocyte culture from liver sample E cultured either in normal medium (□) or in medium containing 2 mM phenobarbitone (▨), as defined in the Methods. Each bar represents the mean result for duplicate plates cultured for 96 hr. PROD—pentoxyresorufin O-depentylase activity. EROD—ethoxyresorufin O-deethylase activity. BROD—benzyloxyresorufin O-debenzylase activity. Enzyme activities were measured as described in the Methods and in each case are pmol resorufin produced/min/mg total cell homogenate protein.

been reported to maintain drug metabolising enzymes to a greater extent during culture [6]. It is of interest to compare the maintenance of MFO and conjugation reactions obtained in human hepatocyte cultures in the present study with that obtained in similar studies carried out in this laboratory using male Sprague–Dawley rats [23–25]. Although Sprague–Dawley is a strain much used for drug metabolism and toxicity studies, there are marked strain differences in the stability of drug metabolizing enzymes in cultured rat hepatocytes [24, 25].

A major difference between human and rat cultured hepatocytes is the markedly greater lability of the NADPH-cytochrome *c* reductase and NADH-cytochrome b_5 reductase in human hepatocytes. Both species show spontaneous increases in MFO activities during culture. In rat EROD increases to a greater extent than PROD, probably reflecting a predominance of cytochrome P-448 during culture [3]. However, in man EROD did not increase, being maintained at 60% of fresh cell values for up to 72 hr, while PROD increased to 160% of initial values. The mechanisms responsible for the changes in MFO activity during culture are not understood, but the shift towards cytochrome P-448 predominance in the rat is thought to reflect de-differentiation of adult hepatocytes [3].

GT activity of both human and rat hepatocytes increases during culture. In man, phenolphthalein glucuronidation increased to a greater extent than that of 1-naphthol. The intracellular GSH content and GSH conjugation of CDNB are maintained at approximately fresh cell values in both species for at least 72 hr. Sulphotransferase activity towards 1-naphthol declines rapidly (by 70% within 72 hr) in cultured rat hepatocytes [23]. In freshly isolated human hepatocytes 1-naphthol sulphation occurred to a small extent (4 times less than glucuronidation), but was not detectable during culture.

The marked increase (2–6-fold) in EH activity observed during human hepatocyte culture has also been reported to occur in rat hepatocyte cultures [26]. However, whereas the increase was transient in human hepatocytes, Razzouk *et al.* [26] found that EH remained elevated (2–3-fold fresh cell values) in rat hepatocytes for up to 96 hr in culture. In rat liver an increase in microsomal EH occurs in the early stages of experimental hepatocarcinogenesis and has been proposed as a marker for pre-neoplastic changes [27]. The increase in GT activity is also thought to reflect de-differentiation of hepatocytes in culture and development of a pre-neoplastic pattern of drug metabolising enzymes [28]. Co-culture of rat hepatocytes with a rat liver epithelial cell line

has recently been shown to prevent the increases in both GT (MH Grant, unpublished observations) and EH [29]. This co-culture technique may stabilise phenotypic expression of drug metabolising enzymes during culture of both rat and human hepatocytes and prevent de-differentiation.

The presence of phenobarbitone in the culture medium increased PROD more than EROD, with no effect on BROD, suggesting that there may have been selective induction of certain isozymes of cytochrome P-450 in human hepatocytes *in vitro*. In the rat phenobarbitone treatment *in vivo* induces both PROD and BROD activities to a much greater extent than EROD [7]. Selective induction of cytochrome P-450 isozyme function *in vitro* has not been demonstrated previously during culture of human hepatocytes. Guillouzo *et al.* [22] reported that the concentration of cytochrome P-450 increased 1.3-fold in human hepatocyte mono-culture and 1.7-fold in co-cultures of human hepatocytes in response to *in vitro* phenobarbitone exposure. Our observation that human hepatocytes respond to phenobarbitone exposure in culture by apparently selective induction of cytochrome P-450 MFO activities suggests that they may provide a useful system for investigating the regulation of drug metabolism and toxicity in man.

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REFERENCES

1. C. H. Walker, *Drug Metab. Rev.* **7**, 295 (1978).
2. A. E. Sirica and H. C. Pitot, *Pharmac. Rev.* **31**, 205 (1980).
3. J. R. Fry and J. W. Bridges, *Rev. Biochem. Toxicol.* **1**, 201 (1979).
4. A. Guillouzo, C. Guguen-Guillouzo and M. Bourel, *Triangle* **20**, 121 (1981).
5. J. A. Holme, E. Soderlund and E. Dybing, *Appl. Pharmac. Toxicol.* **52**, 348 (1983).
6. S. C. Strom, R. L. Jurtle, R. S. Jones, D. L. Novicki, M. R. Rosenberg, A. Novotny, G. Irons, J. R. McLain and G. Michalopoulos, *J. natn. Cancer Inst.* **68**, 771 (1982).
7. M. D. Burke, S. Thompson, C. R. Elcombe, J. Halpert, T. Haaparanta and R. T. Mayer, *Biochem. Pharmac.* **34**, 3337 (1985).
8. H. Morrison and G. M. Hawksworth, *Biochem. Pharmac.* **33**, 3833 (1984).
9. W. H. Habig and W. B. Jakoby, *Meth. Enzym.* **77**, 398 (1981).
10. M. D. Burke and R. T. Mayer, *Chem.-Biol. Interact.* **45**, 243 (1983).
11. P. M. Dansette and D. M. Jerina, *J. Am. chem. Soc.* **96**, 1224 (1974).
12. H. Morrison, V. Hammarskjold and B. Jernstrom, *Chem.-Biol. Interact.* **45**, 235 (1983).
13. M. H. Grant, M. A. L. Melvin, P. Shaw, W. T. Melvin and M. D. Burke, *FEBS Lett.* **190**, 99 (1985).
14. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
15. K. A. M. Johannsen and J. W. DePierre, *Analyt. Biochem.* **86**, 725 (1978).
16. M. Falzon, A. S. Milton and M. D. Burke, *Biochem. Pharmac.* **33**, 1285 (1984).
17. P. M. Dansette, G. C. DuBois and D. M. Jerina, *Analyt. Biochem.* **97**, 340 (1979).
18. A. Karakaya and D. E. Carter, *J. Chromat.* **195**, 431 (1980).
19. P. J. Hissin and R. Hilf, *Analyt. Biochem.* **74**, 214 (1965).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. B. J. Blaauboer, I. van Holsteijn, M. van Graft and A. J. Paine, *Biochem. Pharmac.* **34**, 2405 (1985).
22. A. Guillouzo, P. Beaune, M.-N. Gascoin, J.-M. Begue, J.-P. Campion, F. P. Guengerich and C. Guguen-Guillouzo, *Biochem. Pharmac.* **34**, 2991 (1985).
23. M. H. Grant and G. M. Hawksworth, *Biochem. Pharmac.* **35**, 2979 (1986).
24. M. H. Grant, W. T. Melvin and M. D. Burke, *Food Chem. Toxicol.* **24**, 759 (1986).
25. M. H. Grant, S. J. Smith and M. D. Burke, *Biochem. J.* **239**, 785 (1986).
26. C. Razzouk, M. E. McManus, S. Hayashi and S. S. Thorgeirsson, *Biochem. biophys. Res. Commun.* **116**, 587 (1983).
27. W. Levin, A. Y. H. Lu, P. E. Thomas, D. Ryan, D. E. Kizer and M. J. Griffin, *Proc. natn. Acad. Sci. U.S.A.* **75**, 3240 (1978).
28. N. W. Toribara, R. B. Kirkpatrick, C. N. Falany, D. R. La Brecque and T. R. Tephly, *Hepatology* **4**, 1054 (1984).
29. D. Ratanasavanh, P. Beaune, G. Baffet, M. Rissel, P. Kremers, F. P. Guengerich and A. Guillouzo, *J. Histochem. Cytochem.* **34**, 527 (1986).