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PRIMARY CULTURE OF RAT HEPATOCYTES IN THE PRESENCE OF DIMETHYL SULPHOXIDE

A SYSTEM TO INVESTIGATE THE REGULATION OF CYTOCHROME P450 IA

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Abstract—Primary cultures of adult rat hepatocytes would provide a suitable system for the study of hepatic drug metabolism/toxicity provided that the drug-metabolizing enzymes could be maintained at levels approaching those seen *in vivo*. It has been reported that culture of adult rat hepatocytes in the presence of 2% (v/v) dimethyl sulphoxide (DMSO) allowed partial maintenance of total cytochrome P450 content. However, the levels of the individual isozymes were not determined. Culture of rat hepatocytes in the presence of DMSO did maintain the total cytochrome P450 content at 65% of the fresh cell value after 7 days of culture. This was accompanied by high cytochrome P420 levels suggesting that the solvent was stimulating *de novo* synthesis rather than maintaining existing enzyme. In the presence of DMSO the level of ethoxyresorufin-*O*-deethylase (EROD) rose 4-fold in culture, whilst that of pentoxyresorufin-*O*-dealkylase fell rapidly indicating that the isozyme pattern was altered significantly. The increases in total cytochrome P450 content and EROD activity were prevented by cycloheximide confirming that *de novo* protein synthesis was occurring. Haem oxygenase activity was significantly reduced and aminolaevulinic acid synthetase was significantly increased in the presence of solvent, suggesting increased haem availability for incorporation into cytochrome P450. However increased haem availability is insufficient in explaining the isozyme specificity of cytochrome P450 induction. Hepatocytes cultured in the presence of 2% (v/v) DMSO were markedly more responsive to 1,2-benzanthracene, with EROD increasing approximately 40-fold.

The major routes of oxidative metabolism in the liver, in terms of drug-induced toxicity, involve a multicomponent microsomal system comprising an NADPH-requiring reductase, an essential lipid moiety and a family of inducible proteins known collectively as cytochrome P450. Primary culture of adult rat hepatocytes would provide a suitable system to investigate cytochrome P450 mediated metabolism/toxicity if the loss of drug-metabolizing enzyme activity with time in culture could be overcome.

The most widely adopted system involves culture of hepatocytes in a chemically defined medium on plastic dishes coated with a thin layer of type I collagen to enhance attachment. Within a few days the cells lose several hepatocyte-specific differentiated functions, undergoing a shift towards a more foetal-like state. Guzelian *et al.* [1] demonstrated that the level of cytochrome P450 declined to less than 20% of the fresh cell value within 48 hr of culture whilst NADPH cytochrome *c* reductase was only moderately reduced and glucose 6 phosphate remained unchanged, indicating that a broad deterioration in microsomal enzyme activities was not occurring. The ability to secrete albumin into the culture medium is also lost in pure cultures of hepatocytes [2].

Supplementation of a cysteine/cystine-free culture medium with insulin, hydrocortisone and 5-aminolaevulinic acid prevents the initial decline in cytochrome P450 content [3]. These media modifications do not, however, allow maintenance of cytochrome P450 content beyond 3–4 days of culture. Inclusion of the synthetic hormone analogue, dexamethasone, in the culture medium stabilizes cytochrome P450 content at 65% of the initial value for 21 days [4]. The apparent maintenance of cytochrome P450 in the presence of dexamethasone may be due partly to enzyme induction by the hormone analogue. Spectral measurements of total haem bound protein may hide the selective loss of cytochrome P450 isozymes. Addition of pyridines to the culture medium prevented the decline in cytochrome P450 content but this did not correlate with maintenance of drug-metabolizing activities [5].

In the intact liver, parenchymal cells are in contact with a variety of other cell types which may be involved in the control of hepatocyte differentiation either through cell–cell contact or release of factors into the extracellular fluid. Using a system involving co-culture of both rat and human hepatocytes with a rat liver-derived epithelial cell line, it is possible to maintain cytochrome P450-dependent activity and albumin secretion for prolonged periods [6–8].

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However this model is time consuming and the results are difficult to reproduce, perhaps because of differences in the epithelial cells isolated.

Hepatocytes *in situ* are in contact with a specialized form of extracellular matrix (ECM*) termed basement membrane. Pretreatment of plastic tissue culture plates with components of the ECM, such as collagen or fibronectin, results in enhanced attachment and spreading of the cells but also increases the rate of dedifferentiation [9]. It is now thought that the presence of several, or all, of the components of the ECM simultaneously is required to promote cellular differentiation in culture. A protein mixture can be extracted from the EHS sarcoma and the composition of this extract resembles that of the hepatic ECM [10]. Culture of hepatocytes on a thin layer of matrix derived from the EHS sarcoma does not prevent the decline in total cytochrome P450 content but induction of mRNA and protein for several forms of the enzyme is greatly enhanced [11, 12]. However, the commercially available basement membrane preparation ('Matrigel') is produced only in small quantities and is not readily obtainable.

A third approach involves supplementation of the culture medium with 2% (v/v) DMSO. The solvent can induce characteristics of differentiation in a number of transformed cell lines. Human hepatoma cells cultured in the presence of DMSO have a vastly reduced proliferation rate and regain the ability to secrete albumin into the culture medium [13]. Isom *et al.* [14] provided the first demonstration of this effect in non-transformed cells when it was found that addition of 2% (v/v) DMSO to a chemically defined medium resulted in high albumin secretion for up to 43 days in pure culture. This system was also reported to give partial maintenance of total cytochrome P450 content but levels of the individual isozymes were not determined [15]. DMSO has been shown to increase levels of cytochrome P450 IIE1 protein, in the absence of detectable mRNA, in primary cultures of adult rat hepatocytes suggesting that DMSO binding protects the protein from degradation [16]. No enzyme activities were determined in this study.

We now report that addition of 2% (v/v) DMSO to the tissue culture medium allowed partial maintenance of total cytochrome P450 content and increased significantly the level of EROD over a 7-day period. The cells also exhibited an enhanced response to BA.

MATERIALS AND METHODS

Materials. Hydrocortisone-21-hemisuccinate, ALA, NADH, NADPH, isocitric acid, isocitrate dehydrogenase, Lubrol PX, cycloheximide, and BA were obtained from the Sigma Chemical Co. (Poole, U.K.). 'Myoclone plus' foetal calf serum was obtained from Gibco (Paisley, U.K.) and collagenase A from Boehringer Mannheim, U.K. Resorufin, pentoxyresorufin and ethoxyresorufin were obtained from Molecular Probes Inc. (OR, U.S.A.). DMSO was obtained from BDH (Poole, U.K.). Anti-cytochrome P450 IA1 was purchased from Oxygene (Dallas, TX, U.S.A.).

Primary culture. Hepatocytes were isolated aseptically from the livers of male Sprague–Dawley rats (250–300 g) using Seglen's two-step perfusion technique [17]. Viability greater than 85%, as determined by Trypan blue exclusion, was obtained. Cells were then suspended in modified Earle's medium [3] supplemented with 5% (v/v) foetal calf serum and 2% (v/v) DMSO, where appropriate. Falcon tissue culture plates (100 mm) pre-coated with a thin layer of rat tail collagen were inoculated with 5×10^6 hepatocytes suspended in 10 mL of culture medium. Medium was then changed after 4 hr and thereafter at 24 hr intervals throughout the 7-day culture period.

Enzyme assays. Cell homogenates were obtained from cell culture dishes as follows: the medium was decanted and the plate washed with two 5 mL aliquots of 0.1 M phosphate buffer pH 7.6. The cells were then scraped into 2 mL of either P450 buffer (0.1 M phosphate buffer pH 7.6 containing 1 mM dithiothreitol, 1 mM EDTA, 15% (v/v) glycerol and 2% (v/v) Lubrol PX) or 0.1 M phosphate buffer pH 7.6. The cell suspension was homogenized and stored at −80°. Freshly isolated cells (10^6 cells/mL of buffer) were homogenized and stored at −80°. Total cytochrome P450 content was determined spectrally by the method of Omura and Sato [18]. Cytochrome P420 was quantified by the absorbance of the reduced cytochrome complexed with carbon monoxide at 420 nm.

Mixed-function oxidase activity was measured by the appearance of resorufin fluorescence (excitation at 530 nm and emission at 586 nm) during incubation of cell homogenate with either ethoxyresorufin or pentoxyresorufin [3], and in the presence of 10^{-5} M dicoumarol to inhibit DT-diaphorase [19]. EROD and PROD were used to quantify 3-methylcholanthrene- and phenobarbitone-inducible families, respectively, although they can be metabolized by other forms of cytochrome P450 [20].

Haem oxygenase was measured as the rate of formation of bilirubin from the haem substrate methaemalbumin [21, 22]. The reaction was carried out in the presence of rat liver cytosol to prevent the conversion of bilirubin to biliverdin limiting the reaction rate.

ALA synthetase was measured as the rate of incorporation of [^{14}C]succinate into ALA [23], the labelled ALA being extracted as described by Mauzerall and Granick [24], and Condie and Tephly [25]. Over 90% recovery of labelled ALA was obtained.

Induction of EROD. After 24 hr in culture, hepatocytes were exposed to 25 μM BA continuously.

Hepatocyte survival. Hepatocyte survival was estimated from the protein attached per 100 mm plate, protein being measured by the method of Lowry *et al.* [26]. Cell attachment was estimated as the amount of inoculated protein remaining attached

* Abbreviations: ALA, 5-aminolaevulinic acid; BA, 1,2-benzanthracene; DMSO, dimethyl sulphoxide; ECM, extracellular matrix; EHS, Englebreth Holm Swarm; EROD, ethoxyresorufin-*O*-deethylase; PROD, pentoxyresorufin-*O*-dealkylase; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

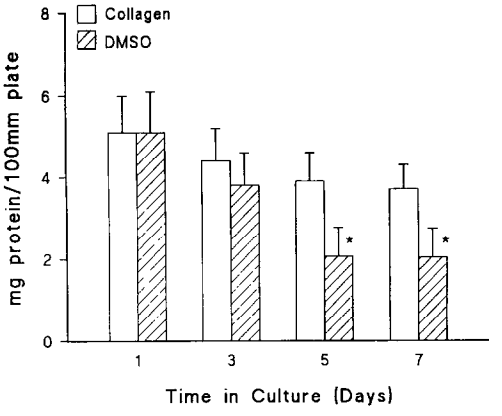

Fig. 1. Protein content of rat hepatocytes cultured in the presence and absence of 2% (v/v) DMSO. Hepatocytes were exposed to 2% (v/v) DMSO after the first 4 hr of culture. Protein content was expressed as mg/100 mm culture plate. The above values represent means of three observations ± SD. *Indicates protein content was significantly lower ($P < 0.05$) compared with the collagen control.

to the culture dish after the first 24 hr of culture. The fall in protein content over the 7-day culture period was taken as an indication of the rate at which cells were detaching.

Protein synthesis was inhibited by culturing hepatocytes in the presence of 10^{-5} M cycloheximide. Cycloheximide was added after the first 48 hr of culture and was present for the remaining 72 hr.

*Immunodetection of cytochrome P*450 IA. Microsomal fractions were prepared from freshly isolated and cultured hepatocytes and the proteins separated by SDS–PAGE as described by Laemmli [27], with minor procedural alterations [28]. For cultured rat hepatocytes approximately 100 plates (100 mm) were used to prepare each microsomal sample. The separated proteins were then blotted against a cytochrome P450 IA1 polyclonal antibody.

The results were analysed using two-way analysis of variance followed by Dunnett's multiple range test. Results were considered significantly different from the collagen controls if $P < 0.05$.

RESULTS

Morphology

Cells cultured on collagen spread to form a complete monolayer within 48 hr of culture. When the plates were examined using inverted light microscopy the cells were found to be more rounded in the presence of 2% (v/v) DMSO.

Attachment

The amount of protein obtained from 100 mm tissue culture plates after the first 24 hr of culture was used as an indicator of cell attachment and was not found to be significantly altered by the incorporation of 2% DMSO in the culture medium (Fig. 1).

The rate at which protein was lost from the plates was taken as a measure of cell death during the culture period. The loss of hepatocytes from culture dishes was negligible when cells were inoculated onto collagen. However, addition of 2% DMSO to the medium caused an approximate halving of protein content over 7 days ($P < 0.05$), suggesting that this concentration of the solvent is toxic to the hepatocytes (Fig. 1).

*Total cytochrome P*450 *content*

The cytochrome P450 content of hepatocytes cultured on a thin layer of collagen declined rapidly, reaching 8% of the fresh cell value by the 7th day of culture (Table 1). There was a concomitant rise in the absorbance at 420 nm suggesting accumulation of cytochrome P420. Although a number of other compounds have absorbance maxima at 420 nm, it is unlikely that the absorbance at 420 nm is due either to haem, since the 420 nm absorbance is very low in fresh cells, or cytochrome *b*5, which has been shown not to increase significantly under these culture conditions [3]. However, when cells were cultured in the presence of 2% DMSO added at either the onset of culture or after 4 hr in culture, total cytochrome P450 content was 54% and 63% of the fresh cell value, respectively, after 7 days ($P = 0.02$ and $P < 0.02$ respectively). Absorbance at 420 nm was found to be equivalent to or greater than that in the absence of solvent, indicating that DMSO does not act by preventing conversion of cytochrome P450 to cytochrome P420 (Table 1). The fraction of total cytochrome present as P420 was decreased in the presence of DMSO.

Mixed-function oxidase activity

EROD declined rapidly when cells were cultured on collagen, falling to 4% of the fresh cell value after 7 days in culture (Table 2). Addition of 2% DMSO at the onset of culture or after 4 hr in culture, caused significant increases in EROD. By 3 days in culture, activity had increased 4–5-fold before falling to 70% of the fresh cell value if solvent was added from the onset of culture whilst addition after 4 hr in culture resulted in EROD activity which was equivalent to the fresh cell value at 7 days.

PROD activity was lower than EROD activity in freshly isolated hepatocytes and declined rapidly in culture (Table 3). The loss of PROD was not significantly altered by the presence of DMSO.

Inhibition of protein synthesis

Hepatocytes were cultured in the presence of DMSO added after 4 hr in culture for 48 hr and were then exposed to cycloheximide for a further 72 hr. This treatment prevented the partial maintenance of total cytochrome P450 content resulting from culture in the presence of the solvent (Table 4). The increase in EROD activity was also prevented by the addition of cycloheximide (Table 5). Cycloheximide at this concentration has no significant effect on hepatocyte viability [29]. There was also no further decrease in attachment when cells were cultured in the presence of 10^{-5} M cycloheximide.

Haem oxygenase

Haem oxygenase was not detected in freshly

Table 1. Cytochrome P450 content over a 7-day culture period

	Time in culture (days)	P450	P420
Fresh cells		0.24 ± 0.03	0.02 ± 0.01
Collagen (N = 6)	1	0.19 ± 0.02	0.31 ± 0.07
	3	0.12 ± 0.01	0.36 ± 0.08
	5	0.04 ± 0.03	0.37 ± 0.06
	7	0.02 ± 0.04	0.32 ± 0.07
DMSO 0 hr (N = 3)	1	0.19 ± 0.03	0.33 ± 0.12
	3	0.08 ± 0.03	0.27 ± 0.04
	5	0.16 ± 0.09	0.56 ± 0.12
	7	0.13 ± 0.06*	0.69 ± 0.19
DMSO 4 hr (N = 5)	1	0.19 ± 0.04	0.32 ± 0.06
	3	0.18 ± 0.11	0.45 ± 0.09
	5	0.14 ± 0.06*	0.46 ± 0.05
	7	0.15 ± 0.05*	0.55 ± 0.20

The above values are expressed as nmol/mg of protein and represent the means ± SD.

* Indicates cytochrome P450 content is significantly different ($P < 0.01$) from the collagen controls.

Table 2. Changes in EROD activity over a 7-day culture period

Time in culture (days)	Collagen (N = 6)	DMSO 0 hr (N = 3)	DMSO 4 hr (N = 5)
1	108.4 ± 30.8	71.9 ± 31.3	81.8 ± 26.7
3	57.5 ± 16.5	930.4 ± 564.9*	880.5 ± 481.1*
5	44.6 ± 19.0	596.4 ± 181.0*	503.9 ± 61.7*
7	16.6 ± 19.0	140.4 ± 81.0	223.5 ± 61.8

The fresh cell value was 198.2 ± 34.0 pmol of resorufin formed/min/mg of protein. The above values are expressed as pmol of resorufin formed/min/mg of protein and represent the means ± SD.

* Indicates activity is significantly different ($P < 0.01$) from collagen controls.

Table 3. Changes in PROD activity over a 7-day culture period

Time in culture (days)	Collagen (N = 6)	DMSO 0 hr (N = 3)	DMSO 4 hr (N = 6)
1	25.9 ± 14.9	36.0 ± 35.9	38.1 ± 28.1
3	5.1 ± 6.0	21.3 ± 22.0	29.9 ± 26.8
5	6.4 ± 7.7	13.7 ± 18.0	21.2 ± 15.2
7	6.3 ± 12.6	10.6 ± 16.2	9.5 ± 13.4

The fresh cell value was 78.2 ± 12.3 pmol resorufin formed/min/mg of protein. The above values are expressed as pmol resorufin formed/min/mg of protein and represent the means ± SD.

isolated hepatocytes. When hepatocytes were cultured on collagen, in the absence of DMSO, haem oxygenase increased to a maximum of 2.88 nmol bilirubin formed/min/mg protein and then fell to 0.02 nmol bilirubin formed/min/mg protein by day 7 (Fig. 2). Culture in the presence of 2% (v/v) DMSO significantly reduced the haem oxygenase activity of hepatocytes on days 1, 3 and 5 of culture (Fig. 2).

ALA synthetase

ALA synthetase in freshly isolated adult rat hepatocytes was 3.04 ± 0.53 nmol ^{14}C-labelled ALA formed/min/mg protein. Culture of hepatocytes in the absence of DMSO resulted in a decline in this activity over the 7-day culture period (Fig. 3). ALA synthetase in hepatocytes cultured in the presence of 2% DMSO was significantly higher ($P < 0.05$) than control cultures on days 3, 5 and 7 (Fig. 3).

Table 4. The effect of cycloheximide on cytochrome P450 content of cultured rat hepatocytes

Time in culture (days)	DMSO 4 hr (N = 2)	DMSO 4 hr + 10 μM cycloheximide (N = 2)
1	0.16	0.16
2	0.15	0.15
3	0.17	0.15
5	0.14	0.07

Hepatocytes were exposed to 10 μM cycloheximide after 2 days in culture. The above values are expressed as nmol of cytochrome P450/mg of protein and represent means of two observations.

The fresh cell value was 0.24 nmol/mg of protein.

Table 5. The effect of cycloheximide on EROD activity in cultured rat hepatocytes

Time in culture (days)	DMSO 4 hr (N = 2)	DMSO 4 hr + 10 μM cycloheximide (N = 2)
1	102.9	102.0
2	611.7	611.7
3	599.1	330.8
5	334.3	29.4

Hepatocytes were exposed to 10 μM cycloheximide after 2 days in culture. The above values are expressed as pmol of resorufin formed/min/mg of protein and represent means of two observations.

The fresh cell value was 176.2 pmol/min/mg of protein.

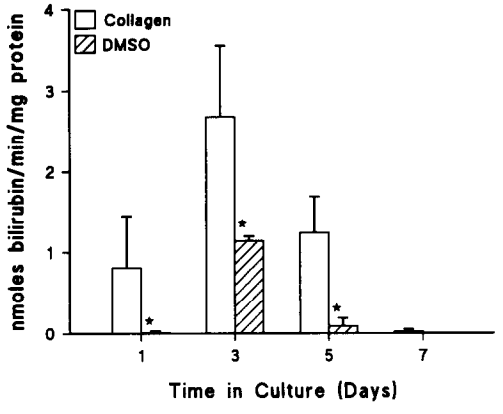

Fig. 2. Haem oxygenase activity of rat hepatocytes cultured in the presence and absence of 2% (v/v) DMSO. Hepatocytes were exposed to 2% (v/v) DMSO after the first 4 hr of culture. Haem oxygenase activity was expressed as nmol bilirubin formed/min/mg protein. No haem oxygenase activity could be detected in freshly isolated rat hepatocytes. The above values represent means of three observations ± SD. *Indicates haem oxygenase activity was significantly lower ($P < 0.05$) compared with control cultures.

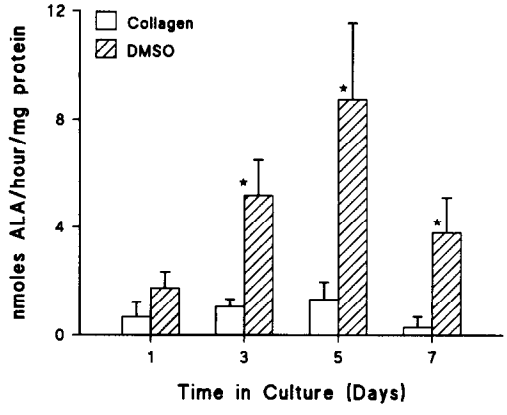

Fig. 3. ALA synthetase activity of rat hepatocytes cultured in the presence and absence of 2% (v/v) DMSO. Hepatocytes were cultured in the presence of 2% (v/v) DMSO added after the first 4 hr of culture. ALA synthetase was expressed as nmol ^{14}C-labelled ALA formed/hr/mg protein. The fresh cell value was 3.04 ± 0.53 nmol ^{14}C-labelled ALA formed/hr/mg protein. The above values represent means of three observations ± SD. *Indicates ALA synthetase was significantly higher ($P < 0.05$) compared with the collagen control.

Response to BA

Exposure of hepatocytes cultured on collagen to BA resulted in partial maintenance of the total cytochrome P450 content, levels being approximately 40% of the fresh cell value after 7 days in culture (Table 6). Culture of hepatocytes in the presence of DMSO and BA simultaneously did not increase

Table 6. The effect of BA on cytochrome P450 levels in cultured rat hepatocytes

Time in culture (days)	Collagen (N = 3)	Collagen + BA (N = 3)	DMSO 4 hr (N = 3)	DMSO 4 hr + BA (N = 3)
1	0.19 ± 0.07	0.19 ± 0.07	0.18 ± 0.04	0.18 ± 0.04
3	0.08 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.02
5	0.05 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.03
7	0.01 ± 0.01	0.09 ± 0.01	0.16 ± 0.05	0.19 ± 0.07

Hepatocytes were exposed to 25 μM BA after 24 hr in culture for the remainder of the culture period. The above values are expressed as nmol of cytochrome P450/mg of protein and represent means of three observations ± SD.

The fresh cell value was 0.24 ± 0.05 nmol P450/mg of protein.

Table 7. The effect of BA on EROD activity in cultured rat hepatocytes

Time in culture (days)	Collagen (N = 3)	Collagen + BA (N =3)	DMSO 4 hr (N = 3)	DMSO 4 hr + BA (N = 3)
1	88 ± 30	88 ± 30	121 ± 39	121 ± 39
3	55 ± 21	670 ± 61	681 ± 216	2277 ± 1581
5	36 ± 18	171 ± 52	486 ± 73	8572 ± 3293
7	15 ± 16	107 ± 16	219 ± 58	6792 ± 1393

Hepatocytes were exposed to 25 μM BA after 24 hr in culture for the remainder of the culture period.

The above values are expressed as pmol of resorufin formed/min/mg of protein and values represent means of three observations ± SD.

The fresh cell value was 172 ± 21 pmol resorufin formed/min/mg of protein.

Table 8. The effect of BA on PROD activity in cultured rat hepatocytes

Time in culture (days)	Collagen + BA (N = 3)	DMSO 4 hr + BA (N = 3)
1	23.6 ± 12.0	38.1 ± 12.4
3	15.2 ± 6.7	43.7 ± 19.2
5	ND	67.3 ± 33.0
7	ND	33.2 ± 2.5

The fresh cell value is 78.2 ± 12.3 pmol resorufin formed/min/mg of protein.

The above values are expressed as pmol of resorufin formed/min/mg of protein and represent means ± SD.

ND, not determined.

cytochrome P450 content above that seen with DMSO alone (Table 6). BA caused significant changes in EROD activity. Cells cultured on collagen showed transient increases in EROD activity to a maximum of three times the fresh cell value whilst culture in the presence of 2% DMSO added after 4 hr in culture caused activity to increase to 40 times the fresh cell value (Table 7). Exposure of hepatocytes to both DMSO and BA reduced the rate at which PROD activity was lost in all three experiments but the extent was variable (Table 8).

Immunodetection of cytochromes P450 in cultured hepatocytes exposed to BA

The polyclonal antibody raised against rat liver cytochrome P450IA1 recognised cytochromes P450 IA1 and IA2 (Fig. 4). The antibody did not cross-react with any of the proteins in microsomes prepared from freshly isolated hepatocytes or from cells cultured on collagen. Hepatocytes cultured in the presence of DMSO contained cytochromes P450 IA1 and IA2 on day 3 but after 7 days in culture no cytochrome P450 IA1 was detectable, and the level of P450 IA2 had decreased [30]. DMSO markedly enhanced the ability of BA to induce cytochromes P450 IA1 and IA2 (Fig. 4).

DISCUSSION

Culture of adult rat hepatocytes on a thin layer of rat tail collagen resulted in a rapid loss of total cytochrome P450 content. The fall in spectrally detectable enzyme was accompanied by large increases in absorbance at 420 nm, suggesting that accelerated degradation may be responsible for the loss of cytochrome P450. Absorbance at 420 nm was high throughout the 7-day culture period, indicating that further degradation of the haemoprotein proceeds slowly, perhaps due to components of the modified Earle's medium. Two activities associated with cytochrome P450, EROD and PROD, declined rapidly under these culture conditions.

Primary culture of adult rat hepatocytes in the presence of 2% (v/v) DMSO resulted in the appearance of a more differentiated phenotype. Total cytochrome P450 content was maintained at 60–70% of the fresh cell value over the 7-day culture

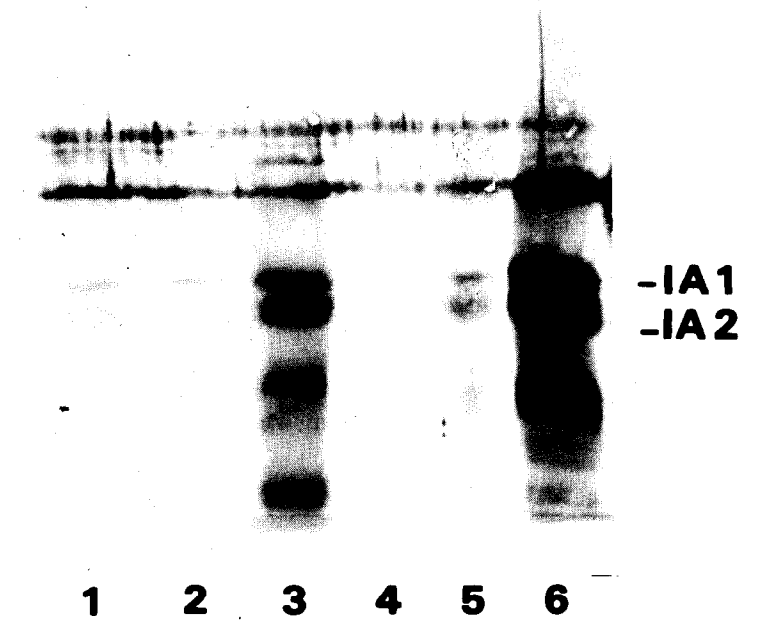

Fig. 4. Immunodetection of cytochrome P450 in rat hepatocytes cultured in the presence of 2.5×10^{-5} M BA and 2% (v/v) DMSO. Hepatocytes were exposed to 2.5×10^{-5} M BA after the first 24 hr of culture. Microsomes were prepared after 5 days of culture. Microsomal proteins were separated using SDS–PAGE and then subjected to immunoblotting using a polyclonal antibody directed against rat liver cytochrome P450 IA1. Lane 1: hepatocytes cultured in the presence of 2% DMSO for 3 days; lane 2: hepatocytes cultured in the presence of 2% DMSO for 5 days; lane 3: hepatocytes cultured in the presence of 2% DMSO and 2.5×10^{-5} M BA for 5 days; lane 4: space; lane 5: hepatocytes cultured on collagen in the presence of 2.5×10^{-5} M BA for 5 days; lane 6: lyophilized cytochrome P450 IA1.

period. Simultaneous exposure to DMSO and cycloheximide abolished the apparent maintenance of cytochrome P450 suggesting that the solvent was stimulating enzyme synthesis rather than stabilizing existing enzyme. The pattern of isozyme expression was altered compared with that of freshly isolated cells. The solvent increased metabolism of ethoxyresorufin probably due to increased levels of cytochromes P450 from the IA family. Culture in the presence of cycloheximide abolished the increase in EROD suggesting that the solvent induces synthesis of cytochrome P450 IA1 and/or IA2. This was confirmed by immunoblotting with an antibody raised against cytochrome P450 IA1 that recognizes both P450 IA1 and IA2. PROD declined rapidly in the presence of DMSO indicating that even within the cytochrome P450 supergene family, the solvent acts selectively to increase levels of particular isozymes. Culture of hepatocytes in the presence of DMSO increased responsiveness to BA, with EROD induced up to 40-fold and a significant increase in immunologically detectable cytochrome P450 IA1 and IA2. Burke and co-workers [20, 31] reported that exposure to the BA-like inducer 3-methylcholanthrene results in a 50–70-fold increase in EROD *in vivo*. It has been suggested that, in untreated rats, EROD is largely catalysed by P450 IA2 but after induction with 3-methylcholanthrene this activity is mainly catalysed by P450 IA1 [32]. The relative contributions of P450 IA1 and IA2 to EROD activity after 3-methylcholanthrene induction in culture have not been determined.

In addition to selectively stimulating synthesis of cytochrome P450 belonging to the IA family, DMSO is also capable of stabilizing existing cytochrome P450 IIE1 [16]. Cytochrome P450 IIE1 was approximately 4-fold higher in DMSO-treated cultures, despite the fact that the mRNA could not be detected. The results suggest that DMSO prevents degradation of cytochrome P450 IIE1 by binding to the enzyme. The authors did not assess the effect of ligand binding on enzyme activity.

Hepatocytes cultured in the presence of DMSO had decreased haem oxygenase and increased ALA synthetase compared with cells cultured on collagen which suggests increased availability of the haem prosthetic group. It is unlikely that increased haem availability can selectively stimulate synthesis of specific cytochrome P450 isozymes. ALA synthetase in cells is tightly regulated with haem itself inhibiting enzyme synthesis. The increase in activity probably reflects loss of haem through incorporation into cytochrome P450 rather than a direct effect of the solvent on enzyme activity or enzyme synthesis.

The increased EROD in the presence of DMSO and the enhanced responsiveness to BA may be due to a direct effect on gene transcription. DMSO and related polar compounds can affect the configuration of biopolymers [33]. DMSO may destabilize DNA–protein interactions causing the chromatin to relax

into a more extended conformation. Altered conformation of the DNA template in the promoter region of specific genes may enhance initiation of gene transcription. It has been demonstrated that exposure of murine virus-infected erythroleukemic cells to DMSO can alter chromatin structure [33]. Regulation of cytochrome P450 IA1 gene transcription has been found to be under the control of a number of positive and negative regulators. Two nuclear proteins XF1 and XF2 have recently been shown to bind to the xenobiotic response element, preventing binding of the liganded aromatic hydrocarbon receptor [34]. Decreased binding or synthesis of these proteins in the presence of DMSO would enhance gene transcription in response to DMSO. However the effects of DMSO on albumin mRNA have been attributed to both enhanced gene transcription and mRNA stabilization, suggesting that both transcriptional and posttranscriptional mechanisms are important [35].

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