Table 1. The effect of mevinolin on bile synthesis in cultured hamster hepatocytes

The amounts of bile acid synthesized were determined using radioimmunoassays. Mevinolin treated plates ( + Mev) were preincubated for 1 h with 100-μM mevinolin. At time '0' the media were replaced with fresh media and mevinolin (100 μM). Results are expressed as nmol/mg of cell protein (mean ± s.d. for four hamsters).

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Cholic acid</th>
<th>Chenodocholic acid</th>
<th>Total amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>3.10 ± 0.38</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Mev +</td>
<td>2.98 ± 0.41</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>6.71 ± 1.16</td>
<td>1.18 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Mev +</td>
<td>6.48 ± 1.58</td>
<td>1.92 ± 0.19</td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>10.66 ± 1.30</td>
<td>1.68 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Mev +</td>
<td>10.43 ± 1.36</td>
<td>1.63 ± 0.29</td>
</tr>
</tbody>
</table>

De novo synthesis of bile acids was investigated by incubating the hepatocytes with [14C]mevalonic acid lactone. As mevalonic acid lies beyond the rate-limiting step of cholesterol biosynthesis, it is efficiently incorporated into cholesterol and subsequently bile acids [4]. After 24 h the cells and media were purified on Bond Elut columns, and spotted on high performance plates and developed as before. To identify and locate the bile acids the plates were developed as described by Grant et al. [4]. After 24 h the amounts of cholic acid and subsequently bile acids [4]. After 24 h the amounts of cholic acid and their taurine and glycine conjugates. The amounts of bile acid synthesized were determined using radioimmunoassays as described previously [5, 6]. The total amounts of bile acids produced were expressed as nmol per mg of cell protein. Protein was determined using as described by Grant et al. [4]. After 24 h the amounts of cholic acid and chenodocholic acid and (and conjugates) synthesized by the hamster hepatocytes were 10.66 ± 1.30 and 1.68 ± 0.20 nmol/mg of cell protein respectively (Table 1).

When mevinolin (100 μM) was added to the hepatocytes a 83 ± 4% (mean ± s.d. from four hamsters) reduction in cholesterol synthesis was determined by [14C]acetate incorporation into cholesterol over 90 min. There was no difference, however, in the amounts of bile acids synthesized between the control and mevinolin treated hepatocytes (Table 1) indicating that de novo cholesterol synthesis may not be important in regulating bile acid synthesis in cultured hamster hepatocytes.

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Isoenzyme-specific induction of cytochromes P-450 in rat hepatocytes cultured in the presence of dimethylsulphoxide

CAROL K. LINDSAY, M. DANNY BURKE, RICHARD J. WEAVER, WILLIAM T. MELVIN and GABRIELLE M. HAWKSWORTH

Clinical Pharmacology Unit, Department of Medicine and Therapeutics, Division of Pharmacology and Department of Molecular and Cell Biology, School of Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD, U.K.

Primary cultures of adult rat hepatocytes would provide a suitable system for drug metabolism/toxicity studies if the drug metabolizing enzymes could be maintained at levels found in vivo. Culture of hepatocytes on collagen-coated plates suspended in a chemically defined medium, supplemented with hormones and serum, results in a loss of hepatocyte-specific differentiated functions. The cells lose the ability to secrete albumin and cytochrome P-450 content declines [1]. Substratum modifications and culture with other liver cell types have been employed to prevent hepatocyte dedifferentiation in culture. Dimethylsulphoxide (DMSO) is a dipolar solvent capable of inducing characteristics of differentiation in a human hepatoma cell line [2]. Isom and co-workers demonstrated that inclusion of 2% (v/v) DMSO in the culture medium allowed hepatocytes to secrete high levels of albumin for at least 43 days [3]. These conditions also resulted in partial maintenance of cytochrome P-450 content [4]. In this study we report the effect of culture in the presence of 2% (v/v) DMSO on cytochrome P-450 content and on the isoenzyme profile.

Hepatocytes were obtained by collagenase perfusion of livers from male Sprague-Dawley rats (200–250 g). Collagen-coated Falcon plates (100 mm) were inoculated with 5 × 106 viable hepatocytes suspended in 10 ml modified Earle's medium [5] supplemented with 5% (v/v) fetal calf serum. After 4 h the culture medium was changed and 2% (v/v) DMSO included, where appropriate. Thereafter medium was changed every 24 h. Cytochrome P-450 content and the dealkylation of ethoxy- and pentoxyresorufin (EROD and PROD, respectively) were determined as described by Grant et al. [5]. EROD

Abbreviations used: DMSO, dimethylsulphoxide; EROD, ethoxyresorufin O-dealkylase; PROD, pentoxyresorufin O-dealkylase; BA, 1,2-benzenanthraene.

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activity was induced by exposing hepatocytes to $2.5 \times 10^{-3}$ \text{M}-1,2-benzanthracene (BA) after the first 24 h of culture. Protein synthesis was inhibited by exposing the cells to $10^{-4}$ M-cycloheximide after the first 48 h of culture. Microsomal proteins, prepared from freshly isolated hepatocytes and cultured cells, were separated using SDS/PAGE, transferred to nitrocellulose paper and then exposed to a polyclonal antibody, raised in rabbits, against purified rat cytochrome $P-450$. The antibody was tested to detect $P-450$ in cultured cells, were separated using SDS/PAGE, transferred to nitrocellulose paper and then exposed to a polyclonal antibody, raised in rabbits, against purified rat cytochrome $P-450$. The results confirm that the increase in EROD activity in hepatocytes cultured in the presence of DMSO is due to a net increase in $P-450$ protein. Exposure of hepatocytes cultured in the presence of DMSO and BA simultaneously caused a 40-fold increase in EROD activity (Table 1). The magnitude of this response to BA approaches that seen in vivo.

The results presented indicate that culture of rat hepatocytes in the presence of $2\%$ (v/v) DMSO causes induction of at least one form of cytochrome $P-450$. The effect of the solvent is isoenzyme specific, since the rate of decline of PROD activity cannot be reduced by culturing in the presence of DMSO. Culture in the presence of DMSO increases availability of the haem prosthetic group, both by an increase in amnolacuvlinic acid synthase and a decrease in haem oxygenase (C. K. Lindsay & G. M. Hawksworth, unpublished work), but this does not explain the isoenzyme-specific response to DMSO.

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