Drug metabolising activity of freshly isolated human hepatocytes

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- 1 Hepatocytes have been isolated from samples of adult human liver by removal of extracellular calcium followed by perfusion with collagenase.
- 2 The hepatocytes were isolated with a yield of up to 39×10^6 cells/g and with a viability of up to 74%.
- 3 The cells were active in the oxidation of aldrin and 7-ethoxycoumarin. They also catalysed the conjugation of 7-hydroxycoumarin.
- 4 Monooxygenase activity of the hepatocytes was linear for at least 60 min.
- 5 Maintenance of the hepatocytes in suspension at 4°C for 19 h resulted in a 15% loss in viability. This was accompanied by a 50% decrease in monooxygenase activity expressed per viable cell.
- 6 It is concluded that human hepatocytes can be isolated in sufficient yield and with satisfactory viability for use in a range of studies on drug metabolism and toxicity.

Keywords human hepatocytes drug metabolism collagenase dispersion aldrin 7-ethoxycoumarin

Introduction

Freshly isolated hepatocytes have been extensively used to study both the processes of drug metabolism and mechanisms of hepatotoxicity (Fry & Bridges, 1977; Thor *et al.*, 1979). These events are often closely associated, in that the hepatotoxicity of many compounds such as isoniazid (Mitchell *et al.*, 1976), paracetamol (Mitchell *et al.*, 1973), halothane (Stier, 1968), and carbon tetrachloride (Slater, 1966), require oxidation to initiate their toxicity. The hepatocyte is thus an ideal model in which to investigate such processes.

There are marked species differences in both the rates and the routes of drug metabolism (Hucker, 1970; Caldwell, 1980). This is true for different substrates and for competing pathways for the same substrate. For example, the oxidation of paracetamol is readily catalysed by mouse liver whereas rat liver catalyses the reaction much more slowly (Jollow *et al.*, 1974). Nevertheless, the sulphation and glucuronida-

tion of paracetamol are catalysed at similar rates in the two species (Jollow *et al.*, 1974). Such species differences in metabolism make extrapolation to man extremely difficult.

The underlying biochemical events, subsequent to metabolism, leading to hepatotoxicity and ultimately to cell death may well show less variation amongst species than the drug metabolizing reactions. However, it is well known that a number of forms of toxicity are confined to a small number of species, and sometimes only to man. These species differences may well reflect differences in events subsequent to metabolism. Examples include the oculomu- cutaneous syndrome caused by practolol (Amos *et al.*, 1978), drug induced lupus erythematosus (Yamauchi *et al.*, 1975) and benoxaprofen hepatotoxicity. Thus, it is ultimately necessary to study toxic reactions in man or in human tissue.

For these reasons it is desirable to be able to

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study metabolism and toxicity in human isolated hepatocytes. Several groups have investigated methods for the isolation of human hepatocytes (Bojar *et al.*, 1976; Miyazaki *et al.*, 1981; Strom *et al.*, 1982) but there have been very few reports on their drug metabolising activity. Bojar *et al.* (1976) perfused the whole liver of a renal transplant donor and obtained cells of high viability. Obvious problems of this method included the amount of collagenase required and the speed with which the perfusion could be performed. Guguen-Guillouzo *et al.* (1982) attempted to overcome these problems by perfusing only a single lobe of the liver (10% of total hepatic mass) via a branch of the portal vein. Cells isolated by this technique had a high viability and catalysed the metabolism of ketotifen by oxidation, reduction and glucuronidation (Begue *et al.*, 1983). An alternative method of obtaining hepatocytes has been to cut biopsy samples into small pieces and incubate with collagenase (Miyazaki *et al.*, 1981). Cells of high viability can be obtained in this way but total yield is small (Maekubo *et al.*, 1982). Attempts to perfuse biopsy samples have so far failed to produce high yields of intact cells (Belfrage *et al.*, 1976).

The current paper reports results of an investigation into the application of the method of Berry & Friend (1969), extensively used in the isolation of rat hepatocytes, in the isolation of hepatocytes from resected samples of human liver. The stability and drug metabolising activity of hepatocytes isolated in this way have been characterised.

Methods

Materials

Dulbecco's phosphate buffered saline (PBS), Earle's balanced salt solution, calcium- and magnesium-free (EBSS) and foetal calf serum (FCS) were purchased from Gibco Ltd, Uxbridge, Middlesex. Gentamicin sulphate, insulin (from bovine pancreas), hydrocortisone 21-hemisuccinate, ethyleneglycol-bis-(β -aminoethyl ether)-*N*, *N*, *N*', *N*'-tetraacetic acid (EGTA), collagenase type 1, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES), Trypan blue, Triton X-100, NADH (disodium salt, grade III) and arylsulphatase (type H-1) were obtained from Sigma Chemical Co Ltd, Poole, Dorset. British Oxygen Company, Mordern, supplied 95% oxygen + 5% carbon dioxide and 100% oxygen. Dimethylsulphoxide (DMSO) was purchased from Flurochem Ltd, Glossop, Derby. Nanograde hexane was obtained from Camlab Ltd, Cambridge, Cambs. Aldrich Chemical Co Ltd, Gillingham, Dorset, supplied 7-ethoxycoumarin (gold label grade) and 7-hydroxycoumarin. The GLC packing SE-30 on Chromosorb W.A.W.DMCS 80-100 was from Perkin-Elmer Ltd, Beaconsfield, Bucks. Veraspor 1200 filters (1-2 μ m), which were used in the perfusion circuit, were supplied by Gelman Scientific Ltd, Northampton, Northants. Aldrin and dieldrin were generous gifts from Dr D. H. Hutson (Shell Toxicology Laboratory, Sittingbourne, Kent). All other reagents were of analytical reagent grade or better.

Table 1 Details of patients from whom liver samples were obtained for the isolation of hepatocytes

a					
Sample	Sex	Age (years)	Weight (kg)	Height (cm)	Indication for surgery
07001	F	23	58.4	163	Malignant mesenchymoma of the liver
07002*					
07003	F	48	57.0	Not known	Metastatic hepatic adenocarcinoma
07004	F	59	49.0	152	Cavernous haemangioma of the liver
07005	M	66	57.9	178	Adenocarcinoma of the gall bladder
07006	F	30	41.5	151	Fibrolamellar type hepatoma
b					
Sample	AST (iu/l)	Alkaline phosphatase (iu/l)	Bilirubin (μ M)	Smoking (cigarettes/day)	Drug ingestion†
07001	42	300	11	0	Pethidine
07002*					
07003	47	210	14	0	Salbutamol, navidrex K, metoprolol, Gaviscon
07004	24	75	10	0	Temazepam, metoclopramide, paracetamol
07005	19	114	16	0	Temazepam, mebeverine
07006	232	179	7	10	Paracetamol, temazepam, metoclopramide, pethidine, prednisolone, dihydrocodeine

*No details were available for this patient

†Drugs listed were taken for a period of at least 7 days prior to obtaining the liver sample.

Liver samples

Human liver samples were obtained from patients undergoing partial hepatectomy for primary or secondary carcinoma of the liver. Local Research Ethics Committee permission was obtained to use such liver samples in these studies. Details of the patients from whom liver samples were obtained are given in Table 1. Tissue as far removed from the neoplastic area as possible, assessed macroscopically, was removed for isolation of hepatocytes. Samples were dissected to have as many capsular faces as possible, usually one or two (Figure 1a). All samples were assessed as non-malignant from histology of a small biopsy taken from the edge of the section used for hepatocyte isolation. All patients were hepatitis B surface antigen negative.

Isolation of hepatocytes

Liver samples were obtained within 45 min of resection and were maintained at 4°C until perfusion was started, usually within 20 min of receipt of the samples in our laboratory. The samples were flushed with ice cold PBS for 2–3 min immediately upon receipt to remove as much blood as possible. One or more branches of the portal vein were identified and cannulated with cannulae of appropriate diameter (Argyle Medicut, Petit Rechain, Belgium). Usually two or three cannulae were inserted into each sample (Figure 1b). The liver sample was then placed on a gauze mesh in the perfusion chamber. The details of the perfusion circuit are shown in Figure 2.

Samples were first perfused with calcium- and magnesium-free Earle's balanced salt solution (EBSS) containing 9.7 mg/100 ml gentamicin sulphate, 26.8 mM sodium bicarbonate and 0.5 mM EGTA, to remove extracellular calcium. The medium was gassed with 95% oxygen + 5% carbon dioxide, to pH 7.4. The pH was constantly monitored and if necessary was adjusted to 7.40 by the addition of 0.1 M sodium hydroxide. During the entire perfusion the pH of the medium was kept between 7.40 and 7.47. The flow rate, dictated by the size and number of cannulae, was between 5 and 10 ml min⁻¹ g⁻¹ liver. During this first stage of the perfusion, which lasted 5–10 min, the medium was not recirculated. The total volume of medium used was 1–3 l.

The EGTA was removed from the liver by perfusion for 7–15 min, without recirculation, with 1–2 l of the above medium omitting the EGTA.

Dispersion of the hepatocytes was achieved by perfusion with EBSS containing 9.7 mg/100 ml gentamicin sulphate, 28.8 mM sodium bicarbonate, 0.05% collagenase type 1 and 5 mM calcium chloride. For the first minute the perfusate was discarded but thereafter it was recirculated, the total volume used being 0.5–1.0 l. This stage of the perfusion lasted 40–55 min. During this time the liver swelled slightly and signs that the hepatocytes were becoming dispersed, such as softening of the cut surfaces of the tissue, were apparent (Figure 1c).

At the end of the perfusion the liver was placed in a petri dish containing dispersion medium. This comprised EBSS with 10% FCS, 8 mM sodium bicarbonate, 10 mM HEPES, 12 mM sodium phosphate, 1.05 mM calcium chloride, 0.73 i.u./ml insulin, 0.1 mM hydrocortisone 21-hemisuccinate and 9.7 mg/100 ml gentamicin sulphate. Any capsule attached to the sample was pierced with a scalpel and dissociated cells teased from the liver by stroking the surfaces of the sample with a glass rod (Figure 1d,e). The crude hepatocyte suspension was filtered through nylon bolting cloth, to remove connective tissue and undissociated cells, and the hepatocytes washed by gentle dispersion in the above medium (dispersion medium) followed by sedimentation at 50–100 × *g*_{av} for 2–4 min. The washing process was repeated three times. The supernatants, containing non-parenchymal cells, were discarded. The hepatocyte pellet was finally resuspended in dispersion medium, and the cells placed on ice. Cell numbers were determined by counting the cells in an aliquot of the suspension using an improved Neubauer counting chamber (depth 0.1 mm; 1/400 mm²) illuminated by phase contrast lighting (10x binocular eyepiece; 10x objective).

Cell viability

The viability of the cells was assessed by Trypan blue exclusion with 0.15% (w/v) final concentration of the dye for staining. Viability was also assessed by leakage of lactate dehydrogenase (LDH) from damaged hepatocytes into the medium. An aliquot of the cell suspension was centrifuged for 1 min at 100 × *g*_{av} to sediment the cells. The supernatant was removed for assay of extracellular LDH. The cells in the pellet were lysed by the addition of 1% Triton X-100 in PBS and used for the assay of intracellular LDH. LDH activity was determined from the rate of oxidation of NADH to NAD in the presence of pyruvate as substrate, at 340 nm on a Model 555 spectrophotometer (Perkin-Elmer Ltd, Beaconsfield, Bucks) thermostated

Figure 1 Photographs of a typical human liver sample before and after collagenase perfusion for the isolation of hepatocytes.

(a) Liver section obtained after dissection from larger resected sample.

(b) Liver section after cannulation of accessible blood vessels. Cannulae were secured by thread stitches but this was not always possible (e.g. cannula at upper right).

c

d

(c) Liver section after perfusion with collagenase-containing medium.

(d) Hepatocytes were dissociated from the tissue matrix by teasing with a glass rod. The suspension of dispersed cells in medium is visible in the petri dish.

(e) Tissue remaining after dissociation of the hepatocytes.

Figure 2 Perfusion circuit used for the isolation of human hepatocytes. The perfusion medium was gassed with 95% O_2 + 5% CO_2 and the pH monitored continuously. If necessary, the pH was adjusted by the addition of 0.1 M sodium hydroxide. Separate peristaltic pumps were used to pump medium into the liver sample, and to return the medium from the perfusion chamber to the reservoir. The temperature of the hepatic sample was maintained at 37°C both by heating the medium and by circulating water at 39–40°C through the jacket of the perfusion chamber.

to 25°C. LDH activity was expressed as change in absorbance/min. Cell viability was calculated from the ratio of intracellular LDH to total (intracellular + extracellular) LDH $\times 100$. Drug metabolising activity was expressed relative to the number of viable cells.

7-Ethoxycoumarin metabolism

Cells were incubated in dispersion medium containing only 5% FCS at a density of $0.5\text{--}1.0 \times 10^6$ cells/ml. The total volume was 40 ml and this was placed in a 100 ml Erlenmeyer flask. The suspension was gassed with 100% oxygen and preincubated at 37°C for several minutes to permit temperature equilibration. The reaction was started by the addition of 7-ethoxycoumarin in DMSO to give a final concentration of 70 μM . The flask was incubated at 37°C in a shaking water bath at 27 cycles/min. Aliquots of the cell suspension (4×1 ml for metabolite assays and 2×0.5 ml for viability assessment) were removed at various times throughout the incubation period which lasted for a total of 60 min. The reaction was terminated by placing the 1 ml aliquots in methanol/solid carbon dioxide mixture. Free 7-hydroxycoumarin was determined in duplicate 1 ml aliquots at each time point, as was total 7-hydroxycoumarin.

Conjugates of 7-hydroxycoumarin were hydrolysed by adjusting the pH of the samples to 5.0 with 0.1 M acetate buffer and incubating with 300 u sulphatase (Sigma type H-1 containing β -glucuronidase) at 37°C for 16–18 h. 7-Hydroxycoumarin was extracted from both sets of samples (free and hydrolysed) into 3 ml of chloroform twice and the chloroform extracts pooled from each sample. One ml of the pooled chloroform extracts from each sample was back extracted into 1.5 ml of 0.01 M sodium hydroxide: 1 M sodium chloride. The amount of 7-hydroxycoumarin in the samples was determined by fluorescence spectroscopy (Aminco-Bowman, American Instruments Co., Silver Spring, Maryland) with an excitation wavelength of 370 nm and an emission wavelength of 450 nm. Quantitation was by reference to 7-hydroxycoumarin standards in the appropriate range taken through the extraction procedure.

Aldrin epoxidation

The incubation medium was dispersion medium without FCS, as this interfered with the determination of dieldrin. Hepatocytes were incubated at a density of $0.5\text{--}1 \times 10^6$ cells/ml in a total volume of 40 ml in a 100 ml Erlenmeyer flask. The suspension was preincubated at 37°C for several minutes to permit equilibration, and

the flask was oxygenated with 100% O_2 . The reaction was started by the addition of aldrin in DMSO, 10 μl /ml incubation medium, to give a final substrate concentration of 200 μM . The flask was incubated at 37°C in a shaking water bath (27 cycles/min) and duplicate 1 ml aliquots of the cell suspension removed at various times up to 60 min for the assay of dieldrin. Duplicate 0.5 ml aliquots of the suspension were removed at the same times for viability assessment.

The reaction was stopped by adding 5 ml nanograde hexane to each 1 ml sample. Dieldrin was extracted into the hexane together with the aldrin, by vortexing for 30 s. The hexane phase was removed and stored until analysis. Dieldrin was assayed by gas liquid chromatography with an electron capture detector (Model F17 GLC Perkin-Elmer Ltd, Beaconsfield, Bucks). The glass column was 2 m $\times$ 2 mm i.d., packed with SE-30 5% silicone rubber on chromosorb (AW-DMCS) 80–100 as stationary phase. The carrier gas was nitrogen at a flow rate of 50 ml/min and an inlet pressure of 42 psi. The oven temperature was 205°C and the detector temperature was 250°C. The electron capture detector was set to an ionisation pulse setting of 6, with a purge flow rate of 25 ml/min. Samples from the hexane extracts, up to a maximum of 5 μl , were injected onto the GC column using a 10 μl high pressure GLC syringe with a PTFE plunger tip (Phase Separations, Queensferry, Clwyd). Quantitation of dieldrin was by reference to external standards taken through the same extraction procedure.

Results

The degree of dissociation of the liver samples and the yield of cells/g tissue varied considerably among the samples (Table 2). The variation in the extent of dissociation was due largely to the morphology of the samples and the extent to which they could be perfused through an accessible branch of the portal vein. It was often apparent from the pattern of blanching during perfusion that large areas of the liver sample were not being perfused. However, because of the number of cut surfaces on the samples, it was often not feasible to improve upon this.

The initial viability of the cells was usually high and the cells appeared bright and well rounded under phase contrast microscopy (Figure 3). Human hepatocytes appear more granular than rat hepatocytes under both light and electron microscopy. A few of the cells showed blebbing of the cell membrane. Electron microscopy of the cells (Figure 4) revealed

Table 2 Dissociation and viability of hepatocytes isolated from liver samples

Sample	Weight (g)	% Dissociation	% Viable cells	Cell yield ($\times 10^{-6}/g$)	Total cell yield ($\times 10^{-6}$)
07001	63	5.0	37.6	0.4	2.1
07002	31	25.4	34.0	3.3	26.3
07003	24	38.8	63.0	5.3	31.0
07004	33	58.1	74.0	39.1	703
07005	14	30.6	67.7	4.0	17.5
07006	13	38.0	64.0	12.6	63.0

that the morphology of the hepatocytes was intact, with well-defined nuclei, endoplasmic reticulum and mitochondria. There were lipid inclusions and many microvesicular bodies. Some of the cells showed evidence of surface blebbing. This ranged from merely a slight extrusion of the cytoplasm to a gross distortion of the cell morphology with invasion of the bleb by intracellular organelles, due presumably to a major disruption of the cytoskeleton.

Monooxygenase activity was assessed in the six samples and activity was detectable in all samples with both of the substrates tested. Only

with three of the samples was it possible to obtain full metabolic profiles. Activity was linear to at least 60 min, expressed per viable cell, for both aldrin (Figure 5) and 7-ethoxycoumarin (Figure 6) oxidation. With 7-ethoxycoumarin as substrate there was substantial conjugation of the oxidation product 7-hydroxycoumarin. Although the data shown are for freshly isolated cells, oxidation of both substrates was linear for 60 min with cells up to 19 h old.

Although the conjugates of 7-hydroxycoumarin could be hydrolysed by a mixed

Figure 3 Photomicrograph of human hepatocytes, exposed to 0.15% (w/v) Trypan blue solution, taken with phase contrast lighting. The cells were photographed with 10x objective and 10x camera lenses. The final magnification in 250x. The lines visible in the photograph are from the Neubauer counting chamber. A few Trypan blue-stained hepatocytes are apparent.

a

b

Figure 4 Electron micrographs of human isolated hepatocytes.

(a) Hepatocyte with no obvious morphological abnormalities. Many microvesicular inclusions are apparent. Magnification $\times 5000$.

(b) Hepatocyte showing slight surface blebbing. Mitochondria and endoplasmic reticulum show no evidence of disruption. Magnification $\times 8000$.

c

d

(c) Binucleate hepatocyte, which constitute approximately 30% of the cells isolated. Magnification $\times 5000$.

(d) Hepatocyte with extensive surface blebbing. Distortions of the mitochondria are apparent. Magnification $\times 5000$.

e

(e) Hepatocyte showing gross distortion of morphology with invasion of the bleb by organelles. Magnification $\times 8000$.

Figure 5 Time course of aldrin epoxidation by human isolated hepatocytes. Aliquots of cell suspension were removed at various times for determination of cell viability and of dieldrin. Activities have been expressed relative to number of viable cells. Results shown are from a single liver sample but are typical of those obtained with three separate samples.

Figure 6 Time course of 7-ethoxycoumarin metabolism by human isolated hepatocytes. Aliquots of cell suspension were removed at various times for the determination of cell viability and of free ($\blacktriangle$) and total ($\bullet$) 7-hydroxycoumarin. Activities have been expressed relative to number of viable cells. The results shown were obtained with a single liver sample but are typical of those obtained with samples from three different livers.

preparation of sulphatase and β -glucuronidase, the exact identity of the conjugates was not established in the present study. The oxidation of 7-ethoxycoumarin was linear for 60 min but the production of free 7-hydroxycoumarin increased only over the first 15–30 min and then plateaued (Figure 6). This presumably reflected the achievement of a steady state of formation and conjugation of this metabolite.

In one of the samples, despite a good overall rate of 7-ethoxycoumarin oxidation, there was poor conversion of 7-hydroxycoumarin to conjugated metabolites (Table 3). Examination of the history of this patient failed to provide any indication as to why this might be so. Nor was it possible to determine whether sulphation, glucuronidation or both reactions were impaired.

During incubation of the cells at 37°C there

was a decrease in viability, assessed both by LDH leakage (Figure 7) and by Trypan blue exclusion. Under similar incubation conditions rat hepatocytes showed no such reduction in viability (unpublished observations).

The viability of human hepatocytes was well maintained when the cells were kept at 4°C in medium containing FCS (Figure 8), with a 15% decrease in viable cells over 19 h. With age the viability profile of the cells during incubation changed (Figure 7). Immediately after isolation the cells maintained their viability well over the first 30 min of incubation. As the cells aged, there was some loss of viability over the first 30 min of incubation. The older the cells, the more substantial was this loss in viability. In contrast to the hepatocytes immediately after isolation, there was a tendency for older cells to maintain

Table 3 Conjugation of 7-hydroxycoumarin by human isolated hepatocytes

7-Ethoxycoumarin O-deethylation			
7-Hydroxycoumarin formation			
Sample	Free (<i>pmol (10⁶ cells)⁻¹ min⁻¹</i>)	Total (<i>pmol (10⁶ cells)⁻¹ min⁻¹</i>)	% Conjugated
07003	5.0	36.0	86.0
07004	9.6	47.5	79.8
07005	18.2	25.8	29.5

Figure 7 Viability of human hepatocytes during incubation at 37°C. Cells were studied immediately after isolation (●) or they were maintained at 4°C for 3 h (▲), 10.75 h (▼), 15.25 h (■) or 19.25 h (◆) prior to incubation at 37°C for up to 60 min. Aliquots of cell suspension were removed at various times for the determination of hepatocyte viability by the lactate dehydrogenase leakage method as described in the text. Results shown are from a single liver sample but similar results were obtained with three separate samples of liver.

Figure 8 Viability of human hepatocytes maintained at 4°C for various time intervals after isolation. Aliquots of the cell suspension were removed and viability determined both by Trypan blue exclusion (■) and by leakage of lactate dehydrogenase into the medium (▲). Although the results shown were obtained with a sample from a single liver they are typical of those obtained with three samples from separate livers.

Table 4 Stability of monooxygenase activity of human isolated hepatocytes maintained in suspension at 4°C

Period of storage at 4°C (h)	Aldrin epoxidase activity (nmol dieldrin formed (10 ⁶ viable cells) ⁻¹ h ⁻¹)	7-Ethoxycoumarin O-deethylase activity (nmol 7-hydroxycoumarin formed (10 ⁶ viable cells) ⁻¹ h ⁻¹)
0	7.98	2.56
3	6.69	2.73
11	4.59	1.93
15	4.41	1.44
19	4.15	1.43

Results shown are from a single liver sample, but similar results were obtained with samples from three different livers.

their viability better over the latter stages of incubation, with no loss of viability occurring from 30–60 min with 19 h old cells.

The metabolic activity of the cells decreased as the cells aged. This was true even if activities were corrected for changes in viability (Table 4). Thus, the loss of activity per viable cell over 19 h was approximately 50%.

Discussion

Several groups have recently reported methods for the isolation of human hepatocytes (Bojar *et al.*, 1976; Miyazaki *et al.*, 1981; Strom *et al.*, 1982). The most successful methods appear to be those using a modification of the collagenase perfusion technique of Berry & Friend (1969). Both yield and viability of cells obtained by the present method compare well with those reported by others. The variability of yield is due presumably to differences in the morphology of the samples. Sometimes it was not possible to cannulate more than one branch of the portal vein. In addition, some samples had only one capsular side, resulting in inadequate perfusion and loss of some of the hepatocytes dissociated initially. However, the method does enable a large number of viable human hepatocytes to be obtained and can easily be scaled up for larger samples of liver.

There have been very few reports on drug metabolising activity of human isolated hepatocytes. Begue *et al.* (1983) have shown that ketotifen is metabolised by a variety of pathways and Reese & Byard (1981) have demonstrated *N*-demethylase activity of a single sample. Strom *et al.* (1982; 1983) demonstrated unscheduled DNA synthesis stimulated by a number of carcinogens such as aflatoxin B₁, 2-acetylaminofluorene and diethylnitrosamine that depend upon oxidation for their genotoxic effects, in hepatocytes isolated from two liver samples. In the present paper the oxidation of

aldrin and 7-ethoxycoumarin by human isolated hepatocytes has been demonstrated. In addition, the cells were capable of oxidising bufuralol and benzo(a)pyrene (data not shown). The cells maintain their metabolic activity for up to 19 h, although they are best used within 6 h of isolation. In addition to oxidation, the cells catalyse the conjugation of 7-hydroxycoumarin with sulphate and/or glucuronide.

The viability of the cells is maintained quite well by storage at 4°C in the presence of 10% FCS. However, the viability profiles of cells of different ages during incubation suggest that as the cells age the more fragile cells are quickly killed at 37°C, leaving a population of cells that is more resistant to the oxidative and mechanical stress of such incubations. The morphology and biochemistry of this sub-population of cells have not yet been investigated, but may have important implications for hepatic function.

Although the cells are still metabolically active at 19 h after isolation, there is a 50% reduction in activity per viable cell during this interval. This may reflect loss of enzyme protein or depletion of essential cofactors. The latter possibility seems less likely since the oxidative reactions are linear for 60 min even 19 h after isolation of the hepatocytes. Cultured human hepatocytes lose approximately 40% of their cytochrome P-450 content expressed per mg microsomal protein (Strom *et al.*, 1982) over the first 24 h. It may be that this process occurs even in cells maintained at 4°C in suspension.

When hepatocytes, particularly from rat, are grown in culture, within 12 h they show dramatic changes in both morphology and monooxygenase activities (Bissel *et al.*, 1973; Guzelian *et al.*, 1977). In contrast, cells kept at 4°C in suspension maintain their morphology and much of their monooxygenase activity over at least 19 h. Although it is not possible to conduct experiments in which continuous metabolic activity is required of the cells over this interval, nevertheless such cells could prove

invaluable in short term studies on the interaction between the processes of drug metabolism and hepatotoxicity. In addition, a number of groups have demonstrated that human hepatocytes isolated by methods similar to that described here can be successfully cultured (Miyazaki *et al.*, 1981; Maekubo *et al.*, 1982; Strom *et al.*, 1982; Guguen-Guillouzo *et al.*, 1982). Recently, Clement *et al.* (1984) have shown that co-culture of human hepatocytes with rat liver epithelial cells enables maintenance of both the morphology and albumin synthesis of the hepatocytes for several weeks. Cytochrome P-450 content of such cells remains at a

level close to that on isolation of the cells. Thus, it should be possible to conduct longer term studies, involving synthetic processes of the cell, to be investigated. Obvious areas for study include enzyme induction, unscheduled DNA synthesis, and DNA repair.

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