Pergamon

0278-6915(94)00067-0

Fd Chem. Toxic. Vol. 32, No. 9, pp. 819–829, 1994
Elsevier Science Ltd. Printed in Great Britain

USE OF PRECISION-CUT LIVER SLICES FOR STUDIES OF UNSCHEDULED DNA SYNTHESIS

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(Accepted 6 April 1994)

Abstract—Precision-cut liver slices were prepared from untreated and Aroclor 1254 (ARO)-treated male Sprague–Dawley rats with a Krumdieck tissue slicer. Liver slices were cultured for 24 hr in medium containing [^{3}H]thymidine and 0–0.1 mM 2-acetylaminofluorene (2-AAF) using a dynamic organ culture system and processed for autoradiographic evaluation of unscheduled DNA synthesis (UDS). Compared with control (i.e. 0 mM 2-AAF) liver slice cultures, 2-AAF produced a concentration-dependent increase in UDS, the effect being more marked in liver slices from ARO-treated than from untreated rats. With liver slices from untreated rats, 2-AAF produced the greatest increase in UDS in centrilobular hepatocytes. 2-AAF-induced UDS in liver slices from ARO-treated rats was most marked in centrilobular hepatocytes but the effect also extended to other areas of the liver lobule. These results demonstrate that precision-cut liver slices may be a valuable alternative *in vitro* system to hepatocyte cultures for screening chemicals for potential genotoxicity. Unlike hepatocyte cultures, liver slices permit the study of zonal differences in UDS. Moreover, this technique could be applied to other tissues and the study of species differences in response.

INTRODUCTION

The liver is the major site of xenobiotic metabolism and is often a target organ for xenobiotic-induced toxicity (Parke, 1968; Zimmerman, 1978). In recent years much effort has been devoted to the development of *in vitro* systems for assessing the hepatic effects of xenobiotics. Such systems reduce the use of laboratory animals and offer the potential to compare effects of chemicals in tissue from both laboratory animals and humans, which may provide valuable data for the human risk assessment of xenobiotics (Frazier *et al.*, 1989).

One such model system is the use of precision-cut tissue slices, a technique developed by Krumdieck, Brendel and coworkers (Brendel *et al.*, 1987; Krumdieck *et al.*, 1980; Sipes *et al.*, 1987; Smith *et al.*, 1989). This technique may be applied to various tissues including the liver, kidney, lung and heart (Azri *et al.*, 1990; Parrish *et al.*, 1992; Ruegg *et al.*, 1989; Smith *et al.*, 1989; Stefaniak *et al.*, 1992). Precision-cut tissue slices have a number of potential advantages over other *in vitro* systems such as primary hepatocyte cultures (Azri *et al.*, 1990; Brendel *et al.*, 1987; Smith *et al.*, 1989). For example, slicing maintains tissue architecture so that all cell types are present, slicing avoids the damage to cells that may occur during cell isolation procedures using proteolytic enzymes and the technique may be applied relatively easily to tissues from different species.

Precision-cut liver slices have been used for studies of xenobiotic metabolism and toxicity (Azri *et al.*, 1990; Dogterom, 1993; Miller *et al.*, 1993; Smith *et al.*, 1987 and 1989) and for studies of induction of peroxisomal and microsomal enzyme activities (Beamand *et al.*, 1993; Lake *et al.*, 1993). With respect to xenobiotic-induced toxicity, previous studies have investigated the effect of various compounds on slice morphology and assays of cytotoxicity including inhibition of protein synthesis and leakage of potassium and enzymes (Azri *et al.*, 1990; Sipes *et al.*, 1987; Smith *et al.*, 1987 and 1989). However, their use to screen chemicals for potential genotoxicity does not appear to have been investigated.

The aim of this investigation was to examine whether precision-cut rat liver slices could be used for studies of unscheduled DNA synthesis (UDS), a technique routinely used with primary hepatocyte cultures (Althaus *et al.*, 1982; Loury *et al.*, 1986; Williams, 1977). Precision-cut tissue slices offer the opportunity to investigate chemically induced UDS in hepatocytes in different areas of the liver lobule and possibly in other cell types (Azri *et al.*, 1990; Brendel *et al.*, 1987; Sipes *et al.*, 1987; Smith *et al.*, 1987 and 1989). The studies were conducted with 2-acetylaminofluorene (2-AAF), a known genotoxic agent that produces UDS in primary hepatocyte cultures (Althaus *et al.*, 1982; Butterworth *et al.*, 1984; Williams, 1977). In addition, studies were conducted with liver slices from control (i.e. untreated)

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Abbreviations: 2-AAF = 2-acetylaminofluorene; ARO = Aroclor 1254; DMSO = dimethyl sulfoxide; EBSS = Earle's balanced salt solution; UDS = unscheduled DNA synthesis.

rats and from rats pretreated with Aroclor 1254 (ARO). This polychlorinated biphenyl mixture is known to induce cytochrome *P*-450 isoenzymes in the CYP1A, CYP2B, CYP3A and other subfamilies (Nelson *et al.*, 1993; Okey, 1990).

MATERIALS AND METHODS

Materials. 2-AAF was purchased from Aldrich Chemical Co. Ltd. (Gillingham, UK) and [*methyl*-^{3}H]thymidine (sp. act. 85 Ci/mmol) from Amersham International plc (Little Chalfont, Bucks, UK). ARO was the generous gift of the Monsanto Company (St Louis, MO, USA). The sources of tissue culture materials, polystyrene incubation vials and stainless-steel wire mesh were as described previously (Beamand *et al.*, 1993; Lake *et al.*, 1993).

Animals and treatment. Male Sprague–Dawley rats were purchased from Harlan Olac (Bicester, Oxon, UK) and allowed free access to R and M No. 1 diet (Special Diets Services, Witham, Essex, UK) and water. The animals were housed in mesh-floored cages in accommodation maintained at 22 ± 3 C with a relative humidity of 40–70%, and were allowed to acclimatize to these conditions for at least 2 wk before use. To induce hepatic xenobiotic metabolism rats were given a single ip 500 mg/kg dose of ARO 5 days before autopsy.

Preparation of liver slices. Untreated and ARO-pretreated rats (16–18 wk old) were killed by carbon dioxide asphyxiation followed by cervical dislocation. The livers were immediately excised and placed in Earle's balanced salt solution (EBSS) containing 25 mM D-glucose, 50 μg gentamicin/ml and 2.5 μg fungizone/ml previously gassed with 95% O_2/5% CO_2. Tissue cylinders were prepared with a 10 mm diameter motor-driven tissue coring tool. From the cylinders, tissue slices (200–300 μm) were prepared in oxygenated (95% O_2/5% CO_2) EBSS containing the above additions at room temperature using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munford, AL, USA).

Culture of liver slices. Liver slices were floated onto stainless-steel mesh inserts (two slices per insert) and cultured in polystyrene vials containing 1 ml culture medium using a dynamic organ culture system as described previously (Beamand *et al.*, 1993; Lake *et al.*, 1993). The culture medium consisted of RPMI 1640 containing 5% foetal calf serum, 0.5 mM (final concentration) L-methionine, 1 μM insulin, 0.1 mM hydrocortisone-21-hemisuccinate, 50 μg gentamicin/ml and 2.5 μg fungizone/ml. The vials were placed on a roller system housed in a humidified incubator. They were incubated at 37 C in an atmosphere of 5% CO_2/95% air and rotated at approximately 9 rpm. After 2 hr, treatment was started by replacing the culture medium with medium containing 10 μCi [*methyl*-^{3}H]thymidine/ml and 0–0.1 mM 2-AAF. 2-AAF was dissolved in dimethyl sulfoxide (DMSO) and added to the culture medium so that the final DMSO concentration was 0.4% (v/v) in all vials including the control cultures. After 24 hr culture the liver slices were harvested for determination of UDS and biochemical investigations.

Measurement of unscheduled DNA synthesis. Liver slices were fixed in neutral buffered formalin and 4 μm paraffin sections were dipped in liquid photographic emulsion and exposed in the dark at 4 C for 14 days. After processing, the developed slices were stained with haematoxylin. Quantitative assessment of silver grains in the nuclei and cytoplasm of centrilobular hepatocytes in the liver slice sections was performed with a Seescan Image Analysis System (Seescan plc, Cambridge, UK). Grains were counted for the whole nucleus area and from three equal-sized adjacent areas of cytoplasm. For each hepatocyte nucleus the mean of the cytoplasmic counts was subtracted from the nuclear count to give the net grain count. For each liver slice 50 centrilobular hepatocytes were scored and for each experiment four control liver slices and two liver slices per concentration of 2-AAF were examined. The slides were coded and examined blind. For each treatment the population average of net grains was calculated (Williams, 1977). In addition, the percentage of centrilobular hepatocytes undergoing DNA repair with both more than five net grains (Williams, 1977) and more than 10 net grains was calculated. The number of hepatocyte nuclei undergoing replicative DNA synthesis (i.e. hepatocytes distinguished by a high density of silver grains over the nucleus) was assessed

Table 1. Levels of protein, DNA and cytochrome *P*-450 in freshly cut and 24-hr cultured liver slices from untreated and ARO treated rats

	Freshly cut slices		24-hr Cultured slices	
Parameter	Untreated	ARO	Untreated	ARO
Protein content (mg protein per slice)	3.6 ± 0.2	3.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
DNA content (μg DNA mg protein)	4.8 ± 0.3	4.7 ± 0.6	5.3 ± 0.6	5.8 ± 0.7
Cytochrome *P*-450 (pmol/mg protein)	164 ± 10	773 ± 32***	60 ± 1	334 ± 30***

ARO = Aroclor 1254

Values are means ± SEM of four to seven experiments.

Asterisks indicate significant differences from the control (untreated) rats (***$P < 0.001$, least significant difference test).

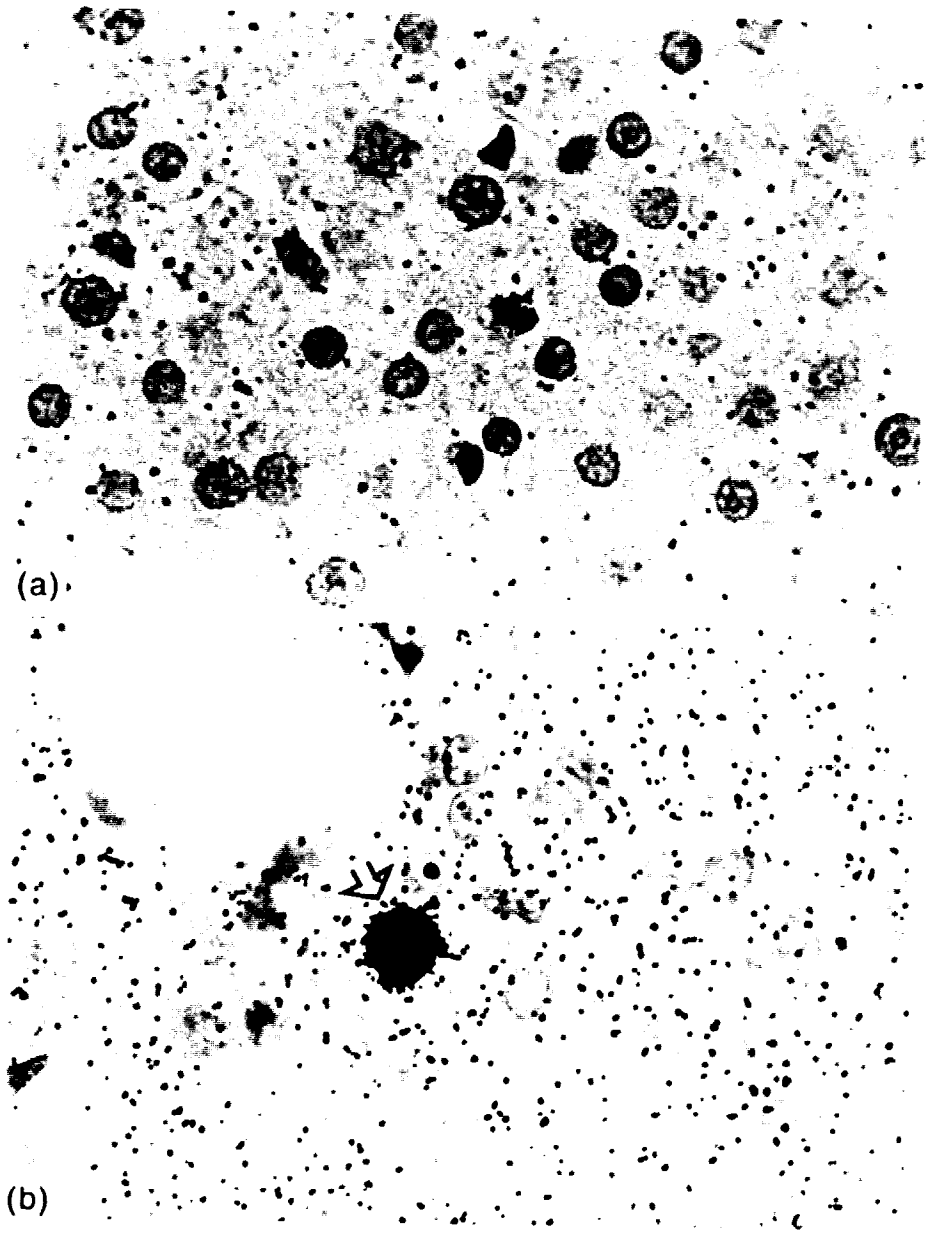

Plate 1(a,b). Legend overleaf.

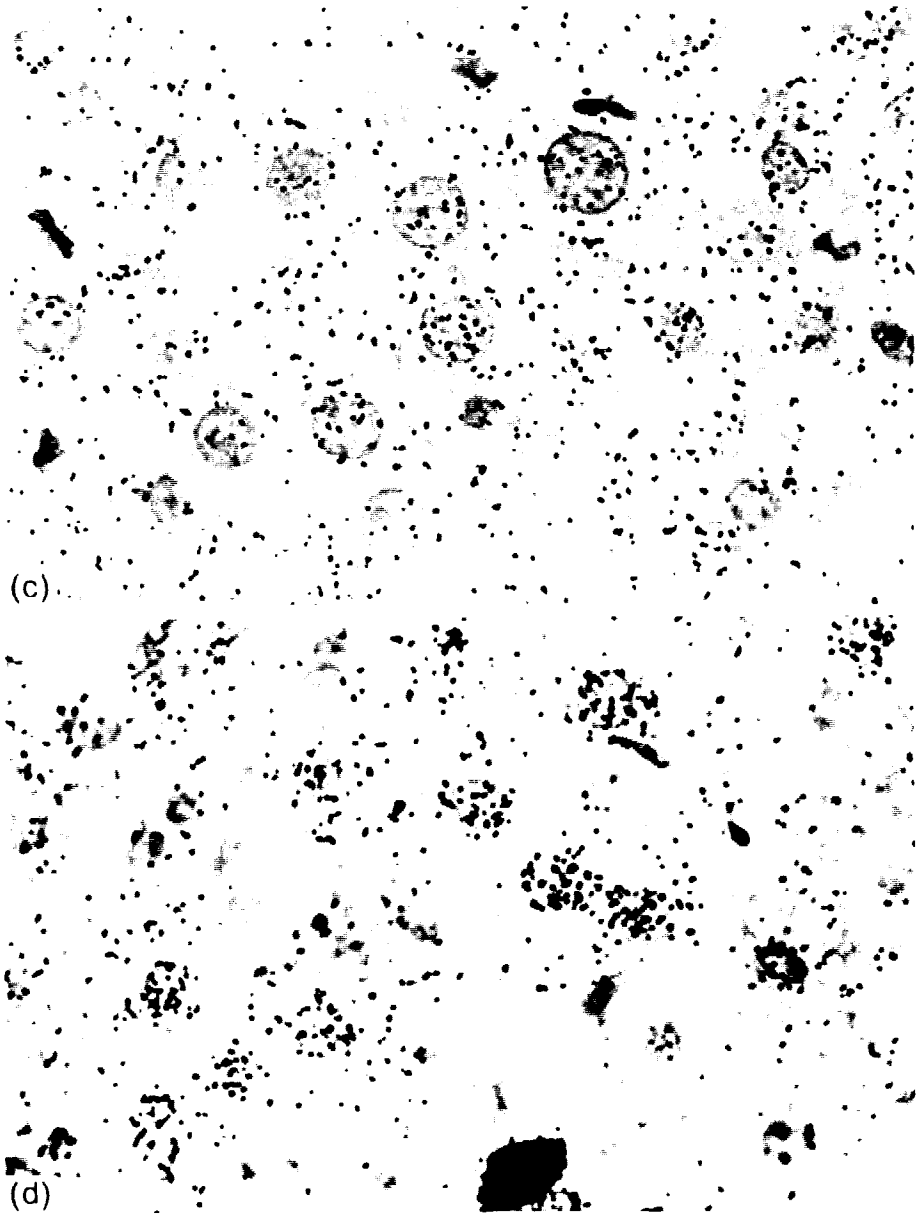

Plate 1. Autoradiographs of centrilobular areas of liver slices from untreated (a, c) and Aroclor 1254 (ARO)-treated (b, d) rats cultured for 24 hr in medium containing [^{3}H]thymidine. Compared with control (dimethyl sulfoxide only) liver slice cultures (a, b), treatment with 0.05 mM 2-acetylaminofluorene produced a marked increase in unscheduled DNA synthesis in liver slices from untreated (c) and particularly from ARO-treated (d) rats. In cultured liver slices from both untreated (not shown) and ARO-treated (b) rats, some hepatocyte nuclei were undergoing replicative DNA synthesis (arrowed). Haematoxylin, magnification ×925.

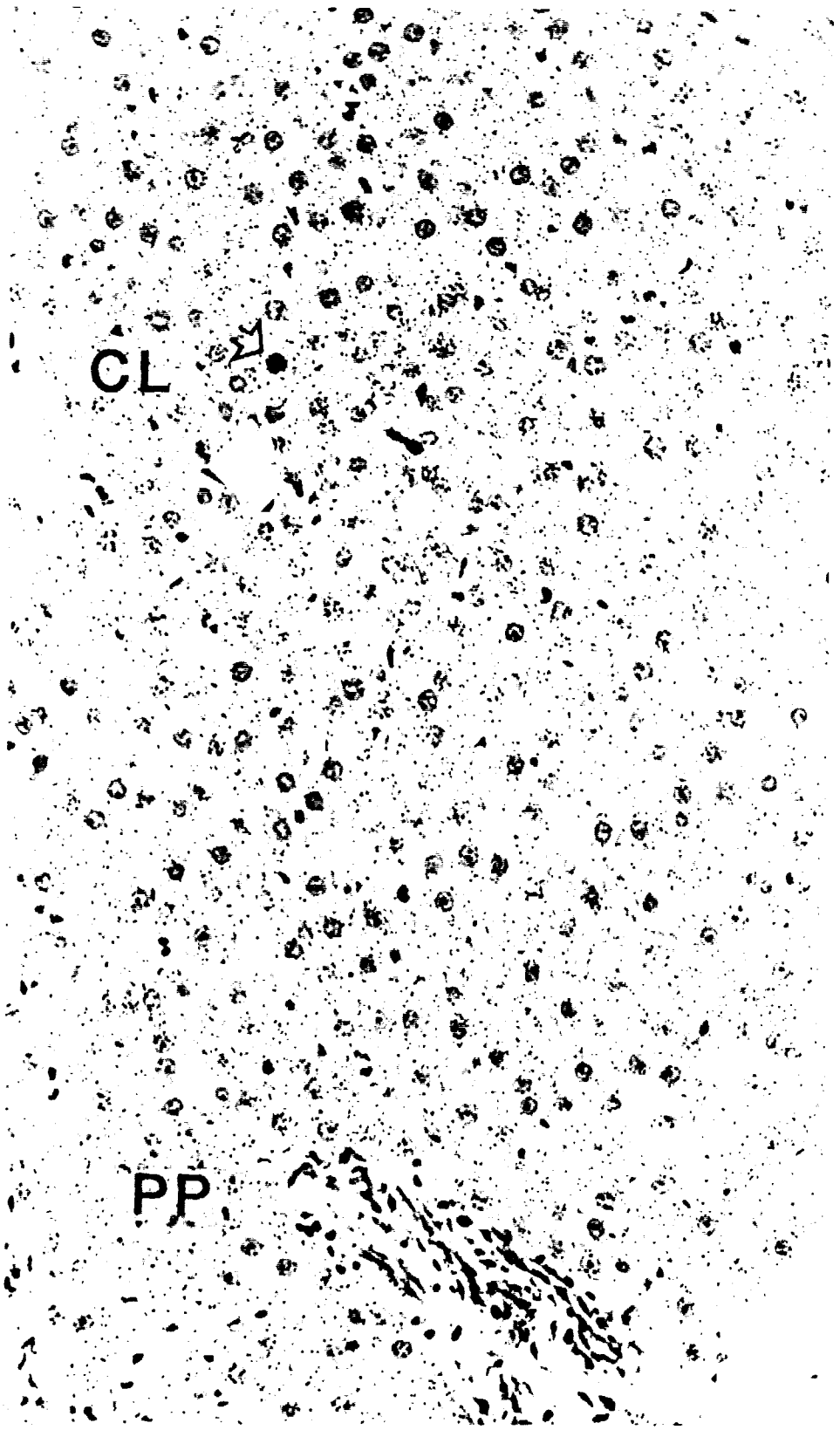

Plate 2. Autoradiograph of a liver slice from an untreated rat cultured for 24 hr in medium containing [H]thymidine and 0·05 mM 2-acetylaminofluorene (2-AAF). 2-AAF-induced unscheduled DNA synthesis is more marked in centrilobular (CL) than in periportal (PP) hepatocytes. Some hepatocyte nuclei were undergoing replicative DNA synthesis (arrowed). Haematoxylin, magnification × 295.

by counting 1000 nuclei per liver slice in random fields across the liver lobule.

Biochemical investigations. Liver slices were washed in 0.154 M KCl containing 50 mM Tris-HCl pH 7.4 and homogenized (two slices in 2 ml) in this medium by sonication (Beamand *et al.*, 1993). Whole homogenate DNA content was determined by the method of Setaro and Morley (1976) and radioactivity incorporated into DNA by scintillation counting. Cytochrome *P*-450 content was determined as described previously (Lake *et al.*, 1993) and protein content was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical analysis. Statistical evaluation of data was performed by a repeated-measure analysis of variance. Comparisons between means were made using the least significant difference test (Snedecor and Cochran, 1968).

RESULTS

Culture of rat liver slices

A 24-hr culture period was selected for these studies as this has been shown to be a suitable time period for studies of chemically-induced UDS in primary hepatocyte cultures (Althaus *et al.*, 1982; Butterworth *et al.*, 1984; Cattley *et al.*, 1986; Loury *et al.*, 1986; Williams, 1977). Moreover, the functional viability of precision-cut rat liver slices cultured under these experimental conditions for periods of 24–72 hr has been demonstrated previously (Beamand *et al.*, 1993; Lake *et al.*, 1993).

Table 1 shows the levels of protein, DNA and cytochrome *P*-450 in freshly cut and 24-hr cultured liver slices from untreated and ARO-treated rats. No significant differences in protein and DNA content were noted between either freshly cut or 24-hr cultured liver slices from untreated and ARO-treated rats. Levels of cytochrome *P*-450 were significantly greater in liver slices from ARO-treated rats, being some 470 and 555% of levels in freshly cut and 24-hr cultured slices from untreated rats, respectively (Table 1). In agreement with previous studies, the culture of rat liver slices for 24 hr led to a reduction in both protein and cytochrome *P*-450 content (Beamand *et al.*, 1993; Lake *et al.*, 1993; Wright and Paine, 1992).

Unscheduled DNA synthesis in cultured rat liver slices

Liver slices from untreated and ARO-treated rats were cultured for 24 hr with 0–0.1 mM 2-AAF. Morphological examination of autoradiographs revealed that compared with control (i.e. DMSO-only treated) liver slice cultures (Plate 1a,b), 2-AAF produced a concentration-dependent increase in UDS, the effect being more marked in liver slices from ARO-treated (Plate 1d) than from untreated (Plate 1c) rats. With liver slices from untreated rats, 2-AAF-induced UDS was more marked in centrilobular than in periportal hepatocytes (Plate 2). 2-AAF-induced UDS in liver

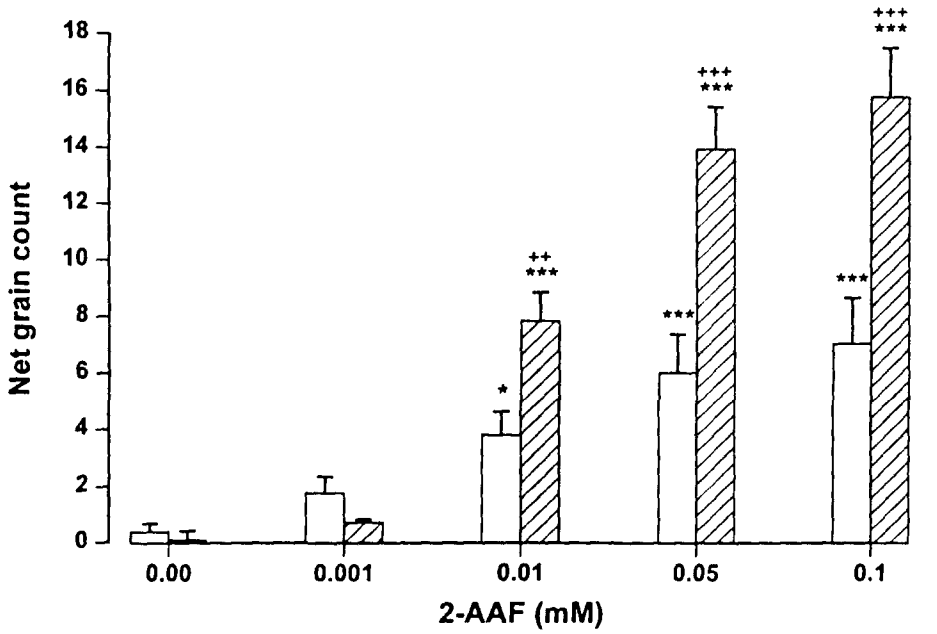

Fig. 1. Effect of 0–0.1 mM 2-acetylaminofluorene (2-AAF) on unscheduled DNA synthesis (UDS) in 24-hr cultured liver slices from untreated (□) and Aroclor 1254 (ARO)-treated rats (▨). UDS was quantified in centrilobular hepatocytes as the net grain count as described in Materials and Methods. Values are means ± SEM of either four (untreated rats) or three (ARO-treated rats) studies. Asterisks indicate significant differences from corresponding control (dimethyl sulfoxide only) cultures (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Significant differences between effects in liver slices from untreated and ARO-treated rats are also indicated (+ + $P < 0.01$; + + + $P < 0.001$).

slices from ARO-treated rats was most marked in centrilobular hepatocytes but the effect also extended to other areas of the liver lobule.

UDS in cultured rat liver slices was quantified both by autoradiography coupled with grain counting and by measurement of [^{3}H]thymidine incorporation into liver slice whole homogenate DNA. Because 2-AAF produced the greatest stimulation of UDS in centrilobular hepatocytes (Plate 2), quantitative autoradiographic assessment of DNA repair was confined to hepatocytes in only this region of the liver lobule. Compared with control (i.e. DMSO only) liver slice cultures, 2-AAF produced a concentration-dependent increase in the net grain count of centrilobular hepatocytes (Fig. 1). The increases were statistically significant at 2-AAF concentrations of 0.01 mM or more, and were significantly more marked in cultured liver slices from ARO-treated than from untreated rats (Fig. 1). The percentage of hepatocyte nuclei undergoing DNA repair with both more than five and more than 10 net grains was also determined (Fig. 2). Previous studies with primary hepatocyte cultures (Cattley *et al.*, 1986; Loury *et al.*, 1986; Williams, 1977) have considered that hepatocytes with more than five net grains per nucleus represent a positive response (i.e. cells undergoing DNA repair). With both cultured liver slices from untreated and ARO-treated rats, 2-AAF produced a concentration-dependent increase in the percentage of centrilobular hepatocyte nuclei with more than five (Fig. 2A) and more than 10 (Fig. 2B) net grains. As with the net grain counts (Fig. 1), the effect of 0.01–0.1 mM 2-AAF was significantly more marked in liver slices from ARO-treated than from untreated rats (Fig. 2).

UDS was also quantified by measurement of [^{3}H]thymidine incorporation into liver slice whole homogenate DNA (Fig. 3). Compared with control (DMSO only) cultured liver slices, treatment with 2-AAF produced only small increases in [^{3}H]thymidine incorporation, with the greatest increase to 126% of control being observed in liver slices from ARO-treated rats at a 2-AAF concentration of 0.1 mM (Fig. 3).

Replicative DNA synthesis in cultured rat liver slices

Examination of 24-hr cultured liver slices from untreated and ARO-treated rats revealed that some nuclei in hepatocytes and other cell types were undergoing replicative DNA synthesis (Plate 1). Cells undergoing replicative DNA synthesis were distinguished from other cells by having a high density of silver grains over the nucleus (Loury *et al.*, 1986; Smith-Oliver and Butterworth, 1987). The hepatocyte labelling index (i.e. percentage of hepatocyte nuclei undergoing replicative DNA synthesis scored in random fields across the liver lobule) in liver slices from

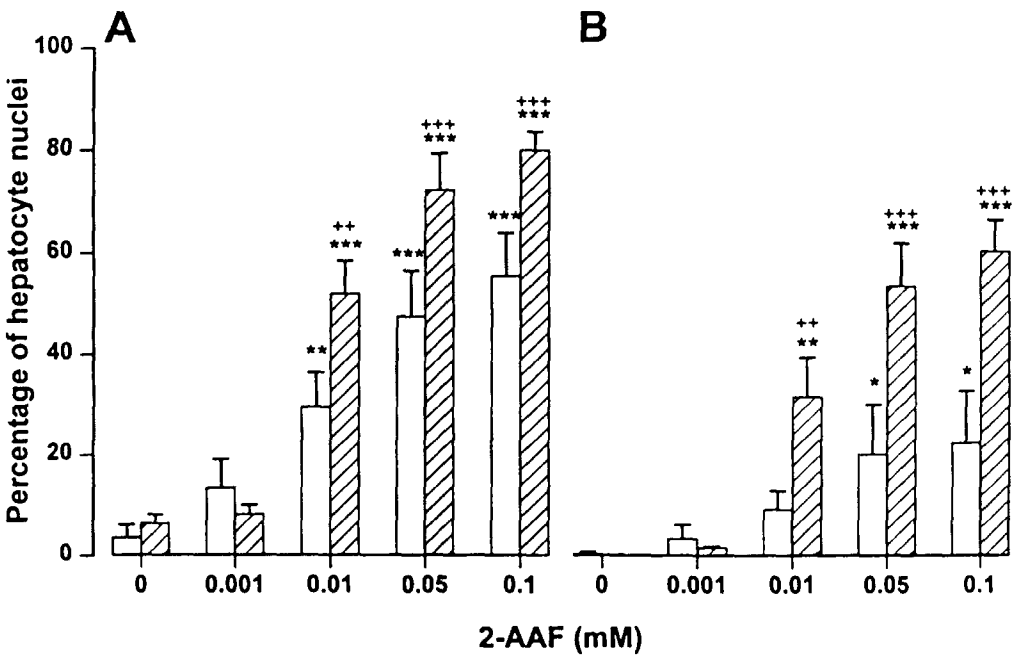

Fig. 2. Effect of 0 0.1 mM 2-acetylaminofluorene (2-AAF) on unscheduled DNA synthesis (UDS) in 24-hr cultured liver slices from untreated (□) and Aroclor 1254 (ARO)-treated rats (▨). UDS was quantified as the percentage of centrilobular hepatocytes undergoing DNA repair with both more than five (A) and more than 10 (B) net grains as described in Materials and Methods. Values are means ± SEM of either four (untreated rats) or three (ARO-treated rats) studies. Asterisks indicate significant differences from corresponding control (dimethyl sulfoxide only) cultures (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Significant differences between effects in liver slices from untreated and ARO-treated rats are also indicated (+ +$P < 0.01$; + + +$P < 0.001$).

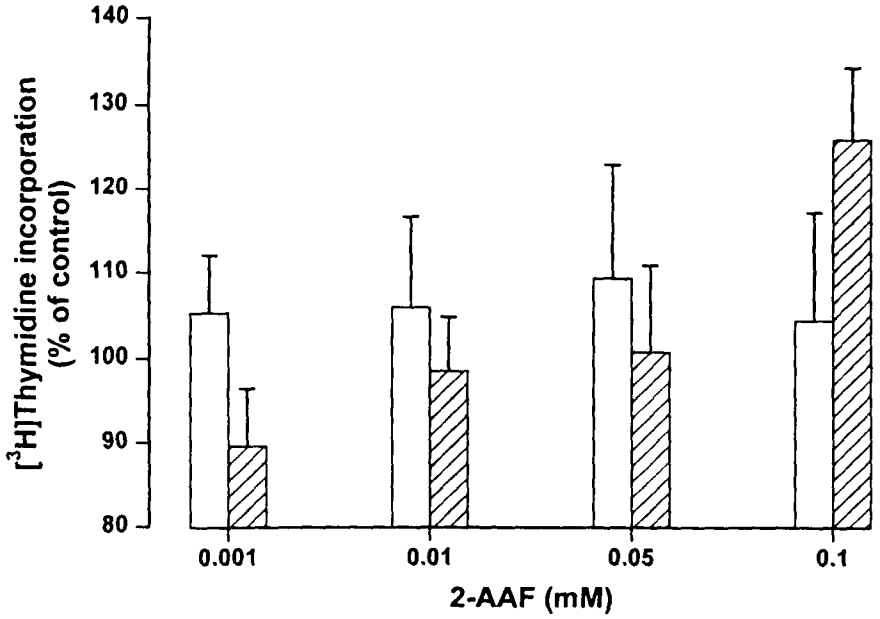

Fig. 3. Effect of 0.001–0.1 mM 2-acetylaminofluorene (2-AAF) on [^{3}H]thymidine incorporation into whole homogenate DNA of 24-hr cultured liver slices from untreated (□) and Aroclor 1254 (ARO)-treated rats (▨). Values [percentage of control (dimethyl sulfoxide only) cultures] are means ± SEM of six studies. Control rates of [^{3}H]thymidine incorporation into DNA of 24 hr cultured liver slices from untreated and ARO-treated rats were 464 ± 113 and 586 ± 77 dpm/mg DNA, respectively.

untreated rats cultured for 24 hr with 0 (control) and 0.05 mM 2-AAF was 0.21 ± 0.06 (mean ± SEM of four experiments) and 0.16 ± 0.07%, respectively ($P > 0.05$). Corresponding values for liver slices from ARO-treated rats cultured for 24 hr with 0 and 0.05 mM 2-AAF were 3.5 ± 0.5 (mean ± SEM of three experiments) and 3.3 ± 0.7%, respectively ($P > 0.05$).

DISCUSSION

Previous studies have demonstrated the usefulness of precision-cut liver slices as an *in vitro* model system for studying xenobiotic metabolism, xenobiotic-induced toxicity and the effects of xenobiotics on certain enzyme activities (Azri *et al.*, 1990; Beamand *et al.*, 1993; Brendel *et al.*, 1987; Dogterom, 1993; Lake *et al.*, 1993; Miller *et al.*, 1993; Smith *et al.*, 1987 and 1989). The results of the present study indicate that cultured precision-cut liver slices may also be used for studies of xenobiotic-induced genotoxicity using the UDS technique previously developed for primary hepatocyte cultures (Althaus *et al.*, 1982; Loury *et al.*, 1986; Williams, 1977).

By using 2-AAF as a model genotoxin, concentration-dependent increases in UDS could be readily demonstrated in 24-hr cultured liver slices from both untreated and ARO-treated rats. With liver slices from untreated rats, 2-AAF-induced UDS was most marked in centrilobular hepatocytes, which are known to have higher levels of total cytochrome *P*-450 than midzonal or periportal hepatocytes (Baron *et al.*, 1978; Gooding *et al.*, 1978). 2-AAF also produced UDS in cultured liver slices from ARO-treated rats, the effect being more marked than in liver slices from untreated rats. Previous studies have demonstrated that ARO induces the metabolism of 2-AAF to mutagenic products by stimulating both rat hepatic microsomal cytochrome *P*-450 isoenzymes in the CYP1A subfamily and cytosolic enzyme activities (Ames *et al.*, 1975; Ioannides *et al.*, 1993; Leist *et al.*, 1992).

Whereas significant concentration-dependent increases were obtained with 2-AAF using autoradiography coupled with grain counting, little effect was observed when UDS was quantified by [^{3}H]thymidine incorporation into liver slice DNA. The comparative insensitivity of the scintillation counting procedure is not surprising. For example, scintillation counting monitors both DNA synthesis and repair in all cell types present (i.e. not just hepatocytes). Indeed, replicative DNA synthesis was observed in both hepatocytes and other cell types present in the cultured liver slices. Moreover, in UDS studies with cultured rat hepatocytes, hydroxyurea is often added to inhibit replicative DNA synthesis and hence to improve the sensitivity of UDS measurements by scintillation counting (Althaus *et al.*, 1982; Cattley *et al.*, 1986; Glauert *et al.*, 1984; Howes *et al.*, 1986). It remains to be elucidated whether hydroxyurea could inhibit replicative DNA synthesis in hepatocytes and other cell types in cultured liver slices. Finally, the grain counting technique permitted the study of zonal distribution of UDS and in these

experiments measurements were confined to centrilobular hepatocytes where the greatest effect was observed in liver slices from untreated rats.

Examination of 24-hr cultured liver slices revealed that the nuclei of some hepatocytes and other cell types were undergoing replicative DNA synthesis. The value of 0.21% for replicative DNA synthesis in hepatocyte nuclei in 24-hr cultured liver slices from untreated rats is in agreement with previous studies with rat and mouse primary hepatocyte cultures where values of 0–0.4% have been reported (Butterworth *et al.*, 1984; Loury *et al.*, 1986; Smith-Oliver and Butterworth, 1987; Williams, 1977). In the present limited studies, replicative DNA synthesis appeared to be greater in 24-hr cultured liver slices from ARO-treated than from untreated rats. This is presumably a reflection of the mitogenic properties of this polychlorinated biphenyl mixture. However, in liver slices from both untreated and ARO-treated rats, the addition of 0.05 mM 2-AAF did not appear to affect the rate of replicative DNA synthesis. Whether cultured liver slices can be used for studies of xenobiotic-induced replicative DNA synthesis merits further investigation. Such studies would need to include investigations of medium composition including presence or absence of serum and variations in concentration of hormones and other components.

In summary, these results demonstrate that precision-cut liver slices may be a valuable alternative *in vitro* system to cultured hepatocytes for UDS studies to screen xenobiotics for potential genotoxicity. Unlike primary hepatocyte cultures, liver slices permit the study of zonal differences in UDS and could be used for UDS studies in cell types other than hepatocytes. Moreover, this technique may be relatively easily applied to other tissues and to other species. For example, we have demonstrated that 2-AAF also produces UDS in cultured human liver slices (Beamand *et al.*, 1994) and that certain cooked food mutagens produce UDS in cultured rat and human liver slices (J. A. Beamand and B. G. Lake, unpublished observations).

Acknowledgements—We are grateful to Dr D. P. Lovell for help with the statistical analysis of data. This work forms part of a research project sponsored by the UK Ministry of Agriculture, Fisheries and Food, to whom our thanks are due. The results of the research are the property of the Ministry of Agriculture, Fisheries and Food and are Crown copyright.

REFERENCES

Althaus F. R., Lawrence S. D., Sattler G. L., Longfellow D. G. and Pitot H. C. (1982) Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential chemical carcinogens. *Cancer Research* **42**, 3010–3015.

Ames B. N., McCann J. and Yamasaki E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella mammalian-microsome mutagenicity test. *Mutation Research* **31**, 347–364.

Azri S., Gandolfi A. J. and Brendel K. (1990) Precision-cut liver slices: an *in vitro* system for profiling potential hepatotoxicants. *In Vitro Toxicology* **3**, 309–320.

Baron J., Redick J. A. and Guengerich F. P. (1978) Immunohistochemical localizations of cytochromes P-450 in rat liver. *Life Sciences* **23**, 2627–2632.

Beamand J. A., Price R. J., Blowers S. D., Wield P. T., Cunninghame M. E. and Lake B. G. (1994) Effect of 2-acetylaminofluorene (2-AAF) on unscheduled DNA synthesis in precision-cut liver slices. *Toxicologist* **14**, 31.

Beamand J. A., Price R. J., Cunninghame M. E. and Lake B. G. (1993) Culture of precision-cut liver slices: effect of some peroxisome proliferators. *Food and Chemical Toxicology* **31**, 137–147.

Brendel K., Gandolfi A. J., Krumdieck C. L. and Smith P. F. (1987) Tissue slicing and culturing revisited. *Trends in Pharmacological Sciences* **8**, 11–15.

Butterworth B. E., Bermudez E., Smith-Oliver T., Earle L., Cattley R., Martin J., Popp J. A., Strom S., Jirtle R. and Michalopoulos G. (1984) Lack of genotoxic activity of di(2-ethylhexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis* **5**, 1329–1335.

Cattley R. C., Richardson K. K., Smith-Oliver T., Popp J. A. and Butterworth B. E. (1986) Effect of peroxisome proliferator carcinogens on unscheduled DNA synthesis in rat hepatocytes determined by autoradiography. *Cancer Letters* **33**, 269–277.

Dogterom P. (1993) Development of a simple incubation system for metabolism studies with precision-cut liver slices. *Drug Metabolism and Disposition* **21**, 699–704.

Frazier J. M., Tyson C. A., McCarthy C., McCormick J. J., Meyer D., Powis G. and Ducat L. (1989) Potential use of human tissues for toxicity research and testing. *Toxicology and Applied Pharmacology* **97**, 387–397.

Glauert H. P., Reddy J. K., Kennan W. S., Sattler G. L., Rao V. S. and Pitot H. C. (1984) Effect of hypolipidemic peroxisome proliferators on unscheduled DNA synthesis in cultured hepatocytes and on mutagenesis in *Salmonella*. *Cancer Letters* **24**, 147–156.

Gooding P. E., Chayen J., Sawyer B. and Slater T. F. (1978) Cytochrome P-450 distribution in rat liver and the effect of sodium phenobarbitone administration. *Chemico-Biological Interactions* **20**, 299–310.

Howes A. J., Beamand J. A. and Rowland I. R. (1986) Induction of unscheduled DNA synthesis in rat and hamster hepatocytes by cooked food mutagens. *Food and Chemical Toxicology* **24**, 383–387.

Ioannides C., Cheung Y.-L., Wilson J., Lewis D. F. V. and Gray T. J. B. (1993) The mutagenicity and interactions of 2- and 4-(acetylamino)fluorene with cytochrome P-450 and the aromatic hydrocarbon receptor may explain the difference in their carcinogenic potency. *Chemical Research in Toxicology* **6**, 535–541.

Krumdieck C. L., dos Santos J. E. and Ho K.-J. (1980) A new instrument for the rapid preparation of tissue slices. *Analytical Biochemistry* **104**, 118–123.

Lake B. G., Beamand J. A., Japenga A. C., Renwick A., Davies S. and Price R. J. (1993) Induction of cytochrome *P*-450-dependent enzyme activities in cultured rat liver slices. *Food and Chemical Toxicology* **31**, 377–386.

Leist M., Ayrton A. D. and Ioannides C. (1992) A cytosolic oxygenase activity involved in the bioactivation of 2-aminofluorene. *Toxicology* **71**, 7–20.

Loury D. J., Smith-Oliver T., Strom S., Jirtle R., Michalopoulos G. and Butterworth B. E. (1986) Assessment of unscheduled and replicative DNA synthesis in hepatocytes treated with unleaded gasoline or 2,2,4-trimethylpentane. *Toxicology and Applied Pharmacology* **85**, 11–23.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Miller M. G., Beyer J., Hall G. L., de Graffenried L. A. and Adams P. E. (1993) Predictive value of liver slices for metabolism and toxicity *in vivo*: use of acetaminophen as a model hepatotoxicant. *Toxicology and Applied Pharmacology* **122**, 108–116.

Nelson D. R., Kamataki T., Waxman D. J., Guengerich F. P., Estabrook R. W., Feyereisen R., Gonzalez F. J., Coon M. J., Gunsalus I. C., Gotoh O., Okuda K. and Nebert D. W. (1993) The P-450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA and Cell Biology* **12**, 1–51.

Okey A. B. (1990) Enzyme induction in the cytochrome P-450 system. *Pharmacology and Therapeutics* **45**, 241–298.

Parke D. V. (1968) *The Biochemistry of Foreign Compounds*. Pergamon, Oxford.

Parrish A. R., Shipp N. G., Spall R. D., Dorr R. T., Krumdieck C. L., Gandolfi A. J. and Brendel K. (1992) Organ culture of rat myocardial slices: an alternative *in vitro* tool in organ-specific toxicology. *Toxicology Methods* **2**, 101–111.

Ruegg C. E., Wolfgang G. H. I., Gandolfi A. J., Brendel K. and Krumdieck C. L. (1989) Preparation and utilization of positional renal slices for *in vitro* nephrotoxicity studies. In *In Vitro Toxicology: Model Systems and Methods*. Edited by C. A. McQueen. pp. 197–230. Telford Press, New Jersey.

Setaro F. and Morley C. G. D. (1976) A modified fluorimetric method for the determination of microgram quantities of DNA from cell or tissue cultures. *Analytical Biochemistry* **7**, 313–317.

Sipes I. G., Fisher R. L., Smith P. F., Stine E. R., Gandolfi A. J. and Brendel K. (1987) A dynamic liver culture system: a tool for studying chemical biotransformation and toxicity. *Archives of Toxicology* (Suppl. 11), 20–33.

Smith P. F., Fisher R., Shubat P. J., Gandolfi A. J., Krumdieck C. L. and Brendel K. (1987) *In vitro* cytotoxicity of allyl alcohol and bromobenzene in a novel organ culture system. *Toxicology and Applied Pharmacology* **87**, 509–522.

Smith P. F., McKee R., Gandolfi A. J., Krumdieck C. L., Fisher R. and Brendel K. (1989) Precision-cut liver slices: a new *in vitro* tool in toxicology. In *In Vitro Toxicology: Model Systems and Methods*. Edited by C. A. McQueen. pp. 93–130. Telford Press, New Jersey.

Smith-Oliver T. and Butterworth, B. E. (1987) Correlation of the carcinogenic potential of di(2-ethylhexyl)phthalate (DEHP) with induced hyperplasia rather than with genotoxic activity. *Mutation Research* **188**, 21–28.

Snedecor G. W. and Cochran W. G. (1968) *Statistical Methods*. 6th Ed. pp. 258–298. Iowa State University Press, Ames, IA.

Stefaniak M. S., Krumdieck C. L., Spall R. D., Gandolfi A. J. and Brendel K. (1992) Biochemical and histological characterization of agar-filled precision-cut rat lung slices in dynamic organ culture as an *in vitro* tool. *In Vitro Toxicology* **5**, 7–19.

Williams G. M. (1977) Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Research* **37**, 1845–1851.

Wright M. C. and Paine A. J. (1992) Evidence that the loss of rat liver cytochrome P-40 *in vitro* is not solely associated with the use of collagenase, the loss of cell cell contacts and/or the absence of an extracellular matrix. *Biochemical Pharmacology* **43**, 237–243.

Zimmerman H. J. (1978) *Hepatotoxicity. The Adverse Effects of Drugs and Other Chemicals on the Liver*. Appleton-Century-Crofts, New York.