THE METABOLISM OF DRUGS BY ISOLATED HEPATOCYTES

Jeffrey R. Fry

Department of Physiology and Pharmacology
Medical School, Queen's Medical Centre,
Clifton Boulevard, Nottingham NG7 2UH,
Great Britain

CONTENTS

0334-2190/82/02&30099-24

ABSTRACT

Isolated hepatocytes are being increasingly used in drug metabolism studies since they possess many of the attributes of both *in vitro* and *in vivo* systems. This paper reviews recent work on this use of isolated hepatocytes, with particular emphasis on their value in the study of (a) species and organ differences in xenobiotic metabolism, (b) the relation of metabolism to toxicity and (c) the balance of Phase I/Phase II metabolism.

INTRODUCTION

A major feature of the *in vivo* metabolism of most drugs and other xenobiotics is the wide range of metabolites that are produced. As our knowledge of the mechanisms underlying the reactions involved in these metabolic pathways has increased considerably over the last twenty years, largely by use of homogenate fractions obtained from liver (the major site of metabolism) and other organs, so a need has arisen to "synthesize" this knowledge to investigate the factors involved in control of these various metabolic pathways and also to assay the response of the intact cell to metabolites produced from these xenobiotics. It is in these areas that the isolated hepatocyte system is a particularly useful tool, as it combines both the inherent simplicity of all *in vitro* systems and the ability to produce the range of metabolites normally produced *in vivo*, at levels of cofactors that approximate to those found *in vivo* /1/. In this respect the isolated hepatocyte model serves as a useful "halfway house" between the homogenate fractions on the one hand and the *in vivo* state on the other hand.

The first generally-accepted technique for the routine isolation of viable mammalian hepatocytes was that of Howard and Pesch /2/ which involved dissociation of rat liver slices with a mixture of the proteolytic enzymes, collagenase and hyaluronidase. Subsequently, this technique has been improved upon by a number of workers, usually by the incorporation of a perfusion stage to increase the cell yield /3, 4/, and the cells isolated by this basic enzyme dissociation technique have been used in an extremely wide range of biochemical studies /1/.

More recently, the isolated hepatocyte system has found increasing use in drug metabolism studies. This paper will largely review these studies published between 1977-1981 and, as such, is complementary to a previous review by this author which covered published studies up

to 1977 /1/. This review will be confined to xenobiotic metabolism in freshly-isolated hepatocytes and will not cover metabolism in cultured isolated hepatocytes. The practical aspects of hepatocyte isolation and of incubation techniques have recently been reviewed by this author /5/ and thus will not be discussed in this review.

REACTIONS STUDIED IN ISOLATED HEPATOCYTES

An extremely wide variety of xenobiotic metabolism reactions and pathways has been studied in isolated hepatocytes (usually derived from rat) and a partial list is given in Table 1. It is apparent that most of the major pathways involved in xenobiotic metabolism have been

TABLE 1. Some Routes of Xenobiotic Metabolism Detected in Isolated Hepatocytes

Reaction	Substrate	Reference
Aromatic hydroxylation	2-Acetylaminofluorene	/6/
	Amphetamine	/7/
	Antipyrine	/8/
	Benzo(a)pyrene	/9,10/
	Biphenyl	/11/
	Butamoxane	/12/
	Naphthalene	/13/
Acyclic hydroxylation	Antipyrine	/8/
	2,4-Dinitrotoluene	/14/
Epoxidation	Aldrin	/15/
N-Hydroxylation	2-Acetylaminofluorene	/6/
O-Dealkylation	Diethyl ether	/16/
	7-Ethoxycoumarin	/17/
	Ethoxyresorufin	/18/
	4-Methoxybiphenyl	/19/
	Phenacetin	/20/
	4-Nitroanisole	/21/
N-Dealkylation	Aminopyrine	/22/
	Antipyrine	/8/
	Cocaine	/23/
	Dansylamide	/24/
	Ethylmorphine	/25/

TABLE 1 (cont)

Reaction	Substrate	Reference
Alcohol oxidation	Ethanol	/26,27,28/
Ester cleavage	Methoprene	/29/
	Hydroprene	
Deacetylation	2-Acetylaminofluorene	/6/
	Phenacetin	/20/
Glucuronidation/ Sulphation	Chloramphenicol (G only)	/30/
	Harmol	/31/
	4-Hydroxybiphenyl	/11/
	7-Hydroxycoumarin	/17/
	2-Naphthol	/31/
	4-Nitrophenol	/21/
	Paracetamol	/31/
Glycine conjugation	Benzoic acid	/32/
	Salicylic acid	/33/
Acetylation	Aniline	/34/
	Sulphonamides	/35,36/
Glutathione conjugation	Paracetamol	/37/

detected in isolated hepatocytes, and the obvious question that must be asked is: how well do the enzyme activities measured in isolated hepatocytes correlate with the activities measured in isolated homogenate fractions and in perfused liver or *in vivo*?

With regard to comparison of drug metabolism in isolated hepatocytes and liver microsomes it is apparent from Table 2 that this is very dependent on the substrate being studied. Thus, the activity in cells may be similar to, greater than or less than the activity in microsomes, and even apparently minor alterations in the structure of the substrate, e.g. the introduction of a chlorine atom in the para-position of N,N-dimethylphenoxyethylamine, can alter the ratio of metabolism in cells to that in microsomes. The reasons for these substrate-dependent differences are largely unknown. It has been suggested that some substrates are better metabolized by the cytochrome P450-dependent mixed function oxidase (MFO) system in cells compared with microsomes because they may be actively transported into the cytosol of intact cells /25/.

TABLE 2. Comparison of Kinetics of Drug Metabolism in Intact Hepatocytes and Liver Microsomes

	Reactions	Reference
Similar in cells and microsomes	Alprenolol metabolism	/38/
	Ethoxybenzamide O-deethylation	/39/
	α-1-Acetylmethadol N-demethylation	/12/
	d-Propoxyphene N-demethylation	
	N,N-Dimethylphenoxyethylamine N-demethylation	
	Biphenyl 4-hydroxylation	/7,40/
	4-Methoxybiphenyl O-demethylation	/40/
	4-Methylumbelliferone glucuronidation	/31/
	Harmol glucuronidation	
Greater in cells compared with microsomes	Ethylmorphine N-demethylation	/25/
	Amphetamine 4-hydroxylation	/7/
Greater in microsomes compared with cells	Cytochrome P450-product complexation with N-hydroxyamphetamine or norbenzphetamine	/41/
	N,N-Dimethyl-p-chlorophenoxyethylamine N-demethylation	/12/
	Ethinimate metabolism	
	Butamoxane hydroxylation	
	8-Methoxybutamoxane metabolism	
	4-Nitroanisole O-demethylation	/7/
	2-Naphthol glucuronidation	/31/

This may, in turn, reflect differences in solubility of the substrates, the more water-soluble substrates being taken up by active transport whereas the more lipid-soluble substrates (which in general have a greater affinity for cytochrome P450) enter the cells by passive diffusion. This argument was based on comparison of ethylmorphine and alprenolol metabolism in cells and microsomes, but it is difficult to reconcile this argument with the physical properties of some of the substrates mentioned in Table 2. This, however, is an interesting argument which should be studied further. Furthermore, this argument cannot explain why some substrates of the MFO system are better metabolized by microsomes than by cells. Other possibilities which may be important in this respect include: NADPH availability (which, as argued by Billings *et al.* /12/, is unlikely to be very important), the presence of alternative

pathways of metabolism in cells, the presence of endogenous inhibitors, differences in enzymic activity between intact endoplasmic reticulum and microsomal vesicles and differences in the extent of non-specific protein binding in the two metabolizing systems. In the case of the cytochrome P450-product complexations of N-hydroxyamphetamine or norbenzphetamine, the reduced activity in intact cells was shown to be due to the presence of active pathways of further metabolism which are not operative in the usual microsomal incubations /41/.

Comparison of the glucuronidation pathway in cells and microsomes has been attempted /31/ but this is a very difficult area of study due to the known latency of the glucuronyltransferase enzyme and its possible activation during preparation of the microsomal fraction.

Attempts have been made to compare the rates of enzyme activity in isolated hepatocytes with those in perfused liver or *in vivo*. Billings *et al* /7, 12/ have demonstrated that the rates of butamoxane and amphetamine hydroxylation in isolated hepatocytes compare well with the activities measured in perfused liver, and Stewart and Inaba /22/ have demonstrated that the rate of aminopyrine N-demethylation (as judged on a g. liver basis) is comparable in isolated hepatocytes and *in vivo*. Bock *et al* /13/ have concluded that the rate of 1-naphthol glucuronidation is comparable in isolated hepatocytes and perfused liver. In a slightly different context, many authors have concluded that the patterns of metabolites produced by isolated hepatocytes resemble more closely those seen *in vivo* than in homogenate fractions. This is well illustrated by the work of Yih and van Rossum /42/ who demonstrated that in liver homogenate fractions the end-products in the metabolism of various barbiturates (e.g. hexobarbital, methylcyclobarbital, methylbrallobarbital) are the hydroxy metabolites while in isolated hepatocytes these metabolites are further oxidized to keto-metabolites (a cytoplasmic reaction) as occurs in perfused liver and *in vivo*. Also, Billings *et al* /12/ reported the discovery of a new metabolite of ethinimate on its incubation with isolated hepatocytes which was subsequently detected in urine of ethinimate-treated rats. These studies indicate that isolated hepatocytes may be useful in metabolic studies of new drugs particularly as the detection, isolation and characterization of drug metabolites in the cell medium is likely to be less complex than detection and isolation of metabolites in body fluids such as blood, urine or bile.

SPECIES AND ORGAN DIFFERENCES IN XENOBIOTIC METABOLISM

One area in which isolated hepatocytes may be of particular value is

in the assessment of species differences in drug metabolism, the obvious hope being that such comparative studies could also include the use of human hepatocytes so that preliminary human metabolic data would be obtained in the early stages in the testing of a new drug. It is likely that cell isolation techniques involving the use of non-perfusion procedures (such as that developed by Fry and co-workers /5,43/) would be more applicable than perfusion procedures in this context because of the relative ease of adapting these techniques to different species and to biopsy samples.

Thus far, this use of isolated hepatocytes has received little attention although preliminary studies by Fry, Jones and Bridges (cited in ref. 44) indicate that the relative extent of biphenyl 2- and 4-hydroxylation is comparable in hepatocytes and liver microsomes isolated from rat, hamster and ferret, the hamster hepatocytes and liver microsomes being more active in both reactions. More recently, it has been demonstrated that species differences exist in the ability of isolated hepatocytes to conjugate benzoic acid with glycine in that viable hepatocytes isolated from omnivores (rat, hamster) possess a good ability to carry out this reaction whereas viable hepatocytes isolated from carnivores (dog, ferret) do not possess this metabolic capability /32/; this data is consistent with previous liver homogenate and slice studies. In contrast, the hepatocytes isolated from all four species possess the ability to conjugate benzoic acid with glucuronic acid. Moldeus has demonstrated differences in paracetamol metabolism between rat and mouse hepatocytes /37/. The rate of glucuronidation was similar in the two species, but the rate of sulphate conjugation was lower in the mouse hepatocytes whereas the rate of glutathione conjugation (which reflects the rate of generation of the toxic intermediate) was approx. 10-fold greater in the mouse cells. In addition, paracetamol was toxic only to the mouse hepatocytes. These data are consistent with the known rat/mouse differences in susceptibility to paracetamol toxicity *in vivo*, and the concept that paracetamol is hepatotoxic by virtue of its metabolism to a reactive intermediate. Also, Bolcsfoldi *et al* /45/ have demonstrated differences in the cytochrome P450-dependent MFO reactions and various conjugation reactions in hepatocytes isolated from rat and dog; whether these species differences are also apparent in isolated microsomes or *in vivo* was not studied by these authors.

Another area in which isolated hepatocytes may be of value is in the study of organ differences in xenobiotic metabolism. Whilst various authors have reported on the metabolism of xenobiotics by cells isola-

ted from extra-hepatic tissues including kidney, intestine and lung, there appear to be few reports of direct comparison between these cells and liver cells. Ideally, such comparative studies should be performed on cell preparations isolated from the same animal but this is difficult if both cell preparations are isolated by perfusion techniques, but can be readily accomplished by use of non-perfusion techniques as has been demonstrated by Fry and co-workers /17, 32/. As with all cell-based systems the advantage inherent in such comparative studies is that these reactions can be studied in conditions approximating to those operating *in vivo*. This is well illustrated if one compares the relative liver and kidney activities of various metabolic pathways assayed in cells and microsomes (Table 3). It appears that in general the relative activities of various cytochrome P450-dependent reactions are similar in cells and microsomes whereas there is a big difference between cells and microsomes with respect to glucuronidation probably because there is a competing pathway of conjugation (viz. sulphation) operative in the intact cells.

TABLE 3. A comparison of drug metabolising capability of rat liver and kidney as judged by microsomal and whole cell studies.

Parameter	Kidney value as % of liver value	
	Microsomes	Whole Cells
Biphenyl 4-hydroxylation	3.6 /46/	<2 /17/
Benzo(a)pyrene 3-hydroxylation	3.3 /47/	<1 /17/
7-Ethoxycoumarin O-deethylation	2.0 /48, 49/	3.0 /17/
4-Methylumbelliferone glucuronidation	140-200 /50, 51/	41.5 /17/

The references to these studies are given in / /.

With respect to a liver and kidney cell comparison it appears that, at least in the rat, kidney cells possess a poor ability for cytochrome P450-dependent reactions (Tables 3 and 4) and are more efficient at conjugation reactions although there are marked substrate differences in this respect (Table 4) this perhaps suggesting the existence of different classes of conjugating enzymes in kidney. A particularly interesting example is afforded by the organ and substrate differences in glycine conjugation. Thus, benzoic acid is well conjugated with glycine in rat

TABLE 4. Comparison of drug metabolism in rat hepatocytes and kidney cells.

Reaction	Rate of Reaction		Reference
	Liver	Kidney	
7-Ethoxycoumarin 0-deethylation[1]	135	4.3	/17/
4-Methylumbelliferone conjugation:			
as glucuronide[1]	289	120	/17/
as sulphate[1]	187	32	
4-Dimethylaminophenol conjugation:			
as glucuronide[2]	3.8	0.4	/52/
as sulphate[2]	3.2	0.2	
Paracetamol conjugation:			
as glucuronide[3]	77.6	2.81	
as sulphate[3]	48.0	1.67	/37,53/
as sulphydryl[3]	7.8	0.79	
Benzoic acid conjugation:			
with glycine[1]	465	260	/17/
as glucuronide[1]	37.5	3.0	
Salicylic acid conjugation:			
with glycine[4]	4.4	16.5	/33/
as glucuronide[4]	123.5	9.3	

Activities expressed as:–
[1] pmol product/mg cell protein/min.
[2] nmol product/mg cell protein/h.
[3] nmol product/10^6 cells/h.
[4] pmol product/mg cell protein/h.

hepatocytes and kidney cells, the better metabolism occurring in the liver, whereas salicylic acid is only poorly conjugated with glycine in liver and kidney cells, the kidney being more active in this respect than liver.

An interesting variation of this approach has been adopted by Moldeus *et al* /54/ who demonstrated that initial cytochrome P450-dependent oxidation and subsequent glutathione conjugation of paracetamol occurred in isolated hepatocytes and, by subsequent re-incubation of the medium from hepatocyte incubation with kidney cells, that kidney cells metabolized this glutathione conjugate further to the mercapturic acid conjugate, a major excretory product of paracetamol metabolism. This study well illustrates the process of initial hepatic and subsequent extrahepatic metabolism.

Fewer studies have been performed to compare the metabolizing capability of isolated liver and intestinal cells. In the study by Shirkey *et al* /34/ it was demonstrated that both the Phase I and Phase II metabolism of xenobiotics was much less apparent in rat intestinal cells compared with rat hepatocytes, although some metabolism was detected; this is illustrated with regard to aniline metabolism in Table 5. Similar conclusions were reached by Stohs *et al* /55/ and Grafstrom *et al* /56/ using different substrates.

TABLE 5. Comparison of metabolic profile of [^{14}C] aniline following incubation with rat isolated hepatocytes or intestinal cells.

	Hepatocytes (%)*	Intestinal cells (%)*
Aniline	0	92
N-Acetyl-p-aminophenol sulphate	20	nd
N-Acetyl-p-aminophenol glucuronide	14	nd
p-Aminophenyl sulphate	14	nd
p-Aminophenyl glucuronide	12	nd
N-Acetyl-p-aminophenol	12	~0.1
Acetanilide	13	8

*Results expressed as % of radioactivity in the incubation mixture corresponding to each metabolite.

nd – not detected

Data from Shirkey *et al* /34/.

RELATION OF METABOLISM TO TOXICITY

Whilst liver microsomal fractions have been widely used in the study of metabolism-mediated toxicity it is only fairly recently that isolated hepatocytes have been used in this context. The role of isolated hepatocytes can be divided into two aspects: a) the generation of the active metabolite and the toxic response both occur in the heaptocyte, and b) the generation of the active metabolite occurs in the heaptocyte but the toxic response is manifest in a second cell-type. These situations approximate to metabolism-mediated heaptotoxicity and extra-hepatic toxicity respectively.

Although a number of chemicals have been shown to exert a toxic effect on isolated hepatocytes, there have been relatively few studies which have attempted to relate xenobiotic toxicity to the involvement of active metabolites. It is obviously difficult to conclusively demonstrate metabolism-mediated hepatotoxicity by this means and it is probably for this reason that most of the attention has been focussed on the already fairly-well documented examples of toxicity arising from glutathione depletion subsequent to generation of an active metabolite. The substrates that have been shown to produce hepatocyte toxicity by this mechanism include iodoacetamide /57/, chloroform /58/, carbon tetrachloride /59/, paracetamol /37/ and bromobenzene /60/, although in some instances fairly drastic measures have had to be used to achieve the desired effect e.g. use of hepatocytes from phenobarbitone – and diethyl maleate-pretreated rats (to induce the cytochrome P450 system and decrease the intracellular glutathione content respectively). Whilst it has been well documented that the generation of large amounts of active metabolite leads to depletion of glutathione and that this leads to cell toxicity, there is a paucity of information regarding the intermediate steps from glutathione depletion to cell lysis. It is likely that derangement of normal cell metabolism is important in this context and this may involve alterations in protein synthesis /61/, glycogen synthesis /62/, ATP levels or lipid peroxidation /57-59/. It is obvious that further work is required in this area and that isolated hepatocytes would be a useful tool to study this problem.

Isolated hepatocytes have also been used as a metabolizing component of a mixed-cell toxicity system. The first reported example of this use was provided by the work of Fry and Bridges /63/ who demonstrated that the antitumour agent cyclophosphamide (CPA) was remarkably non-toxic *per se* to cultured fibroblasts but became very toxic to the fibroblasts if it was pre-incubated with rat isolated hepatocytes. Both the hepatocyte-mediated toxicity of CPA and its overall metabolism was decreased on co-incubation with SKF 525-A, a well-known inhibitor of the cytochrome P450-dependent mixed-function oxidase system. In addition, the level of CPA that produced half maximal toxicity in the hepatocyte-fibroblast system was very similar to that reported for tumour cells by Cox *et al* /64/ when CPA was activated by a mixture of liver microsomes + liver cytosol (CPA toxicity was much greater if it was activated by microsomes alone), this confirming the importance of cytosolic pathways in the deactivation of CPA.

This work was later extended to include a study of the cytotoxicity

of a range of carcinogenic and non-carcinogenic compounds and it was shown that metabolism by hepatocytes greatly increased the toxicity of most compounds tested (e.g. dimethylnitrosamine, 3′- and 2-methyl-4-dimethylaminoazobenzene, 2- and 4-acetylaminofluorene, 2- and 6-aminochrysene and mitomycin c) but that the toxicity of benzo(a)-pyrene (BP) and 4-nitroquinoline N-oxide was reduced by hepatocyte metabolism /65/. It was not possible to distinguish the carcinogens and non-carcinogens by their effect on fibroblast growth.

This mixed-cell approach has been extended by other workers who used suitable bacteria as the "tester" cell-type and were thus able to study the involvement of metabolism of carcinogens in their mutagenic response. This was first reported by Green *et al* /66/ who demonstrated that BP mutagenicity was reduced when the BP was activated by intact liver cells as compared with microsomal activation and it was suggested that this was due to the involvement of competing inactivation pathways of metabolism in the intact cells. These findings have been confirmed by Brouns *et al* /67/ and by Glatt *et al* /68/, the latter workers also demonstrating that the bacterial mutagenicity of BP and four of its major metabolites better correlated with animal carcinogenicity data when the BP was activated by liver cells than by cell homogenate. Whether this conclusion also applies to other classes of chemical carcinogens remains to be determined.

In some studies the mutagenic response of carcinogen metabolites generated by hepatocytes has been compared with the ability of the metabolites to induce DNA repair (as an index of interaction with DNA) within the hepatocytes, and these studies have indicated that the mutagenic response of 2-acetylaminofluorene (2-AAF) is mediated by a deacetylation reaction to produce 2-aminofluorene, the ultimate mutagen probably being N-hydroxy 2-aminogluorene, whereas the DNA repair is mediated via a direct N-hydroxylation of 2-AAF, the ultimate DNA-damaging agent possibly being the sulphate ester of N-hydroxy 2-AAF /69, 70/.

Another index of metabolism-mediated DNA damage that has been used in hepatocyte studies is that of metabolite-DNA binding /71-73/. One interesting example of this type of study is given by the work of Ashurst *et al* /71/ which demonstrated that the major type of BP metabolite-deoxyribonucleoside adduct produced differed when liver microsomes or intact hepatocytes were used for metabolic activation. Thus, the major adduct produced when microsomes were incubated with BP in the presence of exogenously-added DNA was derived from

9-hydroxy BP. In liver cells, 9-hydroxy BP is conjugated with glucuronic acid and in this situation the major adduct produced in hepatocyte DNA was due to a metabolite of 7,8-dihydro-7,8-dihydroxy BP. This study further illustrates the importance of alternative pathways of metabolism in regulating the production of active metabolites.

THE BALANCE OF PHASE I/PHASE II METABOLISM

It is now well recognised that many xenobiotics are metabolized in the liver by sequential Phase I (oxidations, reductions, hydrolyses) and Phase II (conjugation) reactions, and it is becoming increasingly apparent that the balance of these two phases of metabolism may be of great importance in determining the biological effects of a particular xenobiotic /74, 75/. Consequently, great interest has been centred on the factors that may modulate the balance of Phase I and Phase II metabolism, and isolated hepatocytes, for the reasons already discussed, would seem to be an ideal model to study this problem. Most attention has been focussed on the factors that may govern the balance of cytochrome P450-dependent Phase I reactions and subsequent glucuronidation/sulphation reactions, as these reactions are, quantitatively, often the major pathways in xenobiotic metabolism. A number of substrates that are metabolized by this reaction sequence, including biphenyl, 4-methoxybiphenyl, harmine, 7-ethoxycoumarin, 4-nitroanisole and benzo(a)pyrene, have been used as probes to identify those factors that may be important in modulating the balance of these metabolic pathways.

The principal route of biphenyl metabolism in rat isolated hepatocytes is that of initial 4-hydroxylation followed by glucorinidation/sulphation of the 4-hydroxybiphenyl Phase I metabolite. The time course of biphenyl metabolism in rat isolated hepatocytes is illustrated in Figure 1. It is apparent from this graph that at the later time points the Phase I metabolism (i.e. 4-hydroxylation) is well integrated with the subsequent conjugations in that almost all of the metabolites are present as conjugates. Similar findings have been reported with 7-ethoxycoumarin, benzo(a)pyrene, harmine and 4-nitroanisole as substrates /9, 17, 31, 76/. However, there is no obligatory coupling of the two phases of metabolism as has been suggested from microsomal studies. This is indicated by a number of points. Firstly, large amounts of unconjugated Phase I metabolite are produced in the early stages of metabolism (Fig. 1). Studies with benzo(a)pyrene /9/ suggest that this early peak in

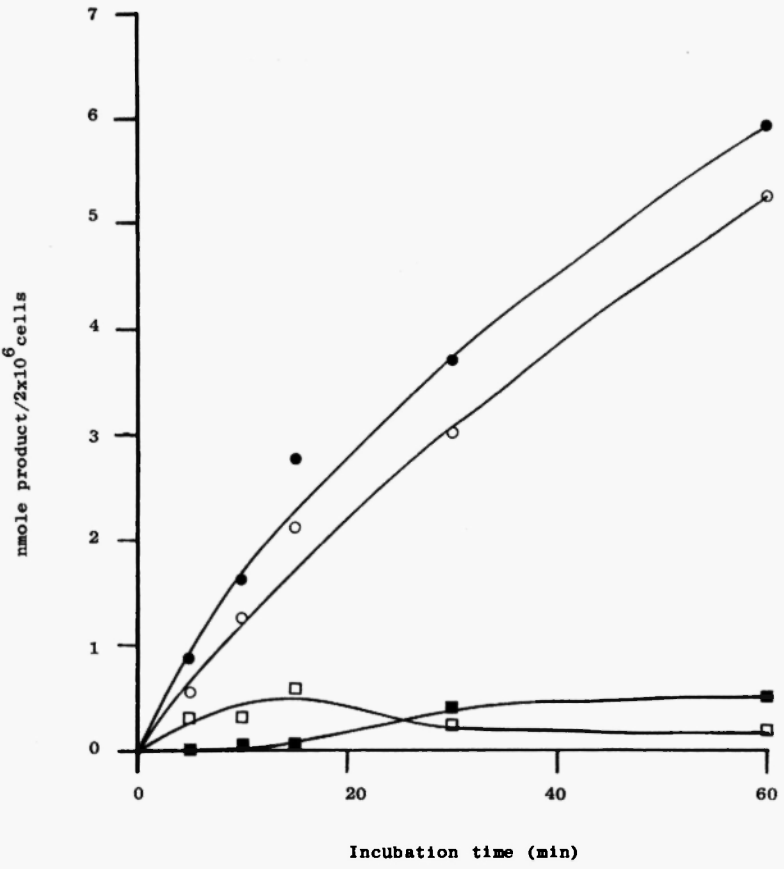

Fig. 1. Time course of metabolism of biphenyl (100 μM) in rat isolated hepatocytes. ●, Total metabolites; ○, 4-hydroxybiphenyl sulphate; ■, 4-hydroxybiphenyl glucuronide; □, unconjugated 4-hydroxybiphenyl.

unconjugated Phase I metabolite is unlikely to represent initial passage of this metabolite from the cells into the medium, followed by re-entry into the cells for subsequent conjugation. Rather, it can be at least partly explained in terms of a 'lag-activation' phenomenon involving one of the conjugating enzymes (see later). Secondly, the conjugation reactions can be inhibited by various substances (e.g. 2,4-dinitrophenol) without influencing the activity of the Phase I reaction /77/. Finally, when high levels of 4-hydroxybiphenyl are produced by 0-demethylation

of 4-methoxybiphenyl, large amounts of unconjugated Phase I metabolite are produced throughout a one hour incubation period (not just during the first 10-15 minutes) but there is no corresponding sharp fall-off in the activity of the Phase I pathway that one might expect if the two phases of metabolism were tightly coupled /40/.

Another interesting aspect of the metabolism of biphenyl is that there is evidence for an initial lag in the activity of the glucuronidation pathway (see Fig. 1). It has been suggested /11/ that this indicates that the glucuronyltransferase involved in 4-hydroxybiphenyl glucuronidation becomes activated (as occurs in microsomes) probably by the substrate itself. This 'lag-activation' phenomenon is even more apparent if the generation of 4-hydroxybiphenyl is greatly increased as after induction of biphenyl 4-hydroxylation with phenobarbitone or 3-methylcholanthrene /11/.

In cells isolated from control animals, the major metabolite of biphenyl that is produced is 4-hydroxybiphenyl sulphate, whereas 4-hydroxybiphenyl glucuronide is the major metabolite produced by hepatocytes isolated from pre-induced animals /11/. This suggests that the rate of generation of 4-hydroxybiphenyl is important in determining the balance of glucuronide/sulphate conjugates produced. This has been confirmed both by direct incubation of hepatocytes with increasing concentrations of 4-hydroxybiphenyl /11/ and by studies in which hepatocytes were incubated with increasing concentrations of 4-methoxybiphenyl to generate 4-hydroxybiphenyl at different rates /40/. Similar findings have been reported with other substrates /76, 78, 79/. It is likely that the increase in glucuronidation relative to sulphation at increasing levels of Phase I metabolite is related more to the kinetic properties of the enzymes involved in these conjugations than to limitation of sulphation by sulphate availability as has been previously supposed /79/. It is probable that the differences between 2- and 4-hydroxybiphenyl in the balance of their glucuronidation/sulphation by liver cells /11/ can also be explained in terms of these enzyme kinetic parameters.

It is known that cytochrome P450 exists as a family of haemoproteins each of different substrate specificity, and the possibility exists that the balance of Phase I/Phase II metabolism is in part dependent on the type of cytochrome P450 involved in the initial Phase I reaction. In order to test this hypothesis, it is necessary to use a pair of substrates that are metabolised by different cytochrome P450-dependent pathways to yield the same Phase I metabolite. Previous studies demonstrated that hepatic microsomal biphenyl 4-hydroxylation and 4-methoxy-

biphenyl O-demethylation are catalyzed by different cytochrome P450 haemoproteins even though the metabolite produced with both substrates is 4-hydroxybiphenyl /80/. Accordingly, isolated hepatocytes were incubated with concentrations of biphenyl and 4-methoxybiphenyl that produced both the same initial velocity of the Phase I reaction and the same overall metabolism to 4-hydroxybiphenyl, and under these conditions, there is a significant difference between the two substrates in the balance of conjugated/unconjugated metabolites produced (Table 6). These preliminary findings thus indicate that the type of cytochrome P450 involved in the initial Phase I metabolism may influence the pattern of subsequent Phase II reactions.

TABLE 6. Comparison of metabolism of biphenyl and 4-methoxybiphenyl in rat isolated hepatocytes.

	Biphenyl (100 μM)	4-Methoxybiphenyl (10 μM)
Initial velocity of Phase I reaction (nmole product/2x10^6 cells/min)	0.200 ± 0.025	0.217 ± 0.048
Total metabolism (nmole products/2x10^6 cells/h)	5.50 ± 0.54	5.33 ± 0.48
Unconjugated metabolites (% of total metabolites)	8.3 ± 1.8	16.8 ± 2.0*
Conjugated metabolites	91.7 ± 1.8	83.2 ± 2.0*

Values are mean ± S.E.M. of 7-11 experiments.

* Values are significantly different ($P < 0.05$) from the values obtained with biphenyl.

The evidence presently available suggests that under normal conditions cofactor availability is unlikely to be limiting in Phase I or Phase II metabolism. However, one abnormal condition in which cofactor availability may influence xenobiotic metabolism is that of diabetes mellitis, since the gross impairment of carbohydrate utilization that characterizes this disease is likely to lead to profound disturbances in cofactor synthesis and supply. We have, therefore, recently undertaken a study of the effect of streptozotocin-induced diabetes on hepatic drug metabolism in female rats. In agreement with other studies, we observed

that the diabetic state was accompanied by an increased activity of the cytochrome P450 system (increased P450 levels and 4-methoxybiphenyl 0-demethylase activity) when measured in microsomes, both on a mg. protein basis and on a g. liver basis /81/. However, this increase was not apparent when the 0-demethylation was measured in intact hepatocytes isolated from similar animals /19/. We interpret this as indicating that the diabetes-induced increase in MFO activity observed in microsomes is counterbalanced in the intact cells by a decreased availability of NADPH that is known to occur in diabetes /82/, probably as a result of diminished activity of the pentose phosphate pathway of glucose metabolism. Also, diabetes produced no change in the 4-hydroxybiphenyl glucuronyltransferase activity of isolated microsomes /81/ whereas the overall glucuronidation of 4-hydroxybiphenyl was lowered in hepatocytes isolated from diabetic rats /19/. We interpret this as indicating that the diabetic state produces a depletion of the cofactor UDPGA, most likely due to decreased activity of the insulin-sensitive UDPG-dehydrogenase enzyme /81/. This diabetes-induced impairment of glucuronidation which is unrelated to any change in glucuronyltransferase activity is consistent with the findings of previous liver slice studies /83, 84/. It should be noted that a similar decreased availability of UDPGA is likely to occur in fasting, and this may explain why a decrease in glucuronidation has been observed in hepatocytes obtained from diabetic rats when compared with fed controls /19/, but was not observed when the diabetic animals were compared with fasted controls /21/.

The studies outlined in the preceding paragraphs have highlighted some of the factors that may be important in the balance of Phase I/ Phase II metabolism in isolated hepatocytes, and it is to be hoped that more work will be carried out in this area in the near future.

CONCLUSIONS

Throughout this review considerable emphasis has been placed on the inherent advantages to the use of isolated hepatocytes in xenobiotic metabolism research, and the ways in which such cells may be profitably used to study many aspects of xenobiotic metabolism. It is important to realise, however, that the isolated hepatocyte system is merely one technique, albeit an increasingly useful technique, in the armamentarium of those studying xenobiotic metabolism, and that probably the greatest value of this system arises when it is used in conjunction with

other metabolic systems e.g. liver microsomes, as is well illustrated in the diabetes study discussed above.

As to the future, it is obviously necessary that techniques must be devised for the isolation of human hepatocytes from small biopsy samples and that this must be coupled to the development of assay techniques sufficiently sensitive to measure metabolite production from such small numbers of cells. Hopefully, this use of human hepatocytes will lead to greater insight into the factors regulating the metabolism and metabolism-mediated biological effects of drugs in the clinical situation. In particular, information is required on the role of endogenous substrates as modulators of drug metabolism.

ACKNOWLEDGEMENTS

The author is indebted to Mrs. Rosemary Goodwin for excellent secretarial assistance during the preparation of this review.

REFERENCES

1. FRY, J.R. and BRIDGES, J.W. The metabolism of xenobiotics in cell suspensions and cell cultures. In: Progress in Drug Metabolism (J.W. Bridges & L.F. Chasseaud, Eds.). John Wiley & Sons, **2**, 71-118, London (1977).
2. HOWARD, R.B. and PESCH, L.A. Respiratory activity of intact, isolated parenchymal cells from rat liver. *J. Biol. Chem.* **243**, 3105-3109 (1968).
3. MOLDEUS, P., HOGBERG, J. and ORRENIUS, S. Isolation and use of liver cells. *Methods in Enzymology* **52** (pt.c), 60-71 (1978).
4. SEGLEN, P.O. Preparation of isolated rat liver cells. *Methods in Cell Biology* **13**, 30-85 (1976).
5. FRY, J.R. The isolation of mammalian hepatocytes and their use in conjugation studies. *Methods in Enzymology* **77**, 130-137 (1981).
6. DYBING, E., SODERLUND, E., HAUG, L.T. and THORGEIRSSON, S.S. Metabolism and activation of 2-acetylaminofluorene in isolated rat hepatocytes. *Can. Res.* **39**, 3268-3275 (1979).
7. BILLINGS, R.E., MURPHY, P.J., McMAHON, R.E. and ASHMORE, J. Aromatic hydroxylation of amphetamine with rat liver microsomes, perfused liver, and isolated hepatocytes. *Biochem. Pharmacol.* **27**, 2525-2529.
8. AARBAKKE, J., BESSESEN, A. and MORLAND, J. Metabolism of ^{14}C-antipyrine in suspensions of isolated hepatocytes. *Acta Pharmacol. et Toxicol.* **41**, 225-234 (1977).
9. JONES, C.A., MOORE, B.P., COHEN, G.M., FRY, J.R. and BRIDGES, J.W. Studies on the metabolism and excretion of benzo(a)pyrene in isolated adult rat hepatocytes. *Biochem. Pharmacol.* **27**, 693-702 (1978).
10. BURKE, M.D., VADI, H., JERNSTROM, B. and ORRENIUS, S. Metabolism of benzo(a)pyrene with isolated hepatocytes and the formation and degradation of DNA-binding derivatives. *J. Biol. Chem.*, **252**, 6424-6431 (1977).
11. WIEBKIN, P., FRY, J.R., JONES, C.A., LOWING, R.K. and BRIDGES, J.W. Biphenyl metabolism in isolated rat hepatocytes. *Biochem. Pharmacol.* **27**, 1899-1907 (1978).
12. BILLINGS, R.E., McMAHON, R.E., ASHMORE, J. and WAGLE, S.R. The metabolism of drugs in isolated hepatocytes. *Drug Metab. Dispos.* **5**, 518-526 (1977).
13. BOCK, K.W., ACKEREN, G.V., LORCH, F. and BIRKE, F.W. Metabolism of naphthalene to naphthalene dihydrodiol glucuronide in isolated hepatocytes and in liver microsomes. *Biochem. Pharmacol.* **25**, 2351-2356 (1976).
14. BOND, J.A. and RICKERT, D.E. Metabolism of 2,4-dinitro ^{14}C toluene by freshly isolated Fischer-344 rat primary hepatocytes. *Drug Metab. Dispos.* **9**, 10-14 (1981).
15. SCHWARZ, L.R., GOTZ, R., WOLFF, T. and WIEBEL, F.J. Monooxygenase and glucuronyltransferase activities in short term cultures of isolated rat hepatocytes. *FEBS Lett.* **98**, 203-206 (1979).

16. STOWELL, A., AUNE, H. and MORLAND, J. Production of acetaldehyde and ethanol by isolated rat liver parenchymal cells in the presence of diethyl ether. *Biochem. Pharmacol.* **30**, 1967-1972 (1981).
17. FRY, J.R., WIEBKIN, P., KAO, J., JONES, C.A., GWYNN, J. and BRIDGES, J.W. A comparison of drug-metabolizing capability in isolated viable rat hepatocytes and renal tubule fragments. *Xenobiotica* **8**, 113-120 (1978).
18. BURKE, M.D. and HALLMAN, H. Microfluorimetric analysis of the cytochrome P448 associated, ethoxyresorufin 0-deethylase activities of individual isolated rat hepatocytes. *Biochem. Pharmacol.* **27**, 1539-1544 (1978).
19. COULSON, R.W.S. and FRY, J.R. Deficient glucuronidation of 4-hydroxybiphenyl in hepatocytes isolated from diabetic rats. *Brit. J. Pharmacol.* **74**, 772P-773P (1981).
20. McLEAN, S. Metabolism of phenacetin and N-hydroxyphenacetin in isolated rat hepatocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **305**, 173-180 (1978).
21. EACHO, P.I., SWEENY, D. and WEINER, M. Conjugation of *p*-nitroanisole and *p*-nitrophenol in hepatocytes isolated from streptozotocin diabetic rats. *J. Pharmacol. Exp. Ther.* **218**, 34-40. (1981).
22. STEWART, D.J. and INABA, T. N-Demethylation of aminopyrine *in vivo* and in the isolated hepatocyte of the rat. *Biochem. Pharmacol.* **28**, 461-464 (1979).
23. STEWART, D.J., INABA, T. and KALOW, W. N-Demethylation of cocaine in the rat and in isolated rat hepatocytes. *J. Pharmacol. Exp. Ther.* **207**, 171-177 (1978).
24. HAYES, J.S. and BRENDEL, K. N-Demethylation as an example of drug metabolism in isolated rat hepatocytes. *Biochem. Pharmacol.* **25**, 1495-1500 (1976).
25. ERICKSON, R.R. and HOLTZMAN, J.L. Kinetic studies on the metabolism of ethylmorphine by isolated hepatocytes from adult rats. *Biochem. Pharmacol.* **25**, 1501-1506 (1976).
26. BERRY, M.N. and KUN, E. Ethanol oxidation by isolated rat-liver cells. *Eur. J. Biochem.* **89**, 237-241 (1978).
27. CROW, K.E., CORNELL, N.W. and VEECH, R.L. The rate of ethanol metabolism in isolated rat hepatocytes. *Alcoholism: Clin. and Exp. Res.* **1**, 43-47 (1977).
28. BERRY, M.N., FANNING, D.C., GRIVELL, A.R. and WALLACE, P.G. Ethanol oxidation by isolated hepatocytes. *Biochem. Pharmacol.* **29**, 2161-2165 (1980).
29. MORELLO, A., REPETTO, Y. and AGOSIN, M. Methoprene and hydroprene are metabolized by ester cleavage in isolated hepatocytes. *Drug. Metab. Dispos.* **8**, 309-315 (1980).
30. SILICIANO, R.F., MARGOLIS, S. and LIETMAN, P.S. Chloramphenicol

metabolism in isolated rat hepatocytes. *Biochem. Pharmacol.* **27**, 2759-2762 (1978).

31. ANDERSSON, B., BERGGREN, M. and MOLDEUS, P. Conjugation of various drugs in isolated hepatocytes. *Drug Metab. Dispos.* **6**, 611-616 (1978).
32. KAO, J., JONES, C.A., FRY, J.R. and BRIDGES, J.W. Species differences in the metabolism of benzoic acid by isolated hepatocytes and kidney tubule fragments. *Life Sci.* **23**, 1221-1228 (1978).
33. PANAYIOTOU, B. and FRY, J.R. Unpublished observations (1980).
34. SHIRKEY, R.J., KAO, J., FRY, J.R. and BRIDGES, J.W. A comparison of xenobiotic metabolism in cells isolated from rat liver and small intestinal mucosa. *Biochem. Pharmacol.* **28**, 1461-1466 (1979).
35. MORLAND, J. and OLSEN, H. Metabolism of sulfadimidine, sulfanilamide, p-aminobenzoic acid, and isoniazid in suspensions of parenchymal and non-parenchymal rat liver cells. *Drug Metab. Dispos.* **5**, 511-517 (1977).
36. SUOLINNA, E.M. Metabolism of sulfanilamide and related drugs in isolated rat and rabbit liver cells. *Drug Metab. Dispos.* **8**, 205-207 (1980).
37. MOLDEUS, P. Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. *Biochem. Pharmacol.* **27**, 2859-2863 (1978).
38. MOLDEUS, P., GRUNDIN, R., VADI, H. and ORRENIUS, S. A study of drug metabolism linked to cytochrome P-450 in isolated rat-liver cells. *Eur. J. Biochem.* **46**, 351-360 (1974).
39. LIN, J.H., SUGIYAMA, Y., AWAZU, S. and HANANO, M. Kinetic studies on the deethylation of ethoxybenzamide. *Biochem. Pharmacol.* **29**, 2825-2830 (1980).
40. FRY, J.R. Unpublished observations (1980).
41. HIRATA, M., HOGBERG, J., THOR, H. and ORRENIUS, S. Cytochrome P-450-product complexes produced by amphetamine derivatives. *Acta Pharmacol. et Toxicol.* **41**, 177-189 (1977).
42. YIH, T.D. and ROSSUM, J.M. Isolated rat hepatocytes and 9,000 g rat liver supernatant as metabolic systems for the study of the pharmacokinetics of barbiturates. *Xenobiotica* **7**, 573-582 (1977).
43. FRY, J.R., JONES, C.A., WIEBKIN, P., BELLEMANN, P. and BRIDGES, J.W. The enzymic isolation of adult rat hepatocytes in a functional and viable state. *Analyt. Biochem.* **71**, 341-350 (1976).
44. BRIDGES, J.W. and FRY, J.R. Drug metabolism in cell suspensions and cultures. In: Drug Metabolism : from Microbe to Man (D.V. Parke & R.L. Smith, Eds.). Taylor and Francis Ltd., 43-54 London (1976).
45. BOLCSFOLDI, G., JOHANSSON, M., ANDERSSON, B. and MOLDEUS, P. Biotransformation of xenobiotics in isolated dog hepatocytes. *Acta Pharmacol. et Toxicol.* **48**, 227-232 (1981).
46. LITTERST, C.L., MIMNAUGH, E.G., REAGAN, R.L. and GRAM, T.E. Comparison of in vitro drug metabolism by lung, liver and kidney of several common laboratory species. *Drug Metab. Dispos.* **3**, 259-265 (1975).

47. CHABRA, R.S. and FOUTS, J.R. Sex differences in the metabolism of xenobiotics by extrahepatic tissue in rats. *Drug. Metab. Dispos.* **2**, 375-379 (1974).
48. LAKE, B.G., COLLINS, M.A. and GANGOLLI, S.D. The effect of treatment with a polychlorinated-biphenyl mixture on hepatic and extrahepatic xenobiotic metabolism in the rat and ferret. *Biochem. Soc. Transac.* **6**, 1251-1252 (1978).
49. AITIO, A. A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Analyt. Biochem.* **85**, 488-492 (1978).
50. AITIO, A. Induction of UDP glucuronyltransferase in the liver and extrahepatic organs in the rat. *Life Sci.* **13**, 1705-1713 (1973).
51. AITIO, A. and VAINIO, H. UDP glucuronosyltransferase and mixed function oxidase activity in microsomes prepared by differential centrifugation and calcium aggregation. *Acta Pharmacol. et Toxicol.* **39**, 555-559 (1976).
52. SZINICZ, L.L. and WEGER, N. Effects of 4-dimethylaminophenol in rat kidneys, isolated rat kidney tubules and hepatocytes. *Xenobiotica* **10**, 611-620 (1980).
53. JONES, D.P., SUNDBY, G.B., ORMSTAD, K. and ORRENIUS, S. Use of isolated kidney cells for study of drug metabolism. *Biochem. Pharmacol.* **28**, 929-935 (1979).
54. MOLDEUS, P., JONES, D.P., ORMSTAD, K. and ORRENIUS, S. Formation and metabolism of a glutathione-S-conjugate in isolated rat liver and kidney cells. *Biochem. Biophys. Res. Comm.* **83**, 195-200 (1978).
55. STOHS, S.J., GRAFSTROM, R.C., BURKE, M.D. and ORRENIUS, S. Benzo (a)pyrene metabolism by isolated rat intestinal epithelial cells. *Arch. Biochem. Biophys.* **179**, 71-80 (1977).
56. GRAFSTROM, R., MOLDEUS, P., ANDERSSON, B. and ORRENIUS, S. Xenobiotic metabolism by isolated rat small intestinal cells. *Medical Biology* **37**, 287-293 (1979).
57. ANUNDI, I., HOGBERG, J. and STEAD, A.H. Glutathione depletion in isolated hepatocytes: its relation to lipid peroxidation and cell damage. *Acta Pharmacol. et Toxicol.* **45**, 45-51 (1979).
58. EKSTROM, T. and HOGBERG, J. Chloroform-induced glutathione depletion and toxicity in freshly isolated hepatocytes. *Biochem. Pharmacol.* **29**, 3059-3065 (1980).
59. LINDSTROM, T.D., ANDERS, M.W. and REMMER, H. Effect of phenobarbital and diethyl maleate on carbon tetrachloride toxicity in isolated rat hepatocytes. *Exp. Molec. Path.* 48-57 (1978).
60. THOR, H., MOLDEUS, P., KRISTOFERSON, A., HOGBERG, J., REED, D.J. and ORRENIUS, S. Metabolism of bromobenzene in isolated hepatocytes. *Arch. Biochem. Biophys.* **188**, 114-121 (1978).
61. GWYNN, J., FRY, J.R. and BRIDGES, J.W. The effect of paracetamol and

other foreign compounds on protein synthesis in isolated adult rat hepatocytes. *Biochem. Soc. Transac.* **7**, 117-119 (1979).

62. KRACK, G., GOETHALS, F., DEBOYSER, D. and ROBERFROID, M. Interference of chemicals with glycogen metabolism in isolated hepatocytes. *Toxicology* **19**, 212-223 (1980).
63. FRY, J.R. and BRIDGES, J.W. A novel mixed hepatocyte-fibroblast culture system and its use as a test for metabolism-mediated cytotoxicity. *Biochem. Pharmacol.* **26**, 969-973 (1977).
64. COX, P.J., PHILLIPS, B.J. and THOMAS, P. The enzymatic basis of the selective action of cyclophosphamide. *Can. Res.* **38**, 3755-3761 (1975).
65. WIEBKIN, P., FRY, J.R. and BRIDGES, J.W. Metabolism-mediated cytotoxicity of chemical carcinogens and non-carcinogens. *Biochem. Pharmacol.* **27**, 1849-1851 (1978).
66. GREEN, M.H.L., BRIDGES, B.A., ROGERS, A.M., HORSPOOL, G., MURIEL, W.J., BRIDGES, J.W. and FRY, J.R. Mutagen screening by a simplified bacterial fluctuation test. *Mut. Res.* **48**, 287-294 (1977).
67. BROUNS, R.E., BOS, R.P., GEMERT, P.J.L., HURK, E.W.M. and HENDERSON, P.T. Mutagenic effects of benzo(a)pyrene after metabolic activation by hepatic 9,000 g supernatants or intact hepatocytes. *Mut. Res.* **62**, 19-26 (1979).
68. GLATT, H.R., BILLINGS, R., PLATT, K.L. and OESCH, F.Improvement of the correlation of bacterial mutagenicity with carcinogenicity of benzo(a)pyrene and four of its major metabolites by activation with intact liver cells instead of homogenate. *Can. Res.* **41**, 270-277 (1981).
69. BROUNS, R.M.E., BOS, R.P., DOORN, R. and HENDERSON, P.T. Metabolic activation of 2-acetylaminofluorene by isolated rat liver cells. *Arch. Toxicol.* **45**, 53-59 (1980).
70. BROUNS, R.M.E., DOORN, R., BOS, R.P., MULLENERS, L.J.S. and HENDERSON, P.T. Metabolic activation of 2-aminofluorene by isolated rat liver cells through different pathways leading to hepato-cellular DNA-repair and bacterial mutagenesis. *Toxicology* **19**, 67-75 (1981).
71. ASHURST, S.W., MEHTA, R. and COHEN, G.M. Importance of conjugation reactions in determining the qualitative nature of polycyclic aromatic hydrocarbon-DNA interactions. *Medical Biology* **57**, 313-320 (1979).
72. JERNSTROM, B., ORRENIUS, S., UNDEMAN, O., GRASLUND, A. and EHRENBERG, A. Fluorescence study of DNA-binding metabolites of benz-(a)pyrene formed in hepatocytes isolated from 3-methylcholanthrene-treated rat. *Can. Res.* **38**, 2600-2607 (1978).
73. SHEN, A.L., FAHL, W.E. and JEFCOATE, C.R. Metabolism of benzo(a)pyrene by isolated hepatocytes and factors affect covalent binding of benzo-(a)pyrene metabolites to DNA in hepatocyte and microsomal systems. *Arch. Biochem. Biophys.* **204**, 511-523 (1980).
74. MULDER, G.J. Detoxification or toxification? Modification of the toxicity

of foreign compounds by conjugation in the liver. *Trends in Biochem. Sci.* **4**, 56-59 (1979).

75. CALDWELL, J. The significance of phase II (conjugation) reactions in drug disposition and toxicity. *Life Sci.* **24**, 571-578 (1979).
76. MOLDEUS, P., VADI, H. and BERGGREN, M. Oxidative and conjugative metabolism of *p*-nitroanisole and *p*-nitrophenol in isolated rat liver cells. *Acta Pharmacol. et Toxicol.* **39**, 17-32 (1976).
77. WIEBKIN, P., PARKER, G.L., FRY, J.R. and BRIDGES, J.W. Effect of various metabolic inhibitors on biphenyl metabolism in isolated rat hepatocytes. *Biochem. Pharmacol.* **28**, 3315-3321 (1979).
78. SUOLINNA, E.M. and MANTYLA, E. Glucuronide and sulphate conjugation in isolated liver cells from control and phenobarbital- or PCB-treated rats. *Biochem. Pharmacol.* **29**, 2963-2968 (1980).
79. KOSTER, H., HALSEMA, I., SCHOLTENS, E., KNIPPERS, M. and MULDER, G.J. Dose-dependent shifts in the sulfation and glucuronidation of phenolic compounds in the rat *in vivo* and in isolated hepatocytes. *Biochem. Pharmacol.* **30**, 2569-2575 (1981).
80. FRY, J.R. A comparison of biphenyl 4-hydroxylation and 4-methoxy-biphenyl O-demethylation in rat liver microsomes. *Biochem. Pharmacol.* **30**, 1915-1919 (1981).
81. KNIGHTS, D.T. and FRY, J.R. Unpublished observations (1981).
82. AL-BADAWY, A.B. and EVANS, M. Effects of streptozotocin on the concentrations of rat liver nicotinamide dinucleotides (phosphates) and the activity of tryptophan pyrrolase. *Biochem. Soc. Transac.* **5**, 1314-1316 (1977).
83. SCHRIEFERS, H., GHRAF, R. and POHL, F. Activity of UDP-glucuronyltransferase during starvation and alloxan diabetes. *Hoppe-Seyler's Z. Physiol. Chem.* **344**, 25-33 (1966).
84. MULLER-OERLINGHAUSEN, B., HASSELBLATT, A. and JAHNS, R. Impaired hepatic synthesis of glucuronic acid conjugates in diabetic rats. *Life Sci. 96*, 1529-1533 (1967).