Pure & Appl. Chem., Vol. 65, No. 10, pp. 2299–2312, 1993.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION
COMMISSION ON AGROCHEMICALS*

IUPAC Reports on Pesticides (30)

USE OF ISOLATED CELLS TO STUDY THE METABOLISM OF AGROCHEMICALS IN ANIMALS

(Technical Report)

Prepared for publication by

N. KURIHARA[1], G. D. PAULSON[2], S. OTTO[3], J. MIYAMOTO[4] and R. M. HOLLINGWORTH[5]

[1]Radioisotope Research Center, Kyoto University, Kyoto 606-01, Japan
[2]USDA, ARS. P.O. Box 5674, Fargo, North Dakota 58105, USA
[3]BASF, Landwirtschaftliche Versuchsstation, D(W)-6703 Limburgerhof, Germany
[4]Sumitomo Chemical Co., 4-2-1 Takatsukasa, Hyogo 665, Japan
[5]Pesticide Research Center, Michigan State University, East Lansing, Michigan 48824, USA

*Membership of the Commission during the preparation of this report (1988–91) was as follows:

Chairman: 1987–89 R. J. Hemingway (UK); 1989–1993 E. Dorn (FRG); *Secretary*: 1985–89 T. R. Roberts (UK); P. T. Holland (New Zealand) 1989–1993; *Titular Members*: N. Aharonson (Israel; 1983–89); A. Ambrus (Hungary; 1983–91); L. A. Golovleva (USSR; 1985–1991); R. M. Hollingworth (USA; 1989–1993); J. W. Vonk (Netherlands; 1985–89); N. Kurihara (Japan; 1989–1993); G. D. Paulson (USA; 1989–1993); *Associate Members*: S. Z. Cohen (USA; 1985–91); B. Donzel (Switzerland; 1987–93); E. Dorn (FRG; 1985–89); P. T. Holland (New Zealand; 1985–89); A. W. Klein (FRG; 1989–1993); S. Otto (FRG; 1983–91); D. B. Sharp (USA; 1985–89); C. V. Eadsforth (UK; 1989–1993); R. Graney (USA; 1989–1993); B. Ohlin (Sweden; 1989–1993); R. D. Wauchope (USA; 1989–1993); *National Representatives*: R. Greenhalgh (Canada; 1985–93); Z. Li (China; 1985–93); J. Kovacikova (Czechoslovakia; 1985–91); J. Iwan (FRG; 1986–91); F. Dutka (Hungary; 1985–89); R. L. Kalra (India; 1986–89); J. Miyamoto (Japan; 1985–93); C. K. Heng (Malaysia; 1985–87); H. S. Tan (Malaysia; 1987–89); S. Lj. Vitorović (Yugoslavia; 1985–89); K. P. Park (Rep. Korea; 1989–1991); T. R. Roberts (UK; 1989–1991); P. C. Kearney (USA; 1989–93).

Correspondence on the report should be addressed to the Secretary (1989–93) of the Commission: Dr P. T. Holland, The Horticulture and Food Research Insitute of New Zealand Ltd., Private Bag, Hamilton, New Zealand.

Names of countries given after Members' names are in accordance with the *IUPAC Handbook 1991–93*; changes will be effected in the 1993–95 edition.

Use of isolated cells to study the metabolism of agrochemicals in animals (Technical Report)

Abstract - Isolated cells derived from various organs of animals can be used to obtain a variety of information on the metabolism of agrochemicals and xenobiotics in intact animals. Isolated cells catalyze various phase I and II reactions and generally more closely mimic intact animal systems than do other *in vitro* systems. However the utility of isolated cells is limited by technical difficulties including isolation techniques, preincubation times, separation of heterogenous cells and the difficulty in maintaining catalytic activity for a long period of time. Thus, improved techniques for isolating cells and for maintaining the viability of isolated cells are needed. Studies on the relationship between results obtained with isolated cells and with intact animals, and results obtained with animal cells and human cells are also required. Studies with cells containing expressed human enzymes are desirable.

Contents

1 INTRODUCTION

Ideally, studies on the animal metabolism of agrochemicals provide a wide variety of information including: (1) rates and routes of absorption; (2) the site(s) of metabolism; (3) interorgan relationships in metabolism; (4) the structures of intermediate metabolites and the end products of metabolism; (5) product-precursor relationships in metabolism; and (6) the rates and routes of excretions of metabolites. Generally, a variety of techniques and procedures must be used to provide this information. *In vivo* studies are generally the best way to study the rates and routes of absorption and excretion and the nature of the end products of metabolism in excreta. *In situ* studies (organ perfusions, bile collections and perfusions, collection of blood at key locations, etc.) often provide useful information concerning intermediary metabolism, interorgan relationships, and product-precursor relationships. *In vitro* studies (isolated enzymes, tissue homogenates, tissue fractions, tissue cultures, and isolated cell systems) have been especially useful to study intermediary metabolism of agrochemicals. Some people advocate much more extensive use of *in vitro* systems to minimize the number of experimental animals used for *in vivo* studies. This is a worthy goal; however the investigator needs to be aware of the limitations of each *in vitro* system used. Unwise use of an *in vitro* system may yield misleading information.

Isolated cells are potentially very useful in xenobiotic metabolism studies because: (1) they retain the various xenobiotic metabolizing enzymes; (2) they contain coenzymes and cosubstrates at physiological concentrations; and (3) they have intact cell membranes and intracellular particles. In this paper we will discuss the advantages, and justifications for the use of isolated cells to study the metabolism of agrochemicals in animals. We will also review the limitations of this technique--especially as they relate to extrapolation of results and predicting metabolism in the intact animal.

2 ADVANTAGES AND DISADVANTAGES OF VARIOUS *IN VITRO* TECHNIQUES

Before discussing the use of isolated cells in metabolism studies, we will briefly review the advantages and disadvantages of various *in vitro* techniques. The *in vitro* models currently used for the study of animal metabolism can be ranked in the order of increasing biological complexity as follows:

1 purified, reconstituted enzyme systems
2 microsomes and other subcellular fractions
3 isolated cells
4 perfused organs (*in situ*)

Every model has certain advantages and disadvantages concerning the availability of the test system, its ease of handling, its reproducibility, and its ability to simulate *in vivo* metabolism.

In general, several consecutive or concurrent and competing enzymatic steps are involved in the metabolism of xenobiotics. Therefore, the incubation of a substrate with a single enzyme or an enzyme system is generally not a suitable model for describing the complexity of *in vivo* metabolism. However, purified enzyme systems have the advantages of being well defined systems which give generally reproducible results and are therefore simple and versatile tools useful for substrate screening tests etc. (e.g. toxicity screening). Isolated enzyme systems are also widely used to study mechanistic aspects of one step metabolic reactions and comparison of isoenzymes (e.g. the genetic polymorphism of cytochrome P-450).

Tissue homogenates, microsomal fractions and other subcellular fractions are the most commonly used *in vitro* systems because they are relatively simple to prepare, they provide generally reproducible results and can be cryopreserved with minimal loss of activity. However,

the metabolic patterns obtained using these systems generally differ (especially quantitatively) from results obtained in intact animals. If conjugation reactions are an important part of the overall *in vivo* metabolism, the qualitative differences obtained with these *in vitro* systems are often quite striking. In spite of these limitations, tissue homogenates and isolated subcellular fractions are the system of choice for certain applications (e.g. production of microgram to milligram amounts of metabolic intermediates) for characterization studies.

The advantages and limitations of isolated cells will be discussed in detail in this paper. However, in brief, isolated cells generally more closely mimic intact animal systems; disadvantages include the difficulty of preparation, the short period of viability and difficulties in cryopreservation. Cell lines in culture avoid several of these problems but generally do not adequately express metabolic enzymes such as P-450s.

In contrast to microsomes and isolated cells, the cellular organization and cellular interactions remain undisturbed in perfused organs. Therefore, perfused organs are excellent models for *in vitro* toxicity testing, the study of inhibitory and inductive effects of xenobiotics, and the investigation of multi-stage metabolic pathways. In some cases they afford information on routes and rates of secretion. Furthermore, perfused organs are particularly useful as 'metabolite factories' to generate large quantities of metabolites for isolation and characterization studies. The disadvantages are the relatively difficult organ preparation and the specialized experimental equipment needed, the short period of viability, and the impossibility of viable cryopreservation. Finally the use of perfused organs does not reduce the number of experimental animals used.

3 METABOLISM STUDIES USING CELLS AS A Model OF WHOLE ANIMAL METABOLISM: ILLUSTRATIVE EXAMPLES WITH ISOLATED HEPATOCYTES

Intact animals have the ability to transform xenobiotics through a variety of phase I (oxidation, reduction, and hydrolysis) and phase II (glucuronidation, sulfation, acetylation, methylation, and glutathione conjugation) reactions. Thus the ideal *in vitro* system must have the ability to catalyze all of these biotransformations. Many studies have been reported which indicate that isolated cells have these capabilities. The following examples of agrochemical metabolism by isolated hepatocytes support this conclusion.

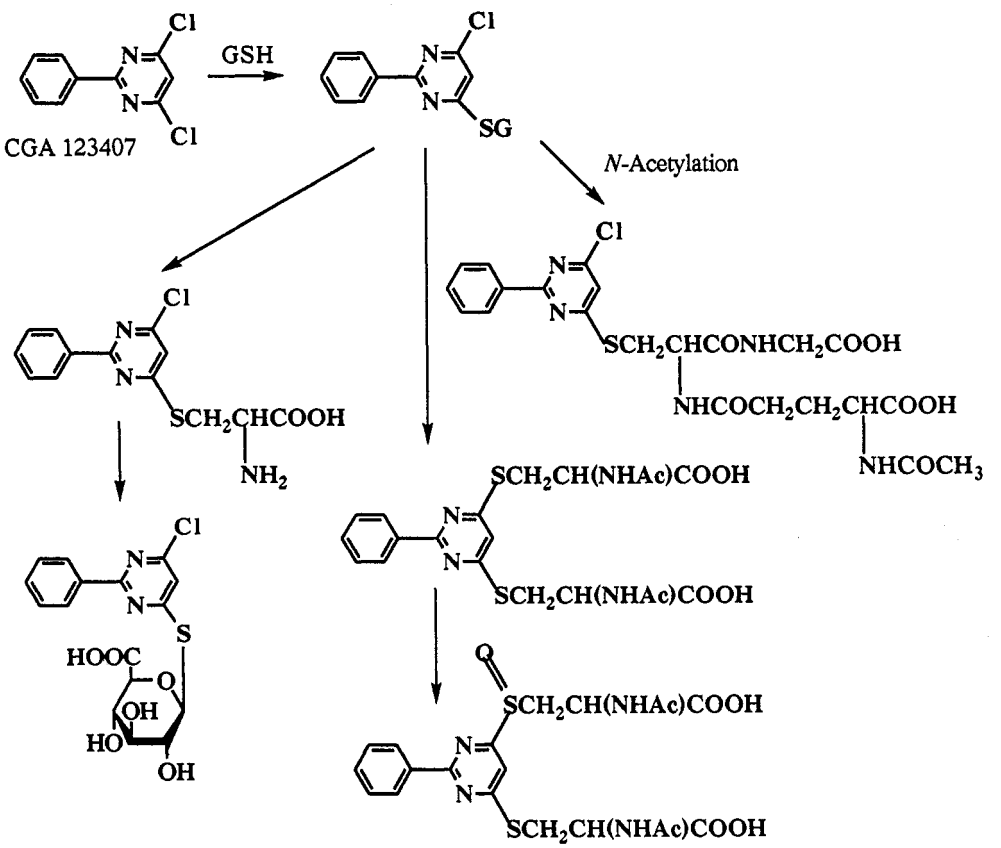
3.1 Studies using rat hepatocytes

The rice safening agent CGA 123407 (against the phytotoxic effects of chloroacetanilide herbicides) was metabolized in the intact rat to mercapturic acids, cysteine conjugates, their sulfoxide oxidation products and glucuronic acid conjugates--all of which were isolated from the bile or urine. Rat hepatocytes metabolized CGA 123407 to the same metabolites. In addition, the glutathione(GSH) conjugate and its *N*-acetyl derivative were detected in the *in vitro* system; these metabolites were predicted intermediates in the conversion of CGA 123407 to the metabolites observed in the urine and bile (ref. 1).

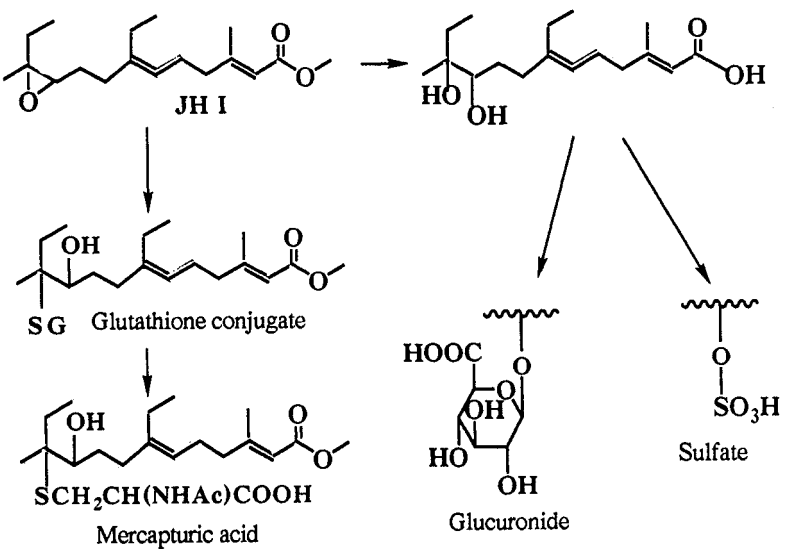
Rat hepatocytes rapidly metabolized insect juvenile hormone I [JH I; methyl (*E, E, Z*)-3,11-dimethyl-7-ethyl-10,11-epoxy-2,6-tridecadienoate] to yield several polar and nonpolar metabolites.

The organic-soluble metabolites included the diol ester and the diol acid, and the polar water soluble metabolites included mercapturic acid, glucuronic acid and sulfate ester conjugates. These metabolites were similar to those produced by intact insects (ref. 2), and there was no evidence that JH I was oxidatively metabolized in intact insects neither in isolated hepatocytes,

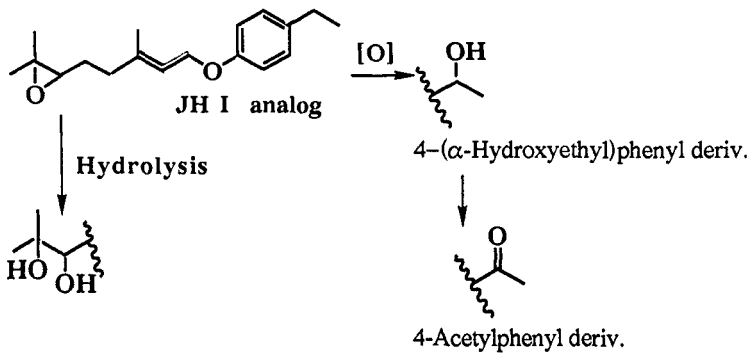
Rice safening agent



JH I Metabolites



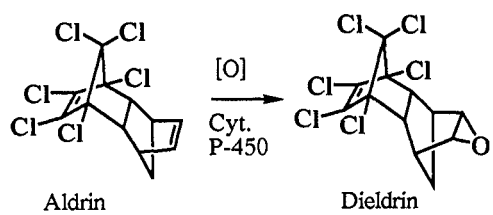
Metabolites of JH I analog



although JH I was a good substrate for a cytochrome P-450 in a reconstituted system. This provides an example of how extrapolation of results obtained by a cell-free system would have given a distorted picture of *in vivo* metabolism; whereas, the results obtained with isolated hepatocytes were in general agreement with the results obtained by *in vivo* studies. A JH I analog [(*E*)-6,7-epoxy-1-(4-ethylphenoxy)-3,7-dimethyl-2-octene] was metabolized primarily by oxidation of the ring ethyl group and epoxide hydrolysis by rat hepatocyte suspensions. It was oxidized to yield the α-hydoxyethyl derivative by cytochrome P-450 in a reconstituted system (ref. 3).

Although the structures of the metabolites were not identified, studies by Yvelin et al. (ref. 4, 5) showed that rat hepatocytes metabolized lindane to an array of products similar to those observed in the bile from lindane treated rats.

The epoxidation of aldrin by rat hepatocyte suspensions was more rapid than by rat liver microsomes when expressed on a liver weight basis or on a cytochrome P-450 basis. Total cytochrome P-450 content (on the liver weight basis) was higher in the hepatocytes than in the rat liver microsomes. These results suggested that some of the cytochrome P-450 components in the liver endoplasmic reticulum were lost or damaged during the isolation of the microsomes and that hepatocytes were therefore a better model than liver microsome systems for studying *in vivo* oxidative metabolism of xenobiotics such as aldrin (ref. 6).



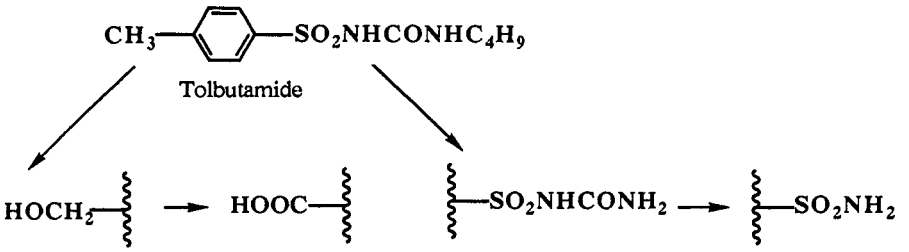
Aldrin Dieldrin

The conversion of 3-phenoxybenzoic acid (a hydrolytic metabolite of some pyrethroids) to lipophilic metabolites was studied using isolated rat hepatocytes. The results obtained indicated that the system was a good model for describing the *in vivo* formation of the nonpolar triacylglycerols (ref. 7).

A recently published study that compared the metabolism of methyl parathion in isolated rat hepatocytes and their cell-free systems demonstrated an influential role of cellular integrity in determining the quantitative metabolic profiles (ref. 8).

3.2 Hepatocytes from various animal species

Gee et al. (ref. 9) used isolated hepatocytes to compare the metabolism of antidiabetic tolbutamide in several species. Tolbutamide was metabolized via two major pathways: oxidation



of the methyl group to yield the alcohol and carboxylic acid, and removal of the butyl moiety. Oxidation of the methyl moiety was the major pathway in rat and rabbit hepatocytes but removal of the butyl moiety was the predominant pathway in dog and squirrel monkey hepatocytes. The overall rates of metabolism in the different hepatocytes were as follows: rat > rabbit > squirrel monkey > dog. The relative rates of total metabolism in hepatocytes agreed with the rates of elimination of this compound from the plasma of intact animals.

Isolated cells, instead of an *in vivo* system, have been used to study enzyme induction by xenobiotics. Isolated cells offer the advantage that they minimize pharmacokinetic influences on the enzyme induction. Cultured rat hepatocytes and chick embryo liver cells were used to study the effects of structurally varied PCBs on the induction of cytochrome P-450 enzymes. In this type of study, the cell systems were also more convenient to use in assessing the structure-activity relationships than an *in vivo* system (ref. 10).

Food producing animals such as cattle and swine are often intentionally treated with therapeutic agents, growth hormones and other drugs. Certain aspects of the metabolism of these drugs have been studied using isolated hepatocytes. Use of bovine hepatocytes for drug metabolism studies has been reported by Shull et al. (ref. 11). Hoogenboom et al. (ref. 12) demonstrated that isolated porcine hepatocytes were capable of deethylation of 7-ethoxycoumarin as well as nitro-group reduction, *N*-acetylation and *N*-deacetylation. However in monolayer culture, the P-450 content and the oxidative activity of the porcine cells gradually decreased. The same group later reported on the metabolic transformation of a steroid hormone 19-nortestosterone(17-hydroxyestr-4-ene-3-one) by cultured porcine hepatocytes (ref. 13, 14). In these studies, oxidation of its 17β-hydroxyl group followed by 15α-hydroxylation and glucuronic acid conjugation was shown with monolayer cultures. These studies were conducted by using relatively small amounts of radio-labeled substrates. In contrast *in vivo* metabolism studies with large animals would be very expensive and require a specially regulated spacious facility. Thus the use of hepatocytes from large farm animals offers a distinct advantage in terms of cost and convenience.

Metabolic studies have also been conducted with primate hepatocytes -- thus providing assessment of species differences without risking direct exposure of human volunteers during the development of new drugs and agrochemicals. For example, hepatocytes and subcellular fractions isolated from the rhesus monkey were used to study the metabolism of styrene oxide (ref. 15), and primary cultures of human hepatocytes were used to study xenobiotic metabolism using ethoxy-, pentyloxy- and benzyloxy-resorufin as substrates (ref. 16). The latter study demonstrated that xenobiotic metabolizing activity varied with time after isolation. For example, epoxide hydrolytic activity increased from 2 to 5 fold 24 hours after isolation; sulfotransferase activity declined after isolation and glutathione *S*-transferase activity remained nearly constant.

Primary hepatocyte cultures derived from rats, hamsters, monkeys and a human patient were used to monitor the change in P-450 activities with time using oxidative *O*-demethylation of 6,7-dimethoxycoumarin as an index (ref. 17). Metabolism studies using *in vitro* systems derived

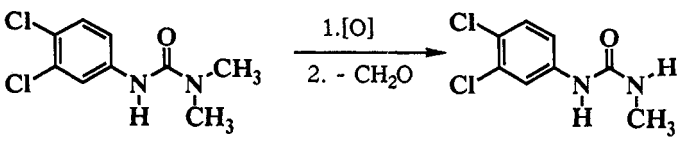
from several species are very useful in assessing the change with time in xenobiotic metabolizing activities. The use of hepatocyte cultures, including those of human origin, may be especially valuable in such studies.

4 METHODOLOGICAL CONSIDEARTIONS WITH ISOLATED HEPATOCYTES

4.1 Time after isolation and incubation conditions

The time after initial isolation and preincubation conditions must be considered when using hepatocytes in agrochemical metabolism studies. For example, in freshly isolated hepatocytes from untreated rat, the level of UDP-glucuronic acid (UDPGA) was low, although the level increased after a 20-min incubation with 1-naphthol. When hepatocytes were incubated in the absence of 1-naphthol, the cofactor level was not significantly altered (ref. 18).

Preincubation treatment of isolated cells may affect conjugation rates -- presumably by changing cofactor levels. For example, preincubation of cells at 30°C for 20 minutes induced cells to biosynthesize UDPGA and PAPS -- cofactors for glucuronic acid and sulfate ester conjugation (ref. 19). The addition of cofactors (or cofactor generating systems) also alters phase I metabolism by isolated hepatocytes. For example, the 4-hydroxylation of biphenyl was enhanced by the addition of NADP, G-6-P and G-6-P dehydrogenase to isolated cells (ref. 20). The addition of NADPH to cryopreserved hepatocytes enhanced the rate of oxidative *N*-demethylation of urea herbicides such as diuron (ref. 21). In both examples, the effect of cofactors could not be explained by the presence of non-viable cells. On the other hand, NADP, G-6-P, and G-6-P dehydrogenase are not known to pass through cell membranes.



Diuron

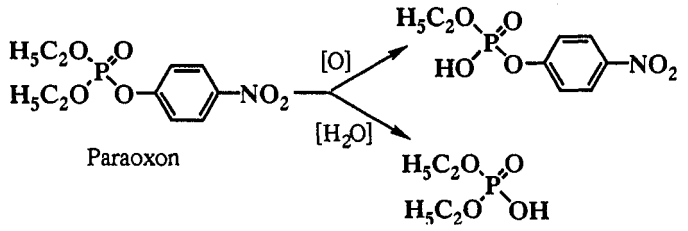
The addition of proteins may alter the metabolic rate and the metabolic profiles in isolated cells. For example, the addition of albumin to the reaction medium enhanced the conversion of ethoxyresorufin by isolated rat hepatocytes. This phenomenon could be explained by the fact that resorufin was bound by the extracellular albumin -- thereby inhibiting the reentry of resorufin into the cells where it was rapidly conjugated with sulfate. Similar extracellular protein-binding may play an important role in the accumulation of unconjugated metabolites *in vivo* (ref. 22).

Uptake of the parent xenobiotic by intact cells can also be altered by extracellular proteins -- thereby changing the apparent kinetics of metabolism. For example, various lipophilic pesticides including chlorinated hydrocarbon insecticides were bound by bovine serum albmin (ref. 23, 24). Thus, the addition of bovine serum albumin normally changed the reaction kinetics of phase I metabolism in various *in vitro* systems (ref. 25, 26). The effect of added protein on the metabolic reactions in isolated cells must be further studied, because in the living animals the xenobiotic-metabolizing liver cells are normally surrounded by blood rich in albumin.

4.2 Heterogeneity of cells

The investigator must be aware that liver contains several different cell types and that agrochemical metabolism may be quite different in the various cell types. For example, phenobarbital-induced cytochrome P-450 was shown to be predominantly located in the centrilobular region (ref. 27). Thus it was not surprising that the ratio of centrilobular to periportal cytochrome P-450 concentration increased from 1.5 to 2.7 after phenobarbital-treatment (ref. 28). The rate of oxidative and hydrolytic metabolism of paraoxon in

centrilobular hepatocytes was nearly twice that in the periportal cells. 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE) treatment of the rat resulted in about a threefold increase in the ratio of oxidative deethylation to hydrolytic dearylation in the centrilobular region of hepatocytes. In contrast, DDE treatment resulted in only a slight increase in this ratio in the periportal region (ref. 29).



Paraoxon

4.3 Maintenance of xenobiotic metabolizing activity

One of the major problems with the use of primary cell cultures is that these systems tend to lose their xenobiotic metabolizing ability within a few days after the initial isolation. Attempts have been made to prevent or reduce this decline in activities with varying degrees of success. For example, a supplementary culture medium with selected hormones (insulin, hydrocortisone 21-acetate, estradiol(17β), testosterone etc.) maintained some xenobiotic oxidizing activities at high levels (but less than the first-day level) for 48 hours, but some activities such as 7-ethoxyresorufin *O*-deethylase were markedly enhanced in 12- and 24-hour cultures (ref. 30).

The removal of cystine, and addition of 5-aminolevulinic acid, was reported to maintain the cytochrome P-450 content at a high level in isolated hepatocytes (ref. 31); the low concentration of glutathione in the medium was alleviated by the addition of methionine (ref. 32). The effect of cystine-removal from the medium on the maintenance of cytochrome P-450 level was confirmed (ref. 33). In the latter study nicotinamide was added to the cystine-free medium. However, it should be noted that hepatocytes maintained in the cystine-free medium (supplemented with 5-aminolevulinic acid) metabolized some common drugs very slowly, even though the P-450 level was maintained. The addition of metyrapone or other substituted pyridines was more effective in preventing the decline of oxidative metabolism activities (e.g. ethylmorphine *N*-demethylation and cytochrome P-450 content) for at least 72 hours. In this study, 7-ethoxyresorufin *O*-deethylase activity was greatly enhanced from the original activity (about 35 times); therefore the induction of some xenobiotic metabolizing activities may have occurred (ref. 34).

Addition of nicotinamide, pyridine and metyrapone maintained 7-ethoxycoumarin *O*-deethylase activity of the rat hepatocytes at near *in vivo* levels for 4 days after cell isolation (ref. 35). Dexamethasone and corticosterone induced the above activity, and some other steroid hormones including hydrocortisone and pregnenolone 16α-carbonitrile also regulated P-450 activities (ref. 35). An appropriate combination of dexamethasone, insulin, glucagon (and hemin and/or phenobarbital) maintained the cytochrome P-450 content in female (but not in male) rat hepatocytes for at least 21 days when the activity was measured with *p*-nitroanisole demethylation (ref. 36).

4.4 Immobilization and cryopreservation of cells

Immobilization of rat hepatocytes in alginate beads maintained the cell viability, and some important activities such as gluconeogenesis and sulfobromophthalein(bromosulfophthalein, BSP) uptake for at least 9 weeks (ref. 37). This immobilization procedure did not change the lindane metabolizing activity (ref. 37). Unfortunately, no further studies using this immobilization technique have been published.

Cryopreservation, which shows promise in maintaining xenobiotic metabolizing ability of hepatocytes, is accomplished by rapid freezing(-70°C) of cells in the presence of 20% DMSO (ref. 38). Cryopreserved rat hepatocytes maintained nearly all of their *N*-demethylation activity for up to 23 days of freezing -- but NADPH addition was required to restore full activity (diuron as substrate) (ref. 21). In contrast when aminopyrine was used as substrate *N*-demethylation activity was retained after two days of storage but decreased to 10% after 21 days of storage (ref. 38)

5 CELLS OTHER THAN HEPATOCYTES

5.1 Liver epithelial cells and bile duct cells

A number of investigators have isolated rat liver epithelial cells (RLEs), which can be targets of chemical carcinogens. A report (ref. 39) noted the similarities between RLEs and oval cells; it has been reported that the latter differentiates into bile duct cells or hepatocytes (ref. 40). The xenobiotic-metabolizing activities of RLEs and bile duct cells were compared to those of hepatocytes. Various conjugating activities such as UDP-glucuronyl transferase, glutathione transferase and sulfotransferase activities were present in RLEs and bile duct cells but the cytochrome P-450-catalyzed *O*-dealkylation was not observed in these cells (ref. 41).

5.2 Lung cells

The mammalian lung contains 40 or more cell types -- and no single type of cell predominates (ref. 42). Moreover, each cell type has its own characteristic combination of drug-metabolizing enzyme activities. For example, the epithelial cells (representing approximately 15% of total cells in lungs) appeared to be most important in xenobiotic metabolism in lung, e.g. benzo[*a*]pyrene hydroxylation and aniline hydroxylation, because the metabolic activity catalyzed by cytochrome P-450 is concentrated in the bronchiolar and alveolar epithelial cells (ref. 43, 44). Clara cells and alveolar type II cells among the epithelial cells are especially rich in P-450 xenobiotic metabolizing enzymes; however, these two cell types appear to have different cytochrome P-450 components (ref. 45).

A few investigators have used lung cells isolated from rabbits (ref. 45) and rats (ref. 46, 47) to study xenobiotic metabolism. One isolated lung cell preparation (84±5% viable) contained 15% alveolar type II epithelial cells, 1.14% ciliated epithelial cells and less than 1% Clara cells (non-ciliated bronchiolar epithelial cells) (ref. 47). Other cell types present were not quantitated. This mixed cell preparation had *O*-deethylation, glucuronyl transferase and sulfate transferase activities indicating potential for use as a model *in vitro* system to study xenobiotic metabolism in mammalian lung.

5.3 Kidney cells

Since important steps in the conversion of glutathione conjugates to mercapturic acid conjugates are catalyzed by the kidney enzymes, the successful preparation of isolated rat kidney cells and their use for studies on mercapturic acid biosynthesis (ref. 48) are important advances. The glutathione conjugate of acetaminophen was catabolized to yield the cysteine conjugate of acetaminophen by kidney cortex tubular cells (ref. 49). Kidney cortex cells were also used to measure phase I and II reactions including enzyme induction by a mixture of PCBs (ref. 50, 51). Studies by several investigators indicated that most of the xenobiotic metabolizing activity of mammalian kidney exists in the cortex region (ref. 44, 52).

5.4 Intestinal cells

Epithelial cells isolated from the small intestine of rats (ref. 53) and guinea pigs (ref. 54) catalyzed the conjugation of phenol, 1-naphthol and hydroxycoumarin (glucuronic acid and sulfate conjugation), the *N*-acetylation of aniline, and the deethylation of 7-ethoxycoumarin. The use of intestinal cells may be especially useful in studying the disposition of orally ingested compounds.

6 USE OF HUMAN CELLS

Because of ethical considerations the evaluation and use of isolated human cells to study xenobiotic metabolism are of special importance. The use of human hepatocytes in selected situations were reviewed (See ref. 16 and 17).

Studies by Guillouzo's group (ref. 55, 56) in which they cocultured human hepatocytes with rat liver epithelial cells and observed the activities of both phase I and II enzymes indicated that their preparations remained viable for at least 8 days. Blaauboer and coworkers reported the maintenance of cytochrome P-450 contents in adult human hepatocytes by the addition of metyrapone (ref. 57). Other recent studies using human hepatocytes in drug-metabolism studies have been summarized in an excellent review by Chenery (ref. 58). The use of isolated human hepatocytes to study metabolism of carcinogens has also been summarized (ref. 59).

Because of the ethical consideration of metabolism studies in humans, the use of isolated cells or cell cultures prepared from human tissues should be evaluated further.

7 TRANSGENIC CELLS

Advances in molecular biology have recently made it possible to modify existing cells by transfection with cDNAs coding for specific metabolic enzymes (ref. 60). The functional expression of these foreign genes creates a transgenic cells with altered metabolic capabilities. Clearly this provides many opportunities to study the functions of these enzymes in isolation, in specific combinations and in different cellular environments. Further, it may be possible to develop cell lines that express critical metabolic enzymes reliably in long-term culture. Cell lines that are currently available, even when derived from hepatic tissue, generally fail to express most forms of cytochrome P-450 and thus lack utility for metabolic studies.

Transfected cells are particularly promising for studying the properties of human metabolic isozymes, particularly those that are not constitutively expressed. Cell lines with closely defined metabolic capabilities will also be useful in routine screening for human metabolism of xenobiotics in risk assessment protocols. This approach circumvents the present severe limitations in obtaining enzymes directly from human tissue samples and the consequent relative ignorance of human metabolic capability in comparison to that of common experimental animals. This reduces confidence in animal-derived risk assessments since species differences in xenobiotic metabolism may be considerable (ref. 60, 61).

Most effort in developing transfected cell lines has so far involved the expression of individual P-450 cDNAs from mouse, rat, or human tissues in yeast or cultured mammalian cell lines. cDNA expression of human P-450s in human cell lines should be one of the best models. Human P-450 cDNAs have been rather readily isolated due to the availability of a number of antibodies and cDNA probes to rodent P-450s and the sequence relatedness among the rodent and human enzymes (ref. 60). Indeed, a metabolically competent human cell line that expresses five human cDNAs (four forms of P-450 and a microsomal epoxide hydrolase) has recently been reported (ref. 62). These forms are known to be involved in xenobiotic activation and the transfected cells showed elevated activative capabilities with a variety of procarcinogens.

Progress in this area has been reviewed by Autrup (ref. 59) and Guengerich (ref. 63) who also points out current limitations in the use and interpretation of data derived from studies with transfected cells. Rapid progress in developing useful cell line for a variety of metabolic studies can be anticipated using this approach.

8 CONCLUSIONS

1. Isolated cells derived from liver, lung, kidney, and intestine catalyze various phase I and phase II reactions, and can therefore be used to obtain a variety of information relevant to *in vivo* metabolism of agrochemicals and xenobiotics.

2. Comparative studies with cells from different organs are sometimes useful in describing intermediary metabolism and interorgan relationships in metabolism.

3. Under the correct conditions, isolated cells provide a more complete and reliable picture of agrochemical metabolism than other *in vitro* techniques such as tissue fractions and isolated enzymes.

4. Because tissues often contain a wide variety of cells, the use of a particular cell type from a tissue may give a distorted picture of *in vivo* metabolism.

5. The utility of isolated cells to study agrochemical metabolism is limited by the methodological considerations including the difficulty in isolating cells, preincubation times required, the difficulty in separation of heterogenous cells and the difficulty in maintaining catalytic activity.

6. Studies with human cells or cells containing expressed human enzymes can provide important comparative information on human metabolic capabilities with significance for risk assessment.

9 RECOMMENDATIONS

The advantages of using isolated cells to complement other *in vitro* and *in vivo* studies to provide a more complete description of the metabolism of agrochemicals in animals have been documented. Judicious use of isolated cells can be expected to decrease the number of animals required to study the metabolism of agrochemicals in the future. However, there are still serious limitations in knowledge concerning the use of isolated cells and interpretations of results obtained from such systems. Studies to determine the best techniques for using isolated cell systems and to improve our ability to interpret information obtained using these systems are needed. Specific recommendations are as follows:

1. Improved, and if possible simplified, methods to isolate viable cells from a range of animal tissues should be developed. Perfecting systems to maintain the viability of these cells for extended periods of time should also be considered a high priority.

2. Additional comparative studies with a wide range of agrochemicals should be conducted to determine the correlation of results obtained using isolated cells, other *in vitro* systems, and especially whole animal studies. These studies will provide a pool of knowledge which can be used in predicting whether or not isolated cells can be expected to describe the *in vivo* metabolism of the same compound in intact animals.

3. Studies to determine the feasibility of using isolated cells to describe the kinetics of agrochemical metabolism in the intact animals should be conducted.

4. Efforts to develop cell lines which express normal amounts of metabolizing enzymes is desirable. Such cells would provide a very convenient means of conducting *in vitro* metabolic studies. Also, when used in quantity, they could be used for metabolite biosynthesis.

5. Comprehensive studies to determine the reliability of using isolated human cells to predict agrochemical metabolism in human should be conducted. These studies should be designed to determine when the use of isolated cells may be expected to generate an artifact(s) (qualitative or quantitative) and the cells should therefore not be used as an estimation of metabolism in the human. Finally comparative studies to determine when isolated cells from experimental animals can be used to mimic metabolism in the human should be considered.

REFERENCES

1. W. Mücke and R. Bissig, Abst. 06B-02, VIIth IUPAC, Hamburg, 1990.
2. A. Morello and M. Agosin, *Biochem. Pharmacol.*, **28**, 1535-1539 (1979).
3. A. Morello, Y. Repetto, R.A. White and M. Agosin, *Pestic. Biochem. Physiol.*, **14**, 72-80 (1980).
4. C. Yvelin, J.M. Yvelin and M. Lièvremont, *Bull. Environ. Contam. Toxicol.*, **32**, 140-147 (1984).
5. J. M. Yvelin, C. Yvelin and M. Lièvremont, *Pestic. Biochem. Physiol.*, **22**, 349-359 (1984).
6. N. Kurihara, N. Hori and R. Ichinose, *Pestic. Biochem. Physiol.*, **21**, 63-73 (1984).
7. K.G. Moorhouse, P. F. Dodds and D. H. Hutson, *Biochem. Pharmacol.*, **41**, 1179-1185 (1991).
8. P. N. Anderson, D. L. Eaton and S. D. Murphy, *Fundam. Appl. Toxicol.*, **18**, 221-226 (1992).
9. S. J. Gee, C.E. Green and C.A. Tyson, *Drug Metab. & Dispos.* **12**, 174-178 (1984).
10. L. E. Rodman, S.I. Shedlofsky, A. Mannschreck, M. Puttmann, A.T.Swim and L.W. Robertson, *Biochem. Pharmacol.*, **41**, 915-922 (1991).
11. L. R. Shull, D. G. Kirsch, C. L. Lohse and J. A. Wisniewski, *Xenobiotica*, **17**, 345-363 (1987).
12. L. A. P. Hoogenboom, F. J. H. Pastoor, W. E. Clous, S. E. Hesse and H. A. Kuiper, *Xenobiotica*, **19**, 1207-1219 (1989).
13. L. A. P. Hoogenboom, M. C. J. Berghmans and W. A. Traag, *J. Chromatog.*, **489**, 105-109 (1989).
14. L. A. P. Hoogenboom, M. C. J. Berghmans, A. van Veldhuizen and H. A. Kuiper, *Drug Metab. Disposition*, **18**, 999-1004 (1990).
15. G. J. Pacifici, B. Lindberg, H. Glaumann and A. Rane, *J. Pharmacol. & Exp. Therap,* **226**, 869-875 (1983).
16. M. H. Grant, M.D. Burke, G.M. Hawksworth, S.J. Duthie, J. Engeset and J.C. Petrie, *Biochem. Pharmacol.*, **36**, 2311-2316 (1987).
17. W. C. Mennes, C. W. M. van Holsteijn, A. Timmerman, J. Noordhoek and B. J. Blaauboer, *Biochem. Pharmacol.*, **41**, 1203-1208 (1991).
18. D. Ullrich and K. W. Bock, *Biochem. Pharmacol.*, **33**, 97-101 (1984).
19. T. Mizuma, M. Hayashi and S. Awazu, *Biochem. Pharmacol.*, **34**, 2573-2575 (1985).
20. R. S. Jones, D. Mendis and D. V. Parke, *Biochem. Biophys. Acta,* **500**, 124-131 (1977).
21. N. Kurihara, K. Kinoshita and M. Fujikawa, *J. Pesticide Sci.*,**15**, 579-584 (1990).
22. M. D. Burke and S. Orrenius, *Biochem. Pharmacol.*, **27**, 1533-1538 (1978).
23. T. Nakatsugawa and S. Tsuda, "Metabolism studies with liver homogenate, hepatocytes suspension and perfused liver", in *Pesticide Chemistry, Human Welfare and the Environment Vol 3* (Eds. J. Miyamoto and P. C. Kearney), pp.395-400, Pergamon Press, Oxford, 1983.
24. R. Ichinose and N. Kurihara, *Pestic. Biochem. Physiol.*, **23**, 116-122 (1985).
25. T. Nakatsugawa, J. Timoszyk and J. M. Becker, "Substrate delivery as a critical element in the study of intermediary metabolites of lipophilic xenobiotics *in vitro*", in *Intermediary Xenobiotic Metabolism in Animals* (Eds. D.H. Hutson, J. Caldwell and G.D. Paulson), pp.335-353, Taylor & Francis, London, 1989.
26. N. Kurihara and A. Oku, *Pestic. Biochem. Physiol.*, **40**, 227-235 (1991).
27. J. Baron, J. A. Redick and F. P. Guengerich, *Life Sci.*, **23**, 2627-2632 (1978).
28. P. E. Gooding, J. Chayen, B. Sayer and T. F. Slater, *Chem.-Biol. Interact.*, **20**, 299-310 (1978).
29. W. L. Bradford and T. Nakatsugawa, *Pestic. Biochem. Physiol.*, **18**, 298-303 (1982).
30. M. Dickins and R. E. Peterson, *Biochem. Pharmacol.*, **29**, 1231-1238 (1980).
31. C. M. Allen and L. J. Hockin, *Biochem. Pharmacol.*, **29**, 3215-3218 (1980).
32. C. M. Allen, L. J. Hockin and A. J. Paine, *Biochem. Pharmacol.*, **30**, 2739-2742 (1981).
33. K. F. Nelson and D. Acosta, *Biochem. Pharmacol.*, **31**, 2211-2214 (1982).
34. B. G. Lake and A. J. Paine, *Biochem. Pharmacol.*, **31**, 2141-2144 (1982).

35. A. M. Edwards, M. L. Glistak, C. M. Lucas and P. A. Wilson, *Biochem. Pharmacol.*, 33, 1537-1546 (1984).
36. C. Vind, J. Dich and N. Grunnet, *Biochem. Pharmacol.*, **37**, 1371-1375 (1988).
37. Pierre-Noël, "Immobilisation d'hépatocytes de rat dans des bille d'alginate: charactérisation des préparations et potentialités biotechnologiques" (Thesis; Laboratoire de Physiologie Biochimique ENS de Cachan et Laboratoire de Technologie Enzymatique UTC Compiegne, 1986).
38. T. Inaba, L. Makowka, L. Rotstein, W. A. Mahon, R. E. Falk, J. E. Falk and M. J. Philips, *Can. J. Physiol. Pharmacol.*, **59**, 408 (1981).
39. M-S. Tsao, J. D. Smith, K. G. Nelson and J. W. Grisham, *Exp. Cell Res.* **154**, 38-52 (1984).
40. R. P. Evarts, P. Nagy, H. Nakatsukasa, E. Marsden and S. S. Thorgeirsson, *Cancer Res.*, **49**, 1541-1547 (1989).
41. D. Schrenk, I. Eisenmann-Tappe, R. Gebhardt, D. Mayer, M. E. Mouelhi, E. Röhrdanz, P. Münzel and K. W. Bock, *Biochem. Pharmacol.*, **41**, 1751-1757 (1991).
42. D. J. Benford and J. W. Bridges, "Xenobiotic metabolism in lung", in *Progress in Drug Metabolism, Vol. 9* (Eds. J. W. Bridges and L. F. Chasseaud), pp.53-94, Taylor & Francis, London, 1986.
43. L. W. Wattenberg and J. L. Leong, *J. Histochem. Cytochem.*, **10**, 412-420 (1962).
44. P. Grasso, M. Williams, R. Hodgson, M. G. Wright and S. D. Gangolli, *Histochem. J.*, 3, 117-126 (1971).
45. T. R. Devereux and J. R. Fouts, *Biochem. Pharmacol.*, **30**, 1231-1237 (1981).
46. R. W. Teel and W. H. J. Douglas, *Experientia*, **36**, 107-107 (1980).
47. J. R. Dawson, K. Norbeck and P. Moldéus, *Biochem. Pharmacol.*, **31**, 3549-3553 (1982).
48. D. P. Jones, G-B. Sundby, K. Ormstad and S. Orrenius, *Biochem. Pharmacol.*, **28**, 929-935 (1979).
49. D. P. Jones, P. Moldéus, A. W. Stead, K. Ormstad, H. Jörnvall and S. Orrenius, *J. Biol. Chem.*, **254**, 2787-2792 (1979).
50. J. Kao, C. A. Jones, J. R. Fry and J. W. Bridges, *Life Sci.*, **23**, 1223-1228 (1978).
51. J. F. Fry and N. K. Perry, *Biochem. Pharmacol.*, **30**, 1197-1201 (1981).
52. H. J. Armbrecht, L. S. Birnbaum, T. V. Zenser, M. B. Mattammal and B. B. Davis, *Arch. Biochem. Biophys.*, **197**, 277-284 (1979).
53. R. J. Shirkey, J. Kao, J. R. Fry and J. W. Bridges, *Biochem. Pharmacol.*, **28**, 1461-1466 (1979).
54. J. R. Dawson and J. W. Bridges, *Biochem. Pharmacol.*, **28**, 3299-3305 (1979).
55. J-M. Begue, J. F. LeBigot, C. Guguen-Guillouzo, J. R. Kiechel and A. Guillouzo, *Biochem. Pharmacol.*, **32**, 1643-1646 (1983).
56. A. Guillouzo, P. Beaune, M-N. Gascoin, J-M. Begue, J-P. Campion, F. P. Guengerich and C. Guguen-Guillouzo, *Biochem. Pharmacol.*, **34**, 2991-2995 (1985).
57. B. J. Blaauboer, I. van Holsteijn, M. van Graft and A. J. Paine, *Biochem. Pharmacol.*, 34, 2405-2408 (1985).
58. R. J. Chenery, "The utility of hepatocytes in drug metabolism studies", in *Progress in Drug Metabolism Vol. 11* (Ed. G. G. Gibson), pp.217-265, Taylor & Francis, London, 1988.
59. H. Autrup, *Carcinogenesis*, **11**, 707-712 (1990).
60. F. J. Gonzalez, C. L. Crespi and H. V. Gelboin, *Mutat. Res.* **247**, 113-127 (1991).
61. F. J. Gonzalez, T. Aoyama and H. V. Gelboin, "Activation of pro-mutagens by human cDNA-expressed cytochrome P450s" in *Prog. Clin. Biol. Res., No. 340, Mutation and the Environment Part B. Metabolism, Testing Methods and Chromosomes,* (Eds. M. L. Mendelsohn and R. J. Albertina), , pp. 77-86, Wiley-Liss, 1990.
62. C. L. Crespi, F. J. Gonzalez, D. T. Steimel, T. R. Turner, H. V. Gelboin, B. W. Penman and R. Langenbach, *Chem. Res. Toxicol.* **4**, 566-572 (1991).
63. F. P. Guengerich, *Chem. Res. Toxicol.* **4**, 391-407 (1991).