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COMMENTARY

CYTOCHROME P-450: A PHARMACOLOGICAL NECESSITY OR A BIOCHEMICAL CURIOSITY?

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Much is known about the biochemical properties of cytochrome P-450* but its pharmacological relevance is less certain. Biochemical studies have generally involved the interaction of P-450 with a small range of model substrates, carefully chosen for their ability to undergo simple metabolism and to yield information about P-450 in uncomplicated experiments. However, very few of these model P-450 substrates are therapeutically used drugs or environmental chemicals (i.e. agriculturally, industrially or domestically used chemicals or their by-products). Hence the question remains: does P-450 play a significant role in the metabolism of the drugs and other chemicals to which mankind is actually exposed?

There are, in reality, two questions: (1) *Can* P-450 metabolize the 'real' drugs and chemicals in animals *in vitro* and *in vivo* and in man *in vitro*? (2) *Does* P-450 metabolize drugs and chemicals significantly in man *in vivo*? Answers to the first question are the more numerous, probably because they are amenable to direct experimental verification. By contrast, the role of P-450 in man *in vivo* can be studied only by using indirect measurements: as yet the answers are few, but they are increasingly needed and will probably require new experimental methods. These two questions are considered below, in turn.

1. CAN P-450 METABOLIZE DRUGS AND ENVIRONMENTAL CHEMICALS IN ANIMALS *IN VITRO* AND *IN VIVO* AND IN MAN *IN VITRO*?

There are several different lines of experimental evidence pertaining to this question, some direct but mostly circumstantial, relating mainly to animals and concerning at least one hundred and eighteen drugs and other important chemicals. Since the strength of the whole argument is only as good as the strength of each piece of evidence, eleven different lines of evidence for chemicals being substrates of P-450 are discussed next in detail. Each line of evidence is summarised in its title and the numbers of compounds complying with the evidence are included to show the scale of the involvement of P-450 in the metabolism of drugs and environmental chemicals. Unfortunately, lack of space precludes a listing of all the compounds by name: it also precludes citation of all the sources of information used and for this I apologise to the many authors concerned.

* For the sake of brevity, cytochrome P-450 is given simply as P-450 for the rest of this review.

(i) *The metabolism of a drug is catalysed by reconstituted purified P-450 systems*

This is the most direct kind of evidence, but it is relatively new and has been reported for only a handful of drugs and environmental chemicals, including benzo(a)pyrene, biphenyl, chlorcyclizine, ethanol, hexobarbitone, methimazole, parathion, pentobarbitone, trichloroethane and warfarin [1–9]. The evidence is to date restricted to animal liver P-450 systems, but similar studies with purified human liver P-450 can be expected.

The main problem with using purified P-450 is that there appear to be several different enzymic forms of P-450, each with a different and characteristic range of substrate and reaction specificities [10]. So the choice of enzymic form of P-450 for testing a given drug becomes both critical and difficult to predict. To complicate matters further, there are great species differences in the specificities of otherwise similar forms of P-450. For example, 3-methylcholanthrene induces an abnormal form of P-450 in both rats and rabbits but the induced form of rat P-450 is much more efficient at hydroxylating benzo(a)pyrene than is the induced form of rabbit P-450 [10]. Another obvious reservation is the extent to which purified enzymes simulate physiological processes.

(ii) *The metabolism of a drug* in vitro *is inhibited by specific antibodies against P-450*

This is the newest evidence and is virtually as direct as using purified P-450. Two important drugs, methimazole [6], and warfarin [11], and two important environmental chemicals, benzo(a)pyrene [12] and parathion [13], have been shown by this method to be P-450 substrates. This will probably be a very important approach for demonstrating P-450-mediated metabolism in future, especially with the advent of antibodies against human P-450 for testing species differences in drug metabolism.

(iii) *The metabolism of a drug* in vitro *is inhibited by carbon monoxide*

This is moderately specific evidence for P-450-mediated metabolism in that, although CO inhibits several other haemoproteins, it does not affect any other enzymes thought likely to be directly involved in hepatic microsomal drug metabolism. A more definitive demonstration of P-450 involvement requires the showing of a photoactivation spectrum for light-reversal of CO inhibition with a maximum

effect at 450 nm [14]. At least thirty-one drugs and environmental chemicals, covering a wide range of different types of metabolism, are metabolized by P-450 on the basis of CO-inhibition of their hepatic microsomal metabolism, although for none of these except benzo(a)pyrene has a photoactivation spectrum been measured. A major problem with this evidence is that different metabolic loci in the same substrate molecule can show different CO-sensitivities, for example the hydroxylations at N-, C5- and C7- of acetylaminofluorene [15]. Furthermore, induction of P-450 can alter the CO-sensitivity of the reactions it catalyses [15].

(iv) *The metabolism or pharmacological effect of a drug is affected by cobaltous chloride*

Evidence indicates that four drugs and environmental chemicals are P-450 substrates, on the basis that their metabolism is inhibited due to supposedly specific depression of P-450 by pretreatment of animals with cobaltous chloride (cannabis, cyclophosphamide, dichloromethane and dioxane [16–19]). There is similar evidence for a further six drugs and chemicals on the basis that cobaltous chloride pretreatment depresses their tissue necrotic actions *in vivo* (cephaloridine, frusemide, iproniazid, isoniazid, paracetamol and trichloroethylene [20, 21]). This latter interpretation implies that the toxicological actions of these compounds are mediated by P-450-catalysed metabolism. However, cobaltous chloride is not specific for P-450: it depresses all hepatic haemoproteins, including catalase and cytochrome b_5 [22]. It also decreases renal function and probably, therefore, the excretion of urinary metabolites. Furthermore, cobalt pretreatment *in vivo* increases liver microsomal ethoxycoumarin *O*-deethylation [27]. For these reasons, evidence involving cobaltous chloride, especially evidence *in vivo*, should be interpreted with caution.

(v) *The metabolism or pharmacological effect of a drug is affected by known inhibitors of P-450*

Moderately good evidence that a compound is a substrate for P-450 would be provided if its metabolism *in vitro* were inhibited by model P-450 inhibitors, for example SKF-525A (diethylaminoethyldiphenylvalerate), metyrapone, α-naphthoflavone, DPEA (dichlorophenylphenoxyethylamine), imidazole and piperonyl butoxide [23–25] (carbon monoxide as a P-450 inhibitor has been discussed earlier). SKF-525A is the most frequently used example, inhibiting the hepatic microsomal metabolism of forty-five drugs and environmental chemicals and the metabolism *in vivo* of eleven drugs and chemicals. There are also nine drugs whose pharmacological or toxicological effects are suppressed by one of these P-450 inhibitors, indicating that the nine drugs are P-450 substrates whose pharmacological actions are due to metabolites. A total of at least fifty-six drugs and environmental chemicals appear to be P-450 substrates on the basis of inhibition of their metabolism or effects by at least one of the inhibitors listed.

Underlying the use of P-450 inhibitors is the assumption that they are highly specific for P-450. This is, however, a false assumption. SKF-525A inhibits a variety of enzymes, some involved in drug metabolism, for example glucuronyl transferase [26], as well as cholesterol esterase [28]. It also inhibits cellular alanine incorporation [29] and renal tubular sodium reabsorption [30], blocks nerve conduction [31], decreases hepatic glycogen [32] and hepatic and renal blood flow [33, 34], increases urinary volume [30], alters non-microsomal membrane structures [35] and actually induces P-450 when administered to animals in repeated doses over at least 48 hours [36]. Most of these actions weaken the case for using SKF-525A as a specific P-450 inhibitor *in vivo*, but probably do not detract from its use with liver microsome preparations. The depression by SKF-525A of hepatic blood flow may decrease the metabolism *in vivo* of drugs with a high hepatic extraction ratio, while the depression of renal blood flow may decrease the excretion of urinary metabolites, as has been shown with morphine sulphate [34].

The mechanisms of P-450 inhibition by SKF-525A and metyrapone are different [88, 89] and metyrapone may be the more specific inhibitor of P-450, since very little evidence to the contrary has been published. But, in truth, metyrapone has not been as widely investigated as SKF-525A. Metyrapone inhibits *in vitro* the hepatic metabolism of dapsone, fluoroxene, halothane, hexobarbitone, morphine, trichloroethylene and trichlorofluromethane [66, 89–92]. Metyrapone-inhibition of some reactions is species-dependent [93]. While it does not inhibit microsomal NADPH–cytochrome *c* reductase [89], evidence suggests that metyrapone does inhibit non-P-450 associated hepatic mitochondrial electron-transport [94]. Metyrapone-inhibition of prostaglandin and thromboxane syntheses [37, 95] and of various physiological effects of arachidonic acid [96, 97] is interesting because it may constitute evidence that P-450 is functional in the synthesis of prostaglandins, although not all prostaglandin synthetases are inhibited by metyrapone [95]. It is already known that P-450 can catalyse the catabolic side-chain hydroxylation of prostaglandins [98]. Alternatively, the metyrapone inhibition may be acting at non-P-450 prostaglandin metabolising enzymes that might have certain characteristics in common with P-450, such as prostaglandin cyclooxygenase, which catalyses xenobiotic metabolism similar to P-450 [38].

There is little evidence to help assess the P-450 specificities of the other inhibitors listed. New arylimidazoles and benzothiadiazoles may possibly be more selective P-450 inhibitors [24, 39, 40]. Octylamine and methimazole preferentially inhibit P-450 and the mixed function amine oxidase respectively [41], which could be useful for delineating the relative drug metabolizing roles of these two hepatic microsomal enzymes. An important factor influencing the use of P-450 inhibitors either *in vitro* or *in vivo* is that they are often selective with respect to the induced or constitutive (i.e. normal) P-450 reactions they inhibit [42, 43].

(vi) *The metabolism or pharmacological effect of a drug is affected by known inducers of P-450*

If the metabolism of a drug is increased by specific induction of P-450, this is further evidence that the metabolism is P-450-mediated. At least fifty-six drugs

and environmental chemicals are indicated as P-450 substrates, on the basis that their metabolism *in vivo* and *in vitro* is increased by the pretreatment of animals with either phenobarbitone (the most commonly used P-450 inducer), 3-methylcholanthrene, benzo(a)pyrene, β-naphthoflavone, PCN (pregnenolone 16α-carbonitrile) or DDT (trichlorobischlorophenylethane) [9, 44–48]. There are also eleven drugs and chemicals whose pharmacological or toxicological effects *in vivo* are altered by the prior treatment of animals with a P-450 inducer. These drugs and chemicals are thereby indicated as P-450 substrates whose pharmacological actions are either mediated or terminated by P-450 metabolism.

The main reservations about this type of evidence are (a) species-variable differential induction and (b) non-P-450 effects of the inducing agents. With differential induction, different inducers preferentially induce different forms of P-450 and different drug metabolizing reactions. For example, in the rat phenobarbitone probably induces codeine *N*-demethylation but not codeine *O*-demethylation [49, 50], and while *R*-warfarin 7-hydroxylation is preferentially induced by phenobarbitone, *R*-warfarin 8-hydroxylation is preferentially induced by 3-methylcholanthrene [51]. Therefore, an inability to show induction of a drug's metabolism may mean that an inappropriate inducer was used, rather than that the drug is not a P-450 substrate. A further consequence is that the metabolism of a drug by an induced animal does not necessarily indicate that it will be metabolized in the normal animal, from which the induced form of P-450 may be absent. This situation is complicated further by the fact that an inducer may act differently in different species. Phenobarbitone, for example, induces mainly *R*-warfarin 7-hydroxylation in the rat but mainly *R*-warfarin 4′-hydroxylation in the rabbit [9]. So the choice of both inducer and species becomes crucial, but at present there is insufficient knowledge to predict the appropriate choice for testing any given drug. Nor can it be assumed that an inducing agent affects only P-450: this is especially important when measuring drug metabolism *in vivo*. Phenobarbitone, for example, increases hepatic size [52], blood flow [52] and glycogen content [53], increases bile flow [54], and induces hepatic microsomal glucuronyl transferase [55], procaine-arylamidase [56], NADH-dependent, CO-resistant, probably non-P-450-mediated *O*-deethylation [57] and cytosolic aldehyde dehydrogenase [58]. One of the consequences of the phenobarbitone-caused increase in hepatic size, blood and bile flow is an increase in the hepatic clearance and biliary excretion of non-metabolized substances, for example indocyanine green [59], and of drug metabolites, for example morphine sulphate [60]. Another consequence of the increased hepatic blood flow is an increase in the metabolism of drugs with a high hepatic extraction ratio.

(vii) *The metabolism of a drug is microsomal, NADPH- and O_2-dependent and of a pattern consistent with the involvement of P-450*

If a compound is metabolized by P-450 the reactions involved should be characteristic of P-450, for example hydroxylation at carbon or *O*-dealkylation of an aryl ether rather than ester-hydrolysis. If the hepatic metabolism of the drug is measured *in vitro* it should (a) be catalysed preferentially by microsomal rather than other subcellular fractions; (b) require NADPH- rather than NADH- as cofactor; and (c) be dependent upon molecular oxygen: also, for most (but not all) P-450 substrates the metabolism will be carried out by the liver in preference to other tissues. The metabolism of at least eighty-eight drugs and environmental chemicals displays some or all of these characteristics indicative of catalysis by P-450.

There are unfortunately many interpretive problems associated with this rather simple evidence. It ignores the fact that some *O*-demethylations are preferentially catalysed by glutathione-alkyltransferase rather than by P-450 and even occur spontaneously in the presence of reduced glutathione, for example *O*-demethylation of the organophosphorus insecticide dimethylvinphos [61]. The heterocyclic ring hydroxylation at C2 of quinoline is catalysed by cytosolic aldehyde oxidase, yet its heterocyclic ring hydroxylation at C3 and its aromatic ring hydroxylation at C6 are catalysed by P-450 [62]. In the pig, hepatic microsomal *N*-demethylation of desipramine is catalysed mainly by the mixed function amine oxidase and only minimally by P-450 [41]. Species differences in the pattern of metabolism cause problems with this type of evidence: diazepam undergoes mainly ring C-hydroxylation with some *N*-demethylation in the rat, mainly *N*-demethylation with some C-hydroxylation in the mouse and solely *N*-demethylation in the guinea-pig [63]. The main responsibility for methimazole metabolism lies with the mixed function amine oxidase in the pig [64] but with P-450 in the rat [6]. Some reactions may show a hepatic microsomal location yet lack the other essential characteristics of P-450-mediation, for example, *p*-nitrophenetole *O*-deethylation is NADH-dependent (and CO-insensitive) [57]. However, an NADPH-dependency of metabolism does not necessarily indicate that P-450 is involved: L-DOPA (dihydroxyphenylalanine), for example, is converted to an unidentified active, protein covalent-binding metabolite by an NADPH-dependent microsomal reaction involving NADPH-cytochrome *c* reductase but not P-450 [65]. Conversely, not all probably P-450-mediated metabolism is oxygen-dependent: the anaerobic dechlorination of trichlorofluromethane is inhibited by oxygen [66]. A P-450-consistent pattern of drug metabolites *in vivo* is not unequivocal proof of the involvement of P-450, since extrahepatic tissues catalyse P-450-style reactions that may or may not involve P-450 itself. 1,3-diphenylisobenzofuran is probably metabolized by P-450 in the kidney cortex but by prostaglandin cyclooxygenase in the kidney medulla, with the same metabolite in both cases [38]. The alkaloid zingerone is *O*-demethylated by gut bacteria and it is unclear whether this involves P-450 [67]. However, major metabolism of a drug by a tissue known to be deficient in P-450 suggests a non-P-450 reaction. Daunorubicin is metabolized by an NADPH-dependent hydroxylation, but this route is unlikely to involve P-450 since the extent of reaction is high in brain and heart cytosol compared to liver microsomes. Hydroxydaunorubicin subsequently undergoes

microsomal NADPH-dependent hydrolysis but this reaction also is unlikely to be P-450-mediated because the rate is high in brain and heart and because hydrolysis is not a typical P-450 reaction [68].

(viii) *A drug is metabolized faster by male than by female rats*

The P-450-dependent metabolism of several model substrates proceeds significantly faster in male rats than in female rats: hepatic metabolism *in vitro* of sixteen drugs and environmental chemicals shares this sex-difference characteristic of P-450 substrates. A major criticism of this evidence is that several model P-450 substrates, for example biphenyl, are metabolized equally rapidly by liver microsomes of male or female rats, while other substrates, for example ethoxyresorufin, are metabolized faster by female than male rats [69]. It should also be remembered that, apart from the rat, tree shrew and mouse (where the female generally metabolizes drugs faster than the male), most species do not show any sex differences in drug metabolism.

(ix) *A drug elicits a Type I, reverse-Type I or Type II P-450-binding spectrum*

Formation of an enzyme–substrate complex must precede metabolism, so further evidence that the metabolism of a drug involves P-450 is that specific binding of the drug to P-450 occurs. It is generally agreed that the so-called Type I, reverse-Type I and Type II changes in the P-450 absorption spectrum result from changes in the spin-state of P-450, caused by the binding of compounds that are usually P-450 substrates. At least forty drugs and environmental chemicals show this effect and might therefore be expected to undergo P-450-mediated metabolism. However, the degree of specificity of the P-450 binding that is manifested in these spectra, and the extent to which these reflect true enzyme–substrate complexes, are matters of debate. Thirty-four of the drugs and chemicals elicit Type I spectra, but this may be a general property of the interaction of any lipid-soluble compound with the membranous apoprotein of P-450 [70]. Phenacetin and theophylline produce reverse-Type I spectra, thought to reflect a different type of apoprotein interaction. Five of the drugs and chemicals give Type II spectra, which may be a general property of any compound containing a suitable nitrogen atom capable of liganding directly with the haem of P-450 [71]. The interpretation of P-450 binding spectra is very complicated and is discussed by Jefcoate [72]. The fact that a drug binds in any of these modes to P-450 is no absolute guarantee that it will be metabolized: TCDD (tetrachlorodibenzo-*p*-dioxin) gives a clear Type I spectrum but is minimally metabolized by P-450. Octylamine binds in a Type II manner, yet is an inhibitor rather than a substrate of P-450. Species variation is a complication: aminopyrine binds Type I to rat liver P-450 but reverse-Type I to human liver P-450 [72]. The mode of binding may also be altered by induction of P-450, thus nortryptiline binds Type I with normal rat liver P-450 but Type II with phenobarbitone-induced rat liver P-450 [73].

(x) *A drug inhibits the metabolism of model P-450 substrates*

On the basis that two alternative substrates for the same enzyme are mutually and competitively inhibitory, evidence that a drug is a P-450 substrate should be given by its inhibition of the metabolism of a model P-450 substrate [74]. Thirty-eight drugs and environmental chemicals inhibit model P-450 reactions, for example ethylmorphine *N*-demethylation, *in vitro*, but in only ten cases was the inhibition shown to be competitive. Three drugs showed non-competitive inhibition, while the type of inhibition was not determined for the remaining twenty-five compounds. Although competitive inhibition *in vitro* probably indicates that a drug binds to the active site of P-450, inhibition *per se* does not necessarily indicate that the drug will be metabolized. For example, the antiamoebic, antischistosomal drug dehydroemetine inhibits model P-450 reactions but is not apparently metabolized by P-450 [75]. It is also highly possible that inhibition of a model P-450 reaction can occur by a drug acting as P-450 reductase, but it is difficult to predict whether this would be competitive or non-competitive in nature. Rifampicin, for example, is *N*-demethylated by (probably) P-450 and competitively inhibits ethylmorphine *N*-demethylation [76], while the spontaneously formed rifampicin-quinone is reduced by P-450 reductase and might be expected to inhibit this enzyme [77]. Several recent reports have stated that the metabolism of certain compounds, for example dimethylnitrosamine, is mediated by monoamine oxidase rather than by P-450, on the basis of inhibition by monoamine oxidase inhibitors. In such experiments the monoamine oxidase inhibitor must be carefully chosen, since several of them also interact with P-450. For example, phenelzine competitively inhibits the P-450-mediated *N*-demethylation of pethidine [78], methylphenethylhydrazine inhibits hexobarbitone hydroxylation [70] and harmine is itself an excellent P-450 substrate.

Inhibition of model P-450 substrate metabolism *in vivo* has been less widely studied, but has been reported for four drugs. Inhibition *in vivo* is less certain evidence of a P-450 substrate than is its ability to act as an inhibitor *in vitro*, because the effects *in vivo* might involve a variety of changes in the pharmacokinetics of the model substrate, as well as inhibition of metabolizing enzymes other than P-450. Inhibition *in vivo*, however, might be a useful guide to the extent of the involvement of P-450 in the metabolism of a drug *in vivo*.

(xi) *A drug prolongs hexobarbitone sleeping time or zoxazolamine paralysis*

An alternative to measuring the ability of a chemical to inhibit model P-450 substrate metabolism *in vivo* is to measure its acute ability to prolong the pharmacological action of a model drug *in vivo*, when this action is known to be terminated through metabolism of the model drug by P-450.

Hexobarbitone-induced sleep and zoxazolamine muscle paralysis are two such widely used model pharmacological actions [80]. Twelve drugs and environmental chemicals prolong hexobarbitone

sleeping time and one drug also prolongs zoxazolamine paralysis, constituting evidence that these twelve compounds inhibit P-450 and are therefore P-450 substrates. A test of theoretically equal value would be that a compound acutely diminished a model pharmacological action which was caused by P-450-derived active metabolites, but such a case has not been reported. There are some major problems of interpretation with the tests for prolongation of pharmacological activity, necessitating careful control of experiments. The chief concern is whether the prolongation is truly caused by inhibition of the metabolic inactivation of the model drug or whether a direct pharmacological action of the prolonging agent is responsible. For example, the agent under investigation might itself depress the central nervous system and prolong sleep. Two suitable experimental controls to show that this is not the case would be that (a) brain levels of the model barbiturate at the moment of waking were not affected by the prolonging agent and (b) the agent did not prolong the sleeping time due to barbitone, which is excreted without significant metabolism. Even if the prolongation of sleep can be shown to result from inhibition of metabolism of the barbiturate, this still does not prove a direct inhibition of P-450; hydrallazine and tolazoline each prolong hexobarbitone sleeping times, but whereas hydrallazine achieves this by a direct inhibition of P-450, tolazoline causes hypothermia, which depresses the activity of P-450 [81]. Ambient temperature has a marked influence on experimental sleeping times.

(xii) *A drug induces P-450 or one of its reactions: a spurious piece of evidence for P-450 substrates*

It is a characteristic feature of P-450 that it is induced by many compounds including at least fifty-one commonly used drugs and environmental chemicals. There are also seven drugs which, when given in several consecutive doses, curtail hexobarbitone sleeping time or zoxazolamine paralysis, presumably by inducing the P-450 metabolism of hexobarbitone or zoxazolamine. Enzyme induction in bacteria was in the past considered an exclusive property of substrates of the enzymes induced, but it is now known that enzyme inducers need not be substrates. Furthermore, in animal experiments using homologous series of barbiturates, polycyclic aromatic hydrocarbons and chlorinated benzo-*p*-dioxins it was shown that the best inducers of P-450 were the poorest substrates and vice versa [82–84]. Therefore, the ability of a drug to induce P-450 is no indication that it is metabolized by P-450.

In more general terms, important pharmacological consequences of the induction of P-450 may arise in the event that the inducible form of P-450 metabolizes essential endogenous substrates. This may occur with the clofibrate-inducible hepatic P-450 that hydroxylates fatty acids [85].

Eleven criteria for metabolism by P-450. The evidence discussed above may be fashioned into eleven experimental criteria for indicating whether a drug or environmental chemical can be metabolized by P-450. The headings used for the evidence will serve as titles for the criteria and they have been discussed in the order of increasing ambiguity of the answer they give. These eleven criteria can be listed alternatively in order of the relative ease of the experiments involved. It is ironic that a listing in order of easiness is virtually the converse of the listing in order of ambiguity: the most unequivocal criteria (metabolism by purified P-450 or inhibition of metabolism by antibodies against P-450) involve the most experimental effort, while the easiest experiments (prolongation of sleeping time or paralysis) furnish the most ambiguous answer as to whether P-450 metabolizes a compound.

2. DOES P-450 PLAY A SIGNIFICANT ROLE IN THE METABOLISM OF DRUGS AND ENVIRONMENTAL CHEMICALS IN MAN *IN VIVO*?

The more difficult of the two questions is whether P-450 *does*, as opposed to *can*, metabolize drugs and environmental chemicals in man, and concerns the extent to which the contribution of P-450 is significant to the total metabolism and clearance of drugs *in vivo*. Definition of the role of P-450 in drug metabolism in man must eventually tackle the problems of multiple forms of P-450, extrahepatic P-450 and interindividual variation in drug metabolism. Again there are two parts to the question, the general and the specific: (i) Is P-450 involved in man as a species? (ii) To what extent is P-450 involved in the metabolism of a particular drug or chemical in a particular individual and for which metabolites is P-450 responsible? The first, general part of the question can be investigated *in vitro* using human liver (or extrahepatic) samples and some of the experimental criteria discussed earlier. There are, of course, many scientific and ethical problems associated with the use of human tissue. In answering the second, specific part of the question, the use of individual liver samples is impracticable. Instead, an approach must be made *in vivo*, for example by considering the induction and inhibition of a drug's metabolism in the individual, whether the pattern of metabolites is consistent with the involvement of P-450 and whether the drug inhibits the metabolism of known P-450 substrates. Interpretation of this sort of evidence relies heavily on a prior understanding of animal P-450 and the assumption that animal P-450 broadly reflects human P-450, an assumption that will need to be verified. Wherever possible a combined study *in vivo* and *in vitro* should be carried out in the same individual, to determine whether interindividual variations in drug metabolism *in vivo* correlate with variations in hepatic P-450 and its reactions *in vitro* [86]. There is a growing and unfulfilled need for model P-450 substrates that can be used in man *in vivo*.

There are at least twelve drugs whose metabolism in man is possibly mediated, at least in part, by P-450, on the basis that their metabolism *in vivo* is induced by phenobarbitone [87]. For three of these drugs (cortisol, cyclophosphamide and digitoxin) an increase in metabolite production was measured but for the other nine drugs (antipyrine, bishydroxycoumarin, chlorpromazine, fenoprofen, griseofulvin, lignocaine, phenylbutazone, phenytoin and warfarin) only an increase in the disappearance of parent drug was measured. That phenobarbitone is able to induce *in vivo* human hepatic microsomal P-450 and

its model reactions has been shown by measuring human liver samples *in vitro* [86]. However, the reservations expressed earlier about interpreting the metabolic effects of phenobarbitone *in vivo* are pertinent. For drugs with a high hepatic extraction ratio, for example lignocaine, their increased clearance may be due as much to a phenobarbitone-promoted increase in hepatic blood flow as to induction of P-450. Furthermore, an increased disappearance of parent drug might result from changes in pharmacokinetic parameters other than metabolism. Many drugs are known to induce drug metabolism in man, but as discussed earlier, this is no indication that the inducer is a P-450 substrate.

CONCLUSION

Circumstantial evidence strongly suggests that animal P-450 can metabolize at least one hundred and eighteen of the important drugs and other chemicals in our environment, but incontrovertible evidence for this is sparse. The evidence is yet to be gathered that confirms the probability of a significant drug-metabolizing role of P-450 in man.

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