Determination of cytochrome *P*-448 activity in biological tissues

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1. Three enzymes used for the determination of cytochrome *P*-448 activity, namely aryl hydrocarbon hydroxylase, biphenyl 2-hydroxylase and ethoxyresorufin *O*-de-ethylase, were evaluated with respect to their specificity, sensitivity and inducibility. 2. Purified cytochrome *P*-448 (LM₄), but not cytochrome *P*-450 (LM₂), catalysed the *O*-de-ethylation of ethoxyresorufin in a reaction that was markedly inhibited by 9-hydroxyellipticine. 3. After the administration of 3-methylcholanthrene to rats all three activities were induced, the extent of induction being highest for ethoxyresorufin *O*-de-ethylase. 4. Administration of very small doses of benzo[*a*]pyrene (50 µg/kg) to rats to induce cytochrome *P*-448 specifically increased only the *O*-de-ethylation of ethoxyresorufin. 5. 3-Hydroxybenzo[*a*]pyrene, the major metabolite determined by the aryl hydrocarbon hydroxylase assay, undergoes further NADPH-dependent oxygenation leading to loss of fluorescence. 6. On the basis of these observations and those by other workers, we conclude that ethoxyresorufin *O*-de-ethylase provides the most specific, sensitive and reproducible means of determining cytochrome *P*-448 activity.

Solubilization and purification of the cytochrome *P*-450-dependent mixed-function oxidase system has provided evidence for the existence of several isoenzymes, differing from each other in their electrophoretic, spectral and immunological properties and exhibiting different substrate-specificities (Guengerich *et al.*, 1982; Ryan *et al.*, 1982). Cytochrome *P*-448, as induced by polycyclic aromatic hydrocarbons, was formerly considered to be one of these cytochrome *P*-450 isoenzymes, but is now recognized as a related but different enzyme system (Parke, 1983; Ioannides *et al.*, 1984). Structural and spectral binding studies have shown that these two enzymes have distinctly different binding sites for the xenobiotic substrate (Dus, 1982; Phillipson *et al.*, 1982a). The different modes of induction of the cytochromes *P*-450 and *P*-448 indicate that they are products of different genomes, thus confirming that they are different enzymes (Parke, 1983).

It is becoming increasingly evident that, whereas oxygenation of toxic chemicals and carcinogens by the cytochromes *P*-450 generally leads to subsequent conjugation reactions and ultimate detoxication, cytochrome *P*-448 generally leads to the formation of reactive intermediates and ultimate carcinogens (Parke & Ioannides,

1982; Parke, 1983). Activation by cytochrome *P*-448, but not by cytochrome *P*-450, has been shown for paracetamol (Ioannides *et al.*, 1983; Steele *et al.*, 1983; Morgan *et al.*, 1983), ipomeanol (Statham & Boyd, 1982), benzo[*a*]pyrene (Levin *et al.*, 1977; Ioannides *et al.*, 1981), the pyrolysis products Trp-P-1 and Trp-P-2 (Ishii *et al.*, 1980, 1981), and many other carcinogens (Parke, 1983; Ioannides *et al.*, 1984). Cytochrome *P*-450 does activate certain toxic chemicals, including the non-oxidative metabolism of carbon tetrachloride (Frank *et al.*, 1982) and the oxidative metabolism of bromobenzene (Jollow *et al.*, 1974).

Thus, in general, metabolism by cytochrome *P*-450 results in detoxication, and by cytochrome *P*-448 leads to the activation of carcinogens and toxic chemicals. It is therefore evident that the biological response to a carcinogen is influenced, not only by the tissue concentration of total microsomal cytochromes, but also by the nature of the cytochromes present, and especially by the cytochrome *P*-450/cytochrome *P*-448 ratio. Species with high concentrations of tissue cytochrome *P*-448 and low concentrations of cytochrome *P*-450 would be expected to be vulnerable to the toxicity of chemicals. The specific determination of cytochrome *P*-448 activity of liver and other tissues is

therefore of considerable importance, as it would provide an indication of susceptibility to the toxicity of carcinogens and certain toxic chemicals.

The selective determination of cytochrome *P*-450 and cytochrome *P*-448 by the CO–ligand spectral difference method (Omura & Sato, 1964) is insufficiently specific, except possibly when purified homogeneous preparations are available. Consequently, the enzymic mixed-function oxidase activities, namely biphenyl 2-hydroxylation (Creaven *et al.*, 1965), benzo[*a*]pyrene hydroxylation or the so-called aryl hydrocarbon hydroxylase (Dehnen *et al.*, 1973), and ethoxyresorufin *O*-de-ethylation (Burke & Mayer, 1974), have been employed for the determination of cytochrome *P*-448 activity in the presence of other forms of the microsomal cytochromes. The methodology of biphenyl hydroxylation has been shown to be insufficiently specific (Tong *et al.*, 1977) unless the high-pressure liquid-chromatographic method is used (Burke *et al.*, 1977a), and similarly the fluorimetric method for determining benzo[*a*]pyrene has been shown to be non-specific (Negishi & Nebert, 1979) and associated with technical problems (DePierre & Ernster, 1978; Yang *et al.*, 1978).

The present study evaluates these three assay methods with respect to their specificity towards cytochrome *P*-448, their sensitivity, and their inducibility by chemical carcinogens. The technical pitfalls associated with each assay method are also discussed.

Materials and methods

3-Methylcholanthrene, benzo[*a*]pyrene, dilauroyl phosphatidylcholine and all cofactors (Sigma Chemical Co., Poole, Dorset, U.K.), biphenyl and its hydroxy derivatives (BDH Chemicals, Poole, Dorset, U.K.) and resorufin and ethoxyresorufin (Pierce Chemicals, Rockford, IL, U.S.A.) were all purchased. 9-Hydroxyellipticine was generously given by Dr. P. Lesca, Laboratoire de Pharmacologie et de Toxicologie Fondamentale du C.N.R.S., Toulouse, France.

Purified solubilized preparations of rabbit liver microsomal cytochromes *P*-450 (LM₂) and *P*-448 (LM₄) from phenobarbital- and β -naphthoflavone-treated animals, and purified rabbit cytochrome *P*-450 reductase, were prepared by Dr. M. Delaforge and Dr. M.-J. Coon, University of Michigan, Ann Arbor, MI, U.S.A.

Male Wistar albino rats (150–200 g) (Animal Breeding Unit, University of Surrey) were used in all experiments. In the study of the time-dependent induction of the mixed-function oxidases, animals received a single intraperitoneal dose of 3-methyl-

cholanthrene (25 mg/kg) and were killed at regular time intervals. In the study of dose-dependence, the animals received a single intraperitoneal administration of benzo[*a*]pyrene (50–300 μ g/kg) and were killed 24 h later.

Liver microsomal preparations were obtained as previously described (Ioannides & Parke, 1975) and the following determinations were carried out: 2- and 4-hydroxylations of biphenyl (McPherson *et al.*, 1976), *O*-de-ethylation of ethoxyresorufin (Burke & Mayer, 1974), the fluorimetric determination of benzo[*a*]pyrene (aryl hydrocarbon hydroxylase; Dehnen *et al.*, 1973), total microsomal cytochromes (Omura & Sato, 1964), and protein (Lowry *et al.*, 1951), with bovine serum albumin as standard.

Results

Ethoxyresorufin was readily de-ethylated by a reconstituted system comprising cytochrome *P*-450 reductase, dilauroyl phosphatidylcholine and cytochrome *P*-448 (LM₄), isolated and purified from rabbits pretreated with β -naphthoflavone (Fig. 1); no activity was detected in the absence of the haemoprotein. In contrast, when cytochrome *P*-450 (LM₂), isolated and purified from phenobarbitone-pretreated rabbits, was similarly used no activity was detected, even at a 10-fold higher concentration of cytochrome. Furthermore, the cytochrome *P*-448 (LM₄)-mediated de-ethylation of ethoxyresorufin was markedly inhibited by 9-hydroxyellipticine ($I_{50} = 9.2 \times 10^{-8}$ M) (Fig. 2).

After the single administration of 3-methylcholanthrene to male Wistar albino rats the patterns of induction of biphenyl 2-hydroxylase, aryl hydrocarbon hydroxylase and ethoxyresorufin *O*-de-ethylase activities were very similar (Fig. 3). However, the extent of induction of ethoxyresorufin *O*-de-ethylase, 48 h after the carcinogen administration, was markedly greater (10-fold) than that of biphenyl 2-hydroxylase (5-fold) and that of aryl hydrocarbon hydroxylase (2-fold); biphenyl 4-hydroxylase activity was unaffected, and the concentrations of total microsomal cytochromes were also increased, but to a much lesser extent (40%) (Fig. 3).

Administration of small intraperitoneal doses (50–300 μ g/kg) of benzo[*a*]pyrene to rats to determine the relative sensitivities of the different assays to the induction of cytochrome *P*-448 resulted in marked induction of ethoxyresorufin *O*-de-ethylase activity, even at the lowest dose, marked induction of biphenyl 2-hydroxylase activity and slight induction of biphenyl 4-hydroxylase activity, but, at these doses, aryl hydrocarbon hydroxylase activity and the total concentration of microsomal cytochromes were unaffected (Fig. 4).

Fig. 1. Ethoxyresorufin *O*-de-ethylation by solubilized purified preparations of cytochrome P-450 (LM₂) and cytochrome P-448 (LM₄)

A reconstituted system comprised haemoprotein (0–1 nmol), cytochrome P-450 reductase (0–1 nmol) and dilauroyl phosphatidylcholine (0–30 μ g) in the proportions 1 nmol of cytochrome to 1 nmol of reductase to 30 μ g of phospholipid. The incubation mixture also contained 250 nM-ethoxyresorufin in 0.1 M-Tris/HCl buffer, pH 7.8, in a total volume of 2.0 ml. The reaction was initiated by addition of NADPH (0.5 μ mol). $\square$, LM₂ preparation; $\bullet$, LM₄ preparation.

Incubation of 3-hydroxybenzo[*a*]pyrene, the major product of the aryl hydrocarbon hydroxylase assay with male rat liver microsomal fraction and an NADPH-generating system resulted in a time-dependent decrease of fluorescence; loss of fluorescence was greater when liver microsomal fraction from 3-methylcholanthrene-pretreated male rats was used (Fig. 5a). In contrast, when resorufin was incubated under the same conditions no loss of fluorescence was observed in the presence of either control or 3-methylcholanthrene-treated microsomal fractions (results not shown). When benzo[*a*]pyrene was used as substrate, the formation of fluorescent oxygenated products increased with time for up to 30 min (Fig. 5b), but was not linear with protein concentration with liver microsomal preparations from either untreated or 3-methylcholanthrene-treated male rats (Fig. 5c). Finally,

Fig. 2. Inhibition of the cytochrome P-448 (LM₄)-mediated *O*-de-ethylation of ethoxyresorufin by 9-hydroxyellipticine

A reconstituted system as indicated in Fig. 1 legend with 0.1 nmol of LM₄ preparation was used. 9-Hydroxyellipticine was dissolved in dimethyl sulphoxide; the total volume of dimethyl sulphoxide in the incubation system never exceeded 1%. Uninhibited activity was 38.8 pmol/min per nmol of cytochrome P-448.

the rate of metabolic oxygenation of benzo[*a*]pyrene into fluorescent products was dependent on substrate concentration with liver microsomal preparations from both control and 3-methylcholanthrene-induced rats, saturation being achieved at substrate concentrations of about 50 and 120 μ M respectively (Fig. 5d).

Discussion

After the administration of a single dose of 3-methylcholanthrene the induction patterns of aryl hydrocarbon hydroxylase, ethoxyresorufin *O*-de-ethylase and biphenyl 2-hydroxylase activities were similar, being decreased at 8 h after administration, probably owing to competitive inhibition of the reaction substrate by 3-methylcholanthrene or a metabolite, and enhanced from 16 to 48 h after administration. Excellent correlations were observed between ethoxyresorufin *O*-de-ethylase and aryl hydrocarbon hydroxylase ($r = 0.99$) and bi-

Fig. 3. Effect of a single dose of 3-methylcholanthrene on rat hepatic microsomal cytochrome P-448-mediated mixed-function oxidases

Animals received a single intraperitoneal dose of 3-methylcholanthrene (25 mg/kg). Each point represents the mean $\pm$ S.D. for at least four animals for ethoxyresorufin *O*-de-ethylase (●), biphenyl 2-hydroxylase (□), aryl hydrocarbon hydroxylase (○) and biphenyl 4-hydroxylase (■) activities and cytochrome P-450 content (▲). Activities of liver microsomal fraction from untreated (control) rats were: ethoxyresorufin *O*-de-ethylase, 28 pmol/min per mg of protein; biphenyl 2-hydroxylase, 21 pmol/min per mg of protein; aryl hydrocarbon hydroxylase, 51 pmol/min per mg of protein; biphenyl 4-hydroxylase, 0.38 nmol/min per mg of protein; cytochrome P-450, 0.88 nmol/mg of protein.

phenyl 2-hydroxylase ($r = 0.96$). However, polycyclic aromatic hydrocarbons and other inducers stimulate the synthesis of more than one isoenzyme of the microsomal cytochromes (Ryan *et al.*, 1979; Lau & Strobel, 1982), so that more than one form may contribute to the observed increased activities. Indeed, indirect evidence indicates that the 2-hydroxylation of biphenyl and the *O*-de-ethylation of ethoxyresorufin are not catalysed by the same isoenzyme; many corticosteroids, when added to a microsomal incubation mixture, increased the 2-hydroxylation of biphenyl (Benford *et al.*, 1980), but had no effect on the *O*-de-ethylation of ethoxyresorufin (D. Benford, personal communication). Moreover, in studies where five fractions containing microsomal cytochromes were purified from non-induced mouse liver, no correlation was

Fig. 4. Hepatic microsomal mixed-function oxidases after the administration of small doses of benzo[a]pyrene. Animals received a single dose of the carcinogen and were killed 24 h later. Each point represents the mean $\pm$ S.D. for four animals. Activities were expressed per nmol of cytochrome P-450, and cytochrome P-450 per mg of microsomal protein, for calculation of the percentage activities shown: ○, ethoxyresorufin *O*-de-ethylase; Δ, biphenyl 2-hydroxylase; ▽, biphenyl 4-hydroxylase; ●, aryl hydrocarbon hydroxylase; □, cytochrome P-450. Control activities were as shown in Fig. 3.

seen between biphenyl 2-hydroxylase, aryl hydrocarbon hydroxylase and ethoxyresorufin *O*-de-ethylase enzyme activities (Levi & Hodgson, 1983).

Specificity for cytochrome P-448

It is essential that any substrate used as a probe for the determination of cytochrome P-448 activity should exhibit high specificity. The 2-hydroxylation of biphenyl fulfils this prerequisite; it is markedly enhanced by administration of inducing agents such as polycyclic aromatic hydrocarbons (Burke & Bridges, 1975; Burke & Mayer, 1975), safrole (Delaforge *et al.*, 1982) and 9-hydroxyellipticine (Phillipson *et al.*, 1982b), which also cause a spectral shift of the CO-reduced cytochrome absorption maximum to 448 nm, but is unaffected by inducers, such as barbiturates or ethanol, that induce cytochrome(s) P-450 (Ioannides & Parke, 1973, 1975). Furthermore, partially purified preparations of rat liver cytochrome P-448, but not cytochrome P-450, catalysed the 2-hydroxylation of biphenyl (Burke & Mayer, 1975).

Similarly, the *O*-de-ethylation of ethoxyresorufin is catalysed exclusively by cytochrome P-448 (Burke & Mayer, 1975). It is induced by many

Fig. 5. Characteristics of aryl hydrocarbon hydroxylase

(a) 3-Hydroxybenzo[a]pyrene (45 pmol) was incubated with male rat liver microsomal fraction (0.65 mg of protein), NADP⁺ (0.5 μmol), glucose 6-phosphate (5 μmol) and glucose-6-phosphate dehydrogenase (1 unit) in a total volume of 1.0 ml. (b) Benzo[a]pyrene (160 μM) was incubated with control (1.2 mg of protein) and 3-methylcholanthrene-treated-rat microsomal fractions (0.96 mg of protein) and the same NADPH-generating system. (c) Microsomal fractions from control and 3-methylcholanthrene-treated animals were incubated with benzo[a]pyrene (160 μM) and the NADPH-generating system for 10 min. (d) Microsomal fractions (0.65 mg of protein) were incubated with various concentrations of benzo[a]pyrene and the NADPH-generating system for 10 min. ▲, Microsomal fraction from control rats; ●, microsomal fraction from 3-methylcholanthrene-treated rats.

carcinogens known to induce cytochrome *P*-448, whereas it is not enhanced, or is even inhibited, by administration of inducers of cytochrome *P*-450 (Ioannides *et al.*, 1984). Solubilized partially purified preparations from rat livers of cytochrome *P*-448, but not cytochrome *P*-450, effect the de-ethylation of ethoxyresorufin (Burke & Mayer, 1975), and this has now been confirmed with the highly purified rabbit liver microsomal preparations LM₄ (cytochrome *P*-448 induced by β-naphthoflavone) and LM₂ (cytochrome *P*-450 induced by phenobarbitone) (Fig. 1). The inhibition of the cytochrome *P*-448 (LM₄)-mediated *O*-de-ethylation of ethoxyresorufin by 9-hydroxy-

ellipticine ($I_{50} = 9.2 \times 10^{-8}$ M) (Fig. 2) confirms previous studies *in vivo* (Delaforge *et al.*, 1980).

The most extensively used assay method for cytochrome *P*-448 activity has been the fluorimetric determination of benzo[a]pyrene hydroxylation (the aryl hydrocarbon hydroxylase assay) (Nebert & Gelboin, 1968). It has been used in possible correlations between aryl hydrocarbon hydroxylase inducibility and polycyclic-aromatic-hydrocarbon-induced tumour formation in human subjects (Kellerman *et al.*, 1973; Paigen *et al.*, 1977; Arnott *et al.*, 1979). However, the validity of this assay method has been questioned, particularly because of the non-linearity of response and

its lack of specificity. The assay of aryl hydrocarbon hydroxylase activity, determined fluorimetrically with benzo[a]pyrene as substrate, favours the determination of the 3- and 9-phenols (Holder *et al.*, 1975), and, since the hydroxylation of benzo[a]pyrene in the various alternative positions is catalysed by different isoenzymes of cytochromes *P*-450 and *P*-448 (Wiebel *et al.*, 1975), this assay must reflect the activity of many forms of the haemoprotein and is not specific for cytochrome *P*-448 (Holder *et al.*, 1974; Rasmussen & Wang, 1974; Nebert & Jensen, 1979). It is therefore not surprising that aryl hydrocarbon hydroxylase activity is enhanced after the administration of inducers not associated with cytochrome *P*-448, such as phenobarbitone and 16 α -cyanopregnenolone (Kremers *et al.*, 1978, 1981), ethanol (Seitz *et al.*, 1978) and flavones (Wattenberg, 1971). Furthermore, correlations between aryl hydrocarbon hydroxylase activity and the hydroxylation of typical cytochrome *P*-450 substrates, such as antipyrine and hexobarbital, have been observed with preparations from human autopsy livers (Kapitulnik *et al.*, 1977). Definitive evidence of the lack of specificity of the benzo[a]pyrene assay for cytochrome *P*-448 has been provided by studies with solubilized purified isoenzymes of the cytochromes of rabbit liver (Wiebel *et al.*, 1975; Guengerich, 1977) and guinea-pig liver (Abe & Watanabe, 1983). Finally, studies with specific antisera to purified cytochromes established unequivocally that 'cytochrome *P*₁-450' and not cytochrome *P*-448 is the most active enzyme catalysing the polycyclic-aromatic-hydrocarbon-induced aryl hydrocarbon hydroxylase activity (Chen *et al.*, 1982; Negishi & Nebert, 1979).

Reproducibility and sensitivity

The microsomal hydroxylation of ethoxyresorufin yields only one product, namely the highly fluorescent resorufin, which does not undergo further metabolism *in vitro*. In contrast, 2-hydroxybiphenyl is further metabolized by an NADPH-dependent reaction, presumably to dihydroxy derivatives, resulting in loss of fluorescence (Burke & Bridges, 1975). Aryl hydrocarbon hydroxylase activity, determined fluorimetrically with benzo[a]pyrene as substrate and 3-hydroxybenzo[a]pyrene as standard, measures primarily those metabolites that fluoresce similarly to 3-hydroxybenzo[a]pyrene, namely, the 3- and 9-phenols (Holder *et al.*, 1975). Furthermore, as demonstrated in the present study, 3-hydroxybenzo[a]pyrene is further metabolized by liver microsomal preparations with loss of fluorescence (Fig. 5a), so that the amount of 3-hydroxybenzo[a]pyrene determined is the net effect of aryl hydrocarbon hydroxylase and of

subsequent enzymic oxygenation. Some of these problems may be partly overcome by the use of radiolabelled substrate, but this leads to loss of sensitivity (DePierre *et al.*, 1975) and lower rates of metabolism (Nebert *et al.*, 1975).

Ethoxyresorufin *O*-de-ethylase activity was linear up to a concentration of 1.0 mg/ml of protein when control rat microsomal fraction was used (Burke & Mayer, 1974), but when the initial phase is considered the specific activity was constant at protein concentrations up to 2.0 mg/ml of protein (Burke *et al.*, 1977b). The 2-hydroxylation of biphenyl with liver microsomal fractions from control and treated hamsters was linear up to 0.5 mg/ml of protein (Burke & Bridges, 1975). However, with the fluorimetric aryl hydrocarbon hydroxylase assay, loss of linearity is observed at protein concentrations of less than 1.5 mg/ml for control microsomal fraction and less than 1 mg/ml with microsomal fraction from 3-methylcholanthrene-treated rats at a benzo[a]pyrene concentration of 160 μ M (Fig. 5c). In a comprehensive study of this enzyme assay method, Hansen & Fouts (1972) demonstrated that reaction rates were highly dependent on protein concentration, and, although enzyme activity by a given concentration of protein followed Michaelis-Menten kinetics, a decrease in K_m was observed with decreased enzyme concentration. The authors suggest that it is imperative that the substrate concentration used is sufficiently high to prevent depletion of free substrate, especially when microsomal fractions from induced animals are used.

The reproducibility of the aryl hydrocarbon hydroxylase assay in different laboratories is poor, e.g. male rat hepatic activities range from 60 (Stohs & Wu, 1981) to 1000 pmol/min per mg of protein (Thorgeirsson *et al.*, 1979). Activity is very much influenced by assay conditions, such as the solvent used for dissolving benzo[a]pyrene and the temperature of the incubation system during its addition (Yang *et al.*, 1978), presumably because they influence free substrate concentration. Modifications of the assay conditions to overcome some of these pitfalls have further lowered reproducibility, and over 20-fold differences occurred when aryl hydrocarbon hydroxylase activity was determined in mouse skin by three different methods (Finnen *et al.*, 1983).

In the assay of the biphenyl hydroxylases Tween 80 is employed routinely as substrate vehicle, and this may also influence reaction rates (Burke *et al.*, 1977c). Furthermore, in the fluorimetric assay 4-hydroxybiphenyl, the major metabolite, also makes a contribution at the wavelengths characteristic of the 2-hydroxy derivative, so that a correction factor must be employed, resulting in loss of sensitivity (Creaven *et al.*, 1965); formation

of 3-hydroxybiphenyl, a minor metabolite, may also interfere with the assay. These problems can be largely overcome by the use of g.l.c. and high-pressure liquid-chromatographic techniques (Burke *et al.*, 1977a), but these are cumbersome and unsuitable for the rapid screening of a large number of samples.

Small changes in cytochrome P-448 concentrations in tissues can only be determined if they result in detectable changes in enzymic activity. In the experiment in which rats were pretreated with low doses of benzo[a]pyrene to induce cytochrome P-448 specifically, the lowest dose (50 µg/kg) resulted in a 100% increase in ethoxyresorufin O-de-ethylase activity but no other activity was enhanced; at 100 µg/kg biphenyl 2-hydroxylase activity was also increased, but aryl hydrocarbon hydroxylase activity only showed a small increase (20%) at the highest dose (300 µg/kg). Total cytochrome P-450 concentrations determined spectrophotometrically were unaffected at all doses (Fig. 4). At a relatively high dose of 3-methylcholanthrene (25 mg/kg), all three activities were increased, but the extent of induction of ethoxyresorufin O-de-ethylase was considerably more pronounced (10-fold) than that of biphenyl 2-hydroxylase (5-fold) or of aryl hydrocarbon hydroxylase (2-fold) (Fig. 3).

The data presented in this paper show that the O-de-ethylation of ethoxyresorufin is the most specific, sensitive, rapid and reproducible means of determining cytochrome P-448 activity. Biphenyl 2-hydroxylase, although specific, is less reproducible because of technical pitfalls, and basal activity disappears rapidly in the adult animal (Basu *et al.*, 1971; Atlas *et al.*, 1977). Aryl hydrocarbon hydroxylase, although surprisingly the most widely used assay, even though attention has been drawn repeatedly to its limitations (Hansen & Fouts, 1972; DePierre & Ernster, 1978), lacks specificity, has poor reproducibility, is insensitive to inducing agents and is associated with major technical difficulties. Conclusions based on data obtained solely from the use of the aryl hydrocarbon hydroxylase assay, even if accompanied by inhibitor studies, must at best be regarded with scepticism.

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