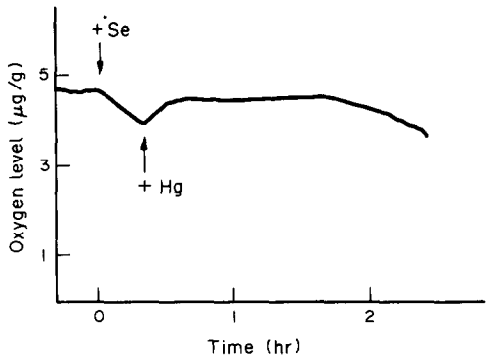

Fig. 3. The effect of $HgCl_2$ on selenite induced decrease in oxygen level. A suspension of hepatocytes was pre-incubated for about 1 hr, and selenite (75 μM) was added at zero time. $HgCl_2$ (15 μM) was added 20 min thereafter. The oxygen level was recorded continuously throughout the experiment.

centration [9] can shorten the lag period in selenite metabolism. We have now shown that the use of air instead of carbogen has the same effect. These data, when combined with the rest of the results presented here, indicate that an essential quality of the hepatocyte model was its marked tendency to become hypoxic. In non-respiring systems, like that used in ref. 3, the tendency to become hypoxic should be less pronounced. This circumstance may explain the inability of a cell free system to produce dimethylselenide under oxygenated conditions.

In summary, in order to elucidate the role of hypoxia in selenium metabolism we have developed a technique to continuously monitor the oxygen tension in a suspension of isolated hepatocytes. It could be shown that in cells, incubated under conditions which permitted selenite metabolism, selenite induced hypoxia. It was also found that dimethylselenide was not formed until hypoxia had been induced. The lag period, which preceded dimethylselenide formation, was shortened by equilibrating the hepatocyte suspension with air instead of carbogen. These data support the hypothesis that selenium metabolites may enter redox cycles and suggest that oxygen acted as modulator of dimethylselenide formation.

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Metabolism of alkoxyphenoxazones by channel catfish liver microsomes: Effects of phenobarbital, Aroclor 1254 and 3-methylcholanthrene

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Cytochrome P-450-dependent monooxygenases (MOs) are biologically ubiquitous enzymes integral in the metabolism of a multitude of organic xenobiotics. In mammals and fishes, there are multiple forms of hepatic microsomal cytochrome P-450, each with slightly different, but usually overlapping, specificities for many substrates [1, 2]. Because of this overlap, substrates that are somewhat specific for a certain cytochrome P-450 are particularly valuable for the elucidation of mechanisms governing changes in the activity of this enzyme system. Unfortunately, relatively few of these types of substrates have been developed.

In mammals, it is well established that certain hepatic microsomal cytochromes P-450 are induced, and associated MO activities increased, by treatment with a variety of compounds [3]. Phenobarbital, a drug, and 3-methylcholanthrene, a polycyclic aromatic hydrocarbon, are representative of two major classes of inducers of cytochrome(s) P-450. It has been known for some time that the rate of O-dealkylation of 7-ethoxyresorufin (7-ethoxyphenoxazone) is increased preferentially up to 50-fold by 3-methylcholanthrene-type compounds, indicating that at least one of the cytochromes P-450 induced by this class of compounds is highly specific for ethoxyresorufin [4]. Recently, a highly specific substrate for cytochrome(s) P-450 induced by phenobarbital-type compounds also has been reported. The rate of O-dealkylation of 7-pentoxyresorufin (7-pentoxyphenoxazone) by rat or mouse hepatic MOs is increased up to 250-fold by phenobarbital-type inducers [5–7]. Hence, pentoxyresorufin should be a powerful tool in the determination of induction by phenobarbital-type compounds.

Hepatic cytochrome P-450-dependent MOs in fishes are functionally quite similar to those in mammals [8, 9]; however, there appear to be differences in their responses to inducers. Although it is well established that hepatic MO activities in fishes are increased greatly by 3-methylcholanthrene-type inducers, there is some question as to the effects of phenobarbital-type compounds. Some reports indicate that hepatic MO activities in fishes are increased by phenobarbital-type inducers [10–12]; however, most studies have shown that these compounds do not affect

MOs of fishes [13–15]. One possible explanation for the discrepancies among these studies is the lack of MO activity assays sensitive or specific enough to detect small changes in levels of phenobarbital-induced cytochrome(s) P-450. Therefore, we decided to examine the effects of phenobarbital on hepatic MOs in a fish species, using pentoxyresorufin as a substrate.

In this study, channel catfish (*Ictalurus punctatus*) were treated with phenobarbital, 3-methylcholanthrene, or the polychlorinated biphenyl mixture Aroclor 1254. In mammals, Aroclor 1254 has properties of both phenobarbital- and 3-methylcholanthrene-type inducers and, therefore, produces a "mixed" response in the MO system [16]. Hepatic microsomal MO activities in the fish were determined using 7-ethoxy-, 7-pentoxy, 7-benzyloxy-, and 7-methoxyresorufin as substrates. The latter two alkoxyphenoxazones were included because previous work indicated they also may be of value in determining the activity of specific cytochromes P-450 [6].

Materials and methods

7-Methoxyresorufin, 7-benzyloxyresorufin, and 7-pentoxyresorufin were synthesized using methods described previously [5]. 7-Ethoxyresorufin was obtained from the Pierce Chemical Co., Rockford, IL, and resorufin was obtained from the Sigma Chemical Co., St. Louis, MO.

Adult channel catfish (90–160 g) were injected intraperitoneally with 2 ml/kg body wt of corn oil or inducers dissolved or suspended in corn oil. Fish were treated on three alternate days with 20 mg of 3-methylcholanthrene/kg, 50 mg Aroclor 1254/kg, or 80 mg phenobarbital/kg, and were killed 1 day after the final injection. After decapitation, livers were removed, washed and minced in 0.15 M KCl solution, and homogenized in 6 vol. of a cytochrome P-450-stabilizing buffer (0.1 M KH_2PO_4 at pH 7.25, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) with a motor-driven glass–Teflon homogenizer. The homogenate was centrifuged for 20 min at 12,000 *g*, and the resultant supernatant fraction was decanted and centrifuged for 90 min at 160,000 *g*. All operations were conducted at 2–4°. Microsomes were stored overnight at −80°.

The O-dealkylation of methoxy-, ethoxy-, benzyloxy-, and pentoxyresorufin was assayed using fluorimetric procedures [4, 6]. Incubation mixtures contained 200–600 μg microsomal protein, 320 nmoles NADPH, 10 nmoles substrate (added in 10 μl dimethyl sulfoxide), and 0.1 M KH_2PO_4 (pH 7.6) in a total volume of 2 ml. Reaction rates were measured directly by following the increase in fluorescence of the incubation mixtures due to the production of resorufin, and activity was quantified by comparison with resorufin standards. Incubations were performed at 30°, under conditions which resulted in linearity over the 3–6 min that the reactions were followed. Microsomal protein was determined by the method of Lowry *et al.* [17], using bovine serum albumin as a standard.

Results

Control (corn oil-treated) catfish exhibited the highest levels of hepatic microsomal MO activity towards methoxyresorufin, followed by ethoxyresorufin, and benzyloxyresorufin (Table 1). There was no detectable O-dealkylation of pentoxyresorufin in the controls. MO activities in liver microsomes from the phenobarbital-treated fish were both quantitatively and qualitatively similar to those of the controls (Table 1). Treatment of the catfish with Aroclor 1254 increased the rate of O-dealkylation of methoxyresorufin 1.6-fold, ethoxyresorufin 9.4-fold, and benzyloxyresorufin 4.7-fold, relative to control values (Table 1). Activity toward pentoxyresorufin in the Aroclor-treated fish was low, but detectable. Treatment of the catfish with 3-methylcholanthrene produced results qualitatively, but not quantitatively, similar to those seen in fish treated with Aroclor 1254 (Table 1). The relative increases in MO activities in the 3-methylcholanthrene-treated fish were much higher than in the Aroclor-treated animals. Increases in the rate of O-dealkylation of methoxyresorufin, ethoxyresorufin, and benzyloxyresorufin were 2.7-, 46.7- and 33.3-fold respectively. Also, hepatic microsomal MO activity toward pentoxyresorufin in the 3-methylcholanthrene-treated fish was easily detectable and was about 17-fold higher than in animals treated with Aroclor 1254.

Discussion

Results obtained in this study support the contention that hepatic cytochrome P-450-dependent MO activities in fishes are not increased by phenobarbital-type inducers. Treatment of catfish with phenobarbital did not affect the rate of O-dealkylation of any of the alkoxyphenoxazones studied, including pentoxyresorufin, a substrate highly specific for phenobarbital-induced cytochrome(s) P-450 in mammals [5–7]. Moreover, treatment of the catfish with the "mixed" inducer, Aroclor 1254, produced results qualitatively similar to those seen in fish injected with 3-methycholanthrene. This result indicates that the 3-methylcholanthrene-type, but not the phenobarbital-type, inducing properties of Aroclor 1254 were expressed in the fish. There have been some reports that phenobarbital-type inducers increase MO activities in fishes [10–12]; however, our results agree with most studies and indicate that MOs of fishes are not affected by these types of inducers [13–15].

Levels of hepatic microsomal ethoxyresorufin O-dealkylase activity found in this study are comparable to those reported for other fish species [18, 19]. At present, there are no published reports concerning fish MO activities

Table 1. Rates of O-dealkylation of four alkoxyphenoxazone compounds by channel catfish liver microsomes: Effects of treatment with phenobarbital, Aroclor 1254 and 3-methylcholanthrene

Treatment	O-Dealkylation by monooxygenases (pmoles resorufin formed/min/mg microsomal protein)			
	7-Methoxyresorufin	7-Ethoxyresorufin	7-Benzyloxyresorufin	7-Pentoxyresorufin
Corn oil	25. 7 ± 6.5	6.8 ± 0.6	2.4 ± 0.3	ND*
Phenobarbital	27.3 ± 5.3 (1.1)	7.4 ± 0.8 (1.1)	2.5 ± 0.1 (1.0)	ND
Aroclor 1254	42.2 ± 5.7 (1.6)	64.1 ± 13.5 (9.4)	11.2 ± 1.4 (4.7)	1.0 ± 0.1
3-Methylcholanthrene	70.6 ± 13.7 (2.7)	317.3 ± 45.6 (46.7)	80.0 ± 8.3 (33.3)	17.5 ± 1.6

Values are given as mean ± SD for three groups of two pooled livers. Numbers in parentheses are #-fold increases in activity, relative to control (corn oil-treated) fish.

* Not detectable. Limit of detection: 0.5 pmole/min/mg.

toward the other alkoxyphenoxazone substrates. Levels of hepatic MO activity in the catfish were lower than those reported for rat liver microsomes assayed under similar conditions [6]. This observation is consistent with previous comparative work in mammals and fishes [8, 9].

In addition to the observed quantitative differences in MO activity between catfish and rat, there were also qualitative differences. In control rats, the highest rate of O-dealkylation activity is toward ethoxyresorufin, followed by benzyloxyresorufin, methoxyresorufin, and pentoxyresorufin [6]. Conversely, O-dealkylation activity in control catfish was highest toward methoxyresorufin, followed by ethoxyresorufin, benzyloxyresorufin, and pentoxyresorufin (not detectable). These results indicate there are differences in the substrate specificities of noninduced cytochromes P-450 in catfish and rat liver.

The most intriguing aspect of our results concerns the effects of 3-methylcholanthrene on the catfish MOs. In mammals, treatment with 3-methylcholanthrene produces marked (50-fold) increases in the rate of O-dealkylation of ethoxyresorufin [4], which is similar to our observations and results obtained with other fish species [20]. Also, in mammals, large increases in MO activity toward pentoxyresorufin, and to a somewhat lesser extent benzyloxyresorufin, are caused by phenobarbital-type but not by 3-methylcholanthrene-type inducers [5–7]. However, treatment of catfish with 3-methylcholanthrene increased MO activity toward benzyloxyresorufin about 33-fold, and also increased the O-dealkylation of pentoxyresorufin from nondetectable levels in controls to a specific activity of 17 pmoles/min/mg in treated fish. The explanation for this observation is not readily apparent. Various cytochromes P-450 have been purified from both untreated fish (scup) and those treated with 3-methylcholanthrene-type inducers (rainbow trout) [2, 21]; however, there were not enough substrates used in common between those studies and ours to speculate on the exact nature of the cytochromes induced in the 3-methylcholanthrene-treated catfish. We hope to purify these induced cytochromes from the channel catfish in order to characterize the catalytic properties of the isoenzymes toward the alkoxyphenoxazone substrates. Overall, the results obtained in the present study do clearly demonstrate that, although alkoxyphenoxazones are useful for determining MO induction by 3-methylcholanthrene-type compounds in fishes, these substrates cannot be used to determine induction by phenobarbital-type compounds.

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Effect of ethanol feeding on hepatic microsomal UDP-glucuronyltransferase activity

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It is well established that chronic ethanol consumption increases hepatic cytochrome P-450 levels [1, 2] and a number of hepatic monooxygenase activities [3, 4]. However, the effect of ethanol feeding on microsomal UDP-glucuronyltransferase (GT) activity has not been fully determined. Since the induction of monooxygenase activities by 3-methylcholanthrene (3-MC) has been shown to result in a coordinated increase in GT activity [5], it would seem possible that a similar phenomenon could occur after ethanol administration. Ethanol feeding has been shown to induce GT activity toward *p*-nitrophenol in rabbits [6] and *p*-nitrophenol and 7-hydroxycoumarin in female Sprague–