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.

School of Forest Resources GERALD T. ANKLEY*
University of Georgia ROBERT E. REINERT
Athens, GA 30602, *U.S.A.*

Horticultural Research Laboratory RICHARD T. MAYER
U.S.D.A.
Orlando, FL 32803, *U.S.A.*

* Author to whom all correspondence should be sent.

Department of Pharmacology M. DANNY BURKE
University of Aberdeen
Aberdeen, AB9 1AS, U.K.

Department of Zoology MOISES AGOSIN
University of Georgia
Athens, GA 30602, *U.S.A.*

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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–

Dawley rats [7], but ethanol does not increase GT activity toward *p*-nitrophenol in male Wistar rats [8]. Bilirubin GT activity is increased by chronic ethanol feeding in Wistar rats [9] but not in Sprague–Dawley rats or humans [10]. Despite occasional reports indicating an increase in GT activities following chronic ethanol administration, it has been suggested that ethanol probably does not induce these enzymes to any great extent [11].

The following study was performed to determine the effect of ethanol feeding on GT activity using the aglycones, 3-hydroxybenzo[*a*]pyrene (3-HBP), 4-methylumbelliferone (4-MU), 4-hydroxybiphenyl (4-HB), acetaminophen (APAP), phenolphthalein (PP) and 4-nitrocatechol (4-NTC). In addition, an HPLC method for determining the amount of 4-HB glucuronidated during the GT assay is presented.

Materials and methods

Female Sprague–Dawley rats (Sasco, Omaha, NE) weighing 140–150 g were placed on nutritionally adequate liquid diets (DeCarli and Lieber [12]) containing ethanol and fat as 36 and 35% of total calories respectively. Pair-fed controls received a liquid diet in which ethanol calories were replaced with dextrin-maltose [12]. The diets were given at 4:00 p.m. daily for 14–16 days prior to experiments, during which time the rats gained 8–12 g in body weight. A 2-week feeding regimen was utilized because related experiments had demonstrated maximal induction of monooxygenase activity during this period. Female rats were chosen to avoid any possible effects that ethanol could have on GT activity as a result of diminished blood testosterone levels.

Hepatic microsomes were isolated by differential centrifugation, washed once, and resuspended in 0.15 M KCl. Incubations were performed in 15 × 100 mm glass test tubes at 37° in a Dubnoff metabolic shaker. The final incubation volume was 1 ml and contained: 0.1 M Tris (pH 7.4), 5 mM $MgCl_2$, microsomes (0.5 mg protein), 0.05% Brij-58, UDP-glucuronic acid (3.0 mM, UDPGA) and the appropriate aglycone. Substrate concentrations are listed in Table 1 (Results). Published methods were used for the determination of GT activity toward 4-MU [13], 3-HBP [14], PP [15], and APAP [16].

The 4-NTC GT reaction was stopped after 10 min by adding 0.5 ml of 0.5 N perchloric acid, after which 0.1 ml of the acidified mixture was added to 0.9 ml of 1.6 M glycine buffer (pH 10.3), and the absorbance of the unmetabolized 4-NTC was measured at 546 nm [4]. For the 4-HB GT assay, the reaction was terminated after 10 min by the addition of 0.5 ml of 0.5 N perchloric acid. Following centrifugation, the supernatant fraction was extracted three times with 2.5 ml of chloroform, after which the aqueous fraction was neutralized by the addition of 0.1 ml of 2.5 N $KHCO_3$ and then recentrifuged. β-Glucuronidase (Sigma Type VIII, 20 units) was added to the supernatant fluid, and the mixture was allowed to incubate for 1.5 hr at room temperature. A 20-μl portion was then analyzed using reversed phase HPLC. The Waters (Milford, MA) HPLC system consisted of a model 510 pump, a U6K injector, a C-18 μBondapak column and a model 481 variable wavelength detector. The mobile phase contained 15% methanol–25% acetonitrile–1% acetic acid (pH 2.6), while the flow rate was 1.3 ml/min. Metabolites were detected at 254 nm, and peak areas were quantitated using a Hewlett–Packard model 3390A integrator.

Microsomal protein was determined by the biuret method [17] using bovine serum albumin as standard. Statistical comparisons were made using Student's *t*-test at $P < 0.05$. All solvents were HPLC grade, and biochemicals used were in the highest grade available.

Results and discussion

The aglycone 4-HB has been widely used as a substrate for studying GT activity. Previous studies have indirectly quantitated the production of the 4-HB glucuronide conjugate by comparing the increase in the fluorescence of the glucuronide conjugate with the disappearance of the unmetabolized phenol [18]. In the present study, an HPLC method was developed for quantitation of the 4-HB glucuronide formed during the GT assay.

Figure 1A shows the chromatogram of an aliquot of the 4-HB reaction mixture after chloroform extraction. The peak eluting at 5.32 min disappeared after incubation with β-glucuronidase (Fig. 1B), indicating that this compound was a glucuronide conjugate. A peak eluting at 14.86 min, the retention time of authentic 4-HB, appeared concomitantly with the disappearance of the glucuronide peak. The chromatographic separation of equal volumes of unhydrolyzed and hydrolyzed reaction mixtures demonstrated that the peak areas of the 4-HB glucuronide and 4-HB were dissimilar. Because 4-HB glucuronide is not commercially available, the 4-HB glucuronide formed during the GT assay was hydrolyzed with β-glucuronidase, and the resultant 4-HB was quantitated with authentic 4-HB standards.

Ethanol feeding significantly increased GT activity toward the aglycones PP, APAP, 3-HBP, 4-NTC and 4-MU, whereas the increase in GT activity toward 4-HB was not significant (Table 1). Furthermore, the degree of induction produced by ethanol differed for all substrates. GT activities toward PP (98%) and APAP (80%) were increased the most, whereas activities toward 3-HBP (60%) and 4-NTC (52%) were increased moderately by ethanol consumption.

The induction of GT activity by ethanol cannot be categorized within any of the traditional classifications of GT acceptor substrates. For example, the induction caused by

Table 1. Effect of chronic ethanol consumption on rat liver microsomal UDP-glucuronyltransferase activity

Substrate	GT activity (nmoles/min/mg protein)			
	Concn (mM)	Control	Ethanol	% Change
Phenolphthalein (PP)	1.5	12.0 ± 0.9	23.8 ± 1.6*	98
Acetaminophen (APAP)	1.5	1.3 ± 0.1	2.3 ± 0.1*	80
3-Hydroxybenzo[*a*]pyrene (3-HBP)	0.05	1.3 ± 0.2	2.0 ± 0.1*	60
4-Nitrocatechol (4-NTC)	0.5	7.9 ± 0.2	12.0 ± 1.0*	52
4-Methylumbelliferone (4-MU)	0.5	36.5 ± 2.0	47.7 ± 2.6*	32
4-Hydroxybiphenyl (4-HB)	0.5	34.1 ± 2.8	41.6 ± 1.8	21

* Values are means ± SE of three to five animals per treatment group. Additional incubation conditions are described in Materials and methods.

* Significantly increased over control, $P < 0.05$.

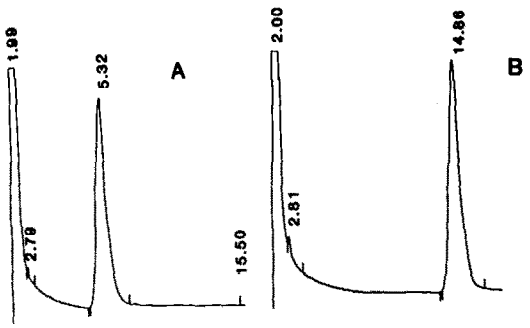

Fig. 1. HPLC separation of 4-hydroxybiphenyl and 4-hydroxybiphenyl-β-D-glucuronide. After extraction of the 4-HB GT reaction mixture with $CHCl_3$, aliquots (20 μl) of the aqueous phase were separated by HPLC as described in Materials and methods. The chromatogram shown in panel A received no additional treatment, whereas the chromatogram in panel B was obtained after incubation of the aqueous phase with β-glucuronidase.

ethanol does not appear similar to that produced by the group 1 inducer 3-MC or the group 2 inducer phenobarbital, since GT activities toward 4-MU (group 1) and 4-HB (group 2) were enhanced only slightly by ethanol feeding. Furthermore, chronic ethanol feeding was shown to have no effect on GT activity toward bilirubin (group 3) in Sprague–Dawley rats [10].

GT activities toward PP and APAP were increased markedly by ethanol administration, supporting the suggestion that these two substrates are glucuronidated by the same form of GT [19]. However, there is still much controversy as to which group of GT acceptors PP belongs, since PP has been classified as a group 1 [20–22] and a group 2 [23] substrate. Results of the present study indicate that PP is not a typical group 1 or group 2 substrate, thereby providing further evidence for functional heterogeneity within these enzymes forms. Moreover, the differential degree of induction within the group 1 substrates (3-HBP and 4-MU) also tends to support the contention of functional heterogeneity.

The inducing agents 3-MC [5] and β-napthoflavone [13] have been shown to produce a coordinated increase (2-fold) in microsomal monooxygenase and GT activities. Two weeks of ethanol feeding was shown previously to cause a 2-fold increase in cytochrome P-450 levels [24] and a 6-fold increase in the microsomal oxidation of *p*-nitrophenol to 4-NTC [4]. In the present study, GT activity toward 4-NTC was enhanced by only 52%, indicating that ethanol feeding caused a large increase in PNP monooxygenation without a coordinated increase in 4-NTC GT activity.

In conclusion, 2 weeks of ethanol feeding produced various degrees of induction of GT activity. However, with the exception of enzyme activity toward PP and APAP, GT activity was increased only moderately or slightly by ethanol.

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Department of Pharmacology
University of Oklahoma Health Science Center
Oklahoma City, OK 73190, U.S.A.

DAVID J. SWEENY
LESTER A. REINKE*

* To whom requests for reprints should be addressed.

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Impairment of the hepatic microsomal drug-metabolizing system in rats parasitized with *Nippostrongylus brasiliensis*

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Nippostrongylus brasiliensis, an intestinal nematode parasite of rats, causes a serious and even fatal disease called nippostrongylosis. The gross pathology of this disease is accompanied by marked physiological and biochemical derangements in the host [1–3]. The hepatic microsomal mixed-function oxidase (MFO) system plays an important role in the metabolism of a variety of xenobiotics and endogenous compounds, such as steroids, fatty acids and