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A MARKEDLY DIMINISHED PLEIOTROPIC RESPONSE TO PHENOBARBITAL AND STRUCTURALLY-RELATED XENOBIOTICS IN ZUCKER RATS IN COMPARISON WITH F344/NCr OR DA RATS

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Abstract—Phenobarbital (PB) and certain structurally-related compounds induce a variety of hepatic drug-metabolizing enzymes in many strains of rats. Thus, following administration of PB (300, 500 ppm), barbital (BB, 1500 ppm) or 5-ethyl-5-phenylhydantoin (EPH, 500 ppm), CYP2B1-mediated benzyloxyresorufin *O*-dealkylase activity and epoxide hydrolase activity were profoundly induced in female DA and F344/NCr rats. In contrast, outbred female lean and obese Zucker rats showed markedly reduced CYP2B1 responses (< 15% and < 5% of those observed in the female DA or F344/NCr rat) to PB (doses ≤ 300 ppm), BB (1500 ppm) or EPH (500 ppm). In parallel studies, profound increases in RNA levels coding for CYP2B1, glutathione *S*-transferases Ya/Yc (α subclass), or epoxide hydrolase were detected in the female F344/NCr rat following treatment with PB (300 ppm), BB (1500 ppm) or EPH (500 ppm). In contrast, lean Zucker rats showed a strong response only to the highest dose of PB (500 ppm), implying that the diminished response in the Zucker rats may occur at some pretranslational level. Similar studies with lower doses of PB, EPH or BB in male lean Zucker rats showed a decreased response, relative to that in male F344/NCr rats. However, this insensitivity was not as profound as that observed in the female Zucker rats. In fact, the response to PB-type inducers in male or female Zucker rats is probably most clearly explained as a shift of the dose–response curve sharply to the right (decreased responsiveness, compared to F344/NCr or DA rats of the same sex). This decreased responsiveness of female lean Zucker rats to induction of CYP2B1, relative to that of F344/NCr rats, was also observed with the structurally-diverse PB-type inducers clonazepam, clotrimazole and 2-hexanone. In contrast, the female Zucker rat (obese or lean) displayed a pronounced response to induction of CYP1A-mediated ethoxyresorufin *O*-deethylase activity by β-naphthoflavone, a prototype inducer of CYP1A1 and CYP1A2. The Zucker rat would thus appear to represent a potentially exploitable genetic model for examining the mechanism of enzyme induction by the myriad xenobiotics which induce a PB-type response.

Studies on the nature of the hepatic pleiotropic response to phenobarbital (PB‖) and PB-type compounds should yield information both on potential drug/drug interactions mediated by altered "induced" metabolism, as well as other, less well understood manifestations of this process, including hepatocytomegaly, liver hypertrophy and liver tumor promotion/carcinogenesis [1–4]. The hepatic pleiotropic response induced by PB-type compounds has been characterized in the past with measurement of aminopyrine or ethylmorphine demethylase, benzo[*a*]pyrene or styrene oxide hydrase, quantitation of total P450 and of cytochrome P450 reductase, and certain phase II activities for which the observed increases have typically been relatively small following drug treatment. More recently, the use of specific immunologic reagents [5, 6], recombinant DNA probes [7, 8], and isozyme-specific P450 substrates [9–11] allow one to more readily examine this pleiotropic response caused by PB and related compounds. Using these techniques, investigators have examined induction in various tissues [12–14] and developmental stages [15, 16] and by a wide variety of compounds [17].

One of the most productive methods for examining specific biological characteristics is to obtain mutant organisms lacking or showing alterations in particular characteristics. This methodology has been employed previously in the study of the induction of drug-metabolizing enzymes in mice by 3-methylcholanthrene/2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-type inducers [18, 19]. The use of different inbred mouse strains has helped in the

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‖ Abbreviations: BB, barbital; β-NF, β-naphthoflavone; BZR, benzyloxyresorufin; CLT, clotrimazole; EPH, 5-ethyl-5-phenylhydantoin; ETR, ethoxyresorufin; PB, phenobarbital; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

recognition of a specific receptor which binds to the various inducing agents [20, 21] and is involved in specific transcriptional control by these compounds [2]. In addition, the use of these mice has enabled investigators to determine the specific role played by receptor mediated induction in mediating the carcinogenicity [2, 22–25] and toxicity [26, 27] of a vast number of chemicals. This topic has recently been reviewed by Nebert [28].

In an attempt to develop an analogous genetic model for PB-type induction in rats, we have now examined induction in three strains or outbred lines of rats. These included the F344/NCr strain, which we considered a normally responsive rat; DA rats, which have been reported to exhibit decreased levels of debrisoquine metabolism [29]; and Zucker rats. The latter rats were examined on the basis of previous reports that male obese Zucker rats display limited CYP2B1-type induction following treatment with PB [30–32]. In the present studies, in which a variety of catalytic or quantitative RNA endpoints were examined, we observed that both female obese and lean Zucker rats show a diminished response to PB and related compounds, such as barbital (BB) or 5-ethyl-5-phenylhydantoin (EPH), with the weakest response seen in the obese Zucker rats. These results demonstrate a potentially exploitable genetic difference in PB-type induction which may prove useful in examining the pharmacologic and toxicologic effects of a wide variety of xenobiotics.

MATERIALS AND METHODS

Treatment of animals. Male and female F344/NCr rats (Animal Production Area, FCRDC) between 7 and 9 weeks of age, obese and lean Zucker rats (Charles River, Kingston, MA) between 10 and 12 weeks of age, or female DA rats (Trudeau Institute, Saragnac Lake, NY) between 11 and 14 weeks of age were used. The various rat strains were administered control diet (Purina Lab Chow No. 5010) or diet containing PB (56–500 ppm), BB (1500 ppm), EPH (500 ppm) or clonazepam (500 ppm) *ad lib.* for 2 weeks. Alternatively, rats were injected i.p. with 50 mg β-naphthoflavone (β-NF) in corn oil/kg body wt at 0 and 24 hr and were killed 24 hr after the final injection. For treatment with 2-hexanone (1500 mg/kg body wt) rats received i.p. injections in corn oil at 0 and 48 hr and were killed 48 hr after the final injection. Clotrimazole (CLT, 75 mg/kg body wt) in corn oil was administered i.g. at 0, 24 and 48 hr, and the rats were killed 48 hr after the final injection. Control rats received i.p. injections of corn oil alone. Individual rats were killed by CO_2 asphyxiation, after which their livers were removed and weighed. Separate portions of the livers were then taken for isolation of RNA and for measurement of catalytic activities. The portions of the livers to be used for enzyme studies were homogenized in 0.15 M KCl/0.2 M sucrose (4 mL/g wet liver wt, 4°). Post-mitochondrial (S-9) and microsomal subfractions were obtained and were characterized for protein content using fluorescamine [33].

Chemicals, biochemical assays and RNA quantitation. The sources for the various chemicals employed, and the methodologies for measuring alkoxyresorufin *O*-dealkylase, epoxide hydrolase and testosterone hydroxylase activities, and for isolating and quantifying total cellular RNAs coding for CYP2B1, glutathione *S*-transferases Ya/Yc, microsomal epoxide hydrolase, or β-actin were described in the preceding paper [34].

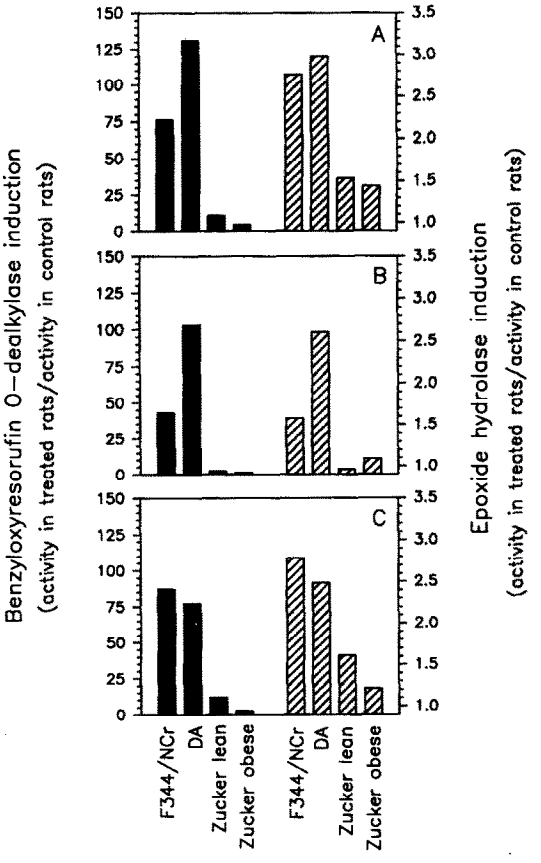

Fig. 1. Relative induction of CYP2B1-mediated benzyloxyresorufin *O*-dealkylase activity (solid bars) and epoxide hydrolase activity (hatched bars) in female rats of various strains/lines. Treatments consisted of two weeks dietary administration of: (A) 500 ppm 5-ethyl-5-phenylhydantoin; (B) 1500 ppm barbital; (C) 300 ppm phenobarbital. Values shown are expressed as the ratio of the mean induced activity to the mean control activity for three rats/treatment. Control BZR *O*-dealkylase activities for the F344/NCr, DA, lean Zucker and obese Zucker rats were 4.7 ± 1.3, 5.1 ± 0.5, 3.9 ± 0.4 and 4.9 ± 0.4 pmol resorufin formed/min/mg S-9 protein at 28°, respectively. Control epoxide hydrolase activities for the F344/NCr, DA, lean Zucker and obese Zucker rats were 315 ± 43, 387 ± 43, 270 ± 17 and 237 ± 31 pmol benzo[*a*]pyrene-4,5-dihydrodiol formed/min/mg S-9 protein at 28° respectively.

RESULTS

Induction of CYP2B1 and epoxide hydrolase. In Fig. 1, the responses to PB, its hydantoin analogue EPH, and BB elicited in female F344/NCr, DA, lean Zucker, and obese Zucker rats are displayed.

Table 1. Induction of BZR *O*-dealkylase and epoxide hydrolase activities in female F344/NCr and female lean Zucker rats by various xenobiotics

Strain/line	Treatment*	BZR *O*-dealkylase activity†	Epoxide hydrolase activity‡
F344/NCr	Control	4.3 ± 0.5	357 ± 36
	Clonazepam	93 ± 16	353 ± 49
	2-Hexanone	158 ± 15	503 ± 29
	Ethylphenylhydantoin	485 ± 37	886 ± 45
	Phenobarbital	625 ± 42	1013 ± 83
	Clotrimazole	943 ± 137	1014 ± 47
Lean Zucker	Control	3.5 ± 0.5	305 ± 27
	Clonazepam	8.8 ± 0.6	353 ± 49
	2-Hexanone	8.9 ± 0.7	337 ± 41
	Ethylphenylhydantoin	83 ± 12	405 ± 63
	Phenobarbital	247 ± 31	736 ± 41
	Clotrimazole	148 ± 21	627 ± 53

* Phenobarbital and 5-ethyl-5-phenylhydantoin were administered in the diet (500 ppm) for 2 weeks. Clotrimazole, 2-hexanone, and clonazepam were administered as described in Materials and Methods. Values shown are the means ± SD for N = 3 rats/treatment.

† BZR *O*-dealkylase activity is given in units of pmol resorufin formed/mg S-9 protein/min at ~28°.

‡ Epoxide hydrolase activity is given in units of pmol benzo[*a*]pyrene-4,5-dihydrodiol formed/mg S-9 protein/min at ~28°.

The O-dealkylation of benzyloxyresorufin (BZR) (mediated by CYP2B1 [9]) was highly induced by PB (300 ppm), EPH (500 ppm) or BB (1500 ppm) in female DA or F344/NCr rats. In contrast, in female lean and obese Zucker rats these xenobiotics caused relatively weak responses. The induction of epoxide hydrolase [35], although much lower in magnitude, paralleled the induction of BZR *O*-dealkylase activity in the various strains of rats (Fig. 1, Table 1). In addition to examining induction by compounds that are structurally related to phenobarbital, we also measured the induction of these drug-metabolizing enzymes by three xenobiotics that are structurally different from the barbiturates and their analogues. CLT, a potent inhibitor of CYP2B1 [36], was an extremely effective inducer of BZR *O*-dealkylase activity in female F344/NCr rats (Table 1), whereas clonazepam (another CYP2B1 inhibitor [37]) and 2-hexanone yielded only 10–20% of maximal activity. In contrast, in female lean Zucker rats, CLT was less effective as an inducer, while clonazepam and 2-hexanone caused extremely weak responses. The induction responses to the various xenobiotics were relatively homogeneous in the Zucker rats. Thus, standard deviations for *O*-dealkylase values in lean or obese Zucker rats generally were limited to < 20% of the mean values, despite the fact that coat color and pattern varied greatly within the individual rats comprising the treatment groups (data not shown), confirming the outbred nature of this line of rats.

Induction of testosterone hydroxylation. Enzymatic testosterone hydroxylation assays for the induction of various cytochrome P450 families (CYP3A, 6β-hydroxylase; CYP2A, 7α-hydroxylase; CYP2B1, 16β-hydroxylase [11, 38]) by PB and EPH yielded results which were in agreement with the benzyloxyresorufin *O*-dealkylase and epoxide hydrolase activities. Thus, these compounds were effective inducers in female F344/NCr rats, but were much less effective in female lean Zucker rats (Table 2). CLT was an effective inducer of both CYP2B1-mediated testosterone 16β-hydroxylase activities and CYP3A-mediated testosterone 6β-hydroxylase activity in F344/NCr rats (Table 2). This xenobiotic retained in large part its effectiveness as an inducer of testosterone 6β-hydroxylase in the female lean Zucker rats, but was a much weaker inducer of testosterone 16β-hydroxylase activity in these rats, in parallel with the weaker induction of BZR *O*-dealkylase activity displayed by CLT in the Zucker rats (Table 1).

Induction of CYP2B1, glutathione S-transferase, and epoxide hydrolase RNAs. The results in Fig. 2 (left panel) demonstrate that for both the female F344/NCr and lean Zucker rats total cellular CYP2B1 RNA was greatly increased in parallel to increases seen in BZR *O*-dealkylase activity. The levels of RNA coding for glutathione *S*-transferases Ya/Yc or epoxide hydrolase were increased to much more limited extents (Fig. 2, center and right panels); however, the relative inductions achieved with both of these genes suggest that induction of these drug-metabolizing enzymes by PB-type inducers occurs at the pretranslational level. The induction of CYP2B1, glutathione *S*-transferase Ya/Yc, and epoxide hydrolase RNA was markedly reduced in the Zucker rat compared to the F344/NCr rat.

Induction of drug-metabolizing enzymes in male rats. The induction of BZR *O*-dealkylase and epoxide hydrolase activities by PB, EPH and BB in male lean Zucker and F344/NCr rats is shown in Table 3. In general, the differences observed between male rats of the two strains/lines were less dramatic than the differences described above for females. The degrees of induction of BZR *O*-dealkylase activity

Table 2. Metabolism of testosterone by microsomes from female F344/NCr and female lean Zucker rats treated with various xenobiotics

Strain/line	Treatment*	Rate of production of selected hydroxylated testosterone products†			
		6β-	7α-	16β-	Androstenedione
F344/NCr	Control	148	551	ND‡	740
	EPH	747	1350	1097	2080
	PB	687	1683	839	2336
	CLT	2320	623	1137	2528
Lean Zucker	Control	130	631	ND	447
	EPH	162	863	221	652
	PB	330	1246	530	1118
	CLT	1097	513	141	662

* Phenobarbital (PB) and 5-ethyl-5-phenylhydantoin (EPH) were administered in diet at 500 ppm for a period of 2 weeks. Clotrimazole (CLT) was administered i.g. in corn oil (75 mg/kg body weight) on days 1, 2 and 3, and the rats were killed 48 hr after the last dose.

† Rates are given in terms of pmol hydroxylated product formed/min/mg microsomal protein at 30°. The samples utilized were microsomes pooled from the individual samples assayed for other biochemical parameters (Table 1).

‡ ND, value is below the limit of detection (25 pmol) under the assay conditions employed.

elicited by EPH, BB, and doses of PB < 500 ppm in the male lean Zucker rat were diminished more than 60%, compared to the F344/NCr rat. Induction of epoxide hydrolase activity in the male lean Zucker rats was quantitatively similar to that observed in the male F344/NCr rats (Table 3).

Dose–response relationships. When the responses of the various rat strains/lines to increasing doses of PB were examined (Fig. 3), two striking points were observed. First, the lean Zucker rats were less responsive to low doses of PB than were the F344/NCr or DA rats of the corresponding sex. Second, maximal levels of BZR *O*-dealkylase induced by PB (500 ppm) were greater in males than in females in both the F344/NCr and Zucker rats.

Induction of P450IA. In contrast to the reduced ability of the female lean or obese Zucker rats to respond to PB-type inducers, the induction of ethoxyresorufin *O*-dealkylase activity (mediated by CYP1A [9]) by the 3-methylcholanthrene/TCDD-type inducer β-naphthoflavone (50 mg/kg body wt), in female Zucker rats was similar in magnitude to that observed in DA rats of the same sex (Fig. 4).

DISCUSSION

Certain investigators have observed that male obese Zucker rats apparently display limited induction of cytochrome P450 following treatment with PB [30–32]. Two of the three studies which showed virtually no induction of cytochrome P450 employed relatively non-specific markers of induction (aminopyrine *N*-demethylase or measurement of total cytochrome P450) [30, 31]. Studies performed by Koch *et al.* [32], employing specific immunological reagents, as well as the more recent work by Bandyopadhyay and coworkers [41] using specific oligonucleotides, have confirmed that a distinctive pattern of induction (characterized by lower induction of CYP2B1) exists in the case of the obese Zucker rat. When we examined induction in female obese Zucker rats employing highly specific substrates for CYP2B1, we observed limited induction (< 3-fold) in obese rats following exposure to 300 ppm PB, in contrast to lean Zucker animals, in which almost 10-fold induction was observed (Fig. 1). However, this dichotomy is difficult to interpret when employing dietary administration of inducing agent because the obese Zucker rats weigh almost twice as much as the lean animals, but consume similar amounts of food, thus receiving a smaller dose calculated on a per kilogram body weight basis (data not shown). Additionally, the high levels of fat in the obese animals make it difficult to determine comparability of the dose actually present in the hepatic parenchyma when employing relatively lipophilic compounds such as PB. Therefore, we felt that the obese Zucker rats had significant limitations as a primary model to study enzyme induction by dietary exposure to PB-type xenobiotics.

Our results concerning induction by non-obese rats (lean Zucker, F344/NCr or DA) can be summarized as follows: (a) in female DA or F344/NCr rats, we observed relatively profound induction of CYP2B1 by high doses of PB (300 or 500 ppm), BB or EPH as measured enzymatically (BZR *O*-dealkylase activity); (b) in female lean Zucker rats, we observed substantial induction of BZR *O*-dealkylase activity following treatment with a high dose of PB (500 ppm) but observed much more limited induction with a lower dose (300 ppm) or with EPH or BB; (c) enzymatically-determined epoxide hydrolase activity and determination of total cellular RNA coding for CYP2B1, glutathione *S*-transferases Ya/Yc, or epoxide hydrolase in the female Zucker rats yielded qualitatively similar results; and (d) a diminished induction of CYP2B1 in male lean Zucker rats was also observed, relative

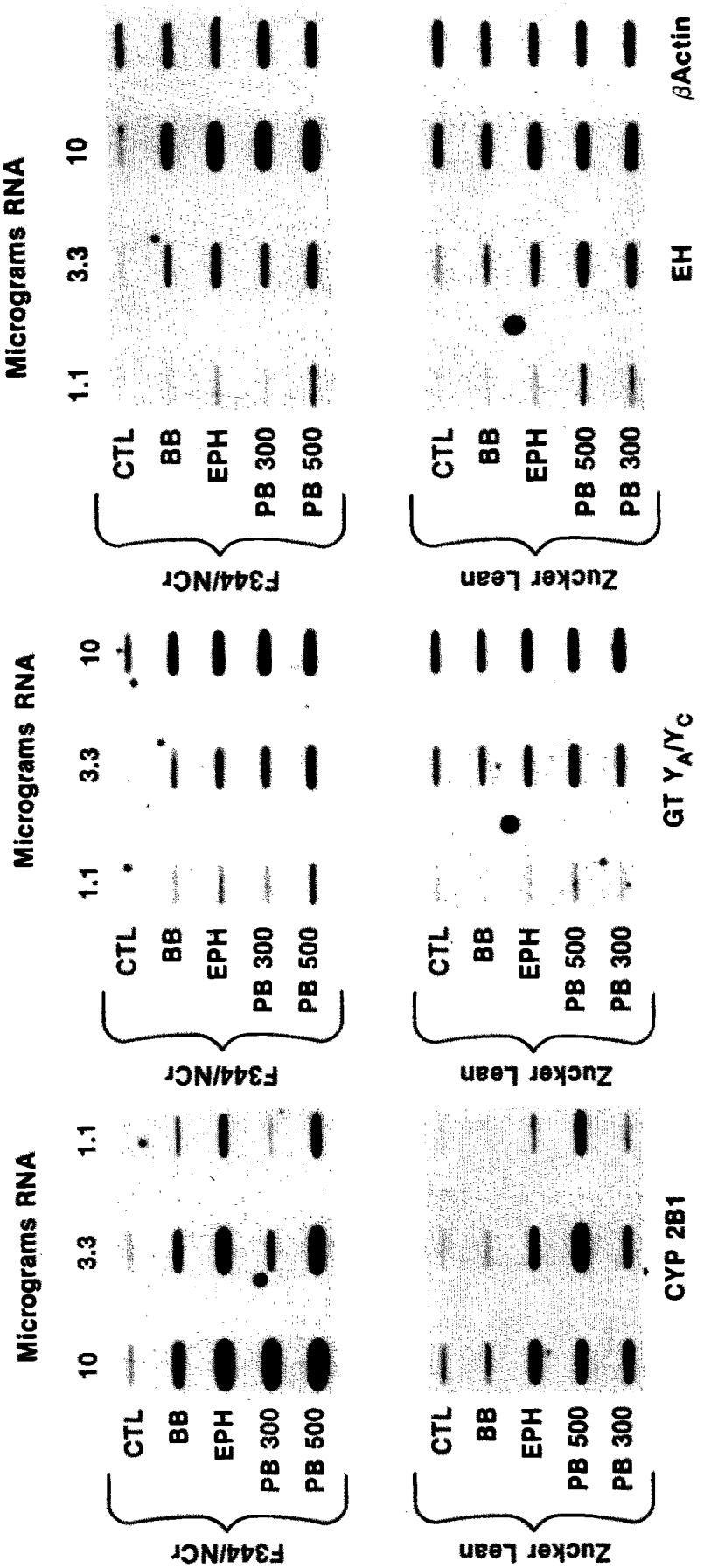

Fig. 2. Total cellular RNAs coding for selected drug-metabolizing enzymes in female lean Zucker or female F344/NCr rats exposed for 14 days to control diet (CTL), or to diet containing 500 ppm 5-ethyl-5-phenylhydantoin (EPH), 1500 ppm barbital (BB), or to 300 or 500 ppm phenobarbital (PB). Slots in the three lanes in each panel contain the indicated amounts of hepatic RNA pooled from three rats/treatment; (left panel) hybridization of RNA to an oligonucleotide (5'-GGTTGGTAGCCGGTGTGA-3') which is specific for CYP2B1 [7]; (center panel) hybridization of RNA to a plasmid (pGTB-38) which codes for the glutathione S-transferase family Ya/Yc [39]; (right panel) hybridization of RNA to a plasmid (pEPH302) which codes for microsomal epoxide hydrolase [40].

Table 3. Induction of BZR *O*-dealkylase and epoxide hydrolase activities in male F344/NCr and male lean Zucker rats

Strain/line	Treatment*	Dose (ppm)	BZR *O*-dealkylase activity†	Epoxide hydrolase activity‡
F344/NCr	Control		21.9 ± 1.7	487 ± 51
	EPH	500	1043 ± 71	1290 ± 87
	BB	1500	933 ± 60	1227 ± 114
	PB	56	462 ± 120	866 ± 73
	PB	167	840 ± 166	1076 ± 68
	PB	500	1025 ± 62	1329 ± 103
Lean Zucker	Control		19.8 ± 4.7	366 ± 57
	EPH	500	365 ± 47	910 ± 73
	BB	1500	281 ± 52	810 ± 101
	PB	300	383 ± 77	822 ± 93
	PB	500	1302 ± 193	1465 ± 147

* Rats were fed the indicated doses of 5-ethyl-5-phenylhydantoin (EPH), barbital (BB) or phenobarbital (PB) in the diet for a period of 2 weeks. Values shown are the means ± SD for N = 3 rats/treatment.

† BZR *O*-dealkylase activity is given in units of pmol resorufin formed/mg S-9 protein/min at ~28°.

‡ Epoxide hydrolase activity is given in units of pmol benzo[*a*]pyrene-4,5-dihydrodiol formed/mg S-9 protein/min at ~28°.

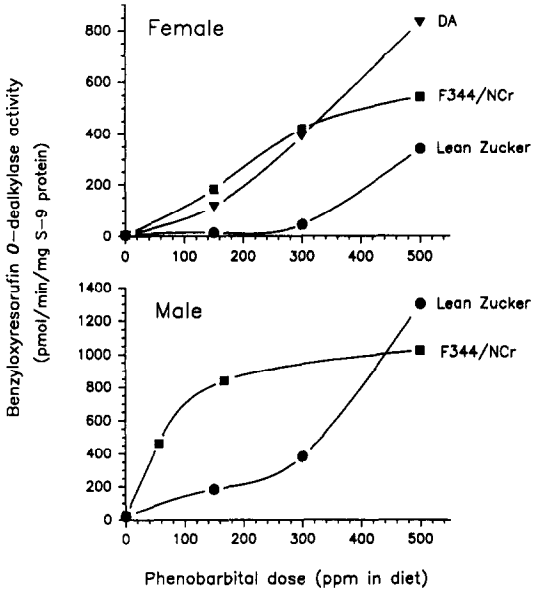

Fig. 3. Dose–response curves for induction of hepatic BZR *O*-dealkylase activity in the various strains/lines of rats. Top panel: induction in female F344/NCr, DA or lean Zucker rats administered dietary phenobarbital for 2 weeks. Bottom panel: induction in male F344/NCr or lean Zucker rats administered dietary phenobarbital for 2 weeks. Each point represents the mean activity for three rats.

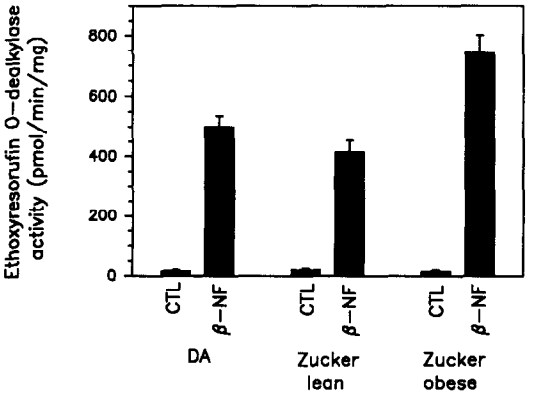

Fig. 4. Induction of CYP1A-mediated ethoxyresorufin (ETR) *O*-dealkylase in female DA or Zucker rats by β-naphthoflavone (β-NF, 50 mg/kg body wt). Each bar represents the mean (± SD) activity for three rats. ETR *O*-dealkylase activity is given in units of pmol resorufin formed/min/mg S-9 protein measured at 28°.

to that observed in male F344/NCr rats. However, the differences observed between the males of the two strains were less profound than those seen with the females. This may reflect the fact that female rats of both strains appear to show reduced absolute *O*-dealkylase activities at doses of PB ≤ 500 ppm, compared to the corresponding males (Fig. 3).

The reduced responses of the lean Zucker rats appear to be mediated at the pretranslational level, based upon the decreased induction of RNA coding for CYP2B1, glutathione *S*-transferases Ya/Yc, and epoxide hydrolase (Fig. 2). It has been shown previously that the *de novo* synthesis of CYP2B1 caused by inducing agents is a reflection of increases in the rate of gene transcription, rather than alterations in stability of the mRNA [42].

When the PB induction data are presented

graphically (Fig. 3), the results imply a dose–response curve in lean Zucker rats that has shifted to the right to produce a less responsive animal. Although our primary efforts in this particular paper have involved the study of induction by a series of compounds related to PB, we have also examined a group of structurally-diverse compounds, all of which are inducers of CYP2B1 activity in the rat (Table 1, see also the accompanying paper). The most striking inducer in female F344/NCr rats was CLT, causing a response even greater than that caused by PB. In contrast, 2-hexanone and clonazepam, although obviously inducers of CYP2B1, were markedly weaker than PB at the doses employed. Each of these xenobiotics yielded markedly weaker inductions in lean Zucker rats than in F344/NCr rats.

Since more than one form of P450 is induced by PB and related compounds, we examined induction of CYP3A (testosterone 6β-hydroxylase), CYP2A (testosterone 7α-hydroxylase) and CYP2B1 (testosterone 16β-hydroxylase) as well as BZR *O*-dealkylase activity. In Zucker rats, we observed diminished induction of each of these activities by PB and EPH (Table 2) as well as by the structurally-unrelated compounds 2-hexanone and clonazepam (data not shown). CLT has been shown to be a potent inducer of both CYP2B1 and CYP3A [43, 44], a result we have now confirmed in female F344/NCr rats. Recent work performed with hepatocytes exposed *in vitro* to CLT indicates that the ED_{50} for induction of CYP2B is lower than that for induction of CYP3A [45]. Thus, our observation in the lean Zucker rats of a relatively normal response with respect to induction of CYP3A (approximately 50% of that observed in F344/NCr rats) at a dose of CLT which resulted in a markedly lower CYP2B1 induction relative to that observed in F344/NCr rats is even more striking. These results with CLT, considered together with those obtained with β-naphthoflavone (50 mg/kg body wt, Fig. 4), suggest a specificity of the defect in the Zucker rat since this strain shows a strong induction of CYP3A by CLT or of CYP1A by β-naphthoflavone or 3-methylcholanthrene [30]. We are presently screening male rats of a variety of inbred strains for evidence of such a diminished responsiveness to PB-type induction. Although we have observed slight differences between the various strains, such differences are minor compared to the diminished responses exhibited by lean and obese Zucker rats (Jones *et al.*, manuscript in preparation).

A variety of recent studies, including those presented here, would appear to be compatible with the idea of a coordinate hepatic response to PB-type inducers. These studies include: (1) the present work, which demonstrated a diminished response to structurally-diverse PB-type inducers in Zucker rats; (2) the qualitatively-similar pleiotropic response caused by structurally-diverse PB-type inducers in male F344/NCr rats [34]; and (3) the ability of relatively high doses of heme arginate to inhibit simultaneously all the various elements of this pleiotropic response in male rats treated with PB or 2-allyl-2-isopropylacetamide, while not affecting the induction of CYP1A by 3-methylcholanthrene [46]. These results would appear to be compatible with the presence of some common biochemical determinant (e.g. receptor mediation) but are no proof of such an entity.

The specific mechanism determining the altered response of the Zucker rat is difficult to assess since the mechanism of induction by PB-type inducers itself is still not defined. Nonetheless, as shown with CYP2A and CYP3A, epoxide hydrolase and the glutathione *S*-transferases, this decreased sensitivity is not limited to the cytochrome(s) P450. Although we have not directly determined the relevant parameters (plasma half-life, steady-state plasma concentration) for each of these inducers, pharmacokinetic differences among the rat strains/lines do not appear to be a likely explanation for the observed differences in relative induction. For instance, BB does not bind to or appear to be a substrate of the P450 monooxygenases [47] and is predominantly excreted unchanged. Therefore, the more limited P450 induction we observed in the Zucker rats would not be expected to alter steady-state BB levels. In fact, we have observed minimal differences in steady-state serum BB levels in female lean Zucker or F344/NCr rats exposed to 1500 ppm BB in the diet (data not shown). In the case of PB, which *is* metabolized by cytochrome P450, higher blood concentrations are observed in obese or lean Zucker rats, relative to other rat strains exposed to the same dose levels [31, 48]. If degree of P450 induction were dependent solely upon the magnitude of the steady-state blood concentration of PB, one would expect higher induction in the Zucker rats, rather than the lower inductions observed, since it is the parent compound, PB, which is active as the P450 inducer and not its hydroxylated product. Thus, *p*-hydroxyphenobarbital, the major metabolite of PB, is totally ineffective as an inducer of CYP2B1 ([49]; Lubet RA, data not shown). Finally, and perhaps most importantly, CLT, which has been shown previously to be an effective inducer of both CYP2B1 and CYP3A1 *in vivo* [36] and *in vitro* [45], was a strong inducer of CYP3A but a much weaker inducer of CYP2B1 in the Zucker rats. This relatively strong induction of CYP3A relative to CYP2B1 is even more impressive in light of the fact that the ED_{50} for induction of CYP2B1 by clotrimazole in hepatocyte cultures has been found to be lower than the ED_{50} for induction of CYP3A [45]. Since the pharmacokinetics must by definition be the same, this implies a markedly reduced response specifically to CYP2B1-type induction.

In the case of the Zucker rats, a diminished response to the entire range of responses associated with PB treatment appears to exist. Previously, investigators have observed a variety of strains of rats which display a diminished CYP2B2 response, while retaining relatively normal CYP2B1 responses [50]. It appears that certain strains of rats fail to express the gene product for CYP2B2, either constitutively or following PB administration. It is not clear whether this reflects a markedly altered structural gene in these rats or rather a "turning off" of this particular gene. In contrast, all strains of rats appear to express a functional CYP2B1 gene, although there are polymorphisms in the peptide

structure of the gene product. A second PB-inducible gene, aldehyde dehydrogenase, also shows a striking strain dependency. Thus, a specific gene can be induced in certain strains of rats but not in others [51]. In contrast to the results with CYP2B2, the lack of aldehyde dehydrogenase induction by PB which is observed in some rat strains (including the F344/NCr) does not result from lack of a functional gene, since the gene is apparently expressed constitutively in all rat strains examined to date.

We initially examined induction of CYP2B1 as part of an extensive study to investigate the tumor-promoting effects of various sedative/anticonvulsant drugs [52–55]. In these studies, we have observed that there is a strong association between tumor-promoting activity and the ability of various compounds (including oxybarbiturates, hydantoins and alkylacetylureas) to induce CYP2B1-mediated catalytic activities. Whether this correlation truly reflects a direct involvement of CYP2B1 in the tumor promotion process or rather reflects CYP2B1 as a marker for an overall, coordinately induced, pleiotropic response which includes induction of P450, epoxide hydrolase, glutathione *S*-transferase, UDP-glucuronyltransferases, liver hypertrophy and hepatocytomegaly is presently under investigation.

In conclusion, the Zucker rat model would appear to be useful for studying the mechanisms of induction of drug-metabolizing enzymes, the phenomenon of drug–drug interactions and the role of induction in tumor-promotion in both the thyroid and liver, by a wide variety of xenobiotics.

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REFERENCES

1. Conney AH, Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**: 317–366, 1967.
2. Nebert DW and Gonzalez FJ, P-450 genes: Structure, evolution and regulation. *Annu Rev Biochem* **56**: 945–993, 1987.
3. Gonzalez FJ, The molecular biology of cytochrome P450s. *Pharmacol Rev* **40**: 243–288, 1988.
4. Kutt H, Phenobarbital: Interactions with other drugs. In: *Antiepileptic Drugs* (Eds. Levy RH, Dreifuss FE, Mattson RH, Meldrum BS and Penry JK), 3rd Edn, pp. 313–328. Raven Press, New York, 1989.
5. Thomas PE, Korzeniowski D, Ryan D and Levin W, Preparation of monospecific antibodies against two forms of rat liver cytochrome P-450 and quantitation of these antigens in microsomes. *Arch Biochem Biophys* **192**: 524–532, 1979.
6. Reik LM, Levin W, Ryan DE, Maines SL and Thomas PE, Monoclonal antibodies distinguish among isozymes of the cytochrome *P*-450b subfamily. *Arch Biochem Biophys* **242**: 365–382, 1985.
7. Giachelli CM and Omiecinski CJ, Regulation of cytochrome P-450b and P-450e mRNA expression in the developing rat: Hybridization to synthetic oligodeoxyribonucleotide probes. *J Biol Chem* **261**: 1359–1363, 1986.
8. Atchison M and Adesnik M, A cytochrome P-450 multigene family: Characterization of a gene activated by phenobarbital administration. *J Biol Chem* **258**: 11285–11295, 1983.
9. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
10. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolff T and Guengerich FP, Dealkylation of pentoxyresorufin: A rapid and sensitive assay for measuring induction of cytochrome(s) *P*-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
11. Wood AW, Ryan DE, Thomas PE and Levin W, Regio- and stereoselective metabolism of two C_{19} steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. *J Biol Chem* **258**: 8839–8847, 1983.
12. Omiecinski CJ, Tissue-specific expression of rat mRNAs homologous to cytochromes P-450b and P-450e. *Nucleic Acids Res* **14**: 1525–1539, 1986.
13. Christou M, Wilson NM and Jefcoate CR, Expression and function of three cytochrome *P*-450 isozymes in rat extrahepatic tissues. *Arch Biochem Biophys* **258**: 519–534, 1987.
14. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Biochemistry* **21**: 1698–1706, 1982.
15. Waxman DJ, Dannan GA and Guengerich FP, Regulation of rat hepatic cytochrome P-450: Age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**: 4409–4417, 1985.
16. Simmons DL and Kasper CB, Quantitation of mRNAs specific for the mixed-function oxidase system in rat liver and extrahepatic tissues during development. *Arch Biochem Biophys* **271**: 10–20, 1989.
17. Traber PG, Chianale J, Florence R, Kim K, Wojcik E and Gumucio JJ, Expression of cytochrome P450b and P450e genes in small intestinal mucosa of rats following treatment with phenobarbital, polyhalogenated biphenyls, and organochlorine pesticides. *J Biol Chem* **263**: 9449–9455, 1988.
18. Thomas PE, Kouri RE and Hutton JJ, The genetics of aryl hydrocarbon hydroxylase induction in mice: A single gene difference between C57BL/6J and DBA/2J. *Biochem Genet* **6**: 157–168, 1972.
19. Nebert DW, Goujon FM and Gielen JE, Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: Simple autosomal dominant trait in the mouse. *Nature New Biol* **236**: 107–109, 1972.
20. Poland A and Glover E, Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Evidence for a receptor mutation in genetically non-responsive mice. *Mol Pharmacol* **11**: 389–398, 1975.
21. Whitlock JP, The regulation of gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Pharmacol Rev* **39**: 147–161, 1987.
22. Anderson LM, Jones AB, Riggs CW and Ohshima M, Fetal mouse susceptibility to transplacental lung and liver carcinogenesis by 3-methylcholanthrene: Positive correlation with responsiveness to inducers of aromatic hydrocarbon metabolism. *Carcinogenesis* **6**: 1389–1393, 1985.

23. Kouri RE and Nebert DW, Genetic regulation of susceptibility to polycyclic hydrocarbon induced tumors in the mouse. In: *Origins of Human Cancer* (Eds. Heath HH, Watson JD and Winstein JN), pp. 811–835. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1977.
24. Kouri RE, Billups LH, Rude TH, Whitmire CE, Sass B and Henry CJ, Correlation of inducibility of aryl hydrocarbon hydroxylase with susceptibility to 3-methylcholanthrene-induced lung cancers. *Cancer Lett* **9**: 277–284, 1980.
25. Lubet RA, Connolly GM, Nebert DW and Kouri RE, Dibenz[a,h]anthracene-induced subcutaneous tumors in mice. Strain sensitivity and the role of carcinogen metabolism. *Carcinogenesis* **4**: 513–517, 1983.
26. Nebert DW, Genetic differences in susceptibility to chemically induced myelotoxicity and leukemia. *Environ Health Perspect* **39**: 11–22, 1981.
27. Lubet RA, Lemaire BN, Avery D and Kouri RE, Induction of immunotoxicity in mice by polyhalogenated biphenyls. *Arch Toxicol* **59**: 71–77, 1986.
28. Nebert DW, The *Ah* locus: Genetic differences in toxicity, cancer, mutation, and birth defects. *CRC Crit Rev Toxicol* **20**: 153–174, 1989.
29. Matsunaga E, Zanger UM, Hardwick JP, Gelboin HV, Meyer UA and Gonzalez FJ, The *CYP2D* gene subfamily: Analysis of the molecular basis of the debrisoquine 4-hydroxylase deficiency in DA rats. *Biochemistry* **28**: 7349–7355, 1989.
30. Litterst CL, *In vitro* hepatic drug metabolism and microsomal enzyme induction in genetically obese rats. *Biochem Pharmacol* **29**: 289–296, 1980.
31. Rowse Brouwer KL, Kostenbauder HB, McNamara PJ and Blouin RA, Phenobarbital in the genetically obese Zucker rat. II. *In vivo* and *in vitro* assessments of microsomal enzyme induction. *J Pharmacol Exp Ther* **231**: 654–659, 1984.
32. Koch JA, Finkelstein JA and Steggles AW, Cytochrome P-450 induction in genetically obese rats. *Biochem Biophys Res Commun* **108**: 247–252, 1982.
33. Böhlen P, Stein S, Dairman W and Udenfriend S, Fluorometric assay of proteins in the nanogram range. *Arch Biochem Biophys* **155**: 213–220, 1973.
34. Lubet RA, Dragnev KH, Chauhan DP, Nims RW, Diwan BA, Ward JM, Jones CR, Rice JM and Miller MS, A pleiotropic response to phenobarbital-type inducers in the F344/NCr rat: Effects of chemicals of varied structure. *Biochem Pharmacol* **43**: 1067–1078 1992.
35. Dansette PM, DuBois GC and Jerina DM, Continuous fluorometric assay of epoxide hydrase activity. *Anal Biochem* **97**: 340–345, 1979.
36. Rodrigues AD, Lewis DFV, Ioannides C and Parke DV, Spectral and kinetic studies of the interaction of imidazole anti-fungal agents with microsomal cytochromes P-450. *Xenobiotica* **17**: 1315–1327, 1987.
37. Nims RW, Prough RA, Stockus DL and Lubet RA, *In vivo* and *in vitro* inhibition of cytochrome P450-mediated enzyme activities by the benzodiazepine, clonazepam. *FASEB J* **4**: A611, 1990.
38. Sonderfan AJ, Arlotto MP, Dutton DR, McMillen SK and Parkinson A, Regulation of testosterone hydroxylation by rat liver microsomal cytochrome *P*-450. *Arch Biochem Biophys* **255**: 27–41, 1987.
39. Pickett CB, Telakowski-Hopkins CA, Ding GJ-F, Argenbright L and Lu AYH, Rat liver glutathione *S*-transferases: Complete nucleotide sequence of a glutathione *S*-transferase mRNA and the regulation of the Ya, Yb, and Yc mRNAs by 3-methylcholanthrene and phenobarbital. *J Biol Chem* **259**: 5182–5188, 1984.
40. Ding VD-H, Cameron R and Pickett CB, Regulation of microsomal, xenobiotic epoxide hydrolase messenger RNA in persistent hepatocyte nodules and hepatomas induced by chemical carcinogens. *Cancer Res* **50**: 256–260, 1990.
41. Bandyopadhyay AM, Chaudhary I and Blouin RA, Molecular mechanism of phenobarbital induction defect of cytochrome P-450IIB1/IIB2 in obese Zucker rats. *FASEB J* **5**: A478, 1991.
42. Hardwick JP, Gonzalez FJ and Kasper CB, Transcriptional regulation of rat liver epoxide hydratase, NADPH–cytochrome P-450 oxidoreductase, and cytochrome P-450b genes by phenobarbital. *J Biol Chem* **258**: 8081–8085, 1983.
43. Hostetler KA, Wrighton SA, Molowa DT, Thomas PE, Levin W and Guzelian PS, Coinduction of multiple hepatic cytochrome P-450 proteins and their mRNAs in rats treated with imidazole antimycotic agents. *Mol Pharmacol* **35**: 279–285, 1989.
44. Ritter JK and Franklin MR, Induction and inhibition of rat hepatic drug metabolism by *N*-substituted imidazole drugs. *Drug Metab Dispos* **15**: 335–343, 1987.
45. Kocarek TA, Schuetz EG and Guzelian PS, Differentiated induction of cytochrome P450b/e and P450p mRNAs by dose of phenobarbital in primary cultures of adult rat hepatocytes. *Mol Pharmacol* **38**: 440–444, 1990.
46. Srivastava G, Hansen AJ, Bawden MJ and May BK, Hemin administration to rats reduces levels of hepatic mRNAs for phenobarbitone-inducible enzymes. *Mol Pharmacol* **38**: 486–493, 1990.
47. Jansson I, Orrenius S and Ernster L, A study of the interaction of a series of substituted barbituric acids with the hepatic microsomal monooxygenase. *Arch Biochem Biophys* **151**: 391–400, 1972.
48. Rowse Brouwer KL, Kostenbauder HB, McNamara PJ and Blouin RA, Phenobarbital in the genetically obese Zucker rat. I. Pharmacokinetics after acute and chronic administration. *J Pharmacol Exp Ther* **231**: 649–653, 1984.
49. Cresteil T, Mahu J-L, Dansette PM and Leroux J-P, *In vivo* administration of hydroxylated phenobarbital metabolites: Effect on rat hepatic cytochromes P-450, epoxide hydrolase and UDP-glucuronyltransferase. *Biochem Pharmacol* **29**: 1127–1133, 1980.
50. Dunn TJ, Koleski AJ, Lindahl R and Pitot HC, Phenobarbital-inducible aldehyde dehydrogenase in the rat. cDNA sequence and regulation of the mRNA by phenobarbital in responsive rats. *J Biol Chem* **264**: 13057–13065, 1989.
51. Deitrich RA, Bludeau P, Stock T and Roper M, Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-*p*-dioxin. *J Biol Chem* **252**: 6169–6176, 1977.
52. Diwan BA, Rice JM, Ohshima M, Ward JM and Dove LF, Comparative tumor-promoting activities of phenobarbital, amobarbital, barbital sodium and barbituric acid on livers and other organs of male F344/NCr rats following initiation with *N*-nitrosodiethylamine. *J Natl Cancer Inst* **74**: 509–516, 1985.
53. Nims RW, Devor DE, Henneman JR and Lubet RA, Induction of alkoxyresorufin *O*-dealkylases, epoxide hydrolase, and liver weight gain: Correlation with liver tumor-promoting potential in a series of barbiturates. *Carcinogenesis* **8**: 67–71, 1987.
54. Diwan BA, Rice JM, Nims RW, Lubet RA, Hu H and Ward JM, P-450 enzyme induction by 5-ethyl-5-phenylhydantoin and 5,5-diethylhydantoin, analogues of barbiturate tumor promoters phenobarbital and barbital, and promotion of liver and thyroid carcinogenesis initiated by *N*-nitrosodiethylamine in rats. *Cancer Res* **48**: 2492–2497, 1988.
55. Lubet RA, Nims RW, Ward JM, Rice JM and Diwan BA, Induction of cytochrome P_{450b} and its relationship to liver tumor promotion. *J Am Coll Toxicol* **8**: 259–268, 1989.