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HEPATIC EPOXIDE HYDROLASE ACTIVITIES AND THEIR INDUCTION BY CLOFIBRATE AND DIETHYLHEXYLPHTHALATE IN VARIOUS STRAINS OF MICE

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Abstract—The presence of epoxide hydrolase activity in cytoplasm, microsomes and mitochondrial fraction in livers from twelve strains of mice (AKR/J, A/J, BALB/cByJ, CBA/J, C3H/HeJ, G57BL/6J, C57BL/10J, DBA/2J, NZB/B1NJ, PL/J, SEC/1ReJ and SW), and the influence of orally administered clofibrate and di(2-ethylhexyl)phthalate (DEHP) (0.5 and 2%, respectively, in diet) on epoxide hydrolase activities, were studied. Significant differences in basal cytosolic epoxide hydrolase activity, which ranged from 5.6 to 11.2 nmol diol · min^{-1} · (mg protein)$^{-1}$ using *trans*-stilbene oxide (TSO) as substrate, were noted among the mice. The highest and lowest enzyme levels were observed in the A/J and DBA/2J strains respectively. Similarly, microsomal epoxide hydrolase activity, monitored with *cis*-stilbene oxide (CSO), varied with the mouse strain, with the highest and lowest microsomal epoxide hydrolase activity being observed in A/J and SW strains respectively. Variations were also noted in the epoxide hydrolase activity in the mitochondrial fraction (monitored with TSO) with the highest and lowest levels observed in C57BL/6J and SW strains respectively. Clofibrate or DEHP treatment induced both cytosolic and microsomal epoxide hydrolases in nearly all of the strains examined. In contrast, the hydrolysis of TSO by the mitochondrial fraction in these strains was either not affected or decreased by clofibrate or DEHP treatment. The induction of cytosolic epoxide hydrolase was found to range between 1.2- and 2.8-fold, with generally a higher level of induction in mouse strains with low basal levels of cytosolic epoxide hydrolase activity. This level of cytosolic epoxide hydrolase activity, monitored with TSO as substrate, closely reflected the level of cytosolic epoxide hydrolase protein detected by immunoblot. There were also no significant differences observed in the molecular weight, immunological characteristics, pH-dependence and heat stability of hepatic cytosolic epoxide hydrolase activities of control and clofibrate-treated mice from various strains. These results suggest that clofibrate and DEHP induce both cytosolic and microsomal epoxide hydrolases but not the epoxide hydrolase in the mitochondrial fraction.

Epoxide hydrolases (EC 3.3.2.3), which metabolize epoxidized compounds or intermediates produced by the conversion of many olefin and aromatic compounds by microsomal monooxygenases [1], are present in most subcellular organelles of mammalian liver [2–4]. Based on a number of criteria, there appear to be two major forms of epoxide hydrolases in mammalian liver, viz. the microsomal and cytosolic forms [2, 4–7].

Genetic factors such as species, strain, sex, and interindividual polygenic and polymorphic variations are known to affect xenobiotic metabolism in laboratory animals [8, 9]. Detailed studies of inbred mouse strain differences have led to a much better understanding of mammalian genetic systems that regulate xenobiotic metabolism, e.g. the *Ah* locus and the *Coh* locus [10]. Differences in hepatic microsomal epoxide hydrolase activity between inbred strains of mice have been noted previously [11, 12]. Using styrene oxide as the substrate, genetic polymorphism of hepatic microsomal epoxide hydrolase activity in mouse has been demonstrated [13]. Two strains of mice, namely C57BL/6J and DBA/2J, exhibited different properties of microsomal epoxide hydrolase and, by crossing the backcrossing of these strains, *Eph-1* was proposed as the locus for the structural gene of the microsomal epoxide hydrolase [13].

Although strain differences exist in the activity of cytosolic epoxide hydrolase in mice [2, 14], no significant attempts have been made to understand and compare the basal levels and properties of cytosolic epoxide hydrolase and its inducibility by an inducer in terms of genetic or enzyme polymorphism in different strains of mice. Administration of clofibrate, di(2-ethyl)phthalate (DEHP) and other compounds, which cause hypolipidermia and peroxisomal proliferation, results in the induction of epoxide hydrolase activity in cytosol and microsomes [15], without causing any alteration in the rate of hydrolysis of *trans*-stillene oxide (TSO) in the mitochondrial fraction and in other subcellular organelles such as peroxisomes [4, 14]. The present study was performed to gain an insight into the regulation of epoxide hydrolase as evaluated by determining the rate of hydrolysis of TSO and *cis*-stilbene oxide (CSO) which served as the substrates for cytosolic and mitochondrial epoxide hydrolases, and the microsomal epoxide hydrolase, respectively. Studies were also extended to evaluate the response of hepatic epoxide hydrolases to clofibrate and

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DEHP treatment in different strains of mice. The status of cytosolic glutathione-*S*-transferase (EC 2.5.1.18), an important epoxide-detoxifying enzyme [16], and its response to clofibrate and DEHP treatment in different strains of mice were also investigated.

MATERIALS AND METHODS

Chemicals. [^{3}H]*Trans*- and [^{3}H] *cis*-stilbene oxides were synthesized, as previously reported [17], and had a >97% geometrical purity and >99% radiochemical purity. Clofibrate (ethyl α-[4-chlorophenoxy]-α-methylpropionate) was synthesized as reported [18]. DEHP was obtained from the Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. Reduced glutathione was obtained from the Sigma Chemical Co. (St. Louis, MO), and goat anti-rabbit IgG-alkaline phosphatase was obtained from HyClone (Logan, UT). Nitrocellulose membrane filters were purchased from BioRad (Richmond, CA). All other chemicals were the best grade commercially available.

Treatment of animals. Different strains of inbred male mice (AKR/J, A/J, BALB/cByJ, CBA/J, C3H/HeJ, C57BL/6J, C57BL/10J, DBA/2J, NZB/B1NJ, PL/J, SEC/1ReJ) were obtained from Jackson Laboratories (Bar Harbor, ME). The outbred strain, Swiss–Webster (SW), was obtained from a colony maintained at the University of California, Riverside. The animals were acclimatized for 5 days to a day–night cycle of 12:12 hr and a temperature of 22° before use. The animals were fed *ad lib.* normal, clofibrate or DEHP diet. Mice of 7 ± 1 weeks of age were fed a diet containing clofibrate (0.5%, w/w) or DEHP (2%, w/w) in corn oil for 10 consecutive days. Control mice were fed on a diet containing corn oil alone.

Preparation of subcellular fractions. Animals were killed without prior fasting by cervical dislocation, and their livers were removed, perfused with chilled 0.25 M sucrose, blotted dry, and weighed. All subsequent procedures were performed at 4°. A 10% homogenate from individual livers (w/v) was prepared in 0.25 M sucrose using a Potter–Elvehjem homogenizer. Centrifugation of the homogenate (600 *g*, 10 min) resulted in the separation of nuclei and cell debris. The postnuclear supernatant fractions were centrifuged (10,000 *g*, 10 min) to obtain the mitochondrial fraction which also contained other subcellular organelles such as lysosomes and peroxisomes. The postmitochondrial fractions were centrifuged (105,000 *g*, 60 min) to obtain the microsomal pellet and cytosolic supernatant fraction. The mitochondrial and microsomal pellets were washed once with homogenizing medium and resuspended in 0.25 M sucrose and 0.1 M sodium phosphate buffer, pH 7.4, respectively, to give a 20% suspension. All the fractions were diluted to appropriate protein concentrations prior to the assay. Protein determinations were determined according to Bradford [19].

Polyacrylamide gel electrophoresis and immunodetection. Cytosolic and mitochondrial fractions of control and clofibrate-treated mice were used for the determination of molecular weight and antigenic properties. The protein samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in discontinuous buffer system in 0.75 mm gels (10%) according to Laemmli [20]. After electrophoresis, the resolved proteins were transferred electrophoretically to nitrocellulose sheets as described previously [21]. The protein bands were probed first with antibody raised against purified cytosolic epoxide hydrolase [22] and then with goat anti-rabbit IgG-alkaline phosphatase. The bands containing cytosolic epoxide hydrolase protein were visualized as described by Blake *et al.* [23].

Ouchterlony double-diffusion analysis. Ouchterlony was performed in 1% agarose gels containing 100 mM Tris–HCl buffer, pH 7.5, 50 mM sodium azide, 1% polyethylene glycol 8000 and 0.9% NaCl layered on glass plates. Crude cytosol (15–20 μl) from induced and uninduced animals was added to the outer wells, and rabbit antiserum (15 μl) was added to the center well. The agarose gels were then incubated overnight at 37° before visualization of immunoprecipitin bands.

Enzyme assays. Cytosolic and mitochondrial epoxide hydrolase activities were monitored following published procedures [17] with TSO (final concentration, 5×10^{-5} M) as substrate in 100 mM sodium phosphate buffer, pH 6.8, while the microsomal epoxide hydrolase was monitored using CSO as substrate (final concentration, 5×10^{-5} M) in 100 mM Tris–HCl buffer, pH 9.0. Using similar methodology, glutathione-*S*-transferase activity was monitored with CSO (final concentration, 5×10^{-5} M) and 5 mM glutathione as substrates in 100 mM sodium phosphate buffer, pH 7.4.

Statistical analysis. All data are expressed as specific activity per mg of cytosolic, mitochondrial or microsomal protein. Comparison of induction of enzyme levels for individual mice strains was performed by paired Student's *t*-test, while comparison for differences in basal values of enzyme levels in untreated mice was performed using Duncan's multiple range test [24].

RESULTS

The level of hepatic cytosolic epoxide hydrolase varied both with the strain of mouse used and with clofibrate or DEHP treatment (Table 1). The specific activities of cytosolic TSO hydrolase from untreated male mice of twelve strains ranged from 5.6 to 11.2 nmol diol $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$. The strains A/J and NZB/B1NJ had high cytosolic epoxide hydrolase activity, whereas the strains C57BL/6J, SW, and DBA/2J had low activity. Other strains had intermediate levels of epoxide hydrolase activity, Treatment of all mice strains with clofibrate or DEHP resulted in induction of cytosolic epoxide hydrolase, and the induction levels ranged between 1.2- and 2.8-fold in the various mouse strains. In general, higher levels of induction were observed in mouse strains with low basal levels of cytosolic epoxide hydrolase and, inversely, lower levels of induction in mouse strains with high basal levels of cytosolic epoxide hydrolase.

The results of the experiments carried out to study

Table 1. Epoxide hydrolase activity in liver cytosol from control, clofibrate, and DEHP-treated mice of various strains

Strain	Epoxide hydrolase activity* [nmol diol formed · min^{-1} · (mg protein)$^{-1}$]		
	Control†	Clofibrate	DEHP
A/J	11.2 ± 0.6^{A}	13.7 ± 1.1‡ (1.2)¶	13.7 ± 1.1‡ (1.2)¶
NZB/B1NJ	9.8 ± 0.4^{AB}	14.4 ± 0.9‡ (1.5)	17.7 ± 0.6§ (1.8)
C3H/HeJ	9.5 ± 0.3^{B}	12.2 ± 0.7§ (1.3)	13.7 ± 0.8‡ (1.4)
AKR/J	9.4 ± 1.5^{BC}	17.5 ± 1.3§ (1.9)	15.8 ± 1.3‖ (1.7)
CBA/J	9.0 ± 2.6^{BC}	17.4 ± 0.3§ (1.9)	17.1 ± 0.3‡ (1.9)
SEC/1ReJ	8.7 ± 0.7^{BC}	13.2 ± 1.6‡ (1.5)	15.3 ± 1.6‡ (1.7)
BALB/cByJ	8.3 ± 0.8^{BCD}	11.9 ± 1.1§ (1.4)	14.5 ± 1.1§ (1.7)
PL/J	8.1 ± 0.7^{BCD}	12.1 ± 0.8§ (1.5)	12.7 ± 0.8‡ (1.6)
C57BL/10J	7.6 ± 0.5^{CDE}	12.3 ± 1.3§ (1.6)	ND**
C57BL/6J	6.7 ± 1.5^{DEF}	12.4 ± 3.3‡ (1.9)	16.2 ± 1.3‖ (2.4)
SW	6.0 ± 0.3^{EF}	12.8 ± 1.4§ (2.1)	13.7 ± 0.9§ (2.3)
DBA/2J	5.6 ± 0.9^{F}	15.7 ± 1.0§ (2.8)	ND

* Activity was measured using TSO as substrate.

† Results are expressed as mean ± standard deviation. Means in the vertical row with the same letter were not significantly different at $P < 0.05$.

‡–‖ Significantly different from control values of the same strain: ‖ $P < 0.05$, ‡ $P < 0.01$ and § $P < 0.001$.

¶ Numbers in parentheses are fold induction of treated animals when compared to basal values obtained from untreated animals.

** Not determined.

cytosolic epoxide hydrolase polymorphism in different strains of mice are presented in Figs. 1 and 2. Molecular weight and antigen-antibody cross-reactivity experiments, as performed by Western blotting and Ouchterlony, revealed that there was no difference in the molecular weight and antigenic reactivity of the cytosolic hydrolase in the different strains of mice studied. Antibodies raised against purified mouse liver cytosolic epoxide hydrolase (SW strain) [22] were able to bind epoxide hydrolase activity from liver cytosolic fractions from different strains of mice, suggesting the presence of similar antigenic determinants of cytosolic epoxide hydrolase in the different strains of control and clofibrate-treated mice. The mitochondrial epoxide hydrolase from control and clofibrate-treated mice of different strains resembled the cytosolic epoxide hydrolase in its molecular weight and antigenic properties ([6], data not shown). The hepatic cytosolic epoxide hydrolase activities from twelve strains in control mice were apparently not very different with respect to heat inactivation and optimum pH. A 10-min treatment of the enzyme in phosphate buffer 0.1 M, pH 6.8, at 60° destroyed 80–95% of the enzyme activity, while incubation at 55° for 10 min either caused a slight increase in activity or destroyed *ca.* 30% of the cytosolic TSO hydrolase activity (data not shown). The pH optima of all strains examined varied between 6.4 and 7.0 with significant variation between individual mice of the same strain.

Table 2 illustrates the basal levels of epoxide hydrolase activity in mitochondrial fraction from

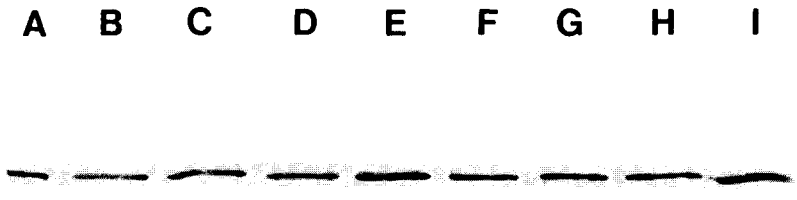

Fig. 1. Detection of hepatic cytosolic epoxide hydrolase by immunoblotting from control and clofibrate-treated male mice of various strains. Clofibrate was administered at a dose of 0.5% (w/w) in diet. (A) Pure cytosolic epoxide hydrolase. (B and C) Crude cytosol from control and clofibrate-treated mice of the BALB/cByJ strain respectively. (D and E) Crude cytosol from control and clofibrate-treated mice of the NLB strain respectively. (F and G) Crude cytosol from control and clofibrate-treated mice of the SEC/1ReJ strain respectively. (H and I) Crude cytosol from control and clofibrate-treated mice of the C3H/HeJ strain respectively.

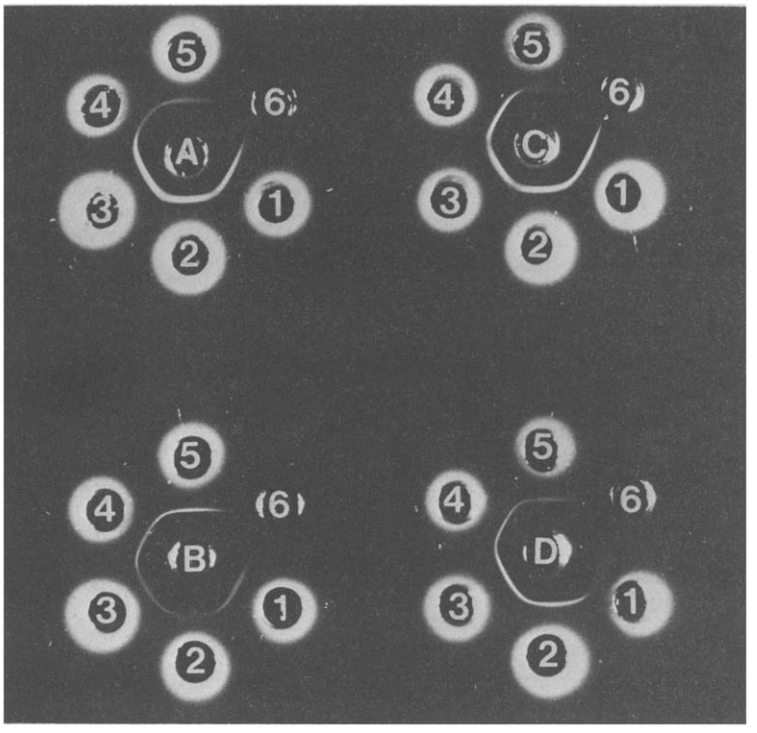

Fig. 2. Double-diffusion analysis of cytosolic epoxide hydrolase from control and clofibrate-treated mice of various strains against antisera to pure cytosolic epoxide hydrolase. All center wells contained 15 μl of antiserum. (Plate A) Wells 1 to 5 clockwise: 20 μl of uninduced cytosol (10%) from NZB/B1NJ, PL/J, A/J, SEC/1ReJ and SW strain of mice respectively. (Plate B) Wells 1 to 4 clockwise: 15 μl of induced cytosol (10%) from NZB/B1NJ, PL/J, A/J, and SEC/1ReJ strains of mice respectively. Well 5 contained 20 μl of uninduced cytosol (10%) from the SW strain. (Plate C) Wells 1 to 5 clockwise: 20 μl of uninduced cytosol (10%) from C57BL/6J, DBA/2J, BALB/cByJ, C57BL/10J and SW strains of mice respectively. (Plate D). Wells 1 to 4 clockwise: 15 μl of induced cytosol (10%) from C57 BL/6, DBA/2J, BALB/cByJ and C57/10J strains of mice respectively. Well 5 contained 20 μl of uninduced cytosol (10%) from the SW strain. Well 6 in all plates contained buffer blanks.

Table 2. Epoxide hydrolase activity in liver mitochondrial fraction from control, clofibrate- and DEHP-treated mice of various strains

Strain	Epoxide hydrolase activity* [nmol diol formed · min^{-1} · (mg protein)$^{-1}$]		
	Control†	Clofibrate	DEHP
C57BL/6J	15.9 ± 1.1^{A}	10.0 ± 2.7‡ (1.6)¶	17.2 ± 5.2 (NS**) (−)¶
CBA/J	14.3 ± 2.4^{AB}	14.6 ± 1.4 (NS) (−)	6.3 ± 1.6§ (2.3)
A/J	12.8 ± 1.0^{BC}	9.6 ± 1.2§ (1.3)	13.2 ± 0.9 (NS) (−)
C57BL/10J	12.0 ± 1.7^{C}	9.6 ± 1.2‡ (1.2)	ND††
PL/J	8.0 ± 0.6^{D}	4.3 ± 0.3‖	6.7 ± 0.7‡ (1.2)
C3H/HeJ	7.1 ± 1.8^{D}	6.2 ± 0.7‡ (1.1)	7.3 ± 0.4 (NS) (−)
SEC/1ReJ	6.9 ± 1.0^{D}	5.2 ± 0.5‡ (1.3)	7.3 ± 0.5 (NS) (−)
DBA/2J	6.5 ± 0.5^{DE}	2.5 ± 0.6‖ (2.6)	ND
BALB/cByJ	6.4 ± 0.7^{DE}	5.8 ± 1.2 (NS) (−)	7.4 ± 0.5 (NS) (−)
NZB/B1NJ	4.8 ± 0.4^{EF}	4.6 ± 0.5 (NS) (−)	4.4 ± 0.6 (NS) (−)
SW	3.9 ± 0.5^{F}	2.0 ± 0.4§ (2.0)	2.8 ± 0.5‡ (1.4)
AKR/J	ND	ND	(−)

* Activity was measured using TSO as substrate.

† Results are expressed as means ± standard deviation. Means in the vertical row with the same letter were not significantly different at $P < 0.05$.

‡–‖ Significantly different from control of the same strain: ‡ $P < 0.05$, § $P < 0.01$, and ‖ $P < 0.001$.

¶ Numbers in parentheses are fold decrease or treated animals when compared to basal values obtained from untreated animals.

** Not significant.

†† Not determined.

control and clofibrate- or DEHP-treated mice. Significant strain variations in the ability of epoxide hydrolase in catalyzing the hydration of TSO were noted in mouse liver mitochondrial fractions. In a number of strains, CBA/J, C57BL/6J and C57BL/10J, the specific activity of TSO hydrolase was higher in the mitochondrial fraction than in the cytosolic fraction. The highest level of mitochondrial epoxide hydrolase activity was found in the C57BL/6J strain, while the lowest level was detected in the SW strain. Dietary administration of clofibrate to mice caused a reduction in epoxide hydrolase activity responsible for the hydrolysis of TSO in the mitochondrial fractions of all the strains examined with the exception of CBA/J, BALB/cByJ and NZB/B1NJ, where no significant alteration in the enzyme activity was observed. In contrast, dietary administration of DEHP caused a significant decrease in TSO hydrolase activity in the mitochondrial fraction only in CBA/J, PL/J and SW mice.

Microsomal epoxide hydrolase activity in twelve strains of mice showed marked variation in their abilities to hydrolyze CSO (Table 3). The enzyme activity ranged between 3.9 and 10.8 nmol diol · min^{-1} · (mg protein)$^{-1}$ among the various strains examined. Strains A/J, AKR/J and C57BL/6J were found to hydrolyze CSO rapidly, whereas strains SEC/1ReJ, PL/J, C3H/HeJ and SW hydrolyzed CSO more slowly. Induction of microsomal epoxide hydrolase activity in all strains of mice, except BALB/cByJ and SEC/1ReJ, was noticed following clofibrate treatment, while with DEHP, induction of microsomal epoxide hydrolase was observed in all strains. However, there was no overall correlation in the metabolism of CSO by microsomal epoxide hydrolase and the metabolism of TSO by epoxide hydrolase in the cytosolic and mitochondrial fractions in the various mouse strains.

The activity of glutathione *S*-transferase using CSO as substrate was also found to vary in control and clofibrate- or DEHP-treated mice from different strains (Table 4). The rate of glutathione conjugate formation was the highest in the C57BL/6J strain as compared to the C3H/HeJ strain which possessed the lowest capability of forming this conjugate. Significant differences, however, were observed in the rate of CSO conjugation among the various strains of mice evaluated in the current study (Table 4). Clofibrate treatment caused an increase in the hepatic cytosolic glutathione-*S*-transferase activity in SW, NZB/B1NJ and PL/J strains of mice but not in any of the other strains studied. However, DEHP treatment caused an increase in cytosolic glutathione *S*-transferase activity in all strains except AKR/J and SW.

DISCUSSION

The present report demonstrates significant inter-strain differences in the activities of hepatic epoxide hydrolases and glutathione *S*-transferase in inbred strains of mice. Although the differences between strains with high and low levels of enzyme activity were statistically significant, the differences do not enable us to classify any strain as having high or low levels of an enzyme purely because of genetic differences. For example, with cytosolic epoxide hydrolase the highest and lowest activities were observed with the A/J and DBA/2J strains respect-

Table 3. Epoxide hydrolase activity in liver microsomes from control, clofibrate- and DEHP-treated mice of various strains

Strain	Epoxide hydrolase activity* [nmol diol formed · min^{-1} · (mg protein)$^{-1}$]		
	Control†	Clofibrate	DEHP
A/J	10.8 ± 1.2^{A}	12.9 ± 0.9‡ (1.2)¶	17.9 ± 1.3§ (1.6)¶
AKR/J	8.2 ± 1.4^{B}	11.7 ± 1.2‖ (1.4)	14.4 ± 0.4‖ (1.7)
C57BL/6J	7.0 ± 1.1^{BC}	9.8 ± 3.5‡ (1.4)	13.4 ± 4.2‡ (1.9)
NZB/B1NJ	6.5 ± 0.5^{CD}	7.5 ± 0.6‡ (1.2)	11.6 ± 1.0§ (1.8)
DBA/2J	6.3 ± 1.1^{CD}	12.5 ± 0.9§ (2.0)	ND**
BALB/cByJ	5.8 ± 0.7^{CDE}	6.6 ± 0.7 (NS††) (−)	8.9 ± 0.6§ (1.5)
CBA/J	5.5 ± 1.7^{DEF}	12.0 ± 0.7§ (2.2)	20.5 ± 3.9§ (3.7)
C57BL/10J	5.4 ± 0.8^{DEF}	6.9 ± 0.5‖ (1.3)	ND
SEC/1ReJ	4.5 ± 0.3^{EFG}	4.8 ± 0.2 (NS) (−)	9.1 ± 0.8§ (2.0)
PL/J	4.2 ± 0.2^{FG}	5.1 ± 0.4‖ (1.2)	6.8 ± 0.5§ (1.6)
C3H/HeJ	3.9 ± 0.5^{G}	5.6 ± 0.1§ (1.4)	8.2 ± 0.9§ (2.1)
SW	3.9 ± 0.3^{G}	6.8 ± 0.3‖ (1.7)	9.3 ± 0.3§ (2.4)

* Activity was measured using C50 as substrate.

† Results are expressed as means ± standard deviation. Means in the vertical row with the same letter were not significantly different at $P < 0.05$.

†–‖ Significantly different from control values of the same strain: † $P < 0.05$, § $P < 0.001$, and ‖ $P < 0.01$.

¶ Numbers in parentheses are fold induction of treated animals when compared to basal values obtained from untreated animals.

** Not determined.

†† Not significant.

Table 4. Effects of clofibrate and DEHP on glutathione-*S*-transferase activity in liver cytosol in various strains of mice

Strain	Gluthathione-*S*-transferase activity* [nmol glutathione conjugate formed·min^{-1}·(mg protein)$^{-1}$]		
	Control†	Clofibrate	DEHP
C57BL/6J	44.0 ± 8.0^{A}	51.5 ± 15.0 (NS‡) (−)	85.0 ± 22.0‖ (1.9)§
AKR/J	41.0 ± 18.0^{AB}	36.0 ± 12.0 (NS) (−)	38.8 ± 6.0 (NS) (−)
DBA/2J	40.0 ± 9.0^{AB}	32.0 ± 8.0 (NS) (−)	ND††
SW	32.0 ± 7.0^{BC}	47.0 ± 8.0‖ (1.5)	37.0 ± 5.0 (NS) (−)
CBA/J	29.8 ± 6.0^{C}	27.2 ± 7.0 (NS) (−)	45.6 ± 21.0‖ (1.5)
A/J	26.2 ± 4.0^{CD}	31.8 ± 6.9 (NS) (−)	54.3 ± 17.3‖ (2.2)
C%[L/10J	17.8 ± 6.0^{DE}	23.0 ± 10.0 (NS) (−)	ND
NZB/B1NJ	13.4 ± 3.1^{EF}	18.2 ± 3.1‖ (1.4)	17.3 ± 1.2‖ (1.3)
SEC/1ReJ	13.1 ± 1.9^{EF}	17.0 ± 3.2 (NS) (−)	28.5 ± 3.4¶ (2.2)
PL/J	13.0 ± 1.1^{EF}	28.0 ± 8.0‖ (2.0)	35.5 ± 8.0** (2.7)
BALB/cByJ	11.5 ± 2.0^{EF}	9.7 ± 0.9 (NS) (−)	35.2 ± 0.6¶ (3.0)
C3H/HeJ	7.1 ± 1.0^{F}	8.5 ± 1.2 (NS) (−)	16.6 ± 3.0¶ (2.3)

* Activity was measured using CSO as substrate.

† Results are expressed as means ± standard deviation. Means in the vertical row with the same letter were not significantly different at $P < 0.05$.

‡ Not significant.

§ Numbers in parentheses are fold induction of treated animals when compared to basal values obtained from untreated animals.

‖–** Significantly different from control values of the same strain: ‖ $P < 0.05$, ¶ $P < 0.001$, and ** $P < 0.01$.

†† Not determined.

ively. While the differences between these two strains were statistically significant, these levels could easily be explained as those distributed about a mean as observed by a range of tissue levels in other strains.

Strain differences in the cytosolic epoxide hydrolase activity have been noted previously and the lowest level of cytosolic epoxide hydrolase activity was recorded in the SW strain, whereas the highest level of enzyme activity was found in the AKR/J and C57BL/6J strains [2]. Although a different substrate was used in the earlier studies, the relative levels reported previously are similar to the values reported here with TSO as substrate.

Striking similarities in the properties of cytosolic epoxide hydrolase were observed when the liver cytosol from various strains was examined for its ability to hydrolyze TSO as the substrate. These similar properties of cytosolic epoxide hydrolase in the different strains were reflected by the similarity in pH optima, heat stability, molecular weight and immunological characteristics. Thus, it appears that quantitative rather than qualitative differences in the activity of epoxide hydrolase in cytosolic fraction are responsible for the strain differences.

Treatment of mice with clofibrate or DEHP resulted in an induction of cytosolic epoxide hydrolase. Although all of the mouse strains were responsive to clofibrate treatment, there was a substantial difference in the induction levels between strains. Similar results were obtained when DEHP was used for induction of cytosolic epoxide hydrolase. It is not possible to conclude, therefore, that this difference in responsiveness in the various mice strains is due to genetic or non-genetic factors because all mice strains responded to clofibrate or DEHP, although to varying levels. Potentially, differences in the rate of metabolism of clofibrate or DEHP in the various mice strains could result in differences in the bioavailability of clofibrate or DEHP, thereby causing varying epoxide hydrolase induction. Alternatively, differences observed in the increase of epoxide hydrolase activity in these mice strains following clofibrate or DEHP treatment suggests the possibility that there may exist quantitative differences in a receptor [25], a product of regulatory genes, to which clofibrate or DEHP may bind to exert its effects. Irrespective of the mechanism, the induction of cytosolic epoxide hydrolase activity by clofibrate and probably also DEHP is due to an increase in the *de novo* synthesis of cytosolic epoxide hydrolase [26].

Differential induction of cytosolic epoxide hydrolase has been noted earlier in four strains of mice following administration of another hypolipidemic drug, nafenopin [14]. Our results are also in general agreement with published reports [15] where clofibrate or DEHP treatment was observed to cause a 2.5- and 1.6-fold induction of cytosolic epoxide hydrolase activity, respectively, in the SW strain.

Epoxide hydrolase activity in the cytosolic and mitochondrial fractions has been shown previously to have similar immunological and other properties [6]. However, in contrast to the cytosolic epoxide hydrolase, epoxide hydrolase activity in the mitochondrial fraction declined or remained unaltered upon clofibrate or DEHP treatment. The extent of reduction of enzyme activity varied among different strains. This decrease in enzyme activity is in agreement with our earlier findings where clofibrate treatment of mice caused an elevation of epoxide hydrolase activity in the liver cytosol but resulted in a decrease

in the crude mitochondrial fraction and in purified peroxisomal fractions [4]. The decrease in enzyme activity in the mitochondrial fraction could be attributed to the large increase in total protein content due to peroxisomal proliferation following clofibrate or DEHP treatment [4, 27].

Interstrain variations were also noted in the distribution pattern of the liver microsomal epoxide hydrolase using CSO as substrate. Differences in the basal levels of mouse liver microsomal epoxide hydrolase in the various mouse strains and their differential induction by clofibrate and DEHP confirm earlier observations of existing variations in different strains of mice. Such variations in inbred mice strains have also been noted when styrene oxide and benzo[*a*]pyrene 4,5-oxide were used as substrates [11, 12, 28]. Like the results obtained here, administration of phenobarbital to various strains of mice resulted in differential induction of microsomal epoxide hydrolase [29]. Similar differential induction of microsomal epoxide hydrolase by TSO and 2-acetylaminofluorene has also been observed in rat strains [29, 30]. Two distinct phenotypes of the microsomal epoxide hydrolase were observed in various mouse strains based on differences in their pH optima and heat sensitivity. The locus of the microsomal epoxide hydrolase, *Eph*-1, was located on chromosome 1, and linked to two loci on that chromosome [13]. However, using a number of criteria (pH optima, heat stability, molecular weight, antigenic similarity, responsiveness to clofibrate or DEHP), we were unable to show polymorphism of the cytosolic epoxide hydrolase in the strains examined.

Marked differences in the basal levels of glutathione-*S*-transferase, an important enzyme for detoxication of many hazardous chemicals, were observed in the cytosolic fractions from various strains of mice. In contrast to the low level of cytosolic epoxide hydrolase observed in the DBA/2J strain, this strain showed a remarkably higher capacity to form glutathione conjugates, a reaction catalyzed by glutathione-*S*-transferase. However, the enzyme in most of the strains showed no change in its ability to metabolize CSO following clofibrate treatment except in the PL/J, NZB/B1NJ and SW strains of mice. In contrast, significant induction of this enzyme was observed in most mouse strains following DEHP treatment.

Although there were significant differences in the basal levels of epoxide-metabolizing enzyme in various mouse strains, and these enzymic activities were increased differentially in the various strains following clofibrate and DEHP treatment, it is uncertain whether these differences can lead to substantial changes in the metabolic pattern of any given compound, and thus produce corresponding differences in biological and toxicological effects.

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