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EFFECT OF DIETARY CLOFIBRATE ON EPOXIDE HYDROLASE ACTIVITY IN TISSUES OF MICE

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Abstract—The effects of dietary clofibrate on the epoxide-metabolizing enzymes of mouse liver, kidney, lung and testis were evaluated using *trans*-stilbene oxide as a selective substrate for the cytosolic epoxide hydrolase, *cis*-stilbene oxide and benzo[*a*]pyrene 4,5-oxide as substrates for the microsomal form, and *cis*-stilbene oxide as a substrate for glutathione *S*-transferase activity. The hydration of *trans*-stilbene oxide was greatest in liver followed by kidney > lung > testis. Its hydrolysis was increased significantly in the cytosolic fraction of liver and kidney of clofibrate-treated mice and in the microsomes from the liver. Isoelectric focusing indicates that the same enzyme is responsible for hydrolysis of *trans*-stilbene oxide in normal and induced liver and kidney. Clofibrate induced glutathione *S*-transferase activity on *cis*-stilbene oxide only in the liver. Hydrolysis of both *cis*-stilbene oxide and benzo[*a*]pyrene 4,5-oxide was highest in testis followed by liver > lung > kidney. Hydration of *cis*-stilbene oxide was induced significantly in both liver and kidney by clofibrate but that of benzo[*a*]pyrene 4,5-oxide was induced only in the liver. These and other data based on ratios of hydration of benzo[*a*]pyrene 4,5-oxide to *cis*-stilbene oxide in tissues of normal and induced animals indicate that there are one or more novel epoxide hydrolase activities which cannot be accounted for by either the classical cytosolic or mirosomal hydrolases. These effects are notable in the microsomes of kidney and especially in the cytosol of testis.

Epoxides occur naturally in the environment or arise from the oxidative metabolism of olefinic and aromatic compounds. Some epoxides are electrophilically reactive metabolites and may bind covalently to cellular macromolecules to produce toxic, mutagenic and/or carcinogenic effects. In the liver, epoxides can undergo enzymatic conjugation with glutathione mediated by cytosolic glutathione *S*-transferases (cGST†, EC 2.5.1.18) or can be hydrolyzed to diols by epoxide ether hydrolases (EHs, EC 3.3.2.3). Two distinct EHs acting on xenobiotics have been described: a predominantly membrane-bound enzyme (mEH) and a predominantly soluble enzyme (cEH). In addition to differences in substrate specificity, pH optima and antigenic determinants, induction of cEH in the liver has been observed with hypolipidemic peroxisome proliferating agents such as clofibrate, di-(2-ethylhexyl)phthalate, and 2-ethyl-1-hexanol [1]. However, cEH activity was not increased following treatment of mice with a variety of other inducers of mEH, cGST and other xenobiotic metabolizing enzymes. In this study, the effect of clofibrate treatment on epoxide-metabolizing enzymes in mouse liver, kidney, lung and testis was investigated to see whether the induction caused by clofibrate is confined to the liver or also is manifested in extrahepatic organs.

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† Abbreviations: cGST, cytosolic glutathione *S*-transferase; mEH, microsomal epoxide hydrolase; cEH, cytosolic epoxide hydrolase; EH, epoxide hydrolase; CSO, *cis*-stilbene oxide; TSO, *trans*-stilbene oxide; BPO, benzo[*a*]pyrene 4,5-oxide; and GSH, glutathione.

MATERIALS AND METHODS

Chemicals. Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) was provided by Ayerst Laboratories (New York, NY). *cis*-Stilbene oxide (CSO) was synthesized by peracid oxidation of the olefin with *m*-chloroperoxybenzoic acid as reported earlier [2]. *trans*-Stilbene oxide (TSO), *cis*-stilbene, *n*-dodecane (99%) and hexanol (98%) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Bovine serum albumin (fraction V) and glutathione (GSH) were obtained from the Sigma Chemical Co. (St. Louis, MO). Bio-Rad protein dye reagent was purchased from Bio-Rad Laboratories (Richmond, CA). ACS scintillation mixture was purchased from Amersham (Arlington Heights, IL).

Radiochemicals. [^{3}H]CSO and [^{3}H]TSO were prepared by base treatment of the corresponding chlorohydrin obtained by reduction of desyl chloride by sodium borotritide [3]. [^{3}H]Benzo[*a*]pyrene 4,5-oxide (BPO) (288 mCi/mmole) and unlabeled BPO were prepared by the Midwest Research Institute (Kansas City, MO).

Animals. Male Swiss–Webster mice (7- to 8-weeks-old, 25–30 g) were purchased from Bantin & Kingman (Fremont, CA) and were housed in steel cages with pine shavings for bedding in an environmentally controlled room (23 ± 1.5°, 14:10 hr light:dark). Food (Purina Rodent Chow) and water were provided *ad lib.* Clofibrate was dissolved in corn oil and mixed into ground chow at a concentration of 0.5% (w/w). Control animals received ground chow with similar amounts of corn oil added (10 ml/100 g). Experimental animals were given clofibrate-containing diet for 14 days.

Enzyme preparation. Mice were killed by cervical dislocation between 9:00 and 10:00 a.m. Livers were removed, perfused with cold 1.15% KCl, and placed in sodium phosphate buffer (76 mM, pH 7.4). Kidneys, testes and lungs were removed, dissected free of adhering tissues and rinsed in phosphate buffer. All organs except liver were pooled from groups of three to four animals. They were weighed and minced and homogenized in phosphate buffer with a Polytron for 20 sec. Homogenates of livers and kidneys were made to 10% (w/w) and those of testes and lungs to 5% (w/w) of the organ weight. They were centrifuged at 10,000 *g* for 20 min and the supernatant fractions were centrifuged at 105,000 *g* for 60 min. The cytosol (105,000 *g* supernatant) was collected after drawing off the fatty layer. Pellets were resuspended in 50 mM Tris–HCl (pH 7.4), 125 mM KCl and recentrifuged. Washed microsomes were resuspended in phosphate buffer (76 mM, pH 7.4) to give 10% or 5% homogenate based on the initial homogenization.

Enzyme assays. cEH, mEH and cGST activities with TSO and CSO as substrate were measured by partition assays as described previously [3]. [^{3}H]-TSO and [^{3}H]CSO were diluted to a specific activity of 4.5 mCi/mmole with a 5 mM concentration of the corresponding unlabeled epoxide. One microliter of substrate in ethanol was added to 100 μl of diluted cell fraction to obtain a final substrate concentration of 0.05 mM. cEH activity was determined with TSO in 76 mM sodium phosphate buffer (pH 7.4), mEH with CSO in 100 mM Tris–HCl (pH 9), and cGST with CSO and 5 mM GSH in 76 mM phosphate buffer (pH 7.4). The hydrolysis of BPO was determined by a modification of the method of Jerina *et al.* [4] as described in Hammock and Ota [1]. [^{3}H]BPO was diluted to 3 mCi/mmole with a 5 mM solution of unlabeled substrate. The final substrate concentration was 0.05 mM. All assays were performed in triplicate. Protein concentration in the various fractions was determined using a modification of the method of Bradford [5].

Isoelectric focusing. Cytosols from livers and kidneys of control and clofibrate-treated animals were analyzed by isoelectric focusing on an LKB 2117 Multiphor apparatus. Twenty microliters of each sample were applied to filter paper squares on a 5% polyacrylamide gel. Samples were analyzed by wide range (pH 3.5 to 9.5) and by narrow range (pH 4 to 6) isoelectric focusing at 4° according to the procedure of Winter *et al.** The gel tracks were sliced into 0.5 cm fractions and eluted in 0.5 ml phosphate buffer for 16 hr at 4° before measuring EH activity. The pH gradient was determined from 0.5 cm gel fractions extracted with glass-distilled water and measured with a Corning (model 125) pH meter at 4°.

RESULTS AND DISCUSSION

The standard assay conditions for measuring cEH, mEH and cGST in mouse liver with TSO and CSO have been described previously [3]. Using these conditions, product formation with incubation time was monitored in the cytosol of kidney, lung and testis (Fig. 1, A–C). Hydrolysis of TSO in the cytosol at pH 7.4 (cEH) was highest in kidney, followed by testis and lung. Activity of cGST measured with CSO also ranked in this order (Fig. 1C), while the conversion of CSO to diol at pH 9.0 (mEH) (Fig. 1B) was higher in testis than in kidney and lung. The formation of product was linear with time for all enzymes except for cGST in kidney, the activity of which did not increase proportionally after 30 min of incubation due to substrate depletion.

Gill and Hammock [6] measured cEH activity in extrahepatic organs of male Swiss–Webster mice using a trisubstituted epoxide [1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene or ethyl epoxide] as substrate. The order of increasing activity was liver > kidney > lung > testis. It is interesting that with both substrates, TSO and the ethyl epoxide, activity in the kidney represented about 57% of that found in liver (Table 1, Fig. 1), whereas the activity detected in testis relative to liver was higher when measured with TSO than with the ethyl epoxide.

The membrane-bound form of epoxide hydrolase (mEH) has been studied extensively and its activity measured in different organs and strains of mice [7, 8]. Since mEH activity usually has been monitored with either styrene oxide and/or BPO, mEH activity in the microsomal fraction of liver, kidney, lung and testis was measured using both CSO and BPO. With both substrates, the specific activity was highest in testis, then liver, lung and kidney (Table 1). The high mEH activity measured in testes of Swiss–Webster mice was present also in the NMR1 strain [8, 9]. Kidneys, on the other hand, possessed very low activity for both CSO and BPO. Oesch and Schmassmann [9] reported a similar tissue distribution pattern in NMR1 mice, while CD-1 mice showed much higher mEH activity in kidneys [10, 11]. With a few exceptions discussed later, the ratio of specific activities with the two substrates used in this study was found to be very similar in all the organs examined (Table 1) and suggests that, in these organs, much of the hydration of both substrates is catalyzed by the same or similar microsomal enzymes. This observation has been demonstrated previously for rat liver using immunoprecipitation [12].

In liver, a membrane-bound enzyme similar to cEH has been characterized in the microsomal fraction [13], and the presence of mEH-like activity has been described in the cytosol of rat [14], primate and human liver [15]. Hydrolysis of TSO at pH 7.4 and CSO and BPO at pH 9 was therefore measured to detect activity of cEH in microsomes and mEH in cytosol, respectively, of liver and extrahepatic tissues. BPO is a more specific substrate for mEH than CSO and is not metabolized by the cytosolic form [16, 17]; thus, its hydrolysis in the cytosol was compared to that of CSO. Enzyme activities are expressed as their concentrations in the various organs (nmoles/min/g tissue) to account for the difference in protein content between the cytosolic and microsomal fractions (Table 1). When affinity purified cEH from mouse liver was incubated in simultaneous experiments with TSO, CSO and BPO

* A. Winter, K. Ek and U. B. Anderson, LKB application note 250 (1977).

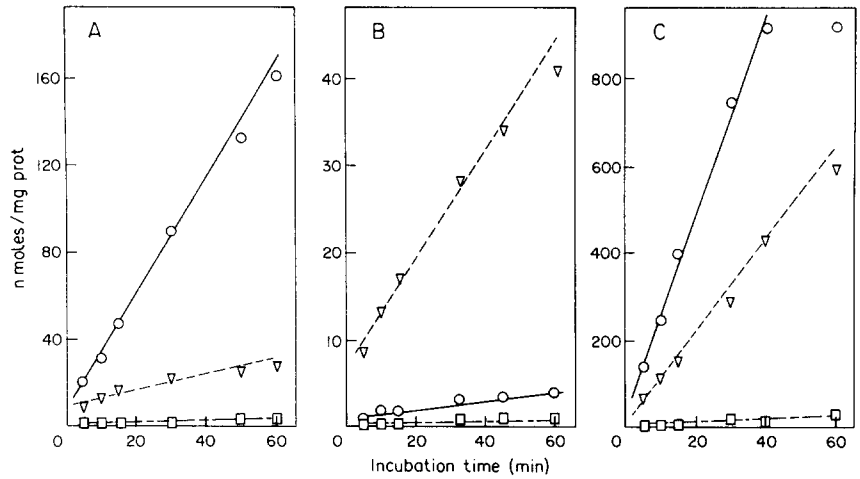

Fig. 1. Product formation with time in extrahepatic cytosol. Cytosols prepared from kidney (○——○), testis (△- - -△), and lung (□-·-·-□) of male mice were incubated at 37° with ^{3}H-labeled epoxides for the times indicated. After incubation, the epoxides were extracted with organic solvent, and aliquots of the aqueous phase were taken for determination of diol formation. (A) cEH, 5×10^{-5} M TSO at pH 7.4, (B) mEH, 5×10^{-5} M CSO at pH 9.0, and (C) cGST, 2.5×10^{-5} M CSO, 2.5 mM GSH, at pH 7.4. Points are the average of triplicate determinations from two experiments which used different preparations of cytosol each pooled from three to four mice. Within run variation of the assays was always less than 10% of the reported value.

at pH 7.4 and 9.0, CSO at pH 9 was found to be metabolized at about 3% of the rate of TSO at pH 7.4 under conditions approaching V_{max} while enzymatic hydration of BPO was not distinguishable from background values (D. N. Loury and G. Prestwich, unpublished observations). This information on the isolated enzymes compares favourably with previous radiochemical and chromatographic measurements [1–3]. Thus, in Table 1 total CSO activity at pH 9 is expressed as well as the CSO activity corrected for the CSO activity expected of the cEH.

In mice treated with 0.5% clofibrate for 14 days, a significant increase in the relative liver weight and cytosolic and microsomal protein was noted (Table 2). This hepatomegaly induced by clofibrate and other hypolipidemic agents is well known in mice [18, 19] and characteristically is associated with the peroxisomal proliferation and increase in smooth endoplasmic reticulum in liver cells [20]. In extrahepatic tissues, however, treatment with clofibrate did not result in an increase in relative organ weights (Table 2). Protein recovery from cytosolic and microsomal fractions of kidneys, testes and lungs remained unchanged following exposure to clofibrate. Clofibrate and other hypolipidemic peroxisome proliferators have been reported to increase the peroxisomal palmitoyl-CoA oxidizing capacity and several other enzyme activities in kidneys from mice as well as rabbits and rats. Since peroxisome proliferation appears restricted to the proximal convoluted tubular epithelium, some clofibrate effects could be masked in homogenates in whole kidneys [21–25].

Based upon previous observations that peroxisomal proliferating drugs can induce cEH activity in hepatic tissues [1], the effect of dietary clofibrate on extrahepatic tissues was observed. In this study, cEH activity in the cytosol measured as TSO hydrolysis was increased by 50% in the kidney of mice treated with clofibrate. This induction was weaker than that observed in liver but more selective since renal mEH and cGST were affected less by clofibrate (Table 1). This significant ($P < 0.05$) induction of cEH activity was observed in kidney whether the results were expressed on a per gram tissue basis (Table 1) or on a nmole/min/mg protein basis (2.90 ± 0.40 and 4.41 ± 0.62 control and induced, respectively) or as nmole/min/organ of 100 g mouse (290 ± 40 and 400 ± 40). Significant induction ($P < 0.05$) was seen also in the liver on a nmole/min/mg protein basis (5.0 ± 0.6 and 10.9 ± 2.7) and on a nmole/min/organ basis (2000 ± 250 and 7700 ± 1900).

In the liver, 0.7% of the TSO activity at pH 7.4 in the cytosol was present in the microsomes, while in other organs the cEH-like activity measured in microsomes only represented 0.3% of the cytosolic activity. Although clearly present and possibly significant in xenobiotic metabolism, the microsomal bound cEH-like activity on TSO represents but a small proportion of the enzymatic activity on TSO in an S9 preparation. This hydrolytic activity on TSO present in washed microsomes also was induced significantly ($P \leq 0.05$) in microsomes of liver regardless of how the data are expressed. The induction observed in the kidney was not significant at this level. These data provide further evidence for the similarity of the enzyme which hydrates TSO in the cytosol and in the microsomes of mouse liver.

No significant induction of TSO hydrolysis was observed in either the cytosol or microsomes of testes or lungs regardless of expression of the data as specific activity, on an organ basis or on a per gram tissue basis as shown in Table 1. Thus, of the tissue examined, clofibrate seems to induce the hydration of TSO selectively in the kidney and liver of mice.

Table 1. Activities (nmoles/min/g organ) of epoxide-metabolizing enzymes in four tissues of Swiss–Webster mice with and without exposure to 0.5% dietary clofibrate

		Microsomes				Cytosol				
		mEH-like activity			cEH	cEH	cGST	mEH-like activity		
Tissue	Treatment	CSO pH 9.0 (corrected)*	BPO pH 9.0	BPO/CSO	TSO pH 7.4	TSO pH 7.4	CSO with GSH, pH 7.4	CSO pH 9.0 (corrected)*	BPO pH 9.0	BPO/CSO
Liver†	Control	29.3 ± 9.2 (29.2)	26.3 ± 10.6	0.90 (0.90)	2.26 ± 0.64	333 ± 41	2140 ± 600	33.5 ± 4.7 (23.5)	4.59 ± 0.68	0.14 (0.19)
	Clofibrate	74.0 ± 14.3‡ (73.8)	61.1 ± 9.5‡	0.82 (0.83)	6.78 ± 2.10‡	810 ± 200‡	3830 ± 340‡	86.2 ± 13.0‡ (61.9)	19.6 ± 9.4‡	0.23 (0.32)
Kidney§	Control	0.44 ± 0.09 (0.42)	0.76 ± 0.17	1.73 (1.80)	0.54 ± 0.08	149 ± 21	2340 ± 190	6.46 ± 0.61 (1.99)	0.68 ± 0.27‖	0.10¶ (0.34)
	Clofibrate	0.55 ± 0.06‡ (0.53)	0.63 ± 0.17	1.14 (1.19)	0.80 ± 0.44	223 ± 33‡	2520 ± 240	8.50 ± 0.18‡ (1.81)	0.25 ± 0.23‡‖	0.03¶ (0.14)
Testis§	Control	22.4 ± 5.4 (22.4)	23.1 ± 6.3	1.03 (1.03)	0.12 ± 0.05	45.5 ± 4.2	1160 ± 290	33.2 ± 7.2 (31.8)	2.56 ± 0.91	0.08 (0.08)
	Clofibrate	24.1 ± 6.3 (24.1)	24.5 ± 5.7	1.02 (1.02)	0.17 ± 0.07	45.7 ± 11.9	1200 ± 320	32.8 ± 17.4 (31.4)	2.40 ± 2.17	0.07 (0.08)
Lung§	Control	2.71 ± 1.0 (2.71)	3.64 ± 1.63	1.34 (1.34)	0.03 ± 0.04	7.57 ± 1.91	860 ± 90	1.70 ± 0.37 (1.47)	1.21 ± 0.24‖	0.71 (0.82)
	Clofibrate	2.74 ± 0.95 (2.74)	2.54 ± 1.36	0.93 (0.93)	0.02 ± 0.01	6.59 ± 1.02	830 ± 100	1.92 ± 0.78 (1.72)	0.29 ± 0.17‖	0.15 (0.17)

* Values in parentheses are corrected for the hydration of CSO caused by the cEH in the subcellular fraction indicated. Homogeneous cEH from the cytosol of mouse liver has been found to hydrate CSO at pH 9 at ~3% of the rate of TSO at 7.4 (D. N. Loury and G. Prestwich, unpublished).

† Values represent mean ± S.D. from three to four mice treated individually from each group.

‡ Significantly different from control values ($P \leq 0.05$, Student's *t*-test).

§ Values represented mean ± S.D. from four to five individual experiments performed on fractions from pooled organs of three to four animals per group.

‖ Values approach limit of sensitivity of assay.

¶ Since the high level of cEH can metabolize CSO at 3% the rate of TSO, these ratios are questionable.

Table 2. Effect of dietary clofibrate on relative organ weight and protein content of four tissues of Swiss–Webster mice

Tissue	Treatment	Relative organ wt* (g/100 g body wt)	Microsomal protein (mg/g)	Cytosolic protein (mg/g)
Liver†	Control	5.97 ± 0.89	10.4 ± 1.1	64.3 ± 3.2
	Clofibrate	9.17 ± 0.51‡	13.0 ± 0.6‡	76.8 ± 8.9‡
Kidney§	Control	1.90 ± 0.04	4.34 ± 0.89	51.3 ± 1.9
	Clofibrate	1.93 ± 0.04	4.60 ± 0.46	50.4 ± 2.6
Testis§	Control	0.56 ± 0.06	4.49 ± 0.71	40.3 ± 5.0
	Clofibrate	0.58 ± 0.05	4.43 ± 0.57	38.1 ± 4.2
Lung§	Control	0.64 ± 0.06	1.90 ± 0.44	63.6 ± 7.2
	Clofibrate	0.68 ± 0.04	1.86 ± 0.47	66.0 ± 4.1

* No difference in body wt was observed between control and clofibrate-treated mice.
† Values represent mean ± S.D. of four mice treated individually from each group.
‡ Significantly different from control ($P \leq 0.05$, Student's t-test).
§ Values represent mean ± S.D. of five experiments using pooled organs from three to four mice in each group.

No induction of the other enzymes examined was observed in extrahepatic tissues. The apparent induction of CSO hydrolysis at pH 9.0 in kidney cytosol can be attributed largely to the increased metabolism of CSO by cEH (Table 1). Although peroxisomes have been identified in Leydig cells of rodent testes [26] and in bronchiolar and alveolar epithelium [27–29], their response to treatment with hypolipidemic compounds in these organs has not been studied. Since the induction of cEH appears to be coupled with peroxisome proliferation in the liver and kidney, the lack of enzyme induction in testis and lung may reflect the absence of proliferation following clofibrate treatment in the latter tissues which may, in turn, be associated with a lack of receptor [30].

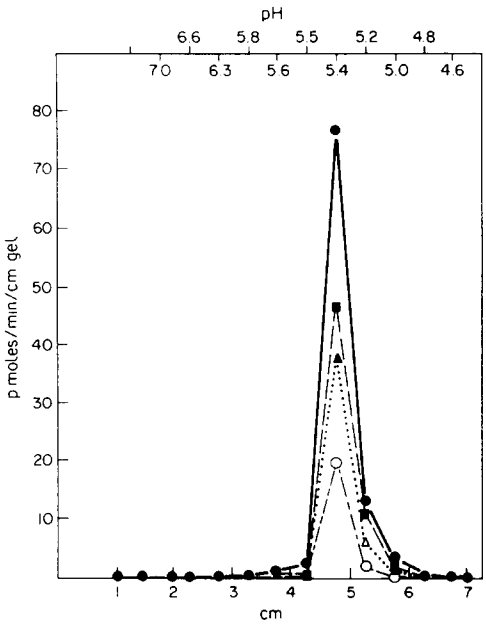

Fig. 2. Epoxide hydrolase activity in fractions from analytical isoelectric focusing. Bottom abscissa indicates centimeter of gel track while top abscissa represents the pH of each fraction. Key: control liver (●——●), induced liver (■– – –■), control kidney (○– – –○) and induced kidney (▲- - - -▲). Recoveries were 30–40% of the enzyme activity applied to the gel.

cGST activity with CSO as a substrate was increased significantly in liver but not other tissues in mice fed clofibrate (Table 1). In an earlier study [1], varying effects on cGST were seen with three peroxisome proliferators, and clofibrate caused a significant decrease ($P < 0.05$) in activity monitored with the more general substrate 1,2-dichloro-4-nitrobenzene. Several explanations are available for this phenomenon including the slight possibility that residual clofibrate or clofibric acid could differentially inhibit or stimulate cGST isozymes as has been observed for related compounds [31, 32] or that clofibrate could differentially induce cGST isozymes or activities [33–36].

Data from narrow range analytical isoelectric focusing indicated that the cytosolic EH from liver and kidney of control and clofibrate-treated animals showed a single peak of activity containing 76–84% of the total activity recovered from the gel (Fig. 2) and focusing at a pH of approximately 5.4. Enzyme activity was recovered in a single fraction from each of the four samples (data not shown) from wide range isoelectric focusing, indicating that all of the enzyme activity would appear on a narrow range gel. These results suggest that the cytosolic enzyme present in kidney is similar to that found in liver and that its activity is induced by clofibrate in both organs. As proposed earlier for liver [1], these data further indicate that the increase in TSO hydrolysis observed in the cytosol of animals exposed to clofibrate is due to an increase in an existing enzyme rather than to the production of a novel protein.

As noted earlier [1], clofibrate induced mEH activity on both CSO and BPO in the livers of mice. Hydration of CSO was also induced significantly in kidney microsomes. This observation is interesting in that there was a corresponding slight decrease in BPO hydrolysis. These trends are illustrated by the largest decrease in the BPO/CSO ratio between control and induced animals seen in the microsomes

and may be evidence for a new enzyme form. This phenomenon did not appear to extend to other tissues within the limits of this experimental design and a significance of $P \leq 0.05$. However, BPO hydrolysis was decreased slightly in lungs as well. Similarly, cGST activity was induced significantly in the liver while significant differences were not found in the other tissues (Table 1).

Substantial hydrolysis of CSO at pH 9.0 was observed in cytosolic fractions. As discussed above, this activity is due partially to a slow hydration of CSO by the cytosolic EH. For instance, assuming that the renal cEH possesses a substrate specificity similar to the hepatic enzyme, the low CSO activity at pH 9.0 in kidney cytosol, which represents 3% of the rate of TSO hydration at pH 7.4, could reflect largely the slow activity of the soluble cEH enzyme on CSO. In the other tissues examined, the relative ratios of hydrolytic activity of CSO to TSO were much higher; thus the classical cEH contributed much less to the metabolism of CSO in the cytosol.

In the liver, lung and especially the testis, however, the activity observed with CSO at pH 9.0 may result from an enzyme that preferentially hydrolyzes CSO over BPO (Table 1). With the hepatic microsomal enzyme, it has been demonstrated that CSO and BPO are hydrated at very similar rates [1, 3]. This similarity is obvious in the microsomes of several of the tissues shown in Table 1, notably the liver and testis. In the liver cytosol, it is clear that a significant proportion of the hydrolytic activity on CSO at pH 9 cannot be accounted for by the activity of the microsomal enzyme measured with BPO, even after the CSO values are corrected for cEH activity. This increase in CSO relative to BPO hydrolysis in liver cytosol is illustrated by the decrease in the BPO/CSO hydrolysis rate from about 1 in microsomes to 0.19 and 0.32 in control and clofibrate-induced cytosol respectively. Also the CSO hydrolysis in the cytosol is significantly higher than that of BPO ($P \leq 0.05$).

Although the absolute enzyme activities were much lower in the lungs, there was again evidence for a novel enzyme activity. Following dietary exposure to clofibrate, there was an observed decrease in the rate of TSO hydrolysis and a significant decrease in the rate of BPO hydrolysis in the cytosol. Surprisingly, hydrolytic activity on CSO remained the same or slightly increased, leading to a dramatic decrease in the BPO/CSO ratio following induction (Table 1).

The situation becomes even more dramatic in the testis because the cEH activity measured on TSO was rather low and thus it contributed a negligible amount to the hydrolysis of CSO at pH 9. In cytosolic fractions from animals on either control or clofibrate-containing diets, the hydrolysis of CSO was significantly higher than the hydrolysis of BPO ($P \leq 0.05$). This observation again was reflected in a BPO/CSO ratio of 0.08 compared to approximately 1 in the microsomal fraction of testis.

This increase could be due to a variety of factors. One possibility is that the microsomal epoxide hydrolase has altered specificity for BPO and CSO as it is removed from the membrane of the endoplasmic reticulum. Possible evidence against this hypothesis is that, if there were but one form of the solubilized mEH, one would expect a similar ratio of BPO to CSO activity in the cytosol of the liver and testis, and this clearly is not the case. One could make a similar argument for a slightly altered form of the cEH. However, the most obvious explanation is that there is a hydrolase present in the cytosol of some tissue which hydrates TSO, CSO, and BPO with rates different from that observed with the classical cEH or mEH.

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REFERENCES

1. B. D. Hammock and K. Ota, *Toxic. appl. Pharmac.* **71**, 254 (1983).
2. C. A. Mullin and B. D. Hammock, *Archs Biochem. Biophys.* **216**, 423 (1982).
3. S. S. Gill, K. Ota and B. D. Hammock, *Analyt. Biochem.* **131**, 273 (1983).
4. D. M. Jerina, P. M. Dansette, A. Y. L. Lu and W. Levin, *Molec. Pharmac.* **13**, 342 (1977).
5. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
6. S. S. Gill and B. D. Hammock, *Biochem. Pharmac.* **29**, 389 (1980).
7. C. H. Walker, P. Bentley and F. Oesch, *Biochim. biophys. Acta* **539**, 427 (1978).
8. F. Oesch, H. Glatt and H. Schmassmann, *Biochem. Pharmac.* **26**, 603 (1977).
9. F. Oesch and H. Schmassmann, *Biochem. Pharmac.* **28**, 171 (1979).
10. L. Cantoni, M. Salmona, T. Facchinetti, C. Pantarotto and G. Belvedere, *Toxic. Lett.* **2**, 179 (1978).
11. G. M. Pacifici, A. R. Boobis, M. J. Brodie, M. E. McManus and D. S. Davies, *Xenobiotica* **11**, 73 (1981).
12. T. M. Guenthner, B. D. Hammock, U. Vogel and F. Oesch, *J. biol. Chem.* **256**, 3163 (1981).
13. T. M. Guenthner and F. Oesch, *J. biol. Chem.* **258**, 15054 (1983).
14. S. S. Gill, S. I. Wie, T. M. Guenthner, F. Oesch and B. D. Hammock, *Carcinogenesis* **3**, 1307 (1982).
15. S. S. Gill, K. Ota, B. Ruebner and B. D. Hammock, *Life Sci.* **32**, 2693 (1983).
16. F. Oesch and M. Golan, *Cancer Lett.* **9**, 169 (1980).
17. B. D. Hammock and L. S. Hasagawa, *Biochem. Pharmac.* **32**, 1155 (1983).
18. D. Svoboda, H. Grady and D. Azarnoff, *J. Cell Biol.* **35**, 127 (1967).
19. J. K. Reddy and T. P. Krishnakantha, *Science* **190**, 787 (1975).
20. J. K. Reddy, J. R. Warren, M. K. Reddy and N. D. Lalwani, *Ann. N.Y. Acad. Sci.* **386**, 81 (1982).
21. J. K. Reddy, T. P. Krishnakantha and M. S. Rao, *Virchows Arch. B Cell Path.* **17**, 295 (1975).
22. T. Hashimoto, T. Shiota, K. Ogawa and K. Hirai, *Acta Histochem. Cytochem.* **8**, 68 (1979).
23. G. M. Small, T. J. Hocking, A. P. Sturdee, K. Burdett and M. J. Connock, *Life Sci.* **28**, 1875 (1981).
24. G. M. Small, K. Burdett and M. J. Connock, *Ann. N.Y. Acad. Sci.* **386**, 460 (1982).
25. N. D. Lalwani, M. K. Reddy, M. Mangkornkanok-Mark and J. K. Reddy, *Biochem. J.* **198**, 177 (1981).
26. J. Reddy and D. Svoboda, *Lab. Invest.* **26**, 657 (1972).

27. P. Petrik, *J. Histochem. Cytochem.* **19**, 339 (1971).
28. E. E. Schneeberger, *J. Histochem. Cytochem.* **20**, 180 (1972).
29. E. E. Schneeberger, *Lab. Invest.* **27**, 581 (1972).
30. N. D. Lalwani, W. E. Fahl and J. K. Reddy, *Biochem. biophys. Res. Commun.* **116**, 388 (1983).
31. P. J. Dierickx, *Fd chem. Toxic.* **21**, 575 (1983).
32. D. A. Vessey and T. D. Boyer, *Toxic. appl. Pharmac.* **73**, 492 (1974).
33. C. A. Lamartinieiri, C. S. Dieringer and G. W. Lucier, *Toxic. appl. Pharmac.* **51**, 233 (1979).
34. A. P. Kulkarni, D. L. Fabacher and E. Hodgson, *Gen. Pharmac.* **11**, 437 (1980).
35. J. S. Felton, J. N. Ketley, W. B. Jakoby, A. Aitio, J. R. Bend and D. W. Nebert, *Molec. Pharmac.* **18**, 559 (1980).
36. R. E. Parchment and A. M. Benson, *Biochem. biophys. Res. Commun.* **119**, 1015 (1984).