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DISTRIBUTION AND PROPERTIES OF A MAMMALIAN SOLUBLE EPOXIDE HYDRASE

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Abstract—Two substrates, 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene and *cis*-epoxymethyl stearate were used to determine the distribution of epoxide hydrase activity in mammals. The highest epoxide hydrase activity in liver subcellular fractions was found in the 100,000 *g* supernatant and the mitochondrial fraction, while activity in washed microsomes is lower. The 100,000 *g* supernatant epoxide hydrase activity is present in all organs studied. This soluble epoxide hydrase activity which is highest in the liver and kidney of mice and rabbits is also present in the duodenum, muscle, colon, lung and spleen in decreasing order of activity. The level of soluble epoxide hydrase activity, although present in all mammalian species studied, is highest in female rabbits and male mice and in comparison, significantly lower in male rats. The level of epoxide hydrase activity varies with the strain of mice used and is higher in male mice than female mice. Epoxide hydrase activity in both male and female mice increases with age particularly after mice are 5 weeks old. The soluble epoxide hydrase activity requires no cofactor and has a molecular weight of approximately 130,000 as estimated by gel filtration on Sephacryl S-200. This molecular weight is about 2.5 times that reported for the solubilized microsomal epoxide hydrase. The soluble epoxide hydrase is inhibited by inorganic ions, particularly Cu^{2+} and stabilized by the addition of diisopropyl fluorophosphate to the incubation mixture. Based on this data the presence of at least two epoxide hydrases in most mammalian tissues, one in the soluble fraction and the other in the microsomal fraction, is evident.

Aromatic and olefinic compounds can be metabolized by mammalian oxidases to give epoxides [1, 2]. In addition, epoxide containing compounds are known to occur naturally [3, 4]. Some epoxides, whether naturally occurring or metabolically formed, are potentially toxic, mutagenic and/or carcinogenic. These epoxides can be metabolically transformed in mammals by conjugation with glutathione, a reaction often catalyzed by glutathione S-epoxide transferases, or hydrated by epoxide hydrases to 1,2-diols [2, 5–7]. These investigative efforts, which have been directed to the role of membrane bound microsomal epoxide hydrases of mammalian liver, are based on studies first reported by Oesch and coworkers [8–10] in which epoxide hydrase activity was found to occur predominantly in the microsomal fraction of mammalian liver using styrene oxide as a substrate. Subsequent studies utilizing other epoxides have rarely investigated the subcellular distribution of epoxide hydrase activity even when using a variety of different epoxide containing substrates [9–12].

In an investigation of the metabolism of a potentially useful insect juvenile hormone mimic, significant epoxide hydrase activity was observed in the 100,000 *g* soluble fraction of mammalian liver [13, 14]. A subsequent study [15] confirmed these initial findings that in contrast to all previous reports, significant epoxide hydrase activity is in the 100,000 *g* soluble fraction. The present study designates some properties and the distribution of the soluble epoxide hydrase in various species of mammals, tissues and subcellular fractions.

MATERIALS AND METHODS

Chemicals. 1-(4′-Ethyl-^{14}C-phenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (ethyl epoxide, 0.63 GBq/mmol, >96 per cent *trans*) was obtained from Stauffer Chemical Company, Mountain View, CA, U.S.A. and was purified to >99 per cent as described earlier [14, 16]. Unlabeled ethyl epoxide and the corresponding ethyl diol were synthesized as reported earlier [13, 16]. Oleic acid (ICN, Irvine CA, 1–^{14}C, 1.11 GBq/mmol) was esterified with diazomethane and oxidized with *m*-chloroperoxybenzoic acid to yield *cis*-9,10-epoxymethyl stearate (epoxymethyl stearate). The radioactive product showed one radioactive spot on thin-layer chromatography (tlc) (Silica gel GF) in several solvent systems including hexane–ethyl acetate–acetic acid (30:20:1) and it cochromatographed with authentic epoxymethyl stearate. All other chemicals used were either of analytical grade or nanograde.

Animals. For routine studies, male Swiss–Webster mice (30–40 g, 9–10 weeks old, Hilltop Laboratories, Chatsworth, CA) were used. Alternatively, Swiss–Webster mice used for studies on the level of soluble epoxide hydrase activity in males and females with respect to age and male Sprague–Dawley rats (150–200 g) were obtained from Simonsen Laboratories, Gilroy, CA. Swiss–Webster, AKR, C57B1 and BALB mice used for studies of soluble epoxide hydrase activity in different strains were obtained from Jackson Laboratories, Bar Harbor, ME. Female New Zealand white rabbits (2–4 kg) were obtained from Vista, CA.

Enzyme preparation. Mice were killed by cervical dislocation, while rats and rabbits were killed by a blow on the head. Liver and other organs, if utilized, were removed immediately, washed with cold buffer and fat or other adhering tissue removed. The organs were weighed then minced and homogenized using sodium phosphate buffer (pH 6.8, ionic strength 0.2 M) in a Potter–Elvehjem homogenizer to give a 10 per cent (w/v) homogenate. Differential centrifugation of the homogenate at 800 *g* for 10 min, the 800 *g* supernatant at 10,000 *g* for 10 min and the 10,000 *g* supernatant at 100,000 *g* for 1 hr gave fractions referred to as the cell nuclei and debris, mitochondria and microsomes, respectively. The 100,000 *g* supernatant was used as the crude soluble fraction. Mitochondria and microsomes were resuspended in buffer and recentrifuged for the appropriate times to give washed mitochondria and microsomes. The washed pellets were resuspended in sufficient buffer to give a 10 per cent homogenate as based on the initial homogenization. The various subcellular fractions were further diluted with cold buffer to give the appropriate protein concentrations before use. Protein concentration of the various fractions was determined by the method of Lowry *et al.*, [17] using bovine serum albumin as the standard.

Assay of enzyme activity. Analysis of epoxide hydrase activity was performed with ethyl epoxide as the substrate by means of a partition assay [18]. The ethyl epoxide in ethanol (2–3 μl) was added to the enzyme (100 μl) and incubated at 37° for varying lengths of time. The incubation reaction was stopped by adding methanol (150 μl) and 2,2,4-trimethylpentane (250 μl), vortexed, centrifuged and the radioactivity in the organic phase (ethyl epoxide) and methanol-aqueous phase (ethyl diol) analyzed by liquid scintillation counting (lsc). Alternatively, diethyl ether (250 μl) was added to the incubation mixture, vortexed, centrifuged and the ether phase spotted on a tlc plate. The plates were developed in benzene–propanol (10:1), the radioactive spots detected by autoradiography and tlc scanning (Berthold, W. Germany), the spots scraped and counted by lsc. Data from the partition and tlc assay procedures agreed closely.

Unless otherwise stated, the ethyl epoxide concentration used in the incubations was 7.4×10^{-6}M and incubation times were of 10 min. The apparent K_m and V_{max} were determined by the Lineweaver–Burk method [19] utilizing six substrate levels between 2.4×10^{-6}M and 2.2×10^{-5}M incubated for 2–20 min with 0.05 per cent w/v crude soluble and 5 per cent (w/v) microsomal fraction (substrate concentrations do not exceed the critical micelle concentration of the substrate).

The hydrolysis of epoxymethyl stearate was similarly analyzed using radiolabeled material followed by tlc analysis using the hexane–ethyl acetate–acetic acid system. This procedure gives complete separation of the substrate ($R_f \cong 0.8$), the diol ($R_f \cong 0.4$), and the two acids (R_f's$\cong$0.6 and 0.2) although ester cleavage was usually avoided by using diisopropyl fluorophosphate (DFP, 1×10^{-3}M, see below). Esterase activity was determined with α-naphthyl acetate as the substrate using slight modifications of classical procedures [20].

Filtration. The effect of low molecular weight factors on epoxide hydrase activity was examined by passing the crude soluble fraction (1 ml) through a carbowax treated glass column (25 × 1.5 cm) filled with Sephadex G-25 and held at 4°. The effluent was monitored at 280 nm and 5.6 ml fractions were collected. Each fraction was then assayed for epoxide hydrase activity and the active (exclusion volume) fractions combined (3–5, 16.8 ml). The fractions comprising the inclusion volume (8–10) were similarly combined. One column was run using sodium phosphate buffer pH 6.8, I = 0.2 M and a second column was run after equilibration with the same phosphate buffer containing 3 per cent sucrose and 1 mM mercaptoethanol. The exclusion volume from each column was diluted 1:1 with each of the two phosphate buffers and inclusion volumes. Similarly, the original crude supernatant was serially diluted with buffer and/or inclusion volumes. Each dilution was assayed in triplicate for epoxide hydrase activity within 1 hr of the column run and again 18 hr later.

Several matrices were examined for use in the partial purification of the soluble epoxide hydrase by gel filtration. The matrices were packed as described by their manufacturers in a Glenco column (86 × 2.5 cm, ID). After application of an aliquot of the crude soluble fraction of mouse liver homogenate, the columns were eluted with pH 6.8, I = 0.2M phosphate buffer delivered at 17 ml/hr with an LKB pump. The columns were eluted in the upward direction with Sephadex G-150 and Ultragel AcA 34 to avoid compressing the gel while Biogel P150 and Sephacryl S-200 were eluted by downward flow. After passing through a U.V. monitor (LKB Unicord II set at 280 nm), the column effluent was collected at 7 ml fractions (LKB Ultrorac). Each fraction was assayed for total protein, epoxide hydrase activity using the ethyl epoxide and epoxymethyl stearate as substrate and α-naphthyl acetate esterase activity. The active fractions were then combined and serial dilutions of the original 100,000 *g* supernatant and the active fraction assayed for total and specific epoxide hydrase activity leading to the data reported in Table 1.

Molecular weight estimation. The molecular weight of the soluble epoxide hydrase was estimated by using a column (86 × 2.5 cm, ID) packed with Sephacryl S-200 and eluted with sodium phosphate buffer, using the system described above. Proteins

Table 1. Effect of gel filtration medium on recovery and purification of the hepatic epoxide hydrase activity in the soluble fraction of male Swiss–Webster mice

Gel filtration medium	Recovery of enzyme activity	Purification factor (increase in specific activity)
Sephadex G-150*	75	30
Biogel P-150†	80	15
Ultragel AcA 34‡	49	25
Sephacryl S-200*	65	44

* Pharmacia Fine Chemicals.
† Bio-Rad Laboratories.
‡ LKB-Produkter.

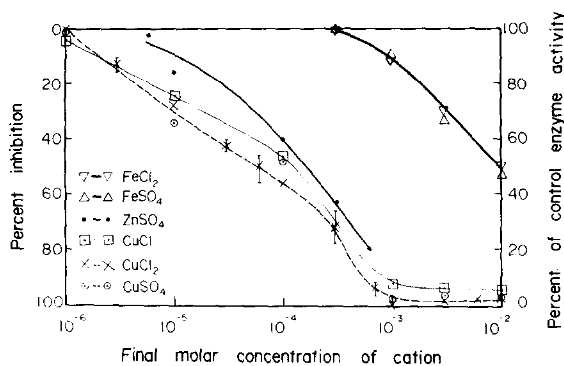

Fig. 1. Inhibition of the epoxide hydrase activity in the soluble fraction of male mouse liver by inorganic ions in Tris buffer. A number of other salts at 1×10^{-2}M, namely NaF, $MgCl_2$, EDTA, $Na_2S_2O_3$, $CaCl_2$, $MnCl_2$ and KCN, did not cause any significant inhibition of epoxide hydrase activity after 10 min preincubation.

of known molecular weight (β-galactosidase, catalase, γ-globulin, bovine serum albumin, ovalbumin, ribonuclease and cytochrome *c*) were used as standards according to the procedure of Andrews [21].

Enzyme stabilization. Aliquots (2 ml) of a 1 per cent soluble fraction were placed in each of several tubes. A potential protease inhibitor was added to each tube in 20 μl of ethanol or acetonitrile to give the following concentrations: *N*-α-*p*-tosyl-*l*-lysine chloromethyl ketone–HCl (TLCK, 2×10^{-4}M), phenylmethyl sulfonylfluoride (PMSF, 1×10^{-3}M), L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK, 1×10^{-3}M), DFP (1×10^{-3}M) and EDTA (added in buffer, 1×10^{-2}M). The tubes were vortexed and incubated with shaking at 37° and at 20 min and each hour subsequently samples were taken for analysis of epoxide hydrase activity.

Effects of inorganic ions. Several salts were dissolved in distilled water at concentrations ranging from 2×10^{-1} to 1×10^{-5}M. Ten microliters of the salt solutions were placed in a 10 × 77 mm tube and 90 μl of soluble fraction added. The tubes were incubated for 10 min at 37°, then substrate was added and epoxide hydrase assays carried out as usual using a 10 min incubation. The soluble fraction was passed through Sephadex G-25 before assay to remove low molecular weight materials and in some cases to change from sodium phosphate buffer (pH 6.8, I = 0.2 M) to Tris–HCl buffer (pH 7.0, I = 0.2M). In phosphate buffer a number of cations precipitated as their phosphate salts. Percent inhibition was determined from water treated controls or from assay of a serial dilution of the enzyme fraction. Both methods yielded identical results. The I_{50}'s were determined from a plot of percent inhibition vs log ion concentration (Fig. 1).

RESULTS AND DISCUSSION

Gel filtration. The inclusion volumes from gel filtration on Sephadex G-25 contained no detectable epoxide hydrase activity, and all detectable epoxide hydrase activity was in the exclusion volume. Recovery of enzyme activity from the column was 77–83 per cent and the addition of the inclusion volume or glutathione (0.1 mM) to the exclusion volumes of the whole enzyme had little effect on activity. The addition of sucrose and mercaptoethanol to the phosphate buffer slightly decreased the enzyme activity and had little effect on the hydrase assay itself. The epoxide hydrase activity in the crude 100,000 *g* supernatant and in the exclusion volumes from Sephadex G-25 using either sodium phosphate buffer alone or with 3 per cent sucrose and 1 mM mercaptoethanol was not observed to deteriorate after 18 hr storage in ice.

This evidence enforces the view that the soluble epoxide hydrase activity observed in mammalian liver is not due to artifacts involving glutathione S-epoxide transferases. The tri-substituted epoxide used as the substrate in this report was previously shown to react very slowly, if at all, with glutathione whether or not the reaction was catalyzed with the soluble fraction from liver preparations [14]. The results from this paper clearly indicate that glutathione does not stimulate the hydrolysis of ethyl epoxide by the mouse liver soluble fraction before or after gel filtration. It also shows that no low molecular weight cofactor which can be removed by rapid gel filtration is needed for enzyme activity.

Of the four matrices examined, Sephacryl S-200 yielded a combination of the best enzyme recovery and purification factor (Table 1). Ultragel gives good resolution of peaks, but the purification factor is low probably due to poor recovery of enzyme activity. Since a lipophilic substrate is used for analysis of the epoxide hydrase, it should be cautioned that nonenzymatic proteins could affect the substrate's distribution in the assay tube. These proteins could either increase or decrease enzyme activity resulting in misleading values for recovery and purification.

The molecular weight of the soluble epoxide hydrase as estimated by means of a Sephacryl S-200 column was found to be approximately 130,000 assuming a close relationship between Stoke's radii and molecular weight. This estimate of molecular weight for the soluble epoxide hydrase, though not strictly comparable since different methods were used in the estimation, is approximately 2.5 times the molecular weight of the artificially solubilized microsomal epoxide hydrase [22, 23]. Glutathione S-epoxide transferase from rat or guinea pig liver is also much smaller with an apparent molecular weight of 46,000 [24, 25]. The coincidence and sharpness of the elution profiles for epoxide hydrase activity acting on the ethyl epoxide and epoxymethyl stearate provide evidence that a single enzyme or enzymes of similar size are hydrolyzing these tri- and di-substituted substrates (Fig. 2). Similar coincidence of elution was found when two tri-substituted epoxides, a di-substituted epoxide and a mono-substituted epoxide were used as substrates [26, 27]. The partial separation of the epoxide hydrase activity from the esterase activity hydrolyzing α-naphthyl acetate (and epoxymethyl stearate) indicates that gel filtration alone is not sufficient to obtain an esterase free preparation and that esterase inhibitors should be used with substrates containing an ester functionality.

Gel filtration through Sephadex G-25 had no effect on the apparent K_m and V_{max} when the ethyl epoxide

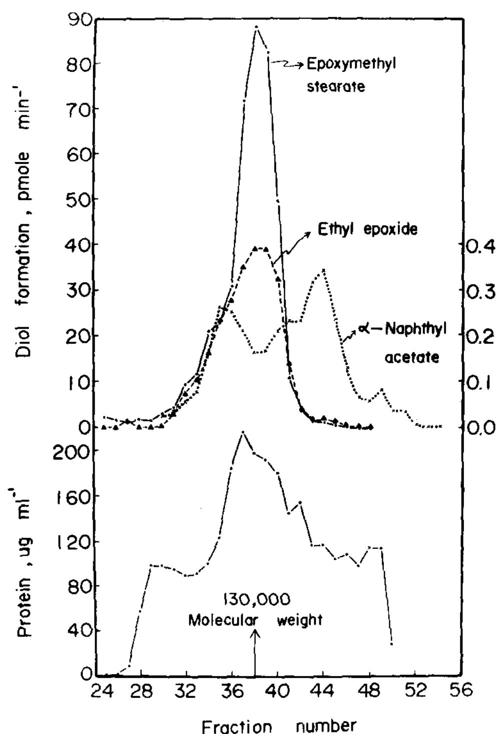

Fig. 2. Elution profiles for epoxide hydrase activity acting on the ethyl epoxide and *cis*-epoxymethyl stearate and esterase activity acting on α-naphthyl acetate (upper tracing). The lower tracing indicates the total protein eluting from a Sephacryl S-200 gel filtration column (86 × 2.5 cm, ID) following application of 5 ml of mouse 100,000 *g* soluble fraction. The fractions (5.6 ml) were assayed for α-naphthyl acetate esterase and hydrase activity on the ethyl epoxide without dilution, but were diluted 20:1 with sodium phosphate buffer for the epoxymethyl stearate assays.

was used as substrate (4.5×10^{-6}M and 1.74 nmol/min/mg protein, respectively). With both the crude soluble and soluble fractions partially purified by Sephacryl S-200 gel filtrations the K_m was unchanged. The apparent affinities and velocities of the soluble epoxide hydrase compare favorably with those reported for the microsomal enzymes [28–32].

Enzyme stabilization. Over a period of 7 hr at 37° control homogenate and homogenate treated with ethanol or acetonitrile lost 35–40 per cent of their original epoxide hydrase activity. DFP treatment resulted in a rapid loss of about 17 per cent of the enzyme activity, but no further loss was detected after 7 hr. TLCK and PMSF slowed the loss of activity to only 25–30 per cent after 7 hr, while PTCK inhibited the enzyme and over half of the activity disappeared after 7 hr. The EDTA treated enzyme lost activity slightly faster than any of the controls although it initially showed slightly higher activity. Subsequent studies indicated that if DFP (1×10^{-3}M) was added to the buffer immediately before the livers were homogenized, slightly higher enzyme activity was found.

Effects of inorganic ions. The results from the two buffer systems are generally comparable although solubility product constants would indicate greater confidence in data obtained with the Tris buffer. Of the ions tested (Fig. 1), the majority showed no effect in either stimulating or inhibiting epoxide hydrase activity. EDTA appears to slightly stimulate soluble epoxide hydrase activity while Fe^{2+} salts are weak inhibitors and Zn^{2+}, Cu^{+} and Cu^{2+} salts are strong inhibitors. Though there was some variation in the I_{50}'s, the I_{50}'s from four different enzyme preparations were between 3×10^{-5}M and 2×10^{-4}M for Cu^{2+}.

Previous studies [14, 15] have shown that 1,1,1-trichloropropene oxide and cyclohexene oxide, potent inhibitors of the microsomal epoxide hydrase [10], do not inhibit the soluble epoxide hydrase even at very high levels. The present observation that inorganic salts, particularly Cu^{2+}, are good inhibitors further suggests that epoxide hydrases with different properties exist in the microsomal and soluble fractions of mammalian liver. The results also indicate that buffers contaminated with some inorganic ions could lead to a sharp reduction in the hydrase activity observed in the soluble fraction.

Subcellular distribution of the epoxide hydrase activity in mouse liver. Epoxide hydrase activity in the subcellular fraction of male Swiss–Webster mouse liver using the ethyl epoxide as substrate is present in all fractions examined. The mitochondrial and soluble fractions demonstrated the highest enzyme activity (Table 2). The epoxide hydrase activity found in the mitochondrial fraction requires further study. It is possible that the mitochondrial epoxide hydrase activity results from contamination by other fractions since mitochondrial and microsomal "marker" enzymes were not followed in this

Table 2. Epoxide hydrase activity in the subcellular fractions of male Swiss–Webster mouse liver*

Subcellular fraction	Protein mg per assay	Specific activity† pmoles min^{-1} mg protein	Specific activity† pmoles min^{-1} mg tissue $equiv.^{-1}$
Cell nuclei and debris	0.060–0.092	297 ± 3.6	31 ± 4.4
Mitochondria	0.005–0.010	2860 ± 402	27 ± 4.7
Microsome, washed	0.018–0.090	131 ± 59	2.4 ± 1.1
Microsomes, unwashed	0.014–0.076	337 ± 182	5.0 ± 2.5
Soluble fraction	0.005–0.010	2990 ± 192	270 ± 66

* Mice used were approximately 10 weeks old.

† Determined at a minimum of two protein concentrations done in triplicate from pooled livers of three mice. Experiment was repeated twice. Values reported are means ± S.D.

study. The presence of high epoxide hydrase activity in the mitochondrial fraction concurs with work previously reported [8] using styrene oxide as the substrate, although these workers were able to significantly reduce the mitochondrial epoxide hydrase activity by use of isotonic buffers [2].

The epoxide hydrase activity is much higher in the soluble than in the microsomal fraction. The level of epoxide hydrase in the protein rich soluble fraction is emphasized relative to the microsomal fraction if specific activity is determined on the basis of tissue equivalents. A significant proportion of the epoxide hydrase activity in the microsomal fraction is lost when microsomes are washed, indicating possible contamination of the microsomal pellet by the soluble fraction. However, some epoxide hydrase activity cannot be washed out of the microsomes. Possibly the microsomal fraction should then be reexamined for epoxide hydrases which have different properties, including substrate specificity and pH optima, than the styrene oxide metabolizing enzyme so intensively studied [8–12, 22, 23, 31–33]. Microsomal contamination in the soluble fraction is insignificant since, negligible hydration of styrene oxide or *cis*-stilbene oxide, two substrates which are readily hydrated by the microsomal epoxide hydrase, is observed in the soluble fraction. Further, the substrate specificity shown by the soluble and microsomal epoxide hydrases differs [26, 27].

The results in Table 2 demonstrate that the levels of epoxide hydrase present in the subcellular fractions are different from those reported by Oesch and coworkers [8] for liver and more recently by Seidegård and others for lung [23]. The differences observed are apparently due, in part, to the difference in substrated utilized [27, 34]. Therefore, studies on epoxide hydrase activity utilizing differing substrates require studies on subcellular localization of epoxide hydrase activity, since utilization of either the 10,000 *g* soluble fraction or the unwashed microsomal pellet as a source of "microsomal" epoxide hydrase could lead to erroneous results. The nuclear epoxide hydrase activity monitored with styrene oxide or benzopyrene oxide appears to be due to the same enzyme that is present in the microsomal membranes [35]. However, the epoxide hydrase activity in the soluble and possibly the mitochondrial fractions is quite distinct from that in the microsomal and nuclear fractions.

Organ distribution of soluble fraction epoxide hydrase. Epoxide hydrase activity that is present in the 100,000 *g* soluble fraction of mouse liver is also found in a number of other organs in mice. As observed with the liver, higher epoxide hydrase activity is observed in the soluble fraction than in the microsomal fraction in the kidney, lung, testes and spleen of mice with the ethyl epoxide as substrate (Table 3). The highest activity is found in the liver and kidney with the lung, testes and spleen showing significantly less activity. Similar tissue distribution was observed for the soluble and microsomal epoxide hydrase activity in rabbit organs (Table 4). As with mice, the highest levels are observed in the liver and kidney. Significant activity was also found in the duodenum and muscle. As with the mouse, the lowest activity was observed in the spleen. Thus, the soluble epoxide hydrase occurs in all tissues tested in both mice and rabbits. Oesch *et al.* [36] similarly reported the ubiquitous nature of the microsomal epoxide hydrase in rat organs. The high level of microsomal epoxide hydrase in the testes of the NMRI strain of mice in comparison to the liver [36] was not observed with Swiss–Webster mice. The high levels observed in the liver and kidney in comparison to the other tissues possibly indicate an important role for the enzyme in the metabolism and excretion of epoxide containing xenobiotics. A thorough study on the levels of the soluble epoxide hydrase in other organs is presently limited by the small differences observed between background and low enzyme activity.

Variations in soluble epoxide hydrase levels in different mammalian species and strains and sexes of mice. The highest level of soluble epoxide hydrase

Table 3. Epoxide hydrase activity of the 100,000 *g* soluble and microsomal fractions in various organs of male Swiss–Webster mice*

		Specific activity†	
Organ	Fraction	pmoles min^{-1} mg $protein^{-1}$	pmoles min^{-1} mg tissue $equiv.^{-1}$
Liver	microsomes	130 ± 58	2.4 ± 1.1
	soluble	1200 ± 350	100 ± 18
Kidney	microsomes	59 ± 2.1	0.7 ± 0.1
	soluble	670 ± 93	36 ± 5.0
Lung	microsomes	not detectable	—
	soluble	77 ± 26	3.9 ± 1.4
Testes	microsomes	not detectable	—
	soluble	58 ± 34	1.9 ± 1.1
Spleen	microsomes	not detectable	—
	soluble	12 ± 9	6.1 ± 3.6

* Mice used were 7–8 weeks old.

† Determined at three protein concentrations in triplicate for all the soluble fractions and the microsomal fraction of liver and repeated once. Only one protein concentration was used for microsomes from the kidney, lung, testes and spleen with the experiment performed in triplicate at 10, 20 and 30 min.

Table 4. Soluble epoxide hydrase levels in various organs of female New Zealand white rabbits

Organ	Specific activity*	
	pmoles min^{-1} mg protein^{-1}	pmoles min^{-1} mg tissue equiv.$^{-1}$
Liver	1400 ± 270	130 ± 18
Kidney	940 ± 140	82 ± 12
Lung	120 ± 36	6.0 ± 1.9
Duodenum	310 ± 150	15 ± 3.7
Colon†	150 ± 6.3	1.8 ± 0.1
Spleen	46 ± 10	2.6 ± 0.6
Muscle†	250 ± 47	1.4 ± 0.3

* Determined at three protein concentrations in triplicate and repeated once.

† Homogenization not total due to muscular structure.

is found to occur in male mice (Table 5). Relatively high activity is also observed in female rabbits and mice. In comparison, significantly lower levels are observed in male rats, although preliminary studies with other species indicate the ubiquitous nature of soluble epoxide hydrase activity in mammals. Clearly, the statement that rats have higher hepatic epoxide hydrase activity than mice must be qualified with a notation of the substrate used [36, 37].

The level of epoxide hydrase in the soluble fraction of mouse liver also varies with the strain of mice utilized in the experiments. Of the four strains utilized, AKR and C57B1 showed higher epoxide hydrase activity when compared with Swiss–Webster and BALB (Table 6). Large variations in the level of soluble epoxide hydrase are also observed with the age and sex of the animal used. In Swiss–Webster mice the level of the hydrase remains essentially constant until 5 weeks at which time an increase in the level of epoxide hydrase is observed (Fig. 3). Increase in activity is more dramatic in males than in females. In males the major increase in soluble

Table 5. Epoxide hydrase activity in the soluble fraction of rat, mouse and rabbit livers

Animal	Sex	Specific activity	
		pmoles min^{-1} mg protein^{-1}	pmoles min^{-1} mg tissue equiv.$^{-1}$
Rat	male	40 ± 7.1*	4.3 ± 0.8
Rabbit	female	1400 ± 270†	130 ± 18
Mouse	male	2700 ± 630‡	210 ± 53
	female	1200 ± 350‡	120 ± 13

* Determined at three protein concentrations in triplicate from pooled livers of three rats and repeated once.

† Determined at three protein concentrations in triplicate from a single rabbit and repeated once.

‡ Determined at three protein concentrations in triplicate from pooled livers of 2–3 mice, 10 weeks in age and repeated twice.

* S. S. Gill and B. D. Hammock, unpublished results (1979).

Table 6. Hydration of ethyl epoxide by the soluble fraction of mouse liver in four strains of male mice

Strain*	Specific activity†	
	pmoles min^{-1} mg protein^{-1}	pmoles min^{-1} mg tissue equiv.$^{-1}$
Swiss–Webster	1200 ± 46	130 ± 4.7
BALB	1300 ± 490	130 ± 51
AKR	1700 ± 270	180 ± 30
C57B1	1700 ± 140	170 ± 13

* All mice used were approximately 7 weeks old.

† Determined at three protein concentrations in triplicate from pooled livers of two mice. Experiment repeated once.

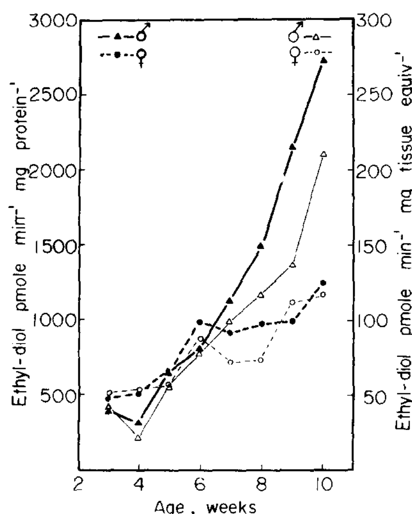

Fig. 3. Epoxide hydrase activity in the soluble fraction of male and female Swiss–Webster mouse livers removed from virgin mice of various ages.

epoxide hydrase activity is observed between 6 and 10 weeks of age (the oldest mice used in the study). In females, on the other hand, the epoxide hydrase activity remains constant after an increase is observed between 5 and 6 weeks. This pattern of increased soluble epoxide hydrase activity with age in male mice differs from the pattern observed by Oesch *et al.* [8] in male rats, supporting the experimental evidence of a distinct epoxide hydrase in the soluble fraction of mouse liver. Based on the data presented here, and substrate preference studies of the soluble epoxide hydrase reported elsewhere [18, 26, 27, 38] and unpublished studies,* it appears that an epoxide hydrase in the soluble fraction may contribute more to the hydration of some epoxides than the hydrases in the microsomal fraction. Since epoxide hydrase activity has been found in the soluble fraction of every organ of the mammals examined, the potential role of the soluble epoxide hydrase should be considered when the metabolism of olefinic or epoxide containing xenobiotics is investigated. Also the soluble epoxide hydrase is clearly in the "S9" fraction used in some mutagenicity assays, so its role as a deactivating factor should be carefully assessed [39].

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