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HEPATIC AND EXTRAHEPATIC METABOLISM, *IN VITRO*, OF AN EPOXIDE (8-^{14}C-STYRENE OXIDE) IN THE RABBIT*

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Abstract—Two epoxide-metabolizing enzymes, glutathione-*S*-epoxide transferase and epoxide hydrase, were studied in subcellular fractions of rabbit liver, lungs, intestinal mucosa and kidney. Glutathione-*S*-epoxide transferase in soluble fraction was assayed by a new specific radiochemical method using styrene oxide (-8-^{14}C) as substrate, and some properties of this enzyme are described. Liver had the highest specific activity of each enzyme, but there was no correlation between the relative specific activities of glutathione-*S*-epoxide transferase and epoxide hydrase in the extrahepatic organs. Feeding rabbits a purified diet did not alter the specific activities of epoxide hydrase or glutathione-*S*-epoxide transferase in liver and intestine. Rat and guinea pig had much higher specific activities of glutathione-*S*-epoxide transferase than rabbit in liver and kidney, and slightly higher specific activities in lung and intestine. Species did not differ markedly in epoxide hydrase activities when the same organ (liver, lung, intestine or kidney) was compared in rabbits, rats and guinea pigs, except that rat intestine had much lower epoxide hydrase activity than intestine from rabbit or guinea pig.

In recent years a large number of reports have suggested that epoxide metabolites may be the mediators of the carcinogenicity of aromatic hydrocarbons. This work is reviewed by Sims and Grover [1].

It has been shown that epoxides are formed by the action of microsomal mixed-function oxidase enzymes on molecules having aromatic rings or alkene bonds [2, 3] and that, in general, an epoxide metabolite is more biologically reactive than the parent hydrocarbon. The epoxides of aromatic hydrocarbons have been found to bind to nucleic acids [4] and other macromolecules and to undergo rearrangement to phenols, hydration to diols [5] or conjugation with glutathione, which can be the first step of mercapturic acid biosynthesis [6]. These last two reactions are catalyzed by microsomal epoxide hydrase (EC4.2.1.63) and soluble fraction glutathione-*S*-epoxide transferase (EC4.4.1.7) respectively. We have studied some properties of these two epoxide-metabolizing enzymes in tissue preparations of several organs of rabbit, guinea pig and rat.

Styrene oxide [-8-^{14}C] was used as a model compound for studying these two pathways of epoxide metabolism, since styrene oxide is stable enough to study in aqueous assay systems, could be obtained labeled with ^{14}C, and has often been studied as a substrate for hepatic microsomal epoxide hydrase in several species [7]. The unlabeled compound is also available, and the compound has comparatively low toxicity and carcinogenicity [8].

EXPERIMENTAL

Styrene oxide-8-^{14}C (sp. act., 0·37 mCi/m-mole) was purchased from Mallinckrodt, St. Louis, Mo., and diluted where necessary with unlabeled styrene oxide purchased from Eastman Organic Chemicals. Glutathione was purchased from Sigma Chemical Co.

Glutathione-*S*-epoxide transferase was assayed by measuring the reaction product, in this case *S*-(2-hydroxy-1-phenylethyl) glutathione. Incubation tubes were set up at 0° containing 160 μmoles HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid) buffer (pH 7·2 for liver, 7·6 for lung, intestine and kidney), 7·5 μmoles glutathione, 0·6 to 1·25 mg soluble fraction protein and water in a final volume of 1·45 ml. Tubes were placed in a bath at 37° and left for 3–4 min to attain this temperature. The reaction was started by adding styrene oxide (1·5 μmoles, $1·5 \times 10^5$ dis./min) in solution in acetonitrile (50 μl). After an incubation time of 5–15 min, the reaction was terminated by adding 4 ml ethyl acetate and vortex mixing. Unreacted styrene oxide and any phenyl-1,2-ethane diol formed were extracted into the organic phase, leaving the glutathione conjugate in the aqueous phase. The ethyl acetate layer was removed and the extraction repeated twice. Thin-layer chromatography of the ethyl acetate phase in chloroform–ethyl acetate (1:1, v/v) and radiochromatographic scanning of the developed plate showed two spots having the same R_f values as unlabeled styrene oxide ($R_f = 0·90$) and diol ($R_f = 0·22$) in this system. The peak at $R_f = 0·22$, the R_f of the diol, was barely detectable after incubation of styrene oxide with the microsomal supernatant (soluble) fraction (in the presence of glutathione). The plates used were purchased from Analtech Inc., Del., and were coated with 250 μ of Silica gel G containing fluorescence indicator. Spots corresponding to unlabeled styrene oxide and phenyl-1,2-ethane diol could be seen on these plates by viewing under ultraviolet light at 254 nm, since both compounds quench the fluorescence of the indicator.

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Paper chromatograms of the aqueous phase (after ethyl acetate extraction) were developed in *n*-butanol–glacial acetic acid–water (2:1:1, v/v) and scanned for ^{14}C. A major peak was seen at $R_f = 0.58$, the same position as non-enzymatically synthesized *S*-(2-hydroxy-1-phenylethyl)glutathione. In some cases, a small peak at $R_f = 0.68$ was also noticed, but no peak appeared at $R_f = 0.88$, the R_f to which both styrene oxide and the diol migrate in this system. The peak at $R_f = 0.68$ is probably due to initial breakdown of the glutathione conjugate to the cysteinylglycine conjugate. This chromatography was evidence that the extraction procedure quantitatively separated the reaction product, *S*-(2-hydroxy-1-phenylethyl)glutathione, from the substrate and its hydrolysis product; hence, the ^{14}C remaining in the aqueous phase after extraction was used to calculate glutathione-*S*-epoxide transferase activities. Specific activities were corrected for nonenzymatic formation of the glutathione conjugate by assaying for conjugation in the absence of tissue and for carryover of radioactivity by assaying a nonincubated blank containing tissue.

The ^{14}C in the aqueous phase after ethyl acetate extraction was counted by washing out the contents of the reaction vial with four (2·5 ml) portions of a Triton–toluene scintillation mixture. The scintillation mixture was prepared by adding 1 liter Triton X-100 (alkyl phenoxy polyethoxy ethanol, New England Nuclear) to 2 liters of a toluene (Baker analyzed) solution containing 16·5 g of 2,5-diphenyloxazole (New England Nuclear, scintillation grade) and 0·5 g of *p*-bis[2-(5-phenyloxazolyl)]benzene (New England Nuclear, scintillation grade).

In order to check that all the ^{14}C added could be accounted for, portions (0·5 ml) of the combined ethyl acetate extracts for each reaction vial were added to 10 ml of the same Triton–toluene scintillation mixture.

All samples were counted in a Nuclear-Chicago isocap/300 liquid scintillation system using program 2 (channels ratio method for finding efficiency) as recommended by the manufacturer for the system to be counted. Appropriate quench curves were set up to find the counting efficiency in the presence of water or of ethyl acetate and these were used to calculate the dis./min for each sample.

Epoxide hydrase activity was determined by measuring the conversion of styrene oxide to phenyl-1,2-ethanediol according to the method of Oesch *et al.* [5]. The usual incubation conditions were: 1 μmole styrene oxide (added last); 100 μmoles HEPES buffer, pH 8·9; 0·4 μl Tween 80; and 1·5 mg microsomal protein in a final volume of 1·0 ml. The reaction was carried out at 37° and terminated after 10–20 min by vortex mixing with 5 ml petroleum ether. Samples for scintillation counting were prepared as described for glutathione-*S*-epoxide transferase.

Young adult, male, New Zealand white rabbits were used having body weights of 2·2 to 2·8 kg. These rabbits were obtained from Pel-Freez animal suppliers (Rogers, Ark.). They were killed by air embolism (marginal ear vein) between 8:00 and 9:00 a.m.; the tissues were removed immediately and placed on ice. The proximal 20 in. of small intestine was used for routine assay. (Reasons for this limit are given in Results.) This portion of intestine was washed with distilled water, cut open, and the mucosa scraped and used for preparation of subcellular fractions. In later experiments, the mucosa was not scraped off, but the washed sections were cut up prior to homogenization.

Washed microsomes were used to assay epoxide hydrase and were prepared as follows. A suitable weight of the minced organ (all in the case of lung and intestinal mucosa) was homogenized in 1·15% KCl 0·02 M HEPES, pH 7·4, using a Teflon motor-driven homogenizer so that 1 g tissue was suspended in a final volume of 4 ml. The homogenate was centrifuged at 600 *g* for 10 min to precipitate nuclear fragments and then at 10,000 *g* for 20 min to precipitate the mitochondrial fraction. The post-mitochondrial supernatant was centrifuged at 176,000 *g* for 45 min using a 60 Ti head in a Beckman L3-50 ultracentrifuge. This microsomal supernatant fraction (soluble fraction) was used to assay glutathione-*S*-epoxide transferase. The pellet was resuspended in 1·15% KCl–0·02 M HEPES and respun at 176,000 *g* for 20 min. The washed microsomes were suspended in 1·15% KCl–0·02 M HEPES to the desired protein concentration.

Experiments to find the K_m and V_{max} values of glutathione-*S*-epoxide transferase with respect to glutathione concentration were performed using soluble fraction which had been dialyzed for 24 hr against two changes of isotonic 0·02 M HEPES, pH 7·4, to remove low molecular weight peptides including glutathione. In these experiments, the concentration of styrene oxide was held at 1 mM and the glutathione concentration varied between 0 and 20 mM.

In studies where the subcellular distribution of the two epoxide-metabolizing enzymes was examined, the preparative medium was 0·25 M sucrose 0·05 M HEPES, pH 7·4, since this led to less disruption of structure as shown by electron microscopy. Protein was assayed by the method of Lowry *et al.* [9].

The distribution of epoxide-metabolizing enzymes along the intestinal tract was studied by cutting the small intestine into 6-in. segments, scraping off mucosa, and preparing subcellular fractions for assay of glutathione-*S*-epoxide transferase and epoxide hydrase.

The effect of starvation on the intestinal enzymes was tested using four groups of three animals each. One group was starved for 48 hr, the second group for 24 hr and the third group for 12 hr. The fourth group was allowed normal access to food and served as control. All rabbits had unrestricted access to water.

Another experiment was carried out to determine the effect that diet might have on epoxide-metabolizing enzymes, especially in intestine. Sixteen 8-week-old male rabbits were fed a purified synthetic diet obtained from General Biochemicals. The composition was as follows: Alphacel (cellulose bulk), 20%; cottonseed oil, 4%; salt mixture, Hegsted, 4%; sucrose, 37%; vitamin-free casein, 30%; magnesium oxide, 0·5%; potassium acetate, 2·5%; and vitamin-diet mixture, 2%.

Fifteen control 8-week-old rabbits were fed standard NIH rabbit feed A with the following composition: alfalfa meal, 38%; whole wheat, 28·9%; whole oats, 17·75%; soybean meal, 13·25%; limestone, 1·1%; sodium chloride (iodized), 0·5%; dicalcium phosphate,

Table 1. Metabolism of styrene oxide in preparations of rabbit liver, lung, kidney and intestine

	Specific activity* (nmoles product formed. min^{-1}. mg $protein^{-1}$)		Total organ activity† (μmoles product formed. min^{-1}. $organ^{-1}$)	
	Epoxide transferase (soluble fraction)‡	Epoxide hydrase (microsomal fraction)‡	Epoxide transferase (soluble fraction)	Epoxide hydrase (microsomal fraction)
Liver	30·5 ± 3·9	5·6 ± 0·9	240·3 ± 73·3	11·3 ± 3·4
Lung	6·5 ± 1·6	0·17 ± 0·02	3·1 ± 0·6	0·02 ± 0·00
Kidney	8·2 ± 2·3	1·4 ± 0·1	3·5 ± 0·5	0·36 ± 0·05
Small intestine mucosa	4·4 ± 0·9	2·8 ± 0·7	2·5 ± 0·3	0·39 ± 0·10

* Values quoted are mean ±S. D. for six young adult male New Zealand white rabbits, body weight 2·2 to 2·8 kg.

† Total activity was calculated by multiplying the specific activity by the total microsomal or soluble fraction protein for that organ and finding the mean ±S. D. for each organ. Even assuming that only 50 per cent of the endoplasmic reticulum is recovered in the microsomal pellet, total epoxide hydrase activity is much lower than total glutathione-*S*-epoxide transferase activity, especially in liver and lung.

‡ See text for preparation of subcellular fractions.

0·1%; Delamix, 0·15%; vitamin D_2 and C fortification, 0·1%.

Four animals from each of these two groups (purified vs regular diet) were sacrificed at 2-week intervals, and levels of epoxide hydrase and glutathione-*S*-epoxide transferase in liver and intestine were measured. Liver and lung microsomal benzphetamine-*N*-demethylase activities were also measured, since it has been reported [10] that the pulmonary mixed-function oxidases are induced in rats by some vegetable components in food.

Benzphetamine demethylase activity of liver, lung and intestinal microsomes was determined as previously described [11].

RESULTS

Glutathione-*S*-epoxide transferase activity was found to be localized in the soluble (microsomal supernatant) fraction of liver, lung, kidney and intestinal mucosa of the rabbit. Liver had the highest specific activity and kidney $\frac{1}{4}$ to $\frac{1}{3}$ of the specific activity of liver. Lung soluble fraction had about $\frac{1}{5}$ of the activity of liver soluble fraction, and intestinal mucosa had the lowest activity of those organs studied—about $\frac{1}{7}$ of the liver's activity. Values obtained for six typical animals are given in Table 1. The total capacity of the organ to metabolize epoxide under the conditions of assay used was also calculated and is shown in Table 1, but it should be noted that the specific activities found under these conditions were lower than the V_{max} for epoxide transferase, so these may not be maximal turnover rates *in vitro*. In the intact animal, rates of conjugation may also depend upon the concentration of glutathione *in vivo* in the various tissues.

The highest epoxide hydrase specific activity and total activity were also found in liver, and this enzyme was localized in the microsomal fraction of each tissue examined, as has been previously reported for liver [5]. Intestinal mucosa microsomes had the second highest specific activity, and kidney microsomes had lower specific activity than intestinal microsomes. Lung microsomes had very low epoxide hydrase activity—about $\frac{1}{20}$ that of the liver. The total activity of lung was about 10^{-3} that of liver; since 1 mM styrene oxide is an order of magnitude greater than the K_m for epoxide hydrase [12], the measured activity should have been close to V_{max} and, therefore, a good indication of the relative maximal potential turnover rates of epoxide hydrase in lung and liver. The epoxide hydrase activity of lung microsomes could be measured accurately only in the presence of 2–3 mg microsomal protein per incubation and by using an incubation time of 15 min or longer. These conditions of assay would result in hydrolysis of more than 50 per cent of the substrate if liver instead of lung microsomes were used. Epoxide hydrase activity in lung microsomes was linear with time up to 30 min and with protein between 2 and 6 mg/incubation vial. Heating lung microsomes to 100° and then cooling before assay abolished the epoxide hydrase activity. Assaying the lung epoxide hydrase activity in the presence of 0·5 and 1·0 mM trichloropropene oxide, a known inhibitor of epoxide hydrase [13], gave progressively less product formation, although the type of inhibition of the enzyme in the lung was not determined.

Incubating lung microsomes together with liver microsomes or with kidney microsomes led to the hydration of the same amount of styrene oxide as would be expected from the sum of the two separate activities in each case, so that the low lung activity does not appear to be due to any potent epoxide hydrase inhibitor present in lung microsomes. For all four organs studied, sonication of homogenate for 10 sec prior to differential centrifugation almost doubled the microsomal protein yield, but led to no change in the measured specific activity of either epoxide hydrase or glutathione-*S*-epoxide transferase.

The rate of formation of the styrene oxide glutathione conjugate was found to be linear with time up to 15 min when catalyzed by liver or intestinal soluble fractions and for at least 15 min with lung soluble fractions under the conditions used. Epoxide hydrase activity was linear with time for 20 min in liver and kidney microsomes, for 15 min in intestinal microsomes, and for at least 30 min in lung microsomes.

Varying the amount of protein added to incubation vials did not change the specific activities of glutathione-*S*-epoxide transferase measured when up to 4·5 mg liver soluble fraction protein was added in the 1·5-ml reaction volume, or when up to 1·8 mg lung or intestinal soluble fraction protein was added in the 1·5-ml reaction volume.

Table 2. Localization of epoxide-metabolizing enzymes in the kidney

	Medulla (nmoles product. min^{-1}. mg protein^{-1})	Total medulla activity*	Cortex (nmoles product. min^{-1}. mg protein^{-1})	Total cortex activity*	Total cortex activity / Total kidney activity
Epoxide transferase (soluble fraction)	21·0 ± 0·8	12·2 ± 3·1	20·8 ± 3·3	2·5 ± 1·0	0·17
Epoxide hydrase (microsomes)	1·30	0·14	0·45	0·004	0·03

* Styrene oxide (μmoles) conjugated (transferase) or hydrolyzed (hydrase) by the total protein in medulla or cortex soluble or microsomal fractions from two kidneys was calculated by multiplying the specific activity by the protein yield for that fraction. Results are from one experiment in which three rabbits were sacrificed. Separate values for each animal were obtained for the soluble fraction enzyme, and the microsomes were pooled for the epoxide hydrase data. In order to verify the distribution of epoxide hydrase between kidney medulla and cortex, the epoxide hydrase assay was repeated using cortex and medulla microsomes prepared from the pooled kidneys of three more rabbits, and the results were similar to those reported here.

Epoxide hydrase activity was linear with a microsomal protein concentration between 0·5 and 3·0 mg/ml of incubation volume for liver, intestine and kidney, and between 2 and 6 mg protein/ml for lung microsomes.

A relatively even distribution of both glutathione-*S*-epoxide transferase and epoxide hydrase is observed along the length of the small intestine, with slightly higher activities of both enzymes in segments from the proximal 24 in. Mixed-function oxidase activity is found to be substantially higher at the proximal end [14].

The localization of the two enzymes in the kidney medulla and cortex is given in Table 2. The specific activity of epoxide transferase was about the same in medulla and cortex, whereas epoxide hydrase specific activity was three times higher in microsomes prepared from medulla than in microsomes prepared from cortex. When the protein yields of the cortex and medulla are taken into consideration, 17 per cent of the kidney transferase activity is associated with the cortex soluble fraction, but only 3 per cent of the kidney hydrase activity is present in cortex microsomes.

Epoxide hydrase was assayed in washed and unwashed microsomes, and the slightly higher specific activity found in washed microsomes corresponded to the slightly lower protein yield in washed microsomes. When calculated per g of tissue, the activities were the same in washed and unwashed microsomes.

Both epoxide-metabolizing enzymes were fairly stable at 0–4° under the conditions of preparation of tissue employed. When glutathione-*S*-epoxide transferase activity was assayed in soluble fraction that had been stored in the refrigerator for 1 day, the activity measured was about 90 per cent of the activity found when the same soluble fraction was assayed immediately after preparation. Thereafter, activity fell off gradually with time of storage at 0–4°.

Epoxide hydrase was an unusually stable enzyme, especially as compared with microsomal mixed-function oxidases. Suspensions of liver or intestinal mucosal microsomes stored in the refrigerator at 0–4° had only slightly lower hydrase activity when assayed 1 month after preparation as compared to freshly prepared microsomes assayed the day the animal was sacrificed. Epoxide hydrase activity in liver, intestine, lung or kidney microsomes was not affected by heating the microsomal suspension at 60° for 5 min prior to assay. However, both microsomal epoxide hydrase and soluble fraction glutathione-*S*-epoxide transferase activities were abolished by heating at 100° for 5 min.

When 0·1 M HEPES buffer (final concentration) was used, the pH optima of the glutathione-*S*-epoxide transferases were found to be pH 7·2 for liver and pH 7·6 for kidney, lung and intestinal mucosa soluble fractions (Fig. 1). At pH 8 or greater, the rate of nonenzymatic synthesis of the glutathione conjugate was high.

Epoxide hydrase in microsomes had a broad pH optimum in the range 6·5 to 10·5 with 0·1 M HEPES (pH 6·5 to 9) or Tris (pH 8·6 to 10·5) buffer. Assays were usually performed in 0·1 M HEPES at pH 8·9, since this pH was quoted as the optimum pH of the purified enzyme [12] in the guinea pig.

Apparent kinetic constants for glutathione-*S*-epoxide transferase were obtained from the Lineweaver–Burk plot by a computerized least squares technique. The regression line drawn by the computer was weighted according to Cleland [15]. Apparent kinetic constants with respect to glutathione were determined using 1 mM styrene oxide concentration in each tissue. Apparent K_m and V_{max} values for styrene oxide were determined at glutathione concentrations of 5 mM (Table 3).

The specific activity of epoxide hydrase in small intestine or liver microsomes from rabbits maintained on the purified diet was not significantly different

Table 3. Apparent kinetic constants for glutathione-*S*-epoxide transferase in soluble fraction*

Tissue	Variable glutathione† (1 mM styrene oxide) K_m apparent (M)	Variable glutathione† (1 mM styrene oxide) V_{max} apparent (nmoles. min^{-1}. mg protein^{-1})	Variable styrene oxide‡ (5 mM glutathione) K_m apparent (M)	Variable styrene oxide‡ (5 mM glutathione) V_{max} apparent (nmoles. min^{-1}. mg protein^{-1})
Liver	$1·5 \times 10^{-3}$	41·2	$1·5 \times 10^{-5}$	93·7
Lung	$2·1 \times 10^{-4}$	10·3	$4·5 \times 10^{-3}$	41·8
Intestine	$1·4 \times 10^{-4}$	7·2	$5·8 \times 10^{-3}$	33·6
Kidney	$5·1 \times 10^{-4}$	22·5	$3·2 \times 10^{-3}$	70·6

* These results are values from one experiment. Repeat experiments gave similar results.
† Glutathione concentrations used were $0·05 \times 10^{-3}$ to 20×10^{-3} M.
‡ Styrene oxide concentration was varied between $0·08 \times 10^{-3}$ and 20×10^{-3} M.

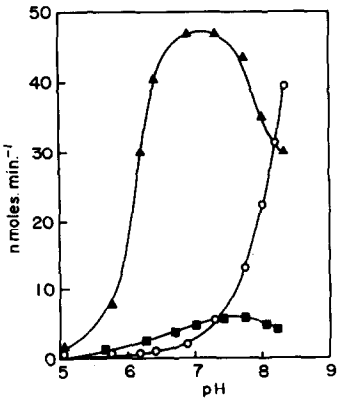

Fig. 1. Variation with pH of liver and lung glutathione-*S*-epoxide transferase activities. Key: (▲) liver soluble fraction: nmoles product formed.min^{-1}.mg of protein^{-1}, corrected for nonenzymatic conjugation at each pH. Highest specific activity was seen when the incubation mixture was at pH 7. (■) lung-soluble fraction: nmoles product formed.min^{-1}.mg of protein^{-1}, corrected for nonenzymatic conjugation at each pH. Highest specific activity was seen when the incubation mixture was at pH 7·4 to 7·6. (○) nonenzymatic conjugation: nmoles conjugate formed .min^{-1}.

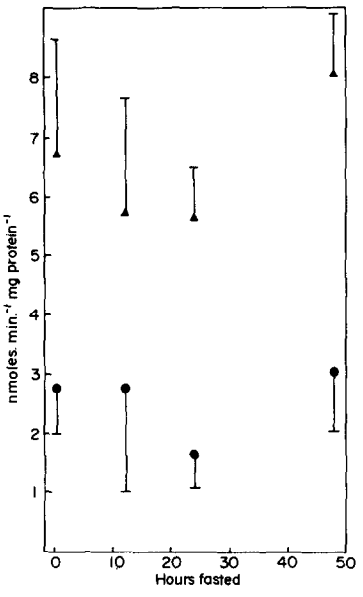

Fig. 2. Effect of starvation on epoxide hydrase activity. Key: (▲) liver, microsomal epoxide hydrase; (●) intestinal mucosa, microsomal epoxide hydrase. Values are mean ±S. D. of four animals.

from that of rabbits on standard feed at any time tested (Table 4). Glutathione-*S*-epoxide transferase in liver and intestine soluble fractions tended to be lower in rabbits fed the purified diet, but the differences were significant at the 5 per cent level (Mann–Whitney U test) for only three of the eight comparisons, namely liver after 6 weeks on the diet and intestine after 2 and 4 weeks on the diet. Benzphetamine-*N*-demethylase also tended to be lower in the liver microsomes from animals fed a purified diet, but the difference was significant at the 5 per cent level only after 6 and 8 weeks on the diet. Lung benzphetamine-*N*-demethylase activity was not affected by the diet differences. Animals maintained on the purified diet gained weight less rapidly than animals fed a standard diet, but the lowering of body weight at the time of sacrifice was significant only at the 2- and 8-week time points.

Table 4. Effect of diet on styrene oxide metabolism *in vitro* by various tissues of the rabbit*

		Weeks on diet (animal age, weeks)							
		2 (10)		4 (12)		6 (14)		8 (16)	
Tissue	Parameter	Regular diet	Purified diet	Regular	Purified	Regular	Purified	Regular	Purified
Liver	Epoxide hydrase (microsomal)†	6·4 ± 1·5	7·6 ± 1·2	6·4 ± 1·0	5·5 ± 0·6	6·0 ± 1·9	4·9 ± 1·7	8·2 ± 1·6	8·2 ± 1·5
	Glutathione-*S*-epoxide transferase (soluble fraction)	29·5 ± 4·2	27·5 ± 2·4	30·0 ± 6·0	24·6 ± 3·5	28·2 ± 2·1	22·2 ± 2·4	27·2 ± 1·3	23·9 ± 5·1
	d-Benzphetamine *N*-demethylase (microsomes)†	4·9 ± 0·8	4·6 ± 0·6	5·2 ± 1·2	4·1 ± 1·3	6·3 ± 1·9	4·2 ± 0·7	6·8 ± 0·9	5·3 ± 0·3
	Average protein yield in microsomes‡	19·1 ± 3·5	13·0 ± 4·3	19·8 ± 1·4	15·6 ± 3·8	20·2 ± 3·3	17·3 ± 1·6	17·1 ± 3·7	20·8 ± 7·1
Lung	Epoxide hydrase	0·18 ± 0·6	ND§	ND	ND	0·15 ± 0·02	ND	ND	ND
	Glutathione-*S*-epoxide transferase	7·1 ± 1·1	ND	6·9 ± 1·5	6·8 ± 1·0	6·4 ± 1·4	5·3 ± 1·1	6·6 ± 1·5	6·8 ± 0·3
	Benzphetamine *N*-demethylase	6·2 ± 2·2	7·2 ± 3·4	3·7 ± 1·4	5·7 ± 1·2	6·7 ± 2·1	5·1 ± 0·6	7·3 ± 3·7	5·2 ± 1·4
	Average protein yield (microsomes)	9·6 ± 1·3	10·0 ± 2·4	9·4 ± 1·0	9·6 ± 2·1	10·5 ± 1·2	9·6 ± 2·1	9·9 ± 0·7	13·0 ± 0·9
Intestine	Epoxide hydrase	5·0 ± 1·1	5·2 ± 0·8	3·6 ± 0·9	3·3 ± 0·4	2·5 ± 0·7	3·2 ± 1·5	3·7 ± 0·4	3·1 ± 0·7
	Glutathione-*S*-epoxide transferase	6·1 ± 0·4	4·1 ± 0·3	4·6 ± 0·6	3·1 ± 0·8	3·8 ± 0·8	2·7 ± 0·4	3·9 ± 1·0	2·8 ± 0·5
	Benzphetamine *N*-demethylase	0·4 ± 0·2	0·5 ± 0·1	0·5 ± 0·3	0·3 ± 0·4	0·6 ± 0·2	0·3 ± 0·1	0·7 ± 0·2	0·6 ± 0·1
	Average protein yield (microsomes)	12·8 ± 0·5	10·9 ± 4·5	10·6 ± 2·6	12·7 ± 1·3	11·9 ± 1·3	12·5 ± 1·3	18·7 ± 2·6	18·0 ± 1·6
	Body wt of rabbits (kg)	1·92 ± 0·13	1·66 ± 0·04	2·26 ± 0·32	2·02 ± 0·2	2·63 ± 0·22	2·23 ± 0·43	3·05 ± 0·27	2·25 ± 0·17
	No. of rabbits	4	4	4	4	4	4	3	4

* See text for composition of each diet.

† Activities are expressed as nmoles product formed.min^{-1}.mg protein^{-1}. The value quoted is the mean ±S. D. Assays were performed as described in the text.

‡ The average protein yield in microsomes is expressed in mg.g wet wt tissue^{-1}.

§ ND means not determined.

Table 5. Species comparison of epoxide metabolizing enzymes*

Animal	Glutathione-*S*-epoxide transferase (nmoles. min^{-1}. mg protein^{-1}) Liver	Lung	Intestine	Kidney	Epoxide hydrase (nmoles. min^{-1}. mg protein^{-1}) Liver	Lung	Intestine	Kidney
Rabbit	30·5 ± 1·6 (15)	6·5 ± 0·6 (15)	4·4 ± 0·4 (15)	7·9 ± 0·9 (15)	5·6 ± 0·3 (15)	0·17 ± 0·02 (15)	2·8 ± 0·3 (15)	1·35 ± 0·05 (15)
Guinea pig	236·5 ± 49·3 (9)	18·6 ± 3·3 (9)	24·6 ± 2·7 (9)	73·5 ± 10·6 (9)	13·7 ± 3·1 (9)	0·47 ± 0·12 (9)	5·8 ± 1·1 (9)	1·06 ± 0·32 (9)
Rat	141·8 ± 38·9 (4)	12·2 ± 4·4 (4 pools)†	12·8 ± 1·1 (4 pools)	82·1 ± 21·2 (4 pools)	4·9 ± 0·5 (4)	0·21 ± 0·06 (4 pools)	0·23 ± 0·09 (4 pools)	0·80 ± 0·14 (4 pools)

* Values are mean ± S.D. Number of animals is given in parentheses.
† Tissue from three rats was pooled for each determination.

Starvation of groups of animals for up to 48 hr also had no effect on the activity of microsomal epoxide hydrase in liver or intestine as compared with that of unfasted animals. However, rabbits fasted for 48 hr had higher microsomal epoxide hydrase activities than the group fasted for 24 hr (Fig. 2), even though the liver weights and protein yields for these two groups were not different.

A species comparison of epoxide-metabolizing enzymes is summarized in Table 5. Both guinea pig and rat have higher hepatic enzyme activities of glutathione-*S*-epoxide transferase than rabbit. Hepatic epoxide hydrase activities are similar in rat and rabbit, while guinea pig has higher activity. The low specific activity of epoxide hydrase in rat intestine is consistent with the results of Oesch *et al.* [5].

DISCUSSION

This study demonstrates that, in the presence of saturating concentrations of glutathione, the specific activity of glutathione-dependent *S*-epoxide transferase in rabbit liver soluble fraction was about five times the specific activity of microsomal epoxide hydrase, using 1 mM styrene oxide as substrate for each enzyme (Table 1). In lung under the same conditions, the specific activity of the glutathione-*S*-epoxide transferase was about 40 times that of epoxide hydrase; in kidney, glutathione-*S*-epoxide transferase activity was about eight times higher than epoxide hydrase activity. However, the specific activity of glutathione-*S*-epoxide transferase in the soluble fraction of intestinal mucosa was only slightly higher than the specific activity of intestinal mucosa microsomal epoxide hydrase. We have not yet attempted to determine how this potential activity correlates with the actual metabolism *in vivo* of epoxides in the different organs. Presumably, how a particular epoxide is metabolized by different organs depends on such factors as the stability of the epoxide in the cell environment, the relative ability of different tissues to transform hydrocarbons to epoxides, and the effective level of epoxide-metabolizing systems. One would speculate that the balance of these factors is crucial in determining localized damage caused by a chemically reactive epoxide to the organ, particularly an epoxide generated *in vivo* by the microsomal mixed-function oxidases. The comparatively low ability of lung to metabolize epoxides, particularly by the epoxide hydrase pathway, may be a significant factor in the comparative vulnerability of lung toward polycyclic hydrocarbon carcinogens.

The preliminary kinetic data for glutathione-*S*-epoxide transferase which are reported here indicate that much higher concentrations of epoxides (than would normally be present *in vivo* from the intake of foreign compounds) are necessary to saturate the enzyme. In addition, the concentration of glutathione available in the organ (about 5 mM in liver, but much less in other organs)* is of the same order as the K_m value for glutathione, at least in the liver. Therefore, in the situation *in vivo*, the rate of glutathione conjugation of epoxides like styrene epoxide would be lower than the V_{max} value for the enzyme *in vitro*, and would vary significantly with the concentration of glutathione in the organ. The amount of glutathione available in any tissue might be expected to affect the toxicity of an epoxide. (This is consistent with studies that show increased tissue necrosis by epoxides after glutathione depletion [16, 17].)

The epoxide hydrase activity of intestinal mucosa was not altered by feeding a synthetic diet for 8 weeks or by starving animals for up to 48 hr, and this agrees with our results, to be reported elsewhere,† that epoxide hydrase is not induced in the rabbit by common microsomal mixed-function oxidase inducing agents as determined in rats, e.g. phenobarbital and 3-methylcholanthrene.

We found marked species differences in glutathione-*S*-epoxide transferase activities between rabbit, guinea pig and rat when the enzyme was assayed under the same conditions of substrate and glutathione concentration in tissue preparations from these three species. We have no information at present as to whether this is a reflection of differences in the amount of enzyme present, if differences in some other enzyme-related factor affect the measurable transferase activity, or if completely different enzymes are present in these three species.

The high specific activities of glutathione-*S*-epoxide transferase found in liver suggest that endogenous compounds or their analogs might be natural substrates for this enzyme. For instance, the metabolite of ethynylestradiol reported by Bolt *et al.* [18] could have been formed by epoxidation at the 1,2-bond and subsequent conjugation with glutathione, catalyzed by glutathione-*S*-epoxide transferase.

Overall, this study indicates that although liver is quantitatively the most important site in the metabolism of epoxides, intestine, lung and kidney were also capable of detoxifying epoxides, since epoxide hydrase and glutathione-*S*-epoxide transferase were found in these organs. Lung and intestine provide routes of

* Unpublished observations in this laboratory.
† R. M. Philpot, J. R. Bend, M. O. James and J. R. Fouts, manuscript in preparation.

entry of foreign chemicals into the body, and kidney is a route of elimination, so the presence of detoxifying enzymes in these tissues may be important to the defense mechanisms of the body. Ratios of activities of glutathione-*S*-epoxide transferase and epoxide hydrase are quite different in the organs studied, and this may be a contributing factor toward selective tissue toxicity after ingestion of xenobiotics which are epoxide precursors.

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