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THE EFFECT OF HEPATIC MICROSOMAL AND CYTOSOLIC SUBCELLULAR FRACTIONS ON THE MUTAGENIC ACTIVITY OF EPOXIDE-CONTAINING COMPOUNDS IN THE SALMONELLA ASSAY

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Summary

7 epoxide-containing compounds: allylbenzene oxide, styrene oxide, *trans*-β-methylstyrene oxide, 4-chlorophenyl glycidyl ether, vinylcyclohexene dioxide, octene dioxide and hexene dioxide were evaluated for mutagenic activity in 4 histidine-requiring strains of *Salmonella typhimurium*, namely: TA1535, TA100, TA1537 and TA98. These epoxides, except *trans*-β-methylstyrene oxide, were mutagenic in TA1535 and TA100 but none of the tested compounds caused mutations in strains TA1537 and TA98. Both the cytosolic (100 000 *g* soluble) and/or microsomal (100 000 *g* pellet) fractions derived from non-induced mouse, guinea pig, and/or rat consistently decreased the mutagenic activity of the 3 most active mutagens: allylbenzene oxide, styrene oxide and 4-chlorophenyl glycidyl ether. This reduction was found to depend on the substrate and the source of the enzyme fraction. Glutathione alone or in combination with the mouse cytosolic fraction resulted in negligible suppression in the mutagenic activity of the 3 epoxides under the conditions reported in this paper. The enzyme(s) in the cytosol responsible for the reduction in mutagenicity co-eluted from gel filtration with the epoxide hydrolase activity. These data are not consistent with the assumption that all epoxide hydrolase activity in an "S9" fraction is microsomal.

The use of in vitro microbial mutagenesis assays either alone or coupled with metabolic activating enzymes is of great importance since there is a close correlation between mutagenicity and carcinogenicity [2—4,27—29]. Electrophilic

attack on DNA by a parent chemical or an active metabolite is a common mechanism of mutagenicity [10,23,30,33,39]. Some epoxides are apparently direct-acting mutagens, and they may be formed in vivo from the corresponding alkene or arene. Besides the activating epoxidation reaction, deactivating reactions are also important in determining mutagenicity [6—9]. Epoxides are converted to more water-soluble materials by conjugation with glutathione with or without catalysis by cytosolic glutathione-*S*-epoxide transferase (EC 2.5.1.18) [8,9,11,20,22]. Enzymes known as epoxide hydrolases convert epoxides to 1,2-diols (EC 3.3.2.3, formerly EC 4.2.1.63 and 64, and referred to as hydrases or hydratases). Epoxide hydrolases have been intensively studied, and this work is covered in numerous reviews [20—22,24,25,34]; however, most reports incorrectly assume that all epoxide hydrolase activity is membrane-bound to the endoplasmic reticulum or nuclear membrane. More recent work has shown that there are highly active cytosolic epoxide hydrolases in numerous tissues from a variety of mammals [13—15,18—21,32]. Since the "S9" fraction commonly used in short-term mutagenicity assays contains glutathione-*S*-epoxide transferases, microsomal epoxide hydrolases and cytosolic epoxide hydrolases, this study was designed to evaluate the influence of these enzymes on the mutagenicity of several epoxides in the Ames' Salmonella assay [2—4,27—29].

Materials and methods

Strains

The *Salmonella typhimurium* strains, TA1535, TA100, TA1537 and TA98 used for the detection of mutagenic activity of the tested chemicals were obtained from Dr. Bruce Ames, University of California at Berkeley. All strains were selected for their sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens [2—4].

Enzyme preparation

The 9000 *g* supernatants of rat, mouse or guinea pig liver homogenates were obtained using a procedure similar to that described by Ames et al. [4], as indicated below. Male Swiss-Webster mice (30—40 g, 9—10 wk old) were from Hilltop Laboratories, Chatsworth (CA), while male Hartley guinea pigs (~600 g, 6 mo. old) and Sprague-Dawley rats (430—560 g) were from Simonsen Laboratories, Gilroy (CA). The animals were not induced prior to sacrifice by cervical dislocation. The 9000 *g* supernatant (equivalent to a 10% w/v liver homogenate) was filtered through a Nalgene disposable membrane filter unit (0.45 μm pore size) to remove potential bacterial contamination. Isolated microsomes were very difficult to filter, so under sterile conditions the filtered "S9" was further centrifuged at 100 000 *g* for 1 h. The supernatant fraction (the cytosol) was decanted into sterilized containers, divided into 3-ml fractions and stored at −80°C until used. No contamination of the plates was detected during these experiments. Epoxide hydrolase activity in the cytosolic fraction was quite stable under these conditions. The insoluble fraction (microsomal) was washed with phosphate buffer (pH 7.4, I = 0.2 M) and recentrifuged at the same rate for 65 min, after which the supernatant was decanted and discarded

while the washed microsomal fraction was suspended in the original amount of phosphate buffer and used the same day. Several marker enzymes indicated that there was negligible cross-contamination of the fractions used although unwashed microsomes may have substantial cytosolic contamination [13,14, 20].

Gel filtration of the cytosolic fraction

The crude soluble fraction was filtered through Sephacryl S-200 as described earlier [13]. Molecular weights were estimated from the elution pattern of cytochrome *c*, ribonuclease, carbonic anhydrase, ovalbumin, lactate dehydrogenase, catalase, phosphorylase *a*, and β-galactosidase. Epoxide hydrolase activity was monitored in the column fractions with radiochemical assays using a terpenoid epoxide [32], methyl *cis*-epoxystearate [14] and *trans*-β-ethylstyrene oxide [31], and with gas-liquid chromatographic assays using allylbenzene oxide, *trans*-stilbene oxide, and *trans*-β-methylstyrene oxide [21]. Fractions corresponding to apparent molecular weights of >140 000 and 140 000—120 000 were concentrated with an Amicon XM50 filter, the 120 000—80 000 and 60 000—15 000 fractions with an Amicon PM10 and the <15 000 fractions were lyophylized to give equal volumes corresponding to a 5% w/v liver homogenate, and 0.5 ml of each fraction were added to each plate.

Chemicals

7 epoxide-containing compounds: allylbenzene oxide (1,2-epoxy-3-phenylpropane), styrene oxide (1,2-epoxyethylbenzene), *trans*-β-methylstyrene oxide (*trans*-1,2-epoxy-1-phenylpropane), 4-chlorophenyl glycidyl ether (*p*-chlorophenyl 2,3-epoxypropyl ether), vinyl cyclohexene dioxide (1-ethyleneoxy-3,4-epoxycyclohexane), hexene dioxide (1,2,5,6-diepoxyhexane), and octene dioxide (1,2,7,8-diepoxyoctane) were obtained from Aldrich Chemical Company or synthesized by peracid or *N*-bromosuccinimide oxidation in the latter case followed by potassium carbonate catalyzed cyclization. Structural assignment was based on the respective nuclear magnetic resonance and infrared spectra. All compounds reacted strongly on silica gel thin-layer chromatography with 4-(*p*-nitrobenzyl)-pyridine [19] and showed only one spot in several solvent systems when visualized with quenching of uv fluorescence or by charring. The compounds showed only one peak on gas-liquid chromatography using flame-ionization detection. All compounds were dissolved in spectral grade or redistilled dimethyl sulfoxide (DMSO) in varying concentrations, and stored in the dark at 5°C until use. Chromatographic analysis indicated that the test compounds were quite stable in DMSO solution, and they were used shortly after dilution.

Mutagenicity assays

Tests for mutagenicity were carried out according to the procedures developed by Ames et al. [4]. To a test-tube containing 2 ml of molten top agar at 45°C were added 0.1 ml of the test compound in DMSO, 0.1 ml of an overnight broth culture of the bacterial tester strain (12 h, 37°C in a shaking incubator) and 0.25 or 0.5 ml (as indicated in the text and tables) of enzyme or co-factor preparations. Glutathione solutions were prepared immediately

before use to prevent disulfide formation. Several glutathione concentrations were tested, but the data presented in this paper was obtained by adding 0.9 mg of glutathione per plate. The control tubes contained the corresponding amount of phosphate buffer and/or 0.1 ml of DMSO. Enzyme fractions held in a boiling water bath for 5 min retained no catalytic activity and were also used as controls. The contents of the tubes containing top agar were mixed and poured immediately onto agar plates. The agar overlay was allowed to harden, and the plates inverted and incubated for about 3 days at 37° C after which the revertant colonies were counted. The number of revertants per plate is an average number of at least 2 separate experiments each comprised of at least 3 plates for each treatment.

Use of mutagenicity factor

For evaluation of the relative efficacy of the tested compounds either alone or in combination with liver homogenates, we propose the use of a mutagenicity factor (MF) = (number of revertant colonies in treatment — number of spontaneous colonies)/number of spontaneous colonies. Use of this factor offers several advantages, some of which are analogous to those proposed by Abbott [1] for the use of a similar factor in classical bioassay. Variations in an overnight culture even when standardized by O.D. and a multitude of minor changes in experimental conditions (such as room or incubator temperature) may lead to moderate variation in the number of spontaneous revertants over a period of weeks or months, although variation on any one day is minimal. Such variation presents no difficulty when a series of experiments are based on a single series of control plates run simultaneously. However, one may experience difficulty in comparing data based on control plates with moderate differences in the number of spontaneous revertants. Use of the mutagenicity factor facilitates direct comparison of such experiments performed in different laboratories or at different times.

The mutagenicity factor is not a statistic which assesses the reliability of data, and such statictical tests of significance should be employed before using the mutagenicity factor. However, once the raw data at different doses of a compound are converted to mutagenicity factors, a plot of MF vs. dose gives higher linearity than a plot of revertant colonies vs. dose. The raw number of revertant colonies are presented in the tables as well as the corresponding mutagenicity factor for the dose used. In accord with the suggestion by Ames et al. [4], a compound could be considered a mutagen if it causes twice the number if spontaneous revertants and thus has a mutagenicity factor of one.

Results and discussion

The mutagenic activity of allylbenzene oxide, styrene oxide, *trans*-β-methylstyrene oxide, 4-chlorophenyl glycidyl ether, vinyl cyclohexene dioxide, octene dioxide and hexene dioxide over a wide range of concentrations in 4 strains of *S. typhymurium* is shown in Table 1. All the tested compounds, except *trans*-β-methylstyrene oxide showed mutagenic activity in strains TA1535 and TA100 but were found to be inactive with the other 2 strains. The lack of detectable mutagenicity of *trans*-β-methylstyrene oxide at the doses examined was surpris-

ing in light of the mutagenicity of the closely related compound, anethole [41]. Anethole presumably acts through its epoxide or "hydroxy-epoxide" metabolite, and possibly 1,2-disubstituted aliphatic epoxides such as *trans*-β-methylstyrene oxide are too sterically hindered or unreactive to be potent mutagens. Subsequent studies in this laboratory have shown that several such 1,2-disubstituted epoxides are only very weak mutagens. In all mutagens, an increase in the concentration up to a certain level (500, 62.5, 1000 μg/plate) for allylbenzene oxide, 4-chlorophenyl glycidyl ether and each of styrene oxide, vinylcyclohexene dioxide, octene dioxide and hexene dioxide, resp., increased the number of revertant colonies. At higher dosages, the number of revertants either slightly or sharply decreased, which may be due to cytotoxicity resulting from inactivation of essential genes or other toxic effects [2]. This screening demonstrates that these epoxides, except *trans*-β-methylstyrene oxide, seem to be base-pair substitution mutagens. The test compounds can be arranged in descending order according to colonies/μmole of compound based on the highest effective dose with TA100 as follows: 4-chlorophenyl glycidyl ether, allylbenzene oxide, styrene oxide, hexene dioxide, octene dioxide and vinylcyclohexene dioxide (hexene dioxide is more mutagenic than styrene oxide in TA1535).

It is interesting to note that when the MF expression is used, mutagenic activity is often higher in TA1535 than in TA100. TA100 is usually throught to be more sensitive since it is derived from TA1535 by the introduction of an R factor plasmid to enhance the sensitivity to certain mutagens [29]. True sensitivity of any assay is the minimum dose detectable from background, so the level of spontaneous recovery as well as the reproducibility of the assay should be considered in a sensitivity assessment.

Allylbenzene oxide, 4-chlorophenyl glycidyl ether and styrene oxide (the most mutagenic compounds, Table 1) were chosen for evaluating the role of deactivating enzyme(s) in the cytosolic and microsomal fractions of liver homogenates from mouse, rat and guinea pig. In each case, compounds were selected which could be readily analyzed by glc for purity and for their relative rates of enzymatic hydration [21]. Styrene oxide is a known mutagen [28,42], it has been extensively used in the investigation of microsomal and nuclear epoxide hydrolase [20,25,26,34—36], and it is poorly metabolized by the cytosolic fraction [20,21,37]. Allylbenzene oxide is rapidly metabolized by cytosolic epoxide hydrolase(s); it is a much better substrate than styrene oxide for microsomal epoxide hydrolase(s), and it is a useful model compound for natural products such as estragole, eugenol and safrole [41] as well as allyl-containing drugs. The report by Andersen et al. [5] that aromatic epoxyresins are weak mutagens has led to a great deal of interest in glycidyl ethers [17,40], in part, because over 260 million pounds of glycidyl ethers were produced in the United States in 1977 alone [40]. The epoxide hydration of *p*-chlorophenyl-glycidyl ether by the microsomal or cytosolic fractions is easily monitored by glc with a flame ionization or an electron-capture detector at increased sensitivity.

As shown in Table 2, both the cytosolic fraction and the microsomal fraction reduced the mutagenicity of the 3 compounds. With the soluble fraction the reduction in mutagenicity was most dramatic with allylbenzene oxide (an

TABLE 1

MUTAGENIC ACTIVITY OF 7 EPOXIDE-CONTAINING COMPOUNDS TOWARD 4 STRAINS OF *S. typhimurium* [a]

Dose (μg/plate)	Number of revertant colonies [b]													
	TA1535							TA100						
	A	S	G	B	V	O	H	A	S	G	B	V	O	H
2000	T	T	—	T	T	T	T	—	T	—	T	T	T	T
1000	740	183	—	15	106	138	273	T	1334	—	179	399	575	721
500	481	103	—	18	61	93	189	3810	673	—	154	359	449	526
250	243	80	T	—	44	55	142	2210	407	T	—	312	295	426
125	161	46	225	—	29	37	83	1085	246	935	—	248	187	307
62.5	90	—	293	—	22	23	51	563	—	984	—	193	153	236
31.25	46	—	112	—	—	—	—	382	—	561	—	—	—	—
15.63	—	—	63	—	—	—	—	—	—	330	—	—	—	—
7.81	—	—	36	—	—	—	—	—	—	269	—	—	—	—
0	27	23	18	18	18	17	26	174	166	156	166	173	155	158

[a] Abbreviations are as follows: A, allylbenzene oxide (MW = 134); S, styrene oxide (120); G, 4-chlorophenyl glycidyl ether (170.5); B, *trans*-β-methylstyrene oxide (134); V, vinylcyclohexene dioxide (114);

TABLE 2

THE EFFECT OF THE SOLUBLE AND MICROSOMAL FRACTIONS OF MOUSE, GUINEA PIG, AND RAT LIVER HOMOGENATES ON THE MUTAGENIC ACTIVITY OF ALLYLBENZENE OXIDE, STYRENE OXIDE, 4-CHLOROPHENYL GLYCIDYL ETHER USING TA100

Mutagens	Dose (μg/plate)	Number of revertant colonies (including spontaneous)					
		Mouse (M)		Guinea pig (G)		Rat (R)	
		— [c]	+ [d]	—	+	—	
I. The cytosolic fraction							
Allylbenzene oxide	250	1845 ± 47	335 ± 32	1845 ± 47	803 ± 11	1911 ± 18	
Styrene oxide	1000	1099 ± 45	890 ± 35	1099 ± 45	966 ± 33	1099 ± 17	
Glycidyl ether	62.5	1027 ± 33	272 ± 30	1027 ± 33	531 ± 11	1033 ± 24	
DMSO	0	161 ± 8	165 ± 8	161 ± 8	165 ± 8	164 ± 21	
II. The microsomal fraction							
Allylbenzene oxide	250	1987 ± 76	894 ± 32	1878 ± 31	281 ± 8	1663 ± 119	
Styrene oxide	1000	1111 ± 51	292 ± 34	1115 ± 54	487 ± 83	812 ± 162	
Glycidyl ether	62.5	966 ± 57	285 ± 56	975 ± 59	259 ± 16	956 ± 98	
DMSO	0	162 ± 12	170 ± 9	166 ± 10	145 ± 22	146 ± 7	

[a] MF (mutagenicity factor) = (number of revertant colonies in the treatment — number of spontaneous colonies/number of spontaneous colonies).

[b] % Reduction in mutagenicity = (number of revertant colonies without enzymes — number of revertant colonies with enzymes/number of revertant colonies without enzymes) × 100.

TABLE 1 (continued)

Number of revertant colonies [b]													
TA1537							TA98						
A	S	G	B	V	O	H	A	S	G	B	V	O	H
—	T	—	T	T	T	T	T	T	—	T	T	T	—
T	8	—	7	11	6	—	50	38	—	36	40	39	51
25	7	—	6	11	5	6	40	30	—	34	40	40	48
18	8	—	—	11	5	5	39	29	—	—	40	30	36
7	6	T	—	8	6	3	38	31	T	—	44	28	41
6	—	2	—	6	5	4	—	—	21	—	43	25	28
7	—	4	—	—	—	—	—	—	44	—	—	—	—
—	—	9	—	—	—	—	—	—	35	—	—	—	—
—	—	10	—	—	—	—	—	—	28	—	—	—	—
9	8	6	8	7	7	7	37	37	43	37	39	39	43

O, octene dioxide (142); H, hexene dioxide (140); T, toxic.
[b] Above data is from at least 3 replicates on 3 separate occasions and includes spontaneous revertants.

TABLE 2 (Continued)

Rat (R)	Mutagenicity factor [a]						% Reduction [b] in mutagenicity		
	Mouse		Guinea pig		Rat				
							M	G	R
+	—	+	—	+	—	+			
1695 ± 70	10.5	1.0	10.5	4.0	10.6	9.0	90	38	12
1058 ± 18	5.8	4.4	5.8	5.1	5.7	5.2	23	15	5
857 ± 29	5.4	0.6	5.4	2.4	5.2	4.0	88	58	20
170 ± 14	—	—	—	—	—	—	—	—	—
377 ± 29	11.3	4.3	10.3	0.9	10.4	1.6	60	92	84
603 ± 52	5.9	0.7	5.7	2.4	4.6	3.2	87	64	31
213 ± 58	5.0	0.7	4.9	0.8	5.5	0.5	86	86	92
145 ± 14	—	—	—	—	—	—	—	—	—

[c] Without enzyme fractions.
[d] With 0.5 ml of 10% tissue weight/homogenate volume equivalent of the respective subcellular fraction.

excellent substrate for cytosolic epoxide hydrolase) and least dramatic with styrene oxide (a very poor substrate for cytosolic epoxide hydrolase). The cytosolic epoxide hydrolase metabolizes glycidyl ethers at an intermediate rate [21]. Maximal reduction in mutagenicity in each case was obtained with the mouse cytosolic fraction and least with rat which correlates well with the cytoplasmic epoxide hydrolase activity observed in the 3 species (mouse > guinea pig > rat) [20,38].

All 3 substrates are hydrated by the hepatic microsomal fractions of the 3 species [21,37], and thus it is not surprising that the mutagenicity was reduced in each case. These results correlate well with glc assays of the metabolism of these substrates in the hepatic subcellular fraction. The rat has very low cytosolic but high microsomal epoxide hydrolase activity, the mouse has high cytosolic and low microsomal epoxide hydrolase activity and the guinea pig demonstrates moderately high epoxide hydrolase activity in both fractions [37,38].

The reduction of mutagenicity by the microsomal fraction was in each case higher than that predicted based on the degradative metabolism of the respective compound. This observation is probably explained, in part, by the hydrophobic binding of microsomes to Salmonella in the Ames' assay [16]. The ability of the cytosolic fraction to reduce the mutagenicity of styrene oxide was surprising. High native protein concentrations may, however, compete with the Salmonella for styrene oxide binding until much of the oxide is hydrated by the cytosolic enzyme or water or until it reacts with other nucleophiles (styrene oxide is quite unstable at pH 7.4 in phosphate buffer). The addition of glutathione alone at up to 0.9 mg/plate had no effect on the mutagenic activity of the 3 compounds (Table 3), and higher doses of glutathione proved toxic. The addition of glutathione in combination with the cytosolic fraction led to no detectable reduction in mutagenicity over that observed with the soluble fraction alone, although glutathione is known to react with these epoxides. A small effect due to glutathione-*S*-epoxide transferases could certainly be masked by a larger contribution from other cytosolic enzymes, and the low mutagenicity of these compounds caused them to be used in amounts which often stoichiometrically exceed the amount of glutathione present. Still, the data summarized in Table 3 indicate that under the conditions used here glutathione has little or no role in reducing the mutagenicity of the 3 compounds studied, and the levels of glutathione employed in the reported tests are higher than the levels found in the livers of most mammals [11] while the levels of cytosolic and microsomal enzymes are much lower. These data are consistent with epoxide hydrolase activity being largely responsible for the reduction in mutagenicity caused by the cytosolic fraction in the studies reported here, and similar results were previously observed using benzo[*a*]pyrene 4,5-oxide as substrate for glutathione-*S*-epoxide transferases [16].

Epoxide hydrolase activity in the cytosolic fraction was observed to elute as a single peak from gel filtration when 6 different substrates, including allylbenzene oxide, were used. Such data should not be taken as proof for the existence of a single cytosolic enzyme but, at least with these 6 substrates, potentially different forms of cytosolic epoxide hydrolase have similar molecular weights. The epoxide hydrolase (apparent MW. $\cong$ 130 000) was assayed in

TABLE 3

RELATIVE EFFICACY OF GLUTATHIONE AND THE SOLUBLE FRACTION OF MOUSE-LIVER HOMOGENATE, EITHER ALONE OR THEIR MIXTURE, ON THE MUTAGENIC ACTIVITY OF STYRENE OXIDE, ALLYLBENZENE OXIDE AND 4-CHLOROPHENYL GLYCIDYL ETHER USING TA100

Biological aspects	Treatments [a]					
	Mut	Mut + Gl	Mut + Cyt		Mut + Gl + Cyt	
			Cytosol 0.25 ml	Cytosol 0.5 ml	Cytosol 0.25 ml	Cytosol 0.5 ml
Styrene oxide (1000 μg/plate)						
Number of revertants/plate [b]	1089 ± 127	989 ± 56	—	874 ± 23	903 ± 68	920 ± 68
MF	6	5.3	—	4.6	4.8	4.9
% reduction in mutagenicity	—	9.1	—	20	17	16
Allylbenzene oxide *(250 μg/plate)*						
Number of revertants/plate	1883 ± 82	1895 ± 45	651 ± 44	213 ± 11	615 ± 93	—
MF	11.1	11.1	3.2	0.4	2.9	—
% Reduction in mutagenicity	—	0	65	89	67	—
4-Chlorophenyl glycidyl ether (62.5 μg/plate)						
Number of revertants/plate	991 ± 86	1096 ± 67	433 ± 42	218 ± 19	450 ± 45	272 ± 47
MF	5.4	5.37	1.6	0.4	1.99	0.6
% Reduction in mutagenicity	—	0	56	78	59	73

[a] Mut, mutagen alone; Mut + Gl, mutagen + glutathione (0.9 mg); Mut + Cyt, mutagen + cytosol (10% w/v equivalent); Mut + Gl + Cyt, mutagen + glutathione + cytosol.

[b] These figures include the spontaneous revertants which are 156 ± 11 and 173 ± 11 with the buffer and glutathione, resp.

a fraction containing proteins of about 120 000—150 000 MW. As shown in Table 4, the fraction containing epoxide hydrolase activity (but no glutathione-*S*-epoxide transferase activity) was the only fraction which showed a highly significant (p 0.01) reduction in the mutagenic activity of allylbenzene oxide using TA100. This fraction showed an 83% suppression of mutagenic activity compared to a 90% suppression with an equivalent amount of crude cytosolic fraction. The recovery of approx. 91% of the ability of the crude cytosol to reduce allylbenzene oxide's mutagenicity agrees well with a 90% recovery of epoxide hydrolase activity from the gel filtration column.

Table 5 shows the relationship between the concentration of the mouse soluble fraction and the number of revertants induced by allylbenzene oxide. The metabolic deactivation of allylbenzene oxide is positively correlated with the amount of the soluble fraction. All concentrations, even the lowest one (1.25% w/v), resulted in a significant decrease in revertant colonies, and the loss of mutagenic response was approx. complete when allylbenzene oxide was incubated with 10% w/v cytosol.

TABLE 4

THE EFFECT OF FRACTIONS FROM GEL FILTRATION OF THE SOLUBLE FRACTION OF MOUSE-LIVER HOMOGENATES ON THE MUTAGENIC ACTIVITY OF ALLYLBENZENE OXIDE (250 μg/PLATE) USING TA100

Range of apparent [a] molecular weight	Number of colonies [b]		MF	% Reduction in mutagenicity
	without mutagen (spontaneous colonies)	with mutagen		
>150 000	159 ± 9	1875 ± 202	10.8	0
150 000—110 000	176 ± 10	458 ± 26	1.6	83.0
110 000— 60 000	174 ± 12	1652 ± 40	8.5	11.0
60 000— 15 000	186 ± 3	1793 ± 266	8.6	3.2
<15 000	187 ± 12	1615 ± 175	7.6	14.0
Buffer	161 ± 8	1821 ± 191	10.3	—

[a] Each molecular weight range was concentrated to be equivalent to a 5% weight/volume liver homogenate, and 0.5 ml was added to each plate.
[b] These figures include spontaneous colonies.

The mutagenicity of a compound is dependent upon the routes of metabolic activation and deactivation as well as the stability and potency of the compound and its metabolites in the test system used. The "S9" or postmitochondrial supernatant often used in short-term assays has been so commonly referred to as "microsomes" that some workers may forget that ~80—90% of the protein present is from the cytosol rather than from the endoplasmic reticulum. Even those authors that recognize the presence of the cytosol are often mistaken in assuming that without NADPH, epoxides are metabolized only by soluble glutathione-*S*-epoxide transferases or microsomal epoxide hydrolases. This study has shown that it is crucial to consider the contribution of cyto-

TABLE 5

THE EFFECT OF DIFFERENT CONCENTRATIONS OF THE CYTOSOLIC FRACTION OF MOUSE-LIVER HOMOGENATE (+) ON THE MUTAGENIC ACTIVITY OF ALLYLBENZENE OXIDE (250 μg/PLATE) USING TA100

Concentration [a] level (w/v%)	Number of revertant colonies [b]		MF [c]		% Reduction in mutagenicity
	(—)	(+)	(—)	(+)	
10	—	276 ± 32	—	0.7	94
5	—	497 ± 75	—	2.2	80
2.5	—	743 ± 77	—	3.7	66
1.25	—	1087 ± 243	—	5.7	45
0 (Buffer)	1679 ± 238	—	10.5	—	—

[a] The concentration refers to the cytosolic equivalent of the tissue wet weight/homogenate volume. In each case 0.5 ml of the enzyme solution was added.
[b] These figures include the spontaneous background; the difference between the spontaneous background with DMSO (160 ± 12) and the above cytosol concentrations were negligible (ranged between 154 and 166). Heat-inactivated protein caused no detectable reduction in mutagenicity.
[c] MF corrects for spontaneous revertants.

plasmic as well as microsomal epoxide hydration when interpreting such tests. Preliminary data from this laboratory indicates that the cytosolic and microsomal epoxide hydrolases and glutathione transferases are under different regulatory control. For instance, in male Swiss-Webster mice Aroclor 1254 appears to be a poor inducer of the cytosolic enzyme. Hydration of several epoxides with low K_m's by the cytosolic epoxide hydrolase is not inhibited by trichloropropene oxide [15,20], and this compound is commonly used in short-term assays to eliminate the contribution of epoxide hydrolases [35]. The activity of both cytosolic and microsomal epoxide hydrolases differs greatly with diet, sex, age and species [13,20,34] and these differences may account, in part, for the difficulty experienced by Ashby and Styles [6,7] and other workers [12] in standardizing the Ames' Salmonella assay. If a standard protocol, careful animal handling, and possibly the use of a mutagenicity factor do not result in sufficient standardization, the quantitation of the cytosolic epoxide hydrolase [20,21,31,32] as well as glutathione transferase and microsomal hydrolase activities should be considered when epoxides are suspected as ultimate mutagens.

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