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Species Differences in Activating and Inactivating Enzymes Related to the Control of Mutagenic Metabolites*

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ıet. Microsomal monooxygenases catalyze the biosynthesis of epoxides lefinic and aromatic compounds whilst microsomal epoxide hydratase oplasmic glutathione S-transferases are responsible for their further bio- mation. Although catalytically very efficient the cytoplasmic glutathione erases play, due to their subcellular localization, a minor role in the vation of epoxides derived from large lipophilic compounds and were, therefore, not included in this study. It was shown with such a lipophilic compound, benzo(a)pyrene, as a model substance and with liver enzyme mediated bacterial mutagenesis as biological endpoint that species and strain differences in epoxide hydratase and monooxygenases are reflected in very dramatic differences in mutagenicity of benzo(a)pyrene which varied from extremely potent to a degree which could easily be overlooked. In order to investigate whether the differences in enzyme activities were causally linked to the observed differences in mutagenicity, the enzyme activities were modulated by inhibition and induction. These manipulations were always accompanied by the corresponding changes in mutagenicity.

It is concluded that species such as mice which possess high monooxygenase activity but very low epoxide hydratase activity are much more susceptible than man to those toxic effects which are mediated by metabolically formed epoxides which are substrates of epoxide hydratase. In this regard, it is especially noteworthy that mice possess a much lower hepatic epoxide hydratase activity than man.

Key words: Epoxide hydratase — Benzo(a)pyrene — Inactivation — Mutagenicity.

Zusammenfassung. Mikrosomale Monooxygenasen oxidieren olefinische und aromatische Stoffe zu Epoxiden, mikrosomale Epoxidhydratase und zytoplas-

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matische Glutathion-S-Transferasen setzen diese Epoxide weiter um. Obwohl katalytisch sehr aktiv, spielen zytoplasmatische Glutathion-S-Transferasen aufgrund ihrer subzellulären Lokalisierung nur eine untergeordnete Rolle bei der Inaktivierung von Epoxiden, die von großen lipophilen Substanzen gebildet werden, und wurden daher in dieser Studie nicht untersucht. Mit Benzo(a)pyren als Modellsubstanz und mit Leberenzym-vermittelter bakterieller Mutagenese als biologischem Endpunkt wurde gezeigt, daß sich Spezies- und Stammesunterschiede von Epoxidhydratase und Monooxygenasen sehr massiv in der Mutagenität widerspiegeln: Je nach Herkunft des aktivierenden Leberpräparates wird für Benzo(a)pyren eine äußerst starke oder eine verschwindend geringe, durch aus übersehbare Mutagenität beobachtet. Um festzustellen, ob die Unterschiede in den Enzymaktivitäten mit den beobachteten Unterschieden der Mutagenität kausal zusammenhängen, wurden die Enzymaktivitäten durch Inhibition und Induktion manipuliert. Diese Manipulationen hatten in jedem Falle entsprechende Veränderungen der Mutagenität zur Folge.

Es wird geschlossen, daß Tierarten wie die Maus, die eine hohe Monooxygenase- und eine sehr niedrige Epoxidhydratase-Aktivität aufweisen, viel anfälliger sind als der Mensch für solche toxischen Wirkungen, welche durch metabolisch gebildete Epoxide verursacht werden, die durch Epoxidhydratase inaktiviert werden. In dieser Hinsicht ist erwähnenswert, daß Mäuse eine sehr viel geringere Epoxidhydratase-Aktivität aufweisen als der Mensch.

Introduction

Evidence is accumulating that a vast array of substances which after application to an animal produce mutagenic and carcinogenic effects need metabolism to electrophilically reactive species before they can exert such effects (for a review see Miller and Miller, 1974). Aromatic and olefinic compounds are metabolized by microsomal monooxygenases to electrophilically reactive epoxides. On the one hand, such epoxides can spontaneously bind with nucleophilic moieties in tissue such as DNA, RNA and proteins. On the other hand there exist enzymes which compete for these epoxides (for reviews see Daly et al., 1972; Oesch, 1973; Sims and Grover, 1974; Heidelberger, 1975). Species differences in activating and inactivating enzymes are likely to play an important role in the determination of the tissue levels of reactive metabolites. For large and lipophilic species such as epoxides derived from polycyclic hydrocarbons, the cytoplasmic glutathione S-transferases play a minor role due to their localization (Glatt and Oesch, 1977). Therefore, in this study, the relationship between species and strain differences in monooxygenases (mixed function oxidases) and epoxide hydratase (epoxide hydrase, E.C. 4.2.1.63), and the relative accumulation of benzo(a)pyrene metabolites mutagenic for *Salmonella typhimurium* strains was investigated.

Materials and Methods

C3H/HeJ mice (18–23 g) wer from the Jackson Laboratory, Bar Harbor, Maine, USA; CF-1 mice (15–20 g) from Issa Credo, Les Oncins, Saint Germain sur L'Arbresle, France; Sprague-Dawley rats

(200–250 g) from the Versuchstier-Zuchtanstalt Wiga, Sulzfeld, West Germany; Fischer rats (220–250 g) from the Zentralinstitut für Versuchstierzucht, Hannover-Linden, West Germany. All animals used in this study were adult males with the exception of the C3H mice, which were adult females[1]. Immediately after killing the animals the livers were removed and all subsequent steps in the preparation of subcellular fractions were performed at 0–4° C. The livers were homogenized in 3 volumes of 150 mM KCl containing 10 mM potassium phosphate buffer, pH 7.4, using a Potter-Elvehjem all glass homogenizer. The homogenates were centrifuged at 9000 *g* for 15 min. The resulting post-mitochondrial supernatant fractions were divided into two portions. One portion was used as such and is called "post-mitochondrial supernatant" in this study. This fraction is analogous to the fraction referred to as "S-9" by Ames et al. (1973). The other portion was centrifuged at 100,000 *g* for 1 h. The resulting pellets were rinsed with 150 mM KCl containing 10 mM potassium phosphate buffer and then resuspended in the same medium. This preparation is called "microsomes".

The mutagenicity was tested as described by Ames et al. (1973) with some minor modifications (Glatt et al., 1975) but without albumin. The test compounds were added in dimethylsulfoxide (20–60 µl) and were, together with the enzyme systems and histidine-dependent *Salmonella typhimurium* TA 1537 or TA 98, poured in a histidine-poor top agar onto a minimal agar plate. After incubation at 37° C for 2 days, the his^+ revertant colonies were counted.

Monooxygenase activity with benzo(a)pyrene as substrate was determined, measuring fluorescent phenolic metabolites and using 3-hydroxybenzo(a)pyrene as standard (Nebert and Gelboin, 1968). Monooxygenase activity with 7-ethoxycoumarin as substrate was determined according to the method of Ullrich and Weber (1972) using 3 diagnostic inhibitors as a crude measure for different monooxygenase forms (Ullrich et al., 1975). Epoxide hydratase activity with styrene oxide (Oesch et al., 1971a) and benzo(a)pyrene 4,5-oxide (Schmassmann et al., 1976) was determined as described. Protein concentrations were determined by the method of Lowry et al. (1951).

[^{3}H]-styrene oxide was prepared as described (Oesch et al., 1971a), [^{3}H]-benzo(a)pyrene 4,5-oxide following the synthetic procedure for the unlabelled compound (Dansette and Jerina, 1974) using generally labelled [^{3}H]-benzo(a)pyrene (Radiochemical Centre, Amersham, England) as starting material. Synthesis, isolation and handling of benzo(a)pyrene and its derivatives were performed under dim light.

Results and Discussion

Species Differences in Enzyme Patterns and Mutagenic Effects. The parent hydrocarbon, benzo(a)pyrene did not revert any of the *Salmonella typhimurium* strains tested (TA 1537, TA 98) to histidine prototrophy in absence of a metabolically activating liver preparation (data not shown). However, in the presence of liver homogenate or liver microsomes and NADPH, the cofactor necessary for monooxygenase activity, benzo(a)pyrene was transformed to metabolites which reverted the *Salmonella* strains investigated (Figs. 1–5).

This was true for the liver preparations from all mammalian species and strains investigated, but the extent of increase in the number of revertant colonies differed dramatically for the various species and strains. As an example, it can seen in Figure 1, that the maximal increase in the number of revertant colonies is 1.7-fold with respect to spontaneous mutations when benzo(a)pyrene is activated with liver microsomes from untreated Sprague-Dawley rats, but more than 20-fold with liver microsomes from untreated C3H mice. The 1.7-fold increase using Sprague-Dawley rat microsomes was statistically highly significant ($P < 0.01$), but would, according to widespread practice, not be considered "biologically" significant – a positive label of

[1] From earlier experiments (unpublished) we know that female C3H mice are not significantly (< 10%) different from males with respect to the parameters of this study

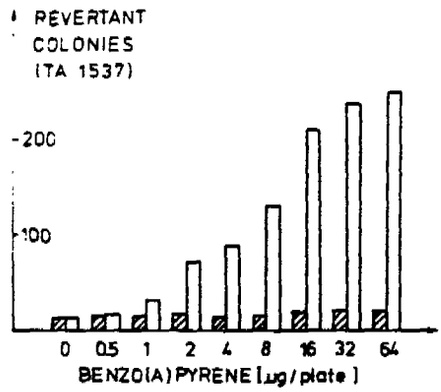

Fig. 1. Activation of benzo(a)pyrene to mutagenic metabolites by rat and mouse liver microsomes. Benzo(a)pyrene in 40 µl dimethylsulfoxide and an enzyme system (liver microsomes corresponding to about 1 mg protein, 1.7 mg $NADP^+$, 1 mg glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase in 0.5 ml 100 mM phosphate buffer pH 7.8, 8 mM $MgCl_2$, 33 mM KCl) and $1-2 \times 10^9$ bacteria (*Salmonella typhimurium* TA 1537) were mixed with 2 ml of a histidine-poor top agar (0.55% NaCl, 0.55% agar, 50 µM biotin, 50 µM histidine) and poured onto a Petri dish with minimal agar. After 48 h at 37° C, the *his*$^+$ revertant colonies were counted. Hatched bars represent activation by Sprague-Dawley rat microsomes, open bars by C3H mouse microsomes. Values are means of 2–4 incubations

"mutagenic" most commonly only being attributed to substances increasing the number of revertant colonies at least twofold. Increasing the amount of benzo(a)pyrene above the maximum shown in Figure 1 did not lead to a further increase in mutagenicity (data not shown). Thus, one of the most potent carcinogens known might have been dismissed as non-mutagenic if submitted to mutagenicity testing as an unknown in a limited screening procedure using only a single metabolically activating system if this system had an enzyme pattern unfavorable to the accumulation of mutagenically active metabolites.

When using liver microsomes from Sprague-Dawley rats which had been pretreated with Arochlor 1254 according to the schedule recommend by Ames et al. (1975)[2] the increase in mutation rate was very high (similar to that after methylcholanthrene pretreatment, data not shown), but the important fact remains obvious (see Fig. 1 and Table 1) that metabolically activating systems possessing different patterns of activating and inactivating enzymes may lead to very drastically different accumulations of mutagenically active metabolites. This may occur to the extent that what is obviously an extremely powerful mutagen when activated by a liver preparation from a particular species or strain may be missed as a mutagen when

[2] Arochlor 1254 induces a wider spectrum of monooxygenase forms (Alvares et al., 1973) than the more classical types of monooxygenase inducers whose prototypes are phenobarbital, 3-methylcholanthrene and pregnenolone 16α-carbonitrile (Conney et al., 1973). Although Arochlor 1254-induction may be expected to lead to a liver preparation which increases the sensitivity of the liver enzyme mediated bacterial mutagenicity test toward many compounds, the opposite may occur in cases where the control monooxygenase forms mediate metabolic pathways leading to more potent mutagens than the induced monooxygenases. Moreover, it is important to realize that Arochlor 1254 also induces epoxide hydratase (Oesch et al., 1977)

Table 1. Enzyme activities in liver microsomes of the untreated Sprague-Dawley rats and C3H mice which were used for the mutagenicity experiment shown in Figure 1

Species and strain	Enzyme activity[a]	
	Epoxide hydratase (nmoles styrene glycol/min/mg protein)	Monooxygenase (pmoles 3-OH-benzo(a)pyrene fluorescence equivalents/ min/mg protein)
Sprague-Dawley rat	7.2 ± 0.2	410 ± 32
C3H mouse	1.0 ± 0.1	850 ± 54

[a] Values represent means ± S.E.M. of 4 incubations (duplicate determinations at 2 protein concentrations)

using a different species or strain. This clearly underlines the crucial importance of differences in enzyme patterns.

Demonstration of Causal Link between Enzyme Patterns and Mutagenic Effect. As indicated in Table 1, epoxide hydratase activity (with styrene oxide as substrate) is lower in liver microsomes from untreated C3H mice compared to Sprague-Dawley rats by a factor of about 7, monooxygenase activity [with benzo(a)pyrene as substrate] is higher by a factor of about 2. Both factors would be expected to lead to a higher accumulation of intermediate epoxides in the former species. Thus, the much higher number of revertant colonies during metabolic activation of benzo(a)pyrene by liver microsomes from C3H mice as compared to Sprague-Dawley rats appears quite logical. However, very many other parameters will also be different between microsomal preparations from two different species. If the difference in mutagenic potential were in fact causally linked to these differences in enzyme patterns, it should be possible to compensate for them by enzyme manipulations. Figure 2 and Table 2 show that inhibition of epoxide hydratase in Sprague-Dawley rat liver microsomes indeed led to a dramatic potentiation of the mutagenic effect. This was true for two quite different types of epoxide hydratase inhibitors: Cyclohexene oxide, a moderately potent inhibitor of the non-competitive type, and 1,1,1-trichloropropene 2,3-oxide, an extremely potent inhibitor of the uncompetitive type (Oesch et al., 1971b). Both inhibitors were used at concentrations where no influence on monooxygenase activity [with benzo(a)pyrene as substrate] was observed.

Similarly, it can be appreciated from the data in Figure 3 and Table 3, that an increase in monooxygenase activity in the liver microsomes from Sprague-Dawley rats due to pretreatment of the animals with the inducer 3-methylcholanthrene also leads to a dramatic potentiation of the mutagenic effect. However, the situation with respect to monooxygenases is complex. While inhibitor and antibody studies had shown that there exists only a single epoxide hydratase catalyzing the hydration of both styrene oxide and benzo(a)pyrene 4,5-oxide (Oesch and Bentley, 1976) (and quite likely of many other epoxides), several different monooxygenase forms exist. They possess overlapping but quantitatively quite different specificities for attack of large molecules at different positions (Lu et al., 1972; Nebert et al., 1973; Holder et

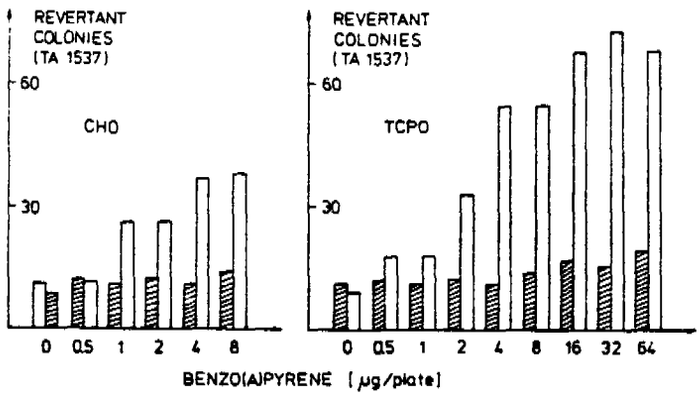

Fig. 2. Activation of benzo(a)pyrene to mutagenic metabolites: Effect of epoxide hydratase inhibitors. Benzo(a)pyrene was activated, as described in Figure 1 to mutagenic metabolites by rat liver microsomes. The open bars show the effect of the epoxide hydratase inhibitors cyclohexene oxide (2.4 mM in the top agar, CHO) and 1,1,1-trichloropropene 2,3-oxide (0.36 mM, TCPO). The hatched bars represent the number of revertant colonies in the absence of the inhibitors. Values are means of 2–4 incubations

Table 2. Effect of cyclohexene oxide and 1,1,1-trichloropropene oxide on microsomal epoxide hydratase and benzo(a)pyrene monooxygenase activities in the untreatet Sprague-Dawley rats which were used in the mutagenicity experiment shown in Figure 2

Inhibitor	Enzyme activity[a]	
	Epoxide hydratase (nmoles styrene glycol/min/mg protein)	Monooxygenase (pmoles 3-OH-benzo(a)pyrene fluorescence equivalents/ min/mg protein)
None (control)	9.8 ± 0.7	364 ± 41
2.4 mM Cyclohexene oxide	1.2 ± 0.6	390 ± 66
0.36 mM 1,1,1-Trichloropropene oxide	0.8 ± 0.5	421 ± 24

[a] Values represent means ± S.E.M. of 6 incubations (duplicate determinations at 3 protein concentrations)

al., 1974; Rasmussen and Wang, 1974; Haugen et al., 1975; Wiebel et al., 1975). Thus modulation of monooxygenase is not only an alteration in the rate of production of oxidative metabolites. Much rather induction or inhibition by different agents will affect differently various monooxygenase forms (Conney et al., 1973; Ullrich et al., 1975) and thereby lead to an alteration of the pattern of metabolites. Thus metabolic activation of benzo(a)pyrene by liver microsomes from untreated (control) or phenobarbital-induced C3H mice ("cytochrome P-450-dependent monooxygenases") leads to mutagenic benzo(a)pyrene metabolites which are very readily inactivated by homogeneous epoxide hydratase while, in contrast, liver microsomes from

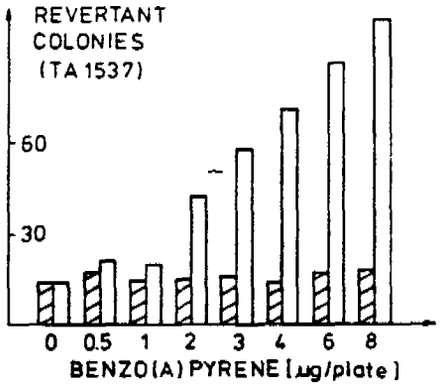

Fig. 3. Activation of benzo(a)pyrene to mutagenic metabolites: Effect of enzyme induction by 3-methylcholanthrene. Benzo(a)pyrene was activated to mutagenic metabolites, as described in Figure 1, by liver microsomes from untreated male Sprague-Dawley rats (hatched bars) or from rats that had been pretreated intraperitoneally with 10 mg/kg of 3-methylcholanthrene in sunflower oil and killed 3 days later (open bars). Values represent means of 2–4 incubations

Table 3. Effect of 3-methylcholanthrene pretreatment on enzyme activities in microsomes of the Sprague-Dawley rats which were used in the mutagenicity experiment shown in Figure 3

Pretreatment[a]	Enzyme activities[b]	
	Epoxide hydratase (nmoles styrene glycol/min/mg protein)	Monooxygenase (pmoles 3-OH-benzo(a)pyrene fluorescence equivalents/ min/mg protein)
None (control)	7.5 ± 0.4	429 ± 36
3-Methylcholanthrene	7.6 ± 0.5	1780 ± 60

[a] Treated rats received a single intraperitoneal injection of 3-methylcholanthrene (10 mg/kg) in sunflower oil 3 days before sacrifice

[b] Values represent means ± S.E.M. of 4 incubations (duplicate determinations at 2 protein concentrations)

3-methylcholanthrene pretreated C3H mice ("cytochrome P-448-dependent monooxygenases") lead to a fundamentally different pattern of mutagenically active benzo(a)pyrene metabolites on which epoxide hydratase has a weaker and more complex (multiphasic) effect (Bentley et al., 1977). Thus, an increase in monooxygenase activity does not necessarily mean that the mutagenic effect will be potentiated. While Figure 3 and Table 3 show that metabolic activation of benzo(a)pyrene by liver microsomes from 3-methylcholanthrene-treated Sprague-Dawley rats leads, indeed, to a dramatic potentiation of the mutagenic effect as compared to control microsomes, Figure 4 and Table 4 show that pretreatment with a dose of phenobarbital leading to a similar increase in "overall" monooxygenase activity (in this experiment determined with 7-ethoxycoumarin as substrate) did not lead to an increase, rather to a slight decrease in mutagenicity. This underlines the importance of keeping the overall complexity of such systems in mind. Phenobarbital induces a pattern of monooxygenase forms which differ greatly from the forms induced by 3-methylcholanthrene. Moreover, the dose of 3-methylcholanthrene used in this study did not lead to any change in epoxide hydratase activity, but phenobarbital led to a consid-

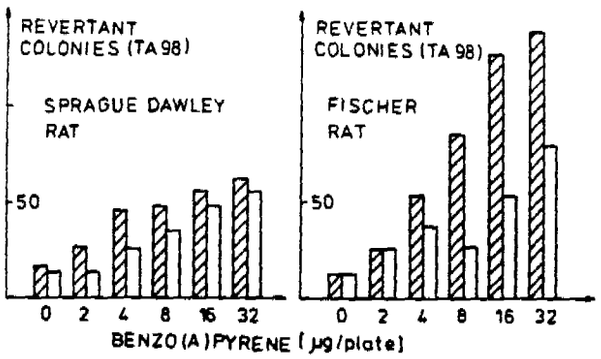

Fig. 4. Activation of benzo(a)pyrene to mutagenic metabolites by rat liver postmitochondrial supernatant fraction: Effect of enzyme induction by phenobarbital. Benzo(a)pyrene in 20 µl dimethylsulfoxide and an enzyme system (liver postmitochondrial supernatant fraction corresponding to about 4 mg protein, 1.7 mg $NADP^+$, 1 mg glucose-6-phosphate in 0.5 ml 100 mM phosphate buffer pH 7.4, 8 mM $MgCl_2$, 33 mM KCl) and $1-2 \times 10^8$ bacteria (*Salmonella typhimurium* TA 98) were mixed with 2 ml of top agar (0.55% NaCl, 0.55% agar, 50 µM biotin, 50 µM histidine) and poured onto a Petri dish with minimal agar. After 48 h at 37° C, the *his*$^+$ revertant colonies were counted. Hatched bars represent the mutagenicity in the presence of the postmitochondrial supernatant fraction from untreated male rats, open bars from rats that had been pretreated intraperitoneally with 80 mg/kg phenobarbital in 0.9% NaCl on the 4th, 3rd, and 2nd day before sacrifice. Values represent means of 2 incubations

erable increase in epoxide hydratase. Both factors would be expected to lead to a diminished mutagenicity of benzo(a)pyrene activated by microsomes from the phenobarbital treated (Fig. 4) as compared to 3-methylcholanthrene treated Sprague-Dawley rats (Fig. 3) which is borne out by the data shown in the figures.

Generalization of the Link between Enzyme Patterns and Mutagenic Effects. The data described so far show that species differences in activating and inactivating enzymes will drastically influence the mutagenicity mediated by liver preparations from these species. All data, except those depicted in Figure 4, were obtained using *S. typhimurium* TA 1537 as detector strain, microsomes as metabolically activating system, Sprague-Dawley rats and C3H mice as source of the liver microsomes, styrene oxide as substrate for epoxide hydratase and benzo(a)pyrene as substrate for monooxygenase. To investigate the generality of the observed phenomena, the following experiments (Fig. 4 and 5) were performed with *S. typhimurium* TA 98 as detector strain, liver post-mitochondrial supernatant as metabolically activating system, Fischer rats and CF-1 mice as source of the liver post-mitochondrial supernatant, benzo(a)pyrene 4,5-oxide as substrate for epoxide hydratase and 7-ethoxycoumarin as substrate for monooxygenase. Moreover, three diagnostic inhibitors (metyrapone, 7,8-benzoflavone and tetrahydrofuran) (Ullrich et al., 1975) were used as a crude measure of the different monooxygenase forms present.

Figure 4 shows that *S. typhimurium* TA 98 was reverted by benzo(a)pyrene metabolically activated by liver post-mitochondrial supernatant from untreated Sprague-Dawley rats more than TA 1537 (Fig. 1) by liver microsomes from the

Table 4. Effect of phenobarbital treatment on microsomal epoxide hydratase (nmoles 4,5-dihydroxy-4,5-dihydrobenzo(a)pyrene/min/mg protein) and monooxygenase activities (pmoles 7-OH-coumarin/min/mg protein) in the adult male Sprague-Dawley rats, Fischer rats and CF-1 mice which were used in the mutagenicity experiment shown in Figures 4 and 5

Animal		Epoxide hydratase[b]	Monooxygenase[b] in presence of modifier			
Species and strain	Pretreatment[a]		None (control)	Metyrapone 10^{-3} M	7,8-Benzoflavone $2 \cdot 10^{-5}$ M	Tetrahydrofuran 10^{-2} M
Sprague-Dawley rat	None	7.4 ± 0.3	372 ± 40 (100%)	344 ± 41 (93%)	451 ± 62 (122%)	104 ± 11 (28%)
	Phenobarbital	13.3 ± 0.7	1210 ± 66 (100%)	593 ± 52 (49%)	1310 ± 110 (108%)	448 ± 36 (37%)
Fischer rat	None	3.2 ± 0.2	414 ± 12 (100%)	369 ± 50 (90%)	504 ± 65 (123%)	123 ± 16 (30%)
	Phenobarbital	10.7 ± 0.5	1640 ± 200 (100%)	623 ± 41 (38%)	1870 ± 210 (114%)	1017 ± 82 (62%)
CF-1 mouse	None	2.7 ± 0.3	900 ± 40 (100%)	693 ± 44 (77%)	855 ± 62 (95%)	423 ± 21 (47%)
	Phenobarbital	2.3 ± 0.1	2410 ± 110 (100%)	988 ± 58 (41%)	2220 ± 160 (92%)	1010 ± 88 (42%)

[a] Phenobarbital treated animals received intraperitoneal injections of phenobarbital (80 mg/kg body weight) in 0.9% NaCl on the 4th, 3rd, and 2nd days before sacrifice

[b] Values represent means ± S.E.M. of 4 incubations (duplicate determinations at 2 different protein concentrations)

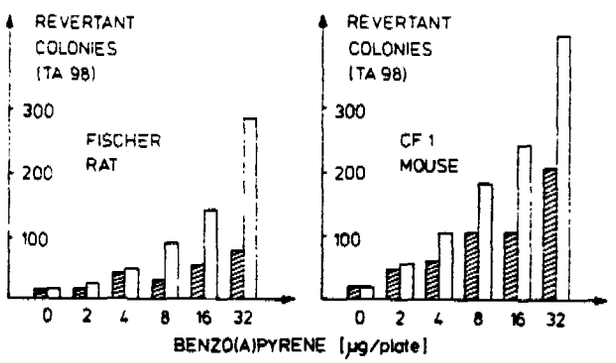

Fig. 5. Activation of benzo(a)pyrene by liver postmitochondrial fraction of phenobarbital treated rats and mice. Benzo(a)pyrene was activated to mutagenic metabolites by liver postmitochondrial supernatant fraction of phenobarbital treated male Fischer rats and CF-1 mice as described in Figure 4. The open bars represent the mutagenicity when the epoxide hydratase inhibitor 1,1,1-trichloropopene 2,3-oxide was added to the top agar at a concentration of 1 mM. The hatched bars show the mutagenicity in the absence of the inhibitor. Values represent means of 2 incubations

same strain (maximally 3-fold as compared to 1.7-fold with respect to the spontaneous rate). When the same metabolic activation was performed with liver postmitochondrial supernatant from Fischer rats, the mutation rate was considerably higher compared to Sprague-Dawley rats (9-fold as compared to 3-fold with respect to the spontaneous rate). Table 4 shows that the "overall" monooxygenase activity (with 7-ethoxycoumarin as substrate) as well as the contributions of the various monooxygenase forms are similar for the two strains. Only the epoxide hydratase is markedly lower in Fischer rats as compared to Sprague-Dawley rats. The higher accumulation of mutagenically active metabolites from benzo(a)pyrene by the liver preparation from Fischer rats is in line with their lower epoxide hydratase activity. Phenobarbital leads to a similar induction of "overall" monooxygenase activity in the two strains and the contribution of the various monooxygenase forms is not drastically different between the two strains after phenobarbital-induction. Interestingly, epoxide hydratase is increased more in the strain with the lower constitutive activity than in the other strain, leading to similar epoxide hydratase activities in the two strains after phenobarbital treatment. Accordingly, the differences in mutagenicity also virtually disappear.

Table 4 shows that the differences in enzyme activities are much less between Fischer rats and CF-1 mice after phenobarbital induction than was the case between untreated Sprague-Dawley rats and C3H mice (Table 1). Accordingly, the differences in mutagenicity are also much less (compare Fig. 5 with Fig. 1). If, in addition, epoxide hydratase activity is inhibited by 1,1,1-trichloropropene oxide, the rather small difference in monooxygenase activity which persits is now accompanied by an accordingly smaller difference in mutagenicity.

In conlusion, differences between species and strains in enzymes activating compounds to electrophilically reactive metabolites and in enzymes inactivating such metabolites, invariably lead to differences in potential of tissue preparations from such species or strains to mediate mutagenic effects of compounds which are sub-

strates of these enzymes. For lipophilic compounds, whose active forms are metabolically produced epoxides which are substrates for epoxide hydratase, liver preparations of several mouse strains (e.g. C3H, CF-1) will lead to an accumulation of such epoxides to an extent much higher than many other mammalian species due to high monooxygenase and to very low epoxide hydratase activities (Oesch, 1973). In some special cases where dihydrodiol-epoxides of an especially high reactivity due to location of the epoxide ring at a bay region can be formed (Lehr et al., 1977) epoxide hydratase will have both activating and inactivating properties (Bentley et al., 1977). However, with the majority of aromatic and olefinic compounds of industrial intrest it is to be expected that epoxide hydratase plays a simple inactivating role. Since man has a much higher epoxide hydratase activity (Oesch et al., 1974) than any of the many investigated mice strains (Oesch et al., 1973) it is to be expected that mice are much more susceptible to toxic effects which are mediated by metabolically produced epoxides which are substrates for epoxide hydratase, at least for compounds large and lipophilic enough to preclude efficient inactivation by glutathione (Glatt and Oesch, 1977). Whether a metabolically produced epoxide will be a substrate for epoxide hydratase can also be predicted for several structural features, since structure-activity relationships have been studied for rodent and human hepatic epoxide hydratase (Oesch, 1974).

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