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STUDIES ON THE NATURE OF THE *IN VITRO* ENHANCEMENT OF BIPHENYL 2-HYDROXYLATION PROVOKED BY SOME CHEMICAL CARCINOGENS

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Abstract—Studies on the metabolism of [^{14}C]biphenyl and of 2-hydroxy and 4-hydroxybiphenyls confirm that preincubation of fresh hepatic microsomal preparations from rats or hamsters with chemical carcinogens such as safrole, benz[a]pyrene and 2-acetamidofluorene and a NADPH regenerating system produces an increase in the levels of 2-hydroxybiphenyl, through a specific increase in its formation from biphenyl. These data also support the validity of the fluorimetric assay method for monitoring this reaction. The addition of oestradiol or glutathione to the incubation mixture and the use of various preincubations and time periods with the carcinogens and NADPH prior to adding biphenyl indicate that production of an active metabolite of the carcinogens is probably a pre-requisite for the *in vitro* enhancement of biphenyl 2-hydroxylase to occur.

The lack of effectiveness of EDTA in enhancing biphenyl 2-hydroxylase and the complete destruction of this enhancement by short-term storage of microsomal preparations at −20° suggests that the phenomenon is different from that of degranulation of the endoplasmic reticulum by carcinogens.

Increased biphenyl 2-hydroxylation activity has been observed in hepatic microsomes isolated from rats treated with safrole or 3,4-benz[a]pyrene 2 hr previously [1]. A similar elevation of this biphenyl 2-hydroxylase was also found following the *in vitro* addition of a number of carcinogenic but not non-carcinogenic chemicals to rat, hamster or mouse hepatic microsomes. Other hepatic microsomal drug metabolising mixed function oxidases were not affected in this manner [2, 3]. Although the mechanism of this enhancement has not been established, NADPH appears to be required in the *in vitro* system and the possibility arises that these carcinogens require metabolism before they can mediate their effect on biphenyl 2-hydroxylase activity. This is compatable with the fact that many carcinogens are known to exert their carcinogenic effect via the formation of active metabolites produced by the enzymes of the endoplasmic reticulum [4, 5].

The purpose of this present paper is primarily to investigate whether metabolism of the carcinogen is required for the *in vitro* enhancement of biphenyl 2-hydroxylase to take place, and to appraise the mechanism by which this enhancement might occur.

MATERIALS AND METHODS

Materials were employed as described previously. Glutathione was purchased from Sigma Chemicals (London) Ltd., oestradiol from Steraloids Ltd., Croydon, Surrey and [^{14}C]biphenyl (sp. act. 2.7 μCi/mg; 98% chemically pure and 99% radiochemically pure) was kindly provided by Dr. A. Monro, Pfizer Limited, Sandwich, Kent.

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Assessment of the influence of test compounds on biphenyl hydroxylation. Freshly prepared rat or hamster hepatic microsomes were added to an $NADPH_2$ regenerating system containing glucose-6-phosphate (25 mM), NADP 500 nM, glucose-6-phosphate dehydrogenase (2 units/ml) together with magnesium sulphate (0.5 mM) to give a final hepatic microsomal protein content of 2 mg/ml.

Test compounds (benz[a]pyrene, 1 mM, safrole or CCl_4, 1 mM–0.1 μM) were added in either ground nut oil (for safrole and 3,4-benzpyrene) or in 1.15% KCl (phenobarbitone, EDTA and 2-acetamidofluorene) and the system preincubated (typically for 10 min) at 37° in a shaking water bath (100 cycles/min). After this preincubation period, biphenyl (13 mM in 1.15% KCl containing 1.5% Tween 80) was added and the incubation continued for a further 5 min. The reaction was then terminated by the addition of 1 ml 4 N HCl, the 2- and 4-hydroxybiphenyl metabolites extracted and determined fluorimetrically [6]. Separate controls were run in which either test substances or biphenyl was omitted from the system, or test compound was added after termination of the reaction.

In vitro *metabolism of* [^{14}C]*biphenyl.* Preincubations with [^{14}C]biphenyl were as above except for the following modifications:

Following preincubation, 0.5 ml of a 1 mM solution of [^{14}C]biphenyl (in 1.15% KCl and 1.5% Tween 80) was added and the reaction terminated after 5 min with HCl. The mixture was then extracted with 7 ml *n*-heptane for 20 min, the heptane extract (5 ml) evaporated to dryness at 40° by bubbling through with nitrogen and the residue redissolved in 0.1 ml of methanol containing 1.5 mg/ml unlabelled 2- and 4-hydroxybiphenyl, 2,2′- and 4,4′-dihydroxybiphenyl. The methanol extract (50 μl) was applied to a Silica gel HF_{254} thin-layer chromatography (t.l.c.) plate, which was then developed in benzene–ethanol (95:5).

The hydroxybiphenyl metabolites and unchanged biphenyl were located under u.v. light by the observed quenching at 254 nm. The relevant areas of the plate were scraped off into 0.8 ml of a 1% Tween 80 solution and shaken for 10 min. The supernatant was then decanted into scintillation vials and mixed with 10 ml toluene–Triton X-100 (2:1) scintillant containing 0.55% (w/v) PPO (2–5 diphenyl oxazole) and counted in a Packard Scintillation Counter (Packard Instruments, Reading). (Addition of 0.1 ml [^{14}C]toluene as internal standard indicated a counting efficiency of 78 ± 4%.)

Conditions of storage. For storage experiments, freshly prepared liver microsomal preparations from hamsters or rats were stored either as microsomal pellets, overlaid with 0.3 M phosphate buffer, pH 7.4, containing 1% glycerol, or as 1-ml aliquots in 1.5% KCl buffered to pH 7.4 with 0.3 M phosphate buffer, to a final microsomal protein content of 15 mg/ml. Samples were initially stored at −40° for 1 hr and then transferred to −20° for the remaining storage period.

In an attempt to reduce damage to the material as much as possible thawing of material was carried out in two stages, firstly the material was allowed to equilibrate to 0° and then to 4°.

Effects of oestradiol and glutathione upon the in vitro *enhancement of biphenyl 2-hydroxylase.* These were investigated by the addition of glutathione (1 mg/ml) or oestradiol (0.5 mg/ml) to the preincubation system simultaneously with the carcinogen test compounds.

RESULTS

Effects of pre-incubation upon the microsomal metabolism of [^{14}C]biphenyl. To confirm that the detectable increase in fluorescence measured at 290 nm excitation and 415 nm emission was attributable solely to 2-hydroxy biphenyl, the effects of pre-incubating rat hepatic microsomes with the carcinogens safrole or 3,4-benz[a]pyrene or the non-carcinogen phenobarbitone upon [^{14}C]biphenyl metabolism was investigated. In control microsomal preparations 5.6 per cent of [^{14}C]biphenyl was metabolised to a product having identical R_f and fluorescence characteristics to 2-hydroxybiphenyl and 30.9 per cent was converted to 4-hydroxybiphenyl. 2,2′-dihydroxybiphenyl and 4,4′-dihydroxybiphenyl were tentatively identified as minor metabolites. However, after pre-incubation of microsomes with 3,4-benz[a]pyrene (1 mM), 2-hydroxybiphenyl accounted for 18.9 per cent of metabolised biphenyl (Table 1) representing approximately a 2.5-fold increase compared to control values, while only a slight decrease in the amount of 4-hydroxy formed (28.8 per cent) was noted. These changes were comparable with those observed using the fluorimetric assay method, thus validating the fluorimetric technique for assessing modifications in biphenyl 2- and 4-hydroxylation. It can be seen from Table 1 that the increase in formation of 2-hydroxybiphenyl following pre-incubation with 3,4-benz[a]pyrene is due to increased metabolism of [^{14}C]biphenyl reflecting an increase in enzyme activity rather than a conversion of 'biphenyl 4-hydroxylase' to 'biphenyl 2-hydroxylase'. The above findings were substantiated using microsomes preincubated with safrole or acetamidofluorene and [^{14}C]biphenyl for which a smaller but significant enhancement of biphenyl 2-hydroxylase was apparent (see Table 3). In the case of safrole, but not of acetamidofluorene, a marked decrease in the amounts of 4-hydroxybiphenyl produced was found. The inhibitory properties of methylenedioxyphenyl compounds such as safrole upon P-450 dependent mixed function oxidase enzyme systems is well documented [7–9] and it seems likely that the inhibition of biphenyl 4-hydroxylase is explicable in these terms. In contrast, pre-incubation with phenobarbitone provoked no significant changes in either 2- or 4-hydroxybiphenyl levels.

Table 1. Kinetics of biphenyl hydroxylation: The effect of preincubation of 3,4-benzpyrene with hamster hepatic microsomes *in vitro* on the characteristics of 2- and 4-biphenyl hydroxylase activities

Biphenyl 2-hydroxylase	$K_m 10^{-4}$	$V_{max} 10^{-2}$
Control	4.3 ± 1.4	1.2 ± 0.2
Benz[a]pyrene incubated	0.14 ± 0.2	6.7 ± 0.6
Biphenyl 4-hydroxylase		
Control	2.6 ± 1.1	2.0 ± 0.3
Benz[a]pyrene preincubated	2.3 ± 1.4	1.9 ± 0.2

Effects of preincubation upon the metabolism of 2-hydroxy and 4-hydroxy biphenyl. The possibility was investigated that carcinogenic compounds might

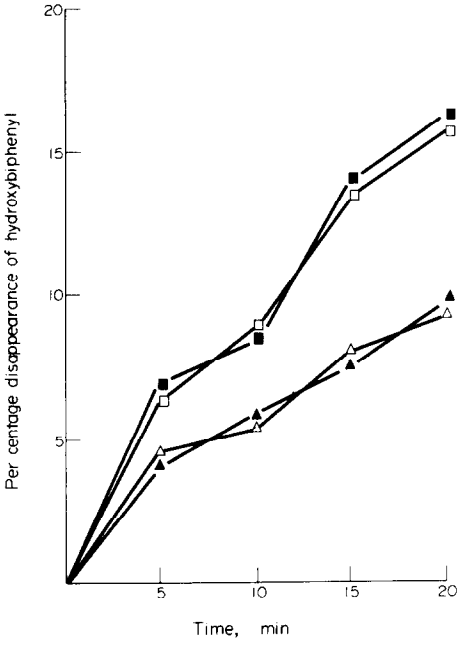

Fig. 1. Effects of preincubation with 3,4-benz[a]pyrene upon the *in vitro* metabolism of 2-hydroxy and 4-hydroxy biphenyl (1 mM). Studies were carried out in male mature Syrian hamsters (6 animals). No significant differences between test and control incubations were detectable for either 2-hydroxy or 4-hydroxy biphenyl metabolism. Results shown are for control microsomes using 2-hydroxy biphenyl as substrate (△——△) or 4-hydroxy biphenyl as substrate (□——□), and for microsomes preincubated with 3,4-benz[a]pyrene (1 mg/ml) in groundnut oil using 2-hydroxy biphenyl as substrate (▲—— ▲) or 4-hydroxy biphenyl as substrate (■·····■).

Table 2. Kinetics of further hydroxylation of hydroxybiphenyls: Effect of *in vitro* preincubation upon kinetic parameters of further microsomal hydroxylation of 2-hydroxy and 4-hydroxy biphenyl

In vitro preincubation	Further metabolism of 2-hydroxybiphenyl		4-hydroxbiphenyl	
Compound	K_m	V_{max}	K_m	V_{max}
Safrole*	13.2 ± 3.4	6.4 ± 1.7	9.9 ± 5.1	2.3 ± 1.7
3,4-benz[a]pyrene†	14.3 ± 6.1	6.2 ± 1.9	8.4 ± 3.8	2.7 ± 1.0
Phenobarbitone*	15.7 ± 3.1	8.4 ± 2.7	6.9 ± 4.1	3.1 ± 1.3
Nikethamide†	14.9 ± 2.1	7.4 ± 1.8	7.4 ± 2.7	2.1 ± 1.2

* Dissolved in 1.15% KCl plus 1.5% Tween 80 to a final concentration of 5×10^{-4} M.
† Added in groundnut oil (Saladin®) 1 mg/ml, 0.5 ml, added to incubate.
Results are as shown for hamster hepatic microsomes, mean ± S.E.M. 8 animals. Values are expressed as K_m 10^6 mole/l and V_{max} 10^9 mole/min/mg microsomal protein.

Table 3. The effects of preincubation of rat liver microsomal preparations with 3,4-benzpyrene, safrole and phenobarbitone on the metabolism of [^{14}C]-biphenyl *in vitro* and the effects of intraperitoneal safrole on the enhancement of [^{14}C]-biphenyl metabolism in male Wistar rats

(a) *In vitro* Preincubation

Preincubation of microsomes	biphenyl	4-hydroxy biphenyl	2-hydroxy biphenyl	4,4'-hydroxy biphenyl	2,2'-hydroxy biphenyl	origin
Control	58.3 ± 0.4	30.7 ± 0.4	6.2 ± 0.3	3.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
3,4-benz[a]pyrene	47.2 ± 0.2	28.8 ± 0.6	18.8 ± 0.5	3.0 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
Safrole	64.0 ± 0.2	21.0 ± 0.3	10.2 ± 0.6	3.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.1
Phenobarbitone	58.6 ± 0.3	30.4 ± 0.4	5.3 ± 0.4	3.2 ± 0.2	1.1 ± 0.1	2.1 ± 0.1

(b) *In vitro* studies 2 hr after intraperitoneal administration of Safrole

Microsomes	biphenyl	4-hydroxy biphenyl	2-hydroxy biphenyl	4,4'hydroxy biphenyl	2,2'-hydroxy biphenyl	origin
Control	58.6 ± 0.5	30.2 ± 0.6	6.5 ± 0.4	3.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.1
Safrole	63.8 ± 0.6	21.1 ± 0.4	11.1 ± 0.5	3.2 ± 0.3	1.2 ± 0.1	1.4 ± 0.3

Results of two experiments (3 animals per experiment) are expressed in terms of percentage of total activity added to the t.l.c. plate.

cause an apparent increase in the *in vitro* biphenyl 2-hydroxylase activity by partially interfering with the further metabolism of 2-hydroxybiphenyl.

Figure 1 shows that on preincubation of microsomes with 3,4-benz[a]pyrene, (the most potent *in vitro* enhancer of biphenyl 2-hydroxylation), and adding 2- or 4-hydroxybiphenyl rather than biphenyl as substrate, no significant effect on the further metabolism of 2-hydroxy- and 4-hydroxybiphenyl was discernable. Moreover, although *in vitro* preincubation with 3,4-benz[a]pyrene caused significant changes in both the K_m and V_{max} of biphenyl 2-hydroxylase (Table 1), when 2-hydroxy- and 4-hydroxybiphenyls were substituted for biphenyl no significant changes in either the K_m or V_{max} for further hydroxylation of either compound was detectable after pre-incubation with either carcinogenic or non-carcinogenic compounds (Table 2).

Effects of preincubation of rat liver microsomal preparations with benz[a]pyrene or safrole on ethyl isocyanide and carbon monoxide difference spectra. To investigate whether the effects of preincubation of liver microsomal preparations with 3,4-benz[a]pyrene or safrole on biphenyl 2-hydroxylation could be manifested by a major modification in cytochrome P-450, the carbon monoxide and ethyl isocyanide binding spectra were determined (Fig. 2) using control micro-

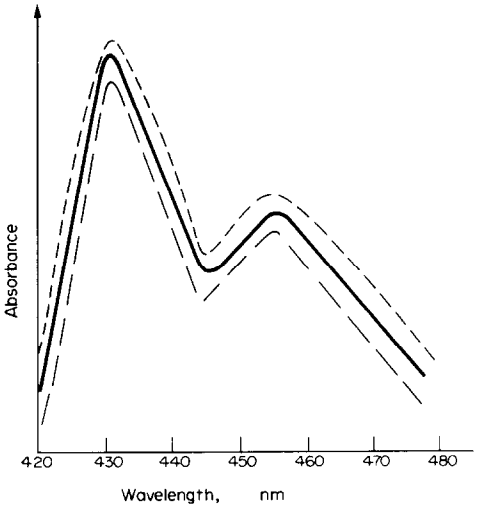

Fig. 2. Effects of preincubation with carcinogenic and non-carcinogenic compounds upon cytochrome P-450-ethylisocyanide binding spectra in hepatic microsomes prepared from male Wistar rats. Results shown are (------) control values; (——) preincubation with phenobarbitone, and (– – – –) preincubation with 3,4-benz[a]pyrene. Plots are arbitarily displaced to facilitate presentation.

Table 4. Effects of storage on the *in vitro* effects of safrole invoked biphenyl 2- and 4-hydroxylase activity in hepatic microsomes prepared from Wistar Rats

Storate time (hr)	Pellet stored biphenyl 2-hydroxylase control	test	biphenyl 4-hydroxylase control	test
0	0.27 ± 0.02	0.46 ± 0.04	2.1 ± 0.09	1.6 ± 0.11
24	0.21 ± 0.03	0.20 ± 0.03	2.0 ± 0.10	1.5 ± 0.07
72	0.19 ± 0.01	0.18 ± 0.02	1.6 ± 0.11	1.2 ± 0.12
120	0.21 ± 0.02	0.22 ± 0.01	1.4 ± 0.08	1.0 ± 0.10
Storate time (hr)	Suspension stored biphenyl 2-hydroxylase control	test	biphenyl 4-hydroxylase control	test
0	0.29 ± 0.03	0.47 ± 0.06	2.3 ± 0.06	1.5 ± 0.08
24	0.20 ± 0.02	0.19 ± 0.05	1.9 ± 0.11	1.1 ± 0.12
72	0.18 ± 0.03	0.16 ± 0.02	1.3 ± 0.18	1.0 ± 0.11
120	0.16 ± 0.01	0.13 ± 0.01	1.2 ± 0.10	1.0 ± 0.09

Materials were stored at −40 either as a suspension of protein concentration 10 mg/ml in media containing 0.01 M β-mercaptoethanol, 0.25 M sucrose and 0.1 M magnesium sulphate or as a microsomal pellet overlaid with 0.1 M phosphate buffer −20% v/v glycerol buffer, pH 7.4.

Results are expressed as nmoles/mg microsomal protein/min. Percentage variation from control are expressed in parentheses.

somal preparations and microsomes preincubated for 10 min with benz[a]pyrene, safrole or phenobarbitone. The compounds were added either to liver homogenates or to microsomes and preincubated. In no instance could a change in the ethyl isocyanide or carbon monoxide spectra be distinguished, in contrast with the situation pertaining 48 hr after pretreating the whole animal with benz[a]pyrene or safrole in which P-448 is formed [10, 11].

Stability studies. To ascertain the stability of the enhancement of biphenyl 2-hydroxylation *in vitro*, studies were made comparing freshly prepared microsomes with those which had been stored at −20° prior to usage. It can be seen from Table 4 that 24 hr after storage of rat microsomes at −20° either as pellets or suspension, no *in vitro* enhancement of biphenyl 2-hydroxylation was detectable following preincubation with either safrole or 3,4-benz[a]pyrene, although the activity of basal levels of biphenyl 2- and 4-hydroxylase were not greatly affected under these storage conditions. Similar results were noted using hamster microsomes.

DISCUSSION

The findings using [^{14}C]labelled biphenyl confirm that the use of fluorimetry to evaluate carcinogen induced changes in 2- and 4-hydroxylation is a valid one. Safrole, benz[a]pyrene and acetamidofluorene all rapidly enhance biphenyl 2-hydroxylation when preincubated with washed microsomes and NADPH. The specific nature of this enhancement is indicated by the fact that no change in biphenyl 4-hydroxylase or in the further hydroxylation of 2- and 4-hydroxybiphenyl was observed with either benz[a]pyrene or acetamidofluorene. Furthermore, no alteration in the CO or ethylisocyanide P-450 binding spectrum was detectable supporting the view that a major change in P-450 is not involved. This agrees with previous studies which showed that the activities of P-450-dependent drug metabolising enzyme systems such as 7-ethoxycoumarin de-ethylase, ethoxyresorufinde-ethylase, *p*-nitroanisole demethylase and aniline hydroxylase are also unchanged under these conditions [1].

It is generally accepted that carcinogens administered *in vivo* elicit damaging effects through their conversion to an active electrophilic or free radical intermediate, this metabolism probably being primarily dependent upon a NADPH cytochrome P-450 hydroxylating system [2, 3]. It is not readily possible to determine directly whether carcinogen metabolism is a pre-requisite for the enhanced biphenyl 2-hydroxylase activity since obvious approaches such as the use of inhibitors of cytochrome P-450 cannot be employed because they would also interfere with the biphenyl 2- and 4-hydroxylation which is also thought to be at least partly cytochrome P-450-dependent. That metabolism of the carcinogen may be required is indicated by the time dependency of preincubation of rat hepatic microsomes with 2-acetamidofluorene or safrole for optimal enhancement of biphenyl 2-hydroxylase (Fig. 3).

Further evidence that the carcinogens require prior metabolism in order to cause an enhancement of biphenyl 2-hydroxylase activity was obtained from an examination of cofactor requirements. An unusual characteristic of biphenyl 2-hydroxylase is that, unlike most drug metabolism systems, it is not entirely dependent upon NADPH, being able to utilise NADH to a much greater extent than typical P-450 substrates such as ethylmorphine. When NADH was substituted for a NADPH regenerating system for safrole, the enhancement of biphenyl 2-hydroxylase was not detectable although the control level activity of this enzyme was not significantly altered (Table 5). In the absence of NADPH, safrole is not metabolised [9], thus the lack of enhancement using NADH is presumably due to the failure to form the active safrole metabolite(s) which provokes the enhancement.

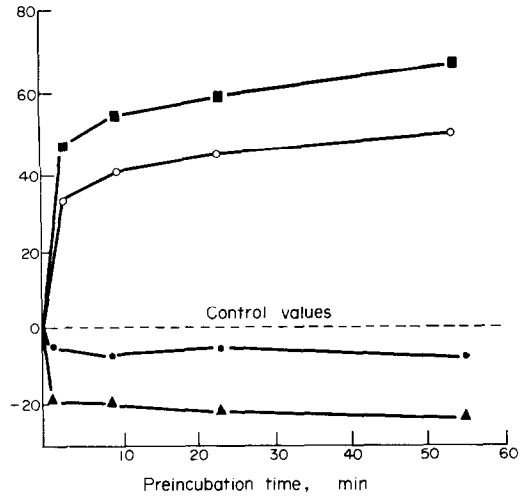

Fig. 3. Variation of preincubation time and its effect upon 2-acetamidofluorene and safrole mediated changes in biphenyl 2-hydroxylase and biphenyl 4-hydroxylase activity in rat hepatic microsomes. Results shown are for changes with time produced by safrole addition (1 mM on biphenyl metabolism indicated by (■——■) for biphenyl 2-hydroxylase and (▲——▲) for biphenyl 4-hydroxylase, whereas changes caused by acetamidofluorene addition (1 mM) are (○——○) for biphenyl 2-hydroxylase and (●——●) for biphenyl 4-hydroxylase).

Glutathione has been shown to exert a protective influence against the toxic effect of various compounds upon microsomal membranes, probably either by a general protective effect on the microsomes against oxidative damage or by reducing the availability of active metabolites to interact with the microsomes [12]. In keeping with this observation, glutathione in high concentrations reduced the *in vitro* enhancement of biphenyl 2-hydroxylase (Table 6) produced by safrole and 3,4-benzy[a]pyrene. Biphenyl 4-hydroxylase on the other hand showed little response to either carcinogens or to glutathione, again emphasing the apparent differences between the inducible biphenyl 2-hydroxylase and biphenyl 4-hydroxylase.

The addition of oestradiol (0.5 mg/ml) (Table 6) with 3,4-benz[a]pyrene also produced a decreased level of enhancement of biphenyl 2-hydroxylase activity although no significant change was observed when safrole rather than benzpyrene was added. This effect of oestradiol upon 3,4-benz[a]pyrene initiated biphenyl 2-hydroxylase enhancement may be because oestradiol can serve as a competitive substrate for benz[a]pyrene metabolism.

However, oestradiol has also been implicated in maintaining the association of ribosomes with the liver endoplasmic reticulum whereas carcinogens in the presence of NADPH cause dissociation of ribo-

Table 5. Showing the effects of Safrole* preincubation with either NADH or NADPH (0.9 m-mole/l) upon biphenyl 2-hydroxylase in rat and hamster hepatic microsomes

	Rat	Hamster
Control + NADPH	0.23 ± 0.04	0.31 ± 0.02
Control + NADH	0.20 ± 0.03	0.32 ± 0.03
Safrole preincubation + NADPH	0.39 ± 0.06 (+70)	0.46 ± 0.04 (+48)
Safrole preincubation + NADH	0.24 ± 0.02 (+17)	0.35 ± 0.02 (+9)

* Dissolved in 1.15% KCl + 1.5% Tween 80 to a final concentration of 5×10^{-4} M.
Results are expressed as nmoles/mg microsomal protein/min. Percentage value relative to control are expressed in parentheses.

Table 6. Showing the effects of glutathione and oestradiol upon biphenyl 2-hydroxylase activity in preincubated microsomes

Glutathione concn. (mg/ml)	Safrole	3,4-Benz[a]pyrene
0	0.50 ± 0.03 (+88)	0.91 ± 0.03 (+237)
0.4	0.39 ± 0.07 (+47)	0.64 ± 0.04 (+140)
0.8	0.38 ± 0.04 (+43)	0.59 ± 0.02 (+118)
1.2	0.37 ± 0.02 (+40)	0.58 ± 0.02 (+115)
1.6	0.36 ± 0.04 (+37)	0.57 ± 0.04 (+112)
2.0	0.33 ± 0.03 (+25)	0.55 ± 0.03 (+106)
Oestradiol concn. (mg/ml)		
0	0.50 ± 0.05 (+88)	0.91 ± 0.05 (+237)
0.4	0.46 ± 0.04 (+73)	0.76 ± 0.06 (+180)
0.8	0.44 ± 0.03 (+69)	0.70 ± 0.04 (+162)
1.2	0.43 ± 0.04 (+58)	0.67 ± 0.03 (+148)
1.6	0.41 ± 0.02 (+50)	0.62 ± 0.07 (+130)
2.0	0.40 ± 0.02 (+49)	0.59 ± 0.04 (+120)

Test compounds and either glutathione or oestradiol were added simultaneously.
Biphenyl 2-hydroxylase activity is expressed as nmoles/mg microsomal protein/min.
Percentage variations from control are expressed in parentheses.

somes (degranulation) [13, 14]. A possible explanation of our results might be that biphenyl 2-hydroxylase is at the ribosomal binding sites and that the active metabolites of the carcinogen cause its enhancement through the displacement of the ribosomes. This hypothesis would rationalise the specificity of the effect for the endoplasmic reticulum, the time dependency of the reaction and the protective effect of oestradiol. However, a closer comparison of our findings with those of Rabin and Williams for degranulation reveals some important dissimularities between the two systems. A very much shorter time period is required for the maximum effect of benz[a]pyrene on biphenyl 2-hydroxylase (<10 min) than for its optimal degranulation activity (~1 hr). Preincubation with EDTA (up to 10 mM) has no effect and carbon tetrachloride (100 mM) [3] a very weak effect on biphenyl 2-hydroxylase whereas these agents cause very extensive degranulation. Furthermore, short term storage of the microsomes does not unduly effect the ability of these carcinogens to cause degranulation whereas it totally destroys biphenyl 2-hydroxylase enhancement.

A less direct relationship between our system and that of degranulation should not be completely discounted because our studies (cited here and elsewhere) like those of Rabin and Williams indicate that a relatively specific, rapid and probably irreversible change in the endoplasmic reticulum membrane is invoked by carcinogens such as benz[a]pyrene, acetamidofluorene and safrole following their metabolism, whereas no such changes are seen using claimed non-carcinogens in the rat like phenobarbitone or 1,2,3,4 dibenz(o)pyrene, the precise nature of these changes and their relationship to carcinogenicity provoked by chemical agents must await further study.

REFERENCES

1. F. J. McPherson, J. W. Bridges and D. V. Parke (1975), in press.
2. J. W. Bridges, F. J. McPherson, L. J. King and D. V. Parke, *Proc. Eur. Soc., Study of Drug Toxicity* **XV**, 98 (London) (1974).
3. F. J. McPherson, J. W. Bridges and D. V. Parke, *Nature* **252**, 488 (1974).
4. P. N. Magee, *Essays in Biochem.* **10**, 105 (1974).
5. R. C. Garner, in *Progress in Drug Metabolism*, Vol. 1. (Eds. J. W. Bridges and L. F. Chasseaud) Wiley, London (1975).
6. P. J. Creaven, D. V. Parke and R. T. Williams, *Biochem. J.* **96**, 879 (1965).
7. H. Jaffee, K. Fujii, H. Guerin, M. Sengupta and S. S. Epstein, *Biochem. Pharmac.* **18**, 1045 (1969).
8. R. M. Philpot and E. Hodgson, *Molec. Pharmac.* **8**, 204 (1972).
9. C. R. Elcombe, J. W. Bridges, T. J. B. Gray, R. H. Nimmo-Smith and K. Netter, *Biochem. Pharmac.* **24**, 1427 (1975).
10. H. Greim, J. B. Schenkman, M. Klotzbucher and H. Remmer, *Biochim. biophys. Acta.* **201**, 20 (1970).
11. M. D. Burke and J. W. Bridges, *Xenobiotica* **5**, 357 (1975).
12. J. R. Gillette, J. R. Mitchell and B. B. Brodie, *A. Rev. Pharmac.* **14**, 271 (1974).
13. B. R. Rabin, C. A. Blyth, D. Doherty, R. B. Freedman, A. Roobol, G. H. Sunshine and D. J. Williams, in *Effects of Drugs on Cellular Control Mechanisms* (Eds. B. R. Rabin and R. B. Freedman) pp. 27 47. Macmillan, London (1971).
14. D. J. Williams and B. R. Rabin, *Nature, Lond.* **232**, 102 (1974).