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Regioselectivity of Rat Liver Microsomal UDP-Glucuronosyltransferase Activities Toward Phenols of Benzo(*a*)pyrene and Dibenz(*a*,*h*)anthracene

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SUMMARY

Inducibility of rat liver microsomal UDP-glucuronosyltransferase was investigated with regard to the substrate structure using 3-, 6-, 7-, 8-, and 9-hydroxybenzo(*a*)pyrene, all seven phenols of dibenz(*a*,*h*)anthracene, 3-hydroxybenz(*a*)anthracene, and 3-hydroxyfluoranthene as substrates. Glucuronide formation of the majority of the planar phenols was preferentially inducible by 3-methylcholanthrene (4- to 8-fold, group 1). However, glucuronidation of 8-hydroxybenzo(*a*)pyrene, 3-hydroxybenz(*a*)anthracene, and 3-hydroxydibenz(*a*,*h*)anthracene was markedly inducible by phenobarbital (3- to 8-fold, group 2). Glucuronidation of 9-hydroxybenzo(*a*)pyrene and 3-hydroxyfluoranthene was only moderately induced by the two inducers (<2-fold, group 3). Glucuronidation was also determined with purified phenol UDP-glucuronosyltransferase from 3-methylcholanthrene-treated rats. A close correlation ($r = 0.95$) was observed between purification factors (ratio of enzyme activities in purified enzyme and microsomes) and induction factors (ratio of microsomal enzyme activities from 3-methylcholanthrene-treated rats and untreated controls). Interestingly, differences in size and shape of group 1 and 2 substrates could be recognized. Group 1 substrates were shorter (< 1.3 nm) and broader (>1.1 nm) than group 2 substrates when viewed from the hydroxy group, along the axis of the C—O bond, to the plane of the polycyclic aromatic hydrocarbon, suggesting a distinct geometry of the binding site of the 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase.

Phenols are major metabolites in the biotransformation of PAHs. It is increasingly recognized that these PAH phenols exhibit a variety of biologic activities. Under certain conditions BP phenols have been found to be mutagenic or carcinogenic (1–7). Under other conditions they inhibit the formation of mutagenic and tumorigenic diol epoxides (8). Phenols have been shown to be oxidized to phenol epoxides, phenoxy radicals, semiquinones, and quinones (9–14). Moreover, quinones are often involved in redox cycles after reduction to semiquinones and quinols whereby toxic oxygen species are generated (15–18). Therefore, conjugation with glucuronic acid or sulfate may be an important cellular defense mechanism to prevent activation of phenolic metabolites of PAHs.

Glucuronide formation is catalyzed by a family of closely related isoenzymes of GT with different but overlapping substrate specificities (19–22). Earlier studies suggested the existence of at least two differentially inducible enzyme forms in rat liver. One form was inducible by phenobarbital and accepted bulky phenols as substrates such as morphine. Another form appeared to be preferentially inducible by MC and accepted planar phenols as substrates (23–28). However, glucuronidation of some planar phenols, such as 9-OH-BP, was only moderately inducible by MC (29), suggesting regioselectivity of the MC-inducible phenol GT [GT_{MC} or GT_1 (19)] for its substrates. These conflicting findings on glucuronidation of planar phenols prompted the present more detailed investigation on the properties of PAH phenols as substrates of differentially inducible GTs. The chosen PAH phenols (Fig. 1) may be particularly useful probes because of their distinct geometry.

Materials and Methods

Chemicals. 7- and 8-OH-BP (30), all DBA phenols (31), and 6-acetoxy-BP (32) were synthesized by published methods. 3-Hydroxybenz(*a*)anthracene was prepared essentially as described for 3-OH-DBA (31), substituting 1,2-naphthalic anhydride by phthalic anhydride. Reductive cyclization of *o*-(6-methoxy-1-naphthoyl)benzoic acid with hydroiodic acid-red phosphorus in boiling glacial acetic acid led, after acetylation, to 3-acetoxy-5,6-dihydrobenz(*a*)anthracene in 75% yield: M.P. 97°; NMR (60 MHz, $CDCl_3$) 2.27 (*s*, 3, acetate), 2.8–3.0 (br. *s*, 4, $H_{5,6}$), 6.9–8.1 (*m*, 9, ArH); MS (70 eV, 100°) *m/z* (%) 288 (24, M^+), 246 (100, $M^+ - C_2H_2O$). Dehydrogenation with 2,3-dichloro-5,6-

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ABBREVIATIONS: PAH, polycyclic aromatic hydrocarbon; BP, benzo(*a*)pyrene; DBA, dibenz(*a*,*h*)anthracene; GT, UDP-glucuronosyltransferase (EC 2.4.1.17); MC, 3-methylcholanthrene; GT_{MC}, 3-methylcholanthrene-inducible UDP-glucuronosyltransferase.

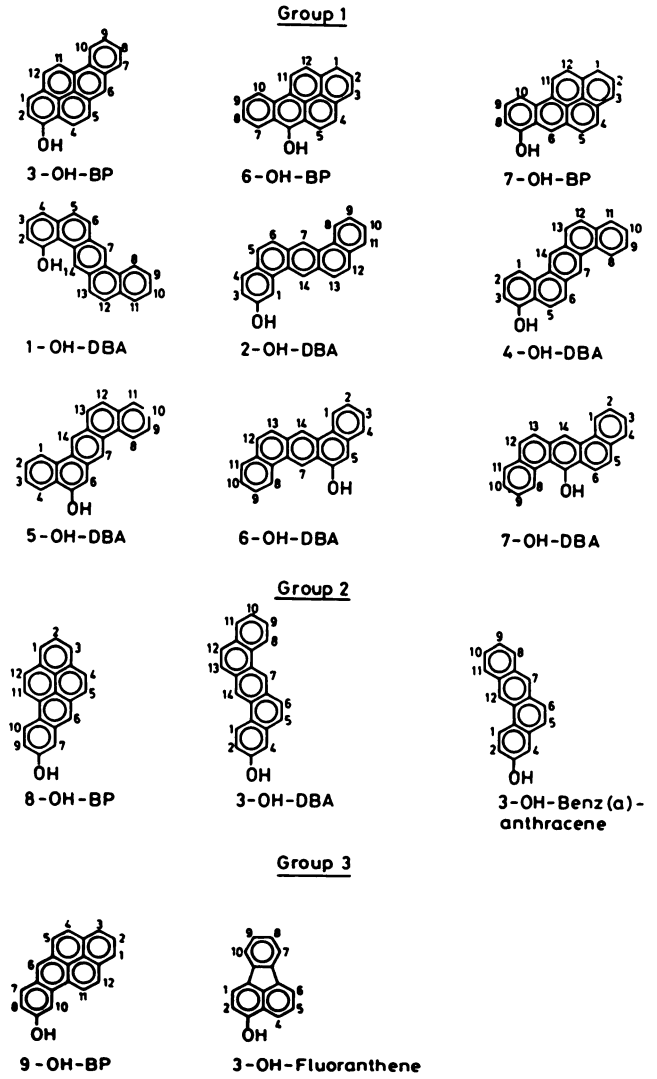

Fig. 1. Chemical structures of investigated phenols of PAHs. The structures of PAH phenols are arranged with the phenolic C—O bond pointing downward in order to illustrate their different molecular dimensions.

dicyano-*p*-benzoquinone in *p*-dioxane at room temperature afforded 3-acetoxybenz(*a*)anthracene which was hydrolyzed yielding 3-hydroxybenz(*a*)anthracene; m.p. 208°, lit. (33) m.p. 209–210°. 3- and 9-OH-BP were obtained from the Chemical Carcinogen Reference Standard Repository, National Institutes of Health, Bethesda, MD. 3-Hydroxyfluoranthene, synthesized according to the method of Shenbor and Cheban (34), was a kind gift from Dr. A. Stier, Goettingen, FRG. All other chemicals were from commercial sources: UDP-[U^{14}C]glucuronic acid (260 mCi/mmol) from Amersham-Buchler, Braunschweig, FRG, and Brij 58 (a condensate of hexadecyl alcohol with 20 mol of ethylene oxide/mol) from Atlas, Essen, FRG.

Treatment of animals. Male Wistar rats (200–250 g) were used. MC (40 mg/kg; dissolved in olive oil) was given once intraperitoneally and the animals were killed after 4 days. Phenobarbital, sodium salt (100 mg/kg; dissolved in saline) was given once intraperitoneally, followed by 0.1% (w/v) of the drug in the drinking water for 4 days. Liver microsomes were prepared as described (23). Microsomal protein was determined according to the method of Lowry *et al.* (35).

Purification of GT$_{MC}$. The enzyme was purified from liver microsomes of rats treated with MC (19). Purity of GT$_{MC}$ was assessed by determining GT activity toward its substrates and selective substrates of other isoenzymes and by gel electrophoresis. GT activity toward testosterone indicated the presence of contaminating testosterone GT with an M_r of 50,000. Densitometry of gels showed that about 20–30% of the protein represented polypeptides other than GT$_{MC}$ (M_r = 54,000).

GT assays. Assay conditions are based on those described previously for 3-OH-BP (36). All experiments with PAH phenols were carried out under subdued light. Incubation mixtures (1.0 ml) contained 0.1 M Tris-HCl, pH 7.4, 5 mM $MgCl_2$, microsomal protein (0.05–1 mg/ml), Brij 58, and 100 μM PAH phenols (added in 10 μl of dimethyl sulfoxide or dimethylformamide). The substrate concentration was close to enzyme saturation.

3-Hydroxyfluoranthene was assayed at a concentration of 0.5 mM. 6-OH-BP was obtained from 6-acetoxy-BP immediately before carrying out the incubation. A solution (10 mM) of the ester in dimethylformamide was mixed with one-fourth the volume of 1 N NaOH. The hydrolysis was accompanied by an immediate change of the yellow color of the ester to the orange-red color of the phenolate. After 30–60 sec, the solution was neutralized by addition of 1 N HCl. Within this time the ester was completely hydrolyzed, indicated by the complete disappearance of the fluorescence of 6-acetoxy-BP at 372 and 408 nm of excitation and emission, respectively (see below). Concomitantly, the phenolate spectrum appeared (18), when an aliquot of the solution was added to 0.4 M glycine/methanol (1:1, v/v), pH 10.3. The assay mixture was preincubated for 5 min at 37° to achieve a homogeneous distribution of the highly lipophilic substrates. Then the reaction was started by addition of UDP-glucuronic acid (3 mM).

At the chosen incubation times and protein concentrations initial reaction rates were measured, and substrate consumption did not exceed 20%. In blanks, UDP-glucuronic acid was omitted. For example, assays toward 6-OH-BP contained 50 μg of protein/ml and were incubated for 1—3 min, whereas 3-OH-DBA was incubated in the presence of 1 mg/ml for 20–60 min. With the purified enzyme, incubation times were similar to those used with microsomes. However, the protein concentration was lower, for example, 4 μg/ml and 20 μg/ml for the assay of GT activity toward 7-OH-BP and 3-OH-DBA, respectively. The reaction was stopped by removing serial aliquots (0.25 ml) and rapidly mixing them with 1.75 ml of chloroform/methanol/water (4:2:1, v/v/v) (37). The samples were extracted, centrifuged (1600 × *g*, 10 min), and aliquots (0.5 ml) of the upper (aqueous) phase were mixed with 1 N NaOH (0.6 ml) and again centrifuged (9000 × *g*, 2 min). Aliquots (0.8 ml) of the upper phase were used for fluorimetric determinations of the glucuronides at the following wavelengths of excitation and emission, respectively: 3-OH-BP, 381 and 425 nm; 6-OH-BP, 375 and 413 nm; 7-OH-BP, 392 and 430 nm; 8-OH-BP, 386 and 410 nm; 9-OH-BP, 377 and 416 nm; 1-OH-DBA, 296 and 398 nm; 2-OH-DBA, 298 and 401 nm; 3-OH-DBA, 299 and 395 nm; 4-OH-DBA, 299 and 399 nm; 5-OH-DBA, 299 and 405 nm; 6-OH-DBA, 298 and 399 nm; 7-OH-DBA, 299 and 402 nm; 3-hydroxybenz(*a*)anthracene, 292 and 390 nm; 3-hydroxyfluoranthene, 363 and 460 nm. Fluorescence was determined using a Perkin-Elmer model 650-10S fluorescence spectrophotometer fitted with a Xenon X BO 150-W lamp. Fluorescence intensity was calibrated with quinine sulfate.

Calibration of glucuronide fluorescence was carried out by incubating the phenols with UDP-[^{14}C]glucuronic acid (1 mCi/mmol) and separation of the labeled glucuronides by thin layer chromatography as described (36). Under our conditions the phenolate fluorescence of traces of unextracted phenols did not interfere with glucuronide fluorescence. Hence, background fluorescence was very low and the fluorimetric determination of PAH phenol glucuronides was very sensitive. Detection limits of these phenol glucuronides were in the nM range.

Calculation of molecular dimensions of PAH phenols. Bond lengths for PAH phenols are not available, except for naphthols. Those of the corresponding PAHs have been determined by theoretical calculations (38) and from crystallographic data (39, 40). The mean carbon-carbon bond length was found to be 0.141 ± 0.003 nm. In the case of 1-naphthol and 2-naphthol the carbon-carbon bond lengths were within the standard deviations of the bond lengths determined for naphthalene (38, 41–43). Therefore, the carbon-carbon bond lengths of the corresponding PAH was used for calculation of the molecular

TABLE 1

Differential induction by MC and phenobarbital of rat liver microsomal GT activities toward PAH phenols

Data represent the means ± standard deviations of four to six experiments.

Substrate	Enzyme activity		
	Untreated controls	MC treatment	Phenobarbital treatment
	nmol/min/mg protein		
Group 1			
3-OH-BP	4.6 ± 0.8	37.4 ± 2.9 (8.1)[a]	7.7 ± 1.8 (1.7)
6-OH-BP	26 ± 4	119 ± 19 (4.6)	53 ± 13 (2.0)
7-OH-BP	14 ± 1	95 ± 6 (6.8)	24 ± 3 (1.7)
1-OH-DBA	1.4 ± 0.2	5.6 ± 0.7 (4.0)	3.3 ± 0.2 (2.4)
2-OH-DBA	1.5 ± 0.1	9.0 ± 1.5 (6.0)	2.9 ± 0.1 (1.9)
4-OH-DBA	0.32 ± 0.03	1.74 ± 0.33 (5.4)	0.42 ± 0.01 (1.3)
5-OH-DBA	2.7 ± 0.5	20.6 ± 1.9 (7.6)	5.6 ± 0.7 (2.1)
6-OH-DBA	7.7 ± 0.2	41.4 ± 2.1 (5.4)	18.3 ± 4.8 (2.4)
7-OH-DBA	2.8 ± 0.2	15.3 ± 0.6 (5.5)	5.3 ± 0.2 (1.9)
Group 2			
8-OH-BP	4.1 ± 0.2	5.4 ± 0.2 (1.3)	23.3 ± 0.8 (5.7)
3-OH-Benz(a)anthracene	3.7 ± 0.4	5.8 ± 0.6 (1.6)	31.2 ± 1.2 (8.4)
3-OH-DBA	0.006 ± 0.001	0.010 ± 0.001 (1.7)	0.016 ± 0.004 (2.8)
Group 3			
9-OH-BP	17 ± 3	29 ± 5 (1.7)	38 ± 4 (2.2)
3-OH-Fluoranthene	86 ± 9	157 ± 26 (1.8)	128 ± 11 (1.5)

[a] Induction factor, i.e., ratio of enzyme activities obtained from inducer-treated rats and untreated control rats.

TABLE 2

Glucuronidation of phenols of BP and DBA by purified GT_{MC}

Substrate	Enzyme activity[a]	Purification factor[b]
	nmol/min/mg protein	
3-OH-BP	733	20
7-OH-BP	1454	15
8-OH-BP	9	2
9-OH-BP	46	2
1-OH-DBA	50	9
2-OH-DBA	136	15
3-OH-DBA	0.02	2
4-OH-DBA	31	18
5-OH-DBA	506	25
6-OH-DBA	550	13
7-OH-DBA	251	16

[a] Data represent the means of two different determinations.
[b] Ratio of enzyme activities obtained with purified GT_{MC} and with microsomes from MC-treated rats (given in Table 1).

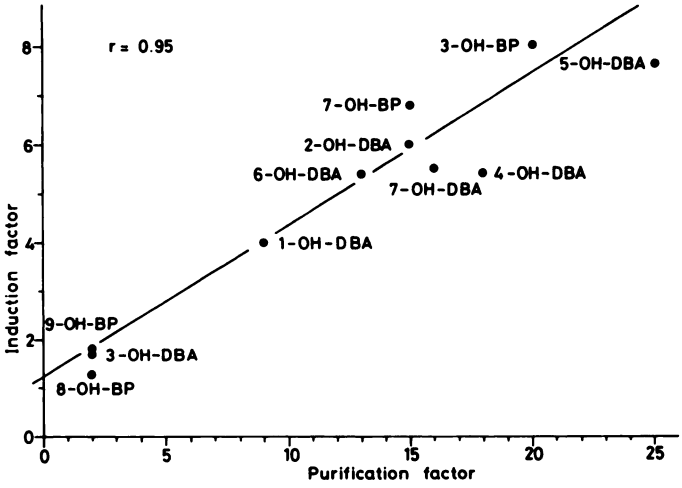

Fig. 2. Correlation between purification factors obtained with purified GT_{MC} and induction factors of enzyme activities toward phenols of BP and DBA. Purification factors represent the ratio of enzyme activities with purified GT_{MC} and with liver microsomes from MC-treated rats. Induction factors represent the ratio of liver microsomal enzyme activities from MC-treated rats and untreated controls. Data were taken from Tables 1 and 2.

dimensions of PAH phenols. In addition, the following bond lengths were used: carbon-oxygen, 0.139 nm (38, 41, 43); carbon-hydrogen, 0.107 nm (44). For the van der Waals radius of hydrogen a value of 0.120 nm was taken (44).

Results

With rat liver microsomes, glucuronide formation of the planar PAH phenols shown in Fig. 1 was differentially induced by MC or phenobarbital (Table 1). Glucuronidation of the majority of the phenols was preferentially inducible by MC (group 1). Surprisingly, glucuronidation of 8-OH-BP, 3-hydroxybenz(*a*)anthracene, and 3-OH-DBA was preferentially inducible by phenobarbital (group 2). However, enzyme activity toward 3-OH-DBA was several orders of magnitude lower than that toward the other substrates. Glucuronidation of 9-OH-BP and 3-OH-fluoranthene was only moderately inducible by either MC or phenobarbital (about 2-fold). Therefore, these substrates were considered as a separate group (group 3).

In studies with purified GT_{MC} it was found that group 1 substrates showed purification factors (ratio of enzyme activities with purified GT_{MC} and with microsomes from MC-treated rats) of about 20 (Table 2). Purification factors obtained with group 2 substrates were much lower (2-fold). A high correlation ($r = 0.95$) was found between purification factors and induction factors (ratio of microsomal enzyme activities from MC-treated rats and untreated controls) (Fig. 2). However, overlapping substrate specificity for simple phenols between GT_{MC} and testosterone GT (21) and the presence of some co-purifying testosterone GT in purified GT_{MC} poses limitations on the interpretation of induction and purification factors. Hence, the selectivity of GT_{MC} for some PAH phenols may be higher than indicated in Table 2.

Group 1 and 2 substrates could also be discriminated on the basis of their molecular dimensions including van der Waals radii. The maximal lengths of the molecules were calculated

TABLE 3

Calculated molecular dimensions of PAH phenols

Molecular dimensions including van der Waals radii were calculated as described in Materials and Methods. Length is given along the axis of the phenolic C—O bond, width at right angles to the axis. Substrates were grouped according to their preferential inducibility as in Table 1.

Substrate	Lengths	Widths
	nm	*nm*
Group 1		
3-OH-BP	1.24	1.16
6-OH-BP	1.03	1.28
7-OH-BP	1.03	1.28
1-OH-DBA	1.16	1.40
2-OH-DBA	1.24	1.40
4-OH-DBA	1.24	1.40
5-OH-DBA	1.24	1.40
6-OH-DBA	1.16	1.40
7-OH-DBA	1.16	1.40
Group 2		
8-OH-BP	1.45	0.91
3-OH-DBA	1.67	0.91
3-OH-Benz(*a*)anthracene	1.45	0.91
Group 3		
9-OH-BP	1.24	1.16
3-OH-Fluoranthene	1.18	0.91

along the axis of the phenolic C—O bond and the maximal widths at right angles to the axis of the C—O bond. As shown in Table 3, group 1 substrates appeared to be shorter (<1.3 nm) and broader (>1.1 nm) than group 2 substrates. The group 3 substrates studied were in between the dimensions of group 1 and 2 substrates.

Discussion

Glucuronidation of a variety of PAH phenols was investigated using the model of differential induction by MC and phenobarbital in rat liver microsomes (19). It was found that most PAH phenols were preferentially inducible by MC (group 1 or GT_1 activities). However, glucuronidation of 8-OH-BP, 3-hydroxybenz(a)anthracene, and 3-OH-DBA was preferentially inducible by phenobarbital (group 2 activities). In support of this distinction, much higher purification factors were found for GT_1 activities (about 20-fold) when assayed with purified GT_{MC} and liver microsomes from MC-treated rats, whereas purification factors for group 2 activities were much lower (about 2-fold). Other GT_1 activities toward simple phenols such as 1-naphthol and 4-methylumbelliferone were also increased 20- to 30-fold with purified GT_{MC} (19). The close correlation between induction and purification factors suggests that the compounds with the highest induction factors may be the preferred substrates of GT_{MC}. On the other hand, we have to assume that there is considerable overlap in the substrate specificity of inducible and constitutive forms of GT. Of course the use of overlapping substrates imposes limitations on the interpretation of induction factors. The level of GT_{MC} is probably low in liver microsomes from untreated rats (18). Hence, in controls and MC-treated rats, phenolic substrates such as 1-naphthol may be conjugated by different isoenzymes, for example, mostly by testosterone GT in untreated rats and mostly by GT_{MC} in MC-treated rats (21). Similarly, co-purifying isoenzymes such as testosterone-GT, together with differential enzyme inactivation during purification, limit the interpretation of purification factors. Hence, detection of GT activities toward group 2 and 3 substrates may be due to small amounts of contaminating isoenzymes.

Interestingly, group 1 and 2 substrates show distinct differences in their geometry. When viewed along the axis of the phenolic C—O bond, group 1 substrates are shorter than 1.3 nm and broader than 1.1 nm, whereas the group 2 substrates investigated appear to be longer (1.45–1.67 nm) and narrower (0.91 nm). These findings suggest distinct molecular dimensions of the binding site of GT_{MC}. Small phenolic substrates of groups 1 and 2 have previously been found to differ with respect to "bulkiness" or "thickness" (extension of the molecule perpendicular to the plane of the aromatic ring) (19, 25, 26, 45). Substrates with a thickness of less than 0.4 nm were preferentially accepted by GT_{MC}, whereas bulkier substrates with a thickness of more than 0.4 nm were preferentially accepted by phenobarbital-inducible GT (26). It would be interesting to study group 2 activities with purified phenobarbital-inducible GT. However, due to its low yield, this isoenzyme (or isoenzymes) is not yet available for such studies.

The highest induction factors for MC-inducible reactions were found so far for the conjugation of BP-3, 6-quinol which, in rat liver, proceeds in two sequential reactions whereby the mono- and diglucuronide are formed (18, 46). With liver microsomes from MC-treated rats, induction factors of 10 and 40 were found for mono- and diglucuronide formation, respectively (18). The widely differing induction factors may suggest more than one GT_{MC} isoenzyme. Diglucuronide formation is probably a selective probe for GT_{MC}. A high rate of diglucuronide formation and a purification factor of about 10-fold were found with purified GT_{MC}.[1] However, this quinol, conjugated in a sequential reaction, may pose special kinetic problems. Therefore, purification factors obtained with the quinol may not be comparable to those obtained with phenols.

In the cytochrome P-450 family an MC-inducible isoenzyme, preferentially oxidizing planar phenols, has been extensively characterized, and termed cytochrome P-450c (47) or P_1-450 (48). In genetic experiments using inbred mouse strains, evidence was obtained for co-induction of MC-inducible isoenzymes of cytochrome P-450 and of GT, using 4-methylumbelliferone as substrate (49). Hence, GT_1 may be part of an adaptive program enabling the organism to more efficiently detoxify PAHs by first pass metabolism in the intestinal mucosa and liver when the organism is exposed to PAHs present in the diet (50). However, much more work is needed to establish this aspect.

Acknowledgments

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References

1. Glatt, H.R., and F. Oesch. Phenolic benzo(*a*)pyrene derivatives are mutagenic. *Mutat. Res.* **36**:379–384 (1976).
2. Wislocki, P.G., A.W. Wood, R.L. Chang, W. Levin, H. Yagi, O. Hernandez, P.M. Dansette, D.M. Jerina, and A.H. Conney. Mutagenicity and cytotoxity of benzo(*a*)pyrene arene oxides, phenols, quinones, and dihydrodiols in bacterial and mammmalian cells. *Cancer Res.* **36**:3350–3357 (1976).
3. Slaga, T.J., W.M. Bracken, S. Desner, W. Levin, H. Yagi, D.M. Jerina, and A.H. Conney. Skin-tumor-initiating activities of the twelve isomeric phenols of benzo(*a*)pyrene. *Cancer Res.* **38**:678–681 (1978).
4. Kinoshita, H., and H.V. Gelboin. β-Glucuronidase-catalyzed hydrolysis of benzo(*a*)pyrene-3-glucuronide and binding to DNA. *Science (Wash. D.C.)* **199**:307–309 (1978).
5. Chang, R.L., P.G. Wislocki, J. Kapitulnik, A.W. Wood, W. Levin, H. Yagi, H.D. Mah, D.M. Jerina, and A.H. Conney. Carcinogenicity of 2-hydroxy-

[1] W. Lilienblum, G. Schirmer, and K.W. Bock, unpublished results.

benzo(*a*)pyrene and 6-hydroxybenzo(*a*)pyrene in newborn mice. *Cancer Res.* **39**:2660–2664 (1979).
6. Owens, I.S., G.M. Koteen, and C. Legraverend. Mutagenesis of certain benzo(*a*)pyrene phenols *in vitro* following further metabolism by mouse liver. *Biochem. Pharmacol.* **28**:1615–1622 (1979).
7. Glatt, H.R., R. Billings, K.L. Platt, and F. Oesch. Improvement of the correlation of bacterial mutagenicity with carcinogenicity of benzo(*a*)pyrene and four of its major metabolites by activation with intact liver cells instead of cell homogenate. *Cancer Res.* **41**:270–277 (1981).
8. Huang, M.-T., A.W. Wood, R.L. Chang, H. Yagi, J.M. Sayer, D.M. Jerina, and A.H. Conney. Inhibitory effect of 3-hydroxybenzo(*a*)pyrene on the mutagenicity and tumorigenicity of (±)-7β-8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene. *Cancer Res.* **46**:558–566 (1986).
9. Wiebel, F.J. Metabolism of monohydroxybenzo(*a*)pyrenes by rat liver microsomes and mammalian cells in culture. *Arch. Biochem. Biophys.* **168**:609–621 (1975).
10. Lorentzen, R.J., W.J. Caspary, S.A. Lesko, and P.O.P. Ts'o. The autoxidation of 6-hydroxybenzo(*a*)pyrene and 6-oxobenzo(*a*)pyrene radical, reactive metabolites of benzo(*a*)-pyrene. *Biochemistry* **14**:3970–3977 (1975).
11. Jernström, B., S. Orrenius, O. Lindemann, A. Gräslund, and A. Ehrenberg. Fluorescence study of DNA-binding metabolites of benzo(*a*)pyrene formed in hepatocytes isolated from 3-methylcholanthrene-treated rats. *Cancer Res.* **38**:2600–2607 (1978).
12. Owens, I.S., C. Legraverend, and O. Pelkonen. Deoxyribonucleic acid binding of 3-hydroxy- and 9-hydroxybenzo(*a*)pyrene following further metabolism by mouse liver microsomal P_1-450. *Biochem. Pharmacol.* **28**:1623–1629 (1979).
13. Harvey, R.G., and C. Cortez. K-region oxidized metabolites of 9-hydroxybenzo(*a*)pyrene. *Carcinogenesis* **4**:941–942 (1983).
14. Bochnitschek, W., A. Seidel, H. Kunz, and F. Oesch. Reactive metabolites of carcinogenic polycyclic hydrocarbons: synthesis and trapping reaction of 9-hydroxybenzo(*a*)pyrene-4,5-oxide. *Angew. Chem. Int. Ed. Engl.* **24**:699–700 (1985).
15. Lorentzen, R.J., and P.O.P. Ts'o. Benzo(*a*)pyrenedione/benzo(*a*)pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen. *Biochemistry* **16**:1467–1473 (1977).
16. Lorentzen, R.J., S.A. Lesko, K. McDonald, and P.O.P. Ts'o. Toxicity of metabolic benzo(*a*)pyrenediones to cultured cells and the dependence upon molecular oxygen. *Cancer Res.* **39**:3194–3198 (1979).
17. Thor, H., M.T. Smith, P. Hartzell, G. Bellomo, S.A. Jewell, and S. Orrenius. The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. *J. Biol. Chem.* **257**:12419–12425 (1982).
18. Lilienblum, W., B.S. Bock-Hennig, and K.W. Bock. Protection against toxic redox cycles between benzo(*a*)pyrene-3,6-quinone and its quinol by 3-methylcholanthrene-inducible formation of the quinol mono- and diglucuronide. *Mol. Pharmacol.* **27**:451–458 (1985).
19. Bock, K.W., D. Josting, W. Lilienblum, and H. Pfeil. Purification of rat-liver microsomal UDP-glucuronosyltransferase. Separation of two enzyme forms inducible by 3-methylcholanthrene or phenobarbital. *Eur. J. Biochem.* **98**:19–26 (1979).
20. Dutton, G.J. *Glucuronidation of Drugs and Other Compounds.* CRC Press, Boca Raton, FL, 23–28 (1980).
21. Falany, C.N., and T.R. Tephly. Separation, purification and characterization of three isoenzymes of UDP-glucuronosyltransferase from rat liver microsomes. *Arch. Biochem. Biophys.* **227**:248–258 (1983).
22. Roy Chowdhury, J., N. Roy Chowdhury, C. Falany, T.R. Tephly, and I.M. Arias. Isolation and characterization of multiple forms of rat liver UDP-glucuronate glucuronosyltransferase. *Biochem. J.* **233**:827–837 (1986).
23. Bock, K.W., W. Fröhling, H. Remmer, and B. Rexer. Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase. *Biochim. Biophys. Acta* **327**:46–56 (1973).
24. Wishart, G.J. Demonstration of functional heterogeneity of hepatic uridine diphosphate glucuronosyltransferase activities after administration of 3-methylcholanthrene and phenobarbital to rats. *Biochem. J.* **174**:671–672 (1978).
25. Wishart, G.J., and M.T. Campbell. Demonstration of two functionally heterogeneous groups within the activities of UDP-glucuronosyltransferase towards a series of 4-alkyl-substituted phenols. *Biochem. J.* **178**:443–447 (1979).
26. Okulicz-Kozaryn, I., M. Schaefer, A.M. Batt, G. Siest, and V. Loppinet. Stereochemical heterogeneity of hepatic UDP-glucuronosyltransferase activity in rat liver microsomes. *Biochem. Pharmacol.* **30**:1457–1461 (1981).
27. Lilienblum, W., A.K. Walli, and K.W. Bock. Differential induction of rat liver microsomal UDP-glucuronosyltransferase activities by various inducing agents. *Biochem. Pharmacol.* **31**:907–913 (1982).
28. Watkins, J.B., Z. Gregus, T.N. Thompson, and C.D. Klaassen. Induction studies on the functional heterogeneity of rat liver UDP-glucuronosyltransferase. *Toxicol. Appl. Pharmacol.* **64**:439–446 (1982).
29. Bock, K.W., W. Lilienblum, D. Ullrich, and G. Fischer. Differential induction of UDP-glucuronosyltransferases and their "permanent induction" in preneoplastic rat liver. *Biochem. Soc. Trans.* **12**:55–58 (1984).
30. Borm, P.J.A., M. Kroon, J. Nordhoek, K.L. Platt, and F. Oesch. Dose-dependent activation of rat small intestinal monooxygenase activity towards benzo(*a*)pyrene and 7-ethoxycoumarin after oral pretreatment with cimetidine. *Res. Commun. Chem. Pathol. Pharmacol.* **44**:99–111 (1984).
31. Platt, K.L., and F. Oesch. Synthesis and properties of the isomeric phenols of dibenz(*a,h*)anthracene. *J. Org. Chem.* **47**:5321–5326 (1982).
32. Fieser, L.F., and E.B. Hershberg. The oxidation of 3-methylcholanthrene and 3,4-benzpyrene with lead tetraacetate: further derivatives of 3,4-benzpyrene. *J. Am. Chem. Soc.* **60**:2542–2548 (1938).
33. Fu, P.P., C. Cortez, K.B. Sukumaran, and R.G. Harvey. Synthesis of the isomeric phenols of benz(*a*)anthracene from benz(*a*)anthracene. *J. Org. Chem.* **44**:4265–4271 (1979).
34. Shenbor, M.I., and G.A. Cheban. Preparation of fluoranthenol-3 by the action of lead tetraacetate on fluoranthene. *J. Org. Chem. (USSR)* **5**:143–144 (1969).
35. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. Protein measurement with the Folin phenol reagent. *J Biol. Chem.* **193**:265–275 (1951).
36. Bock, K.W., and W. Lilienblum. Activation and induction of rat liver microsomal UDP-glucuronyltransferase with 3-hydroxybenzo(*a*)pyrene and *N*-hydroxy-2-naphthylamine as substrates. *Biochem. Pharmacol.* **28**:695–700 (1979).
37. Singh, J., and F.J. Wiebel. A highly sensitive and rapid fluorometric assay for UDP-glucuronyltransferase using 3-hydroxybenzo(*a*)pyrene as substrate. *Anal. Biochem.* **98**:394–401 (1979).
38. Dewar, M.J.S., and N. Trinajstic. Semiempirical SCF-MO treatment of excited states of aromatic compounds. *J. Chem. Soc. Sect. A.* 1220–1237 (1971).
39. Iball, J., S.N. Scrimgeour, and D.W. Young. 3,4-Benzopyrene (a new refinement). *Acta Crystallogr. Sect. B. Struct. Crystallogr. Cryst. Chem.* **32**:328–330 (1976).
40. Iball, J., C.H. Morgan, and D.E. Zacharias. Refinement of the crystal structure of orthorhombic dibenz(*a,h*)anthracene. *J. Chem. Soc. Perkin II* 1271–1272 (1975).
41. Robinson, B., and A. Hargreaves. The crystal structure of α-naphthol. *Acta Crystallogr.* **17**:944–946 (1964).
42. Almenningen, A., O. Bastiansen, and F. Dyvik. An attempt to determine the structure parameters of condensed ring hydrocarbons using the electron-diffraction method in gas molecules. *Acta Crystallogr.* **14**:1056–1065 (1961).
43. Watson, H.C., and A. Hargreaves. The crystal structure of β-naphthol. *Acta Crystallogr.* **11**:556–562 (1958).
44. Pauling, L. *The Nature of the Chemical Bond and the Structure of the Molecules and Crystals*, 3rd ed. Cornell University Press, Ithaca, NY (1960).
45. Bock, K.W., U.C. von Clausbruch, R. Kaufmann, W. Lilienblum, F. Oesch, H. Pfeil, and K.L. Platt. Functional heterogeneity of UDP-glucuronyltransferase in rat tissues. *Biochem. Pharmacol.* **29**:495–500 (1980).
46. Lind, C. Formation of benzo(*a*)pyrene-3,6-quinol mono- and diglucuronides in rat liver microsomes. *Arch. Biochem. Biophys.* **240**:226–235 (1985).
47. Ryan, E.D., P.E. Thomas, D. Korzeniowski, and W. Levin. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, and 3-methylcholanthrene. *J. Biol. Chem.* **254**:1365–1374 (1979).
48. Nebert, D.W., and N.M. Jensen. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals by cytochrome P-450-mediated monooxygenases. *CRC Crit. Rev. Biochem.* **6**:401–437 (1979).
49. Owens, I.S. Genetic regulation of UDP-glucuronosyltransferase induction by polycyclic aromatic compounds in mice. *J. Biol. Chem.* **252**:2827–2833 (1977).
50. Nebert, D.W. The Ah locus. A gene with possible importance in cancer predictability. *Arch. Toxicol.* (suppl. 3) 195–207 (1980).

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