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DEOXYRIBONUCLEIC ACID BINDING OF 3-HYDROXY- AND 9-HYDROXYBENZO[a]PYRENE FOLLOWING FURTHER METABOLISM BY MOUSE LIVER MICROSOMAL CYTOCHROME P_1-450

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Abstract—In the presence of NADPH and microsomes from 3-methylcholanthrene-treated C57BL/6N mice, [^{3}H]3-OH-benzo[a]pyrene is metabolized to reactive intermediates which covalently bind to deproteinized salmon sperm DNA *in vitro*. Enzymatically digested DNA, containing bound [^{3}H]3-OH-benzo[a]pyrene derivatives, generates an elution profile from Sephadex LH20 chromatography which resembles similar chromatograms with [^{3}H]benzo[a]pyrene. All peaks resulting from [^{3}H]benzo[a]pyrene activation appear to be prominently represented in [^{3}H]3-OH-benzo[a]pyrene activation, except that several peaks which emerge near the end of the eluting gradient of methanol and water are much reduced. Notably, a peak designated E, and attributed to benzo[a]pyrene-7,8-diol-9,10-oxide binding in [^{3}H]benzo[a]pyrene incubations, is also prominently represented in incubations with [^{3}H]3-OH-benzo[a]pyrene. Radioactivity in all of these peaks is inhibited effectively if one-seventh the concentration of 1-OH-benzo[a]pyrene is included in the incubation with [^{3}H]3-OH-benzo[a]pyrene. Microsomes from 3-methylcholanthrene-treated DBA/2N mice cause insignificant binding. UDP-glucuronic acid markedly reduces all peaks except E, and 1,2-epoxy-3,3,3-trichloropropane reduces all peaks except C and E. 9-Hydroxybenzo[a]pyrene is further metabolized to DNA binding species by microsomes from either 3-methylcholanthrene-treated DBA/2N or C57BL/6N mice. UDP-glucuronic acid prevents about 50 per cent of the binding with microsomes from DBA/2N mice but not with microsomes from C57BL/6N. In contrast, UDP-glucuronic acid does prevent binding in some of these same peaks when [^{3}H]benzo[a]pyrene is the starting substrate with microsomes from C57BL/6N mice. UDP-glucuronic acid does not prevent binding in peak E in incubations with [^{3}H]benzo[a]pyrene or [^{3}H]3-hydroxybenzo[a]pyrene.

Microsomal mono-oxygenase activity catalyzes the conversion of benzo[a]pyrene (BP)* in a reaction which requires NADPH and molecular oxygen to generate primarily epoxides, quinones and phenols. In most systems, 3-OH-BP and 9-OH-BP combined constitute the major primary metabolites resulting from the oxygenation of BP. These hydroxy derivatives have been considered final metabolites, as such, or substrates for conjugation to glucuronic acid [1] or sulfate [2] for efficient excretion. However, several earlier reports have shown that 3-OH-BP can be further metabolized [3–5] and actually binds to DNA [4]. It has been suggested that 9-OH-BP can be further metabolized *in vitro* to form a DNA binding ligand [6]. Also, labeled phenols generated from [^{3}H]BP were shown to bind to DNA following further metabolism [7]. In agreement with these earlier observations that 3-OH-BP can be further metabolized, the companion report [8] shows that 3-OH-BP and 1-OH-BP can be activated to mutagens in the presence of NADPH and a certain S-9 fraction to such an extent that histidine reversion in *Salmonella typhimurium* tester strain TA98 can rise to values 3-fold higher than for BP. Since the 3-OH- and 9-OH-BP combined constitute the major primary metabolites of BP and since certain phenols are quite mutagenic, it was of interest to examine further the nature of this DNA binding activity and the relative effects of various modifiers on binding.

* Abbreviations used are: BP, benzo[a]pyrene; BP-4,5-oxide, benzo[a]pyrene-4,5-oxide; BP-7,8-oxide, benzo[a]-pyrene-7,8-oxide; BP-9,10-oxide, benzo[a]pyrene-9,10-oxide; BP-7,8-diol-9,10-oxide, benzo[a]pyrene-7,8-diol-9,10-oxide; 9-OH-BP-4,5-oxide, 9-hydroxybenzo[a]pyrene-4,5-oxide; 3-OH-BP-7,8-oxide, 3-hydroxybenzo[a]pyrene-7,8-oxide; 3,7-di-OH-BP, 3,7-dihydroxybenzo[a]pyrene; 3,7-di-OH-BP-9,10-oxide, 3,7-dihydroxybenzo[a]pyrene-9,10-oxide; 3-OH-BP-7,8-diol-9,10-oxide, 3-hydroxybenzo[a]pyrene-7,8-diol-9,10-oxide; 3-OH-BP-9,10-oxide, 3-hydroxybenzo[a]pyrene-9,10-oxide; 3,9-di-OH-BP, 3,9-dihydroxybenzo[a]pyrene; 3,9-di-OH-BP-7,8-oxide, 3,9-dihydroxybenzo[a]pyrene-7,8-oxide; 3-OH-BP-9,10-diol-7,8-oxide, 3-Hydroxybenzo[a]pyrene-9,10-diol-7,8-oxide; TCPE, 1,2-epoxy-3,3,3-trichloropropane; MC, 3-methylcholanthrene; UDPGA, UDP-glucuronic acid; transferase, UDP-glucuronosyl transferase; E.H., epoxide hydratase; and M.O., mono-oxygenase.

MATERIALS AND METHODS

Materials. NADPH was purchased from Cal-Biochem (San Diego, CA); TCPE was from Aldrich (Milwaukee, WI); UDPGA was from Sigma (St. Louis, MO); and MC was from Eastman (Rochester, NY). BP-phenols were obtained from the National Cancer Institute Chemical Repository, National Institutes of Health (Bethseda, MD) through Dr. D. G. Longfellow, Program Manager; 4- to 6-week-old male C57BL/6N

and DBA/2N mice were obtained from Veterinary Resources Branch, NIH. Chemicals and enzymes used in the DNA binding assay were obtained from the sources cited [7]. 3-OH- and 9-OH-BP were tritiated by New England Nuclear (Boston, MA) via catalytic exchange labeling; a specific activity of 7.8 Ci/m-mole was achieved with each phenol. The specific activity of [^{3}H]BP was 25 Ci/m-mole.

Tritiated 3-OH-or 9-OH-BP was purified the day of use by thin-layer chromatography (t.l.c.) on silica gel plates of 0.25 mm thickness eluted with 9:1 benzene–ethanol [9]; 0.9 mCi of [^{3}H]3-OH- or [^{3}H]9-OH-BP or 1 mCi of [^{3}H]BP was added to each incubation for DNA binding. Tritiated phenols were present at 12.0 μM and [^{3}H]BP was present at 4 μM in the DNA binding incubations.

Treatment of mice. C57BL/6N or DBA/2N mice were treated with a single intraperitoneal dose (200 mg/kg) of MC 5 days before death.

Preparation of microsomes. After death the liver was excised and microsomes were prepared as described previously [10]. All further manipulations were done at 0–4°. The microsomal surface was washed in 0.25 M phosphate/30% glycerol buffer, pH 7.2, and resuspended in this same buffer to about 10 mg protein/ml.

Glucuronidation. The effect of 2 mM UDPGA on 3-OH-BP-derived binding to DNA was assessed by a decrease in the DNA binding profile eluted from a Sephadex LH20 column. Triton X-100 (0.1%) or 4 mM UDP-*N*-acetylglucosamine was added to the incubation mixture to activate UDP-glucuronosyltransferase activity.

Hydroxybenzo[a]pyrene binding to DNA. Incubation of microsomes with NADPH, deproteinized salmon sperm DNA, and [^{3}H]BP, [^{3}H]3-OH-BP or [^{3}H]9-OH-BP, and the preparation of the BP metabolite-nucleoside for elution on Sephadex LH20 column chromatography were the same as described previously [7, 11]. Livers from four to six mice for each group were combined for microsomal preparations. Radiolabeled or unlabeled hydrocarbon and TCPE were added to the reaction mixture in 100 μl acetone. The reaction was initiated with radiolabeled BP or phenol and incubated at 37° for 30 min [11]. The reaction flasks were then placed on ice, and the mixture was spun at 105,000 *g* for 1 hr to remove microsomal protein. The hydrocarbon-bound DNA was isolated and purified as described previously [7, 11]. Each incubation was carried out at least two to three times, and the profile presented in each graph represents a typical result for that incubation condition.

RESULTS

Binding of [^{3}H]3-OH-*BP metabolites to DNA.* The companion paper [8] shows that a high level of histidine reversion occurs upon activation of 1-OH-BP and 3-OH-BP with native mono-oxygenase activity contained in certain S-9 fractions. The high level of reversion suggests that metabolites of these phenols bind to critical sites on DNA. It was of interest, therefore, to determine the profile of DNA-adducts from a Sephadex LH20 column following an *in vitro* incubation with a labled phenol and DNA. Figure 1 shows the profile from a Sephadex LH20 column of [^{3}H]3-OH-BP-derived DNA-adducts after incubations in the presence of

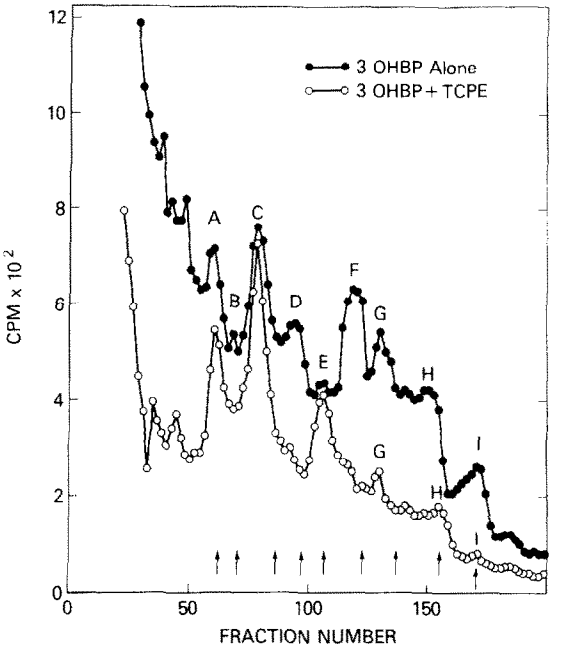

Fig. 1. Chromatogram by Sephadex LH20 of a DNA digest after binding [^{3}H]3-OH-BP metabolites during an *in vitro* incubation with liver microsomes from MC-treated C57BL/6N mice. Closed circles represent binding with [^{3}H]3-OH-BP alone, and open circles represent binding in the presence of 2.0 mM TCPE. Microsomes were prepared as described in Materials and Methods. [^{3}H]3-OH-BP (12.0 μM) and Triton X-100 (0.1%, v/v) were contained in each incubation. The column was eluted with a gradient of 30% methanol: water to 100% methanol. Five ml volumes from alternate tubes were collected and added to an aquasol scintillation mixture for determining radioactivity as described previously [7]. The arrows at the bottom of the figure represent the position of those same peaks (designated by letters) when incubations are carried out with [^{3}H]BP.

microsomes from MC-treated C57BL/6N mice. As the top curve indicates, further metabolism of [^{3}H]3-OH-BP led to the production of peaks A–I as defined with [^{3}H]BP in similar incubations and with the resulting data plotted on an expanded scale [7]. Notably, peaks A–D, F, G and I are as prominent as peak E when compared to these same peaks generated in [^{3}H]BP incubations (see Fig. 4C). It has been shown [7, 11] that BP-7,8-diol-9,10-oxide and BP-4,5-oxide bind to DNA and, after enzymatic hydrolysis, emerge with a gradient eluting system of water and methanol from a Sephadex LH20 column as peaks designated E and G respectively. There is some evidence that peaks A and C are due to dihydrodioloxides [12] and that peaks B, D, F, H and I include metabolites of BP quinones [7]. Of special significance is the presence of prominent peaks E and F′(F). Peak F′ or F has been shown to appear when BP-7,8-oxide or BP-9,10-oxide, respectively, is incubated with DNA [7]. The prominent representation of all peaks which have been previously [7, 12] shown to occur following incubations with [^{3}H]BP and microsomes suggested that [^{3}H]3-OH-BP was being further metabolized by both cytochrome P_1-450 and cytochrome P-450, although peaks G [7, 12] and H [6, 7, 12] attributed to cytochrome P-450 metabo-

lism are less pronounced in this study than in incubations with [^{3}H]BP.

As epoxides are considered the active species involved in covalent binding, the effect of the epoxide hydratase inhibitor, TCPE, on DNA binding was examined, although it was observed that the inhibitor does not diminish the level of histidine revertants due to 3-OH-BP activation [8]. The bottom curve in Fig. 1 shows that the presence of TCPE effectively diminished all peaks significantly except peaks C and E, and it affected peaks D and F–I to the greatest extent.

Since 1-OH-BP was potentially more mutagenic and was considered to be activated by the same enzyme active site as 3-OH-BP, it was of interest to determine if a much lower level of unlabeled 1-OH-BP would inhibit binding of [^{3}H]3-OH-BP during the incubation with microsomes. The bottom curve in Fig. 2 shows that 1.67 μM 1-OH-BP did effectively inhibit all peaks due to 12.0 μM [^{3}H]3-OH-BP shown in the top curve of Fig. 2.

The middle curve in Fig. 2 shows that the addition of the substrate for UDP-glucuronosyltransferase, UDPGA, significantly diminished all peaks except E. Presumably, direct glucuronidation of 3-OH-BP or further metabolized intermediates of 3-OH-BP can occur under these conditions of Triton X-100 activation of the transferase. Glucuronidation in the presence of UDPGA and UDP-*N*-acetylglucosamine as an activator of the transferase (data not shown) generated essentially the same profile of nucleoside-adducts as seen with Triton X-100 as an activator (Figs. 1 and 2).

The results from the mutagenesis assay [8] indicated that 3-OH-BP was not very mutagenic when incubated with the S-9 fraction from the nonresponsive MC-treated DBA/2N mice. Also, Fig. 3A shows that very little binding of [^{3}H]3-OH-BP-metabolites to DNA occurred in the presence of DBA/2N microsomes, and that the effect of UDPGA was practically negligible (Fig. 3B). The absence of a significant peak G in this study with microsomes from DBA/2N mice, while

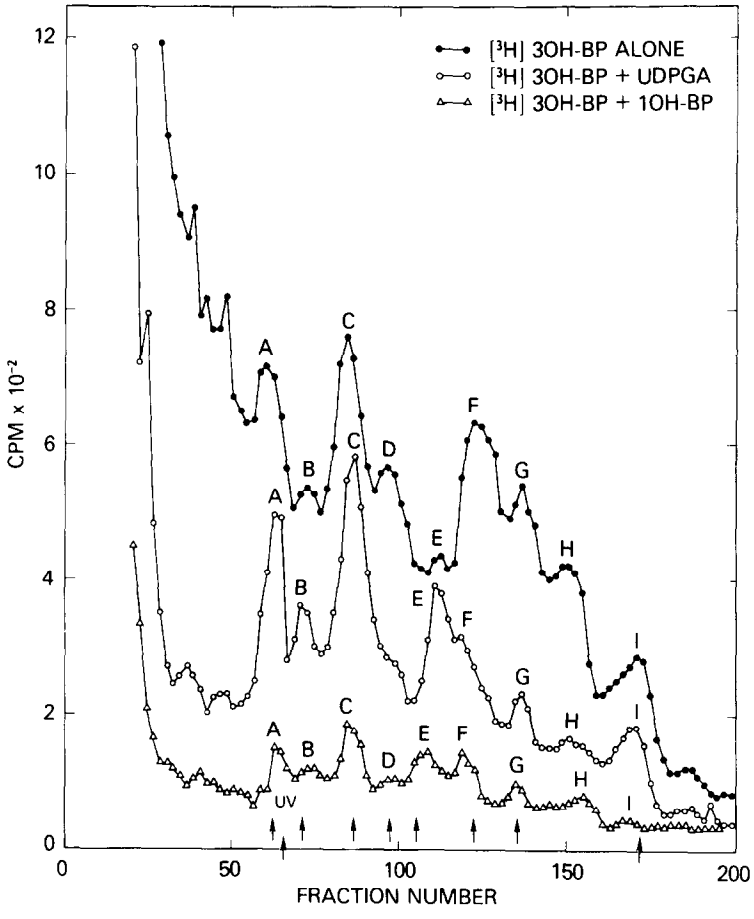

Fig. 2. Chromatogram by Sephadex LH20 of a DNA digest after binding [^{3}H]3-OH-BP metabolites during an *in vitro* incubation with liver microsomes from MC-treated C57BL/6N mice. Closed circles represent binding with [^{3}H]3-OH-BP alone. Open circles represent binding with [^{3}H]3-OH-BP in the presence of 2.0 mM UDPGA. Open triangles represent binding with [^{3}H]3-OH-BP in the presence of 1.67 μM 1-OH-BP. [^{3}H]3-OH-BP (12.0 μM) and Triton X-100 (0.1%, v/v) were contained in each incubation. Microsomes were prepared as described in Materials and Methods. Eluate volume and radioactive determinations are described in the legend to Fig. 1. The arrows at the bottom of the graph represent the position of that same peak (designated by letters) when incubations are carried out with [^{3}H]BP. The scale on the ordinate is the same for all curves.

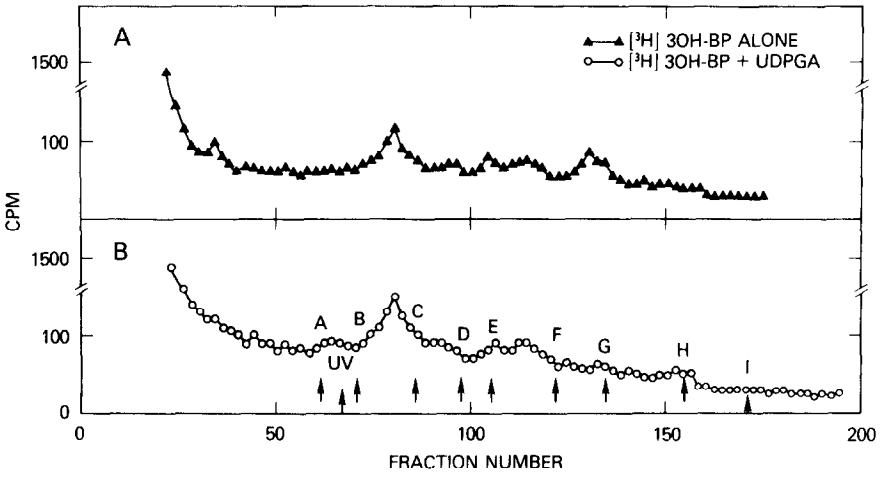

Fig. 3. Chromatogram by Sephadex LH20 of a DNA digest after binding [^{3}H]3-OH-BP metabolites during an *in vitro* incubation with liver microsomes from MC-treated DBA/2N mice. Panel A represents binding with [^{3}H]3-OH-BP alone. Panel B represents binding with [^{3}H]3-OH-BP in the presence of 2.0 mM UDPGA. Microsomes from three mice were prepared as described in Materials and Methods. [^{3}H]3-OH-BP (12.0 μM) and Triton X-100 (0.1%, v/v) were contained in both incubations. Eluate volume and radioactive determinations are described in the legend to Fig. 1. The arrows and letters at the bottom of the graph represent the position of that peak when incubations are carried out with [^{3}H]BP.

peak G did appear with microsomes from C57BL/6N mice, is most likely related to the fact that microsomes from DBA/2N mice are relatively less efficient [7, 12] in generating peak G compared with microsomes from C57BL/6N mice (see Fig. 4, panels C and D). Presumably, cytochrome P-450 of either microsomal preparation is responsible for peak G in incubations with [^{3}H]BP.

Binding of [^{3}H]9-OH-*BP metabolites to DNA*. The further metabolism of [^{3}H]9-OH-BP by microsomes from either MC-treated C57BL/6N or DBA/2N mice is shown in Fig. 4, panels A and B, respectively, to produce a prominent peak H and smaller peak G in each case. The DNA-adduct in peak H is attributed to a 9-OH-BP-oxide which is in agreement with an earlier study which suggested that the equivalent of peak H is due to 9-OH-BP-4,5-oxide [6]. The addition of UDPGA significantly decreased peak H with DBA/2N microsomes but not with C57BL/6N microsomes.

Binding of [^{3}H]*BP metabolites to DNA*. For comparison the profile after incubating DNA with [^{3}H]BP is shown in Fig 4, panels C and D. It can be seen in panel 4C that microsomes from MC-treated C57BL/6N mice generated primarily peaks E and H, and microsomes from MC-treated DBA/2N mice generated primarily peaks G and H, as shown previously [13]. Peak G is attributed to BP-4,5-oxide since the reaction of this metabolite with DNA generates this peak [7]. Panels C and D of Fig. 4 show that UDPGA diminished all prominent peaks due to metabolism of [^{3}H]BP except peak E. Also, Fig. 4C shows that peak H generated with [^{3}H]BP, and *not* peak H generated with [^{3}H]9-OH-BP (Fig. 4A), was diminished in the presence of UDPGA and microsomes from MC-treated C57BL/6N mice. Peak H is attributed to 9-OH-BP-4,5-oxide with either BP [6] or 9-OH-BP as the starting substrate in the incubation medium. An expansion (not shown here, see Refs. 7 and 13) of the scale for Fig. 4C shows detectable peaks for A–D, F, G and I.

DISCUSSION

A high level of mutagenicity after activation of 1-OH- or 3-OH-BP by certain S-9 fractions was observed in a companion report [8] using the *Salmonella* mutagenesis assay. This mutagenicity is probably due to metabolite binding to critical sites on DNA. This study shows that activated [^{3}H]3-OH-BP does bind to DNA and generates a similar series of DNA-adducts as one sees with [^{3}H]BP. Although it cannot be readily seen, there are small shifts in eluting positions for the 3-OH-BP-derived adducts. Since every peak previously defined with [^{3}H]BP metabolites [7] is also prominent after further metabolism of [^{3}H]3-OH-BP, it is not possible to attribute mutagenicity to a specific peak. Binding activity in peaks A–D, F, G or H is as high or higher than binding in peak E, unlike the profile with [^{3}H]BP where peak E is usually higher than most of these peaks. However, it is significant that peak E, attributed to binding of the potent mutagen [14, 15] and carcinogen [16, 17], BP-7,8-diol-9,10-oxide in incubations with [^{3}H]BP, is present in [^{3}H]3-OH-BP-derived binding in the presence of microsomes from MC-treated C57BL/6N mice. It is not possible to make a direct comparison of the quantity of peak E generated by [^{3}H]3-OH-BP and [^{3}H]BP, since the specific activities were 7.8 and 25 Ci/m-mole, respectively, and the substrate concentrations were 12 and 4 μM respectively. Furthermore, metabolism of BP metabolites is very sensitive to BP concentration [18]. If peak E derived from 3-OH-BP is analogous to peak E derived from BP, then two further oxygenations of 3-OH-BP via recycling through the mono-oxygenase system are necessarily required to form the ligand, 3-OH-BP-7,8-diol-9,10-oxide, a compound analogous to BP-7,8-diol-9,10-oxide. The potent mutagenicity [8] resulting from activation and the presence of a peak which emerges almost at the same position as peak E derived from [^{3}H]BP suggest that a highly reactive intermediate, such as 3-OH-BP-7,8-diol-9,10-oxide, is derived from

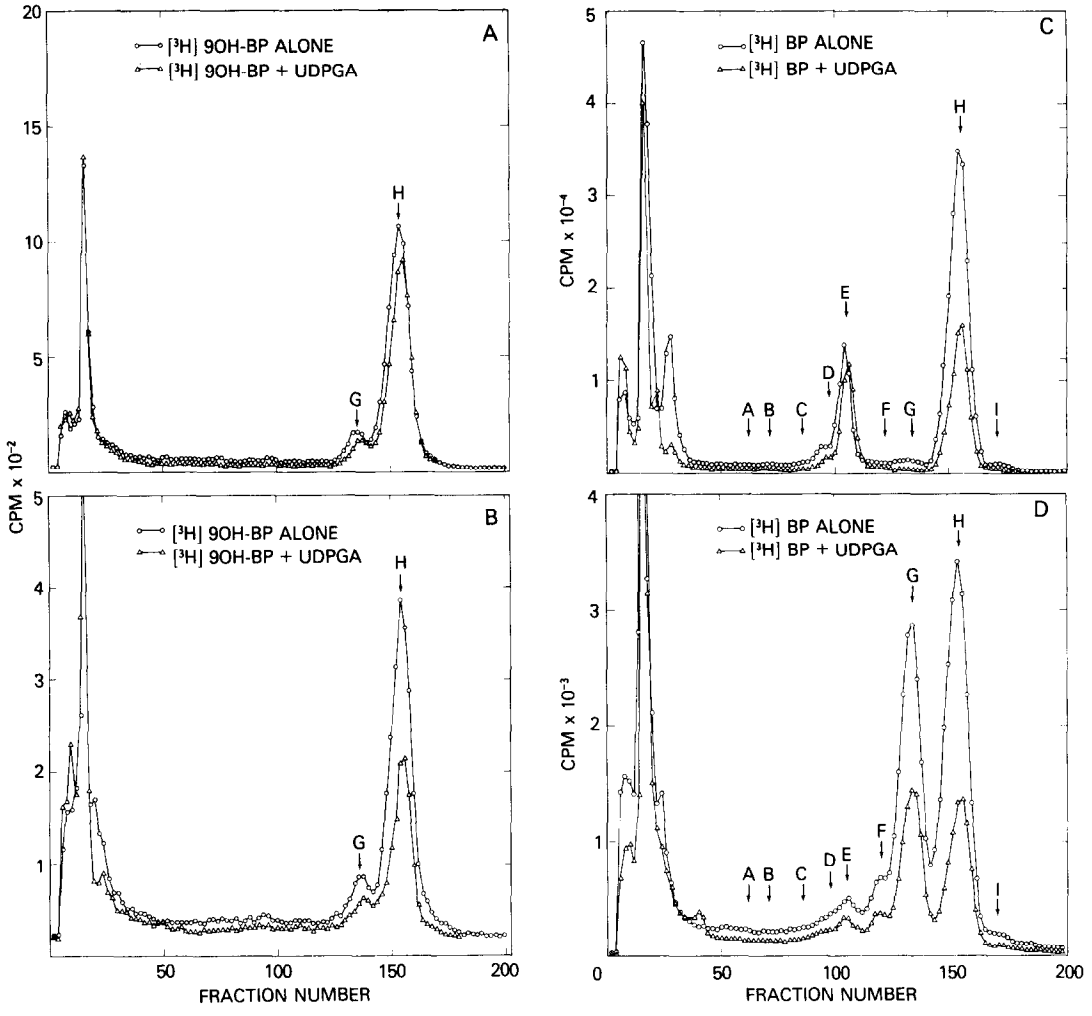

Fig. 4. Chromatogram by Sephadex LH20 of a DNA digest after binding [³H]9-OH-BP metabolites or [³H]BP metabolites during an *in vitro* incubation with liver microsomes from either MC-treated C57BL/6N or MC-treated DBA/2N mice. Panel A represents binding of [³H]9-OH-BP metabolites with and without 2 mM UDPGA using microsomes from C57BL/6N mice. Panel B represents binding of [³H]9-OH-BP metabolites with and without UDPGA using microsomes from DBA/2N mice. The specific activity of [³H]9-OH-BP was 7.8 Ci/m-mole and was present at 12 μM in the incubations. Panel C represents binding of [³H]BP metabolites with and without 2 mM UDPGA using microsomes from C57BL/6N mice. Panel D represents binding of [³H]BP metabolites with and without UDPGA using microsomes from DBA/2N mice. The specific activity of [³H]BP was 25 Ci/m-mole and was present at 4 μM. All incubations contained Triton X-100 at 0.1%, v/v. Microsomes from three mice per group were prepared as described in Materials and Methods. Eluate volume and radioactive determinations are described in the legend to Fig. 1. The arrows and letters at the bottom of the graphs represent the position of those peaks when incubations are carried out with [³H]BP.

3-OH-BP. If this compound does form, it should be possible to observe fluorescence bound to DNA which resembles that for 3-OH-pyrene and to isolate BP pentols by high pressure liquid chromatography after further metabolism of 3-OH-BP by cytochrome P_1-450. Such studies are in progress in order to delineate the nature of binding in peak E.

The lack of 3-OH-BP activation and DNA binding with microsomes from MC-treated DBA/2N mice and the prominent peaks with microsomes from MC-treated C57BL/6N mice are evidence that further metabolism of the phenol is catalyzed by an enzyme which is genetically regulated by the *Ah* locus [19]. Thus, mutagenicity [8] and DNA binding derived from 3-OH-BP are supported by enzymes prepared from MC-treated C57BL/6N mice known to contain primarily cytochrome P_1-450-dependent oxidase and not by enzymes from MC-treated DBA/2N mice, known to contain primarily cytochrome P-450-dependent activity [19, 20].

Previously, 3-OH-BP has been shown to undergo further metabolism in several systems [3–5], and this phenol was shown to bind to DNA in intact liver cells already induced by MC [4]. More recently, a study

indicated that hydrocarbon binding to DNA continues in intact liver cells after [^{3}H]BP is depleted, suggesting that further metabolism of primary metabolites of BP [21] can occur in cells under certain conditions.

In general, glucuronidation appears to decrease significantly all peaks except E; however, peaks D, F, G and H show an even greater decrease in peak heights. If the further metabolism of 3-OH-BP by cytochrome P_1-450-associated activity is analogous to BP metabolism this particular oxidase, then the various derivatives shown in Fig. 5 are possible products. Direct glucuronidation of 3-OH BP should affect all ligands which are primary products of 3-OH-BP, and indirect glucuronidation of 3-OH-BP at the level of an epoxide should result in a further decrease in binding activity such that some peaks, e.g. D, F, G and/or H may be affected, depending upon the substrate specificity of UDP-glucuronosyltransferase. The fact that peak E is not diminished by glucuronidation suggests that this binding is not due to a primary metabolite of 3-OH-BP, but that a secondary reaction is responsible and that the ligand is not a substrate for UDP-glucuronosyltransferase.

As seen in Fig. 1, it is not obvious why TCPE causes a decrease in peaks A, B, D, F, H and I and not in C and E. Perhaps inhibition of the epoxide hydratase enhances spontaneous rearrangement of the epoxides to phenols to a greater extent than DNA binding of the various epoxides, or the ligands generating peaks C and E may have higher affinity for DNA than ligands binding in the affected peaks. On the other hand, TCPE may not inhibit certain components of this system, for it has been proposed that the compound is not an effective inhibitor under certain conditions where the mono-oxygenase and epoxide hydratase activities are highly coupled, e.g. in MC-induced systems [22, 23]. The epoxide hydratase inhibitor, cyclohexeneoxide, was shown to decrease the level of peak E and to increase peaks G and H [12].

Further metabolism of 9-OH-BP by microsomes from either MC-treated C57BL/6N or DBA/2N mice to a form which binds DNA and emerges as peak H on Sephadex LH20 supports an earlier report [6] that peak H is due to metabolism of 9-OH-BP. Metabolism by microsomes from DBA/2N mice is consistent with cytochrome P-450-mediated metabolism of 9-OH-BP. In spite of the high level of DNA binding of activated 9-OH-BP, mutagenesis by this phenol is quite low. It is concluded that in the bacterial genome, at least, the 9-OH-BP metabolite does not readily bind to critical sites necessary to generate a histidine revertant.

With respect to 9-OH-BP metabolism, microsomes from the C57BL/6N and DBA/2N mice appear to differ in some fundamental property. The protection by UDPGA against DNA binding in peak H with microsomes from MC-treated DBA/2N mice (Fig. 4B) and the lack of protection by UDPGA with microsomes from MC-treated C57BL/6N mice (Fig. 4A) suggest that accessibility of the transferase active site in microsomes may be different between the two mouse strains. That is, coupling between the mono-oxygenase and transferase may exist in the C57BL/6N mice for 9-OH-BP, and this coupling is best effected if the phenol is formed in the membrane rather than added exogenously.

These studies support the view that either BP, 1-OH-BP or 3-OH-BP can undergo metabolism by cytochrome P_1-450-dependent mono-oxygenase activity. The extent to which an organ can further metabolism these phenols most likely relates to the amount and type of cytochrome P-450 present, as well as the competition of phenols with the usually higher concentration of BP for the mono-oxygenase active site. Furthermore, phenols must necessarily escape various conjugating activities in order to be further activated. It is not known whether conditions ever exist *in vivo* which favor phenolic metabolism. Cigarette smoke in rats, for example, greatly enhances lung mono-oxygenase activity [24] and 3-OH-BP formation [9], but it is questionable whether UDP-glucuronosyltransferase activity is increased [24, 25, 26], at least for the substrate 4-

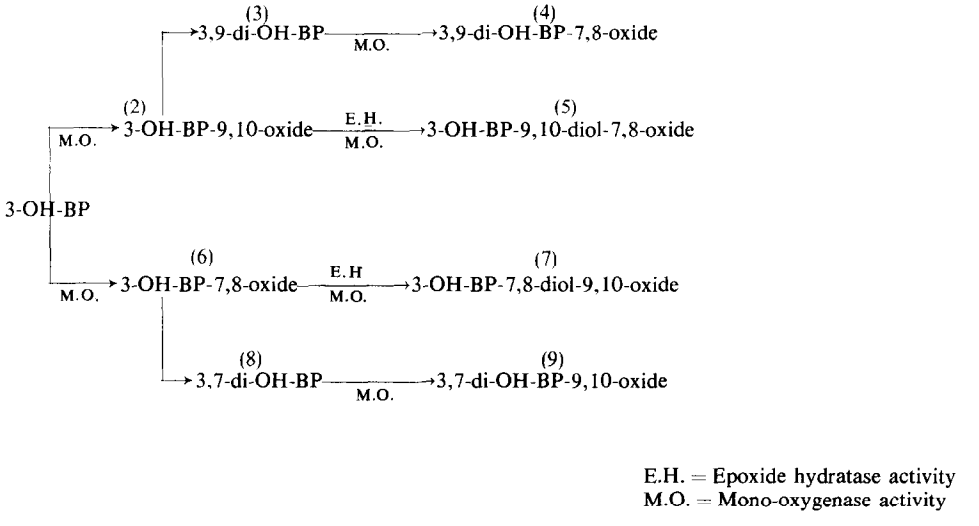

Fig. 5. Some of the possible metabolites of 3-OH-benzo[a]pyrene which may form by cytochrome-$P_1$450-dependent mono-oxygenase activity.

methylumbelliferone. It may, therefore, require special tissue conditions and/or extended incubations to observe recycling of the major phenols through the mono oxygenase system.

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REFERENCES

1. N. Nemoto and H. Gelboin, *Biochem. Pharmac.* **25**, 1221 (1976).
2. G. M. Cohen, S. M. Haws, B. P. Moore and J. W. Bridges, *Biochem. Pharmac.* **25**, 2561 (1976).
3. F. J. Wiebel, *Archs Biochem. Biophys.* **168**, 609 (1975).
4. H. Vadi, B. Jernström and S. Orrenius, in *Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis* (Eds. R. I. Freudenthal and P. W. Jones), pp. 45–61. Raven Press, New York (1976).
5. K. M. Rosie, Y. Cha, R. E. Talcott and J. B. Schenkman, *Chem. Biol. Interact.* **12**, 285 (1976).
6. H. W. S. King, M. H. Thompson and P. Brookes, *Int. J. Cancer* **18**, 339 (1976).
7. O. Pelkonen, A. R. Boobis, H. Yagi, D. Jerina and D. W. Nebert, *Molec. Pharmac.* **14**, 306 (1978).
8. I. S. Owens, G. M. Koteen and C. Legraverend, *Biochem. Pharmac.* **28**, 1615 (1979).
9. G. M. Cohen, P. Uotila, J. Kartiala, E. Suolinna, N. Simberg and O. Pelkonen, *Cancer Res.* **37**, 2147 (1977).
10. I. Owens, *J. biol. Chem.* **252**, 2827 (1977).
11. W. M. Baird and P. Brookes, *Cancer Res.* **33**, 2378 (1973).
12. A. R. Boobis, D. W. Nebert and O. Pelkonen, *Biochem. Pharmac.*, **28**, 111 (1979).
13. D. W. Nebert, A. R. Boobis, H. Yagi, D. M. Jerina and R. E. Kouri, in *Biological Reactive Intermediates* (Eds. D. J. Jollow, J. J. Kocsis, R. Synder and H. Vanio), pp. 125–45. Plenum Press, New York (1977).
14. R. F. Newbold and P. Brookes, *Nature, Lond.* **261**, 52 (1976).
15. P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, H. Yagi, O. Hernandez, D. M. Jerina and A. H. Conney, *Biochem. biophys. Res. Commun.* **68**, 1006 (1976).
16. P. Sims, P. L. Grover, A. Swaisland, K. Pal and A. Hewer, *Nature, Lond.* **252**, 326 (1974).
17. T. J. Slaga, W. M. Bracken, A. Viaje, W. Levin, H. Yagi, D. M. Jerina and A. H. Conney, *Cancer Res.* **37**, 4130 (1977).
18. G. M. Holder, H. Yagi, D. M. Jerina, W. Levin, A. Y. H. Lu and A. H. Conney, *Archs Biochem. Biophys.* **170**, 557 (1975).
19. S. S. Thorgeirsson and D. W. Nebert, *Adv. Cancer Res.* **25**, 149 (1977).
20. F. M. Goujon, D. W. Nebert and J. E. Geilen, *Molec. Pharmac.* **8**, 667 (1972).
21. M. D. Burke, H. Vadi, B. Jernström and S. Orrenius, *J. biol. Chem.* **252**, 6424 (1977).
22. W. E. Fahl, S. Nesnow and C. R. Jefcoate, *Archs Biochem. Biophys.* **181**, 649 (1977).
23. F. Oesch and J. Daly, *Biochem. biophys. Res. Commun.* **46**, 1713 (1972).
24. P. Uotila, O. Pelkonen and G. M. Cohen, *Cancer Res.* **37**, 2156 (1977).
25. P. Uotila, J. Hartiala and A. Aitio, *Toxic. appl. Pharmac.* **41**, 445 (1977).
26. P. Uotila and J. Marniema, *Biochem. Pharmac.* **13**, 259 (1976).