Metabolism and Macromolecular Binding of the Carcinogen Benzo(a)pyrene and Its Relatively Inert Isomer Benzo(e)pyrene by Hamster Embryo Cells

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ABSTRACT

The metabolism and macromolecular binding of the carcinogen benzo(a)pyrene [B(a)P] and its relatively inert structural isomer benzo(e)pyrene [B(e)P] have been studied in order to determine if a metabolic basis exists for their very different biological activities.

B(a)P and B(e)P are metabolized by hamster embryo cells to organic solvent-soluble and water-soluble metabolites. Significant quantitative and qualitative differences are observed in the nature of the metabolites from the different hydrocarbons and the distribution of these metabolites between the cytoplasm and the extracellular medium. B(a)P is metabolized more extensively than B(e)P to both ethyl acetate-soluble and water-soluble metabolites. The major ethyl acetate-soluble metabolite found in the cytoplasm is 9-hydroxybenzo(a)pyrene and 3-hydroxybenzo(a)pyrene (40.7 and 16.5% of metabolites in cytoplasm, respectively) and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (66.7% of total metabolites in medium). Rapid excretion of this product from the cells is indicative of its observed subsequent biological inactivity. Smaller amounts of 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were found, but more of this dihydridiol than the 9,10-dihydridiol was retained intracellularly, where it could be metabolized to an active diol-epoxide. The major metabolites found in the cytoplasm are 9-hydroxybenzo(a)pyrene and 3-hydroxybenzo(a)pyrene (40.7 and 16.5% of metabolites in cytoplasm, respectively) and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (18.4% of metabolites in cytoplasm) with smaller amounts of 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene (9.2% of metabolites in cytoplasm). The low amounts of phenols in the medium are due to their relatively rapid removal as water-soluble, glucuronic conjugates.

The major ethyl acetate-soluble metabolite formed in the extracellular medium, after 24 hr of culture with B(e)P (4 μM), is the K-region dihydridiol, 4,5-dihydro-4,5-dihydroxybenzo(e)pyrene (69.6% of organic solvent-soluble metabolites in medium), with only small amounts of monohydroxybenzo(e)pyrenes (21.9% of organic solvent-soluble metabolites in medium) being formed. Most of the monohydroxybenzo(e)pyrenes formed and significant amounts of 4,5-dihydro-4,5-dihydroxybenzo(e)pyrene are metabolized to their respective water-soluble glucuronic conjugates. The much higher binding of B(a)P than B(e)P to both DNA and RNA of isolated nuclei from cells treated with the parent hydrocarbons reflects the higher biological activity of B(a)P. The metabolic formation of 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene from B(a)P and the apparent lack of formation of 9,10-dihydro-9,10-dihydroxybenzo(e)pyrene from B(e)P suggest a metabolic basis for the relative biological activities of the parent hydrocarbons. While B(a)P forms a dihydridiol which may be metabolized on an adjacent olefinic double bond to a potentially reactive diol-epoxide adjacent to the bay-region, it is apparent that B(e)P does not enzymatically favor this mechanism and forms an inert K-region dihydridiol.

INTRODUCTION

PAHs are ubiquitous environmental contaminants, many of which are capable of inducing tumors in a wide variety of species and tissues as well as causing mutagenesis and malignant transformation of cells in culture (6, 13). Since the synthesis of the first pure chemical carcinogen in this series in 1930 by Kenway and his colleagues, numerous other PAHs have been synthesized in order to determine which structural features are of importance in determining their potential carcinogenicity (22). Since metabolic activation of PAHs is required for their carcinogenic, mutagenic, cytotoxic, and cell transformation activities, much attention has been focused on determining the nature of the reactive metabolites (14, 29). PAHs are metabolized to a wide variety of organic solvent-soluble metabolites, including epoxides, dihydrodiols, quinones, and phenols and their sulfate ester conjugates, as well as to water-soluble glucuronic and glutathione conjugates (5, 14, 24, 29). Recent evidence strongly implicates the further metabolism of certain non-K-region dihydrodiols adjacent to the bay region in the metabolic activation of PAH (30), and it has been suggested that bay-region epoxides of dihydrodiols on angular benzo rings may be the ultimate carcinogenic forms of PAH (15). Perturbational molecular orbital calculations suggest formation of benzylic carbonium ions is easiest when the carbonium ion is part of a bay region of the PAH (15, 16). Such theoretical calculations predict the relative ease of formation of such carbonium ions from 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide or from either of the 2 symmetrical bay regions of B(e)P, e.g., 9,10-dihydro-9,10-dihydroxybenzo(e)pyrene 11,12-oxide (16), and suggest their attraction to cellular nucleophiles.

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In contrast to B(a)P, which is both a potent carcinogen and mutagen (6, 13), B(e)P is inactive or only weakly active when applied either as a tumor initiator on mouse skin (23, 32) or as a mutagen to Chinese hamster V79 cells (9). Cultured hamster embryo cells readily undergo malignant transformation after treatment with PAH (10). Thus, to gain further insight into the markedly different biological activities of these PAH, we have studied the metabolism and binding of the potent carcinogen B(a)P and its relatively inert isomer B(e)P by these cells. Such studies in intact cells containing a full complement of both oxidative and conjugating enzymes should reflect the in vivo situation more accurately than do cell homogenates or microsomes. The balance of these oxidative and conjugating enzymes determines how much of a certain metabolite may be available for conversion to reactive intermediates. Thus, it is important to study both the organic solvent-soluble metabolites (primarily the oxidative metabolites) and the water-soluble metabolites (primarily the conjugated metabolites) in order to determine the relative appearance and removal of the metabolic intermediates under strict physiological conditions. As an additional measure of their potential biological activities, we have determined the binding of these metabolically activated PAH to cellular macromolecules.

B(a)P, but not B(e)P, is metabolized by HEF to a non-K-region dihydrodiol with an isolated olefinic double bond adjacent to the bay region (Chart 1), and B(a)P is converted to a much greater extent than is B(e)P to metabolites which bind to nuclear DNA, RNA, and protein. B(a)P is also converted more readily than is B(e)P to both ethyl acetate-soluble metabolites and water-soluble glucuronide conjugates. Significant differences in the concentrations of intra- and extracellular metabolites were observed. Thus, the apparent inability of HEF to form B(e)P-9,10-dihydrodiol suggests a metabolic basis for the lack of carcinogenicity of B(e)P.

**MATERIALS AND METHODS**

**Chemicals.** Tritium-labeled B(a)P (Amersham; ≥25 Ci/mmol) was diluted with unlabeled B(a)P (Aldrich; gold label) to a specific activity of 3.15 Ci/mmol, dissolved in DMSO at 0.9 mM, and stored at −20°C. A stock solution of tritium-labeled B(e)P (Midwest Research Institute, 5.02 Ci/mmol) was prepared and stored similarly, but at a final specific activity of 2.51 Ci/mmol. Both hydrocarbons were routinely checked by HPLC and were found to exhibit >95% radiochemical purity. Uptake of guanidine·HCl was obtained from Schwarz/Mann (Orangeburg, N. Y.), and density gradient grade Cs2SO4 was from Atomergic Chemetics (Carle Place, N. Y.). Glucuronic acid (a preparation of bovine β-glucuronidase containing very low activity of aryl sulfatase) was obtained from Sigma Chemical Co. (St. Louis, Mo.).

**Cells and Labeling.** HEF were grown in 100-mm dishes as described previously (27). All experiments were performed on confluent tertiary cultures which had been plated at a 1:2 dilution 3 days prior to the beginning of the labeling period. Medium was replaced with 10 ml of fresh medium containing labeled PAH at a final concentration of 4 μM, and incubation was continued for 24 hr. The final concentration of DMSO was 0.44%, a level which gives no measurable cytotoxicity at 24 hr.

**Distribution of PAH Metabolites.** The general plan of the experiments is outlined in Chart 2. The overall distribution of organic solvent-soluble and water-soluble radioactivity among the extracellular medium, the cytoplasm, and the nucleus was determined, and the profiles of organic solvent-soluble metabolites were determined by HPLC. Extracellular water-soluble glucuronide conjugates in the medium were determined by treatments with β-glucuronidase followed by organic solvent to extract the released hydrocarbon moiety, which was identified by cochromatography with authentic standards. Nuclear macromolecules were separated by density, and the amount of bound hydrocarbon was determined by scintillation spectrometry. Determinations were carried out 3 to 5 times with cells prepared from different pregnant females. Although there was variability among determinations in the overall extent of metabolism, the relative ratios of the various metabolites were the same. Accordingly, data in the tables are from one representative experiment.

**Analysis of Extracellular Metabolites.** After 24-hr incubation of PAH with cells, the extracellular medium was aspirated, the cells were gently washed with 5 ml phosphate-buffered saline, and the wash and medium were combined. Organic solvent-soluble metabolites were prepared by twice extracting the medium with 2.5 volumes ethyl acetate. At this and subsequent points throughout the procedure, triplicate aliquots were removed, and their radioactivity was determined by liquid scintillation counting in Aquasol (New England Nuclear, Boston, Mass.), using a Searle Mark III equipped with dpm capability. The distribution of ethyl acetate-soluble metabolites within the medium (Chart 2, Fraction 1) was analyzed by HPLC with a Spectra Physics 3500-B chromatograph fitted with a 1-m Permaprep ODS column (DuPont Instruments, Wilmington, Dela.) and eluted with a linear 30 to 70% methanol:water gradient as described previously (26).

The aqueous phase was reextracted twice with 2.5 volumes ethyl acetate. Metabolites which had been conjugated to glucuronic acid were released by a 16-hr treatment at 37°C with β-glucuronidase as described previously (3). In some cases, α-saccharic acid-1,4-lactone (20 mM) was added prior to the incubation to specifically inhibit β-glucuronidase. The hydroxylated metabolites released from the glucuronide conjugates were extracted twice with 2.5 volumes ethyl acetate and analyzed by HPLC (Chart 2, Fraction 7).

**Subcellular Fractionation.** Since preliminary experiments showed that these cells were refractory to lysis by a variety of standard techniques, the following procedure was devised. The washed cell monolayers were harvested after a 15-min incubation at 37°C with 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline; washed twice at 0–4°C with buffer containing 10 mM NaCl, 10 mM Tris (pH 7.0), and 1.5 mM MgCl2; and resuspended in the same buffer. The solution was adjusted to 0.5% Triton X-100 and 0.5% sodium deoxycholate, and lysis was completed by 10 to 15 strokes of a motor-driven Potter-Elvehjem homogenizer. Nuclei were deposited by centrifugation at 1000 × g. Cytoplasmic organic solvent-soluble metabolites were determined by ethyl acetate extraction of the supernatant followed by HPLC (Chart 2, Fraction 3).

The nuclei were freed of residual cytoplasm by repeating the homogenization step. The final pellet was dissolved in 2.0 ml 6 M guanidine·HCl-10 mM EDTA (pH 7.0), and lysis was completed by sonication. The lysate was extracted 3 times with 3
volumes ethyl acetate, and organic solvent-soluble material was analyzed by HPLC (Chart 2, Fraction 5). The aqueous phase was adjusted to 2.2 ml with the same solution, layered over a 2.8-ml cushion of 2.2 M Cs$_2$SO$_4$-10 mM EDTA (pH 7.0)-9% DMSO in a 0.5- x 2-inch polyallomer tube, and centrifuged at 35,000 rpm for 40 ± 2 hr in a SW 50.1 swinging bucket rotor maintained at 20°. A detailed analysis of this separation method is presented elsewhere (19). The Cs$_2$SO$_4$ gradients were fractionated, and aliquots were used to measure radioactivity and to determine A$_{260}$. Further aliquots were used to determine density by weighing and to determine protein by the fluorescamine method (Roche Diagnostics, Nutley, N. J.); bovine serum albumin (Sigma) was used to construct the standard curves.

RESULTS

B(a)P Metabolism

**Ethyl Acetate-soluble Metabolites.** After 24-hr culture of HEF with B(a)P (4 μM), both organic solvent-soluble radioactivity (32.8% of the original radioactivity, where the original radioactivity represents the total amount of radioactivity added to the cells at the beginning of the experiment) and water-soluble radioactivity (54.5% of the original radioactivity) were determined (Table 1). As in previous studies from this laboratory (25), B(a)P was metabolized to ethyl acetate-soluble metabolites which cochromatographed with B(a)P-9,10-dihydriodiol, B(a)P-7,8-dihydriodiol, quinones, 9-OH-B(a)P, and 3-OH-B(a)P. However, significant differences were observed in the relative amounts of different metabolites found in the extracellular medium and in the cytosol (Chart 3). Particularly striking was the appearance of most of the B(a)P-9,10-dihydriodiol in the extracellular medium (Chart 3A). Relative to B(a)P-9,10-dihydriodiol, significantly more B(a)P-7,8-dihydriodiol and the monohydroxybenzo(a)pyrenes, 3-OH-B(a)P and 9-OH-B(a)P, were found intracellularly (Chart 3A). The relative retention of B(a)P-7,8-dihydriodiol intracellularly strengthens the argument that it may be a major proximate carcinogen in HEF in tissue culture, since its ability to remain intracellular would increase its probability of being metabolized to the reactive diol-epoxide which interacts with critical target sites. Also, the complete removal of B(a)P-9,10-dihydriodiol by the cell into the extracellular medium (Chart 3B; Table 2) would mean that most of this metabolite would be unavailable for further metabolism to its diol-epoxide and may explain the much lower biological activity of this dihydriodiol.

**Water-soluble Metabolites.** B(a)P was also converted extensively to water-soluble radioactivity in the extracellular medium (54.5% of the original radioactivity). When the metabolites associated with the water-soluble radioactivity were hydrolyzed with β-glucuronidase, 20.7% of the original radioactivity was converted to ethyl acetate-soluble radioactivity. When this ethyl acetate-soluble radioactivity was concentrated and separated by HPLC, most of the radioactivity cochromatographed with 9-OH-B(a)P and 3-OH-B(a)P (Chart 4). After hydrolysis of the metabolites associated with the water-soluble radioactivity with β-glucuronylase and 20 mm α-saccharic acid-1,4-lactone (α-glucuronidase), most of the radioactivity was converted to water-soluble radioactivity which cochromatographed with 9-OH-B(a)P and 3-OH-B(a)P (Chart 4). After hydrolysis of the metabolites associated with the water-soluble radioactivity with β-glucuronidase and 20 mm α-saccharic acid-1,4-lactone (α-glucuronidase), most of the radioactivity was converted to water-soluble radioactivity which cochromatographed with 9-OH-B(a)P and 3-OH-B(a)P (Chart 4).
specific inhibitor of β-glucuronidase) and extraction with ethyl acetate, only very small amounts of radioactivity chromatographed with monohydroxybenzo(a)pyrenes (results not shown). Most of the radioactivity chromatographed with an unidentified compound(s) eluting near the breakthrough volume and was probably due to partial extraction of the glucuronide conjugates into the organic solvent at low pH.5,6 The failure of β-glucuronidase to release monohydroxybenzo(a)pyrenes in the presence of the enzyme inhibitor was further evidence of the presence of water-soluble glucuronide conjugates in the medium. Thus, the glucuronide conjugates of 9-OH-B(a)P and 3-OH-B(a)P represent major water-soluble metabolites formed by HEF. This confirms and extends previous work from this laboratory showing the presence of only small amounts of phenolic metabolites in intact cell culture (25, 27).

**B(e)P Metabolism**

**Ethyl Acetate-soluble Metabolites.** After 24-hr culture of HEF with B(e)P (4 μM), both organic solvent-soluble radioactivity (71.7% of the original radioactivity) and water-soluble radioactivity (7.8% of the original radioactivity) were found in the extracellular medium (Table 1). Most of the ethyl acetate-soluble radioactivity in the medium was associated with unmetabolized B(e)P (Table 1; Chart 5). In the medium, B(e)P was metabolized to ethyl acetate-soluble metabolites which chromatographed with B(e)P-4,5-dihydrodiol (D2), quinones, and monohydroxybenzo(e)pyrenes [designated as P1 and P2 (Chart 5)], 4-Hydroxybenzo(e)pyrene, 9-hydroxybenzo(e)pyrene, and 10-hydroxybenzo(e)pyrene chromatograph in the regions designated P1 and P2, using the experimental conditions of the present study. It is not possible at present to exclude the presence in these bands of either other monohydroxybenzo(e)pyrenes or uncharacterized metabolites. However, the major ethyl acetate-soluble metabolite in the medium was the

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Metabolism of B(a)P and B(e)P in Cells

Contamination (less than 1%). Since unbound PAH was extracted with ethyl acetate prior to centrifugation, determination of the mass of macromolecules and of the radioactivity associated with each band gives a direct measure of the binding of the PAH to each class of macromolecules. Chart 7 shows that all classes of nuclear macromolecules bind B(a)P, since significant amounts of radioactivity are found in each of the macromolecular bands. The specific activities attained by the RNA and DNA (56.6 and 40.4 pmol/mg, respectively) were significantly lower than the specific activity of the nuclear protein (147.4 pmol/mg); this is reflected in Chart 7A by a 5-fold difference in the scale of the ordinates for the upper and lower regions of the gradient. In contrast to this, binding of B(e)P was much lower, as is shown in Chart 7B. The specific activities of the RNA and DNA (0.28 and 0.24 pmol/mg, respectively) were over 2 orders of magnitude lower than those obtained with B(a)P and were, in fact, at the limit of detection of this technique (radioactivity in the DNA band was approximately twice that of

Macromolecular Binding

To investigate the binding of the PAH to macromolecules, nuclei from cells incubated with [3H]B(a)P or [3H]B(e)P were lysed in 6 M guanidine-HCl and centrifuged to isopyknic equilibrium in Cs2SO4. As detailed elsewhere (19), RNA, DNA, and protein band at their respective densities with very little cross-

Chart 4. HPLC analysis of water-soluble metabolites of B(e)P rendered organic solvent soluble by treatment with β-glucuronidase.

Chart 5. HPLC analysis of organic solvent-soluble metabolites of B(e)P found (A) in the cytoplasm of HEF or (B) in the extracellular medium.


Chart 7. Separation of [3H]PAH-labeled nuclear macromolecules by isopyknic sedimentation.
background). Binding to protein (1.96 pmol/mg) was easily demonstrable but was also about 2 orders of magnitude lower than that obtained with B(a)P.

**DISCUSSION**

In this study, we have investigated the metabolism to both organic solvent-soluble and water-soluble metabolites and the macromolecular binding of B(a)P and B(e)P. It was believed that such a study utilizing a potent carcinogen [B(a)P] and its relatively inert isomer [B(e)P] might both elucidate the important reactive species and help to clarify the stereoechemical requirement that differentiates the carcinogenic from the noncarcinogenic isomer. The decision to use an intact cell system for metabolic comparison was based on the premise that the parent hydrocarbons as well as their oxygenated intermediates would have different physical and chemical properties that would affect their affinities for the different enzymes involved in their metabolism. The presence of the entire metabolic machinery, in particular the oxidative and conjugating enzymes, should yield a more physiologically accurate biochemical description of the total activation and detoxification schemes acting in concert.

Following incubation of HEF with B(a)P (4 μm) for 24 hr, the major organic solvent-soluble metabolite in the medium was B(a)P-9,10-dihydrodiol with smaller amounts of B(a)P-7,8-dihydrodiol (Chart 3B; Table 2). Only small amounts of monohydroxybenzo(a)pyrenes were detected, in agreement with our previous studies (27), and a large fraction of these monohydroxybenzo(a)-pyrenes were retained intracellularly (Chart 3A). Major phenol peaks in disrupted cell systems simply reflect the absence of the necessary cofactors and intracellular orientation required for the conjugating enzymes, which are removed or altered during the standard microsomal preparations. Although the monohydroxybenzo(a)pyrenes were the major intracellular organic solvent-soluble metabolites (Chart 3A), constituting 57.2% of intracellular metabolites, they constituted only a relatively small percentage (3.6%) of the total, intracellular plus extracellular, organic solvent-soluble metabolites because only a very small percentage of the total radioactivity was associated with the cytoplasm. Similar marked differences in the intracellular and extracellular distribution of metabolites of B(a)P have also been observed in previous studies with isolated rat hepatocytes (17). It seems unlikely that differences in polarity between B(a)P-7,8-dihydrodiol and B(a)P-9,10-dihydrodiol would be sufficient to explain such marked differences in their distribution. The differences may be related to the relative affinities of the dihydrodiols for lipid membranes or intracellular binding proteins.

The results of incubating the extracellular medium with β-glucuronidase indicated that major water-soluble metabolites were the glucuronide conjugates of 9-OH-B(a)P and 3-OH-B(a)P (Chart 4), in agreement with the results of Baird et al. (1). Small amounts of quinones were also observed (Chart 4), but these were most probably due to oxidation of the monohydroxybenzo(a)pyrene rather than to conjugation of the quinones, as quinones were not substrates for a rat liver microsomal system fortified with UDP-glucuronic acid (21). No detectable glucuronide conjugates of dihydrodiols of B(a)P were observed after hydrolysis with β-glucuronidase (Chart 4).

Thus, when one considers the overall metabolic fate of B(a)P in HEF, including both organic solvent-soluble and water-soluble metabolites, the major metabolites formed are monohydroxybenzo(a)pyrenes (Table 2). This is also clearly seen in all microsomal systems studied (27), where in the absence of UDP-glucuronic acid the monohydroxybenzo(a)pyrenes are not further metabolized to their corresponding glucuronide conjugates. Also of interest in the present study was the formation of B(a)P-9,10-dihydrodiol and B(a)P-7,8-dihydrodiol but no detectable K-region 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene was observed. Many studies have shown that B(a)P-7,8-dihydrodiol may be further metabolized to reactive diol-epoxides (30, 31, 34) and that such metabolites are the metabolites bound to DNA and/or RNA in hamster embryo cells (2, 12, 30) mouse skin (8, 18), and human bronchus (8, 33) treated with B(a)P. Thus, HEF cells susceptible to malignant transformation by B(a)P (10) can metabolize B(a)P to 7,8-dihydrodiol which may be further metabolized to the metabolites which are bound to DNA (Chart 8). Although in the present investigation the nature of the adducts bound to DNA was not studied, other work has shown that the major adduct formed in HEF from B(a)P is the 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide bound to DNA (7, 23, 24).

In contrast to B(a)P, after 24 hr of culture with HEF, B(e)P (4 μm) was metabolized primarily to organic solvent-soluble metabolites with only a relatively small percentage (7.8% of original radioactivity) of water-soluble metabolites formed. The major organic solvent-soluble metabolite found in the medium from B(e)P was the K-region dihydrodiol, B(e)P-4,5-dihydrodiol (Chart 5B), with little or no K-region dihydrodiol, B(e)P-9,10-dihydrodiol, detected (Chart 5B). These results are in agreement with an earlier study of B(e)P metabolism in mouse embryo cells when B(e)P-4,5-dihydrodiol was found as the major ethyl acetate-soluble metabolite (28). It is of interest that, while the intermediate in the formation of the dihydrodiol, i.e., 4,5-dihydrobenzo(e)pyrene 4,5-oxide, should be an effective alkylating agent, it is such a good substrate for epoxide hydrolase that it is very readily converted to the dihydrodiol. In addition, the diol is either rapidly excreted from the cells (Chart 5; Table 2) or converted to its glucuronide conjugate (Chart 6), thus minimizing the possibility of further metabolism in the non-K-region area. Only small amounts of monohydroxybenzo(e)pyrenes were found as either intracellular or extracellular organic solvent-soluble metabolites (Chart 5). This was apparently due to the fact that, once formed, some (P) monohydroxybenzo(e)pyrenes were conjugated with UDP-glucuronic acid to their respective glucuronide conjugates (Chart 6). This may be due to the fact that some monohydroxybenzo(e)pyrenes are much better substrates for UDP-glucuronosyltransferase than others; however, all metabolically formed monohydroxybenzo(a)pyrenes are fairly good substrates for this enzyme(s) (21). Thus, it is also possible that P contains other uncharacterized nonphenolic metabolites. Significant amounts of B(e)P-4,5-dihydrodiol were also converted to its glucuronide conjugate; however, no detectable glucuronide conjugate of B(e)P-9,10-dihydrodiol was observed (Chart 6). These results suggest that the relative rate of conjugation of metabolites of B(e)P by HEF is monohydroxybenzo(e)pyrenes ∼ dihydrodiols. Similar results have been observed with the metabolites of B(a)P using short-term organ culture of rodent lung (4) or trachea (20) or using a rat liver microsomal system fortified with UDP-glucuronic acid (21). A summary of
the distribution of the major metabolites formed in HEF from both B(a)P and B(e)P is shown in Chart 8.

Particularly striking was the absence in the present study of any evidence for the formation of B(e)P-9,10-dihydrodiol as either an organic solvent-soluble or water-soluble metabolite (Charts 5 and 6). This is in agreement with another study from this laboratory, in which incubation of B(e)P with hepatic microsomes from either control, phenobarbital-pretreated, or 3-methylcholanthrene-pretreated rats resulted in the formation of significant amounts of B(e)P-4,5-dihydrodiol and monohydroxybenzo(e)pyrenes but little or no B(e)P-9,10-dihydrodiol.7

In the present study, we have correlated the differences in non-K-region diol metabolism with binding to nuclear macromolecules. We have chosen to work with 24-hr incubations in culture, since preliminary kinetic experiments (1.5- to 46-hr incubations) demonstrated that the specific activity attained by nRNA and DNA reached a plateau after 18 to 24 hr of incubation with [3H]B(a)P.5 This is in agreement with the data of Ivanovic et al. (12) for binding to DNA in primary cultures of hamster embryo cells. The concentration of PAH that we have used is below that needed to give maximal binding of [3H]B(a)P (approximately 8 μM)5 but was chosen to minimize cytotoxicity. For all 3 major classes of nuclear macromolecules, we find at least a 100-fold difference in the binding of [3H]B(a)P and [3H]B(e)P. This is in good agreement with the results of previous studies of binding to total cellular macromolecules in primary cultures of mouse embryo fibroblasts (7). In another study, a good correlation was observed between carcinogenicity of PAH and binding to DNA but not to RNA or protein (11).

Thus, these results suggest a metabolic basis for the relative lack of carcinogenic and mutagenic activity of B(e)P. Rodent embryonic fibroblasts and hepatic microsomes cannot form significant amounts of the non-K-region dihydrodiol, B(e)P-9,10-dihydrodiol. The reason why little or no B(e)P-9,10-dihydrodiol is formed is not clear but may be due to physical or chemical factors such as membrane solubility or stereochemical requirements of the active site of the enzyme. The bay-region theory of PAH carcinogenesis predicts that carbonium ion formation from 9,10-dihydro-9,10-dihydroxybenzo(e)pyrene 11,12-oxide, if formed, would be energetically favorable (16). Thus, the inability of HEF and microsomes to form B(e)P-9,10-dihydrodiol, the precursor of its potentially highly reactive diol-epoxide, would explain the relative inertness of B(e)P in several biological systems (9, 23, 32).

As the subtle biochemical interactions of the various carcinogen intermediates become clarified, it becomes apparent that susceptibility and resistance to malignant transformation are based on a complex set of both chemical and physical parameters. Since there is no unique intermediate produced by the monoxygenases in susceptible tissues and since the highly resistant liver enzymes form the highest concentration of proximate carcinogen, it is becoming clear that metabolism kinetics, membrane interaction, and the role of nuclear metabolism help dictate the movements of the carcinogen into and through the metabolic machinery of the cell. Physicochemical calculations that predict biochemical activity cannot utilize all the necessary descriptors, since they are not all known. Therefore, as is the case with B(e)P, the present calculations cannot accurately predict biochemical or carcinogenic activity. Determining and clarifying intracellular physical and chemical parameters will help to correctly assess the risk of any given cell or tissue to potential chemical carcinogens.

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