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Formation and Persistence of Benzo(a)pyrene Metabolite–DNA Adducts

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Benzo(a)pyrene (BP) and other polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants and are suspected to be carcinogenic in man. The *in vivo* formation of BP metabolite–DNA adducts has been characterized in a variety of target and nontarget tissues of mice and rabbits. Tissues included were lung, liver, forestomach, colon, kidney, muscle, and brain. The major adduct identified in each tissue was the (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-BP (BPDEI)–deoxyguanosine adduct. A 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-BP (BPDEII)–deoxyguanosine adduct, a (−)-BPDEI–deoxyguanosine adduct, and an unidentified adduct were also observed. The adduct levels are unexpectedly similar in all the tissues examined from the same BP-treated animal. For example, the BPDEI–DNA adduct levels in muscle and brain of mice were approximately 50% of those in lung and liver at each oral BP dose used. We have also examined adduct levels formed *in vivo* in several cell types of lung and liver. Macrophages, type II cells, and Clara cells from lung and hepatocytes and nonpparenchymal cells from liver were isolated from BP-treated rabbits. BPDEI-deoxyguanosine adduct was observed in each cell type and, moreover, the levels were similar in various cell types. These and previous results strongly suggest that DNA in many human tissues is continuously damaged from known exposure of humans to BP and other PAH. Moreover, DNA adducts formed from BP are persistent in lung and brain. The persistence of adducts in cell types that have slow turnover rates could result in significant accumulation of adducts from long-term exposure to low levels of BP. BPDEI–DNA adducts and other bulky adducts are known to inhibit replication and transcription in *in vitro* systems. Even if environmental exposure to PAH is too low to induce neoplasia, the accumulation of DNA adducts may produce aberrations in transcripts of genetic information in various cells and lead to other toxic effects. Thus, further elucidation of the mechanism by which BP(PAH) metabolites bind to DNA of specific cell types and of the cell-selective repair of the adducts should enhance our understanding of the potential health risk from exposure to this class of environmental pollutants.

Introduction

Benzo(a)pyrene (BP) and other polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants produced mainly by industrial and transportational sources (*1,2*). Since these compounds are carcinogenic in laboratory animals and since human exposure to these compounds in food, air and water has been increasing, these chemicals pose a threat as potential human carcinogens.

Laboratory studies as well as epidemiological studies support this idea (*3–12*). PAH induce tumors in various tissues of animal species, regardless of route of administration. One model of BP-induced neoplasia is the intratracheal administration to hamsters of BP adsorbed to particulate matter such as Fe_2O_3 (*13*). This causes respiratory tract tumors in the hamster and is relevant to human exposure, since atmosphere BP is also adsorbed to particulates. Epidemiological studies on health effects of PAH, though difficult to obtain because of widespread but low or varied exposure, have shown that gas production workers and coal tar pitch workers do have higher incidences of lung cancer in addition to skin and bladder cancers (*14–18*). Living in areas with high pollution increases lung cancer incidence (*19–21*). Studies have also shown that cigarette smoking is the major cause of lung cancer (*20,22*). All of these conditions result in exposure to PAH as well as other classes of carcinogens.

The mechanism(s) by which BP and other PAH induce neoplasia are quite complicated. In the body, these lipophilic compounds are oxidatively metabolized to epoxides, phenols, etc. (Fig. 1), by the cytochrome P-450-dependent monooxygenase and epoxide hydrase. These metabolites are then conjugated to more hydrophillic metabolites by various conjugating enzyme systems, presumably for excretion. Most of the metabolites are excreted but sometimes the enzyme system will convert the parent compound to a more reactive form that can bind extensively and covalently to cellular macromolecules (*23–26*). This covalent binding of reactive metabolites of PAH to DNA appears to be an essential first step in PAH-induced neoplasia (*23–28*). If the cell cannot repair the damaged DNA before synthesis occurs,

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then replication on the damaged template can result in a mutation.

Considerable work has been done to elucidate the structure(s) of the reactive metabolite(s) of PAH which bind to DNA in the intact cell. Many studies have shown that certain diol epoxide derivatives of BP are the predominant metabolites which bind to DNA *in vivo* (*29–32*). These diol epoxide derivatives of BP are formed by the sequential enzymatic action of the cytochrome P-450-dependent monooxygenase system and epoxide hydrase (Fig. 2) (*23*). The (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEI) is the major enzymatic metabolite of (−)-*trans*-7,8-diol (*33*), although some (−)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEII) is also formed. BPDEI and BPDEII predominantly bind to the 2-amino group of guanine residues (Fig. 3). These diol epoxides can also bind to the N-7 of guanine (*34*), adenine (*35–38*) and cytidine (*38*) and to phosphate residues (*39,40*). It should be mentioned that other metabolites of BP can also bind to DNA, especially under *in vitro* conditions; however, in target tissues for BP-induced neoplasia, BPDEI and BPDEII are the predominantly characterized adducts (*41*). Studies have also shown that other PAH, such as 7-methylbenzanthracene (*42,43*), benzanthracene (*44,45*), chrysene (*46,47*), and 5-methylchrysene (*48*), dibenzanthracene (*49*), 3-methylcholanthrene (3-MC) (*50,51*), and dimethylbenzanthracene (DMBA) (*51–55*) are also converted to very reactive diol epoxides like BPDEI and BPDEII that bind to DNA *in vivo*. All of these diol epoxides have a similar structure involving an epoxide ring in the bay region and, therefore, have been termed "bay region diol-epoxides" by Jerina and Daly (*56*).

It is known that bay region diol epoxides are mutagenic (*57–64*), have transforming activity in mammalian cells (*27,61*), are carcinogenic in newborn mice (*60,65–67*), and are initiators in the cells of mouse skin (*42,58–60,65–68*). The mutagenicity and carcinogenicity, added to the fact that these adducts are formed *in vivo* leads to the hypothesis that these adducts play a role in the initiation of PAH-induced cancer. This report will review the *in vivo* binding and persistence of PAH metabolite–DNA adducts and their relationship to PAH-induced neoplasia.

In Vivo Formation of PAH Metabolite–DNA Adducts

Several PAH have been examined for their formation of DNA adducts *in vivo*. In this section, the *in vivo* DNA binding of four PAH: BP, 3-MC, DMBA, and 15,16-dihydro-11-methylcyclopenta[a]phenanthrene-17-one (11-methyl ketone) will be discussed. BP, the most extensively studied PAH, will be the topic of most of this discussion.

BP metabolites are known to bind to DNA in numerous tissues of different animal species. Stowers and

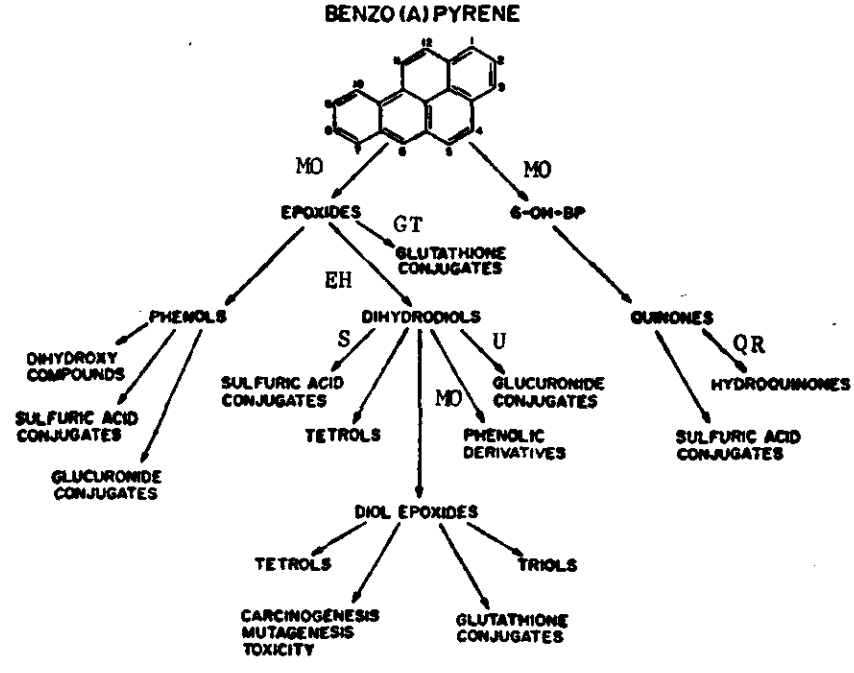

FIGURE 1. The major pathways involved in the metabolism of benzo(a)pyrene. Initial oxidations of BP-catalyzed by monooxygenases (MO) may result in 6-OH-BP or epoxide formation. 6-OH-BP is oxidized to form quinones. Epoxides may spontaneously rearrange to form phenols or form glutathione conjugates, or be further oxidized by epoxide hydrase (EH) to form dihydrodiols. The dihydrodiols may then be activated to the carcinogenic form, the diol epoxide, by the MO. Many of these metabolites may be enzymatically converted to glucuronide sulfate or glutathione conjugates as indicated in the figure. Enzyme notation is as follows: MO, monooxygenase; EH, epoxide hydrase; QR, quinone reductase; U, glucuronyltransferase; GT, glutathione transferase, S, sulfotransferase.

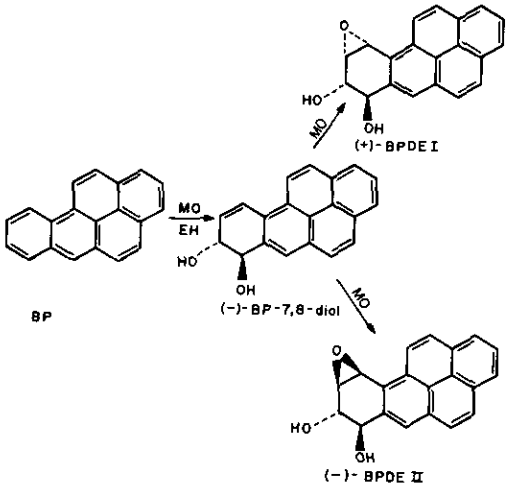

FIGURE 2. Formation of ultimate carcinogenic form of benzo(a)pyrene. Shown are the enzymatic steps involved in the formation of BP-7,8-diol-9,10-oxide. Monooxygenases oxidize BP to form the epoxide intermediate (not shown) and epoxide hydrase converts the epoxide to the dihydrodiol. The (−)-BP-7,8-diol form shown here is the predominate stereoisomeric form produced from this reaction. The second oxidation in the two-step oxidation process is also catalyzed by monooxygenases and results in the production of (+)-BPDEI, the major metabolite that binds to the nucleophilic sites on DNA, and (−)-BPDEII, another metabolite that binds to DNA. Diol epoxides formed from the (+)−BP-7,8-diol enantiomer are (−)-BPDEI and (+)-BPDEII and are found to bind to DNA to a lesser extent.

Anderson (*30*) have shown that BPDEI and BPDEII bind to lung, liver, colon, kidney, muscle, brain, and forestomach of the A/HeJ mouse and to the lung, liver, colon, muscle, brain, and blood of the New Zealand White rabbit (Tables 1 and 2). Eastman et al. (*32*) observed the formation of BP metabolite–DNA adducts in lung, liver, and kidney of mice. Dunn et al. (*31*) showed that BP metabolites bind to DNA in liver, stomach, colon, and intestine of Swiss mice following oral doses of BP. The adduct levels in liver and intestines were similar and these levels were 2 to 4 times higher than those in stomach and colon. Several investigators have shown that BP metabolites also bind to DNA in the skin of several mouse strains (*29,69–75*). In all cases, the major metabolite is the (+)-BPDEI bound to the N-2 of guanine residues as shown in Figure 4. As shown in the chromatogram, other adducts are also consistently observed *in vivo*: (−)-BPDEI–dGuo, BPDEII–dGuo and an unidentified peak. BPDEI and BPDEII may also bind to adenine residues except in smaller amounts. It is important to note that these adducts are formed in both tissues susceptible to PAH-induced neoplasia (target tissues) and tissues resistant to PAH-induced neoplasia. Similar adduct patterns are seen in each examined tissue in mice and rabbits regardless of dose, route of administration, or time of sacrifice after dosing (*30*).

In addition to similar adduct patterns in different tissues and species, it is surprising to find that the levels of the BP metabolite–DNA adducts are similar in tissues of the same BP-treated animal and that this similarity in adduct levels is independent of the oral dose level. As seen in Table 1, Stowers and Anderson (*30*) observed similar (+)-BPDEI–dGuo adduct levels in several tissues of the A/HeJ mouse. At both dose levels of BP, there was no more than a 2-fold difference in the specific activities of the BPDEI–dGuo adduct in the seven tissues examined. Adriaenssens et al. (*76*) showed similar results in the lung, liver, and forestomach of mice over a wider dose range of 2 to 1351 μmole/kg. These studies suggest no first pass effect in the liver for adduct formation after oral administration of BP to mice.

Similar BP metabolite–DNA adduct levels may also be seen after IV administration of BP. Table 2 shows that (+)-BPDEI–dGuo levels in several tissues of the rabbit after an IV dose of 4 μmole BP/kg. The liver, brain, and colon had the same adduct levels while the adduct levels in the muscle and blood were slightly higher. The levels of (+)-BPDEI–dGuo adduct in the lung were even higher, almost three to four times those in the other tissues. Eastman et al. (*32*) saw a similar pattern of binding levels in the A/J mouse after IV administration of BP. The levels of (+)-BPDEI–dGuo in the lung were three to five times those in the liver or kidney. Another study by Eastman and Bresnick (*77*) with a different PAH, 3-MC, shows the similar pattern of relative binding of adducts in various tissues after an IV dose in several mouse strains. In the four mouse strains examined, there is a 2- to 4-fold higher amount

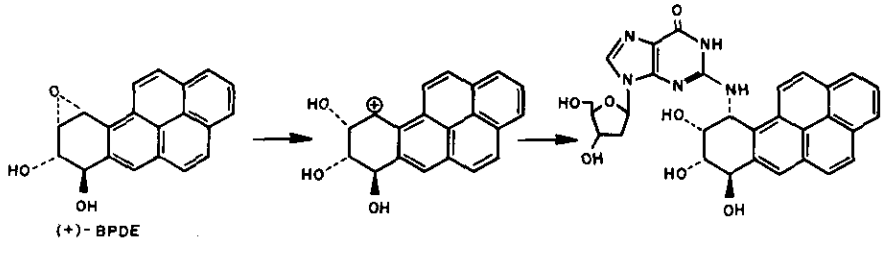

FIGURE 3. Interaction of (+)-BPDEI and deoxyguanosine to form a DNA adduct. (+)-BPDEI spontaneously decomposes to the carbonium ion triol intermediate. The electrophilic intermediate then interacts nonenzymatically with the nucleophilic sites on the DNA. Illustrated here is (+)-BPDEI binding to the N-2 of guanosine.

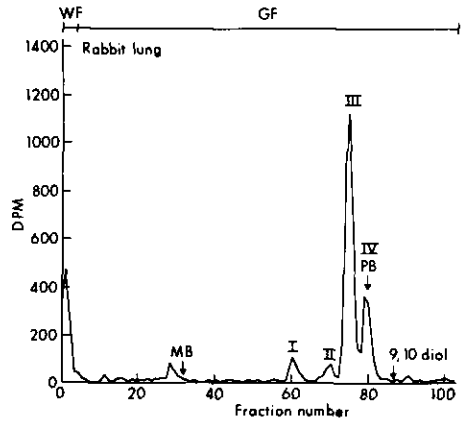

FIGURE 4. HPLC of BP metabolite–deoxyribonucleoside adducts in New Zealand White rabbit lung IV dose of BP. Rabbits were sacrificed 24 hr after an oral dose of ^{3}H-BP (4 μmole/kg). DNA isolated from these tissues was enzymatically digested and the deoxyribonucleosides were chromatographed on HPLC (*30*). WF, water fraction; GF, gradient fractions. Peaks II, III, and IV have been identified as (−)-BPDEI–dGuo, (+)-BPDEI–dGuo, and BPDEII–dGuo adducts, respectively. Peak I is discussed elsewhere (*30*). The specific activities (pmole/mg DNA) of the adducts are given in Table 2. The arrows (↓) denote positions of the internal standards (MB, methyl-*p*-hydroxybenzoate; PB, propyl-*p*-hydroxybenzoate; and 9,10-diol, BP-9,10-diol).

of 3-MC metabolites bound to DNA in the lung than in the liver. These reports indicate that there is a first-pass effect in the lung for adduct formation after IV adminstration of BP as well as other PAH causing the higher relative binding of PAH metabolites to DNA of the lung.

In contrast to mice and rabbits, no generalization can be made at present on the binding of BP metabolites to DNA of rat tissue. In the study by Boroujerdi et al. (*78*), the BPDEI–dGuo adduct was not the major adduct observed in lung and liver 1 hr after an IV dose of BP.

Table 1. BPDEI-dGuo adduct levels in various tissues ofA/HeJ mouse.[a]

Tissue	Adduct levels[b]	
	Low dose (11.9 μmole/kg)	High dose (1190 μmole/kg)
Lung	0.028	6.1
Liver	0.019	5.4
Forestomach	0.031	5.5
Brain	0.012	2.9
Kidney	0.014	3.3
Colon	0.013	2.7
Muscle	0.014	3.2

[a] A/HeJ mice were sacrificed 48 hr after an oral dose of ^{3}H-BP. The mice were divided into two groups of 30 mice: one group received 11.9 μmole/kg and another group received 1190 μmole/kg. DNA was isolated from each pooled tissue (lung, liver, forestomach, brain, colon, kidney, and muscle) and enzymatically digested to deoxyribonucleosides. The deoxyribonucleosides were chromatographed on HPLC.

[b] These numbers represent the specific activity (pmole/mg DNA) of the (+)-BPDEI-dGuo (peak III in the chromatogram in Fig. 2).

The major adduct formed probably results from the interaction of 9-hydroxy-BP-4,5-oxide with DNA. However, Baer-Dubowska and Alexandrov (*79*) observed the same profile in rat skin as seen in mouse after topical application of BP. It is also interesting that studies in cultured rat hepatocytes yield conflicting data: Ashurst and Cohen (*80*) observed that the BPDEI–dGuo as the major adduct whereas Jernstrom et al. (*81*) observed the same major adduct as seen by Boroujerdi et al. (*78*). Obviously, more data are needed to resolve these differences observed in BP metabolite–DNA adduct profiles in rat tissue. In any case, as in rabbit and mouse, adducts were observed in each tissue examined and in the study by Boroujerdi et al. (*78*), the adduct binding levels were similar in lung and liver.

DMBA binding to DNA has been studied in the skin of several mouse strains (*69*). In all mouse strains studied, Sephadex LH20 chromatography of the DMBA metabolite–deoxyribonucleoside adducts in the skin were similar, with three peaks being observed (*69*). The major DMBA–DNA adducts appear to arise through the reaction of the bay region diol epoxide of DMBA with deoxyguanosine and deoxyadenine residues in DNA.

Abbott and Crew have examined the *in vivo* binding of 11-methyl ketone metabolites to DNA in lung, liver and skin of TO mice (*82,83*). The major adduct was formed from the interaction of the *anti*-3,4-dihydro-3,4,*trans*-dihydroxy-1,2-dihydro-1,2-epoxide metabolite with deoxyguanosine (*84*). This diol epoxide bound to deoxyguanosine was observed regardless of tissue susceptibility to PAH-induced neoplasia or route of administration (intramuscular, topical, or intraperitoneal). Similar total carcinogen–DNA binding levels were also seen in the three tissues after intravenous adminstration.

There are several possible explanations for the binding of PAH metabolites to the DNA of all tissue of the mouse and rabbit that have been studied. It is possible that oxidative metabolism of PAH in each tissue is sufficient to account for the observed DNA binding since monooxygenase activity has been detected in most tissues (*85,86*). However, with BP, there is obviously no correlation between the cytochrome P-450-dependent monooxygenase activity and the DNA binding. Because there is a 400-fold difference between mouse brain microsomes and liver microsomes in the metabolism of BP (*87*), it is surprising to see only a 2-fold difference in the BPDEI–dGuo adduct between the two tissues. Another incongruity is that the muscle, a tissue that has no detectable activity, still shows an appreciable amount of binding in the mouse and the rabbit. Another possible cause of similar adduct levels in various tissues could be compartmentalization of some cytochrome P-450 activity in the nucleus; although no data has been obtained to support this theory.

Other studies suggest that transport via a carrier protein can be responsible for the presence of BP metabolites in cells unable to metabolize this carcinogen. Hanson-Painton et al. (*88*) have shown that cytosolic proteins that transport BP from microsomes do exist.

Table 2. BPDEI-dGuo adduct levels in rabbits after IV administration of BP.[a]

Tissue	Adduct levels as specific activity, pmole/mg DNA							
	1	2	3	4	5	6	7	Mean ± SD
Lung	0.071	0.040	0.054	0.054	0.047	0.062	0.077	0.058 ± 0.013
Liver	0.017	0.018	0.020	0.015	0.017	0.014	0.015	0.017 ± 0.0002
Brain	0.018	0.013	0.015	0.011	0.0098	0.024	0.018	0.016 ± 0.005
Colon	0.026	0.014	0.015	[b]—	—	—	—	0.018 ± 0.007
Muscle	0.033	0.012	0.057	—	—	—	—	0.034 ± 0.023
Blood	0.029	0.030	0.038	—	—	—	—	0.032 ± 0.005

[a] New Zealand White rabbits were sacrificed 24 hr after an IV dose of ^{3}H-BP (4 μmole/kg). DNA was isolated from each tissue and was enzymatically digested to the deoxyribonucleosides and chromatographed on HPLC. Shown here are the specific activities (pmole/mg DNA) of the major adduct (+) BPDEI-dGuo.

[b] Specific activity of a peak not quantitated unless the counts in the peak were at least 100 dpm above background.

Others have shown a similar cytosolic protein to bind to and transport 3-MC. These proteins probably bind and transport BP and other PAH to their site of metabolism in microsomes (*89–91*). Lo et al. (*92*) postulate the presence of such a carrier for BPDEI in human and mouse cells. Sebti et al. (*93*) have shown that metabolism of BP in one cell type (activator cell) can result in the binding of BPDEI and BPDEII to DNA in another cell type (target cell). The activated BP metabolites bind to the carrier molecule, are transported from activator cells to target cells and bind to the DNA in the target cells. The transfer of these activated metabolites from one cell to another appears to be very efficient. The transport of diol epoxide metabolites of PAH may be important to the formation of DNA adducts in tissues of low metabolic capabilities.

Several dose-response studies for PAH metabolite–DNA adducts have been reported. Phillips et al. (*69*) treated C57BL/6J mice topically with DMBA at a range of 0.025 to 1.0 μmole/mouse. Three DMBA metabolite–DNA adducts, as determined by Sephadex LH20 chromatography, were present at each dose. They found that the formation of DMBA metabolite–DNA adducts in mouse skin varied nonlinearly with dose. Pereira et al. (*71*) examined the formation of epidermal BP–DNA adducts in ICR/Ha mice after topical doses ranging from 0.01 to 300 mg/mouse. The binding of BP metabolites to DNA was essentially linear throughout the dose range. Adriaenssens et al. (*76*) investigated the binding of BP metabolites to DNA in lung, liver, and forestomach of A/HeJ mice after oral doses ranging from 0.048 to 29.7 μmole/mouse. In lung and liver, the dose-response curve were sigmoidal whereas the forestomach dose-response curve was more linear. In each of these studies the dose-response relationships for PAH metabolite–DNA adducts approached linearity at low doses and, thus, there does not appear to be a threshold dose below which the binding of PAH metabolites to DNA does not occur.

In summary, PAH metabolites have been found to bind to DNA *in vivo* in every tissue that has been examined. This occurs regardless of species, dose, and route of administration. It is also quite surprising to find that similar levels of adducts are formed *in vivo* in the various tissues of the same PAH-treated animal whether the tissue is a target for PAH-induced neoplasia or not. Small differences in the relative binding levels are most likely due to route of administration and probable first-pass effects in particular tissues. The major PAH metabolite–DNA adduct observed has been a bay region diol epoxide metabolite bound to a deoxyguanosine residue. Previous studies have shown that this bay region diol epoxide–deoxyguanosine adduct is also the major adduct formed in human cell culture incubated with BP. These findings have strong toxicological implications because of the low but continuous exposure of humans to BP and other PAH in the environment.

Persistence and Repair of Adducts

Several *in vivo* studies have attempted to determine if persistence of PAH metabolite–DNA adducts in a particular tissue is causally related to its susceptibility to PAH-induced neoplasia. The data of Kulkarni et al. on persistence of BPDE adducts offer no explanation for the strain difference in susceptibility to BP-induced pulmonary adenoma (*94*). The data suggest that adducts may be more persistent in lungs of the resistant C57BL/6J strain than in the susceptible A/HeJ strain (Fig. 5). Several other studies are in agreement with this conclusion. Phillips et al. (*69*) showed that there was no correlation between persistence of DMBA metabolite–DNA adducts in mouse skin and susceptibility of various mice to PAH-induced neoplasia. Similar conclusions were reached with BP and 3-MC, although adduct levels were examined at only two time points (*32*). Pelkonen et al. (*95*) examined the disappearance of BP metabolite–DNA adducts in skin and subcutaneous tissues of C3H and C57BL/6 mice. The rates of disappearance of the adducts do not differentiate between the C57BL/6 resistance and the C3H susceptibility to BP-initiated subcutaneous fibrosarcomas. In contrast, Eastman and Bresnick (*77*) reported that the persistence of 3-MC metabolite–DNA adducts in mouse lung correlated with susceptibility of the various mouse strains to 3-MC-induced pulmonary adenomas (Fig. 5). The reasons for the discrepancy between the results of Eastman and Bresnick (*77*) and the other studies are unclear. In general, persistence of PAH metabolite–DNA adducts does

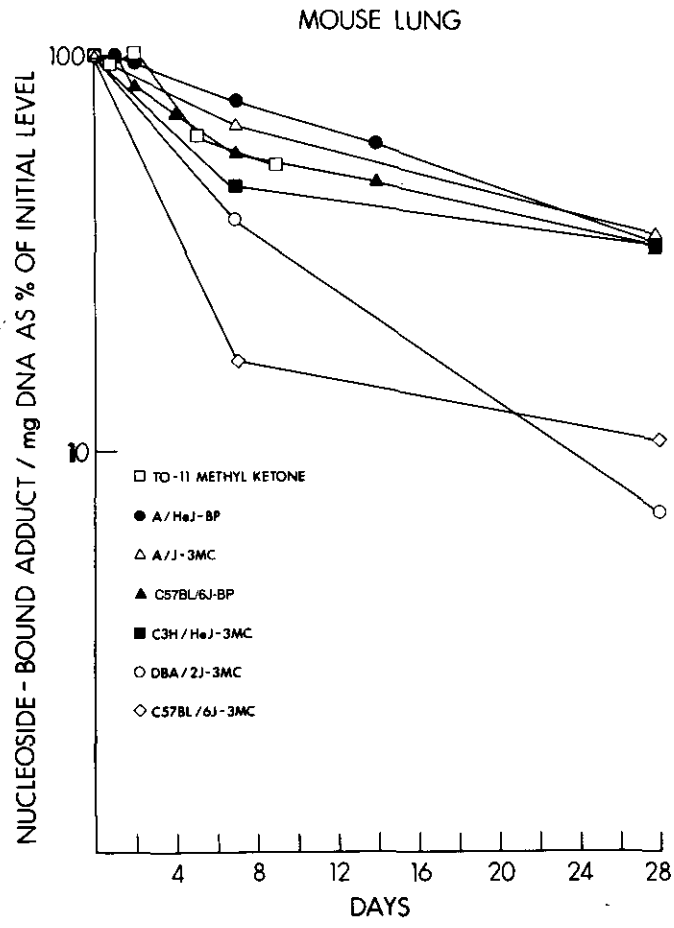

FIGURE 5. Disappearance of PAH metabolite–DNA adduct over time in mouse lung. The nucleoside-bound adduct per milligram of DNA is shown on the ordinate as a percent of the initial level of adduct: (□) Disappearance of 11-methyl ketone metabolite–DNA adduct in lung of TO mice (*83*); (●), (▲) disappearance of BP metabolite–DNA adducts in lungs of A/HeJ and C57BL/6J strains of mice, respectively (*94*); (Δ, ■, ○, ◇) disappearance of 3-MC metabolite–DNA adducts in lungs of A/J, C3H/HeJ, DBA/2J, and C57BL/6J strains of mice, respectively (*77*).

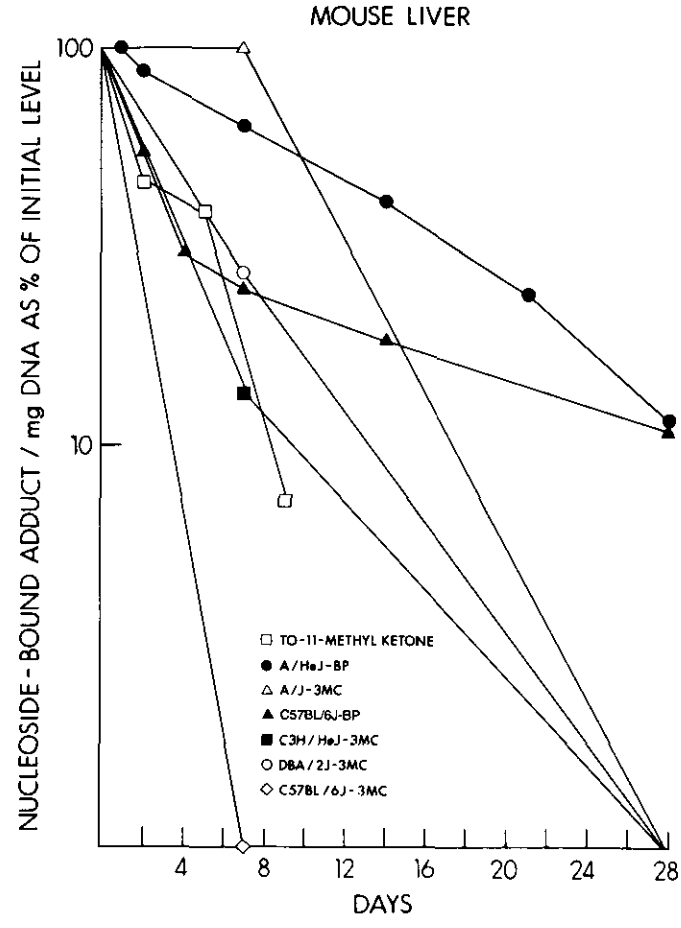

FIGURE 6. Disappearance of PAH metabolite–DNA adduct over time in mouse liver. The nucleoside-bound adduct per milligram of DNA is shown on the ordinate as a percent of the initial level of adduct: (□) disappearance of 11-methyl ketone metabolite–DNA adducts in livers of TO mice (*83*); (●, ▲) disappearance of BP metabolite–DNA adducts in livers of A/HeJ and C57BL/6J strains of mice, respectively (*94*); (Δ, ■, ○, ×) disappearance of 3-MC metabolite–DNA adducts in livers of A/J, C3H/HeJ, DBA/2J, C57BL/6J strains of mice, respectively (*77*).

not explain tissue susceptibility to PAH-induced neoplasia in various mice strains. However, it should be emphasized that the specific activities of the PAH metabolite–DNA adducts reported in the above-mentioned studies are calculated on the basis of the total DNA in the organ. It is possible that the amounts of adducts formed as well as their repair rates in different cell types of the target organ may vary considerably. Examination of the formation and persistence of PAH metabolite–DNA adducts in individual cell types of the target tissue might allow differentiation of tissues with respect to susceptibility and resistance to PAH-induced neoplasia.

A summary of the *in vivo* disappearance of PAH metabolite–DNA adducts in lung and liver of various mice strains is illustrated in Figures 5 and 6. The adduct level present in the tissue at a given time point is expressed as a percent of the initial value measured after administration of the PAH. The variations in disappearance rates are much greater in liver than in lung. In fact, the similarity in disappearance rates in lung is surprising.

Measurements of *in vivo* disappearance rates may reflect enzymatic excision repair; however, cell turnover can also account for adduct disappearance. In a recent study, Kulkarni and Anderson (*96*) examined BP-induced unscheduled DNA synthesis (UDS) in lung and liver of A/HeJ mice. UDS is a direct measurement of excision repair. BP induced UDS in liver but not in lung at the doses and time points examined. The procedure was able to detect UDS in lung, since 4NQO-induced UDS was observed (*96*). Thus the observed disappearance of BPDE adducts in liver of A/HeJ mice (Fig. 6) is due, at least in part, to excision repair whereas the disappearance in lung (Fig. 5) results from cell turnover (*96*). Abbott and Crew (*83*) also showed that normal DNA turnover rates could account for the disappearance of DNA adducts formed from metabolites of 11-

methyl ketone in lungs and skin of mice whereas excision repair was probably involved in removal of adducts from liver. Thus, both excision repair and cell turnover must be considered in assessing the mechanisms of *in vivo* removal of carcinogen metabolite–DNA adducts.

The presence of excision repair in the liver and the low rate of DNA synthesis by this tissue may provide an explanation for the relative resistance of this tissue to carcinogenesis by BP, since, under altered conditions of DNA replication following hepatectomy, tumors can be induced by PAH treatment (*12,97,98*). In contrast to the liver, the lack of excision repair *in vivo* of BP metabolites and the relatively high rate of DNA turnover in lung may be favorable conditions for the fixation of promutagenic lesions. The same arguments would hold for 11-methyl ketone, since lung and skin are target tissues whereas the liver is resistant to carcinogenesis by 11-methyl ketone under normal conditions. Thus, the balance between DNA repair and DNA replication is an important consideration in the study of mutagenesis and carcinogenesis.

In summary, DNA adducts formed from BP and other PAH are relatively persistent in tissues such as lung, skin, and brain. Persistence of PAH metabolite–DNA adducts in these tissues could be the result of particular cell types lacking excision repair. If these cell types also have slow turnover rates, accumulation of significant levels of PAH metabolite–DNA adducts could occur, especially if there is continuous long-term exposure of even low levels of PAH. The result of the persistence of bulky adducts on the DNA template could be inhibition of replication or transcription (*99*). Even if environmental exposure to PAH is too low to be tumorigenic, the persistence of DNA adducts may produce aberrations in transcripts of genetic information in various organs and lead to other toxic effects.

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